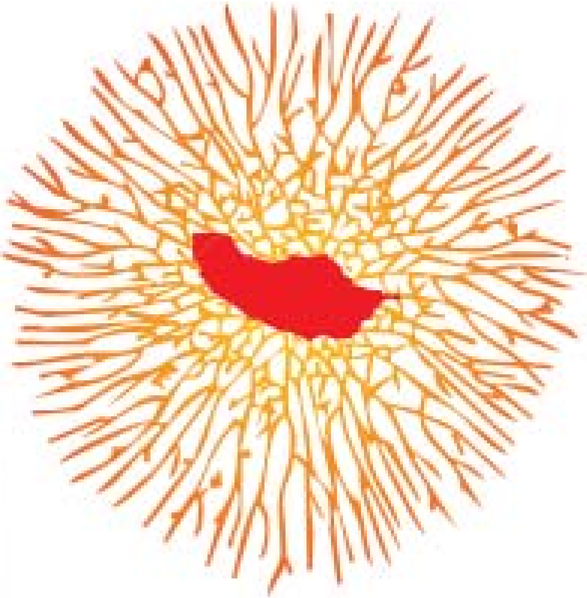

XVII CONGRESS OF EUROPEAN MYCOLOGISTS

Madeira, Portugal
21 - 25 September 2015

ABSTRACTS

28 September, 2015
Final version

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The ***XVII Congress of European Mycologists*** took place in Portugal at the capital of Madeira Island, Funchal, from 21st to 25th September, a meeting that happens every four years.

This Congress, organized under the auspices of the European Mycological Association, was the latest in an unbroken series going back almost sixty years – longer than any other international Congress in mycology. It built on a distinguished record of promoting progress in mycology and understanding the diversity and biology of fungi. Earlier Congresses in this series have been in many different parts of Europe, but this was the first in Portugal, and the first on an Atlantic island beyond the main continental shelf, rich in endemics, of great scientific interest, and further west and south than any before.

Reflecting the inherent difficulties experienced in a time of crisis, this year only 142 participants attended this event. However, it was very encouraging having people coming from all over the world and with a great percentage of young scientists and students, all "working with fungi", the motto of this Congress.

The Congress hosted participants from 32 countries – 21 European countries (Belgium, Bulgaria, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Luxembourg, Poland, Portugal, Russia, Slovakia, Spain, Switzerland, The Netherlands, Ukraine and United Kingdom) and 11 from outside Europe (Brazil, Canada, Egypt, India, Israel, Japan, Korea, México, Nigeria, South Africa and Turkey). In all, 162 scientific papers were presented in a fruitful interchange of knowledge, in an amiable, friendly climate.

At the EMA General Assembly, which took place during the Congress, it was decided that the next Congress will be held in Poland.

On behalf of the president of the *European Mycological Association* and of the Organizing Committee I thank all participants for the enthusiastic adhesion to the proposed programme and their contributions to maintain the usual high scientific standards of these meetings.

João Baptista-Ferreira
Chairman of the XVII CEM

All abstracts are arranged by thematic areas according to the Congress scientific programme.

Oral lectures, symposia and poster presentations are arranged in three sections for easier follow-up.

Only copy-editing and formatting of abstracts have been done, therefore the authors are fully responsible for the scientific contents of their abstracts.

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XVII CONGRESS OF EUROPEAN MYCOLOGISTS
Madeira, Portugal, 20 - 25 September 2015

CONGRESS PROGRAMME

Sunday, 20th September 2015

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19.30-21.00	Former Jesuit College	**Welcome Reception** (offered by Cybertruffle)

Monday, 21st September 2015

08.00-09.00	Congress Centre Reception	**Registration of participants / Talk uploading**
09.00-09.45	Sunrise Auditorium	**Congress Opening Ceremony**
09.50-10.25	Sunrise Auditorium	**Plenary Session:** David Minter – Presidential address: Congresses, the EMA, and infrastructure
10.30-10.55	Congress Centre Foyer	**Coffee break**

Parallel Sessions (oral communications)

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15.45-16.00	Jadwiga Turło	Biosynthesis and structural analysis of mushroom-derived immunosuppresive polysaccharides	Izabela Kalucka	Diversity of pioneer ectomycorrhizal fungi in mine spoil recultivation forests
16.00-16.15	O. Adeogun	Preservation potentials of leaf extracts of three medicinal plants on Citrus sinensis (sweet orange) juice	Maria Rudawska	Fungal communities associated with roots of Scots pine (Pinus sylvestris L.) seedlings from bare-root forest nurseries
16.15-16.30	Vera Tereshina	Adaptation of alkalophilic fungus Sodiomyces tronii to the conditions with different ph	Susana C. Gonçalves	Potential distribution and identity of introduced Amanita muscaria worldwide
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17.45-18.00	Lyubomir Penev	Data papers incentivise biodiversity scientists to publish and re-use their data		
18.00-18.15	Urmas Kõljalg	Species of Fungi		
18.15-18.30	Matias Pasquali	Digging the treasure of hidden data: An open access European database of Fusarium graminearum and F. culmorum from small grain cereals for epidemiological studies		
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Tuesday, 22nd September 2015

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		William Purvis – 20 Year's Monitoring Lichens under Global Environmental Change		
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11.00-11.15	Pedro Talhinhas	A fungal genome at work: Genomic and transcriptomic characterisation of Hemileia vastatrix, the Coffee Leaf Rust pathogen	Eske De Crop	Unravelling the milkcap genus Lactifluus (Russulaceae)
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12.00-12:15			Vladimír Antonín	Taxonomic and molecular studies of the European species of the genus Melanoleuca (Basidiomycota, Tricholomataceae) – a preliminary report
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15.45-16.00	Tatiana Semenova	Ascomycete fungal communities reorganize in response to long-term summer and winter climate warming in moist and dry tundra of Arctic Alaska	Paola Angelini	Current knowledge of Umbrian macrofungi (central Italy)
16.00-16.15	Olga Marfenina	Possible effects for soil microfungal communities in the conditions simulating the global warming	Maria da Luz Calado	Assessment of marine fungal community associated with Spartina maritima (Curtis) Fernald standing plants by morphological and molecular methods
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		Stephen Woodward – Alien invasive pathogens threatening Europe’s forest ecosystems		
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10.45-11.00	Dominik Begerow	The evolution of smut fungi – an update	Hanna Szmidla	Comparison of chemical composition in Tuber aestivum Vittad. of different geographical origin
11.00-11.15	Sofiya A. Bondarenko	A survey of filamentous fungi at the extremely alkaline Magadi Lake	Katerina Sazanova	Geoecological role of acid production by micromycetes on the rock substrates
11.15-11.30	M. Gökhan Halıcı	The Lichenized Fungus Genus Xanthocarpia (Teloschistales, Ascomycota) in Turkey	Guillermo Vidal Diez de Ulzurrun	Spatio-temporal monitoring fungal growth dynamics using image analysis and graph theory
11.30-11.45	Grzegorz Koczyk	Growth on the graveyard of chemical warfare - the plasticity of aromatic polyketide biosynthesis in higher fungi	Jerzy Falandysz	Mercury accumulation and distribution in Suillus bovinus and probable dietary exposure by foragers
11.45-12.00			Innocent Nnorom	Mercury in Suillus granulatus and Suillus variegatus and soil substratum beneath the fruiting bodies
12.00-12:15			Ekaterina Bubnova	Fungi in bottom sediments of the Chukchi Sea: diversity and ecophysiology
12.15-12.30			Maria D'Aguanno	Wood-inhabiting fungi diversity vs. deadwood features: what happens in Mediterranean forests?
12.30-13.00	Discussion			
13.00-14.30	**Lunch** – Tower II Buffet Restaurant			

Thursday, 24th September 2015 (cont.)

14.30-15.30	Tower II Living Lounge	**Poster session** Thematic areas: **Evolution, biodiversity and systematic**; **Genomics, genetics and molecular biology**; **Cell biology, biochemistry and physiology**
15.30-17.30	Sunrise Auditorium	**Symposium on Fungal Conservation and Meeting of the ECCF** – Moderators: Claudia Perini and Beatrice Seen-Irlet
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15.50-16.05	Martyn Ainsworth	The Lost and Found Fungi project: from local treasure hunt to national fungal conservation infrastructure
16.05-16.25	André Fraiture	The publication of the distribution maps of 51 macromycete species in Europe
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10.45-12.15	Sunrise Auditorium	**EMA General Assembly** Electing honorary members, awards for achievement, prizes for posters etc., appointing officers, location of next Congress
12.15-12.30	Sunrise Auditorium	**Congress Closing Ceremony**
13.00-14.30	**Farewell lunch** – Tower II Buffet Restaurant	

INVITED LECTURES

Host shifts in fungi due to climate change

Alan Gange[1], Aqilah Mohammad[2], Thanos Damialis[3]

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[2]Universiti Malaysia, Kuala Terengganu, Terengganu, Malaysia
[3]University of Ioannina, Ioannina, Greece

An accumulating body of evidence suggests that fungi display remarkable responses to climate change, and that across Europe over the last 60 y, significant changes in fruiting patterns have occurred. The majority of these studies have concentrated on phenology, with large changes in the length of fruiting seasons being observed.

Changes in fruiting patterns indicate changes in mycelial growth and have implications for interactions between fungal species and between fungi and their host plants. Has climate change resulted in changes in fungal community structure? Here, we present an analysis of one data set from southern England that has previously been used to show phenological changes in forest fungi. We now extend these analyses to examine whether changes in fungal community structure have occurred, via altered host associations. We compare the responses of saprotrophs with ectomycorrhizal species and show that some remarkable changes have happened, with potential consequences for tree growth and forest dynamics.

20 Year's Monitoring lichens under Global Environmental Change

O. William Purvis

Consultancy 'Lichen Matters', 2 Rambler Close, Taplow, Berkshire, UK

Lichenized fungi are polyphyletic in origin and colonise all major ecosystems apart from the deep sea. Unlike yeasts and filamentous fungi, there are no model lichens and thus knowledge of lichen fungal biology is much less studied than other fungi which may also attract greater commercial interest. Lichens, like other organisms, tend to favour particular climates and under stress may be restricted to specialised ecological niches. Some may be restricted to particular substrates. Why are particular species restricted to particular ecological niches, at least in part of their ranges? Possible explanations have been suggested dating back to the 1970's or are else derived from assumptions through knowledge gained through research on non-lichenised fungi.

Science involves addressing questions and hypotheses in line with research priorities which inevitably change over time. Prior to the 19th Century, botanists throughout much of Europe, even those living in areas remote from cities and learned centres, collected lichens, fungi and other organisms which are now housed in the great herbaria of the

world. A desire to identify and establish the distribution of taxa drove this activity. But today, such historical collections, as exemplified by lichens collected at Epping Forest housed in the Natural History Museum collections, provide a fascinating insight into environmental change. The volume 'Monitoring with lichens – monitoring lichens' was proposed, after some discussion, as the title of a book which arose from a NATO Advanced Research Workshop held at Orielton, South Wales in 2000. Lichen monitoring involves following a method or protocol with a view to obtaining information about lichens or else using lichens to obtain information about environmental change, over space and time. Biologists tend to be keen on statistical correlation. However, correlations do not necessarily imply causality and the lack of a correlation does not imply there is no effect. Further work, including physiological experiments, are required.

The pollution lichen, *Lecanora conizaeoides*, arguably the most familiar lichen to all lichenologists living in urban areas brought up under a high SO_2 pollution regime spread throughout much of lowland Britain and other industrial regions. The demise of other lichen species in response to acidification due to fuel combustion and their return in response to emission reductions is today widely recognised amongst the general public, including school children. Indeed lichens played a role in citizen science engagement long before citizen science became fashionable. Nowadays major stressors on lichen assemblages include both oxidized and reduced nitrogen, not only in rural and urban areas but also remote regions.

In a world where short term funding is the norm, base-line monitoring is unfashionable. However, long-term monitoring can provide unexpected benefits extending well beyond the original remit and highlight the need to address other aspects, e.g. conservation and biogeography. In my talk, I shall focus on recent research carried out in temperate zones in relation to studies carried out in and around London and the Azores, Portugal.

Throughout my talk, I shall emphasise the importance of consulting collections, including historical and 'back-log' collections, early papers and manuscripts. These provide, in conjunction with modern molecular techniques, new and exciting opportunities for innovative research. Young researchers should not only consult available literature on the web but must consider other literature. Future research must capitalise on advances made in non-lichenized fungi and elsewhere through targeted multi-disciplinary approaches involving various methodologies.

Searching for generality in multi-trophic fungal interactions: Implications for invasions and agriculture

Alison E. Bennett

The James Hutton Institute, Dundee, Scotland, UK

Fungi have long been known to influence ecosystems through a wide array of direct interactions, by playing roles in nutrient cycling, decomposition, and even population control via disease. However, more recently we are becoming aware of the multitude of interactions modified by fungi. These indirect interactions can cascade up through food webs, and impact an ever growing group of organisms. In my research group we focus on the indirect interactions promoted by one particular group of fungi, arbuscular mycorrhizal (AM) fungi, in order to understand how common and important these indirect interactions are in a wide array of systems.

Research in my group and others have shown that AM fungi can alter host chemistry through priming of plant hormonal response systems, and these changes can influence plant associations with herbivores, predators of herbivores, and pollinators. As this body of literature grows we search for a consistent pattern of AM fungal influence.

In this talk I will address the generality of AM fungal effects on plant-insect interactions by focusing on research manipulating diversity at multiple levels within these interactions. I will focus on the influence of AM fungi on *Solanum*-aphid-parasitoid interactions as well as other AM fungi-plant-insect systems studied within my group. In particular I will assess variation in AM fungal-plant-insect interactions due to host plant species or genotype and herbivore species and genotype. I will then discuss whether variation introduced by these factors is greater than the variation introduced by AM fungi. Finally, I will suggest when and where variation in response to AM fungi within a group (for example host plant) might increase the influence of AM fungi on plant-insect interactions.

This type of research is particularly important for understanding when AM fungal influenced indirect interactions are most important, and what factors influence this importance. For example, regardless of the importance of species level diversity on influencing these interactions, in invaded and agricultural systems plant genotypic level diversity will become significantly more important. This leads to important questions about how AM fungal influenced indirect interactions are likely to operate in systems with strong variation in species and genotypic level importance.

Alien invasive pathogens threatening Europe's forest ecosystems

Stephen Woodward

Department of Plant and Soil Science, Institute of Biological and Environmental Sciences,
University of Aberdeen, Aberdeen, Scotland, UK

Forest and woodland ecosystems and trees in other situations in Europe are facing unprecedented challenges from an influx of alien invasive pests and pathogens resulting from increased global trade. Moreover, climate change predictions suggest that many additional alien pests and pathogens may become problems in forests as temperatures increase and rainfall patterns change. Recent indications of the potential for pathogens to transfer between hosts and the inter-specific hybridisation possible between related pathogens increase concerns over the possibility of escalations in damage in the future.

Despite multiple warnings over a long period, final confirmation of the presence of ash dieback caused by *Hymenoscyphus fraxineus*, in UK woodlands in late 2012 provided a wake-up call to the authorities, leading to a flurry of activity from the government, sometimes prompted by the noisy clamour raised in the media on the subject. The initial aim of containing the problem soon gave way to an evolving plan for a future without substantial numbers of *Fraxinus excelsior* in the environment. The arrival of this 'new' disease, however, was no surprise: ash dieback was well-known from its steady advance across Europe since the early 1990s.

Ash dieback is only one of many invasive pests and pathogens threatening trees in Europe. Along with unknown pathogens that could arrive, the number of threats is probably very large indeed. Current threats in Europe include a panoply of *Phytophthora* species already present in Europe, along with pathogens such as *Ceratocystis platani* and *Fusarium circinatum*.

Current status of truffle cultivation: recent results and future perspectives

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Keywords: *Tuber melanosporum, Tuber magnatum, Tuber borchii, Tuber aestivum,* cultivation, mycelial inoculation

Introduction

Truffles are ascomyceteous fungi belonging to several families in the Pezizales that have independently evolved a subterranean mode of existence (Læssøe & Hansen, 2007). While some basidiomycetes have also evolved underground fruiting bodies, in *sensu stricto,* only species in the genus *Tuber*, Tuberaceae, are considered the true truffles (Jeandroz *et al.*, 2008). The genus has been estimated to contain 180 to 230 species (Bonito *et al.*, 2010) distributed worldwide. Most species produce strong aromas to attract their dispersal agents and a few have considerable economic value because of their unique aromas and flavors. All the truffles live in mycorrhizal symbiosis with the roots of suitable host plants (Bonito *et al.*, 2013). *Tuber* were thought to only form ectomycorrhizas but recently it was found that they are also able to form arbutoid mycorrhizas (Lancellotti *et al.*, 2014) and endomycorrhizas with orchids (Selosse *et al.*, 2004).

The most valuable truffles are the European species *Tuber melanosporum* (Périgord black truffle), *Tuber magnatum* (Italian white truffle), *Tuber aestivum* (summer or Burgundy truffle) and *Tuber borchii* (bianchetto truffle) (Hall *et al.*, 2007). Other species with a limited market in Europe are *Tuber brumale*, *Tuber macrosporum* and *Tuber mesentericum.* In the U.S.A. several species of truffle are harvested but only the Oregon white truffles (*Tuber oregonense* and *Tuber gibbosum*) and the pecan truffle (*Tuber lyonii*) have a limited local market (Trappe, 2009; Lefevre, 2012). There are many species of truffle in China. Some resemble European species and some are collected and exported to Europe. The Chinese black truffle, *Tuber indicum*, is quite similar to *Tuber melanosporum* but with a relatively poor flavor (Riousset *et al.*, 2001) and *Tuber sinoaestivum* which is similar to the European *T. aestivum* (Zambonelli *et al.*, 2012; Zhang *et al.*, 2012). *Tuber oligosperum* is harvested in North Africa, in particular Morocco, and illegally sold in Italy as *T. magnatum* (Boutahir *et al.*, 2013).

The high economic value of truffles has stimulated researchers to find the most efficient methods for cultivating them. This has not been a simple task because of their complex life cycle involves a symbiotic relationship with suitable host trees and it is entirely completed underground where complex relationships with soil microorganisms are involved.

Here we briefly report on the current status of truffle cultivation, the most recent research information and the future prospects for these diamonds of cuisine.

Early methods

The first Italian and French attempts at cultivating truffles date back to the Renaissance, but successful and commercial cultivation of truffles did not start until the early 1800s when Josef Talon in France developed a crude but effective method for cultivating the Périgord black truffle (*T. melanosporum*) (Hall *et al.*, 2007). This technique involved sowing acorns collected under oaks that were producing truffles. It was very successful in France in areas where truffle spores were already present in soil but when applied in Italy the method failed (Mannozzi Torini, 1984). Modern truffle cultivation was introduced into France and Italy in the 1970's after the discovery of the mycorrhizal nature of truffles.

Initially three methods for inoculating plants were tried: spore inoculation, mother plant technique and mycelial inoculation. The mother plant technique involved planting seedlings into the rooting zone of a plant know to be mycorrhized with the required truffle. While mycelial inocula also showed promise (Chevalier, 1973) spore inoculation soon became the method of choice for the commercial production of plants.

Current status of truffle cultivation

In the second middle of 1900s truffle cultivation became popular had a great impulse not only in Europe but also in non-European countries. *T. melanosporum* is the truffle which has been the most successfully cultivated around the world but the cultivation of *T. aestivum* and *T. borchii* has also been successful in many countries.

T. melanosporum cultivation

Most of the black truffles produced in Italy, France and Spain are now harvested from cultivated truffières and in France only 10% are now harvested from natural areas (Reyna & Garcia-Barreda, 2014). Truffle cultivation gave also very good results in countries like New Zealand, USA and Australia where truffles were introduced by cultivation. In these countries most of the soils are acidic and in their native state unsuited for truffle cultivation. However, Ian Hall showed that some soils could be modified first by intensive liming and then correcting the almost inevitable trace element deficiencies (Hall *et al.*, 2007). This method soon spread to Australia which produced 3 tonnes in 2011 (Hall & Haslam, 2012), which is projected to grow to 8 tonnes in 2015 (Duell, 2012), and could soon overtake French truffle production at least in a poor fruiting year (Hall & Zambonelli, 2012). Cultivated black truffle have also been produced in the USA, Canada, Morocco, Chile and South Africa (Reyna & Garcia-Barreda, 2014; Berch & Bonito, 2014, Zambonelli unpublished data). *T. melanosporum* plantations have also been established in Israel, Argentina and in several European countries but to the best of our knowledge production has yet to begin.

Fig. 1 - *T. melanosporum* plantation in Spain

T. magnatum cultivation

Of the main European truffles only the Italian white truffle (*T. magnatum*), which is the most expensive, has yet to be successfully cultivated. Only few truffières have produced in Italy Despite extensive plantings of inoculated trees in Italy from the early 1980s to the late 1990s few truffières have produced and then only a few kilograms per hectare (Gregori *et al.*, 2010.). The main reason for this seems to be the difficulties in obtaining *Tuber* infected plants in the nursery and significant contamination problems. Before the introduction of molecular methods for the identification of the mycorrhizas, *T. magnatum* inoculated plants were often sold contaminated with other less valuable *Tuber* species such as *Tuber maculatum* and *T. borchii*. In fact these highly infective truffles, which are morphologically similar to *T. magnatum,* when accidentally incorporated in the inoculum colonized the entire root systems (Hall *et al.*, 2007). Another factor limitating the cultivation of *T. magnatum* is that its biology and soil ecology is still a mystery. Its mycorrhizas can be obtained in the nursery (Mello *et al.*, 2001; Rubini *et al.*, 2001), albeit with difficulties, but then they seem to disappear in the field (Hall *et al.*, 2007). *T. magnatum* mycorrhizas are also absent in natural productive areas (Leonardi *et al.*, 2013). Recent molecular techniques like Q-PCR are now able to follow the development of *T. magnatum* mycelium in the soil (Iotti *et al.*, 2012a) and are beginning to give new insights into its soil spatio-temporal development (Iotti *et al.*, 2014). The possible role of associate bacteria, and in particular of rhizobia, has also been hypothesized in the growth, development, nutrition and fructification of *T. magnatum* (Barbieri *et al.*, 2007; Barbieri *et al.*, 2010). Clearly an in depth study of the strange ecology of this truffle will be needed before routine methods for its cultivation can be established.

T. borchii cultivation

The first publication reporting the successful cultivation of *T. borchii* was by Zambonelli and colleagues in 2000 in Marina di Ravenna, Italy. Since then its cultivation has become widespread in Italy and more recently in New Zealand where this truffle is particularly appreciated by gourmets and in particular the Chinese community (Hall *et al.*, 2007; Wong personal communication). The first successful cultivation of *T. borchii* in Australia by Peter Stahle was reported in March 2015 (https://trufflefarming.wordpress.com/2015/03/14/first-tuber-borchii-ever-harvested-in-australia/). Recently, *T. borchii* cultivation was successfully introduced in USA (Isikhuemhen personal communication).

T. borchii cultivation has a great potential because of its broad ecological adaptability, its wide range of angiosperm hosts such as oaks, hazel, and linden, and conifers including pines and cedars (Hall *et al.*, 2007; Zambonelli *et al.*, 2002). Recently it was found to produce mycorrhizas with the pecan tree *Carya illinoinensis* and to fruit when associated with *Arbutus unedo* opening up the possibility of combining both the cultivation of this precious truffle and the production of edible fruits of these plants (Benucci *et al.*, 2012; Lancellotti *et al.*, 2014). However, there will be difficulties because pecan nuts and *A. unedo* fruits are ready for picking just as the truffles are forming in the soil. Also some rather toxic insecticides with residual activity have been used to control pecan pests.

T. aestivum cultivation

T. aestivum cultivation in Italy and in France began a little later than the cultivation of *T. melanosporum* but has grown rapidly and is now widespread. In France the cultivation of *T. aestivum* is concentrated within its natural boundaries: Auvergne, Champagne, Lorraine, Bourgogne, and Franche-Comte (Chevalier and Frochot, 1997). In Italy out of around 120,000 truffle trees planted per year only 15% are inoculated with *T. aestivum* (Bencivenga *et al.*, 2009). *T. aestivum* has also been successfully cultivated in Sweden by Christina Weden (Weden *et al.*, 2009) on the island of Gotland which represents the northernmost boundary of its natural distribution in Europe. Like *T. borchii*, *T. aestivum* has a great potential because of its adaption to a wide range of soils, climate and host plants. Recent inoculation experiments revealed that mycorrhization of pecan tree *Carya illinoinensis* with *T. aestivum* is also possible which makes truffle–nut coproduction a possibility (Benucci *et al.*, 2012).

The problems

Despite the successful cultivation of *T. melanosporum*, *T. borchii* and *T. aestivum* there are still some significant problems with their cultivation. Some truffières are not productive or produce few truffles of poor quality. This is often due to the poor quality of plants produced by some companies. In Europe morphological and molecular certification methods for *Tuber* infected plants are locally applied but an European law and a unique certification protocol is needed to protect truffle farmers from failures (Andrés-Alpuente *et al.*, 2015; Murat, 2014). What also makes truffle cultivation uncertain are the unpredictable genetic characteristics of the truffle strains when plants are inoculated with genetically highly variable spores, which may or may not be the most suited to the edaphic and climatic conditions on a particular site.

The sequencing of the *T. melanosporum* genome has revealed that it is heterothallic so that strains carrying different mating types have to cross for fruiting body production (Martin *et al.*, 2010). Both the mating type genes (MAT1-1-1 and MAT1-2-1) were identified and then characterized (Rubini *et al.*, 2011a). After this discovery, studies were carried out in order to verify if the lack of productivity in some truffières was due to the absence of one of the mating types. However, mating type analyses of seedlings planted in Australia showed that both mating types are present suggesting that there are more factors involved in ascocarp production than just the presence of both mating types on host trees (Linde & Selmes, 2012). Recent studies carried out in Europe showed that even in natural productive areas mating type distribution is patchy and often unbalanced (Rubini *et al.*, 2014). In fact, genetically different truffle strains compete with each other and with few genets of the same mating type colonize distinct patches of the truffières (Rubini *et al.*, 2011b; Murat *et al.*, 2013). Some Authors have hypothesized that such spatial segregation may be due to competitive exclusion, with use of the MAT locus as a marker for self-recognition, probably in addition to other polymorphic loci (Selosse *et al.*, 2013). The presence of a vegetative incompatibility system associated with the mating type genes was demonstrated in *Neurospora crassa* (Shiu & Glass 1999). In this fungal species the *tol* gene, which is turned off during the sexual cycle, interact with MAT A-1 and MAT a-1 to form a heterocomplex that mediates vegetative incompatibility. However, a similar homologous gene has not been found in *T. melanosporum* (Iotti *et al.*, 2012c). In addition the other het domain genes, which in other ascomycetes trigger a programmed cell death after hyphal fusion between incompatible strains (Saupe, 2000), have not been found in *T. melanosporum* (Iotti *et al.*, 2012c). Instead hyphal anastomoses between strains seem to be prevented by other unknown pre-fusion mechanisms (Iotti *et al.*, 2012c) (Fig. 2).

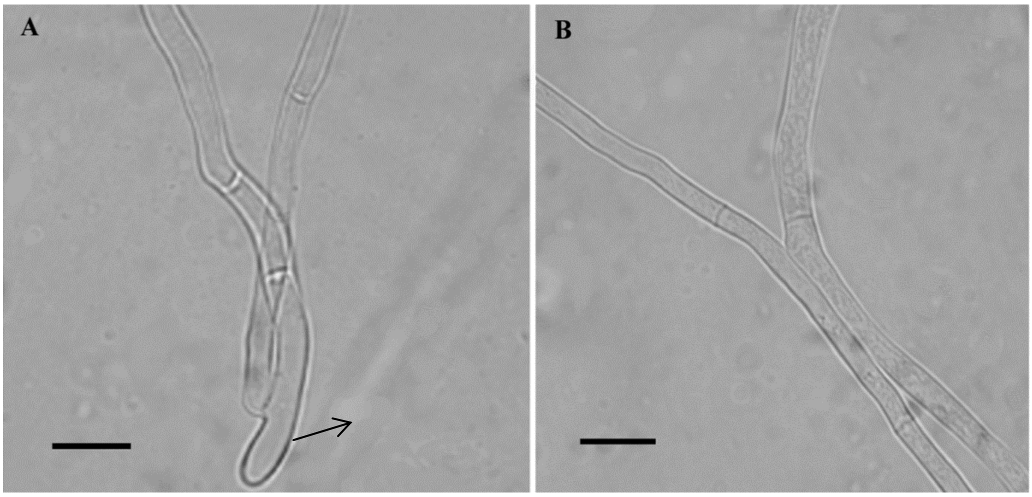

Figure 2 – Interaction between hyphae of *T. melanosporum* : A) hyphal anastomosis between hyphae of the same strain; arrow indicates the fusion point B) interaction between hyphae of different strains: no fusion occurs. Bars = 10 µm

How sexually compatible strains meet and how fertilization occurs is also still an unknown. The discovery of a mitosporic stage in several *Tuber* species suggests the possible role of the conidia in fertilization process like in numerous other ascomycetes (Healy *et al.*, 2012; Carris *et al.*, 2015). Many truffle farmers broadcast low quality truffles onto their truffières in the hope of increasing production. Where this does prove fruitful it might be through introducing new mating strains. However, this practice comes with risks. Often the farmers will spread rotting pieces of truffle which may introduce pathogens. It might also induce a huge genetic load for future generations (Selosse *et al.*, 2013). In nature the role of the farmer spreading mating strains might also be played by mycophagic animals. These are attracted by truffle aromas, eat the truffles and then spread the spores in their feces which remain viable even after passage through the gut (Piattoni *et al.*, 2012; Piattoni *et al.*, 2014) (Fig. 3).

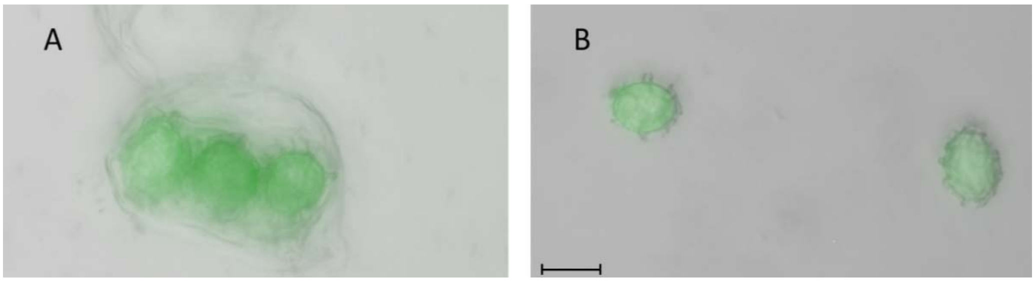

Figure 3 - Fresh spores (a) and spores digested by slugs (b) after vital stain (FDA). The digested spores are free from the asci and vital. Bar = 20 µm (Ori and Zambonelli pictures)

The future

A revolutionary approach to truffle cultivation would be the use selected mycelial inoculum adapted to specific climatic, edaphic and hosts. This technique was explored at the beginning of modern truffle cultivation by Gerard Chevalier (1973) but it has only been used for research exploring the interrelationships the fungi have with their host (Giomaro *et al.*, 2005). One of the biggest limitations to the commercial application of this technique will be the difficulty in isolating and maintaining *Tuber* mycelia in pure culture as well as producing mycelial biomass on a large scale (Iotti *et al.*, 2002; Iotti *et al.*, 2012b). Moreover long-time subculturing in axenic conditions in the absence of host roots results in the loss of isolate infectivity (Boutahir, 2013) and the development of adequate cryopreservation protocols must be used to maintain culture viability (Iotti *et al.*, 2012b). In the past another limit in using mycelial inoculants was the lack of knowledge about truffle sexuality resulting in the possibility of producing plants incapable of producing truffles. This is because pure cultures of mycelia are isolated from the gleba of fruiting bodies and hence only carry the maternal mating type. However, thanks to the characterization of the mating type genes of *T. melanosporum* and of the other edible truffles (Paolocci personal communication) we are now able to produce plants inoculated with strains of both mating types.

Inoculating plants with cultures of *Tuber* spp. adapted to a specific set of conditions would facilitate the selection of better-performing fungal genotypes using the same principles that govern the selection of plant cultivars with improved performance. The extensive *Tuber* genome sequencing program will help to determine the genes controlling the qualitative and quantitative characters of truffles but also their adaptability to different environmental conditions. Recent studies carried out by Zambonelli's research group has shown that different strains of *T. borchii* have a different resistance to high temperature (unpublished data). This aspect is particularly important when truffle cultivation is introduced in countries having different climatic conditions to Europe. It could also help to choose strains having the capacity to adapt to global climatic changes. It is also known that plant genotype can also influence truffle production and so some nurseries now use clonal plants selected for truffle production (Robin & Cammalletti, 2001).

Conclusions

Truffle cultivation is an important agricultural activity in many parts of Europe and is growing in popularity worldwide particularly in Southern Hemisphere countries that aim to produce truffles counter season to the Northern Hemisphere.

However problems still exist and need to be addressed. Basic research is clearly needed to better understand the biology of truffles and in particular to unravel the mystery around the sexual stages in the lifecycles. Studies are also needed to elucidate the mysteries surrounding the soil ecology of *T. magnatum* in order to make its cultivation feasible.

Future challenges for truffle cultivation also include: adapting modern mycelial inoculation technology to large scale production of mycorrhized plants, the creation and identification of elite fungal and plant cultivars; maintaining germplasm collections of these cultivars, and the selection of the best cultivars for different ecological conditions. The possibility to selectively introducing mycorrhizal helper bacteria and perhaps other fungi, which could directly or indirectly affect ectomycorrhizal development and fruit body formation, could be another perspective for future truffle cultivation.

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ORAL COMMUNICATIONS

Applied mycology and fungal biotechnology

Basidiomycetes for bioremediation - a perspective from Canada

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Resource extraction activities for bitumen in parts of Canada exact a heavy price on the environment, particularly soils and water. In numerous studies, bioremediation with fungi (mycoremediation) has been documented as a promising method to degrade DDT, PCBs, PAH (polycyclic aromatic hydrocarbons), pesticides and many other hydrocarbons in vitro. In particular, extracellular phenoloxidases produced by white rot fungi, mainly in the basidiomycetes, may be highly effective in the cleanup of toxic hydrocarbons. The main challenge for a widespread application of mycoremediation lies in the transformation of successful in vitro approaches into a practical in situ application. We have developed wood based inocula with various basidiomycetes that are currently being tested in artificially contaminated soils. Those inocula have the potential for a future in situ application in hydrocarbon contaminated soils.

Applied mycology and fungal biotechnology

The influence of blue and red LED light (BRLED) or Pulsed electromagnetic field (PEMF) on *Hericium erinaceus* (HE) growth

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BRLED light and PEMF favor the proliferation of nucleated cells such as mycelial hyphae or fibroblasts. We investigated the effect of Red and Blue LED Light (BRLED) or PEMF, alone or in combination on HE growth.

Material and Methods

HE was cultivated on barks in plastic bags (Mycelia®/ SacO2®, Belgium).

After 2 months growth in a ventilated sterile chamber, all bags were kept in a non-sterile dark room at 18°C as follows:

- Control : 2 bags were exposed neither to BRLED nor to PEMF
- BRLED : 2 bags were exposed to BRLED (Gled® 120w 1131red:234blue LED; China); four hours/day
- PEMF : 2 bags were exposed to PEMF (Biostim®/Electro-BioMag®; Canada); 30 Hz; modulation 4 ; four hours/day

- PEMF+ PRLED 2 bags were exposed to BRLED and to PEMF simultaneously; four hours/day

Measures of hydrogen emitted through the filter strips were made daily for 2.5 months with a device able to detect 0.1 particle per million (MX6 from Gazdetect®; France).
At the end of the experiment the weight of mushrooms (for each couple of bags) was compared.

Results
The yield of Control was 160 grams. Hydrogen = 1.08 ppm +/- 0.69.
The yield of LED was 290 grams. Hydrogen = 1.02 ppm +/- 0.82.
The yield of PEMF was 490 grams. Hydrogen = 1.41 ppm +/- 0.71.
The yield of PEMF+LED was 270 grams. Hydrogen = 0.91 ppm +/- 0.54.

Conclusions
Maximal yield is obtained with PEMF alone and is associated with an increase in hydrogen production.

Applied mycology and fungal biotechnology

The endophytic entomopathogenic fungus *Beauveria bassiana*: New fermentation and formulation strategies

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A novel approach for a biocontrol agent would be using of the entomopathogenic fungus *Beauveria bassiana* ATP-02 as an endophyte. To commercialize this bioinsecticide, the fungus has to be mass-produced and formulated in such a way that entirely colonizes plants and protects them against insect pests from within.
B. bassiana was raised in shake flasks to produce submerged conidiospores (SCS) which were reported to show a higher shelf life than mycelium and blastospores. In a mineral medium with 5% molasses B. bassiana produced $0.1\text{x}10^{10}$ SCS/g sucrose in 192 h. When 50 g/L NaCl were added 48 h after inoculation, the yield was increased to $1.4\text{x}10^{10}$ SCS/g sucrose. After scale-up to a 2 L stirred tank reactor a yield of $1.7\text{x}10^{10}$ SCS/g sucrose was obtained.
After evaluation of different formulation strategies, namely film coating, encapsulation and spraying, it was found that the most suitable option was application of spores by spray. The novel spray based on 0.1% Triton X-114, 1% molasses, 1% titanium dioxide and 10^6 spores/mL was applied on 6th secondary leaves. After 7 weeks, *B. bassiana* was re-isolated out of the 8th secondary leaves of 24% of the plants whereas hyphae growth was observed in 100% of mid rip cross-sections of these leaves by microscopy. Germination frequency and penetration were investigated with GFP-labeled *B. bassiana*.
To realize the full potential of this biocontrol agent, more systematic research into fermentation, formulations, persistence of fungus, penetration, plant tissue colonization and efficacy in bioassays is needed.

Applied mycology and fungal biotechnology

Simplex real-time PCR assays using hybridisation probes for the detection and the quantification of twelve fungal species commonly recovered from maize

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Maize is one of the most important commodities grown in southern Africa with consumptions of up to 500g per person per day. Unfortunately, maize is susceptible for many fungal pathogens which cause e.g. ear-, root-, crown- and stalk rots. Soil-borne fungi have the ability to infect maize roots and stems, induce stress which can cause severe rots, resulting in lodging of plants and significant economic losses. Traditionally, fungal pathogens causing root- and stem rot in maize were morphologically identified *via* plating-out techniques. In this study, real-time PCR (qPCR) protocols using hybridization probes were developed for the specific identification, detection and quantification of *Curvularia eragostidis* (CE), *Exserohilum pedicellatum* (EP), *Fusarium chlamydosporum* (FC), *F. equiseti* (FE), *F. graminearum* (FG), *F. oxysporum* (FO), *F. verticillioides* (FV), *Macrophomina phaseolina* (MAC), *Phoma* spp. (PHO), *Pythium* spp. (PYT), *Rhizoctonia solani* (RS) and *Trichoderma* spp. (TRI). Primers and hybridization probes were designed to target the translocation elongation factor 1α (*TEF*1) gene of FE, FG, FV, FO and TRI or the internal transcribed spacer region (ITS) of CE, EP, FC, MAC, PHO, PYT and RHI. The specificity of the qPCR protocols was confirmed by sequencing the qPCR product, as well as through the lack of amplification with non-target DNA from other fungal species. The assays were found to be sensitive in the simplex reaction and were able to quantify pg-amount of DNA. This newly developed qPCR assays offers a unique opportunity to investigate and quantify specific fungal pathogens present in the maize root- and stem rot disease complex.

Applied mycology and fungal biotechnology

Spectrum of extracellular proteases of alkaliphilic and alkalitolerant filamentous fungi

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Proteases are widely used and demanded enzymes in industry, medicine, agriculture and waste management. Fungi of extreme environments represent the promising producers

of new proteases. The significant progress in the application of proteases of extremophilic fungi is achieved, but practically nothing is known about their alkali active proteases. The proteolytic activity and spectrum of extracellular proteases of alkaliphilic and alkalitolerant fungi, isolated from soda soils, were assessed. 12 strains of 6 species, *Sodiomyces alkalinus, Chordomyces antarcticum, Acrostalagmus luteoalbus, Verticillium zaregamsianum, Sodiomyces magadii, Gibellulopsis nigrescens* were studied. They were grown in submerged conditions in alkaline medium (pH 10.2) with casein. Total proteolytic activity and spectrum of extracellular proteases were identified with azocasein and specific synthetic para-nitroanilide substrates for different classes of peptidases: GlpAALpNA, GlpFpNA, BzRpNA, GlpFApNA for endopeptidase; LpNA, L-Phenylalanine-4-nitroanilide, L-Glutamic acid 1-(4-nitroanilide) for aminopeptidase; EDTA for metalloprotease. The measurements were done at pH 9.5. 11 fungal strains demonstrated proteolytic activity. The greatest activity was indicated on the substrate with leucine residue – LpNA, it was significantly lower or absent on L-Phenylalanine-4-nitroanilide. Investigated strains did not hydrolyze L-Glutamic acid 1-(4-nitroanilide) or their activity was low. Trypsin and chymotrypsin activities of serine proteases were not detected. However, only 3 strains had low metalloprotease activity. 6 strains had active subtilisin serine proteases. Cysteine proteinases activities were discovered in 3 fungal strains of this ecological group. Perspective strains with high activities of subtilisin serine and cysteine proteases, amino peptidases, total proteolytic activity were detected.

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Applied mycology and fungal biotechnology

Biosynthesis and structural analysis of mushroom-derived immunosuppresive polysaccharides

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Polysaccharides are the best known and most potent mushroom-derived macromolecules with antitumor and immunomodulatory properties. The fruit bodies of *Lentinula edodes* (Berk.) Pegl. are source of immune system enhancer - lentinan, a cell-wall branched β-D-glucan.

The aim of our study was to isolate from the mycelium of *L. edodes*, cultured under submerged conditions a polysaccharide fraction corresponding to lentinan. The Chihara's method of isolation of lentinan was used. In the next step the structure and biological activity of the isolated mycelial fraction were compared with lentinan.

The mycelial fraction significantly differed from lentinan. It proved to be a mannoglucan of molecular weight close to 1000 kDa, containing 4% of protein. The type of glycosidic bounds identified by IR and NMR spectra was mainly α, but also β.

The mycelial polysaccharide showed in HeLa and HUVEC cells no cytotoxic activity, but a strong protective effect against oxidative stress. When assayed in concentrations 1-100 µg/ml caused significant inhibition of human T lymphocyte activation induced by mitogens, without any effect on reactive oxygen species production by granulocytes. The selective immunosuppressive activity of the mycelial polysaccharide fraction, inverse to immunostimulatory activity of lentinan, and not typical for mushroom-derived polysaccharides suggest, that the isolated mannoglucan have to be tested as potential immunosuppresive drug.

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Tests of biological activity were performed in Centre of Molecular and Macromolecular Studies of Polish Academy of Sciences and Department of Clinical Immunology, of Medical University of Warsaw.

Applied mycology and fungal biotechnology

Preservation potentials of leaf extracts of three medicinal plants on *Citrus sinensis* (sweet orange) juice

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Plants remain the most common source of antimicrobial agents and are reliably sources of preservatives in food. Based on this, the preservative potentials of *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellii* leaves were investigated. The freshly squeezed orange juice and orthodox branded fruit juices were assayed for the isolation of fungi. The plants components were extracted with ethanol. The extracts were used to assess the shelf life of the juice for 180 days using quantitative parameters such as total soluble solid, browning potential, pH and turbidity at 4°C, 25°C and at room temperature (28°C-31°C). The fungi isolated from freshly squeezed orange juice and orthodox branded juice include *Aspergillus aculeatus*, *Aspergillus flavus*, *Issatchenkia orientalis, Meyerozyma caribbica, Meyerozyma guilliermondii* and *Penicllium crustosum.* The juice assessment evidently revealed that the freshly squeezed orange juice with the extracts {*M. macrostachyum* (turbidity: 0.35±0.002 stored at 4°C), *T. danielli* (pH: 4.62±0.002, Browning potential: 1.42±0.004 stored at 4°C); and *C. indica* (total soluble solid: 10.07 stored at 4°C)} possess tolerable activity to enhance the shelf life of orange juice. The leaf extract of *M. macrostachyum* preserved the orange juice most, up-to 180 days, better than the leaf extracts of *T. danielli* and *C. indica* respectively. This study was able to validate the usage of the plants for preservation by the natives; it also ascertains the potential preservative qualities of the plants for the enhancement of shelf-life of freshly squeezed orange juice.

Environment, ecology and interactions

Heart rot of deciduous trees

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Wood decay begins while the tree is still standing. Though sapwood functional in water conduction contains many endophytes which are latent decay species they are usually unable to develop overtly as high water content is inimical to their growth. Extensive colonisation begins when the water content is reduced, providing better aeration. In the trunk this occurs in the heartwood and in the vicinity of wounds. Though the species associated with heartrot of trees is known from the presence of fruit bodies, little is known about community structure and development within the wood. Heartrot is much less common than it was one hundred or so years ago, as trees are often removed before decay can develop extensively. Some heartrot fungi are consequently rare. Also, invertebrates are often associated with specific types of heartrot, so fungal community development in heartrot impacts on invertebrates species diversity. This paper reports on our ongoing studies in beech (*Fagus sylvatica*).

Environment, ecology and interactions

A surprising finding underground.

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In the Middle age a net of channels, the so called "Bottini", were created under the historic town of Siena (Tuscany, Italy), in order to bring water into the city as a source of drinking supply. This system of underground tunnels was also used in the craftsmanship of workshops, for the agriculture and for the animals. The "Bottini" were excavated in sands and conglomerates, a geological formations of the Pliocene, that forms the substrate on which Siena was built.

The "Bottino Maestro of Fonte Gaia" is one of the main tract of the undergound channel and passes through the whole city. During recent studies, in this tract localized at about 600 meters from the main entrance at Il Campo, and at a depth of about 10 meters below a square lined with trees, in the darkness a web of roots and well formed ectomycorrhizas were lighted up and bolets hanging from the roof were found. The mushroom had all characteristics of *Xerocomus rubellus*, a widespread species in Europe - but in this case study a singular growth place.

Environment, ecology and interactions

Communities of wood-inhabiting fungi in dead pine logs along a geographical gradient in Japan

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Fungi are the main agents of coarse woody debris decomposition in forest ecosystems. We examined the effects of environmental variables on fungal community structures in dead pine logs. Using 454 pyrosequencing of fungal DNA obtained from 12 geographically distant sites, 575 operational taxonomic units (OTUs) were assembled. Among the known fungal ecological groups, white rot fungi were most abundant as sequence reads, whereas saprotrophic fungi were most frequent and were positively associated with mean annual temperature (MAT) and log diameter. The frequency of wood decay fungi with unknown decay type was positively and negatively associated with pine wilt disease and log diameter, respectively. Ordination analyses of the dataset of 42 widespread or abundant OTUs showed that latitude, MAT and annual precipitation had significant effects on the structure of fungal communities. These results suggested that climatic conditions and pest experience affect fungal communities in pine logs differently among fungal ecological groups.

Environment, ecology and interactions

What is present affects what is to come: priority effects during fungal community establishment in beech wood

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Assembly history of fungal communities plays a crucial role in the decomposition of woody resources, and hence nutrient cycling and ecosystem function. However, it has not been clearly determined whether the fungal species that arrive first may, potentially, dictate the subsequent pathway of community development, i.e. whether there is a priority effect at the species level. We used traditional culture-based techniques coupled with sequencing of amplified genetic markers to profile the communities in beech (Fagus sylvatica) disks that had been pre-colonised separately with nine species from various stages of fungal succession. Clear differences in community composition were evident following pre-colonisation by different species, with three distinct successor communities identified, indicating that individual species may have pivotal effects in community development. However, priority effects were not consistent across different experimental sites, indicating that local species pools are critical determinants of assembly history. Priority effects may depend on the available spora (air- and soil-borne) and mycelia, and may be linked to biochemical alteration of the resource and combative ability of the predecessor.

Environment, ecology and interactions

Molecular ecology of fungus-insect interactions in boreal forests

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Wooded landscapes harbour complex interacting communities of fungi and insects; disclosing ecology of these interactions webs requires massive sorting and identification effort in both explorative and experimental approaches. Traps, e-DNA and molecular identification offer methodological shortcuts and improve efficiency, but pose data handling and interpretation challenges. High-throughput field and lab methods may be harnessed to answer specific questions through customized sampling designs.

Two field experiment case studies of fungus-insect interaction in Finnish boreal forests are presented, **i)** fungus-baited flight-interception traps to study for insect attraction and colonization study using morphological and DNA methods to disclose the identity of the host fungus species and its inhabitants, and **ii)** pheromone traps for bark beetles and Illumina MiSeq high-throughput sequencing of fungal propagules from the beetle bodies vs. forest air to explore role of insect vectors in colonization of trees by wood-decaying fungi. Pleurotoid fungi and fungi associated with non-ambrosia bark beetles are comparatively less studied for their ecological, in particular, trophic links with insects. The scarce information available on Diptera and Coleoptera species colonizing pleurotoid fungi prompted the study of patterns of insect succession, species replacement and diversity. *Pleurotus abieticola* and all its insect associations are reported new to Finland.

Evolution of cheating network interactions: the breakdown of the mutualistic arbuscular mycorrhizal association

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Mutualistic interactions are ubiquitous in all ecosystems. One of the most prevalent mutualisms in nature is the arbuscular mycorrhizal (AM) mutualism between the majority of land plants and fungi of the Glomeromycota. Evolutionary stability is maintained by bidirectional control, such that partners offering the best rate of exchange are rewarded. Nevertheless, the existence of several cheater plant lineages - 'mycoheterotrophic' plants (MHPs) exploiting AM fungi for carbon - demonstrates that the AM 'fair-trade' mutualism is vulnerable to subversion. Many of these MHPs are specialized in their interactions with AM fungi. Thus, to understand the evolution and persistence of cheating in AM networks it is necessary to assess common evolutionary patterns of partner choice leading to these specialized mycoheterotrophic interactions. Nowadays next-generation sequencing techniques offer ample opportunities to unravel the hidden microbial communities. We applied these techniques to characterize the Glomeromycota communities in the roots of mycoheterotrophic plants of the families Burmanniaceae, Triuridaceae, Gentianaceae, and Thismiaceae. We analyse the patterns of specificity for each of these groups and discuss the composition of the communities in a phylogenetic and geographic context. The analysis of the mycorrhizal fungi associated with MHPs will show to which extend this interaction is special and how different it is from the regular mutualism between green plants and their AM fungi. Additionally, focusing on the AM symbiosis provides crucial insights in possible evolutionary pathways to the breakdown of mutualistic interactions creating opportunities for cheaters to persist in stable ecosystems.

Diversity of pioneer ectomycorrhizal fungi in mine spoil recultivation forests

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Open-cast mining results in thousands of hectares of post-industrial barren wastelands, e.g., spoil heaps formed of loosened and mixed overburden from mine pits. Recultivation of such places frequently includes forest restoration. Afforestation success and tree stand development depend on the presence of ectomycorrhizal (ECM) fungi that are capable of forming symbiotic associations with young trees and can survive in the spoil substrate.

Usually not many species are found in young recultivation stands on mine spoils, but these forests are rarely the object of biodiversity studies.

Long term observations of ECM fungi were carried out in monospecific recultivation stands up to 25 years old (*Pinus sylvestris, Quercus robur, Betula pendula, Alnus glutinosa*) on Mt Kamieńsk, an outer spoil heap of the Bełchatów lignite mine (Poland), one of the biggest brown coal suppliers in Europe. Sporocarps of ca. 120 ECM species were recorded. Among them *Hebeloma, Inocybe* and *Cortinarius,* the genera known for their pioneer features, were most species-rich. Species composition and annual sporocarp production were influenced by host identity, tree and stand parameters, and soil conditions. Specific soil environment - extreme pH values, low nutrient and organic matter content, presence of $CaCO_3$, harsh physical features - form a strong environmental filter affecting ECM fungal community. Besides cosmopolitan or highly tolerant species many rare taxa showing specific niche preferences were found. Mine spoil afforestations may provide valuable refuge enabling existence and survival of rare, isolated fungal populations.

Environment, ecology and interactions

Fungal communities associated with roots of Scots pine (*Pinus sylvestris* L.) seedlings from bare-root forest nurseries

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High- quality of tree seedlings produced in forest nurseries is an essential component of a successful reforestation projects. However long lasting bare-root nursery practices might adversely affect beneficial mycobiota of seedling roots and/or promote development of unfavorable groups of microorganisms. The aim of the study was to determine the diversity of different ecological fungal groups (pathogens, saprobes and ectomycorrhizal symbionts) in nurseries being used for a nursery stock production during different time frames. In this study 454 pyrosequencing was applied for the in-depth analysis of fungal communities inhabiting Scots pine seedling roots and surrounding bulk soil in bare-root forest nurseries exploited for 20, 40 and 60 years. Naturally regenerated pine seedlings originating from clear-cuts of nearby forests were used as a reference. In total 917 operational taxonomic units (OTUs) of fungi has been obtained from all roots and soil samples. The number of OTUs from nursery samples (580) was very similar to that from the clear cuts (585). Unexpectedly the number of overall fungal OTUs (293-320) appeared very comparable between nurseries of different age. Overall number of saprobic fungi (around 187 OTUs) was substantially higher than ectomycorrhizal fungi (around 94). No difference between nurseries and natural regeneration stands was found in fungal richness but communities were compositionally clearly distinct in nonmetric multidimensional ordination analyses. Overall number of pagenic fungi in nurseries and in natural regeneration stands was similar (58 vs. 52 OTUs) but higher number of OTUs was found in the oldest nursery in relation to the youngest one.

Environment, ecology and interactions

Potential distribution and identity of introduced *Amanita muscaria* worldwide

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Amanita muscaria sensu lato is an eye-catching and easily noticed fungus, the red-and-white spotted mushroom of fairy tales. However, it comprises different species according to the phylogenetic species concept. Members of this northern hemisphere species complex have been introduced to many locations in the southern hemisphere, and south of its native range in the northern hemisphere. Which species are introductions, and whether more than one species of the *A. muscaria* complex has been introduced to new ranges, is unclear. Nor do we know if multiple species co-occur in introduced ranges. In this study we hypothesized that the cryptic species of *A. muscaria* possess distinct environmental niches, and that environmental niche models of the different clades would predict which species are introduced to different parts of the world. We also used ITS sequencing of *A. muscaria* from across its introduced ranges to ascertain the identity of the occurring species. Species distribution modeling predicts that both clade I (North America) and clade II (Eurasia, Alaska) can grow in parts of the introduced ranges, in places including Chile, southern Brazil, Uruguay, New Zealand and southern Australia. But sequence data from many populations demonstrates that across its introduced ranges *A. muscaria* is mainly (perhaps solely) represented by clade II, the originally described *A. muscaria* from Eurasia. Results are discussed in relation to the current knowledge of the ecology of each cryptic species, as well as in relation to historical information about co-introductions with exotic hosts.

Environment, ecology and interactions

Long-term study of morel proliferation: influences of climate and environmental changes

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In nature, mushroom proliferation in a particular site is affected by a range of environmental factors, some of which are related to changes in climate or to local disruptive activities, such afire events. Moreover, the severity of, and area covered by be dependent on climate. Fires affect the whole ecosystem, including the fungal population and mushroom fruiting. Morels (*Morchella* spp., Pezizales) are important edible mushroos that are known for their delicate taste and aroma, and exhibit a range of mycorrhizal relationships with higher plants. It is important to understand the factors affecting morel fruiting in nature, toward potential manipulation of the morel population for forest conservation and rehabilitation. We conducted a long-term study on the proliferaon of mycorrhizal and free-living morel populations in Israel. This direct follow-up of mushroom proliferation enabled pinpointing the effects of climate change and soil disruption on both mushroom types. Climate changes were found to affect the temporal fruiting of morels, whereas both population types were affected by fire events, such that the morels could be classified as pyrophilous fungi. Forest-management activities performed after the fire event affected both morel ecotypes' populations.

Environment, ecology and interactions

Ascomycete fungal communities reorganize in response to long-term summer and winter climate warming in moist and dry tundra of Arctic Alaska

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Climate warming has already led to a suite of changes in Arctic tundra regions including thawing of the permafrost, alterations in the net exchange of green-house gazes, nutrient cycling in tundra soils and shifts in arctic plant communities. Long-term (18-year) experimental climate manipulations that simulated summer climate warming by open-top chambers and winter warming by snow fences, revealed that warmer conditions lead to significant shifts in tundra vegetation: shrubs and graminoids increased in density and cover, while shade-intolerant lichens and bryophytes declined under warmer conditions. However, changes in arctic ecosystems below-ground remained unknown. In the present work we studied the effects of long-term experimental warming on the most diverse group of arctic soil fungi-ascomycetes. We used deep Ion Torrent sequencing of the ITS2 rDNA region to compare the ascomycete communities across the control and experimentally warmed plots in two main vegetation types found throughout low tundra - dry heath and moist tussock tundra. Ascomycete taxa reorganized in accordance with previously reported shifts in vegetation in the warmed plots: we observed a decline in lichenized and bryophyte-associated ascomycetes corresponding to loss of lichens and bryophytes. Oppositely, root-associated and endophytic ascomycetes increased in accordance with the reported previously increase in shrub cover. Increase in saprotrophic and insect pathogenic ascomycetes was in agreement with litter accumulation and increased insect abundance across the warmed plots. Reorganizations in soil fungal communities may indicate a positive feedback on climate warming through enhanced soil nutrient cycling and increased rates of decomposition in tundra soils.

Environment, ecology and interactions

Possible effects for soil microfungal communities in the conditions simulating the global warming

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Global warming will affect the structure and functioning of soil fungal communities. These trends were observed in laboratory and field experiments which simulated various scenarios of climate change. The investigation was held with two zonal podzolic (Histic Podzol, Umbric Albeluvisol) and two urban soils (Urbic Technosol, Technic Albeluvisol) in laboratory and field experiments. The species and functional diversity of cultivated fungal assemblages was assessed in laboratory experiment under fixed temperature (10, 20, 30°C) and moisture conditions (60%) during fungal succession (3, 7, 14, 21, 35 days). The isolation of microfungi was performed using isolation on CzA. Fungal cultures were identified using cultural methods and ITS1-5.8S-ITS2 sequencing data. The multisubstrate testing method was used for functional assay. Shelters were employed in field experiments (45 days) for determining the reaction of cellulolytic fungi. The isolation of cellulolytic fungi was performed by cellulose-bite technique. The diversity of cellulolytic fungi increased under shelters (increased temperature on average by 2-3°C) in zonal and urban soils. In zonal northern and temperate boreal soils the high temperatures (30°C) had a negative effect on the microfungal assemblages. The microscopic fungi diversity indexes were lower and succession stages were not very explicit at 30°C in comparison to 10, 20°C. The functional diversity of fungal assemblages itself and a response of the functional diversity to elevated temperature differs in urban and zonal soils. Fungal assemblages of urban soils were more resistant to elevated soil temperatures. At elevated temperatures increases the abundance of potentially pathogenic microfungi in all investigated soils.

Environment, ecology and interactions

Melanization as a response in soil fungi to environmental stress

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Melanin is a dark pigment of high molecular weight possessing energy absorbing and dispersing properties accompanied with ability to neutralize cytotoxic free radicals. Beside the most "famous" radioprotective capacity, melanin protects fungi against temperature extremes (both heat and cold), dehydration, chemical and radionuclide pollution, and enzymatic activity of antagonistic microorganisms. Due to such defending abilities, dominance of melanin-containing fungi is characteristic for the upper layers of the Israeli desert soils, open mountain localities (Mount Hermon, both in summer and under snow cover), and highly saline environments of the Dead Sea shore. In the Negev desert, melanized fungi with large thick-walled multicellular conidia increased their abundance southward and overwhelmingly prevailed in southern Negev. They were also dominant in the depth of 30-50-cm in the fine-textured playa sediments with strongly limited aeration and increased salinity. The multicellular spore morphology, together with melanin pigmentation, was found to be an important adaptive feature of desert soil mycobiota helping to carry out both dispersal and resting functions, which are crucial in highly stressful desert habitats. For one of the melanin-containing species prevailing in the soil of the northern Israeli canyons, *Aspergillus niger*, it was shown that the south-facing slope (SFS) strains produced conidia with 1.5-fold higher survival ability after UV-A irradiation and with more than three-fold higher melanin concentration compared to conidia of the north-facing slope (NFS) strains. Such remarkable differences corresponded to the interslope microclimatic divergence caused by much higher solar radiation (200-800%) which the SFS received in comparison with the NFS.

Environment, ecology and interactions

A study on the species diversity of Orbiliaceae in an oceanic archipelago (Canary Islands)

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Within a few decades, the family Orbiliaceae Nannf. has undergone important changes. Being earlier placed in the Helotiales Nannf, the family was transferred in 2003 to a new order (Orbiliales Baral, O.E. Erikss., G. Marson & E. Weber) and a new class(Orbiliomycetes O.E. Erikss. & Baral). The major part of the species generally studied, have been reported in humid ecosystems (Liu et al. 2006, Zhang et al. 2009), but their diversity is more important in arid to semiarid ecosystems (Baral et al. ined.).
The Macaronesian Region is characterized by its high biodiversity and endemism, and the Canary Islands play a key role within these regions (Médail & Quézel 1977, 1999). While Korf (1992) listed 8 species of Orbiliaceae, in the present research the family turned out one of the most diverse families in the Canary Islands (~ 60 spp). Until now, this group have been done in a taxonomical or descriptive way, but, what do we know about them from an ecological point of view? Tenerife is placed in the middle and it is the perfect place to test out how different climatic or biotic parameters influence the diversity of fungi. During three years, the two larger genera Hyalorbilia and Orbilia were monitored in four types of vegetation from sea-level up to the mountains. Here we show the changes in diversity and abundances of species in different levels of complexity: substrate, vegetation, altitude and slope.

The effects of sodium hypochlorite on the control of inter-kingdom biofilm formation by drinking water-isolated microorganisms

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Biofilms in drinking water distribution systems (DWDS) are responsible for several undesirable effects in water. One of the main drawbacks is their potential to protect pathogens from stress conditions. Microbial interactions in biofilms can benefit the survival of co-existing microorganisms, including the increased resistance to antimicrobials. Chlorine disinfection is the main widespread strategy used in DWDS for microbial control. Even if new and alternative strategies are being developed, it is conceivable that the future strategies still persist with chlorine due to economic and safety aspects. Therefore, the understanding on the efficacy of chlorine against biofilms is of utmost importance in order to improve the current strategies. The purpose of this work was to assess the effects of sodium hypochlorite (SHC) on the control of single and dual-species biofilm formation by selected filamentous fungi (*Penicillium expansum* and *Penicillium brevicompactum*) and bacterium (*Acinetobacter calcoaceticus*) isolated from DWDS. Biofilms were developed during 48 h in 96-wells microtiter plates under two hydrodynamic conditions (25 and 150 rpm). The effects of SHC at several concentrations (0.1, 0.5, 1, 10 and 100 mg/L) was tested. The biofilm control was evaluated using crystal violet (removal) and resazurin (inactivation) dyes. The results shown that, *P.brevicompactum* biofilms were extremely resistant to disinfection when compared with single-species biofilms of *P.expansum* and dual-species biofilms of *P.brevicompactum-A.calcoaceticus*. The association of *A.calcoaceticus* with both fungi seems beneficial, since the dual-species biofilms were more resistant to disinfection. The inactivation and removal occurred for high SHC concentrations. However, total biofilm control was not achieved.

Environment, ecology and interactions

Comparison of chemical composition in *Tuber aestivum* Vittad. of different geographical origin

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Truffles are hypogeous and nutrition-rich edible fungi. The aim of this study was a comprehensive investigation of chemical composition of black summer truffle (*Tuber aestivum* Vittad.). We compared lipids, protein, saccharides, polyphenolics, flavonoids, total sterols, ergosterol, volatile flavor and aroma compounds content in fruitbodies of the fungus collected in three different geographical region, i.e. Poland, Slovakia and Italy. A comparison of the mentioned compounds is especially interesting due to environmental and climatic differences between Poland, Slovakia and Italy. It showed that fruitbodies of *T. aestivum* from Poland and Slovakia possessed similar content of proteins, total sterols, and saccharides. The fruiting bodies from Italy contained significantly larger amounts of investigated compounds. In turn, Polish specimens had higher content of lipids and polyphenolics than Slovak and Italian ones. We have found higher similarity of volatile compounds composition between Polish and Italian specimens than those of Polish and Slovak origin.

Environment, ecology and interactions

Geoecological role of acid production by micromycetes on the rock substrates

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A lot of species of fungi from different taxonomic and ecological groups can produce organic acids. Organic acid production by fungi determines the geochemical role of micromycetes, their importance in rock weathering and primary soil formation. In practical terms production of organic acids by fungi regarded as one of the most important factors in the destruction of monuments and building. The growth of rock-inhabited fungi usually occurs in biofilms, including autotrophic and heterotrophic organisms.

The ***aim*** of this study is to expand the data about role of organic acids formation by microscopic fungi in the microbial interactions in lithobiotic communities.

Micromycetes of 24 species isolated from rock substrates were cultivated on the different agar and liquid media. Carbon acids extracted from the cultural fluid were analyzed using

chromatography and mass spectrometry (GC-MS) on Agilent device with MSD5975 mass selective detector, column HP-5MS, 30m X 0.25 mm.
It is established that the acidification fungal activity promotes the easy assimilation of carbons sources by microorganisms in lithobiotic communities. Some acids such as citric, gluconic and malic can be used as a source of nutrition by heterotrophic microorganisms in rock-inhabiting biofilms. It was shown that the oxalic acid production by fungi lead to binding of toxic metals ions and decreasing free cations concentration. It can play adaptive role for biofilms in the technogenic environment. In addition fungi with strong acid production ability increase their acidification activity under influence of some chemical and physical stressors that may promote the viability of the lithobiotic communities.

Environment, ecology and interactions

Spatio-temporal monitoring fungal growth dynamics using image analysis and graph theory

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Due to their ability to grow in complex environments, fungi play an important role in most ecosystems. They are characterized by a unique structure, which allows for efficient internal transportation of nutrients and rapid expansion. Therefore, there have been many attempts to model and understand fungal growth dynamics.
Some of the main obstacles to studying fungal growth are heterogeneity of growth environments and limitations of laboratory experiments. Laboratory experiments are time-consuming and expensive, typically leading to results that depend on specific experimental conditions, so that they cannot be easily compared with other experimental setups. In addition, some in vitro methods are destructive and hence preclude tracking of features of the fungal network through time.
Given the increasing availability of image capturing techniques, a new approach lies in image analysis, i.e., the extraction of significant information from images. Capturing images is easy and does not require expensive machinery. Most previous image analysis studies involve manual labelling of the fungal network, and local or invasive techniques which do not allow for the tracking of the network evolution.
In response, this work presents an automated tool combining image analysis and graph theory to monitor fungal growth through time and space. It is highly versatile; it can be tuned for different fungal species and image resolutions, and can also be adapted for different devices and growth scenarios. It also permits the extraction of the most commonly studied parameters of fungal growth, hence representing a promising alternative to the classical and narrowly focused approaches.

Environment, ecology and interactions

Mercury accumulation and distribution in *Suillus bovinus* and probable dietary exposure by foragers

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Suillus bovinus mushroom is less valued from the culinary and commercial point of view compared to other forest mushroom. However, it is usually mixed with more valued mushroom species and then sold for use in sauce preparation.
This study evaluated the accumulation and distribution of Hg in *S. bovinus* and probable dietary Hg exposure to consumers. Samples of *Suillus bovinus* mushrooms and topsoil (0-10 cm) layer beneath the mushrooms were collected from several sites in the northern part of Poland in 2000–2013 and the mercury contents were determined by CV–AAS.
S. bovinus can be considered as a good accumulator of Hg. The bioconcentration factor (BCF) values were above 1, and highest BCF values for caps and stipes were 40 and 28 respectively. Mercury contents of topsoil samples were low, i.e. below 0.050 mgkg^{-1} dry matter. The content of Hg in fruiting bodies of *S. bovinus* could also be considered "moderately" low. The samples foraged from the region of the Puszcza Darzlubska forest complex showed more Hg in caps and stipes of fruiting bodies (average Hg in caps and stipes were 0.79±0.4 mgkg-1 dm and 0.51±0.22 mgkg^{-1} dry matter respectively) when compared to specimens from all other sites.

Considering the allowable toxicological limits of Hg exposure such as provisionally tolerable weekly intake (PTWI) and reference dose (RfD), the most contaminated mushrooms from the Puszcza Darzlubska site when consumed in amounts around 0.2 kg (dry matter) or 2.0 kg fresh product weekly, the Hg intake would not exceed the recommended limits.

Environment, ecology and interactions

Mercury in *Suillus granulatus* and *Suillus variegatus* and soil substratum beneath the fruiting bodies

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Traditional uses of mushrooms include their use in medicine and as food. In these applications, it is important to have knowledge of toxic metal contents of these species. Mercury uptake and bioconcentration by mushroom may pose health risk to consumers considering the toxicity of mercury.

This study aimed to get insight into the bioconcentration potential and degree of contamination with Hg of fruiting bodies of two species of fungi of the genus *Suillus*: *S. variegatus* and *S. granulatus* that emerged at several spatially distantly distributed places in Poland. Mercury content of the mushroom species and the soil substratum beneath them were determined by cold-vapour atomic absorption spectroscopy.
The soil substratum beneath the fruit bodies of *S. variegatus* and *S. granulatus* in this study showed low degree of contamination with Hg – the range of median values was from 0.014 to 0.026 mg/kg dry matter. The median values of bioconcentration factor calculated for caps of both species did not exceeded 14, which indicate a moderate potential to absorb and bioconcentration Hg in the fruit bodies. The median values of Hg content in caps for both species of *Suillus* mushrooms did not exceed 0.25 $mgkg^{-1}$ dry matter and the values for the stalks were, on the average, from 2 to 4 times lower. The results obtained in this study showed that the fruit bodies of both species when compared to several other species of forest mushrooms collected from the same regions are relatively low in Hg.

Environment, ecology and interactions

Fungi in bottom sediments of the Chukchi Sea: diversity and ecophysiology

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This is a first report on the mycobiota of the Chukchi Sea, one of the most inclement seas of the world. Twenty-two bottom sediment samples were taken at depths ranging from 44 to 110 meters. Fungi were isolated by Warcup's technique using MEA (salinity 35 ‰). Fungal cultures were identified using both cultural methods and ITS1-5.8S-ITS2 sequencing data. The effects of different environmental parameters (temperature, salinity and C sources) on the spore germination and the colony growth rate were examined. The fungal abundance in the analyzed samples was low: in total, as little as 150 fungal strains were isolated from all samples; the number of fungal isolates obtained from 1g individual bottom sediment samples varied from zero to 22. At the same time the fungal diversity was unexpectedly high: we identified 49 morphotypes belonging to 32 genera of Basidiomycota (4 genera, 4 species) and Ascomycota (all the others). Among almost forty non-sporulating cultures initially isolated the majority were identified as Ascomycota, and two – as Basidiomycota species based on DNA sequence data. Most of Ascomycota species in this study were referred to Eurotiales, Pleosporales, Helotiales and Hypocreales; fungi of Eurotiales family were most abundant in all samples examined (41% of all isolates). In experiments with growth and conidial germination all isolated fungi exhibited broad salinity tolerance ranging from 0 to 50‰ and the ability to grow using algal-specific polysaccharides as a sole carbon source. The major growth-limiting factors were low temperature and the lack of available organic matter.

Wood-inhabiting fungi diversity vs. deadwood features: what happens in Mediterranean forests?

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Wood-inhabiting fungi are important organisms in forests ecology, involved in wood decomposition and regulation of food resource for many other groups. Their presence is linked to nutrient turnover and to deadwood availability, then quantity and properties of substrate are fundamental for maintenance of their diversity. Many studies in Northern and Central Europe have been focused on wood-inhabiting fungi and deadwood features. Conversely, in Southern Europe, in particular in Mediterranean forests, although the strong influence of geographic area and forest type on species distribution patterns is well known, there is a lack of data. To gain a better knowledge of their biodiversity in Mediterranean area, we first characterized the species community in different vegetation types; then we analyzed the relationships between species richness and composition and deadwood characteristics (host tree, volume, diversity, decay stage, diameter class).
The results, from 3 years of multiple surveys, indicated that vegetation types have strong influence on species richness and composition and that most of species are strictly linked to a single host. Regarding relationship with wood properties, analysis revealed that: higher number of species was present on coarse woody debris and well decayed wood; fungal composition changed gradually, from smaller woody debris towards coarser ones, and from early to late decay stage, forming distinct groups; the increase of species richness was linked more strictly to variety of woody debris (presence of snags, logs, stumps) than to total deadwood volume.

Adaptation of alkalophilic fungus *Sodiomyces tronii* to the conditions with different pH*.*

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Mycelial alkalophilic fungus *Sodiomyces tronii* was recently isolated from extreme habitats - alkaline soils around the soda lake Magadi. The mechanisms of alkalophilia and adaptation to the conditions with the different pH were not studied. In this research we present the first investigation of changes in composition of membrane lipids and cytosolic soluble carbohydrates in *S. tronii* under different pH conditions.
Optimum conditions for growth of the fungus are pH 9.4 and temperature of 32 ° C. The experiment was carried out according to the following scheme, *S. tronii* was grown in surface culture on the wort agar medium at optimum temperature and different pH (10,2; 9,2; 7,2 and 5,4) during 10 days. The composition of membrane lipids and soluble cytosolic carbohydrates were analyzed in mycelium of the fungus.
It was shown, that during growth under optimal conditions the predominate sugar in cytosol was glucose (65% of ∑), under lowering of pH to 7,2 and 5,4 - trehalose (65%), under increasing of pH to 10,2 - mannitol (52%).
The membrane lipids at optimal conditions consisted mainly of phosphatidylethanolamines (PE), phosphatidylcholines (PC) and sterols. Lowering of the medium pH led to the increase of sphingolipids and sterols shares. The increase of the medium pH was attended by minor changes in lipids composition.
The obtained results point on involvement of cytosolic soluble carbohydrates and membrane lipids in adaptation of alkalophilic fungus to different pH conditions.

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Fungal pathogenesis and disease control

Detection of *Fusarium graminearum* species complex (FGSC) and *F. verticillioides* in maize roots, crowns, stems and ears in South Africa

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Maize is the most important cereal crop produced in South Africa. The *Fusarium graminearum* species complex (FGSC) and *Fusarium verticillioides* cause diseases in maize. FGSC produces mycotoxins such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA), while *F. verticillioides* produces fumonisins (FUM). In this study the occurrence of these pathogens and their respective mycotoxins in maize were evaluated. During the 2012/13 and 2013/14 seasons four fields were identified in the Northern Cape Province, which were visibly infected with *Fusarium* species. The target DNA concentrations of FGSC and *F. verticillioides* were determined in the roots, crown, stems and grain of physiologically mature maize in areas within the field that were visibly green and those that were prematurely senesced using qPCR. The mycotoxins' concentrations in grain were quantified using LC/MS-MS. The target DNA concentrations of *Fusarium* spp. differed between seasons with no significant difference between the visibly green and prematurely senesced plants. DON (1.193 µg/g) was the primary FGSC mycotoxin detected followed by ZEA (0.770 µg/g) and 3 + 15 ADON (0.116 µg/g). No NIV was detected in either season. During the second season the FUM levels were 25 µg/g. Although the prematurely senesced and visibly green plants did not differ significantly from each other, the mycotoxin concentrations was high enough to pose a threat to human and animal health. These results reiterated the importance of good agricultural practices such as crop rotation and tillage practices to reduce *Fusarium* spp. inoculum build-up in order to prevent infection and mycotoxin contamination of grain.

Fungal pathogenesis and disease control

Diversity of *Fusarium* species and mycotoxins accumulated in grain of durum wheat (*Triticum durum* Desf.)

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Fusarium head blight (FHB) is a devastating disease of cereal species, particularly being a major problem to wheat production in many countries. Usually, one or more of the

Fusarium species (*F. graminearum, F. culmorum, F. avenaceum, F. poae and F. sporotrichioides*) are involved as causal agents. These fungi produce various mycotoxins, mainly deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZON) and moniliformin (MON). Plant material used in the study consisted of three durum wheat cultivars: Polish (Komnata), Slovakian (Pentadur) and Austrian (Auradur). Two sowing terms (optimal and delayed) and three different sowing densities (400, 500 and 600 grains/m^2) were used. Fungal strains were isolated and molecularly identified in harvested heads of plants exposed to natural infection by *Fusarium* fungi. Eighty six strains of six *Fusarium* species were isolated during 2014 season and *F. graminearum* and *F. avenaceum* were the most frequent ones. Moreover, DON, NIV, ZON and MON concentrations were measured in the grains of collected wheat using HPLC method. Deoxynivalenol was found with the highest concentration of 3988,57 ng/g in grain samples of cv. Komnata (sowing term: delayed, sowing densities: 600 grains/m^2). Nivalenol was detected in samples of cv. Komnata only. This finding corresponded with the presence of *F. graminearum* of NIV chemotype, identified in grain samples of cv. Komnata. The cultivar has also accumulated the highest amounts of all mycotoxins studied and the delayed sowing date resulted in higher FHB incidence and mycotoxin accumulation.

Fungal pathogenesis and disease control

A fungal genome at work: Genomic and transcriptomic characterisation of *Hemileia vastatrix*, the Coffee Leaf Rust pathogen

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Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens, using elaborated host-pathogen dialogue mechanisms and specialised feeding structures, with complex life cycles, most frequently highly host-specific and with large genomes. *Hemileia vastatrix*, the causal agent of devastating disease Coffee Leaf Rust, represents one of largest fungal genomes (790 Mbp) and one of the most ancestral lineages of Pucciniales. It multiplies asexually, as no aecial host is known (hemicyclic life cycle), but since the first report of the disease (150 years ago), at least 50 races have been identified, following the gene-for-gene host-pathogen interaction theory, suggesting a rapid genotypic adaptability and high plasticity. Intra-specific genomic variability represents ca. 13% of its genome size (764-839 Mbp). Recent advances in transcriptome and genome sequencing have contributed to revealing particular aspects of this fungus. In particular, active metabolism, translational activity and production of new structures in the *H. vastatrix* appressoria and intense signalling, transport, secretory activity and cellular multiplication in the germinating urediniospores, suggesting the onset of a plant-fungus dialogue as early as at the germ tube stage. Gene expression related to the production of carbohydrate-active enzymes and accumulation of glycerol in germinating urediniospores and appressoria suggests that combined lytic and physical mechanisms are involved in appressoria-mediated penetration. Besides contributing to the characterisation of molecular processes leading to appressoria-mediated infection by rust fungi, these results point towards the identification of new *H. vastatrix* candidate virulence factors, with 516 genes predicted to encode secreted proteins.

Fungal pathogenesis and disease control

Diversity of *Rhizoctonia* spp. in Polish forest nurseries

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Rhizoctonia spp. is a severe pathogen of Scots pine (*Pinus sylvestris*) in Polish forest nurseries, causing seedling damping-off. The diversity of *Rhizoctonia* population is little known. From this point of view, seven forest nurseries were examined from 2004 to 2009. Fungi were isolated from diseased seedlings and from soil. 277 *Rhizoctonia* isolates were obtained – multinuclear (prevailing) and binuclear. The multinuclear represented anastomosis groups AG1-IB, AG1-IC, AG2-1, AG2-2, AG2-3, AG4-HGII, AG-5 and WAG-Z (*R. zeae*) and binuclear – AG-E (*R. muneratii*). The anastomosis groups differed significantly from the point of view of their growth rate and pathogenicity to pine seedlings. The biggest growth rate was found for AG1-IB and the smallest for AG2-1and AG4-HG2. All the isolates were strongly pathogenic to Scots pine seedlings: multinuclear caused 92,81-100% mortality and binuclear 65,77%. The most pathogenic isolates belonged to AG2-1 and AG-5. *Rhizoctonia zeae* was obtained for the first time in Poland from a forest nursery. It proved capable of causing damping-off of Scots pine seedlings.

Environment, ecology and interactions

Fungal diseases of goutweed (*Aegopodium podagraria* L.)

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Aegopodium podagraria L. is a perennial plant in the family Apiaceae, native to Eurasia, witch usually grows in shady places, but also can maintain rather high level of insolation. In Russian Federation the species is not only one of the most common forest plants but a great weed for gardens and kitchengardens too. Four fungal disease caused by *Puccinia* *aegopodi*i, *Plasmopara nivea*, *Protomyces macrosporus* and *Septoria podagrariae* are peculiar to the goutweed. We studied the occurrence of these pathogens in Zvenigorod Biological Station (Moscow region) and the influence of diseases on the host plant. Because the goutweed is a perennial rhizomatous plant we used leaves as a count unit. *Puccinia aegopodii* has a very strong locality with solitary infected plants and this fungus may considered as a rare one. *Plasmopara nivea* is widespread, but has great seasonal dynamics with one or two peaks per year. The first flash of disease is in June (with 30% infected leaves) and the second could occur in September.

From 30 to 80% of leaves of goutweed are usually infected by *Septoria podagraria* and *Protomyces macrosporus*. The correlation between two pathogens varied from 20 to 30 % depending on the season. Infected leaves are bigger then uninfected, so that could mean that pathogens more often infect older parts of plants.

Neither of pathogens has real harm to the host plant.

Fungal pathogenesis and disease control

Distribution of Botryosphaeriaceae species on Eucalypt stands in Portugal

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Eucalypts are among the most important planted forest species in Portugal and are affected by various pests and diseases. Among these, some fungi in the Botryosphaeriaceae are generally considered pathogens that can cause tree mortality. In a recent nationwide monitoring program of eucalypt diseases, a total of 42 forest stands were surveyed across the entire country. In this study we investigated the identity and distribution of the Botryosphaeriaceae associated with eucalypt forests in Portugal. Sampled sites were digitally geo-referenced and mapped. Botryosphaeriaceae were detected in 20 stands and a collection of isolates has been established. Representative isolates were identified from their morphological characters in culture and in addition molecular methods were used based on the analysis of the ITS rDNA and partial sequence of the translational elongation factor-1α gene.The two most frequently encountered species were identified as *Neofusicoccum eucalyptorum* and *N. parvum*.

Evolution, biodiversity and systematic

Species identification in corticioid fungi based on a barcoding approach

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DNA barcoding is a standardized technique for assigning biological specimens to their correct species using a short gene sequence (Hebert et al. 2003). Using in conjunction with other characters (morphology, or ecological data), DNA barcoding is allowing to the taxonomists to make species identification more precise (Rubinoff 2005, Hajibabaei et al. 2007).

The present contribution points out how the accepted barcode for fungi (sequences of ITS nrDNA; Schoch et al 2012) is being very useful to assess the circumscription of corticioid fungi. For example, in *Brevicellicium*, *Hypochnicium*, *Hyphoderma*, *Jaapia*, *Sistotremastrum*, a "DNA barcoding gap" is present, allowing to assign species names to specimens, since the intra-specific and the inter-specific distances do not overlap. The reexamination of the morphological features of the specimens proves that, in many cases, the phenotipical variation coincides with the variation of the barcoding sequence (eg. *Jaapia argillacea*/*J. ochroleuca*), but in others, the sequences are key to detect cryptic species, such as in *Hyphoderma macaronesicum* sl.

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Evolution, biodiversity and systematic

Host and geographic speciation in the *Phanerochaete sordida* group (Polyporales, Basidiomycota)

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Specimens of *Phanerochaete sordida* from different regions of temperate Eurasia were studied by morphological and DNA methods, and type material (*Corticium sordidum, Corticium cremeum, Corticium eichlerianum, Grandiniella livescens*) was checked for reestablishing species concepts in this group. *P. sordida* s.str. is widely distributed in boreal and hemiboreal zones of Eurasia, and it is predominantly a gymnosperm-dwelling species. In phylogenetic analysis, *P. sordida* is rather distant from two other species possessing similar morphological characters; those species inhabit angiosperm hosts. One of them, *P. livescens*, is widely distributed in temperate forests of Eurasia, and its distribution ranges from France to Russian Far East. Another species, *P. concrescens*, is limited to temperate East Asia, and inhabits dry, hanging branches of various deciduous trees and shrubs in open, dry habitats. The recently described *P. coniferícola* (Floudas & Hibbett 2015) is also covered by traditional concept of *P. sordida. P. conifericola* is a circumpolar species, common in boreal conifer forests; in addition to gymnosperm hosts mentioned in the original description, it regularly occurs on fallen branches and logs of frondose trees in Fennoscandia and adjacent part of Russia.

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Evolution, biodiversity and systematic

Unravelling the milkcap genus *Lactifluus* (Russulaceae)

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The ectomycorrhizal Russulaceae family consists of four non-corticioid genera, amongst which the milkcap genera *Lactarius* and *Lactifluus*. *Lactifluus* has some well-known European representatives, e.g. *Lf. volemus* and *Lf. piperatus*, but is mainly represented in the tropics. *Lactifluus* is characterized by a high genetic variability and a conserved morphology, which is supported by the occurrence of cryptic species complexes and species with isolated phylogenetic positions. The general relationships within *Lactifluus* are unresolved and synapomorphic characters on higher taxonomic levels are unknown. In a global study of *Lactifluus*, we aimed to get a clear view on the phylogenetic status of the genus, unravel the relationships within the genus and reconstruct its biogeographical history.

Collections covering the entire distribution area and all sections within *Lactifluus* (80% of all known species) were used to construct a four-gene molecular phylogeny. Macro- and microscopical characters were studied for each collection, featuring five key characteristics, and compared with the phylogenetic results. Geographical data, combined with the phylogeny, were used to infer the biogeographic history of *Lactifluus*. Our results confirm the monophyly of *Lactifluus* and support the division of the genus into four subgenera. Within these subgenera, sections were assigned and confirmed by morphological characters if possible. Ten new clades and at least 17 new species were discovered. Our morphological study confirms the importance of the five featured characteristics on different evolutionary levels, however, more characteristics need to be studied to morphologically support each clade. Biogeographically, *Lactifluus* shows a pattern of multiple independent colonisations of the different continents.

Contribution of ITS sequences to the taxonomy of *Lycoperdon*

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Members of the genus *Lycoperdon* (Lycoperdaceae) are mainly distinguished by their pyriform and closed basidiomes, with an opening in the apex through which spores are discharged and a cellular subgleba is normally present. Demoulin (1971-1972) included a list of more than 550 names validly published under *Lycoperdon*; although many taxa have been transferred to other genera. Based on ITS-LSU nrDNA sequences, Larsson & Jeppson (2008) considered that in Europe the genus comprises around 30 species.
In this work, we want to examine whether ITS provides a good proxy for expert morphological identification in *Lycoperdon*. We have obtained ITS sequences from around 160 specimens located at MCF, BPI and MA-Fungi herbaria, mainly from Northern Hemisphere. New sequences were aligned and compared with 83 sequences included in Larsson & Jeppson (2008). The preliminary analyses, of the ITS sequences, revealed in the *Lycoperdon* cluster around 40 molecular taxonomic units (MOTUs), whose relationship to morphologically defined species will be discussed.

The research received support from the projects Flora Micologica Iberica (PB98-0538-C04-01, FMI-REN2002-04068-CO2-01), Programa Ciencia sem Fronteiras (MEC/MCTI/CNPq/FAPs nº71/2013) and Synthesys (ES-TAF-788. ES-TAF-4935).

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Key words: Lycoperdaceae, taxonomy, molecular identification, ITS nrDNA

Evolution, biodiversity and systematic

Discovery of a cryptic taxon among sporocarp collections of the edible *Amanita ponderosa* (Basidiomycota, Agaricales)

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Amanita ponderosa Mal. & Heim is endemic to the Western Mediterranean basin and produces fruitbodies between Winter and Spring. Long-standing gastronomic tradition around this edible species is notable in parts of Spain and Portugal, but in-depth population studies regarding the genetic variability, ecology and conservation of this species still need to be done. We report the discovery of a separate taxon, among collections that would be normally identifed as *Amanita ponderosa*, that is closely related to *Amanita curtipes* Gilbert, as revealed by the analysis of nuclear rDNA internal transcribed spacer (ITS) and large subunit (LSU) sequences. The taxonomic discrimination from previously described species is discussed, based on basidiospore measurements, ecological data and phylogenetic distances, suggesting that this is a cryptic species, provisionally named *Amanita* aff. *curtipes*. Given that it might be common among collected and even marketed *Amanita ponderosa*, genetic markers for this taxon were designed, based on the analysis of the sequence alignments for three genetic regions, and their usefulness for its detection was demonstrated in further DNA samples. The present study also discusses the phylogenetic reconstructions for series *Amidella* (Gilbert) Neville & Poumarat, from the point of view of identifying monophyletic subgroups and revising the names assigned to the sequences.

Evolution, biodiversity and systematic

Reticulate evolution of non-homogenised rDNA arrays prevents the application of ITS and D1/D2 sequences as barcode markers in *Metschnikowia* species

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Ribosomal DNA arranged in arrays of tandem repeats is believed to evolve via concerted evolution, which promotes sequence homogenisation along the array by either removing or amplifying novel variants. Sequences of the repeats are commonly used for determining species identity and inferring phylogenetic relationships. The ITS region has recently been proposed as the primary DNA barcode marker for Fungi. In yeasts, the

D1/D2 segment is preferred. In a recent study we noticed that in two *Metschnikowia* species the D1/D2 segments were not homogenised. Both species had heterogeneous arrays consisting of repeats differing in their D1/D2 domains (PLoS ONE 8:e67384, 2013). Here we report on the extension of the analysis to all pulcherrimin-producing *Metschnikowia* species. We show that none of them have homogenised LSU and ITS regions. In Bayesian and network analyses, the LSU and ITS sequences of the type strains of these species did not form distinct clades but a continuous joint pool. Thus, neither D1/D2 nor ITS can be used as barcode markers in this group of species. The neighbour-net analysis of the sequences then revealed that their rDNA arrays do not evolve in a vertical, tree-like way but by reticulation, probably involving interspecies exchange of repeats as well. These results prompted us to test the type strains of the species for capability of interspecies hybridization. By using complementary auxotrophic mutants, we found that prototrophic interspecies hybrids can be produced with very low efficiency. Thus, interspecies gene flow can also contribute to the reticulate evolution of their rDNA arrays.

Evolution, biodiversity and systematic

Taxonomic and molecular studies of the European species of the genus *Melanoleuca* (Basidiomycota, Tricholomataceae) - a preliminary report

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The genus *Melanoleuca* contains agaricoid fungi having collybioid or tricholomatoid basidiocarps, basidiospores with mostly amyloid ornamentation, often typically shaped cheilocystidia, and lacking clamp connections. However, macro-, micromorphological and also ecological characters overlap in many species, and this makes this genus taxonomically very complicated. The species identification based only on macro- and microscopic characters is usually almost impossible. The taxonomical revision of European *Melanoleuca* taxa has been conducted in collaboration with the Italian mycologists for several years. Type specimens of taxa were revised, and more than 400 specimens, including also type material were sequenced (ITS region of ribosomal RNA gene in most cases). The genus *Melanoleuca* is divided into two subgenera - *Macrocystis* and *Urticocystis* (syn. *Acystis*). In *Macrocystis*, seven clades, arbitrary marked A-F have been recognized till now. So far, only two of them have fixed names - *M. pallidicutis* (A) and *M. strictipes* (D). In total, 19 distinct clades were distinguished in *Urticocystis*, most of them have fixed names. However, one group probably represents an undescribed species growing on montane *pastures and meadows. Detailed studies of two groups, M. brevipes / grammopodia / humilis / malenconii /*, and *M. bresadoliana / subexcentrica / tristis*, were already finished. Two new taxa, *M. juliannae* growing in Hungary and *M. juliannae* var. *decolorans* from the Czech Republic and Italy were published.

Fungal pathogenesis and disease control

A new species, *Ganoderma damiettense* nom. prov., pathogenic to lemon trees in Egypt

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Fungi belonging to the genus *Ganoderma* are important pathogens of diverse tree crops in the tropics causing root and butt rot. *Ganoderma* basidiocarps were observed causing disease on lemon trees during field surveys in the Nile Delta, Egypt. Isolation into pure cultures, DNA barcoding (rRNA operon) and scanning electron microscopy (SEM) was conducted from these basidiocarps. Genetic analysis revealed the presence of two *Ganoderma* species in lemon orchards, one, *G. resinaceum*, is a well-known and widely distributed species forming perennial basidiocarps and found mainly on *Casuarina* shade trees. However, the other species forming annual basidiocarps in the early autumn period only, had a novel ITS sequence, and was found mainly on lemon. Axenic cultures were studied and found to exhibit optimal and maximal growth temperature of 33°C and 39°C, ca. 3°C higher than *G. resinaceum*. SEM showed warty projections on the surface of the inner spore wall, which is a feature not previously reported.
Phylogenetic analysis revealed that this species forms a distinct clade alongside other unnamed *Ganoderma* isolates from Africa. We propose to name this new species *Ganoderma damiettense*.

Environment, ecology and interactions

Exploring Antarctic yellow-green *Rhizocarpon* under Global Environmental Change

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Rhizocarpon geographicum agg. is widely used as an indicator of glacial retreat and, more recently, considered as an indicator of climate change. The taxonomy of members of the genus is poorly understood, not least that very few specimens from Antarctica exist in collections. Intensive, replicated sampling was carried out across altitudinal and nutrient gradients on Signy Island, South Orkney Islands at the tip of the Antarctic Peninsula over the period 12 November to 13 December 2009. Samples ranged considerably in morphology and colour. The collections will enhance knowledge of the distribution of certain taxa. The potential further exists to test the hypothesis that phenotypically distinct units represent evolutionary distinct lineages.

Evolution, biodiversity and systematic

The evolution of smut fungi – an update

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Smut fungi are characterized by host specificity and intimate interactions. Various hypotheses of coevolution and reciprocal adaptation are trying to explain the mechanisms behind. We studied several groups of smut fungi in their respect to host relationship, interaction and diversity revealing new aspects of phylogeny, ecology and taxonomy. Examples from different genera will be used to exemplify the recent advances and to discuss lasting problems and open questions. The talk will follow an integrative approach to combine morphology, molecular phylogenetic data and genomics.
Thus, new data concerning the basis of host specificity will be presented, especially in the genera *Microbotryum* and *Urocystis.* Taxonomic changes accounting for the new code of nomenclature will be discussed.

Evolution, biodiversity and systematic

A survey of filamentous fungi at the extremely alkaline Magadi Lake

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Lake Magadi in Kenya is one of the most alkaline natural habitats on Earth, a place where pH values can exceed 11-12. It has been shown to harbour abundant prokaryotic organisms that form complex communities along with algae and few other eukaryotes. The fungal diversity in such extreme environments remains understudied.
In the current study, we isolated and characterized 22 filamentous fungal species from Magadi Lake edge using systematic approach, which involved selective recovery, morphological analyses, growth experiments estimating pH and temperature preferences, and phylogenetic multi-gene analyses. We demonstrated both alkaliphiles and weak alkalitolerant (presumably, transient) fungi are present in soda soils at Magadi Lake. Our growth experiments suggest that obligate alkaliphiles also exhibit thermotolerant and thermophilic traits. All alkalitolerant and alkaliphilic isolates referred to the families of *Ascomycota - Onygenaceae, Trihocomaceae*, *Pleosporaceae*, yet predominantly to the *Plectosphaerellaceae. Sodiomyces tronii* and *S. magadii* were described as new species within the formerly monotypic obligate alkaliphilic genus *Sodiomyces* (*Plectosphaerellaceae*). These species develop traits that are characteristic for the

majority of fungi capable of growing at high ambient pH - light-coloured mycelium with simply-structured asexual morphology producing slime-covered conidial heads and enclosed fruit bodies containing slime matrix. How do they adapt to extreme environment? Why the *Plectosphaerellaceae* family is enriched with strong alkalitolerants and alkaliphiles? Physiology and genomic studies could provide an insight on the evolution of the alkaliphilic trait in filamentous fungi.

The work was supported by RFBR grants № 15-04-06975, № 15-04-06260, RSCF grant № 14-50-00029 (Bilanenko).

Evolution, biodiversity and systematic

The Lichenized Fungus Genus *Xanthocarpia* (*Teloschistales*, *Ascomycota*) in Turkey

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In this study, hundreds of samples belonging to this genus collected from Turkey. After morphological examinations; molecular analyses of ITS nrDNA were carried in the samples. Fourteen species belonging to this genus were identified in Turkey. They are *X. aquensis*, *X. borysthenica*, *X. crenulatella*, *X. diffusa*, *X. erichansenii*, *X. feracissima*, *X. ferrarii*, *X. interfulgens*, *X. lactea*, *X. marmorata*, *X. ochracea* and *X. tominii*. In this presentation we will discuss the morphological and ecological characters of these species along with distributional data of the species in Turkey.

Evolution, biodiversity and systematic

Growth on the graveyard of chemical warfare - the plasticity of aromatic polyketide biosynthesis in higher fungi.

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We analysed the evolutionary sources of genetic diversity in aromatic polyketide biosynthesis in over a hundred model fungal genomes. By reconciling the history of 413 non-reducing polyketide synthases with corresponding species history, we have found evidence for ancient origin of extant fungal non-reducing polyketide synthases (NR-PKSs). The present-day NR-PKSs are clades of distant siblings, originating from a burst of duplications in early *Pezizomycotina*, thinned by extensive losses and, occassionaly, shaped by more recent duplication rounds and Horizontal Gene Transfer.

Based on reconciliation of gene and species history, the analysis of genomic context, we show that ancient origins of divergent cyclisations have been shaped by strong genetic linkage with key accessory enzymes, as well as cotemporaneous changes in gene structure (modularisation of key protein features, as contrasted to splice junction location). In particular, we show how molecular novelties tied to melanin biosynthesis (such as the modular design of product template domain as well as the involvement of key reductase, laccase and methyltransferase accessory enzymes) have been further adapted towards biosynthesis of alternative naphtopyrone and naphtoquinone-derived compounds including both toxins (fusarubins and aflatoxins) and alternative pigments (such as the *Trichoderma* conidial pigment, aurofusarin and bikaverin).

The research was funded under the Polish National Science Centre research grant "Hybrid, metagenome-based approach to assessing biodiversity and toxigenic potential of fungi in anthropogenic environments" (SONATA/UMO-2011/03/D/NZ2/01435).

Field mycology and conservation

Diversity and abundance of *Mycosphaerella* and its anamorphs in virgin forests of the Ukrainian Carpathians: changes over centenary and conservation effects

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Studying of plant pathogenic fungi distribution and diversity in virgin ecosystems serves to reveal the dynamic tendencies in wild nature and indicators for coenotically stable communities. Modern research of *Mycosphaerella* and its anamorphs has been undertaken as a part of national projects on fungi in preserved forests of the Ukrainian Carpathians, assessing ecosystems stability and threats in 1992-1994, 1997-2000 and 2010-2014. The comparison was done with the data of Namyslowski, Wróblewski and Petrak, pioneers of such investigations in early XXth century, and specimens of dry reference collections (KW, LW, CHU). The obtained results on abundance and diversity of these fungi in oak, beech and sycamore virgin forests demonstrated changes over centenary: microclimate fluctuations and human impact affected the species composition of conidial representatives of *Mycosphaerellaceae*, some abundant species of *Septoria* and *Cercospora-Passalora* complexes were not observed during modern study; representatives of *Ramularia-Pseudocercosporella* complex were much diverse. Decline in the total population of these fungi in mature ecosystems has been mentioned, though virgin forests are the localities for rare or the first time registered species in Ukraine. Observations demonstrate that large territories are not always reliable to conserve biodiversity of these fungi and they have not higher diversity of *Mycosphaerellaceae*. Variety of habitats is more important for these fungi development, along with the presence of virgin ecosystems. Conservation effects of recently organized small national parks in the Ukrainian Carpathians have supported the idea of importance of small conservation areas which provide unique microclimatic conditions and maintain diversity of studied fungi.

Field mycology and conservation

Current knowledge of Umbrian macrofungi (central Italy)

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The Umbria region (area: 8456 km^2, 50-2436 m a.s.l.), thanks to its geographical position and peculiar geo-lithological and climatic conditions, is characterized by a great richness and diversity of habitats and vegetation. A long history have the researches done for

understanding the ecology of hypogeous fungi and this region is famous for the cultivation of truffles. On the other hand, in respect to other Italian regions few was known concerning the presence of epigeous macrofungi. The work presented here is mainly based on observations made during 2010-2014 in five Natura 2000 Sites of Community Importance (SCI) with different vegetation types of the Umbria region. Additional records were obtained from literature of the last thirty years. This up-to-date checklist of the known Umbrian macrofungi reports 815 species (87 Ascomycota, and 728 Basidiomycota) belonging to 248 genera and 91 families. Comparing with the Italian checklist of Onofri et al. (2005), about two-thirds are new records for the Umbria region. Even if not complete, an exhaustive overview of the current understanding of the mycobiota of Umbria is presented. Although a sizeable large amount of the regional territory has yet to be explored for mycological diversity, this study offer an important support in compiling Red Lists of endangered macrofungi, as well as to identify indicator species of particular habitats to be considered for wildlife reserves, as is done in many European countries.

Field mycology and conservation

Assessment of marine fungal community associated with *Spartina maritima* (Curtis) Fernald standing plants by morphological and molecular methods

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Spartina species represent one of the main dominant halophytes and primary producers in temperate salt marshes. The release of nutrients from these plants to surrounding environment strictly depends on the degradative activity of saprobic ascomycetous fungi. Nevertheless, there is still a considerable gap in the knowledge regarding diversity and ecological requirements of the fungal species associated with these plants. In Portugal, only three studies have inventoried the filamentous marine fungi colonizing standing plants and baits of *Spartina maritima*. Thus, the present study mainly intended to complement previous studies by combining morphological and molecular methods, reporting simultaneous the vegetative structures of *Spartina maritima* where each fungus occurred more frequently. The study was conducted in two salt marshes with different environmental characteristics, where were collected 195 standing live plants (per study site) over a 2-year period (October 2010 to August 2012). Thirty-four and 26 fungal taxa were recorded in *Spartina maritima* samples from Castro Marim and Ria de Aveiro salt marshes respectively; 11 (32%) and 6 (23%) fungal taxa were exclusively identified by traditional microscopy-based methods, 14 (41%) and 12 (46%) by analysis of full-length ITS sequences and 9 (26%) and 8 (31%) by the two methods. Both fungal communities were predominantly represented by the Ascomycota, particularly Dothideomycetes and Sordariomycetes. The application of the both methods in this study was demonstrated to provide a more realistic and accurate representation of fungal biodiversity and ecological role of each fungus in the salt marshes, given the unavoidable drawbacks inherent to each method.

Field mycology and conservation

Renewal of Burgundy truffle (*Tuber aestivum* Vittad.) in Poland

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Truffles (*Tuber* spp.) are hypogeous fungi growing in symbiosis with a broad diversity of tree hosts in a variety of habitats. Besides important role in forest ecosystems, truffles are prized due to their aroma and taste and appreciated by gourmets throughout the world. First records regarding truffles’ usage can be found in first Polish Cookbook by Czerniecki (1682). Through the ages, truffles’ fruitbodies have been harvested in certain regions of southern and southeastern Poland and sold in city markets. Until Second World War truffles were quite popular, yet in the time of communism they were completely forgotten as luxury products associated with the aristocracy and therefore unwelcome.

In the last decade research on truffles were retaken and many forgotten historical data from 18th and 19th centuries regarding truffles’ occurrence in Poland have been rediscovered. First fruitbodies of *Tuber aestivum* Vittad. in natural stands were found in 2007 and were used as inoculum of *Quercus robur* and *Corylus avellana* seedlings in first truffle orchard.

The aim of our study was to recognize specific environmental factors driving development of truffle fruitbodies in our country. Our inventory has been conducted in deciduous forests on rendzina soils. So far, our research revealed occurrence of eight *Tuber* species, including *T. aestivum* and *T. macrosporum*.

Currently there are three pioneering plantations established by the Forest Research Institute within the last eight years. Due to our pioneer research we observe increasing interest in truffle orchards and the ultimate fungi as a new source of benefits in agroforestry.

"Fungi in Folk Culture" in Western Black Sea Region of Turkey

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In this study; we deal with fungi species which have an importance in the folk culture in the Western Black Sea Region of Turkey. For this study; we interviewed with nearly 200 people and we asked how they use fungi in their daily lives and how they name those species. According to these interviews; the most favorite species in the region are as follows: *Lactarius deliciosus* (L.) Gray, *Morchella* spp., *Lactarius piperatus* (L.) Pers., *Lactarius volemus* (Fr.) Fr., *Cantharellus cibarius* Fr., *Flammulina velutipes* (Curtis) Singer, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Agaricus campestris* L., *Coprinus comatus* (O.F. Müll.) Pers., *Lepista nuda* (Bull.) Cooke, *Lactarius blennius* (Fr.) Fr., *Boletus edulis*, *Amanita caesarea* (Scop.) Pers., *Hydnum repandum* L., *Clavaria flava* Schaeff., *Clitobilis prunulus* (Scop. ex. Fr.) P.Kumm and *Geastrum* spp.

Genetic analysis of mating type loci in some *Pleurotus* species

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Sexual development in fungi is controlled by genetic loci called *mat* loci or mating type. The most advanced system of genetic control of sexual compatibility is known to have tetrapolar basidiomycete fungi. Tetrapolar heterothallism is based on two unlinked mating loci *matA* and *matB* with multiple alleles each. The edible species of the genus *Pleurotus* are ones of the most cultivated species worldwide. Genetic analysis of sexual compatibility was performed on monokaryotic tester strains that revealed identical alleles at *matB* locus for cultivated commercial strains Sommer and L/4, while the other strains of *P.ostreatus, P.pulmonarius* and *P.citrinopileatus* analysed demonstrated different alleles at both mat loci. In this study, we analyzed in detail the structure of *matA* locus of *P.ostreatus*. It encodes homeodomain transcription factors (HD) that regulate expression of many genes involved in sexual development. The active transcription factor is a heterodimer that consists of two interacted homeodomain proteins (HD1 and HD2) which transcribed from two different *matA* alleles originated from opposite mating partners. We searched for conservative regions in *HD* genes of *Pleurotus*. As a result, some important differences between HD1 and HD2 protein sequences were found. Based on these differences we have predicted *in silico* secondary protein structure and tertiary structure for HD1 and HD2 protein families in *P. ostreatus*. Dimerization sites and DNA-binding domains of HD proteins were found. The structure of DNA-binding domain of HD1 and HD2 proteins was proposed. The DNA-binding model for the heterodimer protein molecule was predicted by analysis *in silico*.

Variations of the nuclear content along the urediniosporic cycle of *Hemileia vastatrix* and other rust fungi suggests unknown biological strategies in rust fungi

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Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens with complex life cycles (up to five spore types). The urediniosporic infection cycle is frequently the most important in disease dissemination as the only stage capable of repeating itself. The cell nuclear content of rust fungi is thought to follow that of other Basidiomycota, with haploid nuclei throughout the life cycle, only becoming diploid upon karyogamy in telia and immediately returning to the haploid state as meiosis takes place leading to the formation of basidiospores. Recently, using genome size quantification techniques, the presence of 1C, 2C and a low proportion of 4C nuclei was detected in different stages of the urediniosporic cycle of the coffee leaf rust pathogen, *Hemileia vastatrix* (a hemicyclic fungus), of the faba bean rust pathogen, *Uromyces fabae* (autoaecious macrocyclic), and in fact of several other rust fungi. These results suggest the presence of diploid nuclei that supposedly only occur in rust fungi telia. Furthermore, the 1C nuclei were not detectable in resting and in germinating urediniospores and reappeared once appressoria were formed. Carvalho et al. (2011) speculated, also based on cytological observations performed by Rajendren et al. (1967), that a criptossexuality phenomenon was occurring in *H. vastatrix* urediniospores. Although we cannot discard this last hypothesis, cytological and transcritomic observations suggest parallel hypotheses, such as endopolyplody or other parasexuality phenomena. This unexpected phenomenon seems to be transversal to the Pucciniales. The different hypotheses explaining it will be discussed together with its putative biological roles.

Genomics, genetics and molecular biology

Next-generation RAD sequencing as a tool for evaluation the phylogeographic patterns of *Amanita ponderosa* Malençon & R. Heim in Iberian Peninsula

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Ectomycorrhizal fungi are one of the most important constituents of forest ecosystems. Besides this little is known about the structure and organization of these organisms in this complex system. Here we investigate the phylogeographic patterns of one of the most charismatic wild and edible mushroom species. *Amanita ponderosa* occurs in southwest of Iberian Peninsula, some regions of North Africa and west coast Italy. Ectomicorhyzical of cork and holm oaks preferentially, it is very typical in Montado ecosystems characterized by agroforestry areas in open woodlands. Here we sequenced the partial genome of 206 individuals across all the distribution area through the Restriction-site Association DNA sequencing (RADseq) using a low frequent cutter restriction enzyme (*SbfI*) to generate a panel of genetic markers (SNP's). The markers were called and analysis conducted using statistical framework estimators from the site frequency spectrum. We clearly detected a longitudinal gradient of clustering populations in the Iberian Peninsula. From the point of view of management and conservation of this natural resource, the utilization of this and other approaches proves to be crucial in evaluating the fitness of populations, so it is important to implement it in future management and conservation plans of mycological resources in Portugal.

Genomics, genetics and molecular biology

The natural history of fungal DNA transposons

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Transposons shape genomes via recombination, transposition events, lead to chromosomal rearrangements, create new gene neighborhoods and alter gene expression. Transposons play a role in adaptation to symbiosis in *Amanita* and to pathogenecity in *Pyrenophota tritici-repentis*. Previous studies focused on the most abundant transposons the LTR retrotransposons. In contrast, the abundance and distribution of mobile elements replicating in a “cut and paste” fashion is barely described, possibly due to inefficient identification. In order to improve our knowledge on this old and ubiquitous class of transposable elements, 217 fungal genomes were scanned

with an inverted repeat finding tool (IRF) what enabled us to identify transposons with flanking repeats (Terminal inverted repeats) regardless of their internal sequence (de novo). Identified transposon candidates possessing DDE nuclease domains were subjected to further expert-driven, manual curation to remove dubious hits. The conjunction of terminal inverted repeats and DDE nuclease presence makes our count conservative and there might be more remnant sequences present in the analysed genomes. DNA TE have been identified in almost all of the 217 analyzed genomes. Unequal distribution of element abundance can be noticed both from DNA TE superfamily perspective and from fungal taxonomy side. Genomes previously described as abundant in mobile elements showed highest numbers of DNA transposons as well, what is in agreement with the notion that many mobile families proliferate simultaneously. Our results show that the core set of fungal transposons formed already in Cryptomycota. However, the whole repertoire of DNA transposons finally developed in land fungi (Mucormycotina and Mortierellomycotina).

SYMPOSIA

Biodiversity, Information and fungal data

Environment, ecology and interactions

Principles and tools on data quality and fitness for use of biodiversity occurrence data

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The Global Biodiversity Information Facility is de facto the reference repository for biodiversity occurrence data, with an impressive 540 million records freely available for all through the internet. While this is a most valuable asset to researchers, the effective number of records used in a study normally is lowered by several orders of magnitude, after trimming data by taxonomic, geographic or temporal filters. But further criteria might be applied to identify suitable records, related to data quality criteria. It is, therefore, important that researchers take most of the information they can from data, using the right procedures and tools, which ultimately will help on the identification of the fitness-for-use of such data.

Data quality principles should be considered in all steps of data fluxes, from capturing to publication. Aspects related to completeness, consistency, precision and accuracy, uncertainty, documenting need to be covered for each of the data dimensions, i.e. taxonomic, spatial and temporal axis. Limitations of quality of data, sometimes, may determine limitations on its use in all dimensions, but other times data can still be used for some applications while preventing use in others. This is also related to the scope of the aimed use, which can be defined in terms of taxonomic, ecological, biogeographical, phylogenetic or other. The use of data for certain purposes may, therefore, benefit from a preliminary tagging of data identifying its fitness-for-use.

The GBIF network, and the wider biodiversity informatics community, already developed a set of standards, documentation, reference datasets, procedures and tools that help researchers to improve and assess data quality, in all levels of the data management cycle. Also at the data portal level, GBIF also runs quality assessments that are visible to the user, helping to detect data quality issues which can be corrected by the data publisher, or used by the data consumer to filter datasets. In this talk, a review of these resources, with special focus on fungal data, will aim to show how mycologists can use them to improve data quality and produce fitness for use assessments for their datasets.

Evolution, biodiversity and systematic

Data papers incentivise biodiversity scientists to publish and re-use their data

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Data Paper is a scholarly journal publication whose primary purpose is to describe a dataset or a group of datasets, rather than to report a research investigation. As such, it contains facts about data, not hypotheses and arguments in support of the data, as found in conventional research articles. Its purposes are three-fold: (a) provide a citable journal publication that brings scholarly credits to data creators; (b) describe the data in a structured human-readable form; and (c) bring the existence of the data to the attention of the scholarly community.

In 2010, GBIF and Pensoft began investigating a mainstream biodiversity data publishing in the form of "data papers". As a result this partnership pioneered a workflow between the GBIF's Integrated Publishing Toolkit (IPT) and Pensoft's journals viz., MycoKeys, Phytokeys, ZooKeys, Nature Conservation, Biodiversity Data Journal, and others. The workflow generates data paper manuscripts from the metadata descriptions in IPT automatically at the "click of a button". Then manuscripts are submitted to a journal and undergo peer review. Within less than four years, nearly 100 data papers have been published in Pensoft journals, in total.

The concept of data paper is rapidly evolving. New features in place are fully XML-based submission of manuscripts from GBIF IPT direct to the Biodiversity Data Journal, as well as easy search, download and import individual specimen records from online resources into a manuscript. The data publishing workflow is extended also to streamline new species descriptions identified as such by gene sequencing.

Evolution, biodiversity and systematic

Species of Fungi

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The nuclear ribosomal internal transcribed spacer (ITS) region has nearly 20 years history of use as a molecular marker for species-level identification in ecological and taxonomic studies of fungi. It offers several advantages over other species-level markers in terms of high information content and ease of amplification, and it was recently designated the official barcode for fungi. To facilitate ITS-based molecular identification and discovery of fungal species the Species Hypotheses (SH) approach has been implemented by the UNITE community (http://unite.ut.ee). The demand for high-quality reference sequences has risen rapidly due to the increasing use of high-throughput sequencing technologies. Several software pipelines are available for overseeing more or less the entire analysis procedure, from data cleaning to sequence clustering and taxonomic assignment. Some of them, e.g. QIIME, mothur, CREST, SCATA, UCHIME, UTAX are now utilizing the downloadable UNITE repository of SHs (http://unite.ut.ee/repository.php). 7th version of the UNITE includes in total 487 435 SHs which are divided between different threshold values as follows (% is a dissimilarity gap between two SHs: 3% 38 353 SHs; 2.5% 42 098 SHs; 2% 47 080 SHs, 1.5% 53 891 SHs, 1% 64 727 SHs, 0.5% 86 824 SHs. In order to provide a stable communication of the SHs UNITE implemented a set of mandatory principles: 1) all SHs are connected to the fungal classification(s); 2) all SHs have a unique ID; 3) all SHs have a globally unique Digital Object Identifier (DOI) which are available at https://plutof.ut.ee/CARDINALI/digital-object-identifiers.

Evolution, biodiversity and systematic

Digging the treasure of hidden data: An open access European database of *Fusarium graminearum* and *F. culmorum* from small grain cereals for epidemiological studies

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Fusarium graminearum and *F. culmorum* on small grain cereals produce, among others, type B trichothecenes mycotoxins whose consumption is under regulation at the European level. Deoxynivalenol and nivalenol, together with their acetylated derivatives differ with respect to their toxicity to humans and animals. Fungal populations producing different profiles of type B trichothecenes can be distinguished by genetic means. Understanding which population is present in the field may help the development of preventive approaches in order to avoid serious losses due to high level of toxin contamination. The prevalence of a certain population able to produce one or another type of toxin is triggered by unknown factors that are currently under investigation. To answer this question, a large dataset on *Fusarium* strains with precise information on environmental descriptors of the area of origin can help addressing epidemiological questions.

For this reason, we have created an open-access database (intended to expand with contributions from actively working laboratories collecting information from European groups working on *Fusarium* species from agricultural crops. With the current dataset it has been possible to draw a map of the distribution of *Fusarium trichothecene* genotypes in small grain cereals and maize in Europe showing geographic and temporal patterns of trichothecenes genotype distribution. A common core set of related variables (sampling method, host cultivar, previous crop, GPS coordinates are collected in the database possibly favouring integrated epidemiological studies at the European scale.

This open-access database also aims to improve the practice of collecting and sharing epidemiological data in fungal studies.

Field mycology and conservation

Biodiversity informatics and fungal data

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[2]*University of Helsinki, Helsinki, Finland*

Mycologists, both explorative and experimental, collect, analyse and interpret mostly their own data. Aggregated data on fungi is essential to answer questions at various temporal, spatial and taxonomic scales. Based on biodiversity and environmental data standards, the potential of massive and heterogeneous digital data on fungi is underutilized in mycological research and in fungal conservation. Global Biodiversity Information Facility (GBIF) is an international open data infrastructure, funded by governments, allowing free and open access to biodiversity data collected and stored anywhere on Earth. GBIF provides a single point of access to more than 500 million records of nearly 1.5 million species, shared freely by hundreds of institutions worldwide (www.gbif.org), including over 400,000 records on fungi. The records are provided both by researchers and citizen scientists, and the undigitized resources are a treasure of biological information. GBIF collaborates with major organisations active in biodiversity conservation and data, including IUCN, Biodiversity Heritage Library, Encyclopedia of Life, International Barcode of Life Consortium, and Catalogue of Life, and is recognised as a complementary partner of the Convention on Biological Diversity (CBD) and Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) for data mobilization, integration and access. There is strong potential for substantial global benefits to improve quality, completeness and accessibility of biodiversity data and information in closer collaboration between institutions and expert communities, data aggregators and research infrastructures, mycology and other biodiversity sciences. Digital data solutions are essential to promote mycology as a discipline and to facilitate use of fungal data in decision making.

Fungal conservation

Field mycology and conservation

Fungal conservation in Europe. What are our governments doing? What can we do?

David Minter[1]

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For years science has recognized fungi have their own biological kingdom separate from animals and plants. As nature's recyclers, fungi are necessary for sustainable life. Without them, plants (the producers) and animals (consumers, including humans) could not exist. Climate change, exploitation, habitat destruction, persecution, pollution and war threaten fungi no less than animals and plants, so protecting fungi is essential for successful nature conservation. Do governments understand this? The answer lies in national strategies and reports to the Convention on Biological Diversity, where governments describe in their own words their contributions to biodiversity conservation. Examples from Europe show that fungi are overlooked or treated as an obscure corner of botany. This is disastrous. Our governments are ignorant about fungi and do nothing to promote their conservation. What can we do? It's a complex problem. Conservation combines science ("this species is threatened") with politics ("something must be done"), but infrastructure and education are also crucial. Governments must be educated. That needs not just scientists and learned societies but also teachers, negotiators, publicists, champions and politically oriented NGOs. Our responsibility is enormous. If we don't shout, who will? We must tell the public and the governments they employ that fungi are important: "flora and fauna" is a totally inadequate description of the living world. Fungi deserve the same attention as animals and plants. Most of all, conservation policy must give fungi the same protection as animals and plants. The presentation ends with some practical steps to help you promote fungal conservation.

Field mycology and conservation

The Lost and Found Fungi project: from local treasure hunt to national fungal conservation infrastructure

Martyn Ainsworth[1], Brian Douglas[1], Paul Cannon[1]

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The UK is a relatively mycologically well-recorded country. This is mainly due to our long tradition of recording fruitbodies on forays organised by the BMS and, more recently, by the two national networks of local recording groups. It is undoubtedly very satisfying to pick a site, foray there and add yet more fungal treasures to a list of names that just keeps on growing. In a significant departure from this tradition, the Lost and Found Fungi project aims to nurture a different, and no less exciting, style of fungal hunt with conservation objectives. Its immediate focus is on targeted searches for selected species that are rarely recorded, or have not been seen recently, and on recording and mapping both successful and unsuccessful searches. Its longer term impact, however, lies in official red-listing, national surveillance/monitoring and the incorporation of fungal ecological requirements within site management planning. This calls for much more pre-foray preparation, data gathering and networking than has been the norm hitherto. We have already prioritised 100 species from a list of almost 400 proposed candidates. Species data sheets are now being prepared for the priority 100 which include descriptions, distribution maps, photos and tips to help volunteers find the target fungi and are available from http://fungi.myspecies.info/content/lost-found-fungi-project. We have around 150 potential participants on the mailing list. The project team is now entering a phase of working with these volunteers in appropriate regions of habitat, at the right time of year, guided by the compiled historical data. We are also supporting the recording community with training and mentoring events. The project started on 1 July 2014 managed by the Royal Botanic Gardens, Kew, and is supported by a very generous donation from the Esmée Fairbairn Foundation.

Field mycology and conservation

The publication of the distribution maps of 51 macromycete species in Europe

André Fraiture[1]

[1]*Botanic Garden Meise, Meise, Belgium*

After a long period of work, the Mapping Programme launched by the European Council for the Conservation of Fungi (ECCF) reached its end, with the publication of the commented European maps of 51 macromycetes, together with a synthesis about the ecology and status of these species in the 38 participating countries (Fraiture & Otto 2015). Most of these species are rare or endangered in some way, at least in a part of

Europe. The programme involved the participation of very numerous contributors.
An overview of the work, its goals and its methods, is given. Several distribution maps are presented. Various types of distribution are recognized and commented. Comparisons with the distribution of host plants are made. The progress made since the maps published by Lange (1974) are commented.

Bibliography:

Fraiture A. & Otto P. (eds) (2015) Distribution, ecology and status of 51 macromycetes in Europe. Scripta botanica belgica 53.
Lange L. (1974) The distribution of Macromycetes in Europe. Dansk Botanisk Arkiv 30 (1): 1-105.

POSTER PRESENTATIONS

PROGRAMME

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Applied mycology and fungal biotechnology

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Poster Session I

Applied mycology and fungal biotechnology

Poster nr. 01

New strategies to produce bioinsecticides by endophytic fungi, bacteria and plant cell cultures

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Recent studies have shown that plants like the Neem tree (*Azadirachta indica*) contain endophytic fungi and bacteria which are able to colonize internal plant tissue without causing visible disease symptoms. The estimated high species diversity of endophytes suggests a rich and almost untapped source of new secondary metabolites. That is why it can be hypothesized that some of the bioinsecticidal compounds of the Neem tree mainly belonging to the azadirachtin family are either produced by endophytes *de novo* or the microbial production is somehow linked to the plant metabolism. Further on, there is increasing evidence that plant cell cultures can produce azadirachtins. Both strategies allow to by-pass the cost intensive transport and extraction of plant metabolites.
The aim of a BMBF project is to develop a competitive process to produce high concentrations of azadirachtins with endophytes and Neem plant cell cultures.
In total, 303 endophytes (196 fungi; 107 bacteria) were isolated from plant material of different origins. Besides, we induced plant cell cultures from various plant tissues with a medium that allows a callus proliferation in more than 50% of explants. Furthermore, we developed a method for *in situ* product removal in liquid media where more than 85% of azadirachtins are bound and stabilized. Moreover, a high throughput bioassay based on *Spodoptora frugiperda* Sf9 cell cultures was developed. Additionally, we will present results on classification of endophytes as well as on induced plant cell lines, submerged cultivation in a novel fully automated microbioreactor and on classification of metabolites via UHPLC-UV/VIS-MS/MS.

Applied mycology and fungal biotechnology

Poster nr. 02

Antimicrobial and mycofumigation potential of novel Indian *Muscodor* species at enhancing the shelf life of fruits and vegetables

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Muscodor is a genus of sterile, volatile organic compounds (VOCs) producing endophytic fungi with antimicrobial properties. In the present study seven novel *Muscodor* species *M.*

kashayum, M. strobelii, M. tigerii, M. darjeelingensis, M. ghoomensis, M. indica and *M. camphora* were isolated from *Cinnamomum zeylanicum*, *Aegle marmelos* and *C. camphora,* respectively growing in Western Ghats and North eastern Himalayan region of India. When tested for their antimicrobial potential, *M. kasahyum* emerges most lethal to the battery plant and human pathogens. It exhibited complete inhibition of 26 pathogenic microorganisms whereas growth of rest of the isolates was reduced to 50-70%. *Muscodor strobelii*, *M. darjeelingensis, M. camphora* also exhibited strong antibacterial and antifungal activity whereas *M. tigerii* only showed antifungal activity. The volatiles produced by *M. kashayum* successfully preserve grapes, jamun, cherry, black gram and wheat from *Botrytis cinerea, Rhizoctonia solani, Collectrotrichum gloeosporioides* and *cercospora beticola* infection till 15 days post treatment. All the isolates produced fruity smell which is attributable to a mixture of volatile compounds predominantly producing 3-cyclohexen-1-ol,1-(1,5-dimethyl-4-hexenyl)-4-methyl; 1,6-dioxacyclododecane- 7,12-dione; 4-octadecylmorpholine; 2, 6-bis (1, 1-dimethylethyl)-4-(1-oxopropyl) phenol, aspidofractinine-3-methanol, tetracontane etc. *Muscodor* species produces sterile ropy mycelia with coiling and non-descript structures and lacks sexual stage. Their ITS sequence also showed high similarity with other *Muscodor* species. Phylogenetic, distance and haplotype analysis confirms their identity as novel *Muscodor* species. Thus, these *Muscodor* isolates can be taken into account to be developed as a myco/biofumigant that acts as a biopreservative for fruits, vegetable and grains and help to reduce post harvest losses.

Applied mycology and fungal biotechnology

Poster nr. 03

Chitinolytic enzymes production by two marine isolates of *Aspergillus pseudoterreus* and *Sydowia polyspora* and investigation of their antagonistic interactions against fungal pathogens

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Marine fungi are one of the most promising group of organisms in terms of ecological diversity, new enzyme and secondary metabolites detection, application in environmental sciences.

In the present work, in order to evaluate the biotechnological potential of two marine strains, *Aspergillus pseudoterreus* and *Sydowia polyspora*, the production and activity of chitinolytic enzymes were investigated. Moreover the *in vitro* antagonistic activity towards plant, human and animal pathogenic fungi was investigated.

The strains were isolated from *Ulva rigida* and *Sargassum muticum*, collected along the coast of the Italian peninsula during a PhD thesis (Garzoli, 2013).

Sydowia polyspora was obtained both from *Ulva rigida* and *Sargassum muticum*; *Aspergillus pseudoterreus* was isolated only from *Ulva rigida*.

The studied fungi were inoculated in mineral medium added with colloidal chitin as the only carbon source; chitinase production was assayed by the DNS method which estimates the concentration of reducing sugars in the sample. The antagonistic activity was investigated by dual cultures (Skidmore & Dickinson, 1976).

Results showed that:

1) both *S. polyspora* and *A. pseudoterreus* produced a good amount of chitinases; the maximum enzymatic yield was detected after 4 days for *S. polyspora* and after 7 days for *A. pseudoterreus*;

2) an interesting antagonistic activity was evidenced for *A. pseudoterreus*, able to inhibit the growth of *A. fumigatus* and *P. oryzae*.

In conclusion, positive data regarding the chitinases production have been highlighted, even if these results cannot completely explain the antagonistic activity of the two marine strains against the tested pathogenic fungi.

Applied mycology and fungal biotechnology

Poster nr. 04

Antiproliferative potential of extracts of microscopic fungi isolated from Crater Lakes of Puebla, Mexico

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The ecosystems in Mexico have great diversity in a variety of microscopic fungi and the use of them in medicine and the use of them in medicine may be have great potential. Therefore, for several years, our group has been researching the bioactive substances from these organisms. One of the strategies used was an isolation of fungi from aquatic habitats of Puebla, Mexico, to obtain organic extracts with antiproliferative activity. The objective of the research presented is to isolate microscopic fungi from water, rooted and floating vegetation, and sludge in the coastal area of crater lakes using two culture media, PDA and Marine Agar pH 8.5. Subsequently, for biomass production, the fungi were cultivated for 15 days in Wickerham's liquid medium; then, the produced biomass was separated, and both the biomass and the culture liquid were extracted with a mixture of chloroform:methanol in 1:1 proportion; the extracts obtained were tested for their cytotoxicity on some cell lines human solid tumors by employing the protocol of the National Cancer Institute. For the results, among 35 fungal strains isolated, eight strains manifested antiproliferative activity with a low rate of growth (GI_{50}) against cancer cell lines tested. These fungi belong to the genera *Monilia*, *Acremonium*, *Morteriella, Alternaria, Trichoderma,* and *Papulaspora.* The study on extracts' purification and structural characterization has to be continued to determine the metabolites responsible for the bioactivity. This is the first study of bio-prospecting of Mexican aquatic fungi with the aim of obtaining new bioactive substances from these organisms.

Applied mycology and fungal biotechnology

Poster nr. 05

Hericium erinaceus (HE) grown on barks produces hydrogen whilst *Laetiporus sulfureus* (LS) produces isobutylene and may capture hydrogen

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Fermentation of carbohydrates by bacteria can produce either hydrogen or isobutylene (an indirect marker of methane production). Bacteria producing isobutylene are known to consume hydrogen. It was investigated whether HE or LS may produce hydrogen or isobutylene when degrading lignin.

Material and Methods

HE and LS were cultivated on barks in plastic bags (Mycelia®/ SacO2®, Belgium).

After 2 months growth in a sterile chamber, 4 bags of each strain were put in a dark non-sterile room at 18°C.

Measures of the gas emitted through the filter strips were made daily with a device able to detect 0.1 ppm (particle per million) of hydrogen or isobutylene (MX6 from Gazdetect®; France) until the first fructification ended: exactly 2.5 months after the start of the experiment.

Results

The gas production was almost constant during the experiment, for HE and for LS; except when a bacterial or a fungal contamination occurred. Fructification did not modify the gas production.

HE produces hydrogen (1.08 ppm +/- 0.69) and very few isobutylene (0.33 ppm +/-0.33).

LS produces isobutylene (0.65 ppm +/- 0.5) and captures hydrogen (-0.73 ppm+/- 0.47).

When LS bags were contaminated, the production of isobutylene decreased (0.44 ppm +/- 0.34) as well as the capture of hydrogen (-0.29 ppm +/- 0.48). No HE bags were contaminated.

Conclusions

HE produces hydrogen whilst LS produces isobutylene. Gas detection can also be used for early detection of contamination.

Applied mycology and fungal biotechnology

Poster nr. 06

Effect of different cultivation techniques and inducers on ligninolytic enzyme production by *Bjerkandera adusta* (Willd.) P. Karst.

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White-rot fungi produce several types of lignin modifying enzymes which have considerable industrial, biotechnological and environmental interest. The present work focuses on the obtaining of higher ligninolytic enzyme production by *Bjerkandera adusta* by selecting cultivation techniques and inducers. For this purpose, the *Bjerkandera adusta* isolate was grown in presence of agricultural (wheat bran, ground orange peelings and tea wastes) and synthetic (xylidine) inducers under three of submerged, static and solid state cultivation types. Glucose was used for comparison in all cultivation types. During 20 days of incubation period, periodically taken samples were examined for laccase, manganese peroxidase and lignin peroxidase enzyme activities. As a result, the studied *Bjerkandera adusta* isolate was defined as a good manganese peroxidase producer under all culture types. The highest activity was obtained as 1327.7±107.1 u/L on static culture type and in the presence of xylidine on 11th days of incubation. The identification of the mushroom was also confirmed with the BLAST analysis of the rDNA-ITS sequence of the isolate.

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Applied mycology and fungal biotechnology

Poster nr. 07

Clitocybe odora (Fr.) P. Kumm. in MMN and PDA culture media: monitoring of mycelial growth and biomass production

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Clitocybe odora (Fr.) P. Kumm. an edible wild mushroom is known for its characteristic flavor and aroma due to the p-anisaldehyde, the compound held responsible for its anise-like fragrance (Rapior S. et al., 2002). Furthermore, this mushroom rings showed a high content of ascorbic acid and interesting antioxidant properties in comparison with other already described wild edible mushrooms (JA Vaz et al., 2010).

The aim of this study was to isolate *C. odora*, from settlements of Fagaceaes and evaluate biomass production and mycelial growth of *C. odora* produced by in vitro culture methods using MMN and PDA culture media.
The primary best result for the growth of mycelium of *C. odora* was obtained in the MMN medium.
For biomass production in MMN medium obtained after 4 weeks presented an average of 83.2mg and 16.1mg in fresh and dry weight, respectively. The average radius of the mycelial growth 2.7 ± 0,23cm. Concerning to PDA, lower values were obtained for growth (1,4 ± 0,21cm) as well as biomass production(53,6mg and 1,65mg in fresh and dry weight, respectively).
Further studies should be conducted to explore this particular fungus, using different culture media to obtain essential oils and understand the differences in composition and oil yield.

References:

S. Rapior et al. (2002). The anise-like odor of *Clitocybe odora, Lentinellus cochleatus and Agaricus essettei*. *Mycologia*. 94(3), 373-376.

J. A. Vaz, et al. (2010). Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Food Chemistry*. 126, 610-616.

Applied mycology and fungal biotechnology
Poster nr. 08

Large-scale production of Arbuscular Mycorrhizal Fungi (AMF) inocula under tropical humid and semiarid climates in Northeast Brazil

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Root inoculation with arbuscular mycorrhizal fungi (AMF) can benefit agricultural crops by reducing the need for chemical inputs. However, this is rarely practical because of the restricted availability of inoculants. Production levels were compared in two simultaneous experiments, under humid tropical (Recife-PE) and semiarid tropical climates (Petrolina-PE). Each experiment was established in a randomized block design following a 2 x 4 factorial arrangement: two inoculation treatments (*Acaulospora longula* or *Claroideoglomus etunicatum*) and four growing substrates (T1: sand + clay, T2: sand + clay + sugarcane bagasse, T3: sand + clay + shredded leucaena (*Leucaena leucocephala*), and T4: sand + clay + shredded leucena + sugarcane bagasse), across two cropping cycles (December-March; April-July), using sorghum (*Sorghum bicolor*) as host. Plants inoculated with both AMF produced greater biomass in the substrate composed of sand + expanded clay + leucaena than in the other substrates, in both humid and semiarid locations, and during both growing seasons. The plants grown on substrates with leucaena also had higher levels of mycorrhizal colonization and generated more glomerospores. The inoculants were infective (> 25% colonization) in all treatments in the humid area, whereas in the dry area they produced an average of 90% colonization. In general, the inocula of both AMF produced in the substrates with leucaena had higher infectivity than those produced with the other substrates. The production of AMF inoculum in horticultural beds using sand + clay + leucaena as substrate can be recommended for small farms in Northeast Brazil and in other similar locations.

Applied mycology and fungal biotechnology

Poster nr. 09

Coprinus comatus: a possible myco-remediator and bio-indicator of soils polluted with mercury?

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Macrofungi are well known for their ability to efficiently bioconcentrated various metallic elements and metalloids from the substrata and sequester same in their fruiting bodies. Hence, fruit bodies of edible and inedible mushrooms can be relatively rich in inorganic constituents. Some authors suggested that macrofungi are good indicators of environmental (soil) contamination with metallic elements (heavy metals), radionuclides and metalloids and that they are suitable in the process of remediation of soils contaminated with heavy metals. However, convincing evidence for these bio-indication properties is lacking though this can not be denied as the possibility of the practical usefulness of macrofungi in the process of restoring degraded soils have so far been limited to experimental studies. Some studies have shown limited (for one element or location) bio-indication ability of *Macrolepiota procera* for Hg in soil at the background area, a few species for methylmercury in a cinnabar polluted soil as well as a weak for Cd by *Boletus edulis.* This study shows that *Coprinus comatus* seems to be a sensitive boindicator of urban soils pollution with Hg that is efficiently sequestered by this species in fruit bodies – both caps and stems and with potential in process of myco-remediation.

Applied mycology and fungal biotechnology

Poster nr. 10

A study on trace elements and minerals composition of *Xerocomus chrysenteron* and *Boletus badius* fruiting bodies emerged from the same background area

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The samples of *Xerocomus chrysenteron* and *Boletus badius* were collected from the same site of the Bory Tucholskie forest complex in northern Poland and examined for similarity and differences in accumulation of Ag, Al, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Rb, Sr and Zn. In order to demonstrate similarities and differences between the trace

elements and minerals composition of the two species of mushrooms that emerged at the same site, the statistical tests such as the non-parametric Mann-Whitney test, cross-correlation analysis, cluster analysis (CA) and principal component analysis (PCA) have been used. Statistically significant differences have been found in the content of Al, Co, Ni, Rb and Hg in caps between *X. chrysenteron* and *B. badius* and in the case of stipes both species differed in the content of Ba, Fe, K, Mn, Na and Rb ($p < 0.0001$).
Acknowledgements. This study was funded by the National Science Centre of Poland (No. UMO-2012/05/N/NZ7/00935).

Applied mycology and fungal biotechnology

Poster nr. 11

Impact of culinary processing on mercury content of three species of mushrooms

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The aim of the study was to evaluate an influence of culinary treatment (drying, freezing, cooking and pickling) on the mercury content in cooked fruiting bodies of two species of edible mushrooms collected in wild (*Boletus edulis* and *Xerocomus chrysenteron*) and one cultivated (*Agaricus bisporus*). Mercury content was determined in pooled samples separately of caps and stems (ten pooled samples per species and morphological part) of the fruiting bodies. Mercury content in dried and freezed caps and stipes of all tested species of fungi does not differ significantly ($p < 0.05$). Maceration, cooking and pickling of a fresh and dried fruiting bodies of *Agaricus bisporus*, *Xerocomus chrysenteron* and *Boletus edulis* does not result in a significant reduction in the concentration of mercury (loss up to 7 %) in final product normalized to a dry matter content.

Acknowledgments
This study was supported by National Science Centre under project: Decision No UMO-2013/11/N/NZ7/01240.

Applied mycology and fungal biotechnology

Poster nr. 12

Heavy metals myco-accumulation by indigenous strains isolated from metal contaminated waste-rock dumps.

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In recent years several researches aimed at looking for new, cheap, and green technologies for the remediation or reclamation of heavy metals contaminated

environments. As concerns green biotechnology, fungi are the most promising organisms, in particular for bioremediation activities. In our work we isolated indigenous microfungal strains from a heavy metal contaminated waste-rock dump in Libiola Mine (Genoa, Italy); later, their biotolerance and bioaccumulation capability were tested.

The Libiola Mine is a derelict Fe-Cu sulphide mine, exploited until 1962. In the whole area Acid Mine Drainage processes occur and persist over time. The mine soils and, in particular, the waste-rock dumps are characterized by extreme edaphic conditions: high metal concentrations, low pH and low availability of essential macronutrients. Among the most critical metals occurring in the waste-rock dumps and in the surrounding soils stand out Cu (≤ 13347 ppm) and Ag (≤ 11000 ppb).

In this peculiar environment, the microfungal strains were isolated from bare dump soil samples and copper and silver biotolerance screeening tests were carried out. Copper and silver bioaccumulation capability of most tolerant strains (*Aspergillus alliaceus* Thom&Church, *Trichoderma harzianum* Rifai, *Clonostachys rosea* (Link)Schroers, Samuels, Seifert & W.Gams) were evaluated.

Among the species tested, *C. rosea* shows a Cu uptake capability of 19628 mg kg^{-1}; *T. harzianum* strain appears the most efficient, showing an uptake capability of 22222 mg kg^{-1} of copper and 153 mg L^{-1} of silver.

In the field of mycoremediation, the study also highlights the possibility to fruitfully employ microfungi for original metal extraction techniques.

Applied mycology and fungal biotechnology

Poster nr. 13

Fungi found in imported commercial sawdust media for Shiitake production

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Shiitake mushroom is a highly priced edible mushroom as well as one of favored medicinal mushrooms in Korean markets. It has been diversely used in Korean traditional cuisines and. Shiitake is produced through either log-based or sawdust media-based cultivation. Recently, compared to log-based cultivation, sawdust media-based cultivation is increasing with the advantage of the easiness of cultivation and shorter cultivation times. With the increase of sawdust media-based cultivation, the demanding of materials for sawdust media is also enhancing. In step with this demanding, the import of sawdust media from foreign sources are also increasing in Korea. However, the imported sawdust media have not been properly checked for their quality so that some cultivators have been confronted with low productivity and low mushroom quality. Therefore researches about cultural adaptability and quality control are needed for the imported sawdust media. In an effort to find the status of imported sawdust media we first checked mycological contamination in this study. We isolated eleven fungal species including *Trichoderma* spp., *Penicillium* spp., *Hypocrea citrine, Ceriporia lacerata, Schizophyllum* *commune, Aspergillus oryzae,* and *Rhizoctonia solani.* Among these species, *Trichoderma* spp. and *Penicillium* spp. are found to potent problematic contaminant sources of shiitake cultivation and *R. solani* is a known plant pathogenic fungus with wide host range and worldwide distribution. Out results suggest that the sawdust materials for import needs to be further checked through administrative process.

Applied mycology and fungal biotechnology

Poster nr. 14

Development of method for detection of the genus *Neosartorya* in strawberry juice

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Neosartorya species produce heat-resistance ascospores, which make them to survive temperatures of 85°C for 50 minutes. They are able to cause the spoilage of heat-processed foods.
We developed the method of *Neosartorya fischeri* detection in strawberry juice based on specific primers, for β-tubulin and calmodulin genes, designed by Yaguchi et al. (2012). This method was used previously just for pure strains identification not for detection of fungi in environmental samples like juice.
Fungal genomic DNA was extracted using extraction buffer based on EDTA, SDS with benzyl chloride, sodium acetate, isopropanol and ethanol. Strawberry juice was mixed in the following doses of 2, 1 and 0.5 µl with 5 µl of extracted fungal DNA. PCR reactions were prepared using primers and PuReTaq-Ready-To-Go-PCR Beads. The sizes of the PCR products were confirmed by electrophoresis. Among the 4 strains used in this experiment, PCR products of approximately 220 bp were detected according to the primers designed for *Neosartorya* and *A. fumigatus*. No PCR products were obtained for the control. The species specific primers detected just *N. fischeri* and not detected *A. fumigatus*. The study showed that just in the mixture of DNA and 0.5 µl of strawberry juice the method was working very well, but for 1 µl of juice we observed very weak bands on the gel and using 2 µl of juice there were no bands on the gel.

Yaguchi et al. (2012) Journal of Food Protection, 75, 10: 1806-1813.

The study was supported by National Science Centre (Poland), grant: DEC-2012/07/D/NZ9

Fungal pathogenesis and disease control

Poster nr. 15

Fusarium as a source of echinocandins

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The incidence of fungal infections, especially candidiasis, is on the rise in human populations. Evidence for increasing fungal resistance to currently used drugs is accumulating. Moreover, many of the antifungal drugs in use today have known side effects, and can harm the patient. Novel potential chemicals are therefore being sought to widen the range of drugs of choice. Drug companies and scientists are seeking new niches the world over for drug discovery, a work which generally includes meta-analysis of microorganisms and compounds. We isolated a soil-inhabiting *Fusarium* strain that produces an anticandidal compound. The isolated strain was identified by molecular means, and the active metabolite was extracted, purified and identified using separation and purification methods that included TLC bioautograms, HPLC and NMR. The active molecule was identified as a novel echinocandin. This finding is important since echinocandins are currently used as antifungal drugs and are considered safer than drugs from azole or polyene compounds.

Fungal pathogenesis and disease control

Poster nr. 16

Fusarium graminearum susceptibility to azoles

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Azoles represent actually the only effective and registered chemical measure for controlling *Fusarium* spp. spread in cereals in many regions. *Fusarium* species cause both direct losses and indirect losses due to accumulation of mycotoxins in the grains and represent at the moment a significant challenge to wheat production worldwide.
The aim of the work was to characterize the level of sensitivity of a *Fusarium graminearum s.s.* population against azoles belonging to different classes: imidazoles and triazoles (five-membered nitrogen heterocyclic ring compounds containing respectively 2 and 3 nitrogen atoms). By a microtiter spectrophotometric test, a total of 27 isolates collected over the period 1981 to 2012 in Luxembourg and abroad were screened for sensitivity to six different azole molecules.
Different levels of sensitivity were found among national and also international isolates. At the moment, in Luxembourgish fungal populations, no complete resistance has been identified but isolates carrying a higher level of resistance against cyproconazole, tebuconazole and tioconazole were found. To link the diverse level of sensitivity to molecular targets, the full sequence of the *cyp51A*, *B* and *C* genes (being cyp51p the

primary target of azoles*)* was carried out for a subset of 19 isolates. Single nucleotide polymorphisms (SNPs) determining aminoacid changes in the primary protein structure of cyp51 genes were identified and are under investigation for their association with resistance levels towards azoles.

Fungal pathogenesis and disease control

Poster nr. 17

Multiple facets of resistance to azoles in the *Fusarium* genus

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The chemical treatments are a key component in the integrated management of plant diseases and coping with increased resistance/tolerance of key plant pathogens has become an important factor in coping with plant diseases of fungal origin. The selection pressure resulting from long-term fungicide application, leads to the emergence of new strains with increased resistance to new groups of compounds. There are several well-known molecular mechanisms directly reducing the efficacy of fungicides. The observed resistance stems from multiple genetic sources (the spread of mutations in the sequences encoding target proteins, overexpression of genes encoding the target proteins and the adaptation of transport proteins to increase the efflux of substances with antifungal activity) which can coexist in the field populations. Based on the combined information on the mechanisms of fungal resistance to fungicide substances, it is now possible to design and deploy targeted diagnostics procedures which allow rapid analysis of samples. Our experiments aim to correlate morphological changes in mycelia with polymorphism of candidate resistance genes and their expression in stress conditions (fungicide treatment). We demonstrate example polymorphisms in CYP51A, CYP51B and CYP51C sterol demethylase homologs and in the homologs (FGSG_02865) of FLR1 gene, transporter known to be implicated in resistance to azoles. In the latter case, our findings underscore the synergistic involvement of different transporters in resistance to toxic compounds, as well as the complex relationships between multidrug resistance-related proteins of common (albeit distant) origin.

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Fungal pathogenesis and disease control

Poster nr. 18

Secretome analysis of the fungal plant pathogen *Botryosphaeria dothidea*

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Eucalyptus species are commercially exploited in many countries around the world. They are also the most abundant forest tree species in Portugal and have an enormous economical significance for the country.

Botryosphaeria dothidea is a widespread and important pathogen of trees around the world, including important fruit crops and forest trees. Although not considered a major pathogen of *Eucalyptus* it is able to cause dieback and canker disease especially under stress conditions. Despite the relevance of this pathogen the mechanism of interaction between this fungus and its hosts is not known. Since the extracellular molecules of fungi are the main effectors of fungus-plant interactions, we mainly centered our analysis on the secretome.

The major challenges in the secretome analysis of filamentous fungi are the low protein concentrations and the presence of many interfering substances. To overcome these problems, we determined the adequate growth conditions as well as protein extraction protocol. Secreted proteins from *B. dothidea* collected from liquid cultures in the presence and absence of *Eucalyptus* stem were separated by 1-DE and 2-DE gel electrophoresis. 1-DE results showed the differences of protein profiles among secretomes. A total of 276 and 285 spots were detected on 2-DE in the secretome from both control and strain grown in the presence of the host, respectively, being 133 spots statistically different. Identification of these spots by mass spectrometry allows determining the proteins involved in fungus-host interaction mechanisms.

Fungal pathogenesis and disease control

Poster nr. 19

Secretion of extracellular enzymes by strains of *Hypholoma* sp. and *Pleurotus* sp.

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White rot fungi are known to secrete enzymes that degrade lignin. The most important of these enzymes are laccases (Lacs), lignin peroxidases (LiPs) and manganese peroxidases (MnPs).

The aim of this study was to examine the activity of laccase, peroxidase, the level of micromolecular compounds, protein concentration in strains of *Pleurotus* sp. and

Hypholoma sp. All the strains were grown on pieces of Norway spruce wood as sole carbon and energy source. The culture supernatants were examined for the specific enzymes using appropriate assays, each 10 days (it means10, 20, 30, 40 and 50 days), at the temperature 28°C.

The preliminary study indicate that *Pleurotus* strains secreted Lac, MnP, LiP and hydroxy-, methoxyphenols, whereas *Hypholoma* strains - the same except for LiP.

Fungal pathogenesis and disease control

Poster nr. 20

Effect of temperature on the plant pathogen *Lasiodiplodia theobromae*: enzyme and secondary metabolites production

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Climate changes are taking place with major consequences for the agricultural and forestry systems. Abiotic factors, such as temperature, can influence the dynamic between host and pathogen that could result in changes in disease impact.

The pathogenicity of fungi has been shown to be associated with the expression of several molecules, such as enzymes or other natural compounds that are known to be involved in host/pathogen interaction in fungi.

Lasiodiplodia theobromae is a phytopathogenic fungus that is found in a diverse range of plant hosts. Although it is typically found in tropical and subtropical regions, it may also occur in other climates. Besides, it can act as an opportunistic pathogen in humans.

The aim of this study was to characterize the expression of extracellular enzymes and secondary metabolites produced by different isolates of *L. theobromae* grown at different temperatures.

The detection of extracellular enzymes was performed at 25ºC, 30ºC and 37ºC for isolates from different hosts (grapevine, mango, coconut and banana trees, avocado tree and human) and the metabolites identification at 25ºC and 37ºC for isolates from coconut tree and human.

The enzymatic activity is different between some isolates and temperatures, especially for CAA019 that exhibited a different profile for all the temperatures and CBS339.90, with higher enzymatic indexes at 37ºC. CBS339.90 and CAA019 produce different secondary metabolites and we show that temperature modulates the expression of secondary metabolites. This alteration on the behavior could be related with host-specificity requirements and may be relevant for the host/pathogen interaction.

Fungal pathogenesis and disease control
Poster nr. 21

The most important fungi on needles and twigs of pine trees in Slovakia

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Austrian pine (*Pinus nigra*) and Scots pine (*P. sylvestris*) trees are an ecologically and economically important tree species in Slovakia. In 2014, fungal endophytes and fungi associated with needle and twig diseases of these two pine species on 67 pine stands were surveyed. A total of 18 fungal taxa were identified. The most dominant fungi identified were *Sphaeropsis sapinea*, *Lophodermium pinastri*, *Cyclaneusma minus*, and *C. niveum*. For both *Pinus* species, *Lophodermium* and *Cyclaneusma* species were the dominant fungi on fallen needles, whereas *S. sapinea* and *Zythiostroma pinastri* dominated on twigs. On dead needles still attached to branches of both *Pinus* species, *Dothistroma septosporum* and *S. sapinea* were observed in frequencies of more than 30% and 55% of examined samples, respectively. The fungus *D. septosporum*, one of the most serious foliar diseases of *Pinus* spp. was found on *P. nigra* as well in the conidial state as in the perfect stage *Mycosphaerella pini*. Species diversity of endophytic mycoflora was enriched with *Anthostomella pedemontana* and *Pestalotiopsis* sp. reported on fallen Scots pine needles. On dead twigs of *P. sylvestris*, the following fungi occurred sporadically: *Cytospora pini*, *Gibberella* sp., *Gremmeniella abietina* and *Therrya fuckelii*. *Sclerophoma pithyophila* was found on dead needles and twigs of *P. nigra*. Ecological aspects of colonization of the needles, especially phenomena of competition for the substrate, were observed.

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Fungal pathogenesis and disease control
Poster nr. 22

Diversity of fungi on dead plane tree branches

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The London plane (*Platanus × hispanica*) is a commonly planted ornamental and parkland tree in cities throughout Slovakia. Fungal pathogens cause mortality of branches still attached to the tree and many fungus species are specialised on dead branches that have fallen to the ground. In 2013 and 2014, the diversity of fungi on dead plane tree branches was studied. In total, 118 samples (branches with necrotic lesions or dead branches in the lower parts of the tree crown, and fallen branches) were collected in urban parks and tree-lined streets in 27 cities in Slovakia. Fungal identification was based on

morphological examination under stereo and compound microscopes. A total of 25 fungal taxa were identified. *Macrodiplodiopsis desmazieri* (73%) and *Cytospora platani* (64%) were the dominant species occurring in necrotic tissue on fallen branches. The frequency of occurrence of fungi in examined samples was as follows: *Splanchnonema platani* (34%), *Hapalocystis berkeleyi* (25%), *Massarina eburnea* (17%), *Discula platani* (15%), *Phomopsis* sp. (14%), *Diplodia* sp. (10%), *Macrodiplodia* sp. (8.5%), *Camarosporium pommersheimii* (7.6%), *Diaporthe ambigua* (5.9%), *Coniothyrium* sp. (4.2%), *Ascochyta* sp. (3.4%), *Nectria* sp. (3.4%), *Melanomma pulvis-pyrius* (2.5%), *Leptosphaeria* cf. *vagabunda* (1.7%), *Melanconium* sp. (1.7%), *Asterosporium asterospermum* (0.8%), *Fusarium* sp. (0.8%), *Libertella* sp. (0.8%), and *Valsa* sp. (0.8%). The activity of fungi found on damaged and dead branches may be related with sudden branch breakage and eventual loss of canopy shape. Dead or damaged branches pose the greatest danger of falling.

This study was supported by the Scientific Grant Agency VEGA, project No. 2/0071/14.

Fungal pathogenesis and disease control

Poster nr. 23

Multidisciplinary studies on the rice blast pathogen *Magnaporthe grisea* in Italy

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The devastating rice blast disease caused by *Magnaporthe grisea* has never been eliminated from a region in which the crop is grown.

In temperate areas, Italy included, it can cause marginal losses (2012 and 2013) as well as devastating outbreaks (2008). Even if the *Oryza sativa* - *M. grisea* pathosystem has become a model for understanding the molecular basis of plant-fungal interactions, practical answers to some key questions regarding it still have not been found.

Thanks to two Italian National projects, RISINNOVA and POLORISO, knowledge concerning the pathogen and the prevention of the disease has been improved.

A third Project, "Fight to Blast" (2009), by the Vercelli Province, allows the continuous bio-monitoring, from June to August, of an about 110.000 ha production area. This results in a disease-forecast free-service: a real time bulletin based on 4 risk levels, from "0 - no risk of blast" to "3 - high risk" (resistant rice varieties and/or unfavourable to blast field management included).

Those projects are focused on: 1) phytosanitary evaluation of both experimental and cultivated plants; 2) collection of the *M. grisea* Italian population; 3) aerosporological monitoring of *M. grisea* spores; 4) optimization of a model (SiRBInt - Simulation of Rice-Blast Interaction), created for temperate rice growing areas.

Results confirm that *M. grisea* is a pathogen able to change in relation to geographical, environmental and agronomical factors. The collection of the pathogen could be a reference point for the international scientific community involved in rice plant protection and improvement.

Fungal pathogenesis and disease control

Poster nr. 24

Wood decomposition ability of strains of *Hypholoma* sp., *Pleurotus* sp. and *Phlebiopsis gigantea*

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Phlebiopsis gigantea (Fr.: Fr.) Jülich is used as biocontrol agent against *Heterobasidion annosum* sensu lato. However, after artificial inoculation *P. gigantea* grows very well in wood of Scots pine stumps, but its growth in Norway spruce is lower.

The aim of this study was to examine the wood decay capacity of some strains of *Hypholoma* sp., *Pleurotus* sp. and *P. gigantea* through 1, 2 and 3 months treatment of Norway spruce wood (sapwood and heartwood) under laboratory conditions.

Strains of *P. gigantea* decomposed spruce wood more effectively than strains of *Hypholoma* sp.and *Pleurotus* sp.

Fungal pathogenesis and disease control

Poster nr. 25

Pathogenicity of Botryosphaeriaceae species to *Eucalyptus* spp. in Portugal

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Several Botryosphaeriaceae species are important endophytes and latent pathogens of *Eucalyptus* spp. in their native and non-native range, being mostly stress-related pathogens. Recently we identified several species of Botryosphaeriaceae associated to *Eucalyptus globulus* plantations in Portugal. Knowledge on the pathogenicity of these fungi and tolerance of different *Eucalyptus* spp. is much needed to develop suitable control and management strategies for plantations.

The aim of this study was to evaluate the pathogenicity of these species towards clones of *E. globulus*, *E. nitens* and a hybrid of *E. globulus x E. cypellocarpa*. For that, 6 months old plants were inoculated with each species and incubated in a non-controlled greenhouse for 2 months. At the end of the experiment lesion lengths were determined and plant physiological parameters were evaluated namely growth rate, water potential, relative water content, chlorophyll fluorescence, leaf gas-exchange, pigments (total chlorophyll and carotenoids) and total soluble sugars.

Considering the size of lesion developed there were obvious differences between fungal species reflecting potential differences in aggressiveness. Further, differences were observed between the different hosts. Concerning physiological parameters related to plant performance, significant differences were observed between control and inoculated plants for most of them. However, no direct relation was found between lesion sizes (aggressiveness) and plant physiological responses. Fungal inoculation affected more *Eucalyptus globulus* and *E. globulus x E. cypellocarpa* hybrid than *E. nitens* which exhibited a somehow more tolerant behaviour. Future studies including more isolates, different host genotypes and abiotic stress effects are needed.

Medical mycology and fungal pharmacology
Poster nr. 26

Screening of submerged culture fluids and mycelia of mushroom isolates from Turkey for antioxidant activity

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The aim of the present study was to select to mushroom isolates demonstrated higher antioxidant activity in their submerged culture fluids and/or mycelia. For this purpose, firstly, growth conditions of the fungal isolates which will be used in screening study were selected. To select the growth medium and cultivation techniques, representative fungal isolates were grown on HAGEM, potato malt peptone, Czapek Dox's -1 and Czapek Dox's -2 media with both of submerged and static cultivation types. Potato malt peptone medium and the static cultivation types were selected the best ones to increase antioxidant activity of the isolates. The selected parameters were used to evaluate the DPPH free radical scavenging activity of the macrofungi isolated from different parts of Turkey.

Totally 132 macrofungi isolates were examined with the using of ascorbic acid and BHT as controls. Eight selected isolates representing best DPPH free radical scavenging activity were further tested for their additional activities. The activities (%) of the selected isolates for scavenging ability on hydrogene peroxide and ABTS radicals, chelating ability on ferrous ions, reducing power activity, β-carotene-linoleic acid activity, and ferric thiocyanate (FTC) and thiobarbituric acid (TBA) assays for lipid peroxidation were determined as 50.29 - 100.00, 49.79 - 87.07, 3.36 - 3.95, 9.03 - 83.50, 0.03 - 3.59, 38.10 - 78.70, 95.20 - 100.00, and 72.25 - 100.00, respectively. Time dependent antioxidant activities of the selected isolates were also studied during incubation period of 20 days.
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Medical mycology and fungal pharmacology
Poster nr. 27

The nutritional and medicinal value of *Pleurotus* species from Italy

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The genus *Pleurotus* is a cosmopolitan group of fungi which comprises ca. 30 species and subspecific taxa. The genus *Pleurotus* also represents the second main group of cultivated edible mushrooms in the world. The *Pleurotus* species are efficient colonizers and bioconverters of lignocellulosic agro-industrial residues into palatable human food with medicinal properties. Besides *Pleurotus* species demonstrates significant nutritional and their bioactive compounds (mainly polysaccharides) possess antibacterial, antibiotic, antitumor, hypocholesterolemic and immunomodulation properties. Extracts of the Mediterranean culinary-medicinal Oyster mushrooms *P. eryngii* var. *eryngii*, *P*. *eryngii* var. *ferulae*, *P*. *eryngii* var. *elaeoselini*, and *P*. *nebrodensis* were tested for their *in vitro* growth inhibitory activity against a group of bacterial reference strains of medical relevance: *Staphylococcus aureus* ATCC 25923, *S*. *epidermidis* RP62A, *Pseudomonas aeruginosa* ATCC 15442, and *Escherichia coli* ATCC10536. All of the *Pleurotus* species analyzed inhibited the tested microorganisms in varying degrees. The cold-water extracts of *P. eryngii* var. *ferulae* and *P. nebrodensis* can affect the tumor phenotype of human colon cancer HCT116 cells. The *in vitro* antitumor effects indicate that some *Pleurotus* species can be considered as possible sources for new alternative therapeutic agents for cancer treatment. Studies carried out on a new productive strains of "cardoncello" mushroom, *P. eryngii*, a prized Italian culinary-medicinal mushroom, demonstrated that this food is very low in saturated fat, dietary fiber and, a good source of vitamins. The vitamin B_{12} content makes *P*. *eryngii* suitable as a possible alternative food for vegetarians or for those with diets lacking in foods of animal origin.

Poster Session II

Environment, ecology and interactions

Poster nr. 28

Rodents as a dispersal vector for hypogeous fungi

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An interdisciplinary and comprehensive ecological studies have been brought into a rising significance in resent years.
Mycophagy and spore dispersion by animals is widely studied in the world, and there are still new discoveries made in this matter. This strategy is beneficent for both animal and fungus, with one gaining a good food source, and the other having the opportunity to spread spores on long distances and into a new environment. Laboratory studies have shown that passing through the animal's digestive system enhances the germination of the spore after it leaves the animal's gut. Mycophagy studies can also be a supplement for mycological studies, adding new records on species distribution.
Currently our studies are concentrated on the degree in which rodents feed on hypogeous fungi, and can they be a important spore dispersal vector. The studies are carried out by live trapping animals from study areas in the forest ecosystems in Spalski Landscape Park (SPK) in Central Poland and in Jura Landscape Park (JPK) in Kraków-Częstochowa Upland. The animals are captured with baited live traps and after releasing the animals, fecal samples are taken from the traps. The samples are preserved in 90% alcohol, and analyzed microscopically in the laboratory. Identified structures as asco- and basidiospores, asci, basidia and their fragments are used to determine the fungal taxa. The ongoing analyzes of the gathered material has shown the presence taxa from *Glomus*, *Elaphomyces, Tuber* and *Melanogaster* in the samples from SPK, and *Hydnobolites cerebriformis* and species of *Genea, Hymenogaster* and *Melanogaster* from JPK.

Environment, ecology and interactions

Poster nr. 29

Interactions between filamentous fungi found in agricultural and other anthropogenic environments

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Many species of filamentous fungi occur in both agricultural environment and other anthropogenic environments (such as dwellings). Depending on the enviromental context, different relationships arise between diverse sets of eukaryotic microorganisms

competing for nutrients, minerals, water, etc. The agricultural environment is strongly shaped by phytopathogenic fungi (eg. *Fusarium* sp., *Alternaria* sp., *Parastagonospora* sp.) competing for access to the plant hosts. Additional contributions, stem from the involvement of saprobic (eg. *Aspergillus* sp.) and mycoparasitic fungi (eg. *Trichoderma* sp.). In other anthropogenic environments, the composition of fungal population is significantly different. Such niches are dominated by saprophytic fungi (e.g. *Mucor* sp.), animal (including humans) pathogens (eg. *Aspergillus* sp., *Penicillium* sp.) and the role of plant pathogens (*Fusarium* sp., *Alternaria* sp.) is considerably lower. The different conditions (higher temperatures, presence of chemical additives and contaminants as well as residual processed food) can affect both the secondary metabolism and the resulting interactions between different species competing for nutrients.
In our work, we investigate the secondary metabolite-mediated interactions between diverse fungal strains originating from different enviromental conditions (fields, agricultural buildings, schools, old tenement house etc.). We examined changes in morphology, growth patterns, gene expression and toxigenic capability after stimulation in both mycotoxin producing and non-producing isolates of divergent isolates, obtained from different niches.

The research was funded under the Polish National Science Centre research grant "Hybrid, metagenome-based approach to assessing biodiversity and toxigenic potential of fungi in anthropogenic environments" (SONATA/UMO-2011/03/D/NZ2/01435).

Environment, ecology and interactions
Poster nr. 30

Fungi associated with the vector of the pinewood nematode and their influence on pine wilt disease

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The insect *Monochamus galloprovincialis* is the vector of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, causal agent of the pine wilt disease that has killed yearly thousands of pines in Portugal since its detection in 1999. The PWN has an obligatory mycetophagous phase being the development of the nematode populations closely associated with fungi that colonize the declining trees. Moreover, recent works highlighted the simultaneous transport of different fungal species by *Monochamus* insects in other European countries. Nevertheless, no such studies were ever performed in Portugal, and it was our aim to characterize the mycobiota vectored by the insect in Portuguese pine stands, both affected and not affected by pine wilt, and begin to evaluate their relevance for the pinewood nematode development and survival. Fungi were collected from adult insects in various locations, and identified by morphological and molecular characteristics. A considerable fungal diversity was found, divided in cosmopolitan (e.g. *Penicillium* spp., *Trichoderma* spp.), entomopathogenic (*Beauveria* sp.)

and phytopathogenic fungi. The role each fungi plays in the pine wilt disease complex is discussed, as some species are known to be antagonists, neutral or favorable for the nematode´s development in the wood, although special attention is given to the *Ophiostoma* genus (blue-stain fungi), which are preferred food sources for the PWN and can condition the abundance and frequency of nematode-infested vector beetles.

Environment, ecology and interactions
Poster nr. 31

Ectomycorrhizal status of spontaneous herbs and shrubs collected in Alentejo

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Several families of angiospermic shrubs and herbs have been reported to have ectomycorrhizas, but the status of many species, especially those present in areas of Mediterranean influence, needs to be confirmed. Such confirmation may help revaluate their ecological role in the Mediterranean forest ecosystems where they occur. To investigate the presence of ectomycorrhizas in the root systems, a careful collection of soil samples or root systems was undertaken, followed by anatomical analyses to detect diagnostic features for ectomycorrhizas, namely the Hartig net. Ecologically relevant members of Rosaceae, Fabaceae, Ulmaceae, Oleaceae, Rhamnacae, Myrtaceae, Polygonaceae, Caryophyllaceae, Juglandaceae and Rubiaceae were of special interest to this investigation. Other types of mycorrhizal symbiosis that were also detected are described.

Environment, ecology and interactions
Poster nr. 32

The effect of dew on flint and limestone lichen communities in the Negev Desert

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Although lichens are important components of arid ecosystems, the influence of microclimatic factors (particularly dew) conducive to their distribution on rock habitats in the Negev Desert are still not sufficiently investigated. Here, we present a study on adjacent lichen communities inhabiting slightly acidic flint (chert) and slightly alkaline limestone cobbles in the north-facing slopes of two sites in the Negev Desert, Nizzana, NIZ (with long-term mean precipitation of 95 mm and average daily dew amount of 0.1 mm) and Sede Boqer, SB (with long-term mean precipitation of 95 mm and average daily

dew amount of 0.2 mm). The communities on flint consisted of either scarce or lush cover of exclusively epilithic species, while endolithic lichens predominated on limestone. Our results pointed to the fact that while non-significant differences characterized the amounts of dew condensed on both substrates, dew duration on some of the flint cobbles was by ~1.2 longer. With longer dew duration, the cover of the epilithic lichens *Aspicilia contorta* subsp. *hoffmanniana* and *Buellia sorediosa* on flint increased along with the cover of the fruticose lichen *Ramalina maciformis*. Some of the species (*Caloplaca circumalbata* var. *circumalbata*, *Caloplaca flavocitrina*, *Caloplaca oasis*, *Candelariella minuta*, *Diplotomma epipolium*) inhabited both substrates pointing to the possibility that dew duration rather than alkalinity determines their distribution. While not explaining the higher lichen cover on limestone, dew duration explains the significantly higher cover of epilithic lichens and chlorophyll content (per inhabited surface) on flint in comparison to limestone.

Environment, ecology and interactions

Poster nr. 33

Impact of environmental conditions on mycelial growth of *Clathrus archeri*

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One of the main causes of biodiversity loss in ecosystems can be attributed to the invasion of alien species. The impact that these introduced species produce depends on their competition ability for space and nutrients. The fungus *Clathrus archeri,* known as devil's fingers or octopus stinkhorn, comes originally from Tasmania and was introduced in 1914 in Europe being afterwards extended throughout many European countries. In particular, in the Basque Country, *C. archeri* has significantly increased its population since 2002.

The aim of our group is to assess whether the entrance and uncontrolled progression of *C. archeri* in the Iberian Peninsula might compromises the survival of the native saprotrophic species through competition. For that purpose, different laboratory experiments will be accomplished. First of all, *C. archeri* will be cultured in different fungal culture media (Potato Dextrose Agar, Modified Melin-Norkrans, Malt Extract Agar and Czapek Agar) to determine its growth rate and nutritional requirements. Subsequently, this species will be undergone at different incubation temperatures (15°C, 25°C and 30°C), water potentials (from -0.62 to -2.85 MPa) and a pH range from 3 to 9 to simulate its ecophysiological potential, and try to know its response to alterations caused by climate change. Finally, an *in vitro* confrontation with native saprotrophic species such as *Lepista nuda*, *Clitocybe nebularis* and *Hebeloma radicosum* will be developed to observe if competition effect does exist.

Environment, ecology and interactions
Poster nr. 34

The survival of micromycetes exposed to space conditions

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The survival of fungal spores exposed to space conditions was studied. The experiment was carried out on the Foton-M4 spacecraft. The fungal spores were kept in sterile quartz sand (10^5 per gram) in Eppendorf tubes: (1) inside the Foton descent capsule and (2) in containers on the external surface of the capsule, exposed to the open space. 20 species from 14 genera recovered from the white-stone and plaster of the cultural monuments were tested. After 45 days of flight, the spores of 14 of 20 species stored in the capsule survived, and 13 of 20 exposed to the open space. *Acremonium furcatum*, *Engyodontium album*, *Epicoccum nigrum*, *Mortierella alpina*, *Sarocladium strictum* and *Verticillium zaregamsianum* did not survive neither in capsule, nor in outer containers. *Trichoderma harzianum* and *Geomyces pannorum* stayed viable only in the capsule (10^5 spores per gram for both). Spores of *Chaetomium globosum*, *Purpureocillium lilacinum*, *Aspergillus niger* and *Lecanicillium kalimantanense* survived better inside (10^4 to 10^5) than out of the capsule (10^2 to 10^4). The rate of viable spores of *Acremonium charticola*, *Cladosporium sphaerospermum*, *Penicillium aurantiogriseum*, *P.chrysogenum*, *P.verrucosum*, *Sarocladium kiliense* was statistically similar in both types of conditions and decreased by 1 to 3 orders after the flight. Another two species, *Acremonium potronii* and *Aspergillus versicolor*, possessed high viability after flight in the capsule (10^4 and 10^5 respectively) and in the open space as well (10^5 for both). So micromycetes can be used as a model for studying eukaryotic organisms' resistance to stress factors.

Environment, ecology and interactions
Poster nr. 35

Diversity of root endophytic fungi from salt marshes plants and their potential benefits on crop salinity tolerance

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Soil salinity is one of the main problems for agriculture nowadays, affecting land degradation, desertification, and crop productivity.
Root fungal endophytes can interact with the plant host in different ways, including by establishing a symbiosis, which provides benefits to plants (increasing tolerance to salt).

The aim of this work is to assess the diversity of root endophytic fungi in different plants of salt marshes and select some of the isolates with benefic effects on plants tolerance to salinity. To achieve this goal the effects of that isolates were assessed by the inoculation of tomato plants and barley.

Four species of halophytic (*Aster tripolium, Arthrocnemum macrostachyum, Halimione portulacoides* and *Spartina maritima*) were collected in different places of two salt marshes along the Tagus estuary known to have distinct soil salinities. After superficial sterilization roots pieces were inoculated on three media, Potato dextrose agar, Malt agar and Modified Melin Norkrans agar (MMNA) to obtain fungal isolates that were grouped in morphotypes according to colony morphology, for further identification. To study the effect of salinity in the growth of the fungi, two of the most frequent morphotypes in each plant species were inoculated in MMN broth containing 0, 0.25, 0.5 and, 0.75 M of NaCl. Fungal biomass was evaluated within 10 days. The fungal morphotypes with larger biomass at higher concentration of NaCl were inoculated in plants with different salinity tolerance: tomato plants and barley. The results are discussed in an applied perspective of root fungal endophytes as potential inoculants to ameliorate crop salinity tolerance.

Environment, ecology and interactions

Poster nr. 36

Mercury in mushrooms of genus *Leccinum* from Yunnan Province of China and Europe in Poland: accumulation, distribution and probable dietary exposure

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Reported are the results of the determination of mercury (Hg) in soil substratum and the accumulation and distribution of Hg in six species of mushrooms of the genus *Leccinum* from geographically diverse regions of Yunnan in China and eight species from Poland. Also assessed was the probable dietary intake of Hg from consumption of Leccinum spp. The results showed that mushrooms such as *L. chromapes, L. extremiorientale, L. griseum* and *L. rugosicepes* are good Hg accumulators and contained Hg in caps ranging from 2.1 to 4.7 mg kg^{-1} dm, and in stipes were from 0.56 to 2.8 mg kg^{-1} dry matter. The species, *L. rufum* and *L. quercinum* from Poland with Hg in caps of about 1.0 mg kg mg kg^{-1} dm were better accumulators than *L. albellum, L. duriusculum, L. melaneum, L. scabrum, L. versipelle* and *L. vulpinum*, which showed about 0.5 mg Hg kg^{-1} dm, on an average. Hg content in caps and stipes of the fruiting bodies of *Leccinum* spp. from locations in China and Poland showed a weak positive tendency for increase with increase of Hg content of soil substratum. Consumption of fresh caps of mushrooms of genus *Leccinum* with elevated Hg from locations in Yunnan at the rate of up to 300 g per week during the foraging season would not result in Hg intake exceeding the provisional weekly tolerance limit of 0.004 mg kg^{-1} body mass, assuming no Hg ingestion from other foods.

Environment, ecology and interactions
Poster nr. 37

Cd, Hg and Pb in mushrooms *Boletus badius*, *Xerocomus chrysenteron* and *Xerocomus subtomentosus*

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Some species of fungi can accumulate toxic metallic elements such as Hg, Cd or Pb in fruiting bodies at elevated concentration even if they emerged at background uncontaminated areas. Intake of Hg, Cd or Pb from food is under surveillance by health authorities and an appropriate tolerance levels for foods and the values of provisionally tolerable weekly intake (PTWI) and tolerable weekly intake (TWI) have been established by World Health Organization (WHO). Mushrooming is a part of gourmet heritage of Poland, while intake rate *per capita* of wild grown mushrooms is associated with family tradition, location and income. This aim of this study was to examine the Hg, Cd and Pb contents of fruiting bodies of *Boletus badius*, *Xerocomus chrysenteron* and *Xerocomus subtomentosus* that emerged at three spatially distant locations in the agricultural and forested regions of Poland. The probable dietary intake of these metallic elements by mushroom consumers and the associated risk to health were also evaluated. Cadmium is a major contaminant in all three species followed by lead and then mercury. The most contaminated species was *X. chrysenteron* followed by *B. badius* and then *X. subtomentosus*.

Environment, ecology and interactions
Poster nr. 38

Soil filamentous fungi with potential to solubilizing phosphate on agroforestry system

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Agroforestry Systems (AFS) are known models of soil exploitation systems that are ecologically more similar to natural forests and, therefore, they are considered an important alternative for sustainable use of tropical ecosystems. The type of soil management influences its physico-chemical conditions and is reflected in the development of microorganisms and their biological properties. Among the macronutrients in soil, phosphorus is outstanding for living beings due to its structural and functional performance and energy transfer; however, it is poorly available in Brazilian soils. Microorganisms are recognized for their ability to promote biochemical

transformations of nutrients and to provide important nutrients to the plants. The presence of phosphate solubilizing fungi and management of their populations in the soil are alternatives for improving phosphorus supply to plants. The aim of this study was to identify fungal species from an agroforestry soil and analyze semi-quantitatively their capacity to solubilizing phosphate sources. The fungal colonies were isolated and identified to species. One colony from each species (totaling 80 individuals) was tested against monobasic and dibasic phosphate. Confirmation of hydrolysis activity on Petri dishes was based on the substrate degradation halo using the Enzyme Relation Index (ERI). Seventy percent of the species tested solubilized the phosphate sources. *Aspergillus niger, Penicillium echinulatum* and *Fusarium solani* showed the greatest potential. The knowledge about PO4 solubilizing potential of soil fungi aids guiding the use of this microbial community to improve plant development while reducing costs with fertilizers and the impacts of excessive chemical release to the environment.

Environment, ecology and interactions

Poster nr. 39

Accumulation of metals and metalloids in ectomycorrhizae from smelter-polluted soil

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Samples of ectomycorrhizal (ECM) tips and non-mycorrhizal fine roots were collected from strongly smelter-polluted Oe soil horizon at Picea abies forest plantation and analyzed for metal/metalloid content. Large variation of element concentrations was observed both among ECM tips and non-mycorrhizal fine roots. The ECM tips were particularly enriched with Ag, As, Cd, and Zn. Fungal species forming the ECM tips were identified by DNA sequencing; *Boletus badius* and *Thelephora terrestris* were the most abundant species in the sample set. Distinctly elevated concentrations of V were found in ECM tips of *Amanita muscaria*, which is known to accumulate this element.

As the concentrations of metals detected in ECM tips refer to both fungal and plant biomass, we have attempted to quantify the fungal biomass in ECM tips of two macrofungal species: *A. muscaria* and *B. badius*. In both species, two independent primer pairs, including species-specific and non-specific primers, were used in qRT-PCR with double-stranded DNA-binding dye. PCR outputs were calibrated against pure mycelial biomass. The results of PCR (expressed as % of sample dry weight) obtained for 11 samples of *A. muscaria* and 19 samples of *B. badius* were similar but varied within a relatively large range of 0.8-30% and 0.5-37%, respectively. However, median values were 4.8% for *A. muscaria* and 5.7% for *B. badius*.

Our results indicate high capacity of ECM mycelia to accumulate toxic metals/metalloids. Besides the protective role in symbiosis with their host plants, ECM fungi apparently contribute to biotransformation and retention of toxic elements in polluted soils.

Environment, ecology and interactions

Poster nr. 40

Notes on trace elements and minerals composition of King Bolete (*Boletus edulis*)

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Presented are data on occurrence multivariate analysis on interrelationships of Ag, As, Ba, Be, Bi, Cd, Co, Cu, Cs, Hg, Li, Mo, Ni, Pb, Rb, Sb, Sn, Sr, Ti, Tl, Th, U, W, V, Zn, Zr and rare earth elements (RREs) in by *Boletus edulis* collected from several distantly distributed forest complexes in Poland as well as is assessed risk to health resulting from probable dietary intake of toxic Hg, Cd and Pb. Particularly large differences in the contents of the elements from fruiting bodies were observed for such elements as Ba, Bi, Ce, Co, Dy, Eu, Er, Gd, Hf, Ho, La, Li, Lu, Nb, Nd, Pr, Sm, Sr, Tb, Th, Tm, Y, Yb, Zr (coefficient of variation was greater than 100%), slightly less variability was observed for Sc, Sr, W and U. All tested consignments from thirteen sites were characterized by low coefficient of variation (less than 20%) for As, Cu, Ge, Hg, Ni, V, Zn. In a view of the values of tolerance limits and tolerated intake available for Cd, Hg and Pb, the most problematic element in fruiting bodies of *B. edulis* collected from background areas in Poland is Hg but if consumed in moderate quantity will not pose a risk to health of consumers.

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Environment, ecology and interactions

Poster nr. 41

A study on impact of added lithium on accumulation of mercury by *Agaricus bisporus*

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The enrichment of fruiting bodies or mycelium of cultivated mushrooms with selenium, lithium or other essential compounds is considered as a promising way of obtaining functional food and nutraceuticals that could be rich in desired constituents [1-3]. The aim of this study was to examine an impact of fortification of compost with increasing

doses of lithium salt on co-bioconcentration of mercury (Hg) in fruiting bodies by edible mushroom *Agaricus bisporus*. Lithium (Li_2CO_3) was added at doses of 0, 1.0, 5.0, 10, 50, 100 and 500 mg kg^{-1} dried compost. Lithium at dose up to 100 mg kg^{-1} had no effect on growth and yield of fructification, while at dose of 500 mg kg^{-1} fructification was completely inhibited. Mercury content was determined separately in caps and stipes of *A. bisporus* using well validated analytical method and cold-vapour atomic absorption spectroscopy (CV-AAS) for final measurement. The analytical data obtained showed that fortification of compost with increasing doses of lithium in form of Li_2CO_3 caused decreased uptake and sequestration of mercury in fruiting bodies of *A. bisporus*.

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Environment, ecology and interactions

Poster nr. 42

Mineral profile of tubers of 'king tuber oyster' (*Pleurotus tuber-regium*) mushroom from Nigeria

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The mushroom *P. tuber-regium* is edible and popular in Nigeria with many medicinal applications. Knowledge of mineral and toxic metal contents of mushrooms is important in evaluating the safety and mineral intake benefits.

Sclerotia of *P. tuber-regium* were collected from rural areas of Isuikwuato (Abia State) and Okigwe (Imo State) towns in Nigeria. The contents of 19 trace elements (Ag, Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, Sr and Zn) were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

The concentrations of K and Mg were observed to be very high (500-10000mg/kg, dry weight), followed by Ca and Na (19-1700mg/kg). The median concentrations of Fe, Zn, Al, and Mn were generally >10mg/kg, followed by Rb, Cu, Sr, Ba, and P with median between 1.1 and 11mg/kg. The median concentrations of Cr, Ni, Ag, Co, and Cd in the sclerotia of *P. tuber-regium* were generally low and <1 µg/g.

The results of this study provide detailed information on the micronutrient benefits of foods prepared with the sclerotia of *P. tuber-regium,* and that there is no toxicological health risk in their consumption. The data show that the sclerotia would serve as a dietary source of Zn, Mn, Mn, Fe, Cu, Ca, Co, Cr, K, and Na. The concentrations of the toxic metals Cd and Pb were generally low and below the European Union limits for these metals in fungi, indicating that the sclerotia of *P. tuber-regium* are safe for human consumption.

Environment, ecology and interactions
Poster nr. 43

Mercury contamination of wild grown *Pleurotus ostreatus* from Nigeria: assessment of bioconcentration potential and intake risks

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Many edible mushroom species are valued in gourmet tradition around the world. In Nigeria, Pleurotus mushroom are considered delicacies and are well foraged. Because of the toxicity of mercury, its contamination of foodstuff is of concern.
To assess mercury accumulation potentials and intakes by the Oyster Mushroom (*P. ostreatus*), samples of *P. ostreatus* and the wooden substrate on which they develop were collected from the wild in Southern Nigeria and evaluated for their Hg contents using CVAAS.
The mushroom samples showed very low Hg contents that varied from 18 to 58 ng g^{-1} for the caps and from 21 to 83 ng g^{-1} for the stipes. The mean Hg contents of the wooden substrate varied from 16±4 ng g^{-1} to 19±8 ng g^{-1} (overall range, 9-38 ng g^{-1}). The Hg distribution was nearly equal between caps and stipes of *P. ostreatus* with the mean values of cap to stipe Hg concentration quotient ($Q_{c/s}$) ranging from 0.9±0.4 to 1.1±0.3. *P. ostreatus* is a weak Hg accumulator as the mean BCF for both caps and stipes ranged from 1.6 to 2.2.
For the sites investigated, consuming about 300 g of *P. ostreatus* will result in Hg intakes ranging from 0.84-0.93 μg for caps and 0.84-1.11 μg for stipes and the weekly consumption will result in Hg intakes less than 0.03% of the recommended provisionally tolerable weekly intake (PTWI) for both caps and stipes.
Therefore, consumption of *P. ostreatus* harvested from the areas investigated pose no toxicological health risks to human health.

Environment, ecology and interactions
Poster nr. 44

Host specificity and geographical distribution of *Fomes fomentarius* genospecies in European woody plants

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For the processing of fungi, species delimitation is a critical point since it is important to characterize and document the starting material for every study conducted. These problems also apply to *Fomes fomentarius* (L.) Fr. (Polyporales, Agaricomycetes, Basidiomycota) which comprises at least two cryptic sympatric species in Europe as evidenced by ITS, LSU, and *efa* sequence analysis. Morphological variability of its basidiocarps is well documented, however recent studies revealed no constant features which would be suitable for reliable separation of *F. fomentarius* species. The host specificity and distribution of both genospecies were critically assessed on the basis of all *F. fomentarius* ITS sequences currently available in GenBank database from different tree hosts and geographical regions in Europe. The genospecies B consists of strains isolated from Slovakia, Russia, Slovenia, France, the United Kingdom, and Italy, whereas the genospecies A consists of strains recently isolated from Slovakia, Russia, Slovenia, Austria, Germany, Lithuania, and Latvia. While genospecies A seems to be linked preferably with *Fagus sylvatica* and *Betula* spp. as a hosts, genospecies B is found mainly on other species such as *Tilia* spp., *Populus* spp., and *Quercus* spp.

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Environment, ecology and interactions

Poster nr. 45

Survey of basidiomycetes in wooden constructions: species and decay visualisation

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Lignicolous basidiomycetes cause enormous damage to wooden constructions in favourable growth conditions. Private, public and heritage buildings are subjected to fungal damage.

According to our latest data, 106 species of the phylum Basidiomycota have been identified in Latvian buildings. The fungi were collected from wooden structures indoors (roofs, walls, ceilings, floors, stairs) and outdoors (roofs, walls, windows, doorsteps, stairs, logs, benches, fences, bridges, stages, beehives).

The brown-rot damage was recorded in 76.3% cases and white-rot – only in 23.7% cases. The most frequent brown-rot basidiomycetes in buildings were *Serpula lacrymans* (47.3%), *Antrodia* spp. (*A. sinuosa, A. vaillantii, A. serialis, A. xantha*) (11.5%) and *Coniophora puteana* (5.3%). These species were found mainly on the interior woodwork. The white rot was caused mainly by corticoid species (Corticiaceae) such as *Athelia* spp., *Hyphoderma* spp., *Hyphodontia* spp., *Botryobasidium* spp., *Hypochnicium* spp., *Tubulicrinis* spp., *Phlebiopsis gigantea* and *Resinicium bicolor.* Corticoids were found primarily on the exterior woodwork. The mycobiota of wooden constructions was equal to that of natural environment with the exception of the exclusive indoor fungus *S. lacrymans.*

The visualisation of the brown-rot degradation pattern of wood cells was performed by light- and fluorescence microscopy, and UV microspectrophotometry (UMSP). The distribution of fungal hyphae within wood and degradation of wood cells was investigated by cultivation of the brown-rot fungus *C. puteana* on pine wood. The results showed very fast initial colonisation of rays followed by tracheid wall degradation. Investigation on wood biodegradation provides useful information for scientifically based heritage restoration and renovation.

Environment, ecology and interactions

Poster nr. 46

Investigation of lichenicolous fungi in Hungary - *Xanthoriicola physciae* (Kalchbr.) D. Hawksw.

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Our knowledge of lichenicolous fungi was poor in the middle of the last century, the majority of the specimens was reported from the historical area of the country. *Xanthoriicola physciae* is interesting because it has one of the oldest reports by a Hungarian mycologist, Hollós, in 1913, from Kiskunság. Although in collections there are more specimens with annotations concerning parasites or black forms, no one mentioned it in flora lists.

In the last decade our research group started to pay more attention to this fungi and collected samples intensively from the whole area of the country. *Xanthoria parietina* was chosen as a first host, because of its wide ecological tolerance and easily recognizeable thallus. Our first preliminary results showed that its most common parasite, *Xanthoriicola physciae,*was distributed mainly in the lowlands, but soon more montainous locations were found.

After 100 years of Hollós finding we recognized that the area of Kiskunság is rich in lichenicolous species, not only *Xanthoriicola physciae*, but many other xanthoriicolous species, wich occours usually together.

Environment, ecology and interactions

Poster nr. 47

Aquatic Hyphomycetes in Pernambuco: a first approach

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Fungi with tetraradiate, sigmoid or star-shaped conidia that live in aquatic environments are known as aquatic Hyphomycetes. The hydrodynamic shape of their conidia aids in their dispersion during asexual reproduction placing these fungi among the most important decomposers in aquatic environments. Diversity of these organisms in the Atlantic Forest of Brazil is as yet poorly studied. To increase knowledge about these fungi in the Northeast of Brazil, several samples of submerged leaf litter were taken from August 2011 to May 2015 from four aquatic systems (River Jaboatão, River Carnijó, artificial lake Dois Irmãos and River Capibaribe) in the metropolitan region of Recife, Pernambuco, where water temperature range from 23 to 32 Celsius. The leaf litter was washed in running tap water, cut into 1cm2 pieces and incubated at room temperature in Petri dishes containing sterile distilled water. Microscope analyses of leaf fragments revealed predominance of *Triscelophorus monosporus* Ingold., *Lunulospora curvula* Ingold., *Monotosporella microaquatica* (Tubaki) S. Nilsson. and *Blodgettia indica* Subram. Species of *Anguillospora, Campylospora, Colispora, Dactylella, Dendrosporium, Endophragmiella, Flabellospora, Idriella, Pyramidospora, Tricladium, Triscelophorus* and *Wiesneriomyces* were also found. Most of the detected species are referred for the first time in the Northeast of Brazil. Water variables such as temperature, pH, O2 saturation and conductivity did not shown any direct relationship with the diversity of the aquatic fungi. However, diversity of submerged leaf litter and moderate water turbulence favour the occurrence and richness of aquatic Hyphomycetes.

Environment, ecology and interactions

Poster nr. 48

Mycodiversity of two Italian caves

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In recent years, many mycological researches have been addressed to the study of microfungal communities inhabitants in extreme environments. Despite caves represents a typical example of such environments, only limited number of studies were conduced and the knowledge of the related mycoflora is overlooked. Caves are particular semi-closed environments characterized by strong and specific ecological parameters: mainly lack of nutrient and darkness.

In this framework, a work package of the CAVELAB project (funded by Compagnia di San Paolo and University of Turin) is aimed at surveying the fungal communities living in two different Italian caves: Bossea Caves (Piedmont), and Hypogean System of Kronio Mountain (Sicily).

All in all, 8 surveys were carried out and 42 samples of soil were collected during the period 2011-2014. Fungi were isolated from the samples using the modified dilution plate protocol on Malt Extract Agar added with Chloramphenicol, Rose Bengal, and Sabouraud. This protocol allows us to count, identify and isolate vital strains from the environment surveyed. On the whole, more than 1700 MTUs belonging to 22 genera of filamentous fungi were found on 212 Petri dishes inoculated. The most frequent species of filamentous fungi belong to the genus *Aspergillus*, *Mucor*, *Penicillium*, *Trichoderma.* Furthermore, a number of interesting species were isolated such as *Metarrhizium flavoviridae* var. *flavoviridae,* which was recorded for the first time in a cave system.

Our results underline the mycoodiversity of hypogean environments and, in particular, shows that the anthropogenic influence strongly affects the mycobiota in such semi-closed environments.

Environment, ecology and interactions

Poster nr. 49

Genetic diversity of the chestnut blight fungus Cryphonectria parasitica and its associated hypovirus in Portugal

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The European chestnut (*Castanea sativa* Mill.) agro-ecosystem has been of high social, economic, and landscape importance in Portugal. Chestnut blight caused by the fungus *Cryphonectria parasitica* is considered a major cause of the decline of chestnut trees across Europe. *C. parasitica* is an ascomycete (Diaporthales) that is native to eastern Asia. Infection of chestnut trees with this pathogen is typically associated with extensive bark necrosis (so-called cankers) on stems and branches, resulting in the subsequent death of the part of the tree above the infection point. Chestnut blight in Portugal was first reported in 1992 and since expanding in distribution. Here, we investigated the invasion history of *C. parasitica* and its associated hypovirus in Portugal. For this, we characterized 137 isolates collected between 2013 and 2014 in four chestnut stands for virus-infection, vegetative compatibility (vc) type, mating type and microsatellite haplotype. A total of 33 haplotypes and four vc types were observed, although the Portuguese *C. parasitica* population is currently dominated by a single haplotype and a single vc type (EU-11). Further diversification may be expected due to ongoing sexual recombination, but eventually also to new migration and additional introductions. *Cryphonectria hypovirus* 1 (CHV-1) was found in two populations. Genetic analysis of the six CHV-1 isolates obtained revealed that three viral strains belong to the Italian subtype and three to the French subtype, which suggest different, independent introductions.

Keywords: Chestnut, *Cryphonectria parasitica*, microsatellite

Environment, ecology and interactions

Poster nr. 50

Effect of ectomycorrhizal symbiosis on the features of *Populus x canescens* seedlings under lead ion stress

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Lead represents toxic elements harmful to living organisms even at very low concentrations. However, some plants, e.g. poplars, are able to withstand exposure to lower Pb^{2+} concentrations due to highly developed defence mechanisms including the protective action of root symbionts. One of well known effects of symbiosis with ectomycorrhizal (ECM) fungi is the increased resistance to heavy metal stress. This increased plant tolerance is commonly believed to be caused e.g. by an improvement in the nutrient and water uptake by the plant (in exchange for its carbohydrates) or direct protection against lead ions, which may be deposited in fungal mantle cells and therefore prevented from penetration into plant cells.

On the other hand, reports on the ECM-driven heavy metal resistance are varied, probably due to the fact that interactions between the two living organisms are complicated and the final protective effect depends on numerous conditions, primarily the characteristics of particular symbiotic partners.

In this work, we present the results of an experiment performed on poplar, *Populus x canescens,* inoculated *in vitro* with two strains of *Paxillus involutus*. The field-collected strains showed significantly different values of indices of tolerance for lead ions as well as different levels of root mycorrhization. The differences were not only reflected in the biometric features of inoculated poplar seedlings, but also in the mineral composition and leaf proteomes of poplars.

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Field mycology and conservation

Poster nr. 51

Local names for common wild edible mushrooms growing in Europe, North Africa and the Kingdom of Saudi Arabia

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Mushroom hunters in rural areas call and identify wild edible mushrooms on the basis of their local or common names. Local names of mushrooms are also widely used in folk medicine and particularly in shamanic and religious rituals. Linking of local names with their respective scientific names is of fundamental importance for the exploitation of their market potential and for prevention of poisoning. We present a list of common names given to 45 wild edible mushroom taxa (28 basidiomycetes and 17 ascomycetes) occurring in Austria, the Czech Republic, France, Greece, Italy, the Kingdom of Saudi Arabia, Morocco, Romania, Serbia, and Spain. The selected taxa are *Agaricus campestris*., *A. crocodilinus*, *Amanita caesarea*, *Boletus aereus*, *B. edulis*, *B. pinophilus*, *B. reticulatus*, *Calocybe gambosa*, *Calvatia gigantea Cantharellus cibarius*, *Craterellus cinereus*, *C. cornucopioides*, *C. tubaeformis*, *Cyclocybe cylindracea*, *Delastria rosea*, *Hydnum repandum*, *Infundibulicybe geotropa*, *Lactarius deliciosus*, *L. salmonicolor*, *L. sanguifluus*, *L. semisanguifluus*, *Macrolepiota procera*, *Morchella elata*, *Picoa juniperi*, *P. lefebvrei*, *Pleurotus eryngii*, *P. eryngii* var. *ferulae*, *P. nebrodensis*, *P. ostreatus*, *Podaxis pistillaris*, *Russula cyanoxantha*, *R. virescens*, *Terfezia arenaria*, *T. boudieri*, *T. claveryi*, *T. leptoderma*, *Tirmania nivea*, *T. pinoyi*, *Tuber aestivum*, *T. asa*, *T. borchii*, *T. brumale*, *T. magnatum*, *T. melanosporum*, and *T. oligospermum*. The national and the regional common names (if known) are also reported.

Field mycology and conservation

Poster nr. 52

Improving edible fungal production: report on the experience gained in a North-Western Italian forest (Liguria)

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Wild edible ectomycorrhizal fungi represent one of the major and valuable non-timber forest products worldwide. The progressive abandon of forest areas has significantly reduced the production of many edible mushrooms. The maintenance and increase of fungal production require suitable management practices and sylvicultural choices. In Italy scarce attention was paid on how the sylvicultural practices affect the fungal production and diversity. The European project ALCOTRA *Amycoforest* have involved Italian and French institutions with the aim to develop a sylviculture that harmonizes wood and fungal production according to the concept of multiple-use forestry. Within this

framework, an experimentation was conducted in two stands of a natural silver fir forest of Liguria (North-western Italy) to test a sylvicultural treatment and management with the final goal to observe possible effects on fungal production. Specifically, two edible ectomycorrhizal species, particularly appreciated in Liguria and in Italy, were chosen: *Boletus edulis,* and *Hygrophorus marzuolus*. The possibility to develop sylvicultural practices devoted to increase their production may positively affect the economy in rural areas.

During the 2-years of the project, mycological investigations were carried out in order to monitor macrofungal communities trends. Specifically, the impact of sylvicultural treatments on mycodiversity and fungal biomass production was observed. Preliminary results (after two years) appear to highlight favorable effects of forestry practices on fungal production. The values of biodiversity indices (i.e. Shannon Index) and biomass were higher after the treatment than before. Geologic and vegetation aspects were also taken into account in order to characterize the favorable growing habitats.

Field mycology and conservation

Poster nr. 53

The OPTIMA (Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area) Commission on Fungi

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A list of proposed activities/objectives by the members of OPTIMA Commission on Fungi is here reported:
Prepare a list of local names related to wild edible mushrooms (WEM); Define a provisional catalogue of macrofungi that could be characterized as typical-representatives of the Mediterranean region (MR); Publish a Checklist of all macrofungi occurring in the MR; Setup of a literature database on fungi occurring in the MR; Promote studies on Mediterranean fungi to be used as food and medicine, and examine their potential in other biotechnological applications (e.g. mushroom cultivation, treatment and detoxification of wastes etc.), incl. large-scale (commercial) use; Document ethnomycological knowledge in the MR (incl. traditional use of mushrooms in local communities); Prepare a list of threatened fungal species (with emphasis on those of economic importance) in the MR and adopt suitable strategies for their conservation. Assess invasion and determine possible risks from alien species. Examine existing legislation and regulations on harvesting of WEM and on quality of fungal products; Promote mycological education and disseminate fungal-related knowledge in a wider audience; Examine existing protocols used for various experimental purposes in fungal taxonomy (e.g. molecular systematics, phylogeny, chemotaxonomy, characterization of secondary metabolites, proximate analyses, etc.) and possibly adopt common improved approaches/techniques to deal with pertinent issues; Examine the possibilities for networking the Institutions of participating members in order to seek and obtain funding from the E.U. or other national and international Organizations. Assess the potential of collaboration with other Societies, SME's and/or NGO's having pertinent interests and priorities.

Innovative silvicultural treatments to enhance soil biodiversity in artificial black pine stands: monitoring mycological diversity

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The data presented in this work are part of a LIFE Biodiversity project (SELPIBIOLIFE) presented by 5 partners with the main goal to demonstrate the positive effects of an innovative silvicultural treatment on black pine forests. The specific management applied improves growth rates and stands stability and enhance the level of biodiversity of various soil components (flora, fungi, bacteria, mesofauna, nematods and microarthropods).

The results regarding the fungal community present before silvicultural treatment in round plots of 314 m2, localized 27 on Pratomagno and 27 on Mount Amiata (Tuscany, Italy), are here reported. The study follows classic mycocoenological method counting and identifying fruit bodies of all macromycetes; fungal biomass (fresh and dry weight) weighed to the nearest 0.01 g, was also detected. Over the study period (September-December 2014) 6704 carpophores, 180 different fungal species, with a fresh and dry weight of 44,953 and 5,395 kg respectively, were observed. The most frequent species on Pratomagno was *Russula xerampelina*, followed by *Chroogomphus rutilus, Clitocybe nebularis, Inocybe geophylla*. On M. Amiata *Galerina marginata* was collected in 23 plots out of 27 and then less frequent *Hemimycena gracilis, Mycena arcangeliana* and *Phellodon niger*. To underline the high fungal biomass found on Pratomagno with nearly 36 kg (on M. Amiata it was only 9 kg) due principally to *Clitocybe nebularis* (13 kg). The values of the Shannon and Pielou indexes indicated that the two communities were characterized by a low diversity and a good evenness of the species.

Field mycology and conservation
Poster nr. 55

Implications of exotic *Pinus radiata* plantations for macrofungal diversity in the Urdaibai Biosphere Reserve (Northern Spain)

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To analyse the implications of exotic radiata pine (*Pinus radiata* D. Don) plantations for macrofungal diversity, a comparative mycocoenological survey was conducted over three consecutive years in pine plantations and native oak forests (*Quercus robur* L.) in the Urdaibai Biosphere Reserve. Macrofungal diversity was analysed at species and community levels, and multivariate techniques were used to obtain insight into the fungal community in all functional groups.
A total of 513 species was recorded, with ectomycorrhizal fungi being the most abundant, followed by the lignicolous saprotrophs. Measurements at both the overall level and the plot level showed that oak forests were richer in macrofungal species than pine plantations. The two ecosystems shared 107 species, but they possessed distinct species assemblages in all functional groups, as confirmed by PERMANOVA analysis. Although more abundant in oak stands, host-specific fungi were recorded in both ecosystems, revealing that exotic pine plantations can easily access fungal inocula from natural pine forests in the vicinity. However, even though the macrofungal richness in plantations is comparable with that in other native conifer forests in Europe, the community was made up of generalists, i.e., species that were not habitat specialists. In contrast, rarely reported, uncommon fungi (e.g. *Aleurodiscus wakefieldiae, Boletus spretus, Xerocomus pelletieri)* were recorded in oak plots, revealing the importance of residual, native forest patches for fungal conservation.

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Field mycology and conservation
Poster nr. 56

Evidence of fungal biodiversity recovery in a model of anthropogenic impact reduction

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At Mitra campus (University of Évora), a part of the forest covering was fenced since 1991, to allow for the reestablishment of natural vegetation away from the impact of

cattle (goats), along with a drastic reduction of human presence. The surrounding areas remained under this impact until 2008. Collections of macrofungi were made in two contiguous areas, separated by the fence, during the Autumn 2014 fruiting season. The comparison between the resulting species lists reveals a strong contrast between the two areas. It is likely that this contrast is due to the difference in successional stage. We discuss the possibility that some of the collected macrofungi are, together with the vegetation, indicators for the different stages.

Field mycology and conservation

Poster nr. 57

Macrofungal communities of two native oak woods (*Quercus faginea* subsp. *broteroi* and *Q. rotundifolia*) in Central Portugal, with a study of sampling methods

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Despite the growing focus of research on fungal diversity in Mediterranean regions, the macrofungal flora of Portugal remains comparatively unexplored. The present study is a preliminary fulfilment of the urgent needs for renewed and expanded mycological studies that cover Portugal's natural habitats. We selected two native oak woods in protected areas of Central Portugal to record the epigeal macrofungal diversity and to test hypotheses on sampling methods and environmental variables. Transects were intensively collected using two methods, plot-based and opportunistic, with standardized sampling efforts. Environmental variables (vegetation, soil, topology, weather) were characterized to derive causal relationships to the diversity patterns observed. Results showed high diversity in both habitats, with a total of 250 and 230 taxa recorded in a single fruiting season, for the *Q. faginea* and *Q. rotundifolia* habitats respectively. Several taxa are first references for Portugal. Significant differences in species richness were found between the two methods, while retaining a similar distribution by major trophic groups. The Sørenson similarity coefficient between the two habitats was 0.4, and the Morisita-Horn index 0.468. The high diversity, and the new records found in this study, emphasize the richness of the macrofungal flora associated with these habitats and the paucity of its knowledge in Portugal. Overall, these results highlight the ecological value of protected habitats, and call for further research as well as a better integration of macrofungi diversity in the development of conservation strategies.

Field mycology and conservation

Poster nr. 58

Spore is in the air – search for a critically endangered fungus *Cryptomyces maximus*

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Cryptomyces maximus (Rhytismatales) is a very rare ascomycete fungus, found on willows (*Salix cinerea*) in Wales and Scandinavia, and which is classed as 'Critically Endangered' (one of the IUCN top 100 threatened organisms). Assessment of the distribution of this fungus, which forms stromata on infected branches in early Spring, is important in order to better focus conservation efforts. The presence of airborne propagules of *C. maximus* was determined using Rotorod-type impaction samplers were used to trap airborne spores onto Vaseline-coatged plastic rods during a sampling campaign in South-West Wales (Pembrokeshire) in March 2014. DNA was extracted from samples and used for NextGen sequencing (Ion Torrent PGM) using fungal-specific primers to amplify the D1 region of the 28S large ribosomal subunit, as well as the ITS2 region of the rRNA operon. Microscopic examination was also conducted on the second replicate of each sampler. Propagules of *C. maximus* were detected at several of the sites which were sampled, and their distribution was correlated with the distribution of ascomata of the fungus. This approach provides a very useful method for the detection of the distribution and abundance of rare fungi. It is possible to determine the pathways of dispersal of such organisms and the environmental conditions conducive to spore release through this method in combination with automatic weather stations deployed in this study.

Field mycology and conservation

Poster nr. 59

Hypogeous fungi of Pienieny Mts. and Gorce Mts., two ranges of the Polish Western Carpathians

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Hypogeous fungi are often neglected in the research on the macroscopic fungi because of their specific ecology. The research carried out in the two montain ranges in Western Carpathians was the first project on diversity of this group of fungi on the local scale. The results presented will concentrate on: (1) the species diversity of hypogeous fungi, (2)

their ecology and distribution in the local scale, (3) comparison of the hypogeous mycobiotabiota of the Pieniny Mts. and Gorce Mts.

Field mycology and conservation

Poster nr. 60

New records of gasteroid fungi from the Brazilian southeastern in Atlantic Forest Biome

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Field expeditions were carried out in Atlantic Forest remnants of the southeastern part of Minas Gerais State, Brazil. Atlantic Forest biome is considered one of the most important hot-spots of the world (Myers & al. 2000). Our goal was to increase knowledge on gasteroid fungi in this biome. The species were analyzed and identified according the specific methodology, available in the literatures of each taxonomic group, as such as Calonge (1998) and Sousa et al. (2014). Eleven species were identified: *Geastrum fimbriatum, G. javanicum*, *G. lageniforme*, *G. morganii*, *G. rufescens, G. saccatum*, *G. schweinitzii*, *G. triplex*, *Bovista cunninghamii, Lycoperdon lambinonii* and *Morganella fuliginea*. There are two first records for Brazil: *B. cunninghamii* and *L. lambinonii.* All specimens were deposited in the UFRN.

Keywords: Geastraceae, Lycoperdaceae, Taxonomy

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Poster Session III

Evolution, biodiversity and systematic
Poster nr. 61

Climatic gradient and soil factors determining the diversity of arbuscular mycorrhizal fungi in corn fields in Northeast Brazil

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The arbuscular mycorrhizal fungi (AMF) aid improving organic matter and energy cycling, leading to increased soil productivity which benefits agricultural and natural ecosystems. The diversity of AMF is influenced by human activities, ecological characteristics, soil factors, type of crop management and climatic differences. This study tested the hypothesis that the communities of AMF in corn fields differ according to the planting locations. Rhizosphere soil samples were taken in plantations located in three areas that characterize a climatic gradient, from rainy area (Zona da Mata) to transition-to-dry (Agreste) to dry semi-arid area (Sertão), in the state of Pernambuco, Northeastern Brazil. The analyses included: quantification of glomerospores, estimation of the most probable number (MPN) of infective propagules, identification of species and ecological evaluations of AMF communities. Morphological analysis of glomerospores allowed the differentiation of 57 species, two of which are new to science: Fuscutata aurea and Paraglomus pernambucanum. The MPN of infective propagules did not differ between areas, however higher density of spores occurred in the area with intermediate moisture, which had lower species richness of AMF. Higher richness and diversity were recorded in the wetter area (Zona da Mata). The structure of the AMF communities differed between locations due to differences in chemical and texture attributes of the soil as shown by the multi-response permutation test procedure (MRPP). Our data confirmed that soils under corn are amenable to establishing AMF and in the areas studied, harbor significant diversity of AMF, with soil and climatic factors determining the occurrence and distribution of species.

Evolution, biodiversity and systematic

Poster nr. 62

Putative and novel fossils of insect-associated fungi from Polish Baltic amber

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Fossil material is very important for better understanding of phylogenetic relationships and evolution of fungi (Beimforde et al. 2014). Unfortunately due to the low preservation potential only few fossilized fungal structures are found among conservation Lagerstätten (as Rhynie Chert) (Barbee&Taylor 2007) where especially conducive taphonomic conditions occured. A very good source of knowledge about ancient organisms including mycocoenosis, relatively rare is fossil resin, amber. Hitherto only few species of fossil fungi from Baltic amber (dated for Eocene epoch), especially those related with insects, were described (Rossi et al. 2005). Our research focused on light-microscopial analyses of inclusions shared by Museum of The Earth of Polish Academy of Sciences in Warsaw - especially those containing beetles, flies and other invertebrates. During investigation we found filamentous fungal forms on plant remains, as well as Mortierella - like structures associated with rove beetles (Staphylnidae: Pselaphinae).

Evolution, biodiversity and systematic

Poster nr. 63

The use of free fatty acids profiling in chemotaxonomy of *Tuber aestivum - T. uncinatum* species complex

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Tuber aestivum Vittad. and *T. uncinatum* Chatin are Ascomycetes producing hypogeus fruiting bodies (truffles). For almost two centuries it has been disputed whether *T. aestivum* and *T. uncinatum* are two distinctive species or a single species producing truffles with different morphologies. *T. aestivum* produces during the summer truffles with a black peridium and a light brown gleba. *T. uncinatum* mainly differed from *T. aestivum* for the presence of hooks in the spore reticulum and because its fruit bodies mature in the late autumn and their gleba generally become more brown than *T. aestivum*. Recently, molecular markers were used to typify ascocarp belonging to these two morphotypes, reaching contrasting conclusions. Free fatty acid (FFA) profiles are used increasingly as a chemotaxonomic tool for the identification and classification of fungi. The objectives of this study were: (i) to analyze the FFA compositions of methanolic extracts of *T. aestivum* and *T. uncinatum* ascocarps, and (ii) to evaluate the use of FFAs profiling to differentiate truffles belonging to the *T. aestivum* and *T. uncinatum* morphotypes. A hierarchical cluster analysis of FFAs present in methanolic extracts from *T. aestivum* and *T. uncinatum* ascocarps evidenced the presence of two ‘natural’ groups according to the harvesting period: summer and autumn. The analyses showed that *T. aestivum* is very homogeneous forming a single group while the *T. uncinatum* samples were quite heterogeneous but all samples are separated from the *T. aestivum* cluster. This research is supported by Fondazione Cassa di Risparmio di Perugia (Perugia, Italy), project code 2014.0094.021.

Evolution, biodiversity and systematic

Poster nr. 64

Mycological taxonomy across five centuries: 239,388 species after *Fungus minimus* Ray (1690)

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The Index Fungorum database (http://www.indexfungorum.org/) records 239,389 fungal species, and another 41,370 infra-specific taxa, most of them being Ascomycota (59%) or Basidiomycota (37%). Their descriptions reflect the work of mycologists and other biologists over the last five centuries, since the first record, *Fungus minimus* Ray (1690). In this communication we analyse the fungal species recorded in Index Fungorum according to their phylogeny, classifiers and year of classification. Some of the most relevant or prolific authors are pointed out, as well as the main trends in taxonomy throughout times. For instance, while the 20th century accounted for over 63% of all fungal species described, the majority of the members of the Peltigerales were described throughout the 19th century, over 50% of the Pucciniales were described in a 50-year period between 1875-1925, most of the Capnodiales, Saccharomycetales, Sordariales, Ustilaginales, Eurotiales and Microascales were described after 1950, and over 60% of the Erysiphales were described in the last 35 years. Attention will also be given to the contribution of Portuguese authors to the progress of Mycology, as well as the relevance of Portugal (Lusitania) to fungal taxonomy. For instance, the olive anthracnose pathogen was first described as *Gloeosporium olivarum* by the Portuguese Phytopathologist Veríssimo de Almeida in 1899. The Portuguese contribution to the mycological taxonomy can also be seen by the use of epithets such as *lusitaniae*, as in the case of the yeast *Candida lusitaniae* that was identified in Portugal, in 1959, by Nicolau van Uden and Lídia do Carmo Souza.

Evolution, biodiversity and systematic
Poster nr. 65

Golovinomyces ambrosiae (Erysiphaceae) associated with *Erigeron annuus* in Korea

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The genus *Golovinomyces* (Erysiphaceae, Erysiphales) is a large genus comprising 52 species in a wide range of different geographical areas throughout the world. Of these, *Golovinomyces ambrosiae* is a destructive fungus causing serious damages on the tribe Heliantheae of family Asteraceae. Within the past 3 years, a powdery mildew fungus morphologically resembling *Golovinomyces* species has been consistently found to be associated with the disease on *Erigeron annuus* in Korea. *Erigeron annuus*, belonging to the tribe Astereae of the family Asteraceae, is an invasive plant, native to North America. The powdery mildew caused by *G. cichoracearum* parasite to the *E. annuus* has been only recorded from North America and this highlights the necessity for a better knowledge of the host spectrum of *Golovinomyces* species. Based on morphological characteristics as well as DNA sequence comparisons of internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA), we confirmed the identity of the Korean powdery mildew fungus as *Golovinomyces ambrosiae.* In conclusion, this is the first case study that provides a significant insight into better understanding of the host spectrum of *Golovinomyces* species from the tribes of Asteraceae or extending geographical distribution in Erysiphales taxonomy.

Evolution, biodiversity and systematic
Poster nr. 66

Corticioid fungi (Basidiomycota) of Madeira: influence of woody host plant diversity on corticioid fungi richness

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The evergreen laurel forest, Laurisilva, is a type of subtropical forest growing on deep soils, in areas with high humidity and mild temperatures. It originated in the Tertiary subtropical forests of southern Europe. Madeira, in particular, harbours one of the largest extant areas of this type of forest, which is designated a UNESCO World Heritage.
In Madeira, this unique forest is mostly located between 300 and 1300m, in the northern part of the island, with a temperate climate, where the prevailing trade winds from the

north-east bring lots of moisture. The trees that compose the canopy, most belong to the Lauraceae family, such as *Laurus novocanariensis, Ocotea foetens, Persea indica*, etc., and are endemic to Macaronesia. In more arid condition dry versions of the moist Laurisilva appear, being *Myrica faya, Erica arborea, Ilex canariensis* or *Apollonias barbujana* more frequent.

The evergreen laurel forest harbour a high diversity of corticioid fungi and this may be explained by the high diversity of woody species of this forest. Of the 113 corticioid species reported from Madeira, 86 were collected in Laurisilva, being *Ocotea foetens, Laurus novocanariensis* and *Erica* spp., the preferred substrata. It is worth to note that two recently described fungi, *Candelabrochaete macaronesica* and *Sistotremastrum guttuliferum*, were reported growing on these substrata, as well as the African species *Gloeocystidiellum kenyense*. The same applies to *Stereum pseudorimosum* (known from the islands of Reunion, Mauritius and Madagascar), and *Subulicystidium perlongisporum* (reported from Reunion Island, Vanuatu Islands and Costa Rica), registered also in this forest.

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Evolution, biodiversity and systematic

Poster nr. 67

Ectomycorrhizal and other sabulicolous macrofungi from sand dune ecosystems of coastal Greece

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Sand dunes ecosystems exist in several locations along the coastline of continental and insular Greece. Although their vegetation has been thoroughly investigated, there is a significant gap as regards pertinent fungal diversity data. In the frame of this work, an extensive sampling of sabulicolous macrofungi was performed in lower, shifting dunes covered by plants of the Ammophilion arenariae and Agropyrion juncei alliances as well as in the upper semi-fixed or fixed dunes dominated by members of the alliances Juniperion lyciae and Oleo-Ceratonion. Sixty collections of larger ascomycetes and basidiomycetes were identified and assigned to 40 species. Among them, the ectomycorrhizal *Geopora arenicola* and *G. foliacea* (Paros Isl., among *Cistus creticus*), and *Inocybe heimii*, *I. juniperina* and *I. rufuloides* (West Peloponnese among *Cistus* spp., *Pinus halepensis*, *P. pinea* and/or *Quercus coccifera*) are reported for the first time in Greece. Moreover, typical for this habitat type saprotrophic species such as *Coprinus xerophilus*, *Geopyxis majalis*, *Entoloma neglectum*, *Hygrocybe conicoides*, *H. pseudo-olivaceonigra*, *Melanoleuca leucophylloides*, *Pithya cupressina* and *Psathyrella ammophila* also constitute new records for Greece.

Acknowledgments
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Evolution, biodiversity and systematic

Poster nr. 68

The taxonomic identity of Odemira's 'pucarinha' (genus *Amanita*, series *Amidella*; Basidiomycota)

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The delimitations among several Mediterranean *Amanita* species belonging to series *Amidella* Neville & Poumarat have been controversial. Macroscopic features within the group are variable and highly overlapping, stressing the importance of relying on microscopy for accurate determination. *Amanita ponderosa* Mal. & Heim is highly regarded for human consumption in parts of Spain and Portugal, but its closest relatives might also be inadvertently collected. DNA sequencing has revealed a cryptic species that occurs commonly among collections of *Amanita ponderosa*, and molecular markers that help discriminate the species within the group have been designed (communication by Oliveira et al.). In Odemira (Southwest Portugal), collections of the locally named 'pucarinha' or 'púcara' of March were found to belong to this cryptic species. New collections from Odemira were made in spring 2015 to clarify its taxonomic identity with a detailed macroscopical, microscopical and ecological characterization. All collections were probed with the mentioned molecular markers. The taxonomic identity of these collections and the cryptic species is discussed.

Evolution, biodiversity and systematic

Poster nr. 69

Pluteus floccipes, a new species from the Czech Republic

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A new species, *Pluteus floccipes*, is described and illustrated based on material from the Czech Republic. It is characterised by a whitish stipe with conspicuous brown floccules, a dark brown pileus with an "opaque" surface, a pileipellis in the form of a euhymeniderm and the presence of cheilocystidia with brown pigment. *Pluteus floccipes* belongs to section *Celluloderma* subsection *Eucellulodermini*. The European species *Pluteus nanus*, *P. phlebophorus* (including *P. luctuosus*) and *P. satur* are morphologically similar to *P. floccipes*. The phylogenetic analysis (ITS rDNA) revealed that *Pluteus floccipes* is closely related to *P. satur*, *P. cinereofuscus*, and *P. eludens*. Distinguishing characters between *Pluteus floccipes* and similar species are described and discussed.

Evolution, biodiversity and systematic

Poster nr. 70

Survey of the mycobiota of mature ears of winter wheat in the main production areas of Slovakia

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Fungi represent one of the major factors which induce deterioration of vitality of ears of winter wheat (*Triticum aestivum*) during growing seasons. A survey of fungal populations in fully mature ears of winter wheat was conducted throughout Slovakia in 2013. A total of 5000 wheat ears in the growth stage of full grain maturity were processed from 118 wheat fields, and 722 isolates of microscopic fungi recovered were identified. Fungal taxa were identified on the basis of the morphology and micrometrical measurements of fruiting bodies and spores found on infected ears. Thirty four species representing 30 genera were recorded; of these 12 were Ascomycetes, 4 Basidiomycetes and 18 Fungi Imperfecti. The fungus with the highest percentage of isolation, and found in the greatest number of fields, was *Fusarium* spp. associated with fusarium head blight, followed by *Stagonospora nodorum* and *Septoria tritici*. Fusarium head blight of small grains caused primarily by the fungus *Fusarium graminearum* and its teleomorph *Gibberella zeae* was found on 39% samples of wheat ears. Fungal saprophytes as *Alternaria* sp., *Cladosporium* sp. and *Epicoccum purpurascens* were also recorded with high frequency. This study is an initial attempt to clarify what ear blight diseases occur on winter wheat in Slovakia, to what extent, and what the fungi are which cause these diseases. This study was supported by the Ministry of Agriculture and Rural Development of the SR, Research and Development project „Genetic improvement of farm traits of important crops".

Evolution, biodiversity and systematic

Poster nr. 71

Hypogeous Basidiomycota of Poland - what we know and what we have to learn.

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Hypogeous basidiomycetes are among the least known groups of macroscopic fungi in Poland. Up to the early 21st century only few localities were known and many species have been treated as rare or even extinct. The observations and research carried out in

the last years revealed high diversity of this group of fungi and revised our opinions on the distribution range, ecology and rarity of several species in this part of Central Europe.

Evolution, biodiversity and systematic

Poster nr. 72

Diaporthe species on *Rosaceae* in Portugal

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The family *Rosaceae* includes a large number of species ranging from herbaceous (*Fragaria*) to woody ornamental plants (*Rosa* and *Pyracantha*) and fruit trees (*Malus* and *Pyrus*). Some *Diaporthe* species have been associated with twig canker, shoot blight, dieback, wood decay and fruit rot on members of the Rosaceae.

Recognition of *Diaporthe* species was originally based on morphology, culture characteristics and host association. However, with the introduction of DNA sequence data for species recognition it became clear that these criteria, especially host association, are not useful to discriminate species. Currently, much effort is being devoted to redefine species using a combination of phylogenetic, morphological and mating data.

In this study we characterised a set of isolates obtained from the following hosts in the *Rosaceae*: *Pyrus communis* (canker), *Malus domestica* (fruit rot), *Pyracantha coccinea* (canker). Isolates were initially subjected to BOX-PCR fingerprinting and representative isolates of each fingerprint type were further characterised by multi-locus sequence analyses (internal transcribed spacer, translation elongation factor 1-alpha, beta-tubulin, histone H3 and calmodulin). These sequences were aligned with sequences retrieved from GenBank and representing known *Diaporthe* species. A maximum likelihood phylogenetic analysis of the combined five loci revealed that the isolates studied were distributed among three clades, of which one corresponds to *Diaporthe foeniculina*. The other two clades, closely related to *D. passiflorae* and *D. leucospermi* represent possible novel species that need to be further characterised in terms of morphology and pathogenicity.

Evolution, biodiversity and systematic
Poster nr. 73

Reconsideration of the genus *Miuraea* based on morphological and phylogenetic analyses

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Miuraea established by Hara(1948) is an anamorphic genus belonging to *Mycosphaerellaceae* and morphologically allied to intermediate between *Pseudocercospora* and *Pseudocercosporella*. Recently, *Miuraea* has been considered as an equivocal genus and excluded within *Mycosphaerellaceae* due to the paucity of information on type species. The objective of this study was to re-establishe the generic concept of *Miuraea* and re-evaluate criteria in species delimitation based on the muti-locus sequences. For this purpose, *Miuraea* isolates and specimens originating from Prunus spp. in Korea were used. Morphological observation showed that great variation in conidial shape and formation used as key characters for identification was induced by changes in environmental condition. Thus, this genus is suggested to be divided into two species by the presence or absence of chlamydospore and host specificity. Molecular phylogenetic analyses performed using the internal trascribed spacer regions, large subunit, actin, calmodulin, histon H3 and traslation elongation factor 1-apha genes revealed *Miuraea* was divided into two species forming distinct clades from *Pseudocercosporella*. Therefore, it is concluded that *Miuraea* should be remained as a separate genus from *Pseudocercosporella* within *Mycosphaerellaceae* including *M. degenerans* and *M. persicae*.

Evolution, biodiversity and systematic
Poster nr. 74

The occurrence of very large genomes in the Pucciniales (Basidiomycota) and genome size variability across fungi

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The completion of genome sequencing for some rust fungi has contributed to suggest a link between biotrophic specialisation and genome size expansion. The measurement of genome sizes for 39 rust fungi using Flow Cytometry has revealed some of the largest genomes among fungi, with nine rust species with haploid genomes between 300 and 893

Mbp (*Uromyces appendiculatus, Phakopsora pachyrhizi, U. transversalis, Hemileia vastatrix* and *Gymnosporangium confusum* genomes were measured as 652, 716, 746, 772 and 893 Mbp, respectively) and one, *U. bidentis*, with a genome size of 2489 Mbp. Genome size information is available for over 1800 fungal species, either arising from Flow Cytometry, genome sequencing, or other methods. Departing from our genome size measurements of Pucciniales fungi, in this work we analyse genome size variability across representatives of the entire fungal phylogeny, relating such variations with relevant biological and genomic traits (life style, sexuality, nutrient use, composition in transposable elements, etc.). The analysis of genome size variation can unveil clues suggesting polyploidisation events or transposable elements activity of evolutionary/adaptive relevance. Such traits can be related to reproduction strategies (sexual, asexual, parasexual and/or rare sexual) and substrate utilization (saprobes, mutualists, obligate/facultative pathogens, biotrophs/necrotrophs and combinations of those).

Evolution, biodiversity and systematic

Poster nr. 75

Taxonomy and phylogeny of the genus *Schizopora* (O. Hymenochaetales)

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The genus *Schizopora* has been traditionally studied as poliporoid or corticioid group, but little is known about the phylogeny between their species. Currently, new methods like molecular biology have demonstrated that classical approaches of morphological or biological species concepts have failed in the task of describe the actual diversity in many corticioid fungi (Schoch et al., 2012). In this study, we address the internal relationships of this genus using two nuclear molecular markers (ITS and LSU) combined with morphological study in more than 150 specimens around the world. Our results show that internal biodiversity of *Schizopora* is higher than those described by exclusively morphological characters and their distribution patterns indicate that ecological conditions could model the evolution of these white rot fungi. This study raises new questions in order to rethink about the hypothesis "Everything is everywhere, but environment selects" (Baas Becking, 1934) in this organisms.

Baas Becking, 1934. Geobiologie of inleiding tot de milieukunde. The Hague, the Netherlands: W.P. Van Stockum & Zoon
Schoch et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal barcode marker for Fungi. PNAS 109 (16): 5907-6354

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Evolution, biodiversity and systematic
Poster nr. 76

Genome wide studies reveals cryptic speciation in *Amanita* Pers. in Iberian Peninsula

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During the past few years several studies have highlighted threats for biodiversity conservation. The scenario that we are now driving species to extinction without even recognize its value as species without knowing what their role in the ecosystem may bring irreversible consequences. Despite the occurrence of cryptic species have been reported over the last decades, their study only has grown exponentially in recent years based due to the advent of relatively inexpensive and rapid DNA sequencing. However the incidence of cryptic species in some group of organisms such as fungi still very poor known. Here we show that cryptic speciation in mushroom forming fungi can actually may be occurring in species that humans have interacted over generations. We found an undescribed sympatric species in Iberian peninsula with macroscopical traits of *Amanita ponderosa* (Malençon & R. Heim) but genetically more related to *Amanita curtipes* (E.-J. Gilbert) through the application of restriction-site associated DNA (RAD-seq) technique. Furthermore we proved that RAD *loci* appear to hold promising results in phylogenetic inference in relatively divergent clades in which sufficient numerous of orthologous *loci* are retained across species. The presence of this new *taxon* illustrate a very challenging scenario to implement policies of management and conservation for the mycological resources mainly concerning *A. ponderosa.*

Evolution, biodiversity and systematic
Poster nr. 77

DNA barcoding allow to identify *Phallus* species

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The genus *Phallus* includes species of gasteroid Basidiomycota with a spongy and cylindrical pseudostipe wearing a bell-shaped receptacle. Based on morphological features, Calonge (2005) published a provisional key to identify species of *Phallus* around the world. In his paper 25 species were accepted.

In this study, we compared barcoding performance of two nuclear ribosomal regions (ITS and LSU) and three protein-coding genes (RPB1, RPB2 and ATP6), based on PCR success and the presence of a barcoding gap (interspecific variation should exceed intraspecific variation), in the genus *Phallus*. After preliminary results, RPB1 and RPB2 were excluded from the general analyses, since many specimens failed to amplify and was not easy to get good quality products (multiple bands). The marker LSU was excluded since not a clear barcoding gap was obtained. For the general analyses, ITS and ATP6 were selected because both markers gave a high PCR success and a high resolving power to discriminate *Phallus* species. Both markers revealed a high number of molecular taxonomic units (MOTUs) that could be related with morphologically defined species (e.g. *Phallus echinovolvatus, P. impudicus, P. hadrianii,* among others). Moreover, sequences obtained from *P. indusiatus* specimens from Brazil, Cameroon, China, Guatemala and India had a high interspecific variability, suggesting that under this name several taxa could be included.

Calonge FD. 2005. A tentative key to identify the species of *Phallus*. *Bol. Soc. Micol. Madrid*. 29: 9-17.

Flora Micologica Iberica (PB98-0538-C04-01, FMI-REN2002-04068-CO2-01), Programa Ciencia sem Fronteiras (MEC/MCTI/CNPq/FAPs nº71/2013), CNPq (473422/2012-3) and FAPEAM (3137/2012).

Evolution, biodiversity and systematic

Poster nr. 78

Diversity and phylogeny of *Neofusicoccum* species from woody hosts in Portugal

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The genus *Neofusicoccum* includes species with a very wide range of geographical and host distribution, including woody plants of agricultural, forestry and economic importance. Neofusicoccum species are typically endophytes but under stress conditions cause decline and dieback symptoms. This genus comprises 27 species that are difficult to identify based on morphological features alone. In recent years much effort is being devoted to redefine species limits using multi locus sequence data, which has resulted in the identification of several cryptic species.

The aim of this study was to establish phylogenetic relationships within a collection of Neofusicoccum isolates obtained from several woody hosts in Portugal. A total of 350 isolates was characterised by BOX-PCR fingerprinting to evaluate their overall genetic diversity. Representatives of each group identified in this analysis were selected for sequence analysis of the ribosomal internal transcribed spacer region and partial sequences of protein-coding genes, namely translation elongation factor 1-alpha and beta-tubulin.

Available sequences from all known and well-characterized *Neofusicoccum* species were retrieved from GenBank and included in the phylogenetic analyses along with the sequences from the isolates under study. Maximum likelihood phylogenetic analysis of the combined three loci clearly resolved all species. Most of the isolates belong to known species, namely *N. australe, N. eucalyptorum, N. luteum* and *N. parvum* and many new host-associations were identified. However, a set of isolates grouped into two different clades closely related to N. parvum that represent two novel cryptic species.

Evolution, biodiversity and systematic

Poster nr. 79

Preliminary studies on molecular phylogeny of Laboulbeniales

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Laboulbeniales is an ascomycetous order of obligatory biotrofic ectoparasites of insects and mites. They are unculturable fungi that lack any forms of asexual reproduction. They occur mostly on small arthropods causing no visible harm to host and are often omitted by both entomologists and mycologists. The order compromises over 2000 species, yet only few have any Genbank record. Laboulbeniomycetes is the only class of Ascomycetes that was not subject to any multilocus phylogenetical study. For the Laboulbeniales only ITS and partial SSU data is available. Obscurity and methodological difficulties has long prevented unravelling relationships inside this enigmatic group.
For this study we obtained SSU and LSU data from several species of Laboulbeniales collected in Poland. We also checked possibility of amplifying different molecular markers (ITSI and ITSII, ACT, EF-1α, RPB1) using available primers from *Monoicomyces invisibilis* Thaxt. and *Laboulbenia pedicellata* Thaxt.
Here we present phylogenetic trees of studied species. We also propose new primers, methods of DNA isolation and discuss perspectives of phylogeny of this group.

Evolution, biodiversity and systematic

Poster nr. 80

Genetic variation and population structure of *Syzygospora bachmannii* (Tremellomycetes, Basidiomycota)

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Lichenicolous fungi are a group of fungi specialized in living on lichens. It has been suggested that one of the main diversification factors of these parasites is their specificity when selecting their host. It is possible that one of the factors functioning in the selection process is the presence of certain secondary metabolites in the host lichen. Hence, when putative hosts with similar chemical and ecological characteristics exist, a greater probability there will be for the parasite to develop in new hosts.

Syzygospora bachmannii is a parasitic fungus that can live on several species of the genus *Cladonia*. This fungus has been found on 32 species of this genus, both in Europe and North America. The genetic variation of two loci (ITS rDNA and LSU rDNA) of *S. bachmannii* is studied here in order to clarify the population structure and which of the following factors are the most influential in it: a) the host species, b) the secondary metabolites of the host, or c) the geographical origin. Specimens from Azores islands, Alaska, Canada, Finland, Russia, Spain and Turkey were studied, and *S. bachmanii* was found on 12 species of *Cladonia* (*C. crispata, C. cornuta, C. furcata, C. foliacea, C. gracilis, C. macroceras, C. ochrochlora, C. pyxidata, C. ramulosa, C. rangiformis, C. squamosa*, and *C. stereoclada*). Some geographically widespread haplotypes were found on several *Cladonia* species with different secondary metabolites, while other haplotypes were restricted to a single *Cladonia* species.

Evolution, biodiversity and systematic

Poster nr. 81

Exploring the phylogenetic and morphological relationships of disregarded genera in Helotiales s.l.

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The order Helotiales Nannf. is one of the major groups of apothecial fungi, recognized within Ascomycota Caval.-Sm. It is the most diverse order in Leotiomycetes O. E. Erikss. & Winka, with ca. 2.036 known species in 487 genera. Until recently, there were 13 families recognized.

After the first comprehensive phylogenetic study of helotialean fungi (Wang et al.2006), only a few changes to the clades proposed (op.cit) occurred. One of the most important phylogenetic studies within the order was published by Han et al. (2014), who showed that the family Hyaloscyphaceae s.l. split into ten different clades, mixed among clades of other families previously reported by Wang et al. (2006). Recently, Crous et al. (2014) introduced a new taxonomic change, he reestablished Phacidiaceae at the ordinal level, Phacidiales Höhn., now a sister clade of Helotiales.

Our morphological and phylogenetic reviews of specimens ascribed previously to Bulgariaceae, Dermateaceae and Helotiaceae, has provided new insights to propose the erection of new family, Tympanidaceae Baral & Quijada, which we tentatively include in the Phacidiales.

Genomics, genetics and molecular biology
Poster nr. 82

Intracellular sequestration of Ag and Cu in *Amanita strobiliformis* and characterization of its Cu and Ag transporting AsCTRs

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Macrofungi can accumulate remarkably high concentrations of Cu and Ag in their sporocarps. We have previously demonstrated that the non-essential Ag is in the ectomycorrhizal Ag-hyperaccumulating *Amanita strobiliformis* sequestered by isomorphic 3.4 kDa metallothioneins AsMT1a, 1b and 1c. Here, we document that As*MT1* genes are expressed in two populations of wild-grown *A. strobiliformis* sporocarps, which showed significant difference in and certain correlation between the concentrations of accumulated Ag and Cu. Metal speciation analysis revealed that the intracellular Cu extracted from the sporocarps of both populations was, like Ag, associated with 3.4-kDa metallothioneins. A search for sequences encoding proteins of the Cu transporter (CTR) family in the *A. strobiliformis* transcriptome revealed four As*CTR* cDNAs, whose transcription was confirmed in both populations. The predicted AsCTR proteins showed homology to vacuolar Cu transporters (AsCTR1 and AsCTR4) and plasma membrane Cu uptake transporters (AsCTR2 and AsCTR3). The ability of AsCTR2 and AsCTR3 to transport the chemically similar Ag and Cu ions was examined in Cu uptake-deficient *S. cerevisiae* in which both AsCTRs complemented growth defects, and AsCTR2 and 3 fused with green fluorescent protein localized to the plasma membrane. Increased uptake rates of Cu and Ag observed with the As*CTR2*- and As*CTR3*-expressing yeasts indicated that these AsCTRs are the functional Cu and Ag uptake transporters. The results point to a certain overlap in Ag and Cu biology in *A. strobiliformis* and provide the first evidence that fungal CTRs can recognize Ag for transport.

Genomics, genetics and molecular biology
Poster nr. 83

Metal-binding peptides of Zn-accumulating *Russula atropurpurea* and characteriation of its RaCDF1 and RaCDF2 transporters

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Ectomycorrhizal *Russula atropurpurea* can accumulate in its sporocarps remarkably high concentrations of zinc (Zn). Several studies indicated that the cellular mechanisms

preventing the toxicity of Zn ions involve binding with cytosolic metal-binding peptides, subcellular compartmentation (e.g. in vacuoles) or the efflux of the excess metal out of the cells. To gain an insight into these mechanisms at the molecular level, the transcriptome of *R. atropurpurea* was analyzed and screened *in silico* for potential Zn transporters and Zn-binding ligands. The homology-based search allowed us to identify cDNAs coding for nine putative Zn transporters and two metallothioneins (MTs) designated RaMT1 and RaMT2. Heterologous complementation assays in metal-sensitive *S. cerevisiae* mutants indicated that RaMTs encode functional metal-binding peptides and can confer increased Zn, cadmium and copper tolerance upon the yeasts. Two predicted transporters of the cation diffusion (CDF) family were characterized in *S. cerevisiae*: RaCDF1, which localized to the vacuolar membrane and protected the yeast againts Zn toxicity and RaCDF2, which localized to the plasma membrane and decreased Zn accumulation and toxicity in the cells. Moreover, screening of the expression cDNA library allowed identification two Zn-/Cd-binding peptides RaZBP1 and RaZBP2, which are only distantly related to MTs and thus escaped from the homology based transcriptome screening. Both RaZBPs, but not RaMTs, were detected in a native Zn complex of *R. atropurpurea* and the recombinant RaZBP1 was found associated with Zn and Cd in yeasts. Altogether, the results improve our understanding of the mechanisms available in *R. atropurpurea* for the handling of excess Zn.

Genomics, genetics and molecular biology

Poster nr. 84

Amanita ovoidea and/or *Amanita proxima*: the end of a mistery?

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The genus *Amanita* includes about 600 species; some are edible and precious such as *Amanita caesarea*, others are really poisonous, such as *Amanita phalloides*. However, the toxicity of some species within this genus is still under debate and whether *Amanita ovoidea* includes only edible mushrooms is one of the most vexing questions among mycologists.

Present study stems from a case of poisoning, classified as allenic norleucine syndrome, occurred in Italy which was likely caused by consumption of *A. ovoidea*. Since *A. ovoidea* shares morphological similarities and habitats with the poisonous species *A. proxima*, it is not surprising that these two species can be confused each other. Yet, according to some authors *A. proxima* is a subspecies or variety of *A. ovoidea*.

Preliminary phytochemical screenings and toxicological tests performed on specimen belonging to *A. proxima* and *A. ovoidea*, provided interesting but not exhaustive differences between these taxa (Biagi et al., 2014).

Here, to get more insights into relatedness between these taxa a molecular approach based on the sequencing of the rDNA ITS (Internal Transcribed Spacer) region was undertaken on 14 mushrooms morphologically ascribed either to *A. ovoidea* or *A. proxima* or other *Amanita* spp. Phylogenetic analyses showed that *A. ovoidea* and *A.*

proxima specimens are separated into distinct clusters with a low level polymorphism within each cluster. This let us argue that they likely belong to two different species. ITS sequence analyses on different *Amanita* spp. are in progress to get a closer look into phylogenetic relationships within this genus.

Genomics, genetics and molecular biology

Poster nr. 85

Serine protease evolution in fungi with variable lifestyles

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Fungi are able to switch between different lifestyles in order to adapt to environmental changes. Their ecological strategy is connected to their secretome as fungi acquire nutrients by secreting hydrolytic enzymes to the surroundings and acquiring the digested molecules. We focus on fungal serine proteases, which phylogenetic distribution is barely described so far. Expanding the repertoire of known proteases in fungal genomes will facilitate evolutionary studies of fungal secretome and will help to understand the relation between secretome and fungal lifestyle.

In order to obtain a complete set of fungal proteases, we performed iterative jackhmmer searches against Uniprot protein sequence database and Blast searches against JGI genomes database. Obtained results suggest that serine proteases are more ubiquitous than expected. From 53 serine protease families described in Merops Peptidase Database, 18 are present in fungi. Interestingly, 17 of them are also present in Metazoa - this suggest that, except one (S64), all fungal serine proteases families evolved before animals and fungi diverged. This hypothesis is supported by the presence of most serine proteases in ancestral fungal groups, i. e. Chytridiomycota, Microsporidia, Mucorales. The number of serine proteases from each family varies among analysed taxa. The most abundant are S8 proteases (560 species), whereas only 19 species encode proteins from the S49 family. Our study shows that S49 is the only one from 18 fungal families not present in Ascomycota.

Here, we present a comprehensive evolutionary history of fungal serine protease families in the context of fungal ecology and the fungal tree of life.

Cell biology, biochemistry and physiology

Poster nr. 86

Membrane lipids and cytosol sugars of *Aspergillus niger* under heat, osmotic, oxidative and cold shocks

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Protection of the membranes under unfavorable conditions occurs by changing the composition of membrane lipids and cytosol carbohydrates. The aim of this work was the comparative investigation of the composition of membrane lipids and soluble cytosol carbohydrates of *A. niger* under different shocks.

The scheme of experiment: trophophase submerged culture of fungus was subjected to different shocks for 3 hours: heat shock - 40-41°C; cold shock - 15-16°C; osmotic stress - 1.0 and 1.5 M NaCl; oxidative stress - 10, 20 and 50 mM H_2O_2.

The major membrane lipids under optimum conditions were phosphatidylethanolamines (PE), phosphatidylcholines (PC), cardiolipines, phosphatidic acids (PA) and sterols (St), while sphingolipids (SL) were the minor components. All shocks resulted in changes in composition of lipids: heat shock caused the increase of PA and SL percentage; cold shock - of PA and St; oxidative stress - of PA; osmotic shock - of PA and St.

Soluble cytosol carbohydrates of the mycelium *A. niger* reached 8-10% and the predominant sugar was mannitol (70-85% of ∑). Heat shock led to significant increase of the trehalose level; cold and osmotic shocks - to glycerol percentage increase. Oxidative stress didn't change the composition of sugars.

Thus, the general pattern of the changes in the membrane lipids composition under all shocks was the percentage of PA increase. Changes in sugars composition were specific for different shocks.

This work was supported by the grant 12-04-00732 of the Russian Foundation for Basic Research.

Cell biology, biochemistry and physiology

Poster nr. 87

Visualization of nuclei and cytoskeleton with different fluorescent proteins in vegetative and mating hyphae in the filamentous basidiomycete *Schizophyllum commune*

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While observations on nuclei and cytoskeletal elements labeled with fluorescent proteins in living hyphae have been very successful in filamentous ascomycetes, in filamentous basidiomycetes there are very few observations on this line. The present work describes the visualization of nuclei in the basidiomycete *Schizophyllum commune* in living homokaryotic (haploid) and dikaryotic hyphae using a histone encoding gene (Schco_3:

PID2605148). The gene was expressed under its native promoter and the carboxyl terminus tagged with a 30 bp linker, *egfp* gene and the *Sccdc42* gene terminator. Combined confocal and phase contrast microscopy revealed one nucleus in each hyphal compartment of the haploid hyphae, two nuclei in dikaryotic hyphae and the conjugate division of the nuclear pair at the clamp cell formation. Comparable cloning ofa red fluorescent protein (mRuby, dTomato, Red)to H2B histone is currently under way. This latter construct will be transformed in a haploid *S. commune* strain either fully or semi compatible with the strain expressing H2B-EGFP. Fluorescent microscopic investigation of differently labeled nuclei in compatible hyphae is expected to give valuable information about the nuclear behavior at hyphal fusions leading to the establishment of dikaryotic hyphae. Microtubules and microfilaments play a central role in nuclear division and movement both at mating and clamp cell formation. For this purpose, the labeling of the cytoskeletal components with fluorescent proteins is also under way. Recently the labeling of microfilaments with the LifeAct construct has succeeded in homokaryotic hyphae, in which the construct visualizes microfilaments at hyphal tips and the formation of septa.

Cell biology, biochemistry and physiology

Poster nr. 88

Lygnomyces vetlinianus (Domański) R.H. Petersen & Zmitr.- a new species producing phthalides

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A new genus *Lygnomyces* was described and *L. vetlinianus* comb. nov. was proposed as a result of recent taxonomic investigation of a pleurotoid fungus from eastern Russia made by R. Petersen and co-authors. Culture characters of three dikarion strains of this species were studied. Some white crystals glowing in UV, produced particular abundant by one of the strain, were observed in mycelial mats on agar media. Investigation of the crystals using metabolomic profiling by GC-MC analysis was the aim of this study. The strains were cultivated in Petri plates on BWA and MEA. Mycelium and basidiomata fruited in culture were extracted with methanol and chloroform. Over 60 compounds including amino acids, organic acid of TCA cycle, sugars, fatty acids, sugar alcohols were detected in the extracts. On 10th -14th days of cultivation 4,6-dimethoxy-phthalide (4,6-dimetoxy-1(3H)-isobenzofuranone) was found in mycelium. The molecular structure of the substance was confirmed by NMR. By 6 weeks of cultivation crystals-like structures (diameter up to 3 mm) accumulated in mycelium. Theses structures were picked up from plates and identified as clusters of 4,6-dimethoxy-phthalide. Fruited basidiomata, however, contained much smaller amount of 4,6-dimethoxy-phthalide as compared with mycelium. Phthalides were reported by several authors as secondary metabolites in a number of fungi, responsible for antimicrobial, antifungal, cytotoxic and enzyme inhibiting bioactivities. Thus *L. vetlinianus* can be considered as a model species for studies of secondary metabolism in fungi and as a perspective phthalide producer for biotechnology.

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