IMC 11 2018

INTERNATIONAL MYCOLOGICAL CONGRESS

July 16-21, 2018 | San Juan, PR

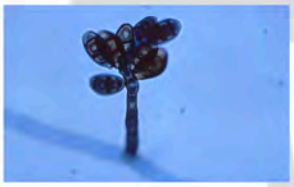
"Mycological Discoveries for a Better World"

San Felipe del Morro Castle, Old San Juan, Puerto Rico

Abstract Book







INTERNATIONAL MYCOLOGICAL ASSOCIATION IMA

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11th International Mycological Congress

Mycological Discoveries for a Better World

July 15-21, 2018

Puerto Rico Convention Center
San Juan, Puerto Rico

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16 July 2018

Dear Delegates of IMC11,

On behalf of the International Mycological Association (IMA) and the host organization the Mycological Society of America (MSA), we welcome you to the 11th International Mycological Congress (IMC11). Every four years, the mycologists of the world gather to share the latest, cutting-edge research on all aspects of fungal biology. We are excited for this opportunity to meet in San Juan in the spectacular Puerto Rico Convention Center for what we know will be a fantastic meeting filled with informative keynote addresses, in-depth symposia, intriguing poster presentations, interesting field trips, and inspiring social events.

The Local Organizing Committee, led by chair Sharon Cantrell and co-chair Jean Lodge, overcame enormous logistical obstacles following Hurricane María in 2017, and we owe them tremendous thanks for their efforts for this congress. The Scientific Programme Committee led by chair Chris Schardl and co-chair Don Pfister, have toiled for the past two years assembling the diverse scientific program of the meeting. The IMA and MSA are extremely grateful to these committees and all who dedicated their time and energy to the organization of this meeting, the University del Turabo for their support of the local organizing committee, and to all the sponsors and exhibitors for their contributions to the success of the meeting.

We expect IMC11 to follow in the footsteps of previous congresses as a life-changing, mycology-affirming experience for all delegates. With its common roots in field biology and laboratory science, mycology provides a unique opportunity for interaction and exchange between scientists with diverse technical backgrounds and from different cultures. We are particularly happy to welcome students to the meeting. Please take the opportunity to interact with as many of your colleagues as you can, whether you are a student, a professor or an emeritus. The world of mycology is here. Embrace it.

Sincerely,

Keith A. Seifert, PhD
President, International Mycological Association
ima-mycology.org || @IMA_Mycology

Thomas J. Volk, PhD
President, Mycological Society of America
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Congress Speakers: Keynote Address and Plenary Lectures

Keynote Speaker
Prof. Dr. Paola Bonfante, Università degli Studi di Torino, Italy.
Keynote address: *Fungi, plants, bacteria: A network of dialogues and interactions*

Pathology Theme
Dr. Anuradha Chowdhary, Univ. of Delhi, India
Lecture: *Fungal human pathogens: From obscure significance to impending disasters*

Applications Theme
Prof. Dr. Russell Cox, Leibniz Univeristät, Hannover, Germany, and University of Bristol, Bristol, UK
Lecture: *Heterologous expression: The key technique for investigating and engineering fungal secondary metabolism*

Genomics Theme
Prof. Dr. Chengshu Wang, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China
Lecture: *From one to many: Fungal genomics and the future of population genetics*

Cell Biology Theme
Dr. Jesús Aguirre, UNAM, Mexico
Lecture: *ROS signaling and fungal development*

Ecology Theme
Dr. Thomas Bruns, University of California, Berkeley
Lecture: *Experimental fungal communities: Tools for testing theory and determining mechanisms*

Evolution Theme
Dr. Priscila Chaverri, University of Maryland, College Park, MD, USA and University of Costa Rica, San José, Costa Rica.
Lecture: *Evolution of protective mutualism in plant-fungal endosymbiosis*

Environment Theme
Dr. Matthew Fisher, Imperial College, London,
Lecture: *Big data & big biology approaches to addressing big fungal problems*

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Andrea Porras-Alfaro, Environment
Jason Stajich, Genomics
Li-Jun Ma, Genomics
Jacque F. Meis, Pathology
Lydia I. Rivera Vargas, Pathology

Our sincere gratitude to Armando Soto (Webmaster), Jack Catheline (Registration), Astrid Concepción (Meeting Coordinator), Carmen Acevedo (Artisan Exhibition), María M. Claudio Rodríguez (Administrative Assistance), Program and Abstract Book Development by D. Jean Lodge, José R. Pérez-Jiménez, Melissa Palmer and Donald Pfister. Field trip guides: Paul Bayman, D. Jean Lodge, Sandra Maldonado, Joel Mercado, Kurt Miller, José R. Pérez-Jiménez and Yaritza Rivera. General collaboration provided by the Puerto Rico Institute for Microbial Ecology Research, Student Chapter at Universidad del Turabo (Gurabo, PR).

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Keynote Address

Fungi, plants, bacteria: a network of dialogues and interactions

P. Bonfante
Life Sciences and Systems Biology, University of Torino, Torino, ITALY

Abstract: Can we live without fungi? Microscopic entities or giants, friends or killers, degraders or producers, they all play crucial roles for the life on our planet. Their presence has accompanied the history of humanity, but for a long time, due their largely hidden and unseen actions, their importance was not fully acknowledged and their phylogenetic relationships with animals and plants were erroneously described. Nowadays we are aware that fungi are powerful organisms, which can offer us new pharmaceuticals, help cleaning up waters and soils from contaminants, and provide crucial support to the green inhabitants of the planet. The aim of this presentation is to illustrate different strategies developed by fungi in order to beneficially interact with land plants. Fossil data reveal that fungi resembling modern Glomeromycotina were already associated with first land plants around 450 MYA. However, only today, thanks to the use of -omics technology, we can decipher their enigmatic genomes, reconstruct their metabolic pathways, describe their impact on plants, and identify the molecules involved in the molecular dialogue with their hosts. Thanks to this huge amount of data, we can finally make hypotheses on the evolution and molecular mechanisms that make fungi so successful in time and space. Mycorrhizal fungi create networks not only with plants, but also with other soil inhabitants like animals, other fungi and bacteria. The dialogue with bacteria is particularly fascinating. Bacteria can live on the surface of mycelia and spores or, in a more intimate way, as endobacteria inside fungal structures. In the past, the interactions between bacteria and fungi were mostly described as of antagonistic nature, however most recent data report on cooperative activities between fungi and bacteria. Increasing attention is currently given to the concepts of microbiota and holobiont. In this context, on the one hand, mycorrhizal fungi are part of the plant microbiota, and represent a key component of the plant holobiont; on the other hand, they also possess their own microbiota. Thus, analogous to animals and plants, the fungal holobiont may be seen as a complex network of inter-kingdom interactions. An example of this tripartite symbiosis is given by *Gigaspora margarita*, an arbuscular mycorrhizal fungus that associates both with many plants and diverse endobacterial populations. Deciphering these multiple interactions will be a future goal, which may provide interesting insights into the capacity of mycorrhizal fungi to modulate their responses depending on the organism with whom they interact.

Plenary Lectures

Plenary Lecture 1 -- Applications

Heterologous Expression: The key technique for investigating and engineering fungal secondary metabolism

Russell Cox
BMWZ, Leibniz Universität Hannover, Hannover, GERMANY

Abstract: Fungi are extremely proficient producers of often complex secondary metabolites. The biosynthesis of these compounds is genetically encoded, usually by groups of clustered genes. Many fungi possess up to 100 biosynthetic gene clusters (BGC), each with the potential to produce one or more compounds, but most clusters are either silent (known product, but not produced) or cryptic (unknown product) or both. Numerous methods have been reported for the 'activation'; of such BGCs, but none is systematic or widely applicable except for heterologous expression. Rapid cloning methods in yeast, combined with a modular vector series and the tractable host *Aspergillus oryzae* now enable fast and reliable analysis of fungal BGCs from diverse sources, as well as the investigation of silent and cryptic clusters and the rational engineering of fungal secondary metabolites.

Plenary Lecture 2 -- Ecology

Experimental fungal communities: tools for testing theory and determining mechanisms

T. D. Bruns
Department of Plant and Microbial Biology, University of California, CA, Berkeley, USA

Abstract: We are in golden age of fungal community ecology. An explosion in research has been driven by a combination of high-throughput sequence methods, expansive public databases, improved statistical tools, and raw computational power. Together these have allowed us to sample and analyze fungal communities at levels that were impossible just a few decades ago. Collectively this work has now provided the first global views of fungi in some of the most important guilds and ecosystems, and has allowed us to sample fungi in forms and habitats that were previously inaccessible. Along with this expansion in our knowledge of patterns has come an increasing integration of fungi into the broader field of community ecology. In particular the fit of observed pattern to theory has become a common theme within this body of work, and this focus has resulted in a greater understanding of the drivers and functional consequences fungal community structure. However, the greatest impact of fungal systems on community ecology may be in their use for testing, revising, and creating new theory. This potential is based in part on the fact that community ecology theory is heavily biased by plant ecology. This is an advantage because like plants, fungi are sessile, territorial, and frequently limited by rates of dispersal and establishment. These shared features mean that much current theory developed in plant ecology applies well to fungi. However, fungi also differ from plants in the dominant types of competition employed and in the wealth of symbiotic interacts in which they are involved. Furthermore, many fungal communities are much more amenable to manipulation and replication on rapid time scales than are plant or animal communities. This means that theory can often be tested more rapidly and under more controlled conditions than is possible with other types of organisms. This talk will illustrate these points with recent and historical fungal research within the broad field of community ecology.

Plenary Lecture 3 -- Pathology

Fungal human pathogens: from obscure significance to impending disasters

A. Chowdhary
Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, INDIA

Abstract: *Candida auris* that exhibits resistance to fluconazole (FLU) and markedly variable susceptibility to other azoles, amphotericin B(AMB), and echinocandins. This yeast has globally emerged as a nosocomial pathogen that can cause invasive infections. *Candida auris* was first described in 2009 by Satoh et al. as a novel *Candida* species, in the *Candida haemulonii* complex (Metchnikowiaceae), from a patient in Japan after its isolation from the external ear canal. Alarmingly, in less than a decade this yeast, which is difficult to treat, has become widespread across several countries causing a broad range of healthcare associated invasive infections that display clonal inter- and intra-hospital transmission. The fact that this yeast exhibit MDR clonal strains which are nosocomially transmitted is unusual in other *Candida* species. Therefore, the possible threat of its rapid spread in affected countries and its emergence in unaffected countries will not only challenge clinicians for its effective therapeutic management but will also bring high economic burden to countries especially in resource limited settings where modern identification facilities and access to antifungals other than FLU are limited. Also, substantial increase in azole resistance among clinical *Aspergillus* isolates is recorded world-wide raising concern if this very effective azole class of antifungals for treatment of invasive aspergillosis will still be effective to use in the coming years. This emergence of resistance has led to the hypothesis that the deployment of demethylation inhibitors fungicides in agriculture select antifungal resistance not only in target crop pathogens but also in those fungal species that co-occur in their environment, and opportunistically infect humans, specifically the saprophytic genus *Aspergillus*. Two hypotheses have been proposed to explain recent increases in triazole resistance in clinical settings: (i) the use of triazoles for prophylaxis and treatment in patients with pulmonary cavities. *A. fumigatus* produce spores in the cavity (asexual sporulation), which is probably an important condition that facilitates resistance selection. (ii) The extensive use of triazole fungicides in agriculture can lead to selection of azole resistant *A. fumigatus* in the environment which may infect the susceptible patient population. Several reported cases of triazole-resistant aspergillosis developing in triazole-naıve human and animal patients support the latter hypothesis. The threats by MDR fungal pathogens will pose real challenge especially in the era witnessing ever increasing susceptible patient population but limited by new/novel antifungal arsenals availability.

Plenary Lecture 4 – Evolution,

The Puerto Rico Mycological Soceity Carlos E. Chardón Lecture

Evolution of protective mutualism in plant-fungal endosymbiosis

P. Chaverri
Plant Science, University of Maryland, College Park, MD, USA

Abstract: *Diaporthe*, *Tolypocladium*, *Trichoderma,* and other Hypocreales). Second, I have studied intensively the evolution of ecological traits in *Trichoderma*, with emphasis on endophytic species. Results from my studies show that *Trichoderma* is not only ubiquitous in the soil, but also in living sapwood of various tropical trees. In addition, living woody plants contain exclusively endophytic *Trichoderma* spp. not found in any other niche. Evolution and radiation of endophytic species likely occurred from host/substrate shifts, from soil saprotroph to plant biotroph. Comparative genome analyses are also showing unique features in the endophytic species *Trichoderma endophyticum*. With solid taxonomy, species delimitation and phylogenetic analyses, it is then possible to infer the cryptic roles endophytes play in their hosts. This could be accomplished by evaluating their closest relatives and determining their most recent ancestors. Findings from these studies have implications for understanding certain evolutionary processes such as species radiations in some hyperdiverse groups of fungi, and for more applied fields such as the discovery and development of novel biological control strategies. Intersecting ecological and evolutionary studies have therefore served me to use the information in applied agriculture. For example, culture-dependent and -independent metabarcoding analyses show that *Trichoderma*, in addition to other antagonistic fungi, dominate the endophytic fungal community in wild tropical trees, demonstrating a protective mutualism that may be altered in monoculture plantations. My studies also indicate that species composition in seedlings in the wild is significantly different from adult trees and that putative pathogens are more frequent in seedlings and absent in adults, whereas mycotrophs are absent in seedlings and abundant in adults. I also show that endophytic fungi in fruits of *Ficus colubrinae* with potential to be plant pathogenic do not survive the digestive tract of the bat *Ectophylla alba*. Dispersed seeds may benefit from frugivores by a reduction in the number of potentially pathogenic taxa. These results support well-known hypotheses, such as the Janzen-Connell, Negative Density Dependence, and the Theory of Pest Pressure. With a better understanding of the evolution and ecology of endophytes, we have used many of these fungi in biological control tests against diseases of various tropical crops. For example, endophytic *Trichoderma* species are significantly more effective against several plant pathogens than non-endophytic isolates, including those from commercial preparations. In addition, some species can even promote growth. My research on phytobiomes in wild relatives of economically important tropical crops will continue to aim to understand their function in natural ecosystems and applications in agriculture.

Plenary Lecture 5 -- Cell Biology

ROS Signaling and fungal development

J. Aguirre, A.E. Mendoza-Martínez, F. Lara-Rojas, O. Sanchez
Cell Biology and Development, Instituto de Fisiología Celular (UNAM), México City, MEXICO

Abstract: *Aspergillus nidulans* as model system, we have shown that transcription factors (TF) SrrA, NapA and AtfA are individually required to survive oxidative stress and that they also regulate asexual and sexual development. *Aspergillus nidulans* transcription factor (TF) NapA is a member of AP-1 family, which includes fungal Yap1 and Pap1 TFs. Just like Yap1 and Pap1 orthologs, NapA accumulates in the nucleus in presence of H_2O_2, a behavior also observed in the presence of menadione, osmotic stress or glucose starvation. NapA is essential for H_2O_2 resistance and normal production of conidia, while, it represses sexual development and regulates cleistothecia pigmentation. By showing that *ΔnapA* mutants are unable to grow in arabinose, fructose and ethanol, we uncovered a novel role for NapA in carbon utilization. This is consistent with a transcriptomic analysis showing that during conidial development NapA is required for the regulation of at least 214 genes, including ethanol utilization genes *alcR*, *alcA* and *aldA*, as well as other genes involved in carbohydrate utilization, transcriptional regulation, drug detoxification and secondary metabolism. Peroxiredoxins are enzymes belonging to a conserved family of ~~peroxidases~~ that have been involved in H_2O_2 sensing and Yap1 and Pap1 activation. The phenotypic characterization of *ΔgpxA*, *ΔtpxA*, and *ΔtpxB* single, double and triple peroxiredoxin mutants in wild type or *ΔnapA* backgrounds shows that none of these Prxs is required for NapA function in H_2O_2 or menadione resistance. However, these Prxs participate in a minor NapA-independent H_2O_2 resistance pathway, while NapA and TpxA appear to regulate conidiation along the same route. While all these peroxiredoxins are not necessary for arabinose and fructose utilization, TpxA and TpxB are important for ethanol utilization, suggesting that the utilization of this carbon source involves a specific type of oxidative stress.

Plenary Lecture 6 -- Genomics

From one to many: genomics and the future of population genetics

Y. Lu, Y. Shang, G. Xiao, C. Wang
Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, CHINA

Abstract: *Metarhizium* spp. and *Beauveria bassiana* have been developed as environmentally friendly biocontrol agents against different insect pests. Phylogenetic analysis revealed that fungal entomopathogenicity is polyphyletic, so similar expansions of insect cuticle degrading proteases and chitinases reflect a convergent evolution during the arms race of fungus-insect interactions. Relative to the advances in understanding fungus-plant interactions, the mechanisms of the molecular pathogenesis of entomopathogenic fungi are rather limitedly understood. In particular, the machinery of effector-mediated inhibition of host immunity has not been well established in fungus-insect interactions. We found that the divergent LysM proteins are present in animal pathogens. By using the insect pathogen *B. bassiana* as a model, we revealed that two of 12 encoded LysM protein genes are required for full fungal virulence against insect hosts to deregulate insect immune responses and protect fungal cells from chitinase hydrolysis. For *M. robertsii*, a collagen-like protein can camouflage cell wall components for evading host immunity. In addition, *in vivo* metabolomics analysis revealed the dynamics of small molecules were produced by both the fungi and insect hosts during fungal invasion of hosts. In particular, the small molecules such as the cyclodepsipeptide destruxins produced by *M. robertsii* and dibenzoquinone oosporein by *B. bassiana* could be deployed by the fungus to inhibit host immune responses to facilitate fungal infection. The understanding of fungal molecular pathogenesis can facilitate the development of cost-effective mycoinsecticides.

Plenary Lecture 7 – Environment

The Mycological Society of America John S. Karling Annual Lecture

Big data and big biology approaches to addressing big fungal problems

Mathew Fisher
MRC Centre for Global Infectious Disease Analysis, Imperial College London, UNITED KINGDOM

Abstract: An unprecedented number of emerging fungal pathogens (EFPs) are emerging and causing disease in animals and plants, putting the resilience of wild and managed ecosystems in jeopardy. While the past decades have seen an increase in the number of EFPs, they have also seen the birth of new big-data technologies and analytical approaches to tackle these emerging pathogens. I explore the methodologies and bioinformatic toolkits that currently exist to rapidly analyse the genomes of unknown fungi, then discuss how these data can be used to address key questions that shed light on their epidemiology. I then show how new high-throughput experimental models, biochemical methods and informatics toolkits are allowing the fuller characterisation of ecological interactions that modify the outcome of EFPs as they occur, and speculate on future 'Big Biology' approaches that will transform our ability to tackle this increasingly important class of emerging pathogens.

Symposium Sessions • Tuesday, July 17, 2018

Symposium Session 1:

Fungi and Fungal Enzymes for a More Sustainable World

L. Lange and A. Tsang

S01-1 Enzymes of halophilic and psychrophilic fungi for a more sustainable world

C. Gostinčar, L. Perini, P. Zalar, N. Gunde-Cimerman
Department of Biology, University of Ljubljana, Biotechnical Faculty, Ljubljana, SLOVENIA

Abstract: Water is crucial for life as we know it. High salinity, drought and freezing all lead to decreased water activity and thus disturb the functioning of biological systems. In addition to this, ions of inorganic salts are directly toxic to the cells. Halophilic/halotolerant and psychrophilic/psychrotolerant fungi have evolved specialized molecular mechanisms for avoiding and managing these detrimental effects. Due to their excellent adaptability many of these fungi have great biotechnological potential, due to two reasons in particular: 1) Hypersaline, arid and polar environments are very specific and promote competition for the scarce resources, and are thus promising sources of novel and unique antibacterial, antifungal and/or antialgal compounds. 2) Enzymes from psychrophiles and halophiles are functional at low temperatures and high salinity and therefore interesting for sustainable cleantech biotechnological applications, such as degradation of macro-algae in cold Arctic, marine, hypersaline waters and snow and ice algae covering the surface of glaciers and ice sheets. Different life strategies of extremophilic fungi will be exemplified by five representative species: *Aureobasidium pullulans*, *Hortaea werneckii*, *A. subglaciale*, *Penicillium* sp. nov. and *Articulospora* sp. nov., which inhabit hypersaline waters of salterns around the world, Arctic glaciers and black Greenland ice sheet, respectively. Environmental data and information on their molecular mechanisms of adaptations combined with the knowledge produced by their genome sequencing will be presented for *Aureobasidium pullulans*, *A. subglaciale* and *Hortaea werneckii*, while *Penicillium* sp. nov. and *Articulospora* sp. nov. will be presented in the context of environmental data related to their recent discovery in the Greenland ice in association with the non-cultivable black ice algae. In the analysis of the genomes and transcriptomes we focused on (i) the presence and characteristics of genes involved in stress tolerance, (ii) the presence of biotechnologically important genes, in particular enzymes relevant for decomposition of abundant algal biomass. Introduction

S01-2 *Aspergillus pseudoterreus*: A fungal platform for organic acid production within the Agile BioFoundry

J. Magnuson[1], K. Burnum-Johnson[1], N. Hillson[2], H. De Paoli[2], K. Pomraning[1], Y.-M. Kim[1], J. Kim[1], S. Tripathi[1], J. Zucker[1], N. Munoz-Munoz[1], M.C. Burnet[1], S. Deng[1], Z. Dai[1], B. Hofstad[1], J. Collett[1], E. Panisko[1], Y. Gao[1]
[1]Biochemistry, Pacific Northwest National Laboratory, Richland, WA, USA; [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Abstract: The Agile BioFoundry (ABF) is a virtual consortium consisting of eight US National Laboratories funded by the Department of Energy, Bioenergy Technologies Office that leverages the combined resources of the partner labs to create an open BioFoundry for utilization by government, academic and industrial entities. Our implementation of the Design Build Test Learn cycle is focused on

the development of non-traditional hosts for the production of bioproducts (chemicals) and hydrocarbon biofuels. *Aspergillus* spp. are widely utilized in industry for the production of organic acids and enzymes, hence incorporating a representative of this genus in our foundry was important for building tools and knowledge for a practical organic acid host. *Aspergillus pseudoterreus* was chosen for its ability to produce high concentrations of organic acids natively (itaconic acid) at low pH and the interesting phosphate depletion condition that coincides with native organic acid production. Approaches to engineering the organism to produce non-native organic acids, such as 3-hydroxypropionic acid, as well as multi-omics approaches to learn more about the organism will be discussed.

S01-3 *Dichomitus squalens* as a model white-rot basidiomycete for plant biomass degradation

M. Mäkelä
Department of Microbiology, University of Helsinki, Helsinki, FINLAND

Abstract: Wood-degrading white-rot basidiomycetes are exclusively found on wood in nature, where they play a significant role in the degradation of all polymeric components of wood cell walls, including both polysaccharides and the extremely recalcitrant aromatic polymer lignin. The increasing number of fungal genome sequences has revealed that white-rot basidiomycete genomes typically possess a wider repertoire of genes predicted to encode diverse plant cell wall modifying enzymes compared to ascomycete fungi. Therefore, wood-degrading white-rot fungi have a high potential as a source of industrially interesting enzymes or enzyme sets. The white-rot fungus *Dichomitus squalens* is an efficient wood degrader, which is commonly found in the northern regions of Europe, Asia and North America. When grown on different wood and non-woody plant biomasses, *D. squalens* upregulates specific sets of genes and secretes the corresponding enzymes matching the composition of the different substrates. The ability of *D. squalens* to respond to the various plant biomass types, including those that do not exist in its natural habitat, makes it an ideal species to study modification and degradation of plant biomass. Four genome sequenced *D. squalens* strains providing the best coverage of a filamentous basidiomycete species to date together with recently established genetic transformation system further facilitate the use of this species to understand basidiomycete gene function and development of improved strains for biotechnological applications. We have also detected differences between mono- and dikaryotic strains of *D. squalens* to grow on and degrade plant biomass. To study this in more detail, we grew four dikaryotic and three monokaryotic strains of *D. squalens* on spruce wood sticks and analysed the cultures after two and four weeks for their transcriptome, proteome and metabolome. Highlights from this study will also be presented.

S01-4 Fungal plant biomass conversion is controlled by an integrated network of transcriptional regulators

R. De Vries
Fungal Physiology, Westerdijk Fungal Biodiversity Institute, Utrecht, NETHERLANDS

Abstract: For many fungi, plant biomass is the predominant carbon source, but also a highly challenging substrate due to its complex and variable composition. It consists mainly of polymers, of which the polysaccharides are the major carbon sources used by fungi. Secreted enzymes degrade these polymeric compounds to mono- and small oligomers that are taken up by the fungal cell. Filamentous fungi typically contain between 120 and 350 genes in their genome that encode plant biomass degrading enzymes. Therefore, it is important that the genes expressed by a fungus encode those enzymes that match the composition of the prevailing substrate. For this fungi have evolved an intricate

regulatory system that responds to the various mono- and disaccharides that are released from plant biomass. This system does not consist of a set of independent regulators, but rather of a network in which links between the individual regulators exist not only with respect to their target genes, but also by influencing each other's expression level. In this presentation the current knowledge on regulation of plant biomass degradation from several well-studied ascomycete fungi will be compared and linked, to provide an overall view of this highly complex process. The recent identification of the L-arabinose responsive regulatory systems in eurotiomycetes and sordariomycetes, which are a clear example of parallel evolution, will also be discussed, as well as the regulatory differences between species.

S01-5 Fungal host strain development: Unique protease regulatory genes from *Aspergillus* and *Trichoderma reesei*

P. J. Punt[1], M. Paloheimo[2], S.M. Mäkinen[2], K. Juntunen[2], T. Puranen[2], J. Vehmaanpera[2], W. De Bonte[1]
[1]DDNA Biotech, Utrecht, NETHERLANDS, [2]Roal Oy, Rajamäki, FINLAND

Abstract: The reduction of unwanted endogenous proteases has already been an important target for strain improvement of fungal host strains used in protein production for many years. Targeted deletion of specific proteases has been used extensively for this purpose. Surprisingly, only very little is known about regulatory circuits that specifically control fungal protease production. The only protease-specific regulator gene discovered to date is the *prtT* gene from *Aspergillus niger*, which encodes a canonical Zn2-Cys6 activator protein (Punt *et al*., 2008) involved in the expression of a wide range of protease genes. Interestingly, homologues of *prtT* are only found in *Aspergillus* species, whereas no *prtT* homologue is present in *Trichoderma reesei*. We aimed to discover a similar protease master switch in our research. *T. reesei* mutants with strongly reduced overall protease levels and strongly reduced expression of a number of protease genes were obtained using a biological screen for the selection of protease-deficient mutants (Braaksma *et al*., 2008). Genome sequencing of a number of these strains followed by SNP analysis revealed that several of these mutants carried mutant alleles from a single gene, which we termed *pea1* for *protease-expression-affected*. Disruption of this gene in both *T. reesei* and *Fusarium sp*. confirmed the role of *pea1* in protease gene expression. Intriguingly, the encoded protein does not show any similarity to known regulatory proteins, indicating that a completely new regulatory circuit may be governing protease gene expression in *T. reesei*, which opens the way to further research in this area.

S01-6 Production of recombinant enzymes mediated by CRISPER/cas9 in the filamentous fungus *Aspergillus niger*

A. Tsang
Department of Biology, Concordia University, Montreal, Quebec, CANADA

Abstract: We tested the utility of a comprehensive set of tRNA promoters in driving gRNA transcription *in vivo* in the CRISPR/Cas9 system in the filamentous fungus *Aspergillus niger*. For the majority tRNA promoters tested, we obtained mutation frequency of 82-97%. We further showed a gene replacement frequency of >90% when the recipient host is defective in non-homologous end joining DNA repair. I will describe the use of this approach for strain improvement and to facilitate the targeted integration of foreign genes to produce recombinant enzymes.

S01-7 How fungi and fungal enzymes can be used to upgrade underutilized bio-resources – exemplified by discoveries from Ascomycetes, Early Lineage Fungi and Yeasts

L. Lange
Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby, DENMARK

Abstract: Fungi and fungal enzymes can contribute significantly to meeting the UN SDG´s and to mitigating climate change by enabling improved use of global bio-resources. Four examples of recent discoveries of both scientific (increased understanding of fungal interaction with their substrate) and applied relevance will be presented: 1. How fungal enzymes (from *Onygena corvina*) can hydrolyze keratin, a recalcitrant proteinaceous substrate, by synergistic action of at least three different types of proteases; possibly also enabled by the AA11 LPMO monooxygenase (hypothesis proposed). 2. Overview of enzyme secretome of early lineage fungi, exemplified by analysis of species from all four phyla; revealing significant differences between the phyla; evidence for horizontal gene transfer from bacteria; and suggested origin of plant cell wall degrading enzymes. 3. How fungal enzymes can convert cell wall components of cereal to gut-health stimulating sugar oligoes (for improved health of man and animals). 4. Yeasts can convert plant biomass which so far has remained underutilized (as e.g. waste from wood processing or macroalgae) into animal feed: a protein rich yeast biomass = yeast cream. This is exemplifying how protein rich animal feed can be produced without use of land, pesticides, fertilizer and irrigation water

Symposium Session 2:

Membrane Dynamics in Fungal Cells

S. Bartnicki-Garciá and B. D. Shaw

S02-1 Endosome-coupled mRNA transport during fungal growth

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Abstract: *Ustilago maydis*. In highly polarized cells of this fungus microtubule-dependent co-transport of mRNAs and endosomes is essential for efficient polar growth. We discuss a novel concept of endosome-coupled translation that loads shuttling endosomes with septin cargo, a process important for correct septin filamentation. Key players are RNA-binding proteins containing RNA recognition motifs for mRNA binding as well as Mademoiselle domains for protein/protein interaction. Here, new insights on protein RNA as well as protein-protein interactions will be presented. Interestingly, evidence is accumulating that RNA and membrane trafficking are also tightly interwoven in higher eukaryotes suggesting that this phenomenon is a common theme and not an exception restricted to fungi.

S02-2 Phospholipid markers and membrane traffic in *Neurospora crassa*

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Abstract: In fungal cells, specialized proteins gather in specific places to break cell symmetry and produce hyphae. This organization includes the orchestration of two distinct vesicle processes, endocytosis, and exocytosis that take place in tandem in different areas of the apical compartment in growing hyphae. Part of the signals for endocytosis and endocytosis include the asymmetry of the plasma membrane phospholipid bilayer. We studied the flippases, DNF-1, DRS-2 and DNF-4 that seems to be responsible for this membrane asymmetry in Golgi, vesicles and the plasma membrane. The mutation of *dnf-1* and *drs-2* genes produced alterations in the maintenance and stability of the Spitzenkörper and affected the actin cytoskeleton organization in the apical compartment. Surprisingly, neither of the flippases DNF-1 and DRS-2 was present in the plasma membrane; both were localized in different layers of the Spitzenkörper, associated to different secretory vesicles. DRS-2 was associated with vesicles transporting chitin synthases. DNF-4 seemed to be present in the Golgi equivalent. Each flippase is in charge of the localization of different phospholipids, their presence in different compartments can predict which phospholipid is more abundant. These results indicate that phospholipid flippases (P4 ATPases) may be important for the polarization of secretory vesicles, Spitzenkörper integrity and thus for the localization of many tip growing proteins.

S02-3 An ultrastructural view of the endomembrane network in hyphae of *Neurospora crassa*

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Abstract: The endomembrane network is a system of cytoplasmic membranes that partition the cell into functional and structural organelles and compartments, which together are involved in biomolecular synthesis, break down, and transport. The endomembrane network of *Neurospora crassa* hyphae was examined using transmission electron microscopy. All hyphae were prepared by cryofixation and freeze substitution protocols. *Neurospora crassa* hyphal tip cells contained three cytoplasmic regions based on content and organization. Region I corresponded to the hyphal apex and contained a well-defined Spitzenkörper composed of macro and microvesicles, plus cytoskeletal elements. Golgi equivalents (GE) and aggregations of cisternae with electron-dense lumen were present in this region. Region II extended behind region I approximately 10 to 20 µm and contained abundant vesicles, GE, and rough endoplasmic reticulum (rER). Smooth, flattened cisternae with electron-transparent contents were also present in region II. Endocytotic profiles along the plasma membrane were not common in regions I and II. The transition into region III was marked by abundant nuclei, multivesicular bodies (MVBs), vacuoles containing granular-like material, rER, GE, and flattened cisternae. Cytosolic surfaces of flattened cisternae were coated with a fibrous, electron-dense material. These coated surfaces were restricted to the edges of the flattened cisternae. Microtubules were in close proximity to GE, MVBs, and flattened cisternae. MVBs were diverse in size and shape. Observations of serial sections revealed that vacuoles and flattened cisternae were continuous

S02-4 Imaging the secretory compartments involved in CHS-4 biosynthesis in *Neurospora crassa*

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Abstract: In *Neurospora crassa* hyphae the localization of all seven chitin synthases (CHSs) at the Spitzenkörper (Spk) and at developing septa has been well analyzed. Hitherto, the mechanisms of CHSs traffic and sorting from synthesis to delivery sites remain largely unexplored. In *Saccharomyces cerevisiae* exit of Chs3p from the endoplasmic reticulum (ER) requires chaperone Chs7p. Here, we analyzed the role of CSE-7, *N. crassa* Chs7p orthologue in the biogenesis of CHS-4 (orthologue of Chs3p). In a *N. crassa Δcse-7* mutant, CHS-4-GFP no longer accumulated at the Spk and septa. Instead, fluorescence was retained in hyphal subapical regions in an extensive network of elongated cisternae (NEC) referred to previously as tubular vacuoles. In a complemented strain expressing a copy of *cse-7* the localization of CHS-4-GFP at the Spk and septa was restored, providing evidence that CSE-7 is necessary for CHS-4 to exit the NEC and for its localization at hyphal tips and septa. CSE-7 was revealed at delimited regions of the ER at the immediacies of nuclei, at the NEC, and remarkably also at septa and the Spk. The organization of the NEC was dependent on the microtubule cytoskeleton. SEC-63, an extensively used ER marker, and NCA-1, a SERCA-type ATPase previously localized at the nuclear envelope, were used as markers to discern the nature of the membranes containing CSE-7. Both SEC-63 and NCA-1 were found at the nuclear envelope, but also at regions of the NEC. However, at the NEC only NCA-1 co-localized extensively with CSE-7. Observations by transmission electron microscopy revealed abundant rough ER sheets and distinct electron translucent smooth flattened cisternae, which could correspond collectively to the NEC, thorough the subapical cytoplasm. This study identifies CSE-7 as the putative ER receptor for its cognate cargo, the polytopic membrane protein CHS-4, and elucidates the complexity of the ER system in filamentous fungi.

S02-5 Mitosis, nuclear migration and actin formation in *Schizophyllum commune*

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Abstract: *Schizophyllum commune* belongs to the white rot basidiomycetes and is relevant for wood degradation worldwide. As early colonizer after forest fire and of tree wounds, the fungus has also phytopathogenic importance. Its high competitive ability is based on the recognition of other bacteria and fungi, the production of specific extracellular metabolites and a strategy of fast growth. The fungal cytoskeleton, composed of a complex network of microtubules and actin structures, has a major impact on transport of vesicles as well as endo- and exocytosis processes. Visualization of the actin cytoskeleton in actively growing hyphae was performed with Lifeact-GFP. Thereby cortical actin patches were visualized at cell tips and clamps and as well as in subapical cells, preceded septation. The actin cytoskeleton in living hyphae during septum development shows close association with nuclear division. Clamp cell formation, typical of many model basidiomycetes including *S. commune*, indicated an aggregation of actin filaments to ring structures at the future site of nuclear division. Additionally, GFP-labeling of histone H2B enables visualization of nuclear movement and mitosis events in monokaryotic and dikaryotic cells. After mating events, fast nuclear exchange in anastomoses and hyphal cells were observed.

S02-6 Trafficking of membrane and endocytic cargo proteins in *A. nidulans*

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Abstract: During growth, filamentous fungi uniquely produce polarized cells called hyphae. It is generally presumed that polarization of hyphae is dependent upon a mechanism called apical recycling, which maintains a balance between the tightly coupled processes of endocytosis and exocytosis. Endocytosis predominates in an annular domain deemed the sub-apical endocytic collar, which is located in the region of plasma membrane 1-5μm distal to the Spitzenkörper (SPK). Here, a bioinformatics approach was utilized to methodically identify 42 *Aspergillus nidulans* proteins that are predicted to be cargo of endocytosis based on the presence of an NPFxD (or similar DPFxD) peptide motif. This motif is a necessary endocytic signal sequence first established in the model yeast *Saccharomyces cerevisiae*, where it marks proteins for endocytosis. The focus of this project is to examine the predicted endocytic association and function of these motif-containing proteins during hyphal growth using fluorescent markers and live-cell imaging. Many of these proteins have orthologs in budding and fission yeasts that have previously been shown to play a role in cell development and regulation of polar cellular morphology. Based on this data, we hypothesize that NPFxD or DPFxD motif-containing proteins in *A. nidulans* that are cargo for endocytosis will localize to at least one of three regions where cargo are characteristically observed. These predicted regions include the sub-apical collar, where cargo is actively endocytosed and internalized into the hypha, as well as the apical crescent, which lines the membrane at the apex of the hypha and terminates roughly where the sub-apical collar is predicted to begin. The third and final anticipated area of localization is the SPK, which contains two subsets of differently sized vesicles, and is considered to be the key determinant of hyphal growth and directionality. Proteins that are cargo for endocytosis can be observed in each of these areas based on the step(s) of the recycling process they are involved with during membrane turnover. At this time, we have observed localization to the predicted regions associated with hyphal growth for 9 of the 42 motif-containing proteins in *A. nidulans*. Mutants in these genes have varied in their ability to establish or maintain polarity and also display atypical development in various cell types, which suggests that the genes in question are involved with membrane turnover.

Symposium Session 3:

Bringing the Dark Taxa into the Light - Prospects and Challenges

D. Hibbett and M. Ryberg

S03-1 Sequence-based diversity of Glomeromycotina – why, how, and where next?

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Abstract: Availability of DNA sequencing approaches to identify fungi in samples from nature revolutionized the research on fungi and are now standard. DNA sequencing of fungal specimens and cultures, and natural soil and root samples has considerably changed the understanding of the diversity of Glomeromycotina, fungi forming arbuscular mycorrhiza (AM). It is now possible to identify AM fungal taxa by DNA sequencing irrespective of the presence of microscopically identifiable structures. Further developments of species proxies (Virtual Taxa, VT; Species Hypotheses, SH) and concurrent systematic organization of information about their occurrences in databases provides tools and data for broad research community to target these fungi in studies from taxonomy to physiology and genomics or to ecosystem sciences and beyond. However, several questions remain unsolved. How to handle "unnamed" diversity? How to appropriately delimit DNA sequence based species proxies? How to maintain databases? Do the unnamed AM fungi carry biological properties that differ from those of cultured "lab rats"? To illustrate some of these issues, I will tackle *Rhizophagus intraradices-irregularis* species group. I'll present the current knowledge of the phylogenetic diversity within the group in the context of recent genotyping data, the global geographical and habitat-wise distribution in the group, and the share of data in culture collections vs DNA sequences from various samples. I will conclude with future prospects in the direction of unravelling the AM fungal diversity and its patterns in the nature.

S03-2 Untapping the diversity and function of novel fungal rhizobiomes

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Abstract: Mycorrhizal and pathogenic fungi dominate curated databases of root-associated fungi limiting our capacity to identify and define specific functions of other fungal symbionts found in plant roots. Next generation sequencing and the use of fungal-plant bioassays can guide studies for the discovery and characterization of novel and specialized fungal species across multiple environments. This talk will discuss the importance of technology integration and collaborations to advance our understanding of plant-fungal interactions, the discovery of dark taxa, and the emergent properties that result from complex microbial interactions. I will focus on the use of targeted culturing efforts based on Illumina sequencing data to discover and characterize abundant but unknown fungal species.

S03-3 *Hawksworthiomyces sequentia* ENAS: a case study in DNA-based taxonomy

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Abstract: The increasing numbers of studies using environmental nucleic acid sequences (ENAS) to assess fungal diversity have revealed the existence of thousands of previously unknown, and in many cases unculturable, species. It is estimated that more than a third of all fungal DNA sequences in GenBank are of environmental origin. But inconsistent annotation of these undescribed, sequence-based taxa limits functional access to the data. Consequently, these ENAS are rarely considered in other

studies, especially not in taxonomic treatments. This problem is confound by the fact that the International Code of Nomenclature for Algae, Fungi, and Plants at present prohibits the description of novel taxa known only from ENAS, which discourages taxonomists to include these sequences in their studies. Various options have been suggested by members of the mycological community to amend the Code to allow the systematic nomenclatural treatment of these 'orphan' taxa. One possibility would be to allow DNA sequences as types instead of the typical herbarium specimens, graphic representations or living cultures. As an example, a new species with an ITS sequence as type was recently described in a study based on two matching ITS sequences of fungi inhabiting conifer wood, but that was generated in two earlier, independent studies. One came from an uncultured fungus clone from spruce in Sweden, and the other from a culture from cedar wood in Canada, that later died. The lineage containing these two sequences was phylogenetically different from related species in the Ophiostomatales and was described as "*Hawksworthiomyces sequentia* sp. nov. ENAS". It was suggested that in cases like these the ENAS acronym should be used with the species name until a specimen is found and designated as type, after which it can be omitted. Of importance is that this novel ENAS species was described adhering to currently accepted phylogenetic standards in the Ophiostomatales. The inclusion of environmental LSU sequences in the same study, furthermore confirmed the presence of a novel genus in the order that was represented previously only by a single taxon. This genus seems to be biologically and ecologically different from other genera in the order, and thus enhances our understanding of evolutionary processes in this group of fungi. In this rather modest case study we showed that much value can be added by including environmental sequences in taxonomic studies. What is clear is that a decision and guidance is needed from the mycological community that will allow for and enable the systematic naming of sequence-based taxa.

S03-4 Dealing with taxa known only from DNA sequences

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Abstract: The number of sequences in public sequence databases without specific taxon name are steadily increasing. It is clear that many of these sequences represent taxa that have previously not been recognized by the scientific community, but that are important for our understanding of fungal diversity, ecology, and evolution. For example, *Archaeorhizomyces* is a group with worldwide distribution and many species, but searching biodiversity databases such as GBIF and Species Fungorum there are only two species listed, since they are the only that have been possible to typify and name according to the rules of the International Code of Nomenclature of algae, fungi, and plants (ICN). Even if sequences without specific taxon names are now being included into some taxonomic studies, and species are being described to accommodate them, they are usually ignored due to the lack of valid name under the ICN. What is included in biodiversity datasets does therefore often depend on nomenclature issues rather than taxonomic issues, even if taxa is what are of interest. There are many possible ways to amend this problem, for example: 1) We can separate our biodiversity data from the dependence of names. We then need some other unique identifier, preferably global identifiers that can be used to link different datasets. UNITE provides identifiers in the form of DOIs for species hypotheses, but not species. The identifiers consequently change with the dataset on which the hypotheses are based, and they are not stable taxon identifiers. We could construct another system based for example on the identifiers provided by taxonomic databases. However, DOIs and accession numbers alike may be suitable for database handling, but are less suitable for human communication. 2) We can use names not valid under the code of nomenclature, which are now being provided for some taxa, and include these in biodiversity datasets. The downside with this is that there is no available, agreed upon, rules to govern these names and resolve conflicts between them, i.e. nothing to fill the very function ICN was designed

for. 3) We can amend the ICN to include names for taxa without any physical voucher. One possible downside with this is that the number of descriptions of taxa that are in fact not new to sciences may increase significantly, and thereby the number of synonyms, making nomenclature and consequently taxonomy more cumbersome. The extent of the problems with names outside the code and names governed by the code will depends on the actions of the community in promoting good taxonomy and nomenclature through discussions, training and peer-review, but also on the actions of individual researchers. There is likely no system without any risks or drawbacks to deal with the issues of species known only from sequences, but we urgently need a solution to be able to present an as true picture of the fungal diversity as possible.

S03-5 A methodical approach to revealing dark taxa: *Hebeloma* as a test case

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Abstract: Today, species are increasingly identified by their ITS sequence. Accordingly, any taxon that can be recognized by its ITS alone can hardly be viewed a "dark taxon" and such taxa are likely to be discovered by concurrently widely applied methods such as barcoding or metagenomics. A different situation applies to taxa that do not have a distinct ITS sequence; these are unlikely to be discovered by these methods. Further, taxa that are represented by types that are not readily accessible to molecular methods often remain neglected. Here, we present an approach that links taxonomy, morphology and molecular data through the use of modern database functions, type studies and multi-locus-sequencing. The use of a digital database allows collection data and species morphology to be recorded as a set of parameters. This facilitates easy comparison of collections. Database queries may be built representing putative species profiles which may then be compared with the results of molecular analyses. Conversely, sets of collections forming clades in molecular analyses may be compared and analysed to determine which characters they have in common and the variation within those characters. After several rounds of comparing results from molecular analyses with sets of collections exhibiting common characters and refining the characters and character value ranges to a query, species descriptions may be assembled automatically from the database, corresponding to clades generated within phylogenetic trees. These descriptions have two major advantages over traditional methods: (i) they are based on easily traceable data, and (ii) they can be re-adjusted if more material becomes available. Keys may also be built, tested and refined through the same process. By entering into the database character values for types, existing names may be linked to sets of collections representing putative taxa. If molecular data is available for a type, this provides another line of evidence. More often than not, as the data sets are enriched, ecological and biogeographical patterns emerge to support the validity of the delimited taxa. Furthermore, multi-locus sequencing can also provide additional evidence on the species delimitation. This methodology allows the identification of taxa that could not be recognized by ITS alone. Our subject is *Hebeloma*, a genus of ectomycorrhizal fungi, which are often similar in appearance and for which molecular species divergence is low. In Europe, we managed to link 55 species to existing names and discover 29 new species through this approach. In North America, based on 944 collections contributed through the mycologist community and type collections loaned from herbaria, we have discovered 38 species not known from Europe. For 18 of these, we have been able to establish that they have been described before under at least one legitimate name.

S03-6 Creatures from the black lagoon: Generating reference sequences for uncultured marine fungi

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Abstract: High-throughput sequencing (HTS) has ushered in a new age of mycological exploration, revealing uncharacterized fungal diversity across disparate habitats. However, efforts to infer the true phylogenetic affinities of many novel fungal phylotypes are hampered by the maximum read lengths of most sequencing platforms (< 500 bp), which often provide little phylogenetic signal. Moreover, the increasing adoption of next-generation sequencing methods to characterize fungal communities supplants traditional culturing surveys, decreasing the generation and availability of high-quality reference sequences. Taxonomic identification of OTUs observed in environmental surveys hinges on well-curated databases of complete or nearly-complete reference sequences. Consequently, despite the detection of wholly unknown groups among the fungi, we are unable to put these taxa into a robust phylogenetic framework, nor can we add them to existing databases to inform future HTS studies. For this study, I explored using a third-generation sequencing platform (PacBio) to generate long reads suitable for phylogenetic analysis and improved taxonomic identification of uncultured fungi from poorly sampled habitats. I targeted a nearly 2,000-bp region of fungal rDNA spanning three loci commonly employed in environmental surveys of fungi: ITS1, ITS2, and LSU. Using a mock community approach, I calculated the error profile for PacBio's default parameters, from which I developed an analysis pipeline for environmental samples. Amplicon libraries from water and sediment samples originating from diverse marine habitats were then sequenced on the PacBio platform. I observed over 200 OTUs, with Ascomycota and Chytridiomycota exhibiting the highest diversity. Phylogenetic analyses placed most OTUs in the Dikarya to known marine genera, but marine OTUs allied to the zoosporic lineages represented novel clades. Locus-specific databases were shown to vary widely in their ability to assign the correct phylum-level taxonomy to marine OTUs outside of the Dikarya, demonstrating that biases in the taxonomic composition of extant reference databases can result in the failure to recognize OTUs from the early-diverging phyla in environmental sequence datasets.

Symposium Session 4:

Molecular mechanisms of human fungal pathogenesis

R. Cramer and L. Ma

S04-1 RNAi-dependent epimutations evoke transient antifungal drug resistance

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Abstract: Microorganisms evolve via sexual/parasexual reproduction, mutators, aneuploidy, Hsp90, or prions. Mechanisms that are detrimental can be repurposed to generate diversity. Microbes are known to evolve resistance to antimicrobial agents via pathways involving both stable and unstable genetic mechanisms, such as aneuploidy underlying azole resistance in *Candida albicans* and *Cryptococcus neoformans*. We discovered a new mechanism conferring antifungal drug resistance in the human fungal pathogen *Mucor circinelloides*. Spontaneous resistance to the antifungal drug FK506 was found to evolve via two distinct mechanisms. One involves Mendelian mutations conferring stable irreversible drug resistance; the other occurs via an epigenetic RNA interference (RNAi)-mediated pathway resulting in unstable, transient drug resistance. The peptidyl-prolyl isomerase FKBP12 interacts with FK506 forming a complex that inhibits the protein phosphatase calcineurin. Calcineurin inhibition by FK506 blocks *M. circinelloides* dimorphic transition to hyphae and enforces growth as yeasts. In some FK506 resistant isolates, mutations in the *fkbA* gene encoding FKBP12 or the calcineurin *cnbR* or *cnaA* genes confer FK506 resistance and restore hyphal growth. In other resistant isolates, no mutations are found in the known drug targets. Instead, RNAi has been triggered to silence the *fkbA* gene, yielding drug-resistant epimutants. FK506-resistant epimutants readily reverted to drug-sensitivity in the absence of FK506. The establishment of epimutants is accompanied by generation of abundant *fkbA* small RNAs and requires some known RNAi pathway components whereas others are dispensable. Surprisingly, epimutants occur at a higher frequency and are more stable in mutants lacking RNA-dependent RNA polymerase 1 (Rdrp1), revealing some RNAi components constrain or reverse epimutation. Silencing of the drug target FKBP12 appears to involve generation of a double-stranded RNA trigger intermediate using the *fkbA* mature mRNA as template to produce antisense *fkbA* RNA. Epimutational silencing may be stochastic, similar to Mendelian mutations, but differs as the altered phenotype is reversible in response to fluctuating environmental conditions. Our recent studies reveal novel components required for epimutation, including orthologs of the *Neurospora crassa* quelling inducing protein (QIP) and Sad-3 helicase (RnhA); interestingly, the *rnhA* gene is linked to the *Mucor sex* locus, suggesting sexual reproduction may activate epimutation similar to sex-induced-silencing in *Cryptococcus*. We found epimutants occur at a higher frequency in mutants lacking RNA-dependent RNA polymerase 3 (Rdrp3) or the RNaseIII-like protein R3B2. Rdrp1, Rdrp3, and R3B2 operate a non-canonical RNA degradation pathway suppressing RNAi-dependent epimutation by competition for targets. We generalized these findings by showing epimutations occur in a second species of *Mucor*, and identifying epimutations in the *pyrF* or *pyrG* genes conferring 5-fluoroorotic acid (5-FOA) resistance. These studies uncover a novel, reversible, transient epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity, with implications for antimicrobial drug resistance and RNAi-regulatory mechanisms in fungi and other eukaryotes. The full impact of epimutations in this and other genetic systems may have eluded discovery previously given their inherently unstable nature.

S04-2 *Fusarium*, the trans-kingdom pathogen

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Abstract: *Fusarium* head blight, *Fusarium* ear rot, and vascular wilting are –despite their names- typical diseases caused by *Fusarium* species in different plant hosts, including many economically important plant species. *Fusaria* have often specialised on a certain host and in some cases this virulence seems to be organised on supernumerary chromosomes containing the essential information for pathogenicity on one or a limited number of host species. As added bonus, *Fusarium* is capable of the production of a large variety of mycotoxins, often acting as virulence factors, contaminating harvested products. However, over the past years we also see *Fusarium* species as emerging pathogens both in human and animal. There they cause from relatively innocent, but actual quite frequent, nail and skin infections to rarer local, deep and in the growing group of immuno-compromised hosts even disseminated infections. These latter infections are connected with high mortality rates. In *Fusarium*, it has become customary to cluster closely related sibling species or lineages with little to no morphological differences in so-called species complexes: The opportunists on human and animal group into seven main species complexes: the *Fusarium solani*, *F. oxysporum*, *F. incarnatum-equiseti*, *F. fujikuroi*, *F. clamydosporum*, *F. dimerum* and *F. sporotrichioides* species complexes. In some cases, the human infections can directly be linked to trauma with infected plant materials, making *Fusarium* a true trans-kingdom pathogen. With the control of both plant and human pathogenic *Fusarium*, we are faced with several major challenges: One foremost being the limited availability of effective treatments as the species are generally very resistant to the available antifungals. Also, some species prove to vary in their susceptibility to those few effective drugs available, emphasizing the need for fast identification and suitable diagnostic tools which are at the moment limited available. Additional confusing factors are that especially for some of the clinically important species there has been limited nomenclatural stability over the years, obscuring at times available data, and the recent discovery of many new species.

S04-3 Exploring possible ecological niches for *Coccidioides* species endemic in New Mexico

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Abstract: Species in the genus *Coccidioides*, *C. posadasii* and *C. immitis,* are the causative agents for coccidioidomycosis, the disease commonly known as Valley Fever. Coccidioidomycosis is estimated to affect more than 150,000 humans each year and is one of the few fungal diseases to affect otherwise healthy individuals. While progress has been made in the clinical understanding of the disease, little is known of the natural biology. Eighteen clinical isolates derived from 17 individuals diagnosed with coccidioidomycosis collected from New Mexico were used in a multi-locus sequencing analysis to explore genetic variation within the state and between neighboring states. While New Mexico is predicted to have *C. posadasii*, results of our analysis indicate that both *C. immitis* and *C. posadasii* are present among clinical isolates in New Mexico. Five of eight infections for which patient ethnicity was known occurred in Native Americans, suggesting that further studies should be conducted to determine if American Indians represent a risk group for coccidioidomycosis. We are also taking a novel approach to screen small rodents for exposure to *Coccidioides* by two means: 1) a survey of frozen mammal lung tissue for fungal infections, and 2) an enzyme immunoassay that detects IgG antibodies against *Coccidioides* in a variety of mammalian species. This will produce critical information regarding animal

infection rates, the geographical distribution of infected animals, and relative spore loads in soils. Characterization of clinical and environmental isolates will allow us to understand the genetic variation that affects the virulence of these pathogens.

S04-4 Emerging pathogen *Candida auris* evades neutrophil attack

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Abstract: *Candida auris*, an emerging fungal pathogen, causes hospital-associated outbreaks of invasive candidiasis with mortality near 60%. Little is known about the pathogenesis of this species that has newly arisen in the last 10 years, and it is unclear why this species is rapidly spreading worldwide. Neutrophils are critical for control of numerous invasive fungal infections, including candidiasis. These leukocytes kill fungi through phagocytosis or the release of neutrophil extracellular traps (NETs), which are structures of DNA, histones, and proteins with antimicrobial activity. The objective of this study was to delineate the neutrophil response to *C. auris*. We hypothesized that an ineffective neutrophil response may account for the poor outcomes observed in patients. We examined interactions of human neutrophils with *C. auris* and included *C. albicans* for comparison. Neutrophil-*Candida* interactions were visualized by time-lapse fluorescent microscopy and scanning electron microscopy (SEM). We utilized oxidative stress indicator CM-H2DCFDA to measure the generation of reactive oxygen species (ROS) in neutrophils. NET formation was quantified by Sytox Green staining and assessed by SEM and immunofluorescent labeling of NET-associated proteins. Fungal viability was evaluated using microbiological counts and viability stains. We utilized a zebrafish larval infection model to evaluate neutrophil-*Candida* interactions in vivo. Imaging revealed the phagocytosis of *C. albicans* by human neutrophils at 1 h, followed by the formation of NETs by 4 h. In contrast, neutrophils appeared rounded upon encountering *C. auris* and rarely engaged in phagocytosis or produced NETs. As shown by Sytox Green staining, *C. auris* triggered negligible NET release by human neutrophils, with levels 7-fold lower when compared to *C. albicans*. *C. auris* did not induce neutrophils to generate ROS, a key signaling mechanism for NET formation. The ineffective neutrophil response to *C. auris* correlated with diminished fungal killing. Imaging of neutrophils in a zebrafish model of invasive candidiasis revealed the recruitment of approximately 50% fewer neutrophils in response to *C. auris* as compared to *C. albicans*. In conclusion, *C. auris* evades neutrophils by altering multiple aspects of their usual anti-candidal responses. This is linked to improved fungal survival. We propose that this diminished innate immune response may contribute to the unexpected virulence of *C. auris*.

S04-5 Identifying fungal determinants of keratitis pathogenesis through a reverse-translational approach

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Abstract: Fungal infections of the cornea (mycotic keratitis) are a significant cause of ocular morbidity and blindness worldwide. These infections are difficult to manage and many cases, despite treatment,

result in corneal perforation or require corneal transplantation. The aim of our work, therefore, is to better understand fungal genes and pathways that drive pathogenesis in the eye, and could consequently inform novel therapeutic strategies. To this end, our multi-site collaboration is analyzing clinical data and fungal isolates from patients enrolled in the Mycotic Ulcer Treatment Trial (MUTT), a large-prospective study completed in India to compare the efficacy of natamycin vs voriconazole monotherapies. We hypothesize that (1) the fungal isolates will be heterogenous with respect to various *in vitro* phenotypes, and (2) some of those phenotypes will correlate with patient outcome. Such phenotypes will then serve as putative virulence determinants and will be the focus of downstream molecular analysis. In this way, our search for fungal virulence determinants can be informed by context-specific clinical data, as opposed to candidate gene analysis based on work in disparate fungal species and/or disease models (e.g. the lung). *Fusarium* is the most common mold associated with keratitis both in the MUTT (50% of culture-positive cases) and in other studies, and so these isolates are the current focus our analyses. We first employed multi-locus sequencing to determine the species-level distribution across the 128 *Fusarium* isolates. The majority were *F. solani* (80%), followed by *F. delphinoides* (10%) and varying others. In addition to its prevalence, *F. solani* was also statistically associated with larger ulcer size across the patients. Initial assessment of the *F. solani* isolates has already revealed marked heterogeneity with respect to colonial morphology (e.g. pigmentation) and growth rates across physiological temperatures (30-37C). Ongoing efforts are aimed at screening metabolic and stress-related phenotypes, secreted protein profiles, as well as immune cell interactions. We predict that that these parameters will also vary widely across the isolates, and that correlation analyses against the clinical data will provide novel insights into *Fusarium* pathogenesis in the eye.

S04-6 Surface proteins and the interaction of *Lichtheimia corymbifera* and phagocytes

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Abstract: Mucoralean fungi can cause mucormycosis, a life-threatening disease in immunocompromised patients. In our study, we analysed the influence of different enzymatic treatments of the spore surface alterations on the phagocytosis by murine alveolar macrophages. Two strains which were shown to be virulent and attenuated in avian, invertebrate and murine infection models were used in this study. The spore surface was treated with different cell wall-degrading cell wall enzymes targeting carbohydrate and protein cell wall components. The highest phagocytosis index was achieved with the proteolytic treatments which encouraged us to do focus our research on the protein surface of spores. Proteomic analysis of the spore surface was conducted for both strains. About four-teen candidate proteins were found which were differentially abundant in either the virulent strain or in the attenuated strain leading to the hypothesis that these proteins may play a role in virulence. One of these candidate proteins is the hydrophobic surface binding protein A (HsbA) which was first found in higher abundance in the virulent strain of *L. corymbifera*. HsbA was first described as an adhesin in *Aspergillus oryzae*. Additionally, HsbA was purified from the insect-killing ascomycete *Beauveria bassiana* and identified to play a role in immunogenicity. The HsbA protein from the virulent strain of *L. corymbifera* was heterologously overexpressed in *Pichia pastoris*. After pretreatment of murine alveolar macrophages and spores with purified fractions of the HsbA protein, the phagocytic index was found to be enhanced

in comparison with unstimulated host cells. The findings presented in this study will open the door for the role of surface protein in the recognition of *L. corymbifera* by phagocytes of the innate immune system which raise important measures to mammalian infection models. Our prospect for the future research will focus on the identification of potential stimulatory effects of *L. corymbifera* surface proteins and their putative receptors on the surface of macrophages which possibly contribute to virulence.

Symposium Session 5:

Evolution and diversity of lichenization in the Basidiomycota

M. Dal Forno and R. Lücking

S05-1 The origin and phylogenetic diversity of lichen-forming fungi in the Basidiomycota

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Abstract: Lichenization is a major evolutionary lifestyle in Fungi, with approximately 20,000 species currently recognized and up to 28,000 estimated. Although most lichenized species (99.7%) are members of the Ascomycota, basidiolichens have evolved independently and relatively recently in at least four separate orders, making them excellent models to study the evolution of lichenization, since descent from non-lichenized ancestors can be reliably established for many groups. Molecular data have revolutionized the classification of Basidiomycota, including lichenized lineages, reflected in the changes to classification attempts made throughout history, starting with Zahlbruckner and during recent decades by Oberwinkler. Two surprising recent classification-altering discoveries are that clavarioid lichens belong to unrelated lineages and that mushroom-forming and corticioid lichens are closely related. Molecular approaches also led to the discovery of many new taxa. At present, over 300 species of basidiolichens are recognized and more than 700 are predicted, compared to only 40 species distinguished less than a decade ago. The highest diversity of basidiolichens is in the agaric family Hygrophoraceae, which harbors hundreds of newly described species in two distinctive lineages representing separate lichenization events. One of these (*Dictyonema* s.l.) is tropical to tropical-alpine and consists of species with a cyanobacterial photobiont, and the other (*Lichenomphalia* s.l.) is mainly arctic-alpine to tropical-alpine with species having a green algal photobiont. The *Dictyonema* s.l. clade is notable for an unusually high diversification rate, given its relatively young phylogenetic age, and a remarkable diversity of thallus morphologies, representing an evolutionary transition from loosely organized filamentous crusts with separate basidiocarps to highly derived foliose forms with integrated basidiocarps. Clades outside the Hygrophoraceae represent lichenization events that led to either little or no subsequent diversification (possibly *Athelia* in the Atheliales), genus-level diversification (*Multiclavula* in the Cantharellales) or family-level diversification with several genera and a growing number of known species (Lepidostromatales). It is expected that increased attention to the lichenized Basidiomycota will yield further insights into the process of lichenization and symbiosis in general, as reflected by the wide variety of topics featured in this symposium on 'basidiolichenology'.

S05-2 The genus *Cora* in Colombia: diversification of a hyperdiverse, basidiolichen-forming clade in the northern Andes

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Abstract: The genus *Cora* comprises foliose basidiolichens with resupinate hymenophore. This group was first revised in a monograph of the genus *Dictyonema* s.lat. in 1978 by Parmasto, at which time a single foliose species was accepted under the name *D. pavonium* (later changed to *D. glabratum*). Before 1978, historically a total of five species had been described, under the epithets *ciferrii*, *glabrata*, *gyrolophia*, *pavonia* (≡ *montana*), and *reticulifera*. The group remained monospecific until 2004, when a new species was discovered. Based on subsequent molecular studies, the genus *Cora* was formally reinstated, and accumulation of data on the ITS barcoding locus, together with detailed field studies, showed that this genus included a much higher number of taxa than previously assumed. A 2013 revision recognized 14 species, including reinstatement of the five historical epithets, and in 2014, 116 species were distinguished phylogenetically, based on 338 ITS accessions. The most recent study, dating from 2017, distinguished 189 species based on 651 ITS accessions and five historical epithets, 92 of which are formally described. In the 2014 study, a grid-map approach predicted that *Cora* may contain more than 450 species, a dramatic increase from a single taxon recognized until a decade before. Most of the currently recognized 189 species are restricted to the northern Andes and particularly Colombia. In the 2014 study, 46 out of 116 species (40%) were from Colombia (corresponding to 145 out of 338 ITS accessions or 43%), with 36 species (31%) exclusively known from that country. In the 2017 work, 87 out of 189 species (46%) were present in Colombia (corresponding to 366 out of 651 ITS accessions or 56%), with 73 species (39%) exclusively known from there. Of the 92 described species, 34 (37%) have their type locality in Colombia. This is in part due to sampling effort: of the 209 sampling grids defined in the 2014 study, 15 (7%) correspond in part or entirely to Colombia, roughly mirroring Colombia's proportional area cover within the target area (6%). Within the target area, 24 grids were sampled, three of which well-sampled, with five sampled (21%) and one well-sampled (33%) corresponding to Colombia, denoting a proportionally higher sampling effort in that country. However, the increased sampling effort also reflects the fact that *Cora* is most diverse in the wet (sub-)paramos of the northern Andes, characterized by the plant genus *Espeletia*, which range from Venezuela to Ecuador and are most extensively developed in Colombia. Molecular dating suggests that the diversification of *Cora* is temporally correlated with the uplift of the northern Andes during the past 10 million years, underlining the importance of Colombia as the center of diversification of the genus. Three examples of current studies in Colombia further highlight this notion: (1) testing the grid prediction method by visiting the southernmost Colombian grid, comparing detected and predicted number of species; (2) analyzing patterns of local endemism in the highly threatened Colombian paramos; and (3) documenting the discovery of novel taxa in the context of urban expansion in the metropolitan area of Bogotá.

S05-3 Diversity and evolution of lichenized Basidiomycota from Colombia

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Abstract: Basidiolichens are the morphological and physiological result of the symbiotic relationship between a basidiomycete fungus and green alga or cyanobacteria. Less than 1% of all lichen fungi are found in the Basidiomycota, whereas over 99% belong in the Ascomycota. However, even if comparatively low in species, basidiolichens appear in independent lineages in three orders: Agaricales (*Acantholichen*, *Cora*, *Corella*, *Cyphellostereum*, *Dictyonema*, *Lichenomphalia*, *Semiomphalina*), Cantharellales (*Multiclavula*) and Lepidostromatales (*Ertzia*, *Lepidostroma*, *Sulzbacheromyces*). Some of these groups are morphologically similar but phylogenetically unrelated, so their phenotypes have evolved independently in similar ways, such as in the genera *Multiclavula* and *Sulzbacheromyces*, both of which have a clavarioid basidiocarps and crustose thalli, hard to distinguish without genetic data. Thus, molecular methods are an important tool to elucidate the evolutionary relationships and classification in these lichen fungi. With the objective of recognizing the phylogenetic position and taxonomic identity of lichenized, mushroom-like Basidiomycota in Colombia, fresh material was collected in major Colombian biomes (Andes, Amazonas, and Chocó) and ITS barcode sequences were obtained, proving the first records of several of the aforementioned genera for Colombia. Based on phylogenetic analysis we propose three new and semi-cryptic species in the genus *Sulzbacheromyces* from the Amazon and the Chocó, a new polymorphic species of *Multiclavula* from the paramo region, a new *Acantholichen* without acanthohyphidia (previously considered a synapomorphy of this group), and a new non-filamentous species in *Dictyonema* clade as a potentially new genus. Their morphology, anatomy and phylogenetic relationships are described and discussed.

S05-4 The basidiolichens in China

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Abstract: Lichenization is a successful strategy of establishing a stable mutualistic relationship of fungi with phycobionts. However, lichens involve almost exclusively ascomycetes associated with either green algae or cyanobacteria, and some basidiomycetes which compose a polyphyletic assemblage of Agaricales, Cantharellales, Corticiales, and Lepidostromatales containing ca. 172 known species with most of the diversity occurring in the Hygrophoraceae (Agaricales), particularly in the genera *Cora*, *Dictyonema*, and *Lichenomphalia*. In China, four genera and twelve species, *Dictyonema* (3 spp.), *Lichenomphalia* (3 spp.), *Lepidostroma* (1 spp.), *Multiclavula* (5 spp.), were previously reported, but most of them have no molecular data and the phylogenetic systematical position is uncertain. In order to clarify basidiolichens species flora in China, field surveys were conducted around China, covering Fujian, Guizhou, Hainan, Sichuan, Taiwan, Yunnan, Xizang province, then morphology of old (collected in last several decades) and newly collected specimens were examined under the microscope. Newly sequenced species (18S, 28S, ITS) were combined with those published on GenBank and used for maximum likelihood analysis and Bayesian inference to investigate the phylogenetic relationships. Based on morphology, phylogeny and literatures, the basidiolichens in China were revised, specimens from China regarded as *Multiclavula fossicola* and *M. sinensis* belong to the Lepidostromatales, and are

transferred to *Sulzbacheromyces*. Chinese reports of *M. clara* and *M. vernalis* belong to species of Lepidostromatales and specimens identified as *M. mucida* belong to the non-lichenized genus *Clavaria*. Hence, evidence of *Multiclavula* occurring in China is lacking. Similarly, *L. calocerum* is excluded from the Chinese flora. The recently described *L. asianum* should be regarded as conspecific with *S. sinensis*, and detail observation on this species variation under different micro-ecology was recorded. Consequently, 2 family, 3 genera and 12 species were confirmed, including three newly described species: *Dictyonema yunnanum*, *Sulzbacheromyces bicolor* and *S. yunnanensis*, and two new combinations: *S. fossicolus* (= *Multiclavula fossicola*), *S. sinensis* (= *M. sinensis*), and one new record species: *Lichenomphalia velutina*. In addition, following basidiolichens were added into the Chinese lichen flora: *Dictyonema scabridum*, *D. sericeum*, *D. thelephora*, *Lichenomphalia hudsoniana*, *L. luteovitellina*, *L. umbellifera*.

S05-5 Unexpected basidiolichen diversity discovered in lowland Brazilian forests

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Abstract: Basidiolichens comprise a diverse ecological assemblage from a few different phylogenetic lineages, mainly in the Hygrophoraceae. Basidiolichens are mostly known from cool climates and are by far most diverse in montane regions in the Neotropics, both at genus and species level. Exceptions are the genus *Sulzbacheromyces* and crustose species of *Dictyonema*, which occur in dry and wet tropical lowland areas around the world. Brazil is the country with the highest lichen biodiversity on Earth, but much of this diversity remains as yet undiscovered. A 5-year programme of field trips to Amazonian and Atlantic rain forests, as well as to Caatinga and Cerrado regions in N and NE Brazil, revealed that basidiolichens are quite common in these lowland forest biomes and more diverse than previously known. *Sulzbacheromyces* is not only occurring in dry biomes, but present everywhere in Amazonian and Atlantic rain forests, often found on termitaria, a substratum that was until recently rarely explored for lichens. Crustose species of *Dictyonema* are omnipresent in all forest types and on all available substrata (including rock and living leaves), also in dry Cerrado forests, where they seem very much out of place. A large conchate *Dictyonema* of the *sericeum*-group was found on twigs just a few kilometres from the Atlantic Ocean; species of this group are characteristic for wet mountain forests, and there is only one previous lowland record from Thailand. *Cora* is the most speciose genus of basidiolichens. It is most speciose on soil, rock and low shrubs in mountain regions of the Neotropics, with only one Palaeotropical species known so far. The new species *Cora itabaiana* was described from a similar habitat in NE Brazil, but from low elevation. Surprisingly, a species of *Cora* was even found seven meters high on a (fallen) tree in an Atlantic rain forest reserve in Alagoas. As there is only rarely access to this specific habitat, it might indicate that *Cora* is more common as epiphyte in lowland areas. A possibly undescribed species of the genus *Lepidostroma*, a genus which was so far known only from the Andes and mountains in Mexico and Central America, was found to be locally common in disturbed places such as road banks in Atlantic rain forest. The basidiolichens found in Brazilian lowland forests are not phylogenetically related, but do belong to at least six different lineages, and the representatives are more related to species from Neotropical mountains than to each other. There is thus no evidence that the Brazilian lowland forest region is a center of speciation in the group. Only a very small percentage of the tropical lowlands has as yet been investigated by lichenologists. Our results suggest the existence of considerable unexplored basidiolichen diversity in Neotropical lowland forests.

S05-6 Microbiome of basidiolichens in the *Dictyonema* clade (Hygrophoraceae, Agaricales)

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Abstract: The *Dictyonema* clade, also known as *Dictyonema* sensu lato, is the most species-rich group of basidiolichens, with 136 currently accepted species in five genera. The clade occurs worldwide, but has its highest diversity in the Neotropics. The group shows a remarkable diversity of morphologies; the basal clade of *Cyphellostereum* and the genus *Dictyonema* sensu stricto are filamentous, while the other three genera (*Acantholichen, Corella* and *Cora*) are squamulose to mostly foliose. The photobionts of these lichens are cyanobacteria of genus *Rhizonema*, but relatively little is known about the associated microbiomes of these basidiolichens. Here, our main objectives were to investigate these microbiomes in different genera and species belonging to the *Dictyonema* clade to reveal whether microbial patterns found in herbarium samples were similar to specimens recently removed from their natural habitat. We first sequenced a partial region of the 16S rRNA gene (covering variable regions one and two) with multi-tag pyrosequencing (MTPS) in a 454 Roche instrument for 697 samples from 22 countries representing all major clades within *Dictyonema*. Then, we sequenced another region of the16S rRNA gene from 192 samples utilizing the Illumina MiSeq platform following the procedures of the Earth Microbiome Project. We found that the most abundant non-photobiont bacteria in these basidiolichens belonged to the Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Chloroflex*i*. The most common Proteobacteria are Alphaproteobacteria, in agreement with previous studies of ascolichens. Our preliminary results show that genera with filamentous morphology include a higher number of bacterial OTUs than foliose samples and that only a small percentage of OTUs are found in both filamentous and foliose basidiolichens. Finally, we observed that historical herbarium samples showed a decreased number of photobiont reads with metabarcoding sequencing, drastically changing the abundance pattern of bacterial taxa. Our results provide, for the first time, important insight into basidiolichen microbiomes and advance the current knowledge of these complex symbioses.

Symposium Session 6:

Evolutionary Genomics

S. Branco and J. Uehling

S06-1 Shared versus independent losses: evolutionary consequences of intracellular parasitism in cryptic Fungi

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Abstract: Phylogenomic analyses have suggested that a clade comprising eukaryotic parasites with the smallest known genomes, Microsporidia, and the phylum known primarily from environmental sequences, Rozellomycota, are at the base of the fungal phylogeny. However, the ecological and genetic similarities between these distant relatives remains unclear. Recently we compared genome data of Rozellomycota and Microsporidia with the newly acquired nuclear and mitochondrial genomes of *Paramicrosporidium saccamoebae* – an intranuclear parasite of amoebae. Our analyses demonstrate

that Microsporidia are nested within Rozellomycota, which forms a paraphyletic clade. Comparative analysis revealed that *P. saccamoebae* shares more gene content with distantly related Fungi than with its closest relatives, suggesting that genome evolution in Rozellomycota and Microsporidia has been affected by repeated and independent gene losses, possibly as a result of variation in parasitic strategies (*e.g.* host and subcellular localization) or due to multiple transitions to parasitism. To understand if this represents a larger pattern of independent gene loss within the clade, we have sequenced the genomes of new rozellids and another amoeba-parasite that previous work suggests may be the closest relative to Microsporidia. Here we will explore the variation in gene content and genome evolution within this clade of intracellular parasites.

S06-2 Using codon usage bias to predict ecologically adaptive metabolic pathways in the budding yeast subphylum

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Abstract: Since diverging about a half-a-billion years ago, the 1,000+ yeast species of the subphylum Saccharomycotina have diversified into every biome on Earth. The diversity of yeast ecological adaptation is underpinned by their ability to utilize a wide range of substrates and grow in a variety of environments. Traditionally, metabolic pathways that are key for yeast ecological adaptation have been identified through functional experiments in the laboratory, statistical analysis of associations between traits and environments, and by examining signatures of selection in the genes encoding metabolic enzymes. One genomic signature that has proven especially powerful at predicting gene activity but has yet to be widely employed in evolutionary ecological research, is codon usage bias or the differential use of synonymous codons within and between genomes. The strongest driver of genome-wide codon usage bias patterns is G/C mutational bias. Codon usage bias at the level of individual genes, however, is a consequence of selection for translational efficiency, and therefore, gene level bias is strongly associated with gene expression. We expect that highly expressed genes will show codon usage bias in favor of optimally translated codons and that networks of co-expressed genes will show bias in favor of the same set of codons. In this work, we use species-specific gene-based estimates of codon usage bias (as a proxy for genes and expression levels) to predict metabolic pathways that are highly active across the genomes of 332 budding yeast species. These active metabolic pathways are then compared to the known habitat features of these 332 budding yeast species to identify significant associations between highly active metabolic pathways and habitat features. In my presentation, I will report the results of these analyses. Identification of significant associations between metabolic activity predicted by codon usage bias analysis and habitat features will provide insight into which metabolic capabilities may be responsible for adaptation to specific environments. More broadly, this work also sheds light on the ability of codon usage bias to be more broadly used to predict ecologically relevant genes and pathways in other microbes--especially those that are currently unculturable.

S06-3 The role of gene flow in rapid adaptive evolution of fungal plant pathogens: a comparative population genomics study

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Abstract: Antagonistic co-evolution between pathogens and their hosts can drive rapid adaptive changes in both partners. Pathogens exert a strong selection pressure on their hosts, in particular on immune defense genes. At the same time, host resistance can be overcome in the pathogen evolving to escape host recognition or to suppress host defenses. The genetic innovations allowing rapid adaptation in this evolutionary "arms-race" can have various origins including mutational events, sexual recombination and gene flow. Genome-based studies of fungal pathogens have revealed a frequent contribution of inter-specific gene exchange in rapid evolution. We used a population genomics approach based on de novo assemblies of genomes and whole genome alignments to characterize the distribution of highly variable regions in the fungal wheat pathogen *Zymoseptoria tritici*. These regions are found throughout the genome, comprise around 5% of the total genome size and overlap with 600 predicted coding sequences. We performed window based phylogenetic analyses align the genome alignment and show that the highly variable regions overlap with regions of showing signature of past interspecific hybridization events. We detect a similar pattern in the closely related wild grass pathogen, *Zymoseptoria ardabiliae*, and some hybridization events have involved these two species. Overall, our results demonstrate a significant impact of frequent interspecific hybridization on the genome evolution of this important wheat pathogen. We speculate that gene flow act to fuel arms race evolution of *Z. tritici* with its host.

S06-4 Coccidioidomycosis in the surrounding landmasses of the Caribbean Sea is caused by cryptic *Coccidioides posadasii* populations

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Abstract: *Coccidioides posadasii* causes coccidioidomycosis in arid regions of the Americas. *C. posadasii* is comprised of at least two populations; Arizona (AZ) and Texas/Mexico/South America (TXMXSA). The exact range of *C. posadasii* in Central and South America is undetermined for many reasons. For one, the disease is sub-notified to local health departments. Second, fewer than 1,000 total cases across the region have been reported. The Caribbean region is bordered by the Caribbean Sea, and the surrounding continental landscape and islands may play an important role in the dispersion of *C. posadasii* through Mexico, Guatemala and Venezuela. To define the distribution of *C. posadasii* populations in Central and South America, we sequenced the genomes of 6 clinical isolates from Venezuela, 1 from Argentina, 2 from Mexico, 1 from Texas and 1 from Florida. References were assembled using the Unmanned Genome Assembly Pipeline using SPAdes as well the Pilon toolkit. Weincorporated 52 published genomes from *C. posadasii* to identify the genetic background of newly sequenced strains and develop hypotheses regarding the dispersion into Central and South America. Maximum Likelihood methods implemented in IQ-TREE software using jModelTest for model selection and 1,000 ultrafast bootstraps with Shimodaira-Hasegawa-like approximate likelihood ratio test were

performed for branch confidence. The genealogical concordance level was tested using the Bayesian concordance analysis implemented in the BUCKy. To avoid linkage disequilibrium effect, the SNP matrix was assessed in 2500bp blocks. Posterior tree distribution of each locus was individually tested via MrBayes under GTR nucleotide substitution model. SNP matrices were run using two independent Markov Chain Monte Carlo simulations and four chains for 10 million generations with samples collected every 1,000 generations. Tracer was used to check convergence between the two chains and individual loci. Time-scaled phylogenies were calculated for timing analyses in BEAST. Trees topologies were visualized using FigTree. Comparative phylogenomic analyses reveal that clinical strains from Guatemala and Venezuela are genetically isolated from the well-described populations AZ and TXMXSA, whereas the new Texan, Mexican and Argentinian isolates cluster with TXMXSA as expected. Analysis indicates that limited gene flow exists between Guatemala and AZ populations, whereas we observe nearly complete reproductive isolation from both AZ and TXMXSA among the newly sequenced Venezuela isolates. Interestingly, the isolate from a Florida patient was paraphyletic to the Venezuela/Guatemala cluster. Based on these observations, we propose new patterns of dispersion and endemicity through Central and South America. We provide strong evidence that the South American continent was colonized by at least two ancestral populations: one by a TXMXSA ancestral genotype, and the second by a Guatemalan ancestral genotype. Isolates from Brazil, Argentina and Paraguay cluster within the TXMXSA cluster whereas the Venezuelan clade shares a common ancestor with the Guatemalan cluster and together forms a newly designated "Caribbean" population, including the isolate from Florida, which is distinct from either AZ or TXMXSA. We propose that the Venezuela lineage was purified during migration through Central America to the semi-arid regions of the Paraguaná peninsula and the depression valleys of Lara and Falcon states.

S06-5 Genome-wide survey for understanding genetic basis of morphological evolution of septal pore cap in Agaricomycetes

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Abstract: Understanding genetic basis of morphological evolution is essential for clarifying the evolutionary history of fungi. To understand the evolution of Agaricomycetes, morphological character of septal pore cap (SPC) is one of the key characters to distinguish taxonomy. In our study, we are focusing three phenotypes of SPC (vesiculate, imperforate and perforate) and searched candidate causal mutation of the differences of SPC types from fungal genome sequences. Among these three phenotypes, vesiculate SPC is known as the most ancestral characters. After the emergence of imperforate SPC from vesiculate SPC, perforate SPC had been evolved from imperforate SPC at multiple times independently (morphological independent evolution). The objective of this research is detecting mutation correlated with morphological evolution of these three types of SPC in amino acid sequence level. As the first step, for detecting the gene correlated with the evolution from imperforate SPC to perforate SPC, we searched genes that has parallel substitutions correlated with the emergence of perforate SPC from imperforate SPC against orthologous gene datasets of 12 fungal genomes. When genes were clustered by SPC type rather than species phylogeny by phylogenetic analysis from each orthologs, the genes were extracted as candidate causal genes. By using these genes, we searched SPC-type specific sites that show differences of amino acid residue depending on the difference of SPC types. We also checked whether the substitution had been derived in the exact ancestral brunch that is reasonable to assume as the period of emergence of perforate SPC by ancestral sequences reconstruction. For detecting the gene correlated with the evolution from vesiculate SPC to imperforate SPC, BLAST search against vesiculate type species was conducted to know gene present/absent pattern

of detected gene. We detected *spc33* as a gene correlated to the morphological evolution of SPC. Amino acid substitutions D254E, K357R, V359I and P402R were observed during morphological independent evolution from imperforate type species to perforate type species in both lineages. When we checked each sites of multiple alignment of *spc33*, we found K357R and M/V359I are remained such differences in extant species. Therefore, same genetic changes were detected from the independent emergence of perforate SPC. The results of BLAST search showed that *spc33* was observed only from imperforate type species and perforate type species. Vesiculate type species and any other organisms did not have *spc33* homologs. In conclusion, correlated evolutionary event in amino acid sequences of *spc33* had been occurred during both the evolution from vesiculate SPC to imperforate SPC and the evolution from imperforate SPC to perforate SPC.

S06-6 Multiple evolutionary origins lead to diversity in the metabolic profiles of ambrosia fungi

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Abstract: Ambrosia fungi are an ecological assemblage of species cultivated by ambrosia beetles in their gallery as required nutrient sources. This nutritional mutualistic relationship with beetles has evolved at least 7 times in Dikarya (Ascomycota and Basidiomycota). However, whether convergence in ecology led to convergent metabolism in ambrosia fungi is still unknown. We compared the assimilation of 190 carbon sources in five independent lineages of ambrosia fungi and closely related, non-ambrosial species. These repeated comparisons, and the use of variation partitioning to separate the effects of phylogeny and ecology, enabled us to assess functional convergence versus phylogenetic divergence in the metabolic diversity of ambrosia fungi. Our results revealed no convergence in carbon utilization capacities among ambrosia fungi. Instead, metabolic variation among fungi was largely explained by phylogenetic relationships. In addition, the range of carbon usage was as diverse in ambrosia fungi as in non-ambrosial species. Our results demonstrate that carbon metabolism of each ambrosia fungus is determined by its inherited metabolism, rather by the transition towards symbiosis. In contrast to other fungus-farming systems of termites and attine ants, the fungal symbionts of ambrosia beetles are functionally diverse, which reflects their independent evolutionary origins.

Symposium Session 7:

Challenges in the Exploitation of Beneficial Fungal Secondary Metabolites

M. Stadler and R. Cox

S07-1 Discovery of new bioactive secondary metabolites from medicinal mushrooms: Identification, biosynthesis and bioactivity evaluation.

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Abstract: The Tibetan plateau is well known for its diversity ecological system and extreme environmental condition and harbors rich medicinal fungi, such as *Ganoderma* sp., *Ophiocordyceps sinensis*, *Tricholoma matsutake*. With the help of chemistry and bioassay-guided separation technology, over 900 natural products, including 460 new compounds (36 new skeletons), have been isolated from the medicinal fungi collected in the Tibetan region. The diterpene eryngiolide A and sesquiterpene

dimer Sterhirsutin A were selected as "hot-off-the-press" by Natural Product Reports. Bioactivity screening revealed 460 compounds possessing antivirus, anticancer, antibacterial, and antiplasmodial activities. More importantly, two diterpenes (cyathin R and Q) with in vivo anti-tumor activity, one diterpene with in vivo anti-inflammatory activity, one sesterterpene with in vivo anti-virus effect, and one meroditerpene with in vivo anti-diabetic and anti-obesity activity were obtained. In the field of the biosynthesis of diterpene, we reported the diterpene cyclase responsible for the synthesis of cyathane skeleton in the mushroom of *Hericium erinaceus*. Furthermore, a new group of diterpene cyclases in the superfamily of UbiA proteins were identified.

S07-2 Reading fungal genomes to discover and engineer antifungal natural products

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Abstract: Natural products from fungi have been critical to the development of antifungal agents in use in the clinic and as agrochemicals, including griseofulvin, echinocandins, and strobilurins. Other outstanding preclinical and clinical antifungal leads (sordarins, enfumafungin, aureobasidin, ASP2397) have originated from fungi. Fungi continue to be a promising source of low molecular weight molecules that regulate fungal growth, interact with essential fungal proteins, or modulate the activities of key fungal pathways. Genome sequencing and bioinformatic prediction of secondary metabolite-encoding genes now enable searches for relatives of antifungal-encoding biosynthetic gene clusters that can lead to discovery of even more antifungal metabolites. But can this prospective approach guide discovery of new antifungals belonging to unrecognized structural classes without the aid of bioactivity-directed data? We will relate our experiences in dissecting the distribution of gene clusters of some major classes of antifungal agents produced by fungi. Although genome mining approaches can reveal the potential chemical diversity encoded by sets of related gene clusters, the approach remains limited by the small number of available fungal genomes. Therefore, tracking down historical records and the strains responsible for producing reported antifungals, e.g., echinocandins, has been critical for completing the genetic map of biosynthetic families and for guiding genome mining studies. An expanded focus on the target pathogens for whole-cell screening can also widen the search for new antifungal metabolites. Most of our historical knowledge regarding antifungal activity of fungal metabolites has been based on data from whole-cells assays of *Candida albicans* and *Aspergillus* spp., and to a lesser extent, on data from some major crop pathogens. Other major pathogens, like *Cryptococcus* species, were typically tested for antifungal susceptibility only when *C. albicans* lead compounds were evaluated for their antifungal spectrum. We contend that *Cryptococcus*-centric screening of fungi for antifungal natural products offers an outstanding opportunity for the discovery of new antifungal therapies. In addition to using non-traditional pathogen as targets for discovery of antifungals, prospects can be complemented by targeting fungi with complex secondary metabolism but that have rarely been included in screening programs to date.

S07-3 Exploiting the secondary metabolome of tropical Basidiomycota

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Abstract: The phylum Basidiomycota comprises the mushroom-forming fungi and various other organisms that represent a considerable part of the global biodiversity. Recent molecular ecology studies have revealed an unprecedented, huge diversity of fungi in different habitats, including soil, plants and invertebrate animals. Most of these organisms remain unknown to Science and have never been cultured and studied for potential beneficial traits such as the production of antibiotics and other useful secondary metabolites. During the course or our search for new anti-infective agents from nature to combat the newly arising multi-resistant human pathogens, we embark on extensive exploitation of tropical fungi whose riches remain largely untapped for new bioactive metabolites. Our approach which has resulted in a rather high discovery rate of novel metabolites, is based on a combination of extensive field work and classical mycological know-how together with sophisticated methods of analytical chemistry and biotechnological process development. Numerous novel compounds with interesting biological activities, which have been discovered from new and hitherto untapped species from Thailand and Kenya will be presented.

S07-4 Molecular genetic studies of alkaloid biosynthesis genes in *Epichloë coenophiala*, a bioprotective symbiont of the forage grass, tall fescue

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Abstract: *Epichloë* species are fungal endophytes of grasses that confer significant defense against vertebrate and invertebrate herbivores, attributed in part to their production of up to four known classes of alkaloids: aminopyrrolizidines such as lolines, pyrrolopyrazines such as peramine, indole-diterpenes such as lolitrems, and ergot alkaloids such as ergovaline. *Epichloë coenophiala* is a common seed-borne symbiont (endophyte) of tall fescue, a popular grass for pastures and forage due to its high productivity, stand longevity, stress tolerance and pest resistance. The endophyte is a significant contributor to these characteristics, produces anti-insect lolines and peramine, but also ergovaline, which is toxic to livestock. Our focus has been to manipulate the ergot alkaloid genes in the endophyte, with the aim of improving forage quality of tall fescue. *Epichloë coenophiala* is a triploid interspecific hybrid with two homeologous ergot alkaloid gene clusters, designated *EAS*1 and *EAS*2. Its genome sequence revealed that *EAS*1 is near a telomere, and that *EAS*2 has an *lpsB*2 pseudogene due to a frame shift mutation. We developed a method to knock off chromosome ends without stable introduction of foreign genes, and we eliminated *EAS*1. The genome sequence of two independent mutants confirmed the elimination of *EAS*1 and absence of any foreign genes. Once reintroduced into tall fescue, these *eas*1 deletion mutants produced the ergot alkaloid intermediate, chanoclavine I, plus high levels of the spur product, ergotryptamine, but lacked ergovaline. Interestingly, intermediates such as agroclavine and lysergic acid were undetected, and this ergot alkaloid profile recapitulated that of some naturally occurring grass-*Epichloë* symbiota. Complementation of the *eas*1 deletion mutant with a functional *lpsB* gene restored ergovaline production, but still gave high levels of ergotryptamine. Comparison of the in symbio gene expression data between wild type, the knockoff and complemented strains was highly

variable, although two of the genes required for conversion of chanoclavine I to agroclavine were poorly expressed in the *eas*1 deletion mutant and *lpsB* complemented strains. Additional gene complementation studies are now underway to further decipher roles and regulation of the pathway genes. In addition, loline-biosynthesis genes have been introduced into the non loline alkaloid producer, *Epichloë hybrida*, and analysis of plants with those transformants have been generated and will be analyzed to investigate the biosynthesis of this important class of anti-insect alkaloids.

S07-5 Secondary metabolites from fungal cultures: The role of mycologists in multidisciplinary teams

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Abstract: Fungi comprise a hyperdiverse group of organisms capable of producing notable secondary metabolites such as Penicillin, cyclosporin A, and the cholesterol lowering class of statins. Approximately, 24, 000 fungal secondary metabolites have been reported in the literature; however, we have only scratched the surface with respect to novel bioactive compound discovery from fungi. In this presentation, I will discuss findings from two projects, which highlight our work on secondary metabolites from fungal cultures. In the first part, I will discuss how isolation and identification of fungal secondary metabolites was used for discovering a quorum-sensing inhibitor in a clinical isolate of methicillin-resistant Staphylococcus aureus (MRSA), which is an antibiotic-resistant pathogen causing global health threat. In the second part, I will discuss how mass spectrometry mapping of secondary metabolite biosynthesis in situ can be used to probe a series of ecological questions about fungi that may be lost through traditional natural products chemistry extraction protocols. Together, our results highlight the importance of interdisciplinary nature of our research on fungal secondary metabolites and its contribution to both basic and applied sciences.

S07-6 Bio-prospection of microfungi from Thailand

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Abstract: Fungi are regarded as prolific sources of novel secondary metabolites with prominent and selective biological activities that can serve as basis for the development of new antimicrobials, agrochemical pesticides and other useful compounds. In particular the mycobiota of tropical countries are still widely unexplored and can yield a plethora of novel chemical entities. Furthermore, fungi represent a rich source of nematicidal compounds, which are natural antagonists of nematode parasites and thus offer novel biocontrol strategies. During the course of the "GOLDEN MYCOLOGICAL TRIANGLE" project, on the functional biodiversity of the mycobiota inhabiting rainforests in Thailand, we recovered several interesting microfungi from plant debris. Plant samples were transported to the lab in plastic or paper bags and treated in moist chambers. Pure cultures were obtained by single spore cultures in WA and transferred to OA and PCA agar plates. Morphological features were obtained from fungi growing on OA or SNA supplemented with fragments of autoclaved pine needles, incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. DNA was extracted from cultures growing in MEA. ITS barcodes and LSU sequences were generated for all species. Additional molecular markers i.e. *rpb2* and *tef* sequences were generated for selected strains. A preliminary maximum likelihood phylogenetic analysis of the LSU sequences placed all fungal taxa in Ascomycota, except for

one isolate that was related to the Basidiomycota. Among the Ascomycota the isolates were distributed in four classes; mainly in Sordariomycetes (57.5%) and Dothideomycetes (32%) and to a lesser extent in the Eurotiomycetes (7.5%) and Leotiomycetes (3%). Familial, generic and species level identification for some of the microfungal strains still remain unknown, and they appear to represent new taxa. Researchers at HZI selected 18 strains for additional studies on biological activity, screening of nematode trapping fungi and secondary metabolites. So far, *Dactylaria hyalotunicata*, *Petrakiopsis* sp. nov. and *Sirothecium* sp. nov. represent three new lineages in Sordariomycetes. Furthermore, some isolates represent a new genus in Sulcatisporaceae introduced as *Pseudobambusicola*, and other isolates represent a potentially new genus related with *Exophiala* in Eurotiomycetes. Other new species include *Anteaglonium* sp. nov., *Brachiosphaera* sp. nov. and *Teichospora* sp. nov. in Dothideomycetes; *Campylocarpon* sp. nov., *Halorosellinia* sp. nov., *Hydea* sp. nov. and *Kionochaeta* sp. nov. in Sordariomycetes. Until now, *Pseudobambusicola thailandica* gen et. sp. nov. revealed strong antagonistic activity against nematodes (*Caenorhabditis elegans*). Six novel and two known compounds were isolated. Compounds 4 and 8 showed strong nematicidal activity, while compounds 1 and 8 also inhibited growth of the pathogenic basiodiomycete *Phellinus tremulae* in a plate diffusion assay.

Symposium Session 8:

Morphogenesis and Invasion (Fungal-Host Interactions)

M. Momany and M. Riquelme

S08-1 Enabling tools for the study of neutrophil-fungi interactions

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Abstract: During infections with *Aspergillus fumigatus* or *Candida albicans in* healthy individuals, neutrophils accumulate fast and in large numbers. The interactions between neutrophils and fungi are key for protecting healthy tissues, by sealing off sites of infection and neutralizing the pathogens. Better understanding of these interactions may provide new capabilities for protection against infections in patients at risk. However, currently, the investigations of the interactions between neutrophil-fungi interactions can only be studied in vitro and in animal models which are limited by lack of temporal and spatial control over interactions. In this presentation, we will discuss new approaches for studying neutrophil-fungi interaction at single-cell resolution over time. These approaches are enabled by microfluidic tools, which create precisely controlled, repeatable conditions for the interactions between neutrophil and growing fungi. In one example, we studied the interactions between human neutrophils and *Aspergillus* and observed an evasive fungal behavior triggered by interaction with neutrophils. Interacting hyphae performed de novo tip formation to generate new hyphal branches, allowing the fungi to avoid the interaction point and continue invasive growth. The consequence of branch induction upon interaction outcome depends on the number and activity of neutrophils available: In the presence of sufficient neutrophils branching makes hyphae more vulnerable to destruction, while in the presence of limited neutrophils the interaction increases the number of hyphal tips, potentially making the infection more aggressive. In another example, we found that human neutrophils swarmed vigorously against *Candida* and significantly delayed the growth of *C. albicans* hyphae for up to 16 hours. Disruption of swarming mediators compromised the ability of neutrophils to swarm and limited the ability to contain *C. albicans*. Neutrophil extracellular traps were formed during neutrophil swarming against both *Candida* and *Aspergillus*. However, the disruption of NETs only compromised the protection against Candida and not against *Aspergillus*. The novel capabilities enabled by microfluidic

devices have implications for our understanding of infections in neutrophil-deficient patients and open new avenues for treatments targeting opportunistic fungi.

S08-2 Septins impact hyphal and nuclear morphology

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Abstract: Septins serve as scaffolds and diffusion barriers, organizing cellular morphology. In several filamentous fungal pathogens loss of septins results in the emergence of extra protrusions from the hypha and decreased virulence. In *A. nidulans* deletion of septins *aspA*, *aspB*, or *aspC* causes extra protrusions to emerge from hyphae. In contrast deletion of *aspD* does not have a major impact of hyphal morphology; instead nuclei take on a "stringy" appearance and colony sectoring increases. A close examination of nuclei from *aspD* deletion strains shows that nuclear organization is disturbed, including improper positioning of the nucleolus.

S08-3 Elucidating three pathways that contribute to directional growth regulation in *Candida albicans* hyphae

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Abstract: The production of hyphae is strongly linked to pathogenesis during superficial mucosal infections and the life-threatening disseminated disease, invasive candidiasis. Hyphae constitute the 'Special Weapons and Tactics' capability deployed by *C. albicans* during mucosal and endothelial cell layer invasion. Hyphae are equipped with adhesins and secreted effectors but these are only effective if hyphal guidance mechanisms are operational to direct penetrative growth into host tissue. Our aim is to elucidate the signaling pathways involved in hyphal guidance. We have identified three distinct hyphal growth phenotypes – kinked, chaotic and straight - in which the ability to respond normally to external cues is attenuated or lost. Each of the three phenotypes is produced by a specific grouping of mutant strains. The functional links within some groupings are emerging and we are undertaking proteomics screens to extend our understanding of each pathway. Our overall aim is to find out how these pathways integrate to regulate the directional growth of hyphae. We have generated null mutant strains for proteins representative of the three phenotypes and GFP-tagged them to establish their cellular localization. We use live-cell imaging and microfabricated topographies to test for aberrant hyphal growth responses in mutant strains. The tagged strains are used as pull-down baits to identify interacting proteins. Hyphae with kinked or chaotic growth trajectories are generated by deletion of the fungal Paxillin homolog (Pxl1) or the small Ras-like GTPase, Rsr1, respectively. Proteomics suggests Pxl1 interacts with the Rho1 GTPase and β-glucan synthase, which are involved in cell-wall biosynthesis, while Rsr1 interacts with proteins involved in membrane organisation. An emerging theme in this study is the involvement of tip-localised GTPases, which cycle through active and inactive states. This mechanism for binding and releasing effector proteins may allow constitutively polarised cells to adjust the site of growth in response to external cues.

S08-4 Chitin assembly in the cell wall of two *Trichoderma* species

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Abstract: The cell wall of (filamentous) fungi serves as armor against attacks by other organisms or hostile environments as well as a disguise in (phyto-)pathogenic and plant beneficial species to circumvent host defense mechanisms. Next to glucans, chitin is one of the major components of the cell wall that confers strength and rigidity but contributes also to the flexibility of the wall. The homopolymeric chitin chains, composed of β-1,4 linked *N*-acetylglucosamine units, constitute the innermost layer of the cell wall and are connected to the glucan matrix via β-1,3 and -1,4 links. Chitosan, the partially deacetylated form of chitin, is another important component of the fungal wall, which is present in minute to high amounts (up to 40% in Mucorales) depending on the fungal family, cellular component or growth stage. *Trichoderma* spp. are a cosmopolitan group of fungi, with a multitude of adaptions to a variety of environmental niches. *Trichoderma reesei*, for example, is a saprophytic fungus, which is used as potent cellulase producer in the industries. *T. atroviride* and *T. virens* are mycoparasites, with plant beneficial attributes, and thus, are important as biocontrol agents. Although *Trichoderma* spp. had been studied extensively over the past decades, little was known about their cell wall composition. Here we provide a first insight in the composition of the cell wall of *T. reesei* and *T. atroviride*. We show that *de novo* chitin and chitosan synthesis involves coordinated regulation of members of chitin synthesizing and chitin modifying enzyme families, rendering the fungi capable of fast adaption to a variety of environmental stresses and growth conditions. Chitin biosynthesis requires a set of chitin synthases that belong to the glycosyl transferase family (GT) 2. Although a central catalytic domain is shared by these isoenzymes the N- and C- terminal ends can vary considerably. *Trichoderma,* furthermore, contains a variety of putatively secreted and intracellular chitin modifying enzymes, which contribute to the cell wall plasticity. We provide new insights into the assembly of chitin and chitosan in *Trichoderma* spp. Eight chitin synthases and more than 15 additional enzymes - deacetylases, chitinolytic enzymes and accessory proteins, which are important for correct assembly and turnover of chitin and chitosan - are involved in chitin metabolism in *Trichoderma* spp. Defining the mechanisms of fungal chitin and chitosan synthesis facilitates a guided approach in cell wall reconstruction, which contributes to understanding the mycoparasitic capability of *Trichoderma* as biocontrol agents and their adaptability to changing environments.

S08-5 Cell-cell contact during fusion triggers self/non-self-recognition in a social microbe

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Abstract: Cell fusion is required for the development of the hyphal network made by *Neurospora crassa*. Fusion involves chemotropism, remodeling of the cell wall and merging of the plasma membranes. In the wild, a cell will encounter numerous prospective partners with variable degrees of genetic similarity and fusing with them can be beneficial or detrimental for fitness. Therefore, in an attempt to judge between 'good' and 'bad' consequences, cells have developed genetic barriers that are put in place during cell fusion events. We have showed that among a wild population of *Neurospora* isolates, a long-distance kind recognition system defined by the allelic variation at the *doc* locus functions at the level of germling communication; only cells that belong to the same *doc* haplotype group are able to

communicate with each other. Interestingly, our recent data indicates that communication specificity is not enough to guarantee successful fusion due to a second recognition system functions at the cell wall dissolution stage. Germling pairs that harbor compatible *doc* genes but are dissimilar at a locus that we called 'cell wall remodeling checkpoint' or *cwr*, display an arrest phenotype following contact accompanied by the accumulation of cell wall material and are unable to proceed with fusion. The *cwr* locus, encompassing *cwr-1*, *cwr-2* and *cwr-3*, showed high allelic diversity and trans-species polymorphism in populations of *Neurospora*, two features consistent with allorecognition mechanisms. Fusion assays revealed that *cwr-2* is dispensable for non-self-recognition, while a Δ*cwr-1*Δ*cwr-3* mutant undergoes cell wall remodeling and fusion with both compatible and formerly incompatible partners. Our current investigations aim to establish the mode of action of CWR proteins during non-self-discrimination. In summary, we show that microbes have developed sophisticated barriers to avoid unwanted confrontations. The novel self/non-self-surveillance system that functions at the cell wall dissolution step demonstrates that in the fungal world talking the same dialect is not sufficient for effective fusion.

S08-6 Investigating appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Abstract: To cause rice blast disease, the fungal pathogen *Magnaporthe oryzae* develops a specialised infection structure called an appressorium. The appressorium is a dome-shaped cell, which accumulates enormous internal turgor that is translated into mechanical force by reorientation of septin-dependent F-actin cytoskeleton at the base of the infection cell. Septin-dependent polarity determinants reorganize at the base of the appressorium to produce a rigid and narrow penetration peg that ruptures the tough, waxy leaf cuticle to allow colonization of the plant tissue. Here, we show that appressorium mediated plant infection by *M. oryzae* is tightly linked with cell cycle control and more specifically, requires two independent S-phase cell cycle checkpoints. The first checkpoint occurs during initial formation of appressoria on the rice leaf surface and acts through the DNA damage response (DDR) pathway, involving the Cds1 kinase. By contrast, appressorium repolarization involves a novel, DDR-independent S-phase checkpoint, triggered by appressorium turgor generation and melanisation. This second S-phase checkpoint regulates septin-dependent, NADPH oxidase-regulated F-actin dynamics to organise the appressorium pore and facilitate entry of the fungus into a plant cell. We show that specific patterns of gene expression are associated with appressorium maturation, under the control of the PMk1 MAP kinase pathway and a set of specific transcription factors that act in a hierarchy to control formation and function of appressoria. We also show that a minimum turgor threshold in the appressorium, which depends on melanin production, is necessary to trigger the unusual S-phase cell cycle checkpoint that is necessary for the appressorium to function and for tissue invasion to commence.

Symposium Session 9:

Hot Fungi in Hot Spots in a Hot Region

A.N. Miller and T. Iturriaga

S09-1 Discovering fungal hot spots through the MyCoPortal

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Abstract: The Symbiota biodiversity data management system is utilized by hundreds of natural history collections to serve biodiversity data online. These collections are organized into 40 portals which serve ~37 million specimen records of algae, bryophytes, fungi, invertebrates, lichens, plants and vertebrates. Specimen records can be linked to images, tissues samples, DNA sequence data, species information, as well as biotic inventories. A core goal of the Symbiota platform is to build a library of web-tools for documenting species occurrences (i.e., from specimens or observations) and for visualizing and analyzing biodiversity data. The Mycology Collections Portal (MyCoPortal) was created in 2011 to serve non-lichenized fungal data online, and is an easy to use, inexpensive, online resource for fungaria to maintain and serve their fungal collections data. There are currently over 3.6 million records in the MyCoPortal from 80 institutions throughout the world, predominately from North America, but also including 236,000 records representing over 47,000 species from Latin America. The MyCoPortal allows access to important fungal diversity and distribution information, and enables discovery of underexplored areas, biodiversity hot spots, as well as biogeographical patterns. Finally, it provides the big data required for documenting changes in fungal distributions over time.

S09-2 A new higher-level classification for the Leotiomycetes - essential resources, both hot and cold

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Abstract: The class Leotiomycetes comprise a biologically and ecologically diverse group of fungi – plant pathogens, animal pathogens, mycorrhizas, endophytes of roots and leaves, aquatic and aeroaquatic hyphomycetes, and saprobic decomposers. This diversity has meant that these fungi have been studied by several different research communities, and several of these groups have developed what are essentially independent classifications, sometimes based on the sexual, sometimes the asexual, morphology. The accumulation of molecular data has revealed often unsuspected links between the species and genera associated with the different lifestyles, and the change in rules of fungal nomenclature to require a single name for a single organism, has meant that the taxonomies used by the different groups must be reconciled. Coinciding with this is the need to align the historical higher-level taxonomy of the class, based largely on morphology, with increasingly detailed understanding of the phylogenetic relationships of these fungi, based on analysis of DNA sequences. Increasingly, users of fungal names identify their specimens using a DNA sequence rather than characters seen through a microscope, so the classifications taxonomists provide need to service that need. This talk will outline some of the major issues in relation to developing a phylogenetic classification for the Leotiomycetes. Input from 'cold' regions will include selection of epitypes. The vast majority of genera were described from northern Europe, and of the more than 1000 generic names attributed to the order, less than 100 have DNA sequences available from type specimens. Most names are too old for DNA to be extracted

from the type specimens themselves. Input from 'hot' regions (either physically or in terms of their undocumented diversity) will be needed to ensure any newly developed classification is relevant on a global scale. Use of 'hot' technologies, such as genome-scale phylogenetics, may be needed to provide strongly supported branches deep in the phylogeny, the branches that will be key to defining phylogenetically robust order-level taxa.

S09-3 Diversity in Pezizomycetes and Orbiliomycetes with a case study

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Abstract: When thinking about hot spots for fungal diversity we often focus on specific geographical regions. Such is the case with this symposium where assumptions are made regarding diversity in tropical areas being greater than in temperate areas of the world. My purpose is to think more broadly about ecology and evolution as drivers of diversity and how we might target some specific fungal groups for our studies. Drawing on several examples from work in our laboratory on the Pezizomycetes and Orbiliomycetes some of the patterns of richness will be discussed. Many of Pezizomycetes and Orbiliomycetes provide examples of high diversity that reflect patterns that diverge from expectations suggested by the assumptions about tropical richness. Hosts, ecologies and hidden diversity all factor into the perception of diversity. A case study in the genus *Cookeina*, assumed to be well known around the tropical world, will be presented as an example of overlooked diversity and assumptions about the patterns of occurrences of these fungi. In this genus some species show a high degree of geographic range but others are widespread in tropical areas. Under a single name a number of species have been amalgamated that have proven to be independent species. Issues related to the need for more extensive field work, better documentation and attention to biogeographical patterns will be discussed. Molecular phylogenetic studies and ancestral reconstruction have helped to elucidate these patterns.

S09-4 What happened to *Phoma*?

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Abstract: The *Didymellaceae* is the largest family in the *Pleosporales* (*Dothideomycetes*), with more than 5400 taxon names listed in MycoBank. It includes three main genera, namely *Ascochyta*, *Didymella* and *Phoma*, and several allied phoma-like genera. Although the genus *Phoma* includes more than 3000 taxa, DNA data presently suggests that it could be monotypic, and that all taxa other than the type need to be allocated elsewhere. Many species have been linked to different sexual morphs, which in turn have also been shown to be poly- and paraphyletic. As part of an ongoing study we are revising the taxonomy of *Didymellaceae* on the basis of multi-locus DNA sequence data including partial beta-tubulin, ITS, LSU and DNA-directed RNA polymerase II second largest subunit gene sequences. In the present study, we investigated 112 *Didymellaceae* isolates newly obtained from 64 host plant species in 38 plant families, and various substrates. Based on these results, we presently accept 33 genera in the family. Of these, seven genera are newly described, while *Heracleicola (= Ascochyta),* and *Neodidymella (= Boeremia)*, *Didymellocamarosporium (= Neomicrosphaeropsis*), are reduced to synonymy. Although our understanding of the systematics of *Didymellaceae is much improved*, the placement and delimitation of several genera still await to be clarified.

S09-5 The Foraging Ascomycete Hypothesis: Spatial ecology of the fungal genus *Xylaria* in a tropical cloud forest

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Abstract: *Xylaria* (Ascomycota) are ubiquitous wood decay organisms exhibiting a physiological white rot, and also ubiquitous leaf endophytes, particularly in tropical systems. Such fungal/plant symbioses are under-explored, and the benefits to fungal symbionts are particularly unknown. The Foraging Ascomycete hypothesis proposes that some wood-decomposing fungi may shift life-history strategies to endophytism to bridge gaps in time and space between suitable substrates. To test this hypothesis we examine spatial relationships of *Xylaria* endophytic fungi in the forest canopy with *Xylaria* decomposer fungi on the forest floor in a remote Ecuadorian cloud forest. All five species of *Xylaria* found as endophytes were also found as fruiting bodies, and we found evidence of spatial linkage between life stages in two species. Additionally, fruiting *Xylaria* displayed differential habitat preference from those in the endophytic life stage; we also demonstrate that direct transmission of endophytes from leaves to woody substrates is possible. These results indicate that endophytism may represent one way for decomposer fungi to escape moisture limitation, and that endophytic fungi may act as sources of dispersal for decomposer fungi consistent with predictions of the Foraging Ascomycete hypothesis.This study, by necessity, also led to a comprehensive description of the biodiversity of this genus at that site. The protected forest where this work took place, Reserva Los Cedros, is now included in new mining concessions from the Ecuadorian government to the Canadian mining company Cornerstone Capital Resources. There are grave conservation implications of metals mining in biodiverse tropical rain forests.

S09-6 DNA-barcoding of rust fungi collected on *Berberis* host species from Argentina, Brazil, Chile, Ecuador, and Uruguay

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Abstract: A three-year study was initiated in 2016 between Agriculture and Agri-Food Canada and collaborators in five South American countries to collect rust fungi occurring on *Berberis* species and generate DNA-barcode data for these fungi and their associated host species. There are approximately 80 rust fungus names on *Berberis* hosts reported in the USDA National Fungus-Host Distributions Database on-line but only a handful are represented by DNA sequences in GenBank. About half of those names were reported from South America, which is one of two centers of diversity for the genus. The role of *Berberis* in the life cycles of *Puccinia graminis* (stem rust) and *P. striiformis* (stripe rust) has received much attention, but little is known about the susceptibility of South American *Berberis* to these cereal rust species. The objectives of the study were two-fold: 1. Generate DNA barcode sequences and morphological descriptions for the diversity of *Berberis* species and their rusts collected in Argentina,

Brazil, Chile, Ecuador, and Uruguay; 2. Determine the pathotype of any *P. graminis* collections that were viable. In this presentation we report on the results for the first objective for year one. Forensic DNA extraction and standard DNA-barcoding protocols were used to generate ITS-28S sequences for the rusts, and rbcL and ITS sequences for the infected and uninfected *Berberis* plant hosts. A small number of rust-infected *Berberis* specimens were available and sampled from the Canadian National Mycological Herbarium in Ottawa (DAOM) to generate reference DNA barcodes for comparison. DNA sequences were aligned with reference sequences for the cereal rusts and other species suggested by in-house and GenBank BLASTn searches and were analyzed using neighbour-joining and PHYML methods. Twenty-four rust infected and forty-six uninfected *Berberis* collections were processed. DNA was extracted from collections with obvious rust infections (none from Uruguay) and all other uninfected collections from all five countries. None of the rusts were identified as *P. graminis* or *P. striiformis*. Some collections from Ecuador matched data previously published for *Edythea quitensis*. The Brazil collections were close to our new reference sequences for *Puccinia meyeri-albertii* from a DAOM herbarium specimen collected in Argentina in 1922. This species has been reported from Brazil, Chile and Argentina on several *Berberis* species. The rest of the rust collections were unidentified and form separate clades from sequences for named reference specimens. The analyses suggested that anywhere from eight to thirteen species were collected, depending on how the analyses are interpreted. The rbcL and ITS sequences for *Berberis* were used to help confirm host identifications and assign provisional names to those that were unidentified.

Symposium Session 10:

Teaching Mycology Around the World: Examples From South America, North America, Europe, Japan and Australasia

M. Piepenbring

S10-1 The importance of line drawings for teaching and research in mycology

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Abstract: Understanding profiles of fungal species is a long-lasting theme of mycology, for its research works and also in the case of mycological teaching. Phylogenetic analyses of DNA sequence data of fungi concerning phylogenetically and taxonomically meaningful regions have been commonly introduced into their classification. This method often elucidated past mistaken interpretation of morphological (or phenotypical) features or their artificial application to the traditional taxonomy. Nowadays even identifying a single fungal species, DNA analyses became a major tool to obtain exact fungal names of working materials. Taxonomic importance of morphological (or phenotypical) studies became apparently reduced than that of those before molecular techniques have been introduced. There might be some tendency to pay less attention to morphological (or phenotypical) studies even in the case of taxonomic or identification studies of fungi. Researchers on fungi, however, are working on various features of them as their connected attributes, e.g., their pathogenicity, host preference, physiology, reproduction, ecology, distribution, substance production, degradation, tolerance, antagonisms, and so on. Although morphological features, including some phenotypic natures, may have been lost their long recognized former status of taxonomic importance, these features are still a part of important attributes of fungi. To recognize and understand the profiles of individual species of fungi, their morphology or phenotypes may outline the species more clearly, by answering the question, what and how are they. For teaching mycology, fungal names should be the first keys but morphology

and phenotypic natures may help recognition and understanding of each of the species. By applying the modified Prof. Oberwinkler's illustration method for drawing fungal structures, microscopic structural details of fungi will be well grasped, extracted and recorded for their recognition, with a simple equipment using a mesh-type eye-piece micrometer and less effort, as introduced in this talk. Microscopic photographs may often be taken to record and represent morphological features of fungi as real images. However, because of shallow focusing depth and relatively narrow area of microscopic pictures, it often becomes rather difficult to cover and represent a whole structure of fungal organ in one shot of picture, including connective hyphal elements surrounding them. Line drawing may improve and supplement such disadvantages of taking microphotographs. Drawing, with a combination of photographs, may often lead to better understanding of the objectives. Usage of illustration for teaching mycology is highly recommended. Practical examples using some *Fusarium* morphology will be presented.

S10-2 Increasing fungal literacy in Australasia: lessons from universities to communities

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Abstract: We appear to be at somewhat of a cross roads in Australasia. Active teaching about the fungal kingdom has gradually been decreasing in Universities. Mycology lectures have been replaced with DNA dogma, coupled with a general shift away from organismal biology reducing the 'intriguing' and 'naturalist' first stage observational skills of students. Instead, dry 'old-facts' are presented by non-mycologists in a couple of lectures and one practical that covers the 'lower plants'. However, we are also seeing curiosity and knowledge seeking increasing through community-based groups, socially funded workshops and forays, and the use of social media (e.g. Facebook fungal appreciation groups, photography, restoration projects, etc.). From our experiences in teaching both tertiary students and the enthusiastic general public, we have noticed that: University students seem to take information and try to retain it for regurgitation rather than trying to apply the knowledge or use the concepts to understand its function. Versus community groups, particularly land management groups, will take new ideas and immediately try to apply them to their day-to-day management actions. Ideally all students would quickly learn to use the concepts to note features that might indicate relationships to other species, consider possible interactions between animals and plants, determine what a fungus' function is in an ecosystem, and how that can be applied to the management of diversity and function of natural systems. This is likely to be partly a reflection of the experience and maturity of "community" participants compared with the younger students, who are often still in a "school" mentality. However, more, and younger people are joining these community-based attempts to seek knowledge about fungi. This is where social media, and applications such as mushroom observer and iNaturalist have had significant impacts on access to fungal knowledge, particularly mushrooms. In our regular socially funded workshops/forays/lectures our experiences lead us to believe the best way to have successful fungal education is to use a "mushroom sandwich approach" by beginning with a short presentation on fungal facts, then going into the bush/local park/ school yard to show fungi in action in the environment and finishing back in the classroom with more discussion. We also share our favourite resources with students including: Fungimap; Bugs site via AMS; Atlas of Living Australia; local iNaturalist projects; Forgotten flora; fun ID cards; mushroom Russian roulette; and more. The educational success is always improved for both university and community groups by that hands on 'field' experience. After time in the field there is

deeper learning, better questions are asked and overall there is a greater appreciation of the Kingdom of fungi.

S10-3 Development of mycology from ground zero in Patagonia

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Abstract: Patagonia offers numerous distinctive biomes that harbor a particular mycobiota, particular ecological relationships and challenging phytopathological problems. Rainfall of 4000 mm at the Pacific seashore drastically drops to 500 mm at the verge of the Patagonian steppe in only 300 kms width or less. Mycodiversity is immense, with hosts having no comparison with other regions of the Americas. In spite that researches were begun by Spegazzini as far as 1880, that were followed by studies of Singer, Horak, Garrido, Wright, Gamundi (among many others), we are only in the fringes of the mycological knowledge in this part of the world. The 11 IMC Symposium 'Gondwana reunited! Fungal biogeography in the Southern Hemisphere' is just one example of this situation. On this basis it is not a surprise that teaching and investigating mycology offers exciting opportunities to leading teachers. Personal establishment in the town of Esquel in 1991 put me in an isolated area in Patagonia, where no mycology had ever been taught nor researched. The known ascomycetologist Dr. Irma Gamundi was the closest colleague …. but 300 kms north. Such 'ground zero' place offered the opportunity to train novel students and to open lines of investigation of many kinds. The regional Universidad Nacional de la Patagonia S.J. Bosco at Esquel seat offered the opportunity to organize a first General Mycology course within a recently created Natural Sciences career. Clever and enthusiastic students joined. Alexopoulus' Introductory Mycology 3rd Edition was available in Spanish (Mexican Omega Edition) for non-English speaking students. A 2nd Field Mycology course followed, concentrated in visits to the forests, collection of specimens and focused on lab determination of specimens. Keys for main fungal groups were used in a pre-internet era, offered in diverse mycobiotas and books such as Dennis' *British Ascomycetes*, Ainsworth-Sparrow &Sussman's series of *The Fungi*, Gilbertson & Ryvarden's *North America Polypores*, Horak's *Agaricales* from Tierra del Fuego, von Arx's *The Genera of Cultivated Fungi*, among others. The Forest Research Center CIEFAP offered opportunities of many sorts to youngsters interested in pathology, and focuses were put in *Nothofagus* wood-rotting fungi and the decline disease of the endemic conifer *Austrocedrus chilensis*. The National Research Council (CONICET), the main engine for research development in Argentina entered into action through the offer of PhD fellowships. These fellowships are regularly offered to young professionals in order to achieve a 3rd level academic degree. Commitment, nature and love to fungi made the rest. To date 12 PhD thesis have been presented as well as numerous Graduate thesis; the Mycology research group has grown and split in two, one at Centro Forestal CIEFAP (6 researchers + 3 doctoral fellows) and one at the regional university (1 researcher + 1 postdoctoral fellow). Research subjects include: Mycorrhizal fungi of *Nothofagus* forests, Native Edible Fungi and development of Commercial production, Diversity and taxonomy of Wood-rotting fungi, Forest Pathology, Biocontrol of *Austrocedrus chilensis* Phytophthora disease, Biocontrol of post-harvest berries diseases, Blue-stain fungi, Etiology of *Nothofagus* forests decline and Secondary metabolites in Fungi, among others.

S10-4 Teaching about fungal diversity in the tropics

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Abstract: Fungi are a hyperdiverse group of organisms far from being adequately documented. Especially in the tropics, we are still in a pioneer phase concerning the scientific analysis of fungal species diversity. Most investigators working with fungi, however, focus on model species, taxonomy often is considered old-fashioned, and early career mycologists tend to prefer modern methods. Meanwhile, areas with natural vegetation are destroyed with numerous fungal species probably lost forever. This disturbing trend is addressed by a kaleidoscope of activities to enhance the attractiveness and valuation of fungal diversity in teaching and research, namely forays, inventory projects, and checklist compilations; microscopic investigation of fresh fungal specimens; mycology lectures with teaching diagrams, funny mushroom pictures, videos; animated life cycles; colourful textbook; diagrams for the illustration of ecosystem services of fungi; eLearning; exhibition on fungal diversity and applied mycology. These activities are performed with students in Latin America, Benin, and Germany in order to increase enthusiasm for fungal diversity that hopefully will lead to contributions to our knowledge and valuation of fungal diversity worldwide. For teaching material see: http://www.goethe-university-frankfurt.de/61705419/digitale-materialien

S10-5 Partners in success: The science library as an active agent in the education of biologists

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Abstract: The **Bologna Accord**, launched with the **Bologna Declaration**, of 1999, is a voluntary higher education reform agreement at European level - now adopted by 48 European and extra-European countries - that nowadays define the European Higher Education Area. The reform implies increased awareness across the higher education sector of transferable skills and professional competencies. Among the challenges faced is the need to design new learning arenas that can support students in developing such skills and competencies, including collaboration skills, scientific writing, finding, evaluating and using relevant literature, and developing an understanding of their own research progress and workflow. It is advocated that the learning of transferable skills and professional competencies should be integrated with subject specific learning, and that this calls for collaboration within the university community. At the University of Oslo, the Science Library, Faculty of Mathematics and Natural Sciences, is forging teaching partnerships with the faculty staff at the various departments. Subject librarians with an academic background from the different disciplines at the Science Library participate in the teaching of students at all levels, from introductory courses through post-graduate studies, being partly responsible for the learning outcomes of transferable skills expected from single courses and study programs. The teaching portfolio of the librarians also includes the training of students in how to perform outreach activities, e. g. how to communicate bioscience and mycology topics to professionals, experts from other disciplines, and the general public. We report here on some specific initiatives that illustrate both benefits and challenges of such partnerships, with examples from collaborations between the Department of Biosciences and the Science Library at the University of Oslo.

S10-6 Bringing species discovery and indigenous knowledge about fungi to school students

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Abstract: Two initiatives were undertaken to educate New Zealand school students about native fungi – focusing on student participation in fungal species discovery, and provision of an introductory student booklet on fungi written in Māori, New Zealand's second official language. Mycologists collaborated with students of different age groups at three schools to collect fungi nearby, discriminate material, and demonstrate aspects of the identification process in the classroom and through visiting our institute. For the three new species, students chose the species epithet as meaningful to them. Student names and photos were included in 2-sided pictorial scientific papers published in the journal Fungal Planet. For students in New Zealand's Māori immersion education system, mycologists worked with a Māori educator and a translator to prepare and distribute a student booklet and a bilingual teacher guide on fungi. These introduced the biology of fungi and reconnected students to ancestral uses of fungi that were otherwise only accessible from early English texts. Some indigenous Māori knowledge of biota has been lost through reduced oral transfer between generations, so this project seeks to restore awareness of the relevance of fungi – for food, medicine, fire-carrying, and tattooing – to Māori students and their families.

Symposium Session 11:

Integrative Approaches to Understand the Ecology and Evolution of Fungi

S. Skrede and J. Hess

S11-1 The polygenic basis of an ancient divergence in yeast thermotolerance

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Abstract: Some of the most unique and compelling survival strategies in the natural world evolved long ago, and are fixed in now-isolated species. Molecular insight into these adaptations has been limited, as classic experimental genetics has focused on the interfertile individuals of a population. Here we dissect a complex thermotolerance difference between yeast species that diverged millions of years ago. Using a new mapping approach that screens mutants in a sterile interspecific hybrid, we identified eight genes that underlie the growth advantage of *Saccharomyces cerevisiae* over its sister species *S. paradoxus* at high temperature. All eight encode housekeeping factors with no known direct function in heat-shock or stress response. Pro-thermotolerance alleles at these mapped loci were required for the adaptive trait in *S. cerevisiae* and sufficient for its partial reconstruction in *S. paradoxus*. Together, our data reveal the genetic mechanism by which *S. cerevisiae* acquired its high-temperature growth advantage in the distant past. And our study lays the groundwork for the mapping of genotype to phenotype in clades of sister species across Eukarya.

S11-2 Comparative genomics of *Microbotryum* to bridge the gap between sytematics and ecology

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Abstract: The evolution of obligate plant pathogens like smut fungi is often characterized by lineage tracking resulting in host specificity and one-to-one relationships. Therefore, adaptation and specialization to the host plant seems to be crucial and should involve genes or regulatory pathways governing host specificity. To identify genes relevant for host specificity of *Microbotryum* species, we produced artificial hybrids between the two host-specific species *M. lychnidis-dioicae* and *M. silenes-acaulis* and applied strong experimental selection on different host plants to identify genes necessary for successful infections. Genome comparison of the two species revealed that most gene families are shared and the majority of genes are conserved, indicating very similar biological features of both species, including host adaptation and infection processes. Lower nucleotide identity of genes encoding for secreted proteins might indicate their importance for host specific interaction, as it is known from other plant pathogens. Moreover, we identified 211 candidate genes that occur in each hybrid and backcross genome that were posed under host-driven selection and might therefore play a crucial role in host specialization. The analysis of hybrid genomes also demonstrates the effect of genetic homogeneity on the fitness of hybrid individuals including the occurrence of species-specific mating type chromosomes. We analyze the evolution of candidate genes in the context of the whole genus and will discuss their potential contribution to host specificity. These studies should contribute to our functional understanding of the evolution of host specificity and its relevance for systematics of the genus *Microbotryum*.

S11-3 Raman spectroscopy as a method to detect changes in cellulose crystallinity during decomposition caused by mushroom forming fungi

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Abstract: Cellulose represents the most abundant carbohydrate in terrestrial ecosystems and a major carbon and energy source for saprotrophic fungi. Plant cell-wall cellulose is found in an amorphous form, where cellulose chains are loosely interwoven and in a crystalline form, where the chains interact with hydrogen bonds and Van der Waals forces to form highly crystalline resistant to decomposition fibers. White-rot wood decayers employ a diverse set of enzymes during decomposition that cause a combination of hydrolysis (cellobiohydrolases, endoglucanases) and oxidation (lytic polysaccharide monooxygenases) of cellulose. While decomposition progresses, large amounts of crystalline cellulose are left behind in the decomposed wood. In contrast, brown-rot fungi secrete mostly endoglucanase during cellulose decomposition, while their genomes mostly lack cellobiohydrolase and lytic polysaccharide monooxygenase genes. In place of the costly enzymes brown-rot fungi are thought to employ a non-enzymatic mechanism that involves the generation of the Fenton reaction and the subsequent generation of hydroxyl radicals. The hydroxyl radicals in return are thought to affect the crystallinity of cellulose disrupting its structure rendering the fibers susceptible to degradation from endoglucanases. Brown-rot wood decay is efficient and results in the complete decomposition of all carbohydrates in wood. To what degree enzymatic versus non-enzymatic decomposition affects the crystallinity of cellulose is not well established. To examine this, we grew a strain of a *Gloeophyllum* species (brown rot) and a strain of *Phanerochaete laevis* (whit rot) on high-quality filter paper as a carbon source for 40 days and we examined the effect of the two fungi on the crystallinity of cellulose using Raman spectroscopy. Our results show that *Gloeophyllum* had a pronounced impact on the structure of

cellulose, while *P. laevis* had a very small effect. This suggests that brown-rot fungi might target the higher structure of cellulose in order to make it more accessible to the action of endoglucanases, while white-rot fungi degrade cellulose without altering its higher structure. To further test this method, we performed the same experiment for eight litter decomposers across Agaricales. The distinction between white-rot and brown-rot fungi cannot be applied for litter decomposers and therefore, a way to explore further the decomposition of cellulose by such species is needed. Six of the species investigated had a very small effect on the structure of cellulose, similarly to *P. laevis*. However, two species modified the structure of cellulose to an intermediate degree between *P. laevis* and *Gloeophyllum*. Our results suggest that using Raman spectroscopy is a promising method to distinguish cellulose degradation between white-rot and brown-rot fungi. Furthermore, the results for litter decomposers indicate that using this method we will be able to explore the mechanisms of cellulose degradation in fungi with less studied decomposition strategies.

S11-4 Integrative comparative approaches to determine the genomic basis of specialized wood decay in the invasive fungus *Serpula lacrymans*

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Abstract: Comparative genomic approaches, such as the comparison of gene content or gene expression levels, among related species with distinct niches offer powerful tools to understand the genomic basis of adaptation in non-model organisms. Combining both classic comparative and functional genomic approaches in an integrated fashion is essential to our ability to distinguish changes of functional importance for a particular phenotype from incidental ones, or to understand the relative contribution of different evolutionary processes. Here, we use an integrated approach, based on phylogenomic reconstruction of gene content in combination with gene expression profiling on key substrates to study specialisation of wood decay in the house invading fungus *Serpula lacrymans* (Basidiomycota, Boletales, Serpulaceae). This fungus is invasive to the built environment in Europe and distinguishes itself from its wild relatives through a particularly aggressive wood decay and ability to infect dry and patchy habitats. Our analysis includes four individuals of three species within the Serpulaceae: two individuals of the invasive house-living *S. lacrymans* var. *lacrymans*, the wild sister species *S. lacrymans* var. *shastensis* and the widely distributed wild relative *S. himantioides* as an outgroup. Using an integrated analysis strategy, we have investigated i) the relative importance of gene duplication and loss compared to changes in gene expression for the fine-tuning of wood decay, and ii) the timing of such changes with respect to invasion of the built-environment. Results show a shift from generalist to specialist decay strategy in the ancestor of the *Serpula lacrymans* varieties. This is reflected in strong, conserved differentiation of gene sets active on spruce or pine substrates in these species, while the generalist *S. himantioides* expresses largely the same genes on both types of wood. Functional analysis of differentially regulated genes indicate that gene sets expressed on spruce and pine may reflect different decay stages. Among all wood-induced genes in the var. *lacrymans* strain from Europe, 7.6% arose in the common ancestor of vars. *lacrymans* and *shastensis*, 2.1% arose in the common ancestor of var. *lacrymans* and 6.1% were strain-specific, suggesting a considerable contribution of gene duplication.

S11-5 Addressing nutrient exchange rate and genome organization in the study of evolutionary stability of AM fungi

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Abstract: Virtually all terrestrial plants depend on symbiotic interactions with fungi. Symbiotic Arbuscular mycorrhizal (AM) fungi evolved over 450 million years ago and were instrumental for the colonization of land. Mediating nutrient uptake and sequestering carbon in soil this symbiosis lies at the core of all terrestrial ecosystems. AM fungi are obligate biotrophs and cannot complete their life cycle without obtaining carbon from host roots. In return they provide their host with nutrients, such as phosphorus. In contrast to the fungi, plants are facultative mycotrophs, but under natural conditions all host roots are colonized as a result of multiple beneficial effects of AM fungi. The evolutionary stability of this symbiosis is exceptional given that both host plants and symbiotic fungi are promiscuous, forming interactions across individuals and species. In the absence of host - symbiont specificity and given their inability to discriminate among partners prior to interaction, evolutionary theory predicts that "free riders" would evolve and spread. Free riders are cheaters that benefit from the interaction without providing significantly in return. However, under natural conditions this has not crippled the function of the AM symbiosis. In our group we combine single nuclei sequencing methods with estimates of symbiotic efficiency experiments to study the evolutionary stability of AM fungi. Specifically, we try to evolve cheating strains by allowing AM fungi to selectively adapt to different host plants and comparing how symbiotic efficiency develops in response to host adaptation. If cheater strains evolve these are expected to trade less phosphorus per unit carbon obtained from the host, because phosphorus is limiting in our study system. The link between rates of trade, RNA expression in roots and strain genome structure will be explored. Earlier hypothesis that AM fungi may be heterokaryotic, meaning that they harbor genetically distinct nuclei in their coenocytic mycelia has been challenged by recent genome sequencing of the AM fungi *Rhizophagus irregularis*. We are now able to explore the generality of these new findings thanks to our single nuclei sequencing method, originally developed to generate reference genomes for our experimental studies.

S11-6 Phylogenetic structure and ecological function of foliar endophytic *Cladosporium* associated with *Populus trichocarpa*

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Abstract: Fungal endophytes are ubiquitous in plant leaves. While some endophytes are commensal symbionts, others can modify plant disease severity either by interacting directly with pathogens (e.g., mycoparisitism, antibiosis) or by altering the plant defense response. It is often assumed that species within common fungal endophyte genera, e.g. *Cladosporium*, *Alternaria*, *Epicoccum,* exhibit cosmopolitan distributions. However, few studies have investigated the phylogeographic structure of common endophytes, or tested for variation in ecological function within clades. We examined *Cladosporium*, a ubiquitous, wind-dispersed endophyte associated with the leaves of the model tree, *Populus trichocarpa* (black cottonwood). *Cladosporium* populations were sampled by amplicon metabarcoding leaf samples (ITS1) and multilocus sequence typing (5 genes: ITS and partial actin, β-tubulin, ef1α, rpb2) of 96 *Cladosporium* cultures collected from eight sites spanning the core of the tree's geographic range and a strong climatic gradient from west (wet) to east (dry) of the Cascade Range in the Pacific Northwest of North America. Our ongoing experiments test the degree of rust pathogen antagonism (via mycoparisitism) across the 96 strains using both *in agaro* and *in planta* assays.

Our multigene phylogeny supports ~15 previously described species within *Cladosporium*, and as many as five undescribed species. While the majority of these species are thought to have near global distributions, ITS1 metabarcodes and multigene data both indicate strong phylogeographic structure across the study area with particular species largely restricted to either west or east of the Cascade Range. We are working to link the phylogenetic structure of *Cladosporium* endophytes to variation in mycoparisitism. In addition, we are sequencing the genomes of a subset of isolates and will use comparative genomics to explore environmental adaptation and the genomic basis for mycoparasitism.

Symposium Session 12:

Breeding for Resistance to Fungal Pathogens of Crops

H. Cuevas and T. Porch

S12-1 Genetics for resistance to ashy stem blight and white mold in 'PC 50'/'Othello' and A 195/'Othello' common bean populations

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Abstract: Ashy stem blight (ASB) [causal agent: *Macrophomina phaseolina* (Tassi) Goidanich] and white mold (WM) [causal agent: *Sclerotinia sclerotiorum* (Lib.) de Bary] are important diseases of common bean (*Phaseolus vulgaris* L.) worldwide. Seed yield losses over 50% to both diseases have been reported in susceptible cultivars. Genes/QTL, conferring partial resistance to ASB and WM, are found in common bean genotypes in the Andean race. The objective of this study was to determine the genetics for resistance to ASB in 'PC 50'/'Othello' and WM in A 195/'Othello' populations. Resistant (R) plants to ASB and WM from Andean genotypes PC 50 and A 195, respectively were crossed with susceptible (S) plants of pinto Othello to both diseases. The F_1 and parents were inoculated with one more-aggressive *S. sclerotiorum* isolate (ND710); while the F_2 and parents were inoculated with one less-aggressive (ARS12D) and ND710 isolates. In the case of ASB, the same filial populations and parents were inoculated with the PRI16 *M. phaseolina* isolate. Evaluations were conducted in greenhouses up to 35 d for WM in Idaho and 50 d for ASB in Puerto Rico. All F_1 had a susceptible reaction to ASB and the F_2 segregated into 15S:1R. Thus, resistance to ASB was controlled by two independent complementary recessive genes. In contrast, the F_1 plants varied in their reaction to WM. Furthermore, F_2 derived from F_1 resistant plants fit a 9R:7S ratio, especially to ARS12D isolate. These results indicated that two independent complementary dominant genes were involved in the WM resistance. Progeny test conducted in the F_3 corroborated the data observed in the F_2 for both populations. This information should help introgress resistance genes to both diseases in susceptible common bean cultivars.

S12-2 Diversity analysis of the Angular Leaf Spot pathogen in Puerto Rico, Central America and Tanzania for informing breeding of common bean

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Abstract: Angular Leaf Spot (ALS), caused by the fungus *Pseudocercospora griseola*, is an important disease of common bean (*Phaseolus vulgaris*) especially in the tropics and subtropics. The pathogen causes significant yield losses of up to 80% in common bean. Different studies have demonstrated that

P. griseola and *Phaseolus vulgaris* have co-evolved resulting in the classification of the fungus into Middle American or Andean groups. Levels of virulence are used to classify *P. griseola* into different races using Andean and Middle American differential cultivars. Normally, Middle-American isolates affect both Middle American and Andean beans while Andean isolates affect mostly Andean beans. However, an unusual group of ALS isolates found in Africa, termed Afro–Andean, was previously found to be pathogenic on Middle American cultivars. The purpose of this study was to evaluate the diversity of *P. griseola* isolates from four different countries and the existence of different races in Puerto Rican ALS isolates. A total of 200 ALS isolates, from Puerto Rico, Honduras (collected from *P. acutifolius*), Guatemala and Tanzania were used. Four nuclear genes, B tubulin and actin genes and the rDNA ITS and small subunit regions were sequenced and used to construct a phylogenetic tree. All isolates from Puerto Rico, Honduras and Guatemala were Middle American. Initial results for Tanzanian isolates indicate that 52 were Middle American and 22 Andean, while a third group was discovered of 36 hybrid isolates, potentially the Afro-Andean group. ALS races in Puerto Rico were evaluated using 31 isolates inoculated on twelve differential cultivars. Twenty days after inoculation symptoms were observed and 12 races were identified. These results suggest the presence of a unique third group in Tanzanian isolates, potentially the Afro-Andean clade. In addition, the variability in races in Puerto Rican isolates also suggests that *P. griseola* contains polymorphisms in virulence genes. These initial results indicating a new group in Tanzania and the diversity of races in Puerto Rico will be considered in terms of how they can inform current and future plant breeding efforts.

S12-3 Evaluation and breeding for host resistance to *Botryosphaeria* pathogens in *Prunus*

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Abstract: Peach fungal gummosis is a disease associated with a complex of species in the family Botryosphaeriaceae. Several genera of these ubiquitous pathogens cause difficult-to-control diseases on a wide host range including multiple commercial woody crops. With no efficacious chemical or horticultural management options, breeding for host resistance to peach fungal gummosis is a major goal of fruit breeding programs. An interdisciplinary approach, focused on pathogen diversity within *Botryosphaeriaceae*. Several genera of these ubiquitous pathogens cause difficult-to-control diseases on a wide host range including multiple commercial woody crops. With no efficacious chemical or horticultural management options, breeding for host resistance to peach fungal gummosis is a major goal of fruit breeding programs. An interdisciplinary approach, focused on pathogen diversity within *Botryosphaeriaceae* on peach in the Southeastern United States while screening for sources of resistance within a diverse germplasm. A survey of symptomatic trees from Florida, Alabama, South Carolina and Georgia identified three Botryosphaeria species as the predominate pathogens associated with fungal gummosis. Fungal isolates were identified using morphological characters as well as sequences of internal transcribed spacer regions and elongation factor 1-α - genes. Relative susceptibility to peach fungal gummosis was evaluated with multiple pathogenicity assays including natural field infection, an enhanced infection trellis system, detached stem assays, and detached leaf assays to facilitate evaluation of Prunus germplasm. Moreover, detached assays confirmed significant differences in lesion lengths caused by the pathogen species tested. Initial Prunus evaluations using QTL analysis with F1 interspecific hybrids and BC_1F_1 populations indicated a major source of resistance to peach fungal gummosis in almond germplasm. Moreover, genetic analysis of F1, BC_1F_1, and F_2 populations of Prunus indicated a dominant inheritance of the resistance. The locus for resistance was called Botd8, and fine mapping with

microsatellites, Single Nucleotide Polymorphism, SNP CAPS and INDEL molecular markers allowed high-throughput screening of seedling populations. Closely-linked molecular markers and recombinant trees identified a narrowed region with candidate genes likely related to resistance. This study provided the tools required to introgress resistance to *B. dothidea* into the UF peach breeding program using marker-assisted selection. At the same time, detached pathogenicity assays have provided a high-throughput evaluation for relative susceptibility to peach fungal gummosis.

S12-4 Mining sorghum genetic diversity to genomic dissect anthracnose resistance response

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Abstract: Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important grain crop behind maize, wheat, rice, and barley. The productivity and profitability of sorghum is reduced by susceptibility to fungal diseases, such as anthracnose, caused by *Colletotrichum sublineolum*. The identification of anthracnose resistance loci from different sorghum accessions is imperative to develop new varieties with broader resistance response and to increase its durability. The USDA-ARS National Plant Germplasm System (NPGS) maintains a sorghum germplasm collection that includes >41,860 accessions from 114 countries, most have not been characterized for anthracnose disease resistance. Due to the large size of this collection, the sorghum association panel (SAP) consisting of 377 diverse sorghum was assembled to capture the majority of genetic diversity present in sorghum breeding programs and NPGS. We evaluated the anthracnose resistance response of 335 accessions from a sorghum association panel (SAP) and 297 exotic sorghum accessions from the NPGS Ethiopian germplasm collection. The evaluation of SAP identified 75 accessions resistant to anthracnose. A phylogenetic analysis of these accessions showed a high genetic diversity and multiple resistant sources. Genome-wide association scans (GWAS) using 268,289 single-nucleotide polymorphisms and logistic regressions for binary measures of resistance responses identified three loci within a region on chromosome 5 that have been previously associated with three sources of anthracnose resistance. The evaluation of NPGS Ethiopian germplasm collection identified 143 resistant accessions. Genetic characterization of this germplasm and its anthracnose resistance response were merged with phenotypic and genetic characterizations of the SAP for a large GWAS comprising of 592 accessions and 219,037 SNPs. Logistic regressions for binary measures of resistant responses identified the previous associated locus on chromosome 5 and an additional locus on chromosome 3, while a mixed linear model using a quantitative resistant response identified a locus on chromosome 9. Candidate genes within loci on chromosome 5 and 3, include a resistant gene belonging to a family of genes encoding *F-box* proteins, while a resistant gene candidate on chromosome 9, is a gene with leucine-rich repeat and NACHT domain (i.e. R-gene family), suggesting resistance response is controlled by multiple defense mechanisms. Resistant alleles for loci on chromosomes 3 and 9 are present in the SAP at low frequency, thus, the integration of NPGS germplasm increased its frequency and power of detection. Therefore, the strategic integration of exotic resistant germplasm into the SAP is needed to identify additional rare resistance alleles via GWAS.

S12-5 Linkage mapping of QTLs associated with resistance to the rice blast causal agent (*Magnaporthe oryzae*)

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Abstract: Rice blast, caused by *Magnaporthe oryzae,* is one of the most important diseases of rice, due to its broad geographic distribution and capacity to destroy the crop. This disease is a challenge to rice farmers and one of the factors which limit rice yield, especially in the Central Region of Brazil, which is considered an area of high genetic diversity of the pathogen. In this region, rice blast resistance has been overcome only one or two years after a new resistant cultivar is commercially released. One of the objectives of the present work was to map blast resistance genes in the rice genome in order to intensify the development of strategies to use genes conferring major or partial resistance by breeding programs. In this study, the evaluation of the phenotypic interaction between *M. oryzae* isolates collected in the Araguaia River Valley and parents of a population of recombinant inbred lines (RIL) allowed the identification of isolate 623, physiological race IA-1, which is able to induce incompatibility reaction (resistance) in the traditional tropical *japonica* variety Puteca, and compatibility (susceptibility) in the traditional tropical *japonica* variety Chorinho. DNA polymorphism analysis in 192 microsatellite and SNP loci, distributed in the rice genome, allowed the construction of a genetic map with 1074.19 cM and average recombination distance of 5.59 cM between markers. Interaction phenotype and linkage analysis allowed the identification of microsatellite locus RM7213, located near the centromere region of chromosome 6, significantly associated with resistance to *M. oryzae* 623. This gene was temporarily called *Pi-Put1*. The region of maker RM7213 has a cluster of blast resistant genes, some of them with broad resistance to blast races. This region can be further explored by breeding programs in order to obtain new cultivars resistant to the pathogen. One of the alternatives for the development of blast resistant cultivars is indirect gene pyramiding, based on the exploration near-isogenic lines with different resistant genes to compose multilines.

S12-6 Linking the indigenous microbiomes with the health of different disease-resistance wheat and rice varieties

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Abstract: Developing and using disease-resistant varieties of cereal are one of the most effective approaches for combating yield loss. The understanding of how such the cereal varieties would affect the microbiome associated with the host, which can be the key determinant to the health and productivity of cereal grains, is lacking. Finding microbes that correlated with different resistance cereal and geographic location will assist in defining a set of core bioindicators reflecting grain and soil health and safety. In this study, grain and rhizosphere samples from resistant varieties of wheat (*Triticum aestivum* L., resistant to *Fusarium* Head Blight) and rice (*Oryza sativa* L., resistant to rice blast) were collected from seven provinces in China during the harvest seasons 2015/2016. The fungal and bacterial flora was recovered by sequencing the amplicons of internal transcribed spacer (ITS) and 16S rRNA gene region, respectively, using Illumina MiSeq sequencing technology. The core microbiota of rice grains are species from genera *Nigrospora*, *Occultifur*, *Sakaguchia* and *Ustilaginodidea* while that of wheat grains are *Cystofilobasidium*, *Rhizopus* and *Sclerostagonospora*. The distinct microbiota profile

associated with wheat or rice reflects host-mediated selection of microbiomes, which is also shaped by geography, for example some important rice pathogens, including *Cercospora*, *Curvularia*, *Pyricularia* and *Ustilaginoidea* were significantly more frequently recognized in grain samples collected in Central and Southern regions of China than samples collected in Northeast China. The occurrence frequency of these pathogenic fungi in different areas of China can be used in making strategy to control the crop diseases and improve yield and quality of rice and wheat grains.

Symposium Session 13:

Food Mycology in the 21st Century: Impacts on Food Security and Safety

G. Perrone and S.N. Chulze

S13-1 Mycotoxins and food security: deciphering the impacts of climate change scenarios

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Abstract: There is interest in the impacts that climate change (CC) factors will have on the infection of staple food commodities by fungal diseases, pre-harvest and post-harvest. This applies to contamination of staple commodities with spoilage and mycotoxigenic moulds. These will have an impact on the food security agenda. CC is due to the interaction between three key environmental factors of elevated CO_2 (400 vs 800/1200 ppm), temperature increases (+2-4°C) and drought stress. There is now some evidence that CC impacts on plant physiology including growth and yield of staple crops. We have been particularly interested in the impact that changes in CC scenarios may have on the growth/mycotoxin production by key spoilage fungi in staple food commodities. Thus, we have examined the effect of CC environmental factors on growth and mycotoxin production by *Fusarium graminearum* and *F.langsethiae* (type B and type A trichothecenes respectively), Aspergillus flavus (aflatoxins) and *A.westerdijkiae* and *A.carbonarius* (ochratoxin A). We have examined the impact that CC factors may have on growth as well as gene clusters involved in mycotoxin production. For example, by using RNAseq and information on aflatoxin B_1 production we have been able to examine the impact that such CC environmental actors may have on functioning of the biosynthesis of aflatoxins and other key secondary metabolites. Studies on mycotoxigenic *Aspergillus* species colonising coffee and pistachio nuts, suggest differential effects on mycotoxin contamination in vitro and in situ when exposed to CC conditions. In addition, acclimatisation to CC conditions needs to be considered. This could also have implications for the legislative limits for mycotoxins in certain food commodities. These results will be discussed in the context of the food security agenda and the implications that CC scenarios may have on the resilience of staple food crops.

S13-2 Biocontrol to reduce the impact of toxigenic fungi and the entry of mycotoxins into the food chain

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Abstract: The occurrence of plant pathogens and toxigenic fungi and subsequently mycotoxin contamination in different crops around the world have significant implications for food and feed safety, food security and international trade. The main mycotoxins detected as natural contaminants in oily seeds and cereals include aflatoxins, trichothecenes, fumonisins , ochratoxin A and zearalenone. Species of *Fusarium* and those within *Aspergillus* sections *Flavi* and *Nigri* are producers of these toxic secondary metabolites. *Fusarium* head blight (FHB) is a devastating disease that causes extensive yield and quality

losses to wheat and other small cereal grains worldwide. Different strategies including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars are used in order to reduce the impact of mycotoxins in these cereal-based food and feed chains. The development of fungicide resistance together with the rising of public concern of risks associated with pesticides use has led to the search for environmentally friendly alternatives. Biocontrol offers an alternative approach that can be used in the framework of an integrated pest management (IPM) strategy to reduce the accumulation of mycotoxins in food and feed chains. *Aspergillus* section *Flavi* can infect peanuts and maize pre-harvest stage, especially during drought stress episodes resulting in aflatoxin contamination. Biocontrol based on competitive exclusion by using atoxigenic *Aspergillus flavus* strains is one of the most promising strategies for minimising aflatoxin contamination in both these commodities. Populations of native atoxigenic *Aspergillus flavus* strains were evaluated based on phenotypic, physiological and genetic characteristics. Selected atoxigenic strains of *A. flavus* with no capacity for aflatoxin or cyclopiazonic acid production were evaluated in field trials. Reductions of aflatoxin contamination were between 78 - 90% in treated plots in comparison with control plots. Two potential biocontrol agents, *Bacillus velezensis* RC 218 and *Streptomyces albidoflavus* RC 87B, have also been evaluated for reduction of *Fusarium* head blight severity and deoxynivalenol (DON) in bread and durum wheats. Both these strains effectively reduced FHB incidence (up to 30%), severity (up to 25%) and DON accumulation (up to 51%) in durum wheat under field conditions.

S13-3 Genomic comparisons of biocontrol *Aspergillus flavus* strains revealed rearrangements that disrupt secondary metabolite gene clusters

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Abstract: *Aspergillus flavus* is a prevalent saprophytic and pythopathogenic fungus that causes loss of billions of dollars globally due to damage to and mycotoxin contamination of pre- and postharvest crops, and negative health effects in humans and domesticated animals. The *A. flavus* mycotoxins of greatest concern are the aflatoxins. Use of non-aflatoxigenic strains of *A. flavus* to compete against aflatoxin-producing strains has emerged as one of the best management practices for reducing aflatoxins contamination. We recently sequenced the genome and transcriptome of a new potential *A. flavus* biocontrol agent isolated from almond. This strain, WRRL 1519, does not produce aflatoxins or cyclopiazonic acid. The genome of WRRL 1519 was similar to other strains in size (38.0 Mb), GC content (47.2%) and number of putative proteins (12,121). Compared to aflatoxigenic *A. flavus* strains, strain WRRL 1519 had low shared identity or deletions for many genes and proteins required for aflatoxins and cyclopiazonic acid (CPA) syntheses. Over half of the aflatoxin synthesis gene cluster was missing, while the CPA gene cluster could not be identified. The new strain also appeared to maintain functional sequences of genes known to be involved in infectivity, particularly a pectinase gene that is thought to be required for aggressive growth in plant hosts. These results indicated that strain WRRL 1519 would be a good candidate for reducing aflatoxins and CPA accumulation by out-competing toxigenic strains in infected host crops, and warrants further experimental study. We additionally compared the genomic arrangements of predicted protein-coding genes of WRRL 1519 and other naturally-occurring biocontrol strains NRRL 21882 (Afla-Guard), NRRL 18543 (AF36) and NRRL 30797 (K49) to those of the aflatoxigenic strain NRRL 3357. While the aflatoxin synthesis gene clusters were disrupted by deletions and point mutations, our work revealed that chromosomal transpositions also appeared to disrupt several secondary metabolite gene clusters in strain WRRL 1519. The loss of secondary metabolites may affect growth rate, toxicity and effectiveness of biocontrol. Continued computational analyses and

experimental work on the *A. flavus* genomes will identify defense, metabolic and infectivity genes of atoxigenic *A. flavus* strains that promote biocontrol-related management of toxin contamination.

S13-4 Molecular detection of *Penicillium nordicum* in cured meat products: food quality and safety implications.

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Abstract: Fermented and cured meat products are unique and often represented as an element of culinary heritage and gastronomic identity. Together with meat enzymes and bacteria, molds are very important in the ripening of some dry fermented meat products. They contribute to the development of the typical sausage flavor, prevent lipid oxidations and counteract undesirable microorganisms. Various genera of fungi could colonize salami but *Penicillium* species are predominant, and above all *P. nalgiovense*, *P. chrysogenum* and a new recently described species *P. salamii*. On the other hand, depending on its peculiar composition, the surface could be colonized by undesirable molds, like *P. nordicum* an important and consistent producer of the potent nephrotoxic ochratoxin A (OTA). Addressing the safety of seasoning of meat products, we developed different molecular approaches to detect the presence of *P. nordicum* and monitor OTA contamination risk. A sensitive and easy to use Loop-mediated isothermal amplification (LAMP) assay for *P. nordicum* detection on salami surface was set up targeting otapksPN gene, a key gene in the biosynthesis of OTA in *P. nordicum*. Positive reactions were detected directly in-tube by color transition of hydroxynaphthol blue from violet to sky blue. The assay was proved to be specific for *P. nordicum* and able to detect down to 100 fg of target DNA. In addition, gene expression of otapksPN gene in *P. nordicum* and OTA production were monitored throughout the seasoning process up to 30 days in a small-scale experiment. The expression of otapksPN gene was already detected after 4 days of seasoning and increased significantly after 7 days, reaching the maximum expression level after 10 days. Consistent with gene expression data, OTA was detected from the 4th day and its content increased significantly from the 7th day, reaching the maximum level after 10 days. Finally, the LAMP assay was tested to detect the persistence of *P. nordicum* during the seasoning process of sausages after co-inoculation of the fungus with *P. nalgiovense* at different contamination rates. After 14 days of seasoning, LAMP assay was able to detect the presence of *P. nordicum down to* 2.5% of *P. nordicum* contamination. The analysis of toxin content at the end of seasoning, revealed that OTA was accumulated both in mycelium and dry-cured meat when *P. nordicum* contamination rate ranged from 25% to 100% of inoculum, while OTA was not detected in dry-cured meat at 2.5% and 0.25%. These results evidenced that contamination of dry-cured meat products by *P. nordicum* could represent a serious concern for salami production and therefore molecular tools, such as LAMP and gene expression assay, should be considered for new HACCP plans in order to prevent and control OTA risk in dry-cured meat production.

S13-5 Ergot alkaloid synthetic capacity of *Penicillium camemberti*

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Abstract: *Penicillium camemberti* plays a major role in the ripening process of brie and camembert type cheeses. Investigation of the recently sequenced *P. camemberti* genome revealed the presence of a cluster of five genes previously shown to be required for ergot alkaloid synthesis in other fungi. Clustered with the five ergot alkaloid synthesis genes (*eas* genes) were two additional genes that had the apparent capacity to encode enzymes involved in secondary metabolism. We analyzed samples of brie and camembert cheeses as well as cultures of *P. camemberti* grown under different conditions by

HPLC with fluorescence detection and LC-MS and did not detect any known ergot alkaloids, indicating the *P. camemberti eas* genes were either not expressed or encoded non-functional enzymes. We used a heterologous expression strategy to investigate the theoretical biosynthetic capacity of *P. camemberti*. Based on studies with the related ergot alkaloid-producing fungus *Neosartorya fumigata* (*Aspergillus fumigatus*), the five known *eas* genes found clustered in the *P. camemberti* genome should give the fungus the capacity to produce the ergot alkaloid chanoclavine-I aldehyde. We used a chanoclavine-I aldehyde-accumulating mutant of *N. fumigata* as a recipient strain in which to express the two uncharacterized *P. camemberti eas* cluster genes (named *easH* and *easQ*) to create a functioning facsimile of the *P. camemberti* cluster. Expression of *easH* and *easQ* in the chanoclavine-I-accumulating *N. fumigata* strain resulted in the accumulation of a pair of compounds of *m/z* 269.1289 in positive mode LC-MS. Since this *m/z* is consistent with the molecular ion of the isomeric pair of [rugulovasine A/B + H]$^+$, we analyzed a culture of the rugulovasine producer *Penicillium biforme* (a recent ancestor of *P. camemberti*) and found the same isomeric pair of analytes with the same retention times. Fragmentation of the analytes yielded fragments typical of those resulting from fragmentation of rugulovasine A/B. The deduced activities of the products of *easH* and *easQ* indicate the capacity to catalyze theoretical reactions that provide a reasonable pathway from the precursor chanoclavine-I aldehyde to the products rugulovasine A/B. The chanoclavine-I aldehyde-accumulating mutant of *N. fumigata* transformed with the *P. camemberti easQ* gene alone yielded an abundant analyte of *m/z* 271.1, consistent with the addition of oxygen to chanoclavine-I aldehyde. Transformation of the *N. fumigata* strain with *P. camemberti easH* alone did not yield a novel product, indicating that EasH acts after EasQ in the pathway. The data indicate that *P. camemberti* has the genes to produce the ergot alkaloids rugulovasine A/B but that during domestication isolates that failed to produce alkaloids were selected.

S13-6 Prevention of mold spoilage and mycotoxin production: using the right methodology.

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Abstract: Fungal spoilage of foods and beverages imposes significant annual global revenue losses. Mold spoilage can also be a food safety issue due to the production of mycotoxins by these molds. To prevent mold spoilage and mycotoxin production, several hurdles can be used: (1) reducing the water activity, (2) thermal processing, (3) addition of preservatives, (4) reduction of oxygen in the packaging using vacuum, oxygen scavengers or modified atmosphere packaging (MAP), and (5) refrigerated storage. These hurdles individually target a different group of spoilage fungi; the use of two or more hurdles will reduce the number of molds that can spoil the product. This is called the associated mycobiota that typically comprises only a few mold species. It is essential that the associated mycobiota be adequately isolated and accurately identified. While classic mycological detection methods can detect a broad range of fungi using well-validated protocols, they are time consuming, require skilled personnel, and some methods have low sensitivity. Molecular methods for the detection of fungi from spoiled foods are faster than conventional methods but require good DNA isolation techniques, expensive equipment and may detect non-viable fungi that are unlikely to spoil a specific product. One of their advantages, especially in PCR-based methods, is the specific detection of small amounts of target organisms by amplifying their DNA in a considerably short time frame. Identification based on phenotypic characters can be time consuming and well-trained staff is needed. It is therefore more prone to erroneous identifications than a sequence-based identification. However, the results of a sequence-based identification heavily depend on the quality of the database. In order to prevent misidentifications, it is strongly recommended to use sequence-based techniques in conjunction with

morphological techniques. Strain typing – distinguishing between different strains of the same species – is used to get insight in the genetic diversity of spoilage agents (is the contamination caused by the same strain?) or can be used to trace the source of the contamination. Although there is no complete or easy method for the detection of fungi in foods it is important to be aware of the limitations of each methodology.

Symposium Session 14:

Light Sensing in Fungi

D. Rangle and L. Larrondo

S14-1 Dual roles of fungal phytochrome: a light receptor and a temperature sensor

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Abstract: To survive in the ever-changing environment, fungi have evolved to sense internal and external signals and adapt to various stresses. Light as one of the most important environmental signals regulates morphogenetic and physiological processes (1). To sense the light, fungi have been equipped with different photoreceptors during the evolution. *Aspergillus nidulans* is able to sense red and blue light with the red-light sensor phytochrome (FphA) and the blue light senor white collar-1 (LreA), respectively. Phytochrome consists of a photosensory, a histidine kinase and a response-regulator domain and uses the SakA/HogA MAP kinase pathway to transmit light signal (2). The phosphotransfer protein YpdA interacted with the response regulator domain of FphA. The conserved histidine (H770) in the histidine kinase domain and aspartate (D1181) in the response regulator (RR) domain are essential for the activity of FphA. When H770 was mutated to glutamic acid (E), the light-regulated genes *ccgA* and *conJ* were already induced in the dark. Thus, the negatively charged amino acid glutamate mimicked phosphorylation of histidine. FphA wild type and mutant forms were further purified from *E. coli*. The H770E mutated form migrated faster in size exclusion chromatography (SEC) than the wild-type form, which implying the negative charges cause the conformational change of FphA. Intriguingly, phytochrome is more than a light receptor. *In vitro*, the spectra properties of recombinant FphA were changed with temperature. The dark reversion of FphA was slightly increased when the temperature was increased. *In vivo*, higher temperature caused the transient activation of SakA/HogA pathway and *ccgA* and *ccgB* were induced. In *fphA*-deletion strain, SakA phosphorylation and the gene induction in higher temperature were reduced in comparison to wildtype strain. These results demonstrate phytochrome plays dual roles in sensing temperature and light.

S14-2 Mycelial growth under light improves conidial stress tolerance and virulence of *Metarhizium robertsii* and up-regulates stress related genes

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Abstract: Light conditions during fungal growth are well known to cause several physiological adaptations in the produced conidia; thus, conidia of the insect-pathogenic fungi *Metarhizium robertsii* were produced on: 1) potato dextrose agar (PDA) medium in the dark; 2) PDA medium under white light; 3) PDA medium under blue light; and 4) PDA medium under red light. The conidial production, the speed of conidial germination, the virulence to the insect *Tenebrio molitor*, as well as gene expression, and tolerances to osmotic stress and to UV radiation were evaluated. Conidia produced

under white light or blue light germinated faster and were the most tolerant to UV radiation and osmotic stress. White light improved conidial virulence as compared with conidia produced in the dark. Growth under blue light produced more conidia than the fungus grown in the dark. The small (*Mrhsp30*) and large (*Mrhsp101*) heat shock protein genes were highly up-regulated under white light condition, suggesting an active role of heat shock proteins in fungal exposition to the different visible spectrum components. The cytosolic catalase *Mrcatc* gene was not induced under all light conditions assayed. Conidia produced under red light germinated slower than conidia produced in the dark and were the least tolerant to osmotic stress and UV radiation. The virulence of conidia produced under red light was similar to conidia produced in the dark. In conclusion, white light produced conidia that germinated faster and killed the insects faster; in addition, blue light afforded the highest conidial production. Both white light and blue light afforded the highest tolerance to both stress conditions.

S14-3 Shining light on a creature of the dark: extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats

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Abstract: Fungi have evolved complex light-sensing and regulatory systems that control core metabolic processes involved in many aspects of fungal biology, including the ability to repair DNA damage from ultra-violet (UV) light. Repair of UV-damaged DNA lesions in fungi is mediated by several different conserved mechanisms and these repair systems have typically been studied in fungi that have evolved in the presence of light. The fungal pathogen causing white-nose syndrome of bats (WNS), *Pseudogymnoascus desctructans*, affords a rare opportunity to study a fungus that has evolved for millions of years in the absence of light. WNS has decimated North American hibernating bats since its introduction to North America in 2006. In order to better understand this disease, we utilized a comparative genomics approach for *P. destructans*, comparing its genome to those of six closely related non-pathogenic *Pseudogymnoascus* species. A large reduction (~ 65%) in carbohydrate utilizing enzymes (CAZYmes), a reduction in the predicted secretome (~50%), an increase in unique gene models, and estimation of last common ancestor of 23.5 MYA indicate that *P. destructans* has a long evolutionary history with bats and likely evolved alongside bats in the absence of light. *P. destructans* has lost a key enzyme, UVE1, in the alternate excision repair (AER) pathway, which functions to repair DNA-lesions induced by ultra-violet (UV) light. Consistent with a non-functional AER pathway, *P. destructans* is extremely sensitive to UV-light as well as the DNA alkylating agent methyl methanesulfonate (MMS). A better understanding of light-sensing and regulatory systems may be gained by examining fungi that have evolved for long periods of time in dark environments, for example the digestive tracts of animals.

S14-4 What makes a zombie ant tick: the daily rhythms in a fungal behavioral manipulator

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Abstract: Adaptive manipulation of host behavior is an effective way for parasites to increase transmission rates. Manipulations resulting in summiting of insect hosts can be observed in Zygomycetes and Ascomycetes of the genera *Entomophthora* and *Ophiocordyceps*. Climbing, biting to fix the host at elevated positions, following death, and spore release all appear to display time-of-day synchronization. Biological clocks of the insect host and the fungal parasite, as well as environmental factors such as light

and temperature, likely play an important role in these parasite-host interactions. To begin to test these hypotheses we use an integrative approach, combining ecology with behavioral analyses and next-generation sequencing, on a variety of *Ophiocordyceps unilateralis sensu lato* - carpenter ant interactions. Field data indicates that light influences the manipulated behavior of *Ophiocordyceps*-infected carpenter ants. This suggests that the fungal parasite, which is fully pulling the strings at this point, can sense light, and has a light entrainable biological clock. To demonstrate this, as well as identify candidate molecular clock components of *Ophiocordyceps*, we made use of bioinformatics and transcriptional profiling. We did this for the recently sequenced behavior-manipulating parasite *Ophiocordyceps kimflemingiae* (a named species of the *O. unilateralis* complex). Through a bioinformatics approach, we identified putative homologs of known clock genes. RNA-Seq was performed on 48 h time courses of *O. kimflemingiae* to determine daily rhythms and enrichment patterns in the transcriptome. Liquid media cultures were entrained under 24 h light-dark (LD) cycles and harvested at 4 h intervals under LD or continuous darkness. We identified a significant number of fungal transcription factors with peaked activity during the light phase (day time). In contrast, a significant number of secreted enzymes and small bioactive compounds, proteases, and toxins peaked during the dark phase or subjective night. These findings support a model whereby *Ophiocordyceps* species use their biological clock for phase-specific activity and light-dependent manipulation. This may be a general mechanism involved in parasite-host interactions across taxa.

S14-5 Light-Sensing, optogenetics and photographic memory: developing biotechnological solutions and pushing the boundaries between science and art

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Abstract: The filamentous fungus *Neurospora crassa* perceives and responds to blue light through a transcriptional heterodimer named White Collar Complex (WCC). One of its components, WC-1, possesses a LOV (Light Oxygen Voltage) domain capable of detecting blue wavelengths, which promotes a conformational change that leads to dimerization, resulting in strong transcriptional activation, in a light-intensity dependent manner. In order to design and improve optogenetic switches that can be utilized in other organisms as orthogonal controllers, we have been exploring the dynamics of light responses in this fungus. Thus, through the development of *Neurospora*-based optogenetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, in yeast, now we can efficiently induce gene expression over 1000-fold and control biotechnological relevant phenotypes such as flocculation by switching on/off the lights. We have also adopted optogenetic approaches to further delve into *Neurospora*'s circadian and light-responses. In doing so, we were able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a *Neurospora* carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image. Thus, we have established a live canvas in which images are genetically processed and reconstituted with real-time dynamics. Such technology not only allows studying light-responses with great resolution, but also provides a powerful artistic substrate. Remarkably, since the live canvas circuit is integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase

responses, but it also allows for the opportunity to ponder on concepts such as vision and memory. MIISSB and FONDECYT 1171151 and HHMI International Research Scholar grant.

S14-6 Identification of genes involved in fruiting body induction in *Coreopsis cinerea*

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Abstract: Light and nutrient are crucial environmental factors influencing fungal sexual reproduction. Gene expressions influenced by such environmental factors in *Coprinopsis cinerea* were investigated. Blue light induces simultaneous hyphal knot formation in mycelia grown on low glucose (0.2%) media, but not in mycelia grown on high glucose (1%) media in *C. cinerea*. Many hyphal knots are visible in arc near the edge of the colony one day after a 15min of blue light stimulation. These suggest that blue light accelerates hyphal knot induction in nutrient limiting condition. Trascriptome (Super-SAGE) analysis revealed that gene expression after light exposure divided into at least two major stages. In the first stage, genes coding for fasciclin (*fas1*), cyclopropane-fatty-acyl-phospholipid synthases (*cfs1* and *cfs2*), and putative lipid exporter (*nod1*) are highly expressed at 1h after light exposure in the mycelial region where the hyphal knot will be developed. It is reported that the *cfs1* mutated strain was defected in fruiting body development. These genes were up-regulated by blue light, and not influenced by glucose condition and mating. These results suggest that although some of the genes are critical for induction of the hyphal knots, they are not sufficient for hyphal knot development. In the second gene expression stage, genes encoding galectins (*cgl1-3*), farnesyl cysteine-carboxyl methyltransferases, mating pheromone containing protein, nucleus protein (*ich1*) and laccase (*lcc1*) are specifically upregulated at 10-16 h after blue light exposure when the mycelia are cultivated on low glucose media. These might be involved in building architecture of hyphal knot or signal transduction for further fruiting body development. These results contribute to the understanding of the effect of environmental factors on sexual reproduction in basidiomycetous fungi.

Symposium Session 15:

A Big Puzzle to Assemble: Using Taxonomy to Unravel Ecology and Biogeography of Ectomycotthizal Symbiosis in the Tropics

M. A. Neves and A. M. Vasco-Palacios

S15-1 Mycorrhizal associations of *Pseudomonotes tropenbosii* (Dipterocarpaceae) in tropical rain forests of Colombia, Amazonia

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Abstract: Dipterocarpaceae is an important tree family in Paleotropics that form ectomycorrhizal (EcM) symbiosis with fungi and it has been hypothesized that dipterocarps have been partnered in this mutualistic association prior to the separation of Gondwana. Two species of trees have been recently

described from Neotropical ecosystems, *Pakaraimae dipterocarp* from Guyana and Venezuela, and *Pseudomonotes tropenbosii* from Colombia. We documented the EcM fungal diversity in a terra-firme forest with the endemic dipterocarp *Pseudomonotes tropenbosii* in the lowlands of Colombian Amazonia by collecting fruit bodies and by using rDNA sequence analysis of root material for identification of both fungal and plant symbionts. We addressed whether the fungal EcM community associated with *P. tropenbosii* exhibited spatial differences and if the fungal community revealed similar composition that other Neotropical hosts. A total of 83 species of EcM macrofungi were identified based on morphology-based techniques. These taxa represented 16 families and 27 genera. The most abundant families were Boletaceae (7 genera; 13 species), Clavulinaceae and Russulaceae (13 species). Fifteen species constituted new reports for Colombia and at least 18 species found in the study area new to science. Two of those have recently been described as *Austroboletus amazonicus*, *Sarcodon colombiensis* and others belong to genera such as *Russula, Coltriciella, Coltricia,* and *Amanita*. Species richness detected from 200 fragments of mycorrhizal roots tips revealed 34 species-level (ITS sequences). Seven species were detected three or more times such as *Craterellus cinereofimbriatus*, Uncultured *Cortinarius* 866root, Uncultured *Tomentella* 1452root and Uncultured *Sebacina* sp. 8. The hosts identified were *P. tropenbosii*, *Coccoloba* sp. and other plants not reported as EcM hosts as for example *Brosimun*, *Ipomoea* and *Protium*. Differences were observed in the composition of the EcM fungal community for 3 populations of *P. tropenbosii* in Colombia Amazonia. Most of the fungal species documented in this study have also been found in symbiotic associations with other legume or dipterocarp species from geographically distant forests located in Brazil, French Guyana, Guyana and Venezuela. The distribution of some fungal species that were previously considered restricted to the Guiana Shield was extended to *P. tropenbosii* forests in Colombia. The result highlights the low specificity of EcM fungi in relation to their host plants in Neotropical lowland forests. It is important to address further studies in understanding how factor such as forest structure, size of the host plants patches, host distributions and edaphic factors may drive the structure of the EcM fungal communities in Neotropical ecosystems.

S15-2 African ectomycorrhizal communities, diversity of three vegetation types compared, focusing on Russulaceae

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Abstract: Ectomycorrhizal (ECM) fungi play a major role in tropical and subtropical African forest ecosystems, where many trees, often growing on N- and P-poor soils, completely depend on these associations. Based on above-ground biodiversity records, Russulaceae are the dominant group of ECM fungi in tropical African ecosystems, followed by Boletales and Cantharellaceae. The first soil diversity studies, however, show that Thelephoraceae follow Russulaceae in below-ground species richness. Sub-Saharan Africa is characterized by three vegetation types dominated by ECM associations: Central African Guineo-Congolian rainforests, West African Sudanian woodlands and East African Zambezian Miombo woodlands. Little is known concerning composition and distribution of ECM fungal communities in these vegetation types. We studied ECM fungal diversity of rainforest in Cameroon, Sudanian woodland in Togo and Miombo woodland in Zambia. Root tips were sampled in multiple plots per vegetation type and IonTorrent was used to sequence the ITS2 region from the root tips. Our results confirm Russulaceae as dominant ECM group below-ground in all three vegetation types, followed by Sebacinaceae and Thelephoraceae. ECM fungal community composition is strongly correlated with

edaphic factors, with many fungi occurring in either woodland (high pH, together with low C, N and organic material) or rainforest (low pH, together with high C, N and organic material). ECM community composition thus differed amongst the three vegetation types, with the main regions of overlap occurring in the riparian forests in between the vegetation types. It is difficult to draw conclusions on species richness based on metagenomic sequences. Due to the absence of a taxonomical reference framework for most fungal groups, the majority of operational taxonomical units (OTU's) could only be identified on genus level (>50%) or higher (>15%). This makes it difficult to see trends in species composition between vegetation types. Only for the milkcap genus *Lactifluus* (Russulaceae), for which we constructed a solid taxonomical framework over the years, most OTU's could be identified on species level and more detailed conclusions on above- versus below-ground species richness could be drawn.

S15-3 Ectomycorrhizal fungal communities associated with *Oreomunnea mexicana* show high beta diversity at local and regional scales in Central America

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Abstract: *Oreomunnea mexicana* (Juglandaceae) is an ectomycorrhizal (ECM) tree distributed from Mexico to Panama. This species forms monodominant stands in tropical montane forests between 1000 and 1900 m a.s.l. Recent studies of *O. mexicana* in Panama indicate that this species is associated with a diverse community of ECM fungi, but this tree has been studied at only a few sites. We collected fruiting bodies and root tip samples from eight localities (four in Panama and four in Mexico) to elucidate the effect of geographic location, soil fertility, precipitation, temperature, and host abundance on the ECM fungal communities associated with *O. mexicana*. Fungal fruiting bodies were preserved and ITS sequences were generated for molecular identification. In addition, ECM roots were sampled from 60 *O. mexicana* trees (40 trees in Mexico and 20 in Panama) and the fungal communities were assessed based on Illumina ITS1 sequencing. We sequenced 874 ECM fruiting bodies and preliminary results suggest a high a diversity of ectomycorrhizal fungi with approximately 360 OTUs. Of these, 17 OTUs were shared between sites in Mexico and Panama. Fungal diversity in *O. mexicana* roots based on Illumina sequencing was high, with approximately 4000 OTUs (97% OTUs cutoff and all OTUs > 10 reads) with 1541 belonging to ECM genera. Alpha diversity of both the root-associated fungi and the ECM fungi were higher in Mexico than in Panama, consistent with a previously documented global pattern of decline in species richness of ECM fungi towards the equator. This lower species diversity of ECM fungi in Panama could be associated with a lower species richness and abundance of ECM plant species in the surrounding forests. Beta diversity was high at both regional and local scales with a significant turnover of fungal species. At a local scale, β-diversity was higher in Panamanian *O. mexicana* populations, with species composition changing rapidly over a short geographical distance. These results are consistent with previous findings based on Sanger sequencing where high β-diversity was found with *O. mexicana* in sites with contrasting soil fertility. Permutation tests determined that total soil nitrogen was the environmental variable that significantly explained the most variation of both the total

root-associated fungal community and the ECM community. However, total soil nitrogen was also highly correlated with total soil carbon (98%). This is the first study to focus on the variation of ECM communities associated with a tropical tree at both regional and subcontinental scales. This work supports the hypothesis that soil nutrient availability is the main factor structuring local ECM fungal communities and that ECM fungal communities are less species rich towards the equator.

S15-4 Ectomycorrhizae in the Neotropical dry forest: mycobionts, new hosts and morphology

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Abstract: Neotropical dry forest extends from Northern Mexico to Colombia, Brazil, and Peru, also including Caribbean islands. This ecosystem is imperiled by the land use change to agriculture or grazing. Plants and their symbionts from this forest have adaptations to resist long dry periods. Our main objective was to detect the ectomycorrhizal hosts and their mycobionts by sampling roots, fruitbodies and soil, and describe their interactions. We sampled roots from two places from the Mexican Pacific coast, Jalisco and Oaxaca. The roots were dissected and all the ectomycorrhizae (ECM) morphotypes were separated for anatomical observations and sequencing. We also sampled fruitbodies and soil, amplified ITS region, and sequenced by Sanger and Illumina respectively. To identify the photobiont, we amplified rbcL, matK and trnL regions from the ECM. We found that ECM were restricted to particular plant genera, such as: *Achatocarpus, Coccoloba, Guapira, Pisonia* (Caryophyllales), and also some legumes, for example *Lonchocarpus*. These plants are frequent to scarce in the forest, none of them forms patches. From 19 ectomycorrhizal fungal species we amplified from roots, 18 were not in NCBI or UNITE data bases. ECM fungi belonged to *Clavulina, Inocybe, Membranomyces, Russula, Sebacina, Thelephora, Tremelloscypha, Tomentella*. We have described three new species including their fruitbodies and mycorrhizal associations: *Thelephora versatilis, T. pseudoversatilis* and *Tomentella brunneoincrustata* (Thelephoraceae). Illumina sequences found 121 ectomycorrhizal species as propagules in the soil. *Tremelloscypha* sp. was the most frequent on root-tips, forming fruit bodies and also in soil sequences. The ECM morphotypes were monopodial and rarely ramified, with a wide range of colors (whitish to dark hyphae), mantle with contact exploration type to mantle with abundant rhizomorphs. *Coccoloba, Guapira* and *Pisonia* morphotypes developed a paraepidermal Hartig net, however *Achatocarpus* does not form Hartig net. Under dry periods, ECM from *Coccoloba* have incomplete mantles, covering just some parts of the root. Our results showed that ECM fungi are mostly specific, its hosts are scarce in the forest, the ectomycorrhizal fungi are probably endemic, their morphotypes do not present the "typical" ectomycorrhizal characteristics and these vary depending on host and environmental conditions.

S15-5 Linking fungal community functions to forest dynamics: a geographical gradient of fungal community and wood decay in pine logs, and its possible effects on forest regeneration

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Abstract: The decay process of dead wood is crucial for biodiversity in forest ecosystems. Wood decay types, traditionally categorized into white, brown, and soft rots, are the consequences of fungal decay activities and strongly affect biotic communities inhabiting dead wood, including tree seedlings. Given that fungal community is affected by climatic conditions, it is important to evaluate the occurrence

patterns of the decay types along a geographical range to understand forest dynamics in wide spatial scale. In 30 sites covering a latitudinal gradient in Japan, I examined the effects of environmental variables on fungal community and the occurrence of wood decay types in logs of *Pinus densiflora*. Fungal community detected by 454 pyrosequencing showed significant associations with mean annual temperature (MAT) and annual precipitation. Among the wood decay types, the frequency of brown rot was negatively correlated with latitudinal gradient and that of soft rot was positively correlated with MAT. In contrast, white rot was negatively correlated with MAT. Incubation experiments using 36 isolates of 17 basidiomycetes obtained from pine dead wood showed that hyphal growth rates of brown rot fungi were significantly higher than that of white rot fungi in warm conditions (25–35˚C), whereas growth rates were not different between white and brown rot fungi in cool conditions (5–20˚C). These results suggested that activity of brown rot fungi is more prominent in the warmer lower-latitude areas than in the cooler higher-latitude areas in pine log decomposition in Japan. I also examined the effects of wood decay type on seedling densities of 14 tree species growing on pine logs and found that responses to brown rotted wood was considerably different among tree species. For example, seedling densities of *Cryptomeria japonica*, *Chamaecyparis obtusa*, *Clethra barbinervis*, and *Rhus trichocarpa* were positively associated with brown rot, whereas seedling density of *Pinus densiflora* was negatively associated with brown rot. Furthermore, survival and growth rates of *Cryptomeria japonica* seedlings also showed positive associations with brown rot. These results suggested that functional diversity in wood decay fungi could induce niche separation among seedling species on dead wood and affect forest regeneration.

S15-6 Grammar and syntax: Decoding the language of mutualistic vs. pathogenic signaling during plant-microbe interactions

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Abstract: All interactions between living organisms are governed by communication, be this the exchange of simple chemicals to proteins to complex vocalizations. In the interaction between plants and microbes we can identify hundreds of communication signals using multi-omic approaches. What these lists do not tell us, however, is the function or specificity of these signals within a specific symbiotic interaction. This gap in our knowledge can be addressed by studying the mechanistic activity of these molecules and the context within which they operate. Within the study of human-human communication, language is bound by grammar and syntax: the rules governing the general use of language and the finer study of how sentences are put together, respectively. Therefore, by characterizing microbial signals, such as when they are produced, the signaling cascades that they operate within, and their targets within the host organism, we may begin to understand the "grammar" and "syntax" of plant-microbe communication. In this talk, I will cover our work into identifying the proteomic and metabolomic signals used by mutualistic and pathogenic fungi during the interaction with their host plant. Further, I will go into work studying their modulation and the pathways that they effect. I will specifically focus on two model interactions: the mutualistic symbiosis between *Pisolithus* and *Eucalyptus* and contrast this with the pathogenic symbiosis between*Armillaria* and *Eucalyptus*. By comparing these two systems, I will conclude with our current theories upon how contrasting microbial lifestyles may communicate differently with their hosts and how perception of these different "languages" by the plant impacts the ultimate fate of the interaction.

Symposium Session 16:

IMC/ISHAM Human Pathogenic Fungi, Taxonomy and Global Emergence

J.F. Meis and A. Lorenz

S16-1 Genetic diversity and phenotypic variability in the emerging fungal pathogen *Candida auris*

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Abstract: *Candida auris* has been identified as a medically relevant, drug-resistant fungus only in 2009, and is thus a rare example of a completely new pathogenic microbe emerging in the spotlight of global health care. Its emergence and population structure are unusual in many respects (according to current research four distinct geographical clades can be discerned). Because of the recent emergence of *C. auris*, its life cycle and general biology is still enigmatic. Inferences from research on the widely-studied *C. albicans* are not always transferable to *C. auris*, because of the large evolutionary distance between them. Research in our laboratory and by other groups indicate that *C. auris* and *C. albicans* display substantial difference in their cell biology. To provide a better understanding of the phenotypic diversity of *C. auris* – which, ultimately, will enable the Medical Mycology community to progress research into novel diagnostics and therapies – our laboratory studied the cellular and chromosomal features of a series of *C. auris* strains representing the four main geographical clades to understand its genome organization and variation on a species-wide level. Genome size measurements and electrophoretic karyotyping revealed *C. auris* to be a haploid species (in line with whole-genome sequencing by us and other groups). *C. auris* isolates have a plastic karyotype containing 5-7 chromosomes with substantial chromosome number and size variation, both within and between geographical clades. This plasticity of karyotypes within a clade was somewhat unexpected considering the uniformity of *C. auris* on a DNA sequence level belonging to the same geographical clade. This indicates that genome rearrangement on a chromosomal level potentially is a mechanism *C. auris* employs to generate genetic diversity during adaptation to environmental challenges. We are interested in how diversity is generated in *C. auris* and how it affects fundamental features, including growth and stress response, and clinically relevant traits, such as virulence and drug resistance. Recent experiments on *C. auris* phenotypic diversity between clinical isolates in terms of cell morphology and stress response will be discussed.

S16-2 Global emergence of the pathogenic yeast *Candida auris*

E. Johnson
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Abstract: Most human systemic yeast infections are endogenous in origin and until recently fungal outbreaks involving yeast species were rare and involved small numbers of patients in single units. The emergence and global spread of the pathogenic yeast *Candida auris,* first recognised in 2009, has been unprecedented; there have now been nosocomial candidaemia outbreaks in high intensity care settings on five continents reported in a relatively short timescale. There are four recognised clades of *Candida auris,* each of which is associated with emergence and initial subsequent spread in a defined geographical area; the first reported Japanese clade, a South Asian clade, a South African clade and a South American clade. However, with global travel and migration now commonplace several different clades have been found circulating in other countries such as the UK and the USA suggesting multiple

independent introductions. Mortality rates as high as 60% associated with deep-seated *Candida auris* infection have been reported in some countries, although attributable mortality is often hard to establish in the high intensity care settings in which most outbreaks have been reported. *Candida auris* often displays drug resistance, most often to fluconazole but many strains are also resistant to other azoles and sometimes several different antifungal classes. In addition to a worrying innate drug resistance profile, drug resistance also appears to develop quite readily on therapy. This yeast has a propensity to spread from patient to patient and to persist in the environment and index cases are often associated with multiple cases of colonisation of patients in adjacent areas, sometimes leading to subsequent infections. Even with rigorous infection control measures outbreaks have been difficult to control. This talk will discuss some of the findings on the biology, pathogenicity, biofilm formation and resistance mechanisms of this emerging pathogen.

S16-3 Using whole genome sequencing to elucidate the origins and timeline of *Cryptococcus gattii* species complex emergence in North America

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Abstract: *Cryptococcus gattii* species complex (SC) is a group of at least four species of Basidiomycete yeasts that are common in the environment in tropical and subtropical regions. A recent increase in surveillance for the SC allowed more frequent recognition of *C. gattii* SC as the cause of human disease in temperate environments as well. In the last two decades *C. gattii* SC has emerged as a major pathogen in the temperate Pacific Northwest (PNW) of North America. The origin and timeline of this emergence was elucidated using Illumina whole genome sequencing (WGS) and SNP calling. Using a worldwide collection of *C. deuterogattii* isolates it was shown that the origin of the emergence of this species in the PNW was most likely the Brazilian Amazon rainforest. Using Bayesian Evolutionary Analysis by Sampling Trees (BEAST) it was shown that the North American introduction and emergence most likely happened within the last 100 years and that there were at least three separate introductions. The PNW emergence of *C. deuterogattii* served as the baseline for analysis of *C. gattii* sensu stricto in the Southeastern United States. Although less frequently encountered clinically, BEAST analysis showed that the introduction of *C. gattii* in the Southeastern US likely took place thousands of years ago as opposed to a hundred years ago for *C. deuterogattii* in the PNW.

S16-4 Maldi-Tof MS for the identification of filamentous fungi; implications for the clinical lab

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Abstract: Though important advances have been made over the past decades at the level of identification of filamentous fungi, it remains quite a challenge. Taking into account that the number of patients at risk for invasive fungal disease (IFD) is increasing and that superficial dermatophyte infections of skin and nails are affecting about 25% of the world's population, there is a need for reliable, fast and cost-effective identification methods. Species-level identification is critical for correct patient treatment, and it is generally accepted that a rapid diagnosis, coupled to the early onset of the appropriate treatment, leads to a better patient outcome. Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has already been widely used in the clinical routine for the identification of bacteria and yeasts. Its implementation for the identification of molds has been delayed, due to several reasons. Because of their tough cell wall, a protein extraction protocol needs to be

applied, and the heterogeneity of the cultures implies that cultivation and sample processing have to be standardized as much as possible. Furthermore, extended reference spectra databases have been lacking. In the frame of the BCCM/IHEM collection of biomedical fungi, and in collaboration with French colleagues, we have developed an in house reference spectra database consisting of 1913 strains, belonging to 938 species and 246 genera. This database is currently, to the best of our knowledge, the largest reference spectra database in the world. This database has been challenged in different clinical settings for its capacity to identify clinical isolates, including closely related species and dermatophytes. Based upon this database, MALDI-TOF MS has moreover been implemented in the routine of the BCCM/IHEM quality control (ISO17025 accreditation). The same set of strains has been used for the development of an online MALDI reference spectra database. This online tool is freely available, and was validated with clinical isolated from Belgium and France. It is now routinely used by many other laboratories throughout the world. In conclusion, MALDI-TOF MS is a fast, reliable and cost effective method for the identification of filamentous fungi, which van successfully be implemented in a clinical routine setting.

S16-5 Global emerging azole resistance in *Aspergillus fumigatus*: one health and the environment

J. F. Meis
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Abstract: Azole antifungals play an important role in the management of fungal infections. However, in the last decade azole resistance in *Aspergillus fumigatus* has been increasingly reported and this is potentially complicating the effective management of these diseases. Started as a clinical rarity in the last decade of the 20th century, less than 15 years later the potential problem of resistance in *Aspergillus* infections has been recognized on all continents. The higher mortality rates observed in patients with invasive aspergillosis caused by azole resistant *A. fumigatus* pose serious challenges to the mycologist for timely identification of resistance and appropriate therapeutic interventions. The 'TR_{34}/L98H' mutation in the *cyp51A* gene of *A. fumigatus*, which is associated with azole fungicide use is still responsible for most multi-azole resistance seen in many European and Asian countries. Azole-resistant isolates carrying this mutation have been reported from both patients and the environment. Further, several newly emerging resistance mutations are recognized with 'TR_{46}/Y121F/T289A', conferring high voriconazole and variable itraconazole MICs, being now the second most common. Many more resistance mutations associated with environmental selection such as 'TR53' and 'TR_{92}/Y121F/T289A' are being described adding to the emerging problem. Environmental screening and routine antifungal susceptibility testing of clinically significant isolates should be considered in order to develop guidelines for local and national purposes. Considering that azole antifungal drugs are the mainstay of (oral) therapy, especially for chronic invasive and allergic aspergillosis, emergence of resistance will have profound impact on healthcare. This presentation highlights the global development of resistance in *A. fumigatus* and the associated impending clinical treatment problem.

S16-6 Risk factors of vulvo-vaginal candidiasis and antifungal susceptibility pattern of *Candida* species isolated from women of reproductive age group

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Abstract: Vulvo-vaginal candidiasis (VVC) is a widespread inflammatory condition of female genital tract and most encountered problem that affects a large fraction of women in a population. The condition is

caused by numerous microorganisms involving yeast. This study was conducted to examine the risk factors of vulvo-vaginal candidiasis and to evaluate the in-vitro sensitivities of the isolated *Candida* species to six antifungal agents. Vaginal, endocervical and urine samples were taken from consenting women. Patients completed a questionnaire assessing the clinico-demographic and risk factors of candidiasis. Standard microbiological techniques such as Lactophenol Cotton Blue Stain, Germ Tube Technique, chlamydospore formation, wet mount and culture were used to analyse the samples. Frequency, percentile and chi- square tests were performed. From a total of 86 respondents, less than one third (30.23 %, 26) were from the outpatient charity department and 69.77 %, 60 came from the outpatient private physician's clinic. Respondents between 41 and 45 years old had the highest frequency of *Candida* infection (9, 28.12%). Majority of the respondents positive for candidiasis were married. Out of 32 respondents, two (6.25%) had sexual contact with someone having candidiasis. *C. krusei* was isolated from patients who had sexual contact with the same sex. A total of three (9.37%) patients had multiple sex partners. There were 11 (34.37 %) *Candida* species isolated from patients with history of candidiasis in the family. Vaginal and labial itchiness and abnormal discharges showed the highest distribution for *Candida* species. Antifungal susceptibility testing revealed that eighty (88.33%) out of the 96 species isolates were susceptible to amphotericin-B, 38 (39.58%) to Griseofulvin, 72 (75.00%) to Sporonox, 21 (21.88%) to Nizoral, 15 (15.63%) to Diflucan and 78 (81.25%) to Nystatin. The results of the study revealed that sexual activities and high level of reported risk behavior contributed to the development of VVC also this finding support literatures that antifungal prescription should be only given once the proper identification of the *Candida* species has been performed.Furthermore, improperly prescribed antifungal agents may lead to drug resistant.

Symposium Session 17:

Rhizobiomes – Their Interactions with the Hosts and Functions in a Changing Environment

A. Jumpponen, C. Kuske, and A. Porras-Alfaro

S17-1 Root endophyte-mediated manipulation of plant responses to pathogen attack

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Abstract: In order to address how plant-fungal interactions in natural soil shape and are shaped by local and systemic responses to beneficial and pathogenic root associated fungi a reductionist approach which takes advantage of a gnotobiotic natural soil-based split root system was established. Phenotyping, cytological and transcriptional analyses during barley infection with the root rot fungal pathogen *Bipolaris sorokiniana*, and colonization with the beneficial root endophyte *Serendipita vermifera* showed remarkably distinct responses of the host. Whereas the root endophyte only marginally affected the expression of plant genes, 2741 host genes were deregulated by pathogen infection. The presence of the root endophyte significantly reduced pathogen infection and disease symptoms increasing host resistance rather than tolerance against the pathogen without markedly altering plant response at the transcriptional level.The pathogenic fungus utilized secondary metabolites to antagonize the beneficial fungus whereas the root endophyte employed proteins with hydrolytic activities to parasitize the other fungus, including a chitinase with a CBM5-12 carbohydrate-binding domain found specifically in Agaricomycotina and chitinolitic bacteria. The identified sebacinoid mycoparasitic genes from soil confrontations are not induced *in planta* during tripartite

interaction, suggesting that the two fungi are not in direct contact inside the root and niche differentiation might occur. Systemic and local plant responses indicate that a plant component is involved in the increased resistance to the pathogen other than priming.

S17-2 Fungal rhizobiomes of foundation grasses shift across the North American Great Plains

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Abstract: Roots and their surfaces host diverse microbial communities. These rhizobiomes are currently attracting vast interest as a resource for designing microbial consortia that could be harnessed to improve restoration and revegetation success, and to improve yields while minimizing inputs in agricultural systems. Such efforts seem justified as the rhizobiomes are intimately associated with plant tissues, have the potential to regulate plant performance, and may be able to modify the host phenotypes and environmental tolerances. However, before such designed, synthetic consortia can be exploited, research to characterize the communities and – particularly – their stable or core members is mandatory. In the course of an ongoing collaboration, we investigated the biogeography of the fungal rhizobiomes associated with foundation grasses (*Andropogon gerardii* (big bluestem), *Bouteloua eriopoda* (black grama), B. gracilis (blue grama), *B.dactyloides* (buffalo grass), and *Schizachyrium scoparium* (little bluestem) across latitudinal gradients within the North American Great Plains. Our primary goal was to gain a deeper understanding of the relative importance and ranking of climatic, edaphic, geographic and host traits on the diversity and composition of the fungal rhizobiome. To do this, we sampled twelve individuals of each of the five grass species from 24 sites spanning across a N-S and W-E gradients in south and south central US. Our data indicate a host grass species effect amongst the grasses, and a distinct latitudinal interaction as driver for the fungal rhizobiome. In terms of the environmental drivers, pH tended to be the strongest predictor, highlighting the edaphic controls of rhizobiomes. This study improves our understanding of the compositional drivers of fungal rhizobiomes associated with these foundation grasses, permitting a better prediction of compositional community shifts in changing environments.

S17-3 CANCELLED

S17-4 Deciphering grassland dark septate endophyte functions: Insights from *Periconia macrospinosa* genomics, transcriptomics and proteomics

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Abstract: Plants are associated with a suite of microbial symbioses, with roots offering a unique niche for fungal endophytes. Among root fungal symbionts, dark septate endophytic (DSE) fungi are common, sometimes abundant but enigmatic with poor clarity on their functional roles. Biogeographical distinctions likely exist in DSE communities from forests and grasslands, with North American and European grasslands predominantly represented by *Periconia macrospinosa*. To understand their endophytic roles, recently the genome of dark septate *P. macrospinosa* and *Cadophora* isolated from *Festuca vaginata* from semi-arid European grassland were sequenced. To further comprehend DSE functional roles, the objectives of this study were to i) compare the North American *P. macrospinosa* genome with that of the European *P. macrospinosa*; ii) gain insights into *P. macrospinosa* global

proteome profiles; and iii) gain insights into *Periconia* transcriptomics under grass symbiosis. *Periconia* was isolated from a stand of Freedom Giant Miscanthus cultivated in Lorman, Mississippi and was confirmed to be a DSE. We hypothesized that despite the geographical distinctions and diverse grass hosts, *P. macrospinosa* associated with grasses would have similar functional roles. *Periconia* genome was sequenced using Illumina and PacBio platforms. Our *Periconia* genome was determined to be ~ 53.5 MB in size with 45% GC content. At least 12,059 ORFs with 9,086 ORFs with introns were identified and nearly 35% of the ORFs were assigned functions. As expected, several plant cell wall degrading enzymes (PCWDEs) like cellulases (12 ORFs), amylases (2 ORFs), pectin esterase (1 ORF), tannase (2 ORFs), laccase (6 ORFs) were identified along with several sugar transport systems such as maltose, lactate, sucrose, maltose, xylose isomaltose, palatinose, etc. However, ORFs for lignin peroxidase, manganese peroxidase, glyoxal oxidase were not observed. For global proteomic profiling, label free quantitative (LFQ) profiling using UPLC-MS/MS was used. For DSE gene expression insights, transcriptomic studies were performed on symbiotic-*Periconia* ten days post inoculation with *Miscanthus sinensis*. *Periconia macrospinosa* genomics, expression and proteomes data will be discussed to draw big picture inferences regarding DSE symbiosis.

S17-5 Fungal community analysis of adventitious rooting systems in canopy soils of *Acer macrophyllum* using the MinIon Nanopore Sequencer

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Abstract: The temperate rainforests of Western Washington are known for their temporally stable old-growth forests and unique ecosystem processes, including seasonal rainfall regimes. In recent years, they have been experiencing more seasonal extremes, including wetter winters and drier summers. Canopy soils, which form on tree branches from mossy epiphytic mats intercepting literfall and decomposing over time, are prevalent throughout these ecosystems. Some of the old-growth tree species have adapted to the presence of canopy soils by growing adventitious roots into this organic soil horizon. The aim of this research is to elucidate the role of fungi associating with adventitious roots in old-growth *Acer macrophylum* trees. Preliminary DNA and microscopy/imaging analyses suggest that these adventitious roots have adapted to form a diversity of fungal root associates that differ from those found in the forest floor rooting networks. Soil microclimatic and nutrient data suggests that canopy and forest floor characteristics are significantly different ($p<.05$), and there are hotspots of available P and N in the canopy soil environment. Soil microclimate and available nutrients could both be factors influencing fungal community structure. Preliminary DNA analysis was performed by cloning and Sanger sequencing, and although highly accurate, only some inferences on the identity of fungi associating with canopy roots could be made. Due to the cryptic nature of fungi and the paucity of information regarding fungi in canopy soils, a protocol was created to approach fungal community analysis using Oxford's high-throughput MinIon Nanopore Sequencer. The MinIon approach was selected based on the technology's potential to provide long-read barcoded libraries (~1,500 bp). DNA was extracted from adventitious and forest floor root-tips of six *A. macrophylum* trees from two old-growth forest stands, using OPS Diagnostics Synergy 2.0 Plant Extraction Kit. Based on the ability to amplify species from Ascomycota, Basidiomycota, and Glomeromycota, primers ITS1F-KYO and a variant of LR3, with custom tails respective to Oxford's 1D barcoding protocol, were selected for PCR. Following PCR, individual root-tips were assigned a unique barcode, pooled together, and the barcoded library was loaded into the MinIon for sequencing. The MinIon Sequencer ran overnight, returning ~800,000 fungal sequences. Currently, we are working on an efficient workflow to filter, process, and analyze the entire library of

long-read sequences. Subsets of this data form a healthy amount of OTUs from Ascomycota, Basidiomycota, and Glomeromycota, and return identities that match as high as 95% in the NCBI and UNITE databases. Results from this fungal community analysis, and the potential capability of the MinIon Nanopore Sequencer being a sufficient approach to fungal community analysis will be reported.

S17-6 A functional assessment of root-endophytic fungal diversity in non-mycorrhizal plants

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Abstract: Root-endophytic fungi are ubiquitous and abundant in terrestrial plants, likely playing roles in plant health and productivity. They are frequently considered key drivers of ecosystems' function and promising tools for agriculture, but important knowledge gaps on their ecology limit the understanding of their place in ecosystems, as well as their exploitation. Fungal endophytes are phylogenetically and functionally diverse, therefore, unraveling their trophic lifestyles, their impacts on plant fitness, and their taxonomy may help infer their various ecological roles. We sought to gain insight into the evolution and functional diversity of these fungi through a comparative study of their traits, and by providing quantitative linkages between sets of traits and endophytes' community ecology. Using both high-throughput sequencing and cultivation methods, we assessed the fungal diversity within roots of non-mycorrhizal plants across Europe to evaluate differences in host-dependency and distribution between cultivable and non-cultivable endophytes. The samplings enabled the assembly of a collection of ca. 2,500 isolates, representing the majority of dominant endophytic lineages, and their subsequent characterization using multilocus genotyping; measurements of morphological, physiological, and chemical traits; and bioassays with different hosts to determine their impacts on plant growth. We found a consistent dominance of root-endophytic communities by few cultivable fungal taxa, implying that facultative plant associations are pervasive in the core fungome of non-mycorrhizal roots. Dominant fungal lineages displayed different ecological preferences and complementary sets of traits, suggesting niche partitioning as an important driver of the assembly of root-endophytic communities. Experiments in which roots were co-inoculated with representative isolates supported this hypothesis by showing little interaction between endophytes. In conclusion, root-endophytic fungi are not functionally homogeneous, but instead form communities where a partitioned exploitation of niches takes place. Our findings enable to postulate hypotheses about the potential ecological roles of different endophytic groups, although they are not specific in attributing particular functions. Further approaches to assess the implication of fungi in specific symbiotic processes will be discussed.

Symposium Session 18: Oral History for Mycology

M. Blackwell and R. Samson

S18-1 Interview

L. Boddy
Biomedical Building, Cardiff School of Biosciences, Cardiff, UNITED KINGDOM

S18-2 Interview

D. Hawksworth
Department of Life Science, The Natural History Museum, London, UNITED KINGDOM

S18-3 Interview

J. W. Taylor
Department of Plant and Microbial Biology, University of California, Berkeley, USA

Symposium Session 19:

Home Life: The Mycobiomes of Built Environments

J.W. Bennett and J. Gilbert

S19-1 Unveiling the mycobiota of indoor environments

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Abstract: For many decades, fungi in indoor environments received much attention and many mycological studies considered the implication of these on the health of the built environment. Our knowledge of these fungi used to be based on traditional techniques by such as culturing on agar media and in some cases direct microscopy. For many years, the mycobiota was considered to be known. New culture-independent sampling methods led a broader view of the diversity of the indoor mycobiota. A study investigating the mycobiota of settled house dust from the built environment in 12 countries using pyrosequencing resulted in 190.000 ITS sequences and from the same samples, about 8000 isolates were obtained by dilution to extinction. This demonstrated that the built environment possesses a much higher fungal diversity than expected. Many taxa were newly described and would have never been discovered using traditional methods. Combining these strains with others, isolated during ongoing studies of the indoor environment in Asia and Europe, and re-examination of type strains of many moulds previously considered synonyms of broadly defined species, led to monographic treatments of important genera of important indoor fungi. In this presentation, several examples will be discussed. The implication and significance of the high biodiversity and the occurrence of many moderately xerophilic species in dry environments will be discussed.

S19-2 A different suite: The assemblage of distinct microbial communities in poorly-maintained public housing

I. Sylvain
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Abstract: A limited number of studies have used high-throughput sequencing technologies to assess the impact of water-damage on microbial communities in residential buildings. In this study we used amplicon sequencing and quantitative-PCR to evaluate fungal communities in a condemned public housing project in the San Francisco Bay Area. With this data we asked whether broad fungal compositional differences could be detected in units with visible mold when compared to units with no visible mold or the outdoors. We found distinct fungal communities in units with visible mold, characterized by lower diversity and predominance of taxa previously reported with water-damaged building materials. The distinction between outdoor microbial communities, units with no visible mold, and units with visible mold, shows that insufficient building maintenance can drastically shift the assemblage of fungi indoors.

S19-3 The mycobiota and healthy buildings – challenges and solutions

U. Thrane
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Abstract: The quality of the indoor environment is very important and affects our daily life and well-being, whether it is at home, at work, at institutions or any other building that we use. The indoor environment is very complex and many individual parameters act together to a given quality. One of the parameters that – for good reasons – have been central for decades is the mycobiota. The fungi, that thrive in the built environment whether we like it or not. A specific focus has been on the molds that do not destroy the physical strength of a building, but have a severe effect on human health. The body of knowledge within this theme is overwhelming, but still it is impossible to synthesize all the data into error-free operations and solutions. There are too many gaps, and new gaps are discovered on a regular basis. A main reason is that mycology is in the midst of a booming technological development. The classical mycological philosophies (values) are being challenged by a massive data-driven wave that has moved mycology into the information-age. Especially the rapid developing DNA technologies have changed the mycological systematics from being phenotypic based to mainly phylogenetic based. In addition, many analyses are more objective than former time's very subjective judgements of phenotypic traits. The resolution between taxonomic entities has also improved dramatically, which means that the number of fungal species are increasing. Often old broad species are split into several new narrower circumscribed species. For the built environment, this means that the number of species detected are increasing by validated identifications. Good. The drawback is that the phenotypic traits – the functionality – are missing, meaning no information on the mycobiota and its effect on human health, or no explanation why it is exactly these species that are present. The way forward to improve this situation is a holistic approach using available powerful computational capabilities. A multi-disciplinary characterization of the fungal phenotypes that should also cover their physiological, ecological and metabolic traits should interact with the modern taxonomic schemes and the DNA sequence databases used by consensus analyzes to obtain a true picture of the fungal species. This will generate a deeper understanding of the fungal organism in itself, how the fungal communities develop in the built environment, and fungal interaction with building users and the effect on human health. The holistic approach will lead to guidelines for remediation and prevention of unwanted mold growth in the built environment. Furthermore, the improvement of technical installations in our buildings that monitor and regulate the physical environment, energy consumption etc. will give us a unique opportunity for a true big-data based understanding of indoor quality with a focus on molds and healthy homes.

S19-4 Flooring tile eating fungi

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Abstract: Fungi are known for their degradation abilities, mainly of organic matter. However, there are increasing evidences of their role in mineral materials deterioration as well including building materials. We have investigated the possible involvement of fungi in the efflorescence phenomenon of floor tiles inside building. Fungi were isolated from efflorescence site and their abilities of degradation and use of the tile materials were studied in axenic culture. The efflorescence was seen as salt migration on top of a tile with a needle-like re-crystallization on the surface. Three black molds fungi were isolated from the site. The fungi were shown to have selective growth and stone dissolution of the different tile fractions (dolomite, calcite or calcite–apatite mineral) in low-nutrition medium. The growth of the fungi in the presence of the stones fraction, and the dissolution the fraction was accompanied by production and

release of organic acids into the culture medium. The fungi were able to use the dissolved tiles materials as nutritient sources. This indicates that fungi may contribute to the natural physio-chemical process of efflorescence.

S19-5 Synergistic interactions facilitate establishment of opportunistic black yeast *Exophiala dermatitidis* in dishwasher biofilm communities

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Abstract: Extreme habitats are not only limited to natural environments, but also exist in manmade systems, for instance, household appliances such as dishwashers. Research on dishwasher microbiome started in 2011 with the discovery that black yeast, especially opportunistic *Exophiala dermatitidis*, colonizes rubber seals of domestic dishwashers. This global phenomenon attracted even more attention with the isolation of other fungal human opportunistic pathogens from dishwashers, such as *Candida parapsilosis*, *Exophiala phaeomuriformis*, *Aureobasidium melanogenum*, *Fusarium dimerum* and *Saprochaete clavata*. Due to the emphasis on fungi, the bacterial community of dishwasher remained initially unexplored. To address this issue, bacterial and fungal diversity in biofilms isolated from rubber seals of 24 different household dishwashers was investigated using next-generation sequencing. Microbiome resulted in bacterial genera such as *Pseudomonas*, *Escherichia*, and *Acinetobacter*, known to include opportunistic pathogens that were represented in most samples. The most frequently encountered fungal genera belong to *Candida*, *Cryptococcus*, and *Rhodotorula*, also known to include opportunistic pathogenic representatives. This study also showed how specific abiotic conditions of the dishwashers impact the abundance of microbial groups and the interkingdom and intrakingdom interactions in these biofilms. The age, usage frequency, and hardness of incoming tap water of dishwashers had the most significant impact on bacterial and fungal community compositions. *Candida* spp., found with the highest prevalence (100%) in all dishwashers, is probably the first colonizers in new dishwashers. In mixed bacterial-fungal biofilms, early adhesion, contact, and interactions were vital in the process of biofilm formation. Mixed complexes of bacteria and fungi provide a preliminary biogenic structure for the establishment of biofilms. Pairwise correlations in tested microbiomes showed that certain bacterial and fungal groups co-occur. Evaluation of fungal abundance per 1 cm^2 of rubber seals revealed the highest levels for *E. dermatitidis*, followed by *E. phaeomuriformis* and *C. parapsilosis*. Isolation of cultivable bacterial and fungal strains and further screening tests showed which bacterial species gain in biomass by incorporating *E. dermatitidis* in the biofilm and thus contribute to its promotion and abundance. The significance of our research is in identifying the microbial composition of biofilms formed in a broadly used household appliance, in describing how diverse abiotic conditions affect the composition of mixed fungal bacterial microbiota, and which key members were represented in early colonization.

S19-6 More than just a funky smell

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Abstract: Following the flooding that ensued in New Orleans after Hurricane Katrina, my home became a lush habitat for mold. They grew on almost every surface and they generated an overpowering smell. Subsequently, my laboratory has pioneered the use of genetic model organisms to study the physiological effects of VOCs produced by filamentous fungi isolated from indoor environments, especially those affected by flooding. The toxigenic potential of these fungal VOCs has been explored

in *Drosophila melanogaster*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae* with a focus on 1-octen-3-ol, the most common odorant produced by molds and mushrooms and a major component of the musty odor found in water-damaged indoor spaces. At certain concentrations, 1-octen-3-ol is neurotoxic in *Drosophila melanogaster* and inhibits seed germination in *Arabidopsis*. Using a lethal concentration of vapors of 1-octen-3-ol, we screened a yeast knock out library. Over 90 resistant strains were isolated and classified using the *Saccharomyces* Genome Database. The most statistically significant biological processes were endosomal transport (24.2%), protein targeting (20.9%) and proteolysis involved in cellular protein catabolic process (17.6%). This ubiquitous fungal metabolite is more than "just a funky smell." It mediates many cellular processes and is important in intraspecific and intraspecific communication.

Symposium Session 20:

Gondwana Reunited! Fungal Biogeography in the Southern Hemisphere

M.E. Smith and C. Truong

S20-1 Australasian truffle-like fungi: patterns of richness, evolution and diversification

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Abstract: Truffle-like fruit body forms have evolved independently at least 59 times from aboveground ancestors in most major fungal lineages. They can be found in a broad range of habitats, are important food sources for native mammals, and functionally significant as mycorrhizae or saprotrophs. Australasia is a global diversity hotspot, with over 400 described, and a further 500-800 estimated species in 55 genera. Extreme variation in truffle-like fruit body forms make it difficult to determine ancestors or sister taxa without supporting molecular data, and large gaps in phylogenetic and distribution data make estimating patterns of evolution and diversification problematic. Phylogenetic analyses were conducted for exemplars of all known Australasian truffle-like genera, utilising existing and novel sequence data, to provide an updated classification of global application, and fill gaps in phylogenetic data. Fifty 'known novel' taxa were also included: seven endemic Australasian genera in the Hysterangiales and Boletales, the first recorded truffle-like species in *Lactifluus*, and 10-12 species of *Lactarius*. This data formed the basis for several different lines of enquiry. **Cryptic diversity** -- Three widespread species from different lineages were examined in an attempt to look at biogeographic patterns. However, all three taxa turned out to be species complexes, in every case going from one to more than seven species. Some of which have highly restricted known distributions, and plant host associations. **Diversification of evolution** -- The Hysterangiales, Russulales, and Bolbiteaceae are diverse lineages in Australasia, with particularly high levels of truffle-like genera and species richness. We refined molecular clock analyses of the timing of emergence of truffle-like forms and rates of diversification. The Hysterangiales is still one of the oldest lineages with truffle-like forms appearing early on, while Russulales is intermediate in timing of emergence of truffle-like forms. Significant gaps still exist for Australasian mushroom-like sister taxa in the Russulales lineage. **Acquisition of truffle-like habit** -- We are

sequencing the genomes of 14 sister pairs of truffle + mushrooms, and comparing expression of genes both between species pairs and within species pairs to identify those associated with morphological development and the origins of animal attracting odours. Three of the truffle taxa are 'secotioid' or more mushroom-like in habit. Incomplete phylogenies and luck of the draw with fruiting and seasonality, means the closest possible species pairs have been sampled.

S20-2 Biogeographical patterns of southern sequestrate Agaricomycetidae

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Abstract: Truffle-like fungi form a heterogeneous group of diverse origin and they are common in temperate ectomycorrhizal (ECM) forests worldwide. The Nothofagaceae forests are restricted to the southern hemisphere and host a high diversity of fungi that are symbiotically associated with a relatively low number of related tree species. This unusual richness includes genera where the transition to hypogeous or subhypogeous forms is known to have occurred many times in their evolutionary history. Different degrees of hymenium exposure, stipe reduction, and loss of forcible spore discharge occur in many lineages of sequestrate ECM fungi in southern temperate forests. Among the hypothetical driving forces of this process are the adaptation to mycophagy, the closeness of the inoculum reservoir to the roots, and an increased resistance to drought or other climatic stressors. Since these factors are the result of biogeographical processes (climate changes, distribution of ECM hosts, and mycophagous animals), different selective pressures might explain differences in the sequestration process. Lineage specific traits in fungi of restricted distribution are an alternative explanation to biogeographical selective pressures leading to sequestration. Here we use newly generated sequences from Patagonian specimens within the Agaricomycetidae to examine the phylogenetic component of sequestration and compare it to biogeographic, climatic, and geologic influences. We discuss the influence of alternative hosts (e.g. Myrtaceae spp.) as well as hypotheses regarding putative historical host shifts and their implications on the evolution of sequestrate fungi.

S20-3 Comparing ectomycorrhizal fungal communities from Neotropical and Paleotropical monodominant forests: vicariance, dispersal, or both?

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Abstract: Compared to temperate and boreal ecosystems, little is known about ectomycorrhizal (EM) fungal diversity and evolution in tropical rainforests. For instance, it remains unknown whether tropical EM fungi have a common ancient origin, or have codispersed with their EM host plants and subsequently evolved. Tropical EM fungal communities with a common, ancient origin should exhibit similar structures, in terms of generic and species richness, in regions now separated by Gondwanan break up.

Conversely, if tropical EM fungi have codispersed with their host plants, then the generic and species richnesses may vary between now-separated Gondwanan EM communities. To address these questions, we intensively examined two northern Gondwana EM fungal communities now separated for ~100 million years. Monodominant forests of *Dicymbe corymbosa* in the South American Guiana Shield and *Gilbertiodendron dewevrei* in Central Africa were the targets of our sampling efforts. These closely related EM host plant species of the Fabaceae subfam. Detarioideae form ecologically similar forest types in both regions. Multi-year matched sporocarp and root tip sampling was conducted in three plots in both forest types. Sporocarps and root tips were sequenced at the fungal barcode and clustered at a 97% sequence identity threshold. Preliminary results suggest that while EM fungal generic richness and composition is similar at both sites, the species diversity is much higher in the *Gilbertiodendron* forests. Such results lend early support to an Africa to South America plant/fungal codispersal hypothesis. Phylogeographic analyses and divergence dating of target EM fungal clades will also be discussed.

S20-4 Southern Gondwanan fungi associated with Nothofagaceae in Patagonia

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Abstract: Current understanding of fungal biodiversity is particularly limited in South America, yet global studies have identified many unique fungal lineages that are present in the Southern Hemisphere but absent from other regions. The ectomycorrhizal tree family Nothofagaceae is one striking example of vicariance associated with the final breakup of Southern Gondwana (part of the supercontinent that included South America, Antarctica and Australia) and the onset of Antarctic glaciation at the Eocene/Oligocene boundary (ca. 32 mya). However, conflicting evidence suggests that long-distance dispersal or migration has continued long after the fragmentation of Southern Gondwana. As expected from previous studies, we detected strong biogeographic connections between South America and Australasia within many ectomycorrhizal fungal lineages collected in our biodiversity assessment of the Patagonian region (Chile and Argentina). We traced the most recent common ancestors of several southern temperate lineages of Basidiomycota and Ascomycota using dated phylogenies built from nuclear ribosomal loci ITS and LSU as well as RPB2 and EF1-alpha markers. We tested alternative hypotheses of vicariance or long distance-dispersal while taking into account the spore dispersal abilities, responses to disturbance, and whether taxa are early or late successional in Nothofagaceae forests. We also tested whether taxa with sequestrate fruiting bodies are more likely to have a restricted distribution and show evidence of a Southern Gondwanan origin. Our global approach using basidiomycete and ascomycete fungi with various life-history strategies will highlight common patterns for ectomycorrhizal fungi in the Patagonian region.

S20-5 Out of Africa: the evolutionary history of the milkcap genus *Lactifluus* (Russulaceae) explored

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Abstract: Compared with other groups of macro-organisms, the evolutionary histories of most groups of fungi are still largely unknown. Fungal molecular phylogenies, which serve as the base of evolutionary history reconstructions, are often incomplete. Fungal fossils are scarce, which makes it hard to make accurate estimates of divergence times. Furthermore, observed distribution patterns are often difficult to explain. Many ectomycorrhizal fungi display disjunct distribution patterns that might be explained by vicariance or long-distance dispersal events. The ectomycorrhizal milkcap genus *Lactifluus* (Russulaceae) is mainly represented in the tropics and is characterized by a high genetic variability combined with a conserved morphology, which is supported by the occurrence of cryptic species complexes and species with isolated phylogenetic positions. The genus displays disjunct distributions and is characterised by many evolutionary divergent lineages in sub-Saharan Africa. In this study, we aim to construct a global phylogeny of the genus *Lactifluus*, reconstruct its evolutionary history and test whether it has originated in the Afrotropics. We carried out an extensive global sampling and assembled a dataset of 1306 *Lactifluus* collections. A four-gene molecular phylogeny was constructed and compared with morphological data. Species delimitation was performed using the GMYC method in R. Divergence times were estimated in BEAST, using a secondary calibration procedure on a dataset containing species from several Basidiomycota orders. Biogeographical ranges were inferred using BioGeoBEARS in R. Our molecular phylogeny confirms the monophyly of *Lactifluus* and supports the division of the genus into four subgenera. Due to an extensive sampling, ten new clades are discovered, which highlight the high diversity in this genus. The traditional infrageneric classification is only partly maintained and nomenclatural changes are proposed. Morphological synapomorphies were verified for five characteristics and appear important at different evolutionary levels. Species delimitation resulted in 369–461 possible *Lactifluus* species, of which the majority are Asian and African species. Only 162 of these species are already described. Our dating analysis estimated the origin of the Russulaceae in the early Cretaceous and its major genera, *Lactifluus*, *Lactarius* and *Russula*, originated near the mid-Cenozoic. Biogeographical analyses indicated an Afrotropical origin for *Lactifluus* to be most likely, with multiple on-land migrations and long-distance dispersal events to other continents.

S20-6 Insight into the origin of fungi in Antarctica: Case studies in endemic and bipolar lichenized species

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Abstract: Conditions for terrestrial life in Antarctica are frequently described as being amongst the most extreme and challenging on Earth. Coping with these conditions, fungi have been shown to be one of the most diverse elements of the Antarctic biota, with approx. 1350 species listed so far. Among fungi, species associating symbiotically with an autotrophic partner (i.e. lichens) show a high diversity, with about 550 species (ca. 2.8% of all known species worldwide). They constitute the most conspicuous component of terrestrial macrobiota even in areas considered analogs for Mars because of their cold and dry climate and topography, such as the McMurdo Dry Valleys (Victoria Land, Continental Antarctica). From a biogeographic perspective, more than a third of the current Antarctic lichen diversity

is shared with the Arctic and Sub-Arctic regions (bipolar distribution). Long-distance dispersion has been commonly invoked to explain this strikingly disjunct distribution pattern, but it is still unknown whether those species originated in or arrived to Antarctica. Similarly, there is also a considerable percentage of endemic Antarctic lichens (ca. 33%) whose origin some authors claimed to be ancient, pre-dating the last glaciations, which implies that these species may have taken refuge in ice-free areas. We selected several phylogenetically-unrelated species of both endemic and bipolar lichen-forming fungi to determine which historical processes may be responsible for the contemporary geographical distribution of alleles. Molecular data from several markers were obtained and analyses included the inference of genetic clusters based on mixture and admixture models, exploration of genealogical relationships between haplotypes, estimation of divergence times and evaluation of migration models under a Bayesian framework. Herein, through these phylogeography analyses, we provide evidence for the colonization of Antarctica in the Pleistocene by some bipolar species, and point to endemics as long-term inhabitants of this continent.

Symposium Session 21:

Resolving Uncharacterized Symbiotic Relationships: The Delicate Balance from Mutualist to Parasite

S21-1 Plant symbiotic status of species in Archaeorhizomycetes

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Abstract: In 2011, the class Archaeorhizomycetes was described from one cultured species and environmental sequences representing over hundred OTUs as proxies for species. Since then one more species has been cultured and described but estimates based on publicly available sequences at the time suggest that the class encompass close to 500 species. Both described representatives of the class have been isolated from surface sterilized root tips of coniferous forest trees. Both grow on various carbon sources and based on pine root colonization experiments they appear to be non-pathogenic. No mycorrhizal structures have yet been observed in colonized roots. Enhanced root growth and branching has been observed in *Arabidopsis thaliana* when grown with either of the two species. Together these results suggest that both *Archaeorhizomyces finlayi* and *Archaeorhizomyces borealis* are facultative root endophytes. But what does this tell us about the un-cultured species in the class? Analysis of below ground compartment specialisation in *A. thaliana* grown in natural soils demonstrated that three Archaeorhizomycetes OTUs were significantly enriched in the rhizosphere compartment rather than in endosphere and bulk soil belonged to. Using UNITE species hypothesis we can learn more about the distribution and habitats of these species, while continuing to develop *A. thaliana* as a model to identify plant host association of un-cultured Archaeorhizomycetes. There are several challenges in resolving ecological roles of uncultured fungal species. Firstly, the delamination of environmental species itself is crucial but not uncontroversial. Secondly defining and testing ecological roles of non-cultured fungi is challenging. In this presentation I will discuss some approaches taken in my group to disentangle the ecological role of species in Archaeorhizomycetes, including preliminary comparative genome analysis. Using long amplicon PacBio sequences from soil samples representing three distinct soil layers at a well-studied field site in Sweden we delimitated thirteen distinct groups of sequences and propose that these represent phylogenetically distinct species. One of these being *A. finlayi*. By comparing the ITS region of the long reads with short read sequences from metabarcoding studies at the site we concluded that species sampling was exhaustive. Based on the distinct chemical

characteristics of different soil horizons we propose that these represent different habitats for soil fungi. We analysed distribution of the thirteen species across three soil horizons as a proxy for species specific niche preference. This analysis supported that the phylogenetic species represented ecologically distinct species. Because rare species have few observations only eleven of the species were firmly supported. In accordance with earlier observations *A. finlayi* was associated with the mineral B horizon and we conclude that closely related species had rather similar distribution. Our approach demonstrates that it is possible to delaminate fungal species using environmental data alone but the absence of type material may limit the relevance of formally naming species based on such data. However, communication and scientific progress in the field of environmental mycology is severely hampered by the lack of names. I think environmental species need names but these should be distinguishable from those associated with biological type material.

S21-2 Host specificity of fungal endophytes uncovers their biodiversity and ecology

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Abstract: Current estimation of fungal biodiversity of 1.5 million species is considered a vast underestimate by many scientists because we have a limited understanding of the diversity of fungi living inside plants. Foliar fungal endophytes (FFE) ubiquitously and asymptomatically inhabit the photosynthetic tissues of all plant phyla, but their biodiversity is only beginning to be fully uncovered with recent advances in high-throughput sequencing. Their ecological roles remain elusive without detailed studies of their natural history. Here we intensively sampled diverse plant communities across a latitudinal gradient in North America while focusing specifically on evergreen pine hosts and their host-specific endophytes. We utilize a collection of cultures in conjunction with high-throughput sequencing to examine previously unexplored patterns of host specificity that help estimate regional diversity and assign potential ecological roles to host-specific endophytic species. We test hypotheses that consider climate and plant-host specialization as causative mechanisms of FFE species diversification with multivariate community and phylogeographic analyses that control for spatial, temporal, and environmental effects previously known to bias species diversity patterns. We show that fungal diversity measured per plant can give qualitatively different biodiversity patterns than when diversity is measured per plant community. Furthermore, we demonstrate how a robust measure of host specificity requires both standardized and intense sampling per plant community. The implications of high host-specificity in the temperate forests and low host-specificity in the tropics on the estimation of alpha, beta and gamma diversity of fungal endophytes will be discussed. Ongoing work will highlight the complementarity of intensively sampling the whole plant community with deep sequencing of single host species to understand the biotic and abiotic influences of fungal endophyte diversification and their ecologies.

S21-3 Evaluating the capabilities of commensal Sporidiobolales yeasts as a bioprotective agent against the establishment of harmful microbes on Romaine lettuce

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Abstract: The agricultural relevance of commensal yeasts inhabiting the phylloplane of leafy green vegetables is still poorly understood. Our current research describing the culturable and total fungal

communities associated with Romaine lettuce reported that basidiomycetous yeasts in Sporidiobolales and Tremellales are the most common fungal groups found in this broadly consumed vegetable. We also discovered that a single undescribed species, the sister of *Sporobolomyces roseus* (*S.* cfr. *roseus*), was constantly present in the majority of lettuce plants examined. Here, we present preliminary results of greenhouse experiments designed to evaluate the capabilities of *S.* cfr. *roseus* as a bioprotective agent against the establishment of fungal pathogens on Romaine lettuce. All of the experiments were conducted in a BL-2 biosecurity green house, where lettuce seedlings were grown in 12 h photoperiod. First, we demonstrated that *S.* cfr. *roseus* is not an inhabitant of lettuce grown under greenhouse conditions. Second, we demonstrated successful colonization of the lettuce phylloplane by *S.* cfr. *roseus* and it did not induce any antagonistic immune response in the host. Third, in co-inoculation experiments, we found that the well-known plant pathogen *Botrytis cinerea* significantly did not induce necrosis on lettuce leaves when seedlings were previously inoculated with *S.* cfr. *roseus*. In sum, our results suggested that *S.* cfr. *roseus* could be used as a protective agent against the establishment of fungal pathogens on lettuce. The inoculation of commensal red yeasts to leafy green vegetables may also increase the nutritional value of pro-vitamin A, a compound obtained from the digestion of the carotenoid pigments present in vacuoles in the Sporidiobolales yeasts.

S21-4 Factors correlated with invasive success in *Moniliophthora roreri*

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Abstract: *Moniliophthora roreri* is the causal agent of frosty pod rot of cacao (FPR). FPR is a disease that, since the 1950's, has progressively and severely invaded cacao growing areas from almost all of Latin America but has not yet reached the major world cacao producing regions of Brazil, West Africa and Southeast Asia. This situation makes *M. roreri* one of the most threatening plant pathogens in the world. The fungus belongs to the Marasmiaceae (Agaricales, Basidiomycota) and no evidence of sexual reproduction has ever been found for this fungus. We have conducted a population genetic study in South America and found that the countries harboring the highest allelic diversity are Colombia and Ecuador, where the first reports of FPR took place. Despite this diversity, thus far we have found that only two clonal lineages appear to be responsible for the majority of invasive FPR. A single clonal lineage is responsible for FPR in Peru and Bolivia, while invasion of this disease throughout Central America, Mexico and Jamaica, was accomplished by another clonal lineage. The factors that contributed to the overall success of these two clones remain unknown, although we have previously determined that they carry separate mating types. In this study, we generated genomic, transcriptomic and comparative culture data to examine the characteristics correlated with the invasive success in this fungus. These and other factors that may explain the overall success of these two clonal lineages will be discussed.

S21-5 The surprising ecological versatility of *Mycena* s.s.

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Abstract: *Mycena* sensu stricto is one of the largest genera in Agaricales, comprising over 500 species, many of which are widespread and commonly found in all types of habitats across all climate zones. They are quantitatively important litter and wood debris decomposers. Traditionally, they have been uniformly described as saprotrophs, and the genus contains several very broad generalist species such as *Mycena epipterygia* and *M. pura* growing nearly everywhere, needle litter specialists (*M. rosella*) and hardwood specialists (*M.haematopus*). In addition there are extreme specialists like *M. belliae* that only grows on dead *Phragmites* stalks. However, recent research has suggested that *Mycena* members may have a biotrophic or symbiotic relationship with ericoid plants. Here, we combine several lines of evidence, including datamining of high-throughput amplicon ITS sequences from plant roots, stable isotope signatures of fungal communities and whole genome sequencing - to construct a new and detailed picture of the evolution and nutrition of *Mycena*. A large datamining study of ITS1 and ITS2 amplicons from roots from *Betula, Salix, Pinus, Cassiope, Bistorta, Dryas* and *Arctostaphylos* from temperate and Arctic latitudes showed that *Mycenas* can be found in often significant quantities inside living plant roots, from 2-4% of the total sequence count in temperate *Pinus silvestris* up to 20-50% in Arctic *Cassiope tetragona*, with apparently higher frequencies towards higher latitudes. We then surveyed the stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of fruit bodies of *Mycena* taxa compared to available host plants and other present known saprotrophic and mycorrhizal taxa at five selected locations (three from temperate Scandinavia and two from subarctic/arctic Norway). We find no *Mycena* species that convincingly displayed the combination of relative ^{13}C depletion and ^{15}N enrichment which could suggest with a mycorrhizal lifestyle, but some that do have a relative ^{13}C depletion, consistent with obtaining carbon from a living source. This suggests that some *Mycena* do have an opportunistically biotrophic, but not mutualistic, lifestyle, which is also in line with recent (unpublished) co-cultivation studies of *Mycena* and *Betula pendula*. Finally, we are currently assembling full genomes from 23 *Mycena* species, 16 of which are complete. Their genome sizes are surprisingly variable, ranging from a moderate size of ~40 Mbp in highly specialised *M. belliae* to over 120 Mbp in generalist *M. epipterygia* and up to a very large 4-500 Mbp for several Arctic *Mycena* species. Several genomes show evidence of transposable element overload, which is mainly known from biotrophic mushrooms and associated genetic bottlenecks. Taken together, our results show *Mycena* s.s. to be ecologically diverse with a much higher adaptive versatility than traditionally believed.

S21-6 Differences in nutrient utilization ability of orchid mycorrhizal fungi from widely and narrowly distributed sebacinoid orchid species in Australia

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Abstract: The interaction between orchid and mycorrhizal fungi is undeniably crucial in orchid establishment and recruitment. The ability of orchid mycorrhizal fungi to access soil nutrient sources for their growth is also worth noting. This is not only critical for their own survival in soil, but also contribute to orchid survival and growth and subsequently contribute in determining orchid distribution ranges and population size. One of the fungal genera that many Australian orchids are associated with is *Serendipita*. *Serendipita* lineages are widespread across Australia and associate with many genera, both widespread and restricted orchid species. We hypothesise that mycorrhizal fungi from widely and narrowly distributed orchid species will differ in their ability to utilize nutrient sources. Furthermore, we

expect fungi from eastern and Western Australia to differ in their nutrient utilizing abilities because of edaphic differences. To effectively test this hypothesis, we tried to determine the diversity of mycorrhizal partners across the orchid's distribution range (multiple geographical ranges). Therefore, our study approaches multiple geographical ranges that represent orchid biodiversity hotspots in Australia by using *Serendipita* fungi as a model. Mycorrhizal fungi representing Australian sebacinoid orchid genera were isolated from widely distributed species: *Caladenia flava, Caladenia tentaculata, Eriochillus cucullatus, Glossodia major, Elyteranthera brunonis, Cyrtostylis reniformis* and *Microtis unifolia*, and narrowly distributed species: *Caladenia atrovespa* and *Caladenia procera*. The accessibility of specific nutrients encompassing carbon sources (monosaccharide, disaccharide, polysaccharide), nitrogen sources (inorganic and organic) and organic phosphorus sources was assessed by comparing dry biomass of orchid mycorrhizal fungi *in vitro*. The knowledge obtained from this study will improve our understanding of the ecological implications of the differences in nutrient utilisation by orchid mycorrhizal fungi. Furthermore, nutrient utilization patterns of mycorrhizal fungi might shed light on the orchid distribution patterns.

Symposium Session 22:

Bringing Awareness of Fungi – for Teachers and the General Public

M.-A. Neves and M. Menolli

S22-1 Re-discovering the forgotten kingdom: An undergraduate course in fungal biology

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Abstract: In 2017, we re-introduced Fungal Biology as a dedicated 3rd level undergraduate course in the BSc program at The University of Queensland (UQ) in Australia. Although courses covering fungi as plant pathogens are continually taught at UQ, given the relevance of such to the state's agricultural industries, it has been two decades since a mycology course was in the BSc program. Small enrolments and economic rationalism sealed the fate of the previous course. *The Intention* Fungal Biology was squeezed back into the crowded course offerings in the BSc program by running it in the "summer semester." The summer ("third") semester is an optional intensive teaching period run over December and January, generally, when academics in Australia would prefer to be dedicating time to research, writing grants or going on vacation. However, there is increasing demand from undergraduates to take on courses during this period to: 1) fast track their degrees; 2) reduce the intensity over the two standard teaching semesters; or 3) catch up for deficiencies in their previous semesters. Having decided on the summer semester the next step was to attract students. For this purpose, the theory was taught online, reducing the need for attendance for the entire summer break. A practical component was still considered essential and this was run in two intensive blocks: two days in mid-December and two days in mid-January. The course had no pre-requisites with the aim of enticing students from all disciplines. *What Actually Happened* -- For the online theory material, voiceovers were recorded onto each PowerPoint slide, and the subsequent presentations were converted into mp4 files and made available in weekly sets of five instalments. The use of a laptop with an interactive screen enhanced the ability to animate the PowerPoint slides. Thirty topics were made, each with a resultant playing time between 20 to 40 minutes. A teaching grant allowed for employment of a student to conduct interviews with experts in a range of fields including taxonomy, industrial fermentation and herpetology. The resultant video

vignettes were interspersed within the PowerPoint presentations. Weekly online quizzes kept the students on track with their studies. Running the practical component over the wetter period of the year, allowed for an abundant supply of fungal fruiting bodies for development of identification skills. We were also very fortunate in luring the taxonomists from the State Herbarium who freely gave their time during these classes. *Who Actually Enrolled* -- Although some non-scientists started the course, after the first tutorial those without a grasp of biology withdrew; the key factor was asking the question if they had heard of meiosis. Otherwise, we did attract a diverse range of scientist from budding medical students, ecologists to chemists. *Future Years* -- A mass online delivery mode has been considered but that would require a higher degree of audio visual presentation. In the meanwhile, 30 young people know a lot more about fungi than they did previously.

S22-2 Fungi and parataxonomy in the Neotropics: Bringing the 3.8 million figure to within reach

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Abstract: Of the conservatively estimated 3.8 million species of fungi, only some 120,000 have been described to science. The vast majority of the remaining species are widely considered to reside in the tropics. At the current rate of species description in the kingdom, it will take mycologists approximately 4000 years to describe them all. One way to increase the rate at which fungi are described is parataxonomy. First conceived in the 1980s by biologist Daniel Janzen, parataxonomy is a system of labor division for use in biodiversity research, in which the "rough sorting" tasks of specimen collection, documentation, preservation and field identification are conducted by primarily local, less specialized individuals, thereby alleviating the workload for the "alpha" or "master" taxonomist. On a continent as politically volatile, environmentally threatened and colonially scarred as Latin America, the parataxonomist has the added benefit of being able to navigate complex cultural and socioeconomic realities in a way which so-called "westerners" with academic biology backgrounds may not be equipped to address. This presentation looks at a contemporary interpretation of the parataxonomic model in Neotropical mycology with a focus on the Andean-Amazonian region, with examples of the bidirectional flow of data along the "taxonomic food chain", along with a summary of the past, present and future of mycological education in Bolivia; one of the most biodiverse and least mycologically understood countries on earth.

S22-3 Fun activities for teaching fungi

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Abstract: Fungal biology is a topic often neglected in high school and undergraduate courses in Brazil, mainly because the time in the official curriculum dedicated to work on fungi is very short, but also because most activities proposed are based on traditional lectures and laboratory format often using examples from other parts of the world. We will present examples of alternative resources to teaching mycology that we are using in undergraduate and graduate courses in Santa Catarina and in São Paulo to increase the interest in fungi. Mycology is taught at the beginning of the second year of the Biological Sciences undergraduate course at Universidade Federal de Santa Catarina (UFSC) in Florianópolis,

South Brazil. The semester is divided into two modules (phycology and mycology) and there are eight weeks to each module. To broaden the understanding of the undergraduate students on different topics on mycology, online tools are one source of information that is wide and diverse. For instance, the Index Fungorum and Tree of Life websites are used to work on morphological diversity and to build a tree based on a list of taxa provided. They are also asked to 1) choose a topic related to any aspect of fungi to present a seminar; and 2) develop a post to be published on the social media. One approach includes websites or blogs that have been built by mycologists (such as Tom Volk's Fungus of the Month and the Cornell Mushroom Blog) that are used as an inspiration for the students to build their own activities, trying to bring to their own reality. The possibility to search for a free fungal topic among a large variety of interesting fun facts results in students reading more than what is expected within the time frame they have. In São Paulo, Southeast Brazil, as part of the Biological Sciences undergraduate course and the Masters course in Science and Mathematics Teaching at Instituto Federal de São Paulo (IFSP), the students have created supporting materials for high school teachers. The topics in mycology are usually selected based on the students' interest from previous questions during the classes or on previous searches that have pointed out poorly addressed subjects in high school textbooks. During these activities the students produced materials on: 1) edible mushroom cultivation; 2) fungal diversity and phylogeny; and 3) history and use of antibiotics. The preliminary search on textbooks and the topics of interest to high school students have contributed to broaden the subject topics available for teachers. This talk will present materials done by the students from São Paulo (IFSP) and some of the best and more creative results presented over the last two years by the students from Santa Catarina (UFSC). We will also discuss how the students have been impacted with the last discoveries in the field of mycology and how the proposed activities could support high school and undergraduate teachers.

S22-4 Learning, coloring and respecting mushrooms – a coloring book to acquaint children and adults to the fungi

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Abstract: Collecting edible wild mushroom is a very popular practice, especially in Italy where the consumption of "porcini" (*Boletus* spp.) as well as other species is very common. Unfortunately, many toxic species are also consumed by mistake and this results every year in several cases of serious intoxications and even death. Most of the times these toxic mushrooms are confused with the edible ones, which underlines the importance of at least a basic knowledge of fungi to avoid this dangerous mistake. The main target of this coloring book is the introduction of children (and also adults) to the most common mushrooms and toadstools present in their local forests. After a preamble where the main parts of a mushroom are described with also some details about its ecological role, a brief explanation of the coloring technics is given as well as the definition of the symbols of edibility/toxicity present for each species. The main part of the book is represented by mushrooms drawings with their amazing shapes and the indications of their fascinating colors in nature. Each figure has also a short comment to help a rapid macroscopic recognition, the scientific and the popular names and the indication of its edibility/toxicity. A special remark is done for the deadly poisonous ones. Besides there are information for the importance of submitting wild mushrooms to the control of an expert before consuming and the recommendation for children and elderly people to avoid eating mushrooms. The Fungi Kingdom is not properly studied in the school and teachers can use this simple instrument to help children to learn about these fascinating organisms in a ludic and funny way.

S22-5 Lessons learned from mycological educational outreach programs for biology and environmental science teachers

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Abstract: Since 1967, the Larry F. Grand Mycological Herbarium (NCSLG) at North Carolina State University has served as a valuable resource for taxonomic research, student education and information for state and federal regulatory agencies concerned with identification of new and invasive fungal species. With support from the National Science Foundation Advancing Digitization of Biodiversity Collections and Thematic Collections Network Macrofungi and Microfungi Consortium Programs, teams of educators and scientists associated with NCSLG have conducted educational outreach workshops for biology and environmental high school science teachers for the past 10 years. Over this timeframe, workshops have evolved to better communicate and transfer mycological knowledge to diverse, underrepresented, and target populations of teachers serving low socioeconomic students. Workshops were established based on the premise that high school teachers have limited time to teach about fungi as isolated taxonomic units. These workshops provided a conceptual framework that empowered high school science teachers to promote greater student initiative and leadership in formulating research questions that encouraged use of inquiry-based, experiential learning investigations. In recent years, workshops were expanded to include opportunities for graduate and undergraduate students, pre-service, and middle school teachers. Teachers who participated in the workshops created activities that aligned with applicable learning frameworks and national and state science teaching standards for use in their classrooms. In this presentation, we will discuss lessons learned through years of workshop implementation and iteration with a focus on challenges associated with workshop evaluation, expansion, recruitment, subject matter content, and sustainability. The development of successful mycological educational outreach-related activities can foster and strengthen linkages between mycologists and society while increasing public awareness of the value of mycological collections and research.

S22-6 Empowering new investigators by allowing grad students to choose their own research projects

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Abstract: Master of Science students in my lab at the University of Wisconsin-La Crosse have worked on a variety of interesting fungal projects. Whenever possible, I like to have my graduate students choose their own projects, to make sure they're working on something they like and are invested in. Of course, there are a few constraints placed on their choices; their projects have to involve fungal topics that I know something about, or that I am interested in learning about. I have a pretty broad background in mycology, so the choices for my twenty-three Master of Science students have been really quite expansive, including systematics, biodiversity, ecology, mutualism, medical mycology, public health, proteomics, molecular biology, drug discovery, chemistry, heavy metals, and even hardcore nuclear physics. I have (what I now consider) the luxury of having an MS program, so experiments do not necessarily need to "work" for the student to get a degree". Students can take a chance on something interesting; sometimes the results are spectacular and sometimes they are not. This choosing of projects

turns out to be great for students' future careers, since they learn to make hypotheses, develop their project, learn the techniques necessary to perform their experiments, and analyze their own results (all with my help, of course). Students come away with great knowledge of how to do original research. I had hoped that by the time I was ready to retire that these topics would have all blended into a coherent research program, but that now seems unlikely to happen.

Symposium Session 23:

Metagenomics: Whole Fungal Genomes from Complex Samples

C. Quandt and J. Stajich

S23-1 Single cell genomics leads us to a better understanding of the evolution of arbuscular mycorrhizal fungi

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Abstract: Arbuscular Mycorrhizal Fungi (AMF) form symbiotic interactions with over 80% of the land plants. Despite this wide-spread interaction, little is known about the biology of the AMF. The study of AMF has been challenging because of the difficulties in obtaining clean and efficient cultures for DNA extraction. Up-to-date, only one species has been sequenced, and multiple questions remain unsolved, including their life cycle, karyosis, and genomic signatures of their long co-evolution with host plants. We have adapted the method of single cell genomics to obtain genomic data from single nuclei of AMF species that were never studied before. Single spores are crushed and nuclei are stained and isolated individually using the Fluorescence-activated Cell Sorting (FACS). Then, multiple nuclei are amplified and sequenced with Illumina. Several new assemblies are being finalized and I will be presenting comparative genomics results.

S23-2 Endogonales genomes reveal imprints of ectomycorrhizal lifestyle

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Abstract: Endogonales (Mucoromycotina) is the only known non-Dikarya ectomycorrhizal (ECM) order of fungi, representing one or multiple origins of the ECM lifestyle outside Dikaryon. Recent discovery of the mycorrhizal association between Endogonales fungi and liverworts and hornworts has led to the hypothesis that Endogonales were one of the earliest mycorrhizal partners with land plants and played an important role in the terrestrialization of land plants. In this study we applied shotgun sequencing to four Endogonales isolates from sporocarp tissue. The metagenomic sequence data was first binned using the combination of oligonucleotide-composition-based and BLAST-based methods. During the binning process we identified a large number of Mollicutes-related endobacteria (MRE) sequences in genome of three isolates, consistent with the observation that many Endogonales species harbor MREs. The sizes of the four Endogonales genomes vary from 90 megabases (MB) to 240 MB, much larger than an average fungal genome. The expansion of Endogonales genome size is due to extensive proliferation of transposable elements, which has been observed in many other ECM genomes. In addition, like most ECM fungi, Endogonales has low diversity and low copy numbers of genes coding plant-cell-wall-

degrading-enzymes (PCWDEs) in its genome. This is consistent with the notion that ECM lifestyle requires small number of PCWDEs to avoid damaging host plant cells and eliciting host plant defense. Our dating analysis estimated that Endogonales originated in lower Cretaceous, much later than the origin of Glomyeromycotina and the origin of land plants, suggesting that Endogonales were unlikely the first mycorrhizal partner of land plants and that association between Endogonales and plants was established later in evolutionary history of land plants.

S23-3 Genome assembly of the fungus *Leucoagaricus gongylophorus* cultivated by the ant *Atta colombica* using long-read MinION sequences

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Abstract: Assembling heterogenous (two very different sets of chromosomes) or polyploid (more than two sets of chromosomes) genomes can be challenging, but in a fast-developing world, new techniques and software are regularly developed. Leaf-cutting ants cultivate a polyploid fungus with an average of 7 different genomes which has proven to be difficult to assemble using regular short-read genome sequencing. In this study, I used the Oxford Nanopore Technologies MinION to produce long-read sequences. I then used a series of different software packages to assemble genomes from long-read sequences (CANU and Nanopolish) as well as software to deal with heterogeneous and polyploid species (Redundans). The quality of the assemblies was tested by extracting a core set of single-copy genes using BUSCO. With this, I developed a pipeline to assemble fungal genomes accurately using long-read sequences. The resulting genome assembly is an improvement of the one currently available and gives new insights into this intriguing mutualism between ants and fungi. Furthermore, I was able to fully assemble the fungal mitochondrial genome with a high coverage (±4000X), which can be used to both understand the mechanistic of this fungus, but which can also be used for more accurate phylogenetic analyses. In conclusion, I was able to show cost-efficient methods and a pipeline to accurately assemble fungal genomes that can be used for a wide variety of analyses.

S23-4 *scgid*, a bioinformatic tool for scaffold binning and genome prediction from metagenomic sequencing libraries

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Abstract: A kind of dark matter within the kingdom, "uncultured" fungi remain largely inaccessible in the context of genomic studies in the age of next-generation sequencing. Resistance to axenic culture techniques makes acquisition of sufficient input tissues for whole-genome sequencing a near impossible endeavor for these fungi. To circumvent these obstacles, extraction techniques that incorporate non-specific amplification steps can be used to generate sufficient final DNA concentrations from low input materials (single-cell genomics). The nature of these non-specific amplification steps makes sequencing libraries generated in this way especially prone to contamination originating from either the environment or the laboratory. There are a variety of post hoc bioinformatic methods available to attempt to eliminate contamination from these sequencing libraries. All of these methods involve binning of genome assembly scaffolds into target and non-target bins, which can be used to call a final genome draft. Interestingly, when confronted with the same data set, each of these different methods can call a very different final genome. This ambiguity and disagreement among methods seems to be dataset-dependent in such a way that no one method is always conservative or always liberal in its inclusion or exclusion of a scaffold from the final genome draft. This issue poses a major issue to the identification of high-confidence final genomes of uncultured fungi and hinders downstream analyses

incorporating them. To address this, we propose the use of a consensus-based approach leveraged toward determining which final bin a scaffold belongs in. Building upon past work and theory developed and implemented by other authors, the python-based bioinformatic tool *scgid* uses three distinct binning methods that bin scaffolds based on independent sets of characteristics. From these three drafts, a final, high-confidence consensus genome draft can be called by majority rule, minimizing contamination while maximizing inclusion of target sequences at the intersection of all three independent methods. This tool should prove useful to genome sequencing efforts in uncultured organisms across the tree of life where the goal is extraction of a high-confidence genome from moderately metagenomic sequencing libraries.

S23-5 Era of the living dead: Resurrecting fungal genomes from fungaria with metagenomics

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Abstract: Fungaria are the most mycologically diverse places on the planet and harbor a wealth of potential genetic data for phylogenetics, comparative genomics, taxonomy, and nomenclature. Yet, little molecular exploitation of this diversity has been realized. Until recently, this lack of development was due to technical challenges in harvesting genetic data from poorly preserved specimens where nucleic acids suffer from mild to severe degradation. High throughput DNA sequencing technologies that utilize short fragments has, in theory, largely overcome this technical impediment, yet progress in liberating these molecules from fungaria has been slow. Here, we show how shotgun sequencing of fungarium specimens, including type specimens from Charles Peck and others, is essentially a metagenomics problem where whole genome coverage of target taxa can yield profound information to generate robust phylogenetic hypotheses, anchor scientific names, and possibly provide high resolution information for comparative genomics, such as biosynthetic gene clusters. The fungarium genomics era has the potential to solve myriad puzzles resulting from our current state of knowledge of fungal diversity, including the enduring predicament posed by application of old names through type authentication, establishing its quintessence in contemporary science by breathing new life into the ancient and recent dead.

S23-6 Metagenomic strategies for inferring biological aspects of fungal endosymbiont systems

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Abstract: As we approach our goal of sequencing 1,000 fungal genomes it is becoming evident we will need to include DNAs from sporocarps, fungi in obligate symbioses, and fungal taxa with bacterial endosymbionts to sample genomic diversity across the Kingdom. These systems represent unique opportunities to study fungal evolution, and to develop comparative '–omic' pipelines that lend insights to hypotheses about how symbioses are initiated and evolve. Obligate, long-term, co-evolved fungal

endosymbionts and their hosts are one example of a study system poised to propel evolutionary questions in mycology in new directions. Our research group has been investigating fungal bacterial symbioses using *Mortierella elongata* (Mortierellomycotina, Mucoromycota) and bacterial endosymbiont *Mycoavidus cysteinexigens* (Burkholderiales). However, fungal endosymbiont interactions and other fungal symbioses are challenging to study for the following reasons. First, genomes must be extracted from metagenomic sequencing efforts requiring novel pipelines and creative quality checks to avoid assembly artifacts. Second, several fungal endosymbiont hosts belong to the former zygomycetes, a group of early diverging fungi lacking homology to current fungal model genetic systems, driving need for next generation annotation strategies and pipelines. Lastly, deriving testable, functional hypotheses about symbiotic interaction mechanisms from these complex data sets will require innovative approaches. We have developed metagenome sequencing and computational sorting, transcriptomics, metabolomics, and volatomics for studying fungal endosymbiont interaction dynamics in the *Mortierella-Mycoavidus* system. Challenges and insights from these endeavors will be discussed.

Symposium Session 24:

Applications and Molecular Aspects of Mycoparasitic Fungi

S. Zeilinge and M. Karlsson

S24-1 Unraveling the mycoparasitic interaction between *Trichoderma atroviride* and a fungal prey

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Abstract: Mycoparasitic species of the fungal genus *Trichoderma* are among the most successful biofungicides in today's agriculture although our understanding of the exact molecular mechanisms of their activity still is fragmentary. The biological control of fungal plant diseases by *Trichoderma* includes direct antagonism of phytopathogenic fungi by mycoparasitism. This mycoparasitic attack comprises pre-contact sensing of the prey followed by activation of "molecular weapons" such as cell wall-lytic enzymes, secondary metabolites, and infection structures finally resulting in attack and killing of the prey fungus. We used the strong mycoparasite *Trichoderma atroviride* as a model to study the mycoparasitic fungus-fungus interaction. Investigation of the early interaction stages employing *T. atroviride* labeled with fluorescent CRIB (Cdc42/Rac1-interactive binding) reporters revealed a switch between positive and negative chemotropism in the mycoparasites' hyphae during the pre-contact sensing phase, a behavior indicative of a stress response probably triggered by prey-derived substances. Accordingly, secondary metabolites released by both interaction partners could be visualized in the interaction zone by mass spectrometry imaging pointing to a chemical cross-talk between *Trichoderma* and the prey fungus. Our data support the current model of pre-contact prey sensing; consequently, the receptors and signaling pathways that are involved in sensing and in governing the mycoparasitic attack are of special interest. As indicated by our studies, *T. atroviride* relies on signaling via the Gpr1 G protein-coupled receptor as well as MAP kinase and TOR kinase pathways for triggering the mycoparasitic response in the presence of a fungal prey.

S24-2 The multi-role of *Trichoderma harzianum* Cerato-platanin Epl-1 protein during fungal, pathogen and plant host interaction

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Abstract: *Trichoderma* is well known for the ability of some species to act as i) important biocontrol agents against phytopathogenic fungi; ii) biofertilizers; iii) increasing tolerance of plants to biotic and abiotic stresses; and iv) inducer of plant defense responses via the production and secretion of elicitor molecules. Proteins of the Cerato-platanin (CP) family are released during the early developmental stages of filamentous fungi. They can act as elicitors and induce defense responses in plants. In this

study, we analyzed the effects of the *Trichoderma harzianum* Epl-1 protein in the interaction process with the phytopathogen Botrytis cinerea and with tomato and common bean plants in short and long periods after Trichoderma strains inoculation. The results showed that *T. harzianum* Epl-1 protein affected the eliciting 1) B. cinerea virulence genes; 2) tomato defense-related genes; 3) the activation of the primer effect in tomato plants; 4) the interaction at the first stage of tomato roots colonization; and 5) the growth promotion of bean plants.

S24-3 *Trichoderma* and the plants: beyond a simple biocontrol strategy

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Abstract: Soil fungi belonging to the ascomycete genus *Trichoderma* have the potential to provide environmental-friendly biocontrol of plant diseases. Biochemical and molecular genetic studies have clearly related the mycoparasitic behaviour of *Trichoderma* against phytopathogenic fungi and oomycetes with the secretion of chitinases, glucanases and proteases with the cell wall degrading activity. In addition, *Trichoderma* proteases can also hydrolyze nematode cuticles and eggs and inhibit enzymes produced by the pathogens to penetrate the plant. Comparative genome sequence analysis of biocontrol species of *Trichoderma* has revealed that the mycoparasitic activity in the rhizosphere facilitates the formation of endophytic associations and the evolution of positive interactions with plants, supporting the application of *Trichoderma* strains as plant biostimulants in agriculture and forestry. In this sense, it has been observed worldwide that, as a general rule, the *Trichoderma* positive impact is more apparent in plants subjected to some stress. Early transcriptomic responses of *Trichoderma* colonizing tomato roots have shown that genes related to the formation of infection structures in plant tissues resulted upregulated, and once the hyphal root attachment has already taken place, nutrient uptake and carbohydrate metabolism would be limited by plant defenses. This means that *Trichoderma* is capable of overcoming plant defense responses during the initial stages of the interaction, when the early systemic defense responses would not be reaching its full potential, allowing *Trichoderma* an intercellular apoplastic colonization. As a result, *Trichoderma* exerts beneficial effects on plants in terms of improvement or maintenance of soil productivity, increased percentages and rates of seed germination, nutrient uptake, growth promotion, alleviation of adverse effects caused by environmental damage and systemic defense stimulation against abiotic stress and pathogen attack, without the need of establishing any contact with the invader. *Trichoderma*-primed plants that have a priming memory are able to react more rapidly and more adequately when challenged by a stressor. We have recently observed that tomato progeny inherit resistance to pathogens linked to plant growth induced by *Trichoderma*, without compromising the level of defense.

S24-4 Investigating the genetic basis of biocontrol in the mycoparasitic fungus *Clonostachys rosea* through functional genomics

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Abstract: Biological control of plant diseases holds great promise for replacing chemical pesticides in future food production, as part of integrated pest management. The mycoparasitic fungus *Clonostachys rosea* is an efficient biological control agent under field conditions for a variety of plant diseases on agricultural crops. In order to improve our understanding of critical components of the mycoparasitic lifestyle of *C. rosea*, we sequenced the genome of *C. rosea* strain IK726 using Illumina/PacBio technology. Comparative genomics revealed a significant increase in the number of certain ABC-

transporters, MFS-transporters, proteases, polyketide synthases, cytochrome P450 monooxygenases, pectin lyases and GMC oxidoreductases compared with other Hypocrealean fungi. Interestingly, the increase of membrane transporter gene number in *C. rosea* was primarily associated with efflux drug resistance transporters. Necrotrophic mycoparasites such as *C. rosea* are assumed to have broad host range with little specificity. However, transcriptomic analyses revealed that *C. rosea* responded with both common and specific gene expression during interactions with the plant pathogenic species *Botrytis cinerea* and *Fusarium graminearum*. In agreement with the data on increased gene copy numbers, the majority of the regulated genes were predicted to encode proteins involved in membrane transport, biosynthesis of secondary metabolites and carbohydrate-active enzymes. Whole-genome re-sequencing of 63 *C. rosea* strains followed by genome wide association studies of phenotypic variation related with biocontrol of *Fusarium* diseases on wheat further identified several membrane transporters, proteases and one polyketide synthase to be associated with biocontrol. Finally, gene deletion studies confirmed the involvement of several ABC-transporters, MFS-transporters and polyketide synthases in in vitro antagonism or biocontrol in *C. rosea*. In summary, our data emphasize the role of antibiosis in determining the outcome of biocontrol interactions. Efflux membrane transporters appear to play an important role in the biology of *C. rosea*, by providing tolerance towards secondary metabolites produced by the fungal prey or *C. rosea* itself.

S24-5 *Ampelomyces* mycoparasites in action - improved visualization of a biocontrol fungus by *Agrobacterium*-mediated transformation

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Abstract: Powdery mildew fungi (*Erysiphales*) are obligate biotrophic plant pathogens, infecting around 10,000 dicot species and also some members of the *Poaceae*. Important crops, including wheat, barley, grapevine, apple and a number of cultivated and ornamental plants, are amongst the major targets of powdery mildew fungi. Pycnidial fungi belonging to the genus *Ampelomyces* are commonly found in powdery mildew colonies in the field, and some selected strains have been developed as biocontrol agents of different powdery mildew species. To improve visualization of the interaction between *Ampelomyces* spp. and their mycohosts, we produced GFP expressing *Ampelomyces* transformants with *Agrobacterium*-mediated transformation using *Agrobacterium tumefaciens* strain AGL1 carrying a plasmid with the hygromycin resistance and GFP genes. Transformants were selected on hygromycin-containing medium and were checked for fluorescence after being grown in culture. Growth characteristics and mycoparasitic activity of transformants were measured and compared to those of the wild type. Selected transformants were used in mycoparasitic tests using five different powdery mildew species. In these experiments, sporulating powdery mildew colonies were inoculated with spore suspensions of transformants. We have also conducted persistence tests, in which experimental plants were inoculated first with GFP expressing transformants, and one week later with powdery mildew conidia. The transformation method was effective as several transformants emerged on the selective medium and exhibited strong green fluorescent signal. Transformants were genetically stable as they emitted strong green fluorescence after several subculturing in the absence of selective pressure. Most transformants did not differ in growth characteristics and mycoparasitic activity from the wild type. In mycoparasitic tests we observed extensive intracellular colonization of powdery mildew hyphae, conidiophores and conidia; intracellular *Ampelomyces* hyphae, as well as pycnidia and conidia produced in powdery mildew structures exhibited strong green signals when examined with

fluorescence microscopy. In persistence tests *Ampelomyces* germinated on plant leaves in the absence of their mycohosts, and parasitized powdery mildew colonies as soon as these were available on the inoculated leaves. This work showed that *Ampelomyces* is amenable to *Agrobacterium tumefaciens*-mediated transformation and the commonly used heterologous marker and reporter genes like hygromycin resistance and GFP can be efficiently used. Transformation with GFP is useful for improving direct observation of this interfungal parasitic relationship. Our persistence tests demonstrated that *Ampelomyces* strains can act as biocontrol agents even if the target pathogen infects plants one week after *Ampelomyces* application. *This work was supported by a grant of the Hungarian Research, Development and Innovation Office (NKFIH NN100415), a grant of the Austrian-Hungarian Action Foundation (90öu16) and Janos Bolyai Research Fellowship to AP.*

S24-6 Ancestral state reconstruction and the occurrence of the killer-toxin phenomenon in the Cystobasidiomycetes

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Abstract: Pucciniomycotina is a subphylum with a high diversity in terms of habitat and life history strategies that include plant parasites, animal associates (including opportunistic human pathogens), saprobes and antagonists of other fungi. Antagonistic interactions can occur through: 1) direct physical contact between two fungi, i.e., mycoparasitism; or, 2) the production of killer toxins and other agents (known as the killer-toxin phenomenon). Killer toxins are the less studied of these two types of interactions, yet may play a significant role in the development of community structure in natural environments. The killer-toxin phenomenon was first described in *Saccharomyces cerevisiae* and has been more extensively studied in ascomycetous yeasts, while in Basidiomycota only 50 yeast species have been reported as producers of killer toxins, including a few species in the Cystobasidiomycetes. In this class, direct physical antagonistic interaction which is associated with sexual states has been reported in species of *Cystobasidium*, *Naohidea, Cyphobasidium* and *Occultifur*. On the other hand, the killer-toxin phenomenon which mainly occurs between the yeast stage of the fungi and other organisms has only been reported in *Cystobasidium minutum, C. pallidum* and *Hasegawazyma lactosa*. We hypothesize that the common ancestor to Cystobasidiomycetes is a mycoparasite that also produced killer toxins. To test this hypothesis, we evaluated the presence of killer toxins for 54 strains belonging to 24 species of Cystobasidiomycetes (including 14 species new to science). A sensitive strain was allowed to grow for 24h on media containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, and 2.0% agar, supplemented with 0.003% methylene blue and pH 4.2. Each strain in the Cystobasidiomycetes tested for killer activity was also incubated for 24h on Yeast Malt agar and inoculated by making a single streak on the plate containing the sensitive strain. Cultures were evaluated every 24h for 5 days for the presence of an inhibition zone with no growth. In addition, we constructed a resolved phylogeny for the class based on six loci (ITS-including 5.8 rDNA, LSU rDNA, SSU rDNA, and the protein coding genes *RPB1*, *RPB2*, *TEFα*) to determine the evolutionary origins of mycoparasitism through ancestral character reconstruction.

Symposium Session 25:

Fungal-Bacterial Interactions and Functions of the Fungal Metaorganism

S. Olsson and T. Pawlowska

S25-1 Interactions between ectomycorrhizal fungi and bacteria in boreal forests

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Abstract: In boreal forest ecosystems, trees allocate photosynthetically derived C to symbiotic ectomycorrhizal fungi that mobilise N and P from organic substrates, as well as base cations and P from mineral substrates. The mycorrhizal mycelium increases the nutrient absorbing surface area of the host plant root systems and provides a direct pathway for translocation of carbon to micro-environments in the soil. The continuous provision of energy-rich C compounds powers the mobilisation and uptake of nutrients by the mycelium and, together with its large surface area, creates an important potential niche for bacterial growth and colonization, as well as interactions with other fungi. However, the underlying mechanisms and functional significance of these microbial interactions are still poorly understood. Recent progress in understanding the functioning of ectomycorrhizal fungal mycelia and their associated bacteria will be reviewed, drawing on results from field and laboratory-microcosm experiments, profiling of fungal and bacterial communities using high-throughput sequencing, analysis of single root-tip microbiomes and measurements of stable and radioactive isotopes. DNA sequencing and stable isotope signatures of ^{13}C and ^{15}N suggest that distinct, functionally specialised communities of bacteria and fungi exist in different mineral and organic substrates. Patterns of bacterial colonization of rock surfaces successively colonized by lichens, mosses and finally, by tree roots and ectomycorrhizal fungi, reveal communities successively enriched by taxa from the families Bradyrhizobiaceae, Mycobacteriaceae and Planctomycetaceae. Studies of bacterial microbiomes associated with single root tips colonized by different ectomycorrhizal fungi have revealed that taxonomically distinct communities of bacteria develop with time and that even roots colonized by closely related ectomycorrhizal species within the same genus have distinct bacterial microbiomes in unfertilized soil. Fertilization with N removes this specificity and reduces bacterial diversity, particularly in B horizon soil. These effects may be related to changed patterns of assimilate allocation and mycelial turnover but the functional implications of the observed results need to be unraveled by further experiments. Stable isotope probing (SIP) enables the identification of active microbial taxa with access to different pools of carbon and ^{13}C-RNA SIP of microbial communities decomposing dead fungal mycelium revealed minimal involvement of ectomycorrhizal fungi, supporting the idea that ectomycorrhizal fungi benefit from organic matter decomposition primarily through increased nitrogen mobilization rather than through release of metabolic C. Additional SIP studies are currently in progress, however, using ^{15}N-labelled organic substrates and plants labelled with $^{13}CO_2$, with the intention of identifying the dominant microbial taxa allocating C to different organic and mineral substrates. Several recent studies suggest that ectomycorrhizal fungi are the dominant drivers of silicate weathering and may influence global CO_2 levels on geological time scales. Little is known about the detailed mechanisms or organisms driving long term carbon sequestration in organic and mineral substrates but we have recently observed the formation of biogenic amorphous mineraloids at sites of active mineral weathering and on-going studies

are in progress combining SIP with nano-scale secondary ion mass spectrometry (NanoSIMS) to visualise spatial patterns of carbon sequestration in different substrates.

S25-2 Distribution and population structure of endobacteria in arbuscular mycorrhizal fungi at North Atlantic dunes

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Abstract: Arbuscular mycorrhizal fungi (AMF, Glomeromycotina), in addition to forming symbioses with the majority of land plants, harbor vertically transmitted endosymbiotic bacteria '*Candidatus* Glomeribacter gigasporarum' (*Ca*Gg) and '*Candidatus* Moeniiplasma glomeromycotorum' (*Ca*Mg). *Ca*Gg is a nonessential mutualist of AMF, whereas the lifestyle of *Ca*Mg is unknown. To start unraveling the interactions between AMF and their endosymbionts in nature, we examined diversity and distribution of AMF-associated endobacteria in North Atlantic dunes at Cape Cod, MA. Of nearly 500 foredune AMF isolates surveyed during a systematic study, 94% were classified as the Gigasporaceae. 2% of all AMF isolates harbored *Ca*Gg, and 88% contained *Ca*Mg. *Ca*Gg was found only in the Gigasporaceae, whereas *Ca*Mg was present in Gigasporaceae, Acaulosporaceae, and Diversisporaceae. Incidence of *Ca*Gg across AMF was not affected by any of the environmental parameters measured, whereas distribution of *Ca*Mg in one of the hosts was impacted by plant density. *Ca*Mg populations associated with AMF individuals displayed high levels of genetic diversity but no evidence of gene flow, suggesting that host physical proximity is not sufficient to facilitate horizontal transmission of *Ca*Mg. Lastly, in addition to a novel lineage of *Ca*Gg, we discovered *Burkholderia*-related bacteria previously not known to associate with Glomeromycotina, and likely living inside AMF. They are closely related to free-living *Burkholderia* and endobacteria of other Mucoromycota fungi. Collectively, we conducted the first ecological study of AMF-associated endobacteria and assessed their diversity and population structure.

S25-3 Mortierellomycotina are excellent living tools to understand the role of *Mycoplasma*-related endobacteria and the functioning of their interaction with Mucoromycota

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Abstract: Fungi can interact with a myriad of organisms, from animals to plants, but also bacteria, other fungi and viruses. Some of these interactions, such as fungal-plant mutualisms, are well-known and have been investigated for a long time. In contrast, only in the last few years has the study of bacterial-fungal interactions blossomed, riding a wave of increasing interest by the scientific community. Bacteria engage in various types of symbiotic associations with fungi, ranging from cooperation to antagonism. These symbioses occur at different levels, with bacteria living inside fungal cells representing the most intimate interaction. Bacterial endosymbionts can be widely found in early-diverging fungi of Mucoromycota, among them *Burkholderia*-related (BRE) and *Mycoplasma*-related endobacteria (MRE). BRE represent the best-studied fungal endobacteria and show behavioral shifts from mutualist to weak pathogens. On the contrary, the knowledge on MRE lifestyle is much more limited. Here, we report

about the existence of a new bacterial-fungal symbiosis that involves MRE and Mortierellomycotina fungi. We carried out a large-scale screening of hundreds of Mortierellomycotina strains searching for MRE. We used a combination of microscopy, molecular phylogeny, next-generation sequencing and qPCR. We detected MRE within the mycelium of Mortierellomycotina fungi and their presence demonstrates that MRE distribute across the whole Mucoromycota phylum, where they may have lived in the common ancestor. We cleared MRE from their fungal hosts, obtaining a unique experimental system whereby pairs of isogenic fungal lines, with and without MRE, can be employed for comparative functional studies. Cured lines devoid of MRE showed fast growth and improved biomass production. Our data demonstrate that the fungal host experiences some fitness costs in accommodating its endosymbionts and, therefore, provides the first functional insight into the lifestyle of MRE. Our findings suggest that MRE may be antagonistic to their hosts and adapted to a non-lethal parasitic lifestyle in the fungal mycelium. Additional measurements aimed at exploring chemotypic and metabolomic variations across different fungal pairs are expected to provide novel insights into the ecological and evolutionary role of MRE within fungi. Mortierellomycotina offer a unique opportunity to expand the knowledge on MRE and pave the way for potential applications for controlling and using Mucoromycota in agriculture and industry.

S25-4 Endofungal bacteria – new insights into bacterial-fungal coexistence

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Abstract: Interactions between bacteria and fungi have likely evolved during the 600 million years history of terrestrial fungi. This has resulted in bacteria and fungi coexisting in various modern microhabitats and establishing interactions ranging from mutualism to antagonism or neutral coexistence. Such interactions can directly or indirectly impact higher trophic levels, as well as nutrient cycling. Moreover, the interaction between fungal-bacterial interacting partners is dynamic and can rapidly change in response to changes in environmental factors. The same is true for interactions occurring at the cellular level. Bacteria and fungi in close physical contact show relationships ranging from random coexistence to nearly total physiological interdependency. The most intimate relationship yet described consists in bacteria colonizing inner hyphae (endobacteria). In this study, we have investigated the diversity of both endobacteria and bacteria firmly attached to hyphae in fungal strains collections (c.a. 130 fungal strains). Amplicon sequencing of the 16S rRNA gene was used to identify bacterial species in DNA extracted from individual fungal cultures. We have discovered that endobacteria are much more frequent than previously assumed. Moreover, they seem to appear equally distributed in the phyla Basidiomycota, Ascomycota and Zygomycota, and also occur in the distinct phylogenetic lineage of the eukaryotic fungus-like Oomycota. In addition to this, we have started to investigate the rules underpinning this close association. In particular, under environmental conditions affecting negatively the fitness of the fungal host (e.g. temperature, biocides, and poor nutrient supply), we have observed for several fungal models that this tight relation turns to a loose coexistence. Defining the conditions triggering changes in the type of interaction between both partners are key to understand the dynamics of bacterial-fungal interactions. Such a discovery is essential for a better definition of the general mechanisms behind these interactions and their role in microbial ecosystem functioning.

S25-5 Microbiomes in decaying *Picea abies* logs: Forest management versus locality effects

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Abstract: The decomposition of dead wood and the resulting nutrient recycling depend on the activity of fungi that has evolved unique enzymatic toolkits. There is also a rich community of bacteria in decaying wood with tentative significant functions. It is well known that forest management practices are important for the maintenance of the diversity of wood decomposing fungi: leaving substrate has a positive effect, whereas forest fragmentation has negative impacts. In this study, we assessed the fungal and bacterial community in downed *Picea abies* logs in differentially managed forests across environmental gradients in order to identify the major factors structuring these communities. A total of 270 sawdust samples from the interior of 45 *P. abies* logs were sampled from 3 different landscapes in southern Norway. Within each landscape a cultured forest, a mature or old forest with absence of our red-listed polyporoid focal species, as well as an old growth forest with the presence of our focal species were included. The samples were meta-barcoded by sequencing ITS2 and 16S amplicons of the samples on the Illumina MiSeq platform. Key environmental variables were collected to infer the drivers of the bacterial and fungal communities using ordination methods. We observed a strong biogeographic structuring of the fungal communities and a weaker structuring of the bacteria, while forest management apparently had more limited effects. The co-occurrence pattern of certain taxa of bacteria and fungi were mapped and inferred to reveal potential interactions between them.

S25-6 Endohyphal bacteria modulate transcriptional and metabolic phenotypes of fungi

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Abstract: Fungi are ubiquitous inhabitants of plant tissues and are major drivers of plant and ecosystem health through symbiotic and saprotrophic contributions to nutrient cycles. Interactions between bacteria and fungi can have drastic impacts on fungal phenotypes such as growth, metabolism, and development and thus may alter plant-fungal interaction dynamics. Bacteria that inhabit intracellular space of fungi (endohyphal bacteria, or EHB) can influence fungal phenotypes relevant to interactions with plant tissues. In some cases, these symbioses are facultative and the bacteria are transferable, providing new opportunities to compare the genetic and chemical interactions of EHB and their fungal partners in isolation, in natural associations, and in novel partnerships. We evaluated chemical and biological traits of facultative EHB and their impacts on fungal development, metabolism, and transcriptional dynamics. Using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) we assessed the impact of diverse EHB on a focal fungal endophyte, *Pestalotiopsis* sp. 9143 (P9143). Comparative metabolic analyses showed that association with EHB induced a unique metabolic profile. The native EHB of P9143, *Luteibacter mycovicinus,* induced a unique metabolic profile containing an analog of the antifungal compound, pestalotether. To evaluate impacts of EHB on fungal gene expression we carried out culture-based phenotypic studies and conducted RNA-seq studies with the Illumina HiSeq platform. We found evidence for a significant alteration in the fungal transcriptional profile and confirm that several primary and secondary metabolic genes are differentially regulated when P9143 and *L. mycovicinus* are grown in association vs. in isolation. These chemical and phenotypic

shifts are potentially important to a competitive lifestyle in plant tissues and illuminate how intimate microbial symbioses may drive polymicrobial interactions. Ongoing work is aimed at analyzing which bacterial genes contribute to the endohyphal lifestyle and elucidating their role in modulating fungal phenotypes and metabolism, especially those that may influence interactions with plant hosts or co-occurring fungi in leaf tissue.

Symposium Session 26:

ICTF Symposium Expanding the Taxonomic Context of Genome Sampled Fungi

C. Schoch and N. Zhang

S26-1 Genome wide analysis of the transition to pathogenic lifestyles in Magnaporthales fungi

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Abstract: The rice blast fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae*, *Magnaporthe grisea*), a member of the order Magnaporthales in the class Sordariomycetes, is an important plant pathogen and a model species for studying pathogen infection and plant-fungal interaction. In this study, we generated genome sequence data from five additional Magnaporthales fungi including non-pathogenic species, and performed comparative genome analysis of a total of 13 fungal species in the class Sordariomycetes to understand the evolutionary history of the Magnaporthales and of fungal pathogenesis. Our results suggest that the Magnaporthales diverged ca. 31 million years ago from other Sordariomycetes, with the phytopathogenic blast clade diverging ca. 21 million years ago. Little evidence of inter-phylum horizontal gene transfer (HGT) was detected in Magnaporthales. In contrast, many genes underwent positive selection in this order and the majority of these sequences are clade-specific. The blast clade genomes contain more secretome and avirulence effector genes, which likely play key roles in the interaction between *Pyricularia* species and their plant hosts. Finally, analysis of transposable elements (TE) showed differing proportions of TE classes among Magnaporthales genomes, suggesting that species-specific patterns may hold clues to the history of host/environmental adaptation in these fungi.

S26-2 Phylogenomics link changes in genome architecture and population structure to ecological shifts in *Neurospora*

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Abstract: How does a species' ecology affect its evolutionary trajectory? We seek to answer this question by studying the evolutionary trends of the diverse model Ascomycete genus, *Neurospora*. Within *Neurospora* there have been at least nine separate transitions from sexual outbreeding to clonal selfing. Additionally, there has been a transition from a soil and/or dung habitat to a plant and fire associated habitat. Concomitant with this habitat shift has been the evolution of asexual spores. Finally, we describe a new species of conidiating *Neurospora* that appears to have reverted back to a soil/dung habitat. We analyzed a collection of 181 *Neurospora* genome sequences from across North America, including populations that vary based on sexual mode, asexual sporulation, and habitat. We found that the transition from sexual outbreeding to clonal selfing leads to a drastic increase in the rate of genomic rearrangements, while the transition to a plant-based habitat (and concomitant evolution of asexual spores) led to reduced diversity within populations. Interestingly, a transition from sexuality to asexuality has not been found within the plant-associated clade. Our study illustrates the profound effects transitions in reproductive systems and habitat can have on the pace and potential for evolution. Furthermore, combined with existing knowledge, we begin to construct a mechanistic model for the four-way interaction between sex, habitat, genome architecture, and population structure.

S26-3 Comparative genomics of host-specialized populations of *Corynespora cassiicola* causing emerging diseases reveals differences in necrotrophic effector genes

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Abstract: Numerous plant-pathogenic fungi secrete necrotrophic effectors, also known as host-selective toxins, that are important determinants of pathogenicity and virulence. *Corynespora cassiicola* is a destructive fungal pathogen causing emerging target spot epidemics on crops in the southeastern U.S. Populations of *C. cassiicola* from cotton, soybean, and tomato have recently been determined to be host specialized. We hypothesize that variation in necrotrophic effectors underlies specificity. The necrotrophic effector cassiicolin was previously identified as a toxin and virulence factor of *C. cassiicola* causing Corynespora Leaf Fall of rubber tree. Among isolates of *C. cassiicola*, cassiicolin was encoded by 6 *cas* gene variants, named *cas1* through *cas6*. To identify variation among putative necrotrophic effector genes in *C. cassiicola* causing outbreaks in the southeastern U.S. we conducted comparative genomic analyses of 12 *C. cassiicola* genomes, with 4 each from cotton, soybean and tomato from different regions of the southeastern U.S. The genomes were compared with the reference genome of *C. cassiicola* (Corca1) from rubber tree. The genomes were assembled *de novo* and searched for known *cas*, *Tox*, and other homologs of effector-encoding genes. Putative secondary metabolite synthetic clusters were identified using antiSMASH. Three *cas* variants were identified among the 12 genomes; however, no *cas* genes were identified among the genomes of the tomato isolates. Of the four genomes from soybean isolates, 2 contained only *cas6*, one contained only *cas2*, and one contained both *cas2* and *cas6* variants. The genomes of the four isolates from cotton all contained both *cas2* and a new, previously undescribed variant we named *cas7*. Interestingly, we identified the genes of the biosynthetic gene clusters for zearalenone and T-toxin in all 12 genomes of the isolates from the U.S., yet they were not present in the genome of the rubber isolate. The presence of different T-toxin genes varied among the 12 genomes depending on the host of origin; however, all 4 isolates from cotton contained the 9 genes identified as being involved in T-toxin production in *Bipolaris maydis*. In *C. cassiicola,* the T-toxin genes showed synteny with *B. maydis*; however, they were clustered in a single locus. Studies are

underway to determine if *C. cassiicola* isolates from the three host specialized populations causing epidemics synthesize T-toxin and if it is involved in pathogenicity or virulence. Knowledge of the evolution and variation in necrotrophic effectors of host specialized populations is critical in understanding the genetic basis of specificity and disease emergence of *C. cassiicola* causing target spot of cotton, soybean, and tomato in the southeastern U.S.

S26-4 Phylogenomics of suborder Agaricineae (Basidiomycota)

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Abstract: The evolutionary history of Fungi is still largely unknown. In this era of genomics, we are no longer limited by the amount of data available to resolve many of their relationships and generate new knowledge. Agaricales is the largest order of mushroom-forming Fungi. The suborder *Agaricineae*, containing mainly the brown and darker spored *Agaricales*, was recently shown to be monophyletic. However, the relationships within the suborder remain still largely unresolved. The group includes many important ectomycorrhizal and saprotrophic fungi, as well as edible and commercially relevant mushrooms, such as species of *Agaricus*. The aim of this study was to generate a robust and well-supported phylogeny of the suborder using genome-wide DNA sequence data. Shallow whole genome sequencing was used to create sequence data of 26 species of Agaricineae. In addition, genomic data of 11 previously published species was obtained from GenBank and JGI databases. Together, these 37 species cover all major families of the group. Selected based on previous studies, 211 single copy genes were extracted from the dataset and used for phylogenomic analysis. Previously published sequence data (ITS, nrLSU, nrSSU, *RPB*1, *RPB*2, TEF1) of 250 species was combined with the genomic dataset to produce the most extensive phylogeny of the group up to date. Our results will enable a more stable classification of the group. They will also bring knowledge on the evolution of mycorrhizal and saprotrophic lifestyles within the group. Other evolutionary patterns will also be discussed.

S26-5 ZyGoLife: Evolutionary genomics and phylogenetic classification of zygomycete fungi

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Abstract: To address longstanding questions in zygomycete evolution, we have initiated a large collaborative project – ZyGoLife – to sequence and analyze genomes across the taxonomic diversity of zygomycete fungi. This project is in collaboration with the Joint Genome Institute and the 1000 Fungal Genome Project, which seeks to sequence genomes across the fungal tree of life, and to date we have sequenced more than 100 zygomycete genomes. Phylogenetic analyses of genome-scale data reject the monophyly of zygomycetes and support the recognition of two phylum level clades, Mucoromycota and Zoopagomycota. Zoopagomycota is sister group to Mucoromycota and Dikarya and comprises three subphyla including Entomophthoromycotina, Kickxellomycotina and Zoopagomycotina. It is characterized by associations and interactions with animals and other fungi, septate hyphae, and asexual reproduction by conidia and merosporangia. Mucoromycota also comprises three subphyla including Glomeromycotina, Mortierellomycotina and Mucoromycotina. It is characterized by associations with

plants, plant-based nutrition, coenocytic hyphae, and asexual reproduction by sporangia. We will discuss evolution of these traits, phylogenomic analyses of corresponding genomic features (e.g., CAZymes, secretome), impacts on classification, and estimates of geologic origin in the context of eukaryotic evolution and colonization of terrestrial ecosystems.

S26-6 Leveraging single-cell genomics to expand the Fungal Tree of Life

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Abstract: Bridging the knowledge gap between the approximately 100,000 described fungal species and the estimated 5 million total fungal species will require novel approaches for studying fungi. One major challenge is that many fungal species have not been isolated into pure culture, yet these uncultured species represent most of the observed diversity in environmental DNA amplicon surveys. This issue is exacerbated in the early-diverging fungal lineages, which comprise numerous biotrophic and generally microscopic groups. Recent advances in whole genome sequencing from single cells promise to overcome major challenges in analyzing the genetic makeup of this unknown diversity at a high throughput scale by eliminating bottlenecks imposed by cultivation requirements and workflows. Here we expand our understanding of uncultured lineages of fungi by using our newly developed single-cell genome sequencing pipeline to analyze the genomes of eight uncultured fungal species, seven of which belong to early-diverging lineages. We show that although there is a large variation in genome assembly and gene space recovery (6-88%) from each single amplified genome (SAG), combining data of multiple SAGs from the same species yields estimated genome recoveries of at least 90%. Using even incomplete genomes derived from individual single cells, our phylogenomic analyses provide robust placement for these unsampled lineages on the fungal tree of life, including the previously difficult to place lineages such as *Dimargaris cristalligena* and *Blyttiomyces helicus*. Analysis of genomes from single cells allows us to detect polymorphic nucleotides as heterozygous sites and to infer that multiple early-diverging species and the ancestor of fungi was likely diploid. Nearly complete single-cell genomes facilitated comparative genomic analyses, such as investigation of common metabolic deficiencies and characterization of mycoparasitism-related gene family expansions. Additionally, we discovered the first known instance of hydrophobins outside of the Dikarya in the chytrid *Caulochytrium protostelioides*. These results show that single-cell genomics holds great promise in facilitating fungal phylogenomics, genetically exploring cryptic biology, characterizing nutritional modes and ecological diversity, and discovering novel genes.

Symposium Session 27:

Polyextremotolerant fungi in natural and urban extreme environments

N. Gunde-Cimermann and L. Muggia

S27-1 Hot, Cold, or Salty? Comparative genomics of halophiles from hot and cold deserts.

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Extreme environments may permit survival for only specialized and adapted life forms. The majority of fungi which colonize cold Antarctica rocky deserts, the hot Mojave desert biological crusts, or hypersaline salterns are from a few phylogenetic lineages. Some of the most common groups isolated by culturing and amplicon sequencing are meristematic and black yeast ascomycetes of the order Chaetothyriales (class Eurotiomycetes) and class Dothideomycetes. We sought to ask if these fungi demonstrate signatures of genetic adaptation to these environments with limited water availability, high UV radiation, or extreme temperature ranges. We have sequenced and compared genomes of strains from extreme and mesophilic environments to test if gene or functional domain content distinguishes extremophile. Comparison of the halophilic *Hortaea werneckii* to an Antarctic isolate of *Hortaea thailandica* revealed few changes that distinguish these species. Examination of the broader trends in gene content among some of these Dothideomycetes using our sequencing of *Cryomyces* and *Rachicladosporium* strains will be presented. In addition, we will report culturing and comparison of genomes of Chaetothyriales fungi including *Knufia/Phaeococcomyces/Sarcinomyces* isolated from Mojave desert biological crusts and *Exophila mesophila* from Antarctica. Finally, a population genomic study on the halophilic *Hortaea werneckii* characterized variation in ploidy and tests as to whether whole genome duplication or hybridization explains genome size differences among strains will be presented.

S27-2 Fungi in human-made water-environments

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Abstract: It is surprising how little we know about the fungal microbiome of human-made water environments that include for instance our drinking water systems and swimming pools. Cleaning regimes and for instance chlorination levels are especially directed at bacterial contaminations. For drinking water just one country has rules for maximum levels of fungal contamination, but this is only tested when the water has a dark or brown color or smells wrong. In this presentation both densities of fungal contaminations and species composition in the fungal populations of different human-made water-environments in the Netherlands are studied and compared to studies done in other countries. In general, fungal density and diversity are assessed based on colonies isolated using different (semi) selective media and culturing conditions and fungal identification based on molecular characterization of barcoding regions like the ITS region. For drinking water both surface water and groundwater can be used, each resulting in a different signature of present species, but generally in low numbers. Season has an impact on observed numbers and species. Besides the observation of generalist fungal species also water- specific lineages are observed, that include low numbers of opportunistic (black) fungal species. However, especially in chlorinated pool water –probably after human contact – numbers of

opportunistic pathogens dramatically increase. Some rarely observed pathogenic species prove be common inhabitants of these chlorinated pools.

S27-3 Bass Becking was right: Polar fungi in refrigerators, tropical in dishwashers

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Abstract: People in developed countries spend most of their time indoors, either in their homes or at work. They are thus exposed to a variety of microorganisms that can survive selectively in indoor environments despite of applied hygiene measures and sanitation procedures. Household appliances running at high or low temperatures and with detergents accommodate selective conditions leading to the enumeration of microorganisms that are specifically adapted to these conditions. Indoor mycobiota comprises either air-borne, mostly filamentous fungi inhabiting surfaces of walls and household appliances, or water-born yeasts or filamentous fungi in tap water or bathroom, kitchen and other household appliances, like dishwashers, washing machines and refrigerators. Recent studies of wet and hot indoor niches using culturable and unculturable approaches, have revealed the occurrence of a diversity of yeast species from the genera *Debaryomyces, Meyerozyma, Pichia, Saccharomyces* and *Yarrowia*, as well as opportunistic pathogenic yeast species from genera *Candida, Naganishia* and *Rhodotorula,* and the black yeast *Aureobasidium* and *Exophiala*. From cold household devices, used to preserve food, like refrigerators and freezers, also an array of other fungi was found, many of them being food unrelated and being often detected in Arctic environments. Prevailing filamentous fungi are from genera *Cladosporium*, *Penicillium*, *Aspergillus*, *Exophiala* and *Aureobasidium,* while yeasts belonged to genera *Candida*, *Debaryomyces*, *Naganishia*, *Cryptococcus*, *Vishniacozyma* and *Dioszegia*. The main characteristics enabling all these fungi to colonize specified domestic environments include production of extracellular polysaccharides, ability to grow on cleaning agents, tolerance to high or low temperatures, high salt concentrations, and alkaline pH. These selected and enriched species can form surface biofilms, and can become causal agents of infections. The way of entry of all these fungi to indoor environments are via air and water. Drinking water, which is typically not accredited for fungi, often contains propaguels of opportunistic human pathogens that can cause (sub)cutaneous and catheter-related infections and infections of respiratory and urinary tract. They belong to genera such as *Exophiala*, *Rhinocladiella*, *Candida* and *Rhodotorula*. The species diversity from all above listed indoor environment is described on the basis of advanced culture dependent and DNA-based NGS techniques.

S27-4 Lichen symbioses as a niche for extremotolerant fungi

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Abstract: Fungi living on lichens (lichenicolous fungi) have since long been studied but there is still little comparative information about their unseen diversity. We studied the whole diversity of lichen-associated fungi using a metabarcoding analysis of the internal-transcribed-spacer regions (ITS). We collected thalli with and without infections of specific lichenicolous fungi. The majority of the detected OTUs represented fungi of the two classes Dothideomycetes and Eurotiomycetes. Many of the fungi are also known form other niches and included rock-inhabitant and extremotolerant lineages. We assigned some of the sequences to morphologically characterized lichenicolous fungi and also assessed their potential asymptomatic presence in lichen thalli. Shared fungal composition in the thalli varied among different lichen species and did not correlate with externally visible fungal infections. In fact, microscopic data, culture-based approaches, and high throughput sequencing revealed discordant pictures of mycobiome diversity. Moreover, we find variation in diversity estimates depending on the primers used,

with particular biases in the detection of Basidiomycota. Preliminary co-culture experiments reveal a potential of some extremotolerant lichen-inhabiting fungi to form interactions with the algal partner of the host.

S27-5 Subterranean Fungi: Diversity within the Soudan Iron Mine in Northern Minnesota

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Abstract: Mines and caves are unusual biomes containing unique fungi and are greatly understudied compared to other environments. These studies focus on the Soudan Mine in Tower, MN, an iron ore mine that closed in 1963 after operating for 80 years. The mine has 27 levels and is 714 m deep. Although mines can be nutrient poor, there are areas in the Soudan mine with large quantities of wooden timbers. This wood was used during the mining operations and now serves as carbon source for fungi and other microorganisms. We sampled the mine to explore fungal diversity with the goal to investigate taxa that tolerate heavy metals for potential bioprocessing technologies as well as bioactive molecules for drug discovery and possible biocontrol for white nose syndrome (WNS) of bats. Fungi were cultured from samples and the ITS region was sequenced for identification and phylogenetic analysis. Results show Ascomycota are the dominating fungi followed by Basidiomycota and Mucoromycota. Out of 175 identified taxa 124 belong to the Ascomycota and 26 and 25 to Basidiomycota and Mucoromycota, respectively. There are also 49 taxa that do not match well (<97% BLAST GenBank identity) with described fungal species. Examples of the most commonly isolated fungi are: Ascomycota: S*cytalidium* sp., *Mariannaea comptospora*, *Hypocrea pachybasidioides*, *Oidiodendron griseum* and *Pochonia bulbillosa*; Basidiomycota: *Postia* sp., *Sistotrema brinkmannii*, *Calocera* sp., *Amylocorticiellum* sp.; Mucoromycota: *Mortierella parvispora*, *M. gamsii*, *M. hyaline*, *M. basiparvispora* and *Mortierella* sp. llumina high throughput sequencing was also used on samples of wood from several levels of the mine and showed that Ascomycota was dominant, including a sample with high copper concentrations. A phylogenetic analysis of identified *Pseudogymnoascus* sp. from the mine shows that they are closely related to *P. destructans* (the causal agent for WNS). Culture studies indicate they are present over extensive areas in the mine. Rhizomorphs of *Armillaria sinapina* were also found throughout the mine. The mine environment, with the presence of high levels of heavy metals, complete darkness and nutrient poor areas, is an extreme environment for fungi. One Ascomycota, *Cadophora* sp., and two Basidiomycota, *Amyloathelia* sp. and *Jaapia argillacea*, discovered in the mine are similar to genera isolated by the authors in other extreme environments. However, phylogenetic analysis shows differences in species between these environments. Many of the mine fungi have heavy metal tolerance, others show possible use for biological control of WNS. *Cadophora* sp. was also found to produce new compounds named soudanones. Results indicate this subterranean environment hosts unusually diverse fungi, many of them not found in above ground environments.

S27-6 Hidden depths: Fungi in permafrost soils and a test of the paleosymbiosis hypothesis in high latitude boreal forests

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Abstract: Fungi are fundamental for driving ecosystem processes of carbon and nutrient cycling, and are particularly important in carbon-rich boreal forests. Studies have demonstrated strong vertical stratification of mycorrhizal and saprotrophic communities in boreal forest soils and permafrost. Although much of the subarctic boreal forest is underlain by permafrost (ground perennially below 0°C), changing climate and increased wildfire activity has led to concerns about permafrost thaw and subsequent impacts on nutrient cycles, carbon emissions, and forest regeneration; processes in which fungi play central roles. In addition, the recently posited paleosymbiosis hypothesis suggests that there may be viable propagules in deep soils that could form effective mutualisms. These propagules could become important in boreal forests with projected drought, requiring deeper penetration of plant roots or repeated extreme wildfire events exposing mycorrhizas in deep soil layers. Northwestern North America is disproportionately impacted by climate change so gaining information on fungal taxa in soil layers can help us understand how ecosystems may function in the future. Our aim is to understand the function of fungi in different soil layers of boreal forest soils across regions. Our first objective is to describe fungal communities at different depths of boreal forest soils, including permafrost, and relate them to biogeochemical cycles (N, C) during permafrost thaw from sites with different fire histories in the Northwest Territories, Canada, and Alaska, USA. Our second objective is to test the paleosymbiosis hypothesis by determining the presence of viable ectomycorrhizal propagules from different depths and assessing their colonisation and growth impacts on seedlings. We collected 12 soil cores from active layer to deep permafrost soils from spatially distributed locations with different fire histories throughout subarctic boreal forests in our study region. Preliminary analyses of surface soils showed that fungal community structure was related to soil moisture, suggesting that changes in soil moisture due to permafrost thaw and/or climate change could have large impacts on fungal communities and subsequent biogeochemical cycling. We will present results on fungal community structure from Illumina sequencing of the active, permafrost, and deep permafrost layers. We will also present results from growth chamber experiments inoculating tree seedlings with soil from each depth to identify viable ectomycorrhizal propagules. Our study provides information on how the initial structure of fungal communities impacts C and N cycling in thawing permafrost soils, and survivability of ectomycorrhizal propagules from in deep soil layers.

Symposium Session 28:

Integrative Approaches to Understanding the Diversity and Function of the Boletatles

N. Nguyen and H. Liao

S28-1 An Overview of bolete systematics: Attention to details

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Abstract: The taxonomy and systematics of Boletes has a long history. For much of that time, concepts of genera and species were based on morphological features and ecological preferences displayed by taxa in the Northern Hemisphere – primarily Europe and North America. Increased exploration of the tropics, southern hemisphere, and other under explored/remote areas has revealed previously undocumented complexity that has challenged existing taxonomic concepts. The application of contemporary technologies and methodologies has shifted the paradigm of bolete classification. The use of cladistic approaches to analyze DNA sequences via powerful computational tools has fundamentally changed bolete systematics from an alleged artificial system to one that is deemed less

subjective based on hypotheses of evolutionary descent. Our recent efforts with the latter approach have resulted in a proliferation of taxonomic hypotheses; some withstanding further testing, others not. A verifiable framework is needed within which the details of morphology, sequence data, biogeographic and ecological factors can be evaluated.

S28-2 Functional and evolutionary genetics of zinc tolerance in the ectomycorrhizal fungus *Suillus luteus*

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Abstract: *Suillus luteus* is a cosmopolitan fungal species, symbiotically associated with pine trees. In primary successions of pines this species is abundant and involved in seedling establishment. On severely metal-contaminated soils, metal tolerant *S. luteus* populations evolved by natural selection. Tolerant individuals effectively protect their host tree from metal toxicity on these soils. However, the molecular and genetic mechanisms underlying adaptive metal tolerance in *S. luteus* are unknown. We hypothesize that tolerance phenotypes are due to an adaptation in the common metal homeostasis network. By comparative and functional genetics, we identified several *S. luteus* genes encoding transporters involved in metal homeostasis. One of these transporters, SlZnT2, a CDF family transporter exhibits a differential gene expression among Zn-tolerant and Zn-sensitive phenotypes. The difference in expression level seems to be due to an extensive gene multiplication and differences in cis-regulation. Analyzing natural populations, tolerance phenotype is correlated with *SlZnT2* gene copy number and associated with particular promoter genotypes. SlZnT2 is predicted to be localized on the tonoplast and to move Zn from the cytoplasm into the vacuole. Comparative genomics of different isolates representing distinct metal tolerance phenotypes is ongoing to identify the genetic loci associated with adaptive Cd and Cu tolerance. Altogether results of this study will be valuable to select ectomycorrhizal genotypes to support restoration of metal polluted soils.

S28-3 Phylogenomics of the Boletaceae using generic level sampling and low coverage whole genome sequencing

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Abstract: Boletaceae is one of the largest and most successful families of ectomycorrhizal fungi. Despite their ubiquitous presence in ECM communities worldwide and high scientific interest among taxonomists, a robust intrafamilial phylogeny that implicates unequivocal generic level clades has remained frustratingly elusive. Bolete genera were traditionally defined based on morphology of north temperate taxa, but such character packages are ill-suited for the wealth of tropical species, many of which are new to science. Recent multigene phylogenetic analyses that included taxa from undersampled regions have revealed discrete terminal clades emerging from a largely unresolved backbone, a result that has persisted since the first Boletaceae phylogenies appeared in the 1990s. This pattern may reflect a recent evolutionary radiation or may be an artifact of inadequate phylogenetic resolution from too few genes and/or incomplete taxon sampling. Despite this lack of resolution between clades, this pattern has resulted in a proliferation of new genera, with more than 1/3 of current generic names having originated within the last eight years. This study attempts to reconcile generic-level classification with improved phylogenetic resolution from a molecular dataset generated using low coverage whole genome sequencing from ~100 species of Boletaceae, representing exemplars of most

of the 65 currently accepted genera. Additional overlooked or undescribed taxa from tropical Africa and South America are included. Phylogenetic hypotheses and their implications for taxonomy and biogeography will be discussed.

S28-4 Environmental adaptation in *Suillus brevipes* (Boletales)

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Abstract: Recent advancements in sequencing technology allowed to better address the patterns and mechanisms involved in fungal environmental adaptation by identifying putative adaptive genes and providing a framework to further investigate the genetic basis of adaptation. Here, we report on ongoing studies investigating the physiological and genomic basis of environmental adaptation in species of the genus *Suillus brevipes* (Boletales). Whole genome scans in this widespread pine-associated species from across North America showed signatures of positive selection and genomic sites significantly associated with climate regimes and soil chemistry. Gene ontology enrichment analyses highlighted genes involved in transmembrane transport of substances and helicase activity that are potentially involved in both salt tolerance and cold stress response. We unveiled genomic regions underlying fungal adaptation and established links between phenotypes and genotypes, contributing for understanding how environmental conditions shape evolution.

S28-5 Mualistic coevolution mediating species and population level biodiversity of ectomycorrhizal fungi

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Abstract: Species of the ectomycorrhizal bolete genus *Suillus* exhibit strong host-specific associations with members of the conifer family Pinaceae, suggesting a long history of fungal-plant coevolution with different species of pines, larches, and Douglas fir. *Suillus* species are often prominent members of the early successional ectomycorrhizal community of forests, and play a key role in their establishment, growth, spread, and ecosystem function. Using genomics-based approaches, we are developing the *Suillus*-Pinaceae symbiosis as a model for the study of ectomycorrhizal fungal diversity and function. Genome sequencing of over 50 *Suillus* species is underway to facilitate the study of the molecular evolutionary history of the entire genus for the first time. Using whole-genome sequencing, we are also

examining finer-scale patterns of divergence within several globally distributed species (*S. brevipes*, *S. luteus*) using population genomics. To address molecular mechanisms involved in *Suillus*-Pinaceae coevolution we are using combined 'omics-based approaches to study the genome-wide expression of interacting plant and fungal symbionts at several levels including (a) mycorrhizal compatibility interactions across a phylogenetically broad range of *Suillus*-Pinaceae species pairings (b) within-species variation (*S. brevipes-Pinus spp.)* across geographic and host ranges. RNA-Seq data is being used to estimate expression and diversity of global genes involved in fungal-host plant crosstalk (effector-receptor interaction), zinc uptake, symbiosis maintenance and C/N/P allocation. Our study indicates the genetic elements required for *Suillus*-plant crosstalk underwent very recent genome evolution. This implies that genetic coevolution has potential to affect both within- and across-species diversity. The research outcomes will have the significant impact on expanding the theory of mutualistic coevolution and allow us to predict the effects of host-driven biological diversity on ecosystem function.

S28-6 Host range and specificity of ectomycorrhizal genus *Suillus* with different Pinaceae hosts

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Abstract: Though many species of ectomycorrhizal fungi are often reported to exhibit host specificity with different plant taxa, experimental evidence is often lacking and the mechanisms controlling mycorrhizal compatibility are still largely unknown. One well-known example of host specificity is the bolete genus *Suillus* that forms ectomycorrhizae (ECM) almost exclusively with members of the conifer family Pinaceae. Many species of *Suillus* spp. are reported to only occur only in association with certain genera and subgenera of Pinaceae including *Pinus* subgenera *Strobus* and *Pinus*, *Larix*, *Picea*, *Abies*, or *Pseudotsuga*. Using basidiospore-seedling bioassays, we addressed the host-range and specificity of 98 collections of *Suillus* representing 37 morpho-species from 25 different locations across North America for their ability to form ECM with diverse host species of Pinaceae. Basidiospore suspensions (5 x10^6 spores/mL) were used to inoculate seedlings of 10 different Pinaceae host genotypes. Inoculated plants were grown in the Duke Phytotron under controlled conditions (20ºC, 16h/8h day/night, with regular overhead watering) and assessed for growth and mycorrhizal colonization after 180 days. Identity of *Suillus* spp. forming ECM was confirmed by PCR and by culturing of ECM-colonized root tips. Most *Suillus* species collected under a given species of *Pinus* also formed ECM with other species of the same host, though with different levels of colonization. *Suillus* spp. collected under *Larix* and *Pseudotsuga* showed high specificity for their original host, however, some strain had limited cross-compatibility with *Pinus sylvestris*. Overall, colonization levels were lower on *Larix* and *Pseudotsuga* with respect to the *Pinus* hosts included in the study. Using multilocus sequence genotyping, both dikaryotic and monokaryotic cultures were isolated from ECM root tips, suggesting that haploid mycelia are capable of forming persistent ECM.

Symposium Session 29:

Fungi in a Changing Environment

L. Boddy and H. Kauserud

S29-1 Macroecology analyses of millions of fruit body records: Environmental drivers of phenology and species assemblies across Europe

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Abstract: Fungal species occurrence observations are increasingly available for scientific analyses through citizen science projects and the digitization of museum records, rendering new and large-scale ecological resources. When combined with open-source data, there is unparalleled potential for addressing new questions in fields such as fungal ecology, biogeography, macroecology and global change biology. We have assembled a pan-European mycological meta-database (ClimFun) that has been integrated with open-source environmental and species traits data. Initially 7.3 million unique fungal species fruit body records, spanning nine countries, were processed and assembled into 6 million records of more than 10,000 fruiting species. We will here present results from two studies where we utilize the data. In the first, we assess the phenology of fungal fruiting at a European scale and relates the phenology to climate variability and the seasonality of fungal fruiting. Mean annual temperature is ubiquitously important, and more so for autumnal fruiting fungi. Spring fruiting fungi, especially ectomycorrhizal fungi, are additionally responsive to primary productivity. There is significant likelihood that further climatic change, especially in temperature, will impact fungal fruiting patterns at large spatial scales. In a second study we identify the major geographical and environmental gradients structuring fungal assemblages throughout Europe for two main nutritional modes, saprotrophic and ectomycorrhizal fungi. For both nutritional modes, mean annual temperature correlated most with the first gradient identified that structured assemblages. Soil organic carbon was the highest correlate of the second compositional gradient for ectomycorrhizal fungi, likely an indicator of vegetative- and pH-related covariance. In contrast, a pollution gradient was of secondary importance for saprotrophic fungi, reflected in a high correlation with nitrogen deposition. The highest rates of compositional change in fungal assemblages by time (1970–1990 versus 1991–2010) suggest targeting higher latitudes and altitudes for a better understanding of fungal dynamics related to climate change. We suggest further examination of the ranges and dispersal abilities of fungi to assess responses to global change and to aid fungal conservation.

S29-2 Using species distribution models to inform conservation translocations

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Abstract: Rates of environmental change can outpace the ability of species to migrate or adapt. When this happens, it can lead to local extirpation, or even extinction in extreme cases. Conservation translocations, moving individuals of a species to bolster existing populations, reintroduce them, or establish populations in new areas, are important methods for rescuing species from extirpation or extinction. In this presentation I will discuss the utility of translocation for lichen conservation, with a particular emphasis on the role that species distribution modeling (SDM) can play in the process. I will present three case studies. The first study focuses on high-elevation endemics in the southern Appalachian Mountains of eastern North America. I used SDMs to predict how suitable habitat may shift for target species in the coming century. The results suggested that most of the distributions for all target species would be lost within their current ranges. I then set up a small transplant study to determine if it would be possible to consider conservation translocations as a method to rescue these species, again using SDMs to select suitable sites to establish the transplants. Unfortunately, most of the transplants failed due to the artificial substrate not withstanding climatic conditions in the study area. The second case study focused on whole coastal lichen communities threatened by sea-level rise. In this study SDMs were built for 193 species and used to determine where the greatest diversity and threats were concentrated in the region. This information was then used to guide the establishment of a whole-community lichen transplant on the edge of being lost to sea-level rise. The third case study is on a single species, *Usnea angulata*, which has been extirpated from the majority of its range in eastern North America. Transplants of over 50 individual thallus fragments were established at Highlands Biological Station to establish an *ex situ* source population that can be harvested sustainably to re-introduce the species throughout its historical range. The transplanted thallus fragments are growing quickly, averaging 2-3 cm of length increase every 6 months. Now that the *ex situ* source is established, SDMs will be used to select sites for reestablishing populations that are suitable now, and will continue to be suitable even as the climate changes in the coming century. All of these studies illustrate the utility of SDMs for conservation translocations, and provide examples to discuss decision making, risk assessment, and measurements of success in planning and executing conservation translocations.

S29-3 Fungal community dynamics following bark beetle infestation in Wyoming coniferous forests

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Abstract: Bark beetle (*Dendroctonus* spp.) outbreaks have impacted large areas in the Western United States in the past decade, resulting in large-scale conifer forest mortality. In these regions, the presence of bark beetles changed from endemic to epidemic levels because of increased mean winter temperatures and changes in precipitation patterns caused by climate change. Upon tree death, there is a change in plant-derived inputs via a large one-time needle drop and reduced root inputs, resulting in altered soil conditions. To understand how bark beetle induced changes in plant-derived inputs affect soil fungal community dynamics and associated biogeochemical cycling, a study was conducted in bark beetle affected coniferous forests in Southeastern Wyoming. Soil samples from healthy tree clusters were compared with infested and dead tree clusters. Impacts on fungal community dynamics were determined by measuring extracellular enzyme activities and sequencing the fungal community. Our study demonstrated increased extracellular enzymatic activity and turnover of the fungal community in dead compared with live stands. Specifically, lignin degrading enzyme activities were increased in

infested tree clusters, while (hemi)cellulose degrading enzymes were only increased in the dead clusters compared to the healthy tree clusters. Furthermore, dead stands had lower proportional abundances of ectomycorrhizal fungi (ECM) as well as a relative increase in saprotrophic taxa compared to the healthy clusters. Taken together, our findings indicate significant changes in the structural and functional dynamics of the soil fungal community, which is correlated with changes in overall biogeochemical cycling, and could have large impacts on forest regeneration following large-scale disturbances caused by climate change.

S29-4 Mycorrhizal fungal necromass decomposition under altered environmental conditions

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Abstract: More carbon (C) is stored globally in soils than in the biotic and atmospheric pools combined. Given the large fluxes of C entering the soil through fungal necromass (i.e. dead biomass) and their substantial contribution to soil organic matter, understanding the decomposition dynamics of these fungal inputs represents a critical gap in our current knowledge of global climate change. With regard to intrinsic factors, it is now well recognized that, like plant materials, the biochemical composition of fungal hyphae is a very strong predictor of mass loss. Compared to the decomposition of plant materials, however, the effect of extrinsic (i.e. environmental conditions) factors on fungal necromass decomposition remains poorly characterized. In this presentation, we will present the results of our incubation of four different types of mycorrhizal fungal necromass in the SPRUCE warming experiment in a boreal peatland in Minnesota, USA. Over the past two years, we have characterized rates of mass loss of each necromass type, the microbial decomposer community present on the different types of necromass as well as the chemical composition of their remaining residues. Collectively, this work provides fundamental new insights about the dynamics of fungal necromass decomposition and its role in soil C sequestration under altered environmental conditions.

S29-5 Tipping-point in C storage related to mode of N cycling across the arctic tundra-to-forest transition

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Abstract: Shrubs and trees are currently increasing in tundra areas across the Arctic, a response linked to recent climate warming. Ecotones between forests, dominated by ectomycorrhizal trees, and heathlands, dominated by ericoid mycorrhizal dwarf shrubs, are naturally found in transitions towards arctic and alpine zones, and may be used as space-for-time substitution to reflect long-term consequences of arctic greening on ecosystem level processes such as nitrogen (N) circulation and carbon (C) sequestration. Here, we present results from a subarctic-to-alpine ecotone from mountain birch forest to heath tundra in northern Sweden. We aimed to test the hypothesis that increasing abundance and activity of ectomycorrhizal fungi with increasing shrubs and trees would lead to faster N cycling through soil pools, and consequently lower C sequestration. We found a strong positive

coupling between tree abundance and ectomycorrhizal fungal growth, both of which were negatively coupled with C sequestration. By DNA-barcode sequencing, we identified a shift in dominance from root-associated ascomycetes (mostly ericoid mycorrhizal) in the heath to cord-forming ectomycorrhizal fungi (mostly *Cortinarius* and *Leccinum* spp.) in the forest. Higher C/N-ratios, lower inorganic N levels and lower abundance of functional genes reflecting inorganic N cycling in the forest suggested prevalence of organic N cycling by ectomycorrhizal fungi here. We also transplanted organic substrates between forest and heath to investigate the decomposition capacity of microbial communities. Heath humus decomposed faster than forest humus, irrespective of incubation site, suggesting that the large carbon sink in the heath was not driven by low quality of the organic matter. Furthermore, when tree roots and ectomycorrhizal fungi – but not ericoid roots and associated fungi – were excluded, incubated sample mass increased, suggesting sustained belowground input, but decreased decomposition rate. Taken together our data suggest that the lower C sequestration rate in forest despite the larger litter inputs here is a consequence of more efficient ectomycorrhizal nutrient mining from organic pools and associated soil C loss. In contrast, when stress-tolerant ericoid mycorrhizal plants and fungi, as in heaths, dominate soil processes, decomposition is slower and more, relatively good quality organic matter accumulates. Our results support the idea that the presence and relative decomposition capacity of mycorrhizal fungi, rather than different litter input quantities or qualities, determine long-term carbon sink strength across northern ecosystems. Such direct coupling between tree production and humus decomposition via mycorrhizal fungal communities is important to include in models predicting future C balance of the region, as the globally important soil C stocks may amplify atmospheric warming potential considerably if released through decomposition.

S29-6 Fungal community shifts along a fire severity gradient in a boreal forest

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Abstract: Boreal forest soils store a major fraction of the global terrestrial carbon. However, fires are pervasive disturbances that may critically trigger the carbon loss from these forest soils and in turn, influence the soil microbial communities with important outcomes over carbon dynamics of northern ecosystems. Here, we assessed the fire impacts on fungal communities, fungal transformations of organic matter and soil nutrients on an ecological gradient in fire severity. The study is part of a larger collaborative venture to investigate ecosystem recovery after the Västmanland fire in 2014, the largest Swedish forest fire in modern times. We sampled 25 and 7 plots stablished in burned (differing in fire severity damage) and un-burned areas respectively. Subplots subjected to logging and non-logging treatments were in turn established in each burned plot. As expected, the fire greatly affected the fungal community composition, with a negative effect on fungal biomass. On the other hand, the tree logging after the fire reduced the pine root biomass affecting the fungal community structure but not the fungal biomass. Our preliminary results suggest that the survival of *Pinus sylvestris* root biomass, indicative of fire severity, is one the major drivers of fungal communities, especially for ectomycorrhizal fungi and those from the group Leotiomycetes and Saccharomycetes. Gaining knowledge about post-fire ecosystem processes and fungal dynamics will contribute to a better understanding of fire ecology and succession of Boreal forests.

Symposium Session 30:

Biology of the Fungal Pigmentation: Advances and Perspectives of the Study of Melanin in Fungi

C.P. Taborda and M.C. Saparrat

S30-1 Proteomic screening of the black rock fungus *Knufia chersonesos* for the identification of polyester degrading enzymes

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Abstract: Understanding the molecular basis for survival in stress tolerant species goes hand in hand with the search for metabolites, compounds, and macromolecules playing a role in mechanisms of adaptation and being, at the same time, of possible biotechnological interest. The constant search for new products by the industry has indeed shifted the attention to extremophilic and extremotolerant organisms as potential producers of compounds with novel and unusual characteristics and functional activities under life-threatening conditions. Reflecting this tendency, the objective of the present study is to deepen the knowledge on ecophysiology and systems biology of black fungi – a group of ascomycetes considered as among the most resistant Eukaryotes know to date – as well as to detect species possessing polymer degradation ability. Hence, the extremotolerant rock-associated species *Knufia chersonesos* and its nonmelanized spontaneous mutant, whose degradation skills have been revealed by preliminary studies, were chosen for a proteomic-based screening towards polyesterases. Induced cultures – characterized by the addition of the biodegradable polyester poly(1,4-butylene adipate-coterephthalate) (PBAT) to the growth media – and control cultures were analysed by HPLC to determine the polymer hydrolysis. Both whole PBAT film and milled were tested. The induction was performed both in rich (2% malt extract, ME) and minimal medium (0.2% ME) aiming to test *K. chersonesos* ability to use PBAT as its sole carbon source. HPLC/MS identification and quantitation of the hydrolysis products terephtalic acid (Ta), mono(4-hydroxybutyl) terephthalate (BTa) and bis(4-hydroxybutyl) terephthalate (BTaB) indicated the presence of esters-hydrolyzing enzymes in the secretome of both strains and under induction with both whole and milled PBAT film. Ta was detected as the most abundant hydrolysis product, thus denoting degradation of PBAT to the smallest building block, especially in culture supernatants from minimal medium, resulting in up to 2-fold higher concentrations as compared to the other experimental conditions. Polymer hydrolysis was detected also when exposing un-induced growth media to the polymer, up to 70°C . Label-free shotgun proteomics and protein profiling showed largest differences in secretome composition and protein levels between minimal and reach growth medium. While at the optimal condition of growth an abundant and diverse set of proteins was detected growth on minimal medium lead to secretion of mostly carboxylic esterases. Our results on the extracellular proteome of *K. chersonesos* demonstrate that the culture supernatant has hydrolytic ability when grown in standard media and when PBAT is added to the media. In conclusion, these analyses of polyester degrading activity show that the proteomic screenings of an organism's extracellular proteome can aid the identification of novel polyesterases.

S30-2 Analysis of melanin as a virulence factor is differentially produced in related species of the dimorphic fungi *Paracoccidioides* spp.

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Abstract: Paracoccidioidomycosis (PCM) is a granulomatous systemic mycosis, whose etiological agents are dimorphic fungi of the genus *Paracoccidioides*. Melanin production by various fungi interferes in the mechanism of pathogenesis, and the same is observed in paracoccidioidomycosis. After analysis of melanin production by *P. lutzii* isolates (Pb01,Pb66, ED01,Pb1578and Pb8334) and *P. brasiliensis* isolates (Pb60855, Pb18 and Pbcão), we verified the ability of macrophages to phagocytose the highly virulent isolate Pb18, and the high and low producers of melanin isolates, Pb60855 and Pb01, respectively. Phagocytosis assay was carried out with C57BL/6 mice peritoneal macrophages that were challenged with antibodies/complement-treated or untreated yeast cells. Results showed that the presence of high concentrations of melanin reduced significantly the percentage of phagocytosed untreated Pb60855 and Pb18 yeast cells when compared to Pb01 isolate. SDS-PAGE protein and enzymatic profiles, including laccase activity, of the isolates Pb18, Pb60855 and Pb01 were also analyzed. The isolated Pb01 produced fewer proteins than Pb18 and Pb60855 in the tested conditions, as well as the laccase enzyme activity was reduced in isolate Pb01. Molecular phylogenetic studies have indicated two distinct clades among the genus *Paracoccidioides*: the *lutzii* clade containing *P. lutzii* species and the *brasiliensis* clade that harbors five phylogenetic cryptic species (**S1a, S1b**, **PS2**, **PS3**, and **PS4**) that were recently reclassified as formal species: *P. americana* (PS2), *P. restrepiensis* (PS3), *P. venezuelensis* (PS4) and *P. brasiliensis sensu strictu* (S1a and S1b). Our study included a representative isolates from each new formal species, that gives a differential production of melanin, lacasse, proteins and enzymatic profiles that could be explain the virulence among them. We conclude that the presence of lower concentrations of melanin and the reduced production of protein/enzymes by *P. lutzii* Pb01 isolate can be related to the augmented phagocytosis of these yeast cells by macrophages *in vitro*, explaining the reduced virulence of this isolate *in vivo,* in front of the other spices. Suggesting that there may be a differential expression of the virulence factors according to species that should be better studied.

S30-3 What do fungal melanins do?

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Abstract: Melanins are complex polymers that are synthesized by members of all biological kingdoms, making them one of the great natural pigments. Despite the abundance of melanins in our planet's biomass, there remains a great deal of mystery surrounding this pigment. Fungi produce and utilize melanins in the environment and during disease conditions. Ongoing studies have revealed remarkable structural characteristics of this enigmatic pigment and have uncovered intriguing associations of melanin to fungal virulence. In this session, we will review what we know about fungal melanin and were this knowledge is taking us.

S30-4 Fungal melanins of human pathogenic fungi: Updates and challenges in cell biology

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Abstract: Melanins are dark pigments ubiquitous in nature. Fungal melanins are insoluble polymers that have a crucial role in protecting the cell against environmental stressors. In infection models and *in vitro* experiments those pigments are related to virulence and survival of several fungi, regardless of biosynthetic pathway taken to produce melanin. Among the metabolic pathways described in fungi, the dihydroxynaphthalene and the DOPA pathway are the most studied, but others, including non-canonical pathways, have also been described. After several advances in spectroscopy and crystallography, and over 20 years of extensive research on melanins in *Aspergillus fumigatus*, *Cryptococcus neoformans* and other medically important black fungi, melanin's structure is still unsolved. In fungi, those polymers are ultimately located on the cell wall. Once thought to be synthesized exclusively in the cytoplasm, today is discussed the participation of secretory vesicles on the melanization of *C. neoformans* and attributed to melanosomes, evidenced in *Fonsecaea pedrosoi*and other fungi, the synthesis and storage of melanin. Our long-term goal has been to unravel the structural and cellular roles of fungal melanins. State of the art techniques of electron microscopy have been used for the comprehension of melanin's participation in fungal cell biology and structure. In *F. pedrosoi*, for example, transmission electron microscopy showed from the synthesis of melanin inside organelles (melanosomes) to the 3D map of iron associated to melanin by energy filtered transmission electron tomography. Recently, an international consortium revealed the genome annotation and analysis of melanin producing fungi (e.g. *Sporothrix schenckii* complex). Those studies revealed the putative ORFs related to melanin synthesis pathways in several fungal species. Such information is of extreme value and might lead the way, together with new transformation techniques, to a new era of melanin studies in fungi with new models and the elucidation of cellular mechanisms. The importance and cellular role of fungal melanins is getting clear. New data and discussion regarding its location on the cell is currently an important topic about melanized fungi and its research should be encouraged to understand its cellular mechanisms related to structure and virulence. Financial support: CNPq, FAPERJ, CAPES and UFRJ.

S30-5 Melanins from the fungus *Pseudocercospora griseola* and their properties

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Abstract: *Pseudocercospora griseola* is the causal agent of angular leaf spot (ALS) of common bean. It has undergone parallel coevolution with its host and two major groups have been defined, ''Andean'' (*P. griseola* f. *griseola*) and ''Mesoamerican'' (*P. griseola* f. *mesoamericana*). The aim of this study was to analyze comparatively the melanins synthesized by selected representatives of each group. Melanins in *P. griseola* f. *griseola* isolate S3b and *P. griseola* f. *mesoamericana* T4 were isolated and characterized. Melanin-like pigment derived from the isolate S3b and the T4 one was isolated with a total yield of 1.66 ± 0.64 and 4.07 ± 0.89 mg of melanin per g of dry biomass, respectively. The physicochemical properties and antioxidant activities of these two melanins were investigated. Although both melanins had similar spectroscopic and redox properties, T4 melanin powder showed lower UV-visible absorption than that from S3b, whose content in active phenolic groups was dependent on the size of the sample. Therefore, melanin deposition is differential in mycelium walls of both isolates, which might explain the physiological behaviours of representatives belonging to two major intraspecific groups of *P. griseola*.

S30-6 Mushrooms reveal dark color adaptation to cold environments

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Abstract: Coloration affects the fitness of organisms, e.g. via changes in thermal properties. Although color has been extensively studied in animals and plants, the role of color in mushrooms, the reproductive organs of many fungi, is unknown. We use citizen-science data consisting of 739 European grid cells with 3,054 fungal species, 3.2 million observations, 29,490 color samples and a mega-phylogeny to show that mushroom assemblage color lightness increases with temperature, meaning that assemblages are darker in cold than in warm climates. Our findings suggest that thermal adaptation via dark mushrooms facilitates reproduction of fungal species and the maintenance of populations that drive carbon and nutrient cycling in cold environments. We thus propose the 'thermal pigmentation hypothesis', stating a thermal-adaptation of mushroom-forming fungi.

Symposium Session 31:

Experimental Approaches to the Conservation of Rare Fungi

G. Griffith and R. Yahr

S31-1 eDNA and DNA metabarcoding in fungal conservation

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Abstract: The profile of fungal conservation in Europe has steadily increased over recent decades and by now several sites have legal protection based on the distinctive/rare macrofungal populations which inhabit them. Fungi inhabiting nutrient-poor grasslands, including waxcaps (Hygrophoraceae), fairy clubs (Clavariaceae), are particularly threatened due to habitat loss. For certain habitats in the UK, surveys of fungal diversity can be a requirement for EIA (Environmental Impact Assessment) in planning applications or proposals for agricultural intensification. When such surveys are required, there can often be costly delays (until fruiting season), and the ephemeral nature of macrofungal fruiting can require

several site visits. We have deployed a DNA metabarcoding approach for the assessment of grassland fungi. This work has involved the generation of additional rDNA barcode sequences from 'local' reference specimens. To date this approach has been used in the determination of planning applications, the notification of Sites of Special Scientific Interest and also in the prosecution of landowners who have undertaken unauthorised land improvement. By use of several well-studied sites where extensive fruitbody survey data exists, it has also been possible to ground-truth the data from NextGen Sequencing analyses. Our success in this venture has also been enhanced through a 'Citizen Science', involving amateur mycologists not only in the collection and verification of reference samples but also in the process of DNA barcoding.

S31-2 Increasing success of pitch pine restoration in the Albany Pine Bush Preserve using suilloid fungi

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Abstract: The goal of this research is to provide tools for restoring pitch pine stands at the Albany Pine Bush Preserve (APBP) using local ectomycorrhizal fungi (EMF). Restoration efforts in the APBP focus on removing invasive black locust, reintroducing periodic fires, and reestablishing pitch pine. Restoration of pitch pine by forest managers has had varied success in different areas of the pine bush, but the factors affecting restoration failures are not clear. Soil fungi and their below-ground mycorrhizal interactions may be influencing restoration success. Most terrestrial plants depend in some way on mycorrhizal fungi for establishment, growth, and survival. Pitch pines and the invasive locust both require fungal partners, but the specific fungi they associate with are mutually exclusive. Research has shown that a lack of EMF compatible with pine can hinder their establishment, but that the presence of Suilloid fungi (EMF in the genera *Suillus* and *Rhizopogon*) is sufficient to enable invasion of pines into uncolonized areas. To investigate potential use of EMF to improve restoration at the APBP a factorial field experiment is underway. Pitch pine seedlings inoculated with either live or autoclaved Suilloid spore slurries were planted into sites either never invaded by black locust or that recently had black locust trees mechanically removed. After four months in the field a significantly greater proportion of seedlings treated with live spores (0.76, SE 0.06) have survived than those inoculated with autoclaved spores (0.48, SE 0.03; ANOVA $p = 0.003$, $df = 1,8$). No differences in survival were observed between the non-invaded sites and those with recent black locust removal ($p = 0.425$, $df = 1,8$) and no interaction between inoculation and site was found. Pitch pine seeds from the APBP were planted into live, dried soils collected from each site type in a laboratory soil bioassay to investigate the EMF inoculum present in soils of each site. Seedlings will be harvested and the fungi on the ectomycorrhizal root tips identified with molecular methods. The soil bioassay selects for resistant propagules, a trait of Suilloid fungi, which are expected to be present in soils from both site types. Roots of field seedlings will also be harvested to compare to those of the bioassay. Field seedling roots from the non-invaded pine stands are expected to have a greater diversity of EMF than bioassay seedlings grown in either soil and field seedlings from the black locust removal sites, indicating greater diversity of EMF in non-invaded sites and the potential for association through existing hyphal networks.

S31-3 Protecting the invisible - identifying proxy indicators for landscape-scale arbuscular mycorrhizal fungal conservation

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Abstract: Mycorrhizas are among the most common symbioses on Earth, impacting plant community structure and ecosystem functioning, yet little landscape-scale data linking arbuscular mycorrhizal (AM) fungal diversity to biotic and abiotic properties exist to inform conservation. Furthermore, practitioners rarely possess the resources or taxonomic expertise to characterize AM fungal diversity at large spatial scales, resulting in a lack of evidence-based conservation strategies. Recent work has called for symbioses among mycologists and conservationists to develop approaches for fungal conservation. We examined AM fungal diversity in tropical and temperate locations and identified potential drivers of species distributions and diversity for use as proxy indicators in biodiversity conservation. AM fungal communities were documented at 60 sites in the Colorado Plateau in southwestern United States and La Gran Sabana in southeastern Venezuela. Communities of plants and AM fungi and abiotic variables were measured along 50-m transects in three vegetation types (shrublands, forests, and traditional agricultural fields). Model selection and multivariate analyses were used to evaluate environmental predictors of AM fungal diversity and community structure. AM fungal species richness (α diversity) and community structure, but not among-site turnover (β diversity), differed between tropical and temperate locations; only 15% of taxa were present in both locations. In unmanaged sites, AM fungal richness was not correlated with plant richness, but instead predicted by soil pH and temperature (temperate) or precipitation and latitude (tropics). The structure of AM fungal communities was influenced by plant identity but not plant diversity in both locations. Traditional, sedentary Hopi agriculture influenced AM fungal communities on the Colorado Plateau, but Pemón shifting slash-and-burn agriculture did not alter AM fungal communities at La Gran Sabana. At both locations, AM fungal community structure was linked to soil texture and nitrogen. Our study demonstrates similarity between tropical and temperate regions in the biotic and abiotic drivers of landscape-scale AM fungal species distributions. Soil and climate as well as plant community types may serve as proxy indicators of AM fungal communities for use in conservation planning to preserve the ecosystem functions and services of mycorrhizas. As a result, conservation plans that incorporate gradients in pH, soil texture, climate and habitat heterogeneity may be more effective at preserving AM fungal diversity.

S31-4 Reproductively contrasting lichen-fungi as an experimental model for conservation under climate change

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Abstract: The climate change risk to biodiversity can be characterised by measuring both 'exposure' and 'vulnerability'. Bioclimatic models are now widely used to quantify a species' exposure, i.e. the extent to which suitable climate space might decline or shift under global change, for a given species and region. Vulnerability is less well explored, and, given a species exposure, it addresses coping mechanisms such as evolutionary adaptation, acclimation, or dispersal allowing migration in response to a shifting climate. This study takes two ecologically similar but reproductively-contrasting lichens – *Nephroma laevigatum* and *N. parile* – and examines key aspects of their climate vulnerability for a steep climatic gradient in Scotland (oceanic-to-continental climates). We show evidence of genetic structure

related to climatic setting, consistent with local population adaptation, and pointing to gene flow as a consideration in management for climate change. We challenge a widely held assumption on species dispersal (distance determined by propagule size) that may be disrupted by at the establishment phase by facilitation effects and the role of photobiont sharing in the lichen symbiosis. We also ask whether the nature of the lichen symbiosis could allow acclimation to different climatic settings (spatial, or temporal under climate change), through photobiont selectivity and switching. We provide an overview of the lichen as a model for understanding how close species interactions can affect our understanding of climate change risk, and suggest future research directions.

S31-5 Using species distribution models to inform conservation translocations

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Abstract: Rates of environmental change can outpace the ability of species to migrate or adapt. When this happens, it can lead to local extirpation, or even extinction in extreme cases. Conservation translocations, moving individuals of a species to bolster existing populations, reintroduce them, or establish populations in new areas, are important methods for rescuing species from extirpation or extinction. In this presentation I will discuss the utility of translocation for lichen conservation, with a particular emphasis on the role that species distribution modeling (SDM) can play in the process. I will present three case studies. The first study focuses on high-elevation endemics in the southern Appalachian Mountains of eastern North America. I used SDMs to predict how suitable habitat may shift for target species in the coming century. The results suggested that most of the distributions for all target species would be lost within their current ranges. I then set up a small transplant study to determine if it would be possible to consider conservation translocations as a method to rescue these species, again using SDMs to select suitable sites to establish the transplants. Unfortunately, most of the transplants failed due to the artificial substrate not withstanding climatic conditions in the study area. The second case study focused on whole coastal lichen communities threatened by sea-level rise. In this study SDMs were built for 193 species and used to determine where the greatest diversity and threats were concentrated in the region. This information was then used to guide the establishment of a whole-community lichen transplant on the edge of being lost to sea-level rise. The third case study is on a single species, *Usnea angulata*, which has been extirpated from the majority of its range in eastern North America. Transplants of over 50 individual thallus fragments were established at Highlands Biological Station to establish an *ex situ* source population that can be harvested sustainably to re-introduce the species throughout its historical range. The transplanted thallus fragments are growing quickly, averaging 2-3 cm of length increase every 6 months. Now that the *ex situ* source is established, SDMs will be used to select sites for reestablishing populations that are suitable now, and will continue to be suitable even as the climate changes in the coming century. All of these studies illustrate the utility of SDMs for conservation translocations, and provide examples to discuss decision making, risk assessment, and measurements of success in planning and executing conservation translocations.

S31-6 Investigating the distribution and population sizes of the endemic macrolichen *Cladonia submitis*

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Abstract: The considerable biodiversity of lichens, their ecological value, and their sensitivities to pollution and habitat degradation, are all well-known and understood. However, little attention has been given to lichen conservation, and the vast majority of species remain poorly studied in terms of their

population sizes, genetics or conservation management. To date, the IUCN red list only lists eight lichen species, only two of which are protected under the U.S. endangered species act (ESA). Many lichen species are data deficient, and cannot be properly assessed without further study. One such species, *Cladonia submitis* (or Beach Broccoli), was proposed for risk assessment by the IUCN. However, the eastern North American endemic macrolichen lacked population size data or up-to-date distribution information, with many historical sites not revisited in several decades. As a result, the assessment was tabled until such data could be obtained. I will present the results of population surveys for the rare species in the core of its range, the pine barren and sand dune habitats of New Jersey, Long Island (New York) and Cape Cod (Massachusetts). Revisiting sites of historical herbarium records and seeking out new, previously unidentified populations in other areas, a comprehensive view of *C. submitis* populations, and how they have changed over time, has facilitated an IUCN risk assessment of species.

Symposium Session 32:

Boosting Diversity in Mycology

D. Haelewaters and the MSA Diversity Committee

S32-1 Diversity in Mycology

S. Branco[1] and E. Vellinga[2]

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Abstract: Strong gender and racial biases in STEM fields are prevalent and well documented. Underrepresented groups face implicit and explicit hurdles in career progression, hampering access to positions of power, and leading to lower wages and low retention rates. As data for such biases in mycology were virtually inexistent, we investigated gender balance in the Mycological Society of America (MSA) by compiling numbers on membership, officers and awards. We found male dominance in membership, officer positions (with 30% female presidents in the last 20 years), and non-student awards (including the most prestigious MSA award, granted to 55 male and 5 female mycologists). Notably, gender was near balanced in student membership and student awards. These results catalyzed the creation of the Diversity and Inclusion Committee, aiming at fostering diverse and inclusive participation in all MSA activities. This active and diverse committee compiles data on the composition of MSA membership, provides recommendations for an equitable and inclusive environment in the society and conducts outreach to foster participation of all MSA members.

S32-2 Diversity in the Mycological Society of America

T. E. Cheeke[1], S. Branco[2], D. Haelewaters[3], D. O. Natvig[4], M. Maltz[5], S. A. Cantrell[6], M. J. Cafaro[7], G. May[8]

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Abstract: Increased awareness of systematic biases across the Sciences, Technology, Engineering and Mathematics (STEM) has fueled calls for action across scientific disciplines. In a recent analysis of gender equality within the Mycological Society of America (MSA), Branco and Vellinga found evidence of gender bias, both in serving officers and awards. At the time of the Branco and Vellinga report, there was no

information on other aspects of diversity (e.g. race, age, professional status) within the MSA, as membership demographic information had never before been collected. Following the publication of this report, the MSA created a Diversity and Inclusion Committee with the goal of identifying and implementing specific actions to counteract potential biases pertaining to diversity within the society. The committee is made up of male and female MSA members, international, and Lesbian/Gay/Bi/Trans/Questioning (LGBTQ) mycologists from across academic rank, including graduate students, postdocs, and faculty. To better understand the demographic make-up of MSA the first membership assessment was conducted in 2016. In an anonymous online survey, MSA members were asked to provide responses to questions in the following categories: gender, age group, professional status, race, ethnicity, citizenship, sexual orientation, disability, family status, and annual income. Each question included a 'choose not to answer' option so members were free to answer (or not answer) any number of questions. Fillable text boxes were also included so members could generate their own responses or elaborate on their response to each question. The response to the survey was positive, with over 330 MSA members participating. Results show areas of relative high diversity within the MSA (e.g. international membership, LGTBQ members) but also highlight areas that could be improved. For instance, although gender ratios tended to be fairly balanced among students, gender bias became more pronounced in the more academically advanced categories. The survey also revealed that although MSA is made up of members from at least 15 different countries, there is low racial diversity within the society, reflecting known trends in the STEM fields. Because only specific actions to counteract diversity biases can make MSA more inclusive, the MSA Diversity and Inclusion Committee developed a set of best practices to be incorporated into the MSA Manual of Operations. For example, when MSA committees are identifying potential speakers to invite for annual meetings, best practices suggest that committees should strive for balance among disciplines, and diversity in gender, race, and ethnicity. An emphasis on providing professional development opportunities to a wide diversity of scientists could be critical for the long-term sustainability of the society. MSA members who are successful professionally will be more likely to maintain active membership, and moving forward, could help to improve recruitment and retention of a diversity of students and professionals within the society.

S32-3 Expanding your niche: Latin American mycologists working abroad

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Abstract: Diversity is a multidimensional concept that, among other aspects, entails age, religion, race, ethnicity, gender and gender identity, and work experiences. Even though this concept is not new, academic and non-academic research institutions have relatively recently implemented policies to assure diversity is reflected in their hiring processes, promoting the recruitment of professionals with diverse backgrounds. In this way, research organizations are acknowledging that diversity contributes to the richness of the environment for teaching and research. Bringing more diversity into science creates a wider variety of views and experiences that can greatly benefit research and education. Even with these efforts put in place, the agricultural sciences, which includes mycology and plant pathology, is still considered a male dominated field with positions traditionally filled by scientists with very specific- and usually applied- research experience. Fortunately, as more institutions recognize the value of diversity, changes are happening and scientists with diverse social, cultural, and intellectual backgrounds are having more opportunities of employment. As a Latin American mycologist, the journey to a stable position has not been easy. I obtained my graduate degrees from American institutions, but conducted most of my research in tropical countries. Moreover, I had dedicated most of my research to answer "basic research" questions. Due to the current low funding rates, experienced by most scientific communities, accessing to resources and/or stable research positions has become a

challenge and a source of frustration for many early career researchers. In this talk, I would like to discuss the topic of diversifying our job search approaches and exploring opportunities within applied agricultural fields. In particular, I would like to share my recent experiences running a small plant diagnostic clinic and developing an integrated research and extension program at a land-grant university affiliated research and education center. Plant diagnostics can be a very rewarding and creative career path with direct positive impacts on the community we live in and on the livelihood of its inhabitants. Agriculture is still the dominant economic activity and income source in many regions, even in developed nations, thus working on solving problems directly associated to this industry makes these jobs relevant and on high demand.

S32-4 LGBT+ in STEM, a must-have conversation

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Abstract: Of the approximate 361.1 million people living in the US, 3.5% identify as part of the LGBT spectrum. Of the 332 MSA members who participated in our 2016 Diversity Survey, 43% identify as female, 56% as male, and 1% as trans. Of those surveyed, 11% identify as LGBTQ. The question is whether we are doing enough to welcome those who identify as LGBT+ within the STEM fields, more specifically, mycology – which are still dominated by cisgender persons. There are many terms to describe persons who identify themselves as other than heterosexual or gender conforming. One umbrella-term is LGBTQQIAAP, which stands for lesbian, gay, bisexual, transgender, queer, questioning, intersex, asexual, allies, pansexual, or polysexual. Here we choose to use LGBT+. Gender identity and sexuality should not matter in the sciences, where instead we should focus on formulating research questions, designing experiments, collecting and analyzing data, publishing results and training students. But they still do. To gain more understanding of current trends on LGBT+ in STEM, an online survey was initiated in 2018 and circulated by membership email, on email listservs and through social media networks. The following questions were posed: Do you feel that you can be open about your sexual orientation and/or gender identity in your academic department or professional workplace? Did or will your being LGBT+, or your gender identity, affect your career decisions? Have you had negative reactions from colleagues about your being LGBT+ or because of your gender identity? Have you ever felt that your being LGBT+ or your gender identity has played a role in missing out on academic or outreach opportunities, assignments, or chances to move forward in your career? Have you had any positive reactions from colleagues about your being LGBT+ or because of your gender identity? Have you ever observed harassment in your work environment dealing with someone else's sexual orientation or gender identity? Do you have STEM role models who are open about being LGBT+ or their gender identity? Have there been times when being LGBT+, or gender non-conforming, felt isolating in your career, academic department, or professional societies? Would you appreciate an LGBT+ social event and/or mentoring session at conferences to promote networking? Would you appreciate a spokesperson within academic conferences to assist with being out and building a professional career as LGBT+? Participants answered yes or no and were requested to elaborate on their answers. A final question was added: How do you feel in your work environment? In their answer, participants selected one of five options from "very comfortable" to "very uncomfortable," and again were requested to elaborate. Altogether, this work will reveal the challenges and perspectives of the LGBT+ in STEM and will provide insights on potential efforts and opportunities that the science community can adopt to create a more inclusive STEM environment, particularly within the MSA.

S32-5 Recognizing and addressing unconscious bias

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Abstract: The scientific method is an organized process for experimentation, used to explore observations and answer questions about the natural world. This method of inquiry aims to be as objective as possible, but the humans conducting research are not themselves immune to bias. Both within scientific culture and beyond, stereotypes about what a 'typical' scientist looks like can result in unconscious bias. Unconscious bias, also called implicit bias, is defined as prejudice in favor of or against one thing, person, or group compared with another, usually in a way considered to be unfair. Unconscious bias has been to reduce bias and its effects. In this talk, I will briefly discuss how stereotypes harm people and impact productivity, and then explain how exposure to stereotypes results in unconscious bias. I will share a number of peer-reviewed studies documenting how different types of unconscious bias impacts diversity, equity and inclusion in STEM. I will then provide evidence-based techniques to reduce unconscious bias and limit its effects. I will conclude with a few approaches for mycologists to reduce unconscious bias in themselves and guidance on how best to intervene when others speak or act with bias.

Symposium Session 33:

Fungal Pan-Genomes

C. Schardl and C. Wang

S33-1 Genetic mechanisms generating population level variation in secondary metabolism

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Abstract: Fungal secondary metabolite core genes including nonribosomal peptide synthetases, polyketide synthases, terpenes, and alkaloids are among the most rapidly evolving of fungal gene families. These core secondary metabolite genes are often co-localized in the genome with accessory genes involved in modifying the backbone product, forming secondary metabolite biosynthetic clusters (SMBC). The rapid evolution of both core secondary metabolite gene families and SMBCs is thought to be in response to selective pressures in the environment and may enable fungi to adapt to new environments or result in rapid host-shifts. We investigate the population genomic variation of secondary metabolite core genes and clusters in two insect pathogenic fungi, the beetle pathogen *Tolypocladium inflatum* and the wide host-range insect pathogen and endophyte *Beauveria bassiana*. Using Pac Bio single molecule real time sequencing, *we* sequenced six geographically diverse strains of *T. inflatum* to investigate the role of genome rearrangement in generating diversity in secondary metabolism. For the previously sequenced NRRL8044 strain we also used a Hi-C chromosome mapping approach to improve the chromosomal level assembly and provide support for rearrangement events. The nearly complete chromosomal assemblies produced using these methods have allowed investigation of fine-scale evolutionary genetic mechanisms contributing to the rapid evolution of secondary metabolite genes and clusters, including transposition, duplication/deletion, cluster rearrangement, and horizontal transfer in cluster evolution. Similarly, using Illumina technology, we have sequenced ten strains of B. bassiana and several outgroup *Beauveria* species *(B. brongniartii, B. asiatica,*

and *B. australis)* to analyze the evolution of secondary metabolite clusters and other genes involved in host-interactions (e.g. G-protein coupled receptors, small cysteine-rich secreted proteins). Specifically, we investigate population level variation in secondary metabolism with potential roles in virulence. The results of our analysis shed light on the role of secondary metabolites in shaping the interaction of these fungi with distinct hosts and conversely on host-interactions in shaping the evolution of secondary metabolism.

S33-2 The origin and genome evolution of the wild and domestic populations of yeast from Far East Asia

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Abstract: The yeast *Saccharomyces cerevisiae* has been an essential component of human civilization and used worldwide for baking, brewing, distilling and winemaking for thousands of years. However, the diversity, origin and evolutionary history of both the wild and domestic populations of the yeast remain elusive. Here we employed a total of 106 wild *S. cerevisiae* isolates from diversified natural sources including primeval forests and 160 domestic isolates from various fermentation processes including traditional fermented foods from countryside in China. We performed phenotypic profiling, sporulation and flow cytometry analyses, and high coverage genome re-sequencing in the natural ploidy of the isolates. Based on phylogenomic analysis, we identified 10 wild and 12 domestic lineages including the oldest lineage of the species. The genetic diversities of both the wild and domestic populations of the species in China are much higher than those revealed in other regions of the world, supporting the Far East Asian origin hypothesis of the species. The domestic lineages which are all heterozygous share a single ancestor which was probably formed by outcrossing between diverse wild lineages which are all homozygous. The domestic isolates evolved into two major groups adapting to solid- and liquid-state fermentation, respectively, and share elevated maltose utilization ability, no matter maltose is present or not in their living environments. The data imply that the domestic population of *S. cerevisiae* might originate from an ancestor initially adapting to maltose-rich niches rather than fruit. The sporulation efficiency and spore viability of domestic isolates were much lower than those of wild isolates, explaining the maintenance of heterozygosity of the domestic population. We found consistent expansion and contraction of genes in domestic lineages, acquisition of new traits through lineage specific introgression and horizontal gene transfer, and metabolic remodeling for adapting to specific fermentation environments. Our integrated phenotypic and genomic analyses based on a set of *S. cerevisiae* isolates representing the largest genetic diversity of the species documented so far show a nearly panoramic view of the evolutionary history of *S. cerevisiae* and provide new insights into the origin and domestication of the species.

S33-3 Population genomics and the evolution of virulence traits in *Cryptococcus neoformans*

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Abstract: *Cryptococcus neoformans* is an opportunistic fungal pathogen of enormous clinical importance. To characterize the population diversity, subdivisions, and the level of genetic exchange of

C. neoformans var *grubii*, we sequenced the genomes of over 400 diverse isolates. While our data supports the three previously identified lineages (VNI, VNII, and VNB), phylogenetic analysis highlighted a deep, non-recombining split in VNB (VNBI and VNBII) and that VNI was further subdivided into three distinct clades, two of which were globally distributed and one of which was restricted to sub-Saharan Africa. Despite the higher prevalence of *MAT***a** isolates in VNB compared to VNI, we find similar levels of linkage disequilibrium in VNI, VNBI and VNBII. While we did not detect recombination between lineages based on genome wide comparisons, we identified introgressions (5 to 260 kb) in all pairwise combinations of VNI, VNBI, and VNBII as recipient and donor. Notably, some haploid isolates show more widespread hybrid ancestry of multiple lineages, including isolates that appear to have originated from recent interbreeding. Excluding hybrid isolates and recombinant regions of other isolates impacts estimates of the timing of VNI global dispersal and of VNBI and VNBII diversification. By assembling and annotating multiple isolates of VNI, VNII, and VNB, we find that gene content is highly conserved, with few examples of lineage-specific genes. Rapidly evolving genes between the lineages include transcription factors and transferases, many of which have been implicated in virulence or oxidative stress resistance. To evaluate how selective pressures in the environment coincidentally adapted *C. neoformans* for human virulence, we focused on a set of clinical and environmental VNB isolates from sub-saharan Africa. We found that the VNBII group was enriched for clinical samples relative to VNBI, while phenotypic profiling of sequenced isolates demonstrated that VNBI isolates were significantly more resistant to oxidative stress and more heavily melanized than VNBII isolates. Lack of melanization in both lineages was associated with loss-of-function mutations in the *BZP4* transcription factor. A genome-wide association study across all VNB isolates revealed sequence differences between clinical and environmental isolates in virulence factors and stress response genes. Inositol transporters and catabolism genes, which process sugars present in plants and the human nervous system, were identified as targets of selection in all three lineages. These data highlight the complex evolutionary interplay between adaptation to natural environments and opportunistic infections, and that

S33-4 Pan genome analysis of *Epichloë typhina*, a pathogen of several grasses and a mutualist of a few

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Abstract: *Epichloë* species (Clavicipitaceae, Hypocreales) are systemic symbionts of cool season grasses (Poaceae subfamily Poöideae), some of which are strictly seed-borne (vertical transmission), whereas others can produce stromata that disrupt host seed production (choke disease) and, following heterothallic mating, give rise to airborne ascospores for horizontal transmission. *Epichloë typhina* is a choke pathogen of *Brachypodium pinnatum, Dactylis glomerata, Holcus lanatus, Lolium perenne, Poa nemoralis, Poa trivialis*, *Puccinellia distans* and other grasses, but of these it is apparently capable of vertical transmission only in *Poa nemoralis* and *P. distans*. The species is also phylogenetically indistinguishable from *Epichloë sylvatica*, strains of which can be either horizontally transmitted choke pathogens or nonpathogenic, vertically transmitted symbionts of *Brachypodium sylvaticum*. A feature of *Epichloë* species is their production of bioprotective alkaloids that deter insect and, in some cases, mammalian herbivores. Four classes of *Epichloë* alkaloids are known: aminopyrrolizidines (e.g., lolines), ergot alkaloids (e.g., ergovaline), indole-diterpenes (e.g., lolitrems) and pyrrolopyrazines (e.g., peramine). Genes for each class are typically arranged in a cluster, and the genes or entire clusters

typically exhibit presence-absence polymorphism, giving a wide diversity of alkaloid profiles. Thus, in the context of the *E. typhina* pan-genome, the alkaloid biosynthesis genes are accessory genes, and part of the accessory (a.k.a., "flexible") genome, in contrast to the core genome of genes present in all genomes of the species (e.g., housekeeping genes). In published pan-genome analyses of bacteria, indications are that the accessory genome may have many times more genes than the core genome. We have begun a similar investigation of the *E. typhina/ E. sylvatica* pan-genome. A total of 21 genomes from hosts listed above were sequenced and annotated with FGENESH and MAKER, and arranged into putative orthologous groups (OGs) with OrthoMCL followed by COCO-CL. In the process, filters for minimum length and maximum A-T content were used to minimize the inclusion of spurious gene calls, although it is likely that some actual genes were also removed by those filters. With a 55% AT maximum and aligned gene lengths of at least 50 amino acid positions, we identified 10,901 OGs with genes shared between at least two genomes. Of these, the core contained 6377 OGs that were shared between all genomes, leaving 4524 accessory OGs. In addition, there were 7818 singletons, identified in no more than one sequenced genome, to give a total of 18,719 genes. Although spot checks suggest that the filters were reasonably effective, we will further assess these and other filtering approaches. Then, we will apply rarefaction, capture-recapture and binomial mixture models to estimate the pan-genome size. Further we will assess how the number of genomes sequenced affects the estimation of the total pan-genome size.

S33-5 Life-history traits coupled with population genomic analyses provide insights into fungal dispersal

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Abstract: Fungi have different life-history traits, which may result in highly variable population genetic structure across species. Polypore fungi producing conspicuous fruiting bodies with measurable species traits may serve as good candidates to elucidate fundamental life-history processes such as dispersal. The aim of this study was to investigate the manner and degree in which life-history traits are affecting both spatial genetic structure and in the long-term demographic history of fungi. We compared population genetic statistics among eleven polypore species, using large SNP datasets generated from RAD sequencing. The eleven species with different life-history traits are phylogenetically, geographically and demographically comparable. Traits, related mainly to reproduction and dispersal have a significant influence on the degree of inbreeding among various species. Species producing perennial fruit bodies exhibited higher levels of inbreeding compared to species with ephemeral fruit bodies. Hence, producing short-lived fruit bodies, possibly coupled to quicker life cycles and faster population turnover, seems associated with lower inbreeding. In correspondence with these observations, species with perennial fruit bodies showed signs of local-scale spatial genetic structuring, implying limited dispersal capacity. Spatial population genetics analyses inferred from recent haplotype coancestry, revealed isolation by distance within the population, with variable response among species. Most of the species have undergone a similar demographic history, showing sign of expansion in the population sizes, likely coupled with the co-migration of their host substrate *Picea abies* after the last glaciation. One rare species, *Amylocystis lapponica*, showed a divergent demographic history, with signatures of a population contraction. Further ecological and evolutionary genomics features of the 11 non-model fungal species will be presented.

S33-6 Global genomic survey of *Nothophaeocryptopus gaeumannii*, causal agent of Swiss needle cast disease of Douglas-fir

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Abstract: *Nothophaeocryptopus gaeumannii* (Mycosphaerellaceae, Mycosphaerellales) causes Swiss needle cast disease (SNC), one of the most damaging foliar diseases of Douglas-fir in its native coastal range. Stomatal occlusion by *N. gaeumannii* pseudothecia impede needle gas exchange, causing chlorosis and decreased needle retention. In severe cases, infected trees exhibit sparse crowns and significantly reduced volume growth. Since the 1980s, SNC has become an important emerging forest disease in the Pacific Northwest and in plantations worldwide. Increased disease incidence and severity are attributed to a changing climate that favors the proliferation of pseudothecia and forest management practices, such as the establishment of Douglas-fir plantations in the Sitka spruce zone. As a component of the CoAdapTree project, we are assessing the genomic diversity of *N. gaeumannii* populations to assess temperature and pathogenic adaption and develop models to predict the future distribution of populations under climate change scenarios. Approximately 900 *N. gaeumannii* strains were isolated or acquired from natural or planted Douglas-fir across a large geographic range, for example from Alaska to New Mexico, Europe, and New Zealand. Preliminary results are presented from the analysis of 100+ *N. gaeumannii* genomes, including insight into mating-type loci, lineage divergence, and population structure.

Symposium Session 34:

Threatening Fungal Plant Pathogens for Tropical Countries - Acting Before the Foes Arrive

E.S. Mizubuti and M.A. Dita-Rodriquez

S34-1 Genetic investigation of the causal agent of South American Leaf Blight of rubber tree: contribution to risk mitigation and disease management.

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Abstract: Acting before foes arrive should consider (at least) a three-layer intervention scheme: preventing pathogen from entering disease-free areas, preventing pathogen establishment, and eradication. One of the most dreadful disease to rubber tree plantations is South American Leaf Blight (SALB) caused by *Pseudocercospora ulei* (formerly, *Microcyclus ulei*). This fungus is indigenous to the Amazon region in South America and can completely wipe out plantations in the humid tropical climate regions. Fortunately, the pathogen is not present in Asia, where more than 90% of the world supply of natural rubber comes from. Nevertheless, the environmental conditions of that region are highly favorable to SALB epidemics. It is anticipated that if SALB gets in Asia, there will be a major impact in the international trade of natural rubber and all manufactured products derived from this commodity. The three-layer strategy aforementioned requires knowledge about the basic biology of the pathogen. Despite its potentially huge negative impacts, SALB is a somewhat neglected disease and the lack of basic information about *P. ulei* is notorious. A research program was set aiming at generating information that can be useful for the full implementation of molecular epidemiology studies of SALB. For instance, preventing pathogen entrance depends on the correct identification of the fungus, which in turn needs correct taxonomy and classification. Using molecular phylogenetics, different stages of the

life cycle were analyzed and the pathogen was re-classified as *Pseudocercospora ulei*, Mycosphaerellaceae. Preventing pathogen establishment can be effectively accomplished by planting resistant host plants, but durability of the resistance is a key aspect that deserves special care. Successful eradication programs usually acting on the early stages of disease introduction, while the restricted geographic distribution of a recently established population can be more affected by mitigation actions. Population genetics can be an interesting approach to generate most of the information to support these actions. Isolates of *P. ulei* from the Amazon and other regions were genotyped using SSR markers and the impacts of recombination, gene flow, selection and mutation were assessed as well as how the population is structured. These topics related to pathogen identification, population genetics and the molecular epidemiology of SALB are going to be discussed in regards to the three-layer strategy for disease-free areas and also for disease management where SALB occurs.

S34-2 The evolution of and diversity in the banana Fusarium wilt fungus *Fusarium oxysporum* f. sp. *cubense*

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Abstract: Plant vascular wilts, caused by soil-borne pathogenic fungi, pose serious threats to agricultural productivity. *Fusarium oxysporum*, an ascomycete fungus, is known to cause vascular wilt in more than 100 plant species, including economically important crops. As a strictly asexual fungus, the diversification of *F. oxysporum* is believed to be the result of mutations, possibly parasexuality, and/or lateral gene-transfer. The race designation of the banana Fusarium wilt fungus *F. oxysporum* f. sp. *cubense* (Foc), which groups strains selective to a specific cultivar or group of cultivars is, however, unresolved. This is due to the small number of differential cultivars used and the effect of temperature on host susceptibility. Vegetative compatibility group (VCG) analysis was thus used to measure diversity in the fungus. It also resolved uncertainties about the origin and global spread of the fungus. The VCG procedure is tedious and based on the recognition of mutated individuals, which does not reflect genetic relatedness among isolates. DNA-based methods, therefore, were used in recent years to study the relatedness of individuals and the evolution of the banana wilt fungus. They showed that VCG analyses often overestimated diversity in Foc due to minor mutations in *vic* or *het* loci of strains that are in fact clonal. However, collections of Foc in Asia and Africa also showed that the Fusarium wilt fungus have more clonal lineages than originally anticipated. The polyphyletic nature of Foc suggests that at least two host specialization events occurred after the domestication of bananas. One event lead to the development of Foc races 1 and 2 strains (pathogenic to non-Cavendish bananas), and the other to the development of Foc race 4 strains (pathogenic to Cavendish bananas). Phylogenomics further revealed distinct horizontal gene transfer events occurred in Foc races 1 and 2, and Foc race 4, respectively. Foc TR4, a group of isolates affecting Cavendish banana in the tropics, also forms a monophyletic lineage distinct from other Foc race 4 strains, seemingly with more recent and independent lateral gene transfer events that may have occurred more than once. An understanding of the evolution and diversity in Foc could assist in developing molecular markers for rapid strain identification, assist quarantine authorities to prevent the introduction of foreign strains into new banana-producing areas, and allow the rapid screening of banana varieties for resistance against all forms of the pathogen.

S34-3 Pathogenicity Factors in *Fusarium oxysporum* f.sp. *cubense*

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Abstract: *Fusarium oxysporum* is a ubiquitous soil borne fungus which can exist as a saprophyte, endophyte or as a pathogen**.** As a pathogen it consists of numerous host specific forms (*formae speciales*) causing vascular wilt or " yellows" on a range of plant species including significant crops such as tomato, banana, ginger, cotton and strawberry. In banana, *F. oxysporum* f.sp. *cubense* (*Foc*) is responsible for Fusarium wilt, also known as Panama disease, which in the mid-20th century destroyed vast plantations of the then cultivar of trade, Gros Michel. That initial Gros Michel-infecting strain of the fungus is now known as race 1, as worldwide the banana industry faces a new threat from an apparent different race of *Foc* known as Tropical Race 4 (TR4) to which the now dominant cultivar of trade, Cavendish, is highly susceptible. It is the ability of the fungus to persist in the soil, as a saprophyte or via its long-lived resting spores (chlamydospores), which makes it particularly problematic; once present in a plantation it is not possible to eradicate *Foc*. Thus, quarantine, along with the application of stringent on-farm biosecurity, are the only effective control measures and for that purpose, an efficient diagnostic is required. As stated previously, *F. oxysporum* exists in many forms including as a saprophyte so it is critical for diagnostics that the pathogenic form can be distinguished. *F. oxysporum,* and more specifically races of *Foc,* can be identified by culture techniques where mutants are induced in metabolic pathways and subsequent paired for complementation to identify specific vegetative compatibility groupings (VCGs). Within a VCG, isolates are assumed to be clonal, or at least closely related. A number of VCGs have been associated with race 1 of *Foc* however, for TR4 it seems that VCG 01213/16 is the dominant Cavendish-infecting pathotype. Based on published work on the *Fusarium*-tomato pathosystem we have sought to establish the ***S****ecreted* ***i****n* ***X****ylem* (*SIX)* gene profile of various VCGs and thereby races of *Foc*. *SIX* genes were first identified by reverse genetics from peptides present in the xylem of *Fusarium*-infected tomato plants. Using whole genome sequencing, we have identified nine of the previously 14 published *SIX* genes to be present in *Foc*. However, we have observed consistent sequence variation, which has allowed the identification of homologues in most of the *SIX* genes identified. For instance, *SIX1* exhibited eight different homologues based on SNP analysis across the 24 VCGs of *Foc*, with homologue *SIX1i* being unique to TR4 and phylogenetically quite distinct from the other eight *SIX1* homologues found within the VCGs of *Foc*. Whereas *SIX9* has a highly conserved sequence across all *Foc* isolates tested. Our study showed evidence of horizontal gene transfer occurring within *Foc*, with TR4 hypothesised to have arisen in such a manner. Additionally, the *SIX* gene analysis has allowed identification of targets in the genome that can be used in the development of a specific molecular diagnostics for TR4 and indeed each of the different VCGs of *Foc*.

S34-4 The threat of *Fusarium* tropical race 4 to Latin American and Caribbean bananas: the challenges of anticipatory actions

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Abstract: Bananas, although not originating in the Western Hemisphere, are an important food and income crop in many Latin American and the Caribbean (LAC) countries. About 20 million tons of bananas (64% of production) are locally consumed in LAC every year and seven countries of the region belong to the top-10 exporting nations globally. Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) has been historically a major threat for this crop in LAC. In the 1950s, Foc race 1 devastated the banana export trade, forcing the substitution of the susceptible cultivar Gros Michel by

resistant Cavendish cultivars, which are now planted in over million hectares globally. Unfortunately, Cavendish along with numerous other cultivars is susceptible to a new Foc strain, tropical race 4 (TR4), identified in the 1980s in Asia. TR4 has destroyed over 100.000 ha of Cavendish in Asia and in the past five years has spread into additional countries in Asia, the Middle East and Africa. TR4 was recognized as a major threat to bananas in LAC in 2007 and since then many preventive actions have been implemented both at national and regional levels. A regional contingency plan was launched by OIRSA (Regional Plant Protection Organization) in 2013 is still the only regional plan for TR4 worldwide. Decreed as quarantine pest in almost all the banana production countries in LAC, initiatives towards TR4 exclusion, such as increased border control and traveler alerts, but national readiness is highly variable across the continent. We discuss the risks of the spread of TR4 to LAC and identify priorities to improve response capacities from farm, national, regional and global perspectives.

S34-5 Intercontinental collaborative research significantly enhances our understanding of an invasive pine needle pathogen

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Abstract: Dothistroma needle blight (DNB) is one of the most important foliar disease of pines world-wide. The disease became well known in the 1960s when outbreaks caused significant growth reduction and mortality in exotic pine plantations in several Southern Hemisphere countries. Recent changes in climate, and the abundant availability of susceptible hosts, have led to a succession of devastating DNB epidemics throughout the Northern Hemisphere on native and non-native hosts. The growing global importance of DNB prompted the formation of a collaboration of scientists through the European COST Action FP1102: DIAROD: Determining Invasiveness And Risk Of Dothistroma. With the overarching research themes of biosecurity and risk, the participants of DIAROD, consisting of 160 scientists from 41 countries, developed a research plan based on the disease triangle aiming to address issues concerning the pathogen (defining the current disease distribution), environment (factors influencing the risk of DNB) and host (resistance and susceptibility). This very successful COST Action culminated in a series of reviews and original research articles in a special issue of Forest Pathology on *Dothistroma*. This presentation will highlight aspects of this research and show how this collective network of forest pathologists has expanded our knowledge on a pathogen of international concern. It will also show, at least in the case of DNB, how this network has gone a long way in answering the call to establish "a better coordinated global strategy to manage pest and diseases".

Symposium Session 35:

Fungi as Biocontrol Agents for Sustainable Agriculture

C. Wang and R.S. St. Leger

S35-1 Microbial competition within the context of entomopathogenic fungi and infection of their insect hosts

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Abstract: Ascomycete (Hypocrealean) insect pathogenic fungi, including *Metarhizium* and *Beauveria sp.* are capable of infecting and killing a wide range of insect host insects. These fungi are able to overcome the formidable array of insect defenses that include the cuticle waxy-layer and various levels of insect humoral and innate immune systems. In order to complete its life-cycle the fungus must sporulate on the insect cadaver to produce the next generation of fungal cells, and development on the dead insect has been recognized as an important and discrete part of the infection stage. One hitherto unexplored aspect of the infection process, however, are the roles insect associated as well as other environmental microbes may play in affecting the infection process. Competing (antagonistic) and/or assisting (i.e. facilitating infection) microbes may be present on the insect cuticle surface, within the insect gut, and potentially in specialized insect microbe hosting structures. Endogenous insect microbes that can impact insect (immune) defenses may also be found within cells, and include a wide array of facultative and/or obligate exo- and endo-symbionts. These microbes contribute critical functions to their insect hosts including providing essential compounds, mediating development, facilitating nutrient acquisition, and potentially proving defense against other microbes; the latter via production of antimicrobial compounds. In turn, there is accumulating evidence that insect pathogenic fungi can exploit or suppressing host microbes via mechanisms including the production of antimicrobial compounds whose expression is controlled as part of the developmental program in the insect cadaver. The insect microbiome and its interactions with fungal insect pathogens represents a new frontier that add to the complexity of the environmental interactions that occur and can provide explanations for why there are such large discrepancies between laboratory and field assays, where often the former are very successful whereas the latter are not. In addition, cues for the production of a set of fungal secondary metabolites likely involves competitive interactions with other microbes for completion of the fungal life-cycle on the insect cadaver.

S35-2 Contribution of nematophagous *Hirsutella* spp. in the suppressive soil of soybean cyst nematode

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Abstract: Soybean cyst nematode (SCN), *Heterodera glycines*, is a devastating pest of the soybean (*Glycine max*) worldwide. Continuously monoculturing soybean generally resulted in SCN suppressiveness which has been reported in a number of locations. We have conducted the studies on

SCN suppressive soils for more than two decades. *Hirsutella rhossiliensis* and *H. minnesotensis* have been found to be the dominant parasites of SCN juveniles in the USA and China respectively. Both *Hirsutella* species have been demonstrated to correspond to the SCN suppressiveness in suppressive soils. OWVT-1, a strain of *H. rhossiliensis* isolated from the suppressive soil in a field in Waseca, Minnesota, USA, has resulted in more than 90% control efficiency for SCN in greenhouse trial and has showed a density dependent parasitism with SCN density. However, field trial by inoculation of *Hirsutella* species into the soils did not obtain stable suppression of SCN. Recently, a comparative analysis of microbiomes in suppressive soil and conducive soil has revealed that more microbes are involved in the SCN-suppressive soil. The detail analysis showed that a bacteria in *Chitinophaga* specifically responded to the SCN suppressiveness. Based on our study, we hypothesize that both *Hirsutella* spp. as key biocontrol agents (characterized by dominant parasites and density-dependent parasitism) and *Chitinophaga* spp. as the functional microbes are contributed to the SCN suppressive soil. Comprehensive understanding of suppressive soils can provide novel strategies for successful control of soil-borne disease.

S35-3 Ergot alkaloids in bioactive *Metarhizium* species

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Abstract: Ergot alkaloids are important agricultural and pharmaceutical chemicals. Publicly available genomic sequence data indicate that fungi in the genus *Metarhizium* have the capacity to produce lysergic acid-derived ergot alkaloids; however, the accumulation of ergot alkaloids in these fungi has not been experimentally demonstrated. *Metarhizium* species colonize soil, roots of many plants, and insects. Because of these properties, some Metarhizium species are used as biocontrol agents. We investigated *Metarhizium* species grown under different conditions for accumulation of ergot alkaloids by high performance liquid chromatography (HPLC) with fluorescence detection. *Metarhizium anisopliae* and *Metarhizium flavoviride* were cultured saprotrophically on three different media: corn meal agar, malt extract agar, and sucrose yeast extract agar. Accumulation of ergot alkaloids varied by medium and fungus. *Metarhizium flavoviride* did not accumulate ergot alkaloids on any of these culture media. *Metarhizium anisopliae* accumulated large quantities of the ergot alkaloids lysergic acid α-hydroxyethylamide (LAH), ergine, ergonovine and chanoclavine-I on sucrose yeast extract agar, lesser quantities on malt extract agar, and none on corn meal agar. The identities of the alkaloids were confirmed by mass spectrometry. Interestingly, *M. anisopliae* secreted over 80% of its alkaloid yield into the medium, whereas the ergot alkaloids of most ergot alkaloid-producing fungi are retained in their hyphae. *Metarhizium robertsii* and *M. brunneum* were cultured only on sucrose yeast extract agar; *M. brunneum* produced the same profile of ergot alkaloids as *M. anisopliae*, but ergot alkaloids were not detected in saprotrophically cultured *M. robertsii*. We also investigated the accumulation of ergot alkaloids under the ecologically relevant conditions of mutualistic growth on plant roots and parasitic growth in infected insects. We inoculated roots of corn (*Zea mays*), bean (*Phaseolus vulgaris*), and *Medicago truncatula* with *M. anisopliae* and *M. flavoviride*, and no ergot alkaloids were produced by either fungus on any of the plants. Larvae of the insect *Galleria mellonella* were inoculated with spore suspensions of *M. anisopliae*, *M. flavoviride*, *M. brunneum*, and *M. robertsii*. Each of the four species produced ergot alkaloids in infected larvae. The mean concentration of LAH (the most abundantly accumulating ergot alkaloid) in *M. anisopliae*-infected larvae was 154 μM, a concentration that was 300-fold greater than the concentration observed in sucrose yeast extract medium. The data demonstrate that several *Metarhizium* species have the ability to produce ergot alkaloids of the lysergic acid amide class and that production of ergot alkaloids is tightly regulated and associated with insect colonization.

S35-4 *Metarhizium* and plants: the symbiosis is mutual

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Abstract: First discovered on insect cadavers, *Metarhizium* spp. are best known as insect killing fungi; however, the ability of these fungi to form epiphytic interactions with plant roots likely contributes to their abundance in soils world-wide. Genomic and molecular techniques have revealed that the early ancestors of the genus *Metarhizium* were plant symbionts, which took on an insect killing lifestyle providing insect-derived nitrogen to the plant in exchange for carbon. This hypothesis has generated a plethora of promising avenues of research investigating commonalities and distinctions between these two lifestyles. These fungi have further demonstrated the capacity to specialize variously with insect and plant hosts. Root colonizing *Metarhizium* strains are now known to promote plant growth by mechanisms that include killing insects, making nutrients available, increasing stress resistance and producing growth-promoting plant hormones. Field trials have confirmed that the ability to adhere to root surfaces plays an important part in maintaining *Metarhizium* population size, and that their endophytic and entomopathogenic lifestyles can be decoupled experimentally. The same molecular and genomic tools developed to probe and manipulate *Metarhizium* spp. to better kill insects are quickly laying the foundation for development of these fungi as comprehensive plant-growth promoters.

S35-5 Phenotypic analysis of nematode-trapping fungi using mathematical methods

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Abstract: Nematode-trapping fungi are unique organisms able to live both as saprophytes and parasites. These fungi switch their behavior according to the available nutrients and the environmental conditions and during their predator stage they develop complex trapping devices able to immobilize and kill nematodes that are subsequently consumed. Moreover, nematode-trapping fungi are able to sense their prey and adjust their parasitic behavior to the amount and location of nematodes. Nematode-trapping fungi are particularly important for biocontrol since many nematodes species are plant, animal or human parasites. Even though nematode-trapping fungi are widely spread, they often fail to establish in agricultural soils and their nematode-killing ability in nature is insufficient to control nematode populations. Therefore, in order to use nematode-trapping fungi as biocontrol agents we need to find nematode-killing efficient fungal strains and to fully understand the fungus-nematodes dynamics. Unfortunately, the lack of techniques able to efficiently quantify the phenotypical characteristics of nematode-trapping fungi hinders the study of these dynamics. In response, we propose the use of innovative and multidisciplinary approaches combining mathematical and biological techniques for the study of nematode-trapping fungi. More specifically, the use of image analysis techniques to study the phenotype and the morphological characteristics of different nematode-trapping fungal strains. By means of images we are able to, among others, follow fungal growth over time, track the changes triggered by the presence of nematodes on the mycelium and evaluate the trapping efficiency of different nematode-trapping fungal strains. Ultimately, resulting in detailed comparisons of several fungal features among different fungal strains allowing us to find stronger strains with enhanced colonization ability and nematode-killing activity. In summary, this research will contribute to the use of nematode-trapping fungi as biocontrol agents against parasitic nematodes.

S35-6 Molecular and chemical strategies employed by insect biocontrol fungi to counteract host immune defenses

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Abstract: Insect pathogenic fungi such as *Metarhizium* spp. and *Beauveria bassiana* have been developed as environmentally friendly biocontrol agents against different insect pests. Phylogenetic analysis revealed that fungal entomopathogenicity is polyphyletic, so similar expansions of insect cuticle degrading proteases and chitinases reflect a convergent evolution during the arms race of fungus-insect interactions. Relative to the advances in understanding fungus-plant interactions, the mechanisms of the molecular pathogenesis of entomopathogenic fungi are rather limitedly understood. In particular, the machinery of effector-mediated inhibition of host immunity has not been well established in fungus-insect interactions. We found that the divergent LysM proteins are present in animal pathogens. By using the insect pathogen *B. bassiana* as a model, we revealed that two of 12 encoded LysM protein genes are required for full fungal virulence against insect hosts to deregulate insect immune responses and protect fungal cells from chitinase hydrolysis. For *M. robertsii*, a collagen-like protein can camouflage cell wall components for evading host immunity. In addition, *in vivo* metabolomics analysis revealed the dynamics of small molecules were produced by both the fungi and insect hosts during fungal invasion of hosts. In particular, the small molecules such as the cyclodepsipeptide destruxins produced by *M. robertsii* and dibenzoquinone oosporein by *B. bassiana* could be deployed by the fungus to inhibit host immune responses to facilitate fungal infection. The understanding of fungal molecular pathogenesis can facilitate the development of cost-effective mycoinsecticides.

Symposium Session 36:

Fungal Sexual Development and Exploitation

P. Dyer and U. Kück

S36-1 Fungal genome and mating system transitions facilitated by chromosomal translocations involving intercentromeric recombination

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Abstract: *Cryptococcus neoformans* is a pathogenic basidiomycete fungus present in the environment globally that infects immunocompromised humans causing significant morbidity/mortality. *Cryptococcus gattii* is a related pathogen, more geographically restricted and causing fewer infections but often in healthy individuals. Two pathogenic species are recognized, but seven distinguishable species populate this monophyletic pathogenic species complex. The pathogens are embedded within two broader species groups (*sensu stricto*, *sensu lato)*, which are saprobic, insect, or plant/tree associated and not animal pathogens. The *sensu stricto* complex includes the *Cryptococcus* pathogenic species complex and three additional species: *Cryptococcus amylolentus*, *Filobasidiella depauperata*, and *Tsuchiyaea wingfieldii*. The *sensu lato* complex includes *Kwoniella mangrovensis*, *Kwoniella botswanensis*, *Cryptococococcus heavenensis*, and others. How pathogenesis evolved is being addressed via comparative genomics employing Illumina, PacBio, and Nanopore sequencing supported by chromoblot analysis. This enables complete genome assembly, revealing genes/gene sets unique to pathogens, or particular pathogenic lineages, higher order genomic architecture, specific chromosomal loci such as centromeres and telomeres, and chromosome number alterations. We discovered extant sexual cycles and defined mating-type loci for multiple species. By comparing chromosome-wide

complete genome assemblies, we find the genome of the closest nonpathogenic sibling species, *Cryptococcus amylolentus*, has 14 chromosomes like *C. neoformans*/*C. gattii,* but with extensive translocations and many intrachromosomal rearrangements (inversions, transpositions). We defined an extant tetrapolar sexual cycle for *C. amylolentus*, and found evidence the *MAT* loci are genetically linked to large regional repetitive centromeres of their host chromosomes (Ch. 10, 11). This supports models in which inter-centromeric recombination may have contributed to drive chromosomal translocations and transition from an ancestral tetrapolar outcrossing saprobic state to the derived pathogenic bipolar configuration. Similar types of inter-centromeric chromosomal translocations are present in the *C. neoformans*/*C. gattii* genomes, those of other species in the complex, and also *Candida* species, suggesting this is a general mechanism of genome rearrangement. Remarkably, other species analyzed have fewer chromosomes, in some cases as few as three, providing insights into origins and evolution of saprobic and pathogenic karyotypes. These findings contribute to show recombination involving repeats within centromeres can occur to impact evolutionary trajectories during mating-type locus evolution and speciation.

S36-2 Proteome diversification by A-to-I RNA editing in fungal sexual development

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Abstract: RNA editing occurs post-transcriptionally and leads to transcripts that differ in sequence from their template DNA. Adenosine (A) to inosine (I) RNA editing is common in metazoa and was recently detected in filamentous fungi. In contrast to metazoa, A-to-I editing in fungi occurs mostly within protein-coding sequences. During translation, I is interpreted as guanosine (G), effectively leading to A-to-G changes in amino acid codons, causing amino acid variation in the encoded proteins. In the filamentous ascomycete *Sordaria macrospora*, we found A-to-I RNA editing to be linked to the sexual phase and to be mostly absent from sterile mutants. Editing leads to putative amino acid changes in 361 "edited in fruiting body formation" (EFD) proteins, 47 of which show an extended C-terminus due to RNA editing of the stop codon to a tryptophan codon (stop-loss). Peptides from these C-terminal extensions were identified by mass spectrometry, and synthetic peptides were used to quantify DNA-encoded and extended protein isoforms. This approach revealed that the abundance of extended proteins increases in late sexual development, similar to the edited RNA. Focusing on EFD2 for further analysis, we found that the *efd2* gene has a role in ascospore formation. Ascospores of Δefd2 were smaller on average, but had a bigger size distribution than wild type ascospores. In addition, 5 % of Δefd2 asci harbored seven spores instead of eight spores, with one spore being significantly bigger than the others. The *efd2* transcript undergoes stop-loss editing, and we performed functional analysis with the native allele, a stop allele that cannot be edited, and an edited allele with a mutation of the stop codon to a tryptophan codon. Our results indicate that the non-edited *efd2* respective the DNA-encoded EFD2 protein isoform has an important role in ascospore formation. However, the extended EFD2 isoform may have a role in ascospore germination. GFP-tagged EFD2 localized to the cytoplasm in vegetative cells, but to the nucleus in ascospores, and editing was necessary, but not sufficient for this localization. Though the distinct role of single editing events needs further investigation, our results show that editing leads to the diversification of the proteome, which may be a prerequisite for the massive cellular reorganization during ascus and ascospore formation.

S36-3 Regulation of mating mechanism and sexual development by mating type loci in the homothallic ascomycetes *Fusarium graminearum* and *Chromocrea spinulosa*

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Abstract: Fungi employ one of two mating tactics for sexual reproduction: self-sterile/heterothallic species can mate only with a genetically distinct partner while self-fertile/homothallic species do not require a partner. In ascomycetes, sexual reproduction is controlled by master regulators encoded by the mating-type (*MAT*) locus. The architecture of *MAT* differs in heterothallic *versus* homothallic species; heterothallics carry one of two forms (*MAT1-1* or *MAT1-2*) per nucleus, whereas most homothallics carry both *MAT* forms in a single nucleus. Here, we describe mating mechanisms controlled by *MAT* loci in two homothallic filamentous ascomycetes, *Fusarium graminearum* and *Chromocrea spinulosa* (*Trichoderma spinulosum*). The former, which is causal agent of Fusarium head blight in cereal crops, employs a true homothallic mating strategy, while the latter exhibits both homothallic and heterothallic behavior and self-fertile strains produce progeny cohorts that are 50% homothallic, 50% heterothallic. Sequencing of the *MAT* region of homothallic and heterothallic strains of *C. spinulosa* revealed that both carry an intact *MAT1-1* locus, but homothallic strains have a second version of *MAT* with the *MAT1-2* locus closely linked to *MAT1-1*. In the second *MAT* version, the *MAT1-1-1* open reading frame is split into a large and small fragment and the truncated ends are bordered by 115bp direct repeats (DR). The *MAT1-2-1* gene and additional sequences are inserted between the repeats. By *MAT* manipulations, we discovered that self-fertility is achieved by DR-mediated loss of *MAT1-2* from most homothallic nuclei and subsequent inter-nuclear recognition between the resulting two, unevenly present, nuclear types in a common cytoplasm. To gain a comprehensive understanding of the regulation of sexual development in *F. graminearum*, we employed in-depth and high-throughput analyses to examine the target genes controlled transcriptionally by two-linked *MAT* loci (*MAT1-1, MAT1-2*). A total of 1,245 differentially expressed genes (DEGs) among all of *MAT* gene-deletion mutants included genes mainly involved in metabolism, cell wall organization, cellular response to stimuli, cell adhesion, fertilization, development, chromatin silencing, and signal transduction. Targeted deletions of 106 DEGs revealed 25 genes that were specifically required for sexual development, most of which were regulated transcriptionally by both the *MAT1-1* and *MAT1-2* loci. Taken together with the other analyses, we propose a regulatory pathway for *MAT*-mediated sexual development, in which both *MAT* loci may be activated by several environmental cues, and then control the expression of at least 1,245 target genes during sexual development via regulatory cascades and/or networks involving several downstream transcription factors and a putative RNA interference pathway. These investigation using two different homothallic species provide new insights into our understanding of regulation of mating mechanisms underlying homothallism and subsequent sexual developmental processes by the *MAT* loci in filamentous ascomycetes.

S36-4 Exploitation of fungal sexual reproduction for strain improvement in the food industry

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Abstract: Fungi are used widely for the production of various foodstuffs either as an edible ingredient themselves, or being used to produce metabolites that are key ingredients within foodstuffs. There is

therefore continued demand for strain improvement for such fungi for example to produce novel flavour profiles, to increase health benefits of fungal foodstuffs, and to increase metabolite yield. A difficulty has been that many of the fungi used in the food industry have classically only been known to reproduce by asexual reproduction, limiting opportunities to exploit the sexual cycle to generate novel genetic variation with the possibility of improved genotypes among the offspring. However, recent advances in understanding of mating-type (*MAT*) gene structure and occurrence, together with improved *in vitro* methods for inducing sexual cycles, has allowed the discovery of sexual cycles in a series of fungal species previously considered to be asexual organisms. These approaches have now been applied to asexual fungi used in the food industry. Results will be presented relating to how the discovery of *MAT* genes, characterisation of idiomorph structure, and manipulation of *in vitro* conditions in certain 'asexual' fungal species has been used to induce sexual cycles under laboratory conditions. A combination of GC-MS 'electronic nose' and LC-mycotoxin testing has then allowed the identification of sexual offspring with new, highly desirable, flavour profiles.

S36-5 A network of MAP-Kinase pathways and transcription factors regulates cell-to-cell communication and cell wall integrity in *Neurospora crassa*

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Abstract: Maintenance of cell integrity and cell-to-cell communication are fundamental biological processes. Filamentous fungi, such as *Neurospora crassa*, depend on cell-to-cell communication to locate compatible cells, coordinate the process of cell fusion, and maintain a robust hyphal network. Shortly after the asexual spores germinate, these genetically identical germlings will collaborate to establish a new colony by engaging in communication that drives chemotropic growth and ultimately results in cell fusion and the formation of a multinucleate syncytium. The signal(s) and receptor(s) remain elusive, but two MAP-Kinase pathways are essential for communication and fusion in *N. crassa*. The MAK1 pathway, also known as the Cell Wall Integrity pathway, receives input from cell wall sensors and coordinates the processes that maintain the integrity of the cell wall during growth, fusion, environmental fluctuations, or external attack from agents such as cell-wall-targeting drugs. The MAK2 pathway is homologous to the pheromone response pathway in *Saccharomyces cerevisiae*. The pheromones and pheromone receptors are not involved in asexual germling communication, but we hypothesize that the MAK2 pathway functions downstream of a different receptor that mediates germling communication. Previous studies have demonstrated several points of cross-talk between the MAK1 and MAK2 pathways, which is likely necessary for coordinating chemotropic growth toward a specific extracellular signal, and then mediating the precise process of cell fusion. Canonical MAP-Kinase pathways begin with signal reception and end in a transcriptional response. Two transcription factors, ADV1 and PP1, are essential for germling communication and fusion. PP1 is the evolutionarily conserved target of MAK2, while ADV1 is less conserved and less well studied. To identify the transcriptional targets of ADV1 and PP1, we did RNAseq on *Δadv1*, *Δpp1*, and Wildtype germlings in addition to DNA-Affinity Purification sequencing (DAPseq). DAPseq is a new high-throughput *in vitro* method for identifying transcription factor binding sites on native gDNA. In an effort to elucidate the MAPK-TF regulatory network, we constitutively expressed each transcription factor in each upstream MAPK deletion mutant. Our data show that MAK1 functions upstream of *adv1* (independently of *pp1*), while MAK2 functions upstream of both transcription factors and is required to activate (or de-repress) PP1. PP1 is necessary for transcription of *adv1*, and then ADV1 is the primary regulator of the genes required for communication, fusion, and post-fusion non-self-recognition. Both PP1 and ADV1 regulate transcription of several genes that are necessary for growth. Our experimental data indicate that PP1 and ADV1 likely

also regulate genes important for maintaining cell wall integrity, and we hypothesize that the catalytic activity of MAK2 is also necessary for maintaining cell wall integrity. Follow-up experiments will investigate the role of MAK2, ADV1, and PP1 in regulating the response to specific cell wall stresses. Collectively, our data demonstrate that the MAK1-ADV1 and MAK2-PP1 pathways form a tight regulatory network that maintains cell wall integrity and responds to cell-to-cell communication.

S36-6 MpkB MAP kinase is required for sexual development but not for mycotoxin production in *Aspergillus nidulans* and *Aspergillus flavus*

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Abstract: Mitogen-activated protein kinase (MAP kinase) pathways regulate growth, development and stress responses in most of eukaryotes. *Aspergillus nidulans* MpkB MAP kinase has been known to coordinate sexual development as well as secondary metabolism, including sterigmatocystin (ST). In this study, however, the results of the TLC analysis of wild type and *mpkB* deletion mutants (Δ*mpkB)* showed that the *mpkB* gene did not affect the ST production and ST related gene expression especially in *veA*$^{+}$ genotypes. In the *veA*$^{+}$ background, ST production of Δ*mpkB*, Δ*mkkB* and Δ*mpkB*Δ*mkkB* mutants were similar with wild type. Furthermore, MpkB constitutive activation or inactivation mutants also showed no significant effect on the ST production. Some genes required for ST production (*aflR, stcE* and *stcU*) were constitutively expressed in each mutant, but, interestingly, ST production of *mpkB* and *mkkB* mutants was remarkably delayed in the *veA1* background, suggesting that the ST production is affected primarily by the *veA* gene. Similarly, in *Aspergillus flavus*, MpkB ortholog *AflmpkB* mutant couldn't produce any sclerotia, but it produced aflatoxin B1 normally. Taken together, the *mpkB* gene alone does not affect the expression of genes involved in mycotoxin production such as ST in *A. nidulans* or aflatoxin B1 in *A. flavus*, indicating that the signaling of MpkB MAP kinase and mycotoxin production were governed by independent pathways.

Symposium Session 37:

Fungal Communities and the Functioning of Forest Ecosystems

P. Baldrian and B. Lindahl

S37-1 Functional diversity of forest microbiomes and their consequences

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Abstract: The forest mycobiome includes generalist as well as specialist fungi that interact with their plant hosts to regulate host physiology and ecosystem processes. To better understand the molecular biology of forest-microbial interactions, we are using integrated approaches (transcriptomics, metabolomics, proteomics) to investigate how forest trees interact with their symbiotic fungi, using soil-bioassays which examine belowground genetic interactions between trees with their symbiotic (generalists and specialist) microbiomes. The questions we ask include: (a) What environmental factors are responsible for shaping the composition and function of the forest microbiomes in different plant species and over time? (b) What kinds of molecular-signaling strategies are used by fungal generalists and specialists during interaction with their tree hosts? (c) How do these dynamic changes in turn affect plant development and ecosystem process? Metatranscriptomic studies employing RNA-seq reveal that: (1) Plant species can manipulate the assembly of their root specialist community (especially ectomycorrhizal fungal species) (2) Symbiotic fungal generalists (e.g. *Mortierella*) use similar strategies

to interact and confer beneficial effects to a broad variety of host species. Fungal specialists (e.g. *Suillus* and *Rhizopogon*) use different genetic mechanisms to interact with their specialized hosts (specific species of *Pinus*) and non-host (other *Pinus* species and *Populus*). Our studies also demonstrate how the underground mycelial highway used by fungal specialists (the so-called wood-wide web) supports communication and nutrient reallocation among different species of neighboring plants. Our metatranscriptomic studies reveal how symbiotic fungi use dual strategies to benefit both host and non-host plant species. Because root-associated microbiomes provide their host plants with limiting nutrients (nitrogen) and help to reallocate plant carbon to soil, these molecular-informed mechanistic studies also provide new insights for understanding microbial-plant-soil interactions which help to maximize those functions for forest ecosystem processes and sustainability.

S37-2 Dynamic fungal landscapes and their consequences

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Abstract: Symbioses between plants and fungi can fundamentally alter the structure of ecosystems, from their species diversity to rates of nutrient cycling. Yet, how differences in the prevalence of symbioses arise are unclear. If co-variation in plant and fungal distributions are primarily determined by abiotic factors then symbioses should exert little independent control over ecosystem structure. We examined biotic determinants of biogeochemical cycling, and microbial community structure and function, in a coastal landscape where historical patterns of vegetation transition are known, allowing us to eliminate abiotic determinism. We found that alternative stable states in fungal community structure, functional traits, and ecosystem processes emerged under different plant species. Greenhouse studies further demonstrated that plant selection of fungi is central to emergence of these alternative states and occurs independent of soil abiotic conditions. Moreover, we show that transition between states is highly dependent on the presence of a small set of ruderal symbionts that are rare in mature systems but act as keystone species. Because differences between these alternative states can be directly linked to plant-fungal symbioses, independent of initial conditions, our results suggesting that biotic feedbacks between symbiotic fungi and plants play a foundational role in the diversity and function of soils.

S37-3 Fungi and the dynamics of forest ecosystems

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Abstract: Globally, forests represent highly productive ecosystems that act as carbon sinks where soil organic matter is formed from residuals after biomass decomposition as well as from rhizodeposited carbon. Fungi are especially important in those forest habitats where decomposition of plant organic matter takes place: in the deadwood and litter and in soil, where they represent the necessary symbionts of trees and other plants, responsible for providing their hosts with nutrition. In temperate forests, fungi significantly contribute to the turnover of organic matter in litter and soil, reflecting the seasonality of tree activity across the warm and cold periods of the year by adopting their growth and utilization of C compounds. Transcription profiling indicates fast turnover of fungal biomass in summer together with decomposition of lignin and cellulose, probably primed by nutrient supply from trees to ectomycorrhizal and other fungi. In winter, utilization of reserve compounds including trehalose and glycogen represents an important source of carbon. Overall, fungal activity, especially the activity of ectomycorrhizal fungi that is dependent on tree photosynthesis decreases in winter and this season is characteristic by higher contribution of bacteria to the overall soil activity. Forest ecosystem development, ranging from

successional stand development to the short-term response to disturbances such as tree harvesting or insect outbreaks all represent processes that affect and are affected by fungi. Importantly, fungi are also important contributors to soil organic matter accumulation due to the large annual production of hyphae that serve as resources for other soil organisms and represent thus an important component in the forest food web. The bilateral dependence between trees as dominant primary producers and fungi as their symbionts and dominant decomposers appears to define the important role of these organisms and their interactions in the functioning of forest ecosystems.

S37-4 Sequencing the mycobiome of boreal forest soils

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Abstract: Boreal forest soils store significant amounts of organic carbon and nutrients, but drivers of decomposition and soil fertility are poorly understood, contributing uncertainty in global models of climatic feedbacks. We systematically sampled soil DNA from the entire latitudinal range of Swedish boreal forests and assessed fungal communities by sequencing ITS markers. Predictors of organic matter accumulation in the topsoil were evaluated by statistical modelling, combining fungal community data with climatic, edaphic and productivity parameters. Fungal community composition was identified as the principal determinant of organic matter accumulation, with a positive impact of an ectomycorrhizal *Piloderma* species but a negative impact of other ectomycorrhizal species within the genus *Cortinarius*. Stress tolerant ascomycetes were associated with larger organic stocks. Organic matter accumulation increased with declining pH and tree biomass, but these effects seemed to be indirect and mediated by their interplay with fungal communities. Our findings highlights the regulatory importance of soil fungi in boreal ecosystems. The results contribute correlative support for the idea that certain ectomycorrhizal fungi and stress tolerant ascomycetes that are poor decomposers drive organic matter accumulation, but that other ectomycorrhizal species (e.g. in the genus *Cortinarius*) are efficient decomposers that counteract organic matter accumulation. Intense forestry has large effects on fungal communities and indirect impacts on organic matter turn-over is to expect. Potentially, forestry may have a positive effect on below-ground carbon storage, but there is a major risk that long-term soil fertility is under threat.

S37-5 Globally distributed beetles drive fungal colonization of dead wood and may slow down, rather than facilitate, wood decomposition

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Abstract: Wood comprises the vast majority of standing biomass in forest ecosystems, but decomposition of its complex structural elements is mostly limited to specialized decay fungi. Thousands of species of wood-boring bark and ambrosia beetles in the weevil subfamilies Scolytinae and Platypodinae seek stress chemicals released by dying trees. They often colonize the wood before the tree has died. The beetles introduce commensal and mutualistic fungal symbionts whose extensive hyphal networks absorb, concentrate, and deliver the diffuse labile nutrients from the wood to the next

generation of beetles. In turn, the beetles are effective vectors of fungal spores to fresh woody resources. For over a century, these relationships have been textbook examples of drivers of decay and the turnover of forest biomass. But does this paradigm really make sense? Beetle symbionts are typically saprotrophic Ascomycota that cannot degrade the complex structural components of wood, but can compete with other fungi for more labile resources. We examined a new hypothesis; that bark and ambrosia beetles actually slow the decay process by introducing competitors that exclude or hamper degradation by decay fungi. This hypothesis was supported by DNA and RNA-based community sequencing and culture-based data. A field survey and experiments using beetle exclosure cages revealed significant effects of bark beetles on fungal community assembly and decomposition of mature loblolly pine tree trunks. The beetles vectored a consortium comprised of many diverse Ascomycota taxa. Infestation by bark and ambrosia beetles tripled fungal diversity. Infested pines were dominated by non-decaying Ascomycota species, and there was a significant negative correlation between the abundance of beetle galleries and the loss of woody biomass. We then conducted competition assays on both agar-based and wood-based media to characterize competitive interactions between beetle-associated fungi and a common wood decay fungus, and their effects on decomposition. Our results suggest that the role of bark and ambrosia beetles in forest ecosystems is more complex than previously envisioned. By introducing diverse consortia of non-decay fungi which compete with decay fungi, the beetles may suppress the decomposition of wood. Bark and ambrosia beetles are abundant and ubiquitous in forests globally and our results provide a better understanding of their important role in global carbon and nutrient economies.

S37-6 Biogeochemical and ecological drivers of fungal biogenic weathering in Patagonian temperate rainforests

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Abstract: Biogenic weathering is the process by which rocks are directly or indirectly degraded by biota. In old forests, biogenic weathering is performed mainly by mycorrhizal hyphae, as they have energy from photosynthesis and can access weatherable surfaces. An inverse relationship between the edaphic bioavailability of nutrients and the degree of biogenic weathering is expected, since the latter is energetically costly, as it involves processes of chelation, complexolysis, redoxolysis, metal precipitation and thigmotropism. These processes involve the releasing of essential nutrients in plant nutrition (P, Ca, K, Mg, Na). North-Patagonian temperate rainforests experience extreme environmental conditions, as high precipitation, soil-nutrient limitation and regular natural disturbances. These forests are located within two mountain systems (Andes and Coast mountains), whit different geological history, soils and biogeochemistry. The main difference, is that the soils of Coast mountains are more physically and chemically weathered than most of the Andes. These forests have three types of vegetation with two mycorrhizal dominance types: *Nothofagus* spp. forest (dominated by ectomycorrhizal trees, EM), Valdivian and native coniferous forests (dominated by arbuscular mycorrhizal trees, AM). There is little information about the biogeochemical and ecological factors influencing biogenic weathering in these forests. Specifically, we wanted to answer three questions, which are non-specific for Patagonian forests: what is the relationship between physicochemical weathering (i.e. stand age) and biogenic weathering?, what is the relationship between the forest nutrient economy (i.e. nutrient cycles, soil nutrient availability, plant nutrients) and biogenic weathering?, and finally, which mycorrhizal types are more efficient at

biogenic weathering? To answer these questions, we selected 13 pristine temperate rainforests in five national parks at the south of Chile -both EM and AM forests in both Andes and Coast mountains, where we installed test minerals *in situ* (muscovite, biotite), which after a year of exposure were analyzed through confocal laser microscopy (percentage of mineral colonization). We also measured the ecosystem nutrient economy: soil chemical analysis, roots and leaves nutrients, and with a resin system (Self Integrating Accumulator) we measured the forests' nutrient input by precipitation and its leaching. Using Illumina sequencing of the fungal ITS2 region we determined the whole soil fungal communities. We founded that biogenic weathering was most significant in the middle age stands, this is, when ecosystems were not too young (physical and chemical weathering takes places in these ecosystems) and not too old (nothing left to be weathered). As expected, we found an inverse relationship between soil nutrient availability and the degree of biogenic weathering, but this and the former results were dependent on: the nutrient inputs, i.e. some nutrients were sufficiently supplied by precipitation (which was also reflected on plant nutrients), and the dominant mycorrhizal type of the forest, as EM-dominated forests always were more efficient at biogenic weathering than AM-dominated forests. In conclusion, biogenic weathering, a process mainly done by mycorrhizal fungi, is an important form of nutrient input to the ecosystems, and as such, should be taken into account in future nutrient modeling. Acknowledgements: CONICYT, Austral University of Chile, EarthShape Project (DFG).

Symposium Session 38:

Fungal Extracellular Vesicles

J.D. Nosanchuk

S38-1 What are fungal extracellular vesicles and do they impact pathobiology?

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Abstract: Fungal extracellular vesicles (EVs) are lipid-bilayered structures that are released by diverse species. EVs are generated by more than one mechanism and vesicular transport enables the export of large molecules across the complex cell wall of fungi. EVs contain proteins, lipids, and polysaccharides, many of which are linked to virulence. In this session, we will first review current knowledge on fungal EVs. We will then describe the use *Histoplasma capsulatum* as a model to study EVs and show how host immune molecules can modify fungal responses. Our data demonstrates that cell wall-binding antibody can directly modify protein loading in vesicles as well as fungal metabolism. Moreover, this process is differentially and dynamically regulated by antibody in a concentration dependent manner. EV biology is clearly associated with the homeostatic maintenance of the fungal cell and can be modified in response to external factors, including host-pathogen interactions.

S38-2 In-depth multi-omics analysis reveal the production of platelet-activating factor by the pathogenic fungus *Histoplasma capsulatum*

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Abstract: Lipids are major components of biological membrane, energy storage molecules and cell signaling transducers. Therefore, not surprisingly, lipids play major function in host-pathogen interactions and are frequently targeted for drug development. Seeking to better understand the function of lipids in fungal pathogenesis and identify potential drug targets, we performed a comprehensive analysis of the lipid metabolic pathway of *Histoplasma capsulatum* yeasts and extracellular vesicles. In-depth proteomic and lipidomic analyses of *H. capsulatum* yeasts were performed and integrated into a metabolic map, comprising of 5 major lipid metabolic pathways and 19 lipid classes, being 371 lipid species detected in yeasts and 104 in extracellular vesicles. Of notice, the analysis showed that unlike *Saccharomyces cerevisiae*, *H. capsulatum* is unable to produce mannosylinositolphosphoceramides, but synthesizes glycosylceramides. The analysis also showed that *H. capsulatum* produces analogs of platelet-activating factor (PAF), a potent regulator of the human immune response. The structural information of the *H. capsulatum* PAF was further validated by tandem mass spectrometry, ion mobility and liquid chromatographic analyses. We also tested if the *H. capsulatum* PAF could stimulate the production of cytokines by treating macrophages with yeast lipids fractionated by liquid chromatography. The lipid fraction potently activated the production of the cytokines interleukin-10 and tumor necrosis factor alpha, which was abolished by the PAF receptor antagonist WEB 2086. Overall, our approach led to the identification of a biologically active lipid in *H. capsulatum*.

S38-3 Galectin-3 impacts *Cryptococcus neoformans* infection through direct antifungal effects

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Abstract: *Cryptococcus neoformans* is an encapsulated fungal pathogen that causes cryptococcosis, which is a major opportunistic infection in immunosuppressed individuals. Mammalian β-galactoside-binding protein Galectin-3 (Gal-3) modulates the host innate and adaptive immunity, and plays significant roles during microbial infections including some fungal diseases. Here we show that this protein plays a role also in *C. neoformans* infection. We find augmented Gal-3 serum levels in human and experimental infections, as well as in spleen, lung, and brain tissues of infected mice. Gal-3-deficient mice are more susceptible to cryptococcosis than WT animals, as demonstrated by the higher fungal burden and lower animal survival. *In vitro* experiments show that Gal-3 inhibits fungal growth and exerts a direct lytic effect on *C. neoformans* extracellular vesicles (EVs). Our results indicate a direct role for Gal-3 in antifungal immunity whereby this molecule affects the outcome of *C. neoformans* infection by inhibiting fungal growth and reducing EV stability, which in turn could benefit the host.

S38-4 Plasma membrane dynamics in the interplay between exocytosis and endocytosis in fungal hyphae.

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Abstract: The apical growth of fungal hyphae is an ideal place to study the relationship between the two primary processes that determine plasma membrane dynamics, its generation by exocytosis and its recycling by endocytosis. Both processes can be neatly imaged by confocal microscopy and fluorescent markers and are located in close proximity, exocytosis in the apex itself and endocytosis in the immediate subapical region. While the role of exocytosis is self-evident, the need for endocytosis is not. In fact, the very existence of endocytosis in mycelial fungi was long questioned but finally proven to exist convincingly. Whereas the amount of exocytosis can be calculated geometrically from values of hyphal diameter and vesicle cycle, estimating endocytosis poses a greater challenge. A mathematical approach led to the prediction that the amount of exocytosis required for plasma membrane growth alone was insufficient to account for cell wall growth and extracellular enzyme secretion, resulting in an excess of plasma membrane formation. To measure this excess, we devised a method to estimate endocytosis experimentally by photobleaching the subapical endocytic collar of hyphae of *Neurospora crassa* tagged with Lifeact-GFP. Accordingly, we determined that about 4% of the plasma membrane generated by exocytosis was endocytosed. Seemingly, exocytosis and endocytosis operate in tandem. By removing excess plasma membrane, endocytosis allows exocytosis to have the intensity needed to sustain the rapid growth rate and abundant enzyme secretion typical of fungal hyphae.

S38-5 Insights from *Candida auris* and *Candida albicans* extracellular vesicles

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Abstract: Extracellular vesicles (EV) are lipid-bilayered organelles released by many types of cells, including bacteria and mammalian cells and as recently described, by fungi. Pathogenic fungi take advantage of EV release, to secrete virulence factors associated with pathogenesis and immune evasion, thus these organelles can be a target of drug development. *Candida auris* is an emerging fungal pathogen described in 2009 after being isolated from a patient in Japan. Since its description, some outbreaks have been reported across the globe, and recently in US. *C. auris* bloodstream infections led to mortality in 30 to 60% of the cases in hospitals, and a common feature among the strains isolated so far, is a remarkable resistance against at least one class of antifungals, and in some cases to the three of them. Our aim was to evaluate if *Candida auris* releases EV and if these structures could have a role in the pathogenesis. In order to evaluate these points, we first addressed the EV release by *C. auris,* and then these EV were submitted to lipidomic and proteomic analyses to identify novel targets and mechanisms of disease. Our findings to date demonstrate significant differences in the characteristics and content of EVs from *C. auris* and other pathogens, including *C. albicans*. *C. auris* EV secretion may be associated with the pathogenesis of this emerging fungus.

S38-6 Dissecting the melanin unit in *Cryptococcus neoformans*

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Abstract: In the presence of catecholamines, *Cryptococcus neoformans* forms a melanin coat around the cell body that protects it against human immune defenses. Beyond its contribution to infection, melanin can protect *Cryptococcus* against lethal doses of ionizing radiation (i.e. X-ray, gamma, and particulate) and mediate a radiation energy transduction process that supports fungal growth. The melanin coat is formed by a network of granules deposited and organized as concentric layers surrounding the inner cell wall. The synthesis of cryptococcal melanin is believed to occur intracellularly within spherical vesicular bodies or fungal 'melanosomes'. One hypothesis is that 'melanosome' represents the fundamental unit of the melanin coat. In this study, we have isolated and characterized melanin granules actively secreted to the culture media during melanization. Preliminary analyses indicate that secreted melanin granules share biochemical and biophysical properties with cell wall-associated melanin. Secreted melanin granules are highly monodispersed nanoparticles with 30-60 nm in hydrodynamic diameter, interesting colloidal properties, and fractal appearance. Further studies aim to dissect the structure and composition of the melanin granules to understand their biogenesis, deposition at the cell wall and molecular mechanisms of melanin-mediated energy transduction. The secretion of melanin granules to the extracellular environment also implies a potential role for these particles as immunomodulators during cryptococcal infection which may also apply to other melanotic fungal pathogens.

Symposium Session 39:

Ethnomycology: Scientists and Shaman on Historic and Current Uses of Fungi

A. Blanchette and N. K. Ishikawa

S39-1 The historic and current ethnomycology of Egypt and Middle East countries

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Abstract: Egypt is considered the cradle of mycology. Ancient Egyptians documented the use of fungi on walls and pillars of temples, within hieroglyphic texts, ear studs and medical prescriptions since 5619 B.C. Ancient Egyptians believed that some mushrooms were plants of immortality and called them "a gift from the God Osiris" and symbolized as Was, Djed pillar of Osiris, and ankh (crux ansata). Egyptian pharaohs proclaimed mushrooms to be food reserved only for royalty; common people were not even allowed to touch them. The Pharaohs of ancient Egypt believed they had magical powers. Egyptian crowns (white and triple) were inspired from the primordia of *Psilocybe cubensis*. The most ancient historical use of truffles probably originated prehistorically in the mideastern and North African cradles of civilization. Species of desert truffles (*Terfezia*, *Tirmania* and *Phaeangium*) probably served to the Pharaohs. Better descriptions of the kind of desert truffles that the pharaohs of Egypt may have consumed, along with an ancient version of traditional truffle preparations still popular in North Africa and the Middle East, can be found in the Bible. In the seventh century Prophet Muhammad peace, be upon him (صلى الله عليه وسلم) said a hadith to his followers "Truffles are a part of manna and its juice is healing for the eyes". This study consists of a survey focusing on the knowledge, use and ethnomycological practices of mushrooms and desert truffles among the native people of the Middle Eastern countries. The presentation will highlight their application in traditional medicine in this part of the world. This work

also explores the biology and ecology of truffles in the Middle East, their importance in fragile desert ecosystems, assess their conservation status and effects of various cultivation practices on sustaining truffle populations. General management principles and considerations to sustain this valuable fungal resource will be discussed.

S39-2 Ethnomycological documentation of the historical region of Mazovia, Poland

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Abstract: The research on traditional knowledge of wild mushroom uses in the Mazovia region is the first research that undertakes complex ethnomycological analysis of entire region laying in one of European countries. The field research based on cooperation with local communities enables the acquisition of new and direct information on the present list of collected mushroom species, the level of local knowledge about Central Poland's mycobiota, species' ecology and their actual protection. The research bases on interviews conducted in evenly dispersed locations defined by previously prepared village grid. The high sample size, over 700 interviews with residents of 38 Mazovian villages, will enable to authenticate the acquired information and statistically eliminate borderline and improbable results. The main objectives of research are to create list of mushrooms collected by people living in this region, find rare and protected mushroom species used by local communities and to acquire information about the purpose, methods and extent of their use. In the context of forestry, the study enables the acquisition of new information about the exploitation of mushrooms as an important part of non-wood forest products. Moreover, assignment of local names to proper taxonomic nomenclature will help in further analysis of local reports related to species composition of mycobiota. So far, after near 700 interviews, more than 60 different taxa were recorded as used for consumption purposes among people living in the Mazovia region.

S39-3 The aesthetics of mushrooming in the Tundra and Taiga: Perspectives from Alaska and the Russian Far East

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Abstract: This presentation draws on the ethnographic material gathered in the course of twenty years of the authors' research on the harvest and use of fungi in northern Alaska and Chukotka, Russian Far East. We share insight from the Yupik maritime communities whose ancestral beliefs regarded mushrooms as ears of the tundra spirits, Chukchi reindeer herders for whom mushrooms are reindeer food or "reindeer drugs," recent Russian and Ukrainian immigrants who are adapting the longstanding mushrooming traditions of their homelands to the landscapes and seasons of the Arctic and Subarctic environments, and members of the Alaskan settler populations who in their vast majority are novices to the experience of the "quiet hunt." We discuss the roles of the fungi currently regarded as the core subsistence species in facilitating the social and environmental adaptation for each of these groups in the time of vast and rapid change. Among the featured examples are various uses of multiple varieties of fleshy mushrooms and chaga (sclerotia of *Inonotus obliquus*). Through a framework we have developed by integrating a set of analytical approaches from the fields of cultural anthropology and contemporary art, we examine the aesthetic relationships that the Alaskan and Chukotkan communities hold integral to the contemporary human-fungi interactions. We also reflect on the impact of our efforts

as educators, which include teaching a university course in ethnomycology conducting extensive outreach in communities and among diverse groups of foragers and hobbyists.

S39-4 Historic uses of polypores as symbols of supernatural power

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Abstract: Forest fungi have been used for traditional medicine and other cultural purposes for thousands of years. Documentation of how various fungi have been historically is often difficult to obtain but natural history museum collections have proven to be a rich source of information. Many museums have fruiting bodies of fungi used by the Indigenous People of North America in the 19th and early 20th centuries along with collection notes indicating their use. Objects made from these fungi are also accessioned providing important ethnological information. The Indigenous People of the North American used many different types of polypores. Before knowledge that illness had biological causes, fungi were being used to treat the sick. In addition to their use as medicine, many tribes used them in an array of art forms that were made to represented symbols of supernatural power. These objects were an important part of the Shaman's paraphernalia that was used during ceremonies and rituals to influence the beliefs of the community. The fruiting bodies of *Laricifomes officinalis* were used by the Indigenous People of the northwest Pacific coast and carved into spirit figures or made into masks. Since sickness was considered to be brought about by supernatural forces, the shaman applied a spiritual remedy using polypores that they considered to have unworldly powers. We now know that the fungus selected for their use has medicinal value. The Plains Indians of North America used a different fungus, *Haploporus odorus*, as a symbol of spiritual power. The fruiting bodies of this fungus, with an exceedingly fragrant anise-like aroma, was used by the Blackfoot, Blood, Cree and other northern plains tribes for protection against illness. It was also a component of medicine bundles and was used to ornament scared robes, necklaces and other cultural properties. Modern day Native American healers continue to use this fungus in ceremonies to purify the air and to call helpful spirits to eliminate harmful influences. In other regions of the world, various polypores were similarly used as symbols of spiritual power. Masks made from the fruiting bodies of *Ganoderma* species were used in the Middle Hills region of the Nepal Himalayas in rituals to cure the sick and were displayed in the rafters of village huts to ward off evil spirits, sickness and bad luck. An extraordinary necklace made from an unusual *Ganoderma* with purported supernatural attributes was used in Dutch New Guinea (West Papua Province of Indonesia). In China, polypores such as *Ganoderma lucidum sensu lato*, have had a long history of use in traditional medicine and were assumed to have mystical properties. Examples of their historic uses by early Emperors will be discussed as well as their current use as symbols of immortality, good health and good luck.

S39-5 Yanomami ethnomycology: Knowledge valorization and income alternative for communities of Awaris region - Roraima, Brazil

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Abstract: The Yanomami are a largely isolated human population of about 40,000 people, living in 550 communities, in the Amazonian rainforests of Brazil and Venezuela. On the Brazilian side, the Yanomami indigenous territory is 9,664,975 hectares of forest which was demarcated in 1992 and has been recognized for its significance in protecting the biodiversity of the Amazon. The Yanomami people speak

five languages and the knowledge concerning the consumption of mushrooms has been passed on orally from generation to generation since pre-Columbian times. The scientific world first learned about the ethnomycology of the Yanomami through some articles published in English in the 1960s and 70s by the Brazilian Oswaldo Fidalgo and the Britain Ghilllean Tolmie Prance, respectively. In 2016, the Awaris communities published their own book, in Sanöma and Portuguese, including about 15 species of mushrooms that they have collected and consumed. This book is just one part of a project that promotes and values of indigenous knowledge and the alternative production chains. The project, launched by the Yanomami Association (HAY) and the Socioenvironmental Institute (ISA) in Brazil, has created an unprecedented production chain for native Brazilian mushrooms which begins with the collection of mushrooms in the Amazonian forests. The mushrooms are subsequently dehydrated in the sun and/or smoked, packaged and marketed; until they finally arrive at renowned restaurants in the state capitals of São Paulo and Manaus. Thus, the mushrooms, that for centuries have been an important part of the Yanomami diet, are now benefitting forest communities as a source of income for the purchase of needed items such as machetes, knives, pots, and other household items as well as other essential items for gardening, building, fishing, and hunting, and hence, promoting physical and cultural development in their places of origin. A video of the Yanomami Shaman David Kopenawa will also be shown providing a message on how the traditional knowledge about mushrooms can be used as an alternative source of income that helps to protect and preserve the Amazon rainforest natural resources.

S39-6 Video of Shaman David Kopenava, Brazil

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Symposium Session 40:

Species Limits in the Age of Genomics

J. Taylor and W. Meyer

S40-1 Fungal species limits: A global tree health perspective

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Abstract: Natural forests and plantations globally are increasingly threatened by diseases caused by fungal tree pathogens. The driving forces behind these diseases are complex. They emerge not only from accidental introductions of pathogens into new areas but also via host shifts and hybrisation events. Our ability to identify species, not only in a practical manner but also one that ensures effective and responsible quarantine is challenged. In the case of tree health, there are growing numbers of examples of pathogens where species names are used that poorly or at least ineffectively reflect the genetic nature of the pathogen. The "age of genomics" makes it possible to identify species to include a comprehensive knowledge of their genetic nature. This will improve our understanding of the global pathways of movement of tree pathogens and the quality of quarantine measures. But it is also realistic to understand that the depth of knowledge regarding species limits will be variable in different countries of the world and it will depend deeply on available resources to study tree pathogens.

S40-2 A re-evaluation of species limits and distribution of the cupulate, blackish *Helvella* spp. in the Nordic countries

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Abstract: This study represents an in-depth systematic study of a species complex of small and medium-sized cupulate-stipitate, blackish *Helvella* species. These species were formerly recognized as a single (but variable) morphospecies, i.e. *Helvella corium*. Recently this complex has been shown to represent a group of phylogenetic species, nested in (at least) two divergent evolutionary lineages of a broadly defined *Helvella* genus. Consequently, historically, the herbarium specimens of this species complex have for decades been stored and annotated as *H. corium* in the University herbaria. In the present project we identified and re-assessed the occurrence and distribution of the true phylogenetic species of this morphospecies complex in the Nordic countries. We examined and barcoded all material stored under the name *Helvella corium* in the Nordic University herbaria (O, TRH, BG, TROM, C, S, UPS, UME, GB). This was supplemented with fresh specimens from primarily under-studied, alpine regions of Norway collected in 2015-2017. DNA was extracted from ca. 500 specimens of which 436 specimens were successfully barcoded (HSP and/or RPB2). The oldest, successfully barcoded specimen was dating back to 1888. Species limits and evolutionary relationships of the complex were re-assessed, using an extended set of genetic markers (LSU, HSP, RPB2, EF-1α and 5.8S for 41 specimens). We performed Maximum likelihood and Bayesian inference analyses, including molecular species delimitation with STACEY (Beast2). Morphological characters of the individual species were subsequently re-evaluated across the identified lineages, followed by a character state analysis. Altogether, seven phylogenetic species were recognized: *H. alpestris, H. macrosperma, H. nannfeldtii, H. alpicola, H. alpina*, *H. pseudoalpina* sp. nov, and *H. corium*. The phylogeny received high bootstrap support for a monophyletic group consisting of *H. nannfeldtii, H. alpestris and H. macrosperma*, with *H. alpicola* as its sister species. *H. alpina, H. pseudoalpina* and *H. corium* represented a second evolutionary lineage separate from the other lineage by long branch length and high bootstrap support. The barcoded specimens have been used to map the distribution of the 7 phylospecies in the Nordic countries. Of the 436 barcoded specimens, 233 represented *H. corium*, 76 *H. nannfeldtii*, 39 *H. alpestris*, 23 *H. alpina*, 14 *H. alpicola*, 8 *H. macrosperma*, and 4 *H. pseudoalpina*. An additional 39 misidentified *Helvella corium* samples belonged to *Helvella* species outside the two lineages in question. *H. corium* is the only species that occur at all levels along a gradient from the temperate to the boreal to the arctic-alpine biome. The other six species seem restricted to the arctic-alpine biome, where they occupy different but overlapping habitats. The most common alpine species in the Nordic country seems to be *H. nannfeldtii*, which is assumed to have a circumpolar distribution.

S40-3 Testing an operational approach to species delimitation in *Hydnum* (Cantharellales, Basidiomycota)

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Abstract: *Hydnum* is a genus of edible, commercially harvested ectomycorrhizal fungi with a global distribution. We constructed a phylogeny with ITS nucleotide sequences from North America, Europe, Asia and Australasia that reveals 64 putative species worldwide. A three-gene phylogeny constructed with ITS, TEF1 and RPB2 nucleotide sequences strongly supports relationships between major clades in the genus. Recently described species of *Hydnum* have been circumscribed on the basis of ITS sequence dissimilarity and morphological variation, this often microscopic in detail. However, several undescribed clades of *Hydnum* are morphologically cryptic with varying amounts of ITS sequence variation. Here, we

test an operational threshold-based species delimitation approach in *Hydnum* in an evolutionary framework using a Bayesian general mixed-Yule coalescent model. In addition, we explore integrating ecological niche modeling into species delimitation methods.

S40-4 What clinical mycologists need for species recognition?

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Abstract: Fungal nomenclature is characterised by name changes, which are due to the fact that fungi where able to have multiple names, describing different asexual and sexual morphological stages, Article 59 of the Code of Botanical Nomenclature, as those different stages propagate independently and thus their shared identify is not always obvious. However, based on the molecular identity of the asexual and sexual stage of a fungus, the dual nomenclature was recently abolished. In addition, the increase in knowledge at the morphological, biochemical and genetic level, the increasing accumulation of strains as a result of systematic field studies, and the application of new, more discriminatory technologies, e.g. single/multiple gene/whole genome sequencing and MALDI-TOF, recognises ever more species-specific characteristics leading to the discovery of molecular sibling species. This has potentially a profound effect on clinical mycology, as it directly impacts on established fungal and disease names. Ideally, in a clinical setting, a fungal name should reflect, 1) a specific disease association; 2) inform about antifungal resistance to guide treatment decisions, 3) confirms with clear cut species-specific characteristics (morphological, molecular, biochemical) for a fast an accurate identification of a disease agent, and 4) reflect the true phylogenetic relationships between species. Molecular techniques are now capable to detect minute differences, in the absence of clear species delimitation data, cut off values and an overall lack of intra- and interspecies variation data. As such, science has to find a compromise between scientific progress and clinical confusion. In many cases the increased information is very helpful, e.g. in the case of antifungal resistant species, e.g. *C. krusei = Pichia kudriavzeii*. On the other hand, the detection of genetic diversity does progress faster than the finding of clinical relevant characteristics, e.g. *Fusarium solani* or *C. neoformans/ C. gattii*, which are now large species complexes, consisting of multiple, closely related and morphologically poorly distinguishable, "cryptic" species with similar antifungal susceptibility and currently unfinished studies to characterise the discovered genetic differences. Here it is necessary to provide nomenclature stability and simultaneously recognise ongoing speciation processes, by using the term “species complexes.”

S40-5 Global phylogenomics and fungal species

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Abstract: Comparative phylogenomics can now provide empirical understanding of the relationships between lineages in fungal taxa. Prior to the advent of high-throughput whole genome sequencing, speciation had been typically accomplished through a variety of morphotyping, phenotyping and genotyping techniques. Given that such analysis is done on isolates gathered from clinical, veterinary or environmental convenience sampling, previous methods have been limited in their abilities to understand population structure or establish true genetic relatedness. Global, phenotypic and ecologic diversity are required to build a full species context. Phylogenomic examinations using next generation sequencing and bioinformatic tools are allowing for empirical high-resolution analysis of current and

historical relationships among populations of fungi of interest. Here we analyze the genomes of representatives of the Coccidioides and Cryptococcus species complexes and model their evolutionary relationships. These analyses clearly identify the previously established subtypes and species and shed light on questions regarding a number of lineages within these species that may also warrant species designations. What is not clear is the genomic differentiation limits that actually distinguish subtypes and species. And while comprehensive taxonomic schemes with clear variation thresholds can now be established, diagnostic and treatment confusion may be inevitable in the move to rename identifiable taxonomic groups.

S40-6 Species recognition: Can one type fit all applications

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Abstract: Under the Evolutionary Species Concept, fungal species have been recognized by phenotype, mating tests and phylogenetics. Using conidial *Neurospora* as an example, phylogeny recognizes an order of magnitude more species than morphology and twice as many as mating tests. Now, genomic resequencing is revealing multiple population of interbreeding individuals within phylogenetic species. These populations lack structure and constitute the smallest taxonomic unit above the individual. Within species, there are no intrinsic barriers to mating among these populations, whose divergence is estimated in 100,000s of years (doi.org/10.1073/pnas.1014971108) rather than the millions of years required for the evolution of intrinsic mating barriers (doi.org/10.1371/journal.pgen.1002204). Therefore, between the biological reality of populations and the biological reality of evolved, intrinsic mating barriers, lie 3 million years where species recognition relies on phylogeny. Phylogenetic species recognition is an approach open to interpretation and adding quantification might make is application more uniform. The aforementioned population genomics offers a means of adding quantification to population-species recognition by simply adding a measure of genomic divergence to the taxon name. An example of such a measure is Nei and Li's π (http://www.pnas.org/content/76/10/5269). Examples of this widely used and easily calculated measure of genetic variation will be presented (doi.org/10.1111/mec.13417, doi.org/10.1111/mec.13132). It may come as a shock that the now dominant method of species recognition shares little with the above-mentioned approaches, that is, species recognition by operational taxonomic units (OTUs) recovered from mycobiomes (doi:10.1038/nrmicro2963). Fungal OTU recognition currently relies on PCR amplification from environmental DNA of the rDNA repeat, which is necessarily inferior to the methods mentioned above. For example, even the most discriminating rDNA region, the internal transcribed spacer (ITS), lumps all *Neurospora* species, conidial and aconidial, into one OTU. Hope for a solution to the OTU problem comes from the recent publication of a fungal genome assembled from metagenomic data (doi:10.1101/gr.228429.117). This approach could provide the fungal genomes needed to recognize populations and species as described above. This approach is already being attempted with bacteria (doi:10.1038/ismej.2017.113), and may be easier to apply to fungi, where sexual reproduction produces discrete populations. If the history of fungal phylogenetic species recognition is a guide, technical obstacles will be easier to overcome than political obstacles, where increased transparency and access will be required to promote acceptance.

Symposium Session 41:

Early Fungi That Changed the World: Phylogenomic and Fossil Evidence

M. Berbee and C. Strullu-Derrien

S41-1 Fossil evidence of early Fungi and their role in the environment

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Abstract: Fungi have been an important part of life on land for over 400 million years, playing crucial roles as decomposers as well as forming mutualistic or parasitic relationships with plants and other organisms. However direct fossil evidence of these associations is not visible before 407Ma because of the exceptional geological conditions required to preserve cellular and subcellular detail (*e.g.* rapid permineralization). Such conditions first occur in the 407 million-year-old Rhynie chert (Scotland, UK), which represents a privileged window into the paleontological past. Our objective is to document the early fungal diversity at this site and to understand the nature of the interactions between the fungi and other organisms. We are working principally with historical collections of thin sections that were made during the early part of the 20th century to document fossil plants. Now these sections have proven to be one of the best sources of material on early fungi. We first examined the fossils with standard light microscopy but have recently found that a combination of tools can be very effective for documenting early fungi and fungal associations. We use light microscopy with z-stacking montage, confocal laser scanning microscopy (CLSM) and digital 3D reconstructions obtained from the CLSM data with iso-surfaces digitally rendered using SPIERS and animations created in Blender™. This investigative approach allows us to characterize structures with a resolution of <1µm and to compare resulting images with relevant living groups and appropriate life history stages. Here we present an overview of our current knowledge regarding early steps taken by fungi in conquering the land. Evidence from the Rhynie chert shows that fungi were already diversified. Symbiotic associations (arbuscular mycorrhizae) attributable to Glomeromycotina have long been known and have been described in both stages of the life cycle of a Rhynie chert plant. Other plants were colonized by Glomeromycotina but did not show all the characteristic features of symbiosis. Zoosporic fungi were diverse with Chytridiomycota occurring in organic-rich sediments in wetter parts of the landscape while Blastocladiomycota were mostly associated with plants or plant debris. We recently demonstrated the occurrence of multiple colonizations of a single plant by different types of mycorrhizal fungi (Glomeromycotina and Mucoromycotina) and described fossil Blastocladiomycota, one of which is the earliest known fungal clade to develop hyphae, which likely served as a saprotrophic adaptation to patchy resource availability. A fossil Chytridiomycota has also been found showing that zoosporic fungi were likely important to the mobilisation of nutrients in early aquatic foodwebs. One of the driving forces behind the diversity of the early fungi could be the environments encountered. In the Rhynie chert, these ranged from terrestrial to fully freshwater and saline. This early fossil record is beginning to reveal fungal diversity and the important roles that fungi were playing in the earliest land communities. Interest in fossils is stimulated by our growing understanding of the diversity of modern early-diverging fungi, their life history stages and their roles in today's ecosystems.

S41-2 Evolution of diverse plant penetration strategies in pathogenic fungi.

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Abstract: Fungal spores are responsible for initiation and propagation of a majority of biotic plant diseases. Although spore germination is the first step in most fungal diseases, the genetics of spore germination has never been comparatively explored across multiple fungal lineages. Here we use comparative transcriptomics of spore germination among six fungi to determine how expression of orthologous genes has changed during evolution. and to predict genes whose knockouts will exhibit phenotypic differences in the spore germination and host penetration processes. We have chosen fungi which represent different approaches to plant penetration, including penetration using melanized appressoria, penetration through natural openings and direct penetration without melanization. To provide a basis for comparison among species and to identify infection-specific expression patterns, we compare transcriptional profiles during germination on a single defined medium as well as differences during germination on hosts. We used estimations of ancestral gene expression for orthologous genes common among all species to identify genes that undergo transcriptional shifts during the spore germination process, as well as those that are unique to infective germination, and those that are unique to specific fungi. Functional assays of a subset of genes exhibiting species-specific and infection-type specific upregulation were performed to determine the roles of these genes in conidial germination of these fungi. These experiments contribute to our understanding of how shifts in gene expression drive the evolution of conidial germination in a wide range of fungi.

S41-3 Reassembling the ancient molecular toolkit for cellular morphogenesis in the Fungi

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Abstract: Advancements in next-generation sequencing technologies and community sequencing initiatives, pairing mycologists with sequencing institutions such as the Joint Genome Institute, have gifted us with an unprecedented amount of sequence data from species all over the fungal tree of life and the rest of the eukaryotic domain. We now have an opportunity to view these data through the lens of evolution, evaluating how fungi have changed since their departure from the common ancestor that they share with animals. Investigations of morphogenetic protein function and localization in model yeast and hyphal fungi have provided a robust foundation for understanding the mechanisms of fungal morphogenesis. While parallel studies of protein function and localization cannot yet be pursued in some of the early diverging lineages including Chytridiomycota, Blastocladiomycota, and Zoopagomycota due to the lack of a tractable genetic transformation systems in target organisms, the first step is to identify and compare genes encoding proteins required for morphogenesis in these taxa. Here, we took a comparative approach, reviewing literature on septins, myosins, actin and actin binding proteins in Dikarya and other early diverging lineages. We conducted phylogenomic surveys revealing that seven families of actin binding proteins examined existed prior to the radiation of fungal phyla. Contrary to conventional notions of complexity in the fungi, most of the examined actin binding protein families were just as diverse in zoosporic Chytridiomycota as in Dikarya that have been examined thus far. While three of the actin binding genes that I surveyed here were maintained as single copy genes, the other four gene families underwent lineage specific duplications, which may have contributed to the evolution of morphological diversity in Chytridiomycota. In combination, phylogenetic analyses,

molecular genetic analysis, and microscopy are beginning to tear away the curtains of time that mask the ever-changing molecular machinery that gave rise to hyphae and multicellularity in modern fungi.

S41-4 The nature of early terrestrial communities

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Abstract: Life on land during the early part of the Palaeozoic Era (396-541Myrs) has been likened to modern cryptogamic ground covers (CGCs), which today are photoautotrophic communities that grow on the surfaces of soils, rocks and plants. They are made up of variable proportions of bryophytes, lichens, algae, fungi and cyanobacteria, and they host various groups of animals, predominantly arthropods. Capable of tolerating widely varying environmental regimes encompassing extremes of aridity, temperature and UV flux, CGCs are widespread, and today they are thought to be responsible for an estimated 7% of net primary productivity and almost 50% of nitrogen fixation globally. Our objective is to investigate the nature of CGCs and their impacts on the environment and soil formation during what is arguably the acme of their development. This followed the origins of land plants, recently estimated to lie in an interval between mid-Cambrian (~515.2 Ma) and early Ordovician (~473.5 Ma), and preceded the evolution of forest ecosystems during the mid-Devonian (~385Ma). Early plant-bearing deposits are mostly allochthonous, so we are focusing initially on the 407 million-year-old Rhynie cherts (Scotland, UK) because the biota is fossilised more or less *in situ* and the preservation of the organisms and their associations is exceptional. As the roles of organisms in the formation of ancient CGC soils are largely unknown, we are developing an approach to characterizing these based on comparative analyses using modern analogues and a suite of analytical methods combining *in situ* imaging using X-ray micro-computed tomography in both the laboratory and synchrotron. The Rhynie cherts and their soil community contain many of the components of modern CGCs, including small-stature cryptogamic plants with rhizoidal rooting systems, fungi, cyanobacteria, green algae, fungus-like oomycetes, nematodes and a diverse community of arthropods. There is limited evidence for lichen-like associations, but more compelling fossils have recently been documented at contemporaneous sites elsewhere. Plant growth forms and symbiotic associations with fungi indicate that moss dominated CGCs—and especially peat forming systems—are not the best modern analogues. Liverworts are a more appropriate model for the plant component. Other key differences to many modern CGCs include the absence of annelids and ants; also, absent were the most aggressive white rot lignin decomposing fungi of the Agaricomycetes, implying that there were key differences in the recycling of soil organic carbon. CGCs were the earliest soil forming communities and the organisms that they contained may have had the capacity to aggregate sediments and to weather minerals and clasts. Imaging and *in situ* chemical analyses of micro-dissolution features in soils and regoliths brings a novel perspective to studying early terrestrial communities and to understanding their broader impacts on Earth systems.

S41-5 Proposal for practical classification of fungi within eukaryotes based on monophyly and comparable divergence time, and communication of higher-level taxa

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Abstract: Much of the ecological, taxonomic and biodiversity research relies on understanding of phylogenetic relationships among organisms. There are multiple available classification systems that all

suffer from differences in naming, incompleteness, presence of multiple non-monophyletic entities and poor correspondence of divergence times. These issues render taxonomic comparisons across the main groups of eukaryotes and all life in general difficult at best. By using the monophyly criterion, roughly comparable time of divergence and information from multiple phylogenetic reconstructions, I propose an alternative classification system for the domain Eukarya to improve hierarchical taxonomical comparability for animals, plants, fungi and multiple protist groups. Following this rationale, I propose 32 kingdoms of eukaryotes that are treated in 10 subdomains. These kingdoms are further separated into 43, 115, 140 and 353 taxa at the level of subkingdom, phylum, subphylum and class, respectively (bioRxiv 2017:240929). In Fungi, nine subkingdoms and 19 fungal phyla are proposed. The kingdom Nucleariae (phyla Nuclearida and Fonticulida) is treated as a sister group to Fungi. In addition to widely accepted phyla, it is proposed to adopt phylum rank to Aphelidiomycota, Basidiobolomycota, Calcarisporiellomycota, Glomeromycota, Entomophthoromycota, Entorrhizomycota, Kickxellomycota, Monoblepharomycota, Mortierellomycota and Olpidiomycota given their deep divergence.

S41-6 Elucidation of the "enigma" of *Aenigmatospora*

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Abstract: Sporulations of enigmatic fungus *Aenigmatospora pulchra,* described by Castañeda et al. (1999), were repeatedly observed on the dungs of millipedes. When the basal part of the conidiophore buried in the dung was gently washed in the water, the conidiophore stalk found to be connected to a large cylindrical cell. These cylindrical cells are superficially similar in shape and size with the arthrospores of *Etenerobryus* sp. (Eccrinales) inhabited in the hindgut of millipedes whose dungs harbor *A. pulchra*. Based on these morphological observation, Degawa (2005) speculated that *A. pulchra* must be an unknown stage of *Enterobryus*. However, as the results of recent careful reinvestigations, this hypothesis was denied. Here, we report the new discoveries. In the gut of millipedes, in addition to the genus *Enterobryus*, a similar shaped organism called *Mononema* often coexists. The genus *Mononema* is a fungus-like organism described by Balbiani (1889) and now included 3 spp. growing in the foregut of centipedes and millipedes. Lichtwardt (1986) excluded *Mononema* from the "Trichomycetes" and its real taxonomnic position is uncertain. Unidentified species of *Mononema* was frequently detected in the oesophagus of the field-collected millipedes individuals. Its arthrospores and the basal cylindrical cells of *A. pulchra* emerged on the dung of the same individuals are similar in shape and size. When the mature arthrospores were carefully picked up and put on the surface of plain agar, it sometimes germinated to produce incomplete conidiophores. As a result of sequencing the partial 18SrDNA of both of the arthrospores of *Mononema* and conidia of *A. pulchra,* they are almost identical and belonged to the Kickxellomycotina. These morphological observations and molecular data strongly support that *A. pulchra* was an unnoticed stage of the genus *Mononema* of the Kickxellomycotina and not *Enterobryus*. When the spore of *A. pulchra* was crashed under the cover glass, the endogenously produced double-walled spore was extruded from the echinulate wall, indicating that spores of *A. pulchra* are not "condia" but "unisporous sporangiole". This feature also supports the phylogenetic position of *A. pulchra-Mononema* in the Kickxellomycotina.

Symposium Session 42:

Decipering Fungi-Archaea/Bacteria Interactions for Biocontrol of Soil-Borne Pathogens

G. Berg and R. Grosch

S42-1 Identification of bioactive volatiles produced by sclerotia-associated bacteria

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Abstract: Plant pathogens with a wide host range such as *Sclerotinia sclerotiorum* (Lib.) de Bary and *Rhizoctonia solani* Kühn cause extensive crop damage across the globe. Both pathogens form resilient sclerotia during their life cycle that can persist in the soil for several years. This is aggravated by the inefficiency of conventional fungicides against these survival structures. We explored sclerotia-associated microbial communities on *Solanum tuberosum* L. to identify prevalent bacteria. Amplicon data that was generated by 16S rRNA gene fragment sequencing indicated that the 'sclerotiome' of *R. solani* is highly similar to the microbiome of surrounding soil. In contrast, microbial communities of the unaffected tuber surface showed significant differences in their structure and composition. We found that distinctive bacterial lineages were associated with both healthy and infected areas. Members of the *Flavobacteriaceae* and *Caulobacteraceae* families were primarily detected in unaffected areas, while *Phyllobacteriaceae* and *Bradyrhizobiaceae* were associated with the presence of sclerotia. A complementary approach aimed at isolating natural antagonists of *S. sclerotiorum* delivered promising candidates for extended interaction studies. We selected isolates that produce bioactive volatiles, which substantially increases their inhibition range. The most efficient antagonists among *Bacillus*, *Buttiauxella, Enterobacter* and *Pseudomonas* isolates were shown to emit various alkylpyrazines. Subsequent experiments with the pure compounds confirmed their fungicidal properties. These findings provide a solid basis for further optimizations of promising biocontrol agents. We envisage a combination of highly active microorganisms and natural 'boosters' that will result in higher efficiencies of biopesticides against fungal diseases.

S42-2 Deciphering Archaea interactions for plant health

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Abstract: Plant holobionts are known to harbour a wide diversity of microorganisms, such as bacteria and fungi, influencing plant nutrition, resistance to stress and fitness. Recent studies have shown that *Archaea* also shape the microbiome of plants, but their functions and interactions with their hosts remain mostly unclear. To get a broader insight into the community structure, habitat preferences and functions of plant-associated *Archaea*, we studied 41 different crops and native plants by a combined approach including 16S rRNA amplicon sequencing, whole metagenome shotgun sequencing and fluorescence *in situ* hybridization confocal laser scanning microscopy (FISH-CLSM). All plants were colonized by *Archaea*; we found plant species-specific diversity and abundances. The highest relative abundances were detected in the endosphere of perennial plants, e.g. olive trees with up to 67.3% of total reads and dwarf shrubs (*Vaccinium myrthillus* and *V. oxycoccus*, with 33.0% and 31.7% respectively). *Archaea* were also found in all plant microhabitats including the spermosphere. Across all habitats, the archaeal community structure was dominated by *Euryarchaeota* or *Thaumarchaeota*, followed by the less abundant phylum of *Crenarchaeota*, except in *O. europaea*, where *Thaumarchaeota* were predominant. On plants, we observed signatures for putative adaptation mechanisms of *Archaea* for their hosts,

including those for higher chemotaxis, nutrient cycling like CO_2 fixation, stress response, especially against oxidative stress, archaeon stability, and possible plant growth promotion through auxin. Moreover, we found a strong correlation with fungal inhabitants. These findings reveal a so far unobserved role of *Archaea* for plant and fungi holobionts.

S42-3 Effect of long-term farming practices on the plant and its associated rhizosphere microbiome

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Abstract: Intensification and inadequate agricultural management result in substantial losses of fertility and yield, and the accumulation of pathogens in soils. In order to maintain soil quality and health for the future in agricultural land, the development of more extensive and sustainable farming strategies is urgently needed. Hence, a better understanding of how agricultural management strategies affect soil and associated rhizosphere properties is the key to propose farming strategies for high plant productivity and plant health. We used three long-term field trials to analyze the impact of various management strategies on soil and its associated rhizosphere microbiome under consideration of plant productivity, plant health and the ability of the soil to suppress soil-borne pathogens. The soils of the long-term field trials were subjected to growth chamber pot experiments with lettuce (*Lactuca sativa*) as model plant. After a growth period of ten weeks, significant differences in lettuce shoot fresh mass and microbial biomass were observed among soils depending on long-term farming strategies. The rhizosphere exhibited different bacterial and fungal community compositions depending on soil sites as well as on the agricultural management history (tillage practice, crop rotation, fertilization strategy) of the soils. These factors influenced also relative abundances of distinct bacterial and fungal taxa. In addition, the root exudation of the antifungal metabolite benzoic acid as well the expression of plant-defense related genes was affected by farming practice. This suggests a relationship between long-term agricultural management, soil microbiome and plant performance.

S42-4 Impact of soil treatments on *Pratylenchus penetrans* densities and microbial community composition in arable soil

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Abstract: The impact of different soil treatments, aimed to reduce the population size of the root-lesion nematode species *Pratylenchus penetrans,* on the soil microbial community composition was investigated. It was already shown before that four of nine applied soil treatments lead to significant reductions in *P. penetrans* population densities in different field plots. In our study we found a strong positive correlation between *P. penetrans* densities and the number of fungal genome equivalents, representative for total fungal biomass, in soils. It is a well-known fact that *P. penetrans* densities positively correlates with the presence of the plant pathogenic fungus *Verticillium dahliae* in arable soils and now we found that presence of the plant parasite positively correlates with total fungal biomass in soil. Further, there was a negative correlation between numbers of bacterial biosynthesis genes, responsible for production of the antifungal compounds 2,4-Diacetylphloroglucinol and Phenazine, and

P. penetrans densities in the investigated soils. This brought us to the hypothesis that soil treatments may impact *P. penetrans* densities in soil by shifting microbial (bacterial and fungal) community compositions. We therefore compared the microbial community composition in two differently treated and one untreated (control) soils by bacterial (16S rRNA V3 - V4 gene regions) and fungal (ITS2 region) amplicon sequencing. Based on bacterial and fungal community compositions among individual samples of each treatment (n=4), annually sampled over 5 consecutive years, it became clear that there was a strong effect of 'treatment' on compositions of both communities by comparing both treated soils with the control soil and the soil before application of the soil treatments. Further, soil microbial community compositions strongly depended on the density levels of *P. penetrans* in these soils. Statistically significant inverse relationships between *P. penetrans* densities and specific bacterial (*Arenibacter, Rheinheimeria, Stenotrophomonas*) and fungal (*Microascales, Mortierella*) taxons were present. Negative correlations between densities of *P. penetrans* on the one, and specific microbial populations on the other hand site indicate that microbial suppression of the plant parasite may occur in soil upon application of the treatments. However, the effect of 'soil treatment' on the soil microbial community composition appeared to be only temporal and tended to fade out over time. Further, a strong positive correlation between *P. penetrans* and the fungal taxon of *Pleosporoceae* was shown to be present, indicating that particular microbial groups also may benefit from the presence of the plant parasite in these treated soil. In conclusion, strong positive and negative correlations between particular soil microbial taxons and *P. penetrans* were present, indicative for the fact that the applied soil treatments had strong impact on particular soil microbial groups. The causal relationships between specific strains belonging to these soil microbial groups and *P. penetrans* is something that will be further explored in following experiments using microbial exposure test assays with *P. penetrans*. These are studies that requires directed cultivation-based approaches aimed to specifically isolate the impacted microbial groups from the treated soils.

S42-5 The probiotic cutaneous microbiome of endangered Tennessee bats

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Abstract: Since its introduction in 2006 into the USA, *Pseudogymnoascus destructans* (*Pd*), the fungal agent of white-nose syndrome (WNS), has rapidly spread killing millions of bats. WNS has negatively impacted populations of three federally listed bat species and has been predicted to have the ability to cause extinctions in the near future. Treatment options for bats infected with *Pd* include anti-fungal chemicals, volatile compounds, and naturally occurring antifungal probiotic bacteria that may be members of the cutaneous microbiome. Studies of the cutaneous microbiome have revealed that specific bacterial strains play dominant roles in the microbiome community, when minor fluctuations occur in abundance of these 'core' microbes, it can alter immune function. The main objectives of this research were 1) to characterize the bat, cave soil, and roost microbiome using high-throughput DNA sequencing, 2) observe spatial variation in the structure of the cutaneous microbiome of bats, 3) determine if the cutaneous microbiome is altered in the presence of a pathogen, and 4) to identify potential probiotic antifungal bacterial strains with activity against *Pd* that are found naturally on both the bat and in the cave ecosystem. To date we have sampled the microbiome of bats (n=95) and corresponding soil samples (n=95) from 21 caves and compared bacterial communities using high-throughput DNA sequencing. Results indicate that there is a significant interaction between cave and species (Pseudo-F = 1.397, p = 0.015), suggesting a complex relationship driving the cutaneous

microbial communities. In addition, the cutaneous microbiome of bats is altered when *Pd* is present (Pseudo-F = 3.1517, $p < 0.01$). We have identified 111 bacterial isolates from the skin of bats that have antifungal activity against *Pd,* 25 of which have been found to be members of the cutaneous microbiome, and the cave soil. Of these, 12 isolates have been determined to be part of the 'core' microbiome of bats. We hypothesize that the cave ecosystem may serve as an environmental reservoir for the core microbiome thus contributing to anti-*Pd* activity.

S42-6 An endophytic *Bacillus* inhibits all endophytic fungi in apple leaves

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Abstract: Apples in general have specifically associated fungi and bacteria that will infect them, including *Alternaria tenuissima*, *A. arborescens*, *Fusarium avenaceum*, *Monilinia fructigena*, *M. laxa*, *Penicillium expansum*, *P. solitum*, *P. crustosum* and the bacterium *Alicyclobacillus acidoterrestris*. We wanted to examine whether these fungi were also present as endophytes in the less acidic apple leaves and twigs, and whether *Alicyclobacillus* was present as an endophyte. After surface disinfection a series of filamentous fungi and a *Bacillus* species was rather consistently isolated. The fungi isolated were not the serious apple rot fungi such as *P. expansum*, *P. solitum* and *Monilinia fructigena*, but rather fungi such as *Alternaria tenuissima*, *Broomella acuta*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Fusarium torulosum*, and *Phoma* spp. These fungi produced secondary metabolites such as antibiotic Y, alternariol, tenuazonic acid, orevactaene, chaetoglobosin A, and chlamydosporol, but none of the fungi were able to inhibit the *Bacillus* species. On the other hand, the *Bacillus* species strongly inhibited all the endophytic fungi isolates without exception. Chemical analysis (HPLC-DAD-MS) of the inhibition zones, using interference competition experiments showed that the *Bacillus* sp. produced antifungal (and antibacterial) lipopeptides such as iturins. Furthermore, some fungi such as *Cladosporium cladosporioides* produced extrolites that enhanced the growth of the Bacilli. We speculate that the apple tree recruit endophytic *Bacillus* spp. to keep the endophytic fungi in check.

Symposium Session 43:

Marine Mycology

A. Walker and P. Vélez Aguilar

S43-1 Microscopic fungi from the ocean: their concept, diversity and future in Mexico in a globalized world

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Abstract: The marine fungal biology is a subdiscipline of Mycology that studies the microscopic fungi that inhabit the ocean, from an ecological perspective. This group of marine microorganisms is classified in the Kingdom Fungi, one of the largest and most diverse of all of the eukaryotic organisms that includes a non-taxonomic group called –microscopic fungi/micromycetes– which develops fungal structures of microscopic size during their whole life cycles. The micromycetes form part of all ecosystems, continental or oceanic, where they perform an endless variety of key ecological functions as saprotrophs, degrading organic remains for nutrient recycling, or symbiotrophs associated with microscopic or macroscopic eukaryotes from other kingdoms. The fungal microorganisms synthesize biochemicals as

a result of their complex and diverse metabolic pathways essential to the ecosystems they inhabit. The concept of marine fungi has changed in accordance with advances in science. At present it is defined as 'any fungus that is recovered repeatedly from marine habitats because: 1) It is able to grow and/or sporulate in marine environments; 2) It forms symbiotic relationships with other marine organisms; 3) It is shown to adapt and evolve at the genetic level or be metabolically active in marine environments' and in addition, 4) they exhibit adaptations necessary in order to live in the oceans that are manifested through the possession of specific morphological characters. The fungal diversity that inhabits the planet remains mainly unknown. At present, only 2% of the total number of fungi is described and the majority of species are from terrestrial ecosystems. Of over 100,000 fungi known worldwide, only 1,112 are marine. The ocean covers 71 percent of Earth and 95% is underwater and unexplored. The possibility that the oceans hold a larger and exclusive fungal diversity than that registered from the continents emphasizes the need to describe the fungi from innumerable marine habitats that range from coastal to open sea regions, including those most adverse to the eukaryotic life style. Mexico is ranked as one of the five megadiverse countries of the world. Overall, not more than 100 species have been recorded from its marine ecoregions. The distinctive ecological characteristics of each Mexican marine ecosystem could result in the presence of a new and/or endemic mycobiota in this country with high applied value. Many potentially useful metabolites from undescribed fungi wait to be discovered. Strategies are then proposed in order to obtain the marine fungi diversity from these valuable ecosystems to isolate and identify the species. The establishment of taxonomic identification training programs with the goal of increased use of traditional methods to obtain and conserve cultures of Mexican microscopic marine fungi are necessary to investigate their potential biotechnological use. Therefore, in order for marine mycology to advance in this era that confronts global, environmental and social challenges, it is required to follow a strategy that integrates taxonomic/phylogenetic, genetic/genomic and ecological/functional aspects. To this end, the mycologists of the future need to possess abilities that permit them to attain high levels of innovation, collaboration and operation of cutting-edge technology.

S43-2 New lineages in the Pezizomycotina from marine ascomycetes described from Thailand

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Abstract: Thailand is a biodiversity rich country; however, only 34% of marine fungi have been documented compared to the figures worldwide. To complement the sporadic knowledge on marine fungi in Thailand, we therefore are developing a baseline research in marine fungi to encourage awareness on the conservation of bioresources in the marine protected areas in the country. Noteworthy lignicolous ascomycetous species were collected from southern Thailand. Firstly, a new marine Sordariomycete, *Lautospora obovoidiella* sp. nov., was found on decaying intertidal mangrove wood, and is characterized by having immersed ellipsoidal ascomata, unitunicate cylindrical asci, obovoid and thickened-wall ascospore. Its morphological features are similar to *L. simillima* and *L. gigantea*, but differ in ascospore shape in possessing a round apical region and elongate basal part of ascospore. Based on our molecular study, *L. obovoidiella* sp. nov. forms a well-supported clade with the other *Lautospora*

species within the Lautosporaceae in the Sordariomycetes. Additionally, strains *of L. obovoidiella* sp. nov. constitute a separate group within the *Lautospora* species subclade with strong statistical supports. Interestingly, the Lautosporaceae forms a highly supported monophyletic clade without any named orders in the Sordariomycetes. Therefore, a potential new order, Lautosporales, is proposed. Secondly, a novel Dothideomycetes species, *Helicascus satunensis* sp. nov., was investigated on nypa palm fruit; its markedly morphological character possesses semi-immersed lenticular ascomata, multi-locules; bitunicate ascus; smooth, dark-brown ascospores, obovoid, 1-septate and unequally 2-celled. Based on molecular phylogenetic evidence, *H. satunensis* sp. nov. formed a well-supported clade within *Helicascus* species and closely related with marine species within the Morosphaeriaceae, order Pleosporales. Consequently, the genus *Helicascus* formed a distinct clade from the genus *Morosphaeria*. Therefore, with the unique morphological and molecular characteristics, a new family Helicascaceae is suggested for *Helicascus* species. In conclusion, this study gives an insight into the distribution and diversity of marine fungi present in Thai mangroves especially in the southern coast. Currently 184 marine fungal species were recorded for Thailand.

S43-3 Deep-sea microfungal degradation of hydrocarbons: Implications for oil spill bioremediation

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Abstract: The Gulf of Mexico basin is a biodiverse and productive environment that provides a wide array of resources. In addition, petroleum exploitation within this area sustains a multi-millionaire industry. However, on numerous occasions these operations have resulted in major environmental disasters of unknown consequences. Nonetheless, the estimation of ecological costs of oil spills on marine ecosystems is limited to the extent of our knowledge on the autochthonous biota and its functional capacities. Fungi are involved in key ecological deep-sea processes, yet these microorganisms have been rarely cultured and preserved from deep-sea samples. Moreover, these microorganisms have been recognized as a characteristic component of post-spill deep-sea communities in sediments. So, the objectives of this work were to analyze cultivable fungal diversity from deep-sea sediment samples from two major oil-drilling sites in the Gulf of Mexico for their response to hexadecane and 1-hexadecene as sole carbon sources, and to evaluate their gene expression profiles in response to the utilization of these hydrocarbons. Deep-sea sediment samples were collected during the Metagenómica-Malla Finacruise, as part of the Consorcio de Investigación del Golfo de México (CIGoM) agenda. Fungal isolates were obtained and identified based on the evaluation of sequence data from the nuclear ribosomal internal transcribed spacer including the 5.8 rDNA region. Isolates were screened for their sensitivity to hydrocarbons, and tolerant OTUs were grown with hexadecane and 1-hexadecene as sole carbon sources to test their ability to break down these long-chain aliphatic hydrocarbons in liquid culture. A differential transcriptome analysis was performed for selected OTUs to evaluate genetic signatures during hydrocarbon utilization. We obtained 25 isolates, which clustered into 7 OTUs, that showed differential sensitivity profiles towards hydrocarbons. Our results agree with previous work on deep-sea fungi reporting low levels of cultivable diversity, with Ascomycota as the dominant phylum. Moreover, six of these taxa proved to metabolize the tested alkane and alkene as sole carbon sources, confirming deep-sea fungal taxa as valuable genetic resources for hydrocarbon bioremediation. Transcriptome data on selected deep-sea fungal isolates revealed differential gene expression between treatments. This work provides the first insights of cultivable fungal diversity from deep-sea sediments in the Gulf of Mexico, and their response to hexadecane and 1-hexadecene inputs,

shedding light on a better understanding of the deep-sea ecosystem dynamics and bioremediation using deep-sea native taxa.

S43-4 Marine fungi (*sensu stricto*) – an underexplored resource of natural products

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Abstract: Marine fungi in the strict sense (*sensu stricto*) remain one of the few underexplored resources of natural products and are a proven source of bioactive and structurally diverse molecules. However, reports of new chemistry from marine fungi *sensu stricto* are sparse; rather the literature is dominated by "marine-derived" isolates of well-known osmotolerant terrestrial genera. 'Omics'-based investigations have shed some light on the role that abiotic/biotic factors play in the regulation of natural product production. Culture conditions are likely responsible for many of the reports of new chemistry from osmotolerant species. Osmotolerant terrestrial isolates under saline stress have been found to produce identical chemistry to previous reports from marine-derived counterparts. To truly explore marine fungi as a source of new natural products, new isolation strategies need to be adopted by natural products chemists as culture isolates remain the primary source from which these scientists discover and obtain new molecules. To refocus efforts away from common "marine-derived" fungi, recommendations will be presented on modern isolation and fermentation techniques used to isolate truly unique linages of marine fungi and to maximize their natural product production.

S43-5 Arctic marine fungi, their diversity and bioactivity

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Abstract: The Arctic Ocean and its adjacent seas are one of the least studied geographical areas in marine mycology. Despite the stressful environment and harsh climatic conditions, numerous marine fungi have evolved mechanisms to survive and function in the Arctic, making them intriguing study objects for basic and applied research. The aim of ongoing efforts is to characterize the richness and diversity of Arctic marine mycobiomes and get insights into the ecology of fungal communities in different substrates. The diversity research has focused on driftwood- and macroalga-associated fungi, using culturing and culture-independent high-throughput sequencing (HTS), as well as morphological examination of fruiting structures. Sampling has been conducted in onshore and offshore locations up to 82 degrees north. The research has revealed a greater than expected species richness. In total, 100 species of filamentous fungi have been morphologically documented from the Arctic, whereas molecular data provide evidence for a far greater diversity consisting of hundreds of operational taxonomic units (OTUs; defined using 97% ITS similarity as threshold) in single substrates. For example, fifty pieces of driftwood studied using culturing combined with Sanger sequencing of pure cultures and a HTS approach, revealed a richness of almost 1000 OTUs, whereas the total richness was extrapolated to be 1500 OTUs. Interestingly, several OTUs showed a significant detection bias for culturing and against the HTS approach. Some groups of fungi, such as the Leotiomycetes and the strictly marine order Lulworthiales, seem to host high richness in Arctic driftwood and macroalgae, including undescribed species. The established culture collection consisting of approximately 1000 fungal isolates has been used for marine fungal bioprospecting. Fungal extracts produced in monocultures as well as in co-cultures with bacteria have been screened for bioactivity, and active extracts subjected to bioassay-guided isolation of active molecules. Co-culturing marine fungi with bacteria has led to increased antibacterial activity, and resulted in the identification of novel compounds. Arctic marine fungi are

counted in thousands, seem to play active ecological roles in their native environment and produce novel bioactive molecules laying ground for a fruitful biodiscovery work.

S43-6 Fungi from Fundy: Biodiversity of intertidal fungi from megatidal Nova Scotia, Canada

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Abstract: The fungal diversity of Nova Scotian's saltmarshes and the megatidal marine environments of the Bay of Fundy is underexplored. Fungi are key decomposers and saltmarsh plant root symbionts in these systems. We are expanding our knowledge of the marine fungal diversity from Nova Scotia and determining which of the fungi present have potential use in marine oil spill remediation and saltmarsh restoration. Collections of seawater, marine sediments and saltmarsh and algal detritus floating in sea water have been taken since the summer of 2015 from sites including Apple River, Kingsport, and Bon Portage Island, Nova Scotia. Samples were placed onto saltwater media (saltwater potato dextrose agar, and saltwater agar containing antibiotics) selective for marine fungi. Detrital marine wood and plant samples were incubated in sterile damp chambers to promote the emergence of fungal reproductive structures used for microscopic identification and single spore isolations. ITS rDNA barcode sequences were identified from axenic cultures using the online reference sequence database NCBI Genbank. From 11 locations sampled in Nova Scotia, 101 fungi were identified from marine habitats and two have been confirmed as new fungal species. We present morphological and multi-locus molecular evidence for a new species of obligate lignicolous marine fungus in the genus *Lulworthia* (Luworthiales, Sordariomycetes, Ascomycota) isolated from naturally occurring submerged wood at Apple River Bay, Cumberland County, Nova Scotia.

Symposium Session 44:

Fusarium: The Genomics of Functional and Ecological Diversity

D. Geiser and E. Steenkamp

S44-1 Diversity and evolution of an accessory chromosome within the *Fusarium fujikuroi* species complex

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Abstract: Members of the plant pathogenic *Fusarium fujikuroi* species complex (FFSC) include a diverse assemblage of fungi. Comparative genomics of the genus *Fusarium* has revealed that their genomic landscape can be divided into a core and an accessory genome. In many *Fusarium* species, the accessory compartment can be dispensable and contains genes that may participate in ecological niche exploitation, which in turn contributes to host specialisation and pathogenicity. Within the FFSC, whole-genome assemblies of *Fusarium* species were compared to identify scaffolds corresponding to chromosome 12, a dispensable chromosome belonging to the accessory compartment. Chromosome 12 from different species exhibited significant length polymorphism compared to core chromosomes, and displayed remarkably low levels of sequence homology and synteny. Similarity in gene content and organization appeared to be limited to closely related species in each of the three main clades of the

FFSC. Phylogenetic analyses of the genes on chromosome 12 homologs indicated that most had diverse and non-orthologous origins. Species-specific unique genes were dispersed throughout the chromosome, with no clustering. The low sequence and genic homology, as well as the diverse evolutionary trajectories of genes present on chromosome 12 highlights the plasticity of this chromosome. Our findings thus emphasize the potential role that these molecules play in the ecologic al diversity of this economically important group of fungi.

S44-2 A phylogenomic view of the *Fusarium oxysporum* species complex provides a robust framework for addressing questions in systematics, ecology and plant pathology

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Abstract: Members of the *Fusarium oxysporum* species complex (FOSC) cause devastating vascular wilt diseases on a broad array of crop plants. In addition, some FOSC are known to inhabit soils and plants as presumed non-pathogens, and others inhabit plumbing systems and are associated with serious human infections. Initial attempts to produce a comprehensive phylogeny of the FOSC were hindered by a lack of concordance between phylogenies inferred from different loci. With the goal of producing a highly resolved phylogeny, we used a phylogenomic approach to extract 41 highly informative orthologous protein-coding genes useful for inferring organismal history in this group. OrthoMCL was used to identify orthologous protein clusters from the genomes of the FOSC*, F graminearum, F. verticillioides,* and an undescribed species in the *F. solani* Species Complex (FSSC 11). From the initial set of 9037 orthologue clusters identified, 4056 were found to represent a single protein from each of the four *Fusarium* genomes. Of these 4056 putative single-copy orthologues, only one mapped to a "lineage-specific" chromosome previously identified in the FOSC, with three additional loci mapping to unassembled contigs. This is hypothesized to reflect a strong connection between gene orthology patterns and the core genome of the FOSC. The coding sequences of each putative orthologue were then extracted from ten additional FOSC genomes and subjected to neighbor-joining bootstrap analysis, as a simple means to assess phylogenetic signal. 41 loci that provided ≥70% bootstrap support at seven or more nodes in the phylogeny of the eleven FOSC were then extracted from complete genome sequences of >100 FOSC isolates. Resulting sequences, covering 69,201 nucleotide sites with 9479 parsimony-informative characters, were aligned and subjected to a phylogenetic analysis. The resulting tree showed highly supported terminal clades, providing evidence for species boundaries within the FOSC*,* along with signatures of historical recombination within terminal species lineages. In addition, relationships between pathogenic and non-pathogenic isolates within clades suggest possible models for acquisition and loss of pathogenicity determinants. This highly resolved view of the evolution of the FOSC based on its core genome provides a powerful vantage point for observing the evolutionary patterns associated with pathogenicity and niche adaptation in this extremely important group of fungi.

S44-3 Characterization of the RNAi pathway required for ascospore formation in the cereal pathogen *Fusarium graminearum*

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Abstract: *Fusarium graminearum*, the causal agent of Fusarium head blight in cereal crops, produces sexual progeny (ascospore) as an important overwintering and dissemination strategy for completing the disease cycle. This homothallic ascomycetous species carries two opposite mating-type (*MAT*) loci in a single nucleus to control sexual development. Recently, we have identified a RNA interference (RNAi) pathway that controls a late stage of sexual development in *F. graminearum*. Using several molecular strategies, we have determined the functions of several genes involved in the putative RNAi pathway. In particular, we have focused on the role of *FgSMS-2* encoding an Argonaute-like protein, which is a part of the RNA-induced silencing complex for specific cleavage of target mRNAs. Both gene deletion- and gene overexpression-strains of *FgSMS-2* were defective in ascospore/asci maturation. A GFP-tagging analysis showed that FgSMS-2 was specifically localized on perinuclear regions inside the immature asci. A BiFC analysis revealed that FgSMS-2 was able to bind to a Dicer-like protein, FgDCL-1 in cytoplasmic region during the early stage of sexual development. In addition, we identified putative target gene sets of FgSMS-2 using transcriptomics and RNA-immunoprecipitation (RIP)-seq analyses. A total of 262 genes were up-regulated in the *FgSMS-2* deletion strain under sexual development compared to its wild-type progenitor, and a total of 525 genes were identified from putative RNA samples co-precipitated with FgSMS-2. Using the same RNA samples used in RIP analysis, we were able to identify a total of 8,922 small RNAs (17~32 bp in size). Overall, it is likely that a RNAi pathway plays an important role during the sexual development in *F. graminearum*, particularly the Argonaute-like protein, FgSMS-2 protein, controls a set of mRNAs that might be unnecessary during meiotic event.

S44-4 The structural and functional diversity of fungi in the sorghum-*Striga* interaction in Ethiopia

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Abstract: *Sorghum bicolor* is a key staple crop for millions of people in sub-Saharan Africa, and grown primarily for household food security in drought-prone and resource-poor regions. *Striga hermonthica* is a parasitic weed of sorghum roots and other crops including maize. In Ethiopia, *Striga* infestation can cause severe yield loss annually. Current measures to control *Striga* include cultural and chemical methods as well as breeding for host resistance, of which none have proved to be singularly effective. Furthermore, several of these control measures are not accessible or too expensive for resource-poor famers. Hence, there is a strong need for novel, effective, affordable, integrated and durable control strategies. Key to developing these control strategies are a better understanding of the structure and functionality of the extant microbial diversity within the sorghum-*Striga* interaction, in which fungi play a major role. For sorghum and *Striga*, however, knowledge of the diversity, biogeography and metabolic potential of microbes is fragmentary or non-existent. Therefore, the goal of the PROMISE (promoting root microbes for integrated *Striga* eradication) research programme is to identify specific groups and functions of known & unknown microbes that, in conjunction with specific sorghum lines and agronomic practices, consistently suppress *Striga* infections, adversely affect the *Striga* seed bank and enhance sorghum productivity for smallholder farmers. To this end, fungal isolations were made from soils collected in different Ethiopian agroecologies displaying both *Striga*-suppressive and -conducive traits. Additionally, endo- and epiphytic fungal isolations were made from *Striga* seeds collected from six

different sorghum growing regions in Ethiopia. To meet the goals of the PROMISE research programme, only fast-growing fungi, excluding members of the *Eurotiales* and *Mucorales*, were characterised using the ribosomal ITS fungal barcode in combination with LSU. Where required, secondary barcodes (*cmdA*, *rpb2*, *tef1* and *tub2*) were used for species-level identification. The majority of the fungi identified from soils and *Striga* seeds belonged to the Hypocreales and Pleosporales. Additionally, several novel taxa were also identified. In conclusion, a high diversity of fungi was isolated from the different agroecological regions sampled in Ethiopia, some displaying their own unique diversity. This diversity in sorghum fields is mostly driven by soil structure and climatic conditions, which in turn could adversely affect the application of a fungus to disrupt the *Striga* life cycle.

S44-5 Genetic linkage maps and genomes provide clues to growth rate differences in *Fusarium*

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Abstract: Sequencing fungal genomes has become almost routine and this is also true in the case of *Fusarium* species. Close to a 100 genome sequences are now available for Fusarium spp. and in some cases numerous isolates of a species have been sequenced. Understanding the functions of the genes coded by these genome sequences, especially in non-model species, will be important in managing plant pathogens in the future. We have used a combination of genetic linkage maps for a hybrid cross between *Fusarium circinatum* and *F. temperatum* and whole genome sequences to identify genes involved in growth rate differences in these two species. Predicted quantitative trait loci (QTLs) for growth in culture were mapped onto the genome sequence of both parents. Two QTLs were investigated. One of these enabled the identification of a five gene indel specific to *F. circinatum* that was not present in any other *Fusarium* for which genome data are available. The other QTL involves an indel in which two genes are absent in some *F. circinatum* isolates. Further investigations of these QTLs will enable a better understanding of growth and potentially, also pathogenicity in *F. circinatum* and *F. temperatum*.

Symposium Session 45:

Lichens on Islands: Evolution, Endemism and Conservation

T. Lumbsch and J. A. Mercado

S45-1 An integrative taxonomic approach to elucidate the diversity of the lichen genus *Sticta* (Ascomycota: Lobariaceae) in Puerto Rico

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Abstract: Tropical islands harbor the highest diversity and the largest proportion of endemics per unit area in many biological groups. In understudied organisms such as lichens, however, these patterns remain poorly understood. In this work, we use integrative taxonomic approaches to elucidate the diversity and degree of endemism of the macrolichen genus *Sticta* in Puerto Rico. A combination of existing multi-locus sequence data from different regions and newly generated data from 64 specimens

from the island was used to evaluate if current morphology-based taxonomy agree with evidence from phylogenies. Results from several molecular-based species delimitation analyses (i.e. bPTP, GMYC, BP&P) were also used for assessing species boundaries. We obtained evidence for at least 11 species of *Sticta* occurring in Puerto Rico. Of these, eight are potentially endemic to the island. All endemic species are nested within larger South American clades, suggesting frequent colonization from the continent. Lastly, the number of species recovered from our phylogenetic analysis broadly agree with previous morphology-based estimates, suggesting that morphology could be a good predictor of lichen species diversity in tropical islands. We discuss how factors such as constraints on diaspore dispersal in tropical forests might explain observed trends. We also suggest that the patterns observed might be generalizable and that further studies will likely reveal more cases of lichen endemism in other island systems.

S45-2 Tasmania: an island lichen biota at the edge of the world

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Abstract: The island of Tasmania lies in the Southern Ocean and displays strong biogeographical relationships with other former components of Gondwanaland. At the same time, it is the southern extremity of the Australian landmass, from which it is separated only occasionally in geological time. That Tasmania is isolated, serves as a refugium, especially for species of cool, moist habitats, presents a wide diversity of lichen habitats in close juxtaposition within a small area, and retains more than half of its land area in a more or less natural state, are discussed. At the same time, although supporting a rich lichen biota of c. 1300 taxa, the level of lichen endemism is relatively low (approximately 10%). The unusual or unique components of the lichen biota are attributed chiefly to the presence of certain equally unusual habitats: the island's geology is dominated by Jurassic dolerite and Precambrian metamorphosed sediments; and the extensive expanses of natural forest may contain very large (e.g. *Eucalyptus*), very old (e.g. conifers) or unusual trees (e.g. *Richea*). In combination with a mild, oceanic climate, these factors create diverse and noteworthy microhabitats for lichens, as illustrated by the endemic genera *Cameronia*, *Meridianelia* and *Siphulella*, as well as by genera such as *Menegazzia*, *Siphula s. lat.*, *Bactrospora* and *Rimularia*, all of which have achieved high levels of speciation in Tasmania in cool, moist habitats. However, somewhat surprisingly, more than a quarter of Tasmania's endemic lichens occur in coastal and dry, open woodland habitats, where direct physical and ecological links to mainland Australia are strongest and most recent.

S45-3 Lichens, molecular barcoding, and island biogeography

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Abstract: Islands are laboratories of evolution, having defined limits for dispersal and gene exchange and well-traceable geological histories. In the 19th century, Darwin and Wallace conceived the theory of evolution through natural selection, both inspired by travels including island archipelagos, such as the Galápagos Islands and the Malay Archipelago. The Galápagos Islands and particularly the Hawaiian Archipelago have become prime examples for evolutionary studies, documenting high levels of endemism and striking radiations particularly in vascular plants and animals. Notably, Darwin wrote to Hooker in 1850: "*Of all places in the world I would like to see a good flora of the Sandwich Islands* [Hawaii]. *I would subscribe 50 pounds to any collector to go there and work at these islands*." A century after Darwin's book *On the Origin of Species*, MacArthur and Wilson developed the theory of island

biogeography, predicting species richness on islands through estimations of immigration and extinction based on island size and distance from a dispersal source. This model is complicated by factors such as island geology, climate, and topography, as well as origin, whether oceanic or continental; whereas oceanic islands go through *de novo* colonization from various sources, continental island start out with a fully developed ecosystem, further evolving through isolation. Lichens were traditionally considered to include widespread taxa, many cosmopolitan or pantropical; as such, the level of endemism among lichens in island ecosystems has been estimated to be low, usually not exceeding 20%, compared to around 80% for vascular plants. With the advent of molecular phylogeny and DNA barcoding, mounting evidence suggests that presumed widespread taxa in reality consist of various, often unrelated species. Here, we reassessed levels of endemism in island ecosystems using Lobariaceae, a family of conspicuous macrolichens, often confined to well-preserved habitats and including well-known examples of presumably widespread species. We focused on four taxa: the genus *Lobariella*, the *Crocodia aurata* complex, the *Pseudocyphellaria crocata* complex, and the *Sticta filix* complex. We tested the theory of island biogeography on three model systems: New Zealand (continental), Galápagos (oceanic, close to source), and Hawaii (oceanic, far from source), using species richness estimates for Lobariaceae before and after assessment with DNA barcoding. We found levels of endemism in lichens in island biota to be remarkably high, comparable to those of vascular plants, at 70–80%, with examples of local microradiations in *Lobariella* and the *P. crocata* complex. Diversification correlates with niche preferences, with tropical species, such as *C. aurata*, showing reduced levels compared to montane-temperate taxa, such as in the *P. crocata* complex. The *Crocodia aurata* complex exhibits an inverse effect of endemism relative to distance between Galápagos and Hawaii, explained by dispersal stochasticity. Galápagos exhibits a reduced richness of Lobariaceae relative to predictions based on the theory of island biogeography, explained by its predominantly dry climate. The example of the *S. filix* complex in New Zealand highlights the importance of accurate species concepts in the use of lichens as bioindicators of forest health in highly threatened island biota.

S45-4 The Galapagos Lichen Inventory – challenging taxonomic bias to address conservation ecology for a highly diverse group of organisms

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Abstract: Ecuador is one of seventeen countries characterized by the highest species diversity world-wide. With this mega-diversity comes the obligation to preserve this heritage for humanity. One of Ecuador's most emblematic natural heritage sites are the Galapagos Islands. Known for their unique and iconic species as much as for their most famous visitor, Charles Darwin. Much isolated, the number of species in this insular province is significantly lower than on the Ecuadorian mainland. Therefore, the archipelago represents a simplified ecosystem, a 'natural laboratory of evolution', well suited to study biodiversity. A substantial part of species in the Galapagos occur nowhere else on earth, some even restricted to particular islands only. Many species are attractive, famous icons of conservation biology. Yet, despite being in the limelight of global attention, the majority of species in the archipelago remains ignored. Taxonomic bias is a significant obstacle towards developing objective conservation strategies. The biodiversity of lichenized fungi in the Galapagos is one example of a long neglected group. In 1985, Bill Weber from the University of Colorado published the first checklist with 229 species, suggesting that less than 8% were endemic. Today, results from more than ten years inventory research (2005-2018) suggest that at least 960 lichen species occur in this archipelago. Though a significant amount of new reports remain unpublished and more than 100 species still have to be described, the data suggest that at least 20% of Galapagos lichens may be endemic. Today lichenized fungi represent the most species-

diverse of all groups of organisms reported from the terrestrial parts of this archipelago – a result immediately relevant to conservation. Although we better understand general diversity in this group, assessing abundance and species rarity, and characterizing habitat requirements becomes important to objectively assess, which species are threatened. Detailed habitat data collected as part of the inventory can be extremely useful for this task. Characterizing the Galapagos landscape, identifying refugia and biodiversity hot spots is also part of this challenge. A preliminary IUCN red-list assessment of endemic species indicates that many lost their original habitat and only managed to survive colonizing alternative sites within ecosystems that have drastically changed. Conservation management of these systems is typically aimed at restoring an 'original aspect' of the landscape, an objective that potentially conflicts with protecting some endemic lichen species. An example is *Acantholichen galapagoensis*, a basidiolichen that on Santa Cruz Island survives only on an introduced, invasive tree: *Cinchona pubescens*. Restoration strategies that include mechanical and chemical control of this tree need to be balanced against potential population loss of *Acantholichen*. Preserving population refugia of rare lichens becomes even more urgent, if climate change further amplifies environmental stress. A phenomenon most recently observed *in situ* was the 2016-17 El Niño, causing an exceptional drought in the Galapagos humid highlands, resulting in massive population collapse of *Acantholichen*. This is just one example that illustrates how restoration ecology can no longer afford to focus on iconic species only. Conservation strategies must be designed to address and eliminate taxonomic bias.

S45-5 Islands as promoters of lichen diversity: Case studies from the Peltigerales

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Abstract: Biologists have long been fascinated by island biogeography, and their understanding of the evolutionary processes associated with insular regions naturally increases over time. Because rates of speciation are generally higher on islands than on their continental counterparts, due to adaptive radiations following initial colonization, islands are usually seen as hotspots of biodiversity. Insular radiations within fungal taxa, including lichenized fungi, are poorly explored, and hence the hypothesis that symbiotic fungal species can radiate on islands or even evolve in unique, endemic, lineages remains basically untested. Here we review three studies from the Peltigerales (Lecanoromycetes, Ascomycota), that highlight how islands can act as important promoters of biodiversity of lichenized fungi. Using multilocus sequence data and model-based phylogenetic methods, along with molecular-clock dating, we wish to establish the biogeographical history of each fungal lineage considered here: *Dendriscosticta* (Lobariaceae) on Taiwan, *Nephroma* (Nephromataceae) in Macaronesia, and *Sticta* (Lobariaceae) on Madagascar and the Mascarenes. Our results reveal remarkable cases of insular endemism and radiation within the Peltigerales, and emphasize the importance of insular regions in fungal biogeography. We show here that these diversification events, although often substantial, may appear concealed by our inability to distinguish closely related species based on morphology.

S45-6 How did the endemic fruticose lichens on St Helena evolve?

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Abstract: St. Helena is the most isolated island on earth. It is situated halfway Africa and America, with no other islands within a radius of 1300 km. The island is very stony and home to many lichens. Almost all rock surfaces are covered with *Ramalina* and *Roccella* species. Despite the fact that St Helena was

always visited by exploring expeditions, and people like Darwin, Cook and Wallace collected there, the lichens remained largely unstudied until the first lichenologist (me) visited there in 2006. The dominant fruticose lichens, and many crustose ones, turned out to be mostly endemic species. They are mostly not considered threatened, as they are locally abundant, even though the whole of St Helena is a scanty 122 km^2. There are no less than four endemic species of *Ramalina*, one of which can attain a length of 80 cm and (probably) a considerable age. One of the *Roccella* species is endemic, and there furthermore is an enigmatic Roccellaceae that is described in the genus *Dolichocarpus* (otherwise only known from the type from Chile) and represents a fruticose *Enterographa s. lat*. Sequencing allowed to reconstruct the history of these enigmatic species. The four endemic *Ramalina* species are closely related (though very different in morphology) and have originated on St Helena, evolving from a common ancestor that arrived there from the North from Macaronesia. The endemic *Roccella* is a non-sorediate species that is usually represented by isolated, gnarled, and probably long-lived specimens that occurs among the abundant sorediate species that is shared with W. Africa. Here the story is slightly different: Although in many other cases sorediate species are secondary to non-sorediate ones, here the situation is the other way around. One lineage of the sorediate *Roccella* species lost its ability to form soralia and hence became stuck on St Helena. Just because it is stuck there, it is a separate species, as its evolutionary fate differs from the sorediate parent species. The history of the *Dolichocarpus* is still mysterious. It is known from two small overhanging rock faces and one of the rarest lichens on earth. In addition to the endemic fruticose lichens, there is a whole range of wider distributed fruticose species on St Helena, including e.g. Macronesian *Ramalina* species like *R. maderensis*, and palaeotropical *Ramalina*, *Usnea* and *Roccella* species. Now that an airport recently opened on the island, conservation measures for the endemic species are becoming urgent as tourism is rocketing.

Symposium Session 46:

Unraveling Ecology and Taxonomy of Fungal Endophytes for Benefits to Humankind

C. Schardl and M. Stadler

S46-1 Correlations between biodiversity and secondary metabolism in endophytic and saprotrophic Xylariales

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Abstract: The Xylariales are arguably among the predominant fungal endophytes, which are the producer organisms of pharmaceutical lead compounds including the antimycotic sordarins and the antiparasitic nodulisporic acids, as well as the marketed drug, emodepside. Moreover, many Xylariales are "macromycetes", which form conspicuous fruiting bodies (stromata), and the metabolite profiles that are predominant in the stromata are often complementary to those encountered in corresponding mycelial cultures of a given species. Secondary metabolite profiles have recently been proven highly informative as additional parameters to support classical morphology and molecular phylogenetic approaches in order to reconstruct evolutionary relationships among these fungi. Even the recent taxonomic rearrangement of the Xylariales has been relying on such approaches, since certain groups of metabolites seem to have significance at the species, genus or family level, respectively, while others are only produced in certain taxa and their production is highly dependent on the culture conditions. The correlations between biological and chemical diversity in this fungal order will be demonstrated, based on some striking examples. Furthermore, future challenges in their exploitation for applied

mycology will be outlined. Those include the studies of their volatiles, as well as the exploitation of their secondary metabolome, using methods of bioinformatics, phylogenomics and transcriptomics. A major challenge for the future will be the development of concise, diagnostic PCR-based methods to identify the endophytic stages to species rank and elucidate their life cycle, using better-suited DNA loci than ITS sequence data, which have proven highly unreliable, leading to bad scientific practice and hence, chaos in the literature. The talk is based on the review "Diversity of biologically active secondary metabolites from endo-phytic and saprotrophic fungi of the ascomycete order Xylariales" by Soleiman E. Helaly, Benjarong Thongbai and Marc Stadler (submitted to Natural Products Reports).

S46-2 *Epichloë* in South America: how many, where and why?

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Abstract: Some grasses in the subfamily Pooideae establish symbiotic associations with endophytes of the genus *Epichloë* (Clavicipitaceae). Whereas some species produce stromata with perithecia that abort the development of the florets causing total or partial sterility in the host, in other cases the endophyte colonizes the ovary to be spread in the seeds of the host. This association is considered to be mutualistic because the endophytes may provide the host with enhanced growth and resistance to abiotic stresses and produce alkaloids against herbivores. In this work, we studied the genus *Epichloë* in native grasses from South America, considering its diversity, host range, distribution and effect for the host plant. The presence of *Epichloë* was studied in field collected plants and herbarium material. When possible, the endophytes were isolated and characterized morphologically and genetically by mean of phylogenies of nuclear genes and detection of alkaloid-biosynthesis genes. The distribution of the different lineages was modelled using climatic variables. The effect of *Epichloë* on the growth of some hosts and its capacity to establish associations with mutualistic, pathogenic and soil fungi was studied by comparing *Epichloë*-infected plants (E+) with *Epichloë*-removed plants (E-). In Argentina and Uruguay, where surveys were performed more intensively, *Epichloë* was detected in 41 grass species, and at least eight more hosts species were identified along Cordillera de los Andes from Venezuela to Argentina. Although sexual species of *Epichloë* have not been reported from this region, molecular phylogenies have revealed that all the host species are associated with endophytes that evolved from the hybridization between different sexual species. Gene sequences and genetic profiles revealed at least 19 genotypes grouped in eight different lineages. In general, each host species can be associated with different endophytes and different endophytes may co-exist in the population as observed in *Phleum alpinum* or *Bromus pictus*, or to present different distribution areas as with endophytes of *Bromus auleticus*. Wide host-range endophyte lineages, endophyte diversity in individual host species, and relationships between endophytes from different host species in the same community suggest the occurrence of horizontal transmission between hosts, multiple independent hybridization events, or both. In *Bromus setifolius* and *B. auleticus* the endophyte increases plant growth and promotes seed germination. The association with *Epichloë* promotes mycorrhizal colonization and growth and confers resistance to the smut fungus, *Ustilago bullata*. *Epichloë* endophytes have also impact on the diversity of other endo-symbionts such as dark septated endophytes, mycorrhizal fungi, endophytic actinomycetes, and on free living soil fungi. Considering the high diversity of grasses and environments as well as the recent and fast radiation of the Pooideae in South America, it is likely that many other host and *Epichloë* species remain to be discovered in South America, a region that offers the opportunity to study evolutionary and ecological aspects of the symbiosis between grasses and *Epichloë* endophytes.

S46-3 Amazing alkaloid diversity in Clavicipitaceae! How and why?

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Abstract: Plant-associated members of the fungal family Clavicipitaceae – including species of *Balansia, Claviceps, Epichloë, Metarhizium, Periglandula* and others – are famous for the production of alkaloids belonging to five different classes: ergot alkaloids (named for the "ergot" sclerotia of *Claviceps* species), indole-diterpenes (e.g., lolitrems), aminopyrrolizidines (e.g., lolines), pyrrolopyrazines (e.g., peramine) and the indolizidines (e.g., swainsonine). As the genes and much of the pathways for biosynthesis of these alkaloids have been elucidated, it has become evident that profiles of alkaloids are extremely diverse among species and among strains, and also with respect to the alkaloid classes and specific end- and spur-products within each class. Furthermore, genomic studies have revealed details of several genetic mechanisms for evolutionary diversification. Surprisingly, loss of certain genes in alkaloid clusters is a very common mechanism to generate variation in alkaloid profiles. For example, some fungi have as many as 14 ergot alkaloid genes for biosynthesis of ergopeptines and other lysergic acid amides, whereas others have only 12, 11 or nine genes, or just the genes for the first four steps resulting in chanoclavine. Analogous situations are evident in the indole-diterpene and aminopyrrolizidine gene clusters. Another diversifying mechanism, gene duplication and neofunctionalization, has characterized certain enzyme classes including nonribosomal peptide synthetases (NRPS) for biosynthesis of ergopeptines and lysergyl amides (ergot alkaloids), as well as cytochrome P450 monooxygenases and prenyltransferases for biosynthesis of indole-diterpenes, thereby contributing to the huge chemical diversity of these alkaloids. Additionally, trans-species polymorphism is a feature of the loline alkaloid and pyrrolopyrazine biosynthesis genes, and in the latter case has worked in concert with interallelic recombination to give an array of pyrrolopyrazines in addition to peramine. Although horizontal gene transfer has been suggested for some of the alkaloid gene clusters, evidence is consistent with recombination, neofunctionalization, diversifying selection and perhaps frequency-dependent selection as causes of their unexpected phylogenies and distribution patterns in the fungi.

S46-4 An ash dieback pathogen, *Hymenoscyphus fraxineus*, endophytically inhabits leaves of manshurian ash in Japan

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Abstract: A helotialean ascomycete, *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al., causes a lethal disease known as 'ash dieback' in the common ash *Fraxinus excelsior* of Europe. The origin of this fungus is considered to be East Asia. The same fungus was found on leaf litter of manshurian ash, *F. mandshurica*, in Japan and reported to produce apothecia on pseudosclerotial plates formed on decomposing leaves. However, occurrence of dieback disease has not been reported in Japan. Therefore, the life cycle of *H. fraxineus* is largely unknown. The aim of this study was to clarify the behavior of *H. fraxineus* on *F. mandshurica* in Japan. Healthy leaves were collected from an adult tree and *F. mandshurica* planted at the Sugadaira Research Station of the University of Tsukuba in Nagano

Prefecture, Japan for every one to two weeks from July to October. The sporulation period of the fungus is from middle July to early September at this site. Collected leaves were processed by surface sterilization method with sodium hypochlorite solution (1% available chlorine) to isolate endophytic fungi and by washing method with 0.005% aerosol OT to isolate both epiphytic and endophytic fungi. Isolates were identified based on morphology and sequence analysis of rDNA ITS regions. To detect the presence of *H. fraxineus* inhabiting leaves, DNA was extracted from leaf samples followed by DNA-based fungal species-specific real-time PCR assays by amplifying rDNA ITS regions with specific primers for *H. fraxineus*. In addition, the behavior of the fungus on fallen leaves was observed by using continuously retrieving fallen leaves from litter bags placed in the field. Results showed that one strain isolated by the surface-sterilization method was *H. fraxineus*. By the DNA detection test, the frequency of fungal DNA was low on fallen leaves collected from July to September, but the frequency sharply increased on adult tree leaves collected in October before defoliation. A high concentration of DNA was detected from all leaflets and rachises. Observations of fungal behavior on the fallen leaves revealed *Chalara* anamorph of *H. fraxineus* on blackish mycelial structure formed on the surface of rachises retrieved in December. Subsequently, a pseudosclerotial layer was found under the cortical layer in the rachises retrieved in January, and then, gradual degradation of the cortical layer was observed on rachises retrieved from February to June. Finally, a blackish pseudosclerotial plate appeared on the rachises. The near-UV light irradiation test successfully induced apothecia were on rachises retrieved from the litter bags in November and December. These results suggest that fertilization occurs swiftly after defoliation. In conclusion, *H. fraxineus* infects the living leaves of *F. mandshurica* by ascospores and endophytically inhabits the leaves until defoliation, and the fungus behaves saprophytically on fallen leaves after fertilization and produces apothecia on pseudosclerotial plates formed on the decomposing leaves.

S46-5 Description of *Darksidea*, a novel genus within Pleosporales, and evaluation of their effects on grasses

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Abstract: Dark septate endophytes are dominant colonizers in arid plants. Pleosporalean fungi that belong to the genus *Darksidea* were recently described, yet their distribution and function in semiarid grasses remains poorly known. The objective of this study was to describe novel *Darksidea* fungi and evaluate their effect on semiarid grasses. A total of 24 sites and 6 grass species were sampled across grassland ecosystems in central south states in the United States. We used a collection of 77 *Darksidea* isolates and characterized them using light microscopy and culturing in different media. Fungi were identified using the ITS rRNA region. The resulting sequences were compared with curated databases such as the Ribosomal Database Project and UNITE. Among the 77 congeneric *Darksidea* isolates, we identified a total of eight Operational Taxonomic Units (OTUs) defined at 97% similarity, representing likely yet unidentified diversity within the genus. Using representative isolates from each OTU, five potential novel clades of *Darksidea* were identified. Most isolates were obtained from the southwest field sites in Texas and were abundant in three of the sampled plant species (*Bouteloua dactyloides, B. eriopoda* and *B. gracilis*). The potential function of the fungal isolates was assessed using grass germination bioassays to evaluate direct contact and volatile organic compounds effects. *Darksidea* isolates vary in their role to improve plant growth and survival after 30 days. However, most fungal isolates produced VOCs that enhanced plant growth.

S46-6 There's treasure everywhere – putatively overlooked slow-growing fungi isolated from cereal cyst nematodes produce nematode-inhibiting compounds

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Abstract: Cereal cyst nematodes (CCNs) can lead to significant yield reductions of grains. The use of nematicidal chemicals is banned in many countries due to their generally high non-target toxicity. Therefore, antagonistic microorganisms controlling nematodes are an important alternative. The search for such microorganisms, especially nematophagous fungi, has a long history extending back some 150 years. These studies provided a long list of nematode-associated fungi some of which showed great potential to be exploited as biological control agents. Microscopic observations of cyst samples of the CCN Heterodera filipjevi obtained from wheat fields in Turkey regularly revealed nematode cysts, which displayed fungal colonisation. The aim of our study was to (i) isolate the fungi from the nematode cysts and fulfil Koch's postulates (ii) to classify the isolated fungi using light microscopic and molecular phylogenetic analyses (iii) to study the nematode-fungus interaction microscopically, (iv) to isolate and identify secondary metabolites produced by these fungi. Fungi were isolated from symptomatic cysts applying a specific single-egg isolation technique developed for this study. Fungal strains were identified using morphological studies and multi-locus molecular phylogenetic analyses. To fulfil Koch's postulates, the pathogenicity of isolated fungal strains was examined against nematode eggs in vitro. Secondary metabolites of fungal isolates of interest were extracted and purified using EtOAc, and HPLC-based techniques. The bioactivity of obtained compounds was evaluated using nematode bioassays. This approach resulted in finding six new fungal species. All species are ascomycetes belonging to the Helotiales, Hypocreales and Pleosporales. The newly described *Ijuhya vitellina* and *Monocillium gamsii* belong to the families of Bionectriaceae and Niessliaceae, respectively. A new fungal genus was proposed to accommodate two new species. One of these, representing a dark septate endophyte (DSE), was isolated from nematode eggs. Two more species were preliminarily characterized as DSEs. These are the first DSEs found to parasitize nematode eggs and they might play a role in the plant defense against nematodes. All newly-found species could be successfully re-isolated from artificially infected nematodes and Koch's postulates were thus fulfilled. Both *I. vitellina* and *M. gamsii* formed microslcerotia within the nematode eggs and in culture. Chaetoglobosin A, 19-O-acetylchaetoglobosin A and four novel compounds, among them cyclodepsipeptides, a class of compounds known for anthelmintic effects were isolated. Nematicidal and nematode-inhibiting activities were demonstrated for the isolated compounds. To conclude, using a specific isolation technique novel fungal species and novel compounds could be discovered from the CCN H. filipjevi that might be harnessed for biological control in the future.

Poster Session 1 • Period 1 • Tuesday, July 17, 2018

1.1-1 Knowledge and use of medicinal and edible mushrooms of the Sierra Tarahumara of Chihuahua, Mexico

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Abstract: Chihuahua is the largest state in Mexico. It takes 12.6% of the country's area. The southwestern portion of the Sierra Madre Occidental in this state also known as Sierra Tarahumara named as it is occupied by an ethnic group known as the Raramuri or Tarahumara, which means "light footed people". The territory consists of canyons and ravines with pine, oak and pine-oak forests in the higher plateaus. There is a great diversity in edible and medicinal mushrooms all-around of the Chihuahua state counties. Their residents are the only consumers of wild mushrooms in the Northern Mexico; they have a long tradition of collecting, using and eating them during the "rainy season." However, despite the wide diversity of edible mushrooms that grow in these areas, residents have a selective preference. This paper aims to record evidence of the knowledge and use of wild potentially edible and medicinal mushroom species by inhabitants of towns in Bocoyna and Urique municipalities of Chihuahua, Mexico. In the forests of the Sierra, there are records of around 450 species of fungi; 50 of them with edible importance at nationwide and apparently only 16 fungi species of those 50 are being consumed by the inhabitants of the municipalities of Bocoyna and Urique with Amanita caesarea complex being the most preferred by mestizos and Raramuris. We observed no apparent differences in the population studied in terms of gender, occupation, or language, regarding the recognition and consumption of species; however, this is not conclusive and so it is important to continue with a greater number of such studies to check whether this knowledge and use is differential. Forty eight percent of the people surveyed reported to collect mushrooms directly from the field or forest areas while the others buy them either from the Raramuris who sell them on the side roads, or at their home as a result of door to door to selling. Seven percent mentioned the "Fungus Fair," which is carried out every year in the month of August provides them with a good opportunity to buy mushrooms and reassures them they are edible. Three percent mentioned that besides selling them, they teach other people how to handle mushrooms in the place known as The Valley of the Mushrooms. As medicinal mushrooms, three species are used for the purpose of healing wounds of the skin, and to remove pimples in the face: *Calvatia, Lycoperdon and Astraeus hygrometricus*.

1.1-2 Edible mushrooms in Mizoram, India: Occurrence and perception in the region

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Abstract: Mizoram is regarded as one of the biodiversity hotspots of the world. A diverse group of flora and fauna have been documented. Knowledge on the edible mushrooms is very limited in the region. Moreover, the local knowledge and perception on the edible species of the region is very low. Knowledge of edible mushrooms among the Mizo people has been there for a long time. However, it has been perceived that the number of edible species known by the people is very few. A study of the

occurrence of the edible mushrooms growing in Mizoram was undertaken. From the study a total of 32 species of edible mushrooms was identified from different districts of Mizoram. Study on the local knowledge and perception on the edible mushrooms was also undertaken from different sections of the local communities. It is found that a small percentage of edible species are presently known to be edible by the Mizos despite the existence of other edible species and distribution in the region.

1.1-3 Determining seasonality and local abundance of edible mushrooms in Puerto Rico using social media as a tool

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Abstract: The knowledge of edible mushrooms in Puerto Rico is limited at least in part due to cultural attitudes, which could be largely characterized as locally "mycophobic". For the past three years, I collected information on the mushrooms that are found in the island, especially edible species in the genera *Cantharellus*, *Calvatia*, *Lentinula,* and *Laetiporus*. These genera are easy to identify based on macromorphological traits and therefore lend themselves well to analyzing photos uploaded by users of a Facebook group dedicated to local mushrooms. These data are supplemented by observations from mushroomobserver.org, as well as inventories on cybertruffle.org. The data are therefore a blend, ranging from local residents' photos to established scientific inventories with a total of 150 observations between the four genera. The goal is to answer questions of seasonality, location and abundance of local edible mushrooms, the harvesting of which is of potentially significant commercial interest. The downfall to this "crowdsourced" data is, of course, demographics, as much of the population lives in the Northeast. It is therefore to be expected that a disproportionate number of observations will come from this region. Puerto Rico is divided into 78 municipalities, which is noted, along with the date and ID, for each observation. For each species of edible mushroom, the data are displayed showing where on the island and during which months a species is most likely encountered. The data shows that *Cantharellus coccolobae* occur mainly in coastal beaches, associated with *Coccoloba uvifera*, but they were also observed growing in karstic areas on the north coast of the island, in the presence of other *Coccoloba* species, such as *C. diversifolia*. Although *C. coccolobae* occurred year-round, they were especially abundant during the "rainy season" (April-November), a pattern repeated for all species treated here. *Calvatia cyathiformis* is the only recorded *Calvatia* species on the island. Its distribution is cosmopolitan within Puerto Rico, but the records show that it prefers grassy pastures and it has adapted well to the metropolitan area and its abundance of maintained lawns. *Lentinula boryana*, or Florida shiitake, proves exceedingly rare or at least is not well documented. This is most likely due to its occurrence in inland municipalities with late successional tropical forests, such as El Yunque; habitat which is not common on the island, and is at once sparsely populated and difficult to traverse, and more observations are needed for this species to determine its seasonality. *Laetiporus caribaea* is observed in similar habitats as *Lentinula boryana*, but are more frequently documented, which could be due to their striking orange-red fruiting bodies.

1.1-4 The knowledge of future teachers of science and biology in basic education about the fungi

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Abstract: Although mycology integrates a component of great relevance in basic education, being present in different moments of the national curricular guidelines or the reference curriculum of the states, recent studies show that this content has been neglected in the courses of teacher training for that level of education, in Brazil. The objective of this study was to investigate the knowledge about fungi among the final students of the Biological Sciences Degree courses in the state of Goiás, Brazil. We sampled 10 of the 32 courses existing in the state, so that the sample universe totaled 123 students, belonging to the last period of these courses. The data collection was done from a semi-structured questionnaire applied to the participants. The analysis of the data shows that most of the future teachers have less knowledge than they should have, lacking in depth, with conceptual inaccuracies, presenting difficulty in developing critical and logical reasoning in formulating answers, besides they showing an anthropocentric view, in which fungi are at the service of the human species, with little attention to their interactions and their ecological role. The best performance was observed among students from courses that offer specific discipline for Mycology content. These frailties are incompatible with an efficient scientific training. Since the quality of the teaching in basic education is intrinsically linked to the quality of the training of the teachers who work in it, the courses in Biological Sciences require special attention in regard to the approach to this subject, in order to promote the consolidation of mycological knowledge among the educators they make available to society.

1.1-9 Cultivation of *Polyporus squamosus* on substrates from residues of wood processing industries in Finland

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Abstract: Demand for non-meat protein sources has grown extensively in Finland as well as in rest of Europe. In Finland wood processing industries produces vast amounts of residues, which could be suitable for production of edible mushrooms thus increasing the local protein production as well as harboring circular economy. Our aim in this study was to test cultivation of *Polyporus squamosus* on substrates from wood processing industries, and evaluate the feasibility of cultivation. *Populus tremula* and *Betula pendula* sawdust and chips were used as substrates. Two strains of *P. squamosus* originating from Finland were used in this study. The substrate bags of 1 kg dry weight were filled as follows: 1) 40% of wood sawdust and 60% of wood chips in the case of *P. tremula* species 2) *B. pendula* substrate bags were filled with 100% wood chips 3) Used coffee and rye bran were tested as nutritional additives, 20% of the total substrate bag weight for both nutrients and wood chips from the two species. A total of ten replicates per each substrate formula were inoculated with 150 ml of two strains of *P. squamosus* barley spawn. When the substrate bags were completely colonized, they were kept at 26˚C with a relative humidity of 80% to support fruiting body formation. The biological efficiency was calculated considering the kilograms of fresh fruit body per kilogram of dry substrate. First flush was first observed one month after inoculation, suggesting that crops can be obtained within 2 months. The substrate formula that was found to be the most suitable for fruit body formation was the one containing 20% of rye addition in both *B. pendula* and *P. tremula* wood residues. Differences between mycelium growth rates were

observed between the fungal strains, emphasizing the importance for strain selection most suitable for commercial cultivation in future. Our results suggest that *B. pendula* and *P. tremula* wood residues serve as equally suitable substrates which can be utilized for the cultivation of *P. squamosus*.

1.1-10 *Pleurotus pulmonarius* production optimized on oil palm bunch

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Abstract: This study was conducted to study the yield and some macro-morphological characters of *Pleurotus pulmonarius* fruit bodies cultivated on Hydrochloric acid (HCl) optimized oil palm bunch (OPB) substrate. Concentrated HCl was diluted in tap water at 0.1%, 0.2%, 0.3%, 0.4% and were used to induce changes on the initial pH (9.5) of OPB to 8.9, 8.2, 7.9, 6.2 and control (9.1) respectively; after soaking for 48hrs. One way Analysis of variance (ANOVA) and Correlation test were adopted for data analysis. Mean separation was also done by Duncan Multiple Range Test (DMRT) at probability level of 5%. Results showed that 0.1%, 0.2%, 0.3% and 0.4% HCl treated OPB substrates produced *P. pulmonarius* primordia after 9, 9, 10, 11 and control (12days) respectively. Results further revealed that 0.4% HCl treated OPB substrate induced the highest (900g/kg) fruit body yield and Biological Efficiency (90%) while control (493g/kg and B.E 49.3%) respectively, produced the lowest quantity of fruit bodies. Some macro-morphological characters of harvested fruit bodies revealed that mean cap size (C.Scm) and Weight (wt.g/kg) of fruit bodies were highest (3.83cm and 3.5g/kg) in 0.4% HCl treated OPB respectively. Mean Stipe Length (S.Lcm) was highest (2.77cm) in 0.3% OPB substrate and was significant at $p \leq 0.05$. S.L and C.S of fruit bodies as well as C.S and Wt. were significantly correlated while there was no correlation between S.L and Wt. of fruit bodies. HCl was found as a suitable acid buffer for the optimization of the pH of the highly alkaline OPB for cultivation of *P. pulmonarius* fruit bodies. Oil palm bunch should therefore be adopted in the commercial production of the Oyster mushroom if certified safe for human consumption.

1.1-11 Utilization of Amazonian waste wood for the production of the edible mushroom *Pleurotus ostreatus* and *Lentinus strigosus* in the Amazon

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Abstract: Found in the Amazon, *Pleurotus ostreatus* and *Lentinus strigosus* are not widely cultivated. Like other edible mushrooms, the use of these fungi to convert regional waste is of little cost and is recommended. They are able to grow in the wood waste of *Simaboura amara* (marupá) and *Anacardium gigantium* (cajuí), supplemented with a mixture of bran: 75% rice (*Oryza* spp.), 20% wheat (*Triticum* spp.) and 5% corn (*Zea mays*). The following formulation was made for the two substrate of wood waste: sawdust (68%), mixture of bran (30%) and calcium carbonate (2%), the same being homogenized and humidified to 75%. All material was kept in a growth chamber in the dark at a constant temperature of 25°C and humidity of 80% until full establishment. Then, a photoperiod of eight hours created the stimulating condition for the production of the mushroom. The following were main areas of evaluation: biological efficiency (BE,%), yield (g kg^{-1}) and organic matter loss (OML%). The results showed that the formulation made with cajuí obtained better results with EB at 221.17% (*P. ostreatus*) and 104.88% (*L. strigosus*); Yield: an average of 220.23g kg^{-1} (*P. ostreatus*) and an average of 72.5g kg^{-1} (*L. strigosus*); OML was not significantly different for the two types of substrates used. The cultivation of *P. ostreatus*

and *L. strigosus* reveals that this is a promising raw material due to its great local availability at low to no cost. In addition, production of the mushroom reduces pollution of the environment.

1.1-12 DNA-based identification of consumer-relevant mushrooms: A partial solution for product certification?

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Abstract: Attributing the correct scientific name to dietary ingredients made from fungal materials remains a challenge, in part due to difficulties in species authentication by chemical means and the nature of fungal taxonomic names, which are undergoing numerous taxonomic revisions with the application of molecular methods. This can be particularly difficult for samples that contain fungal mycelia, where morphological characteristics do not present sufficient variation to differentiate species. This challenge is compounded by the fact that many of those materials maybe heavily processed, including drying, milling, and even extraction, prior to analysis. However, monitoring the safety and quality of such products is a requirement for the protection of consumer health. The main goal of the study, which was performed as a collaboration of academic researchers (University of North Carolina at Greensboro) and industry scientists (Procter and Gamble), was to demonstrate that Sanger sequencing of the ITS region is an appropriate means for verification of species identities. We generated ITS barcodes for 33 representative fungi, which are being used by consumers for food and dietary supplement purposes. After generating ITS barcodes utilizing standard procedures accepted by the Consortium for the Barcode of Life, we tested the utility of the ITS by performing a BLAST search against NCBI GenBank. In some cases, we also downloaded published, homologous sequences of the ITS region of fungi inspected in this study and examined the phylogenetic relationships of barcoded fungal species in light of modern taxonomic and phylogenetic studies. In the majority of cases, we were able to sequence the ITS region from powdered mycelium samples, grocery store mushrooms, and capsules from commercial dietary supplements. Results demonstrated that the ITS region was able to identify the mushrooms used in the present study to species-level. We anticipate that these data will motivate a discussion on DNA based species identification, particularly as it applies to the verification/certification of fungal containing products.

1.1-13 Characteristics of a new cultivar *Grifola frondosa* "Daebak" with bottle cultivation

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Abstract: We aim to introduce a new cultivar of *Grifola frondosa* by crossing of mono-spore. The name of this cultivar is 'Daebak', it means jackpot. As in the case of control cultivar 'Cham', temperature of mycelial growth and fruiting of the 'Daebak' were also same at 25°C and 18°C, respectively. The incubation period was 57days, two days shorter than that of the 'Cham' by bottle cultivation. The rate of fruiting for the 'Daebak' was 98.4%, which was 24.8%p higher than that of the 'Cham'. In addition, the coefficient of variation for the 'Daebak' was 0.6, which was lower than the 'Cham' 5.3, resulting uniform fruiting. The L-value of pileus for the 'Daebak' was lower than that of the 'Cham'. The diameter of pileus and length of stem for the 'Daebak' were larger and higher than those of the 'Cham', respectively. Physical properties (strength, springness, and brittleness) of this cultivar were lower than those of the

'Cham'. The fresh weight of this cultivar was 139g/1,100㎖ and was 28% higher than that of the 'Cham'. Additionally, the new cultivar has greater uniformity due to the coefficient of variation in the quantity being lower than the 'Cham'. Shelf life of this cultivar at 4 °C was 42 days and 6 days longer than that of the 'Cham'. In conclusion, new cultivar 'Daebak' of *Grifola frondosa* was in quantity, quality and storage compared to the previous cultivar 'Cham', but also need to be bred with a more physically strong cultivar for the future.

1.1-14 Analysis of nutritional and neutraceutical properties of selected wild-grown mushrooms of Nepal

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Abstract: Mushrooms are the fleshy spore-bearing fruiting bodies of fungi. Wild mushrooms are source of many different nutraceuticals such as unsatured fatty acids, phenolic compounds, tocopherols, ascorbic acid, carotenoids and alkaloids and nutrients such as proteins, fats, ash, fiber, moisture and carbohydrates. Nepal possesses diverse phytogeographical zones related to altitude and other factors, and rich in wild mushrooms. The information and knowledge about nutritional and nutraceutical values of wild mushrooms is limited and poor. Therefore, the present study is undertaken to document the use of wild edible mushrooms and analyze their nutritive values. Herein, it was reported and compared the nutritional value and neutraceutical values of the wild mushroom species; *Laetiporus sulphureus*, *Polypore* sp., *Trametes elegans*, *Trichaptum biforme, Lenzites betulina, Stereum complicatum, Trametes versicolor, Trichaptum subchartaceum,* and *Ganoderma Lucidium*. *The* nutritional and nutraceutical properties analyzed according Association of Official Analytical Chemists (AOAC) and spectrophotometrically respectively. These mushrooms (samples) were rich in proteins (6.8- 60.23%) and fibers were range from 0.174 - 36.38% and contained fat range from 3.642- 14.6%. The carbohydrate contents ranged from 7.058 to 59% (on the basis of dry weight). Similarly; ash content and moisture content ranges from 10- 19% and10-16% respectively. The protein content was highest in *Ganoderma Lucidium*. (*G. lucidium*) and lowest in *Trametes elegans (T. elegans)*. The fat content was highest in *L. sulphureu* and lowest in *G. lucidium*. The analysis revealed that the total phenolic contents ranged from 3.95 to 10.05 mg ml^{-1}. Similarly, the total flavonoid contents ranged from 2.149 to 11.36 mg ml^{-1}. The result indicated the high levels of antioxidants activity thus making mushrooms suitable to be used as functional foods or nutraceutical sources. Therefore, this study provides new information regarding chemical properties of wild mushrooms, which is very important for the biodiversity characterization.

1.1-15 Productivity of edible *Amanita* at Phusing Agricultural Development Center, Sisaket, Thailand

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Abstract: The cultivation of edible ectomycorrhizal mushrooms associated with forest trees is becoming popular in Thailand. It is currently applied to reforestation projects by forestry officials, and in agroforestry situations by farmers. However, information on the mushroom productivity and sustainability is unavailable. This study investigated and reports on the yield of edible *Amanita* and other

wild edible mushrooms in a 1280m^2 plot at Phusing Agriculture Extension Center, Sisaket Province between 2014 and 2016. The plot was on an area mainly covered by *Dipterocarpus alatus* trees, which were planted on bare land in 2003 and were inoculated with *Amanita* during 2004-2005. In 2014 eight edible mushroom species (total weight 72.6 kg) were found in the plot. Three *Amanita* spp. (60.2 kg, 83%) were the dominant group: red *Amanita* (*Amanita* cf. *hemibapha*; 43.2 kg, 59%), yellow *Amanita* (*Amanita* cf. *hemibapha*; 15.7 kg, 22%), and white *Amanita* (*Amanita* cf. *princeps*; 1.3 kg, 2%). These *Amanita* species morphologically resemble *Amanita hemibapha*, and *A. princeps*, but molecular data based on ITS and LSU show that they are taxonomically new to science, and are currently being described. Other inferior mushrooms found in the plot were *Russula nigricans* (8.1 kg), *Termitomyces microcarpus* (2.4 kg), *Lactarius* sp. (1.5 kg), and *Russula emetica* and *Russula* sp. (less than 1 kg). In 2015, two additional mushroom species, *Russula virescens* and *Termitomyces* sp., were also found, but the total yield of the plot was stable (72.1 kg). Yellow and white *Amanita* increased their yields (40 kg, 56% and 6.3 kg, 9%) but red *Amanita* sharply decreased (2.5 kg, 3%) resulting in decline of the total *Amanita* yield (48.8 kg, 68%). In 2016, nine edible ectomycorrhizal mushrooms were found with the total yield 73.6 kg. *Russula nigricans* became the dominant species (38.4 kg, 52%) while the *Amanita* group (31.6 kg, 43%) decreased: yellow *Amanita* (25.9 kg, 35%), white *Amanita* (3.6 kg, 5%), and red *Amanita* (2.1 kg, 3%). In 2017, seven edible mushrooms were found with the total yield only 17.1 kg. *Yellow Amanita* became the dominant species (6.7 kg, 39%), red *Amanita* (4.5 kg, 26%), white *Amanita* (3.0 kg, 17%), while *Russula nigricans* decreased (0.8 kg, 5%). The presentation will show monthly yields of each mushroom species and some environmental factors throughout the last four years. Declining trend of the *Amanita* productivity, as well as possibility of rehabilitation in correlation of environmental factors, will be also discussed.

1.1-16 Mushroom poisonings in South China and study on the toxin genes of lethal *Amanita*

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Abstract: Poisonous mushrooms are the main factor causing the fatal disasters in food poisoning incidents in China. According to the statistical data of Chinese Center for Disease Control and Prevention, the numbers of deaths from mushroom poisonings were 25-55% of the total numbers of deaths from food poisonings from 2004 to 2014. South China is a high-risk area for mushroom poisonings, the authors investigated and analyzed 113 mushroom poisoning cases in South China from 2000 to 2014, which involved 325 patients and 52 deaths, with an overall mortality of 16 %. About 200 poisonous mushrooms have been reported from South China. More than 50% poisoning cases were caused by *Amanita exitialis* Z.L. Yang and T. H. Li and *Chlorophyllum molybdites* (Meyer: Fr.) Mass., but all the poisoned deaths were caused by lethal *Amanita*. Cyclopeptides are the main fatal toxins in lethal *Amanita*, which encoded by MSDIN family. Based on the transcriptome and genome sequencing with Illumina HiSeq 2000, the author studied the toxin gene family and *POPB* involved in the toxin biosynthesis of the lethal *Amanita* from South China. The results showed that 70 different toxic gene family members were obtained from three lethal *Amanita* species, which encoded 3 toxic peptides (α-amanitin, β-amanitin and phallacidin) and 45 new unknown peptides. The research showed that lethal *Amanita* can produce abundant toxic peptides and related peptides, which will lay a strong foundation for the toxic peptide gene expression and exploitation of new cyclopeptide resources. This work was

funded by grants from the National Natural Science Foundation of China (Nos. 31470155 and 31670018).

1.1-25 A study of the biodiversity and secondary metabolites of fungal endophytes from medicinal plants in Guizhou

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Abstract: In southwest China, Guizhou province has a well-known fame for its prolific biodiversity of medicinal plants. For solving the problems of plant shortage, environmental disruption and obtaining novel structural as well as bio-active natural products, we have been studying the fungal endophytes and their secondary metabolites from medicinal plants in Guizhou province for a number of years. We isolated over five thousand fungal strains from medicinal plants including *Artemisia carvifolia, Artemisia japonica, Blumea balsamifera, Camptotheca acuminata, Dendrobium orchids, Ginkgo biloba, Nothapodytes pittosporoides, Reineckia carnea, Taxus brevifolia*. Dozens even hundreds of different fungal isolates were obtained from each plant species. Most of the fungal endophytes belong to Ascomycota which mainly distribute in Pezizomycetes, Dothediomycetes and Sordariomycetes. A few of them are classified into Basidiomycota and Zygomycota. Fungal endophyte taxa were subjected to vary kinds of factors, for example, the age of host, organ, humidity of sample site, altitude, season, surrounding plant, extent of environmental contamination and so on. We combined bio-activity analysis in vitro, chemical composition identification and gene detection in fungal endophyte to screen and evaluate 1003 fungal strains. Fifty-six of them showed anti-inflammatory, antitumor, antioxidant, anti-pathogen and P-gp inhibitory bio-activity to different extent. At present, we accomplished the study for secondary metabolites of ten fungal strains that possess high bio-activity. More than 200 bio-active compounds were isolated. Ten of them have new structures. However, there is a long way to produce bio-active compounds in large scale because of the low production. Therefore, it is necessary to improve cultivation methods or alter inner gene in fungal endophyte by genetic engineering for achieving novel chemical structure as potential new drug.

1.1-26 Diversity of Medicinal Mushrooms of South India

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Abstract: Tropics are considered as rich repositories of mushroom diversity and most of the new mushrooms reported in recent years are from tropics (Hawksworth, 2001). India is one such tropical country with diverse ecological characteristics for species richness. In India, the survey of literature indicates a total number of 1,160 species are only described in these 2 orders viz., Agaricales and Boletales until now (Manjula, 1983, Lakhanpal 1995 and Natarajan *et al.,* 2005). Basidiomycetes especially mushrooms are unlimited sources of biologically active compounds. There are over 700 species of higher basidiomycetes that have been found to possess significant pharmacological activities (Wasser, 2002). Studies on medicinal mushrooms have exponentially increasing in the last two decades. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments (Jong and Birmingham, 1992). In the present study, biodiversity of medicinal mushroom from South India especially in the Eastern Ghats region was taken up in order to fill up the lacuna. The study resulted in documentation of many medicinal mushrooms from these region which include *Ganoderma lucidum*, *Phellinus badius*, *Gymopilus dilepis*, *Gymnopilus palmata*, *Lentinus tuberregium*, *Calocybe Indica*, *Pleurotus ostreatus*, *L. squarrosulus*, *L.*

cladopus, *Mycena pura*, *Macrolepiota rhacodes*, *Termitomyces microcarpa*, *Termitomyces eurrhizus*, *Auricularia polytricha* and so on and evaluation of few.

1.1-27 Ethnomycology and neutraceutical studies of wild edible fungi of Kamrup district of Assam, northeast India

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Abstract: Wild Edible Fungi (WEF), one of the untapped forest resources, serve as an important protein supplement and palatable food of the future. They are actually macrofungi which possess high nutritional value almost twice that of any vegetable or fruit. The indigenous people of Assam are mostly mycophilous and frequently use macrofungi from the wild. The major tribes residing in the area include Bodos, Karbis, Koch-Rajbongshi, Rabha, Khasis along with non-tribal Assamese people. This investigation was conducted in 15 reserve forests of Kamrup district of Assam, India for the period of two years with the aims of studying the types of WEM available in the area and their utilization patterns. In all, 90 macrofungi were recorded, of which 10 different WEF were ethnomycologically important. Here, tribal people usually exploit 5-6 species i.e. *Agaricus bisporus, Cantharellus cibarius, C. lateritius, Lentinus squarosulus, Termitomyces heimii* and *T. microcarpus* as food and 4 species i.e. *Auricularia judae*, *Ganoderma lucidum, Lentinula edodes and Bovista plumbea* in medicine. Three commonly available WEF that were consumed the most in this region as well as sold in the local markets were selected for neutraceutical analysis. They were *Cantharellus cibarius, C. lateritius* and *Lentinus squarusulus*. They were analysed for their macronutrients properties like carbohydrates, proteins, fibre and lipid content and micronutrients like Fe, Ca, Zn, Cu, Pb and Mg. Their physical properties such as moisture content, dry matter and ash content were also measured. The nutritional composition of these three WEF indicates that they are a good source of protein and other nutrients. Among the three species, *Lentinus squarusulus* was found as most nutritious species with maximum amount of carbohydrates, protein and fibre (47.83%, 35.13% and 11.33% respectively) than the other two species. Fat contents were highest in *C. cibarius* (0.62%) followed by *L. squarusulus* (0.58%) and *C. lateritius* (0.52%). The comparison of nutraceuticals of *L. squarusulus* with two widely cultivated species viz., *Agaricus bisporus* and *Pleurotus sajor-caju* shows higher protein (35.13%) and carbohydrate *(*46.63%) content *(*carbohydrate value of *Agaricus bisporus*: 26.29% and protein value of *Pleurotus sajor-caju*: 23.52%), however, crude fibre content was higher in the two cultivated mushrooms. There is a need to develop an easy and fast artificial production protocol of selected WEF to practice in rural area of this region to benefit the local populace at large.

1.1-28 Salt stress on *Ganoderma lucidum*: morphological, physiological and biochemistry aspects

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Abstract: *Ganoderma lucidum* strains are able to produce biologically active polysaccharides. It is widely known that stresses are inducing alteration of metabolome. However, the salt stress effect on *G. lucidum* has not been studied yet. The purpose of this work was to assess the impact of sodium chloride stress on the mycelia *G. lucidum* growth and polysaccharide production. We have examined several strains from our samples for the main work objective determination. An identification of ITS rDNA

sequences has been conducted with a purpose of specifying the taxonomic position of four strains. For further work we selected *G. lucidum* strains capable of forming an alkali-soluble and water-soluble polysaccharides with high antitumor activity and capability to cytokines induction. Preclinical trials of an alkali-soluble highly branched xylomannan are in progress now. NaCl effect on the growth of *G. lucidum* was studied during its introduction into the dense and liquid nutrient culture medium in an amount of 0.5, 1.0, 1.5 and 2.0%. The sharp reduction of fungus colony diameter was observed in medium containing 1.0% or more of sodium chloride. When we added 2.0% NaCl to the dense medium *G. lucidum* colonies diameter was 21.4% of that of the control. IC50 NaCl estimated concentration was 1.45%. When concentration of chloride in the liquid medium was from 0.5 to 2.0%, we observed the gradual decrease *G. lucidum* biomass from 13.3 to 76.5%, respectively. The process was linear. Estimated concentration of the IC50 NaCl for submerged cultivation was 1.48%. In medium containing 12.5 g/l of sodium chloride and above, we observed the formation of brown pigment. The total polysaccharides in *G. lucidum* mycelium have been gradually reduced to 84.6% with 1,0% NaCl, and then have been increased to 171, 6% with 2,0% NaCl to the control. We assessed the changes of the content of monosaccharides as part of studied polysaccharides. Increase of sodium chloride concentrations in the liquid medium to 1.0% has been accompanied by an increase of the total protein in the mycelium to 167.2% compared with that in the control. Further increase of NaCl in the liquid medium to 2.0% was accompanied by a gradual decrease in protein up to 137.7 % to the benchmark. We used the method of light microscopy and method of scanning electron microscopy to reveal micromorphological changes of immersed *G. lucidum* mycelium when it is grown in medium containing NaCl. In particular, we observed «ball-like» structures, an increase of chlamydospores and hyphae crystals with increase of sodium chloride concentration.

1.1-29 Biodiversity and enzymes bioprospection of Antarctic filamentous fungi

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Abstract: Antarctica is one of the most suitable places for the bioprospecting of psychrotrophic fungi. The aim of this study was to investigate the diversity of filamentous fungi from 25 De Mayo Island, Antarctica and their ability to produce extracellular hydrolytic enzymes at low temperature. A total of 51 fungi isolates were obtained from 31 different samples. We identified twelve different genera, seven taxa belonged to the Ascomycota phylum (*Cadophora, Helotiales, Monographella, Oidodendron, Penicillium, Phialocephala, Phialophora, Phoma* and *Pseudogymnoascus*), one taxa to the Basidiomycota phylum (*Irpex*) and two taxa to the Mucoromycota phylum (*Mortierella* and *Mucor*). Some taxa not previously reported in Antarctica, as *Monographella lycopodina, Mucor zonatus* and *Penicillium kojigenum*, were identify. Nine isolates could not be identified to genus level, and could be representing novel species. Most of the fungi were psychrotrophic (76.5%) rather than psychrophilc. Nevertheless, only five isolates were able to grow at 35°C, and the optimal temperature for growth was 15°C for 65% of the fungal isolates. Results from enzymes production (amylase, cellulase, xylanase, lipase, esterase, laccase, protease) at low temperatures revealed that the Antarctic environment contains metabolically diverse cultivable fungi, which represent potential tools for biotechnological applications in cold regions.

1.1-30 Bioremediation of contaminated land by autochtonous fungi: Life-Biorest strategy

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Abstract: Soil degradation is a serious issue in the European Union, causing the loss of more than 340,000 areas. LIFE BIOREST (LIFE15 ENV/IT/000396, www.lifebiorest.com) is a UE funded project in the framework of the LIFE Project, aimed to treat a soil contaminated by PHAs, BTEX and alkanes. This site (about 80,000 m^2 wide) is located in Italy (Fidenza, Emilia Romagna) and has a long history of industrial exploitation. The project aimed to optimize a biomerediation method where the transformation made by consortia of fungi and bacteria is finalized by the final step of re-vegetation. The first phase of the project is indeed focused to characterize the microbial community that naturally populate this extreme environment and isolate those microbes capable of growing in the presence of pollutants as sole C source. The best performing strains will be used to set up consortia working in microcosms and mesocosms before up-scaling the process at in-situ level (biopiles). A solid screening and a liquid enrichment using few selected contaminants (naphthalene, pyrene, phenanthrene, benzene, alkanes and oil extracted from the soil) were carried out to identify the strains with the best adaptation and degradation skills. Despite the strong contamination, microbial communities were consistently developed: more than 220 fungi belonging to 70 species have been identified. Most of the fungal strains belonged to Ascomycetes (mainly to the genera *Aspergillus, Cladosporium, Fusarium* and *Scedosporium*) even though almost 20 Basidiomycetes were also isolated. A further screening was based on an innovative miniaturized approach in 96 multiwell plates in order to evaluate the growth rate of each strain in the presence of 6 contaminants. During the 3 weeks experiments, several strains were capable of growing on the pollutants (at 200 ppm and 1% v/v) as much as positive controls with glucose, highlighting their capability to exploit complex source of nourishment as far as simple and bioavailable ones. Since the bioavailability of organic pollutants in soil is a recognized issue that often limit the efficiency of bioremediation approaches, strains were also screened for their capability to produce biosurfactants. Some fungi were found capable of producing extracellular broths with both emulsifier and biosurfactants activity. Almost 30 fungi and 30 bacteria have been selected and will be tested in micro and mesocosms singly and in consortia with selected bacteria in order to evaluate also their capability to grow and colonize the contaminated soil, and ultimately decontaminate it within 3 months treatment. According to the degradation skills, one consortia was selected for biopile trials. Fungi demonstrated that they could be successfully coupled with bacteria and plants to sensibly reduce the environment hazard of contaminated land, ultimately restoring their ecological functions.

1.1-32 Biodegradation of BTEX by fungi isolated from the hypersaline lagoon Las Salinas, Punta Cuchara Natural Reserve, Ponce, Puerto Rico

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Abstract: Benzene, toluene, ethylbenzene, and the isomers of xylene (BTEX) are volatile anthropogenic pollutants derived from petroleum products that cause harmful effects in humans and other organisms. Fungi were isolated from a 35-ha hypersaline coastal lagoon, Punta Cuchara Natural Reserve, Ponce, Puerto Rico, which is an important nursery for marine species and serves as an avian refuge. Coastal lagoons frequently are contaminated with BTEX. A total of 25 culture-dependent fungal species from the lagoon included the following: *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Trichoderma* sp. *Fusarium* sp., *Curvularia* sp., *Chaetomium* sp., *Blastoschizomyces capitatus*, *Candida albicans*, *C. glabrata*, *C. rugosa, C. parapsilosis, C. tropicalis, C. zeylanoides*, *Cryptococcus neoformans, C. albidus*, and *C. uniguttulatus, Geotrichum* sp., *Kluyveromyces* sp., *Prototheca zopfii, Rhodotorula minuta, R. mucilaginosa, Saccharomyces cerevisiae, Trichosporon cutaneum,* and *Yarrowia lipolytica*. A static culture system, each consisting of a serum bottle capped with Teflon Mininert™ valves was used in each of three successive trials to determine degradation of BTEX by each fungi species. An aqueous mineral medium stock solution was prepared with NaNO3 (3.0 g/L), KCl (0.5 g/L), MgSO4 (0.5 g/L), FeSO4 (0.01 g/L), and K2HPO4 (1.0 g/L). Each fungal species was inoculated at a concentration of 1 x 104 yeast cells/mL into the static culture system with a mixture of 118.75 mL of the mineral stock solution and 6.25 mL of BTEX. The BTEX was the only source of energy and carbon. The inoculation was incubated for 150 hr at 25oC. HPLC-DAD method was used to determine the biodegradation of the BTEX in each system by each fungus. The majority of the fungi species degraded the BTEX completely, although traces of BTEX were detected for some species. Thus, funguses species appear to serve as biodegradation organisms in hypersaline lagoons, many of which could be contaminated from petroleum spills.

1.1-34 Screening macrofungi for antibacterial compounds

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Abstract: The widespread development of resistance to antibiotics amongst bacteria pathogenic to humans has led to interest in finding new antimicrobials. We hypothesised that Macrofungi are a likely source of novel antibiotics since their mode of nutrition makes them vulnerable to competition. Amongst the macrofungi, saprotrophs export enzymes to digest macromolecules and must compete with bacteria for the breakdown products and ectomycorrhizal species need to protect the zone of nutrient exchange with plant roots. Accordingly, the production of antimicrobial compounds to control competition for nutrients would provide a competitive advantage to macrofungi. To test our hypothesis, extracts of 170 species have been screened for antibiotics including compounds that block the bacterial efflux pumps that expel antibiotics and compounds that impede the formation of bacterial biofilms. We have screened fruiting bodies and, where possible, also cultures for compounds active against a panel of sixteen bacterial species, including the ESKAPE group of pathogens that cause troublesome hospital acquired infections. We found that a substantial proportion of Australian macrofungi screened do produce antibacterial compounds, including some that inhibit the formation of biofilms or a *Staphylococcus aureus* efflux pump. One of the new antibacterial compounds identified is non-toxic to human tissue cultures and is amenable to synthesis, offering the possibility of a new family of antibiotics.

1.1-36 Neuroprotective metabolites from *Hericium*

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Abstract: Antibiotic resistance and neurodegenerative diseases are two major medical issues we have to face and which will become even more serious in the future. In order to cope with them and to develop improved therapies new classes of bioactive natural products with different modes of action are needed urgently. *Hericium* spp. of the phylum basidiomycota are among the most praised medicinal and edible mushrooms, and they have been known to produce secondary metabolites, such as hericenones and erinacines, which were isolated from the fruiting bodies and cultured mycelium, respectively. Many of these compounds were found to promote nerve growth factor (NGF) biosynthesis. Recently, corallocin A-C were isolated from basidiomes of the species *Hericium coralloides* and were the first members of this compound family that were found to be able to modulate both, NGF and brain-derived neurotrophic factor (BDNF) production. This prompted us to extend our studies to the metabolites from cultures of *Hericium* species, where metabolite profiles of a strain of the Lion's Mane mushroom (*Hericium erinaceus*) and a strain of the rare species, *Hericium flagellum* (synonym *H. alpestre*) were examined. Highly similar metabolites were observed in both strains, with cyathane diterpenoids being the predominant ones. Seven metabolites obtained from *H. erinaceus* and *H. flagellum* were evaluated regarding their neurotrophin inducing effects. Although none of the tested compounds showed intrinsic neurothrophic activity, erinacines A, B, C, CJ14.258 and the new derivative Z1 clearly enhanced the neurotrophin production in astrocytic cells. Moreover, for the first time we observed a promoting effect of cyathane diterpenoid derivatives on BDNF expression. As they are able to stimulate the transcription of both neurotrophins, they may act upstream on a common molecular target of both pathways or via a third independent pathway.

1.1-37 Investigation of neurite stimulatory properties of *Hericium erinaues* (Monkey's head mushroom) on rat pheochromocytoma cells (PC12 cells)

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Abstract: Dementia is a neurodegenerative disease that has become one of the major issues in the 21th century. However, till date there is no specific treatment that can cure or prevent dementia from occurring in humans. Commercial drugs for dementia can only reduce the symptoms of the disease and even worse long-term consumptions might pose adverse effects to other bodily functions. Therefore, this study aims to investigate the potential of *Hericium erinaceus* extracts to stimulate neurite outgrowth in PC12 cells. Briefly, fresh *H. erinaceus* was cut to cube, freeze dried and extracted with 20% w/v of 95% ethanol and overnight macerated with deionized water followed by 30min hot water extraction respectively. The crude extracts were rotary evaporated and freeze dried before the assay. PC12 cells were treated with 20ug/ml, 40ug/ml, 60 ug/ml and 80ug/ml of the extracts respectively. Neurite bearing score ranged between 2.5-29.6% with the highest score being 50ng/ml NGF treated cells. Pearson's correlation showed positive correlation between extract concentration and neurite bearing score. Immunofluorescence assay was performed to confirm the neurite outgrowth via staining with rabbit anti-neurofilament 200 polyclonal antibody. The RNAs of the treated cells were also extracted to check the expression level of neurite outgrowth related gene, neuritin via qPCR. This would be the first report of

neuritin gene expression following neurite outgrowth induced by mushroom extracts. Further test will be conducted to investigate the effect of co-incubation of *H. erinaceus* extract with 5ng/ml of NGF. In conclusion, *H. erinaceus* may be applied as potential health and functional food source in management of neuronal related diseases.

1.1-38 *Ganoderma lucidum* as a promising source of anti-virals against non-enveloped enteroviruses

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Abstract: Enterovirus infections are amongst the most common infections affecting people worldwide. These viruses cause severe outbreaks especially among children, with symptoms varying from mild to severe including aseptic meningitis, heart muscle damage and paralysis. They have recently been revealed to contribute to chronic diseases, such as type 1 diabetes, as well as with cardiomyopathies and atherosclerosis. Despite efforts of developing anti-virals against enteroviruses that have been going on for years, no vaccines (except for poliovirus) and drugs have made it past the clinical phase and into the market. So far, antiviral effects of extracts obtained from fruiting bodies and/or mycelia in liquid cultures (fermentation) have been described from several mushroom species, of which Ganoderma lucidium is one of the most extensively studied. Crude extracts as well as fractioned compounds including triterpenes and ganoderic acids from G. lucidum have been noted to exhibit inhibitory effects against enterovirus 71 (EV71), herpes simplex virus type 1 and 2 (HSV-1 and 2), as well hepatitis B virus (HBV). Our aim was to evaluate the antiviral effects of crude extracts (water and alcohol extract) from fruiting bodies collected from wild, and liquid fermentation of different strains of *G. lucidum* isolated from various geographical locations in Finland against enterovirus B group viruses, such as coxsackievirus B3 (CVB3). The Ganoderma extracts and sterile-filtered liquid culture media were incubated for 1 h or shorter periods with the purified virus, and thereafter added on lung carcinoma cells (A549). In addition to infectivity tests, a more detailed investigation to decipher the mechanism of action were performed using confocal microscopy, gradient fractionation and TEM. Our results show a direct inhibitory effect of liquid cultures on enterovirus (CVB3) without cytotoxicity in human cells. We noted a clear difference in the intensity of inhibitory effect between different strains of G. lucidum. In the liquid fermentation, the duration also clearly affected on the results, longer fermentation time giving higher inhibitory effect. These results demonstrate the importance of screening and selection of fungal strains with desired intensity of effect. This is of utmost importance if aiming for production of pharmaceutical products in bioreactors.

1.1-39 Taxonomic analysis and medicinal properties of macromycetes in Shikahogh State Reserve (Republic of Armenia)

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Abstract: Biological diversity is one of the powerful reservoirs of resources. The main tasks of a modern science is the study and preservation of biodiversity and its conservation in separate regions, including special protected nature areas (SPNA), which is one of the main key for humanity sustainable development. The goal of this work is a comprehensive study of macroscopic fungi species composition in Shikahogh State Reserve of Armenia. The reserve, with an area of 12.137 ha, is located in the south-eastern part of Syunik Marz, on the northern macroslope of the Meghri Range, at an altitude of 700 to 2400 meters above the sea level. Shikahogh reserve is represented by different types of landscape due to the difficult mountain terrain, vertical zonation, from the forest to the mountain steppes and alpine meadows ending. As an experimental material was chosen macroscopic fungi, collected from the territory of the Shikahogh Reserve as well as the samples from the herbarium of the Department of Botany and Mycology of Yerevan State University. The studies were carried out during the vegetation period using the route-expedition method from 2009 to 2016. Within the studied area 436 species of macrofungi that belong to 176 genus, 74 families, 22 orders, 7 classes, 2 subdivisions and 2 divisions of Ascomycota and Basidiomycota were revealed. The taxonomic analysis of the main families of macroscopic fungi in Shikahogh Reserve area showed that the following families are characterized with rich number of species: Polyporaceae (47 species), Tricholomataceae (38), Agaricaceae (26), Russulaceae (26), Strophariaceae (23), Hymenochaetaceae (21), Cortinariaceae (19), Inocybaceae (17), Mycenaceae (12) and the rest 65 families including 1-9 species (47.5%). In our studies we also registered 99 species of macromycetes with medicinal properties. They belong to classes of Agaricomycetes, Sordariomycetes and Tremellomycetes and 9 orders. The main part of the fungi with medicinal properties refers to the order of Agaricales (52 species). Most of the species found on the territory of the Reserve have antibacterial, antiviral, antifungal properties, which is associated with such chemicals as terpenoids, purines, phenol derivatives isolated from fruit bodies and fungal mycelium (*Agaricus campestris*, *Coriolus versicolor*, *Ganoderma applanatum*). For example, according to our data, the local population engaged in animal breeding uses *Lycoperdon perlatum* for the treatment of purulent diseases of rabbit ears. Thus, this study indicates that the collected data can be used for an update of the data passports of protected areas of Republic of Armenia and it is giving potential opportunities for the usage of macromycetes in medicine and the national household.

1.1-40 A Taxonomic survey of commercially available reishi products: A buyer beware market

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Abstract: The genus *Ganoderma* contains species that occur commonly around the world and function as primary wood decay fungi of a wide array of tree species. In addition to ecological functions, species of *Ganoderma* have been used in traditional medicine in Asia for thousands of years. They are often referenced with common names such as *reishi* or *lingzhi*. *Ganoderma* species contain suites of triterpenes and polysaccharides that have been reported to have medicinal value, and have gained

interest from the pharmaceutical industries in recent years. Globally, the taxonomy of *Ganoderma* species is chaotic, and the taxon name *G. lucidum* (*sensu lato*) has been used for most laccate (shiny) *Ganoderma* species. However, it is now known that *G. lucidum* (*sensu stricto*) has a limited native distribution in temperate climates of Europe and some parts of China. Furthermore, *reishi* or *lingzhi* products, sold as medicine, are not strictly regulated by the Food and Drug Administration in the United States. To determine what species are being sold in commercially-available products marketed as *reishi* or *lingzhi*, twenty products labeled as containing *G. lucidum* were purchased, DNA was extracted and the internal transcribed spacer (ITS) region was sequenced using Sanger sequencing. Based on Sanger sequencing, the majority of the products (93%) were identified as *G. lingzhi*, which is native to Asia and the most widely cultivated taxon. Microscopic analysis of the products revealed different spore morphologies within individual products, and Illumina sequencing of the ITS1 region was performed on all products to determine if multiple *Ganoderma* species could be present. Of the twenty products tested, none contained the species *G. lucidum*. Similar to the Sanger sequencing results, the Illumina results confirmed that *G. lingzhi* was in most products, but other *Ganoderma* species were also present, including the taxa *G. applanatum G. gibossum, G. sessile,* and *G. sinense*. It is likely that there are differences in the quality and quantity of medicinally-relevant chemicals produced by different *Ganoderma* species. Furthermore, if fruiting bodies are cultivated and/or formulations are manufactured outside of the United States, regulations focusing on the content and quality of these products should be re-evaluated before they are ingested or marketed as medicine.

1.1-49 Temporal stability of the fungal community in a tropical rainforest canopy

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Abstract: The spatio-temporal dynamics of fungal communities are largely unknown, despite their ecological importance. Changes in fungal species composition and relative abundance can have impacts on ecosystem processes, such as decomposition rates. They also affect macroscopic plant communities, through gains or losses of mutualistic or pathogenic fungi. Many ecological studies only capture a single snapshot of the fungal community or compare single time points before and after a major disturbance or experimental manipulation. It is often not known if these communities are stable over time. More research is needed to see if observations at one time-point can be used to make inferences about the future. To address this problem, we examined the temporal variability of the diverse fungal community in a tropical rainforest canopy. We selected five branches of *Saurauia montana* trees in a low montane rainforest in Tapanti National Park, Costa Rica and sampled 64 points across these branches each July for three years. Samples included tree bark and any litter or living bryophytes present on the branch surface. ITS sequencing with Illumina technology was used to assess fungal diversity. We found that there is a small, but statistically significant, effect of year on fungal community. Despite this, the fungal community at a given point was more similar to communities at the same point in different years than to other points in the same year, even when these points are nearby on the same tree branch. In previous research on community spatial structure in this system, we found high turnover between samples at the same time-point at sub-meter spatial scales. Here we found that species composition is consistent at a point between years. We conclude that the fungal communities in this system are structured at a very fine spatial scale but are persistent over time. Thus, observations at one time-point can be representative of points in the future.

1.1-50 Characterizing microbiomes of fungal fruiting bodies across functional guilds and growing habits

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Abstract: Over the past decade, culture-independent approaches employing next-generation DNA sequencing have revolutionized our capacity to investigate the composition and dynamics of microbial communities (microbiomes). Major advances have been made on understanding human, animal, and plant microbiomes, however, less attention has been given to the microbial communities of fungi. Bacteria perform various functions in fungi and their reproductive structures (fruiting bodies), spanning from pathogenicity (e.g., mycoparasites) to growth promotion (e.g., mycorrhizal helper bacteria). However, how mushroom-associated bacteria assemble and function within fungi belonging to different ecological guilds and growing habits has yet to be addressed. To this end, we used Illumina high-throughput 16S rRNA amplicon sequencing to characterize bacterial communities in thirty-two fungal genera across Ascomycota and Basidiomycota. Through bioinformatic analyses of the ~6.4M raw sequences generated we recovered 1384 Operational Taxonomic Units (OTUs) across 160 samples. Proteobacteria (71.8%), Bacteroidetes (23.3%) Firmicutes (1.9%), and Actinobacteria (0.7%) were the most abundant phyla overall. Bacterial communities structured according to different fungal functional guilds (mycorrhizal, endophytic, saprophytic) as well as growing habitus (hypogeous, epigeous, wood-growing). The highest bacterial richness was recorded in *Tuber*, *Chantarellus*, *Hydnum*, and *Morchella*; the lowest in *Ganoderma*, *Hericium*, *Paxillus*, and *Lyophyllum*. The genus *Bradyrhizobium* was abundant in *Tuber*, but also present in *Scleroderma*, *Elaphomyces*, and *Lycoperdon*, all grouped by a similar sequestrate fruiting structure. *Pseudomonas* was highly abundant in *Coprinus*, *Leatiporus*, and *Suillus*, while *Burkholderia* were dominated inside *Clavariadelphus*, *Grifola*, and *Hydnum*. Bacteria belonging to *Polaromonas*, *Pedobacter*, and *Janthinobacterium* were all abundant in *Helvella*, *Gyromitra*, and *Morchella* which all share a similar fruiting morphology and belong to Pezizales. Our data demonstrate that fruiting bodies microbiomes relate to the functional guild of their fungal host, which raises the question of whether these bacteria provide symbiotic functions or depend upon specific ecological requirements. A wider sampling of fungi belonging to the same trophic guild, in different geographic areas, will improve the separation of community variations attributable to ecological habitat filtering from metabolic-based symbiotic interactions.

1.1-51 Potential distribution of South American species of the lichen genus *Parmotrema* (Parmeliaceae, Lecanorales): implications for conservation

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Abstract: Species distribution models have become an important tool to assess different issues in fields as biogeography, ecology, evolution, and climatic change. Although they have been commonly used in other organisms, just a limited number of studies about lichen-forming fungi have included modeling approaches. Species distribution models allow us to recognized those areas with favorable ecological conditions to species could develop outside their known localities, and to determine rare or threatened species, contributing thus to the development of conservation strategies. The aim of this study is to estimate the potential geographic distribution of South American species of *Parmotrema*, to identify which are the environmental variables influencing their distribution, and to analyze their conservation implications. For this, six species were selected: *P. cristobaliae*, *P. flavomedullosum*, P. *homotomum*, *P. laciniellum*, *P. masonii,* and *P. melanochaetum*. A database with recorded localities in literature for each species and also specimens deposited at CTES herbarium was made. Localities for which no geographic

coordinates were available were georeferenced with Google Earth™. The potential distribution was modeled with Maxent version 3.3.k, using the 19 climatic variables of temperature and precipitation and altitude of the WorldClim database, estimating their potential influence in these species distributions. The results were visualized and analyzed using DIVA-GIS version 7.5. The potential distribution maps are presented and the influence of environmental variables of each considered species are analyzed. This study has shown that *P. homotomum* and *P. masonii* has a restricted potential distribution area. The distribution of *P. masonii*, as *P. cristobaliae*, could be related to the distribution of the seasonally dry forests, as other species of the genus. The distribution of *P. laciniellum* has also a similar biogeographic pattern, but it would be adapted to wider environmental conditions. Based on these results, we could identify two priority areas for conservation.

1.1-52 Mapping fungal diversity at the global scale

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Abstract: Fungi represent a fundamental component of ecosystems due to their major ecological and economic roles. The fungal distribution worldwide is not only governed by dispersal limitation but also by the suitability of ecological niches determined by both the abiotic environmental factors, i.e., climate and soil characteristics, and the biotic factors, i.e., the plant communities. While most organisms show a gradient of diversity that increases near the tropics, the gradient of fungal diversity is not yet fully described. Therefore, we aimed to map the biogeographic patterns of soil fungal diversity on global scale. Our objectives were to characterize the fungal taxa richness gradient worldwide and to determine which climatic factors could best explain such a gradient. To explore the geographic patterns of fungal diversity, we assigned the soil samples to 1 x 1 degree grid cells covering the globe. Grid-based, rather than locality-based, analyses standardize the geographic scale of the analysis. Altogether we identified fungal diversity in more than 200 grid cells. We used multiple approaches to evaluate the patterns of global diversity. First, we used non-parametric smoothing to investigate the changes in species diversity (number of taxa) with latitude, using a second-degree polynomial function fitted locally to 75% of the data points. Second, we investigated the changes in species diversity across different regions of the world, using climate-based generalized linear model (GLM). Specifically, we characterized the climate within each grid cell using 19 bioclimatic variables and net primary production. These variables capture temperature, precipitation, seasonality, their mean and variation over the course of the year. To identify the most parsimonious model of fungal diversity, we evaluated 4.5 million candidate GLMs that contained different combinations of the previously compiled variables using an exhaustive search algorithm in the R package leaps, but limiting the maximum size of the model to 10 variables to ensure tractability. The best model was selected based on adjusted R^2, AIC, and BIC and used to predict fungal diversity across all grid cells covering the globe. Namely, we predicted the expected diversity of species, and the upper and lower bounds on the expected diversity (95% prediction interval). Finally, we evaluated the global patterns of species diversity using kriging. Kriging extrapolates the patterns of species diversity from the inferred degree of geographic autocorrelation in species diversity across the sampled regions. The results, across different methods and statistical setups, consistently indicate that fungal diversity declines from high latitudes toward the tropics. Non-parametric smoothing reveals that fungi reach their highest diversity at high latitudes of the northern hemisphere. More detailed results, derived from climate-based GLMs, confirm that fungal diversity peaks in the boreal forests of Eurasia and North America. Tropical diversity is limited in comparison, except for several putative biodiversity hotspots in the Amazon and African savannahs. These results indicate different global diversity patterns

between soil fungi and other previously studied organisms such as plants or animals. Our study should help fungal ecologists to advance the knowledge on fungal diversity and ecology worldwide.

1.1-53 Host specificity differs along the life cycle of *Tremella hypogymniae*

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Abstract: Although traditionally considered the symbiosis between a fungus and green algae or cyanobacteria, lichens are complex systems that harbour a diversity of additional organisms. How these organisms are distributed within the thallus is often poorly known. A variety of bacterial and fungal taxa inhabit the lichen thalli, including *Alphaproteobacteria, Acidobacteria*, *Actinobacteria* or *Betaproteobacteria*, and both ascomycetes and basidiomycetes in fungi. Among the basidiomycetes, the "heterobasidiomycetes" show a high specificity towards their lichenized hosts. These fungi are dimorphic: they have both a dikaryotic filamentous phase and a haploid yeast phase in their life cycle. The filamentous phase is usually host specific in the lichen-inhabiting species but little is known on the requirements of the yeast phase. Recently, it has been shown that both *Cyphobasidium* and at least one *Tremella* species are able to complete their life cycle within the lichen thallus, where the yeast phase grows in the lichen cortex. In this work we further investigated the frequency and host-specificity of the yeast phase of tremellalean fungi growing on lichens. For this we chose *Tremella hypogymniae*, a species that is restricted to one of the most common lichens in boreal and temperate forests of the northern hemisphere (*Hypogymnia physodes*). We used highly specific PCR primers to selectively amplify *T. hypogymniae*, avoiding other organisms including other tremellalean taxa. We searched for *T. hypogymniae* in *H. physodes* and in other common Parmeliaceae species more or less closely related to *H. physodes* in different coniferous forests in Sweden and Spain. In addition, we performed fluorescent *in situ* hybridization (FISH) to identify the location of the different phases of the life cycle of *Tremella* within the host lichen thalli. Our results suggest that the yeast phase of *T. hypogymniae* is very frequent – although not always detected – in the cortex of several closely related lichens, whereas meiosis and subsequent formation of basidia seem to occur only when *Tremella hypogymniae* grows in *Hypogymnia physodes*. This implies that host specificity is related to sexual reproduction, whereas the asexual yeast-phase can develop asymptomatically in a wider range of hosts. Our study represents an important step in the understanding of the life cycle and biology of lichen-inhabiting basidiomycete fungi.

1.1-54 Lichenicolous ascomycetes on *Siphula*-like lichens

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Abstract: A survey of collections of *Siphula*-like lichens [*Siphula* (Icmadophilaceae) and *Parasiphula* (Coccotremataceae)] in the herbaria of H, HO, TNS and UPS revealed a rich and highly specialised lichenicolous biota of *c.* 20 taxa. Of these, we have been able to identify *c.*10 to species rank, including five that we describe as new to science in the genera *Cercidospora*, *Endococcus* and *Pyrenidium*. In

addition, we significantly expand the geographical distribution and the number of host species for siphulicolous fungi. By far the greatest species richness is found in the Southern Hemisphere, the centre of speciation of the host genera. The only lichenicolous species recorded from the Northern Hemisphere is *Sphaerellothecium siphulae*. No lichenicolous fungi are shared between *Siphula sensus stricto* and the morphologically similar genus, *Parasiphula*, providing further support for their separation.

1.1-55 Ebony and algal green: Characterizing fungal-algal interactions in a biological soil crust

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Abstract: Biological soil crusts (BSCs) are a type of biofilm that can withstand a wide range of extreme conditions, including: heat, freezing, desiccation, osmolarity, UV, and heavy metals. Some of the most common fungi in BSCs are the polyextremotolerant black yeasts in the Chaetothyriales, such as those in the genus *Exophaila*. Unique features of these organisms make them intriguing to study alone, but may also provide insight into the evolution of fungal-algal interactions. These polyextremotolerant fungi share similar resistance features with the lichens, therefore black yeasts may enable study of the lichen lifestyle without the challenges of directly working with lichens. In addition to sharing similar traits, black yeasts are also almost always found in the same location as algae, even in desert and polar ecosystems. We hypothesize that black yeasts and algae indeed interact in a mutualistic fashion that resembles lichens, and that observing these interactions will reveal clues about the nature of the fungal-algal communication that underlies the formation of BSCs. To test this hypothesis, we have isolated black yeasts and algae from BSCs and rock surfaces, identified isolates using their ITS sequences, and subjected isolates to multiple stressors and nutritional conditions to investigate their phenotypic diversity. Culturing efforts and Sanger sequencing resulted in the identification of 24 black yeasts and 43 algal isolates from eight locations sampled within BSCs found in a semi-arid sand dune ecosystem (Jackman Flats Provincial Park, BC). In addition to these culturing efforts, we sequenced both ITS1 and 16S amplicons using Illumina sequencing to elucidate the microbial composition of the BSCs. Additionally, we have co-cultured isolated fungi and algae together in a pair-wise manner to observe phenotypes of their interactions. Current efforts are focused on integrating phylogenetic, phenotypic, and microbiome data to understand the functional interactions that enable the formation and maintenance of BSCs.

1.1-56 Ultrastructural study of the algae within the globule of *Multiclavula mucida*

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Abstract: *Multiclavula mucida* (Pers.) R. H. Petersen is a basidiomycete forming tiny white fruit bodies on rotten wood and always exists together with algae. Although the ecological association between *M. mucida* and algae is not elucidated, *M. mucida* is often regarded as one of the basidiolichens. There are many green globules on rotten wood where *M. mucida* produces fruiting bodies. In the light microscopic observation, Geitler (1955) and Oberwinkler (1970) reported that these globules consisted of pseudoparenchymatous hyphae of *M. mucida* and several algal cells. Oberwinkler (1984) also observed these globules by transmission electron microscopy (TEM), but it was not mentioned much about intracellular structures of both fungi and algae. In this study, we observed these globules by TEM, mainly focused on algae within the globules. TEM observation showed that the outer surface of the globules was consisted of hyphae. The cytoplasm of the hyphae was often filled with osmiophilic granules with a

diameter of about 1 µm. Such granules were also observed in the other basidiolichen *Omphalina ericetorum* (*Botrydina vulgaris* globules) and treated as glycogen particles (Honegger and Brunner, 1981). Hyphae were also observed within the globule, but no haustorium was found. Each globule contains several to 15 algal cells. Each algal cell was occupied by several electron-dense storage bodies and a single chloroplast. The chloroplast is biased towards the cell wall, thylakoids overlap in many layers, filling the whole chloroplast. At the center of the chloroplast several electron-dense granules were gathered. These granules were similar to the pyrenoglobuli which are found within the chloroplast of *Trebouxia* and always associated with a pyrenoid. If these granules are pyrenoglobuli, this alga is considered to have a pyrenoid. The osmiphilic granules with in the hyphae and pyrenoglobuli-like granules within the chloroplast can be also recognized in the TEM photographs by Oberwinkler (1984) (but not enough explanation was given). Geitler (1955) reported that the algae within the globule belong to the genus *Coccomyxa*, but Komárek and Fott (1983) and Tschermak-Woess (1988) pointed out that the alga had a pyrenoid and could not belong to *Coccomyxa*, a pyrenoid-lacking genus. We established 24 algal cultures from *M. mucida* globules. As a result of molecular identification using the sequence of ITS, they were divided into at least three species (two *Coccomyxa* spp. and *Elliptochloris subsphaerica*). The chloroplast of *E. subsphaerica* is filled with thylakoids that overlap in many layers, and pyrenoglobuli-like granules exist in the center. These characters are consistent with those of algae within the globule of *M. mucida*, whereas both were absent from the two *Coccomyxa* spp. According to Ettl and Gärtner (2014), *E. subsphaerica* has a pyrenoid. In *M. mucida-E. subsphaerica* co-cultivation, algal cells were closely surrounded by the hyphae under a certain condition, which is similar to the early stage of the globule development in the field as reported by Geitler (1955). Based on the above, *E. subsphaerica* is eligible to be considered as photobiont of *M. mucida*.

1.1-57 Exploring foliar fungal endophyte assemblage, diversity, and host specialization in pine

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Abstract: Host specialization of foliar fungal endophytes (FFE's) remains a cryptic and uncommon phenomenon. Since patterns of host specificity are sensitive to host taxonomic and spatial scales, a field study that investigates the fungal endophytic community of conifers, a taxonomically well-defined and diverse group, across a wide geographic range (spanning much of North America) was conducted. Conifer trees have a high incidence of FFE infection, likely due to the longevity of their evergreen foliage as well as their dominance in some ecosystems. Furthermore, *Lophodermium* (Rhytismataceae), a well-studied FFE genus that seems to be common within needles of the Pinaceae family, indicates high phylogenetic host specificity that is rarely documented for other FFE's. In total, 51 species of conifers were sampled from 69 localities across the United States and Mexico. Illumina MiSeq sequences were collected on the community assemblage of FFE's with a closer investigation of *Lophodermium* OTUs. Host specificity of common OTUs were analyzed and compared across geographic and taxonomic groups of conifers. These findings will be crucial for furthering our understanding of the evolutionary and ecological nature of these mysterious microfungi on their hosts.

1.1-58 A new species of *Lophodermium* (Chevall.) described using a combination of morphological data, ITS-LSU rDNA and ddRAD loci

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Abstract: *Lophodermium* is a common endophyte of pine needles with ca. 30 putative species. The species within this genus have large-within species genetic variation with the possible occurrence of cryptic species. The relatively poor sampling isolates with molecular and morphological characterization, together with the lack of genetic markers to resolve its phylogeny with good support, are problems that need to be addressed before attempting further studies or experiments to better understand their ecological role, beyond their 'endophyte' status. We performed a non-exhaustive survey in five-needle pines of Norther California and the Pacific Northwest obtaining cultures from both monosporic ascocarp isolates and surface-sterilized green needles. Ascocarps were identified using morphological characters, and DNA was extracted from cultures to sequence the internal transcribed spacer and a partial sequence of large rDNA subunit (ITS-LSU) using Sanger technology. Furthermore, we used a subsample of isolates to recover putative homologous loci using a novel restriction site-associated DNA sequencing (RADseq) method, developed by our research group. In total, we recovered 250 isolates from both green needles and ascospores. Most isolates belong to *L. nitens* Darker and a new species, *L. fissumilis* sp. nov. Salas-Lizana and Oono. The new described species resembled *L. nitens* morphology because both present dark subcuticular ascocarps without lips. However, the upper wall of both ascocarps is very different, as the new species forms an inward v-shape folding, not present in *L. nitens*. A phylogeny using ITS-LSU confirmed that *L. fissumilis* represents a divergent species, although the support for deeper branches was low. After several experiments using different combinations of similarity clustering and quality filtering, a final concatenated matrix of ddRAD loci at 70% similarity and up to 30 ambiguous sites per locus was used to infer a phylogeny of a subsample of six putative species, including the new species. The topology of this phylogeny is highly similar to that of ITS-LSU but with high support for all branches. This fine-resolved phylogeny also revealed that the populations of the new species are highly structured as well as the potential occurrence of cryptic species within *L. nitens*. This work shows the potential of combining classical morphological and cutting-edge technologies to describe accurately the diversity of understudied groups, such as *Lophodermium*.

1.1-59 Local habitat, leaf biochemistry and seasonality drive phyllosphere fungal communities of European beech

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Abstract: Comparative studies targeting the drivers of endophytic fungal biodiversity are rare and identified multiple effectors, such as plant chemistry, climate and seasonal attributes. Our project aimed to study the pattern of the leaf-associated mycobiomes of European beech (Fagus sylvatica) at different altitudes to reveal diversity, composition and seasonal dynamics of fungal endophytes by a combination of metabarcoding, cultivation and subsequent ecological analyses. An experimental field site consisting of 100 (2-years old beech) trees was established called 'beech phytometer system' at two altitudes (517 and 975 m a.s.l.) in a German mountain forest. Ten trees from each site were chosen and 10 leaves per tree were sampled. The following workflow included leaf biochemistry measurement and fungal DNA metabarcoding with 97% OTU threshold and biodiversity analysis for two continuous years: five trees

from beech phytometer and five trees from surrounding beech trees. Metabarcoding resulted in a total of 15,703,599 demultiplexed and quality filtered ITS1 reads from 165 samples. Clustering at 97% similarity resulted in 1199 OTUs. In addition, 438 isolates from an autumn sampling event were cultivated via the dilution-to-extinction method; endophytes were identified via barcoding based on ribosomal ITS (internal transcribed spacer) markers and Sanger sequencing. A significant correlation of community composition with elevation was observed. The mycobiome was little affected by the physiological state of the leaves; only a partial shift of taxonomic composition was observed from vital towards senescent leaves. Mycobiome diversity and composition correlated significantly with the origin of the trees, pointing to local habitat condition as the main driver. Under natural conditions, the mycobiome was more diverse at the lower elevation. Additionally, leaf chlorophyll and flavonoid contents showed negative correlations with fungal richness in natural stands. Metabarcoding and cultivation approach resulted in non-overlapping community compositions and pronounced differences in taxonomic classification and trophic stages. However, both methods revealed similar correlations of the fungal communities with local environmental conditions. Our results indicate undeniable advantages of metabarcoding over cultivation in terms of representation of the major functional guilds, rare taxa and diversity signals of leaf-inhabiting fungi. We observed a strong seasonal turnover in phyllosphere fungi in both habitats over the two years of investigation, suggesting that the plant- fungal system not only responds to cyclic climatic conditions but depends as well on various parameters, e.g., geographic position, substrates age and surrounding vegetation. In general, the altitudinal difference is the most important explaining factor for community differences, which shapes many dependent abiotic and biotic habitat factors. Regarding cost and time per sequence, metabarcoding is superior to cultivation approaches and offers surprisingly profound insights by yielding much more data, allowing to test at once multiple hypotheses in fungal ecology.

1.1-60 Habitat modeling of foliar endophytic fungi across the Hawaiian archipelago

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Abstract: A phylogenetically diverse array of fungi live within leaf tissue, and in many cases, these foliar endophytic fungi (FEF) live mutualistically with the plant host by producing secondary metabolites or conferring disease-resistance. Many studies have examined FEF across single plant species, or focused on individual FEF taxa, but the broad-scale distributions of these fungi are not well understood, either across geographic space, across climactic conditions, or in the context of host plant phylogeny. The Hawaiian archipelago represents a uniquely tractable system for studying the habitat preferences of FEF, because the islands each have a wide range of climactic conditions, and host species can be found across islands, but dispersal barriers are significant between islands and between the archipelago and other land-masses. Therefore, the Hawaiian archipelago represents an ideal system to apply island biogeographic principles to the study of FEF. Here, we use high-throughput DNA sequencing of the ITS1 region to characterize the FEF communities of over 1000 individual native plants across 5 islands in the Hawaiian archipelago. Using climactic information, geographic locations, a host-plant phylogeny, and a FEF phylogeny, we characterize the dominant forces structuring FEF communities, and model the environmental preferences of the most cosmopolitan endophytic fungi. We found that host phylogeny and precipitation were strong determinants of community structure, but individual FEF species exhibited a wide array of habitat preferences, suggesting that aggregate beta-diversity analyses represent a narrow view of the factors structuring FEF community assembly. These results are the culmination of a four-year effort to characterize FEF communities across the Hawaiian archipelago, and although our findings are limited to Hawaii, the patterns we show here for communities and FEF taxa may serve as

hypotheses for future studies in other locations. Within Hawaii, our results answer important questions about host- and habitat-specificity of FEF in native plants.

1.1-61 How to encompass epiphytic lichen diversity in a protected area? A case-study in a natural park in NE Iberian Peninsula

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Abstract: Since 2012 a survey has been carried on in order to assess lichen diversity in the Cadí-Moixeró National Park (NE Iberian Peninsula). Like in several protected areas in the region, lichen biota has showed up as one of the underrepresented components of biodiversity. The main goal of the survey was to disclose epihytic lichen diversity from the natural park. To achieve this aim, and with the idea to cover as many habitats as possible, a sampling protocol was designed. The protocol was first tested in three coniferous forests, and results were compared with previous studies from other areas within the Iberian Peninsula. The obtained results prompted the application of the protocol in representative habitats of the Cadí-Moixeró National Park. During five campaigns, epiphytic lichen diversity was sampled from ten forestal and arbustive communities. Wood communities included broadleaved woods; such as beechwood, oak grove, and holm oak forests; and coniferous forests, as fir wood, Scots pine and *Pinus uncinata* forests. Additionally, some shrub dominated communities and fluvial forests were also examined. The sampling campaigns yielded an increase in the number of species mentioned in the natural park. In 2012, the lichen catalogue compiled 105 taxa. Five years later, the list of lichen has risen to 319 taxa. Among them, six taxa were newly quoted for the Iberian Peninsula. The epiphytic lichen diversity was represented by 272 species. The study on the species composition from the habitats showed that there was a clear difference between coniferous and broadleaved forests (including shrub and fluvial forests). However, the comparison of ecological traits such thallus growth or photobiont did not present the same pattern. No significant differences were observed between both main sorts of forests. The species-area curve suggested that the potential epiphytic lichen diversity ranges between 345 and 359 taxa, depending on the applied model (jackniffe or Chao2). Although the sampling protocol has provided a good insight in the lichen diversity in the Cadí-Moixeró Natural Park, the complete knowledge is still far of being achieved. The selection of representative habitats has resulted in a good way to increase the knowledge on lichen diversity; however, more especific habitats, like meadows or rocky places, should be included in order to gain a complete view on lichen biota.

1.1-62 Host specificity of endophytic fungi isolated from roots of *Calanthe discolor* and *Cephalanthera longibracteata* in Korea

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Abstract: Orchidaceae is the most diverse and widespread family of flowering plants, with 25,000 species currently recognized. In Korea, more than 100 species have been reported in the wild. However, currently, many orchids are in danger of extinction due to over-collection and habitat destruction. Orchidaceous plants have symbiotic relationships with endophytic fungi, including mycorrhizal fungi, which play important roles in seed germination and growth of the host plants. In this study, the endophytic fungal communities were isolated from the roots of *Calanthe discolor* Lindl. and *Cephalanthera longibracteata* Blume that were collected from two different sites in Korea. The fungal isolates were identified by sequence analysis of the internal transcribed spacer regions of rDNA. In total, 35 species of endophytic fungi, including two species of mycorrhizal fungi belonging to the genus *Tulasnella* and were identified in *C. discolor*. Furthermore, 29 species of endophytic fungi were

identified in *C. longibracteata*. The species diversity and richness were not significantly different among the two sites. However, the endophytic fungal community was highly specific to the host, suggesting that the host characteristics affected the community composition of the endophytic fungi that colonized the roots of the orchids. Our findings will help in developing methods that use symbiotic fungi for orchid conservation and restoration of native habitats.

1.1-63 Towards deciphering the fungal diversity associated with *Malus domestica* in Germany using high-throughput amplicon sequencing

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Abstract: The holobiont concept and hologenome theory highlight the influence that microbial communities can have on the ecology and evolution of their associated macro-organismal hosts. The outcome of those associations thereby not only depends on interactions between the microbiome and the host, but also on the interactions between members of the microbiome, including mainly bacteria and fungi, but also viruses, nematodes and protozoa. Fungi form an important part of the plant-associated microbiomes and the composition of fungal communities and the resulting interactions are highly relevant for the ecology of plants, affecting them in beneficial and/or harmful ways. Therefore, processes within natural ecosystems (e.g., nutrient cycling), as well as in agricultural systems (e.g., plant health) are strongly influenced by the mycobiome. Apple (*Malus domestica* Borkh) is one of the most widely cultivated fruit crops in temperate regions and in Germany by far the most important fruit tree. A better knowledge about the apple tree associated mycobiome and its functioning could enable a targeted application, resulting in better crop yields or reduction of fungicide usage. In this study, fungal communities associated to apple trees in Germany were assessed by high-throughput sequencing of amplicon barcodes. To characterize the fungal diversity associated with apple trees and the factors controlling the community assembly, we sampled 85 apple trees at seventeen locations along two transects (North-South and West-East) throughout Germany. Sampling locations were consistently selected for lack of pesticides, herbicides and fungicides. From each host, leaves, twigs and surrounding soil were collected. DNA was extracted from three independent replicates, the fungal barcode ITS1 amplified and sequenced by Illumina MiSeq. We obtained 11,311,311 quality-filtered ITS1 sequences for leaf and twig samples, which were clustered into 575 OTUs. Our results revealed that the host organ and location were major factors shaping the detected fungal communities, while the distance between locations did not show a major effect. Leaf samples were more equally dominated by Ascomycetes and Basidiomycetes, while Ascomycetes prevailed by far in twig samples. A slight similarity decay along the North-South transect was also observed. We will provide insights into community assembly and further details on the fungal communities, their structure, the taxonomic distribution according to organ and geographical location.

1.1-64 The structure of fungal communities on clonal *Eucalyptus* shows effects of host genotype and environment

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Abstract: Fungi and plants often form intimate relationships that have influenced the ecology and evolution of both groups in a significant way. The phyllosphere (i.e. the aboveground parts of plants) is one of the interfaces of plant-fungus interaction that has gained recent attention, as it has been hypothesized that the fungi inhabiting it can have beneficial effects on their host. Additionally, high-

throughput amplicon-based diversity assessments have shown that this habitat harbors an enormous species diversity of non-symptomatic fungi. In this study, we applied NGS amplicon sequencing to understand the influence of geography and host genotype on the structure of fungal communities in replicates of three different clonal *Eucalyptus* lineages in two geographic locations of South Africa. Additionally, we studied the formation of fungal communities in seedlings from these *Eucalyptus* clones, grown in an incubator and subsequently in a nursery. Our analyses on the plantation trees show that the structure of fungal communities is influenced by the different geographic locations. The fungal communities of seedlings were unique to their environment and showed a high turnover when moved between locations.

1.1-73 Effect of ectomycorrhizosphere bacteria on growth, ectomycorrhizal formation and sporocarps occurrence of *Laccaria laccata*

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Abstract: Ectomycorrhizal (EcM) fungi colonize plant roots and form symbiotic associations with the host plants. It has been shown that a variety of bacteria inhabits surrounding the symbiosis and several of them affect either the mycelial growth of EcM fungi and/or establishment of EcM symbiosis from positively to negatively. However, we have still limited views of functional meanings of the ectomycorrhiza-associated bacteria on the ecology of EcM fungi, especially how they affect on EcM sporocarps occurrence. In this study, I targeted on *Laccaria laccata* that forms sporocarps in vitro with symbiosis with host plants. First, one soil core was collected from 5 locations where sporocarps of *L. laccata* occurred in a chestnut (*Castanea crenata*) plantation in Yamanashi prefecture, Japan, to understand bacterial community on EcM roots of *L. laccata*. Ten and 50 EcM root tips from each soil core were subjected to bacterial isolation and cloning, respectively. Two-hundred-forty-eight isolates were obtained in total and they were divided into 34 MOTUs (99% similarity threshold). Most of MOTUs were infrequent, while one MOTU of *Rhizobium* sp. and *Bradyrhizobium* sp. were commonly and frequently obtained across sampling locations. In cloning method, 83 MOTUs were detected. Most of MOTUs were infrequently detected, while one MOTU of *Bradyrhizobium* sp. that were also commonly found in isolation-based method, were commonly detected across sampling locations. The results indicate that *Rhizobium* and *Bradyrhizobium* are common bacteria on EcM roots of *L. laccata* in the chestnut plantation. Next, I examined the effect of the ectomycorrhizal-associated bacteria on the hyphal growth of *L. laccata* using dual-culture method. One mycelial disc of *L. laccata* was placed on the center of a plastic dish (9 cm diam) with 15 mL of modified Melin Norkrans (MMN) agar media with ten-fold dilution of glucose, and each bacterial strain was incubated 2 cm apart from the mycelial disc. Areas of hyphal growth were calculated after 1-month incubation. Effects of bacteria on hyphal growth largely differed according to the combination of isolates of bacteria and *L. laccata* but most of bacteria tended to inhibit hyphal growth of *L. laccata*. *Bradyrhizobium* and members of *Rhizobium* and Burkholdeliaceae did not inhibit or rather promote hyphal growth of *L. laccata*. Last, bacterial isolates of *Bradyrhizobium* and *Rhizobium* spp. were introduced into *L. laccata–Pinus densiflora* symbiotic system in vitro, and EcM formation and frequency of sporocarps production of *L. laccata* were examined. Introduction of bacteria did not significantly increase host plant growth, EcM formations and frequency of sporocarps occurrence but addition of one taxon of *Rhizobium* tended to increase host plant growth and frequency of sporocarps occurrence. The results indicated that several members of ectomycorrhizosphere bacteria would support sporocarps occurrence of the EcM fungi.

1.1-74 The importance of declining mammalian fungal specialists for ectomycorrhizal fungal dispersal in Australia

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Abstract: Hypogeous sequestrate (truffle) fungi rely primarily on consumption by mammals for dispersal. Most truffle fungi are ectomycorrhizal (ECM), making mammalian dispersers essential to the maintenance of plant-fungal relationships, soil fungal diversity and ecosystem functioning. Australia has the highest current global rate of mammalian extinctions, including important specialist mycophagists within the family Potoroidae. Knowing the functional redundancy of different mammal species as dispersers helps us to understand how this loss in mammal diversity could affect plant-fungi interactions and fungal diversity. We present data from a meta-analysis of Australian mammalian diets and a field study including data on an endangered specialist mycophagist. Data from the literature support our hypothesis that specialist mycophagists within the family Potoroidae consumed and potentially dispersed a significantly higher abundance and diversity of fungi than other mycophagous mammals with generalist diets. This finding was further corroborated in the field study; the specialist mycophagist, *Bettongia tropica*, consumed a higher diversity and more unique species of ECM truffle fungi than nine co-occurring mycophagous mammal species. This implies that between specialist and generalist mycophagous mammals in Australia, there is little functional redundancy with respect to fungal dispersal. Taken together, the results suggest changes to mammalian communities, particularly the loss of specialist mycophagists, could, over time, induce significant changes to truffle diversity, shifting ECM communities with unknown consequences for plant health and nutrient cycling.

1.1-75 Coupling effects of climate and tree growth on the productivity of the prized *Tricholoma matsutake* mushroom

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Abstract: The on-site observation research on wild edible mycorrhizal mushrooms is rare, which hampers the understanding of the ecological process related to the production of the mushroom. Taking the ecotomycohhrizal fungal, *Tricholoma matsutake* as a study system, we attempted to understand how the production of this prized mushroom is influenced by climate variation and the growth status of their host trees. A 15-year long term monitoring data set about *T. matsutake* fruiting in Southwest China were analyzed against climatic and tree ring data (*Pinus yunnanensis* and *P. armandi*) with a synopsis framework established to characterize the parameters related to *T. matustake* productivity. The Partial Least Squares was used to identify the most relevant climate data and key period of time, and the Path Analysis was used to determine the indirect and direct causes. Our results showed that both climate and tree ring width influence the production of *T. matsutake*, with tree ring width and precipitation in early spring as the most significant, positive and direct effects. The temperature in early spring and in summer also plays significant direct cause to the *T. matsutake* production. These results

suggest that production of mycorrhizal fungal is determined by the coupling effects of climate and the growth status of the host trees. These findings may have major implications for improvement of mycorrhizal mushroom productivity by considering the interplay between climate, mushroom and forest.

1.1-76 Communication in *Tricholoma vaccinum*-spruce ectomycorrhizosphere

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Abstract: The ectomycorrhizospheric habitat contains a diverse pool of organisms, including the host plant, mycorrhizal fungi, and other rhizosphere microorganisms. Different signaling molecules may influence the ectomycorrhizal symbiosis. Here, we show that the mutual symbiosis between the basidiomycete *Tricholoma vaccinum* and Norway spruce (*Picea abies*) shapes the surrounding mycorrhizosphere. In a forest biotope, this was characterized by a high diversity in basidiomycetes and a rich bacterial community. This consisted of mainly bacteria plant growth promoting abilities dominated by Rhizobiales, with *Nitrobacter winogradski* being most abundant (3.9 %). Other taxa mainly were pseudomonads and bacilli. The bacterial isolates showed symbiosis-relevant traits with 74 % producing the phytohormone indole-3-acetic acid, 23 % producing siderophores, and 23 % mobilizing phosphate. The mycorrhizal fungus *T. vaccinum* was able to excrete plant hormones into the medium upon axenic cultivation. These include auxins, salicylic and abscisic acid, and jasmonates. The spruce roots exudated auxins and salicylic acid. With these compounds present in soil of a natural ectomycorrhizospheric habitat, a communication network with a response of *T. vaccinum* to the environmentally available salicylic and abscisic acids, which led to altered hyphal branching relevant for mycorrhization. In addition, the fungus protected the mycorrhizal tree against the spruce pathogens *Botrytis cinerea* and *Heterobasidion annosum*. Thus, the finely tuned phytohormone interactions in the mycorrhizosphere represent a specifically rich system to study microbial communication.

1.1-77 Increasing success of pitch pine restoration in the Albany Pine Bush Preserve using suilloid fungi

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Abstract: The goal of this research is to provide tools for restoring pitch pine stands at the Albany Pine Bush Preserve (APBP) using local ectomycorrhizal fungi (EMF). Restoration efforts in the APBP focus on removing invasive black locust, reintroducing periodic fires, and reestablishing pitch pine. Restoration of pitch pine by forest managers has had varied success in different areas of the pine bush, but the factors affecting restoration failures are not clear. Soil fungi and their below-ground mycorrhizal interactions may be influencing restoration success. Most terrestrial plants depend in some way on mycorrhizal fungi for establishment, growth, and survival. Pitch pines and the invasive locust both require fungal partners, but the specific fungi they associate with are mutually exclusive. Research has shown that a lack of EMF compatible with pine can hinder their establishment, but that the presence of Suilloid fungi (EMF in the genera *Suillus* and *Rhizopogon*) is sufficient to enable invasion of pines into uncolonized areas. To investigate potential use of EMF to improve restoration at the APBP a factorial field experiment is underway. Pitch pine seedlings inoculated with either live or autoclaved Suilloid spore slurries were planted into sites either never invaded by black locust or that recently had black locust trees mechanically removed. After four months in the field a significantly greater proportion of

seedlings treated with live spores (0.76, SE 0.06) have survived than those inoculated with autoclaved spores (0.48, SE 0.03; ANOVA p = 0.003, df = 1,8). No differences in survival were observed between the non-invaded sites and those with recent black locust removal (p = 0.425, df = 1,8) and no interaction between inoculation and site was found. Pitch pine seeds from the APBP were planted into live, dried soils collected from each site type in a laboratory soil bioassay to investigate the EMF inoculum present in soils of each site. Seedlings will be harvested and the fungi on the ectomycorrhizal root tips identified with molecular methods. The soil bioassay selects for resistant propagules, a trait of Suilloid fungi, which are expected to be present in soils from both site types. Roots of field seedlings will also be harvested to compare to those of the bioassay. Field seedling roots from the non-invaded pine stands are expected to have a greater diversity of EMF than bioassay seedlings grown in either soil and field seedlings from the black locust removal sites, indicating greater diversity of EMF in non-invaded sites and the potential for association through existing hyphal networks.

1.1-78 Allocation of nonstructural carbohydrates by *Pinus strobus* colonized by three ectomycorrhizal symbionts: *Suillus*, *Rhizopogon*, and *Cenoccocum geophillum*

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Abstract: The NANAPHID is a newly developed nanobiosensor, which mimics an aphid to enable monitoring of three non-structural carbohydrates (glucose, sucrose, and fructose). By measuring these carbohydrates *in situ,* the NANAPHID will allow researchers to quickly measure plant carbohydrates in tissues under varied environmental conditions. So far, the NANAPHID has been utilized successfully to measure glucose levels (g/ml) over a wide concentration range in a phosphate buffered solution. The NANAPHID is also being beta tested to measure sucrose and fructose. The NANAPHID sensor is integrated with a laptop-based, fully automated data acquisition, processing and display system to enable field use by researchers. My research will utilize NANAPHID technology to measure the allocation of carbohydrates to mycorrhizal roots as a proxy for examining the allocation of carbohydrates by *Pinus strobus* to mycorrhizal fungi with varying carbon demands. I am focusing on mycorrhizae formed by three different fungal partners; *Suillus, Rhizopogon,* and *Cenoccocum geophillum*. Ectomycorrhizal associations with plants have been shown to range from more to less mutualistic. This spectrum is perhaps an outcome of the varied levels of carbon needed to support different symbionts. All three mycorrhizal fungi were collected from the Rome Sand Plains in Rome, New York. The fungi have been cultured in the lab to inoculate white pine seedlings. These root systems will be examined for mycorrhizal structures and the root tips compared for varied levels of glucose, sucrose, and fructose using the NANAPHID. *Suillus* and *Rhizopogon* are both thought to allocate large amounts of carbon to sexual reproductive structures and the growth of extensive mycelial networks. *Cenoccocum,* alternatively, does not produce any known sexual structures or extensive mycelial networks. Therefore, it is predicted that *Suillus* and *Rhizopogon* demand more carbohydrates be delivered to the roots than *Cenococcum*. Thus, using the NANAPHID technology, higher measurements of nonstructural carbohydrates may be observed in the root systems of trees colonized with *Suillus* and *Rhizopogon* than those colonized with *Cenoccocum*. Data collected using the NANAPHID will be analyzed using ANOVA to compare the variation of nonstructural carbohydrate allocation between trees with different fungal partners.

1.1-79 Using MiSeq on DNA from in-growth bags to observe ectomycorrhizal fungi with N, P and N+P additions in mature forest plots in Bartlett Experimental Forest, NH

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Abstract: Forests growing on young, recently glaciated soils are predicted to be N limited in early succession and then become more P limited as they age. Experimental tests of N vs. P limitation in such forest systems are few, and those few have been short-term with very high rates of fertilization. Further, while there has been work on the response of mycorrhizal fungi to N additions, little work been conducted with P. We investigated ectomycorrhizal (EM) fungi in mature forest soils exposed to nutrient additions in the Bartlett Experimental Forest, NH. N (30 kg N/ha/yr as NH_4NO_3), P (10 kg P/ha/yr as NaH_2PO_4) and N+P were added annually starting in 2011 in an experiment to investigate multiple element limitation in northern hardwood ecosystems. We used hyphal ingrowth bags to study the response of soil fungi to nutrient additions in three stands. After two growing seasons, the bags were harvested and DNA extracted from the soil. We generated community profiles from these extracts based on the fungal ITS1 region, using the Illumina MiSeq platform. Sequence data were processed using the BBMap package, VSearch 2.5.1 and Qiime 1.9, while FUNGuild was used to identify EMF taxa. As expected, N additions reduced the richness of OTUs identified as EM taxa. P additions did not change the richness of EM taxa compared to the controls, and plots with N+P additions had intermediate levels of richness. Treatment effects were observed in the assemblages of dominant genera, dominance being measured as the total number of sequences recovered. In control plots the three most dominant genera were Tomentella, Tuber and Tomentellopsis. In N plots the three most dominant genera were Sebacina, Tuber and Tomentella. In P plots the three most dominant genera were Genea, Pseudotomentella and Pachyphleous. In N+P plots the three most dominant genera were Paxillus, Tomentella and an unidentified member of the Ceratobasidiaceae. The majority of sequences recovered for a genus in each nutrient treatment were assigned to one OTU in the genus, the exception being Tomentella in control plots with three high abundance OTUs. These data provide a picture of EM fungi in soils as active mycelial networks under the various nutrient treatments. Further, these are some of the first such data from P addition plots and identify some EM taxa that may be functioning in forest P cycling.

1.1-80 Ectomycorrhizal fungal community with coniferous trees in Mt. Seorak

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Abstract: Ectomycorrhizal fungi (ECM) mainly form a symbiotic relationship with coniferous trees and play a crucial role in nutrient cycling in the forest ecosystem. In this study, we investigated ECM communities of *Abies nephrolepis*, *Abies holophylla,* and *Pinus koraiensis*. Roots of host plants and rhizosphere soil were collected at two sites of different altitudes, higher and lower, of Mt. Seorak, in Korea. ECM were identified using morphological characteristics and sequence analysis of internal transcribed spacer regions of rDNA from ECM root tips. In *A. nephrolepis,* 9 species from higher altitudes and 6 species from lower altitudes were identified. In *A. holophylla,* 15 species from higher altitudes and 14 species from lower altitudes were identified. In *P. koraiensis,* 10 species from higher

altitudes and 17 species from lower altitudes were identified. Shannon's index, species evenness, and number of species of ECM communities were significantly higher for samples collected at the higher altitudes than lower altitudes, and the ECM communities were also correlated with soil characteristics. The results showed that the ECM communities of conifer roots showed differences according to the altitudes and host plants.

1.1-89 Macrofungi communities are shaped by local topography in tropical rainforests of French Guiana

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Abstract: Current knowledge of tropical Fungi distribution remains patchy, biased towards accessible sites, and extremely limited in the dense and hyper-diverse Amazonian forests. Despite an increasing number of checklists, recent evidence confirms that unknown lineages remain to be described and highlights the need to systematically collect fungal specimens from under sampled areas with standardized inventories. French Guiana remains largely an uncharted territory, with existing collections of Fungi confined to a few coastal localities. Regional geomorphological gradients, as well as local soil types and topographies, are correlated with contrasting floristic composition, providing a unique framework to investigate the diversity and the structure of fungal communities at different scales. We hypothesized that differences in topography and soil found in contrasting forest habitats would shape fungal communities, especially fungal guilds with a high specificity toward their host plant. We evaluated the taxonomic and functional diversity of macrofungi assemblages in three forests habitats distinguished by their local topography - hilltops, seasonally-flooded forests and slope forests; and two types of soils - clay-rich (*terra-firme)* and white-sand soils. We sampled Basidiomycetes fructifications in 36 plots distributed in four sites across French Guiana. Results showed significant differences between fungal communities growing on clay-rich and white-sand soils and, to a lesser extent, between habitats: seasonally flooded forests harbored slightly different communities than plateau and slope forests. These results suggest that fungal communities show preferences for soils and that the distribution of biotrophic fungi appears to be particularly linked with the topography and appears to be shaped by the distribution of putative host plants. The dataset constitutes an unprecedented and original collection of Fungi for the region, showing an extraordinarily diversity of Basidiomycetes, with more than 253 Genera belonging to 76 Families broadly distributed across French Guianian forest habitats. These data will contribute significantly to record and describe Fungi in the Guiana Shield known to differ from the Amazonia basin by a high level of endemism, unusual tree species composition and different geomorphological landscapes.

1.1-90 Isolation of microfungi from urea-treated litter in *ex-situ* model

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Abstract: Ammonia fungi are a fungal group that forms a fungal community sequentially at the restricted sites of animal materials (e.g., decomposing carcasses and animal waste) or artificial disturbance of nitrogen compounds. Researches about ammonia fungi are fragmentary at global scale, especially

sparse in tropical areas as Southeast Asia. In Vietnam, ammonia fungal study was firstly conducted by urea application in *Pinus kesiya* forest, DaLat, Lam Dong Province from 2010 with a new record of *Hebeloma vinosophyllum* at Southeast Asia. In 2012, 2 urea treated plots in forest of *Pinus dalatensis* and *Quercus* spp. at BiDoup - Nui Ba National Park, Lam Dong Province were observed in 7 months. *Amblyosporium botrytis*, *Ascobolus denudatus*, *Lyophyllum tylicolor*, and *Coprinopsis* sp. appeared in early phase, followed by *Hebeloma lactariolens*, *Hebeloma* sp., *Laccaria* sp. in late phase of ammonia fungi. For investigating the composition of ammonia microfungi, litter from *Pinus* and *Quercus* forests at BiDoup - Nui Ba National Park was applied urea in ex-situ model. The aqueous urea solution was added to litter for setting up the concentration of 40mgN/g dry litter, 20mgN/g dry litter. Based on morphological characteristics and ITS analyses, 14 isolates were identified to genus as *Talaromyces*, *Fusarium* and *Pseudallescheria*. These preliminary results contributed to ammonia fungi data in Vietnam, especially ammonia microfungi data. In further experiments, physiological characteristics of isolated microfungi will be studied.

1.1-91 Physiological characters support to distributional patterns of pinecone fungi *Strobilurus* spp. in Japan

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Abstract: There are 5 or more species of *Strobilurus* recorded from Honshu Island to southernmost of Japan. From the different substrates and climates, *Strobilurus* spp. in Japan can be divided into 3 groups: group A with basidiomata growing from pinecones in temperate areas such as *S. stephanocystis*, *S. esculentus* and *S. tenacellus*; group B with those growing from pinecones in sub-tropical areas such as the new species *S. luchuensis* nom. prov. in the Yaeyama area and group C with those not growing from pinecones in temperate areas as *S. ohshimae*. We aim to know the effects of substrates and temperatures on the growth of *S. stephanocystis*, *S. luchuensis* and *S. ohshimae* as the representatives of the 3 groups above . Four powder substrates from pinecone of *P. densiflora,* dominate in the Japanese main islands; *P. kesiya* dominate in Southeast Asia; *P. luchuensis* dominate in Okinawa; and beech sawdust (*Fagus* spp.) were used to test the substrate adaptation. Temperature effects were tested on a range of temperature from 5 – 35°C, 5°C difference in each step. Survival tests were applied on all non-growth experiments. In results, *S. stephanocystis* grew in all substrate but not all experiments. However, *S. ohshimae* grew well in all substrates except *P. densiflora* while *S. luchuensis* did not grow in all substrates except *P. luchuensis*. On the other hand, all fungal strains died at 35°C and grew weakly at 5°C. *S. ohshimae* was not able to grow in 30°C and grew weakly in 25°C while *S. luchuensis* and *S. stephanocystis* grew well at those temperatures. These results could be explained in that *S. ohshimae* does not appear in pine forests of main islands and that new species *S. luchuensis* nom. prov. was adapted to Okinawan pine and climate.

1.1-92 Fungal foraging behaviour and space exploration in a pristine, micro-engineered habitat

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Abstract: The soil space is a maze-like habitat for saprotrophic fungi, with walls made of quartz grains and food sources heterogeneously scattered in dead-ends. Because of the micro scale heterogeneity and opaque nature of soils, the hyphal-scale foraging behaviours of these fungi have been intrinsically

hard to study and remained largely unknown. However, increasing our knowledge of what their limitations and abilities are could allow us to better understand fungal growth patterns, how fungi contribute to carbon sequestration in soils, and what role soil micro structures play in these processes. In this study, we developed transparent microfluidic chips in which we manipulated the fungal habitat by introducing microstructures. We further examined how fungal traits and growth patterns were affected by this structural heterogeneity at the micro-scale in real time. Our results show proof of fungi's ability to colonise nutrient free environments, bridge dry pore spaces, and navigate through complex micro-confinements. We also show that even fungal species traditionally classified as having the same nutritional strategy - litter decomposition - express very different growth strategies when colonising pristine habitats, and we present results of how six different species explore restricted spaces and overcome structural heterogeneity (tight channels, nutrient free spaces and obstacles) at the micrometre scale inside the microfluidic chips. When challenged with channels forcing the fungi to turn in angles steeper than 90 degrees, and U-turns, we found that some species cope better than others with re-finding their growth direction or getting out of a dead-end corner. Ecologically, this could mean that in the soil, some litter decomposers would be better at accessing remote soil spaces and resources in cores of complex aggregates, potentially also making a larger contribution towards building a stable carbon pool of fungal necromass in remote soil spaces, protected from other decomposers. To conclude, we believe that the microfluidic devices developed in this study open up for new possibilities to study fungal foraging behaviour and decision making at the resolution of single hyphae, something that could further allow us to decipher the role different fungal species play in shaping the soil environment and whether they all contribute equally to carbon sequestration in the soil.

1.1-93 Which criteria should be considered when appraising ectomycorrhizal communities for forest research?

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Abstract: Forests host a large part of terrestrial biodiversity and provide a wide range of ecosystem services; they regulate local, regional and global climate, store carbon, purify air and freshwater. Plants and their networks with associated ectomycorrhizal fungi play a crucial role in biogeochemical cycles, biodiversity, climate stability and economic growth. Furthermore, ectomycorrhizal fungi are themselves important drivers towards sustainable innovation in many research fields such as food industry, biotechnology, biomedicine and agroforestry. Due to complexity of natural environment, the evaluation of any type of change is often very difficult, since it may not be clear which environmental component will be affected by the stressor, what type of change will occur and what the exposure will be. Before–After Control-Impact (BACI) design overcomes the problem of attributing changes to an impact rather than natural variability. In this context, SelpiBioLife project was established to evaluate the effects of an innovative silvicultural treatment on different biological groups (flora, fungi, bacteria, carabids, nematods and microarthropods) in *Pinus nigra* plantations. In order to analyze management effects, we adopted a BACI design applied to two study areas, one located in Pratomagno and one in Monte Amiata (Appenines, Italy). Here, our main aim was to demonstrate whether the sampling criteria were appropriate to describe exhaustively the composition of ectomycorrhizal communities, presenting the results of sampling activity before any type of silvicultural treatment. Diversity and abundance of ectomycorrhizal fruiting bodies (EMFb) were determined using mycocoenological analysis. Soil sampling was set up to test ectomycorrhizal root tips (EMRt) community. Morphological structure of each

morphotype was examined and molecularly identified by means of a direct PCR approach. Analysis of species richness and composition as well as the effect of spatial scale on EMRt and EMFb communities were assessed using rarefaction technique, permutation tests for multivariate analysis of variance (PERMANOVA), Non-metric multidimensional scaling (NMDS), Mantel's tests and similarity Percentage Analysis (SIMPER). Rarefaction curves concerning variation in species richness among fungal communities supported the lowest species richness in EMRt respect to EMFb community. PERMANOVA revealed that spatial-topographic factors significantly affected community composition. The Pair-wise *t*-test showed significant differences between EMRt and EMFb. NMDS confirmed this trend, showing a clear separation between fungal communities in terms of species composition. Furthermore, Mantel's test resulted in no correlation in distance matrices of community structure. SIMPER analysis indicated that the average dissimilarity between EMRt and EMFb communities was relevant. In conclusion, these results suggest that the adopted sampling criteria are appropriate to exhaustively and quickly appraise the composition of ectomycorrhizal communities for forest research.

1.1-94 Response of microbial community to forest succession in Odaesan National Park, South Korea

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Abstract: Succession is variation of ecological communities involved by environmental changes. There are many types of succession caused by extreme stress factors such as volcanic eruptions, landslides, and floods, whereas a succession that occurs slowly over time. Odaesan National Park is well-preserved forest located in the Taebaek mountain range in South Korea. The forest succession of the national park is progressing from a mixed-wood forest to a hardwood forest. In this study, the microbial community composition was investigated using 454 sequencing of the soil samples collected from 13 different locations in Odaesan National Park. We assessed whether the communities are affected by environmental factors such as water content (WC), nutrient availability (total carbon and nitrogen) and pH, which were caused by forest succession. As results, water content, total carbon (TC), total nitrogen (TN) and pH were statistically different according to the succession stages of the forest. WC, TC and TN of forest soils tended to increase as succession progressed, while pH tended to decrease. In both succession stages, the bacterial genus *Pseudolabrys* was most abundant, followed by *Afipia* and *Bradyrhizobium*. In addition, the fungal genus *Saitozyma* showed the highest abundance in the forest soils. The beta diversity of microbial communities was determined by NMDS analysis, which showed a clear discrimination of microbial communities according to forest succession stages, and soil properties (WC, TC, TN, and pH). Furthermore, a network analysis of both bacterial and fungal taxa showed the strong relationship of the microbial community depending on the soil properties caused by forest succession. Further study about functional profiling of each microbial components will help to understand the succession of forest ecology.

1.1-95 Soil depth matters: Communities of bacteria, fungi and micro-eukaryotes structured along soil stratification

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Abstract: The boreal forest is a key ecosystem for global C sequestration and storage. Microorganisms in soil have crucial functions in regulating these processes. Fungi are typically sharply structured with soil depth, but we largely lack such information for other microorganism, including bacteria and other micro-eukaryotes. To improve our knowledge of how different microorganisms are structured vertically and how they might interact, we investigated the community of bacteria, fungi and micro-eukaryotes in four different soil horizons in natural birch forests in Western Norway. The communities of all three organismal groups were strongly structured along the vertical depth. Our results support the hypothesis that natural decrease in nutrient availability and pH differences between organic and mineral horizons affect the distribution of soil microorganisms. Proteobacteria, Actinobacteria and Planctomycetes dominated in the uppermost organic layer while Acidobacteria and Firmicutes are in mineral layers. Proportionally, fungi dominated in mineral layers whereas other micro-eukaryotes (Metazoa, Apicomplexa, Conosa, Ochrophyta and Chlorophyta) in organic layers. Ascomycota were relatively more abundant in mineral layers compared to Basidiomycota and Cryptomycota. Nematoda, Annelida and Arthropoda showed decreasing trends with depth. Furthermore, separation in the optima of different ectomycorrhizal and saprotrophic genera was observed, supporting the view that different genera are adapted to different niches along the soil depth gradient. Network analyses are used to infer tentative biotic interactions between the microbial groups.

1.1-96 Impact of deadwood on fungal communities in underlying soils

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Abstract: Fungal communities in soil are crucial to ecosystem functioning by decomposing dead organic matter and supplying plants with nutrients. Composition of the communities is predominantly shaped by edaphic parameters and the local plant community. However, recent studies show that application of deadwood locally alters composition of the fungal communities in underlying soil. While the underlying mechanisms have not been studied so far, it is conceivable that nutrient transfer by fungal hyphae connecting deadwood and soil entails changes in the soil community. However, wood and soil are usually inhabited by different fungi and only few species are known to colonize both habitats. In contrast to this perception, we hypothesized that a considerable proportion of wood fungi may invade the underlying soil. To test this hypothesis, we sampled soil directly under 9-years-old deadwood logs of 13 different tree species at 29 plots in three regions of Germany. Since wood inhabiting fungi are often selective for wood of one or few different tree species, occurrence of such fungi in the corresponding underlying wood would support our hypothesis. Composition of the fungal communities was assessed by Next Generation Sequencing (Illumina MiSeq) of the ITS rRNA gene region amplified from DNA extracts. Analyses of the data confirmed that soil fungal communities are predominantly shaped by geographic location, soil type, and plant community. Our hypothesis was rejected due to the wide absence of wood-selective fungi in the underlying soil. Effects on soil fungal communities are therefore more likely to result from soil fungi invading the deadwood. However, species richness was higher in soils beneath deadwood than in the uncovered control soils. Further implications on soil fungal community structure and potentially underlying mechanisms will be discussed.

1.1-105 Coprinoid coprophilous fungi in Vietnam

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Abstract: About 125 species have been recognized and recorded for coprinoid agaricales, consisting of *Coprinus, Coprinopsis*, *Coprinellus* and *Parasola*, (Basidiomycota). Among them, about 40 species are known to have coprophilous habit, which appear on animal excrement (2005 Uljé). In Vietnam, 28 species of coprinoid agaricales have been recorded (Patouillad 1910; Trinh Tam Kiet 1998, 2011, 2013) and 6 species of them were coprophilous. We have studied on the coprophilous fungi in Vietnam (around South area). We and our colleagues have collected dungs of wild animals such as elephant etc. in 2015-2016. By the moist chamber method, the emergence of coprophilous fungi was induced, and the fruiting bodies appeared on the substrates were isolated. The collected samples were morphologically and phylogenetically determined. So far, we have identified 8 coprinoid coprophilous species, including 3 new records in Vietnam and 3 new species from Vietnam. These 3 new taxa, designated as *Coprinopsis* sp. 1, *Coprinopsis* sp. 2 and *Coprinellus* sp. 3, are characterized as follows: *Coprinopsis* sp. 1: This species is similar to *C. nivea* and *C. igarashii*, that all share the mealy powdery veil of globose element but differs in the size of veils and basidiospores, collected from a dung of elephant (*Elephas maximus*); *Coprinopsis* sp. 2: this species is similar to *C. clastophylla* in phylogenetically but differs in having globose type veil element; *Coprinellus* sp. 3: this species is similar to *C. marculentus* but differs in lacking pileo-, pleuro- cystidia, collected from an elephant dung. The phylogenetic analysis of ITS data also supports these new taxa. In the session we will show the morphological details and phylogenic positions of newly recognized coprinoid coprophilous species from Vietnam.

1.1-106 The genus *Coprinellus* in Pakistan with the description of three new species

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Abstract: Mushrooms with thin-fleshed pileus that becoming plicate on opening, deliquescent gills and dark brown to blackish basidiospores are commonly called coprinoid mushrooms. The genus *Coprinellus* is one of the important lineages of coprinoid mushroom in Psathyrellaceae. Species-level taxonomy in *Coprinellus* is based on veil structure and basidiospore morphology. In this study three new species of *Coprinellus* (*C. campanulatum*, *C. dissminatus-similis* and *C. tenuis*) are described from Pakistan. Species description are based on morphological and molecular data. Phylogeny based on nuc rDNA region encompassing the internal transcribed spacers 1 and 2 along with the 5.8S rDNA (ITS) show that the new species *C. campanulatum* sp. nov. and *C. dissminatus-similis* sp. nov. are clustered in a clade formed by the members of section *Micacei*; *C. tenius* sp. nov. falls in section *Domestici* of genus *Coprinellus*. Morpho-anatomical descriptions of the new species and comparison with closely allied taxa are provided. With this study the number of known species of *Coprinellus* in Pakistan reached to seven.

1.1-107 Taxonomy and Phylogeny of genus *Lycoperdon* from Pakistan

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Abstract: Genus *Lycoperdon* was first established by Persoon in 1796 by describing *L. perlatum* as a type species. It is characterized by sub globose to pyriform basidomata with conspicuous sterile base and dehiscence by an apical pore. It is represented by 54 species worldwide. Recent molecular phylogenetic studies have widened the concept of the genus and new limits of *Lycoperdon* have been established by proposing many subgenera within the genus, i.e. *Apioperdon, Bovistella, Lycoperdon, Morganella, Utraria* and *Vascellum*. In Pakistan, twenty-one (21) *Lycoperdon* spp. have been reported so far. During this investigation, eight (8) *Lycoperdon* species have been identified and described using morphological and molecular methods based on ITS-nrDNA region. Among these, three (3) belong to subgenus *Vascellum*, two (2) each to subgenera *Bovistella* and *Lycoperdon* and one (1) to subgenus *Apioperdon*. Out of these, three (3) species have been found previously undescribed viz., *L. lahorense, L. olivoflavum, L. parvisporum*, and reported here as new species. *L. utriforme* is a new record for Pakistan and three (3) species have been reported from new localities of the country. One taxon, previously published as *Bovistella japonica* on morphological basis has been shifted to subgenus *Bovistella* of genus *Lycoperdon* based on molecular analysis in this study.

1.1-108 Diversity and phylogeny of *Leucoagaricus* and *Leucocoprinus* (Agaricales, Basidiomycota) in the Neotropics

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Abstract: Among the lepiotaceous fungi in the Agaricaceae the *Leucoagaricus/Leucocoprinus* clade is particularly diverse in the tropics, with a lot of species awaiting discovery and formal description. The major mycofloristic studies of this group in the American tropical region date back to before the molecular era, and most of the molecular data currently available for this area come from the studies on ant-associated fungi and their free-living relatives, that do not focus on taxonomy. Over the past ten years, one of the authors (C.A.) has collected and studied fungi in the Dominican Republic. More than 300 species of macrofungi have been recorded, and almost all voucher specimens are deposited in the herbarium of the Jardin Botánico Nacional Dr. Rafael Ma. Moscoso (Santo Domingo, Dominican Republic). Approximately 20% of the collections represent lepiotaceous fungi of different genera (*Chlorophyllum, Cystolepiota, Lepiota, Leucoagaricus, Leucocoprinus*) and are currently being studied and sequenced. In this contribution we studied the species of *Leucoagaricus* and *Leucocoprinus* present in the Dominican Republic, using morphological and molecular methods, carefully comparing all our collections to the previously described Neotropical taxa. Seven species of *Leucoagaricus* (*La. bulbiger, La. margaritifer, La. peglerii, La. roseovertens, La silvestris, La. stillatus, La. turgipes*) and three of *Leucocoprinus* (*Lc. antillarum, Lc. fuligineopunctatus, Lc. microlepis*) are proposed as new. Additional records of previously described taxa are also discussed, including the first confirmed occurrence of *La. rubroconfusus* in its putative natural habitats.

1.1-109 The genus *Agaricus* in the Caribbean II. Phylogenetic placement of collections from the eastern Greater Antilles using multigene sequences analyses

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Abstract: The taxonomy of the genus *Agaricus* was recently evaluated and reconstructed using multigene (ITS, LSU, TEF, RBP2) analyses of collections from temperate zones (mostly Europe and United States) plus tropical areas of Africa, Asia and the Americas, including some material from the Caribbean region. Six subgenera and 23 sections were proposed under this new taxonomic arrangement of infrageneric taxa in *Agaricus*. Following the same approach, we conducted phylogenetic analyses using ITS, LSU and TEF data to determine the placement of additional Caribbean collections within this new arrangement. This research was initiated when only ITS sequences were available for comparison, but now with tropical data available for ITS, LSU and TEF we were able to compare 29 collections from Puerto Rico, 11 from the Dominican Republic and 7 from the Virgin Islands in a multigene analysis. Among the studied collections, we found representatives of all 6 subgenera and 13 sections, including sections recently described from material collected in the Dominican Republic. Most of the collections studied fell within *A*. subg. *Minores*, *A*. subg. *Spissicaules* and *A*. subg. *Pseudochitonia*, but since there are not multigene data available for several species of the sections in these subgenera, further studies including more temperate species are needed to define their closest relationships. We also placed collections in sections *Agaricus, Leucocarpi*, *Minores*, *Rarolentes*, *Subrutilescentes* and *Xanthodermatei*; these collections may represent new species, which we will investigate further using morphological comparison. In the present study, morphological and multigene data have confirmed the presence of *A. bisporus*, *A. californicus*, *A. endoxanthus*, *A. martinicensis*, *A. pocillator*, *A. subrufescens* and the two recently described *A. lodgeae* and *A. porphyropos* in the Caribbean region.

1.1-110 New taxa of Agaricomycotina from Brazil

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Abstract: Agaricomycotina is a large group of mostly macroscopic Basidiomycota, usually known as mushrooms, jelly fungi, boletes, earth-stars, corticioid fungi, polypores, clavarioid fungi among others. During the last 20 years, efforts of collecting and identifying them in Brazil have been undertaken and were reinforced in the past 5-10 years. The field trips were mostly conducted in the Atlantic Rain Forest, Amazonia and Cerrado in North and Northeast Brazil and several new taxa are being described using morphological and molecular characteristics. In the corticioid fungi, we present one new species of *Amyloathelia*, three of *Botryodontia*, two of *Byssomerulius*, two of *Gloeocystidiellum*, 13 of *Hyphodontia*, two of *Luteoporia*, one of *Lyomyces*, one of *Meruliopsis*, four of *Phlebiopsis*, one of *Resinicium*, one of *Sistotremastrum*, one of *Subulicystidium*, five of *Trechispora*, two of *Vararia*, two of *Xylobolus*, one of *Xylodon*, and two new genera. Among the jelly fungi, two new species of *Calocera*, two of *Dacryopinax*, two of *Tremella* and two new genera are introduced. In the poroid fungi, we present one new species of *Henningsia* and one of *Ceriporia* and three new genera; additionally, two synonyms are solved, one confirmed and one old name is recovered. Among the clavarioid fungi, one new species of *Clavulinopsis* and one of *Ramariopsis* are introduced. The results indicate the high, but still undiscovered

mycodiversity in the Brazilian forests and also that the addition of Brazilian specimens in the phylogenies improves the delimitation of taxa.

1.1-111 Phylogeny overview of Hygrophoraceae from China

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Abstract: Hygrophoraceae, a large, attractive and divers family in Basidiomycetes, includes mainly the agaricoid white-spored mushrooms with waxy pileus and gills, some basidiolichens and some corticioid fungi. Some important morphological characters of its members are often very susceptible to environments, so it is challenging to do morphological recognition, especially for the Chinese taxa since the knowledge of Chinese Hygrophoraceae is still very limited and there is no monograph about them. Therefore, it is significant to do a systematic study based on Chinese materials. In the past seven years, with over 200 Hygrophoraceae fresh samples collected from China, three gene fragments (ITS, LSU, RPB2) were used to reconstruct the phylogenetic relationships of the family Hygrophoraceae based on both newly generated and downloaded sequences. In the results, phylogenetic framework of worldwide Hygrophoraceae shows it as a monophyly family with three monophyly subfamilies, conforming to the subfamily concepts of the previous studies without Chinese data. Under subfam. Hygrophoroideae, genera *Hygrophorus* and *Haasiella* are strongly supported as sister groups, while *Chrysomphalina* is the basal group. The division of subfam. Hygrocyboideae into three tribes is accepted: tribe Hygrocybeae is made by the largest genus *Hygrocybe* and the rough-spored genus *Hygroaster*; under tribe Humidicuteae, *Humidicutis* and *Gliophorus* are confirmed as sister groups with high support value, *Porpolomopsis* is close to them, while *Neohygrocybe* is located at the base of the tribe; and tribe Chromosereae is a basal group of the subfamily, including two sister genera *Chromosera* and *Gloioxanthomyces* and a new genus from China. Multigene phylogenetic analysis shows that China is rich in Hygrophoraceae resources: 13 known genera and a new genus are present, over 60 species can be recognized and at least 30 of which are new to science. Although the recognized taxa can constitute the basic categories of a monograph, more samples and sequences are still needed in order to make a more comprehensive monographic study on Chinese Hygrophoraceae. This study was financed by the National Natural Science Foundation of China (Nos. 31170026, 31370071, 31493011).

1.1-112 Taxonomy and molecular phylogeny of bioluminescent mushrooms in Taiwan

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Abstract: Bioluminescent mushrooms are capable of emitting light from their basidiocarp, mycelia or both. Eighty-nine species of bioluminescent mushrooms have been recorded worldwide. Twelve of them were found in Taiwan. In this study, specimens were recently collected from mountainous areas in Taiwan, and were identified by both morphological characters and sequences of internal transcribed spacers (ITS). ITS-based phylogenetic tree was inferred by maximum likelihood (ML) and Bayesian inference (BI) algorithms. In this study, 15 species of bioluminescent mushrooms were collected, including three new species of genus *Mycena* and four new records (*M. deeptha*, *M. stellaris*, *Omphalotus japonicus* and *Panellus luxfilamentus*). The phylogenetic analyses showed that the three new species formed a monophyletic group respectively. Key to bioluminescent mushrooms from Taiwan

and morphological characters of the three new species were provided. The report of this study brings the total numbers of bioluminescent mushrooms worldwide to 92 species.

1.1-121 The white elephant in the black box: characterizing ITS copy number variation and its influence on fungal metabarcoding studies

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Abstract: High-throughput (HTS) amplicon-based 'metabarcoding' studies of fungi frequently target the Internal Transcribed Spacer (ITS) region, producing millions of reads from a single sample. These reads are frequently used for both species identification and to quantify relative abundance by using read abundance as a proxy for species abundance. The ITS region is multi-copy and the number of ITS copies may vary between species, altering the amount of template DNA available for amplification. The vast differences between observed and expected read abundance in fungal mock communities have been attributed, in part, to potential differences in ITS CNV. However, the actual CNV between fungal species, and its relative influence on HTS read abundance is largely unknown due to the difficulty in discerning ITS copy number using molecular techniques. Here, we investigate ITS CNV using a genome-based *in silico* read depth approach, applied to 21 species of ectomycorrhizal fungi, and characterize the functional consequences of ITS CNV in HTS datasets using mock community analysis over both the ITS1 and ITS2 regions. We report that ITS CNV varies over an order of magnitude between closely related species with no indication of phylogenetic conservation. However, HTS read abundance shows no clear correlation with ITS CNV. Instead, the primary source of variation in sequence abundance in HTS datasets appears to be associated with the process of PCR, with inhibition or preferential amplification of certain lineages in samples containing mixed communities.

1.1-122 International Collection of Microorganisms from Plants (ICMP)

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Abstract: The International Collection of Microorganisms from Plants (ICMP) is New Zealand's national culture collection of living Bacteria, Fungi, and Chromists. The collection and associated databases considered 'Nationally Significant' by the government and is in part publicly funded. The ICMP holds 20,000 cultures predominantly from plant, soil, and water in the natural environment, as well as important reference and type cultures of the world's plant pathogenic fungi and bacteria. All cultures are databased and available online at cultures are available for a fee to cover retrieval costs. New accessions into the collection are welcome, and recommended when publishing papers on microbes to provide a stable permanent resource for future researchers. The cultures are preserved under liquid nitrogen or in freeze dried ampoules. The ICMP containment and transitional facility conforms to enhanced PC2 Containment criteria, with generic permits to import quarantine and unwanted organisms into New Zealand.

1.1-123 Describing and communicating species

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Abstract: Describing and communicating species are fundamentally different approaches and can be referred to as a "The two faces of taxonomy". Here they stand for Karl Popper and Carl Linnaeus.

According to Popper, scientific theories or hypotheses must be falsifiable. It means all data and criteria used for species descriptions must be presented and available for scrutiny – they must, in short, be reproducible. Otherwise we can't falsify species as hypotheses, which according to Popper, would leave them unscientific. In digital era this means that all data (anatomical, genetical, etc.) of all specimens or individuals of the described species must be available as open data. Even better would be if these data are available in machine readable format, which makes reanalyses quick and comparable over time. There are already repositories that provide services to publish species datasets in such formats. Prior to the digital era, the "face of the taxonomy" was secured by listing all studied specimens lodged in public collection(s). In order to falsify described species, taxonomists reanalysed those specimens through loans or by visiting collections. In conclusion, high quality species descriptions were and are scientific when they make data open. Once a species has been described, the second "face of the taxonomy" - communication - will enter the stage. Communication of species is backed by the Code, and Carl Linnaeus is the face indeed. The Code is built for communication of species only and does not govern their description. It is a brilliant example how communication must be built. However, the digital era and the availability of DNA sequences combine to change the way we describe species. They are also changing the way species are used by the research community in metabarcoding and other studies. It is obvious that current Code should follow these advances in order to serve us today and in future. But what has to be changed in the species communication system? There is a proposal to allow DNA sequences to be served as a type of the species. Proponents argue that this change in the Code will allow us to describe and communicate species based on DNA sequences only - and that this will solve the major problems we are facing in this field. In this talk I will demonstrate that this change will not solve current challenges in taxonomy. An alternative way how we need to develop Code in order to serve modern taxonomy will be presented.

1.1-125 Updating the status of global fungal red-listing and a call for participation

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Abstract: Species of fungi are not immune to the threats that put animal and plant species at risk, i.e., habitat loss, loss of symbiotic hosts, pollution, over exploitation, and climate change. Yet, fungal conservation is only now receiving significant attention. So, it is not surprising that fungi have rarely been included in broader conservation discussions, policy decisions, or land management plans. A critical way to help politicians and citizens be more aware of the importance of fungi and the need to conserve them is to have fungal species included in the IUCN (International Union for Conservation of Nature) Global Red List. The Red List is a compilation of rigorous assessments of the extinction risk of individual species made using strict universal criteria and categories (www.iucnredlist.org). Until recently, some thought that it was not possible to rigorously assess the conservation status of fungal species using IUCN criteria because of the unique biology of fungi and insufficient information on their taxonomy, distribution and ecology. However, much progress has been made to address these challenges. The voluntary Global Fungal Red List Initiative aims to facilitate and coordinate efforts by the global mycological community to get species of threatened fungi assessed and included in the global IUCN Red List. The goal of the initiative is to raise awareness of fungal conservation among mycologists, the conservation community, policy makers and the general public. By the end of 2018, more than 100 fungi will be in. A broader engagement by the mycological community is needed to keep this initiative moving forward with the goal of having a sufficient number of species assessed to provide an indication of the conservation status of fungi in relation to other groups of organisms. The initiative needs additional contributors with knowledge of distributions, ecologies and population trends of individual species as

well as help with checking facts and suggesting species to assess. Gaps in our knowledge of fungal diversity, distributions, phenology, and responses to threats will continue to pose significant challenges to fungal conservation initiatives for the foreseeable future. However, we have sufficient knowledge on an increasing number of species to enable fungi and mycologists to play a larger role in regional, national, and global fungal conservation activities. Contribute by contacting the Global Fungal Red List Initiative or directly through their web-page.

1.1-126 A standard protocol for selecting rare or threatened lichens for IUCN red listing: Results from trial implementation in North America

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Abstract: Lichens are ecologically important and threatened by diverse forces worldwide. Yet their conservation has long been hampered by a widespread perception that 1) species are poorly known, 2) cannot be readily identified by non-experts, 3) direct threats are poorly documented/not identifiable, and 4) immediate actionable conservation items are unclear or impossible to implement. We will present a standard protocol that outlines how species can be selected for IUCN Red Listing and specific criteria that should be used to rank species as priorities for IUCN assessment (vs. those requiring further study). This protocol and associated criteria will be placed in the context of the first IUCN Red List assessment workshop for North American lichens that was held in New York in 2016.

1.1-127 An examination into the efficacies of various genomic analyses using *Ophiocordyceps camponoti-floridani*

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Abstract: Entomopathogenic fungi infect insects of several orders. Some of these fungi have the ability to manipulate the behavior of their insect hosts. Such behavior manipulating fungi can be found in the family Ophiocordycipitaceae, and more specifically the genus *Ophiocordyceps*. This genus contains many species that induce behavioral manipulation in ants, however many species are still undescribed. In the Southeastern United States, a recently discovered endemic species, *Ophiocordyceps camponoti-floridani*, infects and manipulates the native, abundant Florida Carpenter ant. This manipulation occurs with a light-induced biting mechanism that allows the fungus to complete its life cycle. Gaining a better understanding of this species, such as identifying potential genes associated with secreted bioactive compounds, is integral to our understanding of how this parasite is able to control the behavior of its host. To this end, we have isolated the fungus from an infected Florida Carpenter ant collected from Central Florida, and sequenced the genome of *O. camponoti-floridani* using two sequencing platforms: Illumina MiSeq and Nanopore MinIon. Due to the relatively new field of genomics, there are many different approaches to assemble, annotate and analyze the raw data. A stepwise pipeline that leads to an annotated genome can thus combine a variety of approaches, with one approach being more efficient and/or effective than the other. For each of these steps, we have examined several de novo assembly and annotation methods and parameters. These results were compared to data of previously produced *Ophiocordyceps* reference genomes. The various assembly and annotation outcomes are then verified through a completeness analysis. This eventually helps us create the most efficient pipeline for obtaining future de novo assembled and annotated genomes of related, novel *Ophiocordyceps*

species. The most complete *O. camponoti-floridani* genome is subsequently further analyzed by the identification and quantification of various annotated gene functions, focusing on identifying genes that could be contributing towards neurological and immunological regulation/domination on the host species. Our work thus contributes to a better understanding of parasitic manipulation of host behavior by a fungal species, while also generating a robust next-generation sequencing pipeline.

1.1-153 *Botryosphaeria* spp. and *Phomopsis* spp. causing leaf blight and necrotic spots on Rambutan (*Nephelium lappaceum* L.) in Puerto Rico

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Abstract: Rambutan (*Nephelium lappaceum* L.) is a tropical fruit tree that has been cultivated since 1998 on commercial orchards in Mayaguez, Puerto Rico. From 2003 to 2015, during a disease survey, leaf blight and necrotic spots were observed at commercial and experimental orchards throughout the island. Diseased leaves were disinfected and plated onto potato dextrose agar (PDA). Four isolates, A1 and A2, of *Botryosphaeria* spp. and A3 and A4, of *Phomopsis* spp. were purified and identified using taxonomic keys and DNA GeneBank sequence comparison. PCR amplifications of the ITS1-5.8S-ITS2 region and partial sequence of elongation factor 1-alpha (EF1-α) genes were used to support the identification. PCR products were sequenced and compared using BLASTn with other sequences of *Botryosphaeria* spp. and *Phomopsis* spp. submitted to the NCBI GenBank. Pathogenicity test was conducted on eight Rambutan trees, using three healthy non-detached leaves per isolate. Trees were inoculated with two separate or combined isolates for each leaf with 5mm mycelial disks from pure cultures grown on PDA. Leaves were kept in a humid chamber using plastic bags for 8 days under greenhouse conditions. Two of eight trees were untreated, inoculated with PDA disks only, and were used as controls. The test was repeated twice. Eight and 14 days after inoculations (DAI) isolates of *Botryosphaeria* spp. caused leaf blotch and leaf blight, respectively. For all isolates, diseased leaves turned from light brown to dark brown starting from the apex and spreading through the lamina with necrotic tissues ranging from 10mm to 40mm in leaf length. Only one isolate of *Phomopsis* spp. caused necrotic spots on the leaves of 5mm in diameter. To prove the pathogenicity test, *Botryosphaeria* spp. and *Phomopsis* spp. were re-isolated from diseased leaf, fulfilling Koch's postulates. Untreated controls showed no symptoms and no fungi were re-isolated from tissue. By having identified the fungal pathogens involved in leaf blight, a more specific management approach can be implemented against Rambutan tree diseases in Puerto Rico.

1.1-154 Inhibition of seedling establishment of soybean by *Phomopsis longicolla* Hobbs

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Abstract: Severe wilt with stem blight of soybean has been occasionally come across in our trial fields located in Hiroshima Prefecture in western Japan throughout the months of June until July since 2012. Leaves initially droop down to fade in color. The whole plants gradually wilt as turning brownish, and then wither entirely. Subglobose, dark brown to black pycnidia of a fungus frequently appear on lower parts of stems of the diseased plants. White to pale beige masses of conidia come out from pycnidia on

the stems under moist conditions. Four representative isolates of the fungus obtained by single-conidium isolation (isolates A3, Fuk, Sac and Sta) produced pycnidia on cuts out of stems of soybean or common bean on potato dextrose agar (PDA) at 25ºC under irradiation with black light. The isolates grew on PDA in the dark at 5-35ºC with maximum growth of 2.9-6.1 mm/day at 25-28ºC. No sclerotia, chlamydospores or teleomorphs were found with the isolates. The isolates were identified as *Phomopsis longicolla* Hobbs (Hobbs et al., 1985) from the morphological and cultural characters. For isolates A3, Fuk and Sac, the identification was supported sequences of rDNA-ITS amplified with primer pair ITS4 and ITS5 (White et al., 1990). No sequence data of rDNA-ITS could be obtained for isolate Sta. Mycelia of each isolate cultured on PDA at 25ºC in the dark for 6 days were suspended in distillated water, and incubated with seeds of soybean (cv. Sachiyutaka) on petri dishes at 24-26ºC in the laboratory. Seeds incubated with distillated water were served as controls. Seed decay, budding delay and/or sprout decay occurred in the incubation with respective isolates. Control seeds healthily germinated and grew, showing compatibility of the isolates with soybean. When seeds dipped in the same suspension of isolate Sac were planted in pots with normal nursery soil, growth delay of seedlings without stem blight was recognized. Part of them recovered. Seed decay of soybean by *P. longicolla* has been reported (Hobbs et al., 1985). This has also been recorded in Japan (Sato et al., 1989), and compatibility of the fungus with stems of soybean has been noted in the report. After that, stem blight of the plant by *P. longicolla* has been reported from China (Chen et al., 2013; Cui et al., 2009). The present symptom in Japan is likely to be due to *P. longicolla*, though pod and stem blight or bean sprout rot of soybean by *Phomopsis phaseoli* (Desm.) Sacc. [Syn. *Diaporthe phaseolorum* var. *caulivora* Athow and Caldwell, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm., *Phomopsis phaseoli* var. *sojae* (Lehman) Sacc.] have been recorded in Japan (Goto, 1925; Sato et al., 2014). We have not yet led to clear-cut reproduction of the present symptom in Japan by inoculation with the isolates, and will retry it as considering conditions of inoculation tests. The present isolates were deposited to Genetic Resources Center of National Agriculture and Food Research Organization, Japan, with accessions MAFF150072-150075, and DNA sequence data obtained here were registered in the DDBJ/EMBL/GenBank databases as accessions LC333208-333210.

1.1-155 Identification of *Gliocephalotrichum* spp. associated with post-harvest rot of fruits in Brazil

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Abstract: The Brazilian Cerrado has a large quantity of endemics plants and until now more than 150 new species of fungi have been described in association with hosts belonging to several botanical families. Although few studies have been carried out to identify species causing post-harvest rots, several diseased fruits have been observed in the plants of this biome. Thus, this study aimed to identify the fungal species associated with post-harvest rot of different hosts in Brazilian Cerrado. The isolates were obtained from fruits rots of *Caryocar brasiliense* (Pequi), *Syzygium jambos* (Jambo amarelo), *Syzygium cumini* (Jamelão), *Spondias purpurea* (Seriguela), *Spondias mombin* (Cajazinho), *Dypsis madagascariensis* (Areca de locuba), and *Roystonea* sp. Only *Gliocephalotrichum*-like isolates were selected for identification. The morphological characteristics are conidiophores septate, hyaline, erect, consisting of a stipe and stipe extensions subtending a penicillate conidiogenous. The conidiogenesis is phialidic, with phialides hyaline and producing conidia cylindrical, hyaline and aseptate, accumulating in a mucilaginous mass. Total genomic DNA was extracted from cultures grown on 2% malt extract agar (MEA) for 7 d, using the Wizard® Genomic DNA Purification Kit. The partial region of the translation

elongation factor 1-alpha (TEF) gene was amplified and sequenced using the primers EF1F and EF2R. The nucleotide sequences obtained were compared with sequences of type species and specimens available from GenBank. From the phylogenetic analysis by Bayesian inference, it was possible to identify five different species of *Gliocephalotrichum*. A total of 39 isolates were obtained, and from these, 22 specimens from *C. brasiliense* show absent stipe extensions and grouped in a well-supported clade that is phylogenetically distant from the other species while 3 isolates grouped with specimens of *G. longibrachium*. The isolates from *S. cumini* and *S. jambos* grouped in a phylogenetically distinct clade, representing a possible new species too. *G. simplex* was found associated to *D. madagascariensis* while *Gliocephalotrichum bulbilium* was obtained associated to *Roystonea* sp. (n=1), *S. cumini* (n=2) and *S. jambos* (n=5). This study reveals new hosts for *G. longibrachium*, *G. bulbilium* and *G. simplex* and the discovery of two possible new species, which will be described according the current International Code of Nomenclature for algae, fungi, and plants. Financial support: FAPDF, CNPq and UnB.

1.1-156 Phytopathogenic fungi affecting *Mentha nemorosa* in Puerto Rico

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Abstract: *Mentha nemorosa,* an aromatic herb known in the Caribbean as "yerbabuena", belongs to the Lamiaceae family. This species is valuable for its aromatic qualities and culinary use in dishes and tropical drinks. For the fiscal year 2014-2015, the cultivation of aromatic plants in general, contributed $334,000 to the revenue of Puerto Rico. Although this plant is produced under controlled conditions in greenhouses, it is affected by pathogenic microorganisms that cause yield losses. The most common symptoms observed were necrosis of leaves and stems; and chlorotic spots on leaves. Root and delayed plant growth were also observed. Therefore, the objective of this study was to identify phytopathogenic microorganisms of *M. nemorosa*. Symptomatic plants were collected, and microorganisms were isolated on PDA. Koch postulates were completed using healthy plants in a humid chamber. After a week, most of the isolates produced chlorosis on inoculated leaves. Interveinal necrosis was observed after two weeks. Using morphological keys, two pathogenic fungi were identified: *Colletotrichum* spp. and one isolate belonging to the Botryosphaeriaceae's family. *Colletotrichum* sp. colonies were grey with scant aerial mycelium towards the center of the plate and scattered ooze of orange conidial masses. Conidia measured 4.9 x 17µm. The species was molecularly identified as *C. queenslandicum* after DNA analysis of rDNA ITS region, actin, alfa-elongation factor, β-tubulin, and glyceraldehyde-3-phosphate dehydrogenase (GADPH) genes. Our isolate showed 95 to 98% of homology with *C. queenslandicum* sequences from Genbank. The isolate belonging to the Botryosphaeriaceae's family showed *98 to 99%* homology with *Lasiodiplodia parva* using rDNA ITS region, β-tubulin and elongation factor genes. On PDA, *Lasiodiplodia parva* colonies had initial greenish grey mycelia that turn white in the margins of the means, but sporulation was not achieved. This information is very valuable to farmers when considering effective disease management practices under greenhouse conditions.

1.1-157 Characterization of secondary metabolite production among *Cercospora* species associated with Cercospora Leaf Blight

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Abstract: *Cercospora* is a species-rich genus of phytopathogenic fungi with a cosmopolitan distribution and causes disease on a wide range of host species, including many of economic importance. Among the diseases caused by *Cercospora* spp. with a major economic impact in North and South America is Cercospora Leaf Blight (CLB) and Purple Seed Stain (PSS) of soybean (*Glycine max*). Recent work has shown several species are associated with CLB and PSS, but typically a single species dominates in a given geographic region. For example, *C. kikuchii, C. cf. sigesbeckiae,* and *C. cf. flagellaris* have been associated with CLB and PSS in Louisiana, but a single species, *C. cf. flagellaris*, dominates in field populations. Cercosporin, a mycotoxin produced by several species of *Cercospora,* is an important virulence factor capable of increasing disease extent and severity. However, there is variation in the quantity of cercosporin produced among strains within a species and among species and high cercosporin-producing species and isolates may have a competitive advantage under certain conditions. The aim of this study is to quantify cercosporin production in several strains of *Cercospora* and assess variation among and within species. Several isolates from each of three species associated with CLB and PSS in Louisiana were characterized for cercosporin production corrected for differences in growth rate. The cultures were monitored for fifteen days with growth measurements taken every three days. Media variants included potato dextrose agar (PDA), minimal medium with ground soybean leaf, and complete medium. Cercosporin production was quantified by placing mycelial plugs from the margin of actively growing cultures in four milliliters of five molar potassium hydroxide and measuring absorbance at 480 nm. The three species responsible for CLB and PSS show variation in cercosporin production across media. All species produced minute amounts of cercosporin (1-2μM) on soybean leaf minimal medium and complete medium. Cercosporin production on PDA was 5.5 to 29.9 times greater than that produced on other media. *Cercospora flagellaris* produced the highest average cercosporin concentration (25.45 μM), *C. sigesbeckiae* produced 16.65 μM, and *C. kikuchii* produced 7.27 μM. While there is significant variation in cercosporin production within species, an inverse correlation was observed between cercosporin production and relative standard deviation. The relative standard deviation in *Cercospora flagellaris* was 61%, while *C. sigesbeckiae* and *C. kikuchii* were 73% and 78%, respectively. We will characterize the range in cercosporin production within the genus using ex-type strains from an additional 24 species. Our results suggest there is significant variation in cercosporin production both within and among species and characterizing *Cercospora* isolates for cercosporin content is sensitive to the conditions under which the phenotyping is being done.

1.1-158 *Phytophthora nicotianae* causing heart rot of pineapple (*Ananas comosus*) in Puerto Rico

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Abstract: Pineapple is an important crop in Puerto Rico, representing 5 million dollars of annual revenues for the economy of the island. *Phytophthora* spp. strikes pineapple production and is one of the most important pathogens for local farmers. Worldwide, 'Heart rot' is an important disease caused by *P. nicotianae* and has increased concern among producers who have unsuccessfully applied chemical fungicides for its control. The excessive use of the only chemical fungicides registered in Puerto Rico (i.e.

Metalaxyl and Fosetyl Aluminium) has increased pathogen resistance, redounding in an ineffective control. Infected fields were evaluated every month for a year to characterize oomycetes and fungal species from five pineapple production areas of the island. During the rainy season from February to May, 2017, a 40% disease incidence of 'heart root' was observed at the southwestern region of Lajas. In Guanica, 75% disease incidence was observed in all plots sampled. Symptoms were chlorosis, necrotic leaf tips and death of many young plantlets. Diseased tissue sections were transferred to V8 PARPH agar. Two *P. nicotianae* isolates were identified using taxonomic keys from tissue samples collected at Guanica, Puerto Rico. Sharpy papillated, ovoid sporangium measuring 32 x 29µm with abundant chlamydospore production were observed. Sequences of rDNA ITS region and COX gene showed 99% of homology with data of *P. nicotianae* obtained from Gen Bank, confirming our morphological identification. Pathogenicity tests were conducted *in vitro* using tissue culture pineapple plantlets. Fourteen days after inoculation, a heart soft rot was clearly observed. We speculate that the expression of symptoms caused by this pathogen were directly related to high precipitation occurring during April, 2017 with 24.43mm. In seasons of low rainfall, from September to December 2017, *P. nicotianea* was not expressed. This study allowed us to assess the distribution and expression of pineapple heart rot disease during the production cycle to improve disease control. Providing a better idea about the disease management for the pineapple productors for the season.

1.1-159 Root-invading fungus of *Vicia sativa* at different growth stages

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Abstract: To explore Root-invading fungus of *Vicia sativa* at different stages, providing scientific basis for *Vicia sativa* disease prevention and control work, Use Lanjian1、2、3 and 333 / A four kings of *Vicia sativa* varieties as materials, at *Vicia sativa* seedling, branch, flowering and mature period sampling in 2017.Through isolating root invasion fungi by conventional tissue separation method, and identificating by morphological and ITS sequence analysis. The results showed that: (1) A total of 23kinds of root invading fungi were distributed from four kings of *Vicia sativa* varieties throughout the growing season, and eight 8 kinds of root invasion fungi were 4 kinds of *Vicia sativa* varieties shared, including: *Microdochium tabacinum*, *Fusarium acuminhtum*, *F. tricinctum*, *F. acuminatum*, *F. solani*, *F. trichothecioides*, *Rhizoctonia solani* and *Phoma multirostrata*. Both *R. solani* and *F. acuminatum* can be isolated during the whole growth period of four kinds of *Vicia sativa* varieties. (2) The dominant population of root invading fungus are different, but with little *difference at* different growth period. (3) The carrier rate of root segment With the growth period is "low - high - high - low" trend. (5) Pathogenicity testin doors by found that: *Scytalidium thermophilum*, *Mortierella alpine* and *Oidiodendron cerealis* have no pathogenicity. The five most pathogenic fungi are: F. *avenaceum*, *F. oxysporum*, *F. acuminhtum*, *Myrothecium roridum*, and *Clonostachys rosea*. Followed by *R. Solani* and *F. tricinctum*.

1.1-160 Diversity of powdery mildew fungi (Ascomycota, Erysiphales) in Azerbaijan

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Abstract: Powdery mildews are an economically important group of fungi. They cause serious damage on about 10.000 angiosperms including many crops, vegetables, fruits, cereals, and ornamental plants worldwide (Braun, Cook 2012; Amano 1986). Biodiversity of this fungal group has been explored well

in temperate regions of the Northern Hemisphere, such as Europe, North America and East Asia. Taxonomy of this fungal group has significantly changed during the last 20 years. Investigations of powdery mildews in Azerbaijan began in middle of the last century during exploring of mycobiota of the country, when comprehensive numbers of samples were collected and numerous taxa from Erysiphales were recorded. However, the exploration of powdery mildews in Azerbaijan was largely neglected in the past 30 years, because other fungal groups were in the focus of interest. The purpose of the present study was to conduct comprehensive investigations on powdery mildews, in order to identity and clarify taxonomy of the preserved herbarium collections in the Mycological Herbarium of the Institute of Botany, ANAS, and newly collected samples by application of modern morphological and molecular approaches. Samples were examined by means of molecular and morphological methods. Molecular analyses were done by using chelex method according to Meeboon and Takamatsu (2015). Morphological examinations were conducted under the optical microscope, using the lactic acid method (Shin, La 1993) and 3% NaOH for observations of asexual and sexual stages, respectively. Re-examination of herbarium samples and identification of new collections revealed 120 taxa from nine genera (*Erysiphe*, *Podosphaera*, *Sawadaea*, *Phyllactinia*, *Leveillula*, *Golovinomyces*, *Neoërysiphe*, *Arthrocladiella*, *Blumeria*) distributed on more than 400 plant species in Azerbaijan. *Erysiphe* is the largest genus consisting of 43 taxa, followed by genus *Podosphaera* with 22, and *Golovinomyces* with 21 species respectively. The genera *Phyllactinia* and *Leveillula*, each compile 12 species. Remaining genera include one or two species. During our study one new species – *Erysiphe azerbaijanica* Abasova, Aghayeva and Takamatsu was described from the *Microsphaera* lineage, and five species (*Erysiphe arcuata* U. Braun, *E. corylacearum* U. Braun and S. Takam., *E. quercicola* S. Takam. and U. Braun, *E. syringae-japonicae* (U. Braun) U. Braun and S. Takam., *E. viciae-unijugae* (Homma) U. Braun) were new powdery mildew records for Azerbaijan. The list of host plant species was amended by including new host species, such as *Castanea sativa* Mill. for *E. quercicola*, *Lathyrus odorathus* L. for *E. viciae-unijugae*, *Carpinus orientalis* Mill. for *E. arcuata*, *Polygonum alpinum* All. for *Golovinomyces spadiceus* (Berk. and M.A. Curtis) U. Braun, and *Alcea rosea* L. for *G. magnicellulatus* (U. Braun) Heluta.

1.1-161 *Herpomyces* (Laboulbeniomycetes): a new order and a new species spread through pet trade

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Abstract: The class Laboulbeniomycetes comprises fungi that are obligately associated with arthropods for dispersal or as biotrophs. Two orders are currently recognized, Laboulbeniales and Pyxidiophorales. The class is severely understudied and the available class-wide phylogeny is still provisional, because it is based on a single gene (SSU) and excludes a majority of the currently recognized taxa. *Herpomyces* is a morphologically and phylogenetically isolated genus with 25 species that exclusively parasitize cockroaches (Blattodea). Presenting the highest level of taxon sampling across Laboulbeniomycetes to date, we evaluate a three-gene phylogeny (nrSSU, ITS, nrLSU) and propose a new order in the class Laboulbeniomycetes. We describe Herpomycetales to accommodate the single genus *Herpomyces*. In addition, building on the six-gene dataset from the *Ascomycota Tree of Life* monumental paper by Conrad Schoch and colleagues (2009), we confirm that Laboulbeniomycetes and Sordariomycetes are sister orders and we apply 'Laboulbeniomyceta' as a rankless taxon for the now well-resolved node that describes the most recent common ancestor of both classes. A molecular clock analysis of this six-gene dataset, using five fossil calibration points, revealed that Laboulbeniomycetes and Sordariomycetes diverged around the Triassic-Jurassic boundary (206 Mya). Within Laboulbeniomycetes, the earliest split (divergence of Pyxidiophorales) occurred around 160 Mya. Finally, Herpomycetales and Laboulbeniales

diverged around 143 Mya. With this contribution, we add a robust molecular phylogenetic component to a group of fungi that has been almost exclusively defined by morphology. Our analysis of the ITS phylogeny of the genus *Herpomyces* brought to light an undescribed species on *Shelfordella lateralis*. Study of its morphology supports separation from other species in the genus. This new species has been discovered in colonies of *S. lateralis* in Hungary, Poland, and Massachusetts, USA. Interestingly, these colonies were all retrieved from pet stores, suggesting a role of international pet trade in the global distribution of these biotrophic fungi.

1.1-162 Metabarcoding of the pathogenic funus, *Ophidiomyces ophiodiicola*

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Abstract: Onygenales is a medically important order of fungi because it houses various fungal pathogens of humans and animals. Within recent years, emerging infectious diseases perpetuated by fungal pathogens have increased in wildlife, many resulting in currently uncontrolled epidemics such as chytridiomycosis among amphibians and white nose syndrome seen among bats. *Ophidiomyces ophiodiicola* is the primary agent of an infectious disease affecting various species of snakes across midwestern and eastern states. The pathogen colonizes the skin of its host, causing skin lesions, facial swelling, and hardened scales that, depending on the species of snake, can be fatal. There is a large variation in species susceptibility and aspects surrounding the fungus remain unclear; primarily its diversity. The objective of my experiment is to implement high throughput sequencing to understand pathogen diversity among its geographical distribution from cultured samples obtained from the National Wildlife Health Center in Madison, Wisconsin. Targeting and amplifying the internal transcriber spacer (ITS) region, which is universal among fungi, and BLASTing the collected sequences across databases such as the National Center for Biotechnology Information and GenBank will help compare and confirm all documented strains. Understanding diversity and distribution are integral in grasping mechanisms of infectious diseases. Using high throughput sequence analysis will aid in making connections behind fungal ecology and diversity among a relatively new pathogen that is threatening species such as the Eastern Massasauga Rattlesnake and Timber Rattlesnake. Furthermore, while extensive surveying has driven higher awareness, there is a lack of vigilance along western states. Extending this method of analysis to acquired swab samples from western facilities such as the Oregon Department of Fisheries and Wildlife and from universities with invested interests in this disease such as UC Davis, a more thorough and complete geographical distribution can be built. In conclusion, diversity and ecology are two sides of the same coin when attempting to understand infectious diseases of which not only elucidates climate tolerance but also the extent of diversity.

1.1-163 Fungal skin assemblages on hibernating bat species that are susceptible and resistant to white-nose syndrome in North America

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Abstract: North American bats have experienced catastrophic population declines from white-nose syndrome (WNS), an emergent disease caused by the fungus *Pseudogymnoascus destructans* (*Pd*). Although *Pd* has a broad host range on hibernating bats in eastern North America, population-level impacts of WNS vary by host species. For example, little brown (*Myotis lucifugus*), northern long-eared (*M. septentrionalis*), and tricolored (*Perimyotis subflavus*) bats have experienced precipitous declines due to WNS, whereas species such as big brown (*Eptesicus fuscus*), gray (*M. grisescens*), and Virginia

big-eared (*Corynorhinus townsendii virginianus*) bats appear to exhibit some degree of resistance to the disease and have not suffered such devastating losses. Mechanisms of WNS-resistance have not been fully elucidated, but are likely multifactorial. Microbial skin communities can influence host resistance to infectious diseases by inhibiting or competing with pathogens. We compared fungal assemblages on hibernating bat species with low or no WNS-related mortality (n=262) to WNS-susceptible bat species (n=163) using culture-dependent methods. The diversity and abundance of skin fungi differed between many of the bat species. Known WNS-resistant bat species had a mean of 2.5 ± 1.8 fungal genera per bat (mean Shannon index 0.48 ± 0.55), which was significantly higher than WNS-susceptible bats (1.1 ± 1.0 with a Shannon index of 0.12 ± 0.30; χ^2=103.54, p < 2.2e-16). of particular note was an abundance of certain yeast species (namely *Debaryomyces* spp.) on the skin of resistant bat species; these yeasts were absent or rare on the skin of bat species susceptible to WNS. Analyses to investigate potential relationships between fungal assemblages and resistance to WNS are underway.

1.1-164 Speciation by host-switching in two related cutaneous fungal pathogens of pet animals

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Abstract: We selected two closely related skin pathogens transmitted from pets to human (mainly children) with increasing tendency to infect human during the last few years. In Europe, species *Trichophyton benhamiae* and *T. erinacei* are transmitted to human mainly from guinea pigs and hedgehogs, respectively. A considerable genetic and phenotypic variability has been revealed in these emerging pathogens. To substantiate the initial finding, we assembled strains isolated from various hosts in different European countries, Japan and USA. We conducted several analyses to elucidate whether the detected level of variability reflects undescribed species diversity or a high infraspecific variability. A total number of 326 and 146 strains from *T. benhamiae* and *T. erinacei* complexes, respectively, associated with human and animal dermatophytoses were analysed using two sequenced loci, 10 and 7 microsatellites loci, respectively, and morphological and physiological methods. Among *T. benhamiae* isolates, we revealed four very distinct population clusters. Three of them were present in Europe and the last only in America. Among *T. erinacei* isolates, only two poorly differentiated clusters were found. The two *T. erinacei* populations seem to be strongly carrier-specific. First, most frequently human-associated population of *T. erinacei* (89% of human isolates) was specific for favourite African pet hedgehog *Atelerix albiventris*, second population was specific for wildlife European hedgehog *Erinaceus europaeus*. In *T. benhamiae*, we observed not so strong carrier-specific population pattern. As main carrier of American population of *T. benhamiae* we identified a dog and for European–Japan population rabbit. However, guinea pig was also recorded as frequent carrier in case of both populations. Another two populations, including most common population (78 % of all strains), are transmitted exclusively from guinea pig. The most common population of *T. benhamiae*, which is responsible for the current epidemic of dermatophytosis in Europe, was identified as closely related to American population. Based on genetic relatedness of strains of both populations, we suggest that virulent population of *T. benhamiae* was introduced from North America to Europe. There, it began to clonally spread among different host (guinea pig) even though original host (dog) also occurred here. High genetic divergence and phenotypic differences between all four populations of *T. benhamiae* indicate that they can be considered as independent species. In contrast, we found small genetic and

phenotypic differences between populations of *T. erinacei*, although they seem to be more carrier-specific.

1.1-169 The architecture of mucoralean genomes: Lessons from a ploidy point of view

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Abstract: Mucoralean fungi represent one of the most ancient terrestrial fungi. While most species are soil-inhabiting saprotrophic organisms, some species can also cause life-threatening infections in humans, called mucormycosis. While mucormycoses are uncommon infections, they have been increasingly recognized in immunocompromised patients during the last decades. More than 80% of these infections are caused by members of the genera *Rhizopus*, *Mucor* and *Lichtheimia*. Besides their clinical importance, mucoralean fungi also represent an interesting model to study evolution of basal fungi. However, research on this fungal group is still limited and only little is known about genome structure and genome evolution of these fungi. To get insights into the transition from saprobe to pathogen, a tripartite -omics based approach was applied using genomics, transcriptomics and lipidomics approaches of *Lichtheimia* species and specimens. The genomes of a total of six isolates from environmental and clinical background were sequenced and compared to other fully sequenced genomes of mucoralean and non-mucoralean fungi. All genomes were characterised by the presence of extensive gene duplications, which seems to be a universal feature of mucoralean genomes. The increased amount of duplicated genes is believed to be a result of a combination of an ancient whole genome duplication event in the early evolution of mucoralean fungi and additional lineage-specific single gene duplications. However, the exact mechanisms are unknown since all current knowledge relies on phylogenetic reconstruction of rather distantly related species. Our analyses revealed that strains of *Lichtheimia* species are generally haploid with an average genome size between 30 – 35 Mb and are well conserved between isolates. In contrast to these results, we identified a single strain which shows a genome size of about 60 Mb and appears to possess a diploid genome. Results of the analysis of single-spore isolates indicate that the nuclei of the strain are not identical, resulting in strong phenotypic differences between the colonies which are associated with defects in central signaling pathways and virulence. Virulence-associated traits appear to be well-conserved also in non-pathogenic species. However, differences were found in thermal adaptation, which was associated with metabolic changes. Interestingly, the sensitivity of *L. hyalospora* to thermal stress could be reduced by the simultaneous increase of osmotic pressure as a measure for the flexibility of the stress response. The data of this study give first insights into the variability of the genome architecture, the genomic, transcriptomic and lipidomic transition to stress resistance and pathogenicity of a mucormycotic agent. and the early consequences of genome duplication in basal mucoralean fungi. In addition, the availability and analysis of isolates with reduced virulence will contribute to the understanding of virulence-associated traits in these human pathogens.

1.1-170 Fifty strains of black: Resequencing of the melanised polyextremotolerant yeast *Aureobasidium pullulans*

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Abstract: *Aureobasidium pullulans* (de Bary) G. Arnaud is a melanised yeast-like fungus of considerable interest due to its ubiquitous distribution, polyextremotolerant physiology and large biotechnological

potential. It is best known as an epiphyte on various plant surfaces, but it is also frequently found in domestic environments and in food, in hypersaline water, in certain types of glacial ice, and a number of other habitats. It is used for the production of pullulan and aureobasidin A and is commercially available as a biocontrol agent for limiting the damage caused by several plant pathogens (both bacterial and fungal) in agriculture. In 2014 we published a *de novo* genome sequence of *A. pullulans* and three closely related species, revealing a redundancy in several gene families that could be linked to the nutritional versatility of these species and their particular stress tolerance. To build upon these initial genomic discoveries and investigate the population genomics of the species, we recently re-sequenced fifty additional *A. pullulans* strains from our culture collection, which includes isolates from various habitats and with a wide geographic distribution. Initial analyses of the data showed that the genomes share a high degree of similarity in their size and the number of predicted genes. Single nucleotide polymorphisms (relative to the reference genome) cover approximately 2% of the sequence. No clear population structure was detected with several methods and these results could possibly be explained by a fair amount of recombination within the species. The genome of *A. pullulans* contains a well-defined mating locus, but the teleomorph of the fungus has never been described. In addition to population genomics and evidence for recombination, other presented results will be focused on genomic traits linked to the polyextremotolerant character and biotechnological use of this interesting and useful black yeast.

1.1-171 Recent advances on taxonomy and systematics Boletaceae: the contribution of species from Thailand

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Abstract: The taxonomy and systematics of the Boletaceae have long been based on morphological species and genera concepts from Europe and North America. From about 2010 on, molecular (DNA) analyses including tropical boletes have started to profoundly change the systematics this group, with many new genera being published. This revolution is still ongoing, and the purpose of this paper is to present recent advances on Boletaceae systematics based on specimens collected in Thailand. We used both multi-gene phylogenetic analyses and morphological descriptions to better understand the systematics of Thai boletes. The most recent findings are two new genera, *Chromatophyllum* and *Cacaoporus*, and the identification of phylogenetic affinities of the genus *Rhodactina*. *Chromatophyllum* is a new phylloporoid genus forming a clade within the *Pulveroboletus* group, distant from the *Phylloporus* clade, which belong in the Xerocomoideae. Morphologically, *Chromatophyllum* can easily be distinguished from *Phylloporus* by the ovoid spores, lack of bacillate spore ornamentation, and deeply yellowish-orange to red lamellae. We described two new species of *Chromatophyllum* from Thailand, and recombined two *Phylloporus* species from the Americas in this new genus. *Cacaoporus*, which also belongs in the *Pulveroboletus* group, is unusual among Boletaceae in having a completely dark chocolate-brown hymenophore. Finally, with the discovery of a new *Rhodactina* species, *R. rostratispora*, we revealed the phylogenetic affinities of this truffle-like genus. It belongs in the Leccinoideae, and forms a well-supported clade with the other genera showing a purple color change of the spores when in contact with aqueous KOH solution, namely *Borofutus* and *Spongiforma*. The project is ongoing, and the impressive diversity of Boletaceae from Thailand might still bring interesting insights on the systematics and evolution of this family.

1.1-172 Mating genes evolution and population genetics in *Suillus brevipes*

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Abstract: Mating system is a crucial feature in population genetics. Sexual reproduction in fungi is controlled by MAT loci that define different mating types. MAT loci of basidiomycetes are characterized by a high level of polymorphism. The high diversity of mating alleles has attracted much attention, and is explained by strong negative frequency dependent selection in which rare alleles have a selective advantage over popular alleles which have lower fitness. Under this scenario, multiple alleles of MAT loci are predicted to have an extended coalescence time relative to other neutral loci. Previously, mating systems and allele number in basidiomycetes are inferred by pairing of single-basidiospore isolates. In this study, we employed aTRAM (automated target restricted assembly method) to recover haplotypes of the HD MAT locus from genomic shotgun sequencing data in populations of the bolete mushroom *Suillus brevipes* across North America. The HD MAT locus of *S. brevipes* only contains a pair of homeodomain encoding HD protein. De novo assembly of shotgun sequence data shows 56 distinct alleles among 55 dikaryotic samples, where heterozygotes in HD MAT are mostly found. Comparison of different haplotypes shows that the same HD1 allele always coexists with the same HD2 in our samples; multipartite linkages are not observed. Population genetic analysis suggests a distinct mating allele composition for each population, consistent with restricted gene flow among these populations in former studies. When compared to selectively neutral loci, we find HD MAT locus has weaker geographic differentiation among populations as theory predicted. Our results confirm that illumina short reads can be used to recover "idiomorphic" alleles of mating types from dikaryotic individuals, the high mating type diversity under balancing selection, and restricted recombination within the MAT region. However, phylogenetic incongruence between HD1 and HD2 phylogenies and analysis of recombination surprisingly implies that deep recombination also contributes to the generation of MAT allele diversity. More samples and analysis are required to fully reveal the evolutionary process of HD MAT alleles.

1.1-181 What do we know about the associated mycobiota of the built environment?

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Abstract: The list of fungal species reported to be associated with the built environment is long, even when broken down to comparable building characteristics. The reason is that surveys are very diverse in terms of sampling methods, identification protocols and environmental conditions. Moreover, the rapid development in taxonomic schemes is a major challenge. The phylogenetic species concept splits 'old' indoor related species of e.g. *Aspergillus*, *Penicillium*, and *Cladosporium* into numerous new species, often delimitated by differences in gene sequences only. The methods used for detection and identification are frequently updated and fine-tuned, but there will always be a lag-time before implementation is completed. Consequently, many reports and scientific papers on the mycobiota are using yesterday's species concept, which may be beneficial as there is a substantial body of knowledge

describing the functionality and ecology of yesterday's species. However, an updated sequenced based identification will generate results based on today's species concept where no or limited information on physiology, toxicology, ecology etc. is present. The modern molecular tools are getting faster and faster, but the functional characterization of taxonomic novelties cannot match the pace of the taxonomic development. The result is a crucial loss of knowledge of important indoor related species of *Aspergillus*, *Penicillium* and *Cladosporium* and several examples will demonstrate this unfortunate situation. The outcome is that many descriptive scientific papers contain long lists of fungal species detected; but what do these results tell us about the mycobiota of built environment or health impact? In most cases, nothing or only speculations that reflect the limited body of knowledge of today's species. The knowledge gaps represent an overwhelming amount of different types of very important data, far too much for a single research unit to cope with. It is proposed to develop and launch an international infrastructure that supports compilation of data on functionality of the fungal species. Ideally, in an open and easy accessible set-up e.g. like GenBank, and with the possibility to link between databanks to support a much better and deeper understanding of the mycobiota of the built environment and its impact on human health.

1.1-182 Improving precision in the study of the built mycobiome: recent changes and additions in the UNITE database for molecular identification of fungi

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Abstract: Recent molecular ecology studies have found the built environment to contain a surprisingly large number of transient and resident fungi. Some of them are associated with medical conditions in humans, notably asthma and eczema development, rendering the taxonomic affiliation of these fungi important from medical and many other points of view. Molecular (DNA-based) identification of fungi is, however, fraught with technical and biological complications, including lack of reference DNA sequences, incorrectly annotated reference sequences, chimeric or otherwise low-quality molecular data, missing metadata on, e.g., country and substrate of collection, and failure to follow relevant metadata standards. This presentation details the steps that the UNITE database for molecular identification of fungi has taken to facilitate molecular identification of the built mycobiome. The measures include organizing two sequence annotation workshops, sequencing >500 previously unsequenced fungal type specimens, implementing support for the MIxS-BE metadata standard, and devising several new software tools, including a search engine designed to find truly unknown fungi in the corpus of public fungal DNA sequences. This project, funded by the Alfred P. Sloan foundation, resulted in more than 75,000 improvements (such as name changes and metadata additions) to public DNA sequences relevant to the built mycobiome. These improvements were shared with a range of other databases and resources, including GenBank and the ISHAM database.

1.1-183 Fungal diversity of airborne samples is uncovered by DNA metabarcoding

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Abstract: Fungal spores and mycelium fragments become and remain airborne and have been subjects of aerobiological studies. The presence and the abundance of certain taxa in aerobiological samples can be very variable and impaired by changeable weather conditions. In particular, it is of key importance monitoring the presence of those fungi which produce mycotoxins, and both their mycelium fragments and spores are regarded as potential allergens. So far the traditional, morphology-based methodologies used to analyze fungi in aerobiological samples have mainly assessed the few, most abundant and easily identifiable taxa and have focused only on certain environments. This research presents a first, comprehensive assessment of fungal diversity from airborne samples using a DNA metabarcoding analysis. The region ITS2 was selected as fungal barcode to catch fungal diversity in mixed airborne samples gathered for one year in five sites of North-Eastern and Central Italy. Molecular data of fungal diversity within and among the sampled sites was assessed and compared with the identifications performed by traditional microscopy. The molecular analyses find fully correspondence with the morphological inspections and provide an almost ten-fold more accurate determination of the fungal taxa. The results prove that the metabarcoding analysis is a promising approach to increases quality and sensitivity of the aerobiological monitoring and pave the way for an automated fungal identification in airborne samples to be applied in routine aerobiological monitoring. To this aim, laboratory and bioinformatics workflows have been *ad hoc* implemented and are here presented.

1.1-184 Fungal communities associated with a condemned public housing project in Richmond, CA

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Abstract: Water-damaged housing has been associated with a number of negative health outcomes, principally respiratory disease and asthma. Much of what we know about fungi associated with water-damaged buildings has been gleaned from culture-based and immunochemical methods. A limited number of studies have used high-throughput sequencing technologies to assess the impact of water-damage on microbial communities in residential buildings. In this study we used amplicon sequencing and quantitative-PCR to evaluate fungal communities in a condemned public housing building in Richmond, CA, before it's residents were relocated. We recruited 21 households to participate in this study and characterized their apartments as either a unit with visible mold or no visible mold. We collected settled dust from bathrooms, kitchens, bedrooms and living rooms from units with and without visible mold, and from the outdoors. We recovered 5,333 OTUs from 92 samples. We found that fungal biomass was greater outdoors compared to indoors, yet there was no significant difference in fungal biomass in units with visible mold and no visible mold. Fungal richness was significantly reduced in units with visible mold compared to units with no visible mold and the outdoors. We also found that units with visible mold harbored fungal communities distinct from units with no visible mold or from the outdoors. Units with visible mold were dominated by taxa within the classes Eurotiomycetes, Microbotryomycetes, Saccharomycetes, and Wallemiomycetes. A number of the OTUs recovered in significantly greater abundance from units with visible mold, such as *Alternaria alternata, Cladosporium sphaerospermum*, *Rhodotorula mucilaginosa*, and *Wallemia muriae,* have previously been reported with water-damaged building materials. This study demonstrates that long-term negligence and poor building maintenance in low-income public housing impacts not only the human inhabitants, but also the fungi.

1.1-185 Carbon for phosphorus trade in AM fungi in response to host adaptation

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Abstract: The majority of land plants establish mutualistic symbioses with arbuscular mycorrhizal (AM) fungi. An association that benefits both partners by mediating uptake of essential plant nutrients, particularly P and N fueled by recently fixed carbon from host plants. AM fungi develop extraradical hyphal networks that link different plant species. The ability of the same fungal mycelium can colonize different plants at the same time involves an extraordinary level of compatibility and ability to adapt to different hosts by the fungus, though the adaptation to a single host might increase fitness of fungi at the expense of the other host. Our question is how plant and fungal fitness are linked and the genetics behind that. It has been suggested that AM fungi are heterokaryotic, contain genetically distinct haploid nuclei, and in response to a new plant species (changing environment), the developing fungal hyphae could temporarily segregate nucleotypes in the emerging spores to produce adapted offsprings that have different traits than the parents. We are conducting a greenhouse experiment to test if the symbiotic performance of host-adapted fungus spores (generation 1) propagated in a two-plant species system, when inoculated onto their same plant host species (generation 2). Symbiotic performance will be estimated using C to P trade in both generations. We will test for differences in C for P exchange between generations and if the one host-adapted generation will grow better at the expense of plant growth/fitness on the other host. Spores of AM fungus *Claroideoglomus candidum* (NC268, INVAM) were inoculated onto an outer soil between two mesh bags filled with soil and one plant seedling. The developed fungal mycelium has access to two different plants; *Petunia grandiflora* and *Allium ampeloprasum* (leek) split by the mesh bags that allow only the fungal hyphae to pass through and colonize the roots. The mesh bags were filled with low P soil; P in the outer soil is doubled. Four treatments were set up as follows: Treatment 1- two leek seedlings, Treatment 2- two petunia seedlings, Treatment 3- one leek and one petunia seedling, Treatment 4- one leek and one petunia seedling (the mesh bags will be split physically in new pots around month 4). The effect of the fungus will be assessed by comparison with control treatments in which fungus was not added. To investigate preferential plant C allocation and AM fungi P uptake, we will use radioactive isotope probing before the first harvest occasion (week 12) when mycorrhiza association is still physiologically active for all the treatments. ^{33}P isotope will be applied to the soil two weeks before ^{14}C labeling. C to P ratio will be estimated using liquid scintillation counting. At harvest, plant fitness will be assessed by biomass and fungal fitness spore counts. The roots will be stained to estimate the colonization rate. If we observe fungal fitness changes in response to host adaptation the genomic basis for this pattern will be analyzed using single cell genomics technique.

1.1-186 Host specificity of mycorrhizal fungi in Australian *Cryptostylis* orchids

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Abstract: The genus *Cryptostylis* is unique among Australian sexually deceptive orchids in that all five species are pollinated by the same wasp species - *Lissopimpla excelsa*. *Cryptostylis erecta, C. leptochila, C. hunteriana* and *C. subulata* occur sympatrically in eastern Australia, while *C. ovata* is restricted to Western Australia. Despite their sympatry and pollinator sharing, eastern Australian *Cryptostylis* do not hybridise. We investigated the mycorrhizal diversity associated with Australian *Cryptostylis* to determine whether an inter-species difference in mycorrhizal association could be contributing to the failed establishment of hybrids. We examined a minimum of 25 plants from across five populations of each species, except for the rare *C. hunteriana* where we analysed only two populations. Results of both fungal

isolations and direct sequencing of the ITS locus from peloton rich orchid tissue show that all the *Cryptostylis* species are associated with closely related Tulasnella fungi. We discovered four previously unidentified Tulasnella operational taxonomic units (OTUs). Specifically, *Tulasnella* OTU A is shared by four *Cryptostylis* species, *C. erecta*, *C. subulata*, *C. leptochila*, and *C. ovata*. *Tulasnella* OTU B is only associated with *C. ovata*, while *Tulasnella* OTU C has associations both with *C. erecta* and *C. subulata*. *Tulasnella* OTU D is only found in association with the vulnerable species, *C. hunteriana*. In addition to these four new OTUs, *Cryptostylis* were found to associate with *T. prima* and *T. sphagneti*, both of which are known to also associate with multiple species of sexually-deceptive *Chiloglottis* orchids. *Tulasnella sphagneti* was found to only associate with *C. subulata*, whereas *T. prima* also associates with *C. leptochila* and *C. ovata*. The association with *C. ovata* extends the known distribution of *T. prima* from eastern Australia to Western Australia. The *Tulasnella* symbionts of *Cryptostylis* all belong to a closely related group of species that are found with other sexually deceptive orchids in Australia such as *Drakaea, Caleana* and *Arthrochilus*. Due to the shared association with OTU A, mycorrhizal specificity is unlikely to explain the absence of hybrids in Australian *Cryptostylis*.

1.1-187 Mycorrhizal specificity and dependence of the leafless Ghost Orchid, *Dendrophylax lindenii*

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Abstract: *Dendrophylax lindenii*, the Ghost Orchid, is an endangered species that is native to far western Cuba and southern Florida. The leafless morphology of *D. lindenii* suggests that it has a high dependency for orchid mycorrhizal fungi (MF) when growing in its natural habitat because of the reduced photosynthetic capacity compared to orchids with leaves. We investigated the root mycobiota of *D. lindenii* individuals using amplicon sequencing and the potential dependence of the orchid on fungi for carbon resources through stable isotope analyses. We hypothesized that the root mycobiota of *D. lindenii* would be distinct from those of co-occurring epiphytic orchids and that $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ of *D. lindenii* root samples would be consistent with fungus to plant transfer. We collected root samples from *D. lindenii* individuals and several co-occurring epiphytic orchids from the Florida Panther National Wildlife Refuge. We also collected bark samples from several host trees of *D. lindenii*, i.e., *Fraxinus caroliniana* and *Annona glabra,* to investigate fungal composition. In total, we recovered 526 OTUs (i.e. 95% sequence similarity) from root samples. *Dendrophylax lindenii* samples were dominated by a narrow clade of *Ceratobasidium* OTUs, suggesting a high specificity for these fungi. Sequences of this Ceratobasidum clade were also obtained from bark samples of host trees. *D. lindenii* samples were highly enriched for ^{13}C, $\delta^{15}N$, and ^{2}H. We hypothesize that orchid MF of *D. lindenii* may potentially be drivers of their fine scale distribution and rarity, but this needs to be tested.

1.1-188 The ectomycorrhizal relationship between *Tricholoma vaccinum* and its plant host, *Picea abies* and geosmin biosynthesis in the ectomycorrhizal fungus

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Abstract: The relationship between ectomycorrhiza fungi and plant roots has been understood to be mutualistic. It is a relationship where the interacting fungus provides water, inorganic and auxotrophic nutrients and many other benefits to the plant host while the plant provide carbon for the fungus in

return. Recent preliminary findings in *Picea abies-Tricholoma vaccinum* ectomycorrhiza interactions, however, have shown plant defense responses while interacting with the "supposed" mutual partner, the ectomycorrhizal fungus *T. vaccinum*. Metabolomics studies and volatile analyses showed a very high upregulation of alkaloids and methyl-salicylates in the plant when interacting with the fungus. The effect of such plant defense responses was also evident for the fungus. The fungus showed lower fitness and reduced mycelial growth when grown in co-culture with *P. abies*. The plant, on the other hand, shows a significantly higher fitness when grown with the mycorrhizal partner than without it. To test if this is a species-specific scenario, ectomycorrhiza formation between *Paxillus involutus* and *Picea abies* was investigated, the reduced fitness of the fungus was also evident in *P. involutus-P. abies* co-culture. To study the molecular basis of the plant response to the ectomycorrhizal fungus interactions, gene expression analysis of different conifer defense genes while interacting with the ectomycorrhizal fungus will be studied. *T. vaccinum* produces geosmin, a decalol known to contribute to the earth smell observed post rain after a long spell of dryness. A gene in *T. vaccinum* has been shown, using mRNA transcript analyses, to be involved in geosmin biosynthesis in the fungus. Stable isotope labelling using deuterium labelled substrates was used to elucidate the pathway utilized in geosmin biosynthesis in this fungus. Concluding results showed that the basidiomycete fungus produces geosmin using the classical mevalonate pathway and it was shown that the alternative MEP pathway was not used for the biosynthesis of this chemical. Recent efforts are now put into the characterization of geosmin as a communication chemical in ectomycorrhiza interaction and the function of geosmin as a communication signal.

1.1-189 Cophylogeny of the lichenicolous *Tremella* species and their hosts: disentangling a species complex mainly based on cospeciation

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Abstract: Cospeciation between parasites and their hosts is difficult to demonstrate. One of the signs of cospeciation is that hosts and parasites share a congruent phylogenetic history and their tree topologies mirror each other. However, in the majority of host-parasite systems, other events such as host switching, lineage sorting or extinction are more frequent. High host specificity is well acknowledged within the lichenicolous species in the Tremellales (Basidiomycota, Fungi). Previous studies of *Biatoropsis* and their *Usnea* and *Protousnea* hosts revealed that host-switching is the most probable cophylogenetic event explaining host specificity in this system. With this work we extend these studies to another putative tremellalean lichenicolous species complex (*Tremella caloplacae* s. lat.) and its hosts, to evaluate if their joint evolutionary histories can be explained by parallel reciprocal speciation. We base this on a multi-loci matrix with data from Blunt-End Illumina® libraries. For species delineation we combine morphological, ecological and molecular data, as well as different molecular-based species delimitation methods. For the coevolution study we use a combination of event-cost methods, distance methods and dependence between phylogenies testing. Our results suggest that *Tremella caloplacae* is a species complex formed by at least six independent lineages, here interpreted as species. Each of these lineages grows on a distinct host clade, demonstrating high host specificity. Cospeciation is the most plausible cophylogenetic event to explain the joint evolutionary histories of *T. caloplacae* s. lat. and its hosts since it can explain the origin of five of the six independent lineages.

1.1-190 A molecular assessment of ectomycorrhizal diversity of cottonwoods

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Abstract: Poplars (*Populus* ssp.) are an economically important wood and bioenergy crop that are nutritionally supported by both arbuscular mycorrhizal and ectomycorrhizal fungi. While aspens (*P. tremula* and *P. tremuloides*) are known to host a diverse array of ectomycorrhizal fungi, cottonwoods (*P. trichocarpa*, *P. deltoides*, and *P. nigra*) are considered to have a more restricted set of ectomycorrhizal associates. Here we provide a comparative overview of the reported ectomycorrhizal associates of cottonwoods informed by metagenomics and sporocarp sampling from natural stands of *P. trichocarpa* in Washington and Oregon and *P. nigra* in France. Based on our sampling we have identified a well-defined community of cottonwood associates, including specific members of *Cortinarius*, *Hebeloma*, *Inocybe*, *Laccaria*, *Lactarius*, and others. It is our objective to phylogenetically place these species within their generic/species complex context and investigate patterns of their host association in relation to their distribution. We hypothesize that host-restricted species are common associates of cottonwoods as they are co-evolved and selected by their host, whereas ECM invaders, or generalist ECM species, are diverse on cottonwoods but rare.

1.1-191 Insights on the phylogeny of *Phyllachora*-like fungi infecting Myrtaceae

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Abstract: *Phyllachora* is a biotrophic fungus found on many Myrtaceae in Brazil; worldwide with *ca* 50 species recorded on myrtaceous hosts. Molecular phylogeny clearly showed that *Phyllachora* is a polyphyletic genus with species recently transferred to *Telimena* (Telimenaceae, Phyllachorales) and *Neophyllachora* (Phyllachoraceae), the latter considered as a genus with species infecting exclusively members of the Myrtaceae. However, both genera have species on myrtaceous host. To elucidate the relationship among these fungi within the Phyllachorales, six fungal species on *Myrcia*, *Psidium*, *Eugenia* and *Campomanesia* from Brazil had the rDNA (nuclear small subunit 18S, nuclear large subunit 28S and nuclear internal transcribed spacer ITS), and the translation elongation factor 1 (TEF1) nuclear loci partially sequenced and were used in multilocus phylogenetic analyses. The taxonomic identity of the fungal specimens was confirmed by morphological examination. The results suggest that the phyllachora-like fungi on myrtaceous host are not a monophyletic group, with species grouping in distinct families within Phyllachorales. Therefore, the circumscription of *Neophyllachora* might be revised. As an example, *Phyllachora furnasensis* became part of a strongly supported clade within *Telimena* and will eventually be recombined. Besides that, the phylogenetic analyses that led to the establishment of *Neophyllachora* need revision considering the new rules guiding the GenBank in terms of the size of acceptable sequences, and the fact that some sequences of mitochondrial DNA were used as if they were rDNA sequences.

1.1-192 Diversity and biogeography of Botryosphaeriaceae associated with Myrtales trees in Southern Africa and Southern China

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Abstract: Botryosphaeriaceae species are important latent pathogens causing diseases on many woody plants, including trees in the Myrtales. The diversity and biogeography of Botryosphaeriaceae on trees in the Myrtales in regions such as Southern Africa and Southern China is poorly documented. In this study, we identified Botryosphaeriaceae species on more than 30 native and non-native tree and shrub species in the Myrtales in five African countries (Malawi, Mozambique, South Africa, Tanzania, Zimbabwe) and the Guangdong and Hainan Provinces of the Southern China. Isolates were identified based on phylogenetic analyses of ITS rDNA, β-tubulin, TEF-1α, LSU and RPB2 sequences. The isolates originating from African countries resided in the genera *Diplodia, Neofusicoccum* and *Lasiodiplodia* and representing 10 species, of which four are previously unknown. The isolates originating from Southern China resided in four genera namely *Cophinoforma, Lasiodiplodia*. *Neofusicoccum* and *Botryosphaeria*, of which the latter two were absent in the Hainan Province. In this region, fourteen species were identified of which two represented novel taxa. The Botryosphaeriaceae species composition differed significantly between the regions where they were collected. *Lasiodiplodia* species were dominant in Southern China representing 80% of all isolates obtained in this study. *Lasiodiplodia theobromae* was the most common species from the latter region. In contrast, *L. theobromae* was absent from the studied hosts in Southern Africa, while *N. parvum* was the most abundant species collected. Only *L. gonubiensis* and *N. parvum* overlapped between Southern China and the Southern African countries. Other than *Caphinoforma mamane*, which was sporadically identified in both provinces in Southern China, there was no overlap of species identified on Myrtales in the two provinces. It appears that location rather than host association shapes Botryosphaeriaceae species composition on Myrtales hosts considered in this study.

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1.2-5 Fungi of the National Botanic Gardens, Glasnevin, Ireland – A taxonomic species inventory

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Abstract: The National Botanic Gardens habitat is a mature, dynamic arboretum with tended lawns growing a wide range of Temperate Zone garden plants, over calcareous and alluvial soils on Carboniferous limestone bedrock. There are old limestone perimeter walls and some 100-year-old quarried siliceous rock surfaces, with limited habitat for aquatic fungi. The site has experienced land use continuity as a public garden since 1795. The educational use of a mycological inventory, for this 19-hectare urban Botanical Garden, is to guide visitors to specific places in the garden where they can relocate and view perennial, ephemeral or transient species of fungi and lichens. This fungal species re-finder tool empowers our visitor guide staff for outdoor educational demonstration of the visual macroscopic features and other taxonomic characters of these species to visitors to Glasnevin. Macrofungi, microfungi on land plants, and lichenised fungi were identified using mycological literature, with growth visible to the naked eye using a x10 hand lens in the field and compound microscopy. Genetic techniques have not been employed as yet. This site inventory catalogues the contributions of over 100 different people and covers the time between 1795 and 2017. The entire Irish mycological chorological literature and distribution catalogues listing species record abstracts from County Dublin was filtered. Herbarium archives and a card index maintained by the late Maura J.P. Scannell from 1970 to 1989 formed the nucleus of pre-1990 records of 150 species. Original fieldwork since 2011 to extend the inventory was undertaken. Specimens were studied using standard taxonomic literature. An inventory of 540 species was prepared from over 1800 records of fungi and lichens in the National Botanic Gardens. Most species are known from one to a few dates per decade. The very low temporal and spatial density of agaric fungi in this city garden inventory is an interesting phenomenon, with a trend for a recent increase. The increase in corticolous lichen ascomycete diversity and density since the 1970s is shown clearly in the data, and this relates well with the big improvement in air quality in the city since 1989, and by the increased crown diameters of trees in the arboretum. Records of perennial and ephemeral fungi and lichens on site provide an interpretative window to 40% of 1500 species now known from County Dublin. For a student visiting and studying fungi and lichens at Glasnevin, in any week of the year, not more than 20% of the County Dublin mycota can be shown to them in situ. The general spatial rarity, temporal infrequency, and small biomass of colonies of most of the 540 species of fungi in this urban habitat merits further ecological explanation.

1.2-6 The National Institute of Science and Technology Virtual Herbarium of Brazil: a successful network for sharing herbarium data and images

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Abstract: Brazil's flora is considered the richest in the world, and its mycota is also estimated as among the most diverse on the planet. This immense natural wealth constitutes a scientific, economic and cultural heritage that must be scientifically recognized, preserved, and used in a sustainable way. Brazil has more than 200 herbaria; 65% of them have less than 20,000 specimens and only 9% contain more than 100,000 exsiccatae. The National Institute of Science and Technology (INCT) Virtual Herbarium of Brazil project has brought together data from more than 100 of these herbaria, from all States of the country, and from 21 foreign herbaria, by an intensive and extensive networking effort of curators, technicians and researchers. Its mission is to: Expand Brazil's knowledge base on flora and fungal diversity; Improve the quality of Brazilian herbaria; Encourage public policies to ensure sustainability of herbaria, training of taxonomists, and support to biodiversity studies; Make species occurrence data a fundamental instrument for decision making and for the formulation of public policies; Encourage free and open data and information sharing in a comprehensive, useful and usable format; Offer data and information to make environmental sustainability just as important as social and economic development in the formulation and assessment of public policies. In eight years, the INCT-Virtual Herbarium achieved its initial objectives and changed a paradigm by stimulating institutions and curators to share information while reinforcing the networking of collaborators that are willing to improve the collections and data quality, as well as in training personnel. The activities of the project include improvement and upgrading of collections' management and data quality (mainly the accurate identification of fungi and plants – 200 visits were carried out by 80 specialists to 70 herbaria, some more than once); training of students, technicians, and curators (60 courses were given by > 80 specialists, benefiting > 930 people), and technology development (with a range of digital tools: data cleaning, annotation system, lacunas, biogeo and statistics of data use) to make herbarium data and images available on-line. The INCT-Virtual herbarium adopted the *speciesLink* platform as the basis for its information system and the data are also available in other platforms (GBIF, IDigBio, and SiBBr). Through this network it has been possible to increase the knowledge about Brazil's biodiversity, disseminating about 6 million records and 1.9 million images of fungi and plants (http://inct.florabrasil.net). The INCT-Virtual Herbarium of Brazil represents a successful multi-institutional data-sharing network that allows access to the entire world, in a free and open way, to most fungi and plants recorded in the country. The Project, with its continuity, expansion and improvement recently approved for six more years, is supported by: Conselho Nacional de Ciência e Tecnologia (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE).

1.2-7 Utilization gaps: mushrooms as a means of poverty alleviation and forest conservation in the Chin State, Myanmar

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Abstract: The Greater Mekong Subregion (GMS) is well known for the high diversity and abundance of macrofungi, this diversity is coupled to the varied of landscapes and forest types across the region. The GMS also includes numerous rural areas, characterized by low income levels, poor access to markets, poor infrastructure, and high levels of forest degradation. Within this context, we have selected a number of villages in the Chin State of Myanmar to study the extent of traditional knowledge relating to the use of mushrooms, and how mushrooms can be used as a means of poverty alleviation and forest conservation. Preliminary studies on the diversity of mushroom species in the forest systems (fallow forest and natural forests) surrounding the villages and workshops with the villagers to assess their knowledge of the mushrooms collected have been carried out. Dominant tree species in both the fallow and natural forests included *Alnus nepalensis*, *Pinus kesiya*, *Quercus spp.*, *Castonopsis sp*, and *Rhododendron arboretum*. We collected 55 mushroom species of which 19 were edible, and 9 were of commercial interest - *Thelephora ganbajun*, *Termitomyces clypeatus*, *Auricularia auricula-judae*, *Ganoderma leucocontextum*, *Cantherellus sp*., *Lactarius sp*., *Russula albida*, and *R. virescens*. The 30 (14 women, 16 men) villagers interviewed in our study had almost no knowledge regarding the use of the mushrooms collected, and only two individuals had a basic knowledge (they recognized 3 species that could be eaten). All participants expressed a desire for further training and saw the potential of mushrooms as a resource, both for household nutrition and trade. All participants expressed a strong fear of mushrooms poisoning. In order to help address the fears and potential dangers of mushroom poisoning we are currently developing a social media platform for the uploading of images of collected mushrooms to assist in identification of collected specimens. Currently the biggest hurdles to trade are lack of knowledge and limited market access.

1.2-8 Teaching mycology by the methodology of problematization with an Antarctic lecture

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Abstract: There are few researchers in fungi field comparing to the others biological fields. Learning about the Fungi Kingdom in high school is essential for stimulating new researchers and new scientific investigations concerning these living beings. In fact, there are many teaching methods to guide teachers in and out the classroom. However, both novice and experienced scientists must be able to apply the scientific method to investigations. Students learning regarding mycological scientific concepts through the observation of reality, providing key points that lead to theorization and hypothesis collection and later, application to reality which can be an effective procedure. This study aimed to build knowledge about fungi, from an Antarctic experience for the scientific literacy of high school students by the Methodology of Problematization. To achieve this goal, four theoretical-practical classes were developed and delivered, in and out the classroom, using the science-teaching laboratory. This classes were categorized and analyzed by words clouds thru transcription of the classes recorded audios and logbooks. All students in the classroom were separated into three groups providing the learning of the fungi by research, among which the scientific contents were conducted in the form of experiences beginning with a lecture-class, followed by three theoretical-practical classes. These activities show many possible indicators of scientific literacy. Thus, the results of the sequential classes

indicates the importance of social interaction between students-teacher and students-students to promote learning, but also contributed to the continuous improvement in the methods of teaching learning process. In fact, interesting investigations points can be stimulating for student's scientific learning. In short and in conclusion, there is a strong need to learn science in a scientific way, contributing to scientific literacy and, consequently, the substantial learning of scientific concepts, so that future generations can apply such knowledge acquired in basic education to their research in higher education, and may even to leverage exponentially the Brazilian research developed in the Antarctic continent.

1.2-17 New edible fungi from Southeast Asia: discovery to production

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Abstract: The forests of Southeast Asia have the potential to be a rich source of cultivatable edible fungi. Although significant amounts of research on the taxonomy and phylogeny of edible mushrooms have been carried out, far fewer studies have focused on the domestication of wild fungi. Today, the most commonly cultivated strains are temperate species, but tropical and subtropical mushrooms are both abundant and highly diverse, with many species having long histories of human consumption. In addition, many new species have recently been introduced to science, including numerous species of high nutritional and medicinal value. The domestication and cultivation of tropical mushrooms therefore provides an enormous opportunity for Southeast Asian countries. Due to the difficulties of cultivating mycorrhizal species, we have concentrated on saprobic species. Most tropical and subtropical mushrooms, if provided with appropriate conditions, grow and produce fruiting bodies more quickly than temperate species. Tropical and subtropical mushrooms can be produced using cheap, readily available waste products such as sawdust, corn cobs, rice straw, sugarcane bagasse, and other forest and agricultural residues, making them an ideal crop for smallholder farmers. We have collected and isolated numerous strains of wild mushroom species from Southeast Asian forests, and have published some initial results documenting our progress in domesticating these species. Using a variety of steps including sample collection, isolation, spawn production and fruiting body production in sawdust and compost media, we showed for the first time that it is possible to domesticate the following fungi: *Pleurotus giganteus*; a new Thai-French hybrid strain of *Agaricus subrufescens*; *A. flocculosipes*; *A. subtilipes*; *Auricularia thailandica*; *A. cornea* (white); *Panus roseus*; *Macrolepiota dolichaula; Ganoderma australe;* and *G. leucocontextum*. These discoveries may create new opportunities for the mushroom growing industry and for smallholder farmers in Southeast Asia in particular.

1.2-18 Economically valuable mushrooms of Armutlu (Yalova)

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Abstract: In this study a total of 123 economically valuable macrofungi specimens have been collected from different localities of Armutlu (Yalova) province between 2010 and 2014. As a result of field and laboratory studies, 23 species within the 16 families and 6 orders were identified. Two of them belong to the division Ascomycota and 21 to Basidiomycota. The species list is given with the information on localities, habitats, collecting dates and collection numbers.

1.2-19 Edible Fungi of woodlands and plantation forests of Ireland

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Abstract: Sustainable development of edible mushroom foraying and the planting of trees in natural, native mycorrhizal associations with edible fungi in Ireland are explored. A compilation of geographical, botanical and temporal occurrence data on edible species of mushrooms and their forest vegetation contexts from historical and original observations, plus data analysis thereof, were chief objectives. Knowledge development from a low base was important as an educational resource and to contribute to effective policies for fungal conservation and good forest management. The development of safe and respectful ways to interact with the beauty, diversity and flavours of wild, edible Irish fungi were priorities. Ireland has no Red Data List for fungi or any legislative protections for fungal species, apart from one lichen. Care in publication of localities of edible fungi is required as the resource is patchily distributed and vulnerable to greed. Along with extensive personal records since 1999, a nationwide survey of forests was undertaken. Recording of 100 plots of 50x2m were monitored across the main woodland and plantation forest types and ecological settings of Ireland on several occasions between September and December in 2007, 2008 and 2009. Carpophore counts and masses of edible species as well as ground flora and tree information from plots were databased. Historical literature detailing all records of edible species in Ireland was reviewed and herbarium voucher material examined. *Russula ochroleuca* (marginally edible) and *Laccaria laccata* were the most widespread species found. While results were generally positive, a high proportion of the exotic, conifer forested area in Ireland was found to be unproductive for prized edible fungi. Awareness and training for the opportunities are still unsupported. *Craterellus* species in the Winter Chanterelle group currently offer potential and some genetics work was carried out on this group. Continuous cover forest methods would improve continuity of edible fungi in Irish forests. Forest pathology expertise has been limited. Care needs to be taken not to transfer parasitic organisms with nursery stock soil or on wood-cutting and chipping equipment. Truffles are more common than previously thought. Beech provides an important mycorrhizal host association for *Tuber aestivum* in Ireland as well as in England, Sweden and north-western France. The impact of *Armillaria* is of concern for the health of deciduous forests. The lack of control measures for this and other parasitic fungi are challenges for securing healthy populations of edible fungi and their host trees. Imported, inoculated nursery stock for plantation forestry, as well as treatments involving mycorrhizal products have influenced mycorrhizal populations. Alien mycorrhizal inoculants and soil additives with fungal components are potential threats to native fungal biodiversity. It is proposed that inoculated stock and fungal "ameliorants" be subject to legislation for myco- and phytosanitary risks. Lessons were learned from experiences at the National Poisons Information Centre and from serving on the panel for fungal expertise there, as well as from experiences of mushroom poisoning cases in other countries. There have been improvements to education for the public, with medical professionals and staff in advisory positions.

1.2-20 Augmentation of nutritive value of rice using *Rhizopus oligosporus* through fermentation

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Abstract: The production of fermented food is one of the oldest food processing technology. Many of these foods have been manufactured because their unique flavor, aroma and texture attributes are much appreciated by the consumer. Moreover, because filamentous fungi simultaneously decrease anti-nutrient compounds and partially hydrolyzed substrate biopolymers, pre-fermented bio-product may

be used as an inexpensive food and feed supplement that may support marketing claims. The results of fermented rice (variety super 85) with *Rhizopus oligosporus* at 30°C for time period of 28 hours was a new food product consisting of soft rice grains with fruity pleasant smell and sweet taste. Chemical composition of fermented and un-fermented rice in respect of %age of moisture contents, protein, crude fat and crude fiber was 42.43, 1.75, 4.1, 9.106, 1.84 and 7.3. In case of calcium highest value was noted in fermented aerobic rice sample 19.36mg/100g.The highest value of phosphorous was calculated in processed control sample i.e. 3.328mg/100g. The highest value of phytic acid was calculated in simple rice sample 0.856%. GABA (aminobutyric acid) was detected in fermented aerobic and anaerobic rice samples with highest Rf value as compared to simple and control rice samples. Rf value of fermented anaerobic and fermented aerobic rice samples was 7.22 and 7.11 respectively.

1.2-21 Heterologous gene expression of *Aspergillus oryzae* strain isolated from Korean traditional fermented foods

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Abstract: It is important to find an efficient way to enhance the expression of heterologous gene(s) of interest in a fungal expression system for giving the high potential of fungi as genetic resources. Protein expression systems that produce the heterologous gene products using fungi are important for various aspects and it is recommended to use the GRAS strain as a host to ensure product safety. Therefore, here we constructed a heterologous gene expression system for producing foreign gene product including bacterial origin one such as bacterial β-glucosidase by using a GRAS fungus *Aspergillus oryzea*. The produced β-glucosidase is a hydrolytic enzyme and the expression of the gene was stimulated by placing it under the control of the constitutively activated *gpdA* gene promoter or threonine-inducing *alcA* gene promoter of *Aspergillus nidulans*. The pyrithiamine-resistant gene, *ptrA,* was used as the selection marker for *Aspergillus* transformation. The signal peptide of *A. oryzae* α-amylase AmyB was linked to the N-terminus of the bacterial β-glucosidase protein, and 3X FLAG was tagged at the C-terminus. *A. oryzae* transformants successfully overexpressed the β-glucosidase gene, and expression level was monitored by western blot analysis with anti-FLAG antibody. The functional activity of the protein was detected by esculin hydrate converting test and pNP-β-D-glucopyranoside (pNP-Glc) measurement assay. The expression system of *A. oryzae* could be beneficial for industrial applications.

1.2-22 The historic and current ethnomycology of Egypt and Middle East countries

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Abstract: Egypt is considered the cradle of mycology. Ancient Egyptians documented the use of fungi on walls and pillars of temples, within hieroglyphic texts, ear studs and medical prescriptions since 5619 B.C. Ancient Egyptians believed that some mushrooms were plants of immortality and called them "a gift from the God Osiris" and symbolized as Was, Djed pillar of Osiris, and ankh (crux ansata). Egyptian pharaohs proclaimed mushrooms to be food reserved only for royalty; common people were not even allowed to touch them. The Pharaohs of ancient Egypt believed they had magical powers. Egyptian crowns (white and triple) were inspired from the primordia of *Psilocybe cubensis*. The most ancient historical use of truffles probably originated prehistorically in the Mideastern and North African cradles of civilization. Species of desert truffles (*Terfezia*, *Tirmania* and *Phaeangium*) probably served to the Pharaohs. Better descriptions of the kind of desert truffles that the pharaohs of Egypt may have

consumed, along with an ancient version of traditional truffle preparations still popular in North Africa and the Middle East, can be found in the Bible. In the seventh century Prophet Muhammad peace, be upon him (صلى الله عليه وسلم) said a hadith to his followers "Truffles are a part of manna and its juice is healing for the eyes". This study consists of a survey focusing on the knowledge, use and ethnomycological practices of mushrooms and desert truffles among the native people of the Middle Eastern countries. The presentation will highlight their application in traditional medicine in this part of the world. This work also explores the biology and ecology of truffles in the Middle East, their importance in fragile desert ecosystems, assess their conservation status and effects of various cultivation practices on sustaining truffle populations. General management principles and considerations to sustain this valuable fungal resource will be discussed.

1.2-23 First record of edibility and ethnomycological notes of Ustilago maydis in Ecuador

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Abstract: *Ustilago maydis* is known by the Ecuadorian local communities with the vernacular name of "Cuscungo", for a long time its use was unknown for Science, however in the last decade ethnomycological studies have been carried out in corn crops, located in the Ecuadorian mountain range, where informal surveys were applied to people from 40 to 80 years of age to know ethnomycological data. In this way, its uses were found with the categories: edible human, edible animal, ludic among the most representative. This fungus represents balance for human communities, traditional areas of cultivation and ecosystems. Key words: *Ustilago maydis*, ethnomycology, Ecuador.

1.2-41 Evaluation of antimicrobial and cytotoxic activites of secondary metabolites produced by *Aspergillus flavus* isolated from Cerrado Goiano

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Abstract: Microorganisms represent an important source of bioactive compounds with high added economic value. Among the microorganisms, fungi have many advantages of use due to their potential as producers of secondary metabolites. Due to their bioactive properties, many secondary metabolites have been adopted for pharmaceutical use as antibiotics, tumor inhibitors and immunosuppressants among other properties. The goal of this study was to investigate the antimicrobial and cytotoxic activities of secondary metabolites produced by Aspergillus flavus fungus isolated from the Cerrado Goiano soil. The production of the secondary metabolites was evaluated by fermentation in submerged culture in TLE medium and after fermentation, the medium was extraction by liquid-liquid partition with ethyl acetate. The secondary metabolites were analyzed by high performance liquid chromatography (HPLC). The isolates of A. flavus were used to evaluate the toxicity in Artemia salina, evaluation of susceptibility to antimicrobial, and cytotoxic evaluation by the MTT. In the evaluation of the toxicity Artemia salina in The compounds isolated from the fungi were tested in concentrations of 2400, 1200, 300, 75 e 18,75 µg. mL-1. The analysis of the tests tested gives high toxicity (LC50 = 11.49 µg mL mL -1) to potassium dichromate, within the standards presented in the literature, attesting to the suitability of the experimental conditions. According to the classification of Nguta et al. (2012), in this assay the extract of secondary metabolites presented LC50 equivalent to 81 µg.mL-1 this result was considered of high

toxicity according to the parameter adopted. To evaluate the antibacterial activity of the isolated compounds, the inhibition test was carried out by dilution in broth using the concentrations of 40 µg.mL-1; 80 µg.mL-1 e 160 µg.mL-1 was tested against standard bacteria ATCC (S. aures e E. coli) e MRSA (Methicillin-resistant Staphylococcus aureus). As a positive control, Chloramphenicol at 100mg.mL-1 concentration for E. coli and Oxacillin at 100mg.mL-1 concentration for S. aureus were used. The concentration of 80 µg.mL-1 was able to inhibit 100% E. coli and 95% S.aureus and 98% MRSA. The concentration of 160 µg.mL-1 was able to inhibit 100% the bacteria tested. A. flavus was subjected to the cytotoxicity bioassay against WM1366 and A375 tumor cells. WM1366 and A375 lineage cells (5x105 / ml), and human mononuclear cells (1.5 x106 cells / ml) were cultured in the presence of *A. flavus* extracts at concentrations of 6 to 48 µg / mL in 96-well plates. After the 24 h incubation period, the enzymatic activity was determined by the enzymatic reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Secondary metabolites were efficient in promoting cytotoxic effect on WM1366 and A375 cells in a dose-dependent manner. Thus, mitochondrial activity was decreased by 98% and in WM1366 cells mitochondrial activity was decreased by 100%. In conclusion, the metabolites produced by Aspergillus flavus presented potent antimicrobial and antitumor activities, evidencing the importance of future studies.

1.2-42 Elevated CO_2 concentration expands the range of temperature and water activity conditions wherein *Aspergillus flavus* produces aflatoxin B_1

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Abstract: There is a significant interest on understanding the impacts that climate change factors [CO_2 concentration, water availability (a_w) and temperature increases (T)] may have on mycotoxigenic fungi. We have, for the first time, examined on conducive media and maize grain the impact that three-way interactions between these factors (a_w, T and elevated CO_2) have on: (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B_1 (AFB_1) production by *Aspergillus flavus*. On conducive media, interactions between water stress (a_w; 0.97, 0.95, 0.92), temperature (34, 37°C) and CO_2 exposure (350, 650, 1000 ppmv) were considered and the growth, AFB_1 production and expression of biosynthetic genes (*aflD*, *aflR*) studied by RT-qPCR. For maize grains, interactions between water stress (a_w; 0.99, 0.91), T (30, 37°C) and CO_2 exposure (350, 650, 1000 ppmv) were included. Fungal growth, AFB_1 production and expression of the all genes in the aflatoxin gene cluster were studied by RNAseq. The results showed that for growth there was relatively little effect. In contrast, the three-way interacting conditions (elevated CO_2, a_w, T) had a profound effect on AFB_1 production both in media and maize grains. Importantly, under slightly elevated CO_2 conditions there was a stimulation of aflatoxin B_1 production. Although under current CO_2 concentrations at 37°C there was no or minimal production of aflatoxins, our results show that, in conducive media and 650 and 1000 ppm CO_2, there was a significant increase in expression of both *aflD* and *aflR* at 0.95 and 0.92 a_w. There was an associated increase in AFB_1 in these treatments. Similar observations were made in stored maize grain. Differential expression of several genes was found by RNAseq in the aflatoxin gene cluster in relation with these interacting factors. The range of a_w and Ts in which aflatoxin B_1 is produced increased under elevated CO_2 conditions. This is the first study to examine these three-way interacting climatic factors on growth and mycotoxin production in *A.flavus*. This provides data which is necessary to help a better and more accurate

prediction of the real impacts that climate change is and will be having on important staple food and feed chains due to mycotoxigenic fungal spoilage and toxin contamination.

1.2-43 New methodology to improvement the sanitary quality of stored –in pod peanut by in situ application of pure BHA (2 (3) ter-butil-4 hidroxianisol) and their formulation

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Abstract: In Argentina in-pod peanut intended for use as seed for planting are stored from 3 to 6 months (from harvest to planting) where fungal contamination could increase resulting in a decrease of the grain quality during storage. However, only germinative power and vigor of the grains are normally evaluated, while sanitary quality is not considered. For this, the aim of this work was to evaluate the effect of pure BHA and in combination with their formulation (F-BHA) on peanut seeds stored in open containers in the field. The experiment was carried out in a storage company of south of Córdoba, Argentina during 2017. Two hundred kilograms of in-pod peanut treated with: Treatment 1 (T1) pure BHA (10mM); Treatment 2 (T2) the mix of pure BHA and F-BHA (5mM: 5mM); Treatment 3 (T3) pure BHA (5mM) at the bottom of the peanut container and F-BHA mix in the peanut and Treatment 4 (T4) untreated grains, were used. Fungal level, residues of BHA and fluctuations of temperature and humidity during 4 months were determined. Fungal count was significantly affected by treatment and time according to ANOVA test ($p<0.05$). This effect was strongly observed after 30 days where control grains showed fungal level 1.6 times higher respect with the treated grains. At this time, no significant differences ($p<0.05$) were found for CFU/g levels between T1 and T2. However, T3 showed fungal levels 20% higher respect T1 and T2. Among the most frequently isolated fungal genera were *Penicillium*, *Cladosporium, Alternaria* and *Fusarium*. For the both treatment, BHA residual levels showed a pick of the amount of the antioxidant after 30 days of the assay. At this time values of 924.28, 748.72 and 193.2 ng/g were found for T1, T2 and T3 respectively. At the final of the experiment levels decreased to reach 110 ng/g for T1; 330.81 ng/g for T2 and 28.10 ng/g for the T3. The temperatures recorded during the sampling fluctuated between 23 and 35 ° C according to the month in which the sampling was taken, while the humidity of the peanut grains remained around 5% during all assay. The treatments used in this study on in-pod peanut, reduced significantly ($p<0.05$) fungal development of the seeds stored in field. This reduction was strongly observed during the first months of storage coinciding with the highest levels of antioxidant detected in the samples of peanut. On the other hand, the highest levels of BHA present in peanut kernels were observed for the combined treatment (T2) where the final amounts were around 3 times higher compared with T1 (pure antioxidant) and 12 times higher compared with T3. The combined treatment (T2) could be an alternative for control of spoilage fungal on in-pod peanut stored in the field where conditions may not be suitable for storage.

1.2-44 Biodiversity of marine-derived *Trichoderma* spp. and their exploitable biological activities

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Abstract: Natural metabolites derived from microorganisms are an important source of novel medicinal compounds. Since 1950s, secondary metabolites from marine-derived fungi have been discovered,

many of which have unique structures and have remarkable biological and pharmaceutical properties. A genus *Trichoderma* is found in a variety of environments and has been reported to produce diverse biologically active secondary metabolites. However, studies on the secondary metabolites of *Trichoderma* derived from the ocean are relatively rare. This study investigated the diversity and physiological activity of 35 *Trichoderma* spp. isolated from various marine resources such as sea sand, seaweed, tidal flat sediment, and *Arctoscopus japonicus* eggs in South Korea. They were identified using the DNA sequence of the translation elongation factor region and the 50% majority conformational tree was constructed through Bayesian analysis. To make fungal extract, all fungi were cultivated in potato dextrose agar for a week and extracted with MeOH. The MeOH extract was dissolved in EtOAc and D.W. (1:1), and the EtOAc layer was taken and dried using a rotary evaporator. The final extract was dissolved in dimethyl sulfoxide (10 mg/mL) for subsequent bioassays. The antioxidant activity of the extract was measured by analytical methods using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-phenylhydrazide. As a result, they were identified as 18 different *Trichoderma* species including three new species candidates and six species not reported in Korea. The fungal extracts of *Trichoderma bissettii* and *Trichoderma longibrachiatum* exhibited high radical-scavenging activities in both assays. It is expected that marine-derived *Trichoderma* spp. can be a great resource for bioprospecting useful natural compounds. The antifungal activities of the extracts are currently being investigated.

1.2-45 Exploring the diversity and bioactivity of cultivable Arctic marine fungi from the Svalbard archipelago

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Abstract: The diversity of marine fungi in Polar Regions, especially Arctic, is severely understudied and is a potential source for novel marine natural products. It can be hypothesized that due to extreme conditions, such as low temperatures, Arctic marine fungi possess unique chemical adaptions of biotechnological relevance. The aim of the study was to get insights into the diversity of marine fungi at high latitudes and screen fungal extracts for the presence of novel bioactive compounds. Fungi were isolated on three media from driftwood, sediments, macro-algae and pelagic water around the Arctic archipelago of Svalbard in 2016. Subculturing was conducted until pure culture isolates were obtained and these were sequenced using ITS5-4 primer pair amplifying the ITS rRNA region. The sequenced isolates were grouped into operational taxonomic units (OTUs) using a 98% sequence similarity cutoff value and a representative isolate from each OTU was sequenced for SSU and LSU rRNA genes. As a preliminary test of the isolates' potential to produce bioactive compounds, an antibacterial plug assay was performed by testing each OTU against five different pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus* group B). In total, more than 200 isolates were sequenced, and 95 of these yielded fungal ITS sequences. These were grouped into 20 OTUs. The diversity was highest in Ascomycota (17 OTUs), followed by Zygomycota (2) and Basidomycota (1). Within Ascomycota, Lulworthiales was the most frequent order with 6 OTUs. Other orders with several OTUs included Eurotiales (3), Hypocerales (3) and Helotiales (2). Identity of the obtained ITS sequences to sequences in Genbank ranged from 85-100%, indicating that some species have not been sequenced for ITS or represent novel species. Representative isolates from Eurotiales (3), Helotiales (1), Hypocerales (1) and one order of *incertae sedis* as well as one order from Zygomycota showed inhibiting zones against gram-positive bacteria ranging from 8-17 mm. These preliminary results will be used to select strains for further bioactivity screening and potential scale-up

cultivation of isolates producing new compounds. This work will shed light on the Arctic fungal diversity and the potentially undiscovered chemical space they possess. In addition, the results of the project will be used to prioritize strains for genome sequencing with the aim of linking produced bioactive compounds with the biosynthetic gene clusters responsible for their production.

1.2-46 The Mediterranean Sea: an untapped source of fungal diversity

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Abstract: The Mediterranean Sea, with almost 12,000 endemic species, is among the 25 global biodiversity hotspots recognised worldwide. Nonetheless, this estimate does not take into account the microbial diversity that is still largely unknown. In the last years, the *Mycotheca Universitatis Taurinensis* (MUT) carried out several research programs to assess the marine fungal diversity in the Mediterranean Sea to better understand the specificity of the fungal community for different substrates (animals, seagrasses, algae, wood, ecc), and to find new species valuable for biotechnological exploitation (i.e. new drugs, new antifouling agents, ecc). Biotic and abiotic marine substrates were sampled during several campaigns. A culturomic approach was employed for the isolation of marine fungi; specific culture media that mimicked both the marine environment and the original substrates of isolation were developed in order to increase the number of cultivable fungi. Fungal strains were identified by combining morpho-physiological and molecular features with deep phylogenetic analysis. Today, the MUT is the largest public collection of marine Mediterranean fungi, hosting almost 1400 taxa from more than 20 different matrixes. Our results show that Ascomycota are ubiquitous in the Mediterranean Sea, while Basidiomycota and Zygomicota are less frequent. Several species were reported for the first time in marine environment, and each substrate was shown to host a specific fungal community. Interestingly, numerous species displayed unique morphological and molecular features and are currently under investigation. Remarkably, these strains could produce enzymes and molecules relevant in the interaction with the host and the surrounding environment. With the present work, we contribute to increase the knowledge on marine fungal diversity.

1.2-47 Assessment of antioxidant activity of some medicinal fungi harvested in forested and mountainous regions of Armenia

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Abstract: Many fungal strains show strong antioxidant activity, comparable to classic antioxidants, such as Vitamin C or E. In presented research we have investigated some major compounds responsible for antioxidant activity for 6 different species of fungi (*Hypholoma fasciculare, Agaricus bisporus, Pleurotus ostreatus, Trichaptum abietinum, Polyporus squamosus, Schizoplyllum commune*), growing in Republic of Armenia. All species of fungi were harvested in June-August 2015 in forested and mountainous regions of Armenia. Antioxidant activity was assessed via potentiometric method based on oxidation/reduction of Fe^{2+}/Fe^{3+} in acetate buffer media (pH=3.6). Phenolic content of fungi was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Flavonoid content was determined by spectrophotometry via reaction with $AlCl_3$ in methanol at 410nm wavelength, using rutin as standard. β-carotene and lycopene contents were determined by spectrophotometry at 453nm, 505nm and 663nm wavelength. Results indicate that all investigated species show antioxidant activity except *Trichaptum abietinum*. *Agaricus bisporus* and *Pleurotus ostreatus* show highest antioxidant activity (8.8

x 10^{-3} g/l and 6.8 x 10^{-3} g/l accordingly) among investigated species. Phenolic compounds have a significant role, but there are other antioxidants present as well. *Hypholoma fasciculare* has highest phenolic content (0.665 g/l). It also has highest β-carotene and flavonoid content (0.324 g/l and 2.505 g/l accordingly), and *Schizophyllum commune* and *Polyporus squamosus* contain trace amounts of lycopene. Further researches of antioxidant activity on lipid membranes and biological objects, such as tissue, cell cultures are in progress.

1.2-48 Biologically active compounds in lichenized fungi

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Abstract: Lichenized fungi (usually referred to as lichens) are unique symbiotic organisms that grow in spots on substrates that are too harsh or limited for most other organisms such as bare rock, desert sand, cleared soil, dead wood, animal bones, rusty metal, living bark, and so on. Lichens consist of two organisms: a fungus (mycobiont) and an intimately associated photosynthetic partner (photobiont). The photobiont can be either a green alga or a cyanobacterium, and some lichens can contain both alga and cyanobacterium. As adaptations for life in specific habitats, lichens produce a large number of chemical compounds (metabolites); almost all of them are unique as they are unknown in other plant sources. Lichen metabolites possess numerous biological activities including antiviral, antibacterial, anti-inflammatory, analgesic, antitumor, etc. Thus, lichens may be of interest for pharmaceutical sciences and the industry itself. The goal of the present research was to study the metabolites of lichens distributed in Israel. We collected different lichen species dwelling on trees, soil and rocks in the Mediterranean territory of Israel and investigated the composition and structure of the chemical compounds isolated from them. All studied species had a high diversity of lipids, fatty acids, aromatic compounds and other metabolites. Metabolites of two fruticose epiphytic species *(Ramalina lacera* and *Tornabea scutellifera*) and four foliose epilithic species from the genus *Collema* (*C. cristatum, C. callopismum, C. fuscovirens* and *C. flaccidum*) were studied in detail the most. It is to be noted that photobionts of *Ramalina lacera* and *Tornabea scutellifera* are green algae, while photobionts of species from the genus *Collema* are cyanobacteria. Four compounds belonging to monotetrahydrofuranic acetogenins (Tornabeatins A, B, C, and D) have been isolated as new natural products from *Tornabea scutellifera*, and their biological activities were tested. The studied metabolites showed modest activity against different microorganisms and significant antitumor activity. Testing of biological activities of rare fatty acids isolated from *Ramalina lacera* and *Collema* species detected significant antibacterial and antifungal activities of these compounds. A photo protective mycosporine (Collemin A) was found in calcicolous lichens *Collema cristatum* and *C. callopumum*. The study of this compound showed that it prevents UV-B induced cell destruction in a dose-dependent manner, partially prevents pyrimidine dimer formation, and completely prevents UV-B induced erythema when applied to the skin prior to irradiation. Thus, our studies revealed that lichens distributed in Israel might be good potential sources of bioactive phytochemicals.

1.2-65 Differentiation of fungal communities between different crops and substrates in an intercropping trial

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Abstract: Isolation based methods have previously revealed that fungal communities occurring in soil, the plant rhizosphere, within plants and often between plant species are usually different with some

fungi having the ability to occur across niches and plant species. However, very few of these studies consistently and comprehensively compared fungal communities between all of these variable origins, most likely due to the vast number of isolations and morphological or DNA sequence-based identifications that would be needed. The advent of environmental sequencing and high throughput sequencing technologies, however, made such large-scale comparisons doable. This study represents comparisons of the mycobiomes present as endophytes within four legumes and sorghum planted in an intercropping trial. These plant endophyte mycobiomes were also compared with the fungi occurring in the rhizospheres of the plants, as well as bulk soil from the various plots. As expected a great deal of overlap as well as differences could be observed between the mycobiomes of the different crops. Similarly, fungal communities from bulk soil, rhizosphere soil and plant communities were mostly distinct. Moreover, these profiles also differed between crops. The approach developed in this study enables tracking interactions and development of fungal communities during intercropping experiments, as well as the mycobiomes present in different niches. This is important when such planting trials aims to promote beneficial organisms and deter detrimental pathogens.

1.2-66 The maize fungal community under various agricultural practices

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Abstract: Fungal and bacteria communities play important roles in biogeochemical processes. Understanding the role of microbial diversity in agricultural systems is important for developing long-term management plans. While numerous studies have examined forest and grassland ecosystems, less is known about agricultural systems in the Midwest USA. Furthermore, even less is known about microbial communities of conventional and organic agriculture. A goal of this study is to compare fungal diversity in organic vs convention corn rotation cropping systems using different tillage regimes. The University of Wisconsin Extension Farm at Arlington has been assessing various farming practices for several decades. We sampled five cropping treatments, each with four replications. Three soil samples per field were collected using 15 cm x 2.5 cm soil cores. DNA was isolated from 60 soil samples. Microbial communities will be identified using next generation DNA sequencing. PCR was performed using Illumina barcodes and ITS primers for the ITS1 region of the ribosomal DNA repeat. Analysis of fungal communities under different agricultural methods associated with maize farming in Wisconsin will be presented. These results will provide information on how current agricultural practices affect soil fungal biodiversity. Corn is a major crop of Wisconsin and thus this data will provide important information for Wisconsin farmers.

1.2-67 Characterization of the fungal phytobiome of sorghum (*Sorghum bicolor*) using environmental sequencing

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Abstract: The phytobiome consists of microbes associated with external and internal areas of a particular plant. These organisms may interact with each other and with the plant, which may have beneficial of detrimental effects. Environmental sequencing using next generation sequencers enables intensive characterization of the phytobiomes of plants. In this study we characterized the fungal component occurring as endophytes from all plant tissues of sorghum (*Sorghum bicolor*), as well as fungi

from the rhizosphere and surrounding bulk soil across two years. Sorghum is one of the most cultivated crops in the world, and widely used in rural agriculture. Complete plants, rhizosphere soil and bulk soil were sampled from Potchefstroom, South Africa, and processed for environmental sequencing of the ribosomal internal transcribed spacer regions using the MiSeq Illumina sequencer. Results indicated significantly different communities of fungi from the different substrates (plant, rhizosphere, soil) and a level of organization between plant tissues. A number of plant pathogenic genera were also detected. Using environmental sequence, these taxa can be monitored more easily over time and their interactions with agricultural practices and environmental factors can be characterized.

1.2-68 Assembly of Sorghum's mycobiome

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Abstract: Fungal ecologists have long debated whether the assembly of the Mycobiome is deterministic, stochastic or strongly influenced by historical contingency, and are especially curious about the processes responsible for the observed patterns. We investigated the assembly of the Mycobiome from weekly sampled leaves, roots, rhizosphere and soils over the growth season of sorghum [*Sorghum bicolor* (L.) Moench] that had been subjected to pre-flowering drought, post-flowering drought or no drought (control), using fungal ITS2 DNA sequence (Illumina, MiSeq). Stochasticity (Raup-Crick index between -0.95 and 0.95) was demonstrated early in the growth season, when fungi are rare on newly emerged leaves (weeks 1-7) and roots (week 2). The observed stochasticity was attributed to ecological drift because its strength was strongly negatively correlated with fungal community size. The stochasticity could not be attributed to dispersal or historical contingency because there was no evidence of distance-decay, stochastic colonization, or priority effects. Stochasticity gave way to homogenous selection for assembly of the Mycobiome in roots at week 3, in leaves at week 8, and was never prominent in rhizosphere or soil. Consequently, the strong stochasticity increased the observed fungal richness of leaves in weeks 1-7 and roots at week 2, and alleviated the effect of habitat selection for Mycobiomes between leaves and belowground compartments. In contrast to the hypothesis that release from stress favors stochasticity, release from drought (control) did not increase stochasticity of community assembly for the Mycobiome in this study. Instead, drought deterministically caused deviation of fungal community composition from control, and release from preflowering drought (rewatering) reduced root community dissimilarity between preflowering drought and control. Besides, drought respectively increased, not changed and decreased fungal richness in leaves, roots and rhizospheres, which might be attributed to the drought-induced differential changes in fitness of fungal OTUs.

1.2-69 Diversity and phylogenetic assessment of fungal communities in sugarcane plantation

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Abstract: Sugarcane (*Saccharum officinarum* L.) is monocotyledonous cash crop, which belongs to *Andropogoneae* tribe in family *Poaceae*. It is widely grown in tropical and subtropical areas and cultivated in nearly 60 countries of the world. China is the third largest sugar cane producing country after Brazil and India. Fungi are most destructive pest of sugarcane worldwide and responsible for yield reduction and economical losses. In the recent few years, a few species have been firstly reported on

sugarcane in China. However, exclusive fungal species are cause of disease development and few are still unknown with accurate morphological and molecular identification. Here, we collected 370 diseased root and leaf samples of sugarcane from 8 sites located at south of China and obtained 762 fungal strains via single spore and tissue isolation and our preliminary phylogenetic analysis based on ITS locus suggested that they belong to 141 species in 52 different genera. Among these species, 81% (135 species) belongs to Ascomycota; 0.6% (3 species) belongs to Basidiomycota and 0.5% (3 species) belongs to Zygomycota while 17% were not identified to species level. At the family level, most of isolated species belongs to *Apiosporaceae, Chaetomiaceae, Didymellaceae, Hypocreaceae, Nectriaceae, Pleosporaceae* and *Trichocomaceae*. The most common genera associated with sugarcane included *Bipolaris, Chaetomium, Colletotrichum, Curvularia, Epicoccum, Fusarium, Nigrospora* and *Trichoderma*. Further multi-locus phylogenic analysis revealed that at least 22 previously unknown species belongs to genus *Chaetomium, Curvularia, Epicoccum, Nigrospora* and two gen. novel in *Chaetomiaceae*. *B. bicolor, B. setariae, C. geniculata, C. muehlenbeckiae, C. hominis* and *C. verruculosa, N. Lacticolonia, N. aurantiaca, N. gorlenkoana, N. camelliae-sinensis* and *N. pyriformis* were reported first time on sugarcane. Present work significantly improved our understanding of mycobiota associated with sugarcane that dominantly grown in Guangxi (South of China) which provide scientific references for controlling sugarcane diseases.

1.2-70 Monograph of fungi on Pandanaceae

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Abstract: Members of the plant family Pandanaceae are ecologically and economically important. Studies of micro-fungi on Pandanaceae based on morphology and phylogeny in Southeast Asia have been poorly done. Most of previous micro-fungi studies on Pandanaceae were carried out based on only morphology. The saprobes and endophytic fungi on Pandanaceae in Southeast Asia are provided here based on the current morphological and phylogenetic data. This study will be continued to produce a world monograph of fungi on Pandanaceae.

1.2-71 Getting to the grassroots of DSE symbiosis: case study from native and non-native perennial C4 grasses

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Abstract: Grass roots host a suite of microbes of which Dark septate endophytes (DSE) are common non-mycorrhizal fungal endophytes. DSE are abundant in North American and European grasslands with Periconia macrospinosa, Microdochium sp., and Darksidea sp. being the most commonly isolated DSE fungi. Perennial C4 grasses namely Switchgrass (Panicum virgatum) and Giant Miscanthus (Miscanthus X giganteus) are top bioenergy feedstocks due to their abundant biomass yields. While the former is a native North American grass widely used for ecological restoration and soil conservation, the latter is a non-native sterile hybrid. Since Long-term and large-scale cultivation of these two grasses is expected by 2030 to meet U.S. bioenergy mandates, it is interesting to evaluate and compare their DSE symbioses. The objective of this study was to isolate DSE fungi from four native switchgrass varieties and a commercial variety of giant miscanthus and evaluate the outcome of their symbiosis. DSE fungi were isolated from Alamo, Bomaster, Colony and Kanlow varieties of switchgrass and Freedom Giant Miscanthus (FGM) cultivated in Alcorn State University's Lorman Experiment Station. As expected Periconia macrospinosa was the most common DSE from both grasses. To test the outcome of the

symbiosis, Miscanthus sinensis the parent of M. giganteus and switchgrass varieties were cultivated with P. macrospinosa isolated from respective hosts. A total of 20 isolates with 10 each from FGM and switchgrass were evaluated. Seedlings were grown in sand: soil mixture with and without DSE fungus for six weeks. Past studies have indicated outcome of DSE symbiosis is dependent both on host and fungal genotypes and is along the mutualism-parasitism continuum. Our preliminary data also indicate this trend. Plant biomass and N, P, K levels will be recorded as proxy for DSE symbiosis. Data from cultivated grasses like in this study and those from native grasslands seem to indicate that Periconia macrospinosa is the most commonly isolated DSE fungus from grasses and could very well serve as model DSE fungus.

1.2-72 Temporal dynamics of endophytic fungi in grassland systems

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Abstract: The seasonal dynamics of foliar endophytic fungal communities in the iconic prairie grass, *Andropogon gerardii* (big blue stem), were investigated to ask how these communities shift over time and ecological context. If the many and diverse fungi occupying leaves have similar functions as endophytes, we expect that their distribution through seasonal time and over the development of individual leaves and plants should reflect only a stochastic sampling process from the regional pool of taxa available. We sampled leaves of *A. gerardii* from marked plants throughout one season and over two years, in field plots that had received additional nutrients or restricted large herbivores or both ecological treatments. We utilized both NexGen and culturing approaches to estimate fungal communities. Our findings demonstrate the importance of stochastic sampling of taxa available at one time in the environment nonetheless, fungal taxa shift through seasonal time and over the development of leaves on individual plants. In addition, the arrival of novel late-season taxa apparently caused major shifts in these communities. Functional analyses using experimental communities are underway to investigate causes of these shifts in taxa over time and plant development.

1.2-81 Ectomycorrhizal (ECM) fungal community of mixed forest of endemic pines and broad leaves in BiDoup – NuiBa National Park, Vietnam

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Abstract: Two 4 hectares - plots were set up in mixed forest between endemic pines *Pinus dalatensis*, *Pinus krempfii* and broad leaves in Bi Doup Nui Ba National Park, Vietnam. The first plot was set up in the dominant of pines *P. dalatensis* forest and the second plot was in Fagaceae dominant forest. Two underground ECM fungal constituent along a 500 meter transect in each plot were identified by ITS regions. Each transect included 50 soil samples in 25 collected sites. In pine dominant plot, transect was through *P. dalatensis* vegetation. Totally, 4966 ECM root-tips belonged to 76 fungal species were recorded. In Fagaceae dominant plot, 4354 ECM root-tips belonged to 83 fungal species were recorded. In both plot, the community structure of underground ECM fungi is evenly high diversity described by diversity index (Simpson 1/D=19.62 and Shannon-Wiener H'=3.5), species evenness (Pielou J about 0.8 in each plot). *Russula* spp. is a dominant fungal group. In each plot, a 1.5 km length x 1 meter width transect will be set up as the perimeter of 3 smaller concentric squares of 4 has, 1 ha and 0.25ha. All observability fungal reproductive structures along transect will be recorded and collect in each 2 weeks of year 2015 (27 times). Totally, 1320 sporocarps were collected and identified

belonged to more than 150 fungal species by ITS regions. The ECM fungal species were propose from reference. The comparison between the underground ECM underground fungal community and terrestrial showed that the similarity of total species and content of species. This is the first study investigates the ECM fungal community of endemic *P. dalatensis*, the most southern population of 5 needles pine in the world and the very narrow distribution pine *P. krempfii* in both underground and terrestrial. The additional transect through *P. krempfii* vegetation is setting up.

1.2-82 Diversity of temperate species of *Clavulina* and new insights about their ecological role in central Mexico

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Abstract: *Clavulina* J. Schröt. (Clavulinaceae, Basidiomycota) is a genus of macroscopic fungi, mostly with clavarioid basidiocarps. In temperate ecosystems, the most commonly recognized species are: *C. amethystina*, *C. coralloides*, *C. cinerea*, and *C. rugosa*. Although species of *Clavulina* are often categorized as ectomycorrhizal fungi (ECMF), growing evidence suggests that categories such as ECMF or saprotrophic fungi (SAPF) are loose and some plasticity may exist. The aim of this work was to describe the diversity of *Clavulina* species in two sites of a continuous *Abies religiosa* forest, with contrasting disturbance conditions and test their potential role as ECMF/SAPF. Species diversity was explored using basidiocarps and mycorrhizae, which were collected during the rainy seasons from 2011-2015 in La Sierra de las Cruces, in Central Mexico. Basidiocarps were described macro and microscopically, by focal and electron microscopy. DNA was extracted from mycorrhizae and basidiocarps, the nrDNA ITS (ITS) region was amplified, and the products were sequenced by Sanger platform. The sequences were edited in the Geneious program and together with those available in GenBank database, were aligned (MUSCLE) in the Aliview program. A consensus phylogenetic tree was obtained using Bayesian inference and *Hydnum repandum* as outgroup. To test the potential role of the sampled *Clavulina* species we obtained $\partial^{13}C$ and $\partial^{15}N$ isotopic values from *Clavulina* basidiocarps, SAPF, ECMF and fir leaves by means of mass spectrometry. Isotopic mean values were compared with one-way ANOVA. Morphological and phylogenetic analyzes were coincident and species were delimited as *Clavulina reae*, *C.* sp. nov. 1, *C.* sp. nov. 2, y *C.* sp. nov. 3. *Clavulina reae* and *C.* sp. nov. 3 are close to species that have been described in Europe, while *C.* sp. nov. 1 y *C.* sp. nov. 2 do not correspond to *taxa* previously described for the genus. Morphologically, the size of the basidiospores, the pigmentation of the basidiocarps and the presence of cystidia clearly delimited the four species. All *Clavulina* species showed $\partial^{13}C$ and $\partial^{15}N$ values intermediate between ECMF and SAPF at both sampling sites; in $\partial^{13}C$ *Clavulina* species are closer to ECMF ($P<0.01$), meanwhile $\partial^{15}N$ values, groups them together with SAPF ($P<0.001$). Even if $\partial^{13}C$ values strength the position of *Clavulina* in ECMF guild, ecological functions of *Clavulina* species at these sampling sites, points towards a role in the transference of nutrients different from nitrogen. Integrating taxonomic, systematic and ecological data is crucial to understand the whole biology of fungal species like *Clavulina*.

1.2-83 Growth of three mycorrhizal mushrooms from Sierra Tarahumara of Chihuahua, Mexico, under two pH conditions and six nutritive media

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Abstract: In the Chihuahua forest more than 50 species of mycorrhizal mushrooms are known, some of them with particular characteristics. *Astraeus hygrometricus* is an ectomycorrhizal mushroom that is found mainly in juvenile forests and is considered as a disturbance indicator species in Chihuahua. *Laccaria laccata* is an ectomycorrhizal mushroom associated with a large number of pine and oak species and *Pisolithus tinctorius* is a frequently used species in the inoculation of plants of forest importance. However, there are only few studies of its mycorrhizal efficiency of these three mushrooms with *Pinus arizonica*, one of the pine species with the highest economic interest in Chihuahua. Knowing the nutritional and environmental needs that these species require to obtain their mycelial production is fundamental in principle to improve the cultivation conditions and offer an alternative to mycorrhization in greenhouse conditions. In the present work, the growth of three mushrooms were evaluated in six growing media (Potato dextrose agar (PDA), Melin-Norkrans (MN), oats (AV), corn (MZ), trypticase soy (ATS) and dextrose sabouraud (ADS), with the purpose of establishing the best growing conditions as part of the first stage of investigation related to mycorrhization with *P. arizonica*. Mycelial development for *Astraeus hygrometricus* and *Pisolithus tinctorius* was carried out from spores planted in PDA medium, for *Laccaria laccata* was realized using internal tissue until pure mycelium was obtained. Then mycelial fragments (1 cm^2) were transferred into the six growing media under two pH conditions (4.8 and 5.8) with four replicates per treatment. Colonial morphology was determined considering macromorphological characteristics, growing media and pH. The highest colonial diameter for *A. hygrometricus* was obtained in ADS medium at pH 5.8 (8 cm) where it was observed the highest growth rate (16 mmd^{-1}) after seven days of incubation. The highest values for *P. tinctorius* occurred in MN medium at pH 5.8 (8 cm) after seven days of incubation. However, AV at pH 4.8 was the medium where the highest growth rate was reached (12 mmd^{-1}). The colonial morphology of *A. hygrometricus* was particularly different in each medium. Finally, for *Laccaria laccata* the highest values occurred in three growing media ADS, AV and MZ (8 cm) after six days of treatment with the highest growth rate of 16 mmd^{-1}, it was also observed that there were no differences between pH.

1.2-84 Ectomycorrhizal effect of the mushroom *Astraeus hygrometricus* (from Sierra Tarahumara of Chihuaua, Mexico) on the growth of *Pinus arizonica* Engelman seedlings

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Abstract: *Astraeus hygrometricus* is an ectomycorrhizal mushroom easily recognized in the field for its star shape, is frequently consumed in countries such as Thailand and India, and it has been used in the inoculation of different species of pine around the world, but mainly in Asia. In Mexico, especially in Chihuahua, we can find in juvenile forest and it is considered as a disturb indicator species. Chihuahua is one of the states with the highest forest production in Mexico, *Pinus arizona* is the most economically important due to wood use, however it has also been overexploited, so strategies for reforestation have been proposed. One of them is through the inoculation of seedlings destined for reforestation with ectomycorrhizal mushrooms but until now there are not studies with both species. In the present work

the ectomycorrhizal capacity of *Pinus arizonica* with *Astraeus hygrometricus* was evaluated using the spore inoculation technique under three different inoculum volumes as treatments (10, 25 and 50 mL) with the purpose of establishing the best dose of inoculation through the registration of morphometric variables in the pine seedlings. The main variables evaluated were height of the complete seedling, height and width of the foliage and percentage of mycorrhization of the root. Likewise, the mycorrhiza was morphological and histologically characterized. Six months after inoculation the two volumes of spore inoculation (10 and 25 mL) gave similar results compared with the control, however the best treatment was 50 mL, the average height of the plant was 6.33 cm in contrast to the control whose value was 4.99 cm, while the average height of the foliage was 6.03 cm in contrast to the control value 4.74 cm and 5.81 cm for width of the foliage in contrast with 4.75 cm to the control. The percentage of mycorrhiza colonization was 64%, the mycorrhizas of *Astraeus hygrometricus* in *Pinus arizonica* presented a coralloid morphology and white color that was smaller at the tips, the mantle surface was granular and the Hartig net was observed.

1.2-85 *Scleroderma meridionale* ectomycorrhizae on *Halimium halimifolium*: expanding the Mediterranean symbiotic repertoire

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Abstract: *Scleroderma* is a gasteroid genus in the Boletales (Basidiomycota), with a cosmopolitan distribution. Species of *Scleroderma* establish ectomycorrhizal (ECM) symbiosis with a range of coniferous and non-coniferous trees and shrubs, both in temperate and tropical regions, with little tendency to host specificity, a feature that might have facilitated the wide distribution of the genus. With the contribute of confocal laser scanning microscopy, we describe the morpho-anatomical features of the ectomycorrhizae formed by *Scleroderma meridionale* on *Halimium halimifolium*, a cistaceous plant belonging to a small group of woody shrubs occurring in open vegetation types in the Mediterranean region. The mycobiont and host plant identity in ECM was verified through molecular tools. Mycorrhizal system is very small, up to 1.9 mm, mostly coralloid to irregularly pinnate. The mantle surface is felty, whitish with silver patches. Differentiated rhizomorphs occur infrequently. Mantle surface is characterized by a network of branched hyphae organized in hyphal boundles. Hyphae are frequently covered by granules or warts. These characters, except for the presence of granules, are similar to those reported for the only two naturally-occurring *Scleroderma* ECM described so far, i.e. *S. bovista* on *Populus* and *S. citrinum* on *Betula* and *Pinus*. On the other side, the peculiarity of *S. meridionale* + *Halimium* ECM is the particularly small dimension of mycorrhizal system, a character shared with ECM formed by Cistaceae. At the best of our knowledge, this is the first description of an ectomycorrhiza on *Halimium*, a plant whose mycorrhizal biology deserves to be explored in greater detail.

1.2-86 Spatial patterns of ectomycorrhizal fungi in Soudanian woodlands

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Abstract: Trees which form ectomycorrhizal (ECM) symbiosis with fungi are important in the Soudanian zone of West Africa, including Central and Northern Benin, where they are often the dominant canopy trees in savannah woodlands. Many of the fungal partners produce edible mushrooms during the rainy season, which are harvested by the local population for culinary use. Our ongoing study investigates the

processes which spatially structure the ECM fungal community in these woodlands. We exhaustively sample ECM fruitbodies in nine 50x50m plots, each divided into 25 10x10m subplots, in Central Benin during the mushroom season, from June-October. Sampling began in 2015 and will continue at least through 2018. In addition to fruitbodies, we are also characterizing the soil fungal community through DNA metabarcoding. We consider the influence of soil characteristics, size and proximity of four species of ECM host trees, canopy closure, ground cover, and microclimate on the productivity, diversity, and community composition of ECM fungi both above and below-ground. In addition, we compare the results obtained from both short (ITS2) and long (ITS2 + partial LSU) amplicons using both the IonTorrent S5 (short) and Pacific Biosciences RSII (short and long) platforms, to compare tradeoffs between cost, sequencing bias, and the ability to place reads taxonomically. Preliminary results show that the fungal fruitbody community has strong spatial structure with high correlation between years (r^2=0.54), about half of which is explained by variation in soil characteristics, including total Phosphorus, pH, total Nitrogen, and clay fraction. Other environmental variables, including the presence of different host species, explain only a small amount of variance. The total biomass of fruitbodies produced is negatively correlated with several factors that may represent human impacts, including elevated Nitrogen, the incursion of grasses, and evidence of charcoal burning.

1.2-87 Diversity of ectomycorrhizal fungi as an indicator of the forest structure of Chihuahua, Mexico.

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Abstract: The forests of the state of Chihuahua have an area of 2,801.80 km^2, representing 1.13% of the state territorial extension. This is dominated by pine and pine-oak forests, characterized by a wide variety of fungi species that perform different ecological functions. However, the fungi considered of greater ecological importance in the forests are those associated with the roots of the trees forming mycorrhizas. The ectomycorrhizal fungi (EMF) are a key functional group in the regulation of nutrients circulating between the soil and the plants in the most terrestrial ecosystems. Numerous studies describe the relationship between the recovery of disturbed ecosystems and the EMF community. Some research has shown a total reduction in the richness of ectomycorrhizal species and significant changes in the composition of species after felling, as well as a positive correlation between mortality of EMF, increased by fire intensity and trees mortality. However, some results suggest that the composition of the EMF community is not substantially altered by the low intensities of fires or by restoration of burning or scarceness of the organic layer that remains unchanged. In the municipality of Bocoyna, there are highly disturbed areas due mainly to excessive wood production and overexploitation of their forests, generating the deforestation of some natural areas within the pine and oak-oak communities, causing the formation of felling areas and burning, which in turn is also one of the determining factors of the heterogeneous structure of the landscape. The objective of this study was to analyze the changes in the richness and abundance of EMF sporomes along a gradient of forest condition: secondary succession by burning, natural area and forest regeneration. In each site three quadrants of 2500 m^2 were located based on the homogeneity of the site, at intervals of ten days during the months of July to September 2016 and 2017. The number of sporomes per species was counted for each site to obtain ecological parameters. In addition, soil samples were taken for the analysis of texture and mineral elements by study area. The results show that the natural zone presented the greatest richness (n = 39) and abundance of EMF species, highlighting *Amanita muscaria*, *A. rubescens* and *Cantharellus cibarius*. In the area of forest regeneration, *Laccaria laccata* stands out for its abundance and finally in the zone of

secondary succession only three different species were recorded, dominating *Astraeus hygrometricus* with a record of more than 500 sporomes. Soil analyzes showed that the area where *Astraeus hygrometricus* was abundant with high levels of P, K, Ca, Mn and Zinc and a pH of 4.9 considered an acid soil in this forest.

1.2-88 Ectomycorrhiza of pecan in Brazil

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Abstract: Ectomycorrhiza studies on pecan (*Carya illinoinensis* (Wangenh) K. Koch) are becoming more common in recent years, both globally and in Brazil. In greenhouse conditions pecan can be mycorrhized easily with different truffles (*Tuber* spp.) species including several commercial species. On the other hand, the growth dynamic and optimal ecological conditions for pecan nuts production in plantations is not in favor of required truffle ecology. We aim to review the biology of both partners in mycorrhiza, pecan seedling/tree and selected commercial truffle species to foreseen optimal co-cultivation and management conditions. The ectomycorrhiza diversity was reviewed and analyzed in selected pecan plantations in Brazil and the growth requirements of pecan in native sites and commonly used management practices in commercial pecan plantations were reviewed. The main challenge was the bringing closer optimal soil conditions that would be in favor for commercial truffle species used in study (*T. aestivum, T. borchii* and *T. brumale*) and conditions required for normal growth, development, and fruiting of pecans. The ectomycorrhizal community on pecan in plantations in southern Brazil was relative rich, with species common for carbonate-poor sites. Besides other species a novel truffle species was discovered, presumably introduced from N. America and recently described by Grupe et al. (2018) as *Tuber floridanum* spec. nov. The southern regions of Brazil have the appropriate conditions for the development of trufficulture, a market niche that does not yet exist in Brazil, but which can become highly profitable, and pecan can be a beneficial host as revealed by Sulzbacher et al, 2018 in recently submitted paper. We propose a time- and management-sensitive production chain: truffle – pecan seeds – pecan wood, which can be feasible and economically sustainable, if the plantation is properly managed throughout its life-cycle.

1.2-97 Teamwork makes the dream work: disentangling cross-taxon congruence across soil biota in *Pinus nigra* plantations

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Abstract: Soil plays a fundamental role in many ecological processes, interacting with above-ground biodiversity in a complex network of ecosystem functions. Recently, there has been an increasing interest in the use of correlates for biodiversity assessment with evidence showing that the use of surrogate taxa in conservation planning is substantially more effective than that of proxies based on environmental data alone. A large body of literature deals with co-variation of species richness and composition in grassland soils whilst only few studies were developed on forests and even less in forest plantations. In this context,

a multidisciplinary EU-Life project (SelPiBioLife, LIFE13 BIO/IT/000282) was established in 2014 in two mountain areas of the Apennines (Italy) aiming at evaluating the application of an innovative forest management technique along with its effects on soil biodiversity in *Pinus nigra* plantations. Based on data collected before the silvicultural treatment, main aim of our study was to test the robustness of cross-taxon congruence across vascular plants, fungi (macrofungi, microfungi, ectomycorrhizae), carabids, microarthropods, nematodes and bacteria, also exploring how abiotic (soil and spatial-topograhic variables) and biotic (dendrometric variables) predictors drive the community concordances among taxa. Abundance data were obtained at the available taxonomic resolution for each biological group. Correlations between taxa were performed through Mantel and partial Mantel tests, while variation partitioning analysis was used to assess the total variance of each dependent taxon in the pure effect of another taxon, pure effect of soil, pure effect of spatial-topographic factors, pure effects of dendrometric variables, partial shared variation and total shared effect among all sets of predictors. In general, the distribution pattern of almost all the groups analysed showed highly supported inter-group congruence, while nematodes were not significantly correlated with other taxon. All biological groups also detained close relationship with the overall dataset of environmental-spatial variables. Considering the variation partitioning results, the variance attributed solely to pure effect of biotic or abiotic predictors was significant only in some cases (e.g. bacteria); remarkably, in all dependent taxa, total shared and partial shared effect of all sets of predictors always explained the highest portion of total variation. In conclusion, we can affirm that it is not a unique factor but rather the mutualistic relationship of all variables, both biotic and abiotic, to regulate the above-below ground subsystems in *Pinus nigra* plantations.

1.2-98 Wood decomposing fungal diversity explains enzyme patterns and element bioavailability in a long-term field experiment of the German Biodiversity Exploratories

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Abstract: Deadwood is an important structural component in forest ecosystems and its degradation is mainly controlled through microorganisms (fungi and bacteria) and their secreted digestion enzymes, as well as by xylobiontic arthropods. While the biochemical processes of wood decay are fairly well understood, *in situ* data about the factors that explain the heterogeneous distribution patterns of wood-decomposing fungi, their corresponding lignocellulolytic enzyme activities and the resulting changes of wood physico-chemical parameters and element bioavailability are rather scarce. We investigated the deadwood decomposition in a long-term experiment, which has been started within the German Biodiversity Exploratories in 2009. Here, we especially address the following hypotheses: *i)* the structure of fungal communities corresponds to the decomposing tree species and significantly differs between, sap- and heartwood, *ii)* specific ecological groups of fungi (e.g. white-rot Basidiomycota) explain the presence of particular enzymes and their distinct spatial patterns; and *iii)* fungal depolymerization processes increase the bioavailability of essential elements in decomposing wood. Samples were taken from deadwood logs of 13 different tree species (n=3) exposed to decomposition for six years in beech forests in Central Germany. Fungal communities were amplicon-sequenced using Illumina MiSeq. Fifteen extracellular enzyme activities (hydrolases and oxidoreductases) as well as wood parameters

(e.g. water-soluble lignin fragments) and element contents were measured. *i)* The fungal community structure and species richness significantly differed between the 13 decomposing tree species (specifically between deciduous and coniferous tree species), which can partially be explained by the different physico-chemical traits of the logs. For example, the lignin content, pH, organic extractives and element contents were found to considerably vary between deciduous and coniferous wood and thus correlated to distinct fungal sub-communities. However, we found no differences in fungal species richness and community structure between sap- and heartwood, six years after log deployment. In this experiment, fungal succession has seemingly reached the optimal phase of decomposition for several tree species. It means that with few exceptions (e.g. *Ascocoryne sarcoides*), the fungi has grown and colonized the logs from the outer to the inner side. *ii)* The presence of particular fungal families (e.g. Meruliaceae or Coniochaetaceae) was found to correlate with the secretion of characteristic lignin-modifying and/or (hemi)cellulolytic enzymes. Specifically, the abundance of white-rot basidiomycetes was positively correlated with the manganese peroxidase activity, which is the crucial enzyme of incipient lignin degradation. *iii)* Fungal depolymerisation activity evaluated by qualitative and quantitative analysis of small to medium-sized, water-soluble lignin fragments (0.5-5 kDa) significantly correlated with element bioavailability, which may be an important result regarding flux processes in forest ecosystems. Causal agent of this relation are ligninolytic white-rot basidiomycetes. Altogether we gathered an in-depth dataset of the microbial diversity, activity and resulting changes in decaying wood, which enables us to mechanistically understand the complex processes in the course of the biodegradation of woody biomass. In addition, we currently analyse the co-occurring bacterial community in the logs.

1.2-99 Wood decomposition and fungal communities: the interactive influences of soil and substrate nutrients

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Abstract: Wood decomposition is fundamental to carbon and nutrient cycling. Fungi play a critical role in wood decay because they produce lignocellulolytic enzymes that degrade wood polymers. The identity of these decomposers may have a profound effect on wood decomposition rate, as the ability to degrade lignin efficiently is restricted to a subset of fungal lineages. However, decomposition rate may also be influenced by nutrient availability. Increased nutrient availability can speed decay either by allowing increased production of nutrient-rich wood decay enzymes, or by shifting community composition towards taxa with intrinsically higher enzyme production and activity. In turn, enhanced enzymatic decay may also depend on nutrient source, as fungi can use nutrients from both wood and soil. This project explores: (1) Whether nutrients influence decay rate directly, by limiting enzyme production, or indirectly, by affecting fungal community composition; (2) The relative importance of wood and soil nutrient availability as determinants of decay rate. We used a novel experimental approach where saplings of four tree species were grown in the greenhouse under different soil nutrient treatments to induce 4-fold variation in wood phosphorus, nitrogen, and potassium concentrations. Wood samples from these saplings were then placed in different soil nutrient treatments established as part of a long-term soil fertilization experiment in a seasonally moist tropical forest in Panama. We measured the effects of wood nutrients and soil nutrients on wood decay rate and included fungal community analyses and assays of enzymatic activity. We found that wood nutrient treatment explained more variation in decomposition rate than soil nutrient treatment. Cellulose, hemicellulose, and chitin related enzyme activity responded to both wood nutrients and soil nutrients. However, acid phosphatase activity only responded to wood nutrient treatment. Completion of fungal environmental

amplicon sequencing will allow us to determine whether increases in enzyme activity in response to increased nutrient availability represents up-regulation of enzyme production by similar fungal communities, or by shifts in fungal community composition in response to substrate and soil conditions. A more complete understanding of how fungal communities and nutrient limitation interact to influence wood decay, has implications for predicting how global change phenomena, including nitrogen deposition, will impact one of the largest compartments of global carbon cycle.

1.2-100 Life after death: Dynamics of beech wood-associated communities over a gradient of life and decay stages

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Abstract: Wood is an important renewable resource that stores massive amounts of carbon globally, and supports complex food webs comprised of diverse, highly specialized organisms. As such, it is important to understand the processes that control wood decomposition to more accurately predict global carbon cycles and protect biodiversity under increasing pressure due to climate change effects, or habitat loss or fragmentation. Wood-inhabiting endophytes sometimes act as latent saprotrophs and recent experiments suggest that priority effects imposed at the endophyte-saprotroph transition may impact community assembly dynamics that affect species richness, decay rate, decay type, litter quality and carbon release in natural settings. Results will be presented from a study in which amplicon sequencing and physicochemical data are being used to assess community dynamics across a gradient of living (5 growth stages) and dead (6 decay stages) European beech *Fagus sylvatica*) wood from a nature reserve in Denmark. We aim to reconstruct wood-associated community assembly histories using network analysis methods to identify key species or abiotic factors that determine decay progression. We expect that species or factors with high *betweenness centrality* scores will link *modules* that are associated with distinct decay types. Further, these modules are likely to be characterized by species or factors exhibiting high scores for *closeness centrality*.

1.2-101 Fungal carbon utilization in temperate forests

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Abstract: Being one of the major terrestrial carbon pools, forests are extremely important for carbon sequestration and greenhouse gas fluxes. The forest soil processes carried out by microorganisms are thus critical for global carbon cycle and consequently global climate. Boreal and temperate forests provide niches for a diverse community of fungi, both saprotrophic and mycorrhizal. Fungi produce a variety of enzymes responsible for decomposition of even the most recalcitrant plant biopolymers. Furthermore, they play a crucial role in carbon sequestration and mediate allocation of plant-derived carbon into soils. This experiment aimed to track the incorporation of carbon derived from different sources in forest soil into the fungal biomass using stable isotope probing. Six substrates labeled with ^{13}C isotope were added to microcosms containing homogenized soil from a temperate forest in the Czech Republic. The substrates varied in complexity – from simple sugars and organic acids present in root exudates (glucose, citric acid) to more complex carbon sources (cellulose, hemicellulose) and substrates resembling total plant biomass (maize leaf) and fungal biomass (chitin). Microcosms were incubated for 1 and 3 weeks, together with associated controls. Substrate respiration rates were determined by the analysis of the CO_2 released in microcosms. Fungal taxa incorporating the ^{13}C into

their biomass were identified after DNA extraction, ^{13}C-DNA separation and amplicon sequencing of ITS2 on Illumina MiSeq. The highest respiration rate (mmol/g $_{13}CO_2$) after 1 week of incubation was observed in microcosms supplemented with citric acid, followed by glucose, hemicellulose, maize leaf, cellulose and finally chitin. After incubation of 3 weeks, a considerable increase in chitin respiration rate was observed, while the increase in respiration of other substrates occurred at a more similar rate. While all substrates were highly incorporated by the Ascomycota, Zygomycota were detected in chitin microcosms in higher relative abundances compared to the other substrates. In the case of chitin, the most dominant ^{13}C-accummulating genus was *Mortierella*, while in other substrates *Geomyces* seemed to be the most dominant genus. Other highly enriched genera included *Pseudocosmospora* (chitin), *Trichosporon* (citric acid), *Pencillium* (glucose, hemicellulose, citric acid) and *Aureobasidium* (maize leaf). The results of this study show that multiple fungal taxa are capable of utilization of all carbon sources regardless of their complexity. However, the results also indicate a difference in fungal community responsible for chitin decomposition, confirming the specificity and importance of fungal biomass as carbon source in temperate forests.

1.2-102 Assessment of dead wood fungi biodiversity in urban parks and natural reserves across New Jersey

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Abstract: Ecosystems with high species richness such as forests rely on biodiversity of decomposers to boost availability of forest elements. Dead wood is an important component of any forest. It is a decomposition site, it protects against erosion, improves water retention in the ecosystem, and creates ecological niches. Dead wood-inhabiting fungi are saproxylic taxa that decompose cellulose and/or lignin. Here, we assess biodiversity of deadwood-inhabiting fungi in areas with varying degrees of human modification (removal of foliage, snags, logs). We hypothesize that removal of dead wood from urban parks diminishes the diversity of dead wood fungi which may affect the stability of ecosystems. Our goal is to compose a comprehensive database of dead wood inhabiting fungi and compare areas with different forest management practices to assess human impact. We present preliminary results from assessment of different locations spanning across northern to southern NJ. Fungal fruit bodies were collected for two hours at each sampling site and photos and GPS coordinates were recorded and shared via iNaturalist.org. Online database is available for the world community. Fungi identification was performed using both morphology and DNA sequence analysis. The nuclear ribosomal Internal Transcribed Spacer (ITS1, ITS2) region was used as a DNA barcoding marker, allowing identification of fungal species via DNA sequencing and bioinformatics methods.

1.2-103 Plant decapitation alters decomposition processes and initiates complex restructuring of fungal communities in tree roots

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Abstract: Although root biomass represents a significant pool of organic matter in terrestrial ecosystems, information on the factors influencing root decomposition is scarce, in contrast to the vast body of data on aboveground litter decomposition. Here, we assessed changes in fungal community structure, lignin and cellulose concentrations, fungal biomass and decomposition activity in the roots of a Picea abies stand for a two-year period following decapitation of Picea abies seedlings compared to data from non-decapitated seedlings. We found that the termination of photosynthate flow is associated

with profound changes in decomposition processes, fungal biomass as well as fungal community composition. We found no support for the involvement of ectomycorrhizal fungi in the decomposition of roots, but we found some evidence that root endophytic fungi may have an important role in the early stages of this process.

1.2-104 Where mycorrhizal fungi meet leaf-endophytes: Fungal community dynamics during early decomposition of translocated leaf litter

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Abstract: Litter decomposition is a key process in nutrient cycling and therefore crucial for ecosystem functioning. The major decomposers in forest ecosystems are considered to be saprotrophic soil fungi. However, recent evidence suggests that previously endophytic as well as mycorrhizal fungi are involved in the degradation of organic matter. To assess the changes in fungal community composition and the dynamics of different functional guilds (i.e. endophytic, saprotrophic and mycorrhizal fungi) during early decomposition, we designed a litter translocation experiment. Beech trees from a tree nursery were planted at different altitudes (500 m and 1000 m asl) at the 'Untersberg' mountain to exclude effects by genetic pre-adaptation of the hosts. After one growing season the trees accumulated site specific endophytic fungal communities. The leaf-litter of each of five trees was collected separately in the following autumn, portioned and incubated at each altitude. Litterbags were collected regularly for 15 months. Content and stable isotopes of carbon and nitrogen were measured and nucleic acids extracted. The present and active fungal communities were assessed by Next Generation Sequencing of the ITS rRNA gene (DNA) and precursor ITS rRNA (RNA), respectively. Isotopic signatures indicated that decomposition was initially faster at the lower, but after summer faster at the higher altitude. Composition of fungal communities (DNA) started to differ between incubation sites in the following May, while the community activity (RNA) already differed significantly in March, i.e. when the sites were still covered with snow. Origin of the samples significantly shaped the communities throughout the first year of incubation. Only after the second winter, origin of the decaying leaves was no longer reflected by the fungal decomposer communities. Proportion of the previously endophytic fungi decreased only slowly in the first year, while ectomycorrhizal fungi occurred at noteworthy contributions from July on and increased in abundance in most samples until October. Community dynamics will be discussed in dependence from incubation altitude, with a focus on the interaction of saprotrophic soil fungi, previously endophytic fungi and ectomycorrhizal fungi.

1.2-113 *Laetiporus lobatus* (Basidiomycota, Polyporales), a new fungal species from Costa Rica

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Abstract: The genus *Laetiporus* with one species *L. sulphureus* (Bull.:Fr.) Murrill was erected by Murrill for a common and very distinct wood-rotting fungus characterized by poroid hymenophore, dimidiate to flabellate pilei of bright orange to yellow color, soft and fleshy context, dimitic hyphal system with simple septate generative hyphae and characteristic, broad and interlocking binding hyphae, and causing a brown rot. For a long time, the only member of the genus has been considered a cosmopolitan species. However, incompatibility studies revealed 5–6 incompatibility groups/species only in the USA

and this result was confirmed by molecular studies. New American species have been described, all often very similar to *L. sulphureus,* but differing by sequence, ecology and geographical distribution and showing also slight differences in basidiospores size and shape. We have recently collected and studied specimen from Costa Rica showing ITS sequence and morphological traits different from other *Laetiporus* sp., we describe it as new species, *Laetiporus lobatus*. *Laetiporus lobatus* is similar to *L. sulphureus* but with much smaller pores, 7–8 per mm, small spores 3.9–4.4 × 2.8–3.2 µm, strikingly lobed pileus margin and unique ribosomal ITS sequence. *Laetiporus* taxonomic problems are briefly discussed and a key to described species is provided.

1.2-114 New poroid species of Hymenochaetaceae (Hymenochaetales, Basidiomycota) from southern Chile

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Abstract: Polyporoid fungi from Chile are poorly known. Most of the species present in the Patagonian Andes forests of Argentina are also found there, but southern Chile is distinguished by particular phytogeographic formations that host a high diversity of tree plants. Field work in the Valdivian rainforest and the temperate durifoliate forests revealed specimens of four taxa that were studied morphologically, through culture characterization and phylogenetic evidence (sequences comparisons of molecular markers) and that are here shown to represent new taxa in the genera *Fomitiporia* (ITS, LSU, EF, rpb2), *Phylloporia* (ITS, LSU) and *Fomitiporella* (ITS, LSU) (Hymenochaetaceae, Hymenochaetales, Basidiomycota). We describe, characterize and show the phylogenetic position of the following proposed new taxa: *Fomitiporia* "chilensis" (on *Cryptocarya alba* and *Peumus boldus*), *Phylloporia* "boldus" (on living *Peumus boldus*), *Fomitiporella* "muriforme" (on undetermined fallen trunks) and *Fomitiporella* "podocarpus" (on living *Podocarpus nubigena*). *Fomitiporia* "chilensis" is phylogenetically associated to the resupinate South American lineage of the *Fomitiporia punctata* species complex and is morphologically similar to *Fomitiporia dryophila*. *Phylloporia* "boldus" is distinguished by relatively large, dull chestnut basidiospores that lack the typical yellowish color of the wall in other species of the genus; it is related to *Phylloporia dependens*, a taxon described from tropical China that is phylogenetically weakly related to the core species group in *Phylloporia*. *Fomitiporella* "muriforme" and *Fomitiporella* "podocarpus" are represented by single specimens but are otherwise distinguished phylogenetically. We also report the wide presence and distribution of *Fomitiporella americana*, a taxon recently described from SE USA; this taxon is morphologically similar to *Fomitiporella inermis*, it decays trunks of many hosts in southern Chile and Argentina and is responsible of the white heart rot present in standing *Austrocedrus chilensis*. The combined ITS and 28S concatenated analysis showed that the genera *Inocutis*, *Phellinotus* and *Arambarria* are included or fall within the gross phylogeny of *Fomitiporella*. The analyses confirmed that these genera are closely related and form a strong monophyletic group (BA 100, ML 100) and that *Fomitiporella* taxa appear across multiple branches of the phylogeny. Funding: Fondecyt 1151028 and Cooperación bilateral MinCyT (Argentina)-CONICyT (Chile) CH13/06.

1.2-115 Diversity and taxonomy of *Ganoderma* species in South Africa, inferred from morphology and a multi-locus phylogeny

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Abstract: *Ganoderma* is a cosmopolitan genus of Polypores that encompasses species important for forestry, medicine and cultural traditions. Despite the importance of this genus, knowledge pertaining to the species diversity of *Ganoderma* in South Africa is limited. This study aimed at elucidating the identity and phylogenetic placements of a large collection of *Ganoderma* samples (including basidiomes and wood samples) obtained during a wood-rot fungus survey in the Garden Route National Park (GRNP) of South Africa and during earlier fieldwork at other localities. Identification was facilitated by phylogenetic analyses of DNA sequences obtained from the ITS regions, a region of the β-tubulin and Translation Elongation Factor 1-alpha (TEF) genes, respectively, as well as morphological characters. Results from these analyses revealed that isolates from the collections belong to eight *Ganoderma* species. Of these, *G. applanatum*, *G. austroafricanum*, *G. destructans* and *G. enigmaticum* have previously been reported from South Africa, while *G. cupreum* and *G. resinaceum* are new records for the country. The remaining two species are novel taxa belonging to subgen. *Elfvingia* and described as *G. acacicola* sp. nov. and *G. knysnamense* sp. nov. *Ganoderma acacicola* occurs on native and non-native hosts, including *Acacia cyclops*, *Celtis africana*, *Prunus africana* and an unknown palm species in four provinces of the country. The fungus is characterised by a perennial, triquetrous and broadly attached basidiome, a sulcate up to zonate yellowish brown to brown pilear surface, and ovoid to ellipsoid basidiospores. *Ganoderma knysnamense* was collected only in the GRNP where it was also the most abundant fungus among the species identified. It is distinguished by its applanate to ungulate, sometimes convex, and dimidiate to broadly attached basidiome, its chocolate-brown pilear surface covered with a hard woody-like crust and ellipsoid, broadly ellipsoid to ovoid basidiospores. The discovery of two new *Ganoderma* species as well as the two newly-recorded species raises the total known *Ganoderma* species in South Africa to 15. The continual discovery of new species, as is shown in this study and other recent studies, suggest that many more *Ganoderma* species are likely to be discovered in South Africa and indicates that further research is warranted on this important genus in the country and in Africa.

1.2-116 Ectomycorrhizal associations with *Salix humboldtiana* in Southern South America: An ancient cross-continental exchange.

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Abstract: Ectomycorrhizal (ECM) forests in southern South America (SSA) are dominated by Nothofagaceae (southern beech) trees. Based upon the fossil record, Nothofagaceae trees and their ECM fungi have been present in SSA for 60 – 100 million years with little evidence for co-occurring ECM hosts during this time. A second native ECM host tree, *Salix humboldtiana* (Humboldt's willow), colonized SSA from the north between 3 – 15 MYA. This event followed the formation of the Isthmus of

Panama during the Great American Biotic Interchange. While Nothofagaceae species and *S. humboldtiana* overlap in latitudinal range, these trees represent different lineages, occupy different niches, and seldom inhabit the same forests. Both *Salix* and Nothofagaceae associate with a diverse assemblage of ECM fungi but the biogeographic origins of these communities is expected to be different (*Salix* from the Northern Hemisphere and Nothofagaceae from the Southern Hemisphere). However, it is possible the two hosts have exchanged ECM fungi since the colonization of SSA by *Salix*. In this study we characterize ECM communities associated with *S. humboldtiana* in Argentina and compare these communities with Nothofagaceae to investigate the potential for ECM host jumping between these taxa. We sampled rhizosphere soil, ECM root tips, and ECM fungal sporocarps from stands of *S. humboldtiana* at 18 sites throughout its range in Argentina from Parque Nacional Calilegua (Northern Argentina) to the Chubut River (Central Patagonia). Since exotic Eurasian *Salix spp.* were present along the same watercourses throughout much of our sampling range, we also sampled rhizosphere soil and ECM root tips from exotic *Salix* at 5 sites to investigate the potential for shared ECM communities between exotic *Salix* and *S. humboldtiana* . Fungal communities from soil DNA and ECM root tips were identified using Illumina Miseq meta-barcoding of the ITS1 region. Operational taxonomic units (OTUs) were sorted by putative ecological niche (saprobic, pathogenic, mycorrhizal) using a combination of FUNGUILD and Blast searches of NCBI GenBank and our extensive in-house database. Results of our comparative metagenomic analyses indicate that ECM fungal communities of *S. humboldtiana* are composed of fungi primarily from northern hemisphere ECM lineages and lack representatives from endemic southern hemisphere ECM fungal lineages (eg. Descolea, Phaeohelotium, Austropaxillus). There are several species of exotic ECM Basidiomycota that were likely introduced into *S. humboldtiana* ECM communities from exotic European *Salix*. Some lineages of ECM host generalist fungi may also have moved between *S. humboldtiana* stands and Nothofagaceae forests. Exemplar lineages from our dataset include species of ECM host generalists *Hebeloma* (Agaricales), which are likely to have moved into Nothofagaceae forests from *S. humboldtiana* stands or exotic Northern Hemisphere hosts, and *Tomentella* (Thelephorales) which may have undergone bidirectional host shifts between Nothofagaceae and *S. humboldtiana*.

1.2-117 Evolutionary relationships of *Gloeoporus* (Irpicaceae, Basidiomycota) with emphasis on the *G. dichrous* complex

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Abstract: *Gloeoporus* is a cosmopolitan genus with species able to decay a wide range of substrates. The genus include species characterized mainly by the continuous gelatinous hymenium along tube surface and dissepiments, shallow pores, whitish context contrasting with a brightly colored hymenophore, monomitic hyphal system and allantoid basidiospores. Currently, 13 species of *Gloeoporus* are accepted by most mycologist but only four of these [*viz*., *G. citrinoalbus*, *G. dichrous G. hainanensis* and *G. pannocinctus*] have been included in phylogenetic analyses. Despite a long history of formal recognition and research, several aspects of *Gloeoporus* phylogeny have yet to be uncovered. This is because the phylogenetic position of *Polyporus conchoides* (= *G. thelephoroides*, type species of the genus) has long been uncertain, and the diversity of *Gloeoporus* is in large part composed of geographically widespread species. The objective of our study was to assess species limits and infer species-level phylogenetic relationships within *Gloeoporus*, with special emphasis in *G. dichrous* complex, through a combination of detailed morphological studies of type/original collections and molecular phylogenetic analyses of specimens collected in North America, South America, Europe and

Southeast Asia. Different optimality criteria were used to analyze combined molecular matrices of two genes (ITS and nLSU). The dataset includes 74 ITS and nLSU original sequences in addition to 140 ITS and nLSU sequences retrieved from GenBank. Our preliminary results suggest the monophyly of *Gloeoporus* within the *I*rpicaceae family with high support. Sequences of specimens morphologically similar with or identified in GenBank as *G. dichrous* were recovered in three different sub-clades, as follows: 1) a sub-clade of widely distributed specimens, including European specimens collected next to the type locality (*G. dichrous s.s.)*, together with North and South American specimens; 2) a sub-clade of Asian specimens representing three different lineages independent from *G. dichrous s.s.*; and 3) a well-supported clade of *Gloeoporus* specimens from Brazil. *Gloeoporus thelephoroides* was included for the firsts time in a phylogenetic analysis. Our analyses have shown that the species as currently defined represents a complex of species, with at least four different lineages independent of *G. thelephoroides s.s*. Additional collections, meticulous study of morphology and more molecular evidence will allow clarification of the evolutionary history of these species. In light of the large number of synonyms and names available in *Gloeoporus* we will discuss how to best apply the existing names to the clades recovered in the molecular phylogenies.

1.2-118 The genus *Trichaptum* (Agaricomycetes, Basidiomycota) in São Paulo state, Brazil

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Abstract: The genus *Trichaptum* was described by Murril in 1904 based on specimens collected in India, Central and South America. In its original description, it was characterized by annual basidiomes, sessile, dimidiate, with brownish color, short tubes that become labyrinthiform with age, with darkest coloration than the context and smooth spores. Nowadays, the diagnoses of the genus expanded, encompassing widely distributed species, characterized by resupinate and effused-reflexed basidiomes, with poroid to irpicoid or lamellate hymenophore, di- to trimitic hyphal system, generative hyphae with clamps and hymenial cystidia. Sixty-six species names are associated with T*richaptum* in online mycological databases. Nine accepted species were recorded in Brazil and six in São Paulo state, *i.e. T. abietinum*, *T. biforme*, *T. byssogenum*, *T. fumosoavellaneum*, *T. perrottetii* and *T. sector*. This study aims to understand the species distribution on the Southeast of the Brazilian Atlantic forest and its relationships through morphological and phylogenetic studies. Macro and microscopic analyses based on specimens collected in different Conservation Units of the state and on specimens deposited on SP herbarium are in progress. The study of type specimens is also being carried out in order to compare and infer important characters to delimit the species and genus. An identification key of the studied species will be presented. For phylogenetic studies, DNA extraction and amplification of ribosomal regions Internal transcribed spacer (ITS1, 5.8S and ITS2) and Large subunit (nLSU) of fresh or recently collected materials are being made. Phylogenetic analyses were constructed using Maximum Likelihood and Bayesian Inference methods. Initial analysis indicates that *Trichaptum* Murril as currently defined is not monophyletic. The species *T. byssogenum* was described from Java but the type specimen was never sequenced and as far as is known there are no recent collections in the type locality. Sequences of specimens from Brazil and sequences retrieved from GenBank morphologically identified as *T. byssogenum* nest in a separate clade independent from *Trichaptum s.s.*, but related to *Hyphodontia* species. This new clade represents a possible new genus including at least two different lineages, one of them formed by Brazilian specimens and other from New Zealand and Sri Lanka specimens. Other specimen recently collected and examined indicates morphological characteristics intermediary between *T. biforme* and *T. sector*. Phylogenetic results indicate that it could be a new species, but more

comparisons as well as type studies are required to elucidate its proper taxonomic placement. In addition, the morphological studies of the herbarium materials showed that *T. fumosoavellaneum* does not occur on São Paulo state and that *T. sector* has a pseudo-trimitic hyphal system, without true binding hyphae, which differs from descriptions by other authors. The sequences produced in this study are important new additions to the knowledge of the genetic diversity of specimens in the Brazilian Atlantic forest. This project is an important addition to the knowledge of the genus *Trichpatum* in the Neotropics, allowing a better understanding of its taxonomic placement as well as re-appraisal of morphological characters for species differentiation.

1.2-119 *Hydnophanerochaete* and *Odontoefibula*, two new genera of phanerochaetoid fungi (Polyporales, Basidiomycota) from East Asia

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Abstract: The broad generic concept of *Phanerochaete* is one of the largest genera in corticoid fungi, decomposing various woody substrata and causing a white rot. Two new genera of *Phanerochaete* s.l. are presented, namely *Hydnophanerochaete* and *Odontoefibula*. The type species of *Hydnophanerochaete* is *Phanerochaete odontoidea*. *Odontoefibula* is established based on a new species, *O. orientalis* (generic type), and *O. deflectens* (≡ *Grandinia deflectens*). Both genera are characterized by having an effused basidiocarp with odontioid hymenial surface, simple-septate generative hyphae, cystidia lacking, clavate basidia, ellipsoid basidiospores which are smooth, thin-walled, inamyloid, non-dextrinoid, and acyanophilous. *Hydnophanerochaete* is diagnostic by its compact texture of subiculum with thick-walled subicular hyphae, and presence of quasi-binding hyphae. *Odontoefibula* is featured by its basidiocarp turning dark brown in KOH, and dense texture of subiculum with thin- to slightly thick-walled subicular hyphae. Morphological study and multigene phylogenetic analyses based on sequences inferred from two combined datasets (nrITS+nrLSU+*rpb1* and nrITS+nrLSU) respectively, indicate that *Hydnophanerochaete* and *Odontoefibula* are respectively placed in Meruliaceae and *Donkia* clade of Phanerochaetaceae. *Phanerochaete subodontoidea* is a synonym of *P. odontoidea*, according to morphological and molecular evidence.

1.2-120 How many *Steccherinum* (Polyporales) species are there in the Neotropics?

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Abstract: The genus *Steccherinum*, typified by *S. ochraceum*, encompasses species with hydnoid and poroid hymenophore mainly characterized by the presence of a dimitic hyphal system, large and usually heavily encrusted skeletocystidia and small globose to cylindrical basidiospores. Even though the genus is widely accepted, and about 10 species have been recorded in the Neotropics, many of these are identified with names originally given to temperate species or representing synonyms. Furthermore, there is no study specifically focused on this group in the Neotropical region combining morphological and molecular evidence. In order to contribute to this picture, twelve hydnoid specimens of *Stecchetinum* collected in southern Brazil throughout 2017 were studied. DNA extraction was performed using CTAB 2% lysis buffer. The Internal Transcribed Spacer (ITS1, 5.8S and ITS2) and the Large Subunit (LSU) of the nuclear ribosomal RNA (rRNA) regions were amplified and used to infer phylogenetic analysis. The PCR products obtained were sent to Macrogen (Korea) for sequencing. A dataset including the sequences obtained, as well as others available on Genbank database, was prepared. Phylogenetic trees were constructed using Bayesian Inference and Maximum Likelihood

methods. The macro and micromorphological analyses revealed the presence of three morphogroups, none of them fitting the concept of species previously registered from the region. However, the phylogenetic analysis showed that at least five different lineages in *Steccherinum* can be recovered with high support, evidencing a greater diversity and the possible occurrence of cryptic species. One of the morphogroups identified encompasses three similar species, morphologically related to *S. ochraceum*, which are very difficult to discern form one another. While one of them can be distinguished by the formation of small pilei, the other two are completely resupinate. Microscopically, all three species present very small basidiospores (2.6–3.0(3.5) × 1.7–2.5 µm), which can also differentiate them from other species in the genus. The other two species recovered present larger basidiospores (3.5–4.5 × 3–3.8 µm), one of them having strictly resupinate pinkish basidiomes while the other is characterized by effused-reflexed basidiomes with brown pilear surface and whitish hymenophore. The high diversity found shows that many Neotropical *Steccherinum* may have been overlooked, suggesting the possibility of new species in the genus. Further studies, including the addition of more specimens to the molecular analyses and a more detailed morphological comparison of recent collections with type specimens, are being carried out in order to try to elucidate the actual diversity of the genus in the Neotropics. The data obtained shows the importance of critical analyses combining all possible empirical evidence before implementing new taxonomic decisions. In an era of fast changes for the systematics, it is always important to supplement molecular data with a detailed morphological study in order to find characteristics of taxonomic value and keep on reviewing and updating the knowledge of Fungi in the world.

1.2-129 Comparative genomics of the genus *Amanita*

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Abstract: The genus *Amanita* has long been an iconic Basidiomycete lineage – to the point that when most people think of a mushroom they envision the red and white cap of *Amanita muscaria*. There are dazzling arrays of biochemical pathways that are induced across the genus – with clades with edible, psychoactive, and deadly poisonous members. Additionally, previous research has shown the loss of genes responsible for a saprophytic lifestyle and the transition to ectomycorrhizal association with woody plant hosts. In order to understand both life history and phylogenomics of the group, we have initiated a study to compare the genus using genome sequence data. Phylogenomic analysis reveal the relative position of each species across the genus using a suite of genes culled from annotated coding regions. Gene annotation methods were used to identify candidate genes for interaction with plants (effectors and other small secreted protein production), novel biochemical pathways, and secondary metabolite production. Pathway analysis has identified numerous biochemical pathways that have emerged across the *Amanita* lineage.

1.2-130 Phylogenetic position and taxonomy of *Kusaghiporia usambarae* gen. et sp. nov. (Polyporales)

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Abstract: *Polyporales* form a large group of Basidiomycota, containing more than 1,800 species in over 216 genera and 37 families. Seven clades are recognized in *Polyporales*: the 'antrodia', 'core polyporoid', 'residual polyporoid', 'phlebioid', 'tyromyces', 'gelatoporia' and 'fragiliporia' clades. Currently, the 'antrodia clade' contains more than 26 genera, which are of economical importance as

food as well as a source of pharmaceutical and biotechnological products. However, some species are plant pathogens. 'Kusaghizi' is a local name of a large polyporoid mushroom from the West Usambara Mountains in Tanzania. The mushroom produces large dark brown fruiting bodies up to 60 cm wide, which at maturity may weigh more than 10 kg. It has a high rate of mycelial growth and regeneration, and was found growing on both dry and green leaves of shrubs and attached to the bases of living trees. It was also observed to degrade snakes and insects coming into contact with it. This mushroom has a long tradition of being used as food and medicine by local communities although no scientific description of this has been carried out. This study describes the species and infers its phylogenetic position. Morphologically, the mushroom produces dark brown basidiomata with globose to subglobose basiodiospores. Phylogenetic analyses based on individual and concatenated data sets of nrLSU, nrSSU, RPB2 and TEF1 genes grouped the mushroom together with *Laetiporus* and *Wolfiporia,* with strong support to form a monophyletic group in the 'antrodia clade'. A new genus and species is proposed: *Kusaghiporia usambarae* gen. et sp. nov. to accommodate this species.

1.2-131 Macroevolutionary analyses of fruiting body forms and nutritional modes in Agaricomycetes based on an 8500-species phylogeny

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Abstract: The Agaricomycetes contains approximately 35,000 described species and a wide variety of fruiting body morphologies such as resupinate, coralloid/clavarioid, pileate-stipitate, sessile and gasteroid forms, as well as diverse nutritional modes, such as saprotrophy, parasitism and mutualistic symbiosis. Previous studies have hypothesized that the resupinate fruiting body form is plesiomorphic, there is a directional trend that favors pileate-stipitate forms, the gasteroid form is irreversible and pileate-sessile and clavarioid forms are labile. The development of new methodological tools and the increasing amount of public DNA sequences provide an opportunity to test these and other hypotheses about the evolution and diversification dynamics in the Agaricomycetes. The aims of this research are to identify broad evolutionary patterns within this group, and to study the effects of morphological transitions and nutritional strategies on diversification rates. We reconstructed a six-gene phylogeny that contains 8,500 species of Agaricomycetes and estimated trait-independent diversification rates using BAMM, as well as trait-dependent diversification rates using BiSSE and MuSSE. We tested several coding regimes that include transitions from non-gasteroid to gasteroid forms, and multi-state transitions such as resupinate, clavarioid, pileate-stipitate, sessile and gasteroid. Our results suggest that there have been 30-40 major shifts in diversification rates, most of them in the Agaricales, Russulales, and Polyporales, with at least 120 transitions from non-gasteroid to gasteroid forms, from both clavarioid/coralloid and pileate-stipitate ancestors. Gasteromycetation does not affect diversification rates. Analyses of diversification dynamics of nutritional modes are ongoing.

1.2-132 Species boundaries in *Mycosphaerellaceae* s. lat.

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Abstract: The *Mycosphaerellaceae* (*Capnodiales, Dothideomycetes*) contains thousands of species, and currently includes 213 generic names according to MycoBank. These genera encompass many

important plant pathogens, saprobes and endophytes, as well as extremotolerant species. Co-occurrence of multiple species or genotypes within the same lesion is a common phenomenon, as is the movement of species between diverse hosts in different plant families. While some species are highly host-specific, others can occur on several hosts or can temporarily occupy a substrate in search of their optimal host ("pogo-stick hypothesis"). Homo- and heterothallic species occur in the same genus, with species observed as being strictly sexual, asexual, or being able to readily produce both morphs in culture. In the past, morphology and host association played a major role in species identification and naming. However, the decrease in cost of Sanger sequencing and the availability of universal primers for numerous fungal house-keeping genes have changed the way we approach species discovery and identification. The ITS nrDNA was accepted as the official barcode gene for Fungi. However, this locus lacks resolution for species identification in several genera in the *Mycosphaerellaceae*, such as *Cercospora*, *Pseudocercospora*, *Ramularia* and *Septoria*. Commonly used secondary barcode genes for these genera include partial actin, beta-tubulin, calmodulin, histone H3, translation elongation factor 1-alpha and DNA-directed RNA polymerase II second largest protein subunit. What is evident from the resulting individual gene trees is that no single protein-coding gene is able to distinguish all species known from DNA data; and ITS nrDNA is consistently the worst locus for phylogenetic species recognition for several genera in this family. A combination of at least two protein-coding loci is routinely required to resolve most of the species. Although the use of multilocus sequence data provides better resolution for species delineation, there are three major constraints which are encountered when screening new loci. These are 1) ease of amplification for all isolates of the genus, 2) level of resolution, and 3) the availability of material for DNA comparisons. However, the increasing number of publicly available genomes of especially *Dothideomycetes* species could provide a source of alternative phylogenetic markers which could lead to the identification of genes that are easier to amplify universally and have improved resolution at the species level. The impact of multilocus DNA data on generic and species concepts in *Mycosphaerellaceae* are discussed, along with future challenges.

1.2-133 Re-evaluation of the genera in the Chaetomiaceae

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Abstract: The Chaetomiaceae (Ascomycota) are well known for their cellulolytic activity and the production of various biologically active secondary metabolites that have great potential in agriculture, medicine and industry. On the other hand, many species are also able to grow in the indoor environment, causing adverse health effects, or are reported as causal agents of fungal infections in humans. Most genera occurring in Chaetomiaceae have not been revised based on molecular data. In this study, genera of Chaetomiaceae are re-evaluated on the basis of morphology and a four-locus DNA phylogeny. The asexual genera *Botryotrichum*, *Humicola* and *Trichocladium* are redefined to include species that reproduce sexually, and *Chaetomium longicolleum* is shown to represent the sexual morph of *Staphylotrichum*. Furthermore, most of the other known genera in Chaetomiaceae are re-circumscribed. The present study is a first attempt to establish an inclusive modern classification of the Chaetomiaceae composed of monophyletic genera.

1.2-134 *Colletotrichum gloeosporioides s.l.*: a multilocus species tree and a flexible metabarcoding framework for species identification and diversity exploration

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Abstract: *Colletotrichum gloeosporioides* s.l.: a multilocus species tree and a flexible metabarcoding framework for species identification and diversity exploration The *Colletotrichum gloeosporioides* (Cg) complex is an ecologically and economically important lineage of plant-associated fungi that includes diverse pathogen and endophyte species. Prior misperceptions that Cg are host specific yielded a proliferation of species recognized primarily on the basis of host affiliation. However, molecular phylogenetic characterization of Cg associated with single and multiple sympatric plant hosts have shown that many pathogen and endophyte species possess multi-host ranges, while also demonstrating that Cg encompasses extensive terminal phylogenetic diversity. While the community of biologists investigating Cg is in consensus that multilocus sequencing is essential for improved accuracy of species delimitation, the suite of multilocus markers in current usage often vary among studies and individual markers differ widely in phylogenetic informativeness. Based on demonstrations that the Apn2/Matigs nuclear intergenic spacer is one of the most informative phylogenetic markers yet developed for Cg, we introduce eight novel nuclear intergenic markers for Cg selected by comparative genomic analysis, rank their phylogenetic informativeness, and present a multilocus phylogeny of ~80 Cg terminals representative of pathogen and endophyte diversity from the Neotropics, many of which represent lineages with pantropical/global distributions. This new set of markers, which possess robust signal for Cg phylogenetics, will improve our ability to accurately delimit species boundaries and explore the roles of lineage sorting and hybridization as sources of topological conflict in the Cg phylogeny. In addition, the multilocus species tree can serve as a flexible tool for species identification and diversity discovery, which can be achieved by use of any individual marker for metabarcode analyses.

1.2-135 Insights into *Claussenomyces* Kirschst.: Past, Present and Future

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Abstract: The Leotiomycetes are recognized as one of the most diverse classes of Ascomycota. Despite the high diversity in this class, certain orders and families have a high proportion of taxa that have not been studied using molecular methods. A good example of the paucity of molecular data is found in Tympanidaceae (Baral 2015), a poorly studied family containing nine genera, which have previously been placed in the Bulgariaceae, Dermateaceae, and Helotiaceae: *Claussenomyces*, *Collophora*, *Durandiella*, *Grovesiella*, *Holwaya*, *Micraspis*, *Myriodiscus*, *Pragmopora*, and *Tympanis*. Some of them are associated with diseases such as bark and xylem lesions, internal necrosis, vascular streaking, cankers and crown damage (Quijada 2015, Baral 2016). The genus *Claussenomyces* was erected by Kirschstein in 1923 for a single species (*C. jahnianus*). Korf and Abawi (1971) drastically widened the concept of the genus combining and adding three species: *Holwaya salicis* E. Müll. and S. Ahmad, *Corynella prasinula* (P. Karst.) Boud., and *Corynella atrovirens* (Pers.) Boud. Currently, *Claussenomyces* is one of the most diverse genera in Tympanidaceae with 16, mostly conifer-associated species, with

corticolous, lignicolous, fungicolous, and resinicolous lifestyles. A monographic treatment of the genus does not exist, and only a few species – *C. kirschsteinianus*, *C. prasinulus*, *C. olivaceus*, *C.* cf. *hydnicola*, *Claussenomyces* sp. or uncultured *Claussenomyces* – have publicly available sequences (NCBI). Baral and Marson (2005) provided for first time a key to species of the genus, but also a revision of several species and types (*C. jahnianus*, *C. pusillus*, *Tympanis xylophila*). In 2013, the first author started to work in the genus and reviewed more than 40 collections, including most of the types with the exception of *C. kirschsteinianus*, *C. simplex* and *C. pleomorphicus*. Another additional 15 collections have been treated in detail jointly by the second and third author previous to the present work. Current results of our morphological and phylogenetic analyses revealed that *Claussenomyces* is a polyphyletic genus that should be split into at least four genera represented as: *C. jahnianus*, *C. atrovirens* group, *C. prasinulus* group, and *C. kirschsteinianus* group. The three former are related to Phacidiales whereas the latter is related to Helotiales. Although *C. jahnianus* still lacks molecular data, we consider it different from all remaining species based on its deviating morphology, but tentatively place it in the Phacidiales. In addition, several species currently included in *Claussenomyces* belong to other genera (i.e. *Claussenomyces pini* in *Durandiella*, *C. tympanoides* = *C. pusillus* in *Holwaya*), or are synonyms of other species in the *Claussenomyces* (i.e. *C. canariensis* and *C. clavatus* being conspecific with *C. atrovirens*). In the future, the 16 current binominals will be reduced to 10 species, divided into four genera: *Claussenomyces*, restricted to its type species (*C. jahnianus*), and three newly erected genera defined by phylogenic, microscopic, and ontogenetic features as follows: (1) fungicolous, lignicolous and resinicolous species with true ascoconidia (*C. atrovirens* group, 6 species); (2) lignicolous species without ascoconidia (*C. prasinulus*, 1 species) and (3) resinicolous species without ascoconidia (*C. kirschsteinianus* group, 2 species).

1.2-136 Divergence on the side of speciation: a peek at ergot alkaloid gene evolution in *Claviceps purpurea* and relatives

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Abstract: Ergotism, a gruesome malady of humans and animals, is caused by consumption of grains contaminated with sclerotia of *Claviceps* (ergot), which produce a wide spectrum of potent mycotoxins namely ergot alkaloids (EA). These alkaloids have caused significant health, social and economic concerns at different times in history, but have also provided potential cures for various stubborn diseases. The contrary effects of these compounds have attracted a great interest in understanding their production by fungi. Molecular biology has brought to light the ergot alkaloid synthesis (*EAS*) genes and the pathways responsible for EA production. The presence or absence of certain *EAS* genes can be used to predict the production of EA by different species and the variance of *EAS* gene sequences could be used to develop DNA-based assays to predict the production of *EA* substrates. The objective of this study was to examine the sequence variances and to gain an understanding of the evolutionary patterns of *EAS* genes of *Claviceps purpurea* and close relatives to provide background knowledge for developing DNA-based detection tools for EA substrates. The whole genomes of 29 strains of *C. purpurea* and close relatives were sequenced by Illumina platform and assembled. *EAS* gene clusters were extracted from each genome using the reference sequences of *C. purpurea* strain 20.1 (GenBank: JN186799.1) and in-house pipelines. Full span DNA sequences of 11 *EAS* genes were recovered. DNA polymorphism of each individual gene was analyzed by using DnaSP. DNA matrices of each gene were

subjected to three approaches for phylogenetic analysis: maximum parsimony (MP), bayesian (BA) and maximum likelihood (ML). The results indicate that *EAS* genes evolved with different patterns at different rates, and that possible gene duplication events happened before and after speciation.

1.2-137 Rearrangement of *Pestalotiopsis* sensu lato by anastomosis grouping

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Abstract: Filamentous fungi have been classified by molecular phylogenetic analysis. In 2014, the genera of *Pestalotiopsis* sensu strict, *Neopestalotiopsis* and *Pseudopestalotiopsis* which differ minimally in terms of conidial morphs and sexual morphs, were separated from *Pestalotiopsis* sensu lato based on a phylogenetic analysis of the 28S nrRNA gene (LSU) region. Simultaneously, a data set combining the internal transcribed spacer region (ITS), β-tubulin, and the partial translation elongation factor 1-alpha (*TEF1*) were introduced as better molecular set for understanding each species within the genera *Pestalotiopsis* and *Pseudopestalotiopsis,* respectively. However, in the phylogenetic tree of LSU, the boot strap value on the node connected with *Neopestalotiopsis* and *Pseudopestalotiopsis* was low, and both genera appeared to fall into one clade. Regarding species classification, phylogeny based on the combined data set loses stability as the number of found new species increases. Within the concept of natural classification, we have been searching for useful indicators of fungal relationships, other than the molecular data sets conceived for the genera and/or species classification. When classifying *Rhizoctonia*, the lineage obtained by anastomosis grouping reflected that obtained by molecular phylogenetic analysis, using the ITS region (Sharon et al., 2008). We used hyphal anastomosis to classify *Pestalotiopsis* s. lat. within the concept of biological species, since this fungal group does not produce sexual morphs and anastomosis is the first event to distinguish self from non-self in the sexual reproduction process. In our preliminary test, we found that within the same genus the hyphae could fuse within the same species and between different ones. Thus, all species within one genus have an ability for hyphal anastomosis with each other, which expanded the understanding of lineage at the genus level. Hyphal anastomosis experiments were conducted using species from the same genus and from different ones, where evaluation of hyphal anastomosis, was based on the transformation of GFP or RFP into strains of each genus. Additionally, we investigated whether molecular the results of phylogenetic analysis reflected those of anastomosis grouping. For molecular phylogenetic analysis, the LSU, ITS, *TEF1* etc. were used, and the evolutionary distance was estimated by phylogenetic trees, using the methods of maximum likelihood and neighbor joining. Finally, we rearranged the classification of *Pestalotiopsis* s. lat.

1.2-138 *Pseudopestalotiopsis hydeae* sp. nov. a new species from *Diospyros* sp.

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Abstract: We illustrate a taxon of *Pseudopestalotiopsis* that is new to science from the culture collection of National Taiwan University (NTUCC). The genus *Pseudopestalotiopsis* consist of 14 formerly described species and are well recognised for their ability to produce unique medicinal compounds that may have pharmaceutical and agricultural applications. We established multi-disciplinary analyses using single- and multi-gene (ITS, β-tubulin and tef1) phylogenies together with morphology to assess the taxonomic status of the *Pseudopestalotiopsis* species isolated from *Diospyros* sp. in Taiwan. The new taxon compatible with the species of *Pseudopestalotiopsis* in having dark concolourous median cells with knobbed apical appendages. The results suggest that our *Pseudopestalotiopsis* isolate is morphologically and genetically distinct from its closely related species *Ps. camelliae-sinensis* and

should be recognized as *Ps. hydeae* sp. nov. Further, this study increases the base of information regarding the diversity of *Pseudopestalotiopsis* species that occur in Taiwan and to the best of our knowledge, this is the first record of *Pseudopestalotiopsis* species associated with *Diospyros* sp. in Taiwan.

1.2-139 *Penicillium gravinicasei*, a new species isolated from Apulian cave cheese

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Abstract: Several species of the genus *Penicillium* were isolated during a survey of the mycobiota of Apulian cave cheeses ripened in a cave in Gravina di Puglia, Italy. A novel species, *Penicillium gravinicasei*, is described in *Penicillium* section *Cinnamopurpurea*. Its taxonomic novelty was determined using a polyphasic approach, combining phenotypic, molecular (β-tubulin, calmodulin, ITS and DNA dependent RNA polymerase) DNA sequences and mycotoxin production data. The type strain of *Penicillium gravinicasei* is ITEM 17411 = NRRL 66733. Phylogenetic analyses of the *RPB2* data showed that isolates of the novel species form a clade most closely related to *Penicillium cinnamopurpureum* and *P. parvulum* with high bootstrap support. The fungus did not produce ochratoxin A, citrinin, patulin, sterigmatocystin or aflatoxin B1 on PDA, MEA and YES. The novel species had a high growth rate on agar media supplemented with 5% NaCl, and could be distinguished from other section *Cinnamopurpurea* species by phenotypic and molecular characteristics.

1.2-140 *Daldinia* sp. nov, from a community forest in Northern Thailand

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Abstract: Hypoxylaceae is one of the well-known and widely distributed families of pyrenomycetous fungi with unitunicate asci and pigmented ascospores. Our survey and collection of Xylariaceae and Hypoxylaceae in the northern part of Thailand has found many specimens and one of these may be a new species of the genus *Daldinia*. The genus *Daldinia* was described by Cesati and De Notaris in 1863 (Hypoxylaceae, Xylariales, Xylariomycetidae, Sordariomycetes, Pezizomycotina, Ascomycota). In this study *Daldinia* sp. nov was found on decaying wood from a community forest in Chiang Dao district, Chiang Mai province, northern area of Thailand. It is characterized by productions of superficial small to widely effused, pulvinate stromata, with mouse grey to pale mouse grey surface, inconspicuous perithecial mound. The perithecia are monostichous lanceolate with black drop, umbilicate ostiole, containing unitunicate cylindrical asci, unicellular ellipsoid dark brown to blackish brown ascospores, and with straight to slightly curved germ slit much less than the spore length on convex size, smooth perispore dehiscent in 10% KOH. The morphological characteristic of this fungus is very similar to that of *D. placentiformis* in the shape of stromata, but is different in KOH extraction in producing dark vinaceous pigment. However, phylogenetic analysis of the multiple loci including internal transcript spacer region (ITS), large subunit of the rDNA (LSU), second largest subunit of the RNA polymerase II (RPB2), and beta-tubulin (TUB2) shows this fungus is placed in the Hypoxylaceae and is closely related to *D. korfii*. It is clear that this fungus showed inconspicuous horizontal zones but *D. korfii* has conspicuous zones. The stromatal acetonitrile extraction contains BNT, and 2 new compounds (Cytochalasin). Critical examination of the phenotypic characters indicates that this fungus represents a new species in Thailand.

1.2-141 The segregation of Hypoxylaceae as a family: A good example of a profound revision within the ascomycota

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Abstract: Stromatic Xylariales exhibit an extraordinary diversity in almost every aspect: They are distributed over the entire globe and colonize various habitats – although primarily known as predominant endophytes, they show a tremendous morphological plasticity, and produce a wide variety of secondary metabolites. A multi-gene phylogeny was conducted using partial ribosomal (ITS, LSU) and protein coding (RPB2 and TUB2) gene sequences of over 100 fungal strains. The topology of the phylogenetic tree backed-up previous research on morphology and chemotaxonomy of stromatic Xylariales and justified the segregation of the Hypoxylaceae from the Xylariaceae as its own family. Furthermore, two new genera were established within the Hypoxylaceae, *Jackrogersella* (formerly *Annulohypoxylon*) and *Pyrenopolyporus* (formerly *Hypoxylon*). Additionally, the genera *Biscogniauxia*, *Camillea* and *Obolarina* were expelled from the Xylariaceae and included into the Graphostromataceae – due to their high affinities to *Graphostroma platystomum*. In conclusion, the current classification of the Xylariales is in accordance with the asexual morphs and secondary metabolite profiles, rather than with the traditional concept that was based on ascospore morphology as the predominant criterion.

1.2-142 Diversity and distribution of order Pezizales (Ascomycetes) in Pakistan

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Abstract: Pakistan is a square stretch of land between Arabian Sea and Karakorum Mountains covering an area of 87.98 million hectares. The forest covers an area of about 4.2 million hectares which is equivalent to 4.8 % of the total land area. About 40% of the forests of this area are present in one of the province Khyber Pakhtunkhwa due to the moisture of the Himalayan area. These moist areas provide conducive environment for the fungal growth like ascomycetous fungi. Ascomycota is a division or phylum of the kingdom Fungi. Its members are commonly known as the sac fungi or ascomycetes. This is the largest phylum of Fungi which contains 15 classes, 68 orders, 327 families, 6255 genera and 64163 species worldwide. From Pakistan, so far 66 families, 309 genera and 1214 species of ascomycota have been reported. The 14 families and 34 genera belong to Archaeascomycetes lichenized ascomycetes, 2 families and 3 genera from unitunicate lichenized ascomycetes and 50 families 272 genera from bitunicate lichenized ascomycetes. The order Pezizales is the most diverse group of ascomycetes represented in Pakistan. The defining feature of this fungal group is the operculate ascus which is microscopic sexual structure in which non-motile spores, called ascospores, are formed. Most of these Ascomycetes are found in Himalayan moist temperate forests which occupy Kashmir, Murree and Hazara Hills, lower Dir, upper reaches of Kurram Agency, moist parts of upper Swat, Gilgit and Baltistan because of moisture and dense vegetation cover. The maximum occurrence of these fungi is recorded from Khyber Pakhtunkhwa due to high rainfall and humidity. The data has been collected from different research articles as well as from Fungi of Pakistan.

1.2-143 Phylogenetic overview of Basidiomycota with divergence times of higher taxa and a phyloproteomics perspective

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Abstract: We provide a phylogenetic overview of Basidiomycota and related phyla in relation to ten years of DNA based phylogenetic studies since 2007. We selected 529 species to address phylogenetic relationships of higher-level taxa using a maximum-likelihood framework and sequence data from six genes (nrLSU, nrSSU, 5.8S, tef1-a, rpb1 and rpb2). These species represent 18 classes, 62 orders, 183 families, and 392 genera from the phyla Basidiomycota (including the newly recognized subphylum Wallemiomycotina) and Entorrhizomycota, and 13 species of Ascomycota as outgroup taxa. We also conducted a molecular dating analysis for 116 species representing 17 classes and 54 orders of Basidiomycota and Entorrhizomycota. Finally, we performed a phyloproteomics analysis from 109 Basidiomycota species and 6 outgroup taxa using aminoacid sequences retrieved from 396 orthologous genes. The time-tree indicates that the mean of stem ages of phyla are ca. 530 Ma; subphyla of Basidiomycota are 406–490 Ma; most classes are 358–393 Ma for those of Agaricomycotina and 245–356 Ma for those of Pucciniomycotina and Ustilaginomycotina; most orders of those subphyla split 120–290 Ma. Most higher-level taxa of Basidiomycota are generally supported. However, the younger divergence times of Leucosporidiales (Microbotryomycetes) indicate that its order status is not supported, thus we propose combining it under Microbotryales. On the other hand, the families Buckleyzymaceae and Sakaguchiaceae (Cystobasidiomycetes) are raised to Buckleyzymales and Sakaguchiales due to their older divergence times. In general, the six-gene phylogenies are in agreement with the phyloproteomics tree except for the placements of Wallemiomycotina, six orders. These conflicting placements in the six-gene phylogeny vs the phyloproteomics tree are discussed. This leads to future perspectives for assessing gene orthology and problems in decipheringtaxon ranks using divergence times.

1.2-144 Diversity and host specificity in the genus *Sarea* Fr. (Ascomycota)

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Abstract: First published by Fries in 1825, the genus *Sarea* today comprises two accepted species of resinicolous discomycetes. Both species have a very broad range, with *S. difformis* reported from North America, Europe, and northwestern Africa, and *S. resinae* reported from North America, Europe, northern and central Africa, and central and eastern Asia. Both species have also been reported in southern hemisphere locations, such as New Zealand, on non-native trees. Both species also have a broad range of hosts in the Pinaceae, with *S. difformis* reported on *Cedrus atlantica* and both *Sarea* species reported on species of *Pinus*, *Picea*, *Larix*, *Pseudotsuga*, *Abies* and *Tsuga*. In addition, *S. resinae* has been reported on species in the Cupressaceae, including members of the genera *Cupressus*, *Chamaecyparis*, *Juniperus* and *Taxodium*. With few exceptions, specimens of each *Sarea* species share

a very similar macro- and micromorphology, with specimens from multiple hosts fitting the specific concepts published by Hawksworth and Sherwood in 1981. Some molecular work has been done on the genus, but in almost all cases sequences are not associated with a vouchered herbarium specimen including the sexual morph. The objective of this study is to determine the degree of relatedness of geographically distant specimens collected in North America, Europe, and Macaronesia on different native and non-native host species. With permission, collections have been made of both species of *Sarea* from California, Georgia and the New England states in the USA, Northern and Southern Europe and Macaronesia. In addition to detailed measurements of the micromorphological features of specimens, ITS and LSU sequences have been generated using Sanger sequencing for analysis and comparison with published sequences. In contrast to the generally only slight morphological differences noted among specimens, ITS sequences from Europe, Asia, and North America not only differ by about 4% from each other, but also when submitted to phylogenetic analyses form multiple well-supported clades for each continent. These patterns are supported by similar analyses using the LSU sequences and ITS+LSU sequences. These clades also point to host specificity at the host family or genus level. In conclusion, the composition of the genus *Sarea* seems much more complicated than previously reported, with the possibility of multiple cryptic species in both accepted taxa; additional work must be done to further expand geographical and host range sampling of specimens to include in these analyses in order to approach a full picture of the diversity in *Sarea*.

1.2-145 Status of Rust fungi on Polygonaceae in Pakistan: Some new reports and an updated checklist of recorded species

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Abstract: In the present research work, we study Polygonaceae parasitized by three taxa of rust fungi in the Deosai plains, Pakistan. *Puccinia shikotsuensis* and *P. polygoni-alpini* are new records for Pakistan; *Uromyces polygoni-avicularis* is reported first time from Deosai Plains. Original illustrations, line drawings, descriptions and data on distribution and host range of these rust species worldwide along with a checklist of rust fungi of Polygonaceae in Pakistan are given. This work will not only be a baseline for further studies in the selected site but will also help in selection of means to protect this economically important plant family from described specific parasites.

1.2-146 New records of rust fungi (Pucciniales, Basidiomycota) from the Himalayan Forests of Pakistan

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Abstract: Rust fungi are well-known plant pathogens that cause diseases in both agricultural crops and wild vegetation. There are approximately 8,000 described species worldwide. The Himalayan Mountains of Pakistan are among the top 25 hotspots of biodiversity in the world. Here we present results from ongoing exploration of the rust fungi in the region. Many species have been documented for the first time from Pakistan, including *Coleosporium tussilaginis, Melampsora dimorphospora, Puccinia phaeopoda* and *Puccinia polygoni-alpini* and *Macruropyxis fulva* sp. nov. discovered. Full host data have been compiled, morphological features have been recorded from multiple collections, and DNA sequence data generated and analyzed. These and other new records will be presented. The

documentation of new taxon from this region will enhance our understanding of fungal biodiversity in Himalayan ecosystems.

1.2-147 Mysterious species of *Thekopsora* (Pucciniales) producing dimorphic uredinial sori and spores from Jilin, northeast of China

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Abstract: Jilin Province is located in the northeast of China and the east mountainous areas including Changbai mountain ranges are rich in vegetation. However, the inventory and ecology of rust fungi have not been sufficiently investigated in Jilin Province. Therefore, we surveyed rust fungi in several locations in Jilin Province from 2013 to 2017 and collected about 1000 rust specimens. Among these specimens, we found specimens on species of *Galium* (Rubiaceae), *Aster* and *Kalimeris* (Compositae) producing two different types of sori (uredinia?) on the same plant leaves. One type was similar to uredinial sori of *Thekopsora* or *Pucciniastrum*, another one was similar to those of *Coleosporium*. We suspected that two different species of rust fungi infected the same host plants because several rust species belonging to these genera have been recorded on these host plant genera. For clarification of the rust species on these specimens we carried out morphological observations with LM and SEM, and molecular analyses with 28S and ITS regions of rDNA. As the results, different types of uredinial sori on the same host plants were phylogenetically identical and were produced by one species of rust fungi, not caused by contamination of two species, although the morphological structures of these types were different each other. We also found two different types of spores (urediniospores?); echinulate and verrucose spores, inside of the same sori. The phylogenetic analyses showed that these specimens included into the group of *Thekopsora*, but they were separated into two distinct clades, which were different from other species of *Thekopsora* including species reported on the same host genera. Therefore, we suspect that they are new members of *Thekopsora* producing dimorphic uredinial sori and spores. The functions of these spores in their life cycles are still unknown. **1**

1.2-148 *Crossopsora byrsonimae*, a possible type species of a new genus of Cerrado rust fungi

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Abstract: Phakopsoraceae is a well known polyphyletic family of rust fungi. Most of the ca. 13 genera currently placed in Phakopsoraceae *s.l*. have not been subject to phylogenetic analyses. In this study we examined the placement of the genus *Crossopsora* within Pucciniales based on morphological traits and newly generated sequences of rDNA nucLSU. Both the type species, *C. ziziphi*, and 5 specimens of *C. byrsonimae* were phylogenetically compared with representatives of the order Pucciniales. The phylogenetic reconstructions using Bayesian Inference and Maximum Likelihood analyses showed that *C. ziziphi* does not belong to Phakopsoraceae *s.l.*, and remains in an uncertain phylogenetic position in Pucciniales. Besides that, the specimens of *C. byrsonimae* were not congeneric with the type species *C. ziziphi* and, furthermore, not allocated in Phakopsoraceae *s.l.* Thus, our data clearly indicated that *C. byrsonimae* can be considered as the type species of a new genus. They show in addition to the phylogenetic divergence, the two species show clear phenotypic dissimilarities that include diverse

spermogonial morphology (group VI type 7 in the former and group VI type 5 in last), aecial type (*Caeoma*-like in *C, ziziphi* and *Aecidium*-like in *C. byrsonimae*), and different geographical distributions.

1.2-149 Morphological review and phylogeny of *Cerradoa palmaea*, the rust fungus of Arecaceae from the Brazilian Cerrado

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Abstract: *Cerradoa palmaea* was described in 1976 by Ono and Hennen based on the holotype collected in Brasília, DF, Brazil. This was the first rust fungus reported on Arecaceae with well documented teliosporic and uredinosporic phases, although the host species indicated was incorrectly identified as *Attalea ceraensis (sic)* (correct spelling: *Attalea geraensis*). As shown by reexamination of the holotype, PUR F18664, it became clear that the real host is *Syagrus commosa*. In the original description, using light microscopy and excellent drawings, the new genus was compared with morphologically close genera such as: *Edythea, Prospodium, Hemileia*, and *Desmella;* all four also showing supra-stomatal telia and uredia. However, the differences in spore shape, sporogenesis, and hosts were then sufficient to recognize *Cerradoa* as a good genus. However, *Cerradoa* was considered a synonym of *Edythea* in the 4rd Edition of the Illustrated Genera of Rust Fungi-2003, without a solid justification. Here additional morphological data is inserted using SEM images and more updated light microscopy supported by Nomarski´s optics. Now, for the first time a molecular phylogeny is presented using rDNA nucLSU and Cox3 sequences of *Cerradoa palmaea* resulting in the allocation the genus within the limits of the Pucciniaceae, the largest family of the order Pucciniales. Based on the only available nucLSU sequences, *Prospodium*, and *Desmella* were completely segregated from *Cerradoa* when submitted to phylogenetic reconstrution using Bayesian Inference and Maximum Likelihood analyses; the same result was obtained for *Hemileia* using both nucLSU and Cox3. Lacking comparable *Edythea* sequences in GenBank it was impossible to arrive at a conclusion as far as its relationship with *Cerradoa*.

1.2-150 Identity and disease cycle of a smut fungus on wiregrass in a longleaf pine-grassland ecosystem in the southeastern USA

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Abstract: A smut fungus that hinders wiregrass restoration efforts in longleaf pine-grassland ecosystems is being investigated in North and South Carolina and Georgia. These ecosystems are unique to the southeastern USA; they are characterized by an open canopy of primarily longleaf pine (*Pinus palustris*) and a dense ground layer of herbaceous species. Wiregrasses, *Aristida stricta* and *A. beyrichiana*, are perennial bunchgrasses and the dominant grass found in longleaf pine forests. Once the predominant forest type in the southeast, longleaf pine forests have been reduced to a fraction of area they once covered due to land use changes and fire suppression. Seeds of *Aristida* species are required for regeneration efforts, but seed production has been affected adversely by a smut fungus. Smut fungi can be damaging pathogens of grasses and typically infect inflorescences of host plants, replacing the seeds with teliospores. Our objectives are to identify the smut species from *A. beyrichiana* and *A. stricta*, and to investigate the disease cycles. Based on microscopic examinations and comparisons of DNA sequences of the ITS, LSU, and GADPH regions, it is a previously undescribed species of *Langdonia*,

which is a monophyletic genus found on *Aristida* species. Investigations are underway to study infection and colonization of the host by this smut pathogen. Understanding this host-pathogen system will help management efforts to increase the availability of *Aristida* spp. seeds.

1.2-151 Emphasizing South African rust fungi of the genus *Ravenelia*

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Abstract: The rust fungi are the most diverse group of obligate plant parasitic fungi and commonly infect the prevailing number of land plant lineages throughout major climatic regimes. The genus *Ravenelia*, however, appears to be confined to members of the legume family with tropical and sub-tropical distributions. With more than 250 species, it is the third largest genus of rusts and can easily be recognized due to its notably complex and distinguished teliospores. Here, we will present recent research on the diversity and phylogeny of this genus but emphasizing South African representatives. Our studies encompassed light- and electron microscopy as well as molecular phylogenetic techniques based on ribosomal DNA. This enabled us to present the first molecular phylogenetic analyses of *Ravenelia* and to describe 9 new species. The phylogenetic reconstructions show evidence for rust lineages specifically associated to monophyletic host groups but furthermore indicate a polyphyletic origin of *Ravenelia*. These studies serve as a basis to analyse and discuss phylogeographic patterns as well as aspects of the ecology and evolution in major lineages of *Ravenelia* rusts in South Africa. This study will be complemented by population genomic analyses using ddRAD sequencing.

1.2-165 Recent horizontal transfer of eight mitochondrial introns between two unrelated *Ceratocystis* pathogens of ohia in Hawaii

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Abstract: Two undescribed *Ceratocystis* species are associated with a new and alarming disease of native ohia trees (*Meterosideros polymorpha*) in Hawaii, rapid ohia death (ROD). *Ceratocystis* sp. A causes a severe wilt disease and is the primary cause of mortality, and *Ceratocystis* sp. B causes a canker-stain disease. Phylogenetic analyses of mitochondrial and nuclear genes place sp. A in the Latin American Clade (LAC) of *Ceratocystis,* where many other aggressive tree pathogens reside, whereas sp. B is clearly placed in the less-aggressive Asian-Australian Clade (AAC). The two pathogens often co-occur in diseased sapwood of ohia but appear to be sexually incompatible. To explore the possibility of genetic interaction between these two unrelated pathogens, we sequenced the genomes of eight sp. A isolates, one sp. B isolate, and twelve *Ceratocystis* relatives in the LAC and AAC. Circularized mitochondrial genomes extracted from the de novo assemblies of each isolate were all relatively large for fungi (97kb–160kb), and larger in sp. B (155kb) than in sp. A (127–143kb), with variation mostly due to accumulated introns. We identified 87 introns in 16 mitochondrial genes across 25 *Ceratocystis* mitochondrial genomes and compared intron distribution among isolates. The majority of introns in sp. A are shared by other LAC isolates with reduced identity, suggesting ancestral LAC introns. However, eight introns in sp. A appear to be of AAC origin, because they are not found in other members of the LAC but are found with 100% identity in sp. B and most are also found with 99%–100% identity in AAC representative *C. uchidae*. These eight introns vary in size (1082–3079bp) and in the mobile machinery they encode in intronic open reading frames, including reverse transcriptase (RT), maturase, and homing

endonuclease genes (HEG). Four of the eight introns (in *rnl, cox1, nad2,* and *nad6*) are typical Group II introns that encode RT and maturase, one is a Group II intron in *cob* that encodes two HEGs but no RT or maturase, one is a Group IB intron in *nad4* that encodes two HEGs, one is an unclassified twintron that encodes two HEGs and inserts into a Group ID intron of *cox2,* and one is a Group II intron in *rnl* with no apparent mobile machinery. Diagnostic primers designed for each of the eight introns were tested against 63 sp. A isolates and 16 sp. B isolates from Hawaii. All eight introns are present in all sp. B isolates, but they are haphazardly-distributed among populations of sp. A. The intron-laden sp. B appears to predate the recent introduction of sp. A to Hawaii, and as populations of sp. A spread across the island, transient hyphal anastomosis may have allowed the transfer of mobile introns from sp. B mitochondria to those of sp. A during their close proximity in diseased ohia sapwood. If true, this suggests that introgression of additional genetic elements between the two fungal species could have occurred, which might include pathogenicity factors and other parasitic DNA and have implications for the origin and future of ROD.

1.2-166 Ceratocystidaceae exhibit high levels of recombination at the mating-type (MAT) locus

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Abstract: Sexual reproduction is an important part of many fungal life cycles and is controlled by genes at the mating-type (*MAT*) locus. Two idiomorphs are present at this locus and each contain distinct *MAT* genes. The presence of particular *MAT* genes are responsible for whether the fungus is self-sterile (heterothallic) or self-fertile (homothallic). The Ceratocystidaceae is a family of Ascomycetes that includes many economically important plant pathogens and saprobes. These species display different mating strategies, which makes the *MAT* loci in this family particularly interesting. The aim of this study was to determine and compare the gene content and structure of the regions flanking the *MAT* locus in 12 species of Ceratocystidaceae representing five genera. Our results showed that the genes typically found flanking the *MAT* locus, such as *SLA2* and *APN2*, in the Sordariomycetes were present in the genomes of the Ceratocystidaceae analysed in this study. There were, however, differences in gene order and presence of these genes around the *MAT* locus in the various species examined. This was especially evident when synteny in the regions immediately flanking the *MAT* locus was assessed. For example, species of *Huntiella* and *Endoconidiophora* were highly syntenic outside of the *MAT* locus, while the three species of *Ceratocystis* did not display much synteny. *Ceratocystis* spp. also had more transposable elements in the regions flanking this locus compared to other genera in the Ceratocystidaceae. We hypothesise that the transposable elements *Ceratocystis* may have facilitated recombination in the mating type region.

1.2-167 Characterization of the *MAT* locus in species of Ceratocystidaceae utilizing uni-directional mating-type switching

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Abstract: Sexual reproduction is an important part of the life-cycle of most Ascomycetes. This process is controlled by the genes present at a single genomic position, the mating-type or *MAT1* locus. Characterization of the genes at this locus has made it possible to elucidate many unique sexual strategies. These include uni-directional mating-type switching, a form of homothallism characterised

by the loss of DNA from the *MAT1* locus. Although uni-directional mating-type switching is known only in a small number of Ascomycetes, three genera in the family Ceratocystidaceae utilize this switching mechanism as reproductive strategy. This represents the largest assemblage of related species known to undergo uni-directional mating-type switching. In this study, the structure of the *MAT1* locus of nine species in the genera *Ceratocystis*, *Endoconidiophora*, and *Davidsoniella* were elucidated using full genome sequences. The results showed that the structure of the locus is conserved across all three genera. For each species, four genes made up the *MAT1* locus: two *MAT1-1* genes (*MAT1-1-1* and *MAT1-1-2*) and two *MAT1-2* genes (*MAT1-2-1* and *MAT1-2-7*). In addition, two copies of a direct repeat flanked the *MAT1-2* genes in each species. These repeat elements would anchor a recombination event where the *MAT1-2* genes would be deleted from the genome. The loss of *MAT1-2* is a hallmark of uni-directional mating-type switching, allowing self-fertile isolates to produce both self-fertile and self-sterile progeny. The availability of complete *MAT1* loci for these species provides a crucial link to understanding the evolution of sexual reproduction in the Ceratocystidaceae.

1.2-168 Dissecting pathogenicity and virulence in *Ceratocystis albifundus* using a GWAS approach

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Abstract: The African fungus *Ceratocystis albifundus* is an important pathogen of commercially propagated *Acacia mearnsii* and *Protea cynaroides*. It also infects a wide range of native tree species asymptomatically. Although populations of *C. albifundus* vary dramatically in terms of fitness traits such as pathogenicity and virulence, the molecular basis of these phenotypes remains unknown. We aimed to identify the possible pathways and processes encoded in genomic regions associated with pathogenicity and virulence. Accordingly, a collection of 36 genetically diverse *C. albifundus* isolates, originating from a wide range of host species and geographic regions, was used in a Genome Wide Association Study (GWAS). The isolates were phenotyped using pathogenicity assays on one-year old *A. mearnsii* seedlings. To determine their genotypes, individual isolates were subjected to low-coverage genome sequencing using Ion Torrent technology. These sequences were mapped to a high-quality hybrid reference genome for the fungus, which was determined using the PacBio RS II and Illumina HiSeq sequencing platforms. Following quality and frequency-based filtering, *ca*. 17 000 single nucleotide polymorphisms were identified within the collection of isolates. Correlation analysis using these polymorphisms allowed identification of a number of genomic regions significantly associated with pathogenicity and virulence. Sequence analysis revealed that the genes occurring in these regions encode products involved in various functions that likely influence pathogenicity and virulence. Subsequent functional characterization of these genes will confirm their involvement in these fitness traits of *C. albifundus*. Our findings demonstrated the power of GWAS for determining the molecular basis of important phenotypic characters in fungal pathogens.

1.2-173 Co-evolutionary patterns in *Phragmidium* (Pucciniales) are blurred by the reticulate evolutionary history of its Rosaceae hosts

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Abstract: Rust fungi of the genus *Phragmidium* infect genera and species within the Rosaceae, including the economically important genera *Rosa* and *Rubus*. As rust fungi are obligate plant pathogens most species exhibit pronounced host specificity and cospeciation with their hosts is traditionally thought to be common. In *Phragmidium*, however, several species are able to successfully infect a broad number of congeneric hosts. It has been known for a long time that a number of species within the host genera *Rosa* and *Rubus* are prone to hybridization that is reflected by difficulties in terms of morphological species recognition. Recent molecular based studies confirmed not only the tendency for hybridization in nature but also suggested that hybridization was a main driver in the evolution of *Roses* and *Rubus* species. This process is termed reticulate evolution instead of a strict bifurcating evolution. For the species pair of dogrose rusts, *Ph. mucronatum* and *Ph. tuberculatum*, it was postulated that their broad host ranges may be a result of the reticulate history of its hosts by enabling the rusts to shift from one host to another via a "hybrid bridge" of shared infection relevant traits. Due to the common phenomenon of reticulation in Roses and *Rubus*, we hypothesized to find widespread evidence for hybrid bridges in this host – parasite system. In the present study, we focused on rusts collected in China and analyzed a set of 56 specimens that represent 24 species of *Phragmidium*. Molecular phylogenetic analyses of 28S rDNA revealed highly polyphyletic host associations that indicate several independent host colonization events and thus strict cospeciation is unlikely. Our analyses further support the current view that the reticulate evolutionary history of the host species enables the rust pathogen to infect several different host species. This process may also facilitate jumps to even more distantly related hosts and which offers an explanation for the blurred co-evolutionary patterns observed within this genus but also for the broad host ranges frequently found in *Phragmidium*.

1.2-174 Taxonomy and mating strategy of *Thielaviopsis basicola* - a globally important pathogen of agricultural crops

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Abstract: *Thielaviopsis basicola* (Ascomycota: Ceratocystidaceae) is a well-known pathogen that causes the disease commonly known as black root rot on multiple important plant species. The species has been extensively studied since its first description more than 150 years ago, but important questions regarding its taxonomic placement and mating behaviour remained to be answered. The aims of this study were to resolve the taxonomic placement of the species using a multi-gene phylogenetic approach, and to determine the sexual reproductive strategy of the species. Phylogenetic analyses of sequence data from six gene regions from a relatively large number of isolates from 13 geographic regions showed that the species groups as a distinct generic lineage in the Ceratocystidaceae, which we subsequently described as *Berkeleyomyces gen. nov*. Additionally, our analyses also separated this collection of *T. basicola* isolates into two well-supported lineages, which were recognized as two distinct species. One of these lineages was selected to represent the originally described species and was described under the new combination *B. basicola*. The second lineage was described as a novel sister species named *B. rouxiae*. To investigate the sexual reproductive strategy of the two species, we sequenced and assembled the genomes of two isolates of *B. basicola*. Genome analyses following with mating gene specific PCR indicated that *B. basicola* and its sister species have a typical heterothallic mating system in which individual isolates of both species harbour either the *MAT1-1* or the *MAT1-2* idiomorphs. Mating type diagnostics using newly developed PCR-based mating type markers revealed

that both mating types of both species are present in nature, although a sexual state for either of the species remains to be discovered.

1.2-175 Intraspecies genetic variability and mating compatibility in basidiomycete fungus, *Fomitopsis pinicola*

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Abstract: The polypore fungus, *Fomitopsis pinicola,* is a widespread basidiomycete species in forests of central Russia. Polypore fungi are known to be the main decomposers of lignocellulose litter in nature. Besides, they are applied in traditional Chinese and Russian medicine due to a wide range of bioactive metabolites. That is why the investigation of genetic resources of polypore fungi is of importance. The red belt conk, *F. pinicola,* represents an excellent organism for genetic study because of the heterothallic type of sexual compatibility and a haploid-dikaryotic life cycle which can be reproduced under the laboratory conditions. Mating criteria are easily testable by clamps formed on dikaryotic mycelia. Somatic incompatibility mechanism, which regulates intraspecific recognition of self or non-self-partner under somatic contacts between heterokaryotic mycelia, is also easily testable on Petri plates. In this study, genotype diversity, sexual and somatic compatibility were analyzed in *F. pinicola* natural populations. Fruit bodies were collected in forests of Moscow suburbs through summer and autumn in 2013-2015. Mycelial cultures were isolated from fruit bodies, and a total 37 isolates of *F. pinicola* were deposited in the collection MSU_BIO_EBF together with 9 strains from Finland and France. For screening mating compatibility, dikaryotic isolates were subjected to analysis in mon-mon matings against the tester monokaryons. To proceed mon-mon matings, we obtained the fertile hymenium producing sterile basidiospore prints on Petri plates. Monokaryotic testers with different mating type alleles (AxBx, AyBy, AxBy, AyBx) were deposited in the collection. Intraspecies genetic polymorphism was analyzed by using variable ITS-region of rRNA gene cluster. Minisatellite DNA SSR analysis was performed on genomic DNA with four different primers (IS.2-, ISP2, ISP4 and ISP6). Dendrograms of genetic similarity between the strains were constructed based on ITS sequences and minisatellites markers using MEGA7 and TREECON program accordingly. *F. pinicola* was shown to have the tetrapolar mating compatibility system which is controlled by two unlinked loci with multiple alleles. All strains possessed different alleles except those grown within the same substrate (a log) at short distance, not more than 3 meters. Test on somatic compatibility between the natural isolates revealed somatic clones only within the same log. In the *F. pinicola* populations, moderate antagonistic (somatically incompatible) responses were predominated (frequency p=0.48) with the overall diversity index Hvc=0.782. The species showed very low ITS sequence divergence, as well as homogeneity of morphological characters among the strains from geographically distant origins, e.g. Russia, Finland, and France. Similarity between the strains were up to 99-100%, that suggests rather low polymorphism of the species. This trend was also reflected on the phylogram demonstrating little genetic differentiation within the species *F. pinicola*, proving that this is a highly outcrossing heterothallic fungus with panmictic populations. Assessment of genotypic variability based on minisatellite DNA markers showed low genetic polymorphism as well: genetic distances between the strains ranged from 0.05 to 0.18. However, the strains of different origins were separated on the dendrogram and were clustered according their geographical origin. The research was supported by RSF grant No. 14-50-00029 and RFBR grant 15-54-05065.

1.2-176 Cheating in *Neurospora crassa* is caused by inactivating mutations of fusion genes

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Abstract: In multicellular organisms, there is a potential risk that cheating mutants gain access to the germline. Free fusion among individuals would open the door to such cheating mutants. We previously found experimental evidence for this theoretical prediction by showing the selection of cheating variants from *de novo* mutation in the multicellular fungus *Neurospora crassa* (Bastiaans, Debets and Aanen, Nature Communications, 2016). In all eight parallel lines of an evolution experiment where free fusion was possible, cheating mutants were selected and reached a frequency of about 20%. At low frequency, those mutants had a competitive benefit over the wild-type, but a negative impact on total spore yield. We sequenced the genomes of the ancestor and all 20 evolved types including the cheater and non-cheater types of eight parallel evolution lines. Strikingly, in all eight parallel evolution lines, the cheaters have loss-of-function mutations in one of three different genes all characterized as fusion genes (six times *so1*, 1 time *ham5* and 1 time *ham8*). Subsequent functional analysis using isogenic Δ*so* lines shows that this loss-of-function mutation is responsible for the cheater phenotype. Although fusion between fusion mutants is strongly reduced, fusion between wild-type and fusion mutant still occurs at appreciable frequency. We demonstrate that fusion between wild-type and Δ*so* is necessary for cheating, since Δ*so* does not have a competitive benefit in competition with vegetatively incompatible wild-types. We also show that the cheater type gains its benefit within the resulting heterokaryon during spore formation, and that this benefit occurs only at low density of the cheating nuclei in the heterokaryon.

1.2-177 Population genomics of the globetrotter *Serpula lacrymans* reveal tight bottleneck and local adaptations

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Abstract: The bolete *Serpula lacrymans* is likely the most devastating fungal wood-decayer of construction materials in temperate regions worldwide. From its native range in North East Asia is has spread to most other continents, including Europe and Southern Oceania, as well as other parts of Asia (Japan). In this study, we performed population genomics analyses of 39 isolates from these three regions in order to assess differences across the invasive populations. More specific, we wanted to analyse the demographic history and level of intra-geographic structuring and local adaptations in the invasive populations. The three populations, Europe, Japan and New Zealand (the latter only represented by three isolates) were strongly differentiated. The European population had clearly gone through a tight bottleneck during its establishment while the Japanese population is far more diverse. Long genomic blocks without genetic diversity in Europe suggest a single founding event, with no secondary migration from the native regions. In both regions, there was a clear geographic sub-structuring, indicating dispersal limitations. Coalescence simulations was used to study the time since the populations diverged and the changes in effective population size. In scans for genetic adaptations in the two populations, we identified three outlier loci subject to purifying selection. These were linked to transport and decay, and support that these functions are particularly important in the human-made habitats of *S. lacrymans* worldwide. Of the three isolates from New Zealand, one had an admixed genomic make up with genetic elements from both the European and the Japanese populations. Since

the original 'out of Asia' dispersal events, our analyses indicate that the dry rot fungus has travelled between continents, also giving rise to admixed genotypes.

1.2-178 Tracing the naturalization of golden oysters in the United States

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Abstract: Wild fruitings of non-native golden oyster mushrooms (*Pleurotus citrinopileatus*) began appearing in North America approximately 5 years ago. Golden oysters are indigenous to eastern Russia, northern China, and Japan, but appear to have naturalized into American woodlands rapidly. The large fruitings observed by mushroom collectors suggest that indigenous saprobes are being outcompeted and displaced, presenting a potential threat to fungal biodiversity. I aim to infer how *P. citrinopileatus* populations have become naturalized, using population genomic data to infer migration pathways and relatedness between wild and cultivated strains. I will test two competing hypotheses for the spread of this organism: 1) multiple introductions have occurred (i.e., that each naturalized isolate is most closely related to a commercial strain; or 2) a single escape of a commercial strain occurred, followed by spread of wild populations. I will also attempt to determine whether naturalization via spores (i.e., involving recombination and more likely due to accidental escape of basidiospores) or clonal spread (i.e., more likely due to intentional introduction) is better supported by genetic evidence. Genome sequencing of 29 wild specimens from 6 American states, as well as 7 cultivated strains, is currently underway. These data will be used to construct a single-nucleotide polymorphism (SNP) dataset to infer the most likely pathway of naturalization and spread.

1.2-179 Mating and infection strategies in the Botryosphaeriaceae: emerging insights from genome and transcriptome studies

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Abstract: Species in the Botryosphaeriaceae are amongst the most common fungi found in tree microbiomes. While these fungi are common in asymptomatic tissues, they can also cause disease when the plants are under stress. Disease symptoms commonly include dieback and cankers and can have severe negative impacts in forestry and agriculture, as well as in urban and natural forests. DNA sequence-based phylogenetic studies have revealed substantial species diversity in the Botryosphaeriaceae with many species rare and seemingly endemic to certain regions. In contrast, these studies have also revealed that some species have a near global distribution and extensive host ranges. Despite their importance, many aspects of the biology of these fungi remain poorly understood, including their mating strategies and infection biology. In this study, we interrogated 29 of the genomes of Botryosphaeriaceae that have thus far been sequenced, including 12 that were added as part of the study. The analyses revealed that heterothallism is the likely ancestral mating strategy, but that there have been at least five transitions to homothallism in the Botryosphaeriaceae. Unlike other latent pathogens, species in the Botryosphaeriaceae appear to have expanded CAZyme repertoires, possibly contributing to their ability to access a range of carbohydrate resources in a broad range of hosts. Furthermore, transcriptome studies revealed the initiation of a necrotrophic infection, yet without expression of symptoms. While the latter studies are still at a preliminary stage, they are beginning to reveal the mechanisms that mediate a state of tolerance that make it possible for these latent pathogens to be so common in woody plants.

1.2-180 Inheritance of putative virulance genes in *Microbotryum* by artificial hybridisation

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Abstract: Biotrophic fungi are characterized by intimate interactions with highly specific hosts and often cause devastating plant diseases. Growth and proliferation during biotrophic phases necessitates an adapted set of genes to survive *in planta*. Hybridisation of distantly related species should lead to new arrangements of virulence factors like effector and transporter genes enabling fungal hybrids to new or formerly resistant host plant species. We use the model genus *Microbotryum* infecting highly specific species of Caryophyllaceae to provide insights into processes of host adaptation and genome arrangements during hybridisation processes. A total of 2600 *Silene latifolia* POIRET seedlings were inoculated with wildtype, cross and hybrid strains of *Microbotryum* species to analyze the hybridisation potential and the viability of *Microbotryum*. Our preliminary results indicate that homospecific crosses and interspecific hybrids cause infection at lower disease rates than infections with the coevolved parasite *M. lychnidis-dioicae*. Interspecific hybrids do mostly not affect morphological plant traits except the count of buds that was significantly altered by fungal infection. Our data indicate that hybrid infected plants have an altered blooming period compared to the adapted parasite *M. lychnidis-dioicae*. The inheritance of putative virulence factors and its potential impact on host specificity as well as the role in host-pathogen interaction will be discussed.

1.2-193 'Missing links' to the fungi domesticated by termites

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Abstract: The old-world fungus-growing termites (Macrotermitinae) cultivate fungi of the basidiomycete genus *Termitomyces*. The termites propagate their fungal symbionts via asexual spores inoculated on plant material upon gut passage, and most species transmit the fungal symbionts horizontally to new colonies via the occasional production of sexual fruiting bodies, the mushrooms. Phylogenetic reconstructions indicate that the symbiotic fungi all descend from a single domestication event some 30 million years ago, and that no 'escapes' of fungal symbionts to a free-living lifestyle have occurred afterwards. In 2007, three non-symbiotic species closely related to *Termitomyces* have been discovered. Those species have some interesting traits that appear to make them 'missing links' between the domesticated and free-living fungi. First, they combine asexual and sexual reproduction on a single morphological structure, which is exceptional for basidiomycete fungi. Second, they grow on fecal pellets of an as yet unknown animal, probably an insect, which is similar to the way termites cultivate their fungi, viz. on termite dung. We present the results of phylogenetic analyses of mitochondrial genomes to reconstruct the phylogenetic position of those newly described species relative to the domesticated fungi, to reconstruct character-state evolution and attempts to identify the animal species producing the dung on which those fungi are growing.

1.2-194 The echo of a distant time in labyrinths of coral caves: Genomic analysis of *Apterostigma*-farmed Pterulaceae and free-living counterparts

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Abstract: Approximately 60 million years ago, the common ancestor of the fungus-farming ants (subfamily Myrmicinae, tribe Attini, subtribe Attina; hereinafter referred to as "attine") swapped its hunting-gathering behaviour to fungal agriculture. Since then, five different types of fungiculture have emerged, the attine leaf-cutters being the most specialised and advanced. The latter are the most studied group of fungus-farming ants due to the economic loss it causes in crops. However, the poorly studied *Apterostigma* "*pilosum*" group, an early diverging group of attine ants, also presents some clues that lead us to believe their crops are domesticated. Approximately at the same time the leaf-cutters started to domesticate their cultivar, *Leucoagaricus gongylophorus*, these *Apterostigma* switched to cultivation of the very distant coral mushroom family Pterulaceae (Agaricales). As in *L. gongylophorus*, the two *Pterula* cultivars do not produce fertile basidiomes and were never reported growing outside the symbiosis. Therefore, after extensive fieldwork in Brazil, several samples of the pterulaceous cultivars and their free-living counterparts were isolated into axenic cultures. Genome sequencing of these cultures of *Apterostigma* cultivars and free-living *Pterula* has allowed us to explore the evolutionary consequences of domestication in these fungi.

1.2-195 Co-divergence of slimy salamanders (*Plethodon* spp.) and microbial communities of the skin and gut

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Abstract: Recent research recognizes a multicellular host and its symbiotic microorganisms as the 'holobiont'—a single unit of evolution. This study used high-throughput sequencing to characterize the complete skin and gut microbiome including both bacteria and fungi of nine closely related terrestrial slimy salamanders (family Plethodontidae). We characterized the salamanders as a holobiont to determine if microbial communities correlate with the evolutionary divergence of host nuclear and mitochondrial gene trees. We hypothesized that the skin and gut microbiome become more specialized to the host species over time, and that evolutionary divergences in the microbiome occur in tandem with host speciation events. Five host clades were compared within a phylogenetic context, and nine sub-clades were assessed for differences in microbial assemblage across sub-clade and geographic location. We found that the complete microbiome is similar in α- and β-diversity between host sub-clade but differs by geographic collection location. To infer microbiome function, we identified antifungal species in the skin- and gut-bacterial communities. In the fungal-gut microbiome, saprotrophs, pathotrophs, and symbiotrophs were the most abundant functional groups. Using a novel approach based on machine learning, we accurately determined host clade using the microbiome and identified indicator taxa specific to each host clade. We found that salamander individuals with similar mitochondrial gene sequences share similar gut-bacterial, gut-fungal, and skin-bacterial communities, indicating possible codivergence of host-microbial communities. Lastly, phylogenetic divergence of 20

microbial operational taxonomic units correlated with divergence of the salamander mitochondrial gene tree, indicating microbial symbionts may share evolutionary history with their host.

1.2-196 How common is mycovirus host-switching in nature?

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Abstract: Emergent viral diseases are often a result of host-switching, which has conventionally been thought a rare phenomenon. Host-switching in fungal RNA viruses(mycoviruses) is thought to be especially rare since mycovirus transmission is presumably restricted to intracellular strategies. Extracellular transmission is the primary transmission strategy of nearly all other viruses however, and we hypothesize that the absence of extracellular transmission in mycoviruses is unlikely. Further, recent evidence demonstrates that host-switching is more frequent in RNA viruses than previously recognized. Are viruses in fungi really an exception to this? If so, how do they defend themselves from extracellular invasion? Whether, and to what extent, host-switching of mycoviruses occurs in nature has not yet been tested. If mycoviral transmission is as restricted as currently thought, theory predicts coevolution of mycovirus and host. Thus, we take a phylogenetic approach to test our hypothesis that host-switching of mycoviruses occurs. Soils have been collected in Michigan from environments that are similar in composition but spatially distant. Common fungal species are being selectively isolated from each soil, screened for mycoviruses, and the phylogenetic relatedness of mycoviruses infecting those fungi will be compared. If mycoviruses from the same soils, regardless of fungal host, are more genetically similar than viruses in the same species from different soils, then host-switching is supported. The guiding questions of this research address our basic understanding of disease and have implications important to human, wildlife, agricultural, and ecosystem health.

1.2-197 Identification and initial characterization of RNA mycoviruses infecting the chestnut pathogen *Cryphonectria naterciae*

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Abstract: The ascomycete genus *Cryphonectria* includes known tree pathogens. The most studied species is *C. parasitica* – the causal agent of the destructive chestnut blight disease, which was introduced in North America and in Europe in the early 20th century. Some strains of *C. parasitica* however exhibit reduced levels of virulence due to a viral infection. The so-called *Cryphonectria hypovirus* (CHV) attenuates the pathogenicity of *C. parasitica* reducing its parasitic growth and sporulation capacity. CHVs are cytoplasmatic, unencapsidated dsRNA viruses with genome ranges between 9 and 13 kbp. CHVs have no extracellular phase and are transmitted mainly from infected to non-infected fungal strains via hyphal anastomosis. The discovery of CHV sparked off great interest in mycoviruses as natural biocontrol agents. The aim of this study is to screen additional species of *Cryphonectria* for the presence of CHV or other RNA viruses, (1) to characterize them molecularly and (2) to test their capacity in hypovirulence on chestnuts trees. In a pilot study, we detected the presence of conserved CHV-sequences in some *C. naterciae* isolates. These strains were used to extract dsRNA directly from lyophilized mycelium and to generate an Illumina cDNA library by reverse-transcription including specific sequencing adaptors. Currently, a *de novo* assembly of the reads is being aligned to viral sequences to characterize them molecularly. The potential for using these *Cryphonectria* viruses as agents for the biological control on chestnuts trees will be presented.

1.2-198 Phylogenetic inference of coevolution between fungi of the *Asterina* genus (Asterinaceae, Asterinales) and their host plants

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Abstract: Species of *Asterina* genus was proposed by Léveillé in 1845, being *Asterina melastomatis* Lév. collected in living leaves of a Melastomataceae in Brazil, the type species. The genus is characterized by having circular to irregular with a star-shapped fissure, superficial mycelium with lateral appressoria, bitunicate asci, and bicellular pigmented ascospores. Furthermore, the genus is biotrophic, growing superficially on living leaves of their hosts and are distributed on tropical and subtropical regions. The *Asterina* genus and the other members of the Asterinaceae family form specific interactions with the live cells of their hosts and are probably host-specific. Although this specificity is assumed by many mycologists, this hypothesis has never been tested in any experiment or checked by molecular data. Phylogenetic analysis involving *Asterina* spp. were constructed using nuc LSU rDNA (28S) sequences. While for the plants, ribulose-1,5-bisphosphate (RbcL) sequences were used. The phylogeny of the *Asterina* species and their respective hosts were used to generate the co-phylogenetic analysis using the Jane program. The phylogenetic analysis obtained through Bayesian Inference for the *Asterina* species generated a tree with high values of posterior probability. As well as for the *Asterina* species, the phylogenetic analysis obtained through Bayesian Inference for the host plants also generated a tree with high values of posterior probability, which corroborates with previous phylogenies. The Jane program found 1488 possible solutions that group the phylogeny of the parasites (*Asterina* spp.) to the phylogeny of the hosts. All possible solutions have the same cost (cost = 0). These 1488 solutions are very similar between them, and can be grouped into a single isomorphic solution (solutions with the same cost and same number of events, changing just the relative time - before or after a given node). All solutions demonstrate the occurrence of coevolution between the *Asterina* species and their host plants. The results obtained by molecular analysis in the present study corroborate with the history of the taxonomy of Asterinales that, since the first description of the type species in 1845 by Léveillé, strongly takes into account the host in the identification of the fungal species.

1.2-200 Phylogenetic delineation and geographic distribution of *Laccaria nobilis* and phenotypic relatives.

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Abstract: *Laccaria nobilis* Smith is one of the larger and more charismatic species of the genus, originally described from the Rocky Mountains of Colorado. However, its species distribution includes Alaska, the Pacific Northwest, Mexico, the Midwestern United States, and Eastern Canada. Cryptic species within *Laccaria* is a very real possibility so the question is whether this distribution accurately reflects the distribution of *L. nobilis* or whether there are other morphological similar *Laccaria* species within this distribution. The purpose of this paper is to establish the phylogenetic identity of *L. nobilis* from its home range and compare this to specimens of *L. nobilis* from other parts of the continent. Molecular sequence data from the nrITS region, as well as the single protein coding genes RPB2 and EF1-alpha are used for phylogenetic evaluation. Maximum likelihood analysis of molecular sequence data for specimens of *L. nobilis* will identify a monophyletic clade of specimens from Colorado and then evaluate which

specimens from a broader geographic range fit within this clade. This will ultimately help evaluate the hypothesis that large specimens of *L. nobilis* outside of Colorado, actually represent non *L. nobilis* species.

1.2-201 Non-concerted genomic evidence and the *Ophiocordyceps sinensis* ITS pseudogene hypothesis

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Abstract: The "ITS pseudogene" hypothesis for *Ophiocordyceps sinensis* (Os) was raised by Li et al.[2013] on the basis of detections of the ITS1-5.8S-ITS2 DNA sequences of Genotypes #1,#5 Os (Groups A,C) in cultured mycelial genomic DNA of the single ascospore of natural *Cordyceps sinensis* (Cs) and the 5.8S gene cDNA of Genotype #1 (*Hirsutella sinensis*, or Hs) in a cDNA library constructed from total RNA of the ascosporic mycelia, but non-detection of 5.8S gene cDNA of Genotype #5. However, the following scientific findings are inconsistent with the hypothesis. (1)Zhang et al.[2009], Mao et al.[2013] and Wei et al.[2016] reported the detections of Genotypes #3,#4,#5 Os in natural or artificial Cs, but non-detection of Genotype #1 Hs. (2)Three genome sequences (ANOV00000000, LKHE00000000, andLWBQ00000000) of Hs strains Co18, 1229, and ZJB12195 contain the ITS sequences of only Genotype #1, but not Genotypes #3-#6,#15-#17 (transition mutants), Genotypes #7-#11 (transversion mutants), and Genotype #12 (insertion/deletion mutants), and Genotypes #13-#14 (DNA segment reciprocal substitution hereditary variations). (3)PCR with using genotype-specific primers, amplicon-cloning sequencing and SNP mass spec genotyping revealed differential coexists of multiple genotypes of Os in the stroma, caterpillar body, ascocarps and ascospores of Cs during maturation. (4)*Eco*RI digestion assay, Southern blotting and RFLP revealed that the biomasses of the *Eco*RI-sensitive Os (GC-biased Genotypes #1-#3,#7-#14) and the *Eco*RI-resistant Os (AT-biased Genotypes #4-#6,#15-#17) are changing in a dynamic, asynchronous fashion during the Cs maturation. (5)Southern blotting revealed a single DNA moiety (*Eco*RI-sensitive Os) in the mycelia of Genotype #1 Hs, but a doublet (*Eco*RI-sensitive and -resistant Os) in Cs [Zhu et al.2010]. These results indicate that the ITS sequences of mutant Genotypes #2-#17 belong not to the genome of Genotype #1 Hs, but to the genomes of independent Os fungi. The 5.8S-F/R primers used for amplification of 5.8S transcripts [Li et al.2013] also called into questions. (i)The 5.8S-F/R are not Genotype #1 Hs-specific, and have similarities of 61.9%-100% and 31.8%-95.5% with the 17 genotype Os. (ii)Similarities between the 5.8S-F/R primers and the 5.8S gene of the 3 Hs genome sequences are 95.2% and 59.1%, respectively. (iii)Similarities between the 5.8S-F and *Tolypocladium sinense*(KX082970), *Paecilomyces hepiali*(EF555097), and *Fusarium* sp.(KJ735013) are equally high, 95.2%, while *P. hepiali* and *Fusarium* sp. were found in the multicellular heterokaryotic ascospores of Cs [Zhu et al.2016]. (vi)The steric conformational differences of 5.8S rRNA of Genotypes #1,#4,#5 [Li et al. 2013] may significantly affect the reverse-transcription of mutant Os 5.8S genes and the subsequent cloning and sequencing, while consistent failures have been reported [Gao et al.2011,2012]. Thus, the detected 5.8S cDNA by Li et al. [2013] needs to be verified to truly belong to Genotype #1 Hs, and non-detection of the 5.8S cDNA of the mutant Os genotypes need to be confirmed with using different sets of primers, or different molecular approaches. In conclusion, [Li et al.2013] provides incomplete and controversial evidence to support their "ITS pseudogene"

hypothesis, and encourages further studies. (Supported by Grant #2017-SF-118 from the Science-Technology Department of Qinghai Province)

1.2-202 Functional study on a non-ribosomal peptide synthetase in an entomopathogenic fungus *Cordyceps bassiana*

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Abstract: Nonribosomal peptides (NRPs) are a group of fungal and microbial secondary metabolites with diverse properties as immunosuppressant, pigments, antibiotics, medicines, etc. NRPs are synthesized by nonribosomal peptide synthetase (NRPS) enzymes, which consist of repeated domains of adenylation, thiolation and condensation, and some modifying elements. Fungal NRPSs usually cooperate with several regulatory genes in gene cluster to produce a certain peptide. In most cases, however, their functions and biosynthesizing pathways have been still remained unknown due to their cryptic expression and recalcitrant genetics. *Cordyceps bassiana* is an ascomycetous fungus that parasites various arthropod species. It produces the NPRs such as beauvericin and bassianolide that are insecticidal virulence factor. Our research group analyzed the genomic data of *C. bassina* strain C101 to discover novel genes biosynthesizing useful compounds. Using fungiSMASH, the fungal version of antiSMASH, 35 NRPS-encoding genes and their gene clusters were found. Among them our study focused on understanding the function of *Cbnrps6*. We generated the *Cbnrps6* gene deletion and over-expressed mutants, respectively, via *Agrobacterium tumefaciens*-mediated transformation (ATMT). and then the mutants were experimentally verified using qRT-PCR and Southern blotting. The 100-fold increase of *Cbnrps6* gene expression was shown in the overexpression mutant. In comparative metabolic profiling between the n-butanol extracts of normal strain and two mutants, a unique peak which is suspected that of the final product of *Cbnrps6* gene was detected at retention times of 25.1 min. Its chemical structure was determined using GC-MS and NMR analyses.

1.2-203 Exploring the transcriptome-methylome dynamics in *Termitomyces* mushroom

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Abstract: *Termitomyces* mushrooms are found to be superior to all other mushrooms globally because of their biting aroma and fullness of nutrients. In Taiwan, the fruiting body of *Termitomyces eurhizus* is a gourmet basidiomycetous mushroom to many people, produced through mutualistically symbiotic association with black-winged subterranean termite (*Odontotermes formosanus*). Until now, the fruiting body can only be acquired from the wild, but not with the cultivated conditions. While the mystery of fruiting in another basidiomycete *Coprinopsis cinerea* is well studied it is never clear with *T. eurhizus,* possibly because of the precise environmental conditions implying epigenetic co-regulation, confounded with a complicated symbiotic relationship with genotypically unique termite. To understand the fruiting dynamics and the life cycle of *T. eurhizus, we profiled* the transcriptome and genome-wide

DNA methylation across the developmental stages of *T. eurhizus*. As a preliminary result, we found the genome of *T. eurhizus* is approximately 76M bp with DNA methylation mostly in CpG context; comparing to black truffle it is smaller and more methylated. We also found the fruiting tissues show different global methylation profiles different from the other tissues; suggesting DNA methylation may actually play a role in the fruiting formation. Lastly, we found the expression of genes and transposons are anti-correlated with their methylation levels; indicating epigenetic regulation in *T. eurhizus*. This is also different from truffle that the gene expression is independent of the methylation at gene body. Our study provides valuable insight into the epigenome regulation of *Termitomyce with a great potential to uncover its* mystery of fruiting.

1.2-204 Gene regulation changes during heterokaryosis in Agaricomycetes inferred from mRNA-Seq and methylome patterning

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Abstract: The process of heterokaryosis in the mushroom-forming fungi of the Agaricomycetes involves plasmogamy (fusion of cytoplasm) between two mating-compatible haploid mycelium (n). However, unlike most organisms, the fusion of nuclei (karyogamy) does not occur until immediately before meiosis, and much of the mushroom life cycle occurs as a heterokaryon (n+n), in which two unique nuclear genomes coexist in each cell, providing opportunities for conflict or cooperation between nuclei. While there is evidence that the two nuclei in a heterokaryon are actively communicating with each other to modulate gene expression, the genetic basis of gene regulation between nuclei during this important and long-lasting part of the life-cycle remains largely unknown. For this study, we looked at changes in gene regulation during the transition from two individual haploid mycelia to a heterokaryon using a combination of mRNA-Seq and whole-genome bisulfite sequencing (WGBS). Transcriptomes and genome-wide methylation profiles of two haploid homokaryotic isolates of five taxa (*Coprinopsis cinerea, Heterobasidion irregulare, Wolfiporia cocos, Coprinellus disseminatus* and *Cyathus stercoreus)* along with a mated heterokaryon for each strain were generated and correlated. Here, we report on the variation in gene regulation and methylation patterns both between homokaryotic and heterokaryotic isolates of the same species, as well at the general pattern of mRNA changes and methylome profiles during heterokaryosis across the species examined.

1.2-205 Uncovering the role of pseudouridylation in a fungal pathogen *Cryptococcus neoformans*

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Abstract: *Cryptococcus neoformans* is an opportunistic fungal pathogen that causes cryptococcosis in both immunocompromised and immunocompetent individuals. Due to its clinical importance, revealing the factors that can affect its life cycle is critical. Among the various factors, pseudouridylation of RNA is the most abundant type of post-transcriptional modification. Pseudouridylases isomerize uridine into pseudouridine, therefore can affect the stability of RNA structure. In *Saccharomyces cerevisiae*, 8 proteins exist as stand-alone pseudouridylases, and each protein has specific pseudouridylation sites and roles. To discover the features of pseudouridylases, we aim to identify 6 putative pseudouridylases in *C. neoformans*. We sorted out the enzymes based on the database from FungiDB and NCBI. We used BLAST search with protein sequences to find out any corresponding orthologs in multiple organisms, such as *S. cerevisiae*, *Candida albicans*, *Aspergillus fumigatus* and *Neurospora crassa*. To characterize

the function of pseudouridylases, we constructed 10 mutant strains representing 5 putative pseudouridylases and we examined their phenotypic traits under various conditions so far. By using pseudouridylation RNA sequencing, we will identify pseudouridylated RNA transcripts and characterize their role in pathogenicity of *C. neoformans*.

1.2-206 Complex multicellularity in fungi: in search of a minimal genetic toolkit of fruiting body development

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Abstract: Mushroom formation is one of the most spectacular and complex processes in the fungal world, comparable to the development of higher plants and animals in terms of its complexity. Yet, its genetic bases, in particular, conserved developmental genetic events are hardly known. Here we set out to identify conserved developmentally regulated genes in mushroom-forming fungi. We identify developmentally regulated genes by comparing transcriptome data across three to eight developmental stages of six Agaricales, Polyporales and Hymenochaetales species. One of the species included in our analysis is Phanerochaete chrysosporium, which procudes simple, crust-like (resupinate) fruiting bodies, presumably resembling the ancestral fruiting body morphologies in the Agaricomycotina. We find that 10-40% of the genes are differentially regulated during fruiting body development in the examined species, comprising functions related to cell wall synthesis and modification, mRNA stability, cell growth and regulation of transcription. By studying the conservation of developmentally genes and their evolution through a comparative genomics analysis of 202 fungal genomes, we aim to understand the origin of fruiting body-related genes and to zoom in on the minimal gene set required to initiate and develop basidiomycete complex fruiting bodies.

1.2-207 Evolutionary transitions in fungal epigenomics

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Abstract: With an estimated age of up to 1 billion years old, the Fungi have evolved a variety of diverse and creative ways to regulate gene activity. In addition to histone modifications, fungi utilize DNA modifications such as 5-methylcytosine (5mC) and 6-methyladenine (6mA). However, with few exceptions their utilization is taxonomically restricted. Recently, we discovered that the 6mA is highly abundant in early fungi, but is largely absent from Dikarya. 6mA plays a positive role in gene expression and is preferentially deposited at gene promoters based on their function. Interestingly, in the Dikarya and early-diverging fungi lacking 6mA, 5mC is the predominant modification. This indicates that in fungi these modifications cannot readily coexist with one-another. Similar to other studies, we found that 5mC was primarily restricted to repetitive sequence and was involved in transposon suppression. These findings suggest that the two modifications are near complete opposites, differing both in location and role in gene expression, raising interesting questions as to why this pattern has emerged. Here we explore evolutionary transitions in fungi that may have led to this pattern, including selective pressure from transposable elements and gene gain/loss. We have identified a variety of methyltransferases that are significantly different in their presence across fungi which may be involved in 6mA regulation. Lastly, we explore factors involved in nucleosome placement and how DNA modification act to manipulate them.

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2.1-9 Metabolomic analysis of *Ipomoea* species containing or lacking *Periglandula* species symbionts

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Abstract: Several *Ipomoea* species and related plants in the morning glory family (Convolvulaceae) harbor vertically-transmitted, symbiotic fungi in the genus *Periglandula* that produce ergot alkaloids. Many more *Ipomoea* species have been surveyed, and approximately one-third of them contain ergot alkaloids indicating symbiosis with uncharacterized *Periglandula* species. The effects of the *Periglandula* species on plant host biology has not been well characterized. Moreover, the question of whether the ergot alkaloid-negative *Ipomoea* species lack *Periglandula* species symbionts or associate with cryptic *Periglandula* species that simply do not produce ergot alkaloids has not been investigated extensively. We investigated both of these issues through a metabolomics approach. We studied the metabolomes of *Ipomoea tricolor* seeds collected from *Periglandula* sp.-infected plants (P+) or plants from the same lineage that had been cured of the *Periglandula* sp. by treatment with fungicide (P-). Methanol extractions were performed on weight-normalized, pulverized seeds. Seed extracts were screened for ergot alkaloids by HPLC with fluorescence detection. For high resolution accurate mass analysis of metabolites, extracts were analyzed by UHPLC-ESI-QTOF mass spectrometry. Chromatographic peak alignment was performed using quality control criteria, and aligned peaks were filtered by fold-change and minimum abundance. Peak abundances were assessed by a T-test ($p < 0.05$). The ergot alkaloids lysergic acid α-hydroxyethylamide (LAH), ergine, ergonovine, and chanoclavine-I were present in high concentrations in P+ seeds but not detected in the P- seeds. Plant stress hormones jasmonic acid and abscisic acid did not differ significantly between treatments; salicylic acid was not detected. Similarly, amino acid concentrations did not differ significantly between treatments. Several unidentified analytes that were significantly more abundant in P+ seeds compared to P- seeds were compared to metabolomes of a collection of seed extracts from ergot alkaloid-positive and ergot alkaloid-negative seeds of *Ipomoea parasitica* and *Ipomoea pes-caprae*–plants previously shown to contain or lack *Periglandula*-species symbionts on a plant-by-plant basis. Only four metabolites tracked the presence of symbiont in this survey; the identity and symbiont of origin of these compounds remain to be determined. A collection of seeds from nine ergot alkaloid-deficient *Ipomoea* species were screened for the presence of these four non-ergot alkaloid biomarkers of *Periglandula* sp. presence. Extracts of all nine species were negative for the presence of the *Periglandula*-associated biomarkers. The data indicate that apart from the accumulation of abundant ergot alkaloids, *Periglandula* species have a minimal impact on the metabolome of seeds of their host plants. Furthermore, we found no evidence of cryptic, non-ergot alkaloid producing *Periglandula* species in the seeds of the nine additional *Ipomoea* species analyzed.

2.1-10 Evaluating the capabilities of commensal Sporidiobolales yeasts as a bioprotective agent against the establishment of harmful microbes on Romaine lettuce

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Abstract: The agricultural relevance of commensal yeasts inhabiting the phylloplane of leafy green vegetables is still poorly understood. Our current research describing the culturable and total fungal communities associated with Romaine lettuce reported that basidiomycetous yeasts in Sporidiobolales and Tremellales are the most common fungal groups found in this broadly consumed vegetable. We also discovered that a single undescribed species, the sister of *Sporobolomyces roseus* (*S.* cfr. *roseus*), was constantly present in the majority of lettuce plants examined. Here, we present preliminary results of greenhouse experiments designed to evaluate the capabilities of *S.* cfr. *roseus* as a bioprotective agent against the establishment of fungal pathogens on Romaine lettuce. All of the experiments were conducted in a BL-2 biosecurity green house, where lettuce seedlings were grown in 12 h photoperiod. First, we demonstrated that *S.* cfr. *roseus* is not an inhabitant of lettuce grown under greenhouse conditions. Second, we demonstrated successful colonization of the lettuce phylloplane by *S.* cfr. *roseus* and it did not induce any antagonistic immune response in the host. Third, in co-inoculation experiments, we found that the well-known plant pathogen *Botrytis cinerea* significantly did not induce a hypersensitive response (HR) on lettuce when seedlings were previously inoculated with *S.* cfr. *roseus*. In sum, our results suggested that *S.* cfr. *roseus* could be used as a protective agent against the establishment of fungal pathogens on lettuce. The inoculation of commensal red yeasts to leafy green vegetables may also increase the nutritional value of pro-vitamin A, a compound obtained from the digestion of the carotenoid pigments present in vacuoles in the Sporidiobolales yeasts.

2.1-11 Screening for pest resistant activity of *Epichloë* grass edophytes *in vitro*

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Abstract: As in many grass-endophyte symbionts, *Epichloë* grass endophyte produce active alkaloids, found in at least ten of the four major classes. One is pyrrolopyrazine, represented by peramine species that are toxic to certain nematodes and herbivores. To explore the optimal conditions for the production peramine by *Epichloë* grass endophyte *in vitro* and to screen their pest resistant activities in grass endophyte strains, peramine contents of eight strains of *Epichloë* grass endophyte (ATZ, SN, S3-3, PJ, NFS6, ZHYM, YZH, AKH) from *Achnatherum inebrians, Elymus tangutorum, Festuca sinensis* and *Hordeum brevisubulatum* were studied under different pH (8, 9, 10, 11, 12) and their pest resistant activities tested through feeding trail of detached leaves. It was found that: (1) The peramine content of eight *Epichloë* grass endophyte all had peak values at pH 8-9 *in vitro*, and the ZHYM's strain was 2-6 times that of other strains.(2) Compared with the control, eight strains of *Epichloë* grass endophyte had different effects on pest resistant. After 48h incubation, the ZHYM's pest resistance activity was significantly higher than the other seven groups, and the mortality rate reached 46.67%. (3) Compared with the stock solution, the pest resistance activity of the fermented liquid diluted 5, 10 and 15 times were significantly lower than that of the original liquid, and the mortality of *Rhopalosiphum padi* was also increased with the prolonging of the treatment time.

2.1-12 Use of soil spectroscopy to explain soil fungal community properties in different agricultural fields sampled across Europe

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Abstract: Rapid, reliable and cost-effective assessments of microbiological and chemical soil properties are highly essential for monitoring soil quality. Spectroscopy is known for its inexpensiveness, rapidity and accuracy and may be a useful tool for the assessment of soil quality. While chemical properties in agricultural systems can often be reliably assessed via spectroscopy, it is unknown to what extent changes in soil microbial communities can be detected with this technique. Soil fungi play a major role in several soil processes, e.g. organic matter decomposition, aggregate formation, disease suppression and plant growth promotion. The fungal community composition is mainly driven by soil C:N ratio, soil carbon content, pH, soil texture, land use and management. Therefore, it seems plausible to detect shifts in soil fungal communities by measuring soil characteristics measured by spectroscopy. In this study 1) we explain properties of the fungal community composition (obtained with Illumina sequencing) through soil chemistry data (obtained with conventional wet-chemistry analyses) and 2) try to predict elements of the fungal community composition using soil spectroscopy data (obtained with near-infrared reflectance – NIR, mid-infrared reflectance – MIR, and X-ray fluorescence – XRF). Preliminary results show that the fungal diversity and community structure varies in different soils. Moreover, fungal communities are susceptible to chemical factors, as pH, macro- and micronutrients (as P, Ca, Mg and Mn). Lastly, different fungal functional groups, as mycorrhizae, saprotrophs and plant pathogens, are affected by the land use and management. The next step is hence to explain through the combined used of diverse sensor data the variations of composition and functions of the fungal communities. We will discuss our findings in light of advantages and limitations for detecting changes in fungal communities measured through spectroscopy. Implementations of our results, with the delivery of geo-referenced and management advice, are of great interest for farmers, policy makers and nature conservationists.

2.1-14 Identifying *Isaria javanica* with high resolution DNA melting assays

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Abstract: *Isaria javanica* is a commonly isolated entomopathogenic fungi with activity against many insect species. Over the years, strains of this species have been commonly misidentified as *Isaria fumosorosea*. We have developed a simple high-resolution DNA melting assay to discriminate *Isaria javanica* from other *Isaria* species. The assay utilizes a previously identified 103 base pair PCR amplicon, which was reported to be selective for *Isaria fumosorosea*. Our study finds the amplicon selective for *Isaria javanica* and *Isaria poprawskii*, when assayed against all members of the genus. The practical application of this technique was confirmed using a bioassay on whitefly nymphs (*Bemisia tabaci* biotype B) inoculated with *I. javanica*.

2.1-15 Functional analysis of a FAD monooxygenase gene involved in synthesis of lysergic acid amides in the insect pathogen *Metarhizium anisopliae*

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Abstract: Ergot alkaloids are specialized metabolites produced by certain fungi, including species of *Metarhizium*. Many important ergot alkaloids are lysergic acid amides, such as ergonovine and lysergic

acid alpha-hydroxyethylamide (LAH). Lysergic acid amides are important in agriculture, where they can contaminate food and feed but also deter insect feeding and have insecticidal properties. They also are important in medicine, where they serve as the basis of drugs used to treat dementia and migraines. The pathway to create ergonovine is established, but the pathway to LAH is unknown. Based on genome sequence comparisons we hypothesized that a FAD monooxygenase gene (*easO*) was involved in the production of LAH. Using PCR methods, we prepared a gene knock out construct and introduced it into the LAH-producing fungus *Metarhizium anisopliae* by protoplast transformation. Four independent knock outs were identified by PCR strategies that showed the knock-out construct had integrated into the *easO* locus. High performance liquid chromatography (HPLC) and HPLC-mass spectrometry (LC-MS) analyses demonstrated that the knock-out fungal strains lacked LAH and retained ergonovine. The data supported our hypothesis that *easO* was involved in the production of LAH. The ergonovine-accumulating *easO* knockout strain was injected into larvae of *Galleria mellonella* to investigate its virulence relative to wild-type (LAH-accumulating) *M. anisopliae*. Ergonovine-accumulating knockout strains killed larvae faster than the LAH-accumulating wild type; however, the *easO* knockout fungus rarely emerged from dead larvae, whereas the wild-type fungus sporulated profusely on larval cadavers. These data indicate that ergot alkaloids play a role in the interaction of *M. anisopliae* with insects. An understanding of the production of lysergic acid amides is beneficial because of their positive (anti-insect) and negative (anti-mammalian) effect in agriculture and because of the medical uses of lysergic acid derivatives.

2.1-16 Species-specific distribution pattern of secondary metabolites in spider parasitic ascomycetes *Gibellula*, *Hevansia* and related genera

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Abstract: Even though the pathogenic fungi associated with invertebrates are well-known for centuries and have been intensively studied for a long time, certain groups of fungi, and in particular the spider parasitic fungi have not yet received much attention. *Gibellula* and *Hevansia* are classified in the Cordycipitaceae which are known to be obligate parasites of spiders. Most of them have been investigated based solely on morphological descriptions. Over a few decades there has been an increasing interest in the study of phylogenetic relationship between them and their allies, it is nevertheless limited to specific research groups. Besides the limited molecular data, their production of secondary metabolites also remains largely unexplored. In the course of a study on invertebrate-pathogenic fungi in Thailand where is accepted to be a rich source for a diverse range of microorganisms particularly invertebrate-pathogenic fungi, more than a hundred of fungal specimens were collected from various areas and encountered in the study. To extend our understanding of the taxonomic relationship among *Gibellula*, *Hevansia* and allied genera as well as to investigate and explore their secondary metabolites, HPLC- and PCR-based techniques were employed to generate the fungal chemoprofiles and molecular data, respectively. The species identification was relied on morphological features and multigene phylogenetic analysis. Five nuclear gene regions including the internal transcribed spacer (ITS), nuclear ribosomal large subunit DNA (nrLSU), elongation factor 1α (EF-1α), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2), were sequenced and the phylogenetic tree was subsequently reconstructed. The fungal secondary metabolite profiles were

examined using analytical HPLC coupled with diode array and mass spectrometric detection (HPLC-DAD/MS) and compared within species according to the multigene tree. Some species remarkably revealed a unique pattern of secondary metabolite production in particular *H. novoguineensis* in which all nine unprecedented secondary metabolites are specifically produced by the species. This is the first report on secondary metabolite profiling of *Gibellula* and *Hevansia* showing the species-specific distribution pattern of their secondary metabolites as well as unprecedented components that might eventually be used as powerful chemotaxonomic markers in species identification.

2.1-33 Parenchymatous cell divisions in the fungal cortex of Collemataceaea and other lichens

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Abstract: According to a widely held view, fungi do not produce parenchymatous tissues. Following up on a recent TEM study that challenged this paradigm in three lichens representing different orders within two classes of ascomycetes, we apply SEM here to determine the orientation of cell divisions in the single-layered cortex of six species of Collemataceae. This family of gelatinous cyanolichens includes both corticated and uncorticated species showing diverse surface morphologies. Examination of thallus surfaces in four species of Leptogium (L. austromericanum, L. burnetiae, L. chloromelum, L. marginellum) and two species of Scytinium (S. gelatinosum, S. lichenoides) revealed the positions of recently formed cortical septa in the six morphologically distinct taxa. Septa adjoined to preceding septa, usually perpendicularly, indicating parenchymatous division. Such divisions were involved the growth and development of characteristic surface structures, such as thallus wrinkles, folds, isidia, and lobules. Tomentum, by contrast, arose as filamentous outgrowths of the cortical cells. For comparison, we also examined the surfaces of the uncorticated Collema furfuraceum, which closely resembles species of Leptogium morphologically. We conclude that the monostromatic cellular cortex in Collemataceae is actively involved in growth and morphogenesis by means of parenchymatous cell divisions, in a remarkable parallel to plant meristems. However, cortical cell divisions are not likely to be the underlying cause of morphogenesis, as very similar morphologies develop in the closely related genus Collema, which lacks a cortex altogether.

2.1-35 The early asexual development regulator *fluG* encodes a putative bifunctional enzyme involved in nitrogen metabolism

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Abstract: The primal transition from vegetative hyphae to conidiophore development in *Aspergillus nidulans* involves a set of proteins which are expressed in vegetative hyphae (Upstream Developmental Activators). One of the earliest acting UDA factors is *fluG* (An4819), along with the G protein regulator *flbA* (Lee and Adams, 1996). Deletion of *fluG* results in colonies which fail to produce conidiophores and accumulate aerial vegetative hyphae, resulting in a colony morphology commonly known as *fluffy* (Lee et al, 1994). In addition, *ΔfluG* mutants show defects in autolysis (Pocsi et al, 2009) and secretion (Wang et al, 2015). Despite these findings, FluG has not been attributed a specific role in conidiophore development. In this investigation, we have conducted a sequence and structural analysis of the FluG protein, establishing an N-terminal amidohydrolase-like region with sequence and structural similarity to the amidohydrolase 2QPX of *Lactobacillus casei,* and a C-terminal region with sequence and structural similarity to a γ-glutamyl ligase (PauA7) from *Pseudomonas aeruginosa* (Ladner et al., 2012).

Alanine substitutions of the predicted key catalytic residues in each region yielded distinct loss of function phenotypes, and the separate expression of both regions yielded a partially functional phenotype, indicating that coupling of both predicted enzyme activities is required for full biological function. Our findings situate FluG as a putative bifunctional enzyme that may exert changes in the levels of nitrogen-containing signal intermediates that are required for development.

2.1-36 An ultrastructural study of sporangial development in *Pythiogeton* spp.

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Abstract: *Pythiogeton* is a cryptic genus in Pythiaceae. A total of 15 species have been reported in this genus and most of the species are saprophytic. *Pythiogeton* spp. are characterized by their unique way to form the sporangium and zoospores. The shape of sporangia are globose, ovoid, ellipsoidal, bursiform or multilobate, and the long axis of sporangia are mostly at the right angles to the supporting hypha. At the late stage of sporangial development, the incomplete differentiated protoplasm mass was emitted into the water through a discharge tube. Afterwhile, the protoplasm mass differentiated into zoospores. The ultrastructures of the sporangial development and zoospores releasing process of some *Pythiogeton* species will be reported in this study.

2.1-38 D-galactose, L-arabinose and D-xylose cross-induce their respective catabolic pathways in *Aspergillus nidulans*

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Abstract: Ascomycetes are mostly saprobic and/or plant pathogenic fungi, feeding on living or dead plant material. Hemicellulose is a major component of plant biomass. The pentoses D-xylose and L-arabinose, and the hexose D-galactose are frequent building monomers of all hemicelluloses as well as pectin. The Pentose Catabolic Pathway (PCP) is comprised of subsequent reduction and oxidation steps, ultimately followed by a phosphorylation to xylulose-5-phosphate that enters the pentose phosphate pathway (PPP). In *Aspergillus nidulans*, the oxido-reductive pathway (ORP) for D-galactose catabolism employs a similar biochemical strategy, with the resulting fructose-6-phosphate entering glycolysis. In contrast, the Leloir pathway of D-galactose breakdown is specifically epimerizing D-galactose into D-glucose. While there is a palpable overlap between PCP and ORP in *A. nidulans*, genes, enzymes and intermediates of the Leloir pathway are (mostly) exclusive for the downstream catabolism of D-galactose. However, *A. nidulans* conidiospores do not germinate on D-galactose alone, but need to be stimulated first with low concentration of other carbon sources such as D-xylose. Due to these overlapping functions pentoses and D-galactose play in the nutrition of fungi, we hypothesized that each catabolic gene of the ORP, PCP and Leloir pathway will be responding by increased levels of expression to the presence of either sugar, irrespective of whether the enzymes encoded are actually involved in the catabolism of the given sugar. As a proof of evidence, we will demonstrate that the first two genes of the Leloir pathway, i.e., D-galactose kinase (*galE*) and galactose-1-P uridyltransferase (*galD*), as well as a D-galactose mutarotase (*galmB*) recently shown to be an integral part of D-galactose breakdown are all induced by D-xylose and L-arabinose, but the respective deletion mutants display no (growth) phenotype on these pentoses.

2.1-39 Carbon utilization in *Phyllosticta* species

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Abstract: Species of *Phyllosticta* occur on numerous plant hosts, including mangoes, citrus, grapes and bananas, where they have been recorded as both endophytes and plant pathogens. On citrus, *Phyllosticta* spp. can cause disease symptoms ranging from small freckles on leaves (*P. citrichinaensis*), to necrotic lesions on fruits (*P. citricarpa*). The latter has been reported specifically in citrus species in Asia, Australia, Africa and North America, and is considered a serious pathogen of this host. On the contrary, *P. capitalensis* is found as a widespread endophyte in more than 70 different hosts, and is rarely recorded as plant pathogen. The ability to degrade cell walls through Plant Cell Wall Degrading Enzymes (PWCDEs) is considered to play a role in the host preference of fungal species. The difference in host and tissue specificity of *Phyllosticta* spp. makes them interesting subjects to study in relation to carbon utilization. The objective of this study is therefore to analyse the repertoire of PWCDEs present in selected *Phyllosticta* spp., and their ability to grow on different carbon sources. This gives insight as to whether the basis of the interaction and lifestyle mode between these fungi and their plant hosts lies in the ability of *Phyllosticta* spp. to take advantage of its host's carbon sources.

2.1-40 Impact of glyphosate and glufosinate on mycelial growth of truffles, morels, and molds

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Abstract: Herbicides are frequently used to control weeds in both agricultural settings and around residential dwellings. Little is known about the interaction that herbicides have with truffles (*Tuber* species), morels (*Morchella* species), and *Mortierella* species. Understanding how weed management tools such as glyphosate or glufosinate interact with these fungi may better inform the use of these herbicides especially in morel or truffle production locations. For this experiment, *Morchella americana, Morchella importuna, Tuber borchii, Tuber gibbosum,* and *Mortierella elongata* were each grown in stepwise serial dilutions of herbicide containing agar media ranging in concentrations above and below the working ratio recommended by the manufactures. Two brands of herbicide (Roundup® – glyphosate and Liberty® – glufosinate) were diluted and mixed into agar media to determine if there is an interaction between different concentrations of glyphosate or glufosinate-based herbicides and mycelial growth of the aforementioned fungi. The mycelial growth-front the fungi was routinely measured and recorded in four quadrants of each replicate. None of the tested fungi grew at the upper range of herbicide working concentrations used in this experiment but grew unimpeded at the lower range. Differences in susceptibility between the tested genera were negligible as the concentration range where the treatment effect was seen in each isolate varied; however, in each instance susceptibility was near the working concentration recommended for application. Though the working herbicide concentration is near the point of fungal growth inhibition, the herbicide becomes further diluted within the environment during broadcast application. As such, the use of glyphosate or glufosinate at recommended rates would not be expected to interfere with the mycelial growth of the fungi used in this experiment; however, use of these herbicides in higher than recommended concentrations may inhibit mycelial growth.

2.1-41 Using codon usage bias to predict ecologically adaptive metabolic pathways in the budding yeast subphylum

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Abstract: Since diverging about a half-a-billion years ago, the 1,000+ yeast species of the subphylum Saccharomycotina have diversified into every biome on Earth. The diversity of yeast ecological adaptation is underpinned by their ability to utilize a wide range of substrates and grow in a variety of environments. Traditionally, metabolic pathways that are key for yeast ecological adaptation have been identified through functional experiments in the laboratory, statistical analysis of associations between traits and environments, and by examining signatures of selection in the genes encoding metabolic enzymes. One genomic signature that has proven especially powerful at predicting gene activity, but has yet to be widely employed in evolutionary ecological research, is codon usage bias or the differential use of synonymous codons within and between genomes. Codon usage bias at the level of individual genes, is a consequence of both GC mutation bias and selection for translational efficiency. Therefore, gene level bias is strongly associated with gene expression. We expect that highly expressed genes will show codon usage bias in favor of optimally translated codons and that networks of co-expressed genes will show bias in favor of the same set of codons. In this work, we use species-specific gene-based estimates of codon usage bias (as a proxy for genes' expression levels) to predict metabolic pathways that are highly active across the genomes of 332 budding yeast species. These active metabolic pathways are then compared to the known habitat features of these 332 budding yeast species to identify significant associations between highly active metabolic pathways and habitat features. Identification of significant associations between metabolic activity predicted by codon usage bias analysis and habitat features will provide insight into which metabolic capabilities may be responsible for adaptation to specific environments. More broadly, this work also sheds light on the ability of codon usage bias to be more broadly used to predict ecologically relevant genes and pathways in other microbes--especially those that are currently unculturable.

2.1-42 Genomic and proteomic dissection of Xylariaceae species and their role in deadwood decomposition

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Abstract: Soft-rot (type II) fungi belonging to the ascomycetous *family Xylariaceae* are known to substantially degrade hardwood by means of a poorly understood lignocellulolytic enzyme system, which comprises various hydrolases (e.g. glycosidases, esterases) and some oxidoreductases (e.g. laccases). Here, we report on the genomes of four *Xylaria* species, *X. polymorpha*, *X. longipes*, *X. hypoxylon* and *X. grammica*, with the aim to better understand this enzyme system. The complete genomes were sequenced using an Ion Torrent® PGM NGS platform. Assembling and gene prediction was performed based on MIRA, Geneious R11 and AUGUSTUS software; gene annotation was done by Blast2GO and dbCAN or via reference-based annotation. The genome size of the four ascomycetes ranged between 42.8 (*X. hypoxylon*) and 47.0 Mb (*X. grammica*). The highest number of predicted genes was found for *X. longipes* (12,638), the smallest number for *X. polymorpha* (10,285). Among them, is a

large number of putative carbohydrate-active enzymes (CAZymes) and several carbohydrate-binding modules (CBM) as evidenced in all four genomes (e.g. 691 and 58 genes, respectively, in the *X. polymorpha* genome). The CAZymes are represented by glycoside hydrolases (GH; e.g. 296 for *X. polymorpha*), carbohydrate esterases (CE; e.g. 117 for *X. grammica*), polysaccharide lyases (PL; e.g. 18 for *X. grammica and X. longipes*) and glycosyl transferases (GT; e.g. 95 for *X. hypoxylon*). CBM1 members, twelve of them were found in all four species, may foster the attack on crystalline cellulose. On the other hand, the four *Xylaria* genomes revealed the absence of ligninolytic class-II peroxidases (i.e. lignin, manganese or versatile peroxidases), which are characteristic for white-rot basidiomycetes. Instead, sequences of putative unspecific peroxygenases (UPOs; EC1.11.2.1) and dye-decolorizing peroxidases (DyPs; EC1.11.1.19) as well as of lytic polysaccharide monooxygenases (LPMOs; AA9 formerly GH61) were identified. Furthermore, numerous genes encoding for other accessory enzymes (AA) of lignocellulose degradation such as cellobiose dehydrogenase (CDH; AA3_1 with one to three genes) and H_2O_2-supplying enzymes (e.g. galactose, glyoxal and alcohol oxidases) were present in the four *Xylaria* genomes. Our genomic findings have been verified by a proteomic approach with *X. polymorpha* grown on beech wood for several weeks. In total, 125 secreted proteins (18.1%) were identified as CAZymes. Most of them belong to GH families (73; 58%) like GH4 and GH78. The latter contains unique enzymes, which combine catalytic features of α-L-rhamnosidase and feruloyl esterase. Secreted members of the AA group have either cellulolytic activities, generate H_2O_2 (e.g. GMC oxidoreductases, AA3_2 or glucooligosaccharide oxidases, AA7) or oxidize phenolics (laccases, AA1_1). No extracellular peroxidase was found in the fungal wood cultures. Our data give new insights into the enzyme machinery of soft-rot fungi that seemingly ranges – from the biocatalytic point of view – between those of white-rot and brown-rot fungi.

2.1-43 Exploiting biology of an *Annulohypoxylon* sp. strain for terpene-based advanced biofuel

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Abstract: Unknown endophytic fungi from forests are one of substantial resources beneficial substances for our future. Fungal terpenes are bioactive functions and also recently found as potential drop-in biofuels compatible to internal combustion energy. An endophytic fungus identified as *Annulohypoxylon* sp.FPYF3050 from a tree, *Neolitsea pulchella* (Meissn.) Merr., in southern China was reported to produce 1,8-cineole at high quality and major quantity in its VOCs using methods of headspace solid phase microextraction combined with gas chromatography-mass spectrometry. It grew well on seven media with different carbon sources and five raw agro-forest residues. The content of 1,8-cineole in the mixed VOCs maintained up to 94.95% relative area only with four compounds growing in PDA, and 91.25% relative area only with five compounds growing in raw poplar sawdust. The fungus preferred starch and lignocelluloses to cellulose and sucrose to facilitate 1,8-cineole production. To our knowledge, the strain produced exclusively VOCs with 1.8-cineole as the major product even growing lignocelluloses biomass. 1,8-cineole is as a monoterpene is an ideal fuel additive for both diesel and gasoline engines. Comparing to environmental concern on extraction the oil from *Eucalyptus* plants, *Annulohypoxylon* sp. FPYF3050 would be a feasible organism to maximize 1,8-cineole production at industrial scale in a more economically and environmentally friendly way, and also to be an ideal organism-producing 1,8-cineole or monoterpene metabolic pathways for fungi. The genome of the strain was sequenced using PacBio RS II instrument and de novo genome assembly size was 42.3 Mbp. In this genome, three metabolic pathways related to terpene biosynthesis were identified according to

KEGG pathway annotation. Among the pathways is one to synthesize the other jet biofuel sesquiterpene, bisabolene. In addition, twelve members were determined in terpene synthase gene family in the genome through InterPro database.

2.1-44 Comparison of CAZyme profiles of *Colletotrichum* genomes to predict host range of *Colletotrichum tanaceti*

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Abstract: Carbohydrate active enzymes (CAZymes) are responsible for synthesis, modification and degradation of glycosidic bonds and some are associated with pathogenicity and host range of fungal pathogens. The aim of this study was to infer the host range of *Colletotrichum tanaceti*, a major foliar pathogen of *Tanacetum cineraiifolium*, using a CAZyme pathogenicity profile consisting of plant cell wall (lignocellulose, pectin, and hemicellulose) degrading enzymes, cutinases and host immunity disrupting chitin-binding domain (CBM50). The recently sequenced genome of BRIP57314 isolate was used to predict the proteome of *C. tanaceti*. Publically available proteomes of 13 Colletotrichum species were used for comparison. Each proteome was searched for proteins encoding domains homologous to CAZyme-specific Hidden Markov Model profiles in the dbcan database V.6, using cut-off values for fungi (e value-1e-17, coverage-0.45) to identify CAZyme-like proteins across the representative taxa. The gene copy numbers of selected CAZyme families were recorded to generate CAZyme profiles for each taxon, which were then subjected to principal component analysis (PCA) and hierarchical clustering. The taxa were also grouped based on previous knowledge of their host-type (dicot/dicot and monocot/monocot) and host-range (broad/Intermediate/narrow). The "narrow" host range was defined for pathogens that are host-specific or infecting few host species within a plant family. Pathogens that infect many host species within a family or few hosts across families were classified under "intermediate". The "broad" category includes pathogens infecting many host species across plant families. No obvious separation was observed among the host type classes "dicot" and "dicot and monocot" in the PCA scatter plot. However, the two species that exclusively infect monocot plants, *C. graminicola* and *C. sublionela* clustered independently in both PCA plot and the dendrogram, from the rest of the *Colletotrichum* spp which have been reported to infect dicots, exclusively or not. In this instance, *C. tanaceti* grouped with the latter. The two graminaceous pathogens exhibited major contractions in pectinase families, supporting the fact that the pectin content in cell walls of monocot is less than dicots. The three classes of host range were separated in both the scatter plot and the heatmap. Interestingly the clustering appeared to complement the host range more than the host type and the taxonomy. The *Colletotrichum* spp with a broad host range separated from the rest and included two sub-clusters of the acutatum complex and gloeosporioides complex with expansions of certain hemicellulose degrading enzymes and pectinases. *Colletotrichum tanaceti* clustered with *C. chlorophyti* which is known to infect herbaceous plants. Pathogens with an intermediate host range, with which *C. tanaceti* clustered, exhibited expansions in pectinase families PL1, PL4, PL9 and contractions in hemicellulose degrading GH29, GH36, GH43 families with respect to those with a broad host range. These findings suggested that comparison of CAZyme profiles within genera is a good approach to infer the host range of a pathogen and that *C. tanaceti* is likely to have an intermediate host range although to date *C. tanaceti* has only been detected in *T. cineraiifolium*.

2.1-45 Identification and characterization of a novel pathogenicity gene of *Colletotrichum orbiculare CoNPC2*, an ortholog of sterol transporter *Saccharomyces cerevisiae* NPC2

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Abstract: Fungal morphogenesis depends on accurate cell cycle progression. GTPase activating protein complex CoBub2-CoBfa1 interacts with downstream factor, GTPase CoTem1, and is required for G1/S progression and pathogenesis in *Colletotrichum orbiculare* (Fukada and Kubo. 2015). To elucidate the signal cascade of CoTem1, we performed the screening of physical interaction factor with CoTem1 by Yeast Two-Hybrid (Y2H) system and identified *CoNPC2*, that encodes a putative phosphatidylglycerol phosphatidylinositol transfer protein. The amino acid sequence of *CoNPC2* showed high homology to sterol transporter *Saccharomyces cerevisiae NPC2* reported in mammals and yeast. To analyze the function of CoNpc2 in infection related morphogenesis, we obtained *Δconpc2* mutant. On the cellulose membrane, *Δconpc2* formed appressoria and penetration hyphae indistinguishable from those of the wild type. Whereas, on cucumber cotyledons, *Δconpc2* formed normal appressoria, however, formation of penetration hyphae was attenuated, thus the lesion formation of the host plant was markedly reduced. To test whether CoNpc2 is required for invasive growth in plant, we conducted wounded inoculation of *Δconpc2* to the cucumber cotyledon. *Δconpc2* formed lesions similar to that of the wild type, suggesting that CoNpc2 is not essential for invasive hyphae growth, but is essential for the host cuticle infection. In *S. cerevisiae*, Npc2 acts as a sterol transporter (Bergeret *et al*., 2005), thus to test whether CoNpc2 involved in sterol transport, we observed sterol accumulation in conidia by FilipinIII, a sterol staining during appressorium development. In *Δconpc2*, sterol accumulation in vacuoles was shown with high frequency compared with the wild type. Furthermore, to elucidate the functional similarity of CoNpc2 to Npc2, we obtained the CoNpc2-Npc2 partial replacement strain. In this strain, sterol accumulation was reduced compared with *Δconpc2*. Therefore, it was suggested that CoNpc2 is involved in sterol transport as a functional orthologue of *S. cerevisiae* Npc2 in *C. orbiculare*. In addition, the CoNpc2-Npc2 partial replacement strain restored disease symptom formation compared with *Δconpc2*. Thus, CoNpc2 could function as a sterol transporter that involves in appressorial infection.

2.1-46 Genome assembly and annotation of *Raffaelea lauricola*: a comparison with other members of the Ophiostomatales.

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Abstract: *Raffaelea lauricola*, an invasive fungal pathogen, has caused widespread mortality to redbay (*Persea borbonia*) and other species of the Lauraceae family in the southeastern United States. We produced a draft assembly of the *R. lauricola* genome and compared this genome to the genomes of other closely related species in the Ophiostomatales including *R. quercus-mongolicae*, *Grosmannia clavigera, Ophiostoma piceae, O. novo-ulmi*, and *O. ulmi*. Structural and functional annotations and analyses were performed for the assembled genome to determine genes that are potentially involved in disease development by this wilt pathogen. The *R. lauricola* genome assembly resulted in 1,535

scaffolds and a total length of 35 Mb, which is larger than the other genomes evaluated. It also has a larger number of secreted proteins and small secreted proteins, ABC transporters, cytochrome P450, and CAZYmes. Taken together, the number of proteins related to pathogenicity encoded in the *R. lauricola* genome suggests that this species is well adapted as a pathogen, and possibly better equipped for pathogenicity than other related species. These findings explain, at least in part, the success of *R. lauricola* as a lethal pathogen with a wide host range within the Lauraceae.

2.1-47 Species limits of *Aspergillus versicolor* and close relatives

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Abstract: Currently, the *Aspergillus versicolor* clade of the sect. *Nidulantes* harbours 17 species all of which are considered anamorphic, and no sexual stage is known. The common occurrence of these species in the indoor environment is associated with various respiratory problems, some species have been also reported as a cause of opportunistic infections, including invasive aspergillosis. In this project, we assembled approximately 300 strains from various substrates (indoor and cave environment, indoor air, house dust, various food, clinical material, soil) and continents (mostly North America, Europe and Asia) and revised the species boundaries using a multidisciplinary approach combining phylogenetic analysis with phenotypic data. We analyzed DNA sequences of five protein-coding genes with species delimitation methods based on the multispecies coalescent model. New microsatellite typing scheme was developed from available genomes to investigate the population structure. Both mating type gene idiomorphs (MAT1-1-1 and MAT1-1-2) were found in genomes of *A. versicolor* clade members suggesting the heterothallic reproductive mode. Preliminary results from different species delimitation methods showed that number of species within *A. versicolor* clade should be reduced significantly. There is large genetic and morphological intraspecific variability within these species, that we are able to detect due to our large sampling. It permits the detection of recombination and thus gives us chance to understand the species boundaries within this economically important group of fungi.

2.1-48 Genomics and transcriptome analysis of *Colletotrichum fructicola*

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Abstract: *Colletotrichum* species are overwhelmingly successful phytopathogens, causing anthracnose foliar blight or fruit/stem rot on more than 3,000 plant species. *C. fructicola* is a recently established species belonging to the gloeosporioides species complex and is likely a broad host range pathogen being made up of individual host-limited forms. To understand the virulence gene content and their expressional profiles, we sequenced a *C. fructicola* genome (1104-7) derived from an isolate of apple in China and obtained the pathogen transcriptomes derived from tissues of conidium, appressorium, *in vitro* infectious hyphae (cellophane infection), and *in vivo* infectious hyphae (apple leaf infection) based on Illumina RNA-sequencing. In accordance with a broad host association, the *C. fructicola* genome contained the largest number of virulence genes among all known *Colletotrichum* genomes, such as plant cell wall degrading enzymes, secreted proteinases, small secreted proteins, and cytochrome

P450s. By comparing the 1104-7 genome with the reference genome (Nara_gc5) derived from an isolate of strawberry in Japan, we identified 0.62 Mb lineage-specific (LS) genomic regions in 1104-7. The LS region contained dynamically-evolving genes, among which were two gene clusters with fungus-to-fungus horizontal transfer signatures. Transcriptome analysis identified a range of virulence-related genes showing plant infection-specific high expression; transcriptome analysis also indicated a phosphate-limited and quinate-rich *in planta* environment. This study sheds light on a better understanding of the pathogenic adaptations of *C. fructicola* and will provide useful resources for identifying key pathogenicity regulators.

2.1-57 Documenting the saprotrophic fungi of the Congo Basin

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Abstract: Discovering and describing the world's biodiversity is of paramount importance to biologists. Conservative estimates of fungal diversity put the total number of species worldwide at about 1.5 to 5.1 million, yet only 1-10% of these have been described. Predicting where the estimated "missing fungi" are, is being debated among mycologists and one likely hypothesis is that the majority of unrecorded fungi are microfungi especially those inhabiting specialty niches. However, data from poorly studied geographic regions such as the tropics are limited. Not only may these regions harbor unexpected fungal diversity but studies of tropical fungi could even lead to a revised estimate of total fungal species, given that the current estimate is based on data from temperate regions alone. For this study, fresh collections of saprotrophic fungal species were made from previously established study plots within the Dja Biosphere Reserve (DBR) in Cameroon, West Africa. Descriptions of macromorphological features were made from fresh material in the field. Colors were compared with plates in the Online Auction Color Chart. Fresh specimens were also photographed *in situ* and in the field laboratory. We dried the sporocarps in the field using silica gel and duplicates were deposited in the National Herbarium of Yaoundé (YA) and the Kriebel Herbarium at Purdue University (PUL). DNA was extracted and analyzed using established protocols. After two years of intensive sampling from this single region in the Congo Basin of Cameroon, we have documented more than 200 species or morphospecies of saprotrophic fungi. Approximately one quarter of these species appear to be new to science. Phylogenetic analyses of these fungi will also aid in species delimitation, as many of these fungi are part of species complexes. These data indicate that tropical regions may not only be host to many of the world's "missing fungi", but that estimates of total number of fungal species may need to be revised.

2.1-59 Diversity, distribution and ecological pattern of macrofungi in Turgo tropical forest ecosystem National Park Mount Merapi

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Abstract: The macrofungi exploration in Turgo tropical forest ecosystem of Mount Merapi National Park aimed to document the diversity and distribution of edible and inedible macrofungi in that region. Macrofungi sampling was distinguished by different forest elevations i.e. Turgo forest, Tritis forest, and Bingungan forest using adaptive sampling method of plot making. Data collection was conducted in May-June where the month is categorized as the wettest month in Turgo area. The total number of macrofungi obtained 133 specimens, in the Turgo forest found 48 species, in the forest Bingungan got 36 species and in the forest Tritis got 49 species. Species with a high level of macrofungi diversity based on the Shannon-Weiner index was owned by Tritis forest with the lowest elevation among the three is 2.521 followed by the Bingungan forest with an index value of 2.51 and the last of the Turgo forest was

worth the index of 2.01 which means the three tracks have a level of diversity of moderate-high. Comparing similarity between the three tracks based on the Sorenson index value found that the three tracks have a much different similarity. Measuring from the important values, the highest value morphogroups was owned by macrofungi agarics group with 82% -169% value, followed by other groups i.e. jelly, polypore, cup, club, coral, and tooth which are calculated based on relative frequency and relative density values. Found 8 species that are edible, 12 species are inedible 6 species are medicinal 106 still remaining unknown. Some key or important species in Bingungan forest were detected like *Xylaria* spp. and *Auricularia delicata*; *Marasmiellus* sp. in Turgo forest and no species can be concluded as the most important in Tritis forest. The map of macrofungal distribution has been made for each forest tracking.

2.1-60 rDNA nucleotide-based phylogeny of ectomycorrhizal fungi from Guineo-Soudanian ecozone of Benin (West Africa)

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Abstract: Mycology has experienced a rapid development during the last two decades through the application of molecular techniques and phylogenetics to fungal taxonomy, ecology and evolution. Many fungal species display a limited number of morphological and anatomical characters, making species demarcation difficult. It has been demonstrated that misidentification, mostly of cryptic species, has led to the deaths of many people, whilst traditional taxonomical methods hamper our ability to assess global diversity of fungi. To get a clear picture of fungal diversity and community phylogenetics, systematic sampling of fruit bodies of ectomycorrhizal fungi was carried out in species-rich ecosystems of Benin. We recorded a total of 110 morphological species in 33 genera. DNA was extracted from representative specimens of each morphological species using either the QuiaGen DNeasy Plant Mini kit or a protocol of cryogenic disruption followed by extraction in CTAB buffer, cleaning with chloroform, and alcohol precipitation. The internal transcribed spacer (ITS) region of the rDNA was amplified by PCR using, variously, the primer pairs ITS1–F/ITS4, ITS1-F/ITS4-B, or ITS1/LB-w, and sequenced using the Sanger method. We generated a total of 117 sequences sorted into Russulaceae (33 sequences), Amanataceae (43 sequences), Boletaceae (37 sequences), Cortinariaceae (3 sequences) and Tricholomataceae (1 sequence). Similar sequences were downloaded from Genbank to generate a dataset of 3304 sequences. In the present talk, we will showcase the placement of our sequences within the global phylogenetic context, whilst the consistency of traditional delimitation of species and sections within core genera will be tested phylogenetically. Relevance/Significance: We expect to depict strong phylogenetic argument to support the description of numerous putative new species and to support delimitations within cryptic taxa. Our investigations will increase our understanding of species limits within taxonomically complex genera. Results from the present study will nourish the Fungi DNA Centre under construction at the University of Parakou.

2.1-61 Think globally, barcode locally: an overview of the Ordway-Swisher Fungal DNA Barcoding

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Abstract: The Gulf Coast region of the Southeastern USA is known as a hotspot of plant and animal diversity, but the fungi of the region are still highly understudied. The most prolific collector and taxonomic expert in the region was William Alphonso Murrill, who collected from the 1920's until the

1950's. Murrill described approximately 800 fleshy basidiomycetes, mostly within walking distance of the University of Florida in Gainesville. Many taxa that Murrill described have not been re-collected since their original discovery. Sadly, many of Murrill's type specimens are degraded so they are challenging to use for phylogenetic analyses and many of Murrill's original collecting sites near the campus have been lost due to development. As part of a long-term goal to document the mycoflora of Florida and to recollect Murrill's Florida species, we systematically collected fleshy macrofungi over a one-year period at the Ordway-Swisher Biological Station (OSBS). Ordway-Swisher is located approximately 25 miles east of Gainesville and contains many habitats similar to those where Murrill collected. Here we present an overview of our DNA barcoding project and we discuss some of the findings and challenges from this work. We collected approximately 1000 samples and generated >600 ITS sequences, with additional 28S sequences for select genera. The most heavily collected groups were Boletales (111 collections), *Amanita* (94), *Russula* (89), *Cortinarius* (53), *Lactarius* (38), *Pluteus* (31), and *Inocybe* (19). Preliminary morphological analyses of our collections indicates that we successfully recollected several taxa that are thus far known only from the type specimens (e.g. *Inocybe taedophila*) as well as several putative new species (e.g. *Tuber* and *Elaphomyces* spp.). The high number of *Amanita* and Boletales specimens and molecular OTUs confirms that the local biodiversity is high for these groups. BLAST analysis of our ITS sequences from OSBS specimens revealed that more than a third of all sequences had no close match in GenBank (<97% similarity) and many taxa had a closest match that was from an unnamed environmental sample. When sequences did match closely with something in GenBank, they were mostly from eastern states (Georgia north to Massachusetts) or from nearby countries across the Caribbean region. Diversity was also highly structured by season; genera such as *Cortinarius* and *Tricholoma* were most abundant in the winter months (December-February) while *Amanita*, Boletales, *Cantharellus*, *Inocybe*, *Lactarius*, and *Russula* were more diverse and abundant in summer (May-September). This suggests that Florida's winter mycoflora is dominated by temperate taxa that normally fruit during Fall in sites further north. In the summer, Florida's mycoflora is dominated by tropical and subtropical taxa, many of which appear to be restricted to the Gulf Coast and Caribbean regions. The strong seasonal temperature fluctuations and potential for rain throughout the year in Florida may contribute to a high diversity of fungal species. This project serves as a model for how local herbaria can contribute to the MycoFlora 2.0 initiative.

2.1-62 FungiWeb Ecuador, online access to the fungal biodiversity of Ecuador

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Abstract: Ecuador is one of the few megadiverse countries in the world. There is a rich history in the exploration of plants and animals in Ecuador and its fairly well documented. Nevertheless, new species are being described every year even in the better studied groups, such as plants and tetrapods. Fungi, in contrast, are amongst the least studied groups and therefore harbor a large proportion of undescribed species. Access to Ecuadorian fungal species lists and collection data is very limited. Lack of basic information hinders efforts of mycologists in the private and public sectors to develop long-term research programs on Ecuadorian fungi. To alleviate this problem, we are launching FungiWeb, a free, open-access web portal dedicated specifically to publish information on fungal diversity with image guides and lists of species generated as PDFs. FungiWeb also provides access to the database of the biggest fungal collection in Ecuador, the QCAM Fungarium at Pontifical Catholic University of Ecuador (PUCE) in Quito. The collection contains over 7400 specimens with more than 60 orders, 155 families and 472 genera. Most samples are macrofungi from protected areas including understudied National Parks. FungiWeb is part of BIOWEB, a web portal aiming to discover, administer and publish general information about the biological diversity of Ecuador. BIOWEB has been developed by PUCE under the

premise that free and open access to biological information is the best incentive for its study, conservation and sustainable use. BIOWEB gives access to information on over 452,000 specimens, which include plants, animals and fungi. The portal details taxonomic classifications, descriptions, distribution maps, images, and more.

2.1-63 Exploring total fungal diversity in Boyacá, Colombia

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Abstract: Found in virtually every ecological niche, fungi are key organisms to the environment and our lives. From nutrient recyclers, decomposers and beneficial symbiotic partners with plants and animals, to food and medicine providers, they can also represent a devastating threat to ecosystems, our food resources and even to our health. Nevertheless, we still suffer from inadequate sampling and study of the kingdom Fungi in many parts of the world. This is the case for Colombia, and specifically in the region of Boyacá, a virgin land for fungal exploration. For the first time, this project aims to close the gap and assess the unknown fungal diversity in the department of Boyacá, Colombia, by using a field-to-lab approach merging expeditions, standard barcoding techniques, and genomic approaches with traditional specimen-based identification methods. During the first phase of this project, we collected and documented fungal diversity in the six types of habitats present in Boyacá: páramo, sub-páramo, *Weinmannia*-pine forest, dry acacia forest, oak forest, and tropical rainforest. Using plots and transects, we systematically collected specimens grouped in four categories: macrofungi and non-lichenised ascomycetes, lichenised fungi, plant roots for mycorrhizal fungi, and leaf material for endophytic fungi. In total, we collected more than 1,500 samples (more than 400 macrofungi and non-lichenised ascomycetes, 760 lichenised fungi, 160 roots, and 200 leaves). Given that 79 species of macrofungi and 261 species of lichenised fungi have been cited so far for Boyacá region, our exhaustive sampling will surely provide many new records as well as potentially new discoveries to science. Furthermore, species accumulation curves show that more sampling is needed to cover the full diversity of the habitats surveyed in this study as well as other habitats present in the region.

2.1-68 A survey of fungi in Gorongosa National Park, Mozambique.

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Abstract: A review of literature indicated that little mycological work had been done in Mozambique, Africa. Therefore, we conducted a fungal survey of Gorongosa National Park (GNP) for one month in June-July of 2016. The Park had been a frequent battleground during the years of the Mozambican Civil War (1977-1992) and was left unprotected for another decade. In that time, the Park's fauna was decimated. Today, restoration of the wildlife and tourist infrastructure is advancing apace. The establishment of the E. O. Wilson Biodiversity Laboratory in the Park, modeled along the lines of the Smithsonian Tropical Research Institute on Barro Colorado Island in Panama, provided an attractive research destination. We collected voucher specimens at random localities and habitats within the Park, focusing on discomycetes, polypores and anamorphic fungi. Despite a severe drought had affected the region that year, we were able to make over 500 collections. We are currently making determinations of these and will publish our results in the form of a checklist. Since most fleshy fruiting bodies occur during the rainy season, and as our survey was conducted in the beginning of the dry season, our collections represent species in fruit during a season that is not often sampled. Voucher specimens will be deposited at the Biodiversity Collection at the E. O. Wilson Biodiversity Laboratory at GNP, the Herbarium of Eduardo Mondlane University in Maputo (LMU), the Farlow Herbarium (FH) at Harvard University and the University of Illinois Herbarium (ILL). Our work will stimulate future studies of the Park's mycobiota and become the cornerstone of a systematic fungarium within the Biodiversity Collection. This would be the first such collection housed at any African National Park. Furthermore, we hope that a baseline understanding of fungal species and their activities in GNP will help to inform current decisions around Park conservation and allotment of resources.

2.1-73 Arthropods and fungi interact during green litter decomposition in a simulated hurricane experiment

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Abstract: Hurricanes generate disturbances in forests such as canopy opening, fallen trees and leaves which alter physicochemical characteristics of the habitat. Litter decomposition depends primarily on the interaction among climate, litter quality and biota, as a consequence any change in habitats will result in changes in these factors. Understanding the mechanistic processes of litter decay is essential for predicting nutrient cycling dynamics in tropical forests. While models of litter decay mostly rely on climate and litter chemistry, it is increasingly apparent that the decomposer communities (fungi, bacteria and arthropods) interact during leaf litter decomposition significantly influencing decay rates and mineralization of nutrients. White-rot basidiomycetes are the most efficient biodegraders of lignin, breaking down bonds to expose the assimilable cellulose and hemicelluloses surrounding lignin. This assimilable cellulose and hemicellulose is available for the bacterial community to degrade. Litter with more lignocellulose promotes the fungal decomposition pathway, which in turn favors soil and litter food webs dominated by arthropods. Our objective is to evaluate the effects of hurricane driven changes

to forests on green litter decomposition, decomposer communities and nutrient mineralization. For this study, three blocks were selected, each with two plots of 20 m x 20 m, one plot was used as unmanipulated control and the other for canopy trimming. In each subplot, litterbags with different mesh sizes were placed. Each of these litterbags were used as the sampling unit. In each one, decomposer fauna and nutrients were measured, and the weight of green litter from the litterbags was used to measure mass loss through time. Microbial diversity was documented using TRFLP. Diversity and abundance of arthropods was determine using the Berlese funnel technique. Nutrient release was documented using Plant Root Simulators (PRS probes). Preliminary results suggest significant differences in abundance of decomposer fauna and in available nutrient concentration between trim plus debris and unmanipulated control plots, and among litterbags mesh sizes. The fungal community structure and nutrients differed significantly between unmanipulated control and trim plus debris. For example, nitrogen and phosphorous were significantly higher in trim plus debris plots and in large mesh litterbags. Also, decomposer arthropod abundance was higher in large mesh litterbags and the TRFLP showed diversity of fungi in the unmanipulated control. In conclusion, there was a trend for higher arthropod abundance, higher nutrient availability and larger mass loss in large mesh litterbags, suggesting trophic dynamics mediated by all decomposer communities. Arthropod abundance increased with the increase in the diversity of fungi. Nutrient release was higher in the trim plus debris plots during the first 5 weeks after treatment and when all the trophic groups were present. In the future, samples will be sent for Illumina sequencing. These results will be further analyzed, and interpreted in the context of food web dynamics.

2.1-74 Effects of warming on fungal and bacterial activity associated with decaying leaf litter in a stream microcosm experiment

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Abstract: Forested stream networks play a significant role in the storing and processing of terrestrial organic carbon (OC), such as leaves and wood. Fungi are at the forefront of stream OC processing by way of plant litter decomposition, mediating the flow of energy and nutrients to higher trophic levels. Increases in temperature under climate change predictions are expected to affect microbial activity, as well as carbon dynamics in aquatic ecosystems. The objectives of our study are (i) to determine the responses of litter associated decomposers to temperature and (ii) to test whether fungal responses can be explained by the Metabolic Theory of Ecology (MTE) within the temperature ranges found in temperate streams. We performed an experiment in laboratory microcosms simulating stream conditions and measured physiological responses of natural stream microbial assemblages, including fungi and bacteria, to increased temperature. Sterilized leaf disks (*Liriodendron tulipifera*) were inoculated in a stream at the Coweeta Hydrologic Laboratory, NC during peak litter fall. Leaf disks were then incubated at five temperatures (4-20°C) in laboratory microcosms. We determined leaf litter decomposition rates, fungal biomass accrual (from ergosterol), fungal and bacterial production (using radiolabeled tracers), sporulation rates and cumulative spore production by aquatic fungi, microbial respiration rates and activity of enzymes involved in carbon sequestration (β-1,4-glucosidase, β-1,4-xylosidase and phenol oxidase). We found that responses of aquatic litter-associated microorganisms to increases in temperature are more complex than simple predictions of the MTE, with more pronounced responses (higher estimates of apparent activation energy, *E*) at lower temperatures. For some parameters, our estimates of *E* at lower temperatures were higher than canonical values often reported

for respiration (~0.65 eV), suggesting that fungi and microbial OC processing in streams during the cold months could be especially sensitive to temperature increases. These trends may have important implications for stream ecosystems under climate change scenarios, since bulk leaf litter inputs and peak fungal activity coincide with the coldest season (autumn-winter) in temperate streams.

2.1-75 Discovery of a new thermotolerant *Ganoderma* species from the South African deep subsurface

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Abstract: Fungi such as mushrooms occupy some of the most diverse ecosystems on the planet and have been found to grow and proliferate in extreme environments. Fungi thriving in harsh environments are of particular interest since they produce enzymes which possess higher thermostability resulting in activity at higher temperatures. In the current study we describe the sampling and isolation of a possible new fungus species from the South African deep subsurface (3.1 kmbls). The deep subsurface is characterized as an extreme environment due to elevated temperatures, humidity, radiation and limited nutrients. The fungus formed brackets and was isolated growing on wood packs supporting the rocks during the mining process in a stope gully. Stope gullies are found in areas were the reef is being actively mined resulting in high temperatures as well as blasting by explosives on a regular interval. With the aid of morphological and cultural observations, and DNA sequence comparisons the fungus was identified as a possible new species in *Ganoderma*. ITS sequencing (internal transcribed spacer region) resulted in a distinct phylogenetic signature clustering separately from all other *Ganoderma* species, including those from South Africa. This bracket fungus has displayed thermotolerant behaviour as maximum radial growth was achieved at the temperature of 35°C (45 mm), followed by 30°C (42.2 mm), 25°C (31.1 mm) and 40°C (23.8 mm) after 12 days of incubation. The ability to produce fruiting bodies was also observed at 35°C. This bracket fungus has also displayed above average lignolytic abilities degrading supporting wood stumps in days. The origin of this *Ganoderma* species is unknown but it is more likely to have originated as an inhabitant of the woody material than from the mine. Further studies to determine its correct origin, and whether its tolerance to high temperatures are natural or could maybe due to environmental pressures in the mine environment, have important implications for biosecurity within mines.

2.1-76 Effect of changing climate conditions on plant disease distribution in Punjab, Pakistan

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Abstract: In Pakistan climate change has emerged as a serious concern for agriculture especially for vegetable and ornamental plant. Investigations conducted against regular field crops like sunflower, sesame, maize and perishable crops Gladiolus, Tomato, Bitter Gourd and onion against most commonly occurring pathogens viz *Macrophomina phaseolina, Alternara alternata* and *A porri spp, Fussarium spp and Myrothecium roridium* from 2007-2016 on disease distribution pattern of Root rot of Peanut, Stalk rot of maize, Myrothecium leaf spot of Bitter Gourd, Charcoal rot of Sesame and Purple Blotch of Onion (ongoing).Comprehensive surveys were conducted toto identify changes in the distribution pattern of major disesase in relation to different geographical zones. Main objective of these studies was to acquire

knowledge about the variation in terms of prevalence, diseases incidence and severity of the diseases with relation to the environmental conditions of the specific zone. The climate change also affected on Sporulation, morphology, new areas of introduction and lesser occur, adaptability physiology. The severity was measured on a 0-5 visual rating scale where 0 stands for no disease and 5 stands for 80% $^{+}$ disease severity. Some of the studies for post harvest quality deterioration in fruit and vegetable markets were also co related with changing climate. A structured questionnaire was also distributed among crop production and protection stake holders from public and privates sector. Data was collected from five Agro ecological zones in Punjab province in Pakistan that are designated as rice, cotton, mix cropping and rain fed or pothohar zone. In these zone farmers concern was noted as Change in inputs requirement: especially irrigation, pesticides and fertilizers, Marketing instability, Post harvest processing, Crop phenology. There is need for regular monitoring of the cropping systems and isolates of the most commonly occurring pathogens of the country. It would help in designing cropping patterns in accordance with the need.

2.1-77 Climate change alters disease severity in the *Endoconidiophora polonica*-Norway spruce pathosystem

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Abstract: Climate change is already having significant impacts on forest ecosystems and these seem likely to be greater in the future. Understanding pest and pathogen dynamics and their impacts on tree health is essential to predict and potentially mitigate the effects of changing climate on forest stability. Most studies have focused on host tree and pest distribution under changing climate scenarios, but only a few have focused on the modulation of pathogenicity within host-pathogen systems. The European spruce bark beetle (*Ips typographus*) is one of the most aggressive insect pests of conifers in Europe. A warming climate has been shown to enhance *I. typographus* infestations by promoting swarming activity and reproduction, which are mainly temperature-dependent. This insect pest vectors the necrotrophic fungus, *Endoconidiophora polonica*, which is regarded as the most aggressive pathogen of Norway spruce (*Picea abies*). In this study, two powerful inoculation experiments were conducted to test *in vivo* the effects of a changed growing environment on Norway spruce seedlings infected with different strains of the *E. polonica*. The first experiment compared seedling performance under ambient summer temperatures and CO_2 levels with those predicted for the years 2030 and 2100 in Finland. The second compared seedling performance under high and low water availability treatments. For both experiments, seedling mortality was monitored throughout the annual growing season. Seedling growth and lesion length indices were measured at the end of the experiment. The results showed that increased temperatures coupled with elevated CO_2 concentration, and water availability can both enhance disease severity in *P. abies*. Higher temperature increases are likely to be the most detrimental to tree health, and interestingly, disease severity varied markedly among the fungal strains. Our results indicate that predicted climate change has the potential to alter the damage caused to Norway spruce by *E. polonica*. They also highlight the importance for a strain-specific level of understanding of the disease agents. Managing increasingly disturbed forests will be an important challenge in the future. There is consequently an urgent need for systems-based research to better understand the impacts of interactions between biotic agents and climate change in forest ecosystems.

2.1-78 Late Quaternary climate change explains soil fungal community composition rather than richness in forest ecosystems

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Abstract: The Earth's climate has undergone major changes in the past, with massive oscillations between cold glacial and warm interglacial periods during the last 2.6 Myr (Late Quaternary). Late Quaternary climate change can drive migration, extinction and speciation and therefore may influence the modern diversity and composition of macroorganism communities, but how it structures belowground microbial communities is less well known. As a major component of belowground microbial communities, soil fungi exhibit a wide range of strategies including mycorrhizal, pathogenic and saprotrophic species, and make a large contribution to biogeochemical processes and ecosystem functioning. The objective of this study is to reveal the relative effect of late Quaternary climate change and contemporary environment on fungal community in different forest ecosystems. We examined soil fungal communities in temperate, subtropical and tropical forests in China, using Illumina Miseq sequencing of ITS1 sequences. The fungi were divided into different trophic groups, such as saprotrophic, ectomycorrhizal and pathogenic fungi. The total, saprotrophic, ectomycorrhizal and pathogenic fungal richness were influenced primarily by the contemporary environment, but not by the late Quaternary climate change in the temperate, subtropical and tropical forests. By contrast, the late Quaternary climate change acted in concert with the contemporary environment to shape total, saprotrophic, ectomycorrhizal and pathogenic fungal community compositions and with a stronger effect in temperate forest than in tropic-subtropical forest ecosystems. Some contemporary environmental factors influencing total, saprotrophic, ectomycorrhizal and pathogenic fungal communities in the temperate and tropic-subtropical forests were different. In conclusion, we demonstrate that late Quaternary climate change can help to explain the mechanisms maintaining current soil fungal community composition and argue that climatic legacies can help to predict soil fungal responses to climate change in ecosystems.

2.1-79 Can metatranscriptomic be used to measure carbon-use efficiency of fungal community in boreal forests?

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Abstract: Fungal acquisition of metabolic carbon and its subsequent partitioning between biomass production and respiration is a central aspect of forest soils. A frequently used concept to express energy and carbon flow in the ecosystem is carbon use efficiency (CUE), defined as the fraction of acquired carbon that is incorporated in biomass. Fungal communities are central below-ground drivers of ecosystem process, especially in boreal forests, and estimating their CUE is important for understanding their role in the ecosystem. However, current available techniques for estimating CUE in natural systems, such as isotope labelling, biomass markers, and respiration measurements, are all associated with practical and theoretical shortcomings, making assessments unreliable. In this project, we propose that metatranscriptomics can be used to assess CUE of fungi in natural systems. In laboratory experiments, we aim to explore transcriptome profiles of fungal cultures, to select genetic markers of resource acquisition, mycelial growth and respiration. Transcriptomic indexes will be related to measurements of CUE. These indexes will then be used to analyze soil metatranscriptome profiles from

boreal pine forest stand, in order to address questions about fungal adaptations and ecological strategies.

2.1-80 Ecological responses to forest age, habitat, and host vary by mycorrhizal type in boreal peatlands

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Abstract: Despite covering vast areas of boreal North America, the ecological factors structuring mycorrrhizal fungal communities in peatland forests are relatively poorly understood. To assess how these communities vary by age (younger v. mature), habitat (fen v. bog), and host (conifer trees v. ericaceous shrub), we sampled the roots of two canopy trees (*Larix laricina* and *Picea mariana*) and an ericaceous shrub (*Ledum groenlandicum*) at four sites in northern Minnesota, USA. To characterize the specific influence of host co-occurrence on mycorrhizal fungal community structure, we also conducted a greenhouse bioassay using the same three hosts. Root samples were assessed using Illumina-based high-throughput sequencing (HTS) of the ITS1 rRNA gene region. As expected, we found that the relative abundance of ectomycorrhizal fungi was high on both *Larix* and *Picea*, whereas ericoid mycorrhizal fungi had high relative abundance only on *Ledum*. Ericoid mycorrhizal fungal richness was significantly higher in mature forests, in bogs, and on *Ledum* hosts, while ectomycorrhizal fungal richness did not differ significantly across any of these three variables. In terms of community composition, ericoid mycorrhizal fungi were more strongly influenced by host while ectomycorrhizal fungi were more influenced by habitat. In the greenhouse bioassay, the presence of *Ledum* had consistently stronger effects on the composition of ectomycorrhizal, ericoid, and ericoid ectomycorrhizal fungal communities than either *Larix* or *Picea*. Collectively, these results suggest that partitioning HTS-based datasets by mycorrhizal type in boreal peatland forests is important, as their responses to rapidly changing environmental conditions are not likely to be uniform.

2.1-89 Growth morphology and kingdom independently affect microbial beta diversity at a regional scale

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Abstract: Although the response of microbial diversity to environmental factors has been well-studied, it remains unclear how the traits of soil fungi and bacteria determine their biogeographical distributions. With the emergence of functional databases like FUNGuild for fungi and Silva for bacteria, we can extend beyond the fungal-bacterial dichotomy to investigate the relative effects of traits such as growth morphology on soil microbial diversity. Here we assess how both phylogenetic placement (fungi or bacteria) and growth morphology (unicellular or filamentous) independently affect changes in microbial diversity across regional environmental gradients. Fungal and bacterial soil communities from multiple ecosystems across an environmentally heterogeneous region of the Midwestern US were characterized by high-throughput sequencing. We hypothesized that microbial communities would be less similar to one another as geographic distance between them increased, but that microbes with unicellular growth morphologies would be less sensitive to changes in geographic distance or environmental factors. We found that fungal communities, on average, turned over much more quickly (i.e. have higher beta diversity) over a given geographic distance relative to bacterial communities. Additionally, we found that

growth morphology was equally or more important than geographic distance in predicting microbial community diversity. Specifically, unicellular fungal communities (i.e., yeasts) turned over at rates more similar to bacteria than to other fungal communities, whereas filamentous bacterial communities had higher rates of turnover than unicellular bacterial communities. These results suggest that both fungal and bacterial filamentous microbes have higher beta diversity than unicellular microbes at a regional scale. Further, we found that fungal communities were more sensitive to plant community composition while bacterial communities had a higher sensitivity to abiotic environmental characteristics. Collectively, our results support the idea that fungi and bacteria are differentially affected by geographic distance and environment, and also that growth morphology is an important factor determining landscape level patterns of diversity.

2.1-90 Fungal and bacterial communities vary in their carbon cycling response to climate

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Abstract: Microbial communities are the engines of decomposition, a fundamental process regulating the carbon cycle. While much is understood about how changes in abiotic conditions and substrate quality affect decomposition rates, the role of microbial community composition remains elusive. This knowledge gap may be key for predicting how ecosystems will respond to climate change. Here, we first surveyed the microbial community composition of decaying leaf litter along an elevation gradient in southern California, USA. The gradient included five sites with precipitation increasing, and temperature decreasing with elevation. To test the importance of climate for microbial composition, we conducted a transplant experiment. We used "microbial cages" to transplant leaf litter communities to different climates while preventing microbial exchange with the environment. We inoculated transplant communities onto a common, irradiated, grassland litter. In contrast with observational data, this manipulation isolates the effects of climatic conditions versus that of microbial composition on litter decomposition. To characterize fungal and bacterial community composition, we extracted DNA from the inoculum, intact litter at sites, and litter in the microbial cages, and amplified and Illumina sequenced part of the ITS and 16S rRNA regions. We also analyzed microbial biomass with fungal hyphal abundance counts and bacterial flow cytometry. We investigated the functional consequences of these transplants by measuring decomposition as mass loss and analyzing nutrient content. We hypothesized that communities from along the climatic gradient would differ in their abundance and composition, and that communities at the extremes of the gradient would be most affected by climate. We found that microbial communities did indeed differ greatly between the five sites (PERMANOVA; Fungi: $R^2 = 0.58$, $P < 0.01$; Bacteria: $R^2 = 0.61$, $P < 0.01$) with fungi primarily dominated by the Ascomycota. The main axis of community separation appeared to be between the colder and wetter sites versus the hotter and drier sites. After transplantation, fungal communities retained a strong signature of the inoculum whereas bacteria were quickly influenced by local climate. Inoculum source was the strongest factor influencing fungi ($R^2 = 0.57$, $P < 0.01$), but site was also significant ($R^2 = 0.14$, $P < 0.01$). In contrast, site was the strongest factor affecting bacterial composition ($R^2 = 0.34$, $P < 0.01$), although inoculum source was also significant ($R^2 = 0.16$, $P < 0.01$). However, a strong site by inoculum interaction effect for both fungi ($R^2 = 0.15$, $P < 0.01$) and bacteria ($R^2 = 0.19$, $P < 0.01$) indicates that the strength of the inoculum effect varied by site, meaning that not all communities responded similarly to the climate gradient. Unexpectedly, communities from the extremes of the gradient responded similarly. In contrast, communities from the intermediate elevation sites had significantly different impacts on decomposition and types of carbon compounds degraded when transplanted. Moreover, significant site by inoculum

interactions impacting decomposition lasted a year after transplantation. These results demonstrate that microbial communities affect decomposition, but bacteria will shift more rapidly in response to climate than fungi, with fungi retaining a strong inoculum signal even after 1.5 years.

2.1-91 Environmental drivers affecting a global distribution of dominant fungi

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Abstract: It is clear that distribution of fungi is not governed by dispersal limitations only, but also by the availability of suitable ecological niches reflecting the abiotic and biotic conditions of the environment. The abiotic factors, especially temperature, precipitation, habitat type, pH, nitrogen and calcium contents were reported to be the most important drivers of fungal distribution. In this study we have focused on the identification of climatic drivers on the distribution of individual fungal taxa based on the records of their occurrences and abundances across the globe. For the study, we have selected top 200 globally distributed species from environmental samples taking the 98.5% species hypothesis (SH) as a proxy of fungal species. To explore the influence of climatic drivers on the distribution of fungi, CHELSA climatic dataset was combined with GPS coordinates of samples and Gradient Forest algorithm was used. This method extends Random Forest, which fits an ensemble of regression tree models between individual species abundance and environmental variables. Based on this analysis we proved that for more than 75% of dominant fungal species, their distribution is significantly affected by climate. Mean Diurnal Range (Mean of monthly (max temp - min temp)), Mean Temperature of the Wettest Quarter (a quarter is a period of three months), Precipitation of the Coldest Quarter and Precipitation Seasonality (Coefficient of Variation) were most often among the important bioclimatic variables that shape fungal distribution. In average, temperature-related variables explained 18% of the total variation, followed by precipitation variables (9%) and combined variables (7%), but the share of explained total variation ranged up to more than 70%. No difference in the share of the total variation in distribution explained by climatic variables was observed between fungi belonging to different ecophysiological groups, e.g. saprotrophs, ectomycorrhizal fungi, plant pathogens, etc. This study shows that climate belongs among the important factors that shape fungal distribution at a global scale.

2.1-92 Distribution limits of fungi with various trophic strategies in Europe

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Abstract: Interactions between fungi and other living organisms resulted in fungal adaptations to various trophic strategies. Occurrence of such fungi primarily depends on presence of suitable substrate or associated plant (host or symbiotic partner). Fungal distribution areas are known not to copy distribution of hosts (partners) and they are limited by ecological, climatic and geographic factors. However, the relation of trophic strategy and corresponding adaptations with flexibility of fungi to inhabit various climatic zones or geographical areas is unknown. We hypothesize that occurrence of fungi with different trophic strategies depends not only on local ecological conditions, but also on

bioclimatic and geographic influences and that they response differently with various environmental factors. These aspects have been studied on three model fungal groups in Europe: parasitic powdery mildews (family Erysiphaceae) growing on selected forest trees, lichenized fungi of the genus *Solenopsora* and ectomycorrhizal fungi of the genus *Russula*. We have used known records on occurrence of precisely defined fungal taxa (genetically, morphologically) and selected environmental variables to model habitat suitability maps showing potential areas of distribution. Based on this, we identified geographical elements within each trophic group and assigned bioclimatic and geographical factors influencing their area of distribution. In addition, we identified barriers or geographic, ecological or climatic disjunctions limiting migration of fungi of studied trophic groups. The project is funded by the Slovak Research and Development Agency under the grant no. APVV-15-0210.

2.1-93 Does a richness patterns exit in the Agaricales s.l. macrofungi assemblages across Chilean Nothofagus forests? A biogeographic perspective

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Abstract: Global patterns of fungal richness have been associated with edaphic variables and current climate, and it is specific for trophic group: ectomycorrhizae (EcM), saprobiont, and parasitic fungi. In specific, global richness in Agaricales sensu lato is high in tropics, and it tends to decrease towards the poles, correlated with precipitation and carbon availability. The southern of South America is dominated by the genus *Nothofagus*, and it is widely distributed in Chile (32° - 55 °S), with 10 species, where *N. macrocarpa* and *N. obliqua* are the northernmost species. *Nothofagus* has obligated ectomycorrhizal associations, being most of the species of the Agaricales s.l. group. Little is known about factors that determine in the distribution of this group along a latitudinal gradient. For *N. obliqua* it has been described that both richness and abundance of the associated EcM species decrease at high nitrogen levels, however the factors that influence *N.macrocarpa* distribution, remains unknown. In this study we describe both Agaricales s.l. richness and composition in *N. obliqua – N. macrocarpa* dominated forests, depending on plant composition, edaphic variables and climate. This will be evaluated along an increasing precipitation gradient towards the south and along increasing plant diversity, also towards the south. We sample in 6 localities, from Region Metropolitana (33 °S) to Region de la Araucanía (39 °S). We made on average 3 vegetation transects per locality where fungi fruiting bodies were collected. We also measured soil pH, organic matter content and soil nutrients. We found around 420 fungi species, where ~130 species correspond to EcM, ~290 species correspond to saprobiont, and 1 species to parasitic fungi. We also found an increasing diversity gradient toward the south and that variables that positively correlates with total species richness, EcM species richness, saprobiont species richness and wood decomposers richness are: 1) Mean annual precipitation; 2) Total plant species richness; 3) Woody plants species richness index and total soil carbon content. On the other hand, we found that variables that negatively correlate with richness factors mentioned before are: 1) soil pH; 2) percentage of uncovered sky in autumn and winter; 3) mean temperature of summer. Finally, our results also show that saprobiont species richness increase at higher mean annual temperatures, higher mean summer temperatures and minors soil pH. Acknowledgments: FONDECYT , PFB-23 (IEB).

2.1-94 Community assembly of ectomycorrhizal fungi on seedlings of a Neotropical monodominant tree

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Abstract: In the lowland tropics, monospecifically dominant forests are patchily distributed, and their persistently dominant trees are usually ectomycorrhizal (ECM). In such forests, supra-annual mast seeding results in dense cohorts of shade tolerant seedlings that ultimately replace conspecific adults, thereby maintaining monodominance. High seedling survivorship and mitigated density-dependent mortality may depend on rapid ectomycorrhization and resulting enhanced nutrient uptake, protection from root pathogens and rhizovores, and possible resource networking with adult trees. However, both the identities of early-colonizing ECM fungi and their influences on seedling establishment and survival are poorly known. To study these factors, we sampled hundreds of seedlings from an even-aged cohort of the monodominant ECM tree *Dicymbe corymbosa* (Fabaceae subfam. Detarioideae) in a Neotropical lowland forest. Seedlings were sampled at < 1 month, 6 months, and 12 months after a large mast seeding event in 2016. For each age class, the percentage of fine roots colonized by ECM fungi was estimated on a per-seedling basis. Root tips were sorted into distinct ECM fungal morphotypes and Sanger sequenced at the ITS region. To compare seedling and adult ECM fungal communities, ECM morphotypes of 30 nearby adult *D. corymbosa* trees were bulk DNA extracted and sequenced at the ITS locus on an Illumina platform. Ectomycorrhization occurred rapidly on seedlings, covering 26–50% of the roots at ~2 weeks of age, and increased with seedling age over 12 months. Early-colonizing ECM fungi on seedlings < 1 month old were primarily from the /tomentella-thelephora, /boletus, and /clavulina lineages, while the /russula-lactarius lineage appeared later in ECM development. While numerous ECM morphotypes were shared between seedlings and adults, two species of *Tomentella* (Thelephorales) dominated root tips of *D. corymbosa* at all age classes. The ecological impacts of rapid mycorrhization and ECM fungal community assembly on seedling recruitment will be discussed.

2.1-95 Contrasting biogeography of fungal communities associated with roots versus soils of coast redwood forests

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Abstract: Studies investigating patterns of fungal biogeography typically focus on one or two main groups, namely ectomycorrhizal (EcM) and saprotrophic fungi. However, few studies have explored biogeographic patterns of soil and root-associated fungi in arbuscular-mycorrhizal (AMF) compared to these other groups. Additionally, AMF research is generally biased towards herbaceous plant species making it difficult to compare these patterns to the forests in which EcM patterns are observed. AMF play important roles in both plant ecology and ecosystem function, however, the patterns of AMF biogeography and how these patterns compare to other fungal guilds remain largely understudied. Here we report the first comprehensive study of fungal communities associated with coast redwood (*Sequoia sempervirens*) where we tested biogeographic patterns of both soil and root fungal communities to compare patterns of distance-decay across different guilds. Using a nested study design, we sampled throughout the redwood range across 8 different sites spanning a 600km distance. Paired root and rhizosphere soils were collected and sequenced using Illumina amplicon sequencing.

Soil chemistry, physical distance, and climatic variables were all collected in addition to these samples and used to create distance matrices to test the effects of each of these different physical factors using a Multiple-Matrix Regression (MMR). We also used FUNguild, a program designed to recognize different fungal guilds to analyze biogeographic patterns of these different fungal groups. We found that while soil fungal communities showed strong trends of distance-decay, there was no significant distance-decay demonstrated in fungi colonizing roots. After breaking these sample types into their different fungal guilds, we then compared different fungal guilds to see which taxa were driving these patterns. We found that saprobes, endophytes, and other mycorrhizal taxa showed strong patterns of distance-decay in both roots and soils. However, AMF showed no patterns of distance-decay in either sample type across the 600km geographic distance and large climatic gradient. It is clear, however, that AMF communities show different patterns of distance-decay than EcM at similar scales. Additionally, we found that certain AMF taxa were disproportionately selected by roots (33% of AMF taxa were significantly enriched in roots compared to soils). We also found numerous EcM taxa and ericoid taxa associated with coast redwood root samples (root DNA was confirmed via sequencing). Though we have not observed any EcM structures, to our knowledge, these findings mark the first discovery of historically EcM taxa in or on roots of an AMF-associated conifer. We assert that these patterns need to be tested at larger (continental, global) scales using high-resolution primers. Additionally, the occurrence of EcM taxa in or on roots of AMF-associated gymnosperms represents an area of future research in understand how common this might be and if there is any potential functional relationship.

2.1-96 Digging into environmental sequences to investigate ectomycorrhizal symbiosis distribution and diversity: a case study in French Guiana

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Abstract: The ectomycorrhizal symbiosis has been largely understudied in the Neotropics, probably due to the rare observations of ectomycorrhizal root tips and fruiting bodies. Ectomycorrhizal hosts are probably rare, and localized, but curiously, recent studies from taxonomists have revealed a scattered distribution of ectomycorrhizal fungi in the Neotropics. In parallel, since the development of next generation sequencing, the number of studies sequencing soil fungi is growing in the Neotropics, increasing the chance of detecting ectomycorrhizal fungi in soil. However, the lack of reference sequences from fruiting bodies, and the short length of environmental sequences still limit bridges between taxonomists and ecologists. In French Guiana, we spent six years collecting fruiting bodies to increase the number of reference sequences, and environmental sequencing began in parallel in diverse forest habitats. We used new phylogenetic methods developed for short sequences to compare these two sources of sequences. We show that part of short sequences are still difficult to assign, and that environmental sequencing can detect putative new species. This study confirms that ectomycorrhizal fungi can be widespread in French Guiana, but are always rare compare to other fungi. We hope that such an approach could be followed by both ecologists and taxonomists, to deeper investigate the distribution and diversity of ectomycorrhizal symbiosis in the Neotropics.

2.1-101 Shut the front door: seasonal patterns in window operation drive fungal and bacterial community dissimilarity between indoor and outdoor air.

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Abstract: Weatherization typically focuses on air-sealing buildings to reduce energy use, which has the potential to alter indoor microbial communities through reduction of a primary source of indoor microbiota: the outdoors. Indoor sources of microbes may be enriched for human-associated organisms with increased probabilities of potential immune dysfunction and infection. Tightening of the building envelope by weatherization measures may also affect the indoor air quality, as reduced air exchange rates may concentrate harmful pollutants indoors. It is known that ventilation strategy alters indoor microbial signatures, but the role of building operation by occupants has not been well studied. We set out to study the ways that weatherization alters indoor air quality, including the composition and structure of indoor fungal and bacterial communities. Sixty-six households across the greater Portland, OR, metro area were recruited through our local partners to obtain paired weatherized and comparison homes. For both comparison and treatment homes, we collected data in three core research domains: *bioaerosol microbial community composition*, *air quality and building science*, and *home occupant behaviour and perception*. Briefly, each home was equipped for one week with dual sampling units (one indoors, and one out-of-doors), including standard air quality sensors and settling plates for bioaerosol microbial community sampling. Each home was sampled twice: before and after weatherization, or a similar interval for comparison homes; at each sampling, we administered a survey of home operational behaviours and health indicators to residents. Microbial communities were assessed using standard Illumina metabarcoding methods for bacteria (16S) and fungal (ITS) components; analysis used *DADA2*, *phyloseq*, *vegan*, and *codyn* in R. We observed no gross effect of weatherization treatment, indicating that microbiomes of weatherized homes were not more human-associated after treatment. We found that the community dissimilarity between paired indoor/outdoor bioaerosol samples is related to seasonal patterns of window operation, and that the synchrony between indoor/outdoor air quality measures helps explain the dissimilarity in microbial communities. These results, taken together, demonstrate the impact that seasonal choices in home operational mode has on the balance of microbial sourcing, which may play a role in the health of building occupants. While weatherization may impact the air quality and microbial communities of buildings when they are operated in an entirely 'closed' mode, the effect of choice of operational mode, which varies seasonally, has by far the greater impact on the indoor microbiota.

2.1-102 *Desertella microspora* sp. nov., a new hyphomycete from an indoor environment

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Abstract: Indoor environments are distinctive man-made habitats for fungi. Whenever water or moisture is available, building materials, such as wood, clothing, fabric, paper products, foodstuffs, etc. are suitable substrates for fungal infestation. Even house dust is an unusual habitat in which some xerophilic fungi can survive. Despite the fact that a number of new taxa had been described from indoor

environments in the past, fungal diversity in indoor environments has not been sufficiently studied. A unique fungus isolated from a swap sample collected from a residence in Massachusetts, USA was found to be new to science. The objective of the study is to describe it and determine its phylogenetic relationship with allied genera. Both morphological methods and phylogenetic analyses using ITS, LSU and SSU ribosomal sequences were used to study this fungus. The fungus was named *Desertella microspora*. The newly named species has thick-walled conidia globose to subglobose, 17.7 ± 2.6 × 17.3 ± 2.5 µm, and belongs to Chaetomiaceae, Sordariales.

2.1-103 Influence of newly developed chemical treatments based on nanoparticles on selected wood rotting fungi affecting wooden constructions

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Abstract: Following work was focused on physiology wood rotting fungi with special emphasis on potential inhibition of their growth with newly developed chemical agents based on metal nanoparticles. In our survey strains *Serpula lacrymans, Coniophora puteana, Fibroporia vaillantii* and *Gloeophyllum sepiarium*ce have been tested for their ability to grow, colonize new substrate and produce extracelular enzymes in presence of mentioned chemical agents. The work was supported by the GAČR (Czech Science Foundation) grant No.17-05497S.

2.1-104 Fungal colonization and incidence of decay in Scots pine utility poles

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Abstract: Wood utility poles have a long record of providing excellent service for supporting electric transmission and distribution lines under a variety of environmental conditions. The major factors driving fungal decay of utility poles are not restricted to the in-service environment; they can be influenced before pole installation by factors during tree harvest, processing, seasoning, and preservative treatment. Air seasoning is often used for wood species with durable or moderately-durable heartwoods because it is simple and effective. However, long-term exposure increases the risk of fungal colonization. Improper sterilization during preservative treatment can result in utility poles with a high susceptibility to premature decay. An excellent potential example of this problem can be found in the electrical distribution system in Ireland. The Irish electrical system was largely rebuilt in the period between 2000 and 2007 using Scots pine (*Pinus sylvestris*) poles that were air-seasoned for 6 to 12 months in various Scandinavian countries, before being pressure treated with creosote. The specification called for 100% penetration of the sapwood, but there was no requirement for sterilization. This study investigated the presence of decay fungi in 963 creosote-treated Scots pine utility poles from Ireland. Nearly 4,000 increment cores were taken at five heights from groundline to pole-top. Cores were plated on malt benlate agar and unique decay fungi were identified using Sanger sequencing. Fungal data were combined with environmental and preservative treatment metadata, as well as visible pole decay, to identify wood protection problems. Sixty-seven percent of poles contained at least one decay fungus, with the most common isolates being *Phlebiopsis gigantea* (77%), *Neolentinus lepideus* (7%), *Sistotrema brinkmannii* (7%), and *Stereum sanguniolentum* (3%). The remaining 20 fungal species accounted for 6% of total isolations. Isolation frequencies suggested no differences with seasoning method, depth of sapwood treatment penetration, or pole age. It is worth noting that most isolations occurred in poles treated after 1996, which correlated with changes in pole procurement practices. Air seasoning for

longer than 6-months also resulted in higher incidence of decay. Decay fungi were isolated from 96% of poles that contained visible decay and 49% of poles where decay was not visible, suggesting a larger percentage of poles are at risk of decay development. The results led to increased quality control and the inclusion of sterilization procedures prior to or during treatment. These data will be used to help guide process-management decisions in climates conducive to fungal decay.

2.1-113 Historic overview of the Phacidiales and the Austral diversity of Tympanidaceae

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Abstract: The order Phacidiales reflects the chaotic systematic situation within the Leotiomycetes, a class where generic and higher delimitations are subject to ongoing changes. Since the order was established by Bessey in 1907, its taxonomic concept has varied through the time, with authors such as von Höhnel 1917, Korf 1973, Dennis 1978, Crous et al. 2014, and Baral 2016, changing the number of species, genera and families that they accepted. Each author seems to have had a different morphological concept of the order. Nowadays, between 27 and 37 of the genera that have at some stage been placed in the Phacidiales are still accepted, but the majority of them are now belong in other families or orders (Baral 2016, Wijayawardene et al. 2017, Index Fungorum accessed I.2018). Only 24 of them have DNA sequences available in public repositories. We have carried out morphological and phylogenetic analyses to better characterize the order Phacidiales and the relationships among its members. Our molecular data set includes 31 species and three regions of rDNA (SSU, ITS, LSU), covering representative members of Phacidiales according to Baral 2016: Phacidiaceae (5 seq.), Helicogoniaceae (5 seq.) and Tympanidaceae (12 seq.). Also, five sequences of the *Mniaecia* lineage were included, and two sequences representing the genus *Epithamnolia*, which was recently placed in Phacidiales as *Incertae sedis* (Suija et al. 2017). As a result of our investigation, the new genus *Aotearoamyces* is proposed to accommodate specimens from New Zealand previously identified as *Claussenomyces*. *Aotearoamyces* belongs to Tympanidaceae, a recently erected family in Phacidiales. It is differentiated from other Tympanidaceae by its phragmospores with lipid bodies, the lack of conidia or ascoconidia, plectenchymatous excipulum with hyphae strongly spaced and gelatinized, and curved or helicoid paraphyses. Our bibliographic revision resulted in the correction of the authority of Phacidiales. The phylogenetic analyses of combined SSU, ITS and LSU strongly support the relationship between the sexual morph *Aotearoamyces* and the *Collophorina* clade containing species with endoconidia, which resulted in the recombination of *C. paarla* in *Aotearoamyces* due to the molecular similarities. Also, based on our molecular results, we placed the genus *Epithamnolia* in the *Mniaecia* lineage.

2.1-114 Several new species in the *Fusarium fujikuroi* species complex discovered in one location

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Abstract: *Fusarium* occurs ubiquitously in the world and probably in all known niches. Species have a diversity of functions, and include some of the most important beneficial and detrimental fungi influencing humans. Systematic studies revealed an increasing number of new species, species complexes and molecular locus sequence types (MLST) as more and more species from soils, plants,

humans, animals and other niches are characterized. Many *Fusarium* species are known from South Africa, but these mostly include plant, human and animal pathogens, and those related to food storage problems. Those species known from natural environments are largely understudied, and their ecological roles and potential uses for humans are unknown. In a study comparing *Fusarium* spp. occurring inside plant tissues and the rhizospheres from six plant species occurring in one location in Bloemfontein (Free State province) a large number of isolates were obtained from a relatively small sample size. When focusing only on isolates grouping in the *Fusarium fujikuroi* species complex, DNA sequence comparisons of the Translation Elongation Factor 1α and β-Tubulin genes revealed several new species. These included species from the grasses *Eragrostis capensis*, *Themeda triandra* and *Sporobolus fimbriatus*, and the sedge *Cyperus dives*. This unprecedented diversity, including especially new species reports, emphasizes how poor our knowledge of our native fungi and their geographic and substrate associations are. Bioexploration of these *Fusarium* species will also significantly improve the international understanding of the classification of this complex group as the addition of more species will improve species resolutions.

2.1-115 Diversity of Japanese ergot fungi, *Claviceps* spp., inferred by phylogenetic analysis

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Abstract: Ergot fungi (*Claviceps* spp.) have been noted for their toxicity to animals. In Japan, at least 13 species and seven varieties of *Claviceps* species have been recorded on gramineous plants. Most of these species are probably indigenous to Japan or East Asia. However, the classification of these *Claviceps* species needs to be reevaluated based on new data that have emerged from recent studies. Unfortunately, almost all type specimens or cultures of the *Claviceps* species in Japan have been lost. Therefore, we have collected hundreds of new *Claviceps* specimens from across Japan to characterize their relationships with their hosts and their phylogenetic positions. To reveal phylogenetic relationships, molecular phylogenetic studies were conducted using ITS-5.8S rDNA, D1/D2 region of 28S rDNA, and TEF 1α gene regions. So far, we have already found 16 distinct groups. One of them is a worldwide *C. purpurea* var. *purpurea*, found mainly on pasture grasses. Ergot fungus on *Alopecurus*, *Poa*, *Dactylis* and other some grasses in Japan were included in the *C. humidiphila* clade, which probably originated from Europe. They were previously recorded as C. *purpurea* var. *alopecuri* and *C. purpurea* var. *dactylidis*. In the present study, ergot fungus on reed canary grass (*Phalaris arundinacea*) or timothy (*Phleum pratense*) in Japan, which was recorded as *C. purpurea* var. *phalaridis*, was not included in the European *C. humidiphila* clade, although it is a type of *C. humidiphila*. Ergot fungus on *Elymus tsukushiensis* in Japan, which was previously recorded as *C. purpurea* var. *agropyri*, should be a distinct species, although it was synonymized with *C. purpurea* var. *purpurea* by previous study. Ergot fungus on *Leymus mollis* in Japan should be a distinct species, althoug it was synonymized with *C. purpurea* var. *purpurea* by previous study. Ergot fungus on *Sasa* spp., which was previously recorded as *C. purpurea* var. *sasae*, should be a distinct species. Ergot fungi on reed (*Phragmites australis*) in Japan are present as two distinct species that are clearly separate from *C. arundinis* on reeds in Europe. Ergot fungus on *Oplismenus* in Japan is closely related to Indian *C. viridis* on *Oplismenus*. Ergot fungus on Sorghum in Japan is a distinct indigenous species, *C. sorghicola*. Ergot fungus on *Isachne globosa* in Japan is a distincet indigenous species, *C. panicoidearum*. Ergot fungus mainly on *Miscanthus* spp. should be a distinct species, although it has been treated as *C. panicoidearum*. However, this fungus is closely related to *C. sorghicola* on Sorghum. Ergot fungi on *Paspalum* in Japan are present worldwide, known as *C. paspali* and *C. paspali* var. *queenslandica*. Ergot fungus on *Arundinella hirta* in Japan is an

indigenous species, *C. microspora*. Ergot fungus on *Spodiopogon*, which was recorded as *C. microspora* var. *kawatanii*, should be a distinct species. We are yet to find speciemens of five further recorded species: *C. amamiensis*, *C. bothriochloae*, *C. imperatae*, and *C. yanagawaensis*. We will conduct a taxonomic study of these fungi in the future.

2.1-116 Unravelling cryptic species in Cordycipitaceae with isarioid morphs

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Abstract: *Isaria* Pers. is one of the oldest asexually typified genera of invertebrate fungal pathogens, with its species characterized by the formation of branched synnemata, that give rise to flask-shaped phialides produced in whorls. The phialides tend to possess swollen bases that narrow abruptly forming a distinct neck. Conidia are one-celled, hyaline, smooth, subglobose to subcylindrical and are produced in divergent chains. Aspects of this morphology are shared with other genera, resulting in a turbulent taxonomic history. With *Isaria* being polyphyletic throughout Cordycipitaceae and rejected in favor of *Cordyceps*, there is a need to identify the taxonomic placement of isarioid species collected in Thailand. We collected isarioid morphs of invertebrate-pathogenic fungi and other *Cordyceps* and *Torrubiella* species infecting coleopterans, lepidopterans and spiders. Sexually reproductive species that produce isarioid morphs in culture and a *Torrubiella* sp. on a spider, were also included in this study. The purposes of these investigations are to re-examine the taxonomic position of these specimens and to describe new taxa to accommodate the phylogenetic diversity of isarioid fungi. Phylogenetic analyses based on a combined dataset comprising ITS and 28S rDNA, partial sequences of translation elongation factor 1-α gene (*TEF*1) and the genes for RNA polymerase II largest (*RPB*1) and second largest (*RPB*2) subunits were used to clarify their relationships within Cordycipitaceae. A new genus and eight new species, all with isarioid phialides, are described in Cordycipitaceae from Thailand. The new genus, *Samsoniella*, is segregated from *Akanthomyces* based on morphological and molecular evidence. *Samsoniella* differs from *Akanthomyces* in producing orange cylindrical to clavate stromata with superficial perithecia and orange conidiophores with isarioid phialides and white to cream conidia. A new combination for CBS 240.32 and CBS 262.58, originally identified as *Paecilomyces farinosus* (*Isaria farinosa*) and *Penicillium alboaurantium*, respectively, is made in *Samsoniella*. Two new species, *Samsoniella aurantia* and *S. inthanonensis* are described from lepidopteran larvae. Two new species in *Cordyceps, C. blackwelliae* and *C. lepidopterorum*, are also found on coleopteran and lepidopteran larvae. Both species produce isarioid morphs with globose phialides and attenuated long necks and white mycelium in culture. We have established sexual-asexual link of *Cordyceps javanica* (=*Isaria javanica*) on lepidopteran larvae. Four new species described in *Akanthomyces* are pathogenic to spiders or unidentified insect larvae. *Akanthomyces kanyawimiae, A. sulphureus, A. thailandicus* and *A. waltergamsii* were found on spiders with some strains of *A. kanyawimiae* also found on unidentified insect larvae. These four species of *Akanthomyces* are found on the underside of leaves and produce white to cream white powdery conidia, while *S. aurantia* and *S. inthanonensis* were found in the leaf litter and produce bright orange stromata and synnemata with white conidia. Another new combination, *Akanthomyces ryukyuensis* is also proposed. Our results from molecular phylogenetic analyses strongly support these new species in *Cordyceps*, *Akanthomyces*, and in a new genus *Samsoniella* in Cordycipitaceae.

2.1-117 Toward a review of the genus *Cordyceps sensu lato* in the Neotropical region

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Abstract: The Neotropical region is considered one of the biological hot spots of the planet. This recognition has been made based on records of amphibians, birds, butterflies, orchids, palms and some groups of insects. However, as in other regions, a consideration of the diversity of fungi has been relegated to the background. Despite the efforts of Latin-American researchers in recent decades to assess the fungal diversity of the region, it is still a distant goal. Within the great diversity of fungal genera there is one which has aroused more interest recently due to its medicinal properties, its high diversity and its curious way of manipulating its hosts. This is the arthropodopathogenic fungus *Cordyceps* sensu lato (s. l.), an ascomycete with more than 500 species located in three families *Cordycipitaceae*, *Clavicipitaceae* and *Ophiocordycipitaceae*. This group comprises obligate endoparasites that show a high diversity related to the high diversity of its hosts. Because the Neotropical region is rich in diversity of arthropods, one would expect to find a high diversity of Cordyceps. However, to date there has been no survey of the diversity or abundance of species found in the region. The objective of this work was to pull together the large number of records of Cordyceps s.l. from herbariums, public repositories and publications. This review drew information from nine countries, namely, Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guyana, Mexico, Peru, as well as from some collections from Caribbean islands and Central America. Brazil is the country with the greatest number of specimens and records of species, followed by Ecuador and Colombia. The region has yielded 2700 specimens, including 1444 specimens from Brazil of *Metarhizium anisoplae and Beauveria bassiana* found on crops. Mexico and Colombia have 342 and 238 specimens, respectively, coming from natural forest. In total, 133 different species were reported, of which Brazil has 50 % of the species followed by Ecuador, Bolivia and Colombia with 37%, 35% and 34%, respectively. *Ophiocordyceps amazonica*, which parasitizes young grasshoppers, is the unique species collected in all the area reviewed except by Mexico where it is not yet reported. In the countries with Amazon rain forest, the pathogens of ants, *Ophiocordyceps australis* and the *Ophiocordyceps unilateralis* complex are the most common species found, while *Cordyceps militaris,* which parasitizes pupae of Lepidoptera, and *Tolypocladium capitatum,* which parasitizes the false truffle *Elaphomyces,* have more records in Mexico and Costa Rica where oak forest is a dominant vegetation. 55% of the species reported in the region are representing by a single report for the region, and of them more than half are known only from their original description in the beginning of the 19th century. Although, this revision does not reflect the natural diversity of the species of *Cordyceps* s. l. in the Neotropics if shows the dynamics of research in the region. Moreover, this exercise offers a prescription as to where we should prioritize *Cordyceps* s.l. research in the Neotropical zone and which particular areas require greater emphasis.

2.1-118 New species of *Prolixandromyces* (Laboulbeniales) from South America

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Abstract: Four new species of *Prolixandromyces* (Laboulbeniales, Ascomycota) found on Veliidae (Heteroptera) from Bolivia, Brazil, Peru, and Venezuela are described and illustrated. These four species, *Prolixandromyces anseris, P. tritici, P. blackwelliae and P. bromelicola,* represent the first records of this genus from South America and their discovery requires emendation of the original generic circumscription. The newly described fungi are compared to known species and a new key to identification is provided.

2.1-119 Diversity of Zygomycete fungi in Mucorales in Korea

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Abstract: The Zygomycota is a group of related basal clades comprising the subphyla Mucoromycotina, Entomophthoromycotina, Mortierellomycotina, Zoopagomycotina, and Kickxellomycotina. The Mucorales is the largest order of fungi, and classified into the subphylum Mucoromycotina. Many species within the order are used in various important biotechnological areas, such as the production of enzymes and antifungal proteins. Especially, some species are known as causal fungi of human mucormycosis. However, the knowledge about the taxonomy of mucoralean fungi in Korea is limited. In this study, 72 isolates representing 31 species belonging to 13 genera were isolated from different sources, including dung, insect, fruits, freshwater, and soil by using the dilution plating and baiting technique, and standard moist blotter technique. Among of these genera, *Mucor* presented with highest number of species, and followed by *Absidia*. The dominant species was *Mucor circinelloides* and *M. hiemalis*. Herein, the isolates were identified at the level of species based on the morphological characteristics and sequence analyses of internal transcribed spacer (ITS) region, small subunit (18S rDNA), D1-D2 region of large ribosomal subunit (28S rDNA), translation elongation factor-1α (EF-1α), and actin (act-1) genes. Sixteen species were represented as new records and nine species as new species. Especially, *Backusella*, *Blakeslea*, *Gilbertella*, and *Piliobolus* species known as rare species were found in Korea.

2.1-121 Phylogeny of the genus *Phaeochorella* resulting in its migration from Phyllachorales into Diaporthales

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Abstract: *Phaeochorella* is a genus of biotrophic leaf-parasitic fungus of tropical distribution. Traditionally the genus was treated as a member of the family Phyllachoraceae/Phyllachoralles, considering exclusively morphological characteristics, without any molecular evidence. The fungus is morphologically well defined producing bright black epiphyllous pseudostromata of irregular shape, often coalescing to cover major portions of the host leaf; the ascospores are characteristically dark brown with an equatorial hyaline band. To elucidate the phylogenetic relationship of *Phaeochorella* species, with members of the Phyllachorales and other Sordariomycetes, in the present study multilocus

analyses were performed. Nuclear rDNA sequences (small subunit nuc 18S, large subunit nuc 28S, and internal transcribed spacer nuc ITS) were used in combination with partial sequences of the nuclear second largest subunit of RNA polymerase II (RPB2), and the translation elongation factor 1 (TEF1). The results clearly showed that *Phaeochorella* species belong in Diaporthales, forming a clade with high support with the recently described genus *Phaeoappendicospora*, but without close phylogenetic connection with the other families in Diaporthales. Thus, this new position of the genus suggests the possibility of even a future establishment of a new family, depending on the expansion of the diversity base with the addition of sequences of other *Phyllachora*-like specimens that may fit in the Diaporthales, as it happened recently with the genus *Apiosphaeria*, long considered a member of the Phylachoraceae.

2.1-122 Contributions to *Telimena* phylogeny and a new species on *Serjania* sp. from Brazil

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Abstract: Many species previously recognized as *Phyllachora* based exclusively on morphological traits were recently transferred to *Telimena* Racib. (Telimenaceae, Phyllachorales) according to molecular data, but no specimens from Brazil were included in previous studies. Two species, *Phyllachora salaciae* Henn. found on *Salacia crassifolia* (Celastraceae) and an unidentified species found on *Serjania* sp. (Sapidaceae) from the Brazilian Cerrado were morphologically examined. In addition, had the rDNA (nuclear small subunit nuc 18S, nuclear large subunit nuc 28S and nuclear internal transcribed spacer nuc ITS), the second largest subunit of RNA polymerase II (RPB2), and the translation elongation factor 1 (TEF1) nuclear loci partially sequenced. We investigate the phylogenetic relationship between these species and the currently accepted Phyllachorales with sequences publicly available on GenBank to perform multilocus analyses. The results indicated that both species belong in *Telimena*. Although the original description of *P. salaciae* did not identify the host at species level, the specimen presently studied matches perfectly its detailed description shown in the literature. Thus, *P. salaciae* will be recombined into *Telimena salaciae*, showing dark amphigenous lesions, up to 7 cm diam., occupying large parts of the leaf blade; pseudostromata dark-brown to black, occupying all the mesophyll extending between both epidermis; internal mycelium well developed, inter- and intra-cellularly, composed by septate hyphae with melanized, thick-walled toruloid to polyhedric cells (6–8 µm diam.); ascomata perithecial, subglobose, conoid to pyriform (114–195 µm diam.), with perifisate short-neck, ostiolate; asci unitunicate, thin-walled, cylindrical to clavate (69–89 × 10–15,5 µm), with rounded apex and inconspicuous apical structures, with 8 uni- or biseriate spores, paraphysate; paraphyses hyaline, filiform; ascospores hyaline, aseptate, fusiform (9–13 × 3–5 µm), guttulate. The specimen found on *Serjania* sp. proved to be morphologically and phylogenetically completely distinct from the *Phyllachora* species recorded on members of the family Celastraceae, and from all *Telimena* species currently recognized and, therefore it constitutes a new species of *Telimena*.

2.1-123 Characterization of Rhizoctonia-like fungi affecting rice in Uruguay

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Abstract: Rice (*Oryza sativa* L.) is one of the most important crops in Uruguay covering around 170.000 ha with a total production of 1.350.000 tons, which represents approximately 7% of the national agricultural GDP over the past several years. Losses due to diseases are important constraints for rice

production with some sclerotia-forming fungi considered at the most important pathogens in Uruguay. This fact is due to the intensification of the crop in the last years and the accumulation of inoculum in rice soils. The pathogens overwinter as sclerotia or mycelium in the soil or in rice crop residues and infect the next crop after flooding. One of these important diseases in Uruguay and other temperate rice producing areas is the sheath spot of rice. Yield losses for sheath spot can reach 9% in susceptible rice cultivar INIA Tacuarí, a japonica tropical type cultivar that covered around 20% of the rice area. This disease is caused by species of basidiomycete fungi in the genus *Rhizoctonia* or allied genera. Thus, a survey of *Rhizoctonia* spp. associated with sheath spot of rice was conducted in Uruguay to elucidate the species present, distribution and pathogenicity to Uruguayan rice cultivars. In total, 52 isolates of *Rhizoctonia* spp. were recovered from diseased rice plants exhibiting sheath spot symptoms. Isolates were obtained in the last 4 years from symptomatic rice plants from all the geographical rice areas of Uruguay. Also, a collection of isolates obtained in the last 15 years from rice soils was studied. These isolates were characterized molecularly, phenotypically and pathogenically. The species identity of the isolates was determined using phylogenetic analysis of the internal transcribed spacer (ITS) region and large sub-unit (LSU) of ribosomal DNA. Pathology studies were conducted under greenhouse conditions. Plants of 12 common cultivars, including testers, were sowed in plastic pots and plants were inoculated with a disc of potato-dextrose agar containing mycelia of each strain. Three pots with five plants by pot were inoculated with each strain. Plants were maintained under greenhouse conditions and evaluated for symptom development in a 0-9 severity degree scale. Two species were identified and confirmed for the rice area of Uruguay by means of ITS sequencing and phenotypical characterization and resulted *Rhizoctonia oryzae-sativae* and *Waitea circinata* (=*R. oryzae*). Other species suspected to occur in rice in Uruguay, including the commonest R. solani, were not found and its occurrence in the country is discarded. *Waitea circinata* was more common in most rice growing areas, although *R. oryzae-sativae* was common in certain areas of eastern Uruguay. *Rhizoctonia oryzae-sativae* was found to be more pathogenic for the cultivars studied in artificial inoculations. Both species were more pathogenic to japonica tropical type cultivars than to indica type cultivars. *Waitea circinata* isolates exhibited a wide phenotypical variation that deserves a more complete study to recognize types (pathology groups) within the varieties present in rice growing areas of Uruguay.

2.1-124 A novel lineage of *Xylaria* is responsible for taproot decline of soybean in the southern United States

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Abstract: Species within the Xylariaceae (Ascomycota, Sordariomycetes) are predominantly endophytes and/or saprophytes involved in wood decay, although a few species are recognized as plant pathogens. A recently characterized disease of soybean [*Glycine max* (L.) Merr], referred to as taproot

decline, is caused by a *Xylaria* species nested within the *Xylaria arbuscula* species complex. However, little is known about the identity, origin, and life history of the species responsible for this emerging disease, and to date no fertile teleomorph has been observed. In order to understand the origin and distribution of this pathogen, isolates of *Xylaria* were recovered from soybean plant samples exhibiting symptoms of taproot decline collected across the currently known range of the disease in the southern United States (Alabama, Arkansas, Louisiana, Mississippi, and Tennessee). Twenty isolates were used to study the phylogenetic position and genotypic diversity of *Xylaria* species associated with taproot decline. Pure cultures were obtained from root tissue, and mycelia were harvested for DNA extraction, PCR amplification, and Sanger sequencing of four informative loci: nuclear rDNA internal transcribed spacer (ITS1, 5.8S, ITS2 = ITS), partial α-actin, partial β-tubulin, and partial RNA Polymerase II second largest subunit (RPB2). Sequences were aligned with MAFFT and alignments filtered with GBLOCKS prior to maximum likelihood and Bayesian phylogenetic inference. All of the *Xylaria* isolates associated with taproot decline are monophyletic and share a most recent common ancestor with *Xylaria striata*. Specimens of *Xylaria* from the southern United States deposited in several herbaria are being sequenced to see if a connection can be made between historical teleomorph specimens and isolates from soybean. This work should provide insight into the origin of the taproot decline lineage in the southern United States.

2.1-125 Two new species of *Diaporthe* isolated from *Phomopsis* stem blight on tomato

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Abstract: The species of *Diaporthe* (Diaporthaceae, Diaporthales) are well-known as plant pathogens, endophytes or saprobes on the wide range of crops, ornamentals, forest trees and fruit trees. In 2017, Phomopsis stem blight on Tomato (*Solanum lycopersicum* L.) were occurred in Japan. The initial symptoms of the disease are characterized by necroses on cleavage regions of defoliation, and the disease plants are eventually wilted. Two different *Diaporthe* species from seven isolates (*Diaporthe* sp.1: KTH-c11, KTH-c12, KTH-c13; *Diaporthe* sp.2: KTH-c41, KTH-c42, KTH-c43, KTH-c44) were obtained from the symptoms. The results of our pathogenicity tests using these isolates showed that *Diaporthe* sp.2 developed more serious symptoms than *Diaporthe* sp.1. *Diaporthe* sp.1 are characterized by conidiomata with short necks and cream conidial droplets exuding from central ostioles. Its alpha conidia are cylindrical to ellipsoidal and 4.6-8.0 × 1.9-3.2 µm in size. Beta and gamma conidia are not observed. *Diaporthe* sp.2 are characterized by conidiomata with long necks and creamy-luteous conidial droplets exuding from central ostioles. Its alpha conidia are ellipsoidal to fusiform and 4.1-7.0 × 1.8-2.9 µm in size. Beta and gamma conidia are frequently observed. Morphological characters of *Diaporthe* sp.1 and 2 were clearly different from length of conidiomatal neck, shape and length of conidiogenous cells, shape of alpha conidia, and absence/presence of beta and gamma conidia. Our phylogenetic tree of five loci: ITS, *tef1*, *tub2*, *his3* and *cal*, also showed that the two species were distantly related, and each species made a monophyletic clade in the genus of *Diaporthe*. *Diaporthe* sp.1 was phylogenetically close to *D. compacta*. However, they were distinguishable from absence/presence of beta conidia, width of conidiogenous cells and shape of alpha conidia. *Diaporthe* sp.2 was closely related to *D. longicolla*, but absence/presence of beta and gamma conidia, and the size of alpha conidia of the two species were useful characters to distinguish them. Previously, *D. eres*, *D. vexans*, *D. phaseolorum*, *D. sojae* and *Phomopsis fusiformis* have been reported from Tomato. Our two species are, however, clearly different

from the five species based on morphological and phylogenetic evidence. We thus introduce two new species of *Diaporthe* on the stem blight of Tomato.

2.1-126 *Pseudopestalotiopsis ixorae sp. nov.,* and *Pseudopestalotiopsis taiwanensis sp. nov.,* associated with leaf spots of *Ixora*

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Abstract: *Ixora* is a genus of flowering plants in the family Rubiaceae native to the tropical and subtropical regions all over the world. However, its centre of diversity is in tropical Asia. *Pestalotiopsis*-like species occur commonly as plant pathogens and represent a fungal group known to produce a wide range of chemically novel, diverse metabolites. During a survey of fungal diseases associated with *Ixora* species in Taiwan, several *Pestalotiopsis*-like species causing leaf spot were isolated. Based on morphology coupled with single- and multi-gene sequence data of internal transcribed spacer (ITS), partial β-tubulin (TUB) and partial translation elongation factor 1-alpha (TEF) gene regions, revealed two novel taxa of *Pseudopestalotiopsis* namely *Pseudopestalotiopsis ixorae* and *Ps. taiwanensis*. *Pseudopestalotiopsis ixorae* and *Ps. taiwanensis* fit well with the generic concept of *Pseudopestalotiopsis* in having dark concolourous median cells with knobbed apical appendages but differ from the other species of *Pseudopestalotiopsis* based on the size of the conidiogenous cells, conidia and the number of apical appendages. Pathogenicity tests confirmed that the isolated species are the causal agent of the leaf spots on *Ixora*. Keywords: Foliar pathogen, New species, Multi-gene, Phylogeny, *Pseudopestalotiopsis*, Taxonomy

2.1-127 Scientometric study of the scientific literature on *Inonotus rickii* (Pat.) Reid (Basidiomycetes: Hymenochaetaceae)

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Abstract: The objective of this work was to perform a scientometric analysis of the publications involving *Inonotus rickii* (anamorph: *Ptychogaster cubensis* Pat.), an important pathogen of woody species, causing white rot. The research consisted of a review of the works available in the virtual data bases Periódicos CAPES, Scielo and Science Direct. As search terms, it was used the name of the species, both in the anamorphic and teleomorphic stages. All papers published in English, Spanish or Portuguese were considered, totaling 42 publications, being two books and 40 articles. The articles were distributed in 33 journals, highlighting Forest Pathology (9% of total publications), Plant Disease (7%), Mycotaxon (5%), Mexican Journal of Biodiversity (5%), Mycological Progress (5%). The distribution of the works according to the time included the interval from 1942 to 2016, and shows a linear pattern, ranging from 0 to 5 publications per year. A total of 96 authors were found, most participating in a single study. Only six authors presented more than three papers, with emphasis on Annesi, T., with seven, Motta, E. and Lopes, S.E, with six papers each. The main countries to host the surveys were Argentina, Italy and China, representing 19, 19 and 14% of them, respectively. According to the main focus of the approach, the papers were distributed in five categories: morphological, molecular and biochemical characterization (36.4%); geographical distribution and / or new records of locality (33.3%); Phytopathology, new host records and / or economic losses (19.6%); biotechnology application (9.1%); others (1.5%). The data show that despite five more decades of the first studies, *I. rickii i*s still little studied and devoid of

specialists. Most of the work is focused on basic research, usually for taxonomic purposes and restricted to a few countries, thus revealing a gap to be explored, considering its worldwide importance as a pathogen and decomposer of wood, or its potential for application in biotechnological processes.

2.1-128 Re-classification of *Exobasidium camelliae* and *E. gracile*, and their neotypification with topotype specimens

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Abstract: *Exobasidium camelliae* Shirai and *E. camelliae* var. *gracilis* Shirai (current name: *E. gracile*) described from Japan are well known as pathogenic fungi causing unique symptoms on *Camelliae* spp. in the world. *Exobasidium camelliae* infects young flower buds of *Camellia* and transforms the buds into galls like ball or glove covered by white-powdery hymenium. *Exobasidium gracile* infects new leaves or shoots of *Sasanqua* and forms conspicuous watery hypertrophy with white-powdery hymenium inside abaxial epidermis. Although extensive explorations in several Japanese herbaria were conducted, neither any type nor authentic specimens of the two species collected by Shirai were found. Based on art 9.7 in ICN (International Code of Nomenclature for Algae, Fungi, and Plants http://www.iapt-taxon.org/nomen/main.php?page=art9), we therefore neotypified both species with the fresh specimens of *E. camelliae* and *E. camelliae* var. *gracilis* collected from type locality, Tokyo, Japan. Since the description of the two species, several mycologists have pointed out that *E. camelliae* may produce a few muriform basidiospores and relatively divergent sterigmata on basidia, which were not mentioned in its original descriptions. Currently, exobasidiaceous fungi having muriform basidiospores produced from two or more divergent sterigmata on basidia with probasidial swellings were transferred into the new genus, *Muribasidiospora*, in Exobasidiaceae. Members of *Muribasidiospora* are commonly isolated from red leaf spots on *Rhus* (Anacardiaceae) and are phylogenetically closely related to species of *Exobasidium*. Our neotype specimens of *E. camelliae* and *E. gracile* showed muriform basidiospores from two to four divergent sterigmata on basidia with probasidial swellings. In addition, their neotype cultures were included in the genus *Muribasidiospora* clade based on ITS and LSU phylogenetic analyses. Therefore, new combinations, *Muribasidiospora camelliae* and *Muribasidiosora gracile*, are proposed.

2.1-129 Fumonisin production by *Fusarium fujikuroi* species complex from cereals

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Abstract: To assess the risk of fumonisin contamination in cereals, we isolated *Fusarium fujikuroi* species complex (FFSC) strains from barley, maize, rice and soybean samples from 2011 to 2015. A total of 878 FFSC isolates were mostly from maize and rice, and species of the isolates were determined using the DNA sequence of the translation elongation factor 1-α (*TEF-1α*) and RNA polymerase II (*RPB2*) genes. The rice samples were dominated by *F. fujikuroi* (47.4%), *F. proliferatum* (27.3%), and *F. concentricum* (15.1%), whereas maize samples were dominated by *F. verticillioides* (33.9%), *F. fujikuroi* (25.3%), and *F. proliferatum* (21.1%). Seventy representative isolates were analyzed using a maximum likelihood method, showing each species was genealogically exclusive in the phylogenetic tree. Fumonisin

production potential was tested using a PCR assay for *FUM1*, one of the fumonisin biosynthesis genes. Most of the isolates tested (94%) were positive for *FUM1* and all the isolates of *F. fujikuroi, F. proliferatum, F. verticillioides* and *F. thapsinum* were positive regardless of their host origin. Seventy-seven representative isolates positive for *FUM1* were examined for fumonisin production in rice medium. The majority of *F. proliferatum* (26/27, 96.3%), *F. verticillioides* (16/17, 94.1%) and *F. fujikuroi* (19/25, 76.0%) produced both FB1 and FB2. Out of 19 fumonisin-producing *F. fujikuroi*, 16 produced> 1000 µg/g of fumonisins (FB1+FB2) in rice medium. These results suggest that *F. fujikuroi* can produce high levels of fumonisins similar to *F. verticillioides* and *F. proliferatum*.

2.1-130 Quantification and discrimination of *Fusarium oxysporum* isolates using VIS/NIR reflectance spectroscopy

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Abstract: Today's agriculture requires fast, precise and non-destructive methods to quantify and discriminate pathogens in plants before the symptoms of disease are visible. VIS/NIR spectroscopy is a non-invasive analytic method which is very appropriate for various agricultural applications due to its fast data acquisition and capacity to determine multiple parameters. The object of this study was to evaluate a quantification and discrimination method of two isolates of *Fusarium oxysporum* (F05 and F07) in tomato plants using reflectance spectroscopy in the VIS/NIR region. The methodology used shows evidence of an increase of Colony Forming Units (UFC) in the root and leaves of the plant as the incubation time increases, with findings of similar concentrations of the pathogens up to 12 days post infection (dpi). After the first two weeks, a larger growth rate in the roots was observed up to 1590 UFC/gr, but maintaining the high correlations between the UFC measured on both organs (R^2=95.60, p=0.01). There were 9 regions in the spectral range of 380-1000nm that showed a high correlation with the increase of the pathogen in the plant, but of the many wavelengths that had high coefficients of determination (R^2) with the UFC, only a few also had low p values (<0.05), for example, at 994 nm (R^2=89.74 y p=0,01). Finally, the Discriminating Linear Analysis allowed the classification of two strains of *F. oxysporum* isolated from different plant species, increasing the percentage of precision by increasing the incubation time, with strain F05 the most clearly isolated one through the use of this method. The results suggest a high correlation between the concentrations of the pathogen and the changes in the reflectance spectra in the incubation period of the plant disease, when symptoms are still not visible, and that it is possible to quantify and discriminate the pathogen using few wavelengths determined through reflectance spectroscopy.

2.1-131 Demystifying *Fusarium* diseases of tomato

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Abstract: Soil-borne diseases such as those caused by *Fusarium* species are of great concern in tomato production worldwide. Two major soilborne diseases, Fusarium wilt (caused by *F. oxysporum* f. sp. *lycopersici (FOL)* and Fusarium crown and root rot of tomato (caused by *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), are widespread and destructive in major tomato-growing regions worldwide. Based on molecular markers and pathogenicity tests, we isolated and characterized a novel genotype of *F. oxysporum* f. sp. *lycopersici* race 3. Under certain conditions, this novel genotype can cause symptoms

identical to Fusarium crown and root rot, making field diagnosis of Fusarium wilt and Fusarium crown and root rot difficult. This novel genotype, which most likely came from Florida via-seedborne transmission, belongs to VCG 0033. We demonstrated that FOL race 3 was readily recovery from seeds harvested from infected plants in commercial fields. The incidence of FOL race 3 from tomato seed batches was reduced by ~98% without affecting seed germination or vigor when FOL race 3-infected tomato seed was immersed in a hot water bath for 10 min at 55°C. FOL race 3 was eradicated from seed without reducing seed germination or vigor when seeds were immersed in a fungicide (Azoxystrobin or Fludioxonil) hot-water bath for 10 min at 55°C. Currently, two genotypes exist of FOL race 3, as well as the existence of FOL race 2, and FORL in California. We also demonstrated that Fusarium wilt is an inoculum-dependent disease. At a high inoculum level, a FOL race 3 resistant cultivar was asymptomatically colonized by FOL race 3. Host range experiments revealed that California and Florida genotypes of FOL race 3 are able to sustain themselves on the roots of many plant species, including weeds, but their capability of invading xylem tissue is limited to tomato.

2.1-132 Molecular interactions of *Fusarium graminearum* and barley

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Abstract: Understanding the interactions between plant pathogenic fungi and host plants is vital for the management of disease symptoms. *Fusarium graminearum* is a fungal plant pathogen and the primary causal agent of Fusarium Head Blight (FHB) on wheat and barley, which reduces quality and quantity of grain yields. FHB is partially controlled by fungicides, with no strong resistance available in host crops. Because of the inefficiency of fungicides in controlling some plant diseases, new methods for disease prevention and treatment need to be established. Plant penetration and colonization are two important stages of infection, which is where we focused our study. Barley has a moderate resistance response where *F. graminearum* cannot spread from individual infection sites. The plant produces a focal accumulation of plant defense compounds (cellulose and lignin) at the infection sites of trichomes on the surface of barley florets in response to *F. graminearum* inoculation. Previous work has shown greater numbers of focal accumulations (foci) on barley varieties with small, dome trichomes than varieties with long, prickle-like trichomes. The genetic basis of the differential response seen in trichome morphologies is being investigated through the use of near-isogenic barley varieties. *In vitro* and *in planta* assays characterize important plant and fungal responses during infections stages leading to disease development. A locus has been identified in barley as important for the defense response to *F. graminearum*. Light and confocal laser scanning microscopies are used to visualize these interactions. Genes of interest have been identified as important for *F. graminearum* – barley interactions. The molecular interactions studied in this work provide new areas to develop disease management tools.

2.1-133 Biological control of Fusarium head blight: *Bacillus velezensis* RC 218 on wheat yield, grain quality and deoxynivalenol accumulation

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Abstract: Fusarium head blight (FHB) is a worldwide relevant disease that causes important losses in grain yield, quality and safety. FHB in Argentina is mainly caused by *Fusarium graminearum sensu stricto* when high humidity conditions prevail during wheat anthesis stage. Management strategies to control the disease includes fungicide use, crop rotation, soil tilling and planting of less susceptible varieties, none of them being completely effective. Biological control offers an environmentally friendly tool that

could be incorporated in the frame of an integrated pest management. Previous studies by our research group have showed the effectiveness of *Bacillus velezensis* RC 218 on FHB severity and deoxynivalenol (DON) accumulation. The aim of the present study was to analyze the impact of *B. velezensis* RC 218 over yield and quality parameters of wheat infected by *F. graminearum*. Two field trials with a complete randomized design were carried out in Marcos Juárez, Córdoba and Necochea, Buenos Aires, Argentina. During anthesis stage the following treatments were applied: i) *Fusarium graminearum*; ii) *B. velezensis* RC 218; iii) *F. graminearum* + *B. velezensis* RC 218 and iv) water + 0.05% tween (control). At 21 days post inoculation, FHB incidence and severity were evaluated and grains were harvested at maturity. Yield components, quality parameters, *Fusarium*-damage kernel (FDK) and DON content were measured. *B. velezensis* RC 218 showed effectiveness as biocontrol agent in both trials carried out in Marcos Juarez and Necochea. A disease incidence reduction of 29% and 20% was observed in both field trials. Severity was also significantly reduced by 52% and 58%. No significant differences were observed in grain yield nor in 1000-grain weight. A significant reduction of FDK was also observed. The results suggest that the application of *B. velezensis* RC 218 is a valuable tool to maintain good quality and safety of harvested wheat grains.

2.1-134 The coffee berry borer may be a vector of *Fusarium* spp. in coffee fruits

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Abstract: The coffee berry borer (CBB) causes great damage to coffee crops. When the CBB attacks coffee fruits, it also facilitates the entrance of fungi that may cause coffee berry disease (CBD). It is not known if the fungi colonize through the opening made by the CBB or if the CBB is a vector that introduces them to the fruit. Also, it is not clear which fungi cause CBD; only *Colletotrichum* spp. have been identified. To determine if CBB is a vector, CBBs were collected from the field and plated on PDA. A total of 158 fungal colonies were isolated and identified using taxonomic keys and DNA sequences. ITS and EF-1 alpha genes were sequenced and compared using BLASTn tool in GenBank. The fungi identified included several species of *Fusarium*, often considered pathogenic but not known to be pathogens of coffee fruits. Several *Fusarium* spp. were also isolated from fruits with CBD in the field suggesting that the CBB may be a vector of these fungi. These results are important for the management of CBD in Puerto Rico and other coffee-producing countries.

2.1-136 Pathogenicity of *Verticillium dahliae* in Australian cotton cannot be based on VCG alone

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Abstract: *Verticillium dahliae* is a soil-bourne ascomycete responsible for Verticillium wilt, a disease that impacts the billion-dollar cotton industry in Australia. Over the years *V. dahliae* has been classified in several ways including defoliating (D) and non-defoliating (ND) pathotypes, and vegetative compatibility groups (VCGs). In cotton it has appeared that the defoliating VCG1A causes the most severe disease symptoms, while the non-defoliating VCG2A causes less disease symptoms. However, in Australian cotton fields the D VCG1A is causing minimal disease, while the ND VCG2A has been responsible for more severe disease and crop loss. The reason for this difference in virulence expression in Australian isolates compared to global isolates is currently not understood. To get a better understanding of the variation within the NSW *V. dahliae* collection, PCR analysis of inter-simple sequence repeats (ISSR) was undertaken and the results used to map a dendrogram visualising the relationships between samples,

while isolate virulence was analysed in a glasshouse over seven weeks. Australian *V. dahliae* VCG1A and VCG2A isolates were inoculated into four different cotton varieties at a concentration of $1x10^6$ conidia/ml by root dipping, and disease severity scored twice weekly. The assay revealed that while Australian VCG1As do cause severe damage and mortality, it is not exclusive to the 1A VCG type, with VCG2A also causing plant mortality. The resulting dendrogram showed that NSW *V. dahliae* isolates do not entirely cluster according to VCG, but seem to also group according to virulence. These results suggest that the disease potential of an isolate is not tied to VCG, and that perhaps a better classification is needed to fully describe *V. dahliae* isolates. The dendrogram also allows for the identification of virulent and non-virulent *V. dahliae* samples from the NSW historical collection. This capability will allow for a selection of diverse strains for genome sequencing to help us understand the differences between isolates. This will hopefully allow for the development of better diagnostics for a more rapid on farm response to Verticillium wilt.

2.1-153 Anthracnose on *Eugenia uniflora* caused by *Colletotrichum siamense* in Brazil

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Abstract: *Eugenia uniflora* (Myrtaceae), also known as 'pitanga', is a native fruit tree from Brazil cultivated throughout the country in domestic gardens and also commercially in the Brazilian Northeast. Recently, anthracnose has been frequently observed on leaves, flowers and fruits from *E. uniflora* in Brazil. In 2017 symptomatic plants were collected in the Brazilian region of Distrito Federal, and 9 isolates of *Colletotrichum* sp. were obtained from lesions affecting leaves, flowers and fruits. Initially, the isolates were identified as a species of the *C. gloeosporioides* complex, according to the characteristics of conidia (hyaline, aseptate, fusiform with obtuse ends, smooth-walled, guttulate, 13 to 18 µm long and 4.5 to 8 µm wide). After the genomic DNA of all 9 isolates were extracted, sequences were generated for the GAPDH and APMAT regions for an indication of identity of each isolate by means of Bayesian analyses. Subsequently, one isolate of each organ (leaf, flower and fruit) was selected, and the sequences from CAL, TUB2, CHS-1 and ACT were obtained for the phylogenetic analyses. For prove of pathogenicity, Koch's postulates were performed. Fruits and leaves (adaxial and abaxial surface) of *E. uniflora* were inoculated with a 5 µl-drop of a suspension of 10^6 conidia/ml. A drop (5 µl) of distilled water was deposited on control leaves. Fruit rot symptoms were observed on inoculated fruits three days after inoculation and on inoculated leaves five days after inoculation. The control fruits and leaves did not present any symptoms. The fungus was reisolated from the lesion and identified as *C. siamense* using the methods described above. Although *C. siamense* has a wide host range, this pathogen was reported only on *Acca sellowiana*, *Coffea* sp. and *Fragaria* × *ananassa* in Brazil. To our knowledge, this is the first report of *C. siamense* causing anthracnose on *E. uniflora* in Brazil. Financial support: FAP-DF, Capes, CNPq and UnB.

2.1-154 *Colletotrichum* species causing anthracnose of *Capsicum* in South East Asia

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Abstract: *Colletotrichum* is one of the most important groups of pathogenic fungi causing anthracnose in crop plants all over the world. Correct identification and knowledge of the pathogenicity of

Colletotrichum spp has great significance to the Australian fruit and vegetable industries, where there are serious biosecurity implications from incursion by new exotic pathogens entering from SE Asia. Recent redefined taxonomy using multigene phylogenetics of the *Colletotrichum* spp infecting chili fruit (*Capsicum*) in Australia identified a novel species, *C. cairnsense*, unique to Australia. The aim of this study was to identify *Colletotrichum* species isolated from diseased chili fruits collected from Thailand, Indonesia, Malaysia, Sri Lanka and Taiwan. The taxonomy of isolates was confirmed using morphological characters, multi gene phylogenetic analysis and pathogenicity based on disease development in inoculated chili fruit. Taxonomic analysis of isolates from SE Asian countries showed that *Colletotrichum scovillei* and *C. truncatum* were the predominant pathogens causing anthracnose in chili. Other *Colletotrichum* species causing anthracnose in chili included *C. fructicola, C. karstii, C. siamense,* and four new species from the *C. gloeosporioides* complex and one potential new species closely related to *C. cliviae*. The dominant species causing anthracnose of chili in SE Asia was *C. scovillei* which is yet to be identified in Australia. Although *C. siamense* had been reported from many plant species, this is the first report of identification of *C. siamense* causing anthracnose in Thailand, Indonesia and Sri Lanka. This is also the first report of *C. fructicola* infecting chili from Thailand and Taiwan. Identification of several new species from SE Asia increases the risk of incursion of exotic species to Australia which will have a significant impact on agriculture, biosecurity and quarantine. These results encourage more surveys to be made in chili anthracnose in the future and emphasizes the importance of correct disease diagnostic methods and management practices.

2.1-155 Systematic analyses of terrestrial obligate *Synchytrium* species based on molecular characterisation of herbarium specimens

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Abstract: There are almost 300 described species of the chytrid genus *Synchytrium* but currently there is only reference DNA sequence data for 18 of these species. The last systematic treatment of *Synchytrium* was published by Karling in 1964, pre-dating molecular technologies. This genus includes the species *S. endobioticum*, which is the causal agent of potato wart disease. This pathogen is a serious threat to trade and is included on the list of select agents of the USA. The identification and the publication of sequence data of the most closely related species to this high risk pathogen is essential in order to avoid false positive detection in metagenomics testing that could lead to trade disputes. To accomplish these tasks, tissue of herbarium specimens was sampled from both the Canadian National Mycological Herbarium (DAOM) and the United States National Fungus Collections (BPI). This sampling effort covered 75% of all known species of *Synchytrium* as well as some unidentified specimens. All available type specimens and a number of specimens observed and utilised by Karling in his morphological examinations of the genus were included. A DNA extraction protocol for herbarium specimens, previously developed for rusts and downy mildews, was optimised for *Synchytrium*. Portions of the ribosomal cistron (SSU and ITS regions) were amplified and sequenced using Chytridiomycota specific primers. To confirm the identity of the plant host the chloroplastic ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene was also amplified and sequenced. Finally, phylogenetic analyses were performed to assess the relationships of species in the genus and to provide a molecular taxonomic framework for further systematic work on *Synchytrium*.

2.1-156 Genetic diversity of bacterial isolates causing brown blotch disease on cultivated mushrooms and selection of the antagonists in Korea

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Abstract: We examined genetic characterization of 19 isolates identified as *Pseudomonas tolaasii* by the sequence analysis of 16S rRNA and WLA assay. Also two antagonists against P. tolaasii, HC1 and HC5 were selected and their control efficacy of brown blotch disease was investigated in this study. From the mushrooms that shows symptoms of bacterial brown blotch disease, 180 bacterial strains were isolated, and 19 isolates of them were identified as *P. tolaasii* with 16S rRNA analysis. Two isolates (CHM01, CHM02) of the 19 isolates did not form white line in white line test. The 19 isolates formed in the same group with analysis of nucleotide sequence similarity of 16S rRNA gene. However, 2 strains (CHM01, CHM02) formed in different group to the rest 17 isolates with analysis of nucleotide sequence similarity of rpoB gene. The patterns of RAPD and REP-PCR of the 2 strains (CHM01, CHM02) were different to the 17 strains. Also, PCR with the primers designed from tolaasin biosynthesis gene did not show any DNA band from the two isolates while specific band was appeared on the 17 isolates by the PCR. By thin layer chromatography (TLC) analyzing of lipopeptide, the 2 isolates (CHM01, CHM02) showed spots of different Rf value to P. tolaasii's. The CHM01 and CHM02 isolates were weaker than the 17 isolates in inhibition of hyphal growth of *Pleurotus ostreatus* and *Flammulina velutipes*. Based on these results together, 17 isolates are typical *P. tolaasii*, whereas CHM01 and CHM02 are considered as low virulent variants of *P. tolaasii*. Further taxonomic research is needed for correct identification of these two isolates. Control efficacy of brown blotch disease by HC1 treatment was 69% on Agaricus bisporus, 68% on Flammulina velutipes and 55% on pleurotus ostreatus respectively. Control efficacy of brown blotch disease by HC1 treatment was 73% on Agaricus bisporus, 78% on *Flammulina velutipes* and 71% on *Pleurotus ostreatus,* respectively.

2.1-169 Timely evolution of ectomycorrhizal symbiosis spurred adaptive radiation of Boletales

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Abstract: The acquisition of mutualistic hosts may provide symbionts with ecological opportunities, which serve to drive evolutionary diversification. However, it remains unclear and controversial whether the origination of ectomycorrhizal (ECM) symbiosis promotes adaptive diversification of fungi. Importantly, a recent empirical study suggested that the evolution of ECM symbiosis did not necessarily result in adaptive diversification. On the basis of a high-resolution phylogenetic analysis, we herein show how timing of ECM evolution can determine the scale of adaptive divergence in a species-rich fungal lineage. For achieving this, we used nucleotide sequences of 87 single-copy genes and reconstructed the evolutionary history of the fungal order Boletales, which comprise over 1300 species. High-resolution phylogeny of Boletales indicated ECM symbiosis independently evolved at least four times in Boletales, specifically in the stem positions of Suillineae, Sclerodermatineae sensu strict, Paxillaceae, and the clade containing ECM lineages of Boletaceae. Among the four origins, a rapid increase in the net diversification rates (speciation rate minus extinction rate) and a rapid ecological divergence (transition from saprotrophic to mycorrhizal trophic state) appeared to occur only in the clade of Boletaceae, which acquired ECM symbiosis third in time in Boletales. These results contradict the hypothesis that the earliest ECM fungal lineages could have obtained broader niche spaces, as expected by the theory of

evolutionary priority effect. Rather, owing to heterogeneity in host availability over space and time, high potentials for allopatric speciation may be available only for "timely innovators", which successfully expanded their geographical ranges via initiating ECM symbiosis with novel hosts (e.g., ECM rosids) that had not been associated with older fungal lineage. Although further studies are still required to corroborate our results and interpretations, the present study contributes to our understanding of how acquisition of ECM hosts could accelerate the evolutionary diversification of fungi.

2.1-170 Species diversity, phylogeny, divergence time and biogeography of the genus *Sanghuangporus* (Basidiomycota)

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Abstract: 'Sanghuang' is a popular fungus used as a Chinese traditional medicine, but little is known about its origin and biogeography. The aim of this study was to characterize the molecular relationships, origin and biogeographical distribution of *Sanghuangporus*. We used multi-locus phylogenetic analyses to infer the phylogenetic relationships. In addition, based on Bayesian evolutionary analysis using sequences from the internal transcribed spacer (ITS), nuclear large subunit rDNA (nrLSU), translation elongation factor 1-α (EF1-α), and the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2), we used a fungus fossil-based approach to gain insight into the divergence time of species in *Sanghuangporus*. The molecular phylogeny strongly supports monophyly of *Sanghuangporus* (MP = 100%, BS = 100% and BPP = 1.00), and thirteen species are recognized in the genus. The Bayesian uncorrelated lognormal relaxed molecular clock using BEAST and reconstructed ancestral areas indicate that the maximum crown age of *Sanghuangporus* is approximately 30.85 million years. East Asia is the likely ancestral area (38%). Dispersal and differentiation to other continents then occurred during the late Middle Miocene and Pliocene. The ancestor of *Sanghuangporus* probably originated in palaeotropical Northeast Asia and covered Northeast Asia and East Africa during the Oligocene-Miocene, hosted by plants that expanded via the 'Gomphotherium Landbridge'. Six kinds of dispersal routes are proposed, including intercontinental dispersal events of three clades between Northeast Asia and East Africa, between East Asia and North America, and between Northeast Asia and Europe.

2.1-171 Investigation of mosaic fitness in the world's largest individual organism (*Armillaria ostoyae*)

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Abstract: Somatic mutation is the process by which novel genetic variation arises within an individual. For most multicellular organisms, somatic mutations are unlikely to be passed along through the germline because of irreversible differentiation. Filamentous fungi, whose cells are totipotent, hold the potential for an exaggerated rate of somatic mutations to enter the germline. This presents a scenario that could result in differential fitness across an individual, i.e. a mosaic of fitness. At small spatial scales, the potential for somatic mutation leading to differences in fitness across and individual is insignificant. However, at large spatial scales, this potential becomes increasingly probable, resulting in the possibility of a selective mosaic across an individual. The 'Humongous Fungus' in eastern Oregon is an individual genet of *Armillaria ostoyae* that covers an estimated 8.8 km^2, presenting a unique opportunity to test the hypothesis of mosaic fitness and selection in the world's largest individual organism. Initial sample collection of the Humongous Fungus and a nearby genet was conducted in October 2017. Samples were taken from 15 sites within the area of genet D (Humongous Fungus) to canvas as much of the

organism as possible. Along with samples from genet D, 10 samples from genet E, another large *Armillaria ostoyae* individual nearby, was also sampled for comparison. Samples of infected wood were collected from diseased host trees and fungi cultured from them on 2% malt agar containing chloramphenicol and Benomyl. Each of these cultures represent a section of The Humungous Fungus that occupies a distinct spatial area that is different from the other collected samples. Somatic incompatibility between isolates is being assessed using complementation tests to reproduce earlier results. Whole genome sequencing of these isolates is utilizing a combination of short- (Illumina) and long-read (Oxford Nanopore) technologies to generate a highly contiguous assembly that can be used as a reference to identify SNPs from low-coverage strain-level sequencing using Illumina. The occurrence of SNPs in coding regions will be determined from genome annotations, and synonymous and non-synonymous changes will be recorded for evidence of differences in phenotype.

2.1-172 Where do new genes come from? The origin of novelty niche transition in the genus *Amanita*

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Abstract: While ectomycorrhizal symbioses are among the most prominent mutualisms in the tree of life repeatedly and convergently evolved among fungi, the genetic mechanisms enabling the origin of an ectomycorrhizal symbiosis remain a mystery. In particular, little is known about the gain of any gene family actively enabling ectomycorrhizal symbiosis maintained across all species of an ectomycorrhizal lineage. Instead, the focus has been gene loss, especially the loss of decomposition genes. Here we identified 109 gene families unique to three diverse ectomycorrhizal *Amanita* species not found in decomposer *Amanita* species. When expression profiles are quantified for the ectomycorrhizal *Amanita muscaria*, genes in these families were more highly expressed during symbiosis compared to the genes in the families shared by both ectomycorrhizal and saprotrophic species suggesting these unique gene families play a functional role in the symbiosis. To understand the origins of these unique gene families, we built a pipeline to identify mechanisms of acquisition. We tested whether each gene family resulted from horizontal gene transfer (HGT), de novo gene creation, or simply represent quickly evolving gene families whose homologs remain unrecognized in decomposer lineages. Two, six and eight gene families were successfully categorized as resulting from de novo gene creation, HGT, or fast-evolving gene families. We also detected high proportions of genes that originated from HGT and fast-evolving gene families, highly expressed in ectomycorrhizal root tips. De novo genes were present in ectomycorrhizal root tips but not highly expressed. Specifically, one gene family derived from HGT encodes a putative 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can convert ethylene's precursor, ACC, to alpha-ketobutyrate, and reduce the concentration of ethylene in a plant. In past research, ethylene can reduce ectomycorrhizal symbiosis. This evidence supports that acquiring a new gene family may help fungi transition into a new niche.

2.1-173 A systematic revision of pyrophilous species of *Pholiota* described from North America

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Abstract: A systematic investigation of North American pyrophilous species of *Pholiota* is presented based on morphological and molecular examination of primarily type and other historical collections.

Much taxonomic confusion surrounds application of the names *P. brunnescens*, *P. carbonaria*, *P. castanea*, *P. fulvozonata*, *P. highlandensis*, *P. molesta*, and *P. subsaponacea* with multiple names applied to single species, and multiple species described more than once. Molecular annotations using ITS and *rpb2* are used to clarify application of these names in a phylogenetic context. As a result the following heterotypic synonymies are proposed: *P. highlandensis* (syn. *P. carbonaria* and *P. fulvozonata*); *P. molesta* (syn. *P. subsaponacea*); and *P. brunnescens* (syn. *P. luteobadia*). In addition the species *P. castanea*, known previously only from the type collection, is found commonly on burned sites in the Gulf Coast and southeast regions of the U.S. Historical collections of this autonomous species were previously referred to as *P. highlandensis*. Overall, the burn habit appears to have evolved independently at least three times in *Pholiota* in *P. brunnescens*, *P. castanea*, and the complex containing *P. highlandensis* and *P. molesta*. Endophytic and endolichenic stages have been deduced for *P. highlandensis*, the most widely distributed of the four species. Lastly, the 'body snatcher' hypothesis is presented that explains the maintenance of some pyrophilous fungi in ecosystems.

2.1-174 Ceraceosorales—An enigmatic lineage of Ustilaginomycotina that reveals an extreme case of intragenomic variation of ribosomal DNA sequences

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Abstract: The enigmatic Ustilaginomycotina lineage Ceraceosorales consists of only one described genus *Ceraceosorus*. The genus currently has three known species of plant-associated fungi—two producing a plant pathogenic teleomorphic stage and one known only from its saprobic anamorphic stage—each of which is rarely found with limited known geographical distributions. The plant pathogenic species (*C. africanus* and *C. bombacis*) are associated with *Bombax costatum* and *B. ceiba* (Malvaceae) in Africa and India, respectively. A previous study has shown extreme intragenomic variation of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) repeat in *C. bombacis* and *C. guamensis,* seemingly presenting a case where concerted evolution of rDNA sequences has failed. The same study also showed complete mismatch between the primers commonly used for ITS amplification with both tested species of *Ceraceosorus*. Since these findings may have broad implications for metagenomic studies as well as fungal barcoding efforts, it is interesting to deeply investigate this phenomenon on a broader scale by expanding taxon sampling and examined rDNA regions. In this study, we will present a recently discovered anamorphic *Ceraceosorus* species collected from a healthy leaf phylloplane in Louisiana, USA. We incorporated all known *Ceraceosorus* species, as well as representatives of other Ustilaginomycotina lineages to investigate the intragenomic variation of the rDNA region through PCR-cloning and high-throughput sequencing. Our analyses revealed that the sequence heterogeneity of the ITS region exists in all examined *Ceraceosorus* species and thus most likely emerged in the common ancestor to these. The intragenomic variation in *Ceraceosorus* also expands into the small subunit (SSU) and large subunit (LSU) regions, but detected variant sites in the SSU and LSU regions were significantly less than those found in the ITS region. Similar intragenomic variation of the rDNA region was not found in any other investigated Ustilaginomycotina lineage. We will present findings from this study, as well as discuss a hypothesis for rDNA evolution in Ceraceosorales.

2.1-175 First insights into the genomes of *Lichenothelia* and *Saxomyces* (Dothideomycetes, Ascomycota)

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Abstract: Natural rocks and anthropogenic substrates are often colonized by black microcolonial rock-inhabiting fungi (RIF). Due to their polyextremotolerance, RIF can colonize the harshest environments on earth; it was even shown that they can tolerate long-term exposure in outer space. *Lichenothelia* and *Saxomyces* are two genera of RIF within the class Dothideomycetes and for which the evolutionary relationships have been recently clarified. Here two *Lichenothelia* and two *Saxomyces* species were sequenced on an Illumina platform to generate high-throughput data for draft genome assemblies. The four genomes were assembled and together with 250 Dothideomycetes assemblies (retrieved from NCBI and JGI portals) were mined for orthologous single copy genes using Benchmarking Universal Single Copy Orthologs (BUSCO) pipeline. A comprehensive, genome-based phylogeny of the class is reconstructed both with a maximum likelihood concatenation approach (IQ-TREE) and a multispecies coalescent approach (ASTRAL-II). The minimum amount of genetic information needed to produce a reliable phylogeny is also assessed by multiple runs of an increasing number of random selected orthologs. Tree topologies are evaluated and compared with the concatenation tree (3004 genes, ~$2*10^6$ bp alignment) using Internode Certainty (IC), Tree Certainty (TC) and Robinson-Foulds distance metrics. Further, the overall evolutionary rates of fungal genomes are here compared among fungi with different lifestyles (lichens, plant and human pathogens, saprotrophs, RIF) to test whether ecological niches trigger faster or slower genome evolutionary rates. Comparative genomic techniques are also applied using the genomes of the most extremophile fungus *Cryomyces antarticus* and the lichenized fungus *Trypethelium eluteriae* to assess whether features related to extremotolerance and lichenization are present in *Lichenothelia* genome.

2.1-176 The coevolution of *Tremella* species and their hosts

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Abstract: The genus *Tremella* sensu lato is featured by mycoparasitic life style. They can parasitize a variety of species belonging to Basidiomycetes and Ascomycetes. *Tremella* species are delimitated mainly by the phylogenetic relationship because of the morphological characters are scarce. Whether hosts and their parasites speciate by cospeciation, or through host switching, is a key issue in host-parasite evolution. Understanding the evolutionary dynamics of parasitism of *Tremella* spp. and their hosts could provide evidence partly for the taxonomy of *Tremella* species. The phylogenies of *Tremella* spp. and hosts were generated based on the ITS and D1D2 sequences of 45 and 100 species, respectively. We investigated the congruence between parasites and hosts phylogenies using distance-based and event-cost based methods. Distance-based test supported an overall congruence between the phylogenies of *Tremella* spp. and their hosts. Reconciliation reconstructions determined host-switching (27-30) other than cospeciation (10-13) is a major impetus driving *Tremella* species diversity. The number of failure-to-diverge case (62) is also high and stable, independently of the cost regimes, which means that some *Tremella* species are able to parasitize closely related species to be generalists.

2.1-185 Towards a global phylogeny of *Cladonia*, one of the world's most diverse and widespread lineages of lichens

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Abstract: *Cladonia* is one of the world's most charismatic and cosmopolitan lineage of lichens. However, efforts towards explaining why the genus is so widespread and diverse have been stymied due to the lack of a phylogeny with global representation and extensive sampling. This presentation focuses on exploring preliminary research ideas regarding (1) centers of biodiversity for the genus, (2) the use of Next Generation Sequencing to reconstruct evolutionary relationships among species with metagenomic DNA, and (3) future pathways to examine ecological, evolutionary, or historical factors contributing to species radiations.

2.1-186 The mating system and population structure of *Physconia muscigena* (Ach.) Poelt.

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Abstract: The principal goals of this study are to describe the population structure of lichen *Physconia muscigena* (Lecanorales) and to investigate possible phylogenetic relationships among the closely related species *P. muscigena* and *P. isidiomuscigena*. The project is focused on clarification of the mating system of this lichen (homothalic or heterothalic), on characterization and population analysis of the mating-type genes within the populations. Are there reasons for loss of sexual reproduction in many populations? Additionally we investigate phylogenetic relationships within the genus *Physconia* (multigene analysis).

2.1-187 Oligocene origin and drivers of diversification in the genus *Sticta* (Lobariaceae, Ascomycota)

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Abstract: A major challenge to evolutionary biologists is to understand how biodiversity is distributed through space and time and across the tree of life. Diversification of organisms is influenced by many factors that act at different times and geographic locations but it is still not clear which have a significant impact and how drivers interact. To study diversification, we chose the lichen genus *Sticta*, by sampling through most of the global range and producing a time tree. We estimate that *Sticta* originated about 30 million years ago, but biogoegraphic analysis was unclear in estimating the origin of the genus. Furthermore, we investigated the effect of dispersal ability finding that *Sticta* has a high dispersal rate, as collections from Hawaii showed that divergent lineages colonized the islands at least four times. Symbiont interactions were investigated using BiSSE to understand if green-algal or cyanobacterial symbiont interactions influenced diversification, only to find that the results were driven almost completely by Type I error. On the other hand, another BiSSE analysis found that an association with Andean tectonic activity increases the speciation rate of species.

2.1-188 A new lineage of fruticose lichens belonging to the Trapeliaceae (Trapeliales, Ascomycota) from Alagoas, NE Brazil

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Abstract: Brazil is the country with the highest lichen biodiversity on earth, but much of it remains as yet undiscovered. A field trip to rock outcrops in a remnant of Atlantic rain forest in Alagoas, viz. Reserva Biólogica de Pedra Talhada, near the city of Quebrangulo, in the middle of the semiarid region in Northeast Brazil, revealed an unusual fruticose lichen. It forms dense mats on siliceous rock that is influenced by run-off water. It typically grows at the upper ends of gullies that are occupied lower down by cyanophilic lichens such as *Peltula* and *Jenmania*. The habitat is extremely poikilohydric; this lichen is occasionally submerged, but usually completely dry. The morphology of this species is enigmatic: it somewhat resembles a *Leprocaulon s.lat.*, but it differs from all known species in that genus by the chemistry; apothecia are not present, but pycnidia are not uncommon. The chemistry is gyrophoric acid, which was not known from any fruticose lichen with chlorococcoid algae. Sequence work places the new species right in the Trapeliaceae, close to or most probably inside *Trapeliopsis*, with which it indeed shares the chemistry. We propose to describe it in *Trapeliopsis*, and name the species in honour of the owner of the reserve, Anita Studer, as *T. studerae*. It represents a new and independent lineage of fruticose lichens.

2.1-189 A new phylogenetic classification for the lichen family Gomphillaceae

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Abstract: Ascomycota has the highest number of species within the kingdom Fungi, with about one third of these species being lichenized. A particular group of lichenized fungi are those that dwell on leaves of vascular plants, the so-called foliicolous lichens. Gomphillaceae is the most species-rich family of foliicolous lichens, but also contains species growing on other substrata, such as bark, rocks, and soil. Despite that the family features many morphological peculiarities, in particular its conidiomata, the hyphophores, the genus-level classification of Gomphillaceae remained thus far unresolved. This study revised the diversity of foliicolous lichens in the main Brazilian biomes, with emphasis on Gomphillaceae, and made a first attempt at assembling a broad molecular phylogeny using the mitochondrial small subunit and nuclear large subunit rDNA markers (mtSSU, nuLSU), based on new collections from leaves made in areas of Caatinga (in higher altitude forest enclaves), Atlantic Forest, and Amazon forest, completed by collections from other areas in the neotropics (Mexico, Guatemala, Costa Rica, Panama, Cuba). To complete the analysis, we used the method of morphology-based phylogenetic binning, which places species that do not have molecular data into the molecular phylogeny based on phylogeny-weighted assessment of the morphological characters. Four hundred and sixty-seven sequences were obtained for the mtSSU and nuLSU, from three hundred and thirty-seven specimens. As a result, we describe 11 genera: *Spinomyces* (*Aderkomyces albostrigosus* group), *Roselviria* (*Aderkomyces purulhensis* group), *Serusiauxiella* (*Tricharia farinosa* group), *Caleniella* (*Calenia triseptata* group), *Lumbrispora* (*Echinoplaca diffluens* group), *Aptrootidea* (*Echinoplaca marginata* group), *Adelphomyces* (*Gyalideopsis epithallina* group), *Batistomyces* (*Tricharia hyalina* group), *Sipmanidea* (*Echinoplaca verrucifera* group), and *Bezerroplaca* (*Echinoplaca lucernifera* group). We also reinstate four genera and propose new combinations in *Linhartia* (*Psorotheciopsis patellarioides* group),

Microxyphiomyces (*Tricharia vainioi* group), *Psathyromyces* (*Aderkomyces heterellus* group), and *Sporocybomyces* (*Echinoplaca leucotrichoides*). This changes the number of genera recognized within the family from twenty-four to approximately forty.

2.1-190 *Usnea* in mointain rainforests of Tanzania: A study of species diversity and phylogenetic relationships

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Abstract: *Usnea* (*Parmeliaceae*) is a species rich lichen genus with more than 350 species worldwide. It is characterized by a fruticose habit, an elastic central chord inside the branches and the presence of usnic acid in the cortex. Many species are considered to have wide distributions. *Usnea* in Africa has been insufficiently studied and the availability of molecular data is very limited. The taxonomy of *Usnea* is difficult due to the high morphological plasticity and chemical variability of the species. Therefore, molecular data is much needed in order to ascertain species recognition. In this study, a dataset of sequences of five nuclear markers (ITS, LSU, SSU, MCM7 and Beta-tubulin) of *Usnea* samples from Tanzanian mountainous rainforests were generated. These were used for elucidating their phylogenetic relationships in a wider sampling of *Usnea*, covering many of the *Usnea* sections. Bayesian analysis was used to infer phylogenetic relationships among the species. The morphology of the samples was assessed and important features such as apothecia and the occurrence and type of soralia, isidiomorphs and fibrils were recorded, along with features such as the cortex and medulla pigmentation, the branch surface characteristics and the branch anatomy. Thin layer chromatography was used to investigate the secondary chemistry. In this study a presentation of the phylogeny of some Tanzanian *Usnea* from mountainous rainforests is presented, which based on sequence data suggests species identifications and species circumscription.

2.1-191 Exploring the evolution of lichenicolous fungi in a phylogenetic context

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Abstract: Lichenicolous fungi are pathogens, saprotrophs or commensals which grow obligately on lichens. The lichenicolous habit has evolved many times, with species found in many families across the Ascomycota, the phylum to which the majority of lichenicolous fungi belong. They are, however, often missing from the general consciousness of fungal strategies, and so provide a novel avenue of phylogenetic research regarding fungal diversity and evolution. In this study, a range of lichenicolous fungi from various localities and lichen hosts have been sequenced, including new species, and molecular data from previously sequenced lichenicolous species have been collated from literature. Species were placed in a broad, multilocus phylogeny of the Ascomycota, alongside diverse taxa such as endolichenic, mycoparasitic and rock-inhabiting fungi. An ancestral state reconstruction was performed to show how a lichenicolous perspective influences the big picture of fungal evolution within the Ascomycota.

2.1-192 Using a phylogenetic framework to assess the role of symbiotic specificity in shaping evolutionary and spatial patterns of associations in trimembered lichens

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Abstract: Species circumscription is key to characterize patterns of specificity in symbiotic systems. Here, a phylogenetic framework was used to assess the biodiversity and symbiotic patterns of association among partners of the trimembered lichens in the genus *Peltigera*. We used a worldwide sampling to explore phylogenetic relationships among taxa included in the section *Chloropeltigera*. We sequenced six mycobiont loci and performed analyses using species discovery and validation methods to establish species boundaries. Single locus phylogenies were used to establish the identity for both, *Nostoc* and *Coccomyxa* photobionts. Distribution and specificity patterns were examined across all *Peltigera* species from sections *Chloropeltigera* and *Peltidea*. The possible role of the reproductive mode (sexual versus asexual) shaping these patterns was explored. Eight fungal species (including five newly delimited) were found in association with nine *Nostoc* phylogroups and two *Coccomyxa* species for section *Chloropeltigera*. In contrast, eight fungal (including three newly delimited) species in section *Peltidea* were found in association with only four *Nostoc* phylogroups and the same two *Coccomyxa* species as for section *Chloropeltigera*. This difference in cyanobiont biodiversity found between these two sections of the genus *Peltigera* can potentially be explained by a different type of reproduction driving cyanobiont transmission (vertical versus horizontal) in each section. We found a significantly higher frequency of sexual reproductive structures and higher number of ITS haplotypes in species from section *Chloropeltigera* compared to species from section *Peltidia*. This suggests that horizontal transmission might be more prevalent in *Chloropeltigera* species, while vertical transmission might be more common in *Peltidea* species. All species in section *Chloropeltigera* are generalists in their association with *Nostoc* compared to more specialized *Peltigera* species in section *Peltidea*. Constrained distributions of *Peltigera* species that associate strictly with one species of green algae (*Coccomyxa subellipsoidea*) demonstrates that the availability of the green alga and the specificity of the interaction might be important factors limiting geographic ranges of trimembered *Peltigera*, in addition to limitations imposed by their interaction with *Nostoc* partners.

2.1-201 Novel bioactive metabolites from *Hohenbuehelia grisea* and other Thai Basidiomycota

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Abstract: Cultures of Basidiomycota were obtained from specimens collected in the field in northern Thailand and screened for antimicrobial effects in collaboration with a worldwide leading group of experts on natural product derived drug discovery in Germany. From selected strains that showed prominent activities, several novel active compounds were isolated from mycelial cultures during the course of this study. These include new isolates belonging to the genera *Cyathus, Hohenbuehelia, Deconica*, *Gymnopus*, *Marasmius*, and *Panus*. The morphological characteristics of these mushrooms were described and molecular data (nrITS) were used to characterize the producer organisms. *Hohenbuehelia grisea* has been known to produce the antibiotic and anticancer-lead compound pleurotin and dihydropleurotinic acid. Recently, the discovery of a *Hohenbuehelia grisea* led to the isolation and identification of novel bioactive metabolites, by the interpretation of spectral data

(HRESIMS, 2D-NMR). Our results show seven new derivatives of dihydropleurotinic acid, out of which three are novel sulfur-bearing derivatives and their decreased cytotoxicity and antimicrobial activities, compared to the other compounds, hint to a possible glutathione detoxification pathway in filamentous fungi. Additionally, a novel derivative of hydroxypleurotin featuring an exocyclic double bond as well as two compounds with a 4-membered ring scaffold have been isolated. The isolation of new derivatives of the carotane antibiotic fulvoferruginin from a *Marasmius* sp. which also prove to be cysteine-derived conjugates of fulvoferruginin, further support our hypothesis of a glutathione detoxification in filamentous fungi.

2.1-202 Phylogeny and chemical diversity of *Preussia similis*

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Abstract: The genus *Preussia* (Sporormiaceae, Pleosporales) comprises filamentous Ascomycota that live on animal dung, plant debris, soil, and wood or as endophytes. *Preussia similis* (Khan and Cain) Arenal was found to be a rich source of antifungal compounds, such as similins A and B and preussomerins. From our collection of endophytic fungi isolated from the medicinal plant *Globularia alypum* Linn. (Plantaginaceae) collected in Batna, Algeria, three isolates of *Preussia similis* were studied for their phylogenetic relationship and screened in order to search for novel biologically active secondary metabolites. Phylogenetic tree inferred from the multigenes analysis revealed that the three strains fell in one strongly supported monophyletic clade of *Preussia similis* complex. Additionally, the three strains originated from the same host plant, have been shown to generate chemical diversity in secreted secondary metabolites. In total, thirteen compounds have been isolated including six new bicyclic polyketides and one new dimer of 2-aminobenzoid acid along with known cytochalasins and xanthones. These chemical features might be considered as good chemotaxonomic markers of the genus *Preussia*.
The results of this project were published in Noumeur et al. 2017. **Preussilides a–f, bicyclic polyketides from the endophytic fungus *preussia similis* with antiproliferative activity.** *Journal of natural products*. https://pubs.acs.org/doi/10.1021/acs.jnatprod.7b00064

2.1-203 The secondary metabolom of *Hypoxylon rickii* (Hypoxylaceae)

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Abstract: The recently erected Hypoxylaceae comprise a rather large world-wide distributed family of ascomycetes with more than 300 accepted species. The majority of its members is associated with decaying hard wood, where they form stromatal tissue embedding one or more perethicia. It is assumed that most of the species spend part of their life inside of healthy trees as endophytes without causing any visible symptoms. With only few exceptions the Hypoxylaceae are characterized by an unusual abundancy of pigments on the surface and/or within the stromatal tissue. These pigments are traditionally extracted with a potassium hydroxide solution and the resulting color is used as a distinctive feature between species. In the last 20 years natural product researcher began to elucidate the underlying chemical structures and revealed a plethora of carbon skeletons including azaphilones (e.g. mitorubrins, multiformins, lenormandins, daldinins), cytochalasins, tetramic acids (e.g.

hypoxyvermelhotins), binaphthyls and derivatives (e.g. BNT, hypoxylone, urceolone), benzo[j]fluoranthenes (e.g. hypoxylonols, daldinones, truncatone), prenylated indol derivatives (e.g. truncaquinones) or other aromatic polyketides (e.g. macrocarpones). Furthermore, cultures derived from spore isolates and endophytic mycelia were intensively screened for bioactive natural products leading to the discovery of hundreds of additional compounds. Many of them displayed interesting activity such as the topoisomerase I inhibitor hypoxyxylerone, the antioxidative rickenyls, the insecticidal nodulisporic acids, the antifungal hypoxysordarin, the anti-HIV agent concentricolide or the immunosuppressive dalesconols. The production of the seemingly unlimited amount of different secondary metabolites is controlled by biosynthetic gene cluster (BGC) encoded in the genome of the producing organism. The advances in the area of genome sequencing enabled us to study the genomes of various Hypoxylaceae and identify BGC using bioinformatics and molecular tools for gene knockouts and heterologous expression systems. By comparing BGC of related species we are about to reveal conserved as well as unique biosynthetic pathways, helping to trace back the evolution of the secondary metabolom. In the past, we extensively evaluated the production capabilities of the prolific secondary metabolite producer *Hypoxylon rickii* and purified more than 30 compounds of eight structural distinct families. Together with the recently obtained genome of the species we could get an insight into the true diversity of its secondary metabolom, which is showcased in this presentation.

2.1-205 Molecular diversity of *Aspergillus* species and toxin production in wheat and soybeans

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Abstract: Aflatoxin contamination of foods poses great health and economic threats to developing countries and the world at large. Hundreds of million dollars are lost to the US and world economy annually due to invasion of crops by *Aspergillus* species and subsequent aflatoxin contamination of food products. A constant evaluation of aflatoxin levels in a nation's food products must form a fundamental part of control strategies so as to assess the effectiveness of the management interventions by government and non-governmental organizations while an understanding of the distribution of the producing fungi will further encourage a target oriented intervention hence this study. Wheat and Soybeans products were analyzed across 6 geo-political zones of Nigeria for fungal load and diversity using standard mycological techniques. Characterization of *Aspergillus* species was by DNA sequencing of the highly variable TRP C13 genes, which was used to distinguish the *Aspergillus* sect. Flavi group by comparing with that of known isolates while confirmation of aflatoxigenicity was done using Thin Layer Chromatography. Isolates of *Aspergillus* were further culturally differentiated into L and S strains using 5/2 agar. The same wheat and soybeans samples were assessed for aflatoxins using Enzyme Linked Immunosorbent Assay (ELISA). From results, mean fungal counts ranged from $1.0x10^2$cfu/g to 2.3×10^6cfu/g in Wheat and $2.6x10^2$cfu/g to $2.2x10^5$cfu/g in Soybean products, with no significant difference in counts at ($p<0.05$) and ($p<0.01$) respectively. Diverse fungi were identified to include *Aspergillus niger, A. nidulans, A. tereus, A. fumigatus, Penicillium. Italicum, P. oxalicum, P. sp,. Mucor mucedo, M. sp., Neospora sp., Choanophora sp., Cladosporium sp., Rhizopus sp., Rhodotorula sp., Sacharomyces cerevisiae, Fusarium oxysporium, Botrydoplodia theobromae, Helminthosporium sp. and Trichoderma sp. A. niger had highest frequency of occurrence of* 14.1% in Soybean and 9.4% in Wheat from the 6 zones studied. Further characterization differentiated the *Aspergillus* species to include *A. flavus* Strain 1-26; *A. flavus* Strain TX18 and *A. flavus* Strain IC1264. There was a predominance of the L strains over the S strains of aflatoxigenic *Aspegillus* though S strains were found in only the wheat

products. Aflatoxin content ranged from 1.18±0.04ppb to 84.61±2.99ppb in Wheat and -9.76±0.35ppb to 73.08±2.58ppb for soybeans products. 38% of wheat products and 23% of Soybeans products had >20ppb Nigerian and U.S maximum aflatoxin limit across the 6 zones studied. This calls for more effective intervention methods for the control of the deadly toxin in Nigerian foods in order to safe guard local consumers against its health hazards.

2.1-208 Towards a 21st century *Dictionary of the Fungi*

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Abstract: Ten editions of the *Dictionary of the Fungi* were published regularly by the Imperial Mycological Institute and its successor organisations from 1943, providing authoritative information on accepted genera and their diversity (within a much-used higher classification) along with a glossary and biographical entries for mycologists. It is ten years since the last edition of the *Dictionary* was published. It was avowedly "the last 'ink-on-paper' version", which prompts the question: how can the mycological community build on the 'marvelously imperfect' *Dictionary* and create maximally useful on-line resources? Glossaries and biographies could be implemented via on-line collaborative editing spaces (such as a wiki). Dealing with names and classifications is more challenging; especially providing accepted names within the one classification, which is what most users want. While there are significant existing on-line resources (such as Index/Species Fungorum, MycoBank, GenBank, U.S. National Fungus Collections and NZFungi) there is currently much wasteful effort by users in determining the 'correct' name and placement of fungi. There is also misunderstanding of the difference between lists of names (nomenclators, such as *Index Fungorum*) and lists of taxa (compilations of accepted names with their synonyms, such as *Species Fungorum*). For names, there should be one version of each name and its associated publication and typification details, compliant with the *International Code of Nomenclature for algae, fungi and plants*. However, existing nomenclators are not complete, and different databases can have different citation details and even different spellings of names. Full population and checking of nomenclators would benefit from citizen science approaches where volunteers are mobilised, while efficiently utilising scarce nomenclatural and orthographic expertise. For name databases, there is considerable support from hosting institutions and massive voluntary input by key individuals, but a sustainable system is yet to emerge due to lack of clarity around the role and integration of existing databases. For taxa, circumscriptions (such as the delimitation of a species or which genera belong to which families) are opinions by taxonomists, and not governed by rules, but only by peer review (as to whether they appear in the first place) and uptake by mycologists. There are challenges in integrating taxa that lack molecular data into classifications that increasingly do not have morphological synapomorphies. Ideally, knowledgeable mycologists would engage with a simple on-line system that generates a global consensus classification, available at any point in time via web services, with version control. Organisationally, an expanded role of the International Commission on the Taxonomy of Fungi (ICTF) can be envisaged, building on the working groups that have successfully guided the one fungus – one name transition. In addition, existing provisions of the *Code* could be utilised to create lists of protected names to stabilise taxonomy of important groups. For governance, the International Mycological Association could play an increased role through its ownership of MycoBank. Across both names and taxa, it is vital to trap metadata behind decisions, both nomenclatural (e.g. which spelling is adopted) and taxonomic (which circumscription is adopted) to provide clarity and avoid re-covering old ground in the future.

Poster Session 2 • Period 2 • Wednesday, July 18, 2018

2.2-1 Searching for microorganisms with a potential of plastic degradation (PET, LDPE).

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Abstract: The low cost and relatively short amount of time of fabrication of plastics, added to the disposable character of plastic-made products and low rate of recycling, has turned plastic pollution into an environmental concern. Plastic is one of the most remarkable causes of death of marine animals. This is a strong reason for us to do research on how to degrade plastic-made materials and remove them from the ecosystems. This study aims to evaluate the potential for plastic degradation among microorganisms like fungi and bacteria, found in soil and degrading biomass from trees in the Amazon, Rain forest and Andean region in Ecuador.

2.2-2 Differential regulation of *Pleurotus ostreatus* dye peroxidase genes in response to carbon source and dyes

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Abstract: The fungus *Pleurotus ostreatus* is a basidiomycete with worldwide distribution and is able to metabolize a wide variety of lignocellulosic and xenobiotic substrates. It has been the subject of many studies focused on bioremediation processes due to its multi-enzymatic machinery, which essentially comprise laccase (Lac), manganese peroxidase (MnP), versatile peroxidase (VP) and accessory enzymes such as dye peroxidase (DyP) and hydrogen peroxide generating peroxidase (H_2O_2). DyPs (E.C. 1.11.1.19) are hemeperoxidases that catalyze oxygen transfer reactions similar to the oxygenases, using H_2O_2 as a co-substrate electron acceptor and also an electron donor compound, which is oxidized to its respective radical. Its name reflects its ability to oxidize several types of dyes. This research has determined the effect of different carbon sources and dyes on both the expression of Pleos-DyP genes via real time PCR (RT-qPCR) and dye peroxidase activity during the time-course of the submerged fermentation of *Pleurotus ostreatus* supplemented with either yellow azo (AA), remazol brilliant blue R (ARBR) and acid blue 129 (AA129) dyes, as well as glucose or glycerol as a carbon source. With regard to the effect of the carbon source on enzyme activity, it was found that glycerol induced the activity levels up to three times compared to basal fermentation in which glucose was the carbon source. All fermentations supplemented with dyes also presented maximum activity values that were higher than those for the basal fermentation. The maximal activity in the basal fermentation was 1550 IU/L, compared to 4900, 3298, 2828 and 1744 IU/L for glycerol, AA, ARBR and AA129 fermentations, respectively. On the other hand DyP gene expression profiles displayed up/down regulation over the fermentation time in the three DyP genes evaluated (Pleos-DyP1, Pleos-DyP2 and Pleos-DyP4). Glycerol induced Pleos-DyP1 and Pleos-DyP2 genes presented a relative expression (log2) of 10.01 and 11.96-fold increase, respectively, with both findings obtained at 504 h. AA addition caused the highest induction level for the gene Pleos-DyP4 with a relative expression (log2) comprising a 14.1-fold increase at the end of the fermentation process (552 h). Furthermore, the addition of ARBR caused the lowest induction level for the gene Pleos-Dyp2 with a relative expression (log2) of 5.05-fold at 360 h. On the other hand, the addition of AA129 caused the highest repression level for Pleos-DyP1 at 168 h with a negative relative

expression (log2) comprising a -14.6-fold change. In conclusion, the enzyme activity and gene expression profiles obtained in the different conditions evaluated show the influence of the carbon source, the nature of dyes and the growth phase on the differential regulation of the DyP gene expression in *P. ostreatus*.

2.2-3 Anaerobic fungi as a source of novel enzymes and antimicrobials

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Abstract: Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of many herbivorous animals, and play an essential role in degrading food with a range of powerful hydrolytic enzymes. Whilst these enzymes are important to rumen efficiency, they can also be useful to biotechnological and biomedical industries. The rumen microbiome presents an underexplored source for novel microbial enzymes and metabolites, the former with biotechnological potential and the latter as potential drug candidates to target ever-increasing antimicrobial resistance. Rumen and faecal samples were collected from various large herbivores, and fungal cultures were grown and maintained under anaerobic conditions. After roll tube culture to isolate single-zoospore cultures, sequencing of LSU was undertaken to identify the fungi to species level. Analysis of genomic data from these cultures alongside data from several published studies has been undertaken to explore the diversity of lipases and antimicrobial peptides (AMPs) within the genomes of these fungi.

2.2-4 Projections for the production of enzymes of industrial interest by *Ceriporia lacerata*

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Abstract: Mushrooms have been widely studied for their production of extracellular enzymes with lignin-degrading ability. Enzymes are biological catalysts of great importance at cellular level, but also, they are of great interest at industrial level since they are key for certain reactions to occur, as they increase the rate of reactions without changing the equilibrium. Typically, enzymes are produced during fermentation processes of microorganism. However, low efficiencies and high costs are usually associated with their production. Therefore, bioprospecting for new microorganism for the production of enzymes is an important topic of research. In particular, *Ceriporia lacerata*, a native white-rot fungus found in Colombia, has not been largely explored for its ability to produce biologically active metabolites. Given the natural growth conditions of this fungus, it is likely that lignin-degradative enzymes are produced, such as: pectinases, amylases, laccases and cellulases. With this project, we aim at exploring the ability of *C. lacerata* to produce these four lignin-degradative enzymes under submerged fermentation conditions. First, the effect on fungal biomass and enzymatic production were assessed using two different flasks geometries and four different growth media, according to literature reports. Fungal biomass production was measured by dry weight, while enzymatic activity was determined using specific protocols depending on the kind of enzyme that wanted to be evaluated. In general, the enzymatic extract of each medium was added to a substrate solution, depending on the evaluated enzyme and either change in viscosity or absorbance values were recorded. The results of these evaluations showed that the geometry of the flask had a significant effect on biomass production but it did not affect enzymatic production. On the other hand, greater enzymatic activities were found for pectinases and cellulases than for amylases and laccases. In fact, for the latter enzyme, we have not been able to determine the enzymatic activity under submerged fermentation; even after evaluating the

addition of different waste/by-products of food industry to the culture media. However, we found that when adding ABTS-like inductor under solid fermentation conditions, some degradation occurred, suggesting that laccases were produced. In conclusion, we found that *C. lacerata* was able to grow under different flask geometry conditions, while baffled-shake flasks increased the production of biomass. Also, we found that *C. lacerata* produced pectinases, celullases, amylases and laccases, although the greatest potential was observed for the production of cellulases and pectinases under the evaluated conditions.

2.2-5 Designer yeast strains for C1/3/5/6 biorefinery

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Abstract: Heterogeneous structure of lignin imparts plants with structural rigidity and also serves to protect cellulose and hemicellulose from degradation. Thus, prior to fermentative production of ethanol from the cellulose by yeast strains such as *Saccharomyces cerevisiae* and *Pichia pastoris*, the materials are degraded and hydrolyzed to release monomeric sugars. In this study, designer cellulosome was assembled in yeast *Saccharomyces cerevisiae* for utilizing of cellulose as the substrate. For utilizing of cellulose part in lignocellulosic biomass by simultaneous saccharification and fermentation, a recombinant scaffolding protein from *Clostridium cellulovorans* and a chimeric endoglucanase from *Clostridium thermocellum* were assembled as complex system. Compared to the results for single subunit, assembly of cellulosome-based enzyme complexes caused a noticeable increase in the level of enzyme activity. The resulting strain was able to ferment cellulose part in pretreated barley straw into ethanol with the aid of beta-glucosidase from *C. thermocellum*. The use of complexed enzyme systems is one of the strategies for effective lignocellulosic biomass hydrolysis. Enzyme complexes were formed via the interaction of a dockerin domain with cohesin modules in the scaffolding protein. Accelerating the biological degradation of lignocellulosic materials will benefit from the development of useful recombinant enzymes with hydrolysis ability. In future research, construction of designer enzyme complexes containing other lignin degrading enzymes could be used to develop biocatalysts that can completely degrade lignocellulose into single sugars.

2.2-6 *Aspergillus terreus* itaconic acid fermentation technology reflects the physiological requirements of overflow metabolism

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Abstract: Itaconic acid (2-methylenesuccinic acid; IA) is a five-carbon dicarboxylic acid, with one of the carboxyl groups being conjugated to a methylene group – a feature that makes IA easy to (co)polymerize. It is thus used as a building block chemical for the synthesis of a variety of compounds with a broad range of applications, such as manufacturing plastics, coatings and resins. IA is commercially produced by large-scale submerged fermentations employing the filamentous Ascomycete fungus *Aspergillus terreus*. Although production of IA is the result of overflow metabolism similar to that leading to citric acid (CA) production in *A. niger*, available data for IA yields are significantly lower than for CA. CA is known to accumulate to high levels only at high concentrations of rapidly assimilating carbon sources, strong aeration and severe Mn(II) ion limitation. Here we show that these three parameters similarly influence IA production and yield by *A. terreus*. Mn(II) ion concentrations lower than 3 micrograms per liter were shown necessary to obtain highest IA yields.

However, Mn(II) ion concentrations higher than that could be antagonized by 50 milligrams per liter Cu(II) ions. Yields are also dependent on the concentration of the carbon source (D-glucose) – highest yields (0.9) only obtained at concentrations of 12 – 20 % (w/v). As for high aeration requirements, we demonstrated that *A. terreus* is capable of growth-supporting respiration in the presence of KCN. Responsible for this activity is the cyanide-resistant alternative oxidase (AOX) that is located on the matrix side of the inner mitochondrial membrane and branches off the canonical cytochrome-dependent pathway at the level of ubiquinon, leaving complexes III and IV of the electron transport system bypassed. The alternative pathway thus moves much fewer protons across the inner mitochondrial membrane to generate a proton motive force, and provides less ATP for energy conservation. In effect, AOX uncouples the re-oxidization of NADH from ATP synthesis, thereby allowing intense carbon assimilation to continue – a prerequisite for high IA yields. The genome of *A. terreus* specifies two paralog genes (*aodA* and *aodB*), each putatively encoding an AOX. During IA fermentations mimicking industrial conditions, *aodB* displayed constitutive but weak expression at each of the time-points tested. In contrast, the *aodA* expression levels strictly and positively correlated with increasing dissolved oxygen (DO) levels as well as with the final IA yield. Hence, high DO levels required for high-yield IA fermentations on D-glucose may be related to *AodA*, putatively encoding a cyanide-resistant alternative oxidase.

2.2-7 Production and characterization of *Penicillium purpurogenum* MM1 cellulases and its application in biobolishing of cotton textile

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Abstract: **Abstract** The modern textile industry has become familiar with the use of cellulases in a number of mechanical and chemical operations to improve the comfort and quality of textiles. For this purpose, deteriorated cotton textiles samples were used to isolate cellulolytic fungi that produce cellulases in quantity and quality suitable for the biobolosh ing process. Among many isolates, *Penicillium purpureogenium* MM1 recorded the highest enzyme activity reaching to 45.7, 8.0 and 117.8 u/ml for CMCase, Avicelase and b-glucosidase respectively and liberated the largest amount of reducing sugar (144.1 mg/ml) in cotton textile treatment. The partially purified cellulases have optimum temperature of 40°C and optimum pH 4 with good half life time reach to four hours at 60°C for CMCase and Avicelase, three hours at 70°C for b-glucosidase. In biobolishing trials carried out at the Masr textile company, *P. purpureogenium* MM1 cellulases have the same efficiency as the imported ones being used (Denimax 992).

2.2-8 A novel laccase from white rot fungus *Trametes orientalis*: Purification, characterization and application

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Abstract: A novel laccase (*Tolacc-T*) from white rot fungus *Trametes orientalis* was enriched to apparent homogeneity with a specific activity of 20.667 U/mg protein and recovery yield of 47.33%. The SDS-PAGE gave a single band indicating that *Tolacc-T* appears a monomeric protein with a molecular mass of 44.0 kDa. Domain structure analysis revealed that *Tolacc-T* contained a typical copper II binding domain and shared three potential *N*-glycosylation sites, but had no copper I binding domain, demonstrating that the enzyme is really a laccase, but a novel laccase. Optimal pH and temperature of *Tolacc-T* was 4.0 and 80 °C, respectively, and it retained more than 80% of its original activity after 2 h incubation at 10 °C to 50 °C. The enzyme exhibited strict substrate specificity towards ABTS but showed

no or trace activities towards other substrates. Among the metals tested, Mn^{2+} was proved to be the best activator for enhancing the laccase activity. A strongly inhibiting effect was found when NaN_3, L-cysteine, and DTT were added to the enzyme. However, *Tolacc-T* activity was little bit inhibited in the presence of chelator EDTA. Furthermore, the enzyme was capable of degrading structurally different synthetic dyes in the absence of a redox mediator.

2.2-17 DNA-free genome editing in *Beauveria bassiana* (Hypocreales, Ascomyota) with CRISPR-Cas9 ribonucleoproteins

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Abstract: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–Cas9 is an adaptive immune system of prokaryotes. Recently it became the most powerful molecular biology tool, which can induce double strand breaks (DSBs) via non-homologous end joining (NHEJ) or homology-directed repair (HDR). The CRISPR-Cas9 system facilitated the precise genome editing in microorganisms, plants and human, with relatively simple protocol. Recently RNA-guided engineered nucleases (RGEN) delivery method that consists of the preassembled Cas9 protein and gRNA, have been successfully applied in plant and human cells. In this convenient straightforward delivery system, the codon optimization and other genetic manipulation for specific organisms are not necessary. Despite these advantages, the CRISPR-Cas9 system has not been widely adapted for fungi since the routine plasmid-based transformation has the complicated process to generate mutant in laboratory. In the present study, we purposed to improve the fungal transformation efficiency with DNA-free CRISPR Cas9 method, with the entomopathogenic fungus *Beauveria bassiana*. Obtained putative transformants were sub-cultured 3 times to confirm their mitotic stability. The RGEN induced mutations and the genome editing efficiencies for target genes were verified using Sanger and high-throughput deep sequencing. Our results revealed that DNA-free CRISPR-Cas9 is an efficient and useful gene editing system for *B. bassiana*.

2.2-18 *Beauveria bassiana*: Potential Biological Control against Coffee Berry Disease

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Abstract: *Beauveria bassiana* (Bb) has been used successfully as a biological control against the coffee berry borer (CBB). Although interactions of Bb, CBB and Coffee Berry Disease (CBD) are frequent, information about the use of Bb as a biological control for plant pathogens is limited. During 2016 - 2017, a survey was conducted to evaluate the potential of Bb in controlling CBD in commercial and experimental coffee farms in Puerto Rico. A total of 8162 fruits were collected and evaluated. Coffee fruits with and without Bb were dissected to evaluate internal and external fruit rot. Coffee fruits with Bb had significantly less internal and external rot than coffee berry fruits without Bb (12.4% vs. 18.8%), suggesting Bb as a potential biological control against CBD caused by *Colletotrichum* spp. To test effectiveness of Bb against CBD, three isolates of *Colletotrichum* spp. and one isolate of Bb were inoculated on coffee fruits. Experiments were conducted in laboratory and field conditions and were repeated twice. A total of 300 coffee fruits were drilled and inoculated. All rotten coffee fruits were plated on PDA and fungi were identified, fulfilling Koch postulates. In laboratory and field conditions, coffee fruits inoculated with both Bb and *Colletotrichum* spp. had significantly lower percentage rot than fruits with *Colletotrichum* spp. alone (as did control fruits). The protective effect of Bb against CBD

caused by *Colletotrichum* spp. is an important discovery and suggests an additional role for Bb in programs of disease management in Puerto Rico and other coffee-producing countries.

2.2-20 Biological control of Fusarium wilt of tomato by application of *Penicillium* spp. and *Chenopodium murale*

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Abstract: Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici,* is an economically important soil-borne disease of tomato especially in warmer regions of the world. Fungicides used to control this disease also pollute the environment and cause health hazards. In the present study, this disease was managed by application of two antagonistic species of *Penicillium* namely *P. digitatum* and *P. expensum*, and dry biomass of a weed *Chenopodium murale* as soil amendments. The antagonistic fungi and different doses of dry biomass of the weed (1%, 2% and 3%) were applied in pathogen inoculated pot soil either separately or in combinations. The highest disease incidence (100%) was recorded in positive control where only fungal pathogen was applied. Different treatments of soil amendments reduced disease incidence to 3 – 23%. The lowest disease incidence (3%) was recorded in 2% *C. murale* biomass + *P. expensum* treatment. All the soil amendment treatments significantly enhanced shoot and root growth as well as fruit yield as compared to positive control. The highest fruit biomass was recorded in 2% *C. murale* biomass + *P. digitatum* treatment. The highest activities of peroxidase (POX), catalase (CAT) and polyphenol oxidase (PPO) were recorded in positive control. These enzymatic activities were significantly lowered when soil was amended with antagonistic fungi or *C. murale* biomass. Effect was more pronounced where *C. murale* biomass was applied either alone or combined with *Penicillium* spp. In laboratory bioassays, 100 mg mL^{-1} concentration of *n*-hexane and *n*-butanol sub-fractions of methanolic leaf extract completely controlled the growth of *F. oxysporum* f. sp, *lycopersici*. GC-MS analysis of these two fractions revealed that hexadecanoic acid; methyl linolenate and β-sitosterol were the most abundant compounds in *n*-hexane sub-fraction, and 1-heptanol; 3-hydroxyhexanoic acid; 1,2-decanediol and etiracetam were predominant constituents of *n*-butanol sub-fraction which may be responsible for antifungal activity of *C. murale* against the pathogen. This study concludes that application of 2% *C. murale* biomass + *P. digitatum* has the potential to significantly reduce Fusarium wilt of tomato and enhance tomato growth and yield.

2.2-21 There's treasure everywhere – putatively overlooked slow-growing fungi isolated from cereal cyst nematodes produce nematode-inhibiting compounds

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Abstract: Cereal cyst nematodes (CCNs) can lead to significant yield reductions of grains. The use of nematicidal chemicals is banned in many countries due to their generally high non-target toxicity. Therefore, antagonistic microorganisms controlling nematodes are an important alternative. The search for such microorganisms, especially nematophagous fungi, has a long history extending back some 150 years. These studies provided a long list of nematode-associated fungi some of which showed great potential to be exploited as biological control agents. Microscopic observations of cyst samples of the CCN *Heterodera filipjevi* obtained from wheat fields in Turkey regularly revealed nematode cysts, which displayed fungal colonisation. The aim of our study was to (*i*) isolate the fungi from the nematode cysts and fulfil Koch's postulates (*ii*) to classify the isolated fungi using light microscopic and molecular phylogenetic analyses (*iii*) to study the nematode-fungus interaction microscopically, (*iv*) to isolate and identify secondary metabolites produced by these fungi. Fungi were isolated from symptomatic cysts

applying a specific single-egg isolation technique developed for this study. Fungal strains were identified using morphological studies and multi-locus molecular phylogenetic analyses. To fulfil Koch's postulates, the pathogenicity of isolated fungal strains was examined against nematode eggs *in vitro*. Secondary metabolites of fungal isolates of interest were extracted and purified using EtOAc, and HPLC-based techniques. The bioactivity of obtained compounds was evaluated using nematode bioassays. This approach resulted in finding six new fungal species. All species are ascomycetes belonging to the Helotiales, Hypocreales and Pleosporales. The newly described *Ijuhya vitellina* and *Monocillium gamsii* belong to the families of Bionectriaceae and Niessliaceae, respectively. A new fungal genus was proposed to accommodate two new species. One of these, representing a dark septate endophyte (DSE), was isolated from nematode eggs. Two more species were preliminarily characterized as DSEs. These are the first DSEs found to parasitize nematode eggs and they might play a role in the plant defense against nematodes. All newly-found species could be successfully re-isolated from artificially infected nematodes and Koch's postulates were thus fulfilled. Both *I. vitellina* and *M. gamsii* formed microslcerotia within the nematode eggs and in culture. Chaetoglobosin A, 19-*O*-acetylchaetoglobosin A and four novel compounds, among them cyclodepsipeptides, a class of compounds known for anthelmintic effects were isolated. Nematicidal and nematode-inhibiting activities were demonstrated for the isolated compounds. To conclude, using a specific isolation technique novel fungal species and novel compounds could be discovered from the CCN *H. filipjevi* that might be harnessed for biological control in the future.

2.2-22 Exploiting lichen-associated bacteria for biocontrol of soil-borne pathogens and stress protection of plants

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Abstract: Symbioses are a hotspot for cross-kingdom communication and control. Bacterial communities were identified as stable, specific and structurally integrated partners in the classical lichen symbiosis. Their roles in the symbiosis of lichens are supported by metagenomic, -proteomic and metabolomic data. Functions include (i) nutrient supply, especially nitrogen, phosphorous and sulfur, (ii) resistance against biotic stress factors (that is, pathogen defense), (iii) resistance against abiotic factors, (iv) support of photosynthesis by provision of vitamin B12, (v) fungal and algal growth support by provision of hormones, (vi) detoxification of metabolites, and (vii) degradation of older parts of the lichen thallus. The results were confirmed by bridging multi-omics with culture studies. Our findings showed the potential of lichen-associated bacteria to interact with the fungal as well as algal partner to support health, growth and fitness of their hosts. As the microbiome of lichens comprise a high number of bacterial species (>800 in *Lobaria pulmonaria*) we started to exploit this diversity for biotechnological applications. We found that bioactive volatiles produced by lichen-associated bacteria are able to suppress soil-borne pathogens. Metatranscriptomic datasets from bacterial communities of the lung lichen also showed that host-associated bacterial communities are well-adapted to dehydration by stress protective mechanisms and changes of metabolic processes. The results indicate an intense interplay in holobiont function under drought stress. These capacities can be exploited for plant protection strategies.

2.2-23 A first look at culture-dependent endophytic fungal diversity of wild Rubiaceae in Costa Rica.

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Abstract: Global warming effects, like longer rainy seasons and higher temperatures are increasing diseases on coffee plantations around the world and thus affecting the producers' economies. Endophytic fungi with plant-beneficial properties have been proposed as an alternative to the use of fungicides. Because the coffee family, Rubiaceae, is highly diverse in Costa Rica, we recently started to characterize their endophytes with the idea of finding beneficial fungi that could be applied in improving coffee-plant health. The objectives of this preliminary study were to identify the culturable endophytic fungi from several wild Rubiaceae species and do *in planta* antagonism tests against the pathogen *Mycena citricolor*. Culturable endophytes were isolated from 49 Rubiaceae species: *Allenanthus erythrocarpus, Bertiera bractiosa*, *Guettarda macrosperma*, *Palycourea eurycarpa*, *Pentagonia costaricencis*, and *Psychotria pubescens*, among others, collected from two regions in Costa Rica, i.e., Guanacaste Conservation Area and the Osa Peninsula. Cultures were identified using the nrDNA ITS region. The culturable endophyte fungi mainly obtained from leaves were e.g., *Clonostachys* spp., *Endomelanconiopsis endophytica*, *Lasiodiplodia theobromae*, *Simplicillium* aff. *lamellicola*, *Diaporthe* spp., *Phyllosticta* spp.; and others like *Trichoderma deliquescens*, *T. virens* and *T. rifaii* were extracted from the stems. *Psychotria solitodinum* and *P. panamensis* were the most diverse in the Osa Peninsula, and *Bertiera bracteosa*, *Coutarea hexandra*, *Psychotria pubescens*, were the most diverse in the Guanacaste Conservation. At the moment, 419 taxa are identified, 397 from Ascomycota and 21 from Basidiomycota. The most frequent families in the Ascomycota are Botryosphaeriaceae, Glomerellaceae and Pestalotiopsidaceae; and in the Basidiomycota Ceratobasidiaceae and Meripilaceae. *Colletotrichum* was the prevalent genus in both areas and within Rubiaceae species. A preliminary antagonism was performed applying *T. rifaii* on two varieties of coffee plants, "Catuaí" and "Caturra" in order to try to reduce the infection caused by *Mycena citricolor*. Results show a significant reduced infection on the plants treated previously with *T. rifaii* specially on the "Caturra" variety. Additional molecular markers will be used to refine the taxonomy of cryptic species and more *in vivo* essays will be done. We think these preliminary results are capable to predict a noticeable improvement of the coffee plantations health.

2.2-24 The discovery and characterization of Ustilaginomycetes yeasts reveal enzymatic activity, resistance to antifungal drugs and species complexes.

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Abstract: The plant biodiversity of Thailand and Malaysia is estimated at over 10,000 species, each growing in an unique geography and climate. These plants and their surrounding habitats offer opportunities for the discovery of yeasts with novel physiological and enzymatic properties, and in recent years there has been a steady increase of yeast discoveries from these and other developing countries. From November 2012 – January 2013, three unique strains (TAR 509, TAR 520, and TAR 523) of anamorphic, basidiomycete fungi were cultured from a leaf of *Pistia stratiotes* (water lettuce) growing in Bangkok, Thailand and from a *Hibiscus* sp. in Kuala Lumpur, Malaysia. Despite having only one strain for each of the novel fungi reported here, we reveal novel physiological and chemical characteristics as well as phylogenetically distinct fungal lineages. Using concatenated nuclear ribosomal gene sequences of the large subunit and internal transcribed spacer regions, we determine that TAR 509, isolated from *P. stratiotes*, belongs to the recently discovered family Fereydouniaceae, in the Urocystidales. TAR 520, isolated from *Hibiscus*, is a novel species of *Dirkmeia*, and TAR 523 is the first characterization of the anamorphic stage of *Ustilago sparsa,* both in the Ustilaginales. We present evidence that these three strains have strong production of amylase, cutinase, lipase, and xylanase. Furthermore, scanning electron microscopy reveals that *Dirkmeia* sp. strain TAR 520 has resistance to antifungal drugs in the echinocandin class, measured by minimum inhibitory concentration analysis. Moreover, data identify possible species complexes within the genera *Fereydounia* and *Dirkmeia*, based on both maximum likelihood and Bayesian analyses. We believe that *Fereydounia* sp. strain TAR 509 is a new species based on differences in its growth in osmotic pressure and temperature ranges, as well as morphological and ecological differences when compared to the type species of *F. khargensis*. Finally, a recent study showed that foliar applications of *Dirkmeia churashimaensis* strain RGJ1 on pepper generated antiviral and antibacterial resistance against pathogens. However, our phylogenetic analyses suggest that this strain is not *D. churashimaensis*, instead, it clusters with our novel species of *Dirkmeia*. Our newly described species is the second know species of the genus. Our discoveries are evidence that many novel species of Ustilaginomycetes may occur in understudied tropical areas.

2.2-25 Where do you fit in the mycological genealogy?

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Abstract: Do you know where you belong in the great family tree of mycologists? Academic Family Tree (https://academictree.org/) is a collaboratively-edited, continuously updated online database of

scientists connected in a family tree with advisors in place of parents and their students in place of children. Trees are being built in many fields, from law and crystallography to biomechanics and *Drosophila* genetics. An offshoot of this project is Mycotree, a database of mycologists, which is currently highly underpopulated. While we did not initiate Mycotree, we want to raise awareness for this unique resource and encourage participants of IMC11 to contribute their "family" in this unique opportunity to preserve mycological history in one place. The accompanying poster includes a representative segment of Mycotree beginning at the authors' leaves and spanning back to such luminaries as Heinrich Anton de Bary and Ernst Haeckel, as well as an example biographical profile. Scientist profiles are more than just nodes in a tree; they allow additional biographical details to be added, such as university positions, dates of supervision of students, links to published biographical sketches, etc. The poster also covers instructions for helping contribute your lineage and knowledge. Adding or editing biographical entries and connections in Mycotree simply requires a free account and making connections between mentors and students is easy. By leveraging the diverse attendees at this year's meeting to combine their knowledge for the greater mycological good, we hope that Mycotree will grow to accommodate a truly global perspective of the long and storied history of mycology!

2.2-26 Oral history for mycology

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Abstract: Relatively few videos of mycological reminiscences exist. Imagine if Margaret Barr, Lilian Hawker, Richard Korf, Erast Parmasto, Emory Simmons, and C.V. Subramanian had left their stories for us on video in addition to their still images and memories printed in memorials. The Congress offers an opportunity to record interviews with mycologists, including their assessments of changes that occurred over their careers and the experiences of other mycologists who are just beginning their careers. Three participants represent the variety of mycologists who will participate in a scheduled session at IMC 11. General questions will be given to participants in advance to provide direction for thoughtful, smooth-flowing interviews. Videos will be housed at the Hunt Institute for Botanical Documentation, Carnegie Mellon University, which makes botanical and mycological materials of historical interest widely available. Interviews would be available for teaching. Additional interviews will be recorded throughout the Congress, so please sign up here.

2.2-27 Inhibitory activity of limonene against a virulent strain of *Candida albicans in vitro* and *in vivo*

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Abstract: *Candida albicans* is a commensal yeast that is part of the human microbiota but in some cases could cause from mild skin mycosis to severe disseminated infections. The most used treatments against candidiasis are fluconazole, itraconazole, miconazole and in severe cases amphotericin B. However, the number of resistance cases to these antifungal drugs it has increased recently. Limonene belongs to the terpene group and has been shown a broad spectrum of activity such as antitumoral and antiprotozoal activities against *Trypanosoma cruzi*, *Plasmodium falciparum and Leishmania amazonensis*. In this study we evaluated the Limonene concentration capable to inhibit the growth of the yeasts *in vitro*. In addition, damage to the fungal structure was observed through transmission electron microscopy. For *in vivo*

studies, a candidiasis vaginal model was used and Colony Forming Units (CFU), histology and scanning electron microscopy of the vaginal canal were verified to analyze the effect of the Limonene. Our experiments suggest that the Limonene presents a protector role in the infection process caused by the virulent strain used of *Candida albicans*.

2.2-29 Impact of *Aspergillus nidulans* signaling mutants on growth and secretion on non-preferred carbon sources

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Abstract: Filamentous fungi are the leading producers of fermentation enzymes. The use for these industrial enzymes are vast ranging from; food fermentation, textile production, leather modification, and chemical detergent processing. The biotechnological supply for enzyme production is in growing demand, and unlocking the knowledge of enzyme secretion pathways is vital to industrial profits. Secretion pathways are coherently linked to fungal morphology, and studying fungal growth patterns could provide insight into these elusive pathways. In filamentous fungi, the Cdc42 and Rac1 GTPases are required for normal hyphal morphogenesis, but their roles in enzyme secretion and morphological adaptation to carbon source shifts have not been investigated. We characterized the effects of Aspergillus nidulans Cdc42 and Rac1 deletion mutations on the activities of secreted cellulase and xylanase. In addition, we determined how these mutations impact morphological responses to a shift from glucose to non-preferred carbon sources. These small GTPases mutants were grown in rich media then switched to a non-preferred carbon source for further growth. The carbon source shifts were marked with a Wheat Germ Agglutinin (WGA) fluorescein labeling, and pictures where taken with a GFP filter. Using ImageJ, the hyphal growth patterns were analyzed for differences in hyphal dimensions. Our results suggest that Cdc42 regulates the morphological transition to non-preferred carbon sources. Cdc42 may also selectively regulate the expression of specific hydrolytic enzymes (cellulases vs. xylanases). Conversely, Rac1 appears to play a more global role in the expression or trafficking of hydrolytic enzymes. Further study of these GTPases and their effectors should provide additional insights into the relationship between fungal morphology and secretion pathways.

2.2-30 *Neurospora crassa* has AP180, a clathrin adaptor in the endocytic collar but not clathrin

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Abstract: Endocytosis is essential for polarized growth and morphogenesis in filamentous fungi. It is highly concentrated in a specialized collar at the hyphal subapex, very close to the hyphal tip. Whether endocytosis in the subapical collar is clathrin dependent is still not clear. In this work, we examined the dynamics and localization of AP180, an adaptor of the clathrin coat, by fluorescent-protein tagging in *Neurospora crassa*, using laser scanning confocal microscopy, spinning disk confocal microscopy and total internal reflection fluorescence microscopy. Additionally, we analyzed the deletion mutant, and a mutant where the ENTH endocytic domain is absent. We observed that AP180 was located forming patches in the endocytic collar and in septation sites. Fluorescence signal was also detected as highly dynamic scattered patches and tubular structures along the hypha, which moved both in retrograde and

anterograde direction. Some patches seemed to be aligned, and some even fused with each other. The deletion mutation of AP180 was lethal for *N. crassa,* while the deletion of the ENTH domain is not lethal but it affects the protein localization at the subapical collar. It is curious that AP180 localizes at the endocytic collar even if clathrin does not. In conclusion, AP180 is an essential protein, part of the endocytic machinery, the absence of clathrin in the endocytic collar suggest that AP180 might be working as part of a clathrin independent pathway.

2.2-31 Identification and functional testing of Wsc1-3, Mid2 and Mtl1 protein-protein interactions forming novel stress signaling complexes in yeast

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Abstract: Signaling proteins required for activating the yeast PKC1-Cell Wall Integrity (CWI) pathway are attractive targets for antifungal drugs because they contribute to cell viability under stress. The objective of this research is to identify proteins that interact with stress sensor proteins Wsc1p, Wsc2p, Wsc3p, Mid2p and Mtl1p of the PKC1-CWI pathway and determine if they contribute to cell survival under stress conditions. Our hypothesis is that these interacting partners are required for resistance to antifungal drugs and environmental stress. To identify novel interacting partners, the integrated Membrane Yeast Two-Hybrid (iMYTH) technique was applied. Confirmatory tests were Immunoprecipitation coupled to Mass Spectrometry (IP-MS) and Affinity Purification with Western blot (AP-WB). To test the functional importance of specific interactions, viability assays were performed under stress conditions induced by Caspofungin (CS, 75ng/ml), temperature, and an oxidizing agent (1mM H_2O_2). PKC1-CWI pathway activation was assayed by Western blot. At 30°C, 14 novel interactors were confirmed for Wsc1p, 31 for Mid2p, 14 for Wsc2p, 5 for Wsc3p, and 15 for Mtl1p. Interacting proteins were associated with biological processes of signal transduction, stress response, cell wall organization, cytoskeleton organization, and unknown functions. Secondary tests confirmed 14 interactors for Wsc1p, 13 for Mid2p, 2 for Wsc2p and 3 for Wsc3p. Confirmatory tests for Mtl1p interactors are underway. Null mutant strains of stress sensors or their interacting partners acquired sensitivity to H_2O_2 (3) and CS (6). Double mutant strains atx1Δmid2Δ, grx1Δmid2Δ, ras2Δmid2Δ and ras2Δwsc1Δ acquired sensitivity to both CS and H_2O_2. Ras2p expression was required for PKC1-CWI pathway activation by oxidative stress. Novel stress signaling proteins involved in PKC1 regulation were identified. These were shown to be associated with yeast cell survival under stress conditions.

2.2-32 Cleaning a multinuclear single cell during fruiting body formation of myxomycetes

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Abstract: Myxomycetes (true slime molds) have a multinuclear single cell stage, plasmodium, which makes fruiting bodies containing numerous myxomycete spores. It has been fragmentary reported that degradation of cellular comportments such as organelle degeneration and deterioration of a layer of peripheral cytoplasm during fruiting body formation. The possible roles of such degradations in the life cycle of myxomycetes also have been barely discussed, however, the risk of its insufficiency has not been emphasized. For cellular integrity, it is critical to clean the cell of damaged organelles, aggregated

proteins and invaders. The objective of this study is to determine how a single cell itself cleans up own used/aged cell components to generate pre-gamete cells, i.e., myxomycete spores. Sequential steps of cell cleaning behavior in myxomycete fruiting body formation analysed by two- and three-dimensional imaging. Organelle clearances especially on nucleus were observed with broad visual field and electron microscopic 2D/3D. DNA fragmentation was examined with cryo-fixed cell. Cleaning process during fruiting body formation were different among species, and the relation with construction of fruiting body structures was observed. DNA fragmentation was detected in multiple nuclei, and its distribution in a single cell differed in the stages of fruiting body formation. The selection of reduction processes in the number of nuclei was also depended on the stages. The results suggested that a myxomycete cell behave sensibly during the fruiting body formation, and cleans up own single cell without rupture or complete cell death.

2.2-49 *Aureoboletus projectellus* - new invasive bolete, rapidly spreading in Europe as an opportunity to study invasions of macromycetes.

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Abstract: American bolete *Aureoboletus projectellus* was reported in Europe in the beginning of the century. At first it was only observed on the Baltic Sea shore. Since 2014 the occurrence range of the species has increased significantly. It now reaches more than 150 km into land and covers eight countries. Main aim of the study was to establish a framework for studying invasion of macrofungus *in statu nascendi* based on the spread of *A. projectellus*. A model prepared in MaxEnt was used to estimate distribution of potential niches of *A. projectellus* in Europe. Comparing model together with data about species distribution in invasive range were combined to predict way of future dispersion and to help to prepare a list of location that should be monitored. Baltic Sea coast line seems to be one of the most suitable area for invasive range. The continuous ring of favorable conditions all around Baltic sea shows that the invasion is unstoppable but can be suitable for studying invasion process. Other disjunctive areas of favorable habitats include Alpine foothills and small remote mountains located on the south of Europe (for example Apennines and Pyrenees). Invasion in remote islands can be probably slowed down or even stopped by preventing the transport of edible fruit bodies or planting pines which is mycorrhizal partner of *A. projectellus*. Every attempt to stop the spread of invasive fungus is important because *A. projectellus* can be a threat to domestic/native species of mushrooms (eg, *Tricholoma focale* or *T. apium*).

2.2-50 Testing the ecosystem function of fungi with isotopes: A case study of nitrogen pollution and *Russula*

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Abstract: Understanding how soil fungi use nitrogen will be critical to our understanding of fungal diversity in contexts of N deposition, a driver of global change. Recent studies have shown that when nitrogen is added to forest ecosystems, the fungal community changes and ectomycorrhizal fungal (EMF) abundance declines. However, at an experiment based at the Harvard Forest, at least one species of ectomycorrhizal fungus, *Russula vinacea*, proliferates in high nitrogen environments, challenging the general finding that EMF species decline when N is added. Stable isotopes of ^{13}C and ^{15}N have consistently revealed differences in the isotopic signatures of EMF and saprotrophic (SAP) fungi. I am using stable ^{13}C and ^{15}N isotopes from fungal sporocarps to probe how *R. vinacea* accesses nutrients in high nitrogen environments, and to test whether *R. vinacea* is, in fact, an EMF, or if it behaves as a

saprotroph in high N contexts. I am collecting *R. vinacea* sporocarps from multiple N addition treatments at the Harvard Forest, including 0 N added, 50 and 150 kg N/ha/yr added, and extracting tissue for isotopic analysis in elemental analyzer machines. If the isotopic signature of *R. vinacea* is enriched in $\partial^{13}C$ and $\partial^{15}N$, it will infer that the fungus is still accessing nutrients from a live plant symbiont. However, if the signature is depleted in $\partial^{13}C$ and $\partial^{15}N$, a contrast to what is known about the isotopic signatures of EMF in general, data will suggest this fungus has found a new way to access nutrients in high nitrogen environments. The method I am developing is likely to pin point how surviving EMF are functioning in N enriched environments.

2.2-51 Long-term nitrogen fertilization alters nitrogen isotopes and concentrations in ectomycorrhizal fungi, hosts, and soil in pine forests

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Abstract: To assess how nitrogen (N) availability affected functioning of ectomycorrhizal fungi and interactions among ectomycorrhizal fungi and plants, we measured %N and $\delta^{15}N$ in soils, foliage, and nine ectomycorrhizal fungal taxa in six-year (Rosinedalsheden) and 40-year (Norrliden) N addition experiments in *Pinus sylvestris* stands in northern Sweden. The %N of the F horizon correlated strongly with sporocarp %N, reflected nitrogen addition history, and served as a useful proxy for N availability. Both sporocarp %N and soil %N increased with fertilization, implying that nitrogen uptake per unit fungal growth and protein content of sporocarps increased with N fertilization. In addition, foliar %N correlated strongly and positively with foliar $\delta^{15}N$, with both increasing with N additions. Our prior growth chamber studies indicated that this positive relationship is related to declines in carbon allocation to ectomycorrhizal fungi at high N availabilities. Thus, we suggest that $\delta^{15}N$ in ectomycorrhizal *Pinus* can be used as a proxy for carbon allocation patterns to ectomycorrhizal fungi and a useful indicator of the extent of alteration of plant-fungal interactions across N availability gradients. Patterns in fungal and soil $\delta^{15}N$ across the two sites suggested that N acquisition was primarily from the H horizon for *Cortinarius traganus* and *Russula aeruginea*, from the S horizon for *Lactarius rufus* and *Paxillus involutus*, and from the F horizon for the other five taxa. Higher ^{15}N enrichments in *Cortinarius semisanguineus*, *Suillus variegatus*, and *Paxillus involutus* relative to source N with increased N availability suggested enhanced transfer of N to plants (higher transfer ratios), whereas other taxa did not change transfer patterns. Our results indicated that sporocarp nitrogen concentrations and $\delta^{15}N$ patterns were useful integrators of fungal responses to N addition, and should be useful in gauging fungal responses to other environmental perturbations.

2.2-52 Ergosterol and nitrogen levels provided by ophiostomatoid fungi associated with the mountain pine beetle (*Dendroctonus ponderosae*) host tree colonization

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Abstract: Mountain pine beetle (*Dendroctonus ponderosae*, Coleoptera: Curculionidae) has killed pine trees on millions of hectares of forest over the past decade in western North America. In Canada, beetle outbreaks had been historically restricted to central British Columbia, but has been recently expected its host range into jack pine (*Pinus banksiana*) forests in Alberta. The beetle inoculates fungal spores of symbiotic, ophiostomatoid (Ascomycota: Ophiostomataceae) fungi as it colonizes host pines. Three most common fungi species associated with the mountain pine beetle in Alberta are: *Grosmannia clavigera*, *Ophiostoma montium*, and *Leptographium longiclavatum*. These fungi play important roles in

beetle host range expansion and survival as they help to overwhelm tree defenses and are an important source of dietary nitrogen and ergosterol for developing beetles. Nitrogen is an important constituent of proteins, nucleic acids, and hormones and can increase in the phloem when the fungi associated with the mountain pine beetle are present. Sterols, on the other hand, are needed for beetle metamorphosis and reproduction and in the case of the ergosterol, its availability changes depending on the fungus species. Our objective was to determine the relative benefit of each of the fungi to mountain pine beetle in terms of their ability to concentrate nitrogen in pine phloem and produce ergosterol along tree stems. Eighty healthy jack pines occurring in two forest stands in Lac La Biche (Alberta, Canada) were inoculated with live plugs from cultures of the three fungi or non-colonized media (control) at three heights along the tree bole: 1m, 3m, and 5m (twenty trees per treatment). Phloem samples were taken during inoculation process to determine constitutive nitrogen levels prior to fungal growth at each height. Six weeks after inoculation, phloem samples were taken from inside and outside of phloem lesions resulting from fungal infections. These samples were analyzed for total nitrogen and ergosterol levels through dry combustion and ultra-performance liquid chromatography, respectively. Nitrogen and ergosterol levels by fungi species and heights will be presented and discussed.

2.2-53 Mycorrhizas, masting, and monodominance in the Neotropical leguminous canopy tree *Dicymbe corymbosa*

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Abstract: Pulse-like bursts of heavy seeding followed by several low-to-no reproductive years (i.e. "masting") are well known from ectomycorrhizal (ECM) trees which dominate high latitude forests, e.g. Pinaceae, Fagaceae. In tropical rainforests masting has been documented in ECM tree species of Fabaceae subfam. Detarioideae and Dipterocarpaceae, which are often locally dominant or co-dominant. Masting may be an evolved trait based on an "economy of scale" mechanism for enhancing seedling establishment. In remote tropical rainforests of Guyana, from 1998–2017, we investigated annual seeding, seedling survivorship and growth, and ECM colonization of the monodominant canopy tree *Dicymbe corymbosa* (Fabaceae subfam. Detarioideae) – a species with highly synchronous supra-annual seed production. Seed output, predation, and nutrient investment were quantified for each seeding year in primary *D. corymbosa* forests following the 2003 mast. Establishment, survival and mycorrhization of seedling cohorts were monitored, and climatic conditions associated with seeding events were assessed. Mast seeding occurred in 1998, 2003 and 2016, with three intervening low seed years, indicating a strongly bimodal seeding pattern for *D. corymbosa*. El Niño-intensified dry season conditions preceded each masting event and regionally synchronized the flowering. The attendant increased solar irradiance appears to magnify flowering and fruiting through increased carbohydrate production. Similarly, ECM-mediated phosphorus (P) cycling appears paramount to facilitating these masting events by (1) refueling stored foliar P to threshold levels and (2) returning subsequent P losses in reproductive parts. Seedlings require rapid ectomycorrhization to persist in high densities following masting events. While > 300 species of ECM fungi occur in these *D. corymbosa* stands, the ECM fungi dominating seedlings were mainly a handful of *Tomentella* species which also dominate adult trees. Seedlings persist with little growth for decades in the heavily shaded understory, but are able to respond quickly to canopy openings. Masting contributes the greater part of standing seedling crops, and along with efficient nutrient recycling by ECM fungi, is critical for persistent monodominance in *D. corymbosa*.

2.2-54 Ectomycorrhizal fungal communities associated with *Abies koreana* in relation to abiotic environmental factors

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Abstract: Ectomycorrhizal (ECM) fungi have a symbiotic relationship with woody plants and they play an essential role in nutrient cycling and maintaining the ecosystem. *Abies koreana* is an endemic species in Korea and found mainly in the sub-alpine areas of Mt. Halla in Korea. The *A. koreana* population has declined and has been designated as critically endangered, which might be due to climate changes. The climate changes also affect the communities of host plants and ECM fungi. This study was conducted to investigate the effects of abiotic factors on ECM fungal community associated with *A. koreana*. We sampled the roots and rhizosphere soils of two host plant species, *Abies koreana*, *Taxus cuspidate*, in two different climate zones, subalpine and temperate, of Mt. Halla. We identified ECM using morphological characteristics of ECM root tips and sequence analysis of internal transcribed spacer regions of rDNA. ECM fungi in the soil were analysed using Illumina Miseq. We detected a high correspondence between fungal diversity by a molecular analysis of the root tips and soil samples. The ECM fungus communities in the roots and soils were significantly different in terms of biotic factors. The results suggest that ECM fungal communities differed in different climate zones and were affected by abiotic environmental factors.

2.2-55 Biogeographic pattern of Collembola fauna associated with the insecticidal mushroom *Strobilurus ohshimae* in Japan

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Abstract: Organisms interact with various other species, and the differences in the associated species drive diversification. The variability in the interactions between macrofungi and fungivores are poorly understood. The basidiomycete *Strobilurus ohshimae*, which fruits exclusively on fallen twigs of *Cryptomeria japonica*, has sporocarps covered with secretory projecting cystidia that can kill Collembola on contact. Collembola are wingless soil microarthropods, some of which feed on macrofungal sporocarps in huge numbers. Some Collembola species avoid *S. ohshimae* sporocarps, while others feed on them. We investigated the geographic distribution of Collembola species feeding on *S. ohshimae* sporocarps in 12 native *C. japonica* forests in Japan. The presence of potential sporocarp feeders was surveyed using bait-traps containing Shiitake mushrooms. The preference for *S. ohshimae* was evaluated by determining the density of *S. ohshimae* per sporocarp mass relative to that for Shiitake mushrooms. The presence, species, and mode of feeding of Collembola preferring *S. ohshimae* varied with the site. In Akita, *Ceratophysella tergilobata* fed mainly on the gills of *S. ohshimae*, while *Ceratophysella* sp. 1 fed mainly on the interior parts of the stipes. In Yamanashi, Kyoto, Wakayama, Oki Is., Shimane, and Kochi, *Ceratophysella pilosa* or closely related taxa preferred *S. ohshimae* and fed mainly on the interior parts of caps. In Miyagi and Yakushima Is., no Collembola showed preference for *S. ohshimae*. *Ceratophysella denisana*, which is distributed widely, and *Ceratophysella* aff. *horrida* never showed preferences for *S. ohshimae*. *Ceratophysella* sp. 2 preferred *S. ohshimae* in Shizuoka, but not in Kochi. *Morulina alata* fed on S. ohshimae in Akita, Sado Is., Toyama, and Oki Is., but did not show strong preferences. These results suggest that the strength and mode of impact of Collembola grazing on *S. ohshimae* varied geographically.

2.2-56 Fungi of *Mortierella* section *lignicola* as potential partners of ants

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Abstract: *Mortierella* is a genus containing nearly 100 species. It belongs to one of the basal phylogenetic lines of terrestrial fungi (*Mucoromycota*). *Mortierella* species are generally known as prototrophic organisms living in oligotrophic habitats. Wagner et al. (2013) provided a comprehensive molecular phylogeny of the order Mortierellales based on nuclear ribosomal DNA and divided all members of the order into seven clades. The aim of the study was to trace the presence of fungi belonging to *Mortierella* on ant's bodies and check the insect's response to presented mycelia in nature. We used the method of isolation of fungal strains straight from cadavers to artificial media and analyzed films documenting ant's behavior in presence of fungal mycelia. Results of our studies revealed that most *Mortierella* strains isolated from healthy ants belonged to Wagner's clade "3" also called lignicola section. Furthermore, some strains from CBS collection used by Wagner also were sourced from infrabuccal pellets of *Campanotus* and *Formica* ants - both from America and Europe. We described new species – *M. formicae* located in that clade. The strain, characterized by compact clusters of gemmae was isolated from *Formica pratensis* workers. We also obtained *M. beljakovae* from *Formica rufa, F. cinerea* and *F. pratensis* ants, as well *M. gemmifera* from *Lasius flavus*. All of above mentioned species characterize by abundant formation of chlamydospores. The studies of *Myrmica scabrinodis* also revealed unexpectedly high occurrence of *Mortierella* on this species. The behavioral observations with test of choice suggest that ants prefer *Mortierella* mycelium more than mycelia of other species. The tendency for transporting the mycelium particles to the nest was observed. We propose a hypothesis that *Mortierella* is used by different ant species of temperate zone as food or source of enzymes. Insects nests could be used by fungi as a suitable habitat. The workers and queens participate in fungal dispersion. The clusters of chlamydospores produced by fungi of lignicola clade could be an adaptation for such symbiosis. The study was supported by the National Science Centre, Poland under grant No. 2016/23/8/N28/00897.

2.2-65 A twenty-year morphological and molecular study of macrofungi in the driftless (unglaciated) region of southwestern Wisconsin, USA.

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Abstract: Southwestern Wisconsin was unaffected by the glacial drifts that ended some 10,000 years ago, and is now referred to as the driftless area. This area is known for the forested hillsides of limestone-based soils with deep valleys called coulees. These ridges and coulees centered on the Mississippi River offer relicts of forests, prairies, wetlands and grasslands that house a diverse array of organisms. A 20-year survey of fruiting bodies of three older growth forests found approximately 1200 species of macrofungi that include some species with disjunct geographical distributions. Previous graduate students as well as students from the UW-La Crosse Mycology class have been making these collections every year on a three-week rotation. At least 500 students and visitors from three mycological forays have been involved in these collecting efforts. Despite their physical proximity, it is surprising that the total overlap within the three sites is just 303/1200 species (~25%). Some Appalachian relict species have been collected (e.g. *Boletus frostii* and *Ciboria americana*), as well as unusually high population densities of some fungi considered to be rare elsewhere (eg. *Phlebia coccineofulva*). Preliminary data from groundwork DNA sequencing suggest that, because of its isolation, the driftless area may also be home to cryptic species of fungi. In our study, all 1800 specimens of nearly 1200 morphological species

from the three study sites are being sequenced, using the ITS region as the primary region of interest. Primers with reference tags are added to the specimens DNA, with different tags on both the forward and reverse strands. These tags allow the delineation of species after the samples are sequenced through Illumina sequencing. Having both morphological and sequence identification will allow for the better understanding of fungi associated with this area, along with confirmation of the species already identified by morphology. These data will also allow for comparisons of geographical regions and revelations of cryptic species. This study is novel because of the large number of specimens and species involved, as well as the length of time (20 years) over which the collections were made.

2.2-66 Towards a Colorado Mycoflora: Molecular diversity of mushrooms from the Southern Rockies.

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Abstract: The diversity of macrofungi across North America is staggering and, traditionally, morphology has been the primary means of identification. While morphology is still considered useful, the advent of cheap and effective DNA sequencing has enhanced the exploration of taxonomic relationships among mushroom forming fungi. Currently, the identities of many North American macrofungi are based on morphological concepts that borrow from Eurasian species. The North American Mycoflora Project was created to test species concepts of the continent's mushroom forming fungi using DNA sequence data. The North American state of Colorado boasts a wide range of fungal diversity. This project aims to study the specimens vouchered in Vera Evenson's book "Mushrooms of Colorado and the Southern Rocky Mountains," and housed in the Sam Mitchel Herbarium of Fungi at Denver Botanic Gardens, in order to produce a regional mycoflora of Colorado and the Southern Rockies. Using ITS sequence data this project intends to expand upon our understanding of the region's diversity of macrofungi. The results of this project represent the projects first steps, using ITS sequence data of over 100 species Colorado mushrooms and allies, and exploring whether the names traditionally used for the region's fungi hold up to direct scrutiny.

2.2-67 Creating a regional mycoflora – an Indiana case study

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Abstract: Attaining a reasonable understanding of the biodiversity of macrofungi for any given region has mostly eluded researchers working in North America. Despite over 200 years of mycological progress, few regions have up-to-date surveys of the macrofungi that occur in their area; this is especially true for surveys that are broadly supported by genetic data. As a part of the North American Mycoflora Project, a full-scale, state-wide survey of macrofungi is currently underway in Indiana. This includes three primary components – extensive field collecting, broad environmental sequencing, and high-level engagement of citizen scientists. Here we report preliminary progress towards a mycoflora of Indiana involving over 4,000 new vouchered and sequenced specimens deposited in the Purdue Kriebel Fungarium since 2015. These sequenced specimens will provide the "faces" for large-scale environmental sampling efforts being conducted across the state. Methodologies for successful citizen scientist engagements are also discussed. Together, these three components combined will significantly enhance our understanding of regional diversity and phenology. It will also form a core dataset to be used in future ecological research.

2.2-68 More than 80% of fungi in northern Thailand are new to science and have amazing biotechnological potential

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Abstract: Fungi are an understudied, but essential, fascinating and biotechnologically useful group of organisms. We have been studying the fungi of northern Thailand at Mae Fah Luang University since January 2008. During this time, we have grown from a single researcher, to a large team that has published more than 500 SCI papers. Our studies have been diverse, from phylogeny and taxonomy of microfungi, to growing novel mushrooms, and correctly identifying plant pathogens. In this presentation, I will discuss the importance of fungi and the advances we have made in the Center of Excellence in understanding the biodiversity of fungi in the region. We have made huge advances in the understanding of the fungi at the higher levels. We have inventoried a large number of new fungi for the region, but a huge amount is still to be done. For example, in the edible genus *Agaricus*, we have introduced more than 20 new species, and many more are waiting description. In these relatively well-known genera we are finding about 80% of species we collected are new to science. In the microfungi, which are relatively poorly studied, the percentage even higher. At the same time, we have been finding ways to exploit these fungi. Our work has resulted in the discovery of at least ten new species which are being developed as novel industrial mushrooms. We have isolated at least ten novel medicinal compounds from Thai fungi and are also looking at ways to exploit them in biocontrol. Selected examples from this study will be given. Our work is just a beginning. Fungi have been generally neglected over time, despite the fact that they provided Penicillin, Lovastatin and various important medicines. Fungi have been poorly exploited and yet have a huge potential in biocontrol, bioremediation, novel compound discovery as well as basic industrial organisms (mushroom, fertilizers and cosmetics).

2.2-69 Diversity and novel species of Entomopathogenic fungi in a conservation area: Banphao Thai community forest, Thailand

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Abstract: In Thailand, entomopathogenic fungi are one of the species diverse groups. They play an important role in the control of population of insects. Banphao Thai forest in Phitsanulok province is a community forest located in the upper central part of Thailand, and covers an area of 0.64 square kilometers. The ecosystem of Banphao Thai community forest is unique by having cassava plantations surrounded by wilderness. The diversity and molecular phylogenetic studies of entomopathogenic fungi were the objectives that have been established in a collaboration between the Centre of Excellence in Fungal Research (CEFR), Naresuan University and Microbe Interaction and Ecology laboratory, BIOTEC, Thailand. During a field study carried out from 2016 to 2017, specimens were recorded, collected, then preliminarily identified according to their morphological characteristics and then subjected to molecular phylogenetic study. The diversity and abundance of entomopathogenic fungi is high during the rainy season from May to November. A total of 393 entomopathogenic fungi belonging to six genera include *Beauveria, Cordyceps, Conoideocrella, Ophiocordyceps, Metarhizium* and *Polycephalomyces*. Their

insect hosts were classified to Coleoptera (75.3%), Hemiptera (6.9%), Hymenoptera (0.3%), Isoptera (8.7%), Orthoptera (0.5%), Lepidoptera (8.4%). *Shimizuomyces* sp. growing on fruit plants were also found in the ecosystem. Interestingly, the interactions between the hyperparasitic fungus *Polycephalomyces* and their diverse hosts has been observed in this ecosystem. The novel fungal pathogen *Polycephalomyces phaothaiensis* is a parasite of coleopteran larvae which occurs in rainy season. Meanwhile *P. phaothaiensis* also plays a role as hyperparasitic fungus which infects numerous fungal hosts such as *Ophiocordyceps* sp.1 and *Ophiocordyceps* cf. *brunneipunctata,* found predominantly during the rainy season. From our investigations, we found 21 species of entomopathogenic fungi and a first record of the genus *Shimizuomyces*. *Polycephalomyces phaothaiensis* has multiple hosts and little is known about its life cycle and ecology.

2.2-70 Decontamination of aflatoxin B1 and mycocinogenic activity evaluation by using yeasts belonging to the complex *Meyerozyma guilliermondii*

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Abstract: This study pursued understanding the modes of action by which yeast isolates belonging to the complex *Meyerozyma guilliermondii* affect AFB_1 production by *Aspergillus flavus*. Isolates Y16 and Y25 were not able to produce *killer* toxins. However, whole cells and some cellular constituents were able to reduce AFB_1 concentrations in different evaluated conditions. Cell-free supernatant of isolates decreased AFB_1 levels in liquid medium. For this reason, the effect on ground maize grains was studied. The characterization of the cell-free supernatants and the optimization of the biological decontamination activity of the toxin were tested. The experimental design showed that the best conditions for the cell-free supernatant of yeast Y16 to produce a significant reduction of AFB_1 were 39° C, pH 8 and the presence of Mg^+. The cell-free supernatant of yeast Y25 was effective at 28° C, pH 5 and presence of Mg^+. These results made us suppose a possible enzymatic effect in the reduction of AFB_1. To determine whether the effect of the cell-free supernatant of the yeasts on the accumulation of AFB_1 was attributable to a protein activity, they were subjected to heat inactivation and proteinase k treatment. No changes on the reduction of the toxin levels were detected. The toxin reduction doesn't correspond to an enzymatic effect. The cell-free supernatant from the two yeast isolates added to ground maize grains were able to reduce the levels of AFB_1 when the toxin was already present in the substrate. Characterization of cell-free supernatants and optimization of the biological decontamination activity of the toxin are necessary steps in the process of selecting a treatment applicable to stored maize.

2.2-71 Phenotypic and phylogenetic studies of selected Philippine invertebrate pathogenic fungi

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Abstract: Invertebrate pathogenic fungi (IPF), are fungi that grow superficially on invertebrate exoskeleton and progress all throughout the body. They encompass an extensive set of phenotypically, phylogenetically, and ecologically distinct fungal species on invertebrates. The present study involves host specificity, phenotypic and phylogenetic studies of IPF. Isolation was done using Potato Dextrose Agar (PDA) and using Lactophenol Cotton Blue to stain the structures for phenotypic analyses.

Cetyltrimethyl-Ammonium- Bromide (CTAB) method was used for DNA extraction. PCR amplification was performed in a total of 25 ul volume using DNA markers of EF1 and ITS. PCR products were checked using Agarose Gel Electrophoresis and Ethidium Bromide used to stain the gel. Purified PCR products were sent to Macrogen South Korea for sequencing and Alignment was performed using BioEdit (Hall, 1999). Phylogenetic Analyses was executed using a maximum parsimony employed in MEGA 6 (Tamura et al., 2013). Phenotypically and Sequence Phylogeny from ITS gene loci confirmed the identification of IPF to *Simplicillium* sp, *Cordyceps* sp. and *Isaria* sp. same with the EF1 gene marker. Species specificity on spider in the family of Sparassidae was the highest (15, 50.00%), followed by Araneidae (5, 16.66%), Tetragnathidae (4, 13.33%), Salticidae (2, 6.66%) and Oxyopidae (1, 3.33%). The present study discussed the evolutionary relationship of IPF in the country and further study is needed to support the results.

2.2-72 The Rio Claro as species refuge of arthropodopathogenic fungi *Cordyceps* in the Magdalena river valley, Colombia

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Abstract: The Rio Claro canyon is a private reserve located at 350 m.a.s.l. in the middle of the Magdalena River basin which lies between the eastern and central Andean cordillera of Colombia. It lies within the Neotropical region and belongs to the Magdalena biogeographic zone, characterized by having biogeographical units which are considered Pleistocene refugia and are used to delimit areas of endemism, that is, they are the result of a process of fragmentation of the area and the temporal isolation of the species contained in these units. The Rio Claro canyon is part of one of these units and according to research carried out during the last three decades, the area has special geological and geomorphological phenomena, such as tropical karstifications in marble, which are the result of the erosion of the mountain range during the last six million years. The forests therefore grow on very particular soil conditions, with a humidity above 75% and temperatures of 28°C on average, and are distinctive. This special vegetation leads to a wide diversity of species of amphibians, mammals, reptiles, and birds, many of them endemic. With these geomorphological characteristics it would be expected that the fungi would also exhibit a high diversity of species and endemism. To investigate this, the species of the arthropodopathogenic fungus *Cordyceps*, which is linked to the arthropod populations, was sampled in the years 2014 to 2017 during the rainy season. They were sampled along a 7 km portion of the canyon. In total, 70 specimens were collected, comprising 29 different species, two of which were new records for Colombia. The most common species were *Cordyceps tenuipes* and *Ophiocordyceps amazonica,* while *Polycephalomyces ramosus* and *P. nipponica* were the new records for the country. Interestingly, the epizootic species *O. unilateralis* on carpenter ants and *O. araracuarensis* on Cicadae nymphs were always found in an area of the forest containing wild cacao trees. The results show that *Cordyceps* has a high diversity corresponding with the previous findings that of Rio Claro canyon as a center of endemism. The conservation of the Rio Claro basin becomes a priority for the government given the current proposals for mining, with the goal to conserve the germplasm that constitutes this Pleistocene refuge.

2.2-81 Interactions between *Inonotus obliquus* and a successor fungus *Hericium coralloides*

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Abstract: Species of wood-inhabiting fungi succeed each other during the process of tree-killing and wood decomposition. The interactions in deadwood are diverse, including some specific relationships where the successor prefers a resource previously occupied by a certain other fungus. The coral tooth fungus, *Hericium coralloides* (Scop.) Pers., is an edible species widely found in Finland, but restricted to sites with abundant deadwood. This saprotrophic species is considered a successor fungus, occurring often on hardwood trees killed by the pathogenic *Inonotus obliquus* (Fr.) Pilát. However, detailed understanding of the association between these two fungal species is lacking. In this study, the interspecific interactions between the *H. coralloides* and *I. obliquus* were studied in two approaches: 1) in co-cultures on three different agar media, and 2) micromorphological study of the structural features of the interaction, under light and helium ion scanning (HIM) microscopy. The isolates used included three strains of dikaryotic *H. coralloides* obtained from fresh basidioma collected from Southern and Eastern Finland in 2016 and 2017 (H1-3), and one dikaryotic *I. obliquus* strain from Eastern Finland. The agar media used were 2 % Malt Extract Agar (MEA), 2 % MEA enriched with 3% birch sawdust (MEA+SD), and water agar with 3 % birch sawdust (WA+SD). Each pairing was replicated three times on each media. The co-cultures were incubated for 100 days, during which the radial growth was measured, and the interactions were reported as: 1. no contact, 2. deadlock, 3. partial replacement or 4. complete replacement. With the *H. coralloides* strains H1 and H2, the radial growth of *I. obliquus* stopped at a consistent distance from *H. coralloides*, whereas loose mycelium of *H. coralloides* grows through *I. obliquus* mycelium. After contact, *I. obliquus* mycelium in the through growth zone thickens, with abundant aerial hyphae and often increased guttation. This was followed by gradual dying of *I. obliquus* hyphae, with the *H. coralloides* eventually replacing *I. obliquus*. Partial replacement of *I. obliquus* was the result in 56% of cases , whereas in 28 % of cases, alive *I. obliquus* hyphae was not found among now very dense *H. coralloides*. With the *H. coralloides* strain H3, the outcome varied considerably. The strain of *I.obliquus* used replaced H3 partially on MEA, deadlocking on WA+SD, and was partially replaced on MEA+SD. The samples studied by microscopy revealed that *H. coralloides* hyphal tips coming into contact with *I. obliquus* branch and coil around latter's hyphae, which subsequently lose turgor and structure. Lysis and vacuolation of *I. obliquus* hyphae appear to follow. In addition, when in contact with *I. obliquus*, terminal chlamydospores emerge in *H. coralloides* hyphae. Our results support the assumed predecessor-successor relationship between the two species and suggests it may involve a phase of direct physical antagonism, with features typically associated with mycoparasitism. *H. coralloides* occurs also on hardwoods that are not known to host *I. obliquus*, such as *Populus*, so further study in the specificity of this strategy is needed.

2.2-82 *Funnelimoris mosseae* alters soil fungal community dynamics and composition during litter decomposition

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Abstract: Although arbuscular mycorrhizal fungi (AMF) are believed to be non–saprophytic, recent studies have indicated that AMF are able to influence litter decomposition through interacting with the

soil fungal community. However, it remains unclear exactly which constituent groups of the soil fungal community respond to AMF during litter decomposition, and in what ways. In order to fill this knowledge gap, we investigated the effect of AMF on soil fungal communities in a subtropical forest in southwestern China. Our experimental set-up included a dual microcosm unit with two treatments: inoculated with AMF (AM) and uninoculated (NM). Destructive soil sampling was carried out at different times (T_0, T_{90}, T_{120}, T_{150} and T_{180}) and Illumina sequencing was used to detect changes in soil fungal community composition. We found that the composition and operational taxonomic unit richness of the fungal community, at higher taxonomical levels (e.g. phyla, order), remained stable across treatments. However, the relative abundance of some key genera including *Mycena, Glomerella, Pholiotina,* and *Sistotrema* were significantly affected by AMF inoculation. Soil fungal community structure was also significantly altered by AMF inoculation during the later stages of litter decomposition, but the diversity of the soil fungal community was unaffected. Our study provides new insight into understanding the interaction between AMF and soil fungal communities during litter decomposition.

2.2-83 Assembling the phylogenetic tree of life of the parasite *Escovopsis*

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Abstract: Symbiotic interactions play an important role in the evolution of life and allow structuring complex systems in nature. Multipartite symbiotic associations with microbes enabled the evolutionary success of fungus-farming 'attine' ants (Formicidae: Myrmicinae: Attini: Attina). These insects collect various substrates for feeding a mutualistic fungus, which is farmed as food source for their colonies. Nevertheless, fungi in the genus *Escovopsis* can affect this mutualism eventually leading attine ant colonies to death. These fungi are thought to be specialized parasites of the attine ants' fungal cultivar. Until now *Escovopsis* is the only known parasite that exploits the ant-fungus mutualism. However, the lack of a broad phylogenetic study hampered our knowledge of the parasite diversity as well as the phylogenetic position of some strains. Here, we shed light on the *Escovopsis* tree of life. Amassing a large number of isolates (365) spanning from several regions in Brazil and other Latin American countries we carried out a robust phylogenetic analysis using five molecular markers (ITS, LSU, *tef*1, *rpb*1 and *rpb*2). Combining phylogenetic with morphological analysis, our results support that *Escovopsis* (*sensu lato*) comprise multiple fungal genera. It includes *E. kreiselii* and *E. trichodermoides,* which form two well-defined phylogenetic clades distinct from *Escovopsis* (composed by 28 and 1 subclades, respectively). In addition, two other genera were found (with four and five subclades). *Escovopsis* (*sensu stricto*) comprehends at least 32 sub-clades including the five formally described species with vesiculate coniophores. Lack of studies in *Escovopsis* systematics made impossible the recognition of these genera. Our results open several questions whether more than one fungus coevolved as parasites like *Escovopsis* and how multiparasitism relationships modelled the life of attine ants.

2.2-84 Parasites of rust fungi from South America

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Abstract: The genera of fungi and dipterans, *Sphaerellopsis* and *Mycodiplosis*, respectively, are known to be cosmopolitan parasites of a wide range of rust fungi. These have the potential to control rust diseases as one alternative to fungicides. Although geographic distribution and genetic variation have been studied in North America and part of Europe for some species, records and data analysis from South America are currently very scarce. Given the high biodiversity in the tropics, the number of genera

of fungi and insects feeding on rusts may be underestimated. We are currently studying rust specimens collected from South America deposited in the Arthur Fungarium (PUR) at Purdue University in terms of geographic distribution, genetic diversity and species richness. Every rust sorus from each specimen will be scanned on a stereomicroscope, and any sign of parasites, such as pycnidia, mycelium, and/or larvae will be extracted from the rust sori for DNA isolation. Internal transcribed spacer (ITS) region and mitochondrial cytochrome c oxidase subunit 1 (COI) for fungi and insects, respectively, will be used to study the genetic variation of these parasites. From the first 207 collections examined so far out of approximately 10,000 collections from South America, 48 specimens were infected by mycoparasites and 28 specimens by fly larvae, finding even—in some collections—two different parasites per specimen. Fly larvae such as *Mycodiplosis* spp., and mycoparasites such as *Eudarluca caricis*, *Ramularia uredinicola*, *Setophoma* sp., and *Sphaerellopsis paraphysata*, have been so far found on *Uredinopsis* and *Melampsora* species. These parasites were discovered in specimens from Argentina, Bolivia, Brazil, Colombia, and Uruguay. We also found the first report of *R. uredinicola* in Argentina associated with *Melampsora epitea* on *Salix babylonica*, suggesting that scanning more specimens may lead to the discovery of more first reports and maybe new unknown species from South America.

2.2-85 Endophyte communities in limber pine in relation to resistance and susceptibility to white pine blister rust

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Abstract: The non-native pathogen *Cronartium ribicola* causes the lethal disease white pine blister rust (WPBR) on five-needled pines throughout much of North America. A susceptible species, limber pine (*Pinus flexilis*), is a high elevation pine valued for its ability to colonize environmentally harsh sites, its role in early forest succession, and as a food source for wildlife. Limber pine is declining from the disease in the Northern and Central Rocky Mountains and *C. ribicola* continues to spread to the Southern Rockies populations. A single R gene, conferring qualitative complete resistance (aka major gene resistance) to WPBR, is present at low frequencies in limber pine. To better understand mechanisms of limber pine resistance to WPBR, endophyte communities in limber pine trees previously determined to be susceptible or resistant were examined. Endophytes are known to buffer biotic and abiotic stresses in their plant hosts, and may play a role in resistance to WPBR or provide an indication of physiological differences between resistance and susceptible trees that affect the resident endophyte community. The objectives of our study are to (1) document the endophyte communities present in limber pine stands in the Southern Rocky Mountains, and (2) determine whether differences in endophyte communities exists upon geographic gradients, or (3) in the colonization of WPBR resistance and susceptible limber pine. To determine the fungal endophyte diversity in limber pine needles, Illumina Sequencing to amplify the ITS-1 region of the internal transcribed spacer region. Mothur was used to classify sequences into operational taxonomic units, and then we determined overall fungal species diversity, and differences between geographical gradients as well as resistant and susceptible trees.

2.2-86 Mycoparasitic ability and host range of *Buchwaldoboletus hemichrysus*

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Abstract: The majority of the taxa belonging to the family Boletaceae form ectomycorrhizal associations with plants. However, two sister groups (the *Chalciporus* lineage and the *Pseudoboletus* lineage) appear to be non-ectomycorrhizal and putatively mycoparasitic. The genus *Buchwaldoboletus* (*Chalciporus*

lineage) fruits directly on wood and is considered a non-mycorrhizal saprobe. *Buchwaldoboletus lignicola* was recently confirmed as a mycoparasite of *Phaeolus schweinitzii* in laboratory confrontation tests, suggesting that all taxa in this group may be parasites. In the southeastern United States, *Buchwaldoboletus hemichrysus* regularly fruits on pine stumps in association with a wide variety of wood decay saprobes, including *Ganoderma curtisii* and *Gymnopilus* species. These observations suggest that *B. hemichrysus* is also a mycoparasite, but the natural host fungi remain unknown. The objective of this study was to test the mycoparasitic ability of *B. hemichrysus* against 11 species of pine decay fungi and two species of fungi that decay hardwood trees (*Pleurotus* sp. and *Lentinus crinitus*) to determine the potential host range of *B. hemichrysus*. We carried out confrontation experiments in Petri dishes on Modified Melin Norkrans medium (MMN) where we challenged *B. hemichrysus* against each of the 13 different wood-decay fungi species from four different orders (Agaricales, Gloeophyllales, Polyporales and Russulales). We also carried out confrontation experiments with *B. hemichrysus* against itself as a control. Confrotation experiments showed that *B. hemichrysus* was able to parasitize five species (*Ganoderma curtisii*, *G. meredithiae, Gymnopilus* sp., *Leiotrametes lactinea* and *P. schweinitzii*) but was not able to parasitize the other 8 species. Two of the confrontations led to a deadlock where *B. hemichrysus* was able to persist but not grow further (*Gloeophyllum separium* and *Ceriporiopsis* sp.). Six fungi (*Dichomitus* sp., *Lentinus crinitus, Pleurotus* sp., *Pluteus* sp., *Schizophyllum commune* and *Stereum hirsitum*) were able to inhibit the growth of *B. hemichrysus* and to partially overgrow it. In all the confrontations, *B. hemichrysus* grew slower than all tested wood decay fungi. Despite the rapid growth of all wood decay fungi, an inhibition halo was observed at the contact zone in the cases of *Gloeophyllum separium* and *Ceriporiopsis* sp. All wood decay fungi produced notable pigments that permeated the media in the presence of *B. hemichrysus* but these pigments were significantly reduced in control plates. At the contact zone of the 5 species that were parasitized, we observed hyphae of *B. hemichrysus* wrapping around the hyphae of the wood-decay fungi. *Buchwaldoboletus hemichrysus* also produced abundant conidia during confrontations with those fungi it parasitized and in confrontations that led to a deadlock. Conidia were abundant on confrontation plates but production was limited on control plates. These results show that *B. hemichrysus* is a mycoparasite like others species in the *Chalciporus* clade and suggests that all fungi in this group are likely mycoparasitic. Our work also confirmed that *B. hemichrysus* was able to parasitize a wide phylogenetic range of pine decay fungi (Agaricales and Polyporales) but was not able to parasitize taxa that primarily decay hardwoods.

2.2-87 Unearthing ephemeral mycoparasites – molecular studies of Pyxidiophorales (Laboulbeniomycetes, Ascomycota)

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Abstract: Laboulbeniomycetes is a class of ascomycetous fungi consisting of three orders with strikingly different biology and morphology. Two of them – Laboulbeniales and the recently described Herpomycetales – are obligate biotrophic ectoparasites of arthropods. They share a number of characters that makes them unique among ascomycetes: determinate growth, lack of hypha and absence of asexual stage. Less is known about the free-living, mycelial order Pyxidiophorales. Those mycoparasites are rarely recorded but their intricate arthropod-dependent dispersal strategies have been well documented. Being a difficult and mostly unculturable group, Laboulbeniomycetes have been poorly represented or omitted in major molecular phylogenetic studies resolving evolutionary relationships in fungi. Similarly, due to lack of reference sequences no Laboulbeniomycetes have been identified in metabarcoding studies. The main objectives of this study were to resolve phylogenetic

relationships among lineages of Laboulbeniomycetes and to analyse existing DNA sequence databases (such as NCBI GenBank) to assess the diversity of uncultured and undescribed members of Laboulbeniomycetes. Newly generated marker sequences were obtained from cultures and unculturable specimens of Pyxidiophorales. We used these sequences to distinguish Laboulbeniomycetes OTUs in publicly available databases. Phylogenetic trees were generated using maximum likelihood and Bayesian approaches based on three ribosomal DNA markers (SSU, ITS, and LSU) and two protein-coding genes (RPB2 and MCM7). Review of sequence databases revealed many environmental OTUs belonging to Pyxidiophorales. This suggests that the group is much more diverse and widespread than currently accepted. Pyxidiophorales may be often overlooked because of their minute, ephemeral fruitbodies and non-characteristic anamorphs. The addition of sequences from new *Pyxidiophora* isolates significantly increased support for major branches in Laboulbeniomycetes phylogenetic reconstructions. Our analyses provide strong support for the class Laboulbeniomycetes and the orders Herpomycetales and Pyxidiophorales. The basal-most node of the order Laboulbeniales generally has weak support although major clades within are well supported. In our reconstructions, Herpomycetales is basally positioned, sister to both Laboulbeniales and Pyxidiophorales. This evolutionary scenario would imply the reversion from determinate thallus growth to typical ascomycete mycelial lifestyle. More likely would be the independent development of insect ectoparasitism by two lineages.

2.2-88 Low molecular weight organic acids as key molecules in bacterial-fungal interaction

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Abstract: Low molecular weight organic acid (LMWOA) production is widespread in the fungal kingdom and can have important roles in processes as diverse as pathogenesis, competition, mineral weathering or lignocellulose degradation. More recently, LMWOA have been identified as being involved in the interaction between fungi and bacteria. This is particularly true in the case of oxalic acid, especially in soils. Oxalogenic fungi are known to interact with oxalotrophic bacteria in soils within the oxalate-carbonate pathway. Moreover, oxalic acid can also serve as a signaling molecule for mycophagous bacteria to localize their fungal target. Due to this multitude of functions, LMWOA appear crucial in bacterial-fungal interactions and might be part of a general mechanism in the mediation of reciprocal bacterial-fungal behavior. Therefore, the aim of this study is to assess the role of LMWOA, and in particular oxalic acid, as key molecules in bacterial-fungal interactions. We tested the role of LMWOA consumption by oxalotrophic bacteria on fungal growth inhibition. Fungal strains of the phyla Ascomycota, Basidiomycota and Zygomycota were selected and LMWOA production was confirmed by acid detection on a pH-indicator-containing medium followed by HPLC identification. Fungal-bacterial co-cultures confronting the selected fungi to three soil bacteria (two oxalotrophic and one non-oxalotrophic) were performed in media with differing nutrient composition (to trigger differential LMWOA production). Co-cultures were then observed with fluorescence stereoscopy and confocal microscopy. LMWOA consumption by bacteria was assessed by MALDI-Imaging Mass Spectrometry. Half of the fungal strains screened produced large amounts of various LMWOA. For those strains, oxalotrophic bacteria appear to control LMWOA production, but this depends on the media

composition, with nutrient-poor media (closer to soil solution) favoring bacterial control of the fungal growth. Likewise, bacterial survival varied depending on the fungal species and nutrient conditions of the medium, once again with nutrient-poor media favoring bacterial survival. With this study, new insights into the functional role of LMWOA in bacterial-fungal interactions are highlighted.

2.2-97 Hyphomycetes in Pernambuco: a study in the tourism destination Ilha de Itamaracá, Brazil

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Abstract: The tropical Atlantic rain forest in Brazil consists of one of the largest reservoirs of biodiversity in the world. The region occupied by this forest has numerous rivers, streams and lakes surrounded by riparian vegetation in different status of conservation. The water systems of the Atlantic Forest is responsible for the maintenance of almost half of the Brazilian population, however, due to the beautiful landscapes, these regions are also very popular as tourism destinations and the intense flow of visitors in some areas have proven to impact the environment and endanger freshwater supply to some villages, like those in Itamaracá Island. To keep the riparian forest balanced it is important an effective cycling of nutrients from plants to soil/microbial biomass and back to plants. Therefore, a number of fungal species are found decomposing organic matter both in the soil and inside the water bodies. Among the taxa that are found in the aquatic environment and the riparian zone are the hyphomycetes, that are mostly asexual Ascomycota. This study aimed to investigate richness of hyphomycetes colonizing the leaf litter in the riparian zone and submerged in the Lagoa da Mata, Itamaracá Island, Brazil. This lake is approximately 43 Km² and is located inside the Environmental Protection Area (APA) of Santa Cruz, although it is used for tourism and swimming and open fire is not prohibited. Decomposing leaf litter was collected in the riparian zone and inside the lake in 6 sampling stations. The leaf litter was taken to the laboratory and processed according to the recommendations for each type of sample. After 3 days of incubation, microscope slides were mounted with fungal structures for identification of specimens. The analysis of the soil leaf litter resulted in 34 taxa of asexual Ascomycota with *Circinotrichum maculiforme* Nees, *Beltrania rhombica* Piroz. and *Beltraniella portoricensis* (F. Stevens) Piroz. and S.D. Patil as the most abundant species. From the submerged leaf litter, 13 taxa were detected with *Xylomyces acerosisporus* M.S. Oliveira, Malosso e RF Castañeda, *Triscelophorus acuminatus* Nawawi *and Triscelophorus monosporus* Ingold as the most abundant. The lowest number of taxa was found in the dry season. This is the first study reporting asexual hyphomycetes for Itamaracá Island. Financial support: CAPES and CNPq.

2.2-98 Description of aquatic yeasts and their interactions with plants and Aedes mosquitoes

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Abstract: Yeasts play important roles in food webs, degradation of organic matter, and nutrient cycling in aquatic ecosystems. The diversity and abundance of yeasts in aquatic systems remains poorly characterized. In addition, their functional roles and interactions with other aquatic species are becoming increasingly important. The purpose of this project is to describe fungal communities in urban streams and examine potential endophytic links between aquatic yeasts and riparian trees and the potential roles of mosquitos in yeast dispersal. Fungal traps containing apples, pears, and cherries were

placed in an urban stream in west central Illinois from April through November. Fungal communities were characterized using cultured-based methods and DNA sequencing. Three hundred and seventy-three fungal isolates were cultured. ITS rDNA indicated 71 OTUs and further multigene phylogenies will be conducted to determine species level identification. Seventy-eight percent of the fungi were isolated from traps containing pears and 53% of all fungi cultured were yeasts. The greatest yeast diversity was cultured from traps set in November with 19 OTUs (50%). Yeast identified as the same species using ITS rDNA showed wide variation in morphology and color. Tremellomycetes (39%) and Saccharomycetes (29%) were the most frequent fungal classes. Both classes were represented by seven genera. Preliminary phylogenetic analysis indicated that 40% of the OTUs were closely related to plant endophytes or associated with insects. The most abundant species cultured were in the genera *Meyerozyma, Candida, Pichia,* and *Cryptococcus*. These yeasts are commonly associated with mosquito microbiota. Yeast bioassays will also be conducted to determine potential mosquito-fungal interactions including the role of yeasts in oviposition, egg hatching, and larval growth. Mosquito eggs will also be examined using microscopy to determine potential fungal interactions. To test potential endophytic life stage, leaves from the most abundant trees were collected at the same sites where fungi were trapped and will be isolated and characterized using molecular methods. The nature of the interactions between mosquitoes and yeasts and their effects on the abundance of populations remains mostly unknown.

2.2-99 Profiling fungal community diversity at the Martin Peña Channel

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Abstract: The Martín Peña Channel is a tidal creek that connects the San Juan Bay with the San José and Los Corozos Lagoons (San Juan, Puerto Rico, USA). The channel has a long history of pollution as unauthorized communities settled in its banks. However, microbial metabolic activities have been reported in the area, including biodegradation of alkanes. The mix of freshwater and seawater found in estuaries creates an environment where unique microorganism can thrive. Estuaries are suited for fungi distribution studies because of the gradient of environmental conditions, air currents, and water transit. Fungi decomposition of complex substrates, lignin or cellulose, contribute to sustain the ecosystem. The objective of this study is to describe the richness and distribution of fungal communities in soil samples in the Martín Peña Channel. The hypothesis is that diverse fungal communities will be present along the channel. Soil samples were gathered along the Martín Peña Channel. Total genomic DNA was extracted from each sample for the amplification of the ITS region. Terminal restriction fragments length polymorphism (TRFLP) analysis was used to describe fungal communities. TRFLP profiles of the fungal ITS region showed that there is little abundance and diversity of fungal phylotypes among the sites. Most samples displayed one phylotype, while one sample had two. Furthermore, many samples showed phylotypes which fragment length were beyond the detection range. The low abundance and diversity of fungal communities found suggest limited taxa richness and the need of more informative approach, as simple of alternative restriction enzyme. Analysis of soil quality suggests that environmental factors (pH ranges from 5.43-8.33) and nutrient content (organic matter percentage ranges from 3.48%-38.27%) should not restrict fungal proliferation. Apparently, high pollution levels may affect the fungal communities in the sites since similar approaches have been applied to forest soil and greater diversity was reported. Other restriction enzymes may have the potential of better describing fungal communities in this area. Cultivation efforts and the application of next generation sequencing are in progress to better disclose fungal diversity on tidal banks heavily polluted.

2.2-100 Fungal community assembly on the roots of Costa Rican coffee (*Coffea arabica*) and native Rubiaceae species.

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Abstract: Myriad plants are grown as crops far beyond their native ranges, and these plant species have novel interactions with their new surroundings. Understanding the interactions of non-native crops with belowground fungal communities is important for maintaining crop and soil health and for conserving fungal diversity. Soil and root fungal community assembly may be influenced by abiotic factors like light availability and soil nutrients, or by biotic filters manifested as phylogenetically-determined plant traits. In this study, we used coffee (*Coffea arabica*), a plant of African origin which is now cultivated throughout the tropics, to examine how belowground fungal biodiversity of forest and agricultural systems might interact. Root and rhizosphere fungal communities of coffee and of native Rubiaceae species were sampled in the Monteverde region of Costa Rica. Environmental variables included soil nutrients, moisture and temperature, and photosynthetically active radiation. Root samples were taken to assess fungal colonization by arbuscular mycorrhizal (AM) fungi and non-AM fungi. In both habitats (forest or coffee field) at each of three sites, samples were taken of roots and rhizosphere soil, as well as of background soil not associated with any specific plant. DNA was then extracted from all samples. Illumina sequencing of the ITS2 region of the fungal DNA and the PIPITS bioinformatics pipeline gave a dataset of fungal communities based on closest known species matches in the Warcup database. Both AM and non-AM fungi differed between coffee and forest, and between species in the forest. AM and non-AM fungi in coffee were positively correlated with soil K and negatively correlated with Cu and Zn. These differences may be effects of agricultural chemicals since K is a common ingredient in fertilizer and Cu is found in fungicides. In forest Rubiaceae, AM fungal colonization was negatively correlated with Ca and P, while non-AM showed a negative correlation with Fe and pH. Since AM fungi are essential to plants for P uptake, it is logical that under low P conditions, plants benefit from higher AM colonization. Additionally, the relationship between pH and non-AM fungi may exist because acidic soils tend to favor fungal growth. However, these abiotic soil factors did not differ by tree species and thus do not explain the observed fungal differences between species of Rubiaceae. These results suggest that, while abiotic factors influence root fungal communities, a biotic effect of plant host is also important consider.

2.2-105 Thermotolerant mycobiota of Israeli soils

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Abstract: Israel is a country with more than 60% of the territory covered by deserts. Its climate is characterized by hot and dry summers even in the Mediterranean part, thus making temperature one of the most important microclimatic factors influencing development and distribution of fungal communities in the soils. The objective of the study was to reveal the composition, structure, and spatiotemporal dynamics of thermotolerant mycobiota in the soils of Israeli deserts and northern territories. Microfungi from the upper soil layers of 0-2 cm from sun-exposed open localities (bare or crusted) and under the nearby shrub or herb canopies were isolated at 37°C using the soil dilution plate method. A comparatively rich thermotolerant microfungal biota contained 165 species from 82 genera, with *Aspergillus* and *Chaetomium* being the most numerous genera – 25 and 22 species, respectively. Aspergilli (*Aspergillus fumigatus* and *A. niger*), teleomorphic ascomycetes (*Canariomyces notabilis, Chaetomium nigricolor*, and *Ch. strumarium*), and zygomycetous *Rhizopus arrhyzus* comprised the basic

part of the thermotolerant communities. The desert areas remarkably differed from the northern areas by a much higher abundance of *A. fumigatus* known as one of the most frequent and abundant thermotolerant and thermophilic species in a variety of desert regions, and teleomorphic species, as well as by a lower abundance of *A. niger* and *R. arrhyzus*. The cluster analysis based on species' relative abundances showed that the thermotolerant microfungal communities from the geographically distinct Israeli and Spanish deserts were much more similar to each other than the communities from the Israeli desert and northern regions. Seasonal dynamics revealed for a canyon in the southern Negev desert was expressed mainly in the variations of species richness (substantially lower in the winter), and abundances of *A. fumigatus* (dominant in the summer) and *A. niger* (dominant in the winter). Importantly, the composition of thermotolerant mycobiota was almost entirely different from the composition of mesophilic mycobiota isolated at 25°C. It, therefore, can indicate that the melanized species with protective multicellular spore morphology, which overwhelmingly prevailed in the desert soils at 25°C and were almost entirely absent at 37°C, possibly occur in the summer in a dormant spore state. At the same time, aspergilli dominant at 37°C, accompanied by teleomorphic species with perithecial fruit bodies producing comparatively large (10 μ and more) dark-colored ascospores, were apparently able not only to survive but also to germinate at high temperatures and be active during a long hot period in the desert.

2.2-106 Biological soil crusts microbial diversity in Mojave desert, USA

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Abstract: Up to 40% of global terrestrial land surface consists of desert environments. Naturally water availability is limited in these areas, which increase the vulnerabilities of desert organisms especially plants. Desert ecosystems are not usually covered with dense vegetation and any occurring vascular plants are normally found associated with important microbial communities in the plant interspaces called "biological soil crusts (biocrust)." Biological soil crusts (biocrusts) also known as biological, cryptogamic, cryptobiotic or microbiotic soil crusts contain diverse variety of microbial communities that are essential to desert environments. Bryophytes, lichens, eukaryotic algae, cyanobacteria, bacteria, and fungi combine with soil particles to form different types of biocrusts. Biocrusts are now widely recognized as a living skin of dryland environment, and biocrusts research is critical for desert ecosystem protection. Although scientists have recognized crusts as an important part of desert ecosystems, biocrusts are often ignored and disturbed by the general public with substantial effects on ecosystem health. Disturbances on biocrust microbial communities are often not visible and rarely quantified. Likewise, the functions of biocrusts microbiome (especially functions and interactions related to fungi) are poorly characterized. The stability and temporal variations in microbial community composition are also poorly understood. Work to classify biocrusts has been largely focused on external morphology of biocrusts, which may underestimate the functions and diversity of microbial communities. As a result, this project aims to explore the composition of microbial communities, to capture the biocrust microbiome, and to investigate spatial and temporal patterns of the community as well as the impact of disturbance on community composition. Understanding how microbes interact to form crust layers requires detailed accounting of their microbial diversity, community structure, and measurable functions. Therefore, deeper and higher resolution in biocrusts microbial community is needed. We have used amplicon sequencing of environmental DNA to assess the composition of bacterial and fungal communities. Biocrusts were collected from Joshua Tree National Park, Granite Mountain, and Kelso Dunes within Mojave Desert in California, USA. Preliminary results showed that major bacterial

phyla were mainly Cyanobacteria, Proteobacteria, Actinobacteria, and Acidobacteria. Major fungal phyla were mainly Ascomycota (Dothideomycetes, Eurotiomycetes, and Pezizomycetes) and Basidiomycota (Agaricomycetes and Tremellomycete). Although alpha diversity analysis showed no different species richness between sites, beta diversity analysis reviewed geographical pattern among three sites. We also found correlation between increasing species richness and raining event, which was an indication of temporal/seasonal effects on biocrusts microbial community. Core microbiome analysis and also abundant taxa were used to determine and prioritize culturing efforts while further analysis on functional diversity and biotic interactions will be examined on fungi, which will be isolated from biocrusts.

2.2-107 Checklist of fungi from a semi-arid region of South Africa established through environmental and Sanger sequencing approaches

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Abstract: South Africa has a rich fungal biodiversity, which is unfortunately poorly studied due to a lack of human capacity and expertise. Although some species have been identified, the majority are new or dubiously named and fungi are rarely included in conservation and biodiversity initiatives. It also frustrates a large and growing group of citizen scientists eager to identify fungi they encounter. The taxonomic dilemma is too great to wait for species to be adequately named and described before fungi can be rightfully included in national and international initiatives. Molecular tools can be predictively used to determine locations and indicate hotspots, which is useful to plan future surveys and strategies for more complete assessments. This is especially useful in drier environments where fungi will not usually fruit and be visible. In one such location, namely Bloemfontein, South Africa, environmental samples (humus, soil, dead plant material) were sequenced directly using Illumina sequencing. The mini-barcodes were used in phylogenetic diversity analyses to ascertain the diversity for the area and establish, for the first time, a checklist of genera present. In addition, fruiting bodies that could be found over the past two years were sequenced using Sanger sequencing to provide a higher level of resolution for identification. These results were also linked to the data obtained with environmental sequencing. Despite the area being semi-arid, a high level of diversity was detected and more than 60 genera, of which some were never before observed in the area, were detected. The approaches developed in this study can thus be useful in the future to generate much-needed biodiversity data for South African macrofungal species that will stimulate further study.

2.2-109 The thread connection between *Hotaea weneckii* and *Dunaliella atacamensis*: triggering symbiosis from spiderwebs to in vitro culture

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Abstract: Microorganisms have evolved multiple and novel strategies to survive in extreme environments. Iconical examples are lichens, in which the association between fungi and algae enable the symbionts to outcompete in habitats where otherwise the single organisms could not survive. The green algal genus *Dunaliella* and the black yeast *Hortaea* are exemplar in being adapted to extremely saline, aquatic environments. Only one single species of the genus *Dunaliella*, *D. atacamensis*, is known from subaerial environments. It thrives on spiderwebs on walls at the entrance-twilight transition zones of caves in the Atacama desert (Chile). A closer inspection of the colonies has shown the presence of

black yeast cells of *Hortaea werneckii* between the algal cells. As symbiotic interactions between *Dunaliella* species and the *Hortaea* fungi have not been known so far, we performed a series of co-cultivation experiments to test whether the two symbionts can co-grow *in vitro* and develop lichen-like symbiotic structures. We set up co-cultures with the axenic strains of *Hortaea werneckii* isolated from the salterns and *Hortaea werneckii* isolated from the Chilean spiderwebs together with the salt tolerant *Dunaliella salina* and the aerial *D. atacamensis*. We used different growth media and culture approaches to trigger the association between the fungi and the algae and documented our observations by light microscopy.

2.2-110 The early growth of conidia in an extraordinarily stressing environment is influenced by water activity during conidial production.

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Abstract: Fungal growth has negative aesthetical, structural and even medical effects. Growth prevention strategies are therefore desired. The influence of water on the growth of fungi is crucial when considering these strategies. Growth often prevails in a surrounding where both water and nutrients are scarce. The impact of this highly stressing environment during sporogenesis on subsequent growth is often neglected. The goal of this study is to investigate the effect of varying water stresses, in combination with depleted nutrient availability, on the early growth of spores cultivated under various circumstances. A nutrient-depleted substrate was therefore constructed. This was done by treating glass with ozone, thereby deleting carbon as a nutrient source. The water conditions during sporogenesis, as well as during subsequent growth, were varied. Spores of *Penicillium rubens* were harvested from colonies grown on Malt Extract Agar, MEA, plates with a varying water activity, a_w. These spores were placed on the carbon depleted glass plates and placed in incubation chambers. The parameter describing the state of the water in the incubation chamber is the relative humidity, RH. Glycerol solutions were used to generate various RH values. The system under consideration is thus an extremely stressing environment: no carbon source is present, and water is provided solely via the vapour phase. Due to this harsh environment, only few spores could germinate, roughly 1 %. For the germinating spores the germination time, t_g and initial growth rate, μ, were monitored. Despite this stressing environment, the expected proportionality between growth behavior and RH was found: t_g decreases and μ increases with increasing the RH, for any value of a_w during sporogenesis. Varying the spore history, via the a_w, has an effect which depends on the RH of the environment. At low RH, spores produced at low a_w have a lower t_g and higher μ compared to those grown at high a_w; i.e. the spores germinate and grow faster when grown at low a_w. This is not found in a high RH environment, where the growth history had no effect. This last result was remarkably pronounced when the substrate was not only depleted of nutrients, but also made hydrophobic: growth only occurred when spores were developed at low a_w and placed in a high RH. It has recently been found that pores grown on lowered a_w can attract more water. This was linked to a varying amount of compatible solutes in these spores. This is hypothesized to justify the reported growth behavior. By deleting carbon as a nutrient source, the substrate used allows one to investigate the effect of a single parameter: water. Water availability during sporogenesis and during germination was independently varied. The influence of sporulation conditions on germination kinetics, which becomes even more pronounced in a more stressing environment, is clearly demonstrated. The fundamental role of compatible solutes in the survival and growth of fungi under stressed conditions can thus not be overlooked.

2.2-111 Diversity and composition of fungal communities associated to biological soil crusts in a fog oasis of the Peruvian desert

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Abstract: Biological soil crusts (BSCs) are describe as a thin layer of upper soil where soil particles are aggregated by a community of highly specialized organisms such as cyanobacteria, bacteria, microfungi, algae, lichens, and mosses. BSCs are essential components of arid and semiarid ecosystems due to their importance in soil stability, water retention and soil fertility; however, they remain poorly studied with respect to their fungal diversity. The objective of this study was to compare the composition, structure and diversity of fungal communities associated to three different BSCs (site 1 to 3; which are at different stages of biocrust formation, being 1 the earliest stage) and superficial soil with vegetation (SSV;site 4) at Lomas de Lachay Natural Reserve, Lima. Five SSV and BSCs samples were taken from a 100 m transect. Soil moisture was determined using the gravimetric method and pH was analyzed using a digital pH meter. Fungi were isolated using the dilution plate technique (10^{-1}; 10^{-6}). An aliquot of 0.1 ml was taken from each dilution and plated in Petri dishes containing Malt Agar (AM) and Czapeck Agar (ACZ). Pure cultures were identified using the microculture technique. Soil moisture was slower in SSV but pH was higher than in BSCs. A total of 142 strains from 46 species belonging to Zygomycetes (4 species) and Ascomycetes (42 species) were isolated. The most abundant genera isolated from SSV were *Penicillium, Aspergillus* and *Fusarium* while for BSCs were *Aspergillus, Penicillium, Fusarium, Paecilomyces, Cladosporium* and dark-colored species. Likewise, melanin-containing microfungi species were higher in BSCs (33%) compared to SSV (15%). Shannon-Wiener diversity index did not show significant differences among sites where biocrust was found but it does between BSCs and SSV. Moreover, diversity is higher in later stages of biocrust development. A NMDS analysis showed clearly three different fungal communities (site 1, 2 and 4) while site 3 seems to be heterogeneous. These results suggest that diversity of fungal communities increases as biocrusts are in later stages of development and more vascular and non-vascular plants are present. The significantly lower percentage of thermotolerant and UV-resistant species and the higher abundance of cellulose degrading species in SSV were caused by the presence of vegetation and debris. In the same way, annual plants that regrow due to the increase of humidity during seasonal fog allows the dominance of species with high growth rates such as *Penicillium, Aspergillus* and *Fusarium* in BSCs.

2.2-112 Molecular identification of filamentous fungi diversity in north coast beaches of Puerto Rico

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Abstract: Puerto Rican beaches are a significant tourist attraction. The Northern region has a great variety of beaches with diverse microbial characteristics. Beach sands receive direct contamination from the garbage generated by people, which serves as nutrient for fungi growth. The objectives of this investigation were to assess the filamentous fungi diversity of four popular beaches; identify the genus and species; and identify the taxonomic relationship between the most abundant fungi. The beaches studied are located in the towns of Vega Baja, Manatí, Barceloneta and Arecibo. One sample of dry sand per month from three equidistant points were acquired every month for a year in each beach. The samples were homogenized according to dry (December-April) and humid (May-November) seasons, for a total of four composite samples per season. The DNA of each sample was isolated and quantified;

and, upon sequencing, evaluated by metagenomics analysis with MG-RAST. There were 104 fungi species identified by DNA sequencing analysis. The most abundant were: Aspergillus penicillioides, Aspergillus terreus, Microascus sp., Arthrographis kalrae, Paramicrosporidium sp., Dokmaia sp., Gliomastix polychroma and Aspergillus sp. The taxonomic analysis demonstrated that there is no relationship in the genus of the most abundant species. As significant finding, 66 species of new registries were identified, including Malassezia restricta, Arthrographics eremomycyces, and Cephaliophora tropica. Not only were many of the species pathogenic, several genus of filamentous fungi have been previously isolated from patients in nasal culture, and can cause eye-, respiratory- and skin disease. The majority of these fungi use direct contact and air transport as transmission vehicle to the host.

2.2-137 Evaluating *Helminthosporium solani*, causal agent of potato silver scurf blemish disease, for sensitivity to the fungicide azoxystrobin

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Abstract: Globally, potato is the third most important food staple crop, which is critical in this time of population growth and increasing world hunger. Silver scurf of potato, caused by the ascomycetous fungus *Helminthosporium solani*, is a tuber blemish disease causing negative quality and storability impacts of great and increasing concern in the U.S. Potato producers in Wisconsin (WI) and other major potato-producing states have had to rely heavily on fungicides in addition to variably-effective cultural controls for silver scurf management. After *H. solani* developed field resistance to the once effective fungicide thiabendazole, QoI fungicides (such as azoxystrobin) were adopted to improve control. However, recently there has been an increase in disease affecting quality and storability. Genetic mutations associated with QoI fungicide resistance have been identified in other potato pathogen populations, such as *Alternaria solani*. For this reason, we hypothesized that *H. solani* might currently be poorly controlled due to QoI resistance after over 2 decades of use. In our preliminary screening of the pathogen population in Wisconsin, we selected five H. solani isolates representing different production regions and management programs. Five of these isolates were exposed to azoxystrobin at varying dose rates to determine fungicide sensitivity in vitro. Isolates were grown on clarified V8 agar amended with azoxystrobin in various concentrations (0, 0.001, 0.01, 0.1, 1, 10, 50, 100 µg/mL). Relative growth was measured after 20 and 40 days of incubation at 23ºC under dark conditions. There were significant differences in growth between isolates, as well as between treatments. One of the isolates grew on all azoxystrobin concentrations, including the highest concentration, suggesting some level of fungicide insensitivity in vitro. Further studies will focus on evaluating a larger isolate collection for sensitivity and determining presence of mutations associated with QoI sensitivity in other potato pathogens.

2.2-138 Thermal sensitivity of *Calonectria henricotiae* and *Calonectria pseudonaviculata* conidia and microsclerotia: developing a tool for managing boxwood blight in nurseries

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Abstract: An understanding of asexual fungal propagule thermal sensitivity is useful for developing disease management approaches that deploy heat to inactivate conidia and microsclerotia of plant pathogens. Heat treatment of cuttings, plant debris and soil that harbor conidia and microsclerotia would provide a useful management tool for suppressing the pathogenic activity of *Calonectria*

pseudonaviculata (*Cps*; present in the US) and *C. henricotiae* (*Che*; a quarantine pathogen not present in the US) and boxwood blight disease. The objective of this study was to determine the thermal sensitivity of conidia and microsclerotia of *Che* and *Cps* treated in water at 45 C, 47.5 C, 50 C, 52.5 C, and 55 C. For conidia, as time of exposure increased at each temperature, the proportion of germinated conidia decreased. The predicted time required to kill 90% of *Cps* conidia (LD_{90}) decreased as water temperature increased from 45 C to 55 C and ranged from 35.4 to 5.6 min, respectively. Conidia inactivation was dependent on isolate, species of *Calonectria*, and length of exposure at each temperature tested. Microsclerotia of *Che* and *Cps* displayed reduced germination with increasing exposure and higher water temperatures. Microsclerotia of *Che* were significantly more resistant to heat treatment than *Cps* at 47.5 C and 50 C, while microsclerotia of both species were rapidly killed at 55 C. In conclusion, these studies provide baseline knowledge on the thermal sensitivity of *Che* and *Cps* conidia and microsclerotia. This information is currently being used to complement thermal sensitivity data for commonly grown boxwood species and plant-based infection assays with the goal of developing a method for managing blight disease on cuttings during the boxwood propagation process.

2.2-139 ZnO-Nanoparticles as antifungal agent limiting growth and mycotoxins production by *Aspergillus flavus* and *Fusarium proliferatum* on a maize based-medium

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Abstract: In Argentina, around 60% of maize is exported and the remaining is used as feedstuff. *Aspergillus flavus* and *Fusarium proliferatum* are two mycotoxigenic species frequently isolated from maize that produce aflatoxins and fumonisins, respectively. ZnO-Nanoparticles (ZnO-NPs) have been used as efficient antimicrobial agents. ZnO is a non-toxic compound and a strategy of low cost and low environmental impact to reduce mycotoxin accumulation in stored maize. The aims of this study were: (i) to synthesize and characterize ZnO-NPs; (ii) to evaluate their effect under presence/absence of white light during fungal incubation on growth rates and aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1) accumulation by *A. flavus* and *F. proliferatum*, respectively, and (iii) to determine fungal morphological alterations by SEM. ZnO-NPs were synthesized according to the drop by drop mixing method and characterized by SEM. *Aspergillus flavus* RCAF016 and *F. proliferatum* ITEM 15699 strains were grown on a 2% maize based medium (0.995 a_W) containing 0, 10, 50 and 100 mM ZnO-NPs. The inoculated plates (by triplicate) were incubated at 25°C, 21 days in darkness or under 12/12 h photoperiod cold white and black fluorescent lamps. SEM analysis of ZnO-NPs showed thin flakes of 200 × 200 nm and thickness of ~30 nm. *Aspergillus flavus* and *F. proliferatum* strains were able to grow in presence of 0, 10, 50 and 100 mM ZnO-NPs. However, growth rates were reduced (in relation to the control) at the three concentrations evaluated under photoperiod or darkness incubation conditions. Hypha and conidia morphological alterations were observed in both *A. flavus* and *F. proliferatum* treated with ZnO-NPs by SEM analysis (2000-5000 X). The alterations were: hyphal deformations, less conidiation and unusual bulges. Mycotoxin accumulation was also affected by the ZnO-NP treatments. After 3 days of incubation in darkness and 10 mM ZnO-NPs, *Aspergillus flavus* RCAF016 showed AFB_1 production higher than the control, but at 50 and 100 mM the toxin production was inhibited at not detectable (ND) levels. After 7 and 14 days, AFB_1 was reduced at ND levels at 10, 50 and 100 mM ZnO-NPs, whereas, at 21 days the toxin was reduced by 85% and at ND levels at 50 mM and 100 mM, respectively. Under the photoperiod incubation condition AFB_1 was not detected in both controls and treatments. Fumonisin B_1

accumulation by *F. proliferatum* ITEM 15699 was also reduced by ZnO-NP treatments. Both incubations conditions showed reduction in FB_1, in general, at the three ZnO-NPs concentrations evaluated. The reduction was between 73 and 99% after 14 and 21 days of incubation. This study showed that ZnO-NPs could be used for limiting growth and AFB_1 and FB_1 accumulation by *A. flavus* and *F. proliferatum*, respectively in a maize based medium. This environmental friendly strategy of low cost could be applied during maize storage.

2.2-141 Using essential oils as a chemical control for fungal plant pathogens

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Abstract: Fusarium head blight is a devastating disease of wheat and barely caused by the pathogen *Fusarium graminearum*. *F. graminearum* also produces the mycotoxin deoxynevalenol which causes a wide range of toxicological effects in both humans and animal fed contaminated feed. Despite the continued release of cultivars resistant to fungal pathogens and chemical fungicides, *F. graminearum* remains difficult to control. Thus, there is an urgent need to find safe and durable strategies to limit the infection of plants by this pathogen. We tested the ability of ten essential oils; castor oil, fenugreek oil, clove oil, peppermint oil, cinnamon oil, eucalyptus oil, fennel oil, thyme oil, clary sage oil and marjoram oil, to inhibit the growth of *F. graminearum in vitro* and *in planta*. All tested oils inhibited the growth of *F. graminearum in vitro* with MICs ranging from 0.037 to 0.3 µM. Clover oil, eucalyptus oil, Thyme oil and cinnamon oil displayed higher antifungal activity with an MIC<0.0375µM. These results suggest that essential oils have the potential to contribute to the development of new class of antifungal agents to protect crops from fungal diseases.

2.2-142 The influence of fungicides on the grapevine wood mycobiome: a case study on tracheomycotic ascomycete *Phaeomoniella chlamydospora*

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Abstract: Grapevine trunk diseases are the major threat to viticulture of our days. They affect the perennial organs of grapevines (*Vitis* spp.) causing decline, loss in quantity and quality of yield, and early death of the plants, resulting in enormous costs for the industry. *Phaeomoniella chlamydospora* is a tracheomycotic ascomycete which colonizes the xylem of grapevines causing wood discoloration and necrosis, plant decline, and it is believed to be indirectly responsible for other symptoms as well. *Phaeomoniella chlamydospora* is associated mainly with four syndromes of grapevines: brown wood streaking of rooted cuttings, Petri disease, grapevine leaf stripe disease and esca proper. The incidence of these syndromes in vineyards has greatly increased over the last 20-30 years and the most puzzling question remains – why has *P. chlamydospora* become so successful? This work aimed to answer this question by formulating the hypothesis that fungicides commonly used in vineyards to control downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) interact with the wood mycobiome causing a change in its composition, which may favor the wood colonization by *P. chlamydospora*. To test this hypothesis, we worked on one-year old rooted cuttings of 'Cabernet Sauvignon', under greenhouse conditions. Grapevines were inoculated with either a spore suspension of *P. chlamydorpora* (strain CBS 161.90), with an artificial mycobiome or with a combination of both, and treated with three combinations of fungicides and a control. We used metabarcoding (Illumina sequencing) with two sets of universal primers (ITS1F2F/ITS2R and ITS86F/ITS4R) in order to understand the changes that the

wood mycobiome incurs due to the application of fungicides and Real-Time PCR to quantify the abundance of *P. chlamydospora*. Our results show that the application of different fungicides changes the relative abundance of several fungal species in the grapevine wood, including that of *P. chlamydospora*. The colonization of the wood by this ascomycete is also greatly reduced when co-inoculated with the artificial mycobiome. Sequencing results are partially supported by quantitative analyses. This study demonstrates that fungicides applied as foliar sprays can indirectly interfere with the fungal communities in the grapevine wood giving an advantage to some communities over others, allowing speculations on the role that this change plays in triggering disease mechanisms.

2.2-143 Response of potato to the black scurf disease pathogen *Rhizoctonia solani* Kühn AG-3

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Abstract: Black scurf disease on potato caused by *Rhizoctonia solani* AG3 occurs worldwide and is difficult to control. It has been observed that potato cultivars show differences in susceptibility to *R. solani* AG3. The cultivation of potato cultivars with high resistance level to Rhizoctonia diseases represents an ecological and economic sustainable control strategy. Presently, the degree of resistance is based on symptom assessment in the field, but molecular methods could offer a more effective screening procedure for cultivars with high degree in resistance. We hypothesized that field resistance to black scurf disease in potato cultivars is associated with defense-related gene expression levels and salicylic acid (SA) concentration. In a comparative analysis, the expression levels of common defense-related genes of two cultivars with moderate and high degree in resistance to black scurf disease were studied. Besides RNA accumulation, salicylic acid (SA) concentrations in potato tissues were measured. A higher constitutive expression level of defense-related genes was found in the highly resistant cultivar. A significant increased expression level of these genes upon pathogen infection was only observed in the moderately resistant cultivar. In addition, the constitutively higher expression level correlated with increased amounts of SA compared to the moderately resistant cultivar. *R. solani* AG3 DNA density reflected differences in resistance. The results indicate that expression levels of defense-related genes and the amount of SA in potato tissues can potentially be used as indicators of potato field resistance to black scurf disease.

2.2-144 Mycovirus isolated from three species of the genus *Pseudopestalotiopsis*

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Abstract: Mycoviruses are viruses that infect fungi and have been detected as dsRNA in several fungal species since 1999. More than 200 cases have been reported, but the number of reported mycoviruses is extremely small compared with the number of mycoviruses infecting animals and plants. We also know very little about how mycoviruses infect and affect fungi. Our current understanding states that most mycoviruses are transmitted by hyphal anastomosis from carrier fungi to healthy fungi, and have no pathogenicity to fungi. In recent year, the cases were reported the transmission of mycovirus via an insect

and carrier fungi reducing pathogenicity to plant. Interactions between fungi and mycoviruses are therefore not yet fully understood. In this study, we aimed to characterize interactions among fungi, mycoviruses and plants. We obtained mycoviruses from the fungal species of the genus *Pestalotiopsis* sensu lato, composed of the *Pseudopestalotiopsis*, *Pesatlotiopsis,* and *Neopestalotiopsis* genera including pathogenic, endophytic and saprophytic fungi in plants. Of the 130 strains belonging to the genus *Pseudopestalotiopsis*, three strains (F0083, F0015, and F0033) were found to contain dsRNA. DsRNA segments were analyzed using agarose gel electrophoresis and were estimated to be approximately 2.9 kbp in length. In a previous study, we reported used Next-generation sequencing and RT-PCR and reported that dsRNA obtained from *Pseudopestalotiopsis* sp. F0083 belonged to a novel mycovirus within unirnavirus (Kusano et al, 2017). This virus encoded two ORFs (unknown protein and RNA-dependent RNA polymerase) and these were detected using an ORF finder. In the present study, we used specific primer pairs designed on the unirnavirus genome of F0083 to confirm that dsRNA detected in F0015 and MM0033 originated from the same unirnavirus. Both dsRNA sequence data in F0015 and MM0033 were mostly shared with dsRNA sequence data in F0083. A protein BLAST search provided the estimates to encode RdRps similar to ORF2 of Penicillium janczewskii Beauveria bassiana-like virus 1 and Beauveria bassiana RNA virus 1 belonging to unirnavirus. Phylogenetic analysis based on the putative RdRp amino-acid sequence revealed that the dsRNAs found in F0015 and F0033 belonged to the same member of unirnavirus. These results indicate that dsRNAs found in different species of *Pseudopestalotiopsis* are unirnavirus and these mycoviruses are the closest strains to each other.

2.2-145 Intercepted fungi: New and interesting species from the Caribbean and Central and South America

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Abstract: Global commerce and trade continues to increase. With this comes the potential for the unintentional introduction of non-indigenous species of fungi into new environs. During the last decade imports of various host genera have risen greatly. Among these are: *Eucalyptus* (Myrtaceae), numerous genera in the Proteaceae (*Leucadendron*, *Leucospermum*, *Protea*), *Coccinia* (Cucurbitaceaea) and others. In an effort to protect American agriculture, inspection of imported host material is performed as shipments arrive at various ports of entry into the US. Inspected host material suspected of possibly harboring diseases is forwarded to the National Mycology Laboratory in Beltsville, MD. In order to provide more information on the diversity of fungi encountered on the above hosts we have studied more closely the fungi occurring on these hosts intercepted over the past 3 years. Morphological and molecular characterization of several intercepted fungi have provided numerous specimens representing undescribed species. Many others represent species with new distributional records. Genera intercepted on these hosts include *Phyllosticta, Cercospora, Colletotrichum, Diaporthe* (*Phomopsis*), *Neofusicoccum, Pyrenophora* and *Pestalotiopsis*. New records, species, and distributional information can affect quarantine decisions and have trade implications. Many widely traded host genera represent taxa for which there is a lack of information on associated fungi. Information on the fungi associated with these hosts supports informed regulatory decision-making.

2.2-146 Powdery mildews (Erysiphales) in Victorian horticulture: DNA isolation to rediscover an old foe hidden in herbaria

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Abstract: Powdery mildews pose a significant threat to the global food trade and can cause massive crop losses throughout agricultural and horticultural industries. In Victoria, the horticultural industry is focused around the production of almonds, pome, stone fruit, grapes, citrus, and berries, with powdery mildews responsible for reducing crop yields across all of these major crops. Possessing accurate and current plant pathogen records is vital for Australian import and export of horticultural crops to prevent incursions of new and exotic pathogens. Maintaining up to date plant pathogen data is vital for Australian Biosecurity. The Victorian Plant Pathology Herbarium (VPRI) houses over 42,000 plant pathogen specimens. Of these the powdery mildews (Erysiphales) represent ~2700 specimens, collected from agricultural, horticultural and ornamental host plants. The VPRI plant pathogen database feeds directly into the Australian Plant Pathogen Database (APPD) providing specimen-based records of current plant pathogens. Traditionally, morphology and host plant associations were used for the identification of powdery mildews but this has been proven unreliable. The modern approach to fungal taxonomy is to use morphological characters together with genetic analyses. The purpose of this study is to update the VPRI Erysiphale specimen database by traditional fungal morphological character description and genetic analyses. I will present research on the molecular characterisation of VPRI powdery mildew specimens, focusing specifically on horticultural crops of Victoria, Australia. An integral part of this research is the establishment of reliable DNA extraction methods and identification of appropriate gene regions for species identification of preserved powdery mildews specimens. The aim of this project was to test a range of DNA extraction protocols which used varying source material of different ages (fresh, recent -200 years old and ancient) to compare the DNA quantity and quality yielded from the VPRI powdery mildew specimens. We also evaluated fungal gene markers for use in herbarium fungal species identification as it was detected that current markers are too large for powdery mildew herbarium DNA. Once DNA extraction protocols and suitable gene markers have been identified we will first focus on powdery mildews of the Rosaceae family, notably pome and stone fruit, for this study. Once VPRI powdery mildew DNA is obtained we will perform PCR to produce gene marker amplicons for species identification. Confirmation of powdery mildew species will be achieved through amplicon sequencing on the Illumina MiSeq platform. Simultaneously, whole genome sequencing of suitable Rosaceae powdery mildew herbarium DNA will be run on the Illumina HiSeq platform to produce reference genomes for powdery mildews. These reference genomes will fill the current knowledge gap in horticultural powdery mildew genomics as currently there are only three reference genomes available for Blumaria graminis (barley), Erysiphe pisi (pea) and Golovinomyces orontii (cucurbits).

2.2-147 Microsatellite and SNP discovery for population genetic studies of *Gemmamyces piceae*

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Abstract: *Gemmamyces piceae*, a psychrophilic fungus, causes bud blight disease of *Picea* species in Northwestern Europe and Central European mountains. The pathogen was first identified in Alaska in 2016, although symptoms were first noticed in 2013. Ninety percent of the forest land in Alaska is boreal

forest dominated by *Picea* species. By 2017, blighted spruce buds were recorded at over 200 locations in Southeast, South Central and Interior Alaska. However, examination of numerous samples using microscopy and sequencing of the ITS barcode revealed that there are actually three different fungi causing bud blight in Alaska: *G. piceae*, *Dichomera gemmicola*, and *Camarosporium strobilinum*. These fungi are indistinguishable in the field. 182 permanent plots were established to evaluate the statewide distribution and presence/absence of bud blight. The pathogenic fungi causing bud blight occurred in 65 of the 182 plots; 117 plots lacked symptoms of bud blight. The occurrence of *G. piceae* is confirmed from Anchorage to Fairbanks, Alaska. However, we did not find *G. piceae* during surveys of Southeast Alaska. To investigate the origins of *G. piceae* in Alaska and elsewhere, population genetic studies have been initiated utilizing microsatellite and single nucleotide polymorphism (SNP) genetic markers. Whole genomes of isolates of Alaska and Czech Republic were used for microsatellite library development. Genomic DNA of two Alaska and one Czech isolates were purified and subjected to Illumina HiSeq sequencing, following which, assembly of paired reads was performed and bioinformatically mined using msatcommander software, which aids in identifying repeat type, repeat length, and candidate primer pairs.

2.2-148 Population structure of *Teratosphaeria pseudoeucalypti*, causal agent of Teratosphaeria leaf blight on *Eucalyptus* in Uruguay

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Abstract: *Teratosphaeria pseudoeucalypti*, the causal agent of *Teratosphaeria* leaf blight, was recently reported in South America where it causes severe damage on *Euclalyptus camaldulensis, E. tereticornis* and related species in plantations. Genetic resistance is the most effective management option for the disease, but success depends greatly on the population diversity of the pathogen. The aim of this study was to characterize the population structure of *T. pseudoeucalypti* in Uruguay. Isolations were made from leaves on *E. camaldulensis*, *E, tereticornis* and hybrids trees widely distributed throughout the country resulting in a collection of 217 strains. Strains were characterized based on morphology and using molecular markers. The complete genomes of two strains were sequenced, and mating type regions were analyzed. Only the MAT1-1 was found in all analyzed strains, suggesting that the fungus is heterothallic and thus likely asexual in Uruguay. The population was also characterized based on SNP's of four genomic regions. A single haplotype of *T. pseudoeucalypti* was found to be present in Uruguay, corresponding to haplotype KE8 reported from Queensland (Australia) in 2010. The results of this study suggest that *Eucalyptus* breeding and planting stock developed for resistance to *T. pseudoeucalypti* will have effectiveness against the entire population, and its durability will depend on the effectiveness avoiding new introduction. Thus, highlights the importance of reinforcing quarantine regulations such that new genotypes, possibly including a MAT 1-2 mating type are not introduced into Uruguay in the future.

2.2-149 Developing the tools to monitor populations of *Cercospora* species associated with Cercospora Leaf Blight on soybean

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Abstract: The purpose of this research is to develop diagnostic tools that differentiate species of *Cercospora* associated with Cercospora Leaf Blight (CLB) and Purple Seed Stain (PSS) on soybean. *Cercospora* cf. *flagellaris*, *C. kikuchii*, and *C.* cf. *sigesbeckiae* are three closely related species that have been associated with CLB and PSS but are difficult to distinguish based on phenotypic characters. It is imperative that we are able to correctly and rapidly identify these pathogens in order to monitor the pathogen population and better understand the etiology of the disease. We used a bioinformatics approach to identify species-specific primers with sequence data from twenty-three *Cercospora* species across sixteen loci to develop specific primers sets for *C.* cf. *flagellaris*, *C. kikuchii*, and *C.* cf. *sigesbeckiae*. We have successfully developed primers that will discriminate among *C.* cf. *flagellaris*, the dominant species in Louisiana, as well as *C.* cf. *sigesbeckiae* and *C. kikuchii*. These primers will allow us to monitor populations of *Cercospora* associated with CLB and PSS and potentially quantify fungal biomass. These diagnostic tools will be used in ongoing efforts to identify sources of inoculum and understand patterns of dispersal in order to improve disease management in soybean.

2.2-150 Epitipification and phylogenetic relationship of cercosporoid fungi associated with plants of the Brazilian Cerrado

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Abstract: Cercosporoids are commonly associated with leaf lesions in several plant species. Currently, there are over 2000 species, most of which are known in a single host. This group of fungi is the most representative in the Brazilian Cerrado, but to date the majority of species have been described exclusively by morphological characteristics. The inclusion of molecular data in the taxonomy of cercosporoid fungi revealed that the group is polyphyletic and demonstrated the need for sequence comparison for the accurate identification of the species. Therefore, this work has the objective of epitypify the specimens of cercosporoid fungi associated to the Cerrado plants and to understand their phylogenetic relationship. For precise identification, isolates were obtained from leaf lesions on *Piper aduncum* (Piperaceae), *Solanum* sp. (Solanceae), *Palicourea rigida* (Rubiaceae), *Smilax japecanga* (Smilacaceae), *Cybistax antisyphilitica*, *Handroanthus ochraeus*, *Handroanthus heptaphyllus*, *Handroanthus serratifolius, Tabebuia aurea*, (Bignoniaceae), *Passiflora setacea* (Passifloraceae), *Acrocomia aculeata*, *Mauritia flexuosa* (Arecaceae) and *Vellozia squamata* (Velloziaceae). After confirmation of identity by morphological comparisons, the genomic DNA was extracted using the Wizard® Genomic DNA Purification kit. Nucleotide sequences from the ITS region were obtained and compared to sequences of type species and specimens available from GenBank. In total, 31 isolates were obtained from 13 host plants belonging to eight botanical families. The isolates obtained belong to the Mycosphaerellaceae and are grouped into ten distinct clades. This study confirms the report of *Pseudocercospora piperis* associated with *Piper aduncum*, with high phylogenetic support. The epitypes of *Cercospora tabebuiae-impetiginosae, Pseudocercospora passiflorae-setaceae, P. cybistacis, P. tabebuiae-caraibae*, and *P. tabebuiae-ochraeae* associated with *Handroanthus heptaphyllus*, *Passiflora setacea*, *Cybistax antisyphilitica, Tabebuia aurea* and *Handroanthus ochraceus*, respectively, were designated for the first time and a probable new species of *Pseudocercospora associated* with *Vellozia*

squamata will be proposed following the norms of the International Code of Nomenclature for algae, fungi, and plants. The specimens examined in this study represent a small fraction of cercosporoid fungi reported in the Cerrado and demonstrate the need to re-collect these organisms to understand their phylogenetic relationship. Financial support: FAP-DF, Capes, CNPq and UnB.

2.2-151 Multiple lines of evidence suggest alternative hosts are involved in the development of Cercospora Leaf Blight of soybean in Louisiana

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Abstract: Recent phylogenetic studies have changed our understanding of the diversity of *Cercospora* species associated with Cercospora leaf blight (CLB) and Purple seed stain (PSS) of soybean. *Cercospora kikuchii* has long been considered the causal agent of CLB and PSS, but we now recognize a composite of species are associated with the disease. These studies suggest most of the species associated with CLB and PSS are not host specific, in contrast to our current understanding of *C. kikuchii*. Given the limited host range of *C. kikuchii*, the frequently observed uniformity of disease symptoms in the field, and the fact that the pathogen is seed borne, it has been assumed that the primary source of inoculum for the disease is soybean seed. However, this assumption needs to be revisited in light of the diverse host preferences of *C.* cf. *flagellaris* and *C.* cf. *sigesbeckiae*, two of the predominant species associated with CLB and PSS. The aim of our current work is to determine the principal sources of inoculum of CLB and PSS through field trials and molecular ecology/epidemiology. We examined the role of seed borne inoculum by eliminating all microbes from the seed prior to planting and monitoring disease incidence and severity over the course of two field seasons. We also characterized the diversity of *Cercospora* species on soybean seed prior to planting, from blighted leaves, and from harvested seed to examine changes in the composition of the *Cercospora* community over the course of a season. Finally, we looked for direct evidence of gene flow between alternative hosts both proximate to and distant from agricultural fields. Despite eliminating all microbes from the soybean seed prior to planting, we did not observe a reduction in disease incidence or severity at two different sites in Louisiana over two field seasons, consistent with planted seed not being the primary source of inoculum. We also found that the composition of *Cercospora* species present in the community at the time of planting (seed) significantly differs from that responsible for leaf blight and seed stain at the time of harvest. In the first season, regardless of the frequency of each of *C. kikuchii*, *C.* cf. *sigesbeckiae*, and *C.* cf. *flagellaris* on seed at the time of planting, *C.* cf. *flagellaris* dominates the community on blighted leaves and harvested seed. This shift in the community over the course of the field season also indicates seed is not the primary source of inoculum. We are currently working on replicating this study with collections from a second field season. Finally, genotype data of *C.* cf. *flagellaris* (n=72) and *C.* cf. *sigesbeckiae* (n=14) at 19,670 SNP loci indicate ongoing gene flow between alternative hosts proximate to agricultural areas and those on soybean. However, the lack of differentiation among isolates from seed in distant geographical areas coupled with significant differentiation among agricultural and non-agricultural populations (alternative hosts), suggests the movement of seed has historically been a significant source of inoculum in soybean fields.

2.2-152 Exploring the diversity of *Colletotrichum* species in Louisiana and the Gulf South.

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Abstract: The genus *Colletotrichum* is globally distributed with diverse ecological niches, including endophytes, pathogens, and saprobes, and ranks among the most important genera responsible for both pre- and post-harvest losses. According to the Louisiana Plant Disease Management Guide (LA-PDMG), *Colletotrichum* is a pathogen of more than 64 ornamental plant species, 12 major crops, and 9 vegetable groups in Louisiana. The development of agricultural practices that minimize the impact of fungal pathogens to Louisiana's $1.78 billion agricultural industry is reliant on knowing the species responsible for plant diseases. However, many of the species in the LA-PDMG remain unidentified despite significant advances in *Colletotrichum* systematics and taxonomy over the last decade. We aim to identify the *Colletotrichum* species associated with plants in Louisiana and the Gulf South by placing isolates into a broader phylogenetic context. We collected endophytes and pathogens from host species listed in the LA-PDMG as well as other introduced and native plant species in the Gulf South. We isolated *Colletotrichum* from healthy and diseased plant tissue, extracted genomic DNA, and sequenced the APN2-MAT IGS or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) regions. These specific loci have been previously shown to be useful for species identification in *Colletotrichum*. Among the 56 plants collected, we isolated *Colletotrichum* from 21, including *Liriope* spp. (monkey grass), Magnolia (*Magnolia grandiflora*), Paw Paw (*Asimina triloba*), Ligustrum (*Ligustrum* spp.), and ornamental Olive *(Olea europaea* cv. *'Arbequina').* Our results will have implications for fungal systematics, fungal ecology, and plant pathology by providing a more thorough understanding of *Colletotrichum* diversity in Louisiana and the Gulf South and the role of individual hosts in the movement of these endophytes and plant pathogens.

2.2-157 Role of motility and phytohormone biosynthesis of an endohyphal bacterium from *Rhizoctonia solani*.

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Abstract: Fungal-bacterial interactions have profound impact on mycorrhizosphere environment of crops, while the nature of these symbiotic relationships remain understudied. An endohyphal bacterium *Enterobacter* sp., named En-Cren, was isolated from the pathogenic soil fungus *Rhizoctonia solani* AG2-2IIIB that causes brown patch disease on cool-season turf grass (*Agrostis stolonifera* L.). The virulence of the fungal pathogen is associated with the presence of En-cren. En-cren can be released as a free-living bacterium when fungal hyphae are damaged and migrate rapidly along the outside of the fungal mycelia (including the aerial hyphae). The major objectives of this study were to identify the genetic nature of hyphal motility of the bacterium and whether the bacterium contributes to fungal virulence through the production of phenylacetic acid (PAA), a phytohormone that had been hypothesized to play a role in the interaction between *R. solani* and host plants. Single gene mutations for flagella hook protein *flgE* and type IV pilus secretin *pilQ* in En-cren did not prevent hyphal movement. Double mutants of both motility apparati will be tested. En-cren produces PAA and indole acetic acid (IAA) in minimal media supplemented with precursor compounds, while mutation at the gene for indole-3-pyruvate decarboxylase suppressed PAA and IAA production in cultures of the free-living bacterium. The study will provide more information for our understanding of fungal-bacterial interaction in the mycorrhizosphere and of the virulence mechanism of the broad range soil pathogen *Rhizoctonia solani*.

2.2-158 Importance of basement topography as a distribution factor of *Pythium* in rotational paddy fields with barley, wheat and/or soybean in hilly and mountainous areas in Japan

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Abstract: We aimed development of techniques for low cost and stable production of rice, barley, wheat and soybean in hilly and mountainous areas in western Japan, based on their rotational cultivation. Poor growth of barley and wheat was found in experimental fields located in Hiroshima Prefecture, Japan, in December 2016. Severe waterlogging was observed in those areas caused by excess soil water due to poor drainage. *Pythium* spp. were frequently isolated from the plants that showed poor emergence in the waterlogging areas. Aerial photos taken by an unmanned aerial vehicle revealed a clear relationship on the spatial distribution between waterlogging areas and poor growth of barley and wheat. It was also clarified that the waterlogging areas located above former river channels which had been buried in the fields, through spatial analysis with overlapping the aerial photos and former topographical maps using geographical information system. From these results, it was revealed that basement topography should affect to growth of barley and wheat. *Pythium* known as an aquatic microorganism prefers to wet condition such as in-water or water-soaked areas. Understanding of basement topography will be important to know distribution of *Pythium* in fields in hilly and mountainous areas in Japan. The following species of *Pythium* have been recorded as pathogens of browning root rot of barley or wheat in Japan; *Pythium iwayamai*, *P. paddicum*, *P. horinouchiense*, *P. graminicola*, *P. okanoganense*, *P. spinosum*, *P. sylvaticum*, *P. ultimum* var. *ultimum*, *P. vanterpoolii*, and *P. volutum*. The present *Pythium* isolates from barley and wheat were tentatively identified as species similar to *P. aphanidermatum* or *P. myriotylum* based on their morphological and cultural characteristics. In future, we will investigate their pathogenicity to barley and wheat.

2.2-159 A deep sequencing dual RNAseq approach investigating the interaction between *Rhizoctonia solani* AG1-IB and a commercial *Lactuca sativa* cultivar (cv. Tizian)

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Abstract: The phytopathogenic species *Rhizoctonia solani* is capable of infecting a wide range of economically important crop plants like; soybean, rice, sugar beet, potato and lettuce. Isolates of *R. solani* AG1-IB are responsible for bottom rot on lettuce. The genome of *R. solani* AG1-IB (isolate 7/3/14) was recently established. At present, little information regarding the molecular responses in *R. solani* during the interaction with its host plants is available though. Therefore, the transcriptome of *R. solani* AG1-IB (isolate 7/3/14) was studied during the course of its pathogenic interaction with the host plant lettuce using a leaf infection model. Samples were taken from three distinct pathogen-host interaction zones which covered different phases of disease progression on leaf tissue inoculated with the AG1-IB isolate 7/3/14. The zones can be defined by, symptomless leaf tissue in zone 1, zone 2 with light brown discoloration and zone 3 with dark brown necrotic lesions. These zones were also verified by means of microscopy. We found decreased auto fluorescence of chlorophyll in zone 2 and 3 accompanied by

fungal infection structures, whereas zone 1 only contained symptomless runner hyphae. For transcriptome analysis, we sampled these zones for high throughput Illumina RNA sequencing. This approach improved sensitivity and robustness of the dual RNAseq transcriptome analysis, enabling the detection of over 3000 significantly differential expressed genes, by cross comparison of the three sampling zones. A group of 21 previously undescribed protein coding transcripts was detected that were almost exclusively transcribed in zone 1. This group could contain potential novel *R. solani* effectors. Furthermore, we were able to give the first account of *R. solani* RSA lectin transcription levels during infection. Within zone 2 and 3 *R. solani* RSA encoding transcripts were among the overall most abundant. Overall, we found that in zone 1 transcripts related to proliferation, metabolism and energy turnover are low in abundance, in zone 2 transcription of the corresponding genes is highly upregulated whereas in zone 3 proliferation is decreased again and signs indicative of programmed cell death can be found. To date, in a follow up study, we are investigating the transcriptional response of lettuce towards infection by *R. solani*.

2.2-160 Redefining genera in the Ophiostomatales

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Abstract: The Ophiostomatales is an order of Ascomycete fungi that contains many economically important tree pathogens, sap-staining fungi and even a few human pathogens. Most species are associated with tree- or plant infesting arthropods such as mites and bark- and ambrosia beetles, while some occur in soil and other unique niches, for example *Protea* infructescences. The taxonomy of the Ophiostomatales was confused for more than a century due to the dependence on morphological characters for species and genus definitions. The availability of DNA sequences helped to align the taxonomy of these fungi with phylogenetic relatedness, and resolved the confusion between *Ceratocystis* (Microascales) and *Ophiostoma* (Ophiostomatales). However, the sexual and asexual states of many species in the Ophiostomatales were still treated in different genera, with genera typified by sexual states having priority. The abandonment of dual nomenclature necessitated a re-evaluation of nomenclature in the Ophiostomatales. The aim of this study was to reconsider the generic boundaries and unresolved nomenclatural issues in the Ophiostomatales based on phylogenetic analyses. Sequences of 155 gene regions were extracted from whole genomes available for 40 taxa representing all the major lineages in the order, and a maximum likelihood tree was generated based on the concatenated data. In addition, sequences of four gene regions were determined for more than 240 species, and single gene trees as well concatenated trees were generated based on these data. The respective trees provided a much clearer understanding of where generic boundaries, supported by morphology and ecology, should be drawn. Based on the results we accept current definitions for *Sporothrix, Ceratocystiopsis, Aureovirgo, Graphilbum, Fragosphaeria, Hawksworthiomyces, Esteya* and *Afroraffaelea*. The generic boundaries for *Leptographium*, *Raffaelea* and *Ophiostoma* had to be redefined, and the names *Grosmannia* and *Dryadomyces* were re-instated. Six new genera are being described. Based on these results, most genera in the Ophiostomatales can be recognized based on morphology and ecology. However, DNA sequences remain essential to confirm generic placements and species delineation.

2.2-161 Assessing the clinical relevance of fungal genotype in Blastomycosis infections

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Abstract: Blastomycosis is a fungal disease caused by infection with the fungus *Blastomyces* which grows as a mold at ambient temperatures and produces infectious spores. When inhaled, the spores undergo a temperature induced transition into yeast which cause infections such as pneumonia, skin lesions, meningitis, bone infections, and sepsis. In endemic regions in the United States, the incidence of infection of blastomycosis ranges from 1-2 per 100,000 annually. However, some counties in Wisconsin have reached up to 41.9 per 100,000 in humans and up to 1,420 per 100,000 in dogs which makes Wisconsin hyper-endemic for this disease. In 2013, multi-locus sequence typing revealed two distinct species of *Blastomyces* were responsible for blastomycosis—*Blastomyces dermatitidis* and *Blastomyces gilchristii*. Despite differences in genotype and demonstrated differences in clinical presentation between these two species, the clinical relevance of this distinction had yet to be thoroughly explored. As physicians are currently blind to species type, the goal of this study was to assess whether the recent distinction had clinical relevance for care providers. Clinical isolates (N=112) were obtained from patients throughout the state of Wisconsin who were diagnosed with blastomycosis from 2008-2016. Fungal DNA was extracted and the ITS region of the genome was amplified and sequenced. A single nucleotide polymorphism in the ITS sequence was used to distinguish *Blastomyces dermatitidis* from *Blastomyces gilchristii*. Fungal genotype was compared to patient demographics, sensitivity and specificity of the urine antigen test, and patient treatment collected from clinical records. In keeping with previous studies, patients infected with *B. dermatitidis* infections were 15 years older, more likely to have a pre-existing condition, and more likely to be male than those with *B. gilchristii* infections ($p<0.05$). Patients infected with *B. gilchristii* were diagnosed earlier than those with *B. dermatitidis* ($p=0.05$) which may indicate differences in disease severity. The urine antigen test displayed false negatives 38% of the time in *B. dermatitidis* infections as opposed to 18% false negatives in *B. gilchristii* infections. A false negative was defined as failure to display positive result throughout the course of a known blastomycosis case. Quantities of detected antigen were also significantly lower in *B. dermatitidis* infections as opposed to *B. gilchristii* infections ($p<0.05$). While 97% of physicians made use of itraconazole to treat blastomycosis, only in 50% of cases physicians used the itraconazole serum concentration test. We found evidence to support use of therapeutic drug monitoring regardless of fungal genotype as 61% of patients displayed at least one value that merited dose modification or patient education. While in 97% of blastomycosis cases physicians made use of radiographic imaging in diagnosis and post-diagnostic monitoring with a range of 1-66 X-rays per patient, there are currently no guidelines for how these should be performed. Fungal genotype has clinical relevance in terms of urine antigen test performance, patient demographics, and clinical presentation. Knowledge provided by this study will improve clinical decision making and patient care in cases of blastomycosis.

2.2-162 Dimorphic pathogen, *Emergomyces africanus*, identified in Cape Town air samples

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Abstract: *Emergomyces africanus* is a recently described dimorphic species in the Ajellomycetaceae that causes an AIDS-related systemic mycosis in South Africa. Infections are believed to result from inhalation of conidia or other infectious propagules, although no attempts had previously been made to isolate *E. africanus* from the air. The present study was undertaken to develop molecular techniques for the identification and quantification of *E. africanus* propagules from air samples. The atmosphere was monitored with a Hirst-type 7-day spore trap (Burkard Manufacturing, Co. Ltd., Rickmansworth, Hertfordshire, England) equipped with an alternate orifice to increase sampling efficiency for small spores. The sampler was stationed on the roof a building 4 meters above ground in Bellville, an urban area of Cape Town, Western Cape, South Africa and sampling occurred from 15 Sep 2015 to 29 Aug 2016. Melinex tape was attached to the sampler drum and greased with petroleum jelly; sampler drums were changed weekly. One-day (24 hour) segments of the exposed Melinex tape were cut into smaller pieces and DNA extracted. For each week, fractions of the DNA extract from the 7 daily air samples were pooled to produce a weekly air sample; this allowed testing of both daily and weekly periods. Quantitative PCR was performed using *E. africanus* species-specific primers and TaqMan probe that hybridized to a region of the ITS I gene, and propagules were calculated using a standard curve which had a lower limit of detection of 5 propagules. *Emergomyces africanus* DNA was detected in 11 of the 50 weekly samples. Ten of these 11 weeks produced ITS target numbers that were within the range of the standard curve and could be quantified. The week of 26 Jul to 1 Aug 2016 had 63 propagules in the pooled sample, which was the greatest number detected. Aliquots of the DNA from the daily air samples from the 11 positive weeks were also analyzed for *E. africanus*. From these 77 daily samples, 34 were positive; however, only 11 were within the range of the standard curve and quantified. The highest numbers of *E. africanus* propagules were found on 30 July and 1 Aug with 26 and 24 propagules, respectively. This study showed that *E. africanus* propagules were detected in the atmosphere of an urban industrial setting on approximately 10% of the days during the period analyzed and suggests that airborne exposure to the pathogen is common in Cape Town. More research is needed to determine the extent of exposure in other areas where infection has been diagnosed.

2.2-163 Population genomics of the deadly human fungal pathogen, *Coccidoides* (causing Valley Fever) in the southwestern United States

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Abstract: The disease Valley Fever is caused by the fungal pathogen *Coccidioides*, which can kill otherwise healthy humans (i.e., those who are not immunocompromised, <10% of infections) when they inhale fungal spores from soils. Valley Fever (VF) is common in deserts of the Southern United States including California. In the last 10 years, VF infection rates have increased by over 8-fold. Although research is ongoing there are currently no vaccines, and for the deadliest infections treatment with

currently available antifungal drugs (azoles) control infections while the strongest drugs (amphotericin) are not tolerated well by all patients. Elegant observational studies of *Coccidioides* have established its complex life cycle, disease etiology, and its interaction with the mammalian immune system. However, the molecular basis of VF infection biology remains very poorly understood. To date, studies have implicated a handful of *Coccidioides* genes in growth and virulence; but the pace of molecular genetics research has been limited by the requirement for BSL3 containment and the paucity of genomic tools available. Thus, the urgent need in the field is to accelerate the discovery of genes and proteins that underlie virulence-relevant traits in *Coccidioides*, which can ultimately serve as the targets for novel therapies and/or vaccines. Here we use population, evolutionary, and phylo- genetics to ascertain the fungal genetic diversity in *Coccidioides* populations from the Southeastern United States and to characterize target virulence genes with potential to guide the development of novel therapeutics. To evaluate population structure among the strains of this population, we extracted genotypes at microsatellite loci from a previously published study (Teixeira et al. 2015) and re-analyzed them with population-genetics and phylogenetic approaches. We found that clinical and environmental isolates are not genetically isolated and that there has been recent genetic interchange between them. Future data analyses will be centered on the generation of full genomes for individuals in this population and by genome wide association studies (GWAS) to elucidate genetics of virulence measured by growth and sporulation rates, production of melanin, antifungal resistance, and the ability to evade human immune system. Results of the Valley Fever population diversity analyses will be presented and discussed.

2.2-164 MALDI-TOF mass spectrometry, a rapid identification tool for the newly revised *Cryptococcus neoformans/C. gattii* species complex

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Abstract: *Cryptococcus neoformans* and *C. gattii* are clinically important yeasts that can cause infections in humans, including meningoencephalitis and Cryptococcosis. Although the two species are phylogenetically closely related to each other, the former primarily infects immunocompromised patients, while the latter primarily is diagnosed in otherwise healthy persons. Recently, Hagen et al. refined the species complex into seven distinct species and four hybrid genotypes based on phenotypic and genotypic diversity, such from the sequence comparison of eleven genetic loci, serotyping, electrophoretic karyotyping, etc. The ATCC Mycology portfolio contains a significant number of yeasts in this species complex isolated from various sources and regions, and a rapid and accurate identification tool is strongly desired to appropriately classify these yeasts within the revised species complex. In this study, we evaluated MALDI-TOF mass spectrometry as a cost-efficient rapid identification method for the revised *C. neoformans/gattii* species complex. Type strains of the seven newly identified species and an additional five reference strains were used to develop reference spectra. The protein profiles were acquired from cultures on at least two different media (Sabouraud dextrose agar and YM agar) at two different incubation time (24 hrs and 48 hrs). A dendrogram generated from the protein profiles using a VITEK® MS plus system (bioMérieux) showed that each species was clearly distinct from other species in the complex, although a few profiles from 48 hr cultures were clustered with other closely related sister taxa. Reference spectra of each species were evaluated by testing with those strains fully characterized by the multilocus DNA sequencing of ITS, D1D2, IGS, and GPD gene. The results indicate that MALDI-TOF mass spectrometry shows promise as a rapid and efficient tool for identifying these closely related yeasts by their protein fingerprints.

2.2-165 Inflammatory responses in mice after intratracheal instillation of spores of some *Aspergillus* and *Penicillium* spp monitored in Southwest Nigeria.

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Abstract: Fungi are an increasing public health problem worldwide because they have a great impact on human health. In this study, comparative analysis of airborne fungal spores for two year (May 2014 – April 2016) in ten different locations in Lagos and Oyo States, Nigeria. Identification and characterization of fungal species was carried out by amplification of internal transcribed spacer 1 and 4 gene followed by quantification of allergenic gene by reverse transcriptase quantitative polymerase chain reaction in the most abundant fungal isolates. A mouse model was devised to elucidate and compare the adverse effects provoked by the four most abundant fungi isolated from various locations in all locations. Sixty Balb/b albino mice were grouped into nine treatments of six per cage with a control group. The animals were exposed intranasally to the spores of *A. flavus, A. penicilloides, Penicillium citrinum* and *Penicillium chrysogenum* at 2.3 x 10^{7} and 3.2 x 10^{5} m/L. Both dose-response and time-course inflammatory and toxic responses were investigated after a single dose of the microbes. Biochemical parameters and histopathology revealed that all the microbes studied provoked inflammation after a single dose but the magnitude and its characteristic features were different. The spores of *A. flavus,* and *A. penicilloides* provoked a very intense acute inflammation indicated by production of increased malondialdehyde, myeloperoxidase, total protein production in the lungs, recruitment of inflammatory cells into the airways and expression of neutrophils, eosinophils and basophils in the blood. The inflammatory cell response in the lungs was more severe and varies with each organism at different concentrations. Histopathology result for all inoculated organisms on mice lung showed that there was mild thickening of the alveolar interstitium and moderate hemorrhages. There was also accumulation of inflammatory cells around blood vessels suggestive of vasculitis. The results showed that *A. penicilloides* in addition to *A. flavus* had highly significant lethality on lungs of mice than those of *P. citrinum* and *P. chrysogenum*. This study has also confirmed that production of fungal spores is indicative of weather parameters. Environmental conditions such as relative humidity (RH), temperature and wind velocity exert a significant effect on the amount of microorganisms in the air therefore airborne microbial quantity and quality can vary with time, year and location.

2.2-166 Identification of dermatophytosis and onychomycosis etiologic agents by classic and by MALDI-TOF MS phenotypic methods

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Abstract: Skin, nail and hair infections are a frequent cause of dermatological consultation in tropical countries. The fungi involved in these infections are usually dermatophytes, *Candida* spp. and some non-dermatophyte filamentous fungi (NDFF). Within the NDFF group, *Neoscytalidium dimidiatum* has been increase as common agent of onychomycosis. The identification of these etiologic agents, in routine mycological diagnosis, is mainly based on macro- and micro-morphology characters. However, this approach is subjective and the identification could be not accurate. This is an obstacle for the implementation of appropriate therapy, since that antifungal susceptibility profiles may vary among species. Currently, more sensitive, specific, rapid and cost-effective techniques are being introduced for the identification of microorganisms, such as Matrix Assisted Laser Desorption/Ionization Time-Of-Flight

(MALDI-TOF) mass spectrometry (MS) with the protein profile of each microorganism. The aim of our study was to compare the identification by classic and MALDI-TOF MS phenotypic methods fungal isolates collected from patients with a clinical diagnosis of onychomycosis or dermatophytosis. In total, 134 clinical isolates were included in the study and identified by classic macro- and micro-morphology characters from de cultures. In addition, the isolates were analysed by MALDI-TOF MS. An in-house spectra library was establish in order to identify *N. dimidiatum*, because this species is not included in commercial libraries. Identification by classic approach resulted in 76 dermatophytes with *T. rubrum* (41), *T. mentagrophytes* complex (15), *T. tonsurans* (6), *E. flocossum* (2), *M. gypseum* (1) and *M. canis* (11); 26 *Candida* with *C. albicans* and *C. tropicalis* representing 5 and 4 isolates, respectively; for NDFF the prevalent species was *N. dimidiatum* with 25 out of 31 isolates, follow *Fusarium* spp (4*), Aspergilllus flavus* (1) and *Penicillium* sp (1). One *Trichosporon* sp was also identified. For MALDI-TOF MS the dermatophytes isolates identified as *T. rubrum* and *M. canis* reduced to 16 and 4, respectively and the prevalent group became *Trichophyton* spp (37). For NDFF, MALDI-TOF MS confirmed the identification at species level of 19 *N. dimidiatum*, the isolate *A. flavus* and at genus level the 4 *Fusarium* spp. Within the NDFF isolates, the *Penicillium* sp isolate was correctly identified by MALDI-TOF MS as *A. versicolor*. The *Trichosporon* sp isolate was also confirmed. Finally, the identification of *Candida* isolates by MALDI-TOF MS were resolved at species level with *C. parapsilosis* (6), *C. haemulonii* (1) and *C. guilliermondii* (1). The inconsistencies observed in the results from the two approaches used with more evidence for filamentous fungi than for yeasts identifications can be due to: 1) the established score to define the identification up to genus or species level; 2), sample preparation and the quality of the extraction of proteins; and, 3) the lack of spectra or low number of species included in the commercial libraries could interfere in the final outputs. The results of this work clearly show that the classic approach is prone of errors, much more work is needed to make MALDI-TOF MS identification a routine in clinical laboratories and to clarify these results, identification by molecular biology is advised.

2.2-167 Establish in Medellin (Colombia) an in-house library to identify clinically important filamentous fungi by MALDI-TOF MS

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Abstract: Epidemiology of fungal infections has changed in an important manner in the last decades, however timely diagnosis and treatment are still a current challenge. Accurate and swift identification of the agents that cause fungal infections are paramount, since sources of infection, differences between therapeutic regimes, and *in-vitro* susceptibility profiles may vary amongst species and strains. The application of Matrix Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) for the identification of fungal samples is currently well-established based on the remarkable reproducibility for the measurement of constantly expressed and highly abundant proteins, such as ribosomal proteins, that are used to generate a fingerprint profile. However, the use of this tool for filamentous fungi identification in routine clinical laboratories is still limited due to several reasons, such as, 1) the biological variations among fungal clinical isolates or 2) the lack of a spectrum of reference in commercial libraries for the identification of emerging, endemic and prevalent fungi in tropical regions. Consequently, the establishment of an in-house library that will contain spectra for prevalent fungi found in routine mycological diagnosis at a local level, and of those not included in commercial libraries, is a demand. The aim of this research was to establish a library of spectra for the identification of clinically important filamentous fungi through MALDI-TOF MS in a mycological diagnosis laboratory. In order to establish the in-house spectra library, 21 strains were used, including dermatophytes

(*Trichophyton* (3), *Microsporum gypseum* (1) and *Microsporum canis* (1)), *Aspergillus* (7), *Fusarium* (4), *Neoscytalidium dimidiatum* (1) and *Sporothrix* (4). Afterwards, the spectra were validated with 17 strains and 35 clinical isolates identified by classic and molecular methods. In addition, 16 of 17 strains and 29 of 35 clinical isolates were subjected to identification in commercial filamentous fungi libraries and in BDAL (Bruker Daltonics, Germany). The results obtained from the three libraries were compared. For the acquisition of the reference spectra for each genus and for some species, it was necessary to standardize growth and assay conditions. The in-house generated library identified clinical isolates/strains down to species in the proportion of 82.5%/93.0% of the cases, and down to genus in 88.6%/100.0%. In addition, 11.4%/0.0% of the fungi not being identified. With the filamentous fungi library, identification down to genus and species were of 62.0%/64.3% and 31.0%/42.8%, respectively for clinical isolates and strains, while 37.9%/35.0% proportion remained unidentified. With the BDAL library identification the proportion for clinical isolates/strains was of 42.3%/7.1% for genus and 3.4%/0.0% for species; identification was not achieved in 51.7%/92.8% of the cases. Optimal growth time required to obtain proteins varied among genera and among some species. With the in-house built library, it was possible to identify strains and clinical isolates, in some cases, more accurately than with commercial libraries. In addition, fungal spectra that are not included in commercial libraries were included; this foster the standardization of the growth conditions for the different strains, the protein extraction technique, as well as the definition of criteria for the acceptance of a spectrum of reference.

2.2-168 Improving tree potassium content effectively controls the occurrence and development of apple Valsa canker

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Abstract: Valsa canker disease, caused by the fungus *Valsa mali*, seriously impedes apple production in China and other eastern Asian countries. Infection can take place throughout the year, and pathogen colonization produces localized cankers that kill twigs, limbs, and, finally, the entire tree. Because *V. mali* penetrates into the host phloem and xylem, resulting in a perennial canker, fungicide application is ineffective. All commercially important apple varieties are susceptible to the pathogen, and the average disease incidence is over 50% across all tree ages in China. To better understand the reason for the prevalence of Valsa canker and provide a theoretical basis for its control, field disease investigation and fertilization experiments were performed. Field investigation of the occurrence of Valsa canker in 24 apple orchards in concert with foliar nutrient analysis found a significant negative correlation of leaf potassium (K) content with incidence and severity of Valsa canker. Greenhouse fertilization experiments showed that increasing tree K content enhanced resistance to pathogen colonization and establishment. Apple trees with leaf K content greater than 1.30% exhibited almost complete resistance to *V. mali*. Field trials demonstrated that increasing K fertilization could significantly reduce disease incidence. Efforts are ongoing to dissect the underlying mechanisms for the observed K effects on Valsa canker. Preliminary result showed that K deficiency reduced the accumulation of cinnamic acid and 4-coumaric acid upon challenge inoculation, these phenolic compounds inhibited *V. mali* growth on PDA. Our study demonstrates the feasibility of managing Valsa canker on apple with adjustments to K mineral nutrition, and implies that K affects Valsa canker resistance via regulating phenolic compound metabolism.

2.2-177 Will the real pecan truffle please stand up?: Use of molecular tools reveals cryptic diversity within the *Tuber lyonii* complex and an updated phylogeny of the *Tuber rufum* clade

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Abstract: *Tuber* species are among the most iconic mushrooms of the culinary world. They share a rich, cultural history throughout the world. While the European black truffle (*T. melanosporum*) and the European white truffle (*T. magnatum*) are well known, there is a growing market demand for the native North American pecan truffle (*Tuber cf. lyonii*). For 110+ years, the true identity of the pecan truffle has been ambiguous because spore morphology and size are not useful metrics to determine species identity within this lineage. *Tuber lyonii*, originally described by Butters in 1904 from Minnesota, and *T. texense* described by Heimsch in 1958 from Texas, are the names that have been applied to multiple cryptic species of the *T. rufum* clade. The objectives of this study are to elucidate the true identity of the pecan truffle, obtain sequence data from the holotypes of *T. lyonii* and *T. texense*, and build an updated phylogeny for the *Tuber rufum* clade. Through use of herbaria in Asia, Europe, and North America, we obtained specimens of target taxa and holotypes of *T. lyonii* and *T. texense* before sequencing the Internal Transcribed Spacer (ITS) region. Sanger sequencing was successful for generating a preliminary tree as well as obtaining sequence data from the holotype of *T. lyonii*. An Illumina MiSeq metagenomic barcode for the ITS1 region was performed on the holotype of *T. texense* and yielded an ITS1 sequence. Although traditional molecular markers work for species level delineation, they lack the ability to resolve the cryptic species within the "*T. lyonii*" complex. However, an ITS sequence from the holotypes of *T. lyonii* and *T. texense* have shown that these holotypes are distantly related to the commonly recovered species that have been previously called by these names. Here we use Sanger sequencing and sequence capture techniques to resolve the species within this cryptic, yet commonly recovered complex. We present the most robust phylogenetic analysis of the *T. rufum* clade to date. The results and future implications of this research on fungal systematics, utility of herbarium specimens, and the developing market for pecan truffles are also discussed.

2.2-178 Boletes of Bangladesh: taxonomy and phylogeny

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Abstract: Boletes are fascinating mushrooms in the family Boletaceae comprising ca. 70 genera. A good number of them are frequently collected for human consumption. Bangladesh is a part of tropical South Asia, has the widest and most scattered distribution of *Shorea robusta*, a tree of Dipterocarpaceae which plays symbiosis association with many groups of fungi. However, macrofungi in Bangladesh are poorly understood. The aim of the study is to investigate the species diversity of boletes and their evolutionary relationships with other boletes of the word. Boletes samples were collected from eight districts of Bangladesh under the monodominant stands of *Shorea robusta*. Species delimitation was documented using morphological data and molecular approaches of the four genes (28S+*tef1-α*+*rpb1*+*rpb2)*. This

is the first survey and taxonomic report of boletes from Bangladesh. An extensive field survey was conducted during the monsoons of 2011–2016, resulted over 250 boletes samples, representing eight genera and 19 species. From amongst the collection of boletes, herein are reported two new genera, 13 new taxa, and three new lineages at generic level (uncertain phylogenetic position) based on morphology along with molecular evidence. In conclusion, this is the first attempt to discovery of boletes, and their diversity, distribution and evolutionary relationships will be addressed during the congress.

2.2-179 Studies in Floridian boletes

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Abstract: DNA sequencing of macrofungi continually shows that many species are yet to be described and many species belong in different genera than originally thought based on morphology. The boletes are remarkably diverse in Florida, yet very few have been sequenced. Numerous boletes were described my William Alphonso Murrill (1869–1957) and Rolf Singer (1906–1994); the protologues and type specimens of many of these species require critical re-examination to understand their application. We have approached the study of boletes in Florida with field work, herbarium studies, microscopy, and targeting the main phylogenetic loci for fungi (ITS, LSU, TEF1, and RPB), sometimes requiring the design of novel primers for successful amplification. Studies of phylloporoid fungi in Florida revealed one species which requires a new genus, adding another example of the independent gain of gills in boletes. A *Tylopilus*-like species collected on the University of South Florida campus (Tampa, Florida) likely represents another novel bolete genus. Additional sequencing suggests several species are undescribed, some belong in recently described genera, and other species are newly confirmed for Florida.

2.2-180 Morphological characters with phylogenetic signal in *Scleroderma* (Basidiomycota)

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Abstract: *Scleroderma*, formerly in the artificial group of the Gasteromycetes, is now part of the Boletales. The genus comprises subglobose, ectomycorrhizal mushrooms, with 21 species according to the monograph of Guzmán in 1970 or 30 following the Ainsworth and Bisby's dictionary, from temperate and tropical regions worldwide. Microscopically, the genus is characterized by its globose thick-walled ornamented basidiospores. The aim of this work was to analyze some morphological characters on the light of a molecular phylogeny, to understand its evolution. Specimens from IBUG and XAL herbaria were macro and micromorphological studied and described. DNA was extracted from herbarium specimens and amplified the ITS rDNA. The purified products were sent to the Sequencing Department, University of Arizona and LaniVeg (CUCBA, UdeG). Sequences were assembled and edited in Chromas and aligned in MacClade. Furthermore, sequences obtained from GenBank were included. *Pisolithus arrhizus* was selected as outgroup. jModeltest was used to determine the best evolutionary model using the corrected Akaike information criterion. 11 macro and micromorphological characters representing more than 20 taxa were codified and included in the matrix along with 287 DNA bp, and analyzed by

Maximum Parsimony and Bayesian Inference. Morphological characters were mapped with Mesquite 3.2. Additionally, ancestral state reconstructions (ASR) were obtained using BayesTrait 1.0. Some of the morphological characters with phylogenetic signal and taxonomic value were the ornamentation of the basidiospores and clamp connections. Three main linages were recognized, corresponding to the traditional classification proposed by Guzmán, as already detected by Phosri *et al*. in 2009, with three sections: *Scleroderma* (formerly *Aculeatispora*), *Sclerangium,* and *Reticulatae* (formerly *Scleroderma*). The synapomorphies for each clade were echinulated basidiospores for sect. *Scleroderma*, subreticulated for *Sclerangium*, and reticulated for *Reticulatae*. Besides, clamp connections are absent in sect. *Scleroderma* and present in the other two sections.

2.2-181 *Cortinarius clavatus* sp.nov. a new species in section *Hinnulei* genus *Cortinarius* from Pakistan

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Abstract: This study is a part of larger project dealing with exploration of macrofungal species concentrated in forest and woodland ecosystems of northern areas of Khyber Pakhtunkhwa, Pakistan. The study area is predominantly mountainous encompassing well-known ranges of Himalaya and Hindu-kush. In this paper a new species of *Cortinarius* (*C. clavatus* sp.nov.) is reported, described and illustrated. Specimen of this new species were collected from coniferous forest of Miandam valley, district Swat, Pakistan during summer 2015–2017. Morphological characters along with molecular data (nrITS) was used to confirm species identification. Basidiospore ornamentation was checked by using electron microscopy. *Cortinarius clavatus* is characterized by convex to plano-convex, light brown to strong brown umbonate pileus, clavate to slightly thickened fibrillose stipe and broadly ellipsoid to sub-amygdaliform, ornamented basidiospores with suraphilar depression. Maximum likelihood analysis inferred from nrITS data clusters the new species within the *Hinnulei* clade of *Cortinarius* supported by a strong bootstrap value.

2.2-182 The genus *Inocybe* from cedar dominating forests of Pakistan

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Abstract: *Inocybe* is a large genus of mushroom-forming ectomycorrhizal fungi with more than 700 species distributed worldwide. The number is increasing considerably with the exploration of tropical and southern temperate areas. Many species have been reported from Asian continent. From Pakistan, 22 species of *Inocybe* have been reported so far. During the field survey to study the ectomycorrhizal fungal communities associated with *Cedrus deodara* from Pakistan, several fungal taxa have been identified using morphological and molecular techniques. *Inocybe* has been found to be the most diverse ectomycorrhizal genus. It is represented by 20 species, among these 6 species have been found in the form of basidiomata while 13 were found in association with root as ectomycorrhizae. One species has been represented from the above ground and also identified from the host root in the form of morphotype. Among these, four species as basidiomata are previously undescribed while from the belowground data, 12 were identified as operational taxonomic units and given the names with running numeric. This data gives an overview about the species diversity of the genus *Inocybe* from Pakistan. Identification of operational taxonomic units suggests the presence of quite a large number of undescribed *Inocybe* species which were not represented by above ground fruiting bodies.

2.2-183 Multigene phylogenetic analyses and morphology reveals three new species within section *Rimosae* (*Inocybe*) from western Himalayas, Pakistan

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Abstract: *Inocybe* (Inocybaceae) is a highly diverse ectomycorrhizal genus among the gilled mushrooms (Agaricales) comprising some 750 known taxa worldwide. However, a considerable number of taxa have yet to be formally described. *Inocybe* has a widespread distribution and is found commonly in temperate areas and to a lesser extent in the tropics. The objective of the present work is to collect, describe, identify using morphological and multigene phylogenetic approach, and enumerate diversity of *Inocybe* species associated with pines in Pakistan. During fungal surveys conducted between 2012-2014, several species of genus *Inocybe* were collected from pine dominating forests of western Himalayas, Pakistan. These were documented morphologically and phylogenetically. Gene regions examined during this study include the complete ITS region (ITS1 - 5.8S - ITS2) of the nuclear ribosomal RNA gene, the first ca. 900 bp of the 25S rRNA gene that encodes the nuclear large subunit of ribosomal RNA (nLSU), and the mitochondrial small subunit of ribosomal DNA (mtSSU). Three different data matrices for phylogenetic analysis were prepared: (i) Maximum Likelihood (ML) analysis of combined datasets of ITS+LSU+mtSSU of Pseudosperma clade (ii) ML analysis of nLSU region of *I. rimosa* s.l. (iii) Maximum Parsimony (MP) analysis of *I. rimosa* s.l. using combined datasets of ITS+LSU+mtSSU. Both morphological and phylogenetical analyses validate the occurrence of three new species, *I. brunneoumbonata*, *I. pinophila,* and *I. triacicularis*. These species shared an alliance with the Pseudosperma clade (sect. *Rimosae*), one of the seven major clades in the Inocybaceae. Species of the Pseudosperma clade are typically characterized by a rimose pileus; furfuraceous to furfuraceous-fibrillose stipe; absence of metuloids and pleurocystidia; smooth, elliptical to indistinctly phaseoliform basidiospores, and cylindrical to clavate cheilocystidia. Comprehensive morphological and microscopic descriptions, illustrations, and phylogenetic affiliations of the studied taxa are provided. The new species are differentiated from their close relatives by basidiospore size and fruit body coloration. Combined sequence data from ITS, nLSU-rRNA, and mtSSU-RNA gene regions also confirmed the novelty of species and their placement within the Pseudosperma clade. All three newly described taxa likely share an ecological association with pine species. This is the first attempt to describe new taxa of *Inocybe* in clade Pseudosperma from Pakistan based on combined morphological and multi-gene phylogenetic approaches.

2.2-184 Evidence of speciation in North American and European populations of *Thelephora cuticularis*

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Abstract: Fungi in the genus *Thelephora* are macroscopically characterized by their purplish-brownish , tough or slightly fleshy, erect fruitbodies. Microscopically, they have brownish, angular, and echinulate spores, and a monomitic hyphal system, usually with clamped hyphae. One species in the genus, *Thelephora cuticularis*, is a rather small, black, water-absorbent fungus, easily recognizable by its non-angular spores, the absence of clamp connections, and the cyanescence of its subhymenium in KOH.

Because of these morphological features, it is not easily confused with any other species in the genus *Thelephora*. The type of *T. cuticularis* was collected in Ohio (United States) in 1844 and described by Berkeley in 1847. Since then, it has been found in both Europe and North America, but is considered rare on both continents. There are very few recent reports, with most records consisting of old collections deposited in North American herbaria. Morphological and molecular methods have been used to study several specimens of *Thelephora cuticularis* from Europe and North America. Our preliminary study reveals that there is evidence for splitting *T. cuticularis* into two species, one found in North America and the second restricted to Europe. The external morphology of both species is similar, but their spores are slightly different in size, shape, and spine length. Additionally, the two species have different ecologies. The American specimens are described as truly lignicolous, while European specimens have been collected on bare soil or litter debris in hardwood forests. Our results confirm the biogeographic separation of the populations from North America and Europe. The specific epithet *T. cuticularis* belongs to the American species and so a new name will be proposed for the European species.

2.2-193 Worldwide eco-evolutionary dynamics of the *Peltigera-Nostoc* symbiosis

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Abstract: All species of lichen-forming fungi (mycobionts) in the genus *Peltigera* (Ascomycota, Lecanoromycetes) associate with cyanobacteria of the genus *Nostoc*, either as a main or secondary photosynthetic and nitrogen-fixing partner (photobiont). A thorough phylogenetic revision of the fungal genus, based on phylogenomic approaches and several species delimitation analyses, revealed the presence of ~150 species, of which about half are new to science. In addition, we inferred the phylogeny of *Nostoc* associating with *Peltigera,* using a phylogenomic approach, and genotyped the *Nostoc* symbionts associating with *Peltigera* species across the entire genus. This provided a unique opportunity to use the *Peltigera-Nostoc* symbiosis as a model system to study patterns of association (including specificity) and diversification rates geographically and through time. We used this phylogenetic framework to study the evolution of ecological networks, including factors such as interaction dependency, symmetry, and nestedness. We compared the eco-evolutionary dynamics among the main clades of *Peltigera*, and the observed patterns in the context of ecological preferences of each species, the type of interactions (*Nostoc* as main or secondary partner) and dispersal mode (sexual or asexual propagules). This study reveals common trends at the genus level, such as the higher specialization and dependency of the mycobiont, but also differences within and among clades, including drastic differences in specificity.

2.2-194 Species delimitation in the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota)

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Abstract: The *Usnea cornuta* aggregate, as defined here, includes *U. brasiliensis* (Zahlbr.) Motyka, *U. cornuta* Körb. and *U. dasaea* Stirt. These species are mainly corticolous, with an erect-shrubby life form, composed of more or less inflated branches constricted at their point of attachment and relatively small, punctiform soralia usually covered with isidiomorphs. The relative thickness of the cortex, medulla and axis (CMA) is of the *cornuta*-type or the *brasiliensis*-type. However, recent studies on some South American *Usnea* taxa revealed that this group is based on homoplasious characters. The increasing availability of DNA sequences data provides much deeper insight in our understanding of the various biological processes responsible for species formation and their consequences on the biodiversity. However, complexity in the evolutionary mechanisms, as for example the incomplete lineage sorting (ILS), can obscure the signals for the recognition of evolutionary distinct lineages, in particular in the recently diverged groups of organisms. Therefore, evaluation of the evidences from diverse sources may be critical for the accurate assessment of the species boundaries in such groups. The coalescence model takes ILS into account, and a derived multi species coalescent framework is now implemented in several softwares used for species delimitation based on DNA sequences data with or without the requirement of defining putative species a priori. The objective of our study is to investigate how many clades are hidden in the *U. cornuta* aggr. and if they can be characterized by morphological, anatomical or chemical characters. For that purpose, we provide the first multi species coalescent model-based species delimitation for the Neotropical *Usnea* species. Based on the ITS rDNA, two protein-coding genes RPB1 and Mcm7, we estimated the species tree under the multi species coalescent model in a Bayesian framework using the STACEY method. We used DNA sequences, chemical, geographic, and morphological data from 156 specimens, and applied an integrative approach to define species boundaries with a particular focus on the cosmopolitan species group *U. cornuta* aggr. (n = 82 specimens). These specimens span over a broad geographical area including Brazil, Costa Rica, Ecuador, France, Peru, Portugal, Spain, United Kingdom and USA. Our results indicate that the *Usnea cornuta* aggr. represents a complex of nine strongly supported lineages correlated with secondary chemistry, as well as two unsupported groups (possibly including *Usnea cornuta* s. str.) and three singletons making a total of at least 14 distinct lineages. This shows that the diversity in the *U. cornuta* aggr., especially in Brazil, was so far underestimated. Further investigations are on going to show whether this genetic diversity correlates or not with morphology and anatomy.

2.2-195 Thyriothecial fungi and fossil evidence of Dothideomycetes

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Abstract: Critical reinterpretations of morphological characters in fossil fungi have the potential to improve estimates of the geological timing of fungal radiations. Some of the best fungal fossils are thyriothecia. A thyriothecium is a minute ascomatal type, with a flat scutellum, a shield-like upper surface, with a distinctive cell pattern that is formed by a sequence of hyphal branching and septation. The fossil record rarely provides diagnostic characters such as asci but the diversity of fossil scutella offers great potential to calibrate ages of extant fungal lineages. However, both DNA sequence data for genera of

thyriothecia-forming fungi and illustrations of scutella remain sparse in public databases. We present an updated phylogeny of thyriothecial Dothideomycetes based on 4251 positions for 320 taxa, contributing new LSU and SSU rDNA sequence data for 14 thyriothecial fungi. We define thyriothecia as flattened, superficial ascomata lacking a differentiated lower wall, with varied scutellum patterns, many, but not all, radiate. We coded character states for taxa including 60 thyriothecial species and then estimated ancestral character states over the Bayesian posterior distribution of topologies from our dataset to account for the phylogenetic uncertainty. Radiate thyriothecia were only found in Class Dothideomycetes, where they seem to have evolved independently at least five times. Clades containing thyriothecia are mostly folicolous and caulicolous, and it remains unclear whether these ascomata were derived from perithecioid or apothecioid ancestors. Genera traditionally recognized in the order Asterinales were polyphyletic and formed two independent clades (Asterinales and Asterotexiales). Morphological distinction between thyriothecia of these orders was impossible because they share a combination of scutellum and mycelium characters. In contrast, the most typical members of Microthyriales and Aulographaceae were readily recognizable from the organization of cells at their scutellum margins, patterns of septation and branching of the scutellum hyphae. We used our newly defined characters to interpret published fossil thyriothecia. We show which of the lineages now sampled for DNA are represented in the fossil record of fungi and present potential Dothideomycetes calibration points available in fossil occurrences of thyriothecia. A phylogenetically congruent distribution of character states among modern thyriothecial clades will help to improve interpretation of fossil material, and will increase the precision of Ascomycota age estimates.

2.2-196 Exploring the diversity of secondary metabolite clusters in Dothideomycete fungi

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Abstract: Quantifying genotypic diversity is essential for understanding and predicting how organisms adapt to their environment, and in fungi, perhaps no process is as central to adaptation as metabolism. As heterotrophs, fungal growth and survival is necessarily contingent on their ability to extract carbon and energy from a particular niche. Fungi are also adept chemical engineers who synthesize broad arrays of specialized or secondary metabolites (SMs) that shape their interactions with surrounding organisms. For example, many plant pathogens produce toxic SMs to kill their hosts, while plant saprotrophs produce antimicrobial SMs to help them compete for available resources. Although fungi have historically been an important source of economically and medically important SMs (e.g., penicillin), relatively little research has been devoted to studying patterns in the diversity of SMs they produce. Consequently, we lack a comparative framework for understanding both the drivers of fungal metabolic diversity, and the dynamics between metabolic evolution and ecological adaptation. New fungal SM pathways are usually encoded in “gene clusters” made up of neighboring genes in fungal genomes, which facilitates their identification and characterization through whole genome sequencing. Here, we conducted a systematic analysis of SM gene clusters in the largest group of fungi, the Dothideomycetes, in order to evaluate the diversity of their SM gene clusters, and to assess the potential for novel SM discovery within this group. We first developed new bioinformatic algorithms for refining gene cluster prediction that we used to query a database of 101 Dothideomycete genomes. We identified 459 unique types of clusters, of which only 6% produce known SMs, and found that even

closely related species harbor distinct SM cluster repertoires. We then used network analyses to identify genes within these clusters that have played an outsized role in the evolution of SM biosynthesis, and whose presence in these clusters can be used to improve future gene cluster detection methods. Next, we used comparative phylogenetic methods to identify clusters with strong signatures of selection that are present in fungal decomposers and pathogens, and that may encode pathways underlying specific plant-associated lifestyles. Finally, based on the phylogenetic distributions of the clusters we detected, we identified specific lineages within the Dothideomycetes that are projected to harbor the greatest diversity of SM clusters, thus providing a valuable roadmap to direct future SM sampling and characterization efforts.

2.2-198 Mediterranean biodiversity hotspot harbours extraordinary diversity of soil yeasts

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Abstract: Species richness and endemism of land plants and animals are important characteristics of a biodiversity hotspot. These parameters were also used to estimate the global diversity of Fungi, including millions of yet undiscovered species. Hence, biodiversity assessments are important to provide more accurate estimations and sampling in areas considered as biodiversity hotspots. Due to small cell size and enigmatic appearance, biodiversity assessments of microscopic fungi become are generally difficult and become laborious in the case of endophytes and soil-borne species. Here, we provide the first comprehensive inventory of soil yeasts in the Mediterranean biome, which is also the leading biodiversity hotspot for vascular plants and vertebrates in Europe. Studies in the Mediterranean biome included a natural forest vegetation and cultural landscape. The cultural landscape was an agro-silvo-pastoral system with Mediterranean oaks and grassland known as Montado (Portugal) and Dehesa (Spain). Diverse forested sites of Serra da Arrábida Natural Park (Portugal) represented the Mediterranean forests, woodlands, and scrub biome. In sclerophyll scrublands, both cultivation experiments and the subsequent species richness estimations suggest the highest species richness values of soil yeasts reported to date. These values far exceed those reported for other forest soils in Europe using either cultivation or culture-independent techniques. Remarkably, about a third of isolated yeasts represented new species, some of which have been recently described. Precipitation level, above ground biomass and plant projective cover under sclerophyll forest vegetation had strong impact on yeast diversity and on community structure and lower precipitation resulted in an increased number of rare species and decreased evenness values along the gradient from humid broadleaf forests to dry scrublands. In agreement with previous observations made in temperate climate, managed agro-silvo-pastoral system was characterised by lower species diversity. This observation further suggests extraordinary value of natural, unmanaged and low-managed, forests for biodiversity conservation of animals, plants and fungi, including saprobic free-living micromycetes. The Mediterranean forests, woodlands, and scrub biome is not limited to the Mediterranean region. Similar plant formations represented by sclerophyll shrublands are known as maquis in the Mediterranean Basin, chaparral in California, matorral in Chile, fynbos in South Africa, and mallee and kwongan shrublands in Australia. Many of these regions have been proposed for World Heritage status. Both high species richness values and high proportion of potential new species makes studies in natural biotopes in the Mediterranean climate of high priority for mycologists and conservation biologists.

2.2-199 Population variation in post-harvest rot *Rhizopus stolonifer*

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Abstract: Globally postharvest diseases contributes to one third of food losses. *Rhizopus stolonifer*, a Mucoromycota fungus, causes postharvest soft rot in fruits and vegetables. Control is typically focused on quick harvesting, packing at cool temperatures combined with treatment of fungicides. However, many fungicides are not approved for direct use on fruits and understanding mechanisms of resistance development is limited. We are phenotyping and sequencing ~250 geographically and substrate diverse R. stolonifer strains. Strains have been cultured from California and Florida strawberries, from almond hulls from California orchards, from other California fruit farms. A subset were collected from around the world and obtained from culture stocks from the USDA-NRRL collection. Fungicide resistance varies among isolates with reduced sensitivity to Fludioxonil in some strains. Linear growth rates in race tubes at 12C, 23C, and 30C show significant differences among strains that originate from varied climates or isolation substrates. Whole genome sequencing is being performed on 250 strains from this collection and analysis of patterns of genetic variation was performed to test for evidence of population structure, demography, and association of genotype to phenotype, particularly fungicide resistance. Preliminary data from a geographically diverse subset of 100 samples identified more than ~70,000 SNPs across the 38 Mb genome. Variants were analyzed to scan for highly diverse gene loci, evidence of directional selection, or insertion/deletions of transposable elements. The strains group into 3 main clades, but no phylogeographic pattern has emerged yet. Initial GWAS show 3 loci potentially linked to fungicide resistance. Further sequencing will test robustness of these potential sub-populations, examine genotype and phenotype correlations, and identify genetic loci with signatures of rapid evolutionary change.

2.2-200 Combining phylogenetic and ecological evidence for achieving one fungus-one name based taxonomy of Hypocreaceae (Hypocreales, Ascomycota)

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Abstract: The ascomycete order Hypocreales is characterised by remarkable diversity of lifestyles, habitats and substrata with the best-known members representing ubiquitous biotrophs – pathogens and parasites of plants and insects as well as endophytes. However, it is also the group most rich in mycoparasitic or fungicolous fungi, many of which inhabit not only mushrooms but fruitbodies of various taxonomic and ecological groups of fungi. They range from obligate parasites to facultative saprotrophs as well as from biotrophs to necrotrophs characteised by a wide array of secondary metabolites. Majority of such taxa belong to the Hypocreaceae, including *Hypomyces* and *Trichoderma* but also their satellite genera, that comprise sexually and/or asexually reproducing species. The present study aimed at phylogeny-based taxonomic revision of the family to discontinue the use of dual or even triple names currently in use for the mostly pleomorphic species. A multigene dataset was created for a wide array of fungicolous Hypocreaceae and analyzed together with sequences of closely related taxa. The resulting phylogenetic reconstruction enabled to present a hypotheses about the evolution of this intricate group of organisms, focusing on the main shifts in the nutritional mode, lifestyle and specialization to hosts from various lineages of ectomycorrhizal vs saprotrophic Basidiomycota and Ascomycota. Evidence about the phylogeny and ecology was used for proposing taxonomic rearrangements in the group where quarter of a hundred generic names are available and several lineages, distinguished in the new phylogenetic hypothesis, await for being described. Possible solutions for sorting out the genus-level taxonomy according to the one fungus-one name principle, while considering both teleomorph- and

anamorph-typified names, are presented for discussion. Advantages and limitations of the official barcode marker of fungi in building species hypotheses and identifying isolates/specimens will likewise be discussed.

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3.1-1 Ascospores- and conidia-specific differential gene expression analysis in *Aspergillus nidulans*

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Abstract: In the homothallic model filamentous fungus *Aspergillus nidulans*, developmental process and spore formation is environmentally and genetically regulated. Although asexual spores or conidia differentiation is well-characterized by analyzing important genes for controlling orchestrated developmental pathways, including the *brlA* gene-mediated conidiophore and conidia morphogenesis, only a few genes such as *nsdD*, *nsdC* and *veA* have been elucidated for playing important roles in sexual development and ascospore formation. Unlike conidia, however, physiological and genetic studies of ascospores are remained to be characterized. To know more about the ascospores biology as well as conidia, we performed RNA-seq analysis from *A. nidulans* conidia and ascospores RNA samples. Comparative analysis of transcription profiles of conidia and ascospores revealed many genes that are expressed differentially in both spores. Detailed investigation of the differentially expressed genes is in progress.

3.1-2 A genomic-level recombination study of familial *Aspergillus flavus*

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Abstract: *Aspergillus flavus* is an agriculturally-significant, filamentous micro-fungus that has the potential to contaminate food and feed crops with toxic secondary metabolites such as aflatoxin (AF) and cyclopiazonic acid (CPA). Once considered an asexual organism, recent discoveries within the last decade have shown *A. flavus* is capable of overcoming heterokaryon incompatibility to undergo meiotic recombination through sexual out-crossing. Recombination in the aflatoxin gene cluster has been reported, but at the genomic level the impact of recombination has not been studied. Therefore, the goals of this study are: 1) to elucidate the heterokaryon incompatibility loci that give rise to VCG designation; 2) to determine the level of overall recombination impacting a single generation of F1 progeny as well as identify any hotspots for recombination; and 3) to elucidate the impacts recombination can have on chemotype diversity. We paired a toxigenic (AF+/CPA+) *MAT1-1 A. flavus* strain with an atoxigenic (AF-/CPA-) *MAT1-2 A. flavus* strain tagged with green fluorescent protein. Ten F1 progenies (five fluorescent and five non-fluorescent) were randomly selected from single-ascospore colonies and broadly examined for evidence of recombination, as well as inheritance of AF and/or CPA production. We observed progenies that had inherited one or both of the examined mycotoxins (AF and/or CPA) from the *MAT1-1* parent, but had inherited the *MAT1-2* gene from the atoxigenic parent. Other progenies had inherited the mating-type and atoxigenic profile from the *MAT1-2* parent, but did not inherit the eGFP gene that facilitates fluorescence. Traditional vegetative compatibility group (VCG) testing of the progenies with one another, as well as with the parent strains, revealed four of the progeny strains to be a unique VCG. We then sequenced the genomes of this familial population for examination of genetic changes and patterns of inheritance at the genomic level. This project is ongoing and will involve results from genomic-level investigations upon presentation at IMC11.

3.1-3 *Aspergillus flavus ecm33*, a GPI-anchored protein-encoding gene, plays a role in vegetative growth, production of conidia and sclerotia, and resistance to calcofluor white

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Abstract: Many glycosylphosphatidylinositol-anchored proteins (GPI-APs) of fungi are membrane enzymes, organization components, and extracellular matrix adhesins. We analyzed eight *Aspergillus flavus* transcriptome sets for the GPI-AP gene family and identified that AFLA_040110, AFLA_063860 and AFLA_113120 were among the top two highly expressed genes in seven of the eight sets. Disruption of the former two genes did not drastically affect *A. flavus* growth and development. In contrast, disruption of AFLA_113120, an orthologue of *Saccharomyces cerevisiae ECM33*, decreased vegetative growth and conidiation, promoted sclerotial production and altered conidial pigmentation. The developmental defects were remediated by incubation under constant light. The *A. flavus ecm33* null mutant showed decreased sensitivity to congo red at low concentrations (25-50 µg/mL) but had increased sensitivity to calcofluor white at high concentrations (250-500 µg/mL). Analyses of cell wall carbohydrates indicated that the *α*-glucan content was decreased but the contents of chitin and ß-glucan were increased in the mutant strain. *A. flavus* Ecm33 is critical for proper cell wall composition and plays an important role in normal fungal growth and development.

3.1-4 Climate change effect on ochratoxin A production and gene expression in *Aspergillus carbonarius*

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Abstract: Ochratoxin A (OTA) is a potent pentaketide nephrotoxin diffusely distributed in food and feed products (grains, legumes, coffee, dried fruits, meat derived products, beer and wine); it is also carcinogenic, neurotoxic, teratogenic and immunotoxic. This mycotoxin is produced by species belonging to the genus *Aspergillus* and *Penicillium*. OTA is the primary mycotoxin risk in wine and dried vine fruits, its maximum level is regulated by law. Several studies focused on *Aspergillus* section *Nigri*, due to their role as causative agents of black rot of grapes, and subsequently as cause of ochratoxin A contamination. In particular, *Aspergillus carbonarius* has been identified as the major cause of contamination in grape berries. This contamination is strongly related to climatic conditions, geographical regions, grape varieties, damage by insects, growing season; in particular great variations may occur from one year to another. So, climate represents an important key-factor in the agro-ecosystem, influencing fungal colonization and ochratoxin A production in grapes. Climate change is expected to have a profound effect on our landscape worldwide, and also to have an important impact on sustainable food production system. Recent studies have reported how the climate change may affect mycotoxins production in the fields and the relevant risk on economically important crops. In this regard, the interacting effect of water stress (a_w 0.99-0.93) and different day/night climate conditions simulating nowadays (18-31 and 15-28°C) and climate change scenarios (18-34 and 20-37 °C) in high OTA risk area of southern Italy during the ripening season, were studied. Mycelial growth rate, OTA production and molecular expression of key genes (PKS, NRPS, Hal, p450, bZIP) of OTA biosynthetic cluster by *A. carbonarius* ITEM 5010 were measured. Our results showed that, in water stress conditions (0.93 a_w), no

OTA production was observed and, except at 20-37°C, the growth rate was slower compared to 0.99 a_w. A significantly higher amount of OTA was observed at 0.99 a_w and 18-34°C climate change scenario. Gene expression, monitored by quantitative real time RT-PCR, gave evidence of the high expression levels of OTA biosynthetic genes in this condition, in particular NRPS and Hal genes were strongly expressed. These preliminary and new results on *A. carbonarius* in a climate change scenario suggest that a possible slight increase of temperature may lead to higher OTA contamination and to a possible expansion of the risk area in the Mediterranean basin.

3.1-5 Biosynthesis, characterization and anti-lung cancer activity of [60]fullerene nanoparticles using an endophytic fungal extract of *Penicillium simplicissimum*

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Abstract: In the present work, PsFNPs were prepared using a simple bioreduction method. It is economically and environmentally safe. Methanol extract of endophytic fungi, *P. simplicissimum* are used as bioreducing agent in the study. The formation of [60]fullerene nanoparticles was confirmed by UV-visible spectrophometer and characterization of nanoparticles by using FTIR, SEM, XRD and EDS. The *P. simplicissimum* extracts exhibited biologically important phytochemicals viz., alkaloids, tannins, saponins, carbohydrates, flavonoids, terpenoids, phenol and anthraquinones. The PsFNPs have shown significant anticancer activity on lung cancer cell line H1975 through cytotoxicity, increasing caspase-3,7, 8 and 9 activity and reduced expression of COX-2 activity significantly.

3.1-6 Thioredoxins and their allies in the foodborne yeast *Debaryomyces hansenii*

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Abstract: *Debaryomyces hansenii* (Debaryomycetaceae, Saccharomycetales) is a halophilic yeast commonly isolated from cheeses and fermented meats, to which it may be added as a culture organism. A close relative of human commensal and pathogenic *Candida* yeasts, *D. hansenii* does not itself grow at 37 C but multiple strains show killer activity against *Candida albicans* and *C. tropicalis*. In our efforts to identify the killer protein(s) we isolated and purified a single protein of approximately 11 kDa from cell-free culture supernatant using ultrafiltration and successive anion exchange and size exclusion chromatography, and identified the protein as a predicted thioredoxin by tandem mass spectrometry and comparison with the *D. hansenii* genome. We have expressed the gene in *E. coli* and are currently working to understand its mode of action.

3.1-7 Studies on *Cordyceps guangdongensis*, an edible and medicinal fungus with broad application prospects

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Abstract: *Cordyceps guangdongensis* T. H. Li, Q. Y. Lin and B. Song (Cordycipitaceae) was discovered in southern China and formally described in 2008. Biological characteristics, artificial cultivation, safety assessments, bioactive constituents and medicinal properties of the fungus were systematically studied by the authors in recent years. Through a series of tests for optimum conditions, biological studies

showed that *C. guangdongensis* fruitbodies could be domesticated as a cultivated fungus, and with suitable substrates, pH value, temperature, water content and light density. In the study of safety assessment, bacterial reverse mutation (Ames), bone marrow cell micronucleus, sperm aberration, teratogenic action, acute toxicity and 90 day oral toxicity experiments were conducted; and the analyses of the tests demonstrated that the fruitbody of *C. guangdongensis* did not have any mutagenic, clastogenic nor genotoxic effects. The maximal tolerance dose（MTD of the biomass in rats was greater than 20 g/kg bw. The no-observed-adverse-effect-level (NOAEL) was 5.33 g/kg bw according to the 90 day oral toxicity analysis. Therefore, the fruitbody of *C. guangdongensis* is considered safe for long term consumption. In 2013, it became the second novel food of *Cordyceps* approved by the Ministry of Public Health of China. In the study of physiological function evaluation, a series of animal tests were carried out. The researches indicated that the fruitbody of *C. guangdongensis* had antioxidant activities, anti-viral activity against the avian influenza virus H9N2, longevity-increasing activities, anti-fatigue effects, curative effects on chronic renal failure, and anti-inflammatory effects. Moreover, this fungus has rich bioactive compounds including cordycepic acid, adenosine, polysaccharides, and so on, many of them are similar to those in *Cordyceps sinensis* (Berk.) Sacc. The above results show that *C. guangdongensis* has a great potentiality for application in food and medical industries. Recently the whole genome of *C. guangdongensis* was also studied, being sequenced and assembled with Illumina and PacBio sequencing technology. The generated genome is 29.05 Mb in size, comprises 9 scaffolds with an average GC content of 57.01%, and is predicted to contain a total of 9150 protein-coding genes. The genome project provided a beneficial source of molecular information, and will lay a better foundation for elucidating the functional genes and exploring more bioactive components for further studies and application. This work was supported by Natural Science Foundation of Guangdong Province, China (2017GD1351), the Science and Technology Planning Project of Guangdong Province, China (No. 2015A030302052; 2016A030303035), and the Science and Technology Planning Project of Guangzhou, China (No. 201504291620324).

3.1-8 *Cordyceps* biodiversity and industrialization of *Cordyceps militaris*

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Abstract: *Cordyceps sensu lato* create tremendous economic value due to their medicinal, biological and nutritional importance. Hence, it is necessary to have further study on classification and identification of *this taxa*. *Cordyceps militaris* was approved as a functional food and Traditional Chinese Medicine owing to its significant pharmacological activity. cordycepin is one of the most important bioactive compounds produced by *Cordyceps*. However, large scale industrial production of cordycepin is still problematic. The objective of this study is classification of *Cordyceps s.l. and* optimization of large-scale production conditions for *C. militaris* and cordycepin. Furthermore, this study helps in industrialization of *C. militaris*. More than 50 species of *Cordyceps s.l. have been* identified from China, Thailand and Russia based on morphology and multi-gene phylogenetic analyses. Among them, 22 are new to the science. *Cordyceps militaris* has been used as a common mushroom in China and the market demand for artificial *C. militaris* has increased continuously. In the industrial application works, (1) the Liquid Static Culture: cordycepin production by three strains of *C. militaris*; using different working volumes and bioreactors. The best cordycepin production; 3005.83 mg/L was obtained in 5 L-flasks, containing 2 L medium, total cordycepin content reached 6011.66 mg/flask. The utilization ratio of adenine reached 91%; this the highest amount recorded in a single fermenter. (2) Large-Scale Culture Conditions: after using single factor design, Plackett-Burman design, and central composite design. Under the

optimization culture conditions, a maximum production of cordycepin was 2008.48 mg/L for 700 mL working volume in the 1000 mL glass jars and total content of cordycepin reached 1405.94 mg/bottle. (3) Solid-state fermentation for fruit body and cordycepin production: the optimization strategies in solid medium culture lead to a fruit body yield increased 67.96% (about 1.73 g/bottle) and cordycepin yield in fruit body increased 55.36% (0.87%). Larger particle size of rice in the medium offers better fruit body growth, and the cordycepin production prefers smaller particle size. (4) Solid-state fermentation only for cordycepin production: medium components glucose, peptone, adenine and histidine have been examined. The levels of variables for CCD experiments were selected according to the above results of the One-factor-at-a-time method; maximum response of 18.92 mg/g cordycepin at levels of glucose 26.25 g/L, peptone 26.25 g/L, adenine 7.50 g/L, and histidine 4.50 g/L as optimized medium components. This is the first report for improving the cordycepin production by using additives in this method. Isolates from colony sector mutation could be used for screening high-yield strains in cordycepin production and colony colour is one of the markers to detect fruit body and cordycepin production. (5) The separation and purification process of cordycepin: this optimization strategy leads to crystal of 60.45 g with ratio of pure 98.05%, and the recovery rate is 43%. Our production line capacity is about 200 kg/year. This study enriches the biodiversity of *Cordyceps s.l*. This method provides an effective way for increasing the *C. militaris* fruit body and cordycepin production at a large scale in order to improve industrial applications.

3.1-17 New insights into *Nowakowskiella* genus (Chytridiomycota)

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Abstract: The genus *Nowakowskiella* was proposed by J. Schröt. in 1893 to include the type species *Nowakowskiella elegans*, previously named as *Cladochytrium elegans* Nowak. In the following years, several species were described and currently this genus contains 18 legitimate species, which are morphologically recognized by the production of polycentric thalli with non-septate swellings besides operculated zoosporangia. Although this genus represents one of the most numerous in number of species within Cladochytriales, the most recent species, *Nowakowskiella keratinophila* Hassan and Batko, was described in 1988 and since then this group have not been undergoing modifications except by the proposition of the family Nowakowskiellaceae in 2009. Considering that, the aim of this study is to introduce a new species, *Nowakowskiella* sp. nov., clarify the phylogenetic positioning of *Nowakowskiella elongata* Karling and pointed another potential new species of *Nowakowskiella*. Besides that, we will include *Nowakowskiella multispora* Karling and *Nowakowskiella ramosa* Butler for the first time in phylogenetic reconstructions. The strains were isolated during two studies developed in aquatic ecosystems (streams and reservoirs) located in different fragments of Atlantic rainforest at Sao Paulo State, Brazil. Their SSU and LSU regions of rDNA were amplified with specific primers and a concatenate tree was build using Garli software package. The new species, *Nowakowskiella* sp. nov., is characterized by production of operculated zoosporangia with a prominent apophysis and crenulated resting spores. The second taxa, *Nowakowskiella* sp.1, could also represent a new species which produce apophysis in both sides of zoosporangia and a small tube during the zoospores releasing, however, we were not able to observe the resting spore production to confirm this supposition. Our Maximum Likelihood analysis showed that *Nowakowskiella ramosa* is sister group of the type species (*N. elegans*) and *Nowakowskiella* sp. nov. is related to our potential new species (*Nowakowskiella* sp.1). *Nowakowskiella elongata* belongs to another group outside of Nowakowskiellaceae and it is related to *Nephrochytrium* sp. JEL125, but seems to represent a new genus. These results bring important information about the taxonomy and molecular relationship inside of *Nowakowskiella* genus besides to contribute to increase the knowledge about the phylogenetic data of South America isolates. Financial support: FAPESP/CAPES/CNPq

3.1-18 Diversity within a single Peronosporaceae sample and implications for phylogenetic studies.

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Abstract: Downy mildews are plant diseases caused by a diverse group of diploid organisms in the Oomycota (Peronosporaceae). Over 700 species cause downy mildew diseases. Some of these organisms are thought to affect a wide diversity of plants, but most well-described downy mildew species are delimited by host genus. Downy mildews typically produce foliar lesions but some species are also reported to cause stunting, mottling, and in extreme cases, complete defoliation. The obligate biotrophic nature of downy mildew organisms creates difficulties in maintaining strains of these pathogens for research. This is particularly true for newly emergent or under-studied species where ideal environmental conditions and propagation protocols have not been established. As a result, single-spored isolates are rarely used in molecular phylogenetic studies. Common practices for generating DNA sequences from downy mildew samples involve performing PCR on genomic DNA extracted from sporulating pathogen tissue scraped from the leaf surface, or from excised disease lesions. When nucleotide sequences are generated directly from amplicons via Sanger sequencing technology, forward and reverse sequences are used to generate a consensus sequence, and any conflicting peak calls and double peaks in the chromatogram are indicated with IUPAC nucleotide ambiguity codes. However, by eliminating steps to ensure a single isolate is evaluated in a phylogeny, conclusions may not fully capture diversity of the population in one sample, nor the potential for heterozygosity of nuclear markers. In the current study, diversity within individual samples of *Peronospora* infecting *Monarda didyma* and *Hyaloperonospora* infecting *Cleome* sp. plants was evaluated using two approaches. DNA was extracted from sporulating leaf lesions, and amplicons of ITS rDNA and *cox2* mitochondrial DNA markers were cloned to produce haplotypes, and 7 to 10 inserts were bi-directionally sequenced per sample. Amplicons from the same two markers were also sequenced to a high depth of coverage using next generation sequencing on an Illumina MiSeq to evaluate the level of diversity within individual samples. The resultant data demonstrated that the level of diversity within individual downy mildew samples has the potential to be much greater than what is traditionally captured using direct Sanger sequencing of potentially heterogeneous amplicons. These unaccounted for variant sequences may have substantial impacts on phylogenetic analyses and should be considered when making taxonomic decisions.

3.1-20 The lignin degrading systems of Polyporales

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Abstract: The Polyporales order of Basidiomycetes contains most of the known wood-rot fungi, effective degraders of lignocellulosic biomass. While many studies have previously examined examples of these fungi during growth on lignocellulose, measured the mineralization of labelled lignin and mapped the secretion of many carbohydrate- and lignin-active enzyme activities, little is known about their ability to grow directly on a pure lignin substrate and the enzymes directly induced by this polyaromatic material. Previously it had been considered that fungi could not grow well on lignin alone. Eleven species belonging to the Polyporaceae (10 strains) and Hypocreaceae (1 strain) families were obtained from the Centre International de Ressources Microbiennes-Champignons Filamenteux (CIRM-CF) culture collection (https://www6.inra.fr/cirm/Champignons-Filamenteux). Their selection was based on the

availability of a sequenced genome and prior knowledge relating to growth on lignosulphonate. The fungal strains were screened for their ability to grow on both agar plates and liquid media containing a commercial alkaline lignin from grasses, and for the production of ligninolytic activities, such as laccase and heme peroxidase. The strains showed differences in their growth rates in the presence of lignin, especially in liquid cultures, and also differences in the secretion of laccase and/or lignin peroxidase activities. Proteomic analysis of the secretomes produced by five of these eleven fungal strains during growth on the alkaline lignin will be presented and the different enzymatic mechanisms employed by filamentous fungi for the deconstruction of lignocellulosic biomasses, and in particular lignin, will be discussed.

3.1-22 Presence and abundance of nitrogen metabolism genes explain fungal community shifts and ecosystem-carbon dynamics in nitrogen enrichment field experiments

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Abstract: Nitrogen (N) deposition in terrestrial ecosystems is a strong driver of ecosystem-level carbon (C) dynamics. In field experiments, simulated N deposition results in accumulation of soil carbon, an increase in recalcitrant compounds, and a decrease in soil respiration. Because rates of N deposition are increasing globally due to anthropogenic activities, we must understand how ecosystem C dynamics will change in response. Fungi are key drivers of ecosystem C cycling and are highly susceptible to soil N enrichment. In addition, N-induced soil C accumulation is related to disruptions in organic matter decomposition due to disturbances to the fungal community. Simulated N deposition reduces fungal biomass, alters the activities of microbial extracellular enzymes, and restructures the fungal community. However, little is known about the evolutionary adaptation of fungi under N deposition that shapes ecosystem C dynamics. Our objective was to analyze genomes of fungi that have been reported in N amended soil to search for presence and abundance of genes associated with N metabolism. We hypothesized that fungi in N enriched plots would have higher abundance of N metabolism genes, compared to fungi observed in control soils, but a lower abundance of genes associated with decomposition of recalcitrant carbon. We predicted this tradeoff because higher abundance of N metabolism genes in fungi may be selected for and facilitate fungal survival at higher soil N concentrations at the expense of genes associated with an ability to decompose energetically expensive recalcitrant carbon compounds. To this end, we searched for genes associated with ammonium transporters, nitrate transporters, and amino acid permeases. We also searched for genes associated with carbon metabolism to gain a comprehensive understanding of the ecosystem-level observations of the fungal community and of the carbon dynamics under elevated N depositions. We found that on average, fungi from N amended plots had significantly more genes for uptake of organic N, as well as more genes to break down cellulose. Moreover, a higher percentage of fungi in N amended plots had more than one gene for uptake of inorganic N in comparison to fungi from control plots. Similarly, a higher percentage of fungi from N treatments had more than one gene for breakdown of labile carbon in comparison to controls. We also detected a shift in the taxonomical distribution of fungi. The order Agaricales, Polyporales, and Russulales decreased in response to N; whereas, Hypocreales, Pleosporales, and Pezizales increased. Concomitantly, we detected an increase in functional guilds associated to plant pathogens, an overall increase of saprotrophs, and a decrease of ectomycorrhizal fungi in response to N. Finally, we analyzed specific decomposition traits, such as white, soft or brown rot, and found that rot fungi are present under both control and N enriched conditions. However, there

was a slight increase in abundance of soft and white rot in response to N. We provide genome- and functional-level evidence for the response of fungi to simulated N deposition and suggest that N may serve as a selective force on fungal communities, driving changes in C dynamics at the ecosystem-level.

3.1-23 Differential gene expression linked to fungal trophic switches (symbiotrophism and saprotrophism) using the moss *Dicranum scoparium* and its associated fungi

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Abstract: While the plant holobiont concept is becoming more widely accepted, interactions between mosses and fungi remain scarcely explored. Yet, moss-fungi model systems offer advantages that are complementary to other plant-fungi experimental systems. Our goal is to understand moss-fungal interactions by integrating environmental samples and laboratory co-culture experiments. Many naturally occurring mosses have stratified growth forms with a photosynthetic layer, a senescent layer, and a decomposing layer. Therefore, these mosses offer a unique opportunity to study shifts in fungal communities and functions along a senescence gradient, simultaneously, i.e., without the confounding effect of time. We examined fungal communities along the senescence gradient of a perennial moss *Dicranum scoparium* using metatranscriptomic data and a culture-based endophyte isolation approach. By integrating the fungal rRNA sequences generated by metatranscriptomics (i.e., as a proxy for fungal activity) and culture-based approaches, we assigned fungi into three types of association categories: 1) High activity in photosynthetic tissues, 2) High activity in decomposing tissues and 3) Low activity throughout the gametophyte but frequently detected using a culture-based method. Using these three categories, seven fungal strains representing distinct fungal lineages (four Ascomycota, two Basidiomycota, one Mortierellomycotina) were selected to establish fungus-plant re-synthesis experiments. We included both the axenic living and dead moss gametophytes for the re-syntheses. The former enabled us to investigate plant-fungus interactions; the latter allowed us to examine fungal functional switches when inhabiting living vs. dead plant tissues. For every fungus-plant pair, growth rates were monitored for two months after fungal mycelia reached the mosses. Our results showed that various fungal structures were produced on the inoculated moss hosts. However, none of these tested strains caused significant moss growth losses. Subsequently, we selected three fungi potentially representing different ecological guilds and we obtained metatranscriptomes for these three moss-fungal pairs. The three fungal strains selected were: 1) a *Coniochaeta* strain closely related to fungal endophytes from other plant lineages, 2) an unknown strain from the class Leotiomycetes closely related to ericoid mycorrhizal fungi, and 3) a *Rickenella fibula* strain producing fruit-body directly on *D. scoparium*. Based on our metatranscriptomic data, mosses inoculated with fungi had genes related to plant defense (e.g. leucine rich repeat receptor, chitinase) and nutrient transportation (e.g. phosphate transporter) upregulated. We also compared GO (Gene Ontology) categories enriched for fungi growing in living plant tissues versus in dead plant tissues. The former scenario was enriched for genes related to carbohydrate transport, while the latter was enriched for genes responsible for oxidation-reduction process, suggesting functional switches of fungi between symbiotrophy and saprotrophy. Our study sheds light on the importance of understudied plant-fungal systems, and provides evidence for functional lability of individual fungal strains in response to host senescence.

3.1-24 A metatranscriptomic key to open the black box of fungal process in forest soil

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Abstract: Boreal forests represent a large carbon sink; indeed, this ecosystem represent 32% of the global carbon store on Earth. Due to the intensive human activity (e.g. forestry, clear-cutting, land use change, fertilization) occurring today in a vast majority of boreal forest, it is essential to advance our understanding of soil processes, to enable informed future policy decisions about forest management and ensure a maintained, and preferably increased, carbon sink. Soil microbial communities play a central role in regulation of soil organic matter dynamics in forest ecosystems and are therefore subject to particular attention. In boreal forest soils, fungi are the main microbial group in term of biomass, and mycorrhizal fungi, living in symbiosis with plant roots, are particularly important in regulating carbon sequestration. New innovative molecular techniques (metatranscriptomics) allow us to open the black box of microbial process in soils, by obtaining massive data of expressed genes from entire microbial communities, and investigate how microorganisms regulate soil organic matter dynamics, directly in the ecosystem. The objective of this study is to develop an approach to highlight fungal functional traits related to soil fungal community ecology. Specifically, to evaluate the mechanisms relative to carbon storage under variation in ecosystem fertility and production. In this purpose, the role of fungal community in carbon transformation, during decomposition and microbial metabolism, is assessing by analyzing expression of genes involved in the production of enzymes implicated in organic matter degradation, stress tolerance and intracellular CO_2 release, at the ecosystem level. The studied site is a native boreal forest presenting a clear split between an N-poor low productive and an N-rich high productive plot. RNAs have been extracted from soil of 16 plots and rRNAs have been removed for a massive Illumina HiSeq sequencing (more than 100 million reads per sample). To decrease the calculation time during bioinformatic analysis and focus on relevant ecological questions, we develop a targeted markers assembly using the HMMER software. Publically available reference sequence databases (e.g. CAZy or JGI) are used to guide transcript filtering and identification of expressing organisms. This study demonstrates the efficiency of the developed approach, to select specific gene family relative to functional traits, from a raw metatranscriptomic dataset. Moreover, the primary results reveal differences in fungal lifestyle strategies, according to forest fertility and productivity, identified by using expressed genes as marker for fungal process such as decomposition (eg. lignocelluolytic enzymes), biomass production (eg. 1,3-β-glucan synthase), stress tolerance (eg. melanin synthesis) and respiration. To conclude, the purpose of this pioneer approach is to provide novel insights about the interplay between microbial traits, such as decomposer capacity and metabolic efficiency, and ecosystem level processes, such as carbon sequestration, in order to increase the predictive capacity of ecosystem models.

3.1-33 Chitin synthase inhibitors from soil fungi

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Abstract: The fungi are ubiquitous group of Eukaryotes that occur in multiplicity of natural and manmade environment. Isolating microorganisms from the environment is the first step in screening for natural products such as, secondary metabolites and antibiotics. Fungal population in soil usually lies numerically between bacteria and actinomycetes. Therefore, the present studies aimed to exploit the available soil fungal flora of the Hyderabad Karnataka Region (HKR) of India for the production of novel

antifungal molecules, which could inhibit chitin synthase activity in fungal cell wall. Chitin could be the target molecules used in the development of antifungal drugs as it is an important component of fungal cell wall and absent in human cell. A total of 53 fungal strains were isolated from various soil samples collected from different locations using PDA medium and screened them for antimicrobial activity against 12 strains of Candida. Among the fungal isolates, two isolates namely, VSGUF1 and VSGUF 2 were found inhibitory. The extracellular and intracellular crude extracts showed a maximum of 17mm inhibition zone against C. albicans1637. Antifungal susceptibility assay of VSGUF1 and VSGUF 2 performed in RPMI 1640 medium against different strains of Candida after 36 h showed MIC90 at 128µg/ml and 32µg/ml against C. glabarata, respectively. In vitro chitin synthase activity indicated 100% inhibition of Candida albicans ATCC 24433 in intracellular crude extract of VSGUF1 at 64 µg/ml. The biomolecules produced by VSGUF1 and VSGUF2 are the potent molecules could be used in the development of new antifungal drug for Candida infections. Further studies are in progress.

3.1-34 Bioprospecting for chitinases among bacteria in Puerto Rico

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Abstract: Chitinases are enzymes responsible for modification of chitin. A variety of chitinases has been isolated from marine bacteria, as many of them live close to chitin-containing invertebrates. Chitinases have been proposed as antifungal alternative since it distorts the chitin-containing cell wall of the fungi. Our objective has been to determine the chitinolytic activity of bacteria capable of degrading organic pollutants or expressing antibacterial activity. A collection of bacterial strains recently isolated from diverse ecosystems in Puerto Rico was subjected to chitinolytic assays. These strains have demonstrated capabilities to degrade xylene or inhibits bacteria growth. Twenty of them were cultivated in mineral media containing chitin (~0.2% w/v) as sole carbon sole. Incubation proceeded at room temperature on an orbital shaker (130 rpm). After four days, minor turbidity was noted on the liquid enrichment. An aliquot (~100 ml) was spun to concentrate cells for microscopic examination. Gram staining resulted on three Gram-negative rods and twenty-one Gram-positive (eleventh rods and nine coccus) among the putative chitin-degrading bacteria. Among them, strains of *Alcaligenes faecalis*, *Pseudomonas* sp., and *Achromobacter* sp. were represented. Sequencing of their 16S rDNA and specific antifungal assays are in progress. This project contributes additional diverse strains with potential for fungal control based on chitinolytic activity.

3.1-35 Reduction of proteolytic activity in filamentous fungal protein production hosts

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Abstract: Fungal host strains such as *Aspergillus niger*, *Aspergillus sojae* and *Trichoderma reesei* are used for the production of a wide variety of industrially relevant enzymes. About 80% of all industrial enzymes are derived from filamentous fungi, making these organisms also the hosts of choice for the production of new enzymes and proteins. The presence of unwanted proteolytic activity in filamentous fungi is a major bottleneck for (heterologous) protein production. Despite the accumulation of data by all kinds of -omics techniques, the regulation of protease genes in fungi remains largely unexplored. Dutch DNA Biotech is working on several approaches to tackle this problem in industrial fungal strains. All these approaches are focused on identifying regulatory genes specific for the pathways underlying protease production. Based on strain engineering, using both classical screening and targeted

molecular approaches, regulatory mutants are being developed. The research presented here is focused on three methods to find relevant factors that are involved in the regulation of proteolytic genes. The first method is a positive screenings method for protease mutants, we named SUI selection. SUI selection is a growth-based screening method and is much more efficient than activity-based screening methods. The second method is the screening of a transcription factor knock out library in *Aspergillus niger* and *Neurospora crassa* for mutants with a lower proteolytic activity. The third method involves a multicopy suppressor approach based on strains carrying multiple copies of the major *A. niger* protease regulator gene, *prtT*.

3.1-36 Xylariaceae volatiles and their applications in agriculture

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Abstract: The Xylariaceae is one of the largest families within the fungal kingdom. The majority of the 800 identified species are commonly classified as saprobes, with many isolated as endophytes. The family is one of the best studied fungal taxa based on morphological, molecular and chemotaxonomic data. Members of the Xylariaceae are recognised for producing a wide variety of secondary metabolites such as pigments, volatiles and biocidal compounds, many of which have demonstrated bioactivity against important agricultural pests and pathogens. Current agricultural practices rely heavily on the use of agrochemicals for crop protection before and after harvest, however their sustainability is constantly under question. Bioactive secondary metabolites from Xylariaceae fungi represent promising candidates as novel "green" agrochemicals, that are (1) beneficial in breaking current and emergent resistances, (2) less prone to induce toxic side-effects on human and animal health, (3) and are more environmentally sustainable. Using Gas Chromatography – Mass Spectrometry, we profiled the volatolome of various native Australian fungal isolates of the Xylariaceae genera *Nodulisporium (Hypoxylon), Muscodor*, *Daldinia* and *Xylaria*. We identified a number of VOCs and investigated their biocidal properties, synergistic effects and modes of action against common pre- and postharvest pests and pathogens. Here, we present an overview of common and species specific Xylariaceae volatiles, their properties, mode of action, as well as their potential application, environmental fate and use as stored grain disinfestants, soil fumigants and food-grade disinfectants.

3.1-37 Chitinolytic efficacy and secretion of cell wall degrading enzymes from *Trichoderma* spp. in response to phyto-pathological fungi

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Abstract: Chitinolytic activity and major antifungal enzyme secretion form *Trichoderma* spp. was studied. Soil samples were collected from different environmental niche of North Gujarat Region, India and 12 different species of *Trichoderma* were obtained and identified. Among 12 isolates, 4 isolates were identified as *T. harzianum*, 5 isolates were identified as *T. viride* and remaining 3 isolates were as of *T. hamantum*. These isolates were identified by using species specific primers amplification by PCR. All identified isolates were screened for chitinase activity using colloidal chitin derived from commercial chitin on the media supplemented with bromocresol purple. According to results of chitinase activity screening assay, *T. viride* was found to be more potential isolate for chitinase production. From

biocontrol assay by using duel culture method, *T. viride* was found to be more potent antagonist against fungal plant pathogens like *A. niger*, *F. oxysporum* and *S. rolfsii*. *T. viride* was selected for further study of biocontrol potential and production of cell wall degrading enzymes. *T. viride* was inoculated in media containing basal media and mycelia of fungal pathogens for cell wall degrading enzyme production. It was found that *T. viride* secretes three major cell wall degrading enzymes i.e. chitinase, protease and β-Glucanase. Optimum production of all three enzymes was found at 96 hr incubation. Details of antifungal protein secretion are mentioned in this paper.

3.1-39 The *Thkel1* gene of *Trichoderma harzianum* plays a key role in its ability to colonize the roots of *Arabidopsis thaliana*

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Abstract: *Trichoderma* is a genus of filamentous fungi widely studied and used as a biocontrol agent in agriculture due to different mechanisms of action, such as its ability to parasitize and inhibit the growth of phytopathogenic fungi, promote plant growth and improve the response of the plant against both biotic and abiotic stresses. The *Thkel1* gene of *T. harzianum* encodes a protein with kelch domains involved in protein- protein interactions. Expression of *Thkel1* in *Arabidopsis thaliana* enhanced plant tolerance to salt and osmotic stresses, accompanied by an increase in β-glucosidase activity. The aim of the present work was to analyze the role of this gene in *Trichoderma*-plant root interaction, using *Trichoderma* transformants silenced in *Thkel1* and *Arabidopsis* plants that overexpressed this gene. We observed that root colonization ability of *Arabidopsis* wild type plants was dramatically reduced in *Trichoderma* silenced transformants whereas this ability was restored in *Thkel1* overexpressing plants. Similar results were observed in canola (*Brassica napus*) plants. On the other hand, *Thkel1* gene was not relevant in *Trichoderma* root colonization of tomato plants. These results suggest that the *Thkel1* gene can play a crucial role in root colonization of Brassicaceae plants.

3.1-40 Role of fungal volatiles produced by *Trichoderma* on plant growth

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Abstract: Microbial volatile organic compounds (VOCs) play important roles in plants influencing their physiology and development. *Trichoderma* is well known for the production of signal molecules that influence the growth of plants and other fungi. The objective of this research was to compare the effect of different *Trichoderma* species obtained from soil and surface sterilized roots. Fungi were isolated across the USA and *Trichoderma* strains were identified using ITS rRNA, and tested on the dominant arid grass, *Bouteloua gracilis* (blue grama) in a closed chamber experiment. Volatiles emitted by different species of *Trichoderma* exhibited a wide range of effects on plant growth and development. *Trichoderma gamsii* (CK71) and *Trichoderma saturi* (CK1108) showed the greatest growth promoting abilities in *B. gracilis*, with a significant increase on seed germination, plant size, and root development compared to the controls. *Trichoderma* strains were also tested in direct contact germination experiments. The association of the fungi with plant roots was analyzed using microscopy. *B. gracilis* seeds inoculated with *Trichoderma* strains showed an increased root length and proliferation of lateral roots compared to the controls. Microscopy examination of stained roots revealed small round fungal-

like structures in cortex and intercellular hyphal growth. *Trichoderma* high abundance in soils across multiple ecosystems as demonstrated by Illumina sequencing and culturing methods showed important ecological functions of these fungi as regulators of plant growth through multiple mechanisms. Future research will be conducted to evaluate factors that influence *Trichoderma*-plant interactions using different growth conditions (e.g. temperature and media).

3.1-57 Isolation and metabarcoding of aquatic mycodiversity in lakes along a humic substance gradient

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Abstract: Fungi in aquatic environments degrade organic matter and thus transfer nutrients to other trophic levels. This phylogenetically heterogeneous group of fungi is widespread and well adapted to life in aquatic habitats. However, biodiversity assessments and living fungal cultures from lakes are both scarce. We investigated the fungal diversity in three different lakes which cover a gradient in organic carbon (OC) content and quality. Cultivation and meta-genomic barcoding were employed to study freshwater fungi in littoral water, fine particulate organic matter (FPOM), sediment and coarse organic matter (CPOM) such as leaves and wood. Furthermore, isolates were tested for the ability of humic matter degradation and for submerged growth in water. Three isolation methods applied to four substrates collected from three lakes yielded 262 cultures. CPOM showed the highest fungal diversity among analyzed substrates. Compared to other cultivation approaches, multi-well cultivation methods were least successful. The taxonomic diversity was dominated by ascomycete species, most of which are known as plant pathogens or saprobes. Overall, our study yielded 20 potential new species which were able to grow and sporulate submerged in water. The production of laccases and peroxidases was observed in 25 % of all isolated species. The metabarcoding approach of the natural samples yielded 90,772 ITS2 sequences; the highest numbers were obtained from littoral water samples. The taxonomic assignment of Operational Taxonomic Units (OTU) was made employing several common pipelines and, additionally, manually curated. The final dataset contained 572 OTUs of which 74.5 % were classified on species level. Similar to the cultivation approach, metabarcoding detected many species belonging to Ascomycota and Basidiomycota but also Cryptomycota, Chytridiomycota, Zoopagomycota and Neocallimastigomycotina. The diversity of fungal communities was highly variable among lakes and substrates. Community structure differed along the environmental factors; particularly the quality of carbon (humic or not) and the form of available nitrogen were important factors. Most species were associated with a particular lake and substrate type. About 30 % of the species occurred more frequently across the habitats. Sequences of 90 % of the cultivated isolates were also retrieved by metabarcoding and most occurred in more than one metabarcoding sample. Substrates but not lakes explained the occurrence of cultivated fungi, while metabarcoding suggests the distribution of differing fungal communities according to the type of substrate and lake more clearly.

3.1-58 Metabolism of fungal species isolated from the coast off central Chile: the role of respiration and nutrient assimilation processes

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Abstract: Filamentous marine fungi have recently been identified as a functional component of coastal systems, playing an important role in the degradation of organic matter and hydrolysis of large

polymers. However, the ecophysiological and biogeochemical role of these fungi has been scarcely explored. This work seeks to determine the effect of temperature and nutrient availability (such as glucose) on respiration and growth of filamentous fungi, as well as to characterize the use of carbon, nitrogen, phosphorus and sulfur substrates by these organisms. Respiration and growth rates were determined for 5 species of filamentous fungi (*Penicillium decumbens, Penicillium chrysogenum, Sarocladium strictum, Fusarium fujikuroi* and *Fusarium sporotrichioides*) isolated from the coastal upwelling zone, so as to examine the effects of temperature and nutrient availability (such as glucose). Growth was monitored via epifluorescence microscopy, ATP concentrations and Optical Density; while oxygen consumption was recorded using a respirometer with "Optodes". Although responses were species specific, generally respiration and growth increased with temperature and glucose concentration. Growth of *P. decumbens, F. sporotrichioides* and *F. fujikuroi* were most favored by when glucose concentrations remained stable. Substrate use profiles for carbon, nitrogen, phosphorus and sulfur were obtained for three species (*P. decumbens, S. strictum* and *F. fujikuroi*). In order to understand their potential impact on the degradation of carbon compounds, their carbon profiles were characterized using Biolog Filamentous Fungi (FF) MicroPlates. These species were found to be versatile, with a large capacity (57.2 % of total) for using a wide range of carbon sources, principally carbohydrates (monosaccharides, disaccharides, oligosaccharides and polysaccharides), but also amino acids (0.99 %), suggesting the use of metabolic pathways, such as glycolysis /gluconeogenesis. These species also displayed high indices of substrate use complementary to nitrogen, phosphorus and sulfur, where organic components accounted for greater hyphal growth. Here, L-amino acids, amines and nucleotides/nucleosides were preferential sources of nitrogen, suggesting the use of pathways involved in the amino acids and/or purine metabolism. Other compounds, such as urea, allantoin and uric acid produced moderate growth. The ribonucleotides adenosine and guanosine were the main substrate for phosphorus use; and cysteine and methionine were the main sources of sulfur for growth in these species. Moreover, growth was observed with several inorganic substrate sources, such as nitrate, nitrite, thiophosphates, tetrathionate, in the three species. Considering the active heterotrophic role of filamentous fungi, their importance in the degradation of organic matter and their participation in important biogeochemical cycles in the ocean, we suggest the inclusion of the mycoplanktonic community as an integral component of the microbial community in the coastal ocean, to be included in microbial conceptual models of degradation of organic matter and biogeochemical cycles given their use of a wide variety of organic and inorganic carbon, nitrogen, phosphorus and sulfur compounds.

3.1-59 Fungi diversity and enzyme activity associated with sailfin sandfish egg masses in Korea

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Abstract: The aggregation of decaying egg masses of sailfin sandfish along the mid-east coast of Korea is a major environmental problem with concurrent negative economic consequences. In an effort to ameliorate decaying egg masses, we investigated the diversity and community structure of fungi from egg masses and tested for their cellulase and protease activity. A total of 1,108 strains were identified based on morphology and multigene analyses, and found to represent 184 fungal species. *Paradendryphiella salina* was the most dominant species, followed by *Penicillium crustosum* and *Penicillium aurantioviolaceum*. The fungal community displayed a significant degree of variation relative to both egg mass color and locality. Over 50 % of species detected in this study exhibited both cellulase and protease activity. This study suggests that fungi play an important role in nutrient recycling at

intertidal zones and thus may have potential industrial applications that can help resolve the environmental problems associated with egg mass aggregation.

3.1-60 Analysis of coral-associated fungal and microbial communities in Fiji using high throughput amplicon sequencing

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Abstract: Coral reefs are biological hot-spots on Earth and provide important habitats for a wide variety of marine species. For this reason, sections of many reefs have been the targets of conservation projects that aim to protect coral and other species that make up the reef ecosystem. Microbial communities make up an integral part of a healthy reef ecosystem, and therefore must be considered when assessing the success of conservation efforts. To this end, corals were sampled from four different genera: Porites, Montipora, Acropora, and Sinularia. Sampling occurred in two adjacent shallow reef areas: 1) a no-take marine protected area with relatively low disturbance, and 2) a fished area with relatively high disturbance from fishing pressure and watershed discharge. These samples were then analyzed for communities of Fungi, Bacteria, and the endosymbiotic dinoflagellate, Symbiodinium. Microbial communities were targeted using primers specific to the Fungal ITS-1 region, the Bacterial 16s region, and the Symbiodinium ITS-2 rDNA region. Amplicons from these primers were then tagged with barcodes and pooled into libraries for sequencing on an Illumina' Miseq platform. Sequence data were then run through a non-biased bioinformatic pipeline and the unoise algorithm in usearch to cluster OTUs for each group by a single nucleotide difference in sequence. The initial analysis on the entire sample set for Fungi recovered a broad diversity of species dominated by Ascomycota (65%) and Basidiomycota (33%). The Ascomycetes were dominated by Cladosporiaceae (15%) and Aspergillacea (10%); while the Basidiomycetes were predominantly Malasseziaceae (45%) and Nectriaceae (15%). The bacterial communities were predominantly Cyanobacteria (51%), Proteobacteria (37%), and Bacteroidetes (8%). Within the bacterial communities, the Cyanobacteria were dominated by Ulvophyceae (84%), Proteobacteria were dominated by Endozoicimonaceae (36%), Bacteroidetes were most highly represented by Chitinophagacae (30%). Symbiodinium communities were dominated by clade C (96%), but clades D (3%), as well as A and G were also present at low levels. This preliminary data on the sample set shows diverse microbial communities and lays the framework for comparing the microbiomes of corals in protected and unprotected areas.

3.1-61 Marine fungi from India: Range of substrata

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Abstract: The present paper deals with distribution and substratum range of 217 species of marine fungi (14 Labyrinthulomycetes, 4 Chytridiomycetes, 4 Oomycetes, 143 Ascomycetes, 3 Basidiomycetes and 46 Mitosporic / Asexual fungi) reported so far from the marine waters of India. These fungi were reported as parasites / saprophytes on Animal substrates (16 sp.), saprophytic on intertidal and deep sea sediments (7 sp.), on intertidal woody debris (131 sp.), on decaying algae (17 sp.), saprophytic on salt marsh plants (3 sp.), saprophytic on sea grasses (4 sp.), saprophytic on woody debris of mangroves from intertidal region (165 sp.). Ascospores and conidia of 27 species were recorded in foam samples from sandy beaches. Fungal species (165 sp.) recorded on mangrove substrata forms the largest group after intertidal woody debris (131 sp.). It also shows that most of the fungal species have been recorded from

the West coast (154 sp.) after the East coast (152 sp.), Andaman-Nocobar Islands (66 sp.) and Lakshadweep Islands (55 sp.). Maximum number of marine fungi were encountered along the coast of Tamil Nadu state (106 sp.) and followed by Karnataka (100 sp.), Maharashtra (95 sp.), Goa (98 sp.), Kerala (93 sp.), Andhra Pradesh (73 sp.), Gujarat (70 sp.), West Bengal (69 sp.), Orissa (54 sp.), Pondecherry-Mahe (46 sp.), Daman (17 sp.), Diu Island (14 sp.) and Pondecherry (12 sp.). This data will be useful in the compilation of marine fungal biodiversity of India. The taxonomy, morphology and ecology of these fungi are discussed.

3.1-62 Structure of halophilic fungi within hypersaline environments

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Abstract: Very little work has been performed to thoroughly inventory halophilic fungi within hypersaline environments. Although it is known that halophilic fungi live within hypersaline environments, this study will use the Great Salt Lake in Utah due to its extreme salinity gradient to create an inventory of species. By using ITS amplicon sequencing of fifty water column and sediment samples, this study is determining how salinity shapes fungal community structures and examining whether or not there is a "tipping point" in which salinity is the main limit on fungal diversity. These results will be beneficial to understanding the ecosystem dynamics of The Great Salt Lake.

3.1-63 Marine filamentous fungi from Sweden

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Abstract: Marine filamentous fungi have been little studied in Sweden, which is remarkable given the depth and width of mycological studies in the country since the time of Elias Fries. A summary of historical records along with numerous additions is given in a commented list of the marine filamentous fungi so far recorded from Sweden. New records for the country are based on morphological identification of species mainly from marine wood, most of them originating from the Swedish west coast. Identifications have been obtained by morphological studies, where spore structure frequently is a diagnostic character, and also by cultivation of the fungus followed by DNA isolation and amplification, mostly of the ITS region and comparisons with sequences in GenBank. A total of 67 filamentous marine fungi were recognized as occurring in Sweden based on a critical assessment of historical records, and additions contributed during field-work for the past two years. This is a substantial increase of earlier assessments (31 by Henningsson in 1974; 31 species of marine ascomycetes by Eriksson in 2014; and an additional seven by Tibell in 2016). Thirteen species are recorded as new to Sweden, whereas fifteen old records, some of which only identified to genus, were for different reasons not accepted in the present list. The number of species listed is a rather modest and this certainly reflects the fact that marine fungal diversity is poorly known and that the species so far described only represent a small fraction of their diversity. Most of the records were obtained from marine wood undoubtedly leaving a rich diversity occurring on marine algae and plants unaccounted for. The study is part of a project for assessing the diversity of marine fungi of Sweden supported by The Swedish Species Initiative.

3.1-64 Fungi are abundant and uncharacterized members of Arctic marine ecosystems

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Abstract: Fungi are among the most diverse groups of organisms in the world that interface essential nutrient cycling and carbon degradation process in the biosphere. The oceans cover 70% of the world's surface; however, the unknown contributions of fungi to ecosystem processes remains a gap in marine ecology. We used molecular sequencing data generated from the Arctic Ocean to assess the diversity, regional abundances, and functional gene potential of marine fungi. We found the Arctic Ocean is comprised of a diverse fungal community that is predominated by the Chytridiomycota. Phylogenetic analysis of 28S rRNA clone data from sea ice revealed a previously undescribed clade of Chytridiomycota that branches sister to the taxonomic order Lobulomycetales. This clade was detected across the entire Arctic Ocean, underscoring the wide-distribution of Arctic marine fungi, especially the Chytridiomycota. To supplement these analyses, we used a GeoChip microarray to screen for known functional genes involved in biogeochemical cycling. We detected a suit of catalytic genes allied to the fungi that interface nitrogen cycling and carbon degradation. Ultimately, out data continue to help establish an ecological paradigm for marine fungi that remain to be fully integrated into marine sciences.

3.1-73 After the Fire: Fungi and wildfires in the Great Smoky Mountains National Park

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Abstract: The effects of wildfires on fungi are not well documented in eastern deciduous forests and likely depend on intensity of the fire. After the November 2016 wildfires in the Great Smoky Mountains National Park (GSMNP), we undertook a survey of above-ground fungi fruiting in lightly burned, moderately burned and heavily burned areas over a one-year period (January of 2017 through December of 2017). Consistent with studies in the western United States, a number of fire-response fungi were collected. Many had not previously been documented in the GSMNP including *Pyronema omphalodes*, *Anthracobia* (4 putative species), *Pholiota highlandensis*, *Morchella exuberans*, *Peziza echinospora*, *Rhizina undulata* and *Geopyxis carbonaria*. An unexpected mass fruiting of *Hygrocybe conica* was observed in high intensity fire zones in mid-summer. Germinating pine seedlings in high intensity fire zones formed mycorrhizal associations very early in development consisting of a single species, often *Telephora/Tomentella* but by mid-summer, many developing pine seedlings had more than one mycorrhizal species. Forty-eight new GSMNP taxa were identified. At least five are new to science.

3.1-74 Compositional and functional responses of soil and litter fungal communities to prescribed fires in pine savannas of North America

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Abstract: Wildfires threaten many ecosystems, but frequent fires are essential to sustain and renew pine savannas ecosystems. In these threatened ecosystems, repeated fires suppress hardwoods and result in

one of the most biodiverse plant communities on Earth, filled with fire-adapted plants. Longleaf pines that dominate these communities, for example, have bark adaptations to resist fire and produce flammable needles that increase the probability and severity of future fires. In contrast, very little research has explored fire effects on the fungal communities that are both biodiverse and important decomposers of the fuels that pines and other plants produce. In a system adapted to recurrent fire, we know almost nothing about how a new fire might shift fungal communities, and in particular if it eliminates or promotes particular taxa or functional groups of fungi, some of which may be relevant to fuels. Given frequent prescribed fires, are fungal communities relatively immune to a new fire? Do any fire effects on fungi lessen from litter to soils, as soils insulate fungi from heating? To assess these questions, we used DNA-metabarcoding (Illumina MiSeq or ITS2 region) to assess and compare communities of soil and litter fungi following fires in a well-preserved North American pine savanna (The Wade Tract, Thomasville, GA, USA). We compared communities from 56 pairs of plots either experimentally burned or left unburned for at least 1 year. Within each plot we also estimated fungal abundance using digital droplet PCR, collected several different metrics of soil properties, and measured microbial decomposition in a parallel experiment. Despite all sites historically experiencing frequent fires, recent fire strongly altered fungal community composition and reduced fungal biomass. Shifts were stronger in litter (25% of variation in communities explained by fire) compared to soils (10% explained by fire) as expected given pine litter's concentration of flammable compounds (resin) and the greater insulation for soil fungi. Burning strongly reduced the number of saprotrophic species, which paralleled slower litter decomposition in burned plots. Fire also effected specific taxonomic groups of fungi; Coniochaetales increased, but Ostropales, Botryopshaeriales and Lophiostoma (Pleosporales) strongly declined in species richness between burned and unburned plots. Most fungal lineages, however, showed no richness response to fire, including orders with the highest number of species - Pleosporales (22% of fungal OTUs), Agaricales (10%) and Chaetothyriales (7%). Across most taxa, regardless of lineage, OTU's occurred in either burned or unburned areas, with very few taxa present in both. Our data reveal a fire-adapted fungal diversity that can respond rapidly to fire. Rather than being a trait of a specific fungal lineage, fire is present for individual taxa across nearly all lineages, possibly as repeated adaptations to recurrent fires in pine savannas. Future work will address fire-induced changes in the expression of fungal genes involved in heat resistance and decomposition, to provide better understanding of fungal functional responses to prescribed burning in pine savannas.

3.1-75 Increase in the outdoor levels of fungal spores in San Juan, Puerto Rico in the aftermath of Hurricanes Irma and Maria

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Abstract: The effect of hurricanes on the calendars of fungal spores and pollens have not been studied. Hurricanes Irma and Maria hit Puerto Rico on September 6 and 20, 2017 respectively and allowed us to determine their impact on the San Juan, PR fungal spore and pollen calendars. In San Juan, PR peaks of fungal spores occur during April and May and from September through November. In contrast, tree pollens, the most abundant in PR, are present all year long but at lower levels during the summer (June-August). We use the Burkard air sample to take daily air samples on a glass slide to count and identify the fungal spores and pollens a week after Maria hit PR, just at the beginning of the fungal spore season. We observed a significant decrease in the levels of fungal spores and the 2017 fungal spore's season was severely affected. Nevertheless, the fungal spores rapidly rebound reaching unexpected record high levels during January through March 2018 and the April and May season is the highest on record.

Several days of very high fungal spores levels (red alerts) and very rapid release of fungal spores were observed. This could be due to the accumulation of biomass for the fungal growth and an unusual rainy season at the beginning of 2018. In contrast to the fungal spores, and due to the devastation of the vegetation after the hurricanes, the tree-pollen levels reached very low levels during the beginning of 2018. Hurricanes may be an important factor in the increase of outdoor fungal spores. As we described that high levels of fungal spores are significant triggers of asthma and allergies in PR, we will expect an increase in the use of asthma and respiratory medical services for 2018 in the aftermath of hurricanes Irma and Maria.

3.1-76 Different fire severities result in distinct soil fungal community trajectories.

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Abstract: Wildfires burn large areas of forested land annually, and they are projected to increase in frequency and intensity. These wildfires are not uniform; rather, they burn in patches that vary in severity. We compared experimental fires of different severities mimicking landscape mosaics created by a wildfire with patches analogous to whole log combustion within a background burn. The primary aim was to improve our understanding of soil fungal community trajectories following varying fire severities. We established ten pairs of plots in the Pringle Falls Experimental Forest in Oregon, USA. For each pair, one plot served as a background control (low severity burn), whereas another included logs piled in 1.5m x 8m x 1m structure for intense whole log combustion (high severity burn). The soils were sampled from 0-10cm depth within each plot before the burn, one weeks after the burn, as well as 2 and 4 years after the burn. DNA was extracted from these soil samples, the ITS2 barcode region of the ribosomal RNA gene PCR-amplified, and amplicons Illumina MiSeq sequenced to compare community richness, diversity, and composition among the severity treatments and over time. The data show that the fungal communities rapidly change in response to fire, and the recovery time depends on the fire severity. Following a high severity fire, the fungal communities follow trajectories distinct from those in low severity fires, although neither has fully recovered to communities resembling pre-fire conditions. Fire events, especially high severity fires have lasting impacts on the above ground system. Our study indicates that similar lasting impacts also happen to the fungal communities in the soil.

3.1-78 Mycobiomes of boreal forest soils: Unraveling patterns in fungal communities following disturbances

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Abstract: Boreal forest soils support a vast array of fungal communities that play important roles in forest composition and regeneration, plant nutrient uptake, carbon sequestration, and biogeochemical cycles. Lodgepole pine (*Pinus contorta* var. *latifolia*), a dominant and economically important forest tree species in Alberta, depends on communities of ectomycorrhizal fungi for successful establishment and survival through increased access to nutrients and water in exchange of photosynthates. These symbioses are critical in forests affected by a variety of tree-killing disturbances where forest regeneration is impacted

by negative disturbance-soil fungal feedbacks. While the responses of many specific fungal taxonomic groups to individual biotic and abiotic disturbances have been well characterized, how the cumulative effects of successive events impact overall soil fungal diversity is not known. In this DNA-metabarcoding study, we analyze soil fungal communities in lodgepole pine-dominated forests following several forest disturbances: mountain pine beetle (*Dendroctonus ponderosae*) outbreak, clearcut harvesting, wildfire, and clearcut harvesting of previously beetle-killed stands. Utilizing two genetic markers, ITS1 and SSU, in combination with next-generation sequencing, Illumina MiSeq, we unravel alpha- and beta-diversity of fungal functional groups within forest stands and among disturbance types, respectively. Results on how individual and cumulative forest disturbances shape patterns in fungal community composition and structure will be discussed under the hypothesis that both community traits will vary with disturbance types as well as single vs. multiple disturbances.

3.1-79 Effects of prescribed burning on wood-decay fungi in the forests of northwest Arkansas

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Abstract: Prescribed burning is a widely used management technique in the forests of Northwest Arkansas, but it is not known to what extent it can affect the biodiversity of wood-decay fungi. The present study was carried out to characterize the different species of wood-decay fungi present in burned (BPR) and unburned (UPR) areas of Pea Ridge National Military Park (PRMP) and an unburned area of Devil's Den state park (DDP). In order to do this, we extracted genomic DNA from 140 specimens of wood-decay fungi collected from these three study areas. This was done using the Promega DNA isolation kit. The Internal transcribed Spacer (ITS) region of fungal ribosomal DNA was amplified using ITS1 and ITS4 primers and sent for Sanger sequencing after quality checking of amplicons by means of 1% agarose gel electrophoresis. Altogether, 110 out of 140 sequences that passed quality checking were further used for identification of species of fungi by nucleotide BLAST searching against the NCBI database. From all study areas, 61 different species of fungi species were identified, with 30, 23, and 28 different species present on DDP, UPR, and BPR, respectively. Only six species were common between the two forests areas (PRMP and DDP) and only four between BPR and UPR of PRMP, indicating that an appreciable difference appears to exist for burned and unburned areas. The relative abundance of *Stereum ostrea* voucher She2067 was highest in BPR (24%) as compared to the other study areas (UPR, 17% and DDP, 3%). The present study is ongoing and will be continued during the 2018 field season.

3.1-80 Fire and alternate ecosystem states of tallgrass prairie: how do soil fungal communities respond?

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Abstract: The encroachment of woody species is among the greatest threats to tallgrass prairie ecosystems. Management choices including the suppression of fire facilitate a transition from grassland to shrub- and/or woodland, resulting in a shift to an alternate ecosystem state. The woody encroached state affects ecosystem productivity and biodiversity, renders pastureland less suitable for grazing, and alters key ecosystem functions above and below ground. Frequent, recurring fire acts as an attractor for the non-encroached grassland state, but restoration of woody encroached states using fire have been unsuccessful. Soil fungi are critical in determining plant community structure; however, little research exists on potential differences between soil microbial communities associated with these two alternate ecosystem states. To improve our understanding of soil microbial community composition and

dynamics in response to fire, we dissected fungal and bacterial communities before and after introduction of fire in both encroached and non-encroached states in a tallgrass prairie ecosystem and describe soil microbial responses on a high temporal resolution scale. We characterize fungal and bacterial abundance and community composition using qPCR and Illumina MiSeq (16S and ITS), as well as their functional responses using extracellular enzyme assays and soil microbial respiration. These data are amended with responses in nutrient dynamics including total concentrations of carbon, nitrogen and phosphorus as well as pH and inorganic nitrogen. Mycorrhizae and other symbiotic microbes have the potential to hinder or facilitate the rapid transition of grasslands to woodland ecosystem states. Understanding compositional and functional responses of these communities to fire likely offers insights into conservation of remaining grasslands and restoration of encroached woodlands.

3.1-89 Western Ghats of India: A cradle of fungal consortium

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Abstract: India is one of the countries blessed with rich biodiversity. The Western Ghats and Eastern Himalaya are a treasure trove of biological diversity in India. The Western Ghats are one of the eight 'hottest hot spots' of biodiversity and is known to possess, the most diverse, weird habitats of the globe and a high level of endemism in respect to plant, animal and microbial groups. About one-third of the world mycota so far described is known from India. About 5% of Indian fungi are endemic to Western ghats. Therefore, such bio diverse regions need to be protected and regulated in a manner to conserve its uniqueness. The mycoflora and their dynamics consequently became a subject of interesting study in this area and therefore systematic study on taxonomic diversity of micro fungi was undertaken with an objective to explore and characterise the diversity of Floristic microfungi from Western Ghats of India. The Fungi were diagnosed down to species level based on conventional parameters, detailed microscopic features and SEM studies. The present study is the result of the 19 extensive and systematic field fungal sample collection trips made to different geographical areas of Western Ghats of India such as grassland plateaus, deciduous forest, semi-deciduous forests, moist deciduous forests, semi-evergreen forests, subtropical hill forests, scrub jungles and cultivated plantations during the period from 2010 to 2018. This multipronged effort resulted in the collection of 2025 diseased samples with identification of 744 isolates of micro fungi, which were assignable to 267 fungal genera, 523 species and 04 varieties, infecting 342 sp. of host plants. All the fungi documented during the studies are grouped under Phyla- Ascomycota and Basidiomycota. The present study area forms the type locality of two new genera *Sheathnema indicum* Dubey and Moonnambeth, 2014 and *Sawantomyces indica* Dubey and Moonnambeth, 2013; eleven new species *Custingophora ratnagiriensis* Dubey and Moonambeth, 2013*; Goosiomyces bambusicola* Dubey and Moonambeth 2014; *Kamalomyces mahabaleshwarensis* Dubey and Moonambeth, 2013; *Periconia chandoliensis* Dubey*,* 2017*; Solicorynespora matheransis* Dubey and Moonambeth, 2014; *Sporidesmiu*m *biligiriensis* 2015; *Stigmina koyanensis* Dubey and Sengupta, 2016; *Tharoopama livistonae* Dubey and Moonambeth*,* 2013; *Tripospermum melghatensis* Dubey and Sengupta, 2016, *Vermiculariopsiella papaya* Dubey and Moonnambeth 2014 *; Zygosporium cocos* Dubey, 2014 and *Zygosporium dilleni* Dubey, 2014. In addition to this 39 fungal taxa were new additions to Fungi of India and 121 fungal taxa were found to be new to Western Ghats. Some fungi was encountered after a period of 35 years or more viz. *Conidiocarpus betle* T. Bose, *Asterina woodfordiae* V.P. Sahni*, Cercospora blumeicola* S. Das; *Cercospora careyae* T. S. and K. Ramakrishnan, *Meliola diospyri* Yates Syd. and P. Syd, from India. *Helicomina costi* M.A. Salam and P.N. Rao was recorded after a period of 65 years from India. 71 % of the total fungal isolates forms new host records from India. In conclusion, this research investigation presents an overview of fungal diversity existing in the Western Ghats of India and also made here to unravel the cryptic fungal consortium of this region.

3.1-90 Comparative study of wood rotting Fungi from two different forests in Mizoram, India

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Abstract: A three years investigation was carried out to study the diversity of wood rotting fungi from two different forest stands, Hmuifang forest and Tanhril forest of Mizoram, northeast India. A total of 45 species were identified from both the study sites. It was observed that a total of 21 species were common to both the forests whereas 19 species were found only found in the Hmuifang forest and 5 species only in the Tanhril forest. *Auricularia auricula-judae, A. polytricha, Coprinus dessimentus, Cyathus sp., Daldinia concentrica, Fistulina hepatica, Hexagonia tenuis, Lentinus badius, Marasmius sp., Microporus affinis, M. xanthopus, Mycena sp., Schizophyllum commune, Stereum hirsutum, S. rugosum, Tremella fuciformis, T. mesenterica, Trametes hirsutum, T. trogii, Xylaria hypoxylon, X. longipes* are the species/genera common to both study sites. *Microporus xanthopus* represents the most abundant species in both the study sites.

3.1-91 Brazilian fungal diversity represented by DNA markers generated over 20 years

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Abstract: The relatively recent molecular techniques using fungal DNA barcoding (ITS) and other markers have been key to identifying and exploring the biodiversity of different geographic areas, and show even more useful to explore megadiverse countries. Here, we provide an overview of the fungal diversity in Brazil based on the main DNA markers of phylogenetic importance generated since 1998, when the first ITS sequence from a Brazilian sample was submitted to GenBank. We retrieved fungal sequences of ITS, nLSU, nSSU, tef1, β-tubulin, RPB1, RPB2, actin, chitin synthase, and ATP6 from GenBank using different field keywords that indicated their origin in Brazil. A Maximum Likelihood phylogeny based on LSU illustrates the main Brazilian taxa grouped in orders. We obtained a total of 19,564 sequences. ITS is the most representative marker with 57.2%, followed by LSU (14.6%), tef1 (11.5%), β-tubulin (8.7%), RPB2 (3.1%), SSU (2.5%), RPB1 (1.2%), actin (0.7%), chitin synthase (0.3%), and ATP6 (0.2%). Based on all the sequences, there are representatives of Ascomycota (48 orders), Basidiomycota (26 orders), Blastocladiomycota (*Allomyces arbusculus*), Chytridiomycota (4 orders), Microsporidia (3 spp.), Mucoromycota (7 orders), Zoopagomycota (3 orders), and the *incertae sedis* taxon *Olpidium bornovanus*. Among the 11,187 ITS sequences, 70.1% are samples of Ascomycota, 18.6% Basidiomycota, 10.2% unclassified taxa, 1.1% Mucoromycota, 2 sequences of *O. bornovanus*, 1 sequence of Blastocladiomycota, and 1 sequence of Chytridiomycota (*Batrachochytrium dendrobatidis*). Based on ITS using a cut-off of 98%, all the fungal sequences comprise 3,036 OTUs, Ascomycota 2,088 OTUs, Basidiomycota 670 OTUs, and Mucoromycota 69 OTUs. Hypocrelales is the order of Ascomycota with more sequences retrieved (1,286 ITS seq.) and also the most diverse order (263 OUTs). In Basidiomycota, Pucciniales has the largest number of ITS sequences (615 seq.) but they represent only 9 OTUs. Agaricales is the most diverse (202 OTUs) and the second most sampled order in Basidiomycota (437 ITS seq.). Among the most sampled genera in Ascomycota (> 50 ITS seq.), the following are the most diverse: *Phyllosticta* (467 seq., 109 OTUs), *Penicillium* (199 seq., 95 OTUs), *Diaporthe* (257 seq., 90

OTUs), *Candida* (536 seq., 83 OTUs), *Fusarium* (449 seq., 80 OTUs), *Colletotrichum* (969 seq., 76 OTUs), *Phomopsis* (149 seq., 69 OTUs), *Aspergillus* (204 seq., 63 OTUs), *Xylaria* (95 seq., 52 OTUs), and *Trichoderma* (289 seq., 44 OTUs). In Basidiomycota, although *Phakopsora* (444 seq.) and *Puccinia* (168 seq.) are the most sampled genera for ITS, their diversity represents only 3 and 4 OUTs, respectively. *Rhizoctonia* (90 seq., 38 OTUs), *Pluteus* (56 seq., 29 OTUs) and *Cora* (70 seq., 24 OTUs) are the most diverse genera among the most sampled Basidiomycota for ITS. Previous data based mainly on morphological studies and bibliographical records pointed out 5,264 fungal species recorded from Brazil with predominance of Basidiomycota (2,741 spp.) and Ascomycota (1,881 spp.). The discrepancy of that number of species and the OTUs found in this study suggests that Basidiomycota has been well studied with regard to morphological diversity, while Ascomycota has been better investigated for their molecular composition and mainly for taxa of clinical and agronomic importance.

3.1-92 Insight into the diversity of soil fungi in Changbai Mountain by high-throughput sequencing

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Abstract: Soil fungi are an important group of microorganisms in forest ecosystem, they play significant roles in cycling of organic compounds and can affect the underground and upground ecosystems. In contrast to soil bacteria, soil fungi have been poorly investigated and understood in forest ecosytem. The fast development of molecular technologies offers a powerful method to access more functional information on soil fungal diversity. We applied the technique of Illumina Miseq High-Throughput Sequencing to investigate the soil fungal diversity and community structures in the northern slope of Changbai Mountain, Jiling Province, China, which is characterized with an evident vertical vegetation distribution pattern along the altitude. The metagenome sequence analysis was conducted by targeting ITS1f-ITS2 fragments for 80 soil samples collected in the four characteristic forest vegetation belts ranging from the root of 700m to the top of 2600m in altitude, it shows a huge abundance of soil fungi in Changbai Mountain forest. Totally 2,294,552 rDNA fragments of reads are grouped into 25,282 operational taxonomic units (OUTs), they are ascribed to 1056 species, 622 genera, 195 families, 87 orders, 24 classes and 5 phyla of fungi. Among which 182 genera are of Basidiomycota (48.72%), 411 genera of Ascomycota (31.67%), 13 genera of Zygomycota (10.21%), 13 genera of Chytridiomycota (0.27%), 3 genera of Glomeromycota (0.04%), and the left 9.09% are unknown taxa. The species of Basidiomycota are the predominant occupiers in the forest soil of the mountain, especially the genera of *Laccaria* (6.17%), *Inocybe* (5.54%), *Hygrocybe* (3.06%), and *Russula* (2.37%) of Agricales are found to be rich. While the genera of *Mortierella* (6.73%) and *Inocybe* are the most widely distributed in all sampling sites in the mountain. The soil fungal richness evidently tends to decrease from the root to the top of the mountain, and the fungal compositions vary in the four characteristic vegetation belts of the mountain. The novel profile of soil fungi in the mountain uncovered by means of metagenome technique could not be paralled by conventional culture-based fungal research methods.

3.1-93 Fungal and bacterial diversity in soils beneath native and introduced plants in Fiji, South Pacific

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Abstract: The Fiji Islands are an archipelago of more than 330 islands located in the tropics of the South Pacific Ocean, and offer a unique opportunity for the study of fungal and microbial biogeography and dispersal. Here we present the first molecular characterization of fungal and bacterial communities in soils from different habitats within the largest Fijian island, Viti Levu. Soil samples were collected from under native vegetation in maritime, forest, stream, grassland, and casuarina dominated habitats, as well as from agricultural sites of sugarcane, cassava, pine, and mahogany cultivation. Fungal and bacterial communities were analyzed using high-throughput MiSeq amplicon sequencing of ITS, LSU and 16S rRNA genes. We found lower richness of fungi and bacteria under single tree species habitats than under native forest and grassland habitats. ITS and LSU were congruent in β-diversity patterns. Fungal communities were dominated by Ascomycota (~57-64 % of relative abundance), followed by Basidiomycota (~20-23%) and Mucoromycota (~10%) according ITS region, or Chytridiomycota (~9%) according LSU region. Indicator species analysis found *Cenococcum*, *Wilcoxina* and *Rhizopogon* statistically associated to *Pinus caribaea*, and were likely co-introduced with the host. *Entoloma* was statistically associated with the grassland soils, and *Fusarium* and *Lecythophora* with soils under cassava. Bacterial communities were dominated by Proteobacteria (~25%), Acidobacteria (~19%) and Actinobacteria (~17%). Observed richness varied from 65 (*Casuarina*) to 404 OTUs (cassava) for Fungi according ITS region, and from 1268 (*Pinus*) to 2931 OTUs (cassava) for Bacteria and Archaea. This preliminary survey provides important baseline data on fungal and bacterial diversity and biogeography in the Fiji Islands.

3.1-94 The mycobiome of Karee Malformation Disease symptoms on *Searsia lancea* (karee) trees in South Africa

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Abstract: *Searsia lancea* (karee) is a common native tree in South Africa. A new disease called Karee Malformation Disease (KMD) consists of malformations of mostly vegetative and floral tissues. The cause of these malformations is still unknown. Using Illumina-based environmental sequencing, the aim of this study was to compare the mycobiomes found in diseased and healthy floral and vegetative tissues of *Searsia lancea*. Previous studies indicated that the two communities differed vastly in number and diversity of species. Minibarcodes using the ITS regions of the ribosomal operon confirmed results from previous studies. The notable differences found between the healthy and malformed tissues confirmed a succession of the fungal communities. As consistent with the latent pathogenic life cycle of many pathogens, genera such as *Alternaria*, *Botryosphaeria* and *Valsa* were present in all tissues but no dominant fungal group that could be the cause of the disease was detected. It is clear that the malformations greatly changed the fungal communities that would normally be present in an unaffected tree and such disease symptoms thus present a niche of their own within the tree.

3.1-95 Yeast diversity in Neotropical Savannah soils of the *Quadrilátero Ferrífero*, Minas Gerais, Brazil

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Abstract: The *Quadrilátero Ferrífero* occupies an approximate area of 7000 km^2 in the central-southeast portion of the State of Minas Gerais and is considered one of the regions of greater floristic diversity of South America inserted in the transition zone of the two Brazilian hotspots: Atlantic Forest and Neotropical Savannah. This region is recognized for its 'special biological importance' due to the occurrence of phytophysiognomies with plant species restricted to the region, as a consequence of the peculiar characteristics of soils that are ferruginous, acidic and of low fertility. It is a unique environment in the state with a great diversity of microorganisms still unexplored, mainly regarding the diversity of fungi in the soil. Yeasts are single-celled fungi and in the soil participate in important ecological processes. The objective of this work was to describe the yeast diversity and to compare the composition of the communities in ecosystems of the *Quadrilátero Ferrífero* under different seasonal seasons. The yeast diversity was analyzed in a total of 40 soil samples from two ecosystems (*Cerrado "latu senso"* and *Campo Rupestre*) in two seasonal seasons (dry and rainy). Soil samples were characterized by their physical and chemical properties and were grouped by Principal Component Analysis (PCA). Yeast diversity was assessed by culture technique and isolated species were identified by sequencing the D1/D2 region of the 26S rRNA gene. A total of 64 yeast isolates were recovered and identified in 20 species belonging to 10 genera. The Ascomycota Phylum (75%) predominated over the Filo Basidiomycota (25%). *Candida melibiosica*, *Meyerozyma guilliermondii* and *Cryptococcus laurentii* were the dominant species. In the *Cerrado* area, only one species was shared among the evaluated seasons, five were detected only in the dry season and only one species in the rainy season. In the *Campo Rupestre* area, two species were detected in both seasons, five only in the dry season and four species only in the rainy season. Four species were shared between the two analyzed ecosystems. The environmental variables explained 76% of the data variation, grouping the samples by ecosystem, but there was no separation between seasonal season. The soil texture was positively correlated with the *Campo Rupestre* samples (dry and rainy), while the organic matter content and soil acidity were correlated with the *Cerrado* (dry and rainy) soil samples. In conclusion, the ecosystems analyzed did not show differences in soil attributes, but showed differences in the yeast diversity between the areas and between the seasonal seasons.

3.1-96 Preliminary study of macrofungal diversity in three Private Reserves of San Pedro (Misiones province, Argentina)

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Abstract: The Atlantic Forest is an ecoregions complex that extends from the northeast of Brazil, by the coastal mountain ranges to Rio Grande do Sul. In the south, it enters to the east of Paraguay and northeast of Argentina, forming the Interior Atlantic Forest or the Paranaense forest. Although it occupies less than 1% of the planet's surface, it contains 7% of the known species on earth. "Juntos por la Selva" is an initiative of a small group of people in order to protect a fragment of the Paranaense forest. This is how, in 2011, the Itaovy, La Coral, and Yacutoro Private Reserves were created. They are located in Argentina, in the center of Misiones Province, in the Guaraní Department, just 15 km from San Pedro

city. They conserve about 500 Ha of forest with an important number of plant and animal species. In order to document the macrofungi within these protected areas, 2 samplings were performed during the 2016 - 2017 period. A total of 190 samples were collected in different environments of the reserves. The material was dried, kept in freezer for a week, and deposited as reference in the CTES herbarium. The morphological analysis was made based on observations of macroscopic and microscopical characters with stereoscopic and optical microscopes. The species found were identified consulting specific literature. The Basidiomycota was the most diverse group and from which a larger number of specimens was obtained, representing 93% of the collections, belonging most of them to the Agaricales, Polyporales, and Hymenochaetales. The remaining species belong to Ascomycota and Myxomycota, representing 7% of the fungi of the reserve. The presence of common and widely distributed genera, such as *Schyzophyllum, Fuscoporia, Pycnoporus, Trametes, Hexagonia, Marasmius, Mycena*, was observed, as well as rare species that have been registered only few times (e.g. *Ascopolyporus polychrous, Marasmiellus volvatus*) and others that has been cited only in the original description (e.g. *Mycena moconensis*). A list of species is presented, with comments about its habitat and distribution, together with a catalog of photographs to illustrate the species. It is expected to move forward with the determination of the collected species and to continue with the samplings at favorable seasons for the development of basidiomata in order to achieve a more complete inventory. It is highlighted that although the reserves harbor a restricted fragment of forest, they protect a high diversity of fungi, being an important region for its conservation.

3.1-97 Revisiting the secotioid and gasteroid *Cortinarius* species from Patagonia

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Abstract: The diversity of secotioid taxa within *Cortinarius* in the Nothofagaceae forests of Patagonia has drawn attention of mycologists during the last century. In the Patagonian region of Argentina and Chile *Cortinarius* is among the most diverse and abundant genera of ectomycorrhizal fungi with at least 240 species from the Andean mountains. Secotioid and gasteroid forms were until recently considered primarily within *Thaxterogaster*, resulting in a confusing intrageneric classification system. Moser and Horak suggested that *Thaxterogaster* was nested within *Cortinarius*. The modern molecular analysis of Peintner et al. investigated the multiple origins of sequestrate taxa related to *Cortinarius* and consequently synonymized *Thaxterogaster* to *Cortinarius*. Subsequent molecular phylogenies have resolved the polyphyletic nature of *Thaxterogaster* and other "cortinarioid" taxa within *Cortinarius* but have also highlighted the fact that most sequestrate Patagonian taxa lack molecular data. Original descriptions of these fungi are available mostly in German and Spanish and the interpretations of morphological structures are outdated considering the current state of knowledge about spore morphology and ontogeny. For example, verrucae on spores were illustrated as globose structures whereas SEM shows that they are complex conical structures that are sometimes interconnected by reticula or sub-reticula. External walls or episporia were sometimes pictured in original descriptions but our analyses suggest that these may have been optical illusions due to non-DIC microscopy. Recently, the incorrect interpretation of this episporium in the "cortinariod" fungi was found to be a misleading character. Despite recent advances in *Cortinarius* systematics, the current classification, diversity and ecology of secotioid and hypogeous "cortinarioid" fungi in the Nothofagaceae forests of southern South America remains unclear. The objective of this study is to update descriptions with diagnostic characters, including color photos of basidiomata, SEM images of spores, and ITS sequence data to clarify the

biodiversity of these fungi in Patagonia. Original descriptions of secotioid and gasteroid taxa were also revised and translated to English. Our analyses based on SEM and ITS rDNA resolves at least 15 species with names that need to be considered as synonyms. The use of these tools combined with an extensive database of described species also facilitated the recognition of several new and undescribed Patagonian species. Analysis of spore ultrastructure across many specimens clearly shows that sequestrate species of *Cortinarius* always lack a perisporium. It also indicates that there is a transition process in shape and ornamentation that occurs in taxa as they switch from ballistosporic to statimosporic spore dispersal.

3.1-98 Study on tropical and subtropical *Marasmius*: Four new species of the sect. *Spinulossi*

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Abstract: *Marasmius* is a large genus with worldwide distribution with more than 500 species, which are characterized by their generally small to medium-sized basidiomata, often membranaceous pileus, and for the ability to be revivescent when are rehydrated. This last feature makes them tolerate conditions of seasonal drought or high temperatures, what allows them to be diverse and abundant in tropical and subtropical forests. *Marasmius* comprises many sections and subsections (e.g. *Globulares, Marasmius*, *Neosessiles*, *Sicci*,). However, recently studies tested the monophyly of the sections traditionally proposed by Singer and confirm that they are highly homoplasic. A small group of species of *Marasmius*, characterized by having setae in the pileus and stipe surface, and even in the hymenophore (e.g. *M. actinopus, M. jalapensis, M. coharens*), belong to section *Spinulosi*. The species of *Marasmius* with setae are not common, being better known from southeastern Asia and Neotropical region. The aim of this study is to propose four new species and to present a worldwide key of *Marasmius* sect. *Spinulosi*. We studied specimens identified as *M. jalapensis, M. spiculosus, M. echinulatus*, and *M. flammans* deposited in CTES, K, LIL, NY and XAL mycological collections, including type specimens. For microscopic characters, a light microscopy (LM) and a scanning electron microscopy (SEM) were used. We discovered two species never described before from northern Argentina and we segregated other two species from the *M. jalapensis* concept based on type material analyses. *Marasmius* sp. 1, resembling *M. chrysoblepharis*, is characterized by its yellowish-orange pileus, with a sulcate-striate margin, entirely pilose orange-brown stipe, setiform caulocystidia with a tapering and thick-walled apex and bacilliform to fusiform large spores. *Marasmius* sp. 2 has characters between *M. trichotus* and *M. ciliatus*, but differs in its large setiform cystidia on the pileus and stipe surface, the absence of broom cells in the stipitipellis and spores size. Both species are collected in northern Argentina. *Marasmius* sp. 3 and *Marasmius* sp. 4 are segregated from the *M. jalapensis* concept. *Marasmius* sp. 3 differs by its narrower spores and two cheilocystidia types and it is distributed in the tropical and subtropical regions of South America. *Marasmius* sp. 4 is restricted to northern Africa and differs mainly by its smaller spores. *Marasmius jalapensis* is confined to the mesophilic mountain forests in Mexico. A key of the tropical and subtropical species of *Marasmius* sect. *Spinulosi* is presented. In conclusion, based on the morphological, biogeographic and phylogenetic characteristics, we propose these four species as new for science.

3.1-99 *Fomes fomentarius* lineages throughout the world

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Abstract: Currently, the existence of three distinct ITS lineages/sublineages among *Fomes fomentarius* isolates has been established. The sublineage A1 occurs only in North America, whereas the other two lineages/sublineages have a wider distribution: the sublineage A2 and the lineage B occur in Europe and Asia. This study represents the first description of limited sympatry between the Southern and Northern phylogeographical groups. The line passes through Central-western Europe, Central Europe, and Central-western Asia. A clear correlation was observed between lineage (sublineage) and host range. The North American sublineage A1 follows the geographical distribution of its main hosts: North American birches *Betula* spp. and *Fagus grandifolia*. The two Eurasian lineages/sublineages, sublineage A2 and lineage B, have different host species preferences (*Acer negundo*, *Alnus*, *Betula*, and *Picea*, vs. *Abies*, *Acer platanoides*, *Aesculus*, *Platanus*, *Prunus*, *Salix*, and *Tilia*). European beech (*Fagus sylvatica*) is only host of both Eurasian lineages/sublineages.

3.1-100 A revision of the genus *Graphis* (lichenized ascomycete) in Colombia

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Abstract: *Graphis* is the type genus of the family Graphidaceae. It contains nearly 400 species and is considered the lichen genus with the highest number of species in the Neotropics, and the number of species described within the genus has grown steadily in the last 15 years. Based on the 2015 *Catalogue of the Plants and Lichens of Colombia*, 67 species of the genus are registered for Colombia, compared to 115 for Costa Rica. Since Colombia is twenty times larger than Costa Rica and has a broad array of ecosystems to harbor a diverse lichen biota, the number of species of *Graphis* is expected to be substantially higher that in Costa Rica. We studied collections of *Graphis* deposited in the herbarium of the District University in Bogotá (UDBC-Cryptogamic Section), which harbors about 700 specimens of this genus from different regions of Colombia, in order to obtain a first assessment of the true richness of *Graphis* in the country. As a result of this study 65 new records of *Graphis* for the country are presented and six new species are described, increasing the total to 138 species known from Colombia. Additionally, the first key to species of the genus *Graphis* in Colombia was elaborated, as well as a rapid color guide following the models of the Field Museum, Chicago.

3.1-101 Three new white sporulating species of *Aspergillus* sect. *Terrei*

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Abstract: The objective of our study was to perform morphological and molecular analyses on a number of white sporulating *Aspergillus* strains from section *Terrei* in order to confirm any undescribed species. The strains were isolated from clinical material, soil and from the built environment (residential home, hospital) by active air and swab sampling. Cultures were grown on Czapek's yeast autolysate agar (CYA) (25 °C and 37 °C), malt extract agar (MEA), potato dextrose agar (PDA), Czapek's agar with 20% sucrose (CY20S), dichloran 18% glycerol agar (DG18), and oatmeal agar (OA) (25 °C) for 7 days in darkness.

Cultures were then described by morphological, physiological (maximum growth temperature) and microscopic analysis followed by multilocus DNA sequencing of four unlinked genetic loci. Based on morphological analysis and molecular confirmation, the unknown isolates were described as new species in the *Aspergillus* section *Terrei*. Historically, members of *Aspergillus* sect. *Terrei* have been isolated from soil, indoor environments, various food and feeds, and have been associated with many health issues of humans and animals. Also, some species have been known to produce a wide range of exometabolites. Future studies are required to determine any pathogenic roles of these new species and exometabolite production.

3.1-103 Current status of the genus *Entoloma* in Ecuador based on the QCAM Fungarium collections

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Abstract: The genus *Entoloma* is very rich and complex, with close to 2,500 described species divided in 36 sections, subgenera and subsections. A megadiverse country like Ecuador potentially harbors a large number of *Entoloma* species. The QCAM Fungarium at Pontifical Catholic University of Ecuador in Quito currently houses over 7400 fungal specimens, a number that has almost tripled in the past six years. It is the largest macrofungi collection of Ecuador, and therefore, where most information related to the genus status can be found. Of the 76 *Entoloma* collections made since 1983, 35 specimens have been identified to 12 different species and 8 aff. open nomenclature designations. Forty-one specimens have yet to be identified to species. Some important findings include the holotype of *Alboleptonia sulcata* T.J. Baroni and Lodge (1998), currently *Entoloma sulcatum*, and six new species. The new species were found after morphological and molecular analyses of a limited number of specimens chosen at random from the QCAM collection in 2017. These new species are listed here with tentative names until they are formally described and published: *E. yanaumense*, *E. astroasprellum*, and *E. yanacolor* that belong to the subgenus *Leptonia* (Fr.) Noordel, section *Cyanula* (Romagn.) Noordel; *Entoloma squamosum* within the subgenus *Trichopilus* (Romagn.) Noordel; *Entoloma crinipellis*, a very close species to *Pouzarella ferreri* T.J. Baroni, Perd.-Sánch. and S.A. Cantrell (2008), and *E. umbellatum* which belongs to the subgenus *Inocephalus*, section *Staurospora* Largent and Thiers (1972). Almost all *Entoloma* species in the QCAM collection are represented by only one specimen, except *E. austroasprellum* with two collections, and *E. serrulatum,* with three. This limited amount of data shows not only the high diversity of the genus in Ecuador, but also the need to devote specific studies to catalogue and discover new species of *Entoloma* in tropical and subtropical areas.

3.1-104 The *Rhodocybe/Clitopilus* clade (Entolomataceae, Agaricomyetes) in the Dominican Republic: a new genus, new species and first reports for Hispaniola

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Abstract: This report serves as the first ever to outline the diversity of the *Rhodocybe/Clitopilus* clade for the Dominican Republic. Based on collections made during several expeditions exploring for basidiomycete macrofungi over a span of 20+ years, we now have a reasonable understanding of the biodiversity of these saprotrophic fungi in the Dominican Republic. Diverse ecological habitats were

sampled during these studies, ranging from tropical seashore vegetation to the endemic pine covered high peaks in the central mountainous regions. Based on morphological and phylogenetic analyses, we document one new genus, three new species, one new variety, one new combination and four first reports for members of the *Rhodocybe/Clitopilus* clade on the island of Hispaniola. Images of the new taxa and the three gene phylogenetic data to support these taxonomic conclusions will be presented. A broader biogeographic analysis of the *Rhodocybe/Clitopilus* clade around the Caribbean region will also be considered.

3.1-105 Use of hyphal image analysis and machine learning to classify Mucoromycota soil fungal isolates

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Abstract: Fungi belonging to the Mucoromycota are abundant in soil communities globally; these fungi are also important industrially for the production of lipids, and some isolates are known to harbor endobacteria. Isolation and identification of fungal strains from soil is an intensive process involving culturing, DNA extraction, PCR, and sequencing. Classification of filamentous fungi is typically dependent on reproductive structures; however, reproductive structures are not always present when isolates are grown in culture. Image classification of hyphae using machine learning algorithms offers a method to streamline prospecting for novel fungal strains. Micrographs of hyphae obtained while isolates were growing in Petri dishes were used to avoid additional sample preparation. We wrote a Python 3.6 script, in which images were converted to grayscale, then fast Fourier transformation was applied to detect distinctive patterns in hyphae. Taxonomic labels were assigned to images based on ITS sequences. A Random Forest Classifier object from the Scikit-learn library was trained using a subset of images and validated on a separate set. Images of Mucoromycota strains were identified with a weighted F-score exceeding 92%. Our method can effectively be used to classify fungal isolates using only hyphal imagery for accelerated identification. Future research will include improving accuracy and specificity to a wider range of taxonomic ranks and diversity. Image classification is a promising tool to aid in the prospecting fungal strains by helping to reduce the number of samples requiring sequencing and intensive culturing.

3.1-106 Updating the taxonomy of the genera *Aspergillus* and *Penicillium* in South Africa

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Abstract: South Africa is a well-known biodiversity hotspot with high endemicity. This is also true for *Penicillium*, with 29 of 61 isolated species described as new from a 2012 survey from the fynbos biome in the Western Cape. Much of our local knowledge are solely based on morphology. However, morphological interpretations are very difficult, unreliable and inconsistent considering the large number of accepted species (378 *Aspergillus* and 427 *Penicillium*). This makes morphological identifications impossible for many species. Recent taxonomic changes, combined with their diverse nature and economic importance necessitates the modernization and updating of local *Aspergillus* and *Penicillium* knowledge. The PPRI collection of the National Collection of Fungi has ~1000 accessions of

Aspergillus and *Penicillium*. These represents the best resource for obtaining base line knowledge on what species occur in the country. Additional strains are also regularly isolated during diagnostic work done at the ARC-Plant Health and Protection. This project has the goal of (1) placing PPRI strains into morphogroups and then (2) sequencing the secondary identification markers for representatives from each group. Sequences will be compared to a curated database and identifications updated. For new/rare species, additional genes will be sequenced (ITS, *BenA*, *CaM* and *RPB2*). This presentation will be focused on recent developments in the taxonomy of *Aspergillus* and *Penicillium*, and how we can apply taxonomy to benefit the scientific community with special focus on future identifications based on culture dependent and independent techniques.

3.1-107 Arthropod infecting fungi in the genus *Cordyceps sensu lato* (Hvpocreales, Ascomycota) and related genera from Australian tropical rainforests

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Abstract: Our collections have shown that Australian tropical rainforests are rich in arthropod infecting fungi, especially on ants, flies, spiders and scales. The aim of our Australian Biological Resources Study project was to systematically catalogue the Australian arthropod infecting fungi in the genus *Cordyceps sensu lato* (Hypocreales, Ascomycota) and related genera, from tropical and sub-tropical rainforests. Specifically, we investigated the systematics of Australian *Hypocrella* (Clavicipitaceae) on scale insects; *Cordyceps sensu stricto* (Cordycipitaceae) on ants and flies; *Ophiocordyceps* (Ophiocordycipitaceae) on ants; and *Akanthomyces*, *Gibellula* and *Hirsutella* that are mostly spider pathogens. Specimens held in Australian mycological herbaria were examined alongside collections made during field trips in the Wet Tropics World Heritage Area (WTWHA) and Cape York Peninsula over a three-year period, 2014 - 2016. We revised the taxonomy based on multigene (ITS, SSU and LSU) DNA sequence phylogenies, fungal morphology and arthropod/host plant associations. We yielded 991 specimens of entomopathogenic fungi, including 337 living cultures. Of these DNA sequence data has been obtained from 138 specimens. Those specimens represented 112 species in 35 genera. The genera most commonly encountered were, *Akanthomyces, Aschersonia, Beauveria, Gibellula, Hirsutella, Hymenostilbe*, *Isaria, Lecanicillium, Metarhizium, Ophiocordyceps, Paeciliomyces* and *Torrubiella*. Many of the species identified in these genera either represent species complexes and/or novel taxa that have not been recorded previously in Australia. One new genus, *Hyweljonesia*, and four new species *H. queenslandica*, *Ophiocordyceps norreniae*, *O. dawkinsii and O. oecophyllae* have been described so far. Fact sheets including high resolution images and descriptions have been completed for 40 target species. Collectively, this information will form the basis for an online, interactive key of Australian entomopathogenic fungi using Lucid software that is currently under development. Taxonomic knowledge about Australian arthropod infecting fungi has increased substantially from this project. This will underpin future systematics and ecological studies on plant-arthropod-fungi interactions, e.g. the role of fungi in insect morbidity in rainforests, as well as the potential of arthropod infecting fungi as biocontrol agents for agricultural insect pests.

3.1-108 Molecular identification of *Aureobasidium* and *Rhodotorula* from spore trap air samples

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Abstract: *Aureobasidium* and *Rhodotorula* are both allergenic yeasts that have been previously identified in viable air samples; however, the daily exposures to these aeroallergens are not known. Though viable sampling is a reliable method to distinguish which yeasts are airborne and the immediate concentrations, the samples are collected for a short period of time (1-2 minutes). Consequently, the daily exposure to airborne *Aureobasidium* and *Rhodotorula* are not known, because yeasts are not morphologically distinct in nonviable (24 hour) spore trap air samples. The objective of our study is to determine the frequency and concentrations of *Aureobasidium* and *Rhodotorula* in daily air samples by using genus-specific DNA targets for molecular identification. Air samples were collected at The University of Tulsa (Tulsa, Oklahoma) on the roof of a building, 12 m above the ground, using a Burkard 7-day nonviable spore trap sampler (Burkard Manufacturing, Co. Ltd., Rickmansworth, Hertfordshire, England). Samples were collected from 22 July to 22 November 2017. DNA was extracted from daily samples and quantified with genus-specific TaqMan assays. The *Aureobasidium* assay hybridized to a region of the fatty acid elongase gene and the *Rhodotorula* assay hybridized to the ITS1 region of the ribosomal RNA operon. The daily concentrations (cells per cubic meter) of *Aureobasidium* and *Rhodotorula* were calculated using a standard curve of known yeast cell concentrations. Real-Time PCR indicated the frequency of *Aureobasidium* was 70% and *Rhodotorula* was 46%. *Aureobasidium* had a higher daily concentration when compared to *Rhodotorula* with maximum concentrations of 25 cells/m^3 and 7 cells/m^3 respectively. In conclusion, the daily exposure to allergenic *Aureobasidium* and *Rhodotorula* occur frequently, but at low daily concentrations. More sample collection is warranted before annual trends or predictive meteorological variables may be identified.

3.1-109 Molecular approach to clarify taxonomy and ecology of *Puccinia* species on Gramineae and Cyperaceae in Jilin, China

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Abstract: Jilin Province is located in the northeast of China and geographically divided into three main areas: an eastern mountainous area, a western dry plain area and the rolling hilly area between them. The natural vegetation consists of prairie grasses in the western plains and broad-leaved deciduous forests mainly consisting of species of *Betula, Populus* and *Quercus* in the hilly and mountainous areas. These areas are rich in vegetation and other suitable environmental conditions to predict a proliferation of rust fungi, especially in the Changbai mountain ranges located at the border of North Korea that harbor conserved natural forests. However, the inventory and ecology of rust fungi have not been sufficiently investigated in Jilin Province. Therefore, we surveyed rust fungi in several locations in Jilin Province from 2013 to 2017 and collected about 1000 specimens. Among them, about 300 specimens were *Puccinia* species on Gramineae and Cyperaceae which were dominantly growing in Jilin Province. We also collected about 50 specimens which are hypothesized to represent the spermogonial and aecial stages of these *Puccinia* species. However, identifications of these specimens are very difficult because of morphological similarity among specimens and difficulties of host identification. Although many are suspected to be heteromacrocyclic species the connections among different stages (spermogonial, aecial, uredinial and telial stages) cannot be confirmed because of difficulties of inoculation experiments

and also difficulties of obtaining plants from conservation areas. For resolving these problems molecular analyses were applied. ITS and 28S regions of rDNA from specimens were amplified and sequenced. After constructing phylogenetic trees by the ML and BS methods about 35 clades were detected. These clades were suspected as species based on morphological observations and host relations. Species of these clades were identified and life cycle connections among stages were clarified based on the species reported in China although many cryptic species were found among these clades.

3.1-110 Cryptic speciation within the *Puccinia hieracii* and *P. calcitrapae* species complexes

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Abstract: There has been an long-standing inconsistency within the scientific literature regarding the nomenclature and taxonomic identification of the autoecious macrocyclic rust fungus species *Puccinia hieracii s.l.* and *Puccinia calcitrapae s.l.* within the order Pucciniales. Some concepts of the species consider that this species infects hosts within two distinct sub-tribes of Asteraceae, Cichoriae and Cardueae, whereas others separate the complex into several distinct host specific taxa. The aim of this study was to undertake phylogenetic and morphological analyses of representatives of the various taxa in order to generate a consistent taxonomic arrangement. DNA barcoding of the ITS2 and LSU (28S; V1 and V2 domains) of the rRNA locus was undertaken from specimens obtained from diverse hosts across the UK. The rusts present on hosts belonging to different genera (*Hieracium*, *Leontodon*, *Scorzoneroides* and *Taraxacum*) were genetically distinct from each other. However, quantitative morphological analyses using both light and scanning electron microscopy could only distinguish a few of these taxa based on features of urediniospores and teliospores, for instance spine density and the presence of a spineless tonsure region. These analyses also identified a new clade of *P. hieracii* infecting *Scorzonderoides autumnalis* and also a distinct variety of *P. jaceae* infecting *Centaurea nigra*.

3.1-111 Molecular bio-markers and phenotypic characterization as a means of determining genetic diversity within *Aspergillus flavus* isolates

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Abstract: Toxigenic *Aspergillus* species produce mycotoxins that are carcinogenic, hepatotoxic and teratogenic immunosuppressing agents in both human and animals. Kenya frequently experiences outbreaks of aflatoxicosis with the worst occurring in 2004, which resulted in 215 deaths. We examined the possible reasons for these frequent aflatoxicosis outbreaks in Kenya by studying *A. flavus* diversity, phenotypes and mycotoxin profiles across various agricultural regions. Using diagonal transect random sampling, maize kernels were collected from Makueni, Homa Bay, Nandi, and Kisumu counties. Out of 37 isolates, nitrate non-utilizing auxotrophs complementation test revealed 20 vegetative compatibility groups. We designated these groups by the prefix ''KVCG'', where ''K'' represented Kenya and consequently assigned numbers 1 to 20 based on our findings. KVCG14 and KVCG15 had highest distribution frequency (n = 13; 10.8 %). The distribution of the L, S and S/L-morphotypes across the regions were 57 % (n = 21); 7 % (n = 3) and 36 % (n = 13) respectively. Furthermore, a unique isolate (KSM015) was identified that had characteristics of S-morphotype, but produced both aflatoxins B and G. Coconut agar medium (CAM) assay, TLC and HPLC analyses confirmed the presence or absence of

aflatoxins in selected toxigenic and atoxigenic isolates. Diversity Index (*H*) analyses ranged from 0.11 (Nandi samples) to 0.32 (Kisumu samples). Heterokaryon compatibility ranged from 33 % (for the Makueni samples, *n* = 3) to 67 % (Nandi samples, *n* = 6). To our knowledge, this is the first reported findings for *A. flavus* diversity and distribution in Nandi, Homa Bay and Kisumu counties and may assist current and future researchers in the selection of biocontrol strategies to mitigate aflatoxin contamination as has been researched in Makueni and neighbouring counties.

3.1-137 Regional variation of endophyte community diversity in *Dalea purpurea* (purple prairie-clover)

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Abstract: Fungal endophytes are found living asymptomatically in the tissues of most plants, and some endophytes may provide benefits to their hosts. The factors affecting the assembly of endophyte communities are not well understood, since the results of current studies differ regarding the relative importance of host species and genotype, abiotic factors, and random sampling of inocula from the environment. To understand the ecological factors affecting the diversity and taxonomic composition of endophyte communities, we sampled foliar endophytes from sixteen populations of purple prairie-clover (*Dalea purpurea*) in remnant prairies in four regions in Minnesota: northwest, west-central, southwest and southeast. We compared the beta diversity among these communities and found strong clustering in the northwest compared the other regions, indicating that the northwest communities were more similar in composition to each other than to other sites. The genetic results are consistent with our observations of morphological traits such as pigmentation because cultures sampled from the northwest were consistently more similar than cultures sampled from other regions. Since distances between sites in the northwest are comparable to or greater than distances between sites within other regions, abiotic conditions particular to these northwest sites may be responsible for the similarity of their endophyte communities.

3.1-138 Characterization of root fungal endophytes of native and invasive *Phragmites australis* along a salinity gradient

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Abstract: In the last 150 years an invasive lineage of the wetland plant *Phragmites australis* has spread aggressively throughout many areas in the United States. Some of the problems associated with the rapid expansion of *Phragmites* are changes in wetland hydrology, biogeochemistry, reduction of wildlife habitat, and the loss of biodiversity, including a native lineage of the same species *Phragmites australis* subsp. *americanus*. Studying the microbiome associated with invasive and native species can lead to new insights into invasive species success, because host microbiome associations can greatly influence plant performance. The objectives of our study were to characterize the fungal endophyte communities associated with native and invasive *Phragmites*, determine the prevalence of dark septate endophytes (DSE) during the growing season, and examine the role of salinity in fungal root colonization. We identified three sites along a salinity gradient in the Choptank River, an estuary of the Chesapeake Bay (MD, USA), and collected roots from contiguous stands of native and invasive *Phragmites* every two weeks from June to October. We used microscopy to determine percent colonization of DSE, and Illumina sequencing of the ITS1 region to characterize the root endophyte communities of each lineage.

DSE colonization did not vary during the growing season, but the invasive lineage was consistently more colonized than the native. Fungal colonization of invasive *Phragmites* also increased with salinity. All identified, sequenced OTUs matched the phylum Ascomycota, and the endophyte communities differed between lineages and among sites. In conclusion, invasive and native *Phragmites* have distinct root endophyte communities that vary across a salinity gradient, and might play a role in aiding the spread of the invasive lineage into higher salinity sites.

3.1-139 Effect of the leaf developmental stage on the chemical and fungal endophytic composition in wild Rubiaceae.

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Abstract: Plants host a complex internal microbiome from which endophytic fungi (EF) represent an important component. This highly diverse group is assumed to have profound impacts on plants, therefore, much attention is now being paid to understand the interactions and relationship between endophyte colonization and leaf traits. Plant-associated environments are highly dynamic, constantly exposed to factors that can affect the structure and composition of colonizing species. In this study we aim to determine the effect of the leaf developmental stage (young vs mature) on the EF biodiversity, leaf chemistry and metabolites bioactivity as facets of the relationship between endophytes and their host. We hypothesize that the prevalence of antifungal secondary compounds is higher in young leaves which makes them more chemically protected than mature leaves, and such defenses may limit endophytic colonization. The sampling took place in the tropical forest of Golfito, southeast Costa Rica, where plants belonging to the *Rubiaceae* family were collected and then processed to eliminate epiphytic and environmental contamination. Fungal diversity was assessed using metabarcoding by amplification of the ITS4 and ITS5 regions and library sequencing was completed by Ion Torrent technology. Data was then analyzed using Geneious and USEARCH. For the metabolomic assessment, whole leaves were lyophilized and then ground for quantification of phenolics and other untargeted metabolites. The powder was extracted, derived, and then analyzed using LC-MS and GC-MS workflows. High-throughput sequencing identified most operational taxonomical units (OTUs) belonging primarily to the Ascomycota phylum. *Pleosporales* and *Capnodiales* were the orders contributing the most species to the endophytic assemblages. The total colonization frequency and species richness of endophytic fungi were higher in mature leaves than in juvenile, meaning, the structure of fungal communities differed significantly by developmental stages of leaf. For secondary metabolites a T-test between young and mature leaves showed little to no significant changes in constitutive concentrations of most compounds through the juvenile-mature stages, but instead showed greater metabolic differences between families of plants sampled. These results suggest that neither phenolics, nor other chemical compounds vary sufficiently with leaf age to be identified as causal agents of the changes in endophyte richness at the leaf stage. Moreover, in tropical forests, leaves of different developmental stages differ in duration of exposure to environmental fungi, which could be influencing these results, along with other structural and chemical properties that change during the leaf's life cycle. In conclusion, it is imperative to continue efforts to understand the degree to which apparent patterns of host colonization are dictated by the host, the endophyte or other ecological mechanisms.

3.1-140 *Fusarium* prevalence on *Vanilla* from different cultivation systems using culture-based methods

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Abstract: *Vanilla* is widely cultivated for its economic importance for the production of natural vanillin. This crop has been extensively studied in different countries but little research has been conducted at its center of origin regarding fungal associations. Our goal was to determine fungal diversity in plants from different agrosystems. Sampling was conducted in agrosystems located at Totonacapan (Puebla and Veracruz, Mexico). Samples were collected from four species of *Vanilla* (*V. planifolia*, *V. pompona*, *V. insignis*, *V. rayada*) on traditional and shade house systems that used dead, alive and concrete tutors. Fungal isolates were obtained from surface-sterilized plant material (i.e. terrestrial and adventitious roots, pelotons, and leaves) and using baiting techniques for soil, humus, litter and vanilla support tree cortex. Isolates were then identified using ITS nrDNA sequencing. Culturing yielded a total of 382 isolates, with 47% identified as *Fusarium* mainly recovered from roots (140 isolates). Other commonly cultured genera include *Trichoderma* (6%), *Penicillium* (5%), and *Aspergillus* (4%). Further characterization of representative *Fusarium* isolates will be performed using the TEF-1α and RPB2 regions. This study reveals the presence of fungi on *Vanilla* from Mexico and reports a high dominance of *Fusarium* in a culture-based study in *V. planifolia*.

3.1-141 Diversity and temporal dynamics of endophytic fungi in leaves, and roots and associated soil of *Coffea arabica* in Panama

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Abstract: Endophytic fungal communities contain species that can confer their host plants with benefits such as tolerance to pathogens and pests. Understanding how these communities assemble, their temporal dynamics, and the factors that affect them is key for developing strategies for using applications of these fungi as protectors of plant crops. In this work, we used classic techniques of microbiology and next generation sequencing (NGS) of amplicons of the ITS1 region to identify endophytic fungi that make up the microbiome of leaves and roots of three coffee varieties, *Coffea arabica* (Geisha, Catuai and Typica). We compare these fungal communities with those in soil associated to the sampled roots, to generate information on the temporal dynamics of these communities and possible factors that influence them. Our results suggest a high diversity of endophytic fungi associated with *Coffea arabica* in Panama, with > 9,000 fungal OTUs and community composition more influenced by plant organ or substrate and source locality than the genetics of the studied varieties. Additionally, we found variation in the relative abundance of taxa over time (one sample per month, per substrate, for six months, for each of 45 sampled trees) and isolated fungi that inhibit the growth of coffee pathogens. We will discuss these results in the context of their relevance for the development of endophytic fungi as plant protectors, incorporating data on agronomic management, plant genetics, disease incidence and climate. This juxtaposition of classical and NGS techniques provides robust and valuable information that can be used to develop optimized regimes of application of endophytic fungi as plant protectors.

3.1-142 Investigations into the diversity and function of *Mortierella* fungi with plants and bacteria

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Abstract: Plants are host to thousands of fungi and bacteria, which constitute the plant microbiome. Some of these microbes appear to contribute to plant development, growth and resilience to stress. *Mortierella* is an early-diverging lineage of fungi that are common in soils and rhizospheres of diverse plants. Many *Mortierella* species host intracellular bacteria including *Burkholderia*- and *Mollicutes/Mycoplasma*-related endobacteria. The function of *Mortierella* and its endobacteria in the plant microbiome remain unknown. The goals of this research are two-fold: (1) assess the diversity of *Mortierella* and its endobacteria; (2) determine the impact of different *Mortierella* species on plant growth and their resilience to drought stress. We first isolated *Mortierella* from Puerto Rican and US soils. To identify the fungi, ITS rDNA regions was amplified and Sanger sequenced. NCBI BLAST queries and phylogenetic analysis conducted. Isolates were screened for the presence of endobacteria using 16S rDNA primers and PCR, and identified by sequencing amplicons. Endobacteria were cleared from their host with antibiotics. To assess impacts of fungi and their endobacteria on plant growth isolates were inoculated onto *Raphanus raphanistrum* (radish) and *Phaseolus vulgaris* (bush bean). Above- and below-ground biomass was weighed and measured. At week 5 plants were exposed to severe drought and their photosynthetic efficiency (Phi2) was monitored. In this research, 11 new isolates of *Mortierella* were obtained. Endobacteria were detected in two isolates (18%). Both new *Mortierella* fungi and endobacteria detected, form part of possible new clades within its phylogeny. Only *M. humiliss* PMI1414 strain appeared to improve the growth of radish bulb biomass and Phi2 measurements in bush bean plants. Additional studies are needed to assess impact of endobacteria on plant-fungal interactions. Response variables additional to Phi2 and dry biomass ought to be measured in future studies.

3.1-143 Characterization of novel endophytic Pleosporales fungus from grasses

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Abstract: Dark septate fungi (DSF) are commonly found in semi-arid regions of Southwest America. Grasses benefit from symbiotic DSF that are well adapted to survive stressful environmental conditions such as UV radiation, drought and heat. A vast diversity of DSF belong to the Pleosporales order, though studied, impact on plants are poorly understood. The objective of this study is to evaluate the effect of a novel Pleosporales fungus on plant growth. Fungal specimens were isolated on MEA with antibiotics from sterile roots. Using the ITS rRNA region isolates were identified to the order level of Pleosporales with low similarity to other described fungal genera. Six isolates were chosen for morphological characterization of the fungus by growing them on multiple media. Direct contact bioassays were conducted on *Bouteloua gracilis* and *B. dactyloides* to evaluate the effect of fungal isolates on plant growth. LSU sequences show evidence of a potential novel fungus closely related to Didymocrea found in the internal tissue of the South China Sea sponges *Clathrina luteoculcitella* and *Holoxea sp*. Additionally, the morphology of the isolates varies between media (MEA, Emerson, PDA, Czapek dox, soil agar) in terms of conidia production. Sexual structures were not observed. Isolates showed a range of effects on plant growth showing signs of pathogenicity to stimulation of root growth. Fungal colonization was observed in plant roots as hyphae and spores. Additional bioassays and sequencing will be conducted with the six isolates to gain a better understanding of their function and taxonomic placement.

3.1-144 Arbuscular Mycorrhizal and dark septate endophytic fungal association in finger millet (*Eleusine coracana*) varieties in Southern India

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Abstract: We investigated the extent on root morphology in finger millet varieties cultivated in southern India. Root samples of cultivated finger millet varieties were collected from Agricultural field GKVK campus at Bangalore. All the 17 varieties were root morphology characteristics. They are characterized by intercellular and intracellular hyphal and arbuscular coils. Moreover, our results suggest that AM and DSE fungal association were significant among the 16 varieties of finger millet roots. We also found spores of 4 species of AM fungi were associated with finger millet varieties and recorded in different rhizospheres. It suggests that AM and DSE fungal association were significant with finger millet varieties that showed strong symbiotic association found between mycorrhizal fungi and finger millet varieties.

3.1-145 Arbuscular mycorrhizal fungi suppression of *Fusarium verticillioides* in maize

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Abstract: The protection of plants from pathogenic organism results to better performances of their growth and yield characters. Hence, the efficacy of *Glomus clarum* and *G. deserticola* against *Fusarium verticillioides* (AKR 05, ILR 06 and ERW 05 strains) on maize T2L COMP.4 was investigated. *Glomus clarum* and *G. deserticola* were inoculated separately at concentrations of 10 g (20 spores), 20 g (48 spores) and 30 g (72 spores) per 8 kg of soil at 4 wk after planting (WAP) with a control. In addition, spore suspension (1.0×10^6 spores/mL) of the pathogen *Fusarium verticillioides* was also inoculated at 8 WAP while the effects of these treatments were observed based on the plant's morphological, biomass and yield traits. Effect of *F. verticillioides* on plant height and shoot weight was significantly reduced by 20 g (48 spores) and 30 g (72 spores) treatments of *G. clarum* and *G. deserticola*, also the treatment at 10 g (20 spores) had biocontrol effect on the husk cover while the percentage ear rot severity ranged from 3.0% to 22.2% across the treatments. Therefore, this result established biocontrol potential of the tested arbuscular mycorrhizal against *Fusarium verticillioides* at 30 g (72 spores) concentration.

3.1-147 Effects of arbuscular mycorrhizal fungi on growth of *Medicago sativa* in acidic soil

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Abstract: Acidic soils are harsh environments for plants. Arbuscular mycorrhizal (AM) fungi play an important role in protecting plant growth against such stresses as acidic soil. To understands the relationship between AM fungi colonisation and soil acidity to evaluate the possibility that AM fungi facilitate the existence of plants on acidic soils, the effects of arbuscular mycorrhizal (AM) fungi *Claroideoglomus etunicatum*, *Rhizophagus intraradices* and the mix of the two AM fungi on the growth of alfalfa (*Medicago sativa)* were assessed at three of soil pH by growing plants in a greenhouse experiments, with and without AM fungi inoculation, individually in pots. The different acid growth medium were established with H_2SO_4 to pH 3.0, 5.0 and 6.58. The results showed that the *C. etunicatum*, *R. intraradices* individual and the mix of the two AMF increased plants dry weight and P uptake at all acid treatments compared with un-inoculation of AM fungi treatment ($P<0.05$). Plants shoot height, leaf numbers, shoot dry weight, root dry weight and total dry weight were averagely increased by 214.77%,

312.67%, 105.39%, 95.46% and 101.85%, respectively by the mix of two AMF compared with un-inoculated treatments. Acid decreased the total dry weight, chlorophyll content, superoxide dismutase (SOD) and peroxidase (POD) activity, of which the above-mentioned index were 17.59%, 21.94%, 29.51% and 51.26% lower at pH 3 than that at pH 6.58. The inoculation of AM fungi averagely increased SOD and POD activity of plant by 108.7% and 49.68%, respectively, and decreased malondialdehyde (MDA) of plants dramatically compared to un-inoculated plants (P<0.05).

3.1-148 Arbuscular mycorrhizal fungi alter plant interspecific interaction under the addition of nitrogen

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Abstract: Arbuscular mycorrhizal fungi (AMF) form linkage with plants and ubiquitous in agricultural ecosystems. AMF are acknowledged to contribute to plant nitrogen (N) uptake, but it is critical to understand how N-addition affects AMF and plant interspecific interaction. However, AMF inoculation response to N-addition for plant competitive interaction are still unclear. Thus, we studied the competitive interaction between mycorrhizal and non-mycorrhizal individuals of *Vicia faba*, *Hordeum vulgare*, and *Brassica napus* species differing in both biomass allocation and mycotrophic degree. Two nitrogen fertilizer treatments (N0 and N15 g N m^{-2} yr^{-1}) were used to originate nutritional differences across the three plant species. Species-specific variation in mycotrophy revealed evident differences in root/shoot biomass allocation in *B. napus*, higher mycorrhizal dependency in *V. faba*, and higher aggressivity of *H. vulgare* with *B. napus* (host vs non-host specie) in AMF inoculation under N0 treatment. This pattern was supported by our study where solitary *B. napus* displayed pronounced investments into root and shoot growth rather than in competition. In addition, *V. faba* indicated as a best competitor and obtained greater biomass across the treatments for the relative yield total. This is predominantly vital for sustainable agriculture for food security because dealing for higher AMF abundance and function may lessen or eliminate incentives for environmentally and costly problematic nitrogen.

3.1-149 Host genotype and plant phenological growth stage are important drivers of root-associated mycobiome

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Abstract: The importance of microbial communities in plant nutrition and health is repeatedly noticed. Microorganisms are ubiquitous in the ecosystem; they interact positively or negatively with plant roots in the rhizosphere or with above-ground plant parts. Fungal assemblage changes in the soil are affected by many factors. It's known that different plant species host specific microbial communities when grown on the same soil, and are able to suppress pathogenic organisms in the rhizosphere. In this study, the indigenous fungal pathogen and symbiotic arbuscular mycorrhizal fungal (AMF) community were studied in conventionally treated field soil by Illumina MISeq sequencing of ITS region. In total 315 soil and root samples were collected on different plant phenological growth stages of 21 potato cultivars. The interactions, possible pathogen suppression ability and the variation of mycobiome structure were determined. The results showed the variable richness of AMF and pathogenic fungi throughout the growing season. The study indicated that potato cultivar and plant growth stage were essential factors that altered pathogenic fungal community composition. In contrast, AMF community was not influenced either by cultivar nor plant growth stage. Despite the applied conventional management regimes, plant

roots were highly colonised by pathogenic fungi. Compared to the pathogens, the abundance of AMF in the plant roots was relatively low and insufficient to suppress pathogenic organisms.

3.1-150 Arbuscular mycorrhizal fungi of areas in different stages of natural regeneration in a tropical dry forest

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Abstract: Considering the importance of conservation of Caatinga, one of the most diverse arid biomes in the world, and the role played by the arbuscular mycorrhizal fungi (AMF) that provide to their hosts an adaptive strategy for surviving in the stressful conditions of semiarid environments, the aim of this study was to assess the impact of forest age on the FMA assemblages in the National Park of Catimbau, Pernambuco, Brazil. Soil samples were collected in July/2016 in four areas, one with natural vegetation and three in process of regeneration, each of them with 3 subareas: (A) area with native vegetation of Caatinga with ages estimated to be over 100 years old; (B) area in initial stage of natural regeneration with ages of 4, 6 and 10 years; (C) intermediate area in stage of natural regeneration with 17, 23 and 30 years; (D) area in late stage of regeneration that are 37, 40 and 45 years old. We compared the species richness of the community of spores, composition, abundance, and similarity among areas under different successional stages. Fifty-two species of AMF were identified, representing 18 genera and 11 families. *Acaulospora* and *Glomus* were the most recorded genera, with 25% and 16% of the taxa, respectively. In general, *G. macrocarpum* was the most abundant species with >78% of relative frequency. The highest FMA richness was recorded in the area of initial regeneration stage with 42 species, followed by the intermediate recovery area (31 species), the area of natural vegetation (28 species) and the area under late regeneration (24 species). Most of the taxa were exclusive (42.30%): 13 for the area of initial regeneration; four for the intermediate regeneration area; three for the native vegetation area and two for the late regeneration area. Members of Paraglomerales were only recorded in areas of initial regeneration. The results showed that forest age has a significant effect on FMA community; however, the age of the areas is not the main modulator in the succession of FMA assemblage, with an interdependent succession occurring, where the ecological succession of fungi occurs independently of the succession of the plant. While the complexity of the plant community occurs with increasing age, that of the FMA community tends to decrease with the age of the areas.

3.1-151 The diversity and distribution pattern of arbuscular mycorrhizal fungi in Taiwan conifer ecosystem

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Abstract: Arbuscular mycorrhizal (AM) fungi influence plant diversity and productivity. To elucidate their biodiversity patterns is fundamental to understand their community assembly mechanism and ecosystem functions. Using morphological identification and high-through put sequencing, we investigated distribution patterns and ecology of AM fungal diversity to address the relative effects of altitude and soil properties on their diversity and community compositions in *Chamaecyparis formosensis* forests. Classical method analysis revealed 26 AM fungal species including *Acaulospora*, *Glomus*, *Entrophospora*, *Sclerocystis*, *Cetraspora*, *Diversispora*, *Paraglomus*, *Racocetra* and *Scutellospora* along three altitudinal transects. Number of total AM fungal species and spore density

were not correlated with the altitude of the study sites. The composition of the AM fungal community was significantly different at each location and altitude. Changes of soil properties across an altitudinal gradient played an important role in shaping AM fungal diversity and community. Species richness was negatively correlated with total organic carbon. Total organic carbon also contributed remarkably to the variations of AM fungal communities. The dominated species, *Acaulospora laevis*, *A. morrowiae* and *Sclerocystis rubiformis* were found at about all elevations and locations, and their spore densities were not correlated with the altitude of the study site. Some taxa were more restricted in particular elevation and their spore density showed a correlation with the altitude. For example, spore density of *A. koskei* increased with increasing altitude. Primary result of molecular analysis showed that *Acaulopora*, *Rhizophagus* and *Sclerocystis* was the dominant AM fungal genus associated with *C. formosensis* roots.

3.1-152 Ecological networks between arbuscular mycorrhizal fungi and plant hosts following restoration in a tropical forest

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Abstract: A fundamental goal during ecological restoration is to return communities to comparable diversity as their natural counterparts. Prior studies of ecological restoration have tended to have an aboveground bias that neglects changes in diversity belowground following restoration efforts. This is an issue because often restoration efforts are unsuccessful, due to either biotic and/or abiotic constraints, both of which can be mitigated by soil microbial organisms. Multiple studies have also shown that soil-borne microbes, especially arbuscular mycorrhizal (AM) fungi, are important drivers of plant community development, accentuating the importance of examining changes in AM fungal diversity during ecological restoration. Here, expand our understanding of belowground community responses to ecological restoration by examining changes in AM fungal community structure following ecological restoration efforts in a degraded Hawaiian tropical forest. For this study, both intact and restored sites were sampled within the Hakalau Forest National Wildlife Refuge on the Island of Hawai`i, where ecosystem recovery attempts have been ongoing since 1985. Within sites we sampled the roots and soil of seven AM fungal plant hosts that were found in both intact and restored patches, and characterized AM fungal diversity using Illumina sequencing. In addition to examining general AM fungal community composition patterns, we also examined interaction networks between plants and AM fungi to determine how network architecture differs between remnant and restored forest patches. We predicted that communities would be significantly influenced by patch type, and that interaction networks between plants and AM fungi would vary in robustness between remnant and restored patches, where networks in restored patches would be significantly less complex, making them more susceptible to perturbation than intact forest networks. Furthermore, we predict that a few species of AM fungi will be dominant within both patch types, where taxa with ruderal life history strategies would be dominant within restored patches, while taxa with competitive life history strategies would be dominant within intact patches.

3.1-173 Expanding the known diversity of North American fetid *Russula*

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Abstract: *Russula* is a highly diverse genus of ectomycorrhizal fungus. Over 700 species of this genus are known making up about 10% of the known ECM fungi in world. One especially intriguing group of *Russula* are the members of subsection Foetentineae, commonly referred by some as the fetid *Russula* since their brown to light yellow capped sporocarps regularly have unpleasant smells and tastes. Their

ecological attributes include the ability to be "dominant" (i.e. numerically abundant) in temperate, boreal and tropical ecosystems worldwide, to persist in high nitrogen environments, and to associate with mycoheterotrophic plants. Despite these ecological attributes, much of the diversity of this group remains unknown and/or fully documented. In this presentation, we will provide a thorough overview of the molecular diversity (using ITS and RPB based phylogenies) of fetid *Russula* in North America and place this overview within a context of the current understanding of subsection Foetentineae globally. We will also highlight a set morphological and molecular characteristics of two groups of North American fetids, *Russula garyensis* and *R. amerorecondita* currently under consideration as species new to science.

3.1-174 Diversity and phylogeny of *Russula* subgenus *Compactae*

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Abstract: Within the genus *Russula*, one of the major ectomycorrhizal fungal genera in all ecosystems worldwide, *R.* subg. *Compactae* is a large subgenus, mainly characterised by the presence of lamellulae which are absent in the rest of the genus. The fruitbodies are rather big, firm and compact with a short and thick stipe and a white, yellowish or brown cap. The context is often browning or blackening, sometimes reddening. The subgenus consists of three main groups: *R.* sect. *Compactae*, *R.* sect. *Lactarioides* and the oldest group *R.* sect. *Archaeinae*. Especially the first two groups are characterized by a high diversity, with several species complexes and undescribed species. In a first part of this project, we aim to make a general phylogeny of *Russula* to get a clear placement of each of these groups. Hence, we will test the hypothesis that *R.* subg. *Compactae* is monophyletic, placed at the base of the *Russula* phylogeny. The preliminar data suggest that our hypothesis about the monophyly of *R.* subg. *Compactae* will be rejected, but the position of the different sections is still unclear. The second objective of this project is to delimit species within *R.* subg. *Compactae* in Europe. We aim to unravel the species complexes and describe new species through phylogenetic and morphological analysis. As it is thought that ecology might play an important role in speciation, we also focus on mycorrhizal host associations. An analysis was done on the existing data and a phylogenetic tree was created. Based on this tree we assume the existence of at least 15 undescribed species within *R.* subg. *Compactae* in Europe in our current sequence dataset. This dataset contains sequences mostly from Europe, some from Brazil, Martinique, Thailand, North-America and Africa.

3.1-175 Molecular phylogenetic analysis reveals new and noteworthy *Lactarius* spp. in different subgenera from Pakistan

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Abstract: *Lactarius* is a large genus of agaric fungi in Russulaceae (Basidiomycota, Agaricales) with more than 600 species, commonly known as milk-caps. The members of the genus are characterized by the milky fluid they exude when cut or damaged. They are ectomycorrhizal and form symbiosis with trees species belonging to different families. Due to these ectomycorrhizal associations, *Lactarius* is one of the dominant agaric genus in many ecosystems, from the boreal forests to the temperate ones, subtropical woodlands and tropical low lands in South-East Asia. The estimated number of species in the genus is up to 700. From Pakistan, six species have been reported to date. These species have been reported on the basis morphological characters. The molecular method of characterization of these

species spilt the genus into several subgenera. The objective of our study is to document fungal flora of Pakistan on the basis of molecular phylogenetic analysis and morpho-anatomical characterization. Genomic DNA was extracted using 2% CTAB method. The polymerase chain reaction (PCR) was carried out using the fungal-specific ITS1F primer and the eukaryotic ITS4 primer to amplify the nuclear ribosomal internal transcribed spacer region. For 28S region amplification, LR0R and LR5 primers were used. For anatomical studies, tissues from pileus, stipe and gills were mounted in 5% KOH and observed under microscope (LABOMED, Labo America, Inc. USA). During this investigation, a total of seven collections have been examined, out of these, four *Lactarius* species have been identified which fall in all subgenera of *Lactarius* well supported by morphological characters and molecular phylogenetic analysis based on ITS sequences. *Lactarius* sp. ST-01, ST-04 and ST-05 fall in subgenus *Piperitus*, *Lactarius* sp. SH-01 falls in subgenus *Russularia* and *Lactarius* sp. ST-10 and ST-26 falls in subgenus *Plinthogalus*. All these species found to be distinct morphologically and phylogenetic data based on ITS region confirmed their identity. *Lactarius scrobiculatus* has been identified on the basis of morphological characters and its molecular phylogenetic analysis supported its systematics in subgenus *Piperitus*. The work on these taxa is in progress, more collections are being examined which will lead to the provision of nomenclature to the previously undescribed species.

3.1-176 Filling the gaps: completing the *Lactifluus* phylogeny by assessment of its Neotropical biodiversity and building a bRIDge between taxonomists and environmental ecologists.

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Abstract: The Research Group Mycology from Ghent University has a large expertise in the Russulaceae family, a family of important ectomycorrhizal fungi. Traditionally the two main groups were russulas and milkcaps, but since 2008 we know that milkcaps are paraphyletic and divide them over the genera *Lactarius*, *Lactifluus* and to a lesser extent *Multifurca*. There are some striking differences between *Lactarius* and *Lactifluus* concerning their distribution, phylogeny and evolutionary history. *Lactifluus* has a mainly tropical distribution and is well-studied in tropical Africa and Southeast Asia. However, little is known about its diversity in the Neotropics because for a long time ectomycorrhizal fungi were wrongfully assumed absent in most South American ecosystems, leaving one big gap left in our world-wide phylogeny. Since 2015 we have been working to assemble a dataset based on herbarium specimens, specimens collected by collaborators and specimens collected during expeditions in Martinique, French Guiana and Brazil. Sequence data indicates the presence of at least 58 *Lactifluus* species in the Neotropics, so it is clear that there is in fact a large diversity of *Lactifluus* species in the Neotropics contrary to previous expectations. These neotropical species occur in three out of four subgenera and they form entirely new clades/sections. So far, the Lesser Antilles have been studied in detail. This area was already well studied morphologically before and our phylogenetic study revealed a total of 10 species in the Lesser Antilles, of which 3 new species. A new identification key to the species was constructed. The completed worldwide phylogeny will provide a much-needed taxonomical framework for environmental ecologists. In function of this, a well-documented database is being established with historical and recent data of Neotropical *Lactifluus* specimens, which will in the future be extended to Russulaceae worldwide. The database will be called bRIDge (Russulaceae Identification Database) and is built with BioloMICS Software for biological data management, identification, classification and statistics. In the database the records are linked to photographs, microscopical illustrations, ecological data and sequences. Environmental ecologists will be able to consult this database to interpret (part of) their datasets.

3.1-193 Survival and growth of western white pine (*Pinus monticola*) is not impeded by association with *Armillaria altimontana* in northern Idaho

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Abstract: *Armillaria solidipes* is well known as a cause of root disease on diverse conifers in areas of inland, western North America, where *A. altimontana* also frequently co-occurs. In contrast, impacts of *A. altimontana* on tree health are not well known. At the Priest River Experimental Forest in northern Idaho, USA, a provenance planting of western white pine (*Pinus monticola*) was established in 1971 in a 0.8-ha plot to examine growth and survival. In 1987 (16 years post-planting), measurements, inspections, and sampling were conducted on 1215 living/recently dead trees to determine potential influences of *A. solidipes* and *A. altimontana* on growth and survival of western white pine. Of these trees, 48% were associated with *A. solidipes* and/or *A. altimontana*. Somatic pairing tests were used to condense the *Armillaria* isolates into unique genets and translation elongation factor 1-α sequencing was used for species identification. Results demonstrated that the plot contained two genets of *A. altimontana* comprising ca. 83% of the isolates and five genets of *A. solidipes* comprising ca. 17% of the isolates. As expected, *A. solidipes* was associated with decreased tree growth and survival. In contrast, *A. altimontana* was not associated with increased tree mortality or decreased tree growth, which suggests that *A. altimontana* was not harmful to western white pine within this northern Idaho planting. Maps of *Armillaria* distribution within the plot indicate that *A. solidipes* was uncommon in areas dominated by *A. altimontana*. Furthermore, the wide spatial span of individual *Armillaria* genets suggest that this site has been occupied by both *Armillaria* spp. for >250 years. Interactions between these two *Armillaria* species appear critical to understanding Armillaria root disease in this region.

3.1-194 *Armillaria mexicana*, a recently described species from Mexico

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Abstract: *Armillaria mexicana* (Agaricales, Physalacriaceae), a recently characterized species from Mexico (Elías-Román et al. 2018), was described on the basis of morphology and phylogenetic analyses that show it is clearly distinct from previously reported species in North, Central, and South America. Morphological characteristics of *A. mexicana* include the absence of fibulae in the basidioma, abundant cheilocystidia, and ellipsoidal, hyaline basidiospores that appear smooth with light microscopy, but slightly to moderately rugulose with scanning electron microscopy. Macro-morphological characters, such as annulus structure, pileus coloration, stipe coloration, and other structures, are useful to distinguish *A. mexicana* from other *Armillaria* spp. Furthermore, phylogenetic analyses of the nuc rDNA internal transcribed spacers (ITS1-5.8S-ITS2 = ITS), 28S D-domain, 3′ end of 28S-Intergenic spacer 1, and translation elongation factor 1-α (*TEF*1) clearly show that *A. mexicana* sequences are quite distinct from sequences of other *Armillaria* species. Interestingly, sequences of ITS sequences of the *A. mexicana* ex-type culture reveal an ITS1 of 1,299 bp and ITS2 of 582 bp, which represent the longest ITS regions reported thus far in fungi. Phylogenetic analysis based on *TEF*1-based phylogenetic analyses place *A. mexicana* within a well-separated, monophyletic clade basal to the polyphyletic *A. mellea* complex,

which may represent the earliest evolving lineage of *Armillaria* in the Northern Hemisphere. Presently, *A. mexicana* is known to be highly virulent on planted peach (*Prunus persica*) and other tree species in central Mexico.

3.1-195 Improving the management of *Armillaria* in gardens

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Abstract: Honey fungus (*Armillaria* spp.) is one of the most common diseases troubling gardeners in the UK. Seven species are found in the UK but only three were found during our four-year garden survey (2004-7): *A. mellea* (83.1%); *A. gallica* (15.8%); and *A. ostoyae* (1.1%). Honey fungus has a large host range, causing death and decline of trees, shrubs and non-woody plants such as bulbs and vegetables. Because of its broad host range, garden choices are restricted if susceptible hosts are to be avoided when replanting. Gardeners want to know the best ways to manage and control the disease. Currently management and control options for gardeners are costly, often difficult to perform in gardens and labour intensive, and thus they are often unsuccessful. Our research aims to improve understanding of the disease and increase the success of *Armillaria* management in gardens. We are engaged in projects to examine (1) a new approach to disease prevention, (2) the importance of species-specific diagnosis for gardeners, and (3) the efficacy of current management protocols. Firstly, the potential of *Trichoderma* endophytes to improve host resilience and promote growth during plant establishment was studied. The introduction of suitable isolates would enable gardeners to replant in *Armillaria*-infected beds. Endophytes sampled from root systems of susceptible hosts within infection foci at RHS Garden Wisley, were cultured, identified and screened for beneficial effects on common garden plants (*Rosmarinus officinalis* and *Thymus vulgaris*). Isolation of *T. atrobrunneum* occurred most frequently, and showed improved host growth and *in vitro* inhibition of *A. mellea*. Secondly, the interactions of different *Armillaria* species was studied. We hypothesised that the less virulent but highly rhizomorphic species *Armillaria gallica* can occupy the same niche and prevent invasion from the aggressive species *A. mellea*. The implication being that gardens with *A. gallica* could be 'protected' from more pathogenic species. Privet plants were inoculated twice with infected hazel stick inoculum. The first inoculation was either a control (sterile hazel stick) or with *A. gallica*. The second inoculation (3 or 6 months later) was either a control or with *A. mellea*. Plant vigour and mortality is being measured over time, and isolations and molecular analysis will be used to determine the *Armillaria* species colonising host tissues at harvest. Our final research direction concerns the growth and survival of rhizomorphs once severed from their food source. Present advice to gardeners is to remove as much of an *Armillaria*-infected plant as possible and cultivate the soil. However, it's virtually impossible to remove all small fragments, including rhizomorphs, so information on survival and growth of severed rhizomorphs would be beneficial to validate our advice to gardeners. In order to test this, rhizomorphs of *A. mellea* and *A. gallica* were cut from their food source and their growth and viability in compost and loam were examined compared to rhizomorphs of the same length still attached to a woody substrate. Following the completion of these projects we aim to use a combination of information from the results to create a suitable Armillaria management program.

3.1-196 Pathogenicity of a parasitic macrofungus in the southern regions of Tanzania

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Abstract: The southern regions of Tanzania are highly vulnerable to food insecurity, and a collapse of the agroforest industry and poverty due to main cash crop failure is threatening. This is caused by a pathogenic macrofungus. The fast expanding outbreak has devastating consequences since the fungus attacks and causes wilting of cashew trees, which is the main cash crop in the region, cassava which is the main staple food, eucalypts and some other trees. This work has investigated the pathogenicity of the fungus by isolating its germ plasm and re-inoculating it to uninfected plant cultivars. The re-inoculated cultivars showed the same disease symptoms as those of the wilting plants. DNA of the pathogenic macrofungus was re-isolated from the plant cultivars which showed symptoms of being infected. Anatomical study of the infested plants from the field showed that the fungus is present in the plant vascular tissues as evidenced by the presence of mycelium and extra-cellular polysaccharides in the xylem vessels. Excessive growth of the mycelium in these vessels most probably interferes with the translocation of nutrients by blocking the flow in phloem and xylem vascular bundles which leads to plant stress, then chlorosis, wilting and ultimately to death. The spread of this pathogenic fungus has to be tackled immediately and with great concern since the Southern regions are the main producers of Cashew in Tanzania, and the population depends heavily on the cassava as their staple food and both of these crops are heavily affected.

3.1-197 Pathogens or Associates? *Leptographium* and *Grosmannia* blue-stain fungi associated with dying loblolly pine in Georgia

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Abstract: *Leptographium* and *Grosmannia* spp. (order Ophiostomatales) and their root-feeding beetle vectors (e.g., *Hylastes, Hylobius*, and *Pachylobius* spp.) have been reported from areas of loblolly pine (*Pinus taeda* - the most commercially important tree species in the southeastern United States) dieback in recent decades. However, species composition of the fungal complex associated with these beetles, and the frequency of association between individual fungal and vector species is currently unknown, with unclear implications for forest management. Our research objectives are to: 1) determine the phenology of loblolly pine root-feeding beetles in Georgia loblolly pine stands; 2) assess the diversity and composition of the Ophiostomatoid fungal complex associated with these root-feeding taxa; and 3) analyze whether pathogen pressure or vector species composition varies in stands with various management practices and across multiple seasons. Root-feeding beetles were live-trapped around girdled loblolly pine trees at two sites in central Georgia with differing management histories [regularly prescribe burned (every 2-3 years) versus unburned (at least for 5 years)] and across seasons (spring, summer, and fall). A subset of collected beetles from each target species was used to isolate and identify associated Ophiostomatoid fungi based on morphological and molecular features. During 2017, we collected *H. porculus*, *H. salebrosus*, *H. tenuis*, *H. pales* and *P. picivorus* beetles with notable variation in numbers of *H. pales,* the most abundant beetle species. Total number of beetles was higher in the burned than unburned sites, suggesting possible attraction of these root-feeding beetles to the burned areas. Three *Leptographium* and *Grosmannia* species with varying reported degrees of virulence (i.e., *L.*

procerum, G. alacris, and *G. huntii)* have been identified via sequencing of the β-tubulin gene, with the potential for other species to be identified. Species-specific PCR primers will be developed for each fungal species identified via sequencing and fungal DNA extracted directly from the integument of the adult beetles to determine fungal species composition and incidence per beetle species. Determination of the species composition of the fungal complex associated with loblolly pine root-feeding beetles in Georgia is a crucial first step to determining their role, if any, in affecting tree and stand health.

3.1-198 Genetic and genomic approaches toward understanding the biology of the Korean oak wilt fungus (*Raffaelea quercus-mongolicae*)

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Abstract: *Raffaelea quercus-mongolicae* is associated with oak wilt disease in Korea. To date, this ambrosia beetle-vectored fungus has only been found in South Korea, and it is phylogenetically distinct from *R. quercivora*, which causes a similar oak wilt disease in Japan, and other *Raffaelea* spp. When this fungus was discovered on a dead Mongolian oak (*Quercus mongolica*) in 2004, the disease epiphytotic was centered around Seoul and the adjacent Gyeonggi Province; however, it has continued to spread southwards since then. Despite continued expansion of the disease and associated major impacts on forest ecosystems, little genetic and genomic information of *R. quercus-mongolicae* available for understanding its biology, evaluating pathways of spread, and developing improved disease prediction and management methods. The objectives of this study were to assess genetic diversity and population structure of the Korean oak wilt fungus, *R. quercus-mongolicae*, using Restriction-site-Associated-DNA sequencing (RAD-seq); sequence the whole genome of *R. quercus-mongolicae*; and analyze the transcriptome (expressed genes) of *R. quercus-mongolicae* by RNA sequencing and reference genome-based assembly. Fifty-four isolates of *R. quercus-mongolicae* were collected from five regions of South Korea. RAD-tag libraries and DNA sequencing were conducted at Floragenex, Inc. (Eugene, OR, USA). The draft genome of *R. quercus-mongolicae* (KACC44405) was sequenced by Illumina NextSeq and MiSeq systems for paired-end reads and HiSeq2000 for mate-pair reads. Total RNA was extracted from *R. quercus-mongolicae* (KACC44405) grown under *in vitro* conditions on two artificial media [potato dextrose agar (PDA) and water agar (WA)]. The mRNA was sequenced using Illumina HiSeq™2000 with a read length of 101 bp. Trimmed sequences were mapped to reference genome. Sequencing the RAD-tag libraries generated 143,696,855 reads using Illumina HiSeq. In total, 179 SNPs were identified among 2,639 RAD loci across the nuclear genome of the 54 *R. quercus-mongolicae* isolates (0.00080 SNPs per bp). Overall low expected heterozygosity and no apparent population structure were found among South Korean populations *R. quercus-mongolicae*, which supports the hypothesis that this ambrosia beetle-vectored fungus was introduced to South Korea. The genomic sequence of *R. quercus-mongolicae* (KACC44405; 27-Mb), along with other *Raffaelea* spp., will provide valuable resources for comparative genomic analyses and identifying genes that contribute to potential pathogenic relationships between the fungus and host, and potential symbiotic relationships between the fungus and insect vector. After mapping with a reference genome, 7,739 transcripts were identified as the *R. quercus-mongolicae* transcriptome dataset. The predicted gene products are associated with diverse functions, such as production of ATP for growth, recovery under stressed conditions, fluidity of cell membrane, maintenance of cell membrane, and regulation of fungal virulence. The transcripts that differed significantly in expression levels between PDA and WA media were associated with localization within subcellular components, hydrolase activity, and intrinsic membrane components.

3.1-199 Diversity of potential emerging pathogens of conifers in Colorado, USA

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Abstract: Conifer foliage possess dynamic communities of microorganisms that may have either beneficial or parasitic relationships with its host plant. The increasing prevalence of disease emergence factors like climate change and globalization can favor the pathogenicity of introduced fungal species and/or enhance virulence of pathogens within the needle community structure. The lack of distinct morphological and molecular information on these conifer foliage pathogens make it also challenging to identify them and recommend appropriate control measures. This ongoing study initially surveyed the health condition of pine forests in Colorado and evaluated the diversity of pathogens in needles of selected conifer host species. Five conifer species (i.e. limber, ponderosa, lodgepole, bristlecone and Himalayan pine) which were recorded to exhibit disease symptoms were sampled from various regions in Colorado, USA. Morphological and genetic characteristics were used to identify pathogenic and non-pathogenic fungal isolates. Known pathogens were assessed based on symptoms, life history, and its distribution and spread. Disease symptoms of host species included needle cast, blight, and red bands. Black fruiting bodies were recorded in some needles of limber and ponderosa. In general, the most common pathogenic species isolated included *Sydowia polyspora*, *Lophodermium* sp., *Rhizosphaera* sp., and *Cytospora* sp. Most of these pathogenic species were associated with necrotic diseases. *S. polyspora* were found in three host species (limber, ponderosa, and Himalayan pine) while *Lophodermium* and *Rhizosphaera* were commonly observed only in limber. Known pathogens of several angiosperms such as *Alternaria alternata* and *Phaeomoniella effusa* were also isolated from lodgepole and limber. Results of the study are relevant for the development of molecular tools for pathogen identification and disease risk assessment. Further studies on interactions of pathogens within host species and its impacts on disease development are also recommended.

3.1-200 Microsatellite characterization of *Penicillium digitatum*, causal agent of green mold of citrus

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Abstract: *Penicillium digitatum* is one of the most important post harvest pathogens of citrus on a global scale causing significant annual losses due to fruit rot. However, little is known about the diversity of *P. digitatum* populations both within the field and subsequently after processing where significant post harvest treatments for control could influence the population dynamics. The genome of *P. digitatum* was recently sequenced, providing an opportunity to determine the microsatellite distribution within *P. digitatum* to develop markers that could be valuable tools for studying the population biology of this pathogen. In the analyses, mono, di, tri, tetra, penta, and hexanucleotide microsatellites within the genome of *P. digitatum* were restricted to 12 repeats for mononucleotides and above and the rest were restricted to 5 repeats and above. A total of 3,134 microsatellite loci were detected; 66.73, 23.23, 8.23, 1.24, 0.16, and 0.77% were detected as mono, di, tri, tetra, penta, and hexanucleotide repeats, respectively. As consistent with other ascomycete fungi, the genome size of *P. digitatum* does not seem to correlate with the density of microsatellite loci. However, significantly longer motifs of mono, di, and tetranucleotide repeats were identified in *P. digitatum* compared to 10 other published ascomycete species with repeats of over 800, 300, and 900 motifs found, respectively. One isolate from southern

California and 5 additional isolates from other countries ('global isolates') were used to initially screen microsatellite markers utilizing the genome to find candidate loci and Primer3 to design primers. Twelve additional isolates, referred to as the 'local isolates', were also collected from the University of California Riverside citrus collection and were subsequently used to screen the primers that sequenced well and were polymorphic based on the global isolates. Thirty-six primers were screened but nine trinucleotide loci and one hexanucleotide locus were chosen as robust markers. These loci yielded 2 to 3 alleles within the global isolates and 2 to 7 alleles in the local isolates. These markers will be useful to study population genetic differentiation, migration, genetic drift, mutation rates, and other ecological and evolutionary processes that shape *P. digitatum* populations.

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3.2-9 Intraspecies genetic variations of *Hirsutella sinensis* (Genotype #1 of *Ophiocordyceps sinensis*) and transcriptome expression variations

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Abstract: Genetic heterogeneity of *Ophiocordyceps sinensis* with 17 genotypes of *O. sinensis* in natural *Cordyceps sinensis* has been documented. But intraspecies genetic variations of Genotype #1 *Hirsutella sinensis* are unknown. The objective was to analyze the intraspecies genetic variations of *H. sinensis*. Methods: To compare 3 assembled *H.* sinensis genome sequences, ANOV00000000 of strain Co18 [Hu et al.2013], LKHE00000000 of strain 1229 [Li et al.2016], and LWBQ00000000 of strain ZJB12195 [Liu et al.2016], a set of unassembled shotgun genome sequences (JM973567-JM973820) of strain YN07-8 [Zhang et al.2012], 2 sets of transcriptome sequences, GAGW00000000 of natural *C. sinensis* [Xiang et al.2014] and GCQL00000000 of strain L0106 [Liu et al.2015]. Results: (1) There are 2 large DNA segment insertions/deletions in the genome sequences ANOV01002305, LWBQ01000138, and LKHE01001489. (2) Multiple, scattered point mutations were found between ANOV01021101 and LKHE01000642. (3) Multiple, scattered transition and transversion point mutations and DNA segment insertion/deletion mutations were noted between the genome sequences LKHE01000676, LWBQ01000084 and ANOV01001676, between the sequences LWBQ01000028, ANOV01000226/ANOV01006525, and LKHE01002847, and between the genome sequences LWBQ01000037, LKHE01000176/LKHE01002503/LKHE01003221, and ANOV0100526/ANOV01001961/ANOV01009408/ANOV01009409/ANOV01013329. (4) There are 42 of 254 unassembled shotgun sequences having low similarities with the 3 assembled genome sequences. (5) Unassembled shotgun sequences, JM973567, JM973713, JM973797 and JM973816, are absent in the 3 assembled genome sequences, while JM973567 is 100% homologous to *Stenotrophomonas maltophilia* CP011010. (6) JM973601 shares 96.5% and 94.2% similarities with the 2 transcriptome sequences of natural *C. sinensis* (GAGW01002159) and strain L0106 (GCQL01017221), and 91.7% similarities with the 3 genome sequences. (7) JM973579 is >99% homologous with the transcriptome sequence GAGW01000465 of natural *C. sinensis* and the genome sequence LKHE01000582, but only 87.2% and 86.2% with the transcriptome sequence GCQL01019215 of strain L0106 and the genome sequence ANOV01022831. (8) JM973799 is 99.7% homologous with the transcriptome sequence GCQL01011878 of strain L0106, but there is a 89-nt segment deletion in the transcriptome sequence GAGW01001648 of natural *C. sinensis*. (9) Aligning with translated genome and transcriptome sequences, the amino acid sequence EQL03991 of strain Co18 was found >97.5% homologous with the transcriptome sequence GCQL01017221 of strain L0106 and the 3 genome sequences, but 79.5% and 85.9% with the transcriptome sequence GAGW01002159 of natural *C. sinensis* and the unassembled shotgun sequence JM973601. Discussions: There are apparent intraspecies genetic variations among the *H. sinensis* strains, warning future molecular studies on the type strain HMAS 55469 of *H. sinensis* [Liu et al.1989], and suggesting avoidance of arbitrary selection

of *H. sinensis* strains in research and probably also in industrial fermentation. (Supported by Grant #2017-SF-118 from the Science-Technology Department of Qinghai Province)

3.2-10 Several genotypes of *Ophiocordyceps sinensis* and *Paecilomyces hepiali* coexist in the two types of heterokaryotic *Cordyceps sinensis* ascospores

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Abstract: The scientific literature reported the detections of 17 genotypes of *Ophiocordyceps sinensis* and the changes of their biomasses in a dynamic, asynchronous fashion in the stroma and caterpillar body of natural *Cordyceps sinensis* during maturation. The objective was to examine the ejection and fungal compositions of the *C. sinensis* ascospores. We collected and cultivated mature *C. sinensis*, to observe the ejections of the ascospores, and to examine the morphology of the ascocarps and ascospores under a microscope and the ascosporic fungal components, including the reported *C. sinensis* associated fungi: *Geomyces pannorum*, *Hirsutella sinensis, Paecilomyces hepiali*, *Paecilomyces sinensis*, *Pseudogymnoascus roseus*, and *Tolypocladium sinense*, and mutant genotypes of *O. sinensis* with using fungal- and genotype-specific primer PCR, amplicon cloning-sequencing, and SNP mass-spec genotyping. Results: Two types of the ascospores were observed from the same specimen of *C. sinensis*: the fully ejected ascospores and the semi-ejected ascospores that hang on and tightly connected to the outside surface of the openings of perithecia. Microscopic examination revealed multicellular heterokaryotic structure of the ascospores, consistent with the findings by Bushley et al.[2013] of the multicellular heterokaryotic ascospores with mono-, bi-, and tri-nucleates. PCR with using fungal specific primers and amplicon cloning-sequencing did not detect the ITS sequences of *G. pannorum*, *P. chrysogenum*, *P. roseus*, *P. sinensis*, or *T. sinense* in the genome DNA samples extracted from the 2 types of the *C. sinensis* ascospores, although these fungi were detected in *C. sinensis* and some of them dominate in the stroma or caterpillar body [Zhang et al.2010]. The ITS sequences of Genotypes #1 *H. sinensis*, #5-#6, #14, #16 of *O. sinensis*, AB067719-type *O. sinensis* and *P. hepiali* were identified in the fully ejected ascospores, but Genotype #13 instead of Genotypes #6, #14, #16 in the semi-ejected ascospores. The biomass of *P. hepiali* was significantly greater in semi-ejected ascospores than in fully ejected ascospores. Two newly discovered Genotypes #13 and #14 of *O. sinensis* feature hereditary variations with DNA segment reciprocal substitution between 2 parental fungi, Genotype #1 *H. sinensis* and AB067719-type *O. sinensis*. The 2 types of multicellular heterokaryotic ascospores feature genetic heterogeneity, containing multiple genotypes of *O. sinensis*, AB067719-type *O. sinensis*, and *P. hepiali*. The findings of the Genotypes #13 and #14 of *O. sinensis* with the DNA segment substitution hereditary variations suggest fusions of chromosomes of 2 parental *O. sinensis* fungi, and even possible mating type(s) (heterothallic or hybrid mating) or hyperparasitism. The fugal components, both the various biomasses of *P. hepiali* and the presence of Genotype #13 or #14 in either type of the ascospores, appear to be of biological importance that determine the formation, maturation, and ejection processes of the *C. sinensis* ascospores. (Supported by Grant #2017-SF-118 from the Science-Technology Department of Qinghai Province)

3.2-11 Indiscriminate use of the name *Ophiocordyceps sinensis* for multiple genotypes of *O. sinensis* fungi and the wild insect-fungi complex

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Abstract: Numerous papers reported detections of 17 genotypes of *Ophiocordyceps sinensis* in natural *Cordyceps sinensis* that consists of a dead larva from the Family *Hepialidae* and multiple intrinsic fungi. Natural *C. sinensis*, a precious traditional Chinese substance, has been used in clinic as a folk medicine in China for 6 centuries. The 17 genotypes of *O.* sinensis include Genotype #1 *Hirsutella sinensis*, Genotypes #3-#6,#15-#17 (transition mutants), Genotypes #7-#11 (transversion mutants), Genotypes #2,#12 (insertion/deletion mutants), and Genotypes #13-#14 (DNA segment reciprocal substitution hereditary variations) [Zhu et al.2016]; Genotypes #1-#3,#7-#12 are GC-biased, while Genotypes #4-#6,#15-#17 are AT-biased. The multiple genotypes of *O. sinensis* coexist differentially in the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis*; their biomasses are changing in a dynamic, asynchronous fashion during the maturation of *C. sinensis*. The sequences of Genotypes #2-#17 belong not to the genome of Genotype #1 *H. sinensis*, but to the genomes of independent *O. sinensis* fungi. All 17 *O. sinensis* genotype fungi share the same Latin name, which has compounded the historical problems since the middle of 19th century, associated with these fungi indiscriminately sharing the same Latin name with the natural insect-fungi complex. The history of and current perspectives on the indiscriminate use of the Latin names *C. sinensis* and *O. sinensis* are now for the multiple genotypes of *O. sinensis* fungi, for the anamorphs and teleomorphs of *O. sinensis* according to the fungal nomenclature rule established by the Amsterdam Declaration of "One Fungus=One Name", and for the natural *C. sinensis* insect-fungi complex. For partially solving the problem of the indiscriminate practice, Zhang et al.[2012] proposed for using *O. sinensis* for the fungi and a non-Latin name "Chinese cordyceps" for *C. sinensis* insect-fungi complex. This proposal, however, did not reach a consensus because government regulations worldwide require every natural product used as a folk medicine or a dietary supplement to have an unique, exclusive Latin name and because of unnecessity of having an additional non-Latin name for natural *C. sinensis* when several such names have been used for centuries. Alternatively Ren et al.[2013] proposed for using "*Ophiocordyceps* and *Hepialidae*" to reflect the nature of *C. sinensis* fungal-insect complex. Discussion on the second proposal has to be postponed because all 17 genotype fungi currently under the name of *O. sinensis* may or may not be variants within the *O. sinensis* species or within the genus *Ophiocordyceps* Petch, also because *Tolypocladium sinensis* has been confirmed as one of the anamorphs of *O. sinensis* [Barseghyan et al.2011], and Quandt et al.[2014] have proposed "for *Ophiocordycipitaceae* (*Hypocreales*) with new combinations in *Tolypocladium*". This taxonomy-nomenclature problem invites taxonomists across disciplines to characterize the multiple mutant genotype fungi, most of which are currently difficult to be cultured and identified through PCR with using common primers, and to end the centuries-old academic confusion on the indiscriminate use of the Latin name *O. sinensis* for multiple *O. sinensis* fungi and the natural insect-fungi complex. (Supported by Grant #2017-SF-118 from the Science-Technology Department of Qinghai Province)

3.2-12 Outcome of blue, red, and white light on *Metarhizium robertsii* during mycelial growth on virulence, conidial stress tolerance, and gene expression

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Abstract: Light conditions during fungal growth are well known to cause several physiological adaptations in the produced conidia; thus, conidia of the insect-pathogenic fungi *Metarhizium robertsii* were produced on: 1) potato dextrose agar (PDA) medium in the dark; 2) PDA medium under white light; 3) PDA medium under blue light; and 4) PDA medium under red light. The conidial production, the speed of conidial germination, the virulence to the insect *Tenebrio molitor*, as well as gene expression, and tolerances to osmotic stress and to UV radiation were evaluated. Conidia produced under white light or blue light germinated faster and were the most tolerant to UV radiation and osmotic stress. White light improved conidial virulence as compared with conidia produced in the dark. Growth under blue light produced more conidia than the fungus grown in the dark. The small (*Mrhsp30*) and large (*Mrhsp101*) heat shock protein genes were highly up-regulated under white light condition, suggesting an active role of heat shock proteins in fungal exposition to the different visible spectrum components. The cytosolic catalase *Mrcatc* gene was not induced under all light conditions assayed. Conidia produced under red light germinated slower than conidia produced in the dark and were the least tolerant to osmotic stress and UV radiation. The virulence of conidia produced under red light was similar to conidia produced in the dark. In conclusion, white light produced conidia that germinated faster and killed the insects faster; in addition, blue light afforded the highest conidial production. Both white light and blue light afforded the highest tolerance to both stress conditions. This research was supported by grants from the National Council for Scientific and Technological Development (CNPq) of Brazil PQ2 302312/2011-0, and PQ1D 308436/2014-8 and the São Paulo Research Foundation (FAPESP) 2010/06374-1 and 2013/50518-6.

3.2-13 Systematic functional profiling of phosphatases in the fungal pathogen *Cryptococcus neoformans*

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Abstract: *Cryptococcus neoformans* causes fatal cryptococcal meningoencephalitis in immunocompromised patients as well as immunocompetent people. Despite its clinical importance, the signaling networks governing its virulence remains elusive and therapeutic options for treatment of systemic cryptococcosis are limited. Here, to understand signaling networks regulating the virulence of *C. neoformans*, we aim to identify and functionally characterize the 139 putative phosphatases, which are major signaling components in the basidiomycete fungal pathogens. We selected putative phosphatases based on annotation in the *C. neoformans* var. *grubii* genome database provided by the Broad Institute and National center for Biotechnology Information (NCBI) and performed a BLAST search with their protein sequences to identify any corresponding orthologs in *S. cerevisiae*, *A. nidulans*, *C. albicans* and *F. graminearum*. We classified putative phosphatases into 16 groups based on InterPro phosphatase domain annotation. Thus far, we have successfully constructed 227 signature-tagged gene-deletion strains representing 114 putative phosphatases through homologous recombination

methods. We are in the middle of examining their phenotypic traits under 30 different in vitro conditions, including growth, differentiation, stress response, antifungal resistance and virulence-factor production. Along with our previous functional genetic studies for *C. neoformans* transcription factors and kinases, this study will provide a comprehensive insight into the fungal signaling networks.

3.2-14 Functional characterization of transcription factors, Hob1 and Sre1, regulating sterol-homeostasis in *Cryptococcus* species complex

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Abstract: Sterol lipid is essential for cell membrane structure in eukaryotic cells. In mammalian cells, sterol regulatory element binding proteins (SREBPs) act as principal regulators of cellular cholesterol which is essential for proper cell membrane fluidity and structure. SREBP and sterol regulation are related to levels of cellular oxygen because it is a major substrate for sterol synthesis. Upon cellular sterol and oxygen levels are depleted, SREBP is translocated to the Golgi where it undergoes proteolytic cleavage of N terminus, then it travels to the nucleus to play a role as transcription factor. In this study, we observed phenotypes in other strains of *Cryptococcus* species by constructing *hob1*Δ and *sre1*Δ mutants to confirm whether the functions of both genes are conserved in most serotypes. As a result, *hob1*Δ showed no noticeable phenotype under treatment of antifungal drugs and most environmental stresses in R265 (serotype C) and XL280 (serotype D), suggesting that Hob1 is related to sterol regulation only in H99 (serotype A). On the other hand, the function of Sre1 was found to be conserved in most serotypes. In conclusion, HOB1 and SRE1 play crucial role in regulating sterol-homeostasis in *Cryptococcus neoformans*, moreover, Hob1 is specific gene in *Cryptococcus neoformans*. It suggests that Hob1 is considered as potent factor-targeted new safety antifungal drug.

3.2-16 What causes the leaf blight of strawberry? Molecular systematics and comparative genomic analysis of the pathogenic fungus

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Abstract: The leaf blight fungus, formerly known as "*Phomopsis*" *obscurans* originally described from the United States, is a severe pathogen of strawberry. Morphological characters are often inadequate for accurate identification of this species, confusing it with other taxa in the order Diaporthales. Although this fungus is currently classified as a *Phomopsis* sp. (=*Diaporthe*), preliminary studies indicated it is not congeneric with *Diaporthe*. Phylogenetic analysis with representative genera from each family in the Diaporthales using five nuclear loci was performed to infer its evolutionary relationships and generic affinities. In addition, whole genome sequencing was performed using a freshly isolated culture from diseased plants. Results of phylogenetic analyses confirmed "*Phomopsis*" *obscurans* represents a unique evolutionary lineage and forms a monophyletic clade with recently described genus, *Microascospora* in *Melanconiellaceae*. The whole genome assembly consisted of 5638 contigs (≥500 bp) with an estimated genome size of 48 Mbp. The N_{50} contig length is 13853 bp and the G+C content is 52%. The output resulting from MAKER-p gene prediction indicated a total of 12431 genes and was modeled with *Cryphonectria parasitica* genome EP155. The estimated complete mitochondrial genome size is 98658 bp, which is comparatively large for those previously reported in fungi. Analysis with the CAZy database (dbCAN) identified in total 778 putative carbohydrate active enzyme encoding genes, including 341 glycoside hydrolases (GHs), 112 glycosyl transferases, 25 polysaccharide lyases, 138 carbohydrate esterases, 51 carbohydrate-binding modules, and 111 auxiliary activities. Therefore,

Microascospora obscurans (syn, *Phomopsis obscurans*) is equipped with all necessary enzymes to breach plant cell wall, penetrate and successfully infect and cause severe plant disease. Among GHs, majority (i.e. 20 from each) belong to GH3, GH5, GH16, and GH28 families that possess xylanase, cellulase, chitinase, polygalacturanases which also potentially involved in maceration and soft-rot of plant tissues including fruits. Knowledge of the enzymatic capabilities of this pathogen provide much needed information for potential targeting of the pathways involved in the disease process.

3.2-25 Fungal metabarcoding – can it be a new archaeological tool?

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Abstract: Methods of molecular biology are used in many different fields of scientific research. Archaeology is one of the disciplines that can benefit the most from it. Realization of main goals of archaeological research, which are i.e. drawing conclusions concerning people living in ancient ages, is a very difficult task since plethora of archaeological objects and traces was destroyed or significantly damaged. Our research focused on applying a molecular technique for studying late Neolitic barrows located in south-eastern Poland. Our main goal was to verify whether high throughput sequencing of ITS2 fragment of rDNA can provide a full picture of fungal diversity and thus, provide new information concerning mankind history. Fungi are suitable organisms for studying the past because they interact with other organisms on every trophic level and moreover, there is an almost universal molecular barcode which can differentiate most fungi on at least genus level. We used MiSeq Illumina platform to sequence ITS2 amplicons and then, publicly accessible database Unite.ut.ee to assign taxonomic position of obtained sequences. We compared female and male graves, vessels buried alongside the non-preserved bodies, traces of wooden constructional element and ancient and modern soil from nearby. Although it is only preliminary study, some substantial differences between all the samples may be observed. The traces of *Fomes fomentarius* (common bracket fungus) were found only in the place where the wood was thousands of years ago. There are also differences between vessels and other samples – aquatic fungi (representatives of Rozellomycota and Chytridiomycota) and cereal pathogens (such as *Giberella tricincta* or *G. zeae*) were found only in the vessels. Concluding, analyzing patterns in fungal distribution on archaeological sites may lead us to creation of a complex tool which can be used to answer the questions about ancient diet, activities and funeral rituals.

3.2-26 Behind the scenes at NCBI Taxonomy.

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Abstract: The International Nucleotide Sequence Database Collaboration (INSDC), is now more than 30 years old and comprise of three partners. These are the DNA Data Bank of Japan (DDBJ) at the National Institute for Genetics in Mishima, Japan; the European Nucleotide Archive (ENA) at the European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI) in Hinxton, UK; and GenBank at the National Center for Biotechnology Information (NCBI), National Library of Medicine (NLM), National Institutes of Health (NIH) in Bethesda, Maryland, USA. A 1997 agreement to resolve taxonomic issues prior to the release of new data paved the way for the NCBI Taxonomy database to serve as a central organizing hub for the INSDC members. This database was thus intended for a specific,

practical purpose - to provide nomenclature and classification information for the source organisms in the public sequence databases. This presentation will highlight and clarify recent improvements introduced to attain this goal, with a focus on Fungi.

3.2-27 Combined meta'omics reveal links among fungal community composition, gene expression, and chemical changes in decomposing leaf litter

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Abstract: Decomposition of plant litter is essential for nutrient cycling and therefore a key process for ecosystem functioning. Our current understanding of this complex process suggests that leaf litter decomposition is driven by climate, litter quality and decomposer communities. Fungi are considered to be the main decomposers of leaf litter in forest ecosystems. They synthesize and secrete enzymes that change the chemical composition of the litter, and thus represent a major effect of the fungal community. However, fungal community composition and their metabolic activity have been rarely analyzed together, and so far never in combination with litter chemistry. To link fungal activity to decomposition chemistry, we characterized the chemical composition of freshly fallen autumn leaves of European beech (*Fagus sylvatica*) and the corresponding leaf litter after one year of decomposition by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The composition and transcriptional activity was assessed for fungal communities by high-throughput sequencing of amplicon barcodes and metatranscriptomes, respectively, in the same litter samples. These analyses were highly replicated across 14 different forest plots and cover three distant regions in Germany. We were able to successfully distinguish freshly fallen leaves from one-year-old litter with respect to their chemical composition. Leaves were chemically more distinct among regions than one-year-old litter. Fungal communities were locally structured, however, functionally redundant among regions, i.e. expressing genes coding for similar litter-degrading enzymes. We identified changes in the abundance of putative chemical compounds between freshly fallen autumn leaves and one-year-old that correlated to the transcription level of litter-degrading enzymes. Furthermore, transcription patterns were also correlated with the abundance of certain fungal species. Overall, we provide strong evidence of a dynamic interaction between substrate chemistry, expression of enzyme coding genes, and fungal community structure in nature.

3.2-28 The accurate identification of fungal and other eukaryotic sequences from complex metagenomic samples

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Abstract: Although significant progress has been made in developing computational methods of binning and taxonomic profiling of metagenomic and microbiome samples, so far they were mostly restricted to the analysis of prokaryotic sequences. However, many recent surveys of environmental metagenomic samples also include sequences from microbial eukaryotes, such as fungi, metazoans and protists in addition to usual bacterial and archael sequences. The accurate identification of eukaryotic sequences, in the large sets of short sequences is an important step for further characterization of their role in the microbial community and evaluation of their metabolic potential. To aid with this task we conducted comparative analysis of ~900 eukaryotic genomes together with several thousand non-redundant reference prokaryotic genomes from IMG database in order to identify PFAM domains

unique either to eukaryota or prokaryota domains. Additionally, other identified unique eukaryotic and prokaryotic sequences without PFAM annotations were clustered into families, with subsequent building of their corresponding Hidden Markov Models (HMMs). These HMMs were calibrated and tested to predict with high accuracy eukaryote and prokaryote specific sequences. Based on found unique PFAMs and constructed new HMMs we developed a program that identifies and bins the potential eukaryotic sequences from metagenomic projects.

3.2-29 Mining big data in fungal genomics

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Abstract: The first genomes of *Saccharomyces cerevisiae* and *Neurospora crassa* were transformational for biology and development of molecular tools. The sequenced genomes of over 1000 fungal species offer new challenges and perspectives. In collaboration with research community from around the world the US Department of Energy Joint Genome Institute (JGI) has scaled-up fungal genomics in large-scale genomics initiatives like the 1000 Fungal Genomes project, which samples diversity across the entire Fungal Tree of Life; the 300 *Aspergillus* genomes, which drills down a single industrially important genus; the Fungal ENCODE, which produces multi-omics datasets to add new dimensions to the genomic data. These data equipped with analytical tools available from the JGI MycoCosm portal (jgi.doe.gov/fungi) are now offered for over 1000 fungal genomes. What can we learn from the 1000 fungal genomes that we cannot from one or ten? Can we predict species lifestyles from their genome sequences after interpreting large collections of genomes? How can we reduce dimensionality and visualize Big Data to make new discoveries? Big Data in fungal genomics has a huge unexplored potential and we'll explore groups of fungi to answer these questions using new computational approaches.

3.2-30 Leveraging fungal genome sequencing projects to better understand the bacterial diversity associated with fungi

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Abstract: Fungi are known to form associations with bacteria that grow either outside or within their hyphae. These associations can be functionally diverse, ranging from fungi that farm bacteria for nutrients to obligate endobacteria with dramatically reduced genomes that live within fungal hyphae. While there is an increasing number of examples of these bacterial/fungal interactions, not enough is known about the overall diversity of these fungal microbiomes or the physiological and ecological functions that result from the close interactions between fungal and bacterial species. Our objective here is to better understand the diversity of bacteria that are potentially associated with a wide range of fungal taxa. In order to do this, we have taken a bioinformatics approach by searching for bacteria-derived sequences within existing publicly available fungal genome sequencing data from a phylogenetically broad sampling of fungi, generated primarily through the 1000 Fungal Genomes project. Not all bacteria identified from fungal sequencing efforts will represent actual fungal associates, as some are almost certainly contaminants or mis-identified sequence reads. Therefore, we will use statistical and bioinformatics tools to attempt to identify and remove these reads from the analysis. To-date, we have analyzed genome sequencing data from a subset of fungal taxa that contain relatively few septa in their

hyphae, which may foster the establishment and long-term persistence of endobacteria. We use standard sequence taxonomy classification methods to assemble a list of bacterial taxa represented in each fungal genome sequencing project. When these bacterial composition lists are analyzed in tandem with fungal phylogenetic relationships, we find that within bacterial groups such as the family *Burkholderiaceae*, some species can occur in a range of fungal genera, while others display statistically significant phylogenetic clustering in their associated fungal species. We are in the process of expanding this analysis to several hundred fungal genomes to gain a more complete understanding of the phylogenetic patterns and functional significance of these putative bacterial associates identified from fungal sequencing data. Our results begin to catalog patterns in bacterial/fungal associations that occur in diverse fungal species and that in the future may help to better understand and predict the functions of these bacterial/fungal associations.

3.2-31 Towards FAIR data: Use case for managing and publishing mycological occurrence and community barcoding data

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Abstract: The FAIR guiding principles for data management and publication as described by Wilkinson et al. (2016) and Mons et al. (2017) are recommendations to make research data sustainable, which means long-term **F**indable, **A**ccessible, **I**nteroperable and **R**eusable. Currently, these principles are widely discussed and largely accepted by major stakeholders and initiatives of the various science domains, e.g., the European Science Cloud and national initiatives for research data publication, like the German Federation for Biological Data (GFBio; (https://www.gfbio.org/). These principles also directly affect primary research data producers and scientists managing mycological research data from their generation onwards. This is demonstrated by a work- and dataflow with the use case '*Mycological occurrence and community barcoding data*'. For organizing such kind of data according FAIR principles, the relational database application DiversityDescriptions (DD) (https://diversityworkbench.net/Portal/DiversityDescriptions), a generic component of the Diversity Workbench environment (https://diversityworkbench.net), is applied. The newly published conceptual schema MOD-CO for 'Meta-Omics Data of Collection Objects' provides concepts and concept collections being appropriate as DD descriptor structure. MOD-CO (http://www.mod-co.net) has been established with the aim to describe operations and object properties along the work- and dataflow from gathering environmental samples, to the various transformation and measurement steps in the laboratory up to sample and data publication and archiving. By supporting parent-child-relationship, the MOD-CO schema (http://www.mod-co.net/wiki/Schema_Representations) allows for the entering and storage of individual records of each operational step (transformation, measurement and transaction) along a workflow. Based on a MOD-CO descriptor structure, DiversityDescriptions might be used as LIMS (Laboratory Information Management System), e.g., for organising '*Mycological occurrence and community barcoding data*'. A DD data export interface enables data managers to provide content data in the formats CSV (https://en.wikipedia.org/wiki/Comma-separated_values) and XML (https://www.w3.org/XML/) according SDD metadata schema with EML and ABCD metadata schemata extensions (for biodiversity schemata and standards see https://gfbio.biowikifarm.net/wiki/Data_exchange_standards,_protocols_and_formats_relevant_for_the_collection_data_domain_within_the_GFBio_network). Domain-specific, service-oriented infrastructures for data publication like GFBio with its submission and brokering services and relation to EMBL-ENA and to several other recognized biodiversity and environmental data centers

(https://www.gfbio.org/about/data-centers) rely on scientists with good data management skills, using such tools and workbenches as described above. In the addressed use case, data and metadata, curated in DD, will be checked by GFBio compliance and consistency tools for being published according to the FAIR data principles. The GFBio data submission and publication process includes terminology and parameter assignment and provides advanced search options for datasets ('findable'), data download services ('accessible'), services for machine-readability of data ('accessible', 'interoperable') and several visualization and analysis options ('accessible', 'interoperable', 'reusable'). The data package from the use case '*Mycological occurrence and community barcoding data*' will be published and offered for reuse under a creative common license. A suggestion for standardized citation is provided, a DOI assignment is feasible and the long-term data archiving is ensured. In the GFBio context, mechanisms to provide metrics, count the impact of data publications and give credits to authors are going to be established.

3.2-32 Modernization and molecular characterization of Canada's National Mycological Collections

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Abstract: Agriculture and Agri-Food Canada (AAFC) has recently invested in a 5-year initiative to update and upgrade its vast collection of live and preserved fungal specimens. The Canadian Collection of Fungal Cultures houses over 19,000 live cultures and is a repository and distributor of fungal genetic resources. The Canadian National Mycological Herbarium contains over 350,000 preserved specimens, representing a historical archive of the existence and distribution of fungi and plant disease in Canada. Plans include full inventories, digitization of specimen metadata, and renovated infrastructure. Furthermore, selected specimens will subjected to characterization at the molecular level through DNA sequencing and analyses. Partial and whole genomes will be sequenced for ~2,500 and ~350 fungal species, respectively. These data will be leveraged by researchers for the development of tools for precise identification and detection of agriculturally important fungi and quarantine species, surveys of agricultural commodities, and the resolution of taxonomic and nomenclatural issues. DNA sequences for universal barcodes (eg ribosomal internal transcribed spacer) will be obtained from ~16,000 live fungal strains. These data will significantly expand the reference sequence databases that may be used in foundational work in the metagenomic and microbiome research fields. For example, these barcode sequences will support metabarcoding studies on topics that are currently of high interest to the agricultural and environmental sectors, such as the impacts of climate change and land use on soil microbial communities. All collection, specimen, and molecular data will be integrated into an information sharing system that will provide rapid and electronic, public access to AAFC's biological collections.

3.2-41 Production of selenium-enriched mycelial biomass by *Ophiocordyceps sinensis* fungus

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Abstract: Selenium (Se) is an essential trace element in healthcare. It has many important bioactivities such as anti-oxidant, boosting immune function, anti-tumor and anti-metastatic. This study proposes a

new cultivation method to produce Se-enriched mycelial biomass of *Ophiocordyceps sinensis* fungus. The result shown that the Se-adaptive cultivation method improved the ability of biomass production of *O. sinensis* AS (with a yield of 17.31 ± 1.12 g L^{-1}) at 25 mg L^{-1} of Se^{+6} ($Na_2SeO_4.12H_2O$). The Se content of *O. sinensis* AS biomass reached about 1354.97 ± 54.54 μgSe g^{-1} dry weight (dw). In addition, selenium-containing compounds of *O. sinensis* AS including selono-exopolysaccharides (Se-EPS), seleno-polysaccharides (Se-PS) and seleno-proteins (Se-Pr) were extracted. The fourier transform infrared spectroscopy (FT-IR) analysis demonstrated that these molecules had the presence of Se=O and C-O-Se stretching vibrations at the characteristic infrared absorption peaks, 1100 - 1050 cm^{-1} and 550 – 620 cm^{-1} bands respectively. Furthermore, there were a considerable rise in the anti-oxidant activities of Se-EPS such as $ABTS^{\bullet}$ and $OH^{\bullet}$ radical scavenging potential as well as reducing the intracellular ROS levels of HepG2 cells. In conclusion, not only does this study improve the pharmacological properties of the *O. sinensis* AS biomass, it also makes the supplementary Se source with high bioavailability.

3.2-42 Metals tolerance by filamentous fungus *Aspergillus fasciculatus* isolated from mine soil in Sonora Mexico

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Abstract: The aim of this research is to find a filamentous fungus with potential of bioremediation of metals, such as Pb, Cr, Zn, Hg, Ag, and Cd. For this, soil samples were taken from a mine, a strain of filamentous fungus was isolated and purified. The filamentous fungus was identified as *Aspergillus fasciculatus* based on molecular data. The tolerance index and minimum inhibitory concentration (MIC) for the metals Pb, Cr, Zn, Ag, Hg, and Cd were determined. *A. fasciculatus* was highly tolerant to 1, 5, 10, 15 and 20 mM of Pb, Cr, and Zn, respectively. While for metals Hg, Ag and Cd were highly tolerant to concentrations of 1 and 5 mM of these metals. Their MIC ranged from 5-10 mM for Hg, Ag, and Cd, moreover MIC from Pb, Cr, and Zn up 20 mM. With these results, it can be concluded that *A. fasciculatus* presents high potential of use to bioremediation the following metals: Pb, Cr, Zn, Ag, Hg, and Cd.

3.2-43 Phylogenetic analysis and the impact of heavy metal contaminants on wild isolates of the ubiquitous ectomycorrhizal species *Cenococcum geophilum*

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Abstract: Agriculture has become a multifaceted industry as the production of biofuels and the need for greater sustainability grow in prevalence. There is a finite amount of land available for either biofuel or food crop systems, and land availability is further limited due to the presence of major pollutants including heavy metal contaminants, leading to phytotoxicity and other concerns. Plants serve as hosts to a diverse community of microbes including fungi, which are capable of metabolizing and/or immobilizing soil compounds which the plant cannot. Some mycorrhizal fungi directly interact with heavy metal contaminants within the soil, and a fungal-plant symbiosis may increase a plant's ability to survive in a soil containing high heavy metal concentrations. These relationships are often difficult to disentangle as a single plant species may be associated with hundreds of fungal species and each of these associations can display varying influences on host plant survival and growth that co-vary with the environmental and physical conditions of soil. One such symbiont, the *Cenococcum geophilum*, is ubiquitously distributed across multiple climates, soil types and plant species. The genome of *C. geophilum* is among the largest in the fungal kingdom, with a total estimated size of 178 megabases

due to an abundance of transposable elements. This large size and highly repetitive genomic data have historically increased the difficulty of both sequencing and analyzing *C. geophilum*. New technologies, such as restriction enzyme associated DNA sequencing (RADseq) decrease both time and financial investment required for such analyses, and will be used to analyze over 200 wild isolates of *C. geophilum* obtained from soil samples across a range of 296 miles in the United States Pacific Northwest. These isolates will be used to determine if established host population structure will be reflected in the symbiont genomes, despite the generalist nature of *C. geophilum*. These wild isolates will also be screened to determine phylogenomic related correlates with the uptake and growth effects of heavy metal contaminants lead, strontium, copper, cadmium and zinc using laboratory growth assays. Heavy metal uptake will be analyzed using an X-Ray fluorescence device (XRF) on dried fungal biomass, and metabolomics analyses will be performed on representative isolates using gas chromatography–mass spectrometry (GC-MS). Finally, individual strains will be selected based on heavy metal resistance and inoculated onto living plant hosts of the potential biofuel crop *Populus* (Poplar). Plant physiological responses, growth patterns, and overall uptake of the contaminants will be monitored to determine if heavy metal resistant *C. geophilum* strains confer increased heavy metal contamination resistance to Poplar. If increases resistance is observed, this type of manipulation of host plant symbionts may increase plant tolerance to soil conditions that would otherwise be toxic to the plant, increasing overall land availability for use in agriculture. This would encourage the planting of biofuel crops within fields that may not be optimized for the growth of crops intended for consumption due to the contamination within the soil, thereby avoiding the "food-for-fuel" tradeoff that has driven agronomic policy concerns in the biofuel industry.

3.2-44 Extraordinary arsenic accumulation and speciation in *Cyanoboletus pulverulentus* and *Elaphomyces* spp.

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Abstract: Many mushroom species have been found to accumulate arsenic in fruit-bodies. Hyperaccumulation of this toxic metalloid was described in the ectomycorrhizal ascomycete *Sarcosphaera coronaria*. A common and often major chemical species of arsenic found in fruit-bodies is arsenobetaine (AB), a non-toxic methylated arsenic compound also known from marine biota. However, several other methylated arsenic species have been identified in fungal tissues, e.g., methylarsonic acid (MA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO). Based on a long-term analytical screening of arsenic in macrofungi by instrumental neutron activation analysis, we have revealed striking arsenic accumulation in *Cyanoboletus pulverulentus* and *Elaphomyces* spp. More samples of these two species were then collected and total arsenic concentrations were additionally determined with inductively coupled plasma mass spectrometry (ICPMS) after microwave assisted acid digestion. The chemical form of arsenic in fruit-bodies was investigated in aqueous extracts by high performance liquid chromatography coupled to ICPMS. In *C. pulverulentus*, arsenic concentrations in sporocarps may reach 1300 mg kg^{-1} (dry mass, d.m.). No significant correlation between the soil arsenic content and arsenic concentrations in the associated sporocarps was found. Within the individual parts of the fruit-body, we found the majority of arsenic accumulated in the hymenium. Besides occasional traces of MA, the arsenic speciation in all *Cyanoboletus* samples consisted solely of DMA and no inorganic arsenic was detected. Because of the carcinogenic potential of DMA, *C. pulverulentus* should not be recommended as an

edible mushroom and its consumption should be restricted. In *Elaphomyces* collected from unpolluted spruce plantations in the Czech Republic, the total arsenic concentrations ranged from 12 to 42 mg kg^{-1} d.m. in samples of *E. asperulus* and from 120 to 660 mg kg^{-1} d.m. in *E. granulatus* and *E. muricatus*. These concentrations are remarkably high for terrestrial organisms and demonstrate the arsenic accumulating ability of these ascomycetes. The dominating arsenic species in all samples was MA which accounted for more than 30 % of the extractable arsenic. AB, DMA, and inorganic arsenic were present as well, but only at trace concentrations. Surprisingly, we found high amounts of TMAO in all samples (0.32 – 28 % of the extractable arsenic). Even more remarkable was that the majority of samples contained significant amounts of the highly toxic trivalent arsenic compound methylarsonous acid (0.08 – 0.73 % of the extractable arsenic). This is the first report of the occurrence of trimethylarsine oxide and methylarsonous acid at significant concentrations in a terrestrial organism. From our results it becomes obvious that the arsenic speciation in fungal fruit-bodies is much more complicated than previously thought and a lot of work needs to be done before we understand the biological importance of arsenic accumulation and speciation in macrofungi.

3.2-45 Microbial interactions within ectomycorrhizosphere in heavy metal contaminated soils

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Abstract: The ongoing increase of heavy metal concentration in soil environments requires the development and the improvement of soil remediation approaches. Microorganism-enhanced phytoremediation has been shown to be very efficient for soil rehabilitation. Here, we focus on ectomycorrhizosphere as a close interaction between plant roots, ectomycorrhizal fungi, microorganisms and soil to understand mechanisms of phyto- and bioremediation. To investigate microbial interactions, two-year pot experiment with 3 variants has been established using trees (birch, oak, pine), and soil substrate with natural occurring microflora from a former mining uranium site (Thuringia, Germany): (1) pots with soil substrate, (2) pots with soil substrate and planted tree seedlings, and (3) pots with soil substrate, planted and additionally inoculated with ectomycorrhizal fungi tree seedlings. The probable changes in metagenome and metatranscriptome of soil communities as well as heavy metal content in plant biomass and in soil adjacent to ectomycorrhizal roots after inoculation will be determined. Moreover, molecular mechanisms of interactions between mycorrhiza helper bacteria and ectomycorrhiza will be investigated using metal resistant *Streptomyces* strains isolated from test field on *Tricholoma vaccinum – Picea abies* symbiosis. Hydrophobins are unique surface-active fungal proteins. Up-regulation of several hydrophobin genes in the presence of heavy metals was demonstrated earlier; however, direct participation of hydrophobins in the alleviation of metal stress has not been addressed so far. *Agrobacterium tumefaciens* mediated transformation has been performed to produce hydrophobin 8 overexpressing mutants of *T. vaccinum*. The properties of hydrophobin 8 found in aerial mycelium of *T. vaccinum* and its participation in formation of ectomycorrhiza as well as in alleviation of metal stress will be studied. The preliminary results will allow to connect the microbial diversity of ectomycorrhizosphere of plants at contaminated site and performed functions. Furthermore, profound analysis of microbial communities will give insight into the molecular mechanisms of microbial biogeochemical functions which are related in many respects to microbial resistance mechanisms. In the future, the given results might be used for optimization of phyto- and bioremediation of soils contaminated with heavy metals.

3.2-46 Induction of glutathione biosynthesis genes in response to cadmium and arsenic stress from the ectomycorrhizal fungus *Laccaria bicolor*

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Abstract: Ectomycorrhizal fungi plays an important role in protecting the host plants in heavy metal contaminated sites. They prevent the uptake of heavy metals into the cytosol by extracellular chelation through extruded ligands like tricarboxylic acid, oxalic acid or by biosorption of these metals to the fungal cell wall through chitin and glucosamine. Further, the metal accumulated in the cytosol is detoxified by synthesizing the range of thiol rich ligands like glutathione (GSH) and metallothioneins (MT). GSH is biosynthesized in two sequential ATP dependent reactions mediated by two enzymes, γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase (GS). In the present investigation, genes involved in GSH synthesis were cloned from the ectomycorrhizal fungus *Laccaria bicolor* and studied their roles in metal detoxification. *L. bicolor* was subjected to different concentrations of cadmium and arsenic and their response was recorded using different parameters like dry weight, total metal uptake, the total glutathione produced, the enzyme activity of γ-GCS and GS and the relative expression of both γ-GCS and GS genes. The two genes involved in glutathione biosynthesis (γ-GCS and GS) were further expressed in *E.coli* cells and were characterized. Uptake of Cd increased up to 9 µM and then decreased due to the lethal effects, whereas in case of As the metal uptake increased with increase in arsenic concentration up to 15 mM. Total glutathione production increased as a function of external Cd. Cd induced the expression of γ-GCS 18-fold and GS 12-fold higher than control while As increased the expression of γ-GCS 10 fold and GS 6 fold higher compared to control mycelium. The functional characterization of the two genes, γ-GCS and GS in *E. coli* cells increased their metal tolerance up to 3 folds justifying the potential role of these genes in metal tolerance. The present study clearly depicts the potential role of glutathione in protecting *L. bicolor* under cadmium and arsenic stress, thus segregated the response of different thiols to different metals.

3.2-47 Cellular processes involved in the handling of heavy metal ions in ectomycorrhizal *Amanita*, *Russula* and *Hebeloma* species

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Abstract: Studies have revealed that mycorrhizal fungi, including ectomycorrhizal (EM) species, effectively mobilize heavy metals from soils and minerals. Mycorrhizal fungi further play an important dual role in plant metal homeostasis: scavenging of metal micronutrients and their supply to the host; detoxification of both the excess essential and physiologically irrelevant metals. The delineation of molecular basis of metal uptake and tolerance in metal-accumulating species may allow rating their metal cycling and host-protection/stimulation capacity, with certain significance for bioremediation purposes. This report focuses on the handling of metals in the EM species accumulating remarkably high concentrations of heavy metals. Searches of sequenced transcriptomes for metal related determinants, gene complementation studies in yeasts, metal speciation analyses in fungal tissues by using size exclusion chromatography and mass spectrometry, and gene expression analyses in the cultured mycelia allowed us to obtain the evidences supporting the following conclusions. In Ag-hyperaccumulating *Amanita strobiliformis*, at least two CTR transporters can efficiently recognize for import Ag in addition to Cu, P_{1B-1}-ATPase transporter can export Ag and Cu from the cytoplasm (presumably into the vacuoles) but virtually all the accumulated Ag occurs complexed by cytosolic

metallothioneins (MTs). In Zn accumulating *Russula atropurpurea*, which has a high affinity ZIP transporter for the acquisition of Zn and CDF transporter for vacuolar sequestration of overaccumulated Zn, is 40% of accumulated Zn bound with unusual MT-like RaZBP peptides; RaZBP homologues are involved in binding of the excess of intracellular Zn also in other Zn accumulators of *Russula* spp., *R. pumila*, *R. ochroleuca*, and *R. viscida*. In contrast, *Hebeloma mesophaeum*, like several other *Hebeloma* species, preferentially funnels excess Zn (and Cd) into subcellular compartments, although it has the capacity to produce MTs. Altogether, our data suggest that there might be a link between cellular biology of Ag and Cu in EM fungi and show that EM fungi of different genera (*Russula* vs. *Hebeloma*) may employ different strategy to handle the excess of particular metal (Zn). *Work currently supported by the Czech Science Foundation (16-15065S).*

3.2-48 Genetic investigation of potential for radionuclide protection by *Schizophyllum commune*

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Abstract: The pollution of the environment with radionuclides is mainly caused by nuclear explosions. The most serious accident to date occurred on April 26, 1986 in Chernobyl in the Ukraine, when approximately 3 x 10^6 trillion Bq were released into the environment. Although more than 30 years later, thousands of hectares of land are still contaminated, especially with cesium. Since fungi are known to have high radiotolerance and are capable of accumulating various radionuclides, they are a good means of bioremediating the contaminated soil. Within this study, tolerance or rather accumulation mechanisms for radionuclides should be identified in fungi. For this purpose, an experimental set-up was established with the saprophytic white rot fungus *Schizophyllum commune*. It absorbs effectively radionuclides, grows well in soil, also under lab conditions, and mycelium free of soil particles can be harvested. Thus, RNA for mRNA-Seq can be isolated from the mycelium after growth on Chernobyl soil, control soil and on minimal medium with and without heavy metal composition found in Chernobyl soil. Thus, first conclusions can be drawn on the molecular mechanism behind the mycoremediation of *S. commune*.

3.2-49 Effect of long-term farming practices on the plant and its associated rhizosphere microbiome

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Abstract: Intensification and inadequate agricultural management result in substantial losses of fertility and yield, and the accumulation of pathogens in soils. In order to maintain soil quality and health for the future in agricultural land, the development of more extensive and sustainable farming strategies is urgently needed. Hence, a better understanding of how agricultural management strategies affect soil and associated rhizosphere properties is the key to propose farming strategies for high plant productivity and plant health. We used three long-term field trials to analyze the impact of various management strategies on soil and its associated rhizosphere microbiome under consideration of plant productivity, plant health and the ability of the soil to suppress soil-borne pathogens. The soils of the

long-term field trials were subjected to growth chamber pot experiments with lettuce (*Lactuca sativa*) as model plant. After a growth period of ten weeks, significant differences in lettuce shoot fresh mass and microbial biomass were observed among soils depending on long-term farming strategies. The rhizosphere exhibited different bacterial and fungal community compositions depending on soil sites as well as on the agricultural management history (tillage practice, crop rotation, fertilization strategy) of the soils. These factors influenced also relative abundances of distinct bacterial and fungal taxa. In addition the root exudation of the antifungal metabolite benzoic acid as well the expression of plant-defense related genes was affected by farming practice. This suggests a relationship between long-term agricultural management, soil microbiome and plant performance.

3.2-51 Novel fungal degradation of bromoalkanes

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Abstract: Bromoalkanes, as 1-bromobutane, are persistent organic pollutants in water, sediments, and soils. They are commonly found in pesticides, herbicides, and industrial solvents. Recently, an aerobic pathway of bromoalkane degradation, based on initial dehalogenation, by an eukaryotic microbe was describe for the yeast *Yarrowia lipolytica* 3589. Our objective is to isolate fungi capable of degrading bromoalkanes. 1-bromobutane was added into mineral media as sole carbon on agar plates. The mineral media was used with and without nitrogen supplementation. Plates were randomly exposed to air at six locations in Puerto Rico and incubated at room temperature. After ten days, small fungal colonies were noted for three sites. Microscopic examination revealed six isolates exhibiting septated fine mycelium and conidiospore arrangements resembling different *Penicillium* strains. The growth suggest that 1-bromobutane can be used as carbon source with faint development. Biochemical characterization are in progress to demonstrate chemical transformations using and kinetics parameters and spectroscopy assays. Further studies will use haloalkanes differing in carbon chain length and position as sole carbon source to continue bioprospecting efforts. The ultimate goal is to propose strategies for decontamination of haloalkanes based on extended mycelial networks.

3.2-52 Improving crop phosphorous uptake through use of fungal bioinoculants

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Abstract: Soil fungi play an essential role in plant growth and development, and the benefits they afford have recently seen them exploited commercially as bioinoculants. Improved phosphorous cycling is one such key benefit fungi can afford to crop production through a variety of modes of action, an understanding of which is important given the context of the unsustainability of rock P supply and the global food security situation. This study used a PSI scanner system to examine the efficacy of several commercial bioinoculants on *Lolium perenne* growth over a three-month period, growth increments recorded daily by a monitored conveyor system and automated scales. Non-destructive cuts were taken at three-week intervals. Plants treated with bioinoculants comprised of zeolite carriers demonstrated as much as 10x dry leaf and root mass of competitor products and controls. Soil, leaf and root P were recorded after a destructive cut to quantify P reclamation, which indicated soluble P concentrations up to six times higher in the soils where plants were treated with zeolite carrier bioinoculants, suggesting that the mode of action responsible for the growth improvements involved P mobilisation. Root:shoot ratio, root staining and elemental analysis were carried out to quantify other possible modes of action. Chlorophyll fluorescence from plants undergoing each treatment suggested lower amounts of non-

photochemical quenching in zeolite-containing bioinoculant treated plants, suggesting greater resilience to climactic changes.

3.2-53 A novel inoculation method of mycorrhizae in wheat fields has remarkable effects on crop yield and soil properties

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Abstract: ABSTRACT In recent years there is growing trend towards organic farming to meet food security and food safety. Inorganic fertilizers are available to boost yield but there are environmental and public health concerns. Amongst various organic alternatives, using mycorrhizal species is one of the most promising option. Therefore, the present research was conducted to evaluate the influence of indigenous mycorrhizal species on the growth of wheat and soil properties under field conditions. In total 11 species of mycorrhizae were identified from the experimental areas, the most prominent genera being, *Claroideoglomus, Rhizophagus* and *Funneliformis*. For inoculation of mycorrhizae, their native density was maintained with a novel idea that these species work better in consortia when their native population density was maintained. There were eight different treatments having plot sizes as 6 meters x 2 meters, employing Randomized Complete Block Design (RCBD). The whole set of experiment was repeated at two different sites. Identified mycorrhizal species were mass cultured in pots using same variety of wheat that was used in field experiments. Bio-inoculation of consortia of different mycorrhizal species showed a significant increase in all growth parameters studied e.g., number of tiller per plant (up to 39 %), plant height (up to 13 %), dry biomass (up to 15 %), grain yield (up to 18 %) and hay weight (up to 15 %). Moreover, remarkable effects were recorded on soil fertility such as soil organic matter, available phosphorus and potassium were increased up to 143 %, 53 % and 25 %, respectively. The enhanced effects of different mycorrhizal species on different growth parameters in wheat were attributed to more number of infections these species caused in wheat roots in field experimentations. Moreover, it is also envisaged that these mycorrhizal species worked better while their native density was maintained in the field that also increased solubilization process of bound minerals. This increased solubilization resulted in pronounced increase of mineral content in the soil that was evident from soil tests of individual replicates from each treatment. The present study concludes that increasing the mycorrhizal inoculum in crop fields by keeping their density as native has tremendous effects on crop productivity and soil fertility status.

3.2-54 Nematicidal culture filtrates of corn and soybean root endophytic fungi

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Abstract: The soybean cyst nematode (SCN) is a root pathogen of major concern for soybean growers worldwide and causes substantial yield losses in infested fields. A common practice to help manage SCN is rotation of soybeans with corn, a non-host of the SCN. Long-term soybean monoculture leads to a proliferation of fungal antagonists of the SCN in soil and cysts, but few studies have examined the root endophytic community for fungi with SCN-antagonistic activity. The objective of our study is to test whether metabolites produced by soybean and corn root endophytes can kill SCN juveniles, the life-stage most commonly encountered in soybean roots. Fungal endophytes were isolated from surface-

sterilized corn and soybean roots from experimental plots in which plants had been grown under annual rotation and under 1, 3, 5, and 35 years of continuous soybean and corn monoculture. Fungal isolates were grouped into 413 morphotypes, and the full ITS region of one representative from each morphotype was sequenced using Sanger sequencing. Isolates with ITS regions sharing 99% sequence similarity were clustered using USearch, giving a total of 114 unique OTUs. Taxonomy was assigned to OTU representatives by using BLAST searches against the UNITE and NCBI databases. One or more isolates from each OTU were grown in malt extract broth and in a secondary metabolite-inducing medium for two weeks, and their culture filtrates were tested for nematicidal activity against SCN juveniles at 6, 24, 48, and 72 hours. Statistical analyses were performed to identify highly nematicidal isolates and to examine relationships between cropping sequences and overall nematicidal bioactivity of root endophytic communities. Results of this study will be used to identify potential biocontrol agents of SCN and to inform farming practices that foster the development of SCN-suppressive soils.

3.2-55 During low water availability, field inoculation with isolates of arbuscular mycorrhizal fungi provide different growth benefits according to carrot genotype

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Abstract: Arbuscular mycorrhizal fungi (AMF) provide growth benefits to water-stressed plants. Carrots (*Daucus carota*) readily host AMF and are commonly used to model symbiosis as root organ cultures, but little is known of carrot-AMF symbiosis regarding whole plant outcomes. Water scarcity threatens production of highly nutritious vegetables due to global climate change; low- input systems are particularly vulnerable. Single inoculant greenhouse studies in sterile media often show that AMF benefit drought-stressed plants, but it is not known how field inoculation impacts carrot performance under low-water conditions. AMF contribution to plant water uptake likely differs among species, but these differences between intraspecific isolates remain unknown. The present study screened four popular carrot cultivars grown in low-nutrient soils inoculated with AMF isolates from geographically distinct locations. Carrots grew in an organic field site in plots amended with whole inoculants of eight AMF isolates. Mock inoculants served as negative controls. Four field studies occurred in 2016 and 2017 with early and late plantings in rain- excluding tunnels that enabled experimental control of water inputs. Carrots received ample water during establishment, and water-limitation occurred during taproot maturation in the six weeks preceding harvest. Biometric data were recorded for roots and shoots. Heirloom carrots differed from hybrid carrots in their response to water limitation and propensity to increase biomass with inoculation by AMF. Fungal isolates differed within species under both low- and high-water conditions. Carrot biomass allocation in response to mycorrhizal colonization differs from other plant models commonly used in mycorrhizal research. Inherent tolerance to water limitation influenced carrot benefits from AMF.

3.2-56 Conversion of *Pueraria montana* to a fish feed by *Pleurotus ostreatus*

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Abstract: The annual yield of fishery industries worldwide is approximately 200 million tons, of which, the catch using fishing boats amounts to approximately 90 million tons and has not increased since 1980. Pisciculture is required to support the increasing demand of fish. For securing a quantity of feedstock for the fish cultivation, harnessing new sources and methods converting them to feedstock is

an urgent requirement. Currently, soybean meal (containing neutral detergent fiber [NDF] and crude protein [CP] of approximately 8% and 54%, respectively) and rapeseed meal (containing NDF and CP about at approximately 35% and 48%, respectively) are frequently used as alternative material for fish feed; these are ingredients of compound feeds for aquaculture. However, plant material is also used in animal husbandry; hence, there is limited prospect of securing a sufficient amount of plant material at a low price for aquaculture in future. *Pueraria montana* is a weed that grows in the temperate zones. The present study aimed to improve the performance of *Pueraria montana* on the raw ingredients of feedstock, using white rot fungus *Pleurotus ostreatus*. *P. ostreatus* was expected to degrade NDF and increase relative CP content. *P. ostreatus* was inoculated in 100 mL of ddH_2O or 100 mM urea containing 25 g (dry weight) of leaves of *P. montana* and incubated at 28ºC for 30 d. During incubation without urea, NDF content of *P. montana* leaves decreased from 38.9% to 23.3%, while CP content rate increased from 23.0% to 25.8%. Urea decreased NDF (21.9%) and increased CP (28.1%), more. Subsequently, *P. ostreatus* was cultured in glucose peptone (GP) medium with and without additional nitrogen at 28ºC for 7 d. Protease activity of the filtrates of the additional nitrogen-free culture was 0.128 unit/mL. 4 mM urea and 4 mM ammonia (2 mM ammonium tartrate) repressed protease activity to 0.022 unit/mL and 0.059 unit/ mL, respectively. To our knowledge, this is first study to report that basidomycete has a system of nitrogen-metabolism repression and the point at which urea was more effective than ammonia differs from that in ascomycetes such as *Aspergillus*. We are constructing a *ΔAreA* strain and a strain containing *AreA-eGFP*.

3.2-65 Nitrogen fixation and nitrogen enrichment in montane Britain: a case study with the lichen *Stereocaulon vesuvianum*.

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Abstract: The tripartite fruticose lichen *Stereocaulon paschale* is widespread and locally abundant in montane-alpine areas on the British Isles. This species forms cephalodia containing the cyanobacterium *Stigonema* and cephalodiate thalli have the capacity for moderate levels of nitrogenase activity. However, in upland regions exposed to high atmospheric N deposition cepalodia in *S. vesuvianum* are infrequent or absent and thalli are often covered by microbial biofilms. Nonetheless, *S. vesuvianum* remains plentiful in N-enriched areas in contrast to some cyanobacterium-containing lichens which are considered highly sensitive to N and acid-deposition. Accordingly, we have examined the relationship between nitrogen fixation and N-deposition in *S. vesuvianum* by comparing thallus chemistry (including ^{15}N natural abundance), thallus morphology and nitrogenase activity among 10 montane sites with modelled N wet deposition (nitrate + ammonium) in the range 2- 36 kg per hectare per year. The common non-nitrogen fixing foliose lichen *Parmelia saxatilis* has been included in the study as a reference species for which thallus chemistry data have also been collected. Both *S. vesuvianum* and *P. saxatilis* occur on rock outcrops and boulders and intercept atmospheric deposits directly without modification by overhanging plant canopies. Data presented suggest that the N supply for *S. vesuvianum* switches from nitrogen fixation to combined nitrogen as atmospheric N load increases.

3.2-66 Factors shaping the distribution of *Peltigera* spp. (Lecanoromycetes) and their *Nostoc* symbionts at an inter-biome scale in Alberta, Canada

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Abstract: *Peltigera* is a diverse and conspicuous genus of lichen-forming fungi, particularly in cold-temperate and boreal regions where macrolichens can constitute an important component of biomass and nutrient cycles. Previous studies on distribution and specificity of the main fungal and *Nostoc* partners in this genus have typically involved opportunistic sampling on a global scale or intensive sampling on a small spatial scale. In this study, we took a middle ground between these two approaches and evaluated the diversity and co-distribution of *Peltigera* species and their *Nostoc* symbionts across the Canadian province of Alberta, taking advantage of an existing collection of ca. 8500 *Peltigera* specimens collected from 1656 Alberta Biodiversity Monitoring Institute (ABMI) permanent monitoring sites spaced approximately 20 km apart across the province. In particular, we sought to understand: (1) which *Peltigera* species are present in the province, in light of recent work which has revealed numerous cryptic species within traditional morphological species; (2) what *Nostoc* phylogroups are associated with Alberta *Peltigera* species, as part of a global effort to understand patterns of *Peltigera–Nostoc* specificity; and (3) whether the distribution of *Nostoc* phylogroups has an influence, independent of environment, on the range of *Peltigera* species. We sequenced the ITS (*Peltigera*) and *rbcLX* (*Nostoc*) loci for c. 150 specimens and utilized ABMI's existing environmental data for the monitoring plots. ITS sequences showed that *Peltigera* identifications based on morphology were not always consistent with phylogenetic data. Sequence data indicate that *P. britannica* and *P. wulingensis* are more common in the province than previously thought, but distinguishing them from morphologically similar species remains a challenge. There is high genetic diversity among specimens morphologically identified as *P. rufescens*, *P. leucophlebia*, and *P. canina*. This diversity includes several potential new species (i.e., ITS genotypes distinct from anything else in our global database of *Peltigera* sequences) which will require further investigation using multiple loci, phylogenetic methods, and detailed morphological study. Globally common *Nostoc* phylogroups tend to be common within Alberta as well. There does not appear to be a relationship between *Peltigera* species richness at a particular site and the presence of specialist or generalist *Nostoc* phylogroups, contrary to our early working hypothesis. We present maps of the co-distribution of *Peltigera* species and *Nostoc* phylogroups across vegetation types and physiographic regions of Alberta. Our molecular analyses will be used to improve the morphological identifications of the unsequenced ABMI specimens, contributing to better monitoring and protection of lichen communities in the province.

3.2-67 Major biomes across North America drive patterns of lichen biodiversity and traits: A case study across all 5,400 lichen species

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Abstract: Lichens are dynamic and important components of ecosystems worldwide, yet large-scale understanding of functional trait patterns and how these relate to overall patterns of species richness across biomes is largely lacking. North America is a biologically diverse continent spanning numerous biomes from the arctic to the subtropics, and deserts to rainforests. We will present the results of analyses of a new trait dataset of 5,400 lichens and lichenicolous fungi known from the region. We will 1) address how patterns of species richness and endemism are distributed across the continent (north

of Mexico) and whether these patterns mirror known hotspots of diversity for other groups, 2) examine correlations between traits, 3) examine patterns and correlations among traits across North American biomes, including as they relate to species range size.

3.2-68 Lichen symbiont interactions along ecological and climatic gradients

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Abstract: Mutualistic symbiotic relationships play important roles in the evolution of many ecologically successful groups of organisms despite their complexity and vulnerability. Lichens are well-known and reasonably well-studied examples of mutualistic symbiosis, consisting of a heterotrophic fungal partner, also called the mycobiont, and photosynthetic algae or cyanobacteria (photobiont). The relationships between the symbionts are shaped by, among others, environmental and evolutionary factors. However, it remains largely unanswered how and in what extent ecological preferences and phylogenetic constrains contribute in interactions between these intimate and long-term partners. We investigated genetic variation and symbiont network patterns of six common lichens along ecological and climatic gradients. Six epiphytic lichens, *Hypogymnia physodes*, *Hypogymnia tubulosa*, *Lecanora aff. chlarotera*, *Parmelia sulcata*, *Physcia adscendens/tenella*, and *Pseudevernia furfuracea*, were collected from the Swiss Alps and Estonia. The sampling sites were selected to include ecological (vegetation openness and host tree species) and climatic variation (mean yearly precipitation and temperature). We sequenced the full nuclear internal transcribed spacer region (ITS) for the mycobiont and the photobiont from the 852 collected specimens, resulting in total of 1622 sequences. We estimated genetic variation and calculated haplotypes for symbiotic partners in each species to evaluate network patterns in lichen microbiomes. *Lecanora* and *Physcia* showed the highest variation in mycobiont ITS sequences, with *Lecanora* dataset also including additional species besides *L. aff. chlarotera*. *Lecanora pulicaris* was the dominant species in colder and more humid sites, growing on acidic bark (mostly conifers), while *Lecanora chlarotera* dominated in dry valley bottoms in the Swiss Alps. The lichen photobionts divided into two groups – *Trebouxia impressa* and *T. jamesii s.lat*. *Parmelia sulcata*, *Physcia adscendens/tenella*, and *Lecanora chlarotera* included species-specific lineages of *Trebouxia impressa*. *Pseudevernia furfuracea*, *Hypogymnia physodes*, and *H. tubulosa* all included photobiont from a single *Trebouxia jamesii* haplogroup with very little genetic variation and no geographic or ecological distinction. *Lecanora* species, being the only sexually reproducing lichens among the studied, showed the lowest selectivity for photobiont, with four different photobiont lineages in *Lecanora chlarotera* and *L. pulicaris*. Symbiotic interactions in studied lichens seem to be determined by phylogenetic constrains, and are likely dependent on reproductive mode and availability of suitable partners in specific habitats.

3.2-69 Bioclimatic factors at an intra-biome scale are more limiting than cyanobiont availability for the lichen-forming genus *Peltigera*

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Abstract: Factors shaping spatiotemporal patterns of associations in mutualistic systems are poorly understood. Here we used the lichen-forming fungi *Peltigera* and their cyanobacterial partners *Nostoc* to investigate the spatial structure of this symbiosis at an intra-biome scale and to identify potential factors shaping these associations. Ninety-three thalli were sampled in Québec, Canada along a South–

North and an East–West transect of ca. 1300 km each. We identified the two main partners (*Peltigera* species and *Nostoc* phylogroups) and modeled the effect of environmental variables and partner occurrence on *Peltigera*/*Nostoc* distributions. *Peltigera* species showed a high degree of specialization towards cyanobionts, whereas two *Nostoc* phylogroups dominated both transects by associating with several *Peltigera* species. *Peltigera* species had narrower ranges than these two main cyanobionts. Distributions of three *Peltigera* species were highly associated with precipitation and temperature variables, which was not detected for *Nostoc* phylogroups at this spatial scale. For these cyanolichens, factors driving patterns of symbiotic associations are scale dependent. Contrary to global-scale findings, generalist *Peltigera* species were not more widely spread within the boreal biome than specialists, and *Nostoc* availability was not the only driver of *Peltigera* species geographic ranges, because environmental factors were also contributing to their intra-biome distributions.

3.2-70 Ecological factors determining symbionts in temperate rainforest cyanolichen communities

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Abstract: A range of selectivity for cyanobacterial symbionts has been demonstrated across investigated lichens, with some species apparently sharing *Nostoc* widely, and others highly selective, with little to no switching so far observed. We ask how ecological factors affect this symbiotic composition, testing climate, microsite, and environmental availability of symbionts, including substrate sampling and co-occurring 'companion' cyanolichens. In the Atlantic forest of Scotland, two *Nephroma* species often co-occur, the primarily sorediate *N. parile* and the primarily sexually-reproducing *N. laevigatum*. Data from rbcLX from Sanger sequences and partial sequences from using Illumina metabarcoding including bryophyte mats on trees, and bare bark were compared. We used ordination and analysis of variance to test sources of variation in the data from a nested sample comprising 6 sites across a steep climatic gradient, those with and without cyanolichen populations and including controls at several levels. In addition, several types of controls were included to check reliability of results and methods. No biases were detected between one-step and two-step PCR amplifications, nor among amplification of different length templates; and consistent results were found with repeat PCRs, and no template controls. Washed and unwashed single thallus extractions were compared. From more than 700K reads, we found ca 300 OTUs at 97% similiarity. There is strong *Nostoc* genotypic association with climate, and clear patterns of environmental availability of lichen symbionts from bryophyte mats in addition to co-occurring lichens with shared symbiont specificity.

3.2-71 Ecological patterns of symbiotic specificity in *Leptogium* (Lichenized Ascomycetes: Collemataceae) in four Colombian biomes

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Abstract: The specificity of *Nostoc* cyanobionts and lichenized fungi has been explored mostly in temperate and paleotropical regions. So far, little attention has been paid to neotropical environments, which are known for their ecological heterogeneity and high species diversity. Here we focused on exploring patterns of phylogenetic and ecological signals in symbiotic specificity of lichen specimens collected in four contrasting biomes of the Caldas department, in the center of Colombia: Andean

Western Cordillera (2400-4000 m), Andean Central Cordillera (1670-3045 m), High Andean Central Cordillera (3470-4200 m) and Magdalena Inter Andean Valley (220-1100 m). To address the issue, we generated rbcLX sequences of *Nostoc* from 45 *Leptogium* thalli (representing 22 spp.) collected in those four biomes and aligned them with GenBank sequences of *Collema* and *Leptogium* cyanobionts. Phylogenetic analysis in a Bayesian framework were done using MrBayes 3. We found one haplotype of *Nostoc* per thallus, which suggests that each lichen included a single cyanobacterial species, at least at a detectable level. The phylogram obtained showed that the species follow a generalist pattern, as it has been shown before. However, we found that most of the cyanobacterial sequences of individuals from the Inter Andean Valley formed a monophyletic group, suggesting that the fungus associates with a locally adapted cyanobacterium. These results suggest that, whereas species from most habitats followed a similar generalist pattern that has been found in previous studies in temperate and paleotropical regions, the Inter Andean Valley differs. Potential explanations include adaptational value of locally adapted *Nostoc* strains or decreased cyaobiont diversity due to environmental stress, including high deforestation rate and the constant expansion of the agricultural border.

3.2-72 Niche modeling of lichens in the Brazilian Atlantic Forest: will global climate change move the tropical-temperate transition southward?

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Abstract: Biodiversity hotspots such as the Atlantic Forest are among the areas most threatened by climate change. Loss of biodiversity is one possible effect, while species displacement and niche changes are others. Based on current niche preferences, future climate models allow to predict such changes. The Atlantic Forest in Brazil is the only region in the world where a continuous, tropical-temperate gradient can be observed in a narrow strip of closed forest from the equator to south of the Tropic of Capricorn, with the added advantage that this gradient can be studied within the confines of a single country. The Atlantic Forest is therefore an ideal target area to analyze the effects of climate change on shifts in species niches and displacements of metacommunities as a whole. Epiphytic lichens are an excellent study object in this context as they are highly sensitive to environmental changes. We thus used epiphytic lichens to estimate a potential spatial shift in the tropical-temperate transition of lichen metacommunities in the Atlantic Forest, selecting the families Graphidaceae, Lobariaceae, Parmeliaceae, and Trypetheliaceae, as these include the two largest families of lichen-forming fungi and two additional families that contain species typical of either tropical or temperate climates. Based on literature review, herbarium revisions, mining of occurrence data in online repositories [GBIF, *species*Link], and new field assessments in the north, southeast, and south of Brazil, we assembled a list of nearly 8,600 occurrence data for the four families in the Atlantic Forest, representing 1,055 taxa. Since existing records often lacked georeferences but included municipality data, we standardized geolocation by using centroid coordinates of municipalities representing the target area [https://www.ibge.gov.br], for a total of 3,241 municipalities. We performed niche modeling on present climate data using a grid raster of 30 degrees [QuantumGIS], resulting in 614 grids covering the target area, with one grid corresponding to approximately five to six municipalities on average. In addition to altitude and forest cover layers, bioclim variables [www.worldclim.org/current] were selected based on prior PCA [PC-Ord] to reduce redundancy effects, and niche modeling [MaxEnt] was performed on 251

taxa present in at least five municipalities each. Modeled, grid-based probability values for each taxon were used to assemble a two-dimensional matrix for grid cluster analysis [PC-Ord], with the main split indicating the line of maximum species turnover between tropical and temperate areas of the Atlantic Forest. We found that the main split based on present climate data corresponded precisely to the Tropic of Capricorn. Projecting the data onto the HadGEM2-ES future climate model [www.worldclim.org/cmip5_10m], we estimated a significant southward shift of the tropical-temperate transition, suggesting an expansion of tropical epiphytic microlichen communities southward and a reduction of available niche space for temperate macrolichen communities. Present niche models for the 251 lichen taxa were further used to characterize within-tropical and within-temperate meta-communities along the latitudinal gradient of the Atlantic Forest, with five meta-communities partially corresponding to the division of the Atlantic Forest into north, southeast, and south.

3.2-81 Assemblage structure of ectomycorrhizal fungi on scrub oak (*Quercus ilicifolia*) roots in fire adapted pine barrens

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Abstract: Ectomycorrhizal (EM) fungi are thought to contribute to establishment of host plants after disturbance such as fire, especially in low-fertility xeric soils. Previous work has shown that while a diverse group of fungi are found on roots of pines in undisturbed settings, a different group of fungi are found on pine seedlings after a fire. The post-fire pine symbionts occur as a resistant spore bank that can be observed using soil bioassays. The objective of my study is to investigate if this same dynamic occurs in scrub oak (*Quercus ilicifolia*) ectomycorrhizal communities**.** I compare EM fungal assemblages on scrub oak roots collected from the Albany Pine Bush Preserve (APBP) where fire is an integral part of the plant community and is being reintroduced as a restoration tool. The APBP in east-central New York is a fire-managed, globally-rare inland pine barrens ecosystem that, like many fire-dependent ecosystems in the northeastern U.S., supports a disproportionally large number of rare or declining species. I collect scrub oak roots along with the surrounding soil from relatively undisturbed sites. The roots are carefully removed from oak root systems and sorted into morphological types (morphotypes). The soil associated with these roots is air dried to select resistant spore inoculum and used in a bioassay with lab grown scrub oak seedlings in a paired design with field and laboratory bioassay data linked. Fungi on root tips from field and bioassay samples are identified using the fungal barcode (nrITS region). Fungal DNA will be extracted to determine RFLP patterns. The DNA of each unique RFLP pattern is sequenced and submitted to GenBank. The mean proportion of root tips with EM fungi per seedling is analyzed using a paired t-test between field and bioassay seedlings to determine if there is a difference in EM fungal colonization on roots. Additionally, mean Simpson's species diversity is compared to see if there is a difference between treatments. Based on the results with pine I expect bioassay seedlings to be colonized by a different assemblage of EM fungi than seedlings harvested from the mature field sites, suggesting early successional fungi occur in soils as a resistant spore bank. I expect a different assemblage of EM fungi on oak roots collected from the field than roots harvested from the bioassay seedlings, with the field assemblage having a higher richness and a different assemblage of fungi on the roots. Preliminary analyses are promising, supporting this hypothesis. When looking only at morphology, field roots had greater morphological variety than bioassay seedlings alluding to this assemblage change. Field roots had an average of 5.000 (SE = 0.503) morphotypes per seedling while bioassay samples averaged 3.800 (SE = 0.115) morphotypes. Results of this experiment may elucidate

how forests recover from disturbance and provide insight on restoration efforts of these threatened young forest habitats.

3.2-82 Variation in mycorrhizal infection across a topographic-fire matrix in a tropical mesic forest.

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Abstract: Topography and fire can influence ecosystems' belowground attributes, including mycorrhizal fungi. In temperate and tropical regions, it has been well documented that soil physical and chemical characteristics change in complex ways with topographic position (ridge -valley positions) but that these changes can be modulated by fire. In contrast, we know little about the combined influence of topographic position and fire on mycorrhizal fungi in fine roots, particularly in montane tropical ecosystems. Understanding these complex interactions may be important in tropical montane forests that experience marked seasonal changes in precipitation given that global warming is converting fire in an important driver of change. The main goal of our work was to examine the combined effect of topographic position and fire on mycorrhizal fungi in fine roots of *Pinus tecunumanii*, including soil nutrients and root biomass in a tropical mesic forest located in the Sierra Las Minas, Guatemala. In paired ridges and slope positions affected and not affected by fire we identified two *Pinus tecunumanii* individuals and collected soil cores at two distances of the dripline. Roots were cleared and stained to estimated mycorrhizal abundance, whereas soils were analyzed for organic carbon and nitrogen, available phosphorus, extractable cations, and cation exchange capacity (CEC). A preliminary analysis of mycorrhizae showed that burned site have more abundance than unburned sites. On the other hand, most soil chemical attributes were greater in unburned than in burned sites. At the scale of this study fire, but not topographic position, influenced soil chemical properties. Based on these results, mycorrhizal fungi abundance increases with decrease of nutrient availability.

3.2-83 Forest disturbances influence the functional roles soil fungi play in defense-related chemical induction of a boreal pine

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Abstract: Mycorrhizal fungi can affect the ability of their host plants to produce chemicals with which to resist insect and pathogen attack. How such resistance is affected by communities of soil fungi that include mycorrhizal, saprophytic, and phytopathogenic species is poorly understood. The composition of these communities can be altered by tree-killing forest disturbances, but how the resulting changes in soil fungi impact the functional roles these communities play in tree resistance is unknown. Clarifying such relationships is important to predicting the susceptibility of recovering post-disturbance forests to insect outbreaks and disease under predicted climate change-associated increases in the frequency of forest disturbances. We investigated how soil fungal communities altered by forest mortality caused by wildfire, clearcut logging, mountain pine beetle (*Dendroctonus ponderosae*) outbreak, and salvage logging following beetle outbreak in Alberta differentially affect the induced resistance of greenhouse-grown lodgepole pine (*Pinus contorta* var. *latifolia*) seedlings. Seedlings grown in pots inoculated with soil from disturbed forest stands were treated with defense-signaling hormones to elicit metabolomic induction responses associated with insect and pathogen attacks. The resulting seedling metabolomes

were analyzed separately for above- and belowground tissues using untargeted metabolomic techniques. Results on how soil fungal community composition influences tree resistance-related metabolites will be discussed.

3.2-84 Effects of forest fertilization and thinning on fungal communities and associated enzyme activities in *Pinus sylvestris* forests

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Abstract: The boreal forest is characterized and limited by the closed circulation of nutrients, related to abiotic factors such as low temperatures and low pH and biotic factors such as recalcitrant organic matter and suppression of saprotrophic activity. Nutrient cycling is regulated by ectomycorrhizal fungi, who associate with plants to trade nutrients for photosynthetically derived sugars. A large part of the boreal forest is managed for timber production, and common practice for increased production is a combination of thinning and fertilization. We used metabarcoding of the ITS region to investigate how the ectomycorrhizal fungal community and associated enzymatic activities are affected by different combinations of fertilization and thinning. We sampled forests along a latitudinal gradient across the entire boreal biome in Sweden. Fungal community assembly was characterized by metabarcoding of the ITS2 region using the PacBio Sequel platform. Ergosterol was measured to evaluate over-all effects on fungal biomass. Changes in fungal community composition were related to increased below-ground accumulation of organic matter in fertilized plots. Relationships are discussed in the context of functional guilds and ectomycorrhizal exploration types.

3.2-85 What is the impact of kauri dieback on fungi associated with *Agathis australis* roots and leaf litter?

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Abstract: Kauri (*Agathis australis*, Araucariaceae) is restricted in distribution to the northern tip of the North Island of New Zealand. Living to 1,500 years or more and having trunks up to 3 m diam., *A. australis* exerts enormous influence on surrounding forest composition and structure, providing varying habitat niches for complex fungal communities. However, information on the diversity of fungi associated with *A. australis* is sparse. Since the 1970s these trees have been under threat from the exotic invasive pathogen *Phytophthora agathidicida* that causes kauri dieback. Our study characterised the fungal leaf litter and root endophytic community of *A. australis*. We obtained root and leaf litter samples from diseased and asymptomatic trees around Auckland, New Zealand. We isolated over 400 cultures from kauri roots and obtained DNA sequences from all of the fungal cultures. We clustered the sequences as OTUs and assigned identities using BLAST. We also used metagenomics to investigate the kauri leaf litter fungi under asymptomatic trees and diseased trees. QIIME and UPARSE were used to assign sequence reads to OTUs. Root endophytic and litter-associated species were distributed throughout the fungal kingdom; most of the endophytic community were in Helotiales and most of the litter-associated community were in Mortierellales. The OTUs were analysed to understand the impact of the invasion of

P. agathidicida on fungal diversity at a site, and to increase knowledge of kauri-influenced biodiversity. We compared the OTUs on the basis of habitat niche, geographic location, and disease status. These results give us an insight into the diversity of fungi associated with *A. australis* and suggest the possible impacts of *P. agathidicida* on the fungal community.

3.2-86 Spatial distribution of leaf litter fungal communities in a simulated hurricane experiment

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Abstract: Fungal communities play important roles in litter decomposition and nutrient cycling. The Canopy Trimming Experiment (CTE) began in 2003 at the Luquillo Experimental Forest in Puerto Rico, with the intention of collecting samples that would provide relevant data. The CTE focused on the immediate effects of hurricanes on forest floor processes and their recovery, in a tropical wet forest ecosystem. Changes to the forest's fungal community structure of litter layers may influence ecosystem recovery. Canopy trimming was performed again in October-November 2014 with the purpose of understanding long-term effects of increased hurricane frequency on forest productivity and carbon sequestration. Our objective was to evaluate if, and how, a hurricane affected fungal communities in the litter. Leaf litter samples were collected in three blocks, at various times up to two years. Based in the results of the first trimming, two treatments were considered for the second: unmanipulated control and trim plus debris. DNA was extracted using MoBio Power Soil DNA Isolation kit. The TRFLP technique was used to obtain profiles of the fungal communities in each sample using the fungal ITS region. Changes in fungal community structure between samples were analyzed using NMDS and Two-Way PERMANOVA. The fungal diversity in the leaf litter increased with the addition of canopy deposits. Fungal diversity decreases as the decomposition of litter progresses. The results indicate significant differences in fungal communities between treatments and though time. Fungal communities were heterogeneous among the treatments and through time indicative of a high turnover of species during the decomposition process. The results support previous observations obtained with the first trimming. In the future, we will analyze the effect of a recent hurricane on the structure of leaf litter fungal communities and the characterization of specific taxa. Climate change will cause an increase in intense hurricanes and understanding their effect in leaf and soil microbial communities will help us understand how resilient or vulnerable tropical forest are to natural disturbances.

3.2-87 Dynamics of microbial groups in response to simulated hurricane at El Yunque Rain Forest in Puerto Rico

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Abstract: Climate variability models predict increase in incidence and intensity of hurricanes. In Puerto Rico, hurricanes have impacted the Luquillo Rain Forest in many occasions resulting on canopy debris deposited in the forest floor. As consequence, the microclimate of the forest floor changed by the increase of direct sunlight, addition of complex plant biomass, alteration of microbial activity and, ultimately, the operation of biogeochemical cycles. A Canopy Trimming Experiment, that simulated the pass of a hurricane, has been done in the Tabonuco forest. It was designed to understand the effect, resistance and resilience of a tropical forest ecosystem after the impact of a hurricane. Our objective is to determine temporal heterogeneity of three microbial groups (fungi, bacteria, and sulfidogens) in response to detritus deposition of simulated hurricane effect. Two treatments are considered: with and without detritus deposition trimmed from the local canopy. Soil samples are being collected from plots,

at various times for a period of two years. Bacteria, fungi, and sulfidogens are being characterized independently by the molecular analyses of three distinctive genes (16S rDNA, ITS, and dissimilatory sulfite reductase) using Terminal Restriction Fragment Length Polymorphisms. Fungal diversity was greater than the bacteria over time. Bacteria was homogeneous over time for the same plot suggesting microbial succession in which rare microbiota became more prevalent over time. Bacterial and fungal communities exhibited spatial variation regardless the availability of plant debris. Diversity of sulfidogenic bacteria decreased over time where detritus was added. Richness for sulfidogens was lower in the absence of detritus. Diversity trends describe the dominance of fungi after deposition of debris and the gradual involvement of the anaerobic sulfidogenic bacteria. Fungal decomposition of complex substrates in the canopy debris seems to foster anoxic conditions where anaerobes thrive using more labile carbon sources. Further studies include the effect of recent hurricanes on the structure of microbial communities, characterization of specific taxa and quantification of microbial guilds examined.

3.2-88 Forest dieback affects spruce seedling regeneration on coarse woody debris by altering wood decomposer fungal community

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Abstract: Seedling regeneration after forest dieback events is crucial for forest recovery. In a subalpine coniferous forest in Kii Peninsula in Japan, however, spruce seedling regeneration has been seldom observed after a wide-range forest disturbance caused by a typhoon in 1959. Deer-proof fence constructed to reduce grazing by deer has not improved regeneration. Given that spruce seedlings need coarse woody debris (CWD) to colonize, their establishment is greatly affected by CWD condition which largely depending on decay activity of decomposer fungal community. Recent studies in Europe found that CWD decayed by brown rot fungi negatively affects spruce seedling density, and that frequency of occurrence of brown rot fungi tends to be increase after forest dieback. These results lead a hypothesis that forest dieback in Kii Peninsula also lead dominance of brown rot fungi and the logs decayed by them reduce spruce seedling regeneration. To test this hypothesis, we compared fungal communities within CWD and spruce seedling density among three sites of different dieback levels (control, intermediately dieback, and intensively dieback). Fungal communities within CWDs were documented using Illumina sequencing. Seedling and epiphytic bryophyte communities were recorded and were analyzed with CWD properties such as wood decay type (white rot, brown rot, and soft rot), pH, moisture, and bryophyte coverage. Sequencing showed that the frequency of brown rot fungi was higher in intensively dieback site than intermediately dieback site and control site. Among the CWD properties, frequency of brown rot wood showed the same trend found in brown rot fungi. Spruce seedlings and bryophyte coverage were higher on CWDs in the control and intermediately dieback sites than that in the intensively dieback site. Spruce seedling density was negatively associated with brown rot in sapwood and positively with bryophyte coverage. Brown rot in heartwood negatively associated with bryophyte coverage. These results suggest that dominance of brown rot fungi after forest dieback event in Kii Peninsula negatively affected spruce seedling regeneration.

3.2-113 Resolving the Mortierellaceae phylogeny: a test of Multi-Locus Sequence Typing (MLST) and phylogenomic approaches

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Abstract: The Mortierellaceae are an ecologically and industrially relevant lineage of early diverging fungi comprised of six genera classified within what is now recognized as phylum Mucoromycota. This polyphyletic family is estimated to contain at least 100 species, the phylogeny of which cannot be fully resolved with ribosomal markers. The ITS region is too divergent to align across the entire lineage and while the 28S region can be aligned across the family, there is poor backbone support for the resulting phylogenetic tree. In this research, we tested the use of high-throughput targeted amplicon sequencing for generating multi-locus sequence data to improve phylogenetic resolution of diverse lineages within the Mortierellaceae. First, we analyzed three *de novo* sequenced *Mortierella* genomes with a non-biased bioinformatic pipeline to identify potential non-ribosomal markers and designed PCR primers for multiplexed PCR amplification. We identified 13 loci that performed well across a diverse test panel of *Mortierella* isolates. We amplified these loci across 330 Mortierellaceae isolates, prepared libraries with an Illumina Nextera kit, and sequenced them on an Illumina MiSeq platform. Our non-biased locus selection successfully identified established phylogenetic markers (i.e., *TEF1* and *RPB1*); however, 7 loci were members of gene families or under selective pressure and therefore inappropriate to use as phylogenetic markers. This multilocus sequence typing (MLST) approach is very robust in extracting phylogenetic information from isolates contaminated by non-target organisms (e.g., *Fusarium* or Mucorales), an advantage over Sanger sequencing of universal barcodes or genome sequencing. However, we were less successful in resolving cross-contamination by other Mortierellaceae. Low-coverage genomes that were available for 60 of our isolates were analyzed to identify 400 informative markers. These markers were used to build a strongly supported genome-based phylogeny. We are identifying MLST loci within the low coverage genomes to serve as a backbone constraint for MLST phylogenetic analyses. This combination of approaches leverages both the sequencing depth of genomics and the sampling depth of amplicon-based sequencing. A resolved phylogeny and identification of non-ribosomal markers will improve identification and placement of novel species and genotypes as they are isolated, and inform higher taxonomy of this family. We will discuss the resulting phylogeny, informative non-ribosomal loci, our evaluation of the MLST approach, and implications for other lineages and organisms.

3.2-114 SMRT sequencing of a 2.5 kb rDNA fragment spanning SSU, ITS and part of LSU of arbuscular mycorrhizal fungi

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Abstract: Arbuscular mycorrhizal fungi (AMF) are ecologically and agronomically important, but global data on their species abundance and diversity are scarce. From ~290 morphologically described AMF species only a part is sequenced or available in culture collections, due to their obligate symbiotic lifestyle. The amount of 'taxa' based on environmental DNA sequences largely exceeds the number of described species. Different research teams often use different DNA marker regions for AMF diversity and taxonomy studies, therefore it is impossible to compare their results and make conclusions on global AMF species distribution, ecology and biogeography. Thus, research is needed to further define

the AMF species on the molecular level and to understand their genetic variability. We optimized an AMF-specific nested PCR approach to amplify part of the rDNA spanning majority of the SSU, complete ITS region and part of the LSU (~2.5 kb). This target fragment was amplified from morphologically described taxa in cultures representing main AMF lineages and selected AMF species-rich root and soil field samples from across the globe. PCR products were multiplexed into libraries and sequenced on the PacBio platform using the SMRT (Single Molecule Real Time) sequencing. High accuracy of such long reads was achieved due to construction of circular consensus reads (>5 passes) with 99% predicted accuracy. Sequences spanning the target fragment will enable us to evaluate genetic variability and determine bioinformatic thresholds for species, genus, family and order- level resolution for the different rDNA marker regions used (SSU, ITS and LSU) for all main AMF lineages, to conduct a robust backbone phylogeny, to compare results of AMF community studies targeting different marker regions and search for general patterns in AMF biogeographic and ecological distribution at global scale. The results will improve the environmental sequence assignment to the species-level and thus serve for a better interpretation of AMF community data from next generation sequencing studies.

3.2-115 Neocallimastigomycota, a gold mine to study the genomic evolution of the early-diverging fungi

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Abstract: Neocallimastigomycota is a distinct fungal phylum consist of micro-fungal symbionts in mammalian rumen and gastrointestinal tracts (e.g. Elephant, Horse, Sheep etc.). Neocallimastigomycota differ from other zoosporic fungi (Chytridiomycota and Blastocladiomycota) in many aspects including the strict requirement for the anaerobic environment. There is still limited knowledge about the biology and ecology of these fungi, except for their pivotal role in the plant biomass degradation for their animal hosts. To better understand the evolutionary history and genomic context of the Neocallimastigomycota we sequenced 22 transcriptomes from 7 distinct taxonomic groups (*Anaeromyces, Caecomyces, Feramyces, Neocallimastix, Orpinomyces, Pecoramyces,* and *Piromyces*). Combining publicly available genomes of Neocallimastigomycota and Chytridiomycota, we conducted phylogenomics and comparative genomic analyses of 32 taxa to better understand gene content and phylogenetic relationships of these lineages. Neocallimastigomycota is estimated to emerge as a monophyletic group (~74 Ma) with the herbivory in mammals (72-100 Ma) based on 426 conserved orthologous using BEAST analyses. Comparative genomics identified that Neocallimastigomycota fungi have lost functional Protein Family (Pfam) domains retained in Chytridiomycota related to oxygenase, dioxygenase, photolyase, and uric acid metabolism. We also identified Pfam domains that are specific to Neocallimastigomycota but missing in other chytrids. These include many carbohydrate binding modules, glycoside hydrolase, metal transporters, and plant cell wall binding domains. Three domains ("Cthe_2159", "Gal_lectin", "YoeB_toxin") are uniquely found in these lineages and have not been identified in any other fungi. One anaerobic functional domain HemN (oxygen-independent coproporphyrinogen III oxidase) is found to be missing from the higher classes of Animal and Fungi (Dikarya). Using phylogenetic methods, we aim to better understand the biological functions of these domains which appear most dramatically different in these fungi. These include galactose-binding lectin, oxygen-independent coproporphyrinogen III oxidase, and the polysaccharide lyase Cthe_2159 domains. Using these approaches we hope to better understand what roles they play in the animal

rumen ecosystem, whether these originate through horizontal gene transfer, and how these dynamic gene content changes inform evolution and emergence of the enigmatic Neocallimastigomycota fungi.

3.2-116 Optimizing high molecular weight genomic DNA extraction for Mucoromycota fungi

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Abstract: There are an estimated 10 million extant species of fungi, each of which contains a genome with unique functional genetic diversity and capacity. Genomic resources provide research opportunities for medicine, toxins/anti-toxins, and food, and a deeper understanding of the world in which we live. While genomes are being sequenced at an unprecedented rate, genomic sequencing is still contingent upon cellular extractions of high molecular weight DNA that are neither sheared nor degraded, and are free from RNA and other contaminants. Factors known to impact DNA quality are the pre-extraction culture conditions, including media contents and concentration, mycelial growth rate, and age of the culture. The NSF-funded ZyGoLife consortium has prioritized the genome sequencing of Mucoromycota fungi, since these fungi are under-sampled and less studied than Dikarya (Ascomycota, Basidiomycota). For these reasons, we optimized a CTAB-PVP genomic DNA extraction method for generating high-quality DNA extractions from fungi in the Mucoromycota (*Rhizopus* and six clades of *Mortierella*) for genomic sequencing through the Department of Energy Joint Genome Institute. DNA quality was measured using a Nanodrop to compare the ratio of absorbance of nucleic acids versus protein (optimal 260/280nm ratio: 1.6-2.0), and agarose gel electrophoresis for ensuring concentrated high-molecular weight DNA free from RNA contamination. DNA quantity was assessed with a Qubit fluorimeter (target amount: 12 µg). Overall, we found that the quality of DNA extraction varied with species and taxonomic group, with the best results from *Mortierella lignicola* and *M. ambigua*. Some of the factors that improved DNA quality involved: abundant fungal tissue, multiple replicates of a sample – with less tissue per tube, 'Snap-freezing' tissues in liquid nitrogen to prevent DNA degradation, and treating the DNA with RNase before precipitating. This approach has yielded pure, high molecular weight genomic DNA and improved upon previous CTAB-PVP protocols. We anticipate that this approach will enable future work with previously unsequenced fungi in Mucoromycota, and will be applicable to genome sequencing in other Phyla.

3.2-117 Revealing metagenomic methodological biases that limit the detection of parasitic fungi in the Zoopagomycotina

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Abstract: The adoption of metagenomic environmental sampling technology by mycologists for community analyses have uncovered entirely new lineages within the fungal tree of life such as the Archaeorhizomycetes and the Cryptomycota. Metagenomic ecological analyses of fungi have also enabled broad insight into the global and large-scale distribution of fungi. These studies have led to testable hypotheses about the factors that influence biogeographic patterns in fungi, such as fungal distributions shaped by dispersal limitation and climate. However, awareness of inherent methodological biases such as primer mismatch, taxonomic representation in reference databases used for operational taxonomic unit (OTU) assignment, and sequencing and barcoding errors have resulted in modified protocols for certain groups. Many surveys of soil-inhabiting fungi have focused primarily on ectomycorrhizae or other fungi in Dikarya. Despite being diverse and ecologically important

members of the soil environment, Mucoromycota and Zoopagomycota are often briefly mentioned or included only as a taxonomic rank in OTU richness figures in metagenomics studies. Though members of the Zoopagomycotina are often encountered in culture-based surveys, their detection in environmental sampling surveys is limited. Here we present an empirical test of Illumina sequencing which demonstrates the strong bias against long internal transcribed spacer regions (ITS) of the mycoparasitic Zoopagomycotina taxa *Piptocephalis* and *Syncephalis*. Previously-analyzed soil DNA samples were spiked with DNAs of isolates of known ITS length ranging from short (~600 bp) to medium (~750-900 bp) to long (>900 bp). A treatment comprised of a mixture of *Piptocephalis* and *Syncephalis* DNAs with varying ITS lengths and a negative control were added to the experimental design, resulting in five treatments that were each replicated three times. All treatments were amplified with the fungal ITS primers ITS1F and ITS2 and sequenced with Illumina MiSeq. Our results show minimal to no detection of the longest sequences, variable detection of medium sequences, and preferential detection of short sequences. We also provide preliminary analyses of the Zoopagomycotina community in environmental samples from Florida based on group-specific, modified metagenomic protocols.

3.2-118 Diversity of *Phytophthora* from water catchments in Auckland, New Zealand: the potential of stream baiting for catchment-scale surveillance of forest *Phytophthora*

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Abstract: Incursions from exotic *Phytophthora* species are considered one of the principal threats to forest ecosystems around the world. New Zealand kauri (*Agathis australis*) is currently threatened by a recently described, exotic *Phytophthora* species, *P. agathidicida*. Pre-emptive detection of novel *Phytophthora* species could provide an early warning of potential disease threats before they become established. While we have knowledge of the post-border soilborne *Phytophthora* species present in the kauri forest, we lack a complete understanding of the diversity of *Phytophthora* species naturally occurring in this system. Research published in Western Australia, Europe and the USA has demonstrated that stream-based catchment-wide surveys of *Phytophthora* can provided useful baseline data on the diversity of *Phytophthora* species, and have identified novel taxa not previously known to science. In this study, we used leaves of either native species (e.g. *Agathis australis* and *Pittosporum tenuifolium*) or exotic species (e.g. Cedrus atlantica, *Cedrus deodara*, *Pinus radiata*, *Rhododendron arboretum* and/or blue lupin cotyledons *Lupinus angustifolius* L.) to capture the diversity of *Phytophthora* species occurring in the Cascades Kauri Park (west Auckland) and Whangaparoa (Coromandel) catchments – both catchments contain forests with the kauri dieback disease, and include kauri in the riparian zone. We found that stream baiting can detect *Phytophthora* species year-around, and in total 12 different species of *Phytophthora* were retrieved across the west-Auckland and Coromandel catchments. The diversity of species recovered included species considered to be part of the "aquatic" Clade 6, however, the further isolation of *P. multivora* and *P. cinnamomi* from the baits means that soil-borne *Phytophthora* species can also be detected via stream surveillance. Species not previously recovered in New Zealand from soil baiting in the kauri forest were also detected in the streams. A previously undescribed species in clade 9 was also characterised, and a member of the recently described *Nothophytophthora*. The "bait" species with the most utility to attract the greatest diversity of *Phytophthora* species were shown to be the Himalayan cedar Cedrus deodara and kauri *Agathis australis*. This study demonstrates the potential for stream baiting to be used for passive-surveillance, and to monitor changes in *Phytophthora* diversity in forested water catchments. As part of the research

project, we developed a re-usable, plastic "bait-cassette" which helped standardise the experimental approach, and made the technique easily operated by school students and "citizen scientists". This will help build awareness around kauri dieback and the pathways through which it is spread utilising a participatory, science platform.

3.2-119 Applied DNA metabarcoding: Towards a streamlined approach for DNA based profiling of indoor mycobiomes

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Abstract: Health risks connected to the indoor microbiome are mostly associated with poor indoor air quality. Wherever excess moisture is available, fungi and other microorganisms start to grow. These microorganisms can act as a source of indoor pollutants and cause poor indoor air quality that is associated with adverse health effects, such as allergies, asthma and other respiratory symptoms. In other parts of the world, several studies have been performed trying to identify the main determinants of the indoor mycobiome and important factors influencing the mycobiome. These factors commonly include building type, geography, ventilation and outdoor air influence amongst others. Therefore, the overarching aim of our project is to improve the knowledge about the indoor mycobiome in the Northern Europe using DNA metabarcoding analyses. By analyses of dust samples, the indoor mycobiomes are characterized at different spatiotemporal scales; within buildings, across buildings at larger geographical scales, as well as throughout different seasons. Sampling has been performed within Norwegian Kindergartens throughout a year by biweekly sampling at four different floors. Dust has been collected from specific areas on identical glass plates by using floq swabs and tape. Real-time PCR has been performed covering the 20 most common fungal species in the indoor air, including Aspergillus, Penicillium, Alternaria and Chaetomium amongst others. The Real-time PCR results can then be compared to the metabarcoding data. We are also evaluating the usage of internal standards for improved quantitative information from DNA metabarcoding data. Compositional differences in the indoor mycobiomes will be coupled to local and regional environmental variation and building characteristics through multivariate analyses.

3.2-120 Fungal biomass in the leaf litter and soil of riparian regions in the rain forest of Pernambuco, Brazil

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Abstract: In the riparian forests, a number of extremely important functions of the ecosystems are carried out maintaining the quality of water and stability of soils. In the riparian portions of rain forest fragments, large quantities of organic matter are produced that guarantee nutrients to the maintenance of these ecosystems, besides offering essential environmental conditions for fungal development. Among the fungi capable of decomposing organic matter are the hyphomycetes, the largest (morpho-)group of conidial fungi which main importance to the ecosystems is to promote nutrient cycling through decomposition of dead organic matter. Therefore, the aim of this work is to quantify fungal biomass in the leaf litter and in the soil of riparian regions in areas of the rain forest that are under environmental protection in the Northeast of Brazil. Three sets of samples (soil leaf litter, submerged leaf litter and soil) were taken between August 2014 and July 2017, in four conservation unities (REBIO-Saltinho, RVS-Gurjaú, REBIOmu-Mata da Chuva and APA-Lagoa da Mata) in Pernambuco, Northeast of Brazil. Each area was represented by six sampling points that were sampled at least six times. These samples were kept in the freezer until extraction of ergosterol and analysis of this biomarker by HPLC were carried out.

Statistical analysis showed that there are differences between the areas for each type of substrate. The soil presented largest amounts of fungal biomass than the decomposing leaf litter independent of litter origin. Submerged leaf litter presented the lowest amounts of fungal biomass. The amplitude of variation between samples of each type of substrate was largest for the soil samples, indicating the importance of microhabitats. There was alternating higher and lower values of ergosterol in all substrates along the period of study, therefore no pattern of ergosterol amounts could be established when comparing with the occurrence of fungal species that have been detected by morphological analysis. The use of a multiplicative conversion factor proposed in the literature resulted in a variation of fungal biomass in the studied natural substrates ranging from 21.84 to 409.5 micrograms of mycelial mass per gram of soil or leaf litter, however, these values can be considered overestimated by a factor of approximately 20%, according to the literature. Nonetheless, ergosterol is considered a good biomarker to estimate fungal biomass in complex substrates. Financial support: CAPES and CNPq.

3.2-123 Host genetics and ecological interactions structure amphibian skin microbiome diversity

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Abstract: The host-associated microbiome, the community of microbes living on or within a host organism, has become a popular area of study in animal health research. Recent breakthroughs in DNA sequencing technology have greatly expanded our understanding of the diversity, distribution, and abundance of host-associated microbes. However, basic biological questions about microbiome structure remain to be answered. For example, it remains to be understood what role host genetics plays in determining microbiome assembly and diversity. In addition, microbiome studies rarely examine how both eukaryotes and bacteria contribute to overall microbiome structure. In this study, we (1) determined the impacts of host genetics on the microbiome, and (2) examined the positive and negative associations between bacterial and eukaryotic taxa found in the microbiome. We used a model system of Brazilian land-bridge island frog (*Thoropa taophora*) populations. Isolated island *T. taophora* frog populations were previously shown to contain very low genetic diversity relative to mainland populations. We collected skin swabs and genetic samples from frogs to respectively analyze the skin microbiome and frog host genetics using DNA sequencing. To determine associations between microbiome structure and host genetics structure, we examined the effects on microbiome assembly and diversity of two aspects of host genetics: (1) population-level neutral genetic diversity, and (2) genotype at the MHC immune locus, which we hypothesized would play a significant role in the ecological selection of microbes. Microbiome structure was significantly related to both host neutral genetic diversity and individual MHC immune genotype. Frogs from genetically diverse populations hosted a higher diversity of microbes. In addition, within each population, frogs that were heterozygous at the MHC locus hosted a higher proportion of likely commensal (non- parasitic) microbes. We then employed a network-based analysis to determine the positive and negative associations between bacterial and eukaryotic taxa across hosts. Specifically, we were interested in testing whether bacteria previously shown to inhibit fungal growth were negatively associated with fungal diversity. This analysis is still in progress, but preliminary results suggest no negative associations between the presence of "antifungal" bacteria and the abundance of microbiome fungi. The results of this study contribute to our understanding of how host genetics and ecological interactions between microbes impact the structure of the host-associated microbiome.

3.2-124 Core members of the cutaneous microbiome of Appalachian salamanders affect pathogenicity of chytrid fungi

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Abstract: *Batrachochytrium dendrobatidis* (*Bd*) is a dermatophytic pathogen that has caused enigmatic declines in amphibian populations worldwide. Salamander populations in the eastern US persist in the presence of *Bd* with surprisingly low rates of infection, despite relatively high rates in co-occurring anuran populations, which may be due to cutaneous probiotic defenses. We conducted a foundational study to better understand effects of microbial community dynamics on pathogenicity of *Bd*, and to identify keystone probiotic members of the salamander skin microbiome. Objectives were to (1) use high-throughput DNA sequencing to characterize the structure of the skin microbiome, (2) isolate and identify bacterial isolates that inhibit *Bd*, and (3) evaluate dominance of antifungal isolates within the microbial community as a whole. During May and July in both 2016 and 2017, salamanders from the genera *Plethodon* (n=67), *Eurycea* (n=65), and *Desmognathus* (n=83) were captured from nine sites in Tennessee, and skin swab samples were obtained. We used high-throughput DNA sequencing techniques and bioinformatics analyses to define the composition and structure of the resident skin microbial community across salamander taxa, seasons, and ecoregions. Results indicate that both geography and host taxon significantly affect the structure of the microbiome, but seasonality does not. Additionally, we isolated a total of 476 bacterial colonies in pure culture. Of the 63 isolates that have been challenged against *Bd*, 16 formed measurable zones of inhibition and were identified through Sanger sequencing. We compared genotypes to a published database of antifungal isolates, and we identified three new anti-*Bd* isolates. After conducting indicator species analyses within salamander skin communities, we pinpointed a core member of the microbiome that is also a strong antagonist of *Bd*. During future research, we will identify additional core probiotic members of the skin microbiome, infer their ecological roles through construction of interaction networks, and continue screening to evaluate candidacy for *in vivo* treatment trials. Results may directly impact future conservation efforts for North American wildlife.

3.2-125 Ecological genomics of divergence and hybridization in the amphibian chytrid

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Abstract: Understanding the generation, maintenance, and distribution of genetic diversity is fundamental to predicting the ecological trajectory of emerging diseases. Chytridiomycosis, caused by the chytrid *Batrachochytrium dendrobatidis* (*Bd*), is the emerging infectious disease implicated in recent population declines and extinctions of amphibian species worldwide. In regions where *Bd* has been recently introduced to naïve host populations resulting in disease outbreaks, only a single hypervirulent clonal genotype has been observed (*Bd*GPL). In the Atlantic Forest of southeastern Brazil, however, a deeply divergent – and potentially endemic – lineage has been recently described (*Bd*Brazil). This newly discovered population of *Bd* provides a critical opportunity to characterize standing genetic variation, and to probe the dynamics of virulence evolution in this emerging pathogen. Here we characterize the population dynamics of *Bd* lineages in the Brazilian Atlantic Forest using whole genome resequencing of field-collected isolates. Our sequencing reveals a high degree of genomic plasticity, with variable

chromosomal copy number and mitotic recombination as major drivers of variation among isolates. Our population genomic analyses suggest that the two lineages have been brought into secondary contact by human activity, with the *Bd*GPL having recently arrived in the historical range of *Bd*Brazil. While the long-term consequences of this secondary contact remain largely unknown, our field studies recovered a number of hybrid strains resulting from outcrossing events between *Bd*GPL and *Bd*Brazil. Using our genomic dataset, we assess the patterns of inheritance in hybrid strains to address the degree of incipient reproductive incompatibility between these *Bd* lineages. Our study provides insight into the genetic basis of divergence, adaptation, and establishment of fungal pathogens; and we highlight the utility of comparative genomic tools in understanding the evolutionary ecology of novel emerging diseases.

3.2-126 Ecophysiology, phylogeography, and virulence of *Pseudogymnoascus destructans*

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Abstract: The fungus *Pseudogymnoascus destructans* (Ascomycota: Leotiomycetes) is invades the skin of European and Asian hibernating bats. This pathogen was recently introduced to USA, where it causes so called "white nose syndrome", resulting in the bat mortality. Our team has long time interest in the multidisciplinary research on *P. desctructans* genetics and physiology and ecology. We studied the genetic relatedness of European and Asian (Ural) strains using six variable genetic loci including the mating type factors. Both mating types were found in the Czech Republic in the equal proportions, what showed to its cryptic sexuality. Among the five recognised haplotypes, one was found in the Czech R., USA as well as in Ural, thereby expanding the source region of this fungus. Further, we compared the extracellular enzymatic activities and secondary metabolite profiles of six virulent strains (Europe, Asia, USA) and six non-virulent *Pseudogymnoascus* species. The characters specific to *P. destructans* were the lipase activity, production of siderophores and hyper production of the riboflavin (vitamin B_2). This shows, that siderophores, which are common virulence factors in microorganisms, play also the important role in *P. destructans* pathogenesis. Riboflavin is hyper accumulated in the skin lesions, and is responsible for their distinctive yellow-orange fluorescence. Its local concentrations reached the values toxic for the bats cells and its massive presence can significantly weaken the infected bats. The other aim of our study was to clarify properties responsible for unique ecology of *P. destructans* by comparison with ecological related or unrelated pathogenic or nonpathogenic fungi. This part includes study of tolerance to physiological stresses and recognition of spectrum of utilizating nutrients (compounds of carbon, nitrogen, phosphorus, sulphur and nutrient supplements). Influence of several types of physiological stress (e.g. UVA, UVA with UVB, 25 °C, 30 °C, 37 °C and dryness) was investigated with fluorescent stain propidium iodide by flow cytometry. The spores of *Pseudogymnoascus destructans* and three fungi from underground spaces were not viable after 3 weeks at 37 °C. Other stresses did not cause a decreasing of viability or some stresses caused a decreasing of viability only in some strains of fungi. System Biolog showed, that isolates of *P. destructans* differ in utilization of sources of carbon,

nitrogen, phosphorus, sulphur and nutrient supplements from other testing fungi. The selective isolations medium was developed in the course of the study and tested in real conditions.

3.2-127 Forensic Mycology: Determining postmortem interval based on fungal flora composition in human cadaver samples

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Abstract: There are many symbiotic and parasitic relationships known between fungi and humans. For instance, some fungi are components of the normal flora in mammalian intestinal tracks, and some are parasitic inside the body and/or on skin tissues. Additionally, micro- and macro-fungi are prominent components of soil. Some of these soil fungi prefer ammonia and lipid rich soils, such as are produced by decomposing bodies. Knowing these previously mentioned attributes of fungi, we asked the question: how can mycology help solve problems in forensic science? Forensics is used in every day crime investigation. Many sub-disciplines are well known and researched, such as forensic entomology; however, mycology is rarely used and is not extensively researched in a forensics context. Fungi can be used to determine time of death by looking at fungal succession on deceased individuals. Also, they can serve as trace evidence to support location of a crime by linking perpetrator to a crime scene by comparing fungal traces, such as spores and mycelium, and yeasts in soil and on materials such as clothes or tires. Our goal is to determine a relationship between fungal flora changes, in terms of community composition, and post-mortem interval (PMI) based on human swab samples. Samples were obtained from a medical examiner from 65 different bodies that were undergoing natural decomposition (were not embalmed). Individuals selected were of varied PMI (between 12 and 456) hours. To broaden our sampling, individuals were included of differing sexes, races and ages, and from different seasons, locations, and causes of death. These samples were collected from different regions of the body, including ears, nose, umbilicus, mouth, eyes, rectum, arms, and skull. The DNA extracted from the body samples was amplified at the fungal barcode (ITS region), and sequenced using Illumina MiSeq. The fungal flora associated with human decomposition will be presented and correlations with PMI will be shown.

3.2-128 Efficacy of *Verticillium lecanii* and *Beauveria bassiana* of commercial source against cattle tick, *Rhipicephalus (Boophilus) annulatus*

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Abstract: Two entomopathogenic fungi, *Verticillium lecanii* and *Beauveria bassiana*, were tested against *Rhipicephalus annulatus*. Mycotal® was the source of *Verticillium lecanii* while Biosect® was the source of *Beauveria bassiana*. Five concentrations (1×10^7, 5×10^8, 2.5×10^9, 1×10^{10} and 4×10^{10} spore/ml) of *Verticillium lecanii* as well as five different concentrations (5×10^7, 2×10^8, $8\times10^{9,}$ 3.2×10^{10} and $12.8\text{x}10^{10}$/ml) of *Beauveria bassiana* were prepared and tested against adult female tick, eggs and larvae. The mortality in adult ticks was 60.60 to 72.00% after 2 weeks of application for *V. lecanii* at concentration ≥ $5\text{x}10^8$spore/ml, while *B. bassiana* showed no mortality at any concentrations. The treated tick revealed nutritional index significantly lower than control untreated one for both fungi. Furthermore, *V. lecanii* showed no effect on eggs, while, *B. bassiana* delayed and reduced the egg hatching. In addition, both fungi caused 100% mortality of larvae. The effective concentration was ≥ 10^8spore/ml for both fungi with no significant difference among the highest concentrations. Moreover,

the fungal extract had no effect on adult tick. In conclusion, *V. lecanii* is lethal to adult tick and *B. bassiana* caused larvae mortality and reduced egg hatching. A prospective application of fungi in the pasture or animal farm is possible for tick control.

3.2-129 Members of the invasive shot hole borer cryptic species complex (*Euwallacea fornicatus*) exhibit promiscuous mutualism with ambrosia Fusaria clade symbionts in Taiwan

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Abstract: Tea shot hole borer (TSHB, *Euwallacea fornicatus*) and polyphagous shot hole borer (PSHB, *Euwallacea* sp. nr. *fornicatus*) are morphologically identical wood boring ambrosia beetles that vector *Fusarium* spp. from the ambrosia Fusaria clade (AFC) in female mycangia, and have been reported as invasive pests in multiple regions around the world including: Madagascar, Costa Rica, Guatemala, Panama, Hawaii, Florida, California and Israel. These xylomycetophagous pests are native to southeast Asia and are a cause for concern as they attack multiple hosts in natural, urban and agricultural environments using a suite of fungal mutualists including *Fusarium* spp., *Graphium* spp., and *Paracremonium* spp. In agriculture, TSHB is best known for causing damage on tea (*Camellia sinensis*) in India and Sri Lanka, while PSHB has been reported causing damage on avocado in California and Israel. In these locations, TSHB and PSHB have been found to be associated with heterothallic *Fusarium ambrosium* and *Fusarium euwallaceae* respectively, suggesting an exclusive mutualism with these AFC members. The aim of this study was to investigate the symbiotic association of AFC fungi and *Euwallacea* spp. from a field location in Taiwan where the two species are sympatric. Ten female beetles from Danei District Tainan City, Taiwan were sampled from six infested avocado groves. The females were surface sterilized with 70% ethanol and the heads, containing mycangia, were removed from the bodies to isolate fungal mutualists. Single colonies of *Fusarium* spp. were recovered from each respective beetle and identified using multi-gene phylogenetic analyses of the internal transcribed spacer region (ITS), translation elongation factor-1 alpha (TEF1-α), and RNA polymerase II subunit (RPB1, RPB2) regions. The body of each beetle was subsequently used to establish its specific identity via a high-resolution melting assay. and the recovered specimens from Taiwan, four TSHB and six PSHB were determined to be vectoring two *Fusarium* spp., from two divergent clades within the AFC. Within the two AFC clades, PSHB and TSHB were found to vector the same *Fusarium* spp., indicating the mutualism within *Euwallacea* spp. is not exclusive, but promiscuous in this region. The *Fusarium* spp. sampled also indicate that both *Fusarium* phylogenetic species are heterothallic and both mating types are present within the *Fusarium* spp. recovered from the beetles. These findings show evidence for a non-exclusive mutualism in heterothallic *Fusarium* spp. vectored by *Euwallacea* spp. in native areas within Taiwan.

3.2-130 New diversity in the mutualism between ambrosia beetles and aggressive wood decay fungi (Polyporales: *Flavodon*)

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Abstract: Fungus-farming ambrosia beetles are a globally distributed, abundant, and diverse group of wood-boring beetles, boasting more than 3,000 species in the weevil subfamilies Platypodinae and

Scolytinae. Ambrosia beetles cultivate gardens of nutritional fungi to feed their young. They have complex organs, termed mycangia, to carry living fungal colonies while dispersing from their natal galleries to new trees. The fungal symbionts of less than 5% of these beetles have been described and aside from a few known plant pathogens, the ecological impacts of these fungi are largely unknown. The majority of the described fungal symbionts of ambrosia beetles belong to Ascomycota and are not known to decay the complex structural components of wood. Recently, however, an aggressive wood-decaying member of Basidiomycota, *Flavodon ambrosius,* has been described as the nutritional symbiont of a diverse, globally-distributed clade of ambrosia beetles (*Ambrosiodmus* and *Ambrosiophilus*). These beetles are common and infest a diverse range of host trees. Thus, this symbiosis may have broad implications for wood-decay processes world-wide. Previous culture-based work on beetle-associated *Flavodon ambrosius* has been largely restricted to Asian beetles in Asia and introduced to North America and suggested that these beetles farm a single *Flavodon* species worldwide. We examined *Ambrosiodmus* and *Ambrosiophilus* beetles native to multiple continents, as well as the closely related genus *Beaverium*. In addition to culture-based assays on freshly-collected beetles, we use next-generation sequencing (Illumina and PacBio) approaches on both fresh and preserved specimens from existing collections. This combined approach allows us to look for multiple symbionts within each beetle and also facilitates broader geographic and taxonomic sampling. Moreover, we compare the results obtained from each method and we suggest an efficient combined approach for future surveys. We incorporated single-copy fungal mock communities to help parameterize our bioinformatics pipelines for both sequencing platforms. We further support our results with mock communities composed of fungal taxa that are closely related to *Flavodon ambrosius*. We discovered new diversity in *Flavodon* from beetles native to both Asia and North America, including a new association between an undescribed *Flavodon* and the beetle *Beaverium*. We also found the presence of Asian *Flavodon* within the mycangia of North American beetle species, suggesting an introgression of invasive fungi into native beetles. By shedding light on the dark diversity of decay fungi present in beetle mycangia, our results demonstrate that the symbiosis between globally distributed ambrosia beetles and aggressive wood decay fungi is more diverse and widespread than previously thought. These interactions may be important to decomposition and nutrient cycles in forest ecosystems. Our results call attention to the potential ecological impacts of widespread introductions of these beetles and their symbionts.

3.2-131 Age does matter - the mycobiota of *Myrmica scabrinodis* ants

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Abstract: Recent studies have shown that ant-fungi interactions may be much more common, than previously thought. Studies on 'carton ants' and 'black yeasts'; *Formica aquilonia* and its yeast community; or *F. pratensis* and the fungus *Mortierella formicae* lead us to believe that a number of fungal species engaged in interactions with ants still could be undiscovered. Moreover, previous research on ant-fungus interactions, with the notable exception of leaf-cutting ants and their fungal gardens, focused mostly on visible, lethal, ant-parasitic fungal species (e.g. *Pandora*, *Cordyceps* spp.), neglecting the mycobiota that inhabits the cuticular surface of ants. Furthermore, in ants occur an age-dependent division of labour which, more or less, goes hand in hand with spatial distribution of individuals, with young ones being confined mostly to the inner part of the colony and old ones foraging outside, coming in contact with different fungal species. Thus, an age-dependent compositional change of mycobiota would be expected. The purpose of this study was to investigate the yet unknown, mycobiota of the ant *Myrmica scabrinodis* and to compare the mycobiota of young and old individuals. We analyzed the

mycobiota of 324 *M. scabrinodis* ants from nine ant colonies. Five colonies were also infected with another fungus, *Rickia wasmanii*, a mild parasite belonging to the family Laboulbeniales. Ants were classified as either young (yellowish) or old (brownish) and then successfully placed on culture media (SDA). Fungal colonies, which grew from the ants' cadavers were verified and assigned to 66 morphotypes. The strains from the genera *Cunninghamela*, *Penicillium*, *Mortierella*, *Absidia* and *Cladosporium* were the most frequently isolated and occurred in all colonies. Additionally, rare Mucoromycota fungal species (e.g. *Gongronella*) were also identified in the mycobiota of ants. No differences were found between the mycobiota growing from infected with *R. wasmanii* and uninfected ants. On average 1,75 fungal colony was growing from every individual, and old individuals contained significantly more colonies. Interestingly, *Mortierella* strains were growing more often from young ants, while *Absidia* colonies were isolated mostly from old individuals. This is the first study to show that the mycobiota of ants changes with age.

3.2-132 Discovery of psychoactive drugs psilocybin and cathinone in the cicada pathogen *Massospora* (Zoopagomycota) using metabolomics

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Abstract: The obligate lifestyle and large genome size of many members of the Zoopagomycota have long precluded their study. Modern –omics-based tools including metagenomics, transciptomics and metabolomics have facilitated a better understanding of these fungi despite barriers that still prevent traditional lab-based studies. *Massospora* is one of several members of the Entomophthorales that have not yet been studied with a systems biology approach. Global and targeted metabolomics were used to compare two contemporary *Massospora* – cicada symbioses, *M. cicadina*-infected periodical cicadas (*Magicicada* spp.) and *M. platypediae*-infected banger-wing cicadas (*Platypedia putnami*) to identify candidate secondary metabolites influencing host colonization and recently described behavioral modification. Results of the global metabolomics uncovered a diverse assortment of monoamine alkaloids including psilocybin, a psychedelic compound, from freshly collected *M. platypediae* and archived samples of *M. levispora* and cathinone, an amphetamine, from *M. cicadina*. Fragmentation of these analytes in pooled samples further validated their identification and targeted quantification confirmed absolute concentration of cathinone, psilocybin and, and psilocin. Additional MS/MS analysis will be performed to confirm intermediates within the psilocybin biosynthesis pathway across all samples. Metagenomics and targeted LC-MS proteomics are currently underway to further validate these findings. These psychotropic compounds may enhance stamina of infected cicadas to ensure continued conidial dispersal over several days despite debilitating infections that likely result in fatigue, decreased muscle strength and general malaise. The presence of these compounds, particularly psilocybin, may also support the hypothesis that they serve to deter predation. Phylogenetic studies showed *M. platypediae* and *M. levispora* were not genealogically exclusive but instead a single clonal

lineage with both species yielding psilocybin. Such discoveries as shown here have only begun to scratch the surface of the vast metabolic capacity of these enigmatic fungi. Follow-up studies on *Strongwellsea*, another member of the Zoopagomycota, are currently underway.

3.2-134 Fungi and their associated insects: ecological observations in a tropical cloud forest, Ecuadorian Amazonia

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Abstract: We carried out this pilot study in a tropical cloud forest at Estación Biológica Yanayacu, Napo province, western Amazonia. During three field campaigns in 2006 we established two 20 m^2 permanent plots where all envisaged sporocarps (fruiting bodies) growing on the ground and over rotting tree-trunks were collected. We classified the fungi in four "developmental states": immature, mature, decomposed and highly decomposed. *In situ*, we manually extracted from each sporocarp as much adult hexapods (insects and allies) as possible which were preserved in ethanol for posterior taxonomic treatment. Posterior extractions were carried out under controlled conditions (artificial light, temperature and humidity) in the laboratory, allowing thus remaining larvae to emerge as adults. Our goals were to i. describe the invertebrate fauna hosted in the sporocarps, and ii. evaluate the preference for the guests to their hosts. We recorded ca. 400 specimens in eight exapod groups and identified 65 morphospecies in 30 families. The beetles were dominant representing ca. 50% of the specimens collected, and among these the rove beetles (Staphylinidae) were highly abundant. Regarding the fungi, we identified 12 families, 21 genera and 25 species, plus ca. 10 morphospecies whose identification is pending. The insects showed an apparent preference for mostly three fungi families: Marasmiaceae (white-hut mushrooms), Polyporaceae (poroid mushrooms) and the paraphyletic Tricholomataceae (white, yellow, pink-spored agaricales). We found more flies (mainly Drosophilidae) in the first group possibly because the Diptera have high protein requirements during fight foraging, whereas the beetles were abundant in the other two fungi families. We have no current ecological clues for the latter apparent preference. Principal component analyses showed a tendency for the beetles (mostly Staphylinidae) to "select" mature fruiting bodies, while the flies were more frequently collected at highly decomposed and mature fruiting bodies. Based on literature search we classified the insect families in the following trophic guilds: primary fungivores (2), secondary fungivores (15), detritivores (9) and sporocaps predators (4). This study was supported by Escuela Politécnica Nacional and Instituto Nacional de Biodiversidad (INABIO_QCNE).

3.2-135 Two species in Legeriomycetaceae (Harpellales) derived from Capniidae (Plecoptera) in Japan

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Abstract: Harpellales is a fungal group which are inhabiting in the digestive tracts of aquatic insects (Ephemeroptera, Plecoptera, Diptera). More than 250 species have been recorded in the world. On the other hand, only 17 species have been recorded in Japan, which have been derived mainly Diptera. In this study, we focused on the family Capniidae (Plecoptera) which has not been examined as host for Harpellales in Japan. Nymphs of Capniidae appear in late autumn and emerge in winter, which are living between the submerged leaves in the very slow flow of the streams. We made collection in Fukushima, Ibaraki and Nagano Prefectures. Submerged leaves were collected and brought back with ice to the

laboratory. The nymphs were sorted from the leaves, and were dissected by a forceps to derive the hindgut. Derived gut was rinsed in a drop of distilled water on a glass slide and was observed with water mount by a light microscope with differential interference apparatus. The distilled water was replaced by lactophenol for preservation. Here we report the two noteworthy undescribed species. The first one was a species of *Ejectosporus* (Legeriomycetaceae). Thallus consisted of a central axis, thin branches and thick branches. The basal part of the main axis attaches to the host gut lining with holdfast mass of secretion. The lateral part of the main axis has several protuberances in a line and attaches to gut lining secreted materials. Two types (long and short) of trichospores were produced (ca 30 x 5μm and 10 x 3μm). The generative cells in the distal end of the branch produced only one type of the trichospores. A branch with long trichospores and a branch with the short trichospores were occasionally produced from one common branch. Long trichospore has two appendages. Thick branches produce long cylindrical spores (vegetative spores) (ca 80 x 10μm). Zygospores have not been observed. As all of the known species of *Ejectosporus* do not have two types of trichospores, this species appears to be undescribed. The second undescribed species also belongs to Legeriomycetaceae. This species also has a main axis and with thin branches. The basal part of the main axis attaches to the host lining with mass of secreted holdfast. The lateral part of the main axis also has protuberances with secretion. In this species, many trichospores are produced. More than 20 generative cells are in a line at the distal end of a branch and produces long elliptical trichospores, ca 10 x 2.5μm, with one appendage. Zygosporophores arises from the conjugation tube, triangle in shape, between two parent hyphae. Zygospore is biconical, 30 x 8.5μm, and connected to the zygosporophore at right angle. The most remarkable character is detached zygospores. Detached spores were surrounded by round clear zone. This zone can be regarded as an expanded appendage. This clear zone reminds us a skirt-like appendage of zygospore of *Zygopolaris* but can be clearly distinguished from it, which is a very unique character and has never been known in Harpellales.

3.2-136 Tally of Harpellales gut fungi in Idaho mosquitoes and dispersal ecology of *Zancudomyces culisetae* in a mountain stream

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Abstract: The trichomycetes is an ecological group of fungi (and protists) associated with arthropods. One fungal order Harpellales has been the target of collecting Idaho, where some dry regions make collecting these fungi challenging. Representatives of two genera, *Smittium* and *Zancudomyces*, were not uncommon in Idaho mosquito (Culicidae) larvae when their puddle (lentic) habitat was associated with stream (lotic) systems. The same was not true in lentic systems free of lotic input, which revealed no fungal infestation. Surveys of gut fungi in Idaho have led to further unraveling of one species that attaches precociously in the midgut, but does not produce spores. Pure isolates were obtained by suspending the larval mosquito midgut linings with immature unbranched thalli on BHI agar plates to promote the continued growth, branching and maturation of thalli and spores for identification as *Zancudomyces culisetae*. Curiously, each particular gut fungus seems to present consistently with its own unique penetrating holdfast during precocious extrusion in the midgut, a type of holdfast which is not found during growth in the hindgut. Among trichomycetes, *Z. culisetae* is a common gut fungus associated with multiple mosquito genera in freshwater lentic and slow moving lotic systems and serves as a model organism for study. Other hosts of this microfungus include: Simuliidae, Chironomidae, Psychodidae, Ceratopogonidae (Diptera), Ephemeroptera and a new report of *Z. culisetae* for the first time in solitary midges (Thaumaleidae). Although its life history and capacity to infest has not been fully recognized, it is loosely held that *Z. culisetae* should be found in almost any type of lentic system

(including discarded bottles, tires, roadside puddles, etc.) due to a putative ovarian cyst or chlamydospore stages in the flying adult female. However, our findings indicate that adult inoculation of puddles is either not as common or perhaps even rare in mosquitoes and could suggest some other factors are in play. When mosquito larvae were collected from puddles associated with lotic systems, *Z. culisetae* was recovered with nearly a 100% infestation rate. This fosters the idea that fungal spores in some way depend on lotic systems for their successful dispersal to adjacent lentic systems, and leaves us pondering the ecological relevance this might have in dry regions such as in southern Idaho.

3.2-153 Arburscular mycorrhizal fungal communities in the Upo wetland

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Abstract: Arbuscular mycorrhizal fungi (AMF) are the most ubiquitous plant-symbiotic fungiin the global ecosystem. Owing to their enhanced nutrient absorption capacity, AMF significantly contribute to the survival of individual plants and the ecosystem functioning. Community structures of AMF are affected by many environmental factors Inland wetlands have a different environment from common forest soils, therefore, plants inhabiting wetlands may have characteristic AMF communities. The purpose of this study was to compare the AMF communities in wetlands, among the species of host plants. We sampled the roots of 3 host plant species, *Phragmites communis*, *Miscanthus sacchariflorus*, and *Trisetum bifidum,* inhabiting the Upo wetland, Korea. We extracted DNA from the roots and amplified 18S rDNA of AMF using AMF specific primers and identified 5 AMF species from 3 genera. *Diversispora aurantia* was the most dominant species, and the AMF community of *P. communis* was different from other host plants. The results showed that the AMF community had specific to host plants in the inland wetland.

3.2-154 Impact of differential flooding regimes on the arbuscular mycorrhizal fungi and root fungal endophytes of Baldcypress seedlings (*Taxodium distichum*)

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Abstract: Arbuscular mycorrhizal fungi (AMF) are known to be beneficial symbionts of most terrestrial plants, exchanging soil-derived nutrients for photosynthates within the root. Dark septate endophytes (DSE) colonize plant roots in a variety of environments, though their ecological role is less understood. Due to the anoxic conditions and variable inundation regimes characteristic of wetland ecosystems, AMF and DSE functional traits in these environments are not well known. Studies of Southeastern USA wetlands show that baldcypress (*Taxodium distichum*), an important foundational species in swamps, has extensive AMF and DSE root colonization. However, no study has previously assessed root fungal infection of baldcypress under environmental stress, and the implication of colonization to plant performance. Our main goal was to evaluate the role of mycorrhizal fungi and root fungal endophytes in alleviating drought and inundation stress of *T. distichum* seedlings. We predicted to observe higher AMF and DSE colonization in drought versus flood-stressed seedlings, due to preference for an aerobic environment. We also expected a decline in plant performance under stressful hydrological conditions in the absence of AMF and DSE. A manipulative flooding experiment was conducted under controlled temperature and humidity conditions. Two-month-old seedlings were inoculated with live and sterile swamp soil from Bayou Chevreuil, a Louisiana swamp with seasonally flooded soil banks. After a month of inoculation, seedlings were exposed to three simulated hydrological regimes: flood, drought, and regular water levels. Water levels were calibrated from two decades of hydrological data from Bayou

Chevreuil. Plant growth rate and physiological performance were tracked during the course of the experiment, and plant relative fitness in terms of total dry biomass was estimated post-harvest. Roots were stained for microscopy and AMF/DSE were quantified using a modified magnified intersects method. Contrary to our predictions, plant performance of non-inoculated plants was significantly higher than plants grown with live soil. Interestingly, plants grown in the presence of AMF and DSE showed equal fitness under the different hydrological regimes, while plant growth and fitness was reduced under inundation stress in the absence of AMF and DSE. This result suggests that fungi may help regulate plant response to extreme conditions. No significant differences were found in total AMF and DSE colonization among hydrological regimes of inoculated plants, demonstrating that the presence of AMF and DSE is not affected by an anaerobic environment. Our findings show that seedling root fungi in variably anoxic environments play a more important role than previously estimated, and may help regulate seedling establishment and success under extreme conditions. This study will inform current restoration efforts of Louisiana coastal swamps by providing a mechanism (i.e. AMF inoculation) that can confer baldcypress seedling resilience under threatening climate events.

3.2-155 Arbuscular mycorrhizal fungi in *Sporobolus pumilus* of the Minas Basin, Nova Scotia; identification, abundance and role in restoration

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Abstract: Saltmarshes are highly productive ecosystems in decline globally. Historically, many saltmarshes worldwide became areas for waste disposal or were converted to agricultural, commercial or recreational lands due to their relative flatness and fertile soil. These activities have had negative effects on saltmarsh ecosystems which are essential nursery and refuge habitat for many juvenile fishes, invertebrates, and birds. Saltmarshes also stabilize coastlines, provide a means of storm buffering and nutrient recycling, and are crucial contributors to primary production in marine ecosystems. Restoration efforts of saltmarsh habitats are of interest worldwide due to new threats imposed by current climate change patterns. Unfortunately, these restoration efforts have had mixed success, with many being unsuccessful in long term coastal stability. This research will improve restoration efforts by assessing the role of fungal communities within saltmarsh sediments and the roots of the dominant high saltmarsh species *Sporobolus pumilus* (Poaceae) in Nova Scotia. Arbuscular mycorrhizal fungi (AMF) can improve the salt tolerance of many plant species, but are often overlooked in saltmarsh restoration projects. Our research focuses on the identification and abundance of AMF species in the roots of *S. pumilus,* from three saltmarsh sites around the Minas Basin, Nova Scotia. We are also interested in the role of AMF in the establishment and growth of *S. pumilus*. We used ITS rDNA barcoding, ink/vinegar staining and microscopy, and qPCR to assess the mycorrhizal status of *S. pumilus* in the field. An AMF species (*Funneliformis geosporum*, Glomeraceae), we previously identified from *S. pumilus* in Nova Scotia, was propagated in trap pots and used in inoculation experiments to determine whether this fungus can increase the establishment and growth of *S. pumilus* under simulated field conditions (tidal mesocosms). As the only identified AMF species currently known to colonize *S. pumilus* in Nova Scotia, this species may play a crucial role in the grass's ability to tolerate the dynamic, saline environment of Nova Scotia's megatidal saltmarshes.

3.2-156 Seasonal diversity of vesicular-arbuscular mycorrhizal (VAM) fungi in banana from three different jhum fallows in Mizoram, India

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Abstract: A system of agriculture called jhumming which involves clear cutting and burning of plant debris thereof is a customary practice in Mizoram, Northeast India. Once the land becomes unproductive, it is left to be reclaimed by regeneration of natural vegetation, or sometimes converted to different long-term cyclical farming practices. Seasonal dynamics of arbuscular mycorrhizal (AM) fungal community composition in three different jhum fallows in Mizoram were investigated. The jhum fallows are of three different years i.e., 1-3 years, 4-6 years and 7-10 years. In all three seasons variation in AM fungal spore density was observed. Maximum spore density and AM species richness were recorded during the monsoon season. A total of 15 AM fungal species representing 4 genera were recorded where *Glomus* species were the dominant species.

3.2-158 High mountain mycorrhizal fungi

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Abstract: This macroproject studies the interaction of symbiotic or endophytic high mountain fungi species associated with *Abies religiosa* in temperate forest in Veracruz, Mexico. We study mycorrhizal colonization in adult trees and seedlings. The diversity of arbuscular mycorrhizal fungi (AMF) spores in the adult rhizosphere was monitored and the effect of autochthonous mycorrhizal fungus inoculation on the development of seedlings was studied. Ectomycorrhizal fungi were also studied. We present the first catalog with more than 30 species of AMF and 35 species of ectomycorrhizal fungi associated with *A. religiosa* in high mountain. The molecular and morphological identification of the associated mycorrhizal fungi is presented. When estimating mycorrhizal colonization, the characteristic structures of AMF were observed in roots of seedlings and adult trees. Colonization by endophytic fungi was very low. Inoculation of AMF in seedlings increases its growth rate. Arbuscular mycorrhiza is present in many woody perennial species, including the Pinaceae family. The AM can be important during the establishment of the seedlings in places where the nutrients are limited, because their contribution to increase the absorption of mineral nutrients of the soil, which finally is reflected in a greater growth and development of the plants. The ectomycorrhizal colonization was very high in seedlings and adults of A. religiosa. To date little is known about the effects that HSOs have on the seedlings of A. religiosa, although it has been reported that these organisms can be similar to mycorrhizal fungi. These results allow to select species to produce inoculated seedlings for restoration or plantations.

3.2-159 Slope aspect modifies phylogenetic structure of a arbuscular mycorrhizal fungal community in a boreal alpine ecosystems

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Abstract: Slopes strongly modify biogeographical patterns of species and ecosystem functions through causing significant climate change and spatial heterogeneity. The effect of slope aspect on the composition of belowground microoranisms of arbuscular mycorrhizal (AM) fungi is still unclear. Here, we investigated the distribution patterns of AM fungi, a type of functionally important soil microorganism, along an alpine ecosystem of a steep environmental gradient from northwest-facing slope (SS) to southeast-facing slope (NS), and inferred the ecological processes assembling the fungal

communities according to the phylogenetic patterns. We detected 32 distinct AM fungal virtual taxa (VT) totally, which mainly belong to the genus *Glomus*. Both the species and phylogenetic composition of AM fungi differed evidently between SS and NS, the majorly being driven by niche (environment and host) filter, including soil pH, microclimate and plant species composition. Instead, the role of geographical distance was a very slightly contributed factor. With slope aspect conversion from SS to NS. Significantly reduced AM fungal richness was observed and such distinct niche shift caused a marked loss of *Glomus* taxa, and increase of *Rhizophagus* spp. In addition, AM fungal communities were phylogenetically clustered on SS and random on NS, respectively, suggesting that the central ecological process governing AM fungal assemblage shifted from niche-dominated filter to one both of niche filter and competitive exclusion. These findings, of the strong local environmental effects of structuring AM fungal community, shed light on the significant susceptibility of these fungi to environmental change.

3.2-161 Ericoid mycorrhizal potential in the Hymenochaetales

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Abstract: Ericoid mycorrhiza is arguably the least investigated mycorrhizal type specific for Ericaceae. It has traditionally been considered as a domain of ascomycetous fungi, e.g., *Meliniomyces* spp., *Oidiodendron maius* and *Rhizoscyphus* (=*Pezoloma*) *ericae*. Recently, however, a member of Serendipitaceae (Sebacinales, Basidiomycota) has been experimentally confirmed as ericoid mycorrhizal and anatomically and morphologically unique sheathed ericoid mycorrhiza formed by a basidiomycetous mycobiont has been discovered and described in Ericaceae from mid-Norway. Subsequently, molecular tools revealed that this mycobiont belongs to the *Kurtia argillacea* (=*Hyphoderma argillaceum*) complex within the Hymenochaetales. Such an intriguing placement among mostly saprobic and parasitic fungi begs further investigation on the ecophysiology of *K. argillacea* and related fungi. A series of resynthesis experiments with ericaceous hosts has been therefore set up to confirm/reveal ericoid mycorrhizal potential of *K. argillacea*, its close relative *Hyphoderma orphanellum*, *Hyphodontia subalutacea* (all Hymenochaetales) and *Hyphoderma cf. medioburiense* (Polyporales). *Kurtia argillacea* formed typical sheathed ericoid mycorrhiza with vigorous intracellular hyphal colonization and hyphal sheaths on the root surface but also *H. orphanellum* formed superficial hyphal sheaths and intracellular colonization typical for ericoid mycorrhiza. Additionally, also the two remaining fungi colonized host rhizodermal cells forming loose hyphal loops with no harm to the host plants. These results indicate that certain lineages in Hymenochaetales, and possibly also in Polyporales, have an apparent but hitherto overlooked potential to colonize Ericaceae roots and form ericoid mycorrhizae. This should be taken into account when investigating these mostly saprobic/parasitic groups of fungi.

3.2-162 Microniche characterization of a rare terrestrial orchid explains its population dynamics

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Abstract: We present one of the first few reports providing evidence of strong interaction between the soil environment, mycorrhizal communities within roots, and population demography of a rare terrestrial orchid. Orchids utilize complex ecological niches including obligate mycorrhizal interaction. The assembly of orchid mycorrhizal fungal (OMF) communities within roots likely reflects a combination of influences from host-fungus compatibility, the structure of OMF in soil, edaphic characters, and the

microenvironment. Untangling the direct and/or indirect effects of one or more of these explanatory variables on plant populations is challenging yet necessary to understand the fundamental niche selection strategies in orchids. We sought to reveal the interaction among an array of biotic and abiotic traits of soil, OMF communities in roots, and population demography of a terrestrial orchid endemic to the California Floristic Province. *Platanthera (Piperia) cooperi* displays wide spatial and temporal variation in population size and demography and presents an opportunity to address our specific questions including if: 1) soil OMF communities, microclimate and edaphic regimes interact to drive the assembly of root OMF communities? and 2) if spatio-temporal differences in root OMF assemblages lead to distinct populations demographic patterns? To answer these questions, *P. cooperi* roots were sampled in February (seedlings and vegetative individuals) and April (reproductive individuals) in each of the three consecutive years (2015-2017) from six disjunct populations. Simultaneously, soil samples were collected to profile OMF communities in soil from the study populations and two sites where plants of *P. cooperi* do not occur. Soil samples were also collected to generate physicochemical profiles and physical environment data was recorded at four populations and one control site. Fungal metabarcoding libraries were generated with ITS3/ITS4OF primer pair which primarily amplifies the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA and were sequenced on Illumina MiSeq platform. Subsequently, data were analyzed using QIIME and VSEARCH bioinformatic tools along with biostatical analyses in R. We identified 955 OMF operational taxonomic units (OTUs) after clustering ITS2 sequences from *P. cooperi* roots at 97% similarity threshold. The 30 most abundant OTUs contributed to 75-95% of abundances across sampling events. The most abundant OTU in roots belonged to Tulasnellaceae and the second most to Ceratobasidiaceae. Further, permutational analyses of variance (PERMAVOVA) revealed the differences in distribution and abundance of root OTUs across populations and years (*P=0.001* for both), whereas OMF communities were similar across three phenological stages. Further, PERMANOVA also revealed that 30 most abundant root-associated OTUs had differential distribution and abundances in soil across populations (*P=0.001*), and years (*P=0.004*). Additionally, hierarchical clustering grouped populations with similar dynamics (size and demography) together for both, root and soil OMF communities. Similarly, redundant analyses (RDA) also clustered populations with similar dynamics together based on edaphic factors and environmental variables. In conclusion, our data suggest that physical, biological and chemical soil environment influence the assembly of root OMF communities, and the spatial and temporal fluctuations in root OMF communities in response to these drivers likely influence the spatial and temporal populations dynamics of *P. cooperi*.

3.2-163 Are EMF plants AMF plants as well?

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Abstract: Subalpine forests in subtropical Taiwan are bounded by Taiwan fir (*Abies kawakami*i) tree-line at their upper margin. Taiwan fir is native to Taiwan and one of the southernmost true firs. Sharing of mycorrhizal fungi could facilitate the coexistence of plant species and the stability of the multispecies assemblage. We evaluated how varied arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) were shared among 9 plant taxa in a forest using denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing techniques. In this study, the long-term research plot (3,000 m a.s.l.) on Mt. He-Huan is an *Abies kawakamii* - *Tsuga chinensis* var. *formosana* (Taiwan hemlock) forest, *Yushania niitakayamensis* (Yushan arrow bamboo) fills in the middle level, and *Rubus pungens*, *Viola* sp., ferns, moss, and mycoheterotrophic plants *Monotropastrum humile* var. *humile* and *M. humile* var. *glaberrimum* are on the forest floor. Results of DGGE indicated that about one-quarter mycorrhizal OTUs associated with single host, 16 of total 20 AMF and 32 of total 43 EMF OTUs were cross-host and some of them interacted with 8 plant taxa. The architecture of AMF and EMF networks seemed to be nested

structure. We provided the evidences that i) ectomycorrhizal plant *T. chinensis* and *A. kawakamii* host AMF simultaneously. ii) *Yushania niitakayamensis* harbored high diversity of mycorrhizal fungi, 12 AMF and 27 EMF OTUs, including half AMF/EMF specialists in the forest. iii) Two mycoheterotrophic plants, *M. humile* var. *humile* and *M. humile* var. *glaberrimum* were reported as EM plants. We found they were AM plants as well, they associated with similar AMF community. They shared the same habitat but their EMF communities are quite different, it could be the mechanism of sympatric speciation of these two mycoheterotrophic species. We are going to describe the mycorrhizal fungal diversity and the AMF, EMF interaction network topologies among 9 plant taxa at a higher resolution level with NGS data.

3.2-164 Global transcriptomes suggest death and dismemberment of *Tomentella fuscocinerea* at the hands of the mycoheterotrophic orchid *Corallorhiza striata*

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Abstract: Members of the orchid family form mycorrhizal associations defined by anatomy, nutrient exchanges and fungal taxa that are distinct from other types of mycorrhizae. In particular, mycoheterotrophy at the protocorm stage of photosynthetic orchids, and lifelong in non-photosynthetic orchids, demonstrates unusual fungus to plant net carbon flow. Significant advances have been made in identifying the fungi associated with non-photosynthetic orchids and understanding the ecology and evolution of these associations. However, the cellular and molecular mechanisms by which orchids achieve narrow specificity and carbon acquisition are unknown. In a search for clues, we undertook an RNA-Seq global transcriptome analysis of interactions between the fully mycoheterotrophic orchid *Corallorhiza striata* and its specific mycobiont, *Tomentella fuscocinerea*. Here we report differential gene expression in the fungus. Using de novo transcriptome assembly combined with mapping to a draft genome, we identified 281 differentially regulated genes when comparing pure culture, early mycorrhizal colonization and late mycorrhizal colonization. Transporters and other genes related to nutrient exchange that are often upregulated in mycorrhizal symbioses were not identified. Instead, genes belonging to families associated with apoptotic programmed cell death (PCD) signal cascades, such as Het-C, WD-40, STYKc, tetratricopeptide repeat domain, and NACHT were strongly upregulated when the fungus colonized the orchid. These results raise the possibility that orchids enhance their mycotrophic nutrition by triggering fungal PCD pathways.

3.2-165 The ubiquitous mycenas – purely saprotrophs or potential plant root symbionts?

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Abstract: The transition from saprotrophic to mycorrhizal lifestyles has occurred several times independently during evolution in the fungal kingdom. Recent studies also indicate that the border between a saprotrophic and mycorrhizal lifestyle is far blurrier than earlier appreciated and that numerous saprotrophic fungi can associate with plant roots as facultative biotrophs *in vitro*. *Mycena*, one of the most speciose genera in Agaricales, has uniformly been described as saprotrophic, and have profound ecological importance in forest ecosystems as litter decomposers. However, several recent studies have suggested that some *Mycena* spp. may have a biotropic lifestyle. Firstly, recent high-throughput sequencing surveys have revealed high abundances of *Mycena* spp. in living

ectomycorrhizal plant roots, secondly, some *Mycena* species are difficult to culture, what is typical for biotrophic fungi, and lastly, one *Mycena* species was suggested to form beneficial associations with ericoid plant roots *in vitro*. These findings encourage us to further investigate *Mycena's* association with plant roots. This study is part of a larger project, where we aim to disentangle the ecology of the genus *Mycena* using genomic, isotopic, physiological and imaging analyses. In this study, we investigate the putative interaction between *Mycena* spp. and plant roots using *in vitro* growth experiments. Sterile seedlings of the ectomycorrhizal plant *Betula pendula* and axenic cultures of 17 *Mycena* species were grown together in microcosms for eight weeks. Images were obtained biweekly, to record changes in seedling growth. We used cryomicrotome sectioning, differential staining and fluorescent microscope imaging to visualize the extent of fungal growth in the fine roots. *Mycena* spp. associated closely with plant roots in all microcosms; we observed evidence of hyphal penetration into the plant fine roots in all microcosms, and in some cases intracellularly. A few *Mycena* spp. formed mantel-like structures and caused shortening of plant fine roots. Our preliminarily data suggest that with most species of *Mycena*, seedling growth was impaired, and there was no evidence of growth benefits for the seedlings in associating with *Mycena* spp. Several *Mycena* spp. seem to be facultative biotrophic *in vitro*. Results from a second, ongoing experiment investigating potential nutrient transfer between *B. pendula* and *Mycena* spp. using ^{14}C and ^{32}P radioactive isotopes will be presented. Our preliminary results show the need to reconsider the previous view on the genus *Mycena* as being uniformly saprotrophic. The ecology and nutritional modes of *Mycena* is likely far more complex, plastic and flexible than earlier believed.

3.2-167 Comparative and functional genomics of the Morchellaceae

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Abstract: The Ascomycete genus *Morchella* is fascinating for a number of reasons. *Morchella* species are globally popular mushrooms collected for the table so they represent a curious recreational activity and a potential income source for mushroom growers. Despite this popularity and a concerted effort to understand their propagation, they have not been consistently cultivated on a large scale. Perhaps this is because the life history of *Morchella* species remains elusive. It has been hypothesized that they form mycorrhizal associations with plant roots, but these associations have not been unequivocally proven. By using a comparative genomics approach, we are sequencing numerous taxa, including *Morchella esculenta*, *Morchella conica*, *Morchella rufobrunnea*, *Morchella americana*, and three strains on unknown lineage. In addition to this sequencing, we have used numerous members of the Morchellaceae to characterize the evolution of family specific metabolic pathways. Phylogenetic analyses reveal the relative position of each strain within *Morchella* and the ascomycetes. Gene annotation methods were used to identify candidate genes for interaction with plants (effectors and other small secreted protein production), novel biochemical pathways and secondary metabolite production. In addition to these genomic approaches, we have data mined 18S and ITS amplicon data, as well as binned metagenomic reads, from plant endophyte studies. This data clarifies the role of *Morchella* species as plant endophytes in the Poaceae and other plant lineages.

3.2-168 Fungal functional ecology: Bringing a trait-based approach to plant-associated fungi

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Abstract: Fungi play essential roles in ecosystems. They facilitate plant access to nutrients and water, serve as decay agents that cycle carbon and nutrients through soil and atmosphere, and are major regulators of macro-organismal populations. Although technological advances are improving the detection and identification of fungi, there still exist key gaps in our ecological knowledge of this kingdom, especially related to function. Trait-based approaches have been instrumental in strengthening our understanding of plant and animal functional ecology and, as such, provide excellent models for deepening understanding of fungal functional ecology in ways that complement insights being gained from traditional and -omics-based techniques. Here we present a synthesis of the current knowledge of the functional ecology, taxonomy, and systematics of plant-associated fungi by addressing three fundamental questions: what is currently 1) known and 2) unknown about fungal guilds, and 3) how we can use a trait-based approach to fill in gaps in our knowledge. We then introduce a novel database of fungal functional traits (Fun^{Fun}). Fun^{Fun} is designed to be a living dataset for which taxonomy and guild definitions update as they change and new information can be easily incorporated, as trait data currently lags behind other databases. We include approximately 870 traits encompassing genetic, enzymatic, morphological, stoichiometric, life history, and physiological aspects of fungi to highlight the state of empirical fungal functional ecology. The current version of this database uses Index Fungorum taxonomy and contains 25,864 measurements for 3,104 species distributed across 1,611 genera, 267 families, and 107 orders. Fun^{Fun} also directly links to the curated and open annotation fungal ecological guild database (FUNGuild), which provides tools for researchers to explore and predict how fungal functional diversity varies across fungal guilds. Finally, we will present several examples of insights that can be gained from our database and a trait-based approach. Our new database will allow researchers to explore critical dimensions of fungal functional diversity and map taxonomic, genomic, and functional data within and across fungal guilds, and will lay the framework for rapid progress on fungal functional ecology in the years to come.

3.2-169 Gut-inhabiting and ectosymbiotic arthropod-associated fungi from a neotropical rainforest, including two new species of yeasts

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Abstract: Yeasts and other microfungi are highly diverse and almost ubiquitous members of the microbiota in nearly all habitats on the planet. The study of fungal microbiota (mycobiome) of multicellular organisms and their complex relationships with their hosts is a relatively new and rapidly developing field. Data on host-microbe interactions are still very sporadic considering the sheer number of plants and animals that potentially sustain diverse and dynamic mycobiomes. The immense diversity of tropical arthropods represents an interesting target to study the species richness and host-relationships of microfungi, but detailed observations have only focused on a few arthropod groups, such as passalid beetles (Coleoptera: Passalidae) and flower-visiting insects. Isolation from the host enables the characterization of culturable members of the mycobiome, but their interactions with the host are often unclear. During fieldwork in Panama, we collected arthropods by hand and using a mouth-operated aspirator. Habitats surveyed include caves (La Cueva de Chilibre) and tropical rainforests (Isla Barro Colorado, Gamboa, Parque Nacional Soberanía). We collected hosts belonging to various orders (Arthropoda: Araneae, Blattodea, Coleoptera, Julida). Host were surface-sterilized, and midguts were removed and homogenized before streaking onto plates with acidified yeast-extract malt (AYM) agar. Colonies were isolated and species were identified based on ribosomal DNA. Here, we report several species of yeasts, dimorphic fungi and filamentous species (Fungi: Ascomycota and Mucoromycotina) isolated from some of these hosts. Among the cultures of yeasts, we discovered two new species, one related to *Diutina* and one to *Kodamaea*. Using the *Galleria melonella* larva pathogenicity model, we found that most yeast and dimorphic species isolated during this study cause high mortality, despite the versatile immune system and antifungal defense mechanisms described for these larvae. Our results suggest that easily culturable microfungi in arthropod guts may represent pathogens capable of circumventing insect antifungal response. With these findings we show that the *Galleria* model is suitable to make implications about the ecology of arthropod-associated yeasts. Some of the hosts from our survey carried fruiting bodies of non-filamentous, ectosymbiotic fungi (Ascomycota: Laboulbeniomycetes). collected *Coreomycetopsis oedipus* on a *Nasutitermes* sp. termite (Blattodea: Termitidae); *Herpomyces paranensis* on *Blaberus giganteus* cockroaches (Blattodea: Blaberidae), which represents a new country record; and *Laboulbenia* sp. nov. on semiaquatic bugs (Heteroptera: Veliidae). Of 328 screened *Nasutitermes* sp. termites, 20 (= 6.1%) carried fruiting bodies of *Antennopsis gallica*, a „minute mycological mystery" with uncertain affinities. Finally, we found a single fruiting body of *Amphoromorpha* sp. on a millipede (Julida).

3.2-170 Diversity and function of fungi associated with the fungivorous millipede, *Brachycybe lecontii*

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Abstract: *Brachycybe* (Wood) is a genus of fungivorous millipedes. To date, the fungal associates of these millipedes have never been characterized. In an attempt to resolve these relationships, culture-based approaches combined with DNA barcode sequencing were used. Sampling of 313 individuals collected from three of four *B. lecontii* clades and 20 sites across seven states uncovered at least 183 genera in 40 orders from four fungal phyla. At least seven putative new species were recovered in this study, despite the use of more classical culture-based approaches. Three of these fungi were phylogenetically resolved using ITS + LSU and include two new species, aff. *Fonsecaea* sp. and *Mortierella* aff. *ambigua,* and a new genus related to *Apophysomyces*. Overall, the results of this study highlight the vast amount of undescribed fungal biodiversity associated with millipedes. Twelve fungal genera from nine orders showed high connectivity across the entire *B. lecontii*-associated fungal network, indicating a central role for these fungi in their association with these millipedes. These twelve include the two putative new species described above. The ecology of these and other fungal associates were also explored, using fungal cohort pairings and entomopathogenicity trials. Over 40% of all fungal pairings resulted in competitive interactions, a majority of which involved inhibition or overgrowth by fungi in the Hypocreales and Polyporales, respectively. The abundance of these competitive interactions in these two orders indicated differing ecological strategies. Interactions with Hypocreales frequently resulted in a zone of growth inhibition around the Hypocrealean colony, indicating accumulation of chemicals to competitively exclude other fungi, while Polyporales physically overgrew their competitors, indicating greater competitiveness for resources and resistance to potential antifungal chemicals. Mucoromycotan fungi used a similar strategy to the Polyporales. Results of a series of entomopathogenicity trials indicated that *B. lecontii* was less susceptible to entomopathogenic Hypocreales than an insect model (*Galleria mellonella*), even though these fungi are known to attack several classes of arthropods. Furthermore, the absence of a negative interaction between *B. lecontii* and entomopathogenic Hypocreales may allow *B. lecontii* to associate with these fungi in way that provides them with a benefit, such as protection from predators. When challenged with some Polyporales, *B. lecontii* exhibited high mortality, while *G. mellonella* was unaffected. This stands in sharp contrast to previous casual observations of the feeding behavior of *B. lecontii*. Recent discoveries of previously overlooked fungal diversity have been groundbreaking and hint at substantial cryptic fungal biodiversity across the globe. The 200-300 million-year-old association between fungi and the Colobognatha, which includes *Brachycybe lecontii,* provides an ideal system to uncover biodiversity and examine function of these fungi in a highly understudied and ancient association.

3.2-171 A new species in the Mycosphaerellaceae from Cecidomyiidae leaf galls on *Avicennia marina* in South Africa

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Abstract: Leaf galls can be formed by a variety of agents, including bacteria, insects and fungi. There is very little known regarding formation of leaf galls on mangrove trees. In studies to determine the incidence of diseases of mangrove trees in South Africa, several *Avicennia marina* trees with leaf galls were observed. Unidentified adults and larvae of midges (Cecidomyiidae) were consistently associated with the galls. In addition, fungal fruiting structures were observed colonizing the galls and isolations were made from these. Phylogenetic analyses of multigene sequence data from the isolates, including the internal transcribed spacer (ITS), a portion of the actin gene region (ACT), mitochondrial large

subunit (LSU) and translation elongation factor -1α (TEF-1α), revealed that the fungal fruiting structures represent a new taxon in the Mycosphaerellaceae. Morphological observations supported the separation of the new species, which is in the process of being described as *Periconiella galla* sp. nov. This is the first report of a species in the Mycosphaerellaceae isolated from cecidomyiid galls on leaves of *A. marina*. Furthermore, the results of this study contribute to limited knowledge of fungi associated with mangrove trees in Africa and worldwide.

3.2-172 Species-specific interactions between myxomycete plasmodia and collembola

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Abstract: Soils play important roles in forests, such as organic matter decomposition and mineral nutrient cycling. The interactions among soil organisms are critical for understanding underlying soil mechanics. Myxomycetes and Collembola are abundant and play an essential role in organic matter decomposition in forest soils. As such, their interactions are important for soils. Some Collembola species of the family Neanuridae feed on myxomycete plasmodia, while some plasmodia ride on Collembola and suppress their movement in response to Collembola grazing. However, the consequences of their species-specific interactions are poorly understood. We investigated whether other Collembola species graze on plasmodia and whether the behaviors of plasmodia differ depending on the Collembola and Myxomycetes species. We cultured different combinations of the plasmodia of two Myxomycetes species (*Physarum melleum* and Didymiaceae sp.) and three Collembola species (*Vitronura pygmaea*, *Ceratophysella denticulata*, and *Sinella umesaoi*) for 3 weeks and measured the changes in body size, egg number, and survival rate for the Collembola, and size, body color brightness, frequency of sclerotium formation, and fragmentation for the Myxomycetes. The response to the plasmodia varied with the Collembola species. *Vitronura pygmaea* showed significantly higher growth and egg production with the plasmodia treatments. Although *C. denticulata* grew and produced eggs in the plasmodia treatments, these were significantly increased in the dry yeast treatment. *Ceratophysella denticulata* appeared to eat the waste products of plasmodia. *Sinella umesaoi* did not show any differences between the treatments. The responses of the plasmodia varied with the Collembola species. Although the frequency of sclerotium formation was higher in *P. melleum* than in Didymiaceae sp., it was not affected by the presence or species of Collembola. In the presence of *V. pygmaea*, both *P. melleum* and Didymiaceae sp. frequently dropped the attacked parts of their bodies. These results suggest that *V. pygmaea* feeds on living plasmodia, whereas *C. denticulata* feeds mainly on fungi, but can utilize the waste products of plasmodia. Dropping the attacked part of the plasmodia body may be a strategy for avoiding consumption by *V. pygmaea*.

3.2-177 Study of *Amanita* from Thailand based on multiple gene phylogeny and morphology

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Abstract: Recent studies of mushroom diversity in the forests of northern Thailand have documented a great number of new species as well as new distribution records of known species. The present study is part of an inventory of *Amanita* in Thailand over a five-year period (2012–2016). As a result, the number

of *Amanita* species from various sections, both new to science or new to Thailand has dramatically increased. This study dealt with section *Vaginatae* and section *Phalloideae*. Remarkably, fifteen specimens studied belong to nine new species in sect. *Vaginatae*, namely, *A. brunneoprocera*, *A. brunneosquamata*, *A. brunneoumbonata*, *A. cinnamomea*, *A. flavidocerea*, *A. flavidogrisea*, *A. luteoparva*, *A. subovalispora*, and *A. suborientifulva*, confirming that the diversity of *Amanita* in Thailand is high, with likely many more remaining undescribed. Specimens were identified based on morphology and DNA sequence analyses of β-tubulin, LSU, nrITS, and rpb2 loci. Those nine species are fully described and illustrated with line drawings and color photographs. Morphological characteristics of related taxa are compared and discussed, and a dichotomous key of *Amanita* sect. *Vaginatae* in Thailand is provided. Several new species and records were also found that belong to sect. *Phalloideae*. Two species, namely *Amanita ballerina* and *A. brunneitoxicaria* spp. nov., were described as new. Their taxonomic placement is supported by both morphological and phylogenetic evidence. Moreover, *A. fuligineoides* is also reported for the first time from Thailand, increasing the known distribution of this taxon, which supports our view that many taxa are likely yet to be discovered in the region. The three taxa are placed in sect. *Phalloideae* based on a multigene phylogeny (β-tubulin, rpb2, nrITS, and nr5.8S), morphological descriptions, photographs, and line drawings. This section contains many of the deadliest poisonous mushrooms in the world. We therefore screened for two of the most notorious toxins by HPLC-MS analysis of methanolic extracts from the basidiomata. *Amanita fuligineoides* was found to contain both deadly toxins α-amanitin and phalloidin, while *A. brunneitoxicaria* contained only α-amanitin. Interestingly, neither α-amanitin or phalloidin was found in *A. ballerina*, the first taxon with white basidioma lacking these toxins in sect. *Phalloideae*. Together with unique morphological characteristics, the basal position in the phylogeny indicates that *A. ballerina* is either an important link in the evolution of the deadly poisonous *Amanita* species, or perhaps a member of a new section also including *A. zangii*. In conclusion, further exploration of *Amanita* diversity and historical biogeography in South-East Asia and Australia, which seems to be a hotspot of early diverging *Amanita* lineages, is critical and could reveal more members of this clade, and help elucidate morphological and molecular synapomorphies to support or refute the hypothesis of a new section.

3.2-178 The genus *Amanita* from northwestern Pakistan with the description of two new species

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Abstract: Two new species in the mushroom genus *Amanita* are described and illustrated from Northwestern Pakistan. Phylogenetic data derived from nuclear ribosomal ITS and LSU regions along with morphological characterizations indicate these species are novel. *Amanita cinis* is a member of *Amanita* section *Lepidella,* while *Amanita olivovaginata* is a representative of section *Vaginatae*. *Amanita emodotrygon* was recently described from the state of Uttarakhand, India; a new record of this species is reported herein for the first time from Pakistan.

3.2-179 Genus *Amanita* (Amanitaceae): Report from Mizoram, India

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Abstract: Mizoram lies in Northeast India. It is regarded as one of the biodiversity hotspots of the world. However, study of mushrooms from this region is very limited. A taxonomic study of the genus *Amanita* from Mizoram was undertaken by morphological characteristics. Specimens were collected from the different forest of Mizoram. From the study, five species of the genus *Amanita* were collected and

identified, which were *Amanita jacksonii, A. pachycolea, A. vaginata, A. cokeri* and *A. spissacea*. The study is a first detailed report on the genus from this region.

3.2-180 Molecular phylogeny based on ribosomal RNA gene and morphological characterization of species in *Amanita* from cedar dominating forests of Pakistan

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Abstract: *Amanita* is a species rich genus of fungi in Amanitaceae (Basidiomycota, Agaricales) with more than 500 known species distributed worldwide. The genus is important because of its mycorrhizal symbiosis with trees belonging to different genera and families. Many taxa of the genus have been reported as edibles, though some others are deadly poisonous. During the investigation for community studies of ectomycorrhizal macrofungi, several species of *Amanita* have been collected and described on the basis of morphological characters and molecular data based on Internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene and 28S nuclear ribosomal large subunit RNA gene (LSU). For the characterization of *Amanita* species, combining ITS with LSU data have been proved best to discriminates species. During this study, five *Amanita* species were identified. Among these three species have not been described previously while the two species have been reported from Pakistan on the basis of morphological characters. This investigation provides a detailed account of their morphology as well as molecular phylogeny.

3.2-181 Tales of the Pale Panther: Phylogenetic delineation and placement of *Amanita pantherina* var. *multisquamosa* within the *Amanita pantherina* complex

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Abstract: The purpose of this research project is to use genetic sequence data to understand more about the *Amanita pantherina* var. *multisquamosa*, that is endemic to the Southern Rockies of North America. Compared to other *A. pantherina* species, var. *multisquamosa* is set apart by its pale beige phenotype. Previous studies have demonstrated that the N. American *A. pantherina* is a separate clade from the *A. pantherina* sensu stricto, which originates from Eurasia. The purpose of this study is to explore whether North American *A. pantherina* var. *multisquamosa* is a species that is phylogenetically distinct from other North American *A. pantherina* sensu lato. More broadly this study will explore the relatedness of North American *A. pantherina* relative to their Eurasean counterparts. To evaluate these questions, molecular sequence data from ITS, 28s and RPB2 regions will be studied under maximum likelihood and Bayesian methods of phylogenetic inference. This data will help to better understand the "*A. pantherina* Complex" and fungal biodiversity in North America.

3.2-182 New tropical *Amanita* species from the Guiana Shield and Central Africa

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Abstract: The primarily ectomycorrhizal (ECM) mushroom genus *Amanita* (Amanitaceae, Agaricales, Basidiomycota) may number ~1000 species worldwide, though only ~600 species have been formally

described. Many *Amanita* species have broad distributions in higher latitude forests, while *Amanita* species in the Neo- and Afro-tropics have restricted ranges in lowland rainforests primarily dominated by ECM trees of Fabaceae subfam. Detarioideae ("detarioids"). Collecting expeditions in rainforests dominated by the ECM detarioids *Dicymbe corymbosa* in Guyana from 2000–2017 and *Gilbertiodendron dewevrei* in Cameroon from 2014–2017 indicated that at local spatial scales both regions are diverse for *Amanita*: 25 morphospecies were discovered in an area of 10 km^2 in Guyana, and 35 morphospecies from an area of ~3 km^2 in Cameroon. Collections were compared with published descriptions and fungarium specimens of previously described *Amanita* taxa. This preliminary work indicates that numerous *Amanita* morphospecies from Guyana and Cameroon are currently undescribed and common in their local habitats. Macro- and micromorphological features and multi-locus DNA sequence data have been compiled from collections of each species, and multiple species from each region will be formally described or epitypified. This work will enhance a broader understanding of fungal biodiversity in tropical ecosystems, and contribute to phylogeographic analyses of post-Gondwanan ECM fungal communities. An overview of the proposed new taxa will be presented.

3.2-183 Gondwanan connections in Tuberaceae-Helvellaceae: Discovery of the first *Gymnohydnotrya* species from South America

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Abstract: Species of *Gymnohydnotrya* are of ectomycorrhizal, exothecial fungi in the order Pezizales. Exothecial fungi are basically 'inside-out truffles', i.e. their fruit bodies lack a peridium and are covered with a hymenium layer instead. *Gymnohydnotrya* species have ornamented ascospores and asci that lack opercula. Morphologically, *Gymnohydnotrya* is similar to species of *Hydnotrya* except that the *Hydnotrya* species typically have a peridium. *Gymnohydnotrya* species are also characterized by hyaline, ornamented ascospores that lack an outer spore wall. The genus was described by Zhang and Minter in 1989 based on herbarium specimens from Australia. Currently, there are three recognized species in this genus: *G. australiana*, *G. echinulata*, and *G. ellipsospora*. Zhang and Minter placed *Gymnohydnotrya* in the Helvellaceae. However, a recent study showed that these species were part of a distinct monophyletic lineage that also included Southern Hemisphere species of the epigeous genus *Underwoodia*. This group was identified as the /gymnohydnotrya lineage, but no further taxonomic or phylogenetic work has addressed the systematics of this group. We recently discovered specimens of an unknown exothecial fungus in Patagonian Nothofagus forests in South America. These fungi are morphologically similar to taxa in the genus *Gymnohydnotrya* but there are no previous reports of this genus from South America. Morphological and molecular analyses indicate that there is at least one novel species of *Gymnohydnotrya* among our collections from South America. Here we describe the morphology of this South American species and place it within a multi-locus phylogenetic framework that also includes new samples of *Underwoodia* and Helvellaceae species. We also discuss the implications of this work on the evolution of truffle-like fungi, the biogeography of the /gymnohynotrya lineage, and the taxonomy of Helvellaceae.

3.2-184 *Amylascus*: an ectomycorrhizal truffle genus with a southern Gondwanan distribution

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Abstract: The genus *Amylascus* Trappe was described from Australia in 1971. The only two described species, *A. herbertianus* and *A. tasmanicus* have globose spiny spores, an ascocarp with a basal tuft, and a gleba with meandering veins or chambers lined with an epithecium, similar to what is seen in the Northern Hemisphere genus *Genea*. Depending on the species, the spores may or may not have a perispore. The warted excipulum is composed of large-cells and the amyloid asci (for which this genus was named) are distributed throughout the gleba. Molecular phylogenetic studies of *Amylascus* indicate that this genus is closely related to the Northern Hemisphere ectomycorrhizal genera *Pachyphlodes, Plicariella* (=*Scabropezia*) and *Luteoamylascus*. *Amylascus* species have been collected in forests dominated by *Eucalyptus* and other ectomycorrhizal Myrtaceae and are assumed to form ectomycorrhizas. ITS sequences from ectomycorrhizal root tips of South American Nothofagaceae are also phylogenetically close to sequences from Australian *Amylascus* specimens, suggesting that the genus has a wider geographic and host range than has been previously documented. Over several decades we have obtained specimens from Australia and southern South America that generally fit the morphology of *Amylascus*. We have also discovered diverse collections of mitospore mats from South American Nothofagaceae forests that are phylogenetically affiliated with *Amylascus*, and morphologically similar to mitospore mats of *Pachyphlodes* and *Plicariella*. Using molecular and morphological techniques, we document our findings of new *Amylascus* species from Australia and South America and document the range of morphologies that we observed in ascomata and mitospore mats. The morphological characteristics of our new species expand the definition of the genus to include taxa with a wide range of morphological features, including inamyloid asci. We also present a phylogenetic reconstruction of *Amylascus* and related lineages based on ITS, LSU, RPB2 and TEF markers, to trace the most recent common ancestors of these lineages and infer the evolutionary history of *Amylascus* in the southern hemisphere. We compare the morphology, biogeography and host associations of this genus with those of related genera.

3.2-185 The luckiest spore: short distance vs. long distance dispersal as tested within a biophysical and physiological framework using two *Alternaria* species

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Abstract: The most obvious and perhaps most critical instrument of fungal dispersal is the spore, whose size and shape may critically affect movement over large distances. There is likely a compromise between small spore size, which can enable dispersal over longer distances, and large spore size, which can facilitate settling onto a favorable substrate. In principle smaller spores should remain aloft for greater time intervals, but their reduced mass makes landing more difficult and increases susceptibility to adverse environmental stresses. But while data on spore size and shape are available for many species, greater and improved data on a range of other spore parameters--emphasizing physiological resilience to atmospheric stresses including: UV radiation, relative humidity (RH), and temperature (T)--

may be required to more carefully model dispersal. This study focuses on two *Alternaria* species with dramatically different conidial dimensions: *A. solani* and *A. alternata*. Conidia of *A. solani* are nearly 10x larger than those of *A. alternata* (~50-100 vs. ~10µm, respectively), yet little is known about their conidial aerodynamics or survival under atmospheric conditions; the larger *A. solani* is hypothesized to be a short distance disperser and the smaller *A. alternata* a long-distance disperser. These *Alternaria* species have emerged as major threats to Wisconsin potato crops in recent years, and despite known differences in spore size, conidia of both species often co-infect the same plant, challenging current hypotheses and suggesting other dispersal dynamics and strategies are at play, including perhaps strategies associated with evolved tolerance to atmospheric stresses. We quantified spore mortality under varied UV intensities and wavelengths (365 nm and 302 nm; 5024±23 to 742±17 µm/cm2), relative humidities (10-90%), and temperatures (10-25°C) typical of the lower-mid troposphere in order to identify the "optimal" dispersal conditions of the longest surviving, "luckiest spores." A machine learning approach was used to count live and dead spores from digital images of experimental spores according to the presence or absence, respectively, of a hyphal germ tube 24 hours after treatment. In-house scripts were used to measure germ tube length and to map spore viability as a function of conspecific spore proximity. Biophysical simulations were then performed using NOAA's HYSPLIT trajectory models, and in-field dispersal measurements collected from lesions on live potato plants arranged radially about an infected host plant. Conidial mortality data was then used to inform both simulated and field-based dispersal measurements to provide a physiologically constrained dispersal kernel of both *A. solani* and *A. alternata*.

3.2-186 High diversity of two *Avr* genes is caused by strong selection on specific codon sites

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Abstract: Adaptation of fungal pathogens to colonize plants often involves escape from host recognition. This can be mediated by sequence polymorphism in avirulence factors that evolve to prevent recognition by host resistance genes. Despite the ubiquity and significance of avirulence factors for the infection outcome, many of them are uncharacterized and the mechanisms behind their evolution remain largely unexplored. We analyzed the population genetic diversity for *AvrStb6* and *Avr3D1,* the first avirulence genes cloned and functionally validated from the important wheat pathogen *Zymoseptoria tritici*, using 142 strains sampled from four naturally infected wheat fields growing on three continents. Both Avr genes were found in every *Z. tritici* strain, but orthologs were only detected in the most closely related sister species. This suggests that these genes are likely to have important non-redundant functions and probably emerged in *Zymoseptoria* very recently. Paralogs of both Avr genes were present in all *Z. tritici* isolates, suggesting that they belong to multigene families of candidate effectors that have expanded recently through gene duplications. Intragenic recombination appears to have affected the diversity of *AvrStb6*, while *Avr3D1* is located in a highly plastic genomic region, in which independent transposable element insertions occurred in the global sample of *Z. tritici* strains. The *Avr* sequences exhibited strong evidence for non-neutral evolution, including a large number of non-synonymous mutations, with significant positive, diversifying selection operating on many of the codons. Despite the high sequence polymorphism, features considered essential for effector function, including signal peptides and the cysteine residues, were highly conserved, even among homologs found in the sister species, supporting the conservation of these effectors in evolution and their crucial role in the life history of *Zymoseptoria* species.

3.2-187 Analyzing sequence variation of avirulence Avr-Pita1 gene of rice blast isolates, *Magnaporthe oryzae* in Vietnam

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Abstract: Rice blast disease caused by a filamentous fungus, *Magnaporthe oryzae,* has been one of the most devastating of all cereal diseases worldwide. It is estimated that each year rice blast causes harvest losses of 10-30% of the global rice yield and leading to serious epidemics throughout rice-growing regions of the world including VIETNAM. The interaction between rice plant, *Oryza sativa* and rice blast fungus, *Magnaporthe oryzae* was activated by the interaction between protein products of the resistant gene of rice and the avirulence gene of fungus. The resistant gene can protect rice from infection of rice blast fungus. However, the resistant genes are usually broken down several years due to the evolution of new fungal races. Thus, the finding of rice blast isolates containing avirulence *Avr-Pita1* gene and analyzing sequence variation of this gene are necessary for further research in reducing rice blast infection. In this study, avirulence *Avr-Pita1* gene of 25 rice blast isolates collected from the northern, middle and southern of VIETNAM were amplified and analyzed. The phylogenetic trees were constructed using Neighbor-Joining and Maximum likelihood methods in MEGA 6.0 program. The result showed that 18 rice blast isolates have *Avr-Pita1* gene and most of them were collected from the middle and southern of VIETNAM. The result of phylogenetic analysis and polymorphism analysis also showed the diversity of nucleotide sequence of *Avr-Pita1* gene among 18 rice blast isolates and differences between *Avr-Pita1* of Vietnam isolates and other countries.

3.2-188 Macroevolutionary patterns in the nutritional modes of Coltricia-Coltriciella clade, with emphasis in Mexican taxa

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Abstract: *Coltricia* and *Coltricialla* are a monophyletic clade in the Hymenochaetales, which includes more than 66 species, but monophyly of each genus is still controversial. Main morphological difference between both genera is that *Coltricia* has smooth spores and *Coltriciella* has ornamented ones. Even when both genera were traditionally considered saprotrophs, several species have been reported with ectomycorrhizal habit. In Mexico five species of Coltricia and three of Coltriciella are known, and one of those is only known from Mexico (Coltriciella sonorensis). At the present work, we inferred a phylogeny for the known species of both genus, with emphasis in Mexican and neotropical taxa, including recently discovered species not previously included in a phylogeny, and one new species from Mexico. Some of the Mexican species of *Coltriciella* were recovered in a monophyletic clade, next with species from North American and Caribe. One of the Mexican species was recovered as sister group with one of the *Coltriciella* ecomychorrhizal sequences from Africa, raising doubt regarding its ecological habit. We performed a preliminary study on the evolution of the ectomycorrhizal habit in the *Coltricia-Coltriciella* clade. Using BayesTraits v3 we estimate the evolutionary rates of gainings and losings of the ectomycorrhizal condition, and we inferred the ancestral condition for selected nodes. Our results indicate at least seven independent origins of the ectomycorrhizal condition in the clade, as well as equal rate of gainings and loses. Previous reports had documented a dual ecological habit on some species of allegedly saprophytic Agaricomycetes (i.e. species in *Phellinus*), and this may be the case for species in the clade like *Coltriciella dependens*. We consider that our result may be product of the dual nature

of several species in the clade, if that were the case, the apparent instability in the habit (equal rate of gains/losses) may be just lack of information regarding the full capabilities of the species in the clade. Probably mycologist need to re-think ecological roles and nutritional modesmycorrhizal habit in the *Coltricia-Coltriciella* clade. Using BayesTraits v3 we estimate the evolutionary rates of gainings and losings of the ectomycorrhizal condition, and we inferred the ancestral condition for selected nodes. Our results indicate at least seven independent origins of the ectomycorrhizal condition in the clade, as well as equal rate of gainings and loses. Previous reports had documented a dual ecological habit on some species of allegedly saprophytic Agaricomycetes (i.e. species in *Phellinus*), and this may be the case for species in the clade like *Coltriciella dependens*. We consider that our result may be product of the dual nature of several species in the clade, if that were the case, the apparent instability in the habit (equal rate of gains/losses) may be just lack of information regarding the full capabilities of the species in the clade. Probably mycologist need to re-think ecological roles and nutritional modes. If the condition of ectomycorrizal type of nutrition for *Coltricia* and *Coltricialla* is present in several specimens of México, the diversity of species will be higher than the known species.

3.2-189 *Xylaria karyophthora*: unravelling the life history strategy of a novel seed pathogen of Greenheart (*Chlorocardium* species)

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Abstract: *Xylaria karyophthora* was documented as a colonizer of seeds of Greenheart (*Chlorocardium* spp.), one of Guyana's most important timber trees. Much is unclear or unknown about this fungus including its life history strategy and this makes our understanding of its transmission, role in disease formation, and strategies for management inadequate. Our previous molecular study of *X. karyophthora* showed that it was derived from within a clade of wood-inhibiting species, suggesting a possible shift from woody to seed substrates. From this premise, we suggest two conceivable hypotheses for the ecology of this fungus. First, is the foraging ascomycete strategy which theorizes that saprotrophic fungi can present as dormant micro-thalli in healthy plant tissue as a means for dispersing across challenging environments. In this scenario, *X. karyophthora* possess the ability to move beyond the endophytic state to utilize another substrate, i.e., the woody seed pods of Greenheart. Second, *X. karyophthora* could represent an emerging infectious organism due to a new host-pathogen interaction that resulted from a recent host jump to Greenheart from another unidentified host. In this presentation, we will examine evidence for both of these hypotheses.

3.2-190 Evolution of carbon assimilation abilities within Mucorales

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Abstract: Representatives of Mucorales belong to one of the oldest lineages of terrestrial fungi. They probably diverged from other fungal groups already in Precambrian and since then they became ubiquitously distributed worldwide. Together with representatives of Glomeromycota, they probably played crucial role in plant terrestrialization. During their long evolutionary history, they evolved various modes of nutrition. At present, Mucorales representatives are mostly saprotrophic organisms which derive their nourishment from decaying organic matter. However, some animal pathogens (e.g. *Saksenaea vasiformis*) and phytopathogens (e.g. *Choanephora cucurbitarum*) are also among this group. Fungi, like all other organisms, may vary in their abilities to use different nutritional compounds (carbon in this study). Their ability to use different compounds available in the substrate can be perceived as one

of the main factors shaping the potential for a given fungal taxon to occupy a particular niche. These capacities are shaped by either the permeability of the cell wall or the presence of specific enzymes. Mucorales representatives are commonly called 'sugar fungi' as, according to literature, they grow well on media rich in simple organic compounds but are unable to assimilate more complex organic compounds. However, recently some studies showed that it is not true for all Mucorales representatives. Therefore, we hypothesize that carbon assimilation capacities within Mucorales representatives depend on their phylogenetic position. The ability of 74 strains belonging to 63 pathogenic and non-pathogenic species of Mucorales to use 95 different carbon sources was tested using FF Phenotypic Microarray Plates (Biolog™). Analysed strains differ significantly in their ability to use varying carbon sources. On average approx. 57 substrates were absorbed per strain and several strains showed capacity to assimilate more complex organic compounds (e.g. cellobiose). *Saksenaea oblongispora* had the highest capacities to assimilate different carbon sources (approx. 90% of all substrates). However, the ability of the various strains to use the analysed substrates did not show correlation with the evolutionary history of the group. Instead, carbon assimilation profiles are probably shaped by environmental conditions. In the hyphae of strains which had the highest capacities to assimilate different carbon sources, endohyphal bacteria were also detected. The interactions of Mucorales representatives with other microorganisms influencing carbon assimilation capacities is of particular interest and needs further study. The study was supported by the National Science Centre, Poland under grant No. 2015/17/D/NZ8/00778 and 2017/25/B/NZ8/00473.

3.2-191 Investigating the secotioid syndrome hypothesis across many climate variables: a case study using a global scale phylogeny of *Cortinarius*

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Abstract: Sequestrate fungi do not forcibly discharge their spores. They evolved from gilled (or pored) taxa independently in many different lineages. In 1984, Harry Thiers presented the Secotioid Syndrome Hypothesis where the sequestrate state is thought to have evolved as an adaptation to arid climates. Bougher and Lebel hypothesized that there may also be a selection pressure to protect the spore-bearing surface from excessively moist conditions. We test these two hypotheses by correlating the state of being gilled or being sequestrate with numerous BioClim variables in the globally distributed genus *Cortinarius*. We found that 'mean diurnal temperature' and 'mean maximum temperature in the hottest month' were significant in estimating the probability of being sequestrate. None of the precipitation variables were significant. A global map of the distribution of sequestrate *Cortinarius* species shows that sequestrate taxa are absent from the tropics in contrast to their persistence in temperate regions, further supporting the finding that sequestrate taxa are found in habitats with variable temperatures. This study casts doubt on the proposition that moisture is the sole driving force for the evolution of sequestrate taxa.

3.2-192 *Aspergillus* becomes a truffle – enigmatic, bright yellow, hypogeous ascomata found from Japan

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Abstract: Convergence of truffle-like fruitbodies form other types of mushrooms is one of the major evolutionary tendencies in Ascomycota and Basidiomycota. During our recent intensive field surveys of truffle-like (sequestrate) fungi in Japan, we frequently encountered tiny (up to 3 mm in diam.), subglobose, bright yellow, hypogeous fruitbodies growing in humus in mature Fagaceae forests. Phylogenetic analyses of nuclear ITS and 28S rDNA datasets unexpectedly revealed that this truffle-like fungus is a member of *Aspergillus s. str.* The multigene analysis suggested that the species fell into *Aspergillus* subg. *Nidulantes* and formed one of earlier diverging lineages within the subgenus. We are currently adding other DNA regions to the dataset to obtain a more robust phylogeny. Morphologically, as in the sexual stages of other *Aspergillus* species, ascospores are produced inside the cleistothecial fruitbody. Ascospores 4–5 µm in diam., ellipsoid to broadly lenticular, colorless, with two equatorial crests. Asci 8-spored, evanescent, uniseriate, cylindrical, which is rare in the genus. Hülle cells ovoid to tubular with irregular inflation, thick walled, surrounding the internal gleba. Peridium composed of loosely interwoven, non-inflated, branched filamentous hyphae. We confirmed germination of the hülle cells on the MNC medium. We further obtained cultures from the fruitbody tissues and successfully induced whitish brown conidiophores with a globose terminal vesicle, typical of the genus *Aspergillus*, on the MEA medium. Furthermore, we also succeeded to induce fructification of the characteristic yellow hypogeous ascomata after incubation of the culture in a pot stuffed with sterilized humus and brans for about 1 week at 20 ˚C. These findings imply that there still remain many unknown wild species of *Aspergillus* species that have unique, interesting characteristics in the nature. Further intensive field surveys must be needed to detect such hidden, characteristic taxa.

3.2-201 Fungi and Oomycetes associated with declining *Ceiba pentandra* in Guatemala

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Abstract: *Ceiba pentandra*, commonly known as Ceiba or Kapok, is a tree in the Malvaceae grown in tropical regions throughout the world. The Ceiba was designated as the national tree of Guatemala on March 8, 1955. Ceiba trees grow throughout Guatemala and their wood has been used to make canoes, the floss of the fruit is used for stuffing cushions and various parts of the tree have been utilized as medicine. Ceiba trees often mark the center of life in villages, as sites of markets and gathering places. In 2013, one of two Ceiba trees in the botanical garden of the University of San Carlos (USAC) failed to leaf out normally and was declared dead by April 2014. Most of the trunk was removed in 2016. At the same time it was noted that a tree in zone 1 of the city with similar symptoms had been removed, and the trees in the central plaza of the university also exhibited similar symptoms. No symptomatic Ceiba trees were reported from any other area of Guatemala. Fourteen Ceiba trees were surveyed in the city of Guatemala, with 5 of these showing definite lesions or dieback symptoms. Based on a review of the literature, approximately 50 fungi and oomycetes have been reported in association with Ceiba trees

throughout the world. None of these organisms have been reported in association with Ceiba trees in Guatemala, although some have been reported on other hosts in Guatemala. In July 2017, samples were taken from the soil surrounding Ceiba trees located on the USAC campus, and trunk samples were taken from the symptomatic trees. The samples were assayed for oomycetes and fungi through baiting and direct plating. The sampling and baiting resulted in 22 total isolates representing multiple organisms. Notably, *Phytopythium* is reported for the first time from Guatemala. This is also the first record of *Albonectria rigidiuscula* associated with Ceiba trees in Guatemala. The relative importance of the isolated organisms in the development of the symptoms observed is unknown, but their presence underscores the importance of maintaining the health of urban trees in Guatemala.

3.2-202 Identification of genetic groups of *Erysiphe necator* in Hungarian vineyards

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Abstract: Studies of Australian and European populations of *Erysiphe necator*, causing grapevine powdery mildew, have revealed the existence of two genetically differentiated groups, A and B. Group A was usually widespread early in the growing season, and was associated with flag shoots, that is powdery mildew covered shoots emerging from overwintered buds infected in the previous growing season. The two groups were shown to have different temporal distributions: group A was rarely found later in the season, when group B became more prevalent. In Europe, most studies focused on Western European *E. necator* populations. We sampled grape powdery mildew populations in Hungarian vineyards, in Central-Eastern Europe, to genotype the local populations and to reveal (i) the spatial and temporal dynamics of the local genotypes; and (ii) the genotype(s) responsible for flag shoot symptoms. Samplings were done in two wine regions in May, August and September 2015, to cover the three main stages of host plant growth. Flag shoots were collected in May; in addition, samples were also collected from close vicinity of the original flag shoots in August and September. Following DNA extraction, powdery mildew samples were genotyped based on partial beta tubulin (*TUB2*) gene sequences. Most sequences were obtained by direct sequencing while some amplicons were sequenced after cloning. About 85% of a total of 183 *TUB2* sequences determined in this work showed polymorphism at one or two nucleotide positions, which were revealed by double peaks on chromatograms of direct sequencing. Sequences obtained after cloning of selected PCR products clearly revealed the presence of three different *TUB2* genotypes in *E. necator* populations in Hungary. Two of these were identified as the A and B groups described in earlier studies, while the third one was designated as B2 in this work, based on a single nucleotide polymorphism (SNP) in the 6th exon of *TUB2*. All flag shoot samples belonged to B and/or B2 groups. Genotype A was present only in 5.5% of the samples and it has always been present in combination with genotype B and/or B2, in August and September. This study revealed three different *E. necator* genotypes in Hungarian vineyards in 2015. Flag shoot samples represented groups B and/or B2, but not A; this finding provides additional evidence of overwintering of B genotypes in buds, and causing flag shoot symptoms in spring. Our results show that co-infections of the same leaves and/or vines by different genotypes are common. Also, the detection of genotype A later in the growing season indicates that the temporal variation of A and B genotypes may not be as strict as suggested by a number of earlier studies. *This work was funded by the Széchenyi 2020 programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00061).*

3.2-203 Resistance to QoI fungicides in the grape black rot pathogen, *Guignardia bidwellii* and related species, in the light of the *CYTB* gene structure

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Abstract: Strobilurins, belonging to the group of Quinone outside Inhibitors (QoIs), are considered as single-site of action fungicides which inhibit the electron transfer in mitochondria by binding to the cytochrome *bc1* enzyme complex. It has repeatedly been shown that a single point mutation in codon 143 of the mitochondrial gene *CYTB*, which encodes cytochrome b, confers complete resistance to QoI fungicides in many plant pathogenic fungi. However, in some species, such as *Puccinia* spp., neither QoI resistance nor this mutation, designated as G143A, have been detected so far. This was explained by the presence of an intron in the *CYTB* gene right after codon 143 in these plant pathogens: it was predicted that a G143A mutation would prevent the splicing of this intron and, thus, the production of functional cytochrome b proteins. Consequently, in these intron-containing species the G143A mutation is considered to be lethal and the risk for QoI resistance is predicted to be low. *Guignardia bidwellii* (anamorph: *Phyllosticta ampelicida*), the causal agent of grape black rot, is considered as a *CYTB* intron-containing species with low risk for the development of QoI resistance in the field. We amplified and cloned *CYTB* fragments in several *G. bidwellii* strains, and also in some other *Guignardia* spp., including authentic strains of *G. citricarpa*, the causal agent of citrus black spot, and also *G. gaultheriae, G. mangiferae* and *G. aesculi* obtained from CBS, to sequence the intron located after codon 143. Surprisingly, no intron was detected in the predicted position in several *G. bidwellii* strains isolated from different grape varieties in Hungarian vineyards. Also, the intron was not found in either an authentic *G. bidwellii* strain obtained from LGC ATCC, or the *G. aesculi* and a *G. gaultheriae* strains included in this study, while the intron was identified, and sequenced, in all other *Guignardia* spp. strains examined by us. *In vitro* fungicide resistance tests did not show a clear correlation between the presence/absence of the intron in *Guignardia* spp. strains and their sensibility to QoI compounds. This might suggest that other mechanisms may also be involved in their QoI resistance. So far, our results indicate that at least some *G. bidwellii* strains causing grape black rot could contain the G143A mutation and might be able to develop QoI resistance in this way in the field. *This work was funded by the Széchenyi 2020 programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00061). Zsolt Bereczky acknowledges the support of a Janos Bolyai Research Fellowship and a grant (NKFIH PD-100724) of the Hungarian Research, Development and Innovation Office.*

3.2-204 The diversity of trunk disease pathogens within the Botryosphaeriaceae from the winelands of South Africa with new additions

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Abstract: The Botryosphaeriaceae are an important group of trunk disease pathogens that affect wine and fruit industries worldwide. These fungi infect the woody tissues of plants, causing necrosis, cankers, stunted growth and dieback which result in lower yields and can eventually contribute to the death of the plant. Some species display broad host ranges, making it difficult to control these pathogens if they persist in the environment by inhabiting alternative hosts. The threats posed by such reservoirs of trunk disease pathogens to the grapevine industry are unknown. Therefore, this study aimed to survey the

diversity of Botryosphaeriaceae species associated with disease symptoms of woody hosts that commonly surround vineyards in the winelands of South Africa. Wood samples were collected from visibly diseased trees and shrubs in the direct vicinity of vineyards and Botryosphaeriaceae strains were isolated. Identifications were made by performing phylogenetic analyses based on ITS and EF1α DNA sequence data. In total, 989 plants from 39 different host species yielded 1674 isolates belonging to 29 different species, eight of which were found to be novel and were described. In addition, 64 of the host/pathogen combinations encountered during this study appear to be novel. Prior to this study a total of 38 different Botryosphaeriaceae species were reported in South Africa. This survey introduces an additional 16 species to this list including the new species. The Botryosphaeriaceae species that were the most frequently encountered during this survey were Diplodia seriata and Neofusicoccum australe. These species are important grapevine trunk disease pathogens and also displayed broad host ranges. This survey clearly shows the prevalence of important grapevine trunk disease pathogens in close proximity to vineyards and lays the groundwork for further studies to investigate the significance of alternative hosts as reservoirs of grapevine trunk disease pathogens.

3.2-205 The Botryosphaeriaceae in China

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Abstract: The Botryosphaeriaceae (Botryosphaeriales, Dothideomycetes) represents one of the most important families within Ascomycota, being widely distributed in the non-polar regions of the world. Some species are pathogens of economically important plants, and several are of quarantine concern in China, such as "*Botryosphaeria laricina*" causing shoot blight or twig dieback of larch, and *Diplodia mutila* causing cankers on apple as well as black dead arm on grapevines. Of the 23 genera currently accommodated within the Botryosphaeriaceae, 11 have been reported from China, namely *Botryosphaeria*, *Cophinforma*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Macrophomina*, *Neodeightonia*, *Neofusicoccum*, *Neoscytalidium*, *Phaeobotryon* and *Sphaeropsis*. Based on the 700 strains of Botryosphaeriaceae isolated from woody hosts in China during this study, the isolation frequency of *Lasiodiplodia* was 43%, followed by *Botryosphaeria* (33%), *Neofusococcum* (10%), and other minor genera (14%). More than 2000 taxa have previously been assigned within Botryosphaeriaceae, while only 123 epithets have been reported from China. Of these 123 epithets, 25 (20%) were first described from China, and 43 have DNA data available. Forty-eight (39%) of the taxa belong to *Diplodia*, 22 (18%) to *Sphaeropsis*, 17 (14%) to *Botryosphaeria*, 13 (11%) to *Dothiorella*, 12 (10%) to *Lasiodiplodia*, 4 (3%) to *Neofusicoccum*, 2 (2%) to *Phaeobotryon*, 2 (2%) to *Neoscytalidium*, 1 (1%) to *Neodeightonia*, *Macrophomina* and *Cophinforma*, respectively. Most of the species (65%) have been reported from tropical or subtropical areas, such as Jiangsu (13%) and Guangdong (13%) Provinces, while the northern or western part of China, such as Xinjiang (1%), Qinghai (0%) and Tibet (1%) have been rather poorly studied. Most of the species (>80%) have been reported as plant pathogens. With extended sampling as well as the development of new molecular identification techniques, more taxa are continuously being recovered.

3.2-206 New *Collophorina* species from *Prunus* trees and vineyards in Germany

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Abstract: *Collophorina* (syn. *Collophora*, *Leotiomycetes*) was described in 2010 from necrotic wood of stone fruit trees in South Africa as a genus forming small aseptate conidia from integrated conidiogenous cells resembling those of the not closely related genus *Lecythophora*. To date, six species have been described and the genus has been reported from necrotic or symptomless tissue mainly of *Prunus* spp., but also of *Castanea sativa*, *Acer glabrum* var. *douglasii* and *Euphorbia polycaulis* in South Africa, Europe, Northwestern United States and Iran. In a survey aiming to reveal the diversity of fungi associated with wood necroses of *Prunus* trees in Germany as well as from spore traps in vineyards, 84 and 10 isolates, respectively, with reduced conidiogenous cells were obtained. ITS sequence data placed these strains in the genus *Collophorina*. In a multi-locus molecular phylogenetic analysis (ITS, TEF and GAPDH), 11 species were differentiated. Most isolates studied were identified as *C. paarla* and *C. africana*, while further nine species were revealed as new to science and characterized by means of morphological and molecular data.

3.2-207 Direct evidence of *Hymenoscyphus fraxineus* infection pathway through the petiole-shoot junction

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Abstract: The symptoms of ash dieback caused by the fungus *Hymenoscyphus fraxineus* include wilting of the foliage followed by dieback of shoots, twigs and branches. Necroses in shoots are assumed to develop after infection through leaf petioles; however, clear evidence of this infection pathway has not yet been provided. Considering the co-occurrence of multiple pathogen genotypes in dead ash petioles, we aimed to obtain a spatial overview of all *H. fraxineus* genotypes colonizing individual shoots and their corresponding petioles. We labelled selected shoots and corresponding petioles whilst on the tree and reconstructed their position after their natural shedding. This design allowed us to acquire precise information about the infection biology of *H. fraxineus* and its ability to cross the petiole-shoot junction. Individual genotypes of *H. fraxineus* were characterized by the analysis of microsatellites using DNA extracted directly from petiole segments or cultures isolated from the segments. We detected 150 different multilocus genotypes in 10 analysed shoots and their corresponding petioles; the highest number of genotypes was eight for a single petiole and three for a single shoot. The genotypes of most shoot lesions were identical to particular genotypes from the proximal segments of petioles, implicating the main pathway of shoot infections. To test whether the amount of colonized substrate or intraspecific competition have an effect on successful infection, genotypes that reached the most proximal end of the petioles were scored for the number of invaded petiole segments and for the number of other *H. fraxineus* genotypes co-occurring in the segments. However, the extent of colonization of the scored genotypes and intraspecific competition with other *H. fraxineus* strains did not enhance nor limited pathogen success in entering the shoot. This study confirms for the first time that the majority of ash shoot infections are caused by *H. fraxineus* strains originating from petioles. Scaling up the counts from petioles to a whole tree suggests that a mature tree must host thousands of *H. fraxineus* individuals.

However, compared to petioles, the number of *H. fraxineus* genotypes in shoots was much lower, which points to the evolutionary benefit of the petiole representing a strong bottleneck for spread of fungal pathogens.

3.2-208 Identification of *Tubaquia* spp. causing foliar spots on oak (*Quercus eduardii*) leaves in Aguascalientes, Mexico

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Abstract: Oaks (*Quercus* spp.), are some of the most important forest species of the mountainous regions of the northern hemisphere. They are found distributed from southern Canada to Colombia. The center of diversification of the *Quercus* genus is in Mexico. and the 531 species described worldwide between 150 to 161 species are found in our country and of them close to 90 are considered to be endemic. Like other forest trees, oaks provide many environmental services that benefit man. Oak trees, like many other plants, can be affected by phytopathogenic fungi. In 2016, foliar spots caused by a fungus not reported in Mexico were observed in *Quercus eduardii*. The objective of this study was to identify the phytopathogenic fungus responsible for the foliar spots. The description of the symptoms caused by the fungus was made. To be able to identify the fungus, fungal structures were taken from the leaves and were placed between a slide and a cover slips with lactophenol cotton blue. Structures and spores were measured using a Carl Zeiss microscope. The microscope was fitted with an ocular micrometer ruler in one of its eyepieces. The fungus was also isolated and the description of the colonies, that were grown on malt extract agar culture medium, was made. The leaf spots were amphigenous, subcircular to angular-irregular and on average measured 2–13 × 2–9 mm, usually ochraceous brown to medium dark brown, margin indefinite or with a narrow darker brown margin or marginal line. Conidiomata (pycnothyria) borne on a stalk or columella, dimidiate, shield-shaped, black, coalescing or scattered, conidiophores simple; conidia hyaline, I-celled, ovoid, oblong or fusoid. In culture: Colony (after 20 days on malt yeast extract agar, at 22°C) 80–82 mm diam. Mycelium with a undulated margin white at first, with concentric rings of aerial mycelium, the center was olive green with stripes of brown mycelium, no color change was observed on the underside after 20 days, there was also no sporulation. Mycelium branching, septate, 3.0–5.0 µm diam., hyaline and brown. All the above characteristics agree with the genus *Tubakia* (*Actinopelte* Sacc.). The isolated fungus was sent to Dr. Pedro Crous (Westerdijk Fungal Institute, Utrecht, The Netherlands), for molecular identification of the species. In conclusion, the phytopathogenic fungus causing the leaf spots on *Qu. eduardii* was identified as *Tubakia* spp.

IMC 11
2018

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July 16-21, 2018 - PR Convention Center

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