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What do we learn from cultures in the omics age? High-throughput sequencing and cultivation of leaf-inhabiting endophytes from beech (*Fagus sylvatica* L.) revealed complementary community composition but similar correlations with local habitat conditions

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Abstract

Comparative simultaneous studies of environmental high-throughput sequencing (HTS) and cultivation of plant-associated fungi have rarely been conducted in the past years. For the present contribution, HTS and extinction culturing were applied for the same leaf samples of European beech (*Fagus sylvatica*) in order to trace both "real" environmental drivers as well as method-dependent signals of the observed mycobiomes. Both approaches resulted in non-overlapping community composition and pronounced differences in taxonomic classification and trophic stages. However, both methods revealed similar correlations of the fungal communities with local environmental conditions. Our results indicate undeniable advantages of HTS over cultivation in terms of revealing a good representation of the major functional guilds, rare taxa and biodiversity signals of leaf-inhabiting fungi. On the other hand our results demonstrate that the immense body of literature about cultivable endophytic fungi can and should be used for the interpretation of community signals and environmental correlations obtained from HTS studies and that cultivation studies should be continued at the highest standards, e.g. when sequencing facilities are not available or if such surveys are expanded into functional aspects with experiments on living isolates.

Key words

Cultivation, high-throughput sequencing, metabarcoding, fungal endophytes, biodiversity

Introduction

Fungal endophytes reside in the living tissues of plants without causing visible disease symptoms (e.g. Christian et al. 2015). Particular research interest is given to endophytes of the phyllosphere and other photosynthetic organs due to the enormous availability and environmental importance of leafy habitats (Lindow and Brandl 2003), the complex biochemical processes in these plant tissues and the generally close interconnectivity of leaf mycobiome with their hosts (reviewed in Rodriguez et al. 2009, Peršoh 2015). Within the last decade, significant progress has been made in unraveling plant-associated mycobiomes by using both cultivation (Collado et al. 2007, Unterseher and Schnittler 2009, Gazis and Chaverri 2015) and direct high-throughput sequencing (HTS) techniques (Bálint et al. 2016). To date, most knowledge about endophyte richness, composition or host preferences is still based on traditional culturing approaches (Arnold 2007, Sieber 2007, Albrectsen et al. 2010, Unterseher et al. 2013a). On the other hand, direct environmental assessment of mycobiomes provides unprecedented details of community diversity, composition, taxonomy and interactions (e.g. Peršoh 2013, Jumpponen and Brown 2014, Bálint et al. 2015, Eusemann et al. 2016, Purahong et al. 2016). These improvements go side by side with the ever increasing accuracy of reference sequence databases (Nilsson et al. 2014, 2015, Abarenkov et al. 2016)

It is well recognized that interpretation of diversity of endophytes and other fungi depends on the applied methods (Unterseher 2011). On the one hand, cultivation data are often biased by under-sampling and the use of selective media. On the other hand, amplicon library preparation can differ strongly among studies (Salter et al. 2014) and HTS data are generally squeezed through complex and highly customisable bioinformatic pipelines, leading to variable data analysis (Meiser et al. 2014, Bálint et al. 2016), even if the same plant-fungus system is used in independent studies (Cordier et al. 2012, Siddique and Unterseher 2016).

To date, published studies about the comparative assessment of fungal biodiversity are rare. Allmér et al. (2006) demonstrated the advantages and limitations of fruit body observation, mycelial cultivation and T-RFLP identification of wood-inhabiting fungi. Zhang et al. (2014) assessed microbial communities from deep-sea sediments with cultivation and environmental molecular cloning and identified two complementary assemblages. Similar conclusions were made by Langarica-Fuentes et al. (2014) who identified a biased composition of compost fungi by cultivation. Recently, HTS was rated superior over cultivation, given its ability to detect more obligate, slow growing and rare fungi (Al-Sadi et al. 2015, Oono et al. 2015). Whereas the general lack of congruence between mycobiomes generated by cultivation and HTS can be safely postulated nowadays (Pitkaranta et al. 2008), much less is known about environmental correlations of these differing data sets.

In this study, we investigated endophytic phyllosphere fungi with dilution-to-extinction cultivation (and ITS barcoding) and Illumina sequencing of the same DNA region and from the same material. In accordance with existing knowledge, we expected lower OTU (operational taxonomic unit) richness in the cultivation data and a

preferential isolation of ubiquitous, primary saprobic taxa. Consequently, we hypothesized that the cultivable mycobiome exhibits different ecological signals compared with the mycobiome obtained by HTS.

Materials and methods

Study sites and sampling

The samples were obtained from an experimental site established in 2013 (Unterseher et al. 2016), consisting of two plots of 4 years-old *Fagus sylvatica* trees from a different origin. The tree seeds originated from the Lustian lowland area (central eastern Germany, HKG 81005) and were grown in a nursery in northern Germany (near Hamburg) for two years. (Unterseher et al. 2016). The plots are located at the same slope of the mountain massif "Untersberg" in Bavaria, Germany at 517 m asl. (above sea level, respectively) (Valley site; Lat: 47.712946 Long: 13.040101) and at 975 m asl. (Mountain site; Lat: 47.683158 Long: 13.002102) (Siddique and Unterseher 2016). It is humus-rich with a well developed topsoil layer (A horizon) at the valley site, while the A horizon is only weakly developed at the mountain site. The ground and understory vegetation of the mountain site was mainly composed of *Acer pseudoplatanus*, *Picea abies*, *Daphne mezereum*, *Cardamine* (= *Dentaria*) *enneaphyllos*, *Helleborus niger* and *Hepatica nobilis* (Siddique and Unterseher 2016). At the valley site, ground vegetation was different with dominance of *Acer pseudoplatanus, Mentha* spp., *Petasites hybridus, Equisetum sylvaticum* and *Rubus* sp (Unterseher et al. 2016). Five trees each from each site were selected randomly in October 2014, exactly one year after planting. Ten symptomless green leaves per tree were removed and processed as described in Unterseher et al. (2016).

Cultivation of endophytes using the dilution-to-extinction method

Isolation of endophytes followed the dilution-to-extinction cultivation (Solis et al. 2016). In brief, samples were blended into tiny particles and filtered. Smallest particles (ø < 0.2 mm) were resuspended and strongly diluted before plating onto malt extract agar (MEA, 1.5%) containing 48-well plates (Carl Roth, Karlsruhe, Germany). The plates were inspected regularly for four weeks. Emerging colonies were transferred into Petri dishes containing the same growth medium.

DNA extraction and ITS sequencing from axenic cultures

Instead of classifying the fungal cultures according to macroscopic and microscopic characters (Khoyratty et al. 2015) and selecting only a few representative strains per

morphotype for downstream processing, genomic DNA was extracted from all isolates using a traditional, chloroform-based protocol (e.g. Solis et al. 2016). The ITS region was amplified with the primer pair V9G - ITS4 (de Hoog and Gerrits van den Ende 1998) using approved amplification kits (MangoTaq; Bioline, Germany) and cycling conditions (Solis et al. 2016). Unpurified PCR products were shipped to Beckman Coulter Genomics (Takeley, UK) for sequencing. Sequences were discarded if their corresponding chromatograms showed pronounced signs of ambiguous base calling after end trimming.

Library preparation for high-throughput Illumina sequencing

Total genomic DNA was extracted with the Charge Switch® gDNA Plant Kit (Invitrogen, Germany) from the same fresh leaf particle mass that was used for cultivation. Library preparation consisted of two consecutive amplification steps in order to add sample-specific tag combination for multiplexing. Please refer to Siddique and Unterseher (2016) and Unterseher et al. (2016) for the detailed description of this procedure. Amplicons were sequenced in pair-end mode on an Illumina MiSeq platform (Illumina Inc.) at the Genetics Section, Biocentre of the LMU Munich, Germany.

Processing of Illumina reads and Sanger sequences

Demultiplexing and quality filtering of Illumina reads relied on QIIME (Navas-Molina et al. 2013; see Suppl. material 1) and is also detailed in Unterseher et al. (2016) and Eusemann et al. (2016). Extraction of ITS1 (forward R1 Illumina reads) was done with ITSx (Bengtsson-Palme et al. 2013) followed by reference-based chimera checking (Nilsson et al. 2015), open-reference OTU picking (complete-linkage clustering at 97% similarity; Rideout et al. 2014), selection of representative sequences and taxon assignment (Kõljalg et al. 2013). These last steps were also performed with all high-quality Sanger sequences to guarantee best comparability. Final quality filtering of HTS OTUs consisted of the removal of unique (occurring in only one sample) and rare OTUs (having less than five reads, cf. Brown et al. 2015). In contrast, all OTUs from cultivation data were retained, knowing that they belonged to true fungi. The reasons for using only the ITS1 region for subsequent analyses is comprehensively explained in recent papers (e.g. Unterseher et al. 2016, Eusemann et al. 2016).

Biodiversity analysis and assessment of functional guilds

The analysis of fungal biodiversity comprised the assessment of OTU richness and further indicators of diversity (Fisher's Alpha, Shannon index and three numbers of Hill's series of diversity, the latter considering different levels of rarity) (Hill 1973). Given the strong positive correlation of OTU richness and read numbers (data not shown, but compare

Siddique and Unterseher (2016)) as well as the strongly different sequencing depth between the two approaches, read counts were standardised (divided) by sample totals.

Community composition was assessed with PCoA (principal coordinate analysis) and NMDS (non-metric multidimensional scaling) and tested with PERMANOVA (permutational multivariate analysis of variance). Functional guild analysis was performed according to Nguyen et al. (2016). The entire biodiversity analysis was performed with R (freely available at www.r-project.org, last accessed 11/2016). The corresponding command script and necessary input files are available as "Suppl. material 2". Curated Sanger sequences were taxonomically annotated as far as possible and made available in ENA/GenBank through accession numbers LT604837–LT604881. High-throughput data are available under SRA accession number SRX1211311.

Results

Basic data exploration, diversity and community composition

Data volumes differed strongly between Illumina sequencing and cultivation. Illumina sequencing resulted in 597 OTUs from 170480 curated ITS1 reads and cultivation revealed 70 OTUs from 438 culture-based Sanger sequences with the same settings for OTU clustering. The combined data set comprised 630 OTUs (+ 33 OTUs compared with Illumina data). Thirty-seven OTUs were detected with both methods (see Table S1 on Suppl. material 3 and Suppl. material 4).

An insignificant trend of lower fungal diversity at the mountain site across all indexes was observed for HTS data (Fig. 1A). Richness analysis of cultivation data was partly contradictory with significant differences of Fisher's Alpha between valley and mountain samples ($p < 0.01$, see Table S2 and S3 "Suppl. material 3") but nearly identical accumulation curves (Fig. 1B). In addition and contrary to HTS data, Hill numbers N2 and N3 were significantly different for cultivation data (Fig. 1B, for statistical details see Table S2 and S3; "Suppl. material 3"). The accumulation curves for HTS data (Fig. 1A) revealed a clearly lower fungal richness for mountain than for valley samples, whereas the cultivation data failed to show such differences (Fig. 1B).

The analysis of community composition with non-metric multidimensional scaling (NMDS), principal coordinate analysis (PCoA) and PERMANOVA discovered a significant influence of the isolation method ($df = 1$, $F = 7.58$, $R^2 = 0.30$, $p = 0.001$) as well as of locality ($df = 1$, $F = 2.87$, $R^2 = 0.14$, $p = 0.014$) (Fig. 2). The differences between valley and mountain site were more pronounced for HTS ($F = 3.94$, $R^2 = 0.33$, $p = 0.007$) than for cultivation data ($F = 1.96$, $R^2 = 0.20$, $p = 0.027$) (Fig. 2).

Taxonomic composition of HTS and cultivation data

Three of the five most abundant orders from Illumina data were also most abundant in cultivation data (Capnodiales – both methods, Helotiales – both methods, Saccharomy-

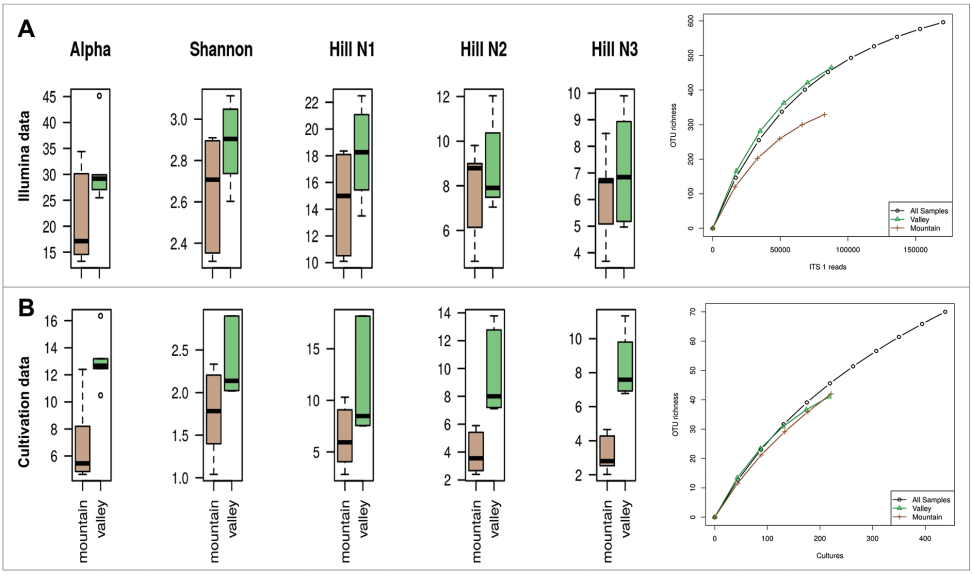

Figure 1. Diversity indexes and accumulation curves for **a** Illumina and **b** cultivation data of fungal leaf-inhabiting endophytes of beech. Except of the accumulation curves of cultivation data, both methods revealed a clear and partly significant trend of higher fungal diversity at the valley site.

cetales – Illumina only, Pleosporales – both methods; all Ascomycota) (Fig. 3). In addition, HTS revealed further and abundant orders from both Asco- and Basidiomycota, which were not recovered during cultivation (Fig. 3), such as the yeast fungi Saccharomycetales (Ascomycota) and Malasseziales (Basidiomycota). The Xylariales (Ascomycota, present with one isolate) was the only order from the cultivation data that was not detected with HTS.

Composition of trophic guilds revealed by HTS and cultivation

The five main guilds (pathotrophs, patho-saprotrophs, patho-symbiotrophs, saprotrophs and symbiotrophs) were all detected by HTS. Cultivation largely failed to detect pathotrophs (including patho-saprotrophs and -symbiotrophs). The relative abundance of saprotrophs was clearly higher in cultivation than in HTS data (Fig. 4B, C).

When analysing the influence of locality for the occurrence of different ecological guilds, it turned out that the abundance of pathotrophs was significantly higher in leaves of mountain trees than of valley trees (Fig. 5A). Saprotrophs were also more abundant in leaves of mountain trees, whereas symbiotrophs were more abundant in valley than in mountain trees (Fig. 5A, B).

Results from HTS and cultivation data were congruent in as much as saprotrophs and symbiotrophs revealed similar abundance patterns for both methods.

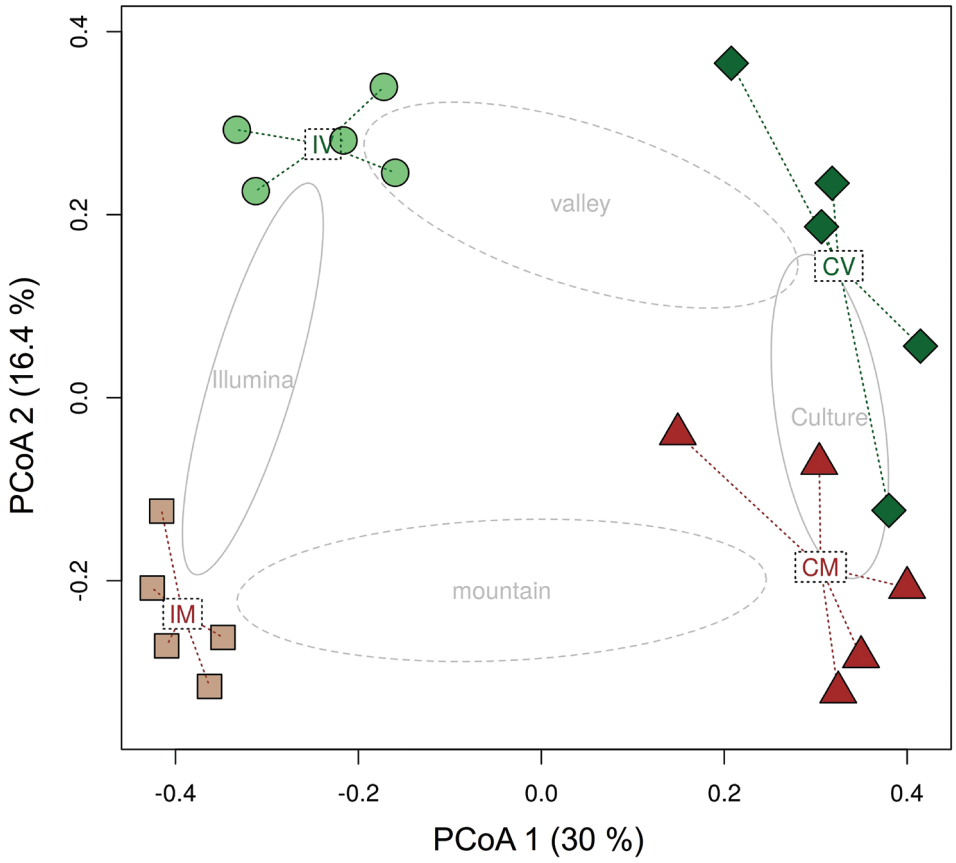

Figure 2. Principal coordinate analysis (PCoA) of fungal leaf-inhabiting endophytes of beech display strongly differing assemblages obtained with Illumina sequencing and cultivation. Both methods revealed differing mycobiomes from valley and from mountain leaves, although these differences were less pronounced for cultivation data. Abbreviations: **IM** = Illumina data from mountain samples, **IV** = Illumina data from valley samples, **CM** = cultivation data from mountain samples, **CV** = cultivation data from valley samples

Discussion

The methodology of biodiversity assessment influences the interpretation of community composition and community ecology

The most abundant orders were the same for both cultivation and HTS, namely Capnodiales, Helotiales and Pleosporales (Fig. 3). Ascomycota clearly dominated the cultivation data thus confirming results of many earlier cultivation studies (e.g. U' Ren et al. 2012, Scholtysik et al. 2013, Unterseher et al. 2013b). Many of own isolates, such as those with highest sequence similarities to the genus *Mycosphaerella* and its anamorphs, are often described in literature as frequent asymptomatic inhabitants of living leaves. In this study, taxa generally known as saprotrophs were the dominant trophic

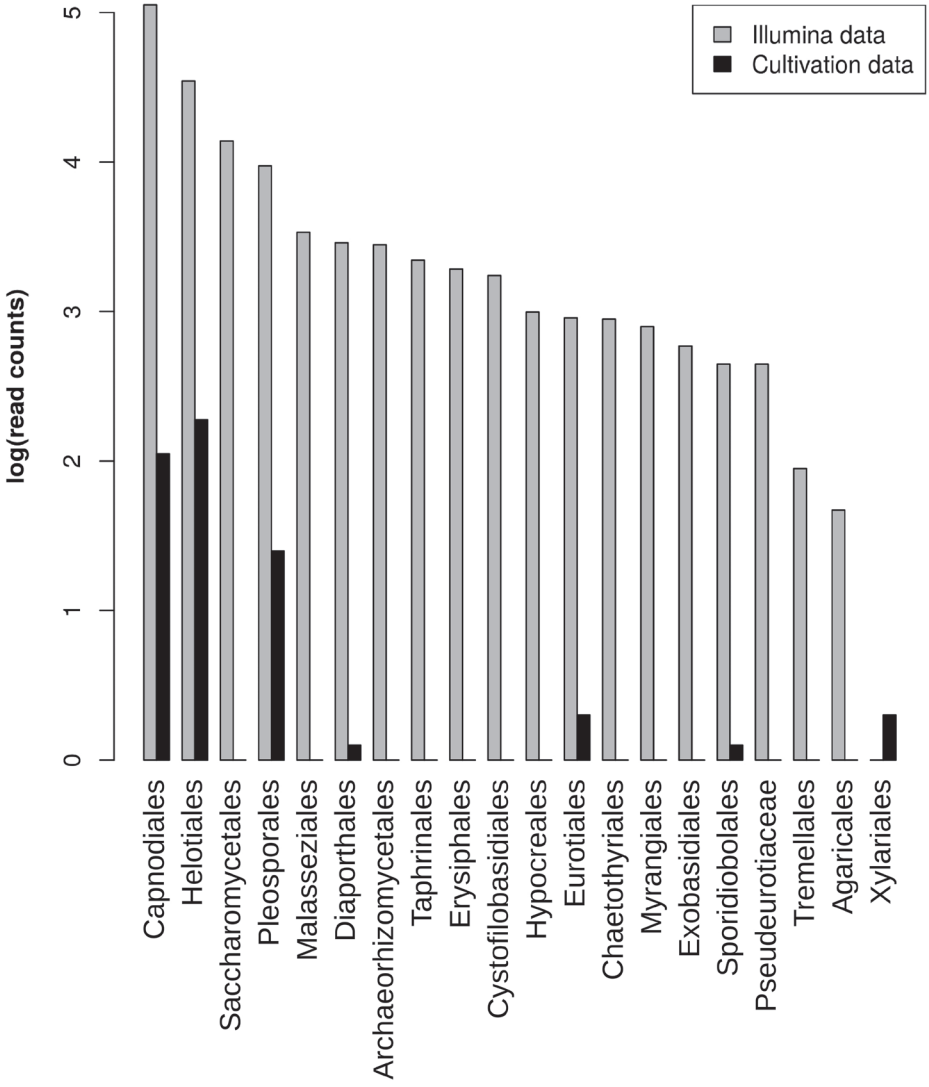

Figure 3. Abundance distribution of the 20 most abundant orders of fungal leaf-inhabiting endophytes of beech on a logarithmic scale. Three of the five most abundant orders from high-throughput sequencing were also most abundant in cultivation data.

guild of the cultivable part (see below) and, in terms of relative abundance, surpassed this guild as detected with HTS by far (Fig. 4B, C).

The compositional difference in the two mycobiomes also corresponded to the presence of parasitic taxa (Taphrinales, Erysiphales) and yeast-like fungi (Saccharomycetales and Tremellales) in the HTS data, whereas the cultivation data were devoid of fungi with obligate parasitic, biotrophic or pathogenic lifestyle. The latter guilds usually cannot be cultivated, and yeasts are often detected only during cultivation studies when growth of

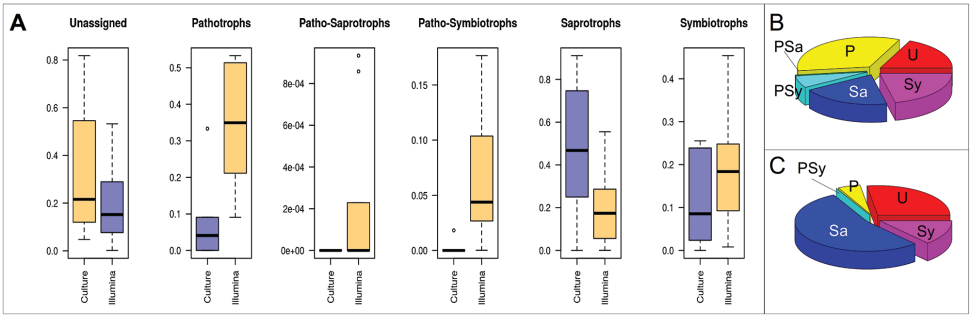

Figure 4. Relative abundance of fungal leaf-inhabiting endophytes of beech among the five main trophic guilds as revealed by analysis with FUNGuild (Nguyen et al. 2016). **A** compares the two methods for each trophic guild and unassigned data. **B** displays the trophic guilds and unassigned taxa for Illumina data, **C** for cultivation data. Abbreviations in [**B** and **C**]: U = Unassigned, P = Pathotrophs, PSa = Patho-Saprotrophs, PSy = Patho-Symbiotrophs, Sa = Saprotrophs, Sy = Symbiotrophs

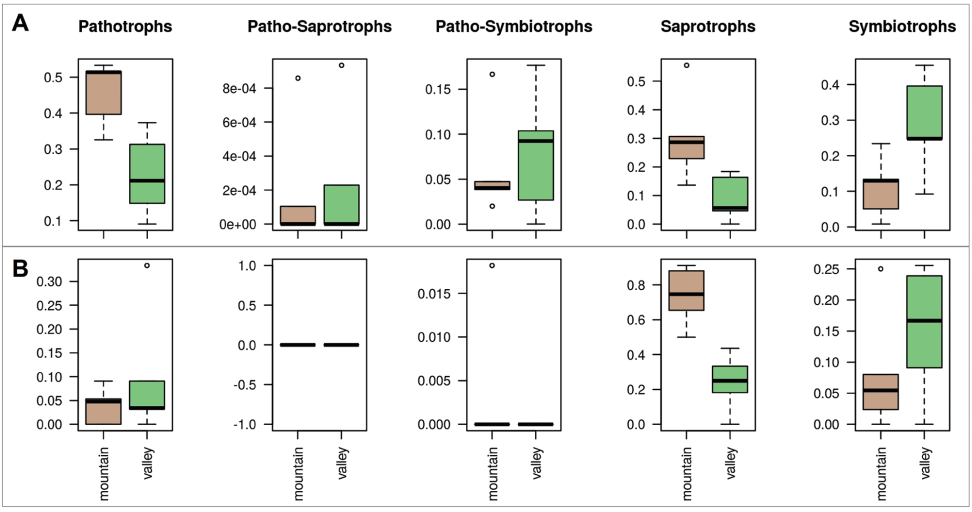

Figure 5. Relative abundance distribution of fungal leaf-inhabiting endophytes of beech among the five main trophic guilds as revealed by analysis with FUNGuild (Nguyen et al. 2016). **A** compares the two localities for each trophic guild on the basis of Illumina data **B** compares the two localities for each trophic guild on the basis of cultivation data.

filamentous fungi is slowed down with low-temperature incubation. In this study, HTS retained a wide range of taxa (compare Al-Sadi et al. 2015) and all guilds available in the FUNGuild reference data base (Nguyen et al. 2016) as expected (Figs 4, 5).

A poor comparability of cultivation and HTS data, as it is presented here, was recently reported for a microbiome study (Eevers et al. 2016). It was caused by the fundamentally different sample coverage with the most abundant OTU counting 28621 reads for HTS (Mycosphaerellaceae, *Sphaerulina*) and 102 isolates for cultivation (Hyaloscyphaceae, *Lachnum*) (Suppl. material 4).

The two methods revealed consistent signals of both community data to environmental conditions

On the one hand side our results clearly demonstrate the limitations and biases of cultivation approaches for comprehensive biodiversity assessments. On the other hand, the results did not meet our expectations (see above), because significant correlations to environmental parameters (here, it was the difference between valley and mountain samples) were still recognized. The present cultivation data are in concordance with similar studies (Unterseher et al. 2013b) and confirm general knowledge about the community ecology of leaf endophytes (Cordier et al. 2012, Zimmermann and Vitousek 2012, Meng et al. 2013, Glynou et al. 2015, Rojas-Jimenez et al. 2016). Moreover, similar results were observed in the present comparative study on the basis of HTS data (see also Siddique and Unterseher 2016, Unterseher et al. 2016).

Conclusions

Our results clearly justify the co-existence of cultivation and high-throughput approaches. Despite the fast improvement and diversification of HTS technologies with many undeniable advantages in microbial biodiversity assessments (Bálint et al. 2016), cultivation should be retained at highest standards (e.g. Gazis and Chaverri 2015), given, among others, the availability of living cultures for genome, metabolome or bioprospecting analyses (Gazis et al. 2016, Kolarik et al. 2016).

Our results suggest that the immense body of literature about cultivable endophytic fungi can and should be consulted for the interpretation of community signals obtained from HTS studies.

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References

Abarenkov K, Adams RI, Laszlo I, Agan A, Ambrosio E, Antonelli A, Bahram M, Bengtsson-Palme J, Bok G, Cangren P, Coimbra V, Coleine C, Gustafsson C, He J, Hofmann T, Kristiansson E, Larsson E, Larsson T, Liu Y, Martinsson S, Meyer W, Panova M, Pombubpa N, Ritter C, Ryberg M, Svantesson S, Scharn R, Svensson O, Töpel M, Unterseher M, Visagie C, Wurzbacher C, Taylor AFS, Kõljalg U, Schriml L, Nilsson RH (2016) Annotating public fungal ITS sequences from the built environment according

to the MIxS-Built Environment standard – a report from a May 23-24, 2016 workshop (Gothenburg, Sweden). MycoKeys 16: 1–15. https://doi.org/10.3897/mycokeys.16.10000

Albrectsen BR, Björkén LL, Varad A, Hagner A, Wedin M, Karlsson J, Jansson S (2010) Endophytic fungi in European aspen (*Populus tremula*) leaves – diversity, detection, and a suggested correlation with herbivory resistance. Fungal Diversity 41: 17–28. https://doi.org/10.1007/s13225-009-0011-y

Allmér J, Vasiliauskas R, Ihrmark K, Stenlid J, Dahlberg A (2006) Wood-inhabiting fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as reflected by sporocarps, mycelial isolations and T-RFLP identification: Wood-inhabiting fungal communities in woody debris of Norway spruce. FEMS Microbiology Ecology 55: 57–67. https://doi.org/10.1111/j.1574-6941.2005.00010.x

Al-Sadi AM, Al-Mazroui SS, Phillips AJL (2015) Evaluation of culture-based techniques and 454 pyrosequencing for the analysis of fungal diversity in potting media and organic fertilizers. Journal of Applied Microbiology 119: 500–509. https://doi.org/10.1111/jam.12854

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biology Reviews 21: 51–66. https://doi.org/10.1016/j.fbr.2007.05.003

Bálint M, Bahram M, Eren AM, Faust K, Fuhrman JA, Lindahl B, O'Hara RB, Öpik M, Sogin ML, Unterseher M, Tedersoo L (2016) Millions of reads, thousands of taxa: microbial community structure and associations analyzed via marker genes. FEMS Microbiology Reviews 40(5): 686–700. https://doi.org/10.1093/femsre/fuw017

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I (2015) Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. Molecular Ecology 24: 235–248. https://doi.org/10.1111/mec.13018

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, Amend AS, Jumpponen A, Unterseher M, Kristiansson E, Abarenkov K, Bertrand YJK, Sanli K, Eriksson KM, Vik U, Veldre V, Nilsson RH (2013) Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4: 914–919. https://doi.org/10.1111/2041-210X.12073

Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Lickteig SK, Lothamer K, Oliver AK, Jumpponen A (2015) Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecology 13: 221–225. https://doi.org/10.1016/j.funeco.2014.08.006

Christian N, Whitaker BK, Clay K (2015) Microbiomes: unifying animal and plant systems through the lens of community ecology theory. Frontiers in Microbiology 6. https://doi.org/10.3389/fmicb.2015.00869

Collado J, Platas G, Paulus B, Bills G (2007) High-throughput culturing of fungi from plant litter by a dilution-to-extinction technique. FEMS Microbiology Ecology 60: 521–533. https://doi.org/10.1111/j.1574-6941.2007.00294.x

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau M, Vacher C (2012) The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*)

varies significantly along an elevation gradient. New Phytologist 196: 510–519. https://doi.org/10.1111/j.1469-8137.2012.04284.x

de Hoog GS, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. Mycoses 41: 183–189. https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

Eevers N, Beckers B, Beeck M, White JC, Vangronsveld J, Weyens N (2016) Comparison between cultivated and total bacterial communities associated with Cucurbita pepo using cultivation-dependent techniques and 454 pyrosequencing. Systematic and Applied Microbiology 39: 58–66. https://doi.org/10.1016/j.syapm.2015.11.001

Eusemann P, Schnittler M, Nilsson RH, Jumpponen A, Dahl MB, Würth DG, Buras A, Wilmking M, Unterseher M (2016) Habitat conditions and phenological tree traits overrule the influence of tree genotype in the needle mycobiome- *Picea glauca* system at an arctic treeline ecotone. New Phytologist 211: 1221–1231. https://doi.org/10.1111/nph.13988

Gazis R, Chaverri P (2015) Wild trees in the Amazon basin harbor a great diversity of beneficial endosymbiotic fungi: is this evidence of protective mutualism? Fungal Ecology 17: 18–29. https://doi.org/10.1016/j.funeco.2015.04.001

Gazis R, Kuo A, Riley R, LaButti K, Lipzen A, Lin J, Amirebrahimi M, Hesse CN, Spatafora JW, Henrissat B, Hainaut M, Grigoriev IV, Hibbett DS (2016) The genome of *Xylona heveae* provides a window into fungal endophytism. Fungal Biology 120: 26–42. https://doi.org/10.1016/j.funbio.2015.10.002

Glynou K, Ali T, Buch A-K, Haghi Kia S, Ploch S, Xia X, Çelik A, Thines M, Maciá-Vicente JG (2016) The local environment determines the assembly of root endophytic fungi at a continental scale: Continental-scale distribution of root endophytes. Environmental Microbiology 18: 2418–2434. https://doi.org/10.1111/1462-2920.13112

Hill MO (1973) Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 54: 427–432. https://doi.org/10.2307/1934352

Jumpponen A, Brown SP (2014) The rich and the sensitive: diverse fungal communities change functionally with the warming Arctic. Molecular Ecology 23: 3127–3129. https://doi.org/10.1111/mec.12805

Khoyratty S, Dupont J, Lacoste S, Palama TL, Choi YH, Kim HK, Payet B, Grisoni M, Fouillaud M, Verpoorte R, Kodja H (2015) Fungal endophytes of *Vanilla planifolia* across Réunion Island: isolation, distribution and biotransformation. BMC Plant Biology 15. https://doi.org/10.1186/s12870-015-0522-5

Kolařík M, Spakowicz DJ, Gazis R, Shaw J, Kubátová A, Nováková A, Chudíčková M, Forcina GC, Kang KW, Kelnarová I, Skaltsas D, Portero CE, Strobel SA, Narváez-Trujillo A (2016) *Biatriospora* (Ascomycota: Pleosporales) is an ecologically diverse genus including facultative marine fungi and endophytes with biotechnological potential. Plant Systematics and Evolution. https://doi.org/10.1007/s00606-016-1350-2

Koljalg U, Nilsson R, Abarenkov K, Tedersoo L, Taylor A, Bahram M, Bates S, Bruns T, Bengtsson-Palme J, Callaghan T, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc T, Griffith G, Hartmann M, Kirk P, Kohout P, Larsson E, Lindahl B, Lucking R, Martin M, Matheny P, Nguyen N, Niskanen T, Oja J, Peay K, Peintner U, Peterson M, Poldmaa K, Saag L, Saar I, Schussler A, Scott J, Senes C, Smith M, Suija A, Taylor D, Telleria M,

Weiss M, Larsson K (2013) Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22: 5271–5277. https://doi.org/10.1111/mec.12481

Langarica-Fuentes A, Handley PS, Houlden A, Fox G, Robson GD (2014) An investigation of the biodiversity of thermophilic and thermotolerant fungal species in composts using culture-based and molecular techniques. Fungal Ecology 11: 132–144. https://doi.org/10.1016/j.funeco.2014.05.007

Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Applied and Environmental Microbiology 69: 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

Meiser A, Bálint M, Schmitt I (2014) Meta-analysis of deep-sequenced fungal communities indicates limited taxon sharing between studies and the presence of biogeographic patterns. New Phytologist 201: 623–635. https://doi.org/10.1111/nph.12532

Meng H, Li K, Nie M, Wan J-R, Quan Z-X, Fang C-M, Chen J-K, Gu J-D, Li B (2013) Responses of bacterial and fungal communities to an elevation gradient in a subtropical montane forest of China. Applied Microbiology and Biotechnology 97: 2219–2230. https://doi.org/10.1007/s00253-012-4063-7

Navas-Molina J, Peralta-Sanchez J, Gonzalez A, McMurdie P, Vazquez-Baeza Y, Xu Z, Ursell L, Lauber C, Zhou H, Song S, Huntley J, Ackermann G, Berg-Lyons D, Holmes S, Caporaso J, Knight R (2013) Advancing our understanding of the human microbiome using QIIME. Methods in Enzymology 531: 371–444. https://doi.org/10.1016/B978-0-12-407863-5.00019-8

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology. 20: 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson RH, Hyde KD, Pawłowska J, Ryberg M, Tedersoo L, Aas AB, Alias SA, Alves A, Anderson CL, Antonelli A, Arnold AE, Bahnmann B, Bahram M, Bengtsson-Palme J, Berlin A, Branco S, Chomnunti P, Dissanayake A, Drenkhan R, Friberg H, Frøslev TG, Halwachs B, Hartmann M, Henricot B, Jayawardena R, Jumpponen A, Kauserud H, Koskela S, Kulik T, Liimatainen K, Lindahl BD, Lindner D, Liu J-K, Maharachchikumbura S, Manamgoda D, Martinsson S, Neves MA, Niskanen T, Nylinder S, Pereira OL, Pinho DB, Porter TM, Queloz V, Riit T, Sánchez-García M, de Sousa F, Stefańczyk E, Tadych M, Takamatsu S, Tian Q, Udayanga D, Unterseher M, Wang Z, Wikee S, Yan J, Larsson E, Larsson K-H, Kõljalg U, Abarenkov K (2014) Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Diversity 67: 11–19. https://doi.org/10.1007/s13225-014-0291-8

Nilsson RH, Tedersoo L, Ryberg M, Kristiansson E, Hartmann M, Unterseher M, Porter TM, Bengtsson-Palme J, Walker DM, de Sousa F, Gamper HA, Larsson E, Larsson K-H, Kõljalg U, Edgar RC, Abarenkov K (2015) A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. Microbes and Environments 30: 145–150. https://doi.org/10.1264/jsme2.ME14121

Oono R, Lefèvre E, Simha A, Lutzoni F (2015) A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*). Fungal Biology 119: 917–928. https://doi.org/10.1016/j.funbio.2015.07.003

Peršoh D (2013) Factors shaping community structure of endophytic fungi–evidence from the *Pinus-Viscum*-system. Fungal Diversity 60: 55–69. https://doi.org/10.1007/s13225-013-0225-x

Peršoh, D (2015) Plant-associated fungal communities in the light of meta'omics. Fungal Diversity 75: 1–25. https://doi.org/10.1007/s13225-015-0334-9

Pitkaranta M, Meklin T, Hyvarinen A, Paulin L, Auvinen P, Nevalainen A, Rintala H (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. Applied and Environmental Microbiology 74: 233–244. https://doi.org/10.1128/AEM.00692-07

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F (2016) Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Molecular Ecology 25: 4059–4074. https://doi.org/10.1111/mec.13739

Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, Chase J, McDonald D, Gonzalez A, Robbins-Pianka A, Clemente JC, Gilbert JA, Huse SM, Zhou H-W, Knight R, Caporaso JG (2014) Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. PeerJ 2, e545. https://doi.org/10.7717/peerj.545

Rodriguez R, White JJ, Arnold A, Redman R (2009) Fungal endophytes: diversity and functional roles. New Phytologist 182: 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Rojas-Jimenez K, Hernandez M, Blanco J, Vargas LD, Acosta-Vargas LG, Tamayo G (2016) Richness of cultivable endophytic fungi along an altitudinal gradient in wet forests of Costa Rica. Fungal Ecology 20: 124–131. https://doi.org/10.1016/j.funeco.2015.12.006

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology 12: 87. https://doi.org/10.1186/s12915-014-0087-z

Scholtysik A, Unterseher M, Otto P, Wirth C (2013) Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*). Mycological Progress 12: 291–304. https://doi.org/10.1007/s11557-012-0835-9

Siddique AB, Unterseher M (2016) A cost-effective and efficient strategy for Illumina sequencing of fungal communities: A case study of beech endophytes identified elevation as main explanatory factor for diversity and community composition. Fungal Ecology 20: 175–185. https://doi.org/10.1016/j.funeco.2015.12.009

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? Fungal Biology Reviews 21: 75–89. https://doi.org/10.1016/j.fbr.2007.05.004

Solis MJL, dela Cruz TE, Schnittler M, Unterseher M (2016) The diverse community of leaf-inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina, F. elastica* and *F. religiosa*. Mycoscience. https://doi.org/10.1016/j.myc.2015.10.002

Unterseher M (2011) Diversity of Fungal Endophytes in Temperate Forest Trees, in: Pirttilä AM (Ed.) Endophytes of Forest Trees: Biology and Applications. Springer Netherlands, 31–46. https://doi.org/10.1007/978-94-007-1599-8_2

Unterseher M, Gazis R, Chaverri P, Guarniz CFG, Tenorio DHZ (2013a) Endophytic fungi from Peruvian highland and lowland habitats form distinctive and host plant-specific assemblages. Biodiversity and Conservation 22: 999–1016. https://doi.org/10.1007/s10531-013-0464-x

Unterseher M, Peršoh D, Schnittler M (2013b) Leaf-inhabiting endophytic fungi of European Beech (*Fagus sylvatica* L.) co-occur in leaf litter but are rare on decaying wood of the same host. Fungal Diversity 60: 43–54. https://doi.org/10.1007/s13225-013-0222-0

Unterseher M, Schnittler M (2009) Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.)--different cultivation techniques influence fungal biodiversity assessment. Mycological Research 113: 645–654. https://doi.org/10.1016/j.mycres.2009.02.002

Unterseher M, Siddique AB, Brachmann A, Peršoh D (2016) Diversity and composition of the leaf mycobiome of beech (*Fagus sylvatica*) are affected by local habitat conditions and leaf biochemistry. PLoS ONE 11: e0152878. https://doi.org/10.1371/journal.pone.0152878

U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch AD, Arnold AE (2012) Host and geographic structure of endophytic and endolichenic fungi at a continental scale. American Journal of Botany 99: 898–914. https://doi.org/10.3732/ajb.1100459

Zhang X, Tang G, Xu X, Nong X, Qi S-H (2014) Insights into deep-sea sediment fungal communities from the East Indian Ocean using targeted environmental sequencing combined with traditional cultivation. PLoS ONE 9, e109118. https://doi.org/10.1371/journal.pone.0109118

Zimmerman NB, Vitousek PM (2012) Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proceedings of the National Academy of Sciences 109: 13022–13027. https://doi.org/10.1073/pnas.1209872109

Supplementary material 1

Bioinformatics pipeline

Authors: Abu Bakar Siddique, Anis Mahmud Khokon, Martin Unterseher

Data type: scripts, spreadsheet

Explanation note: This file provides all steps and commands necessary for quality filtering and demultiplexing of raw paired fastq sequences.

Supplementary material 2

Biodiversity workflow in R

Authors: Abu Bakar Siddique, Anis Mahmud Khokon, Martin Unterseher

Data type: Scripts, measurement

Explanation note: Bundle of files for biodiversity analysis in R. All necessary input files and a commented script of R-commands are provided.

Supplementary material 3

Common OTU lists and Statistical analysis

Authors: Abu Bakar Siddique, Anis Mahmud Khokon, Martin Unterseher

Data type: measurements

Explanation note: This file contains detected OTUs in both methods and biodiversity analysis (GLM and t-test)

Supplementary material 4

Master data sheet

Authors: Abu Bakar Siddique, Anis Mahmud Khokon, Martin Unterseher

Data type: spreadsheet

Explanation note: Spreadsheet file containing information about read abundances of operational taxonomic units (OTUs) and sample metadata. Here, data were prepared for subsequent biodiversity analysis in R.

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RESEARCH ARTICLE

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The genus *Podaxis* in arid regions of Mexico: preliminary ITS phylogeny and ethnomycological use

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Abstract

Identification of *Podaxis* species to species-level based on morphology alone is problematic. Thus, species of the genus *Podaxis* are in dire need of taxonomic and phylogenetic evaluations using molecular data to develop a consensus between morphological taxonomy and more robust molecular analyses. In Mexico, most of the collected specimens of *Podaxis* have been morphologically identified as *Podaxis pistillaris sensu lato* and are locally used for its culinary value. In this study, the internal transcribed spacer region of *Podaxis* specimens from the MEXU fungarium collected between 1948 and 2014 from arid regions of Mexico were sequenced and these collections placed into a molecular phylogenetic framework using Maximum Likelihood analysis. In addition, the ethnomycological use of *Podaxis* in Mexico (utility, traditional handling, economic importance, etc.) is described by observations, interviews, and sampling of *Podaxis* species with local people from three areas of the region of the Cañada of Oaxaca, which belongs to the Tehuacán-Cuicatlán Biosphere Reserve. These results indicate that the Mexican *Podaxis* were divided into two clades. Specimens collected in the northern region showed phylogenetic affinities to clade D, while specimens from the south of Mexico clustered within clade E. Morphological data, such as spore length and width, showed significant differences between the two phylogenetic clades, implying that these clades represent different species. None of the Mexican specimens were found in association with termite mounds, which might indicate an adaptation to desert-like regions. This study provides the first ethnomycological use of *Podaxis* from Mexico.

Key words

Basidiomycota, edible mushroom, *Podaxis pistillaris*

Introduction

Podaxis has been collected from numerous arid regions around world; approximately 44 species have been described to date (Conlon et al. 2016). This genus encompasses a wide range of morphological characters such as variation in color, size and shapes in fruit body morphology, as well as a wide range of spore length, width and wall thickness, and has often been confused with *Coprinus comatus* (Morse 1933; Morse 1941; Herrera 1950). Earlier classifications have placed *Podaxis* within the family Podaxaceae (Morse 1933); however, modern taxonomic classification places it within the family Agaricaceae (Kirk et al. 2008). Recently, Conlon and collaborators (2016) studied 45 specimens labeled as *Podaxis pistillaris*, mainly from South Africa, and based on combined internal transcribed spacer (ITS) region and LSU rDNA phylogeny analyses demonstrated that the genus contained at least six clades (A-F) representing different putative *Podaxis* spp.

In Mexico, *Podaxis* was reported for the first time in 1893 as *P. mexicanus* from Agiabampo, Sonora (Ellis 1893). Then, in 1908, N. T. Patouillard identified *P. farlowii* also from Sonora (Morse 1933), and in 1938, D. H. Linder identified *P. farlowii* from Hipolito, Coahuila (http://mycoportal.org). Since then, all the specimens, including the ones deposited at the fungarium of the Herbario Nacional de Mexico (MEXU), have been described as *P. pistillaris* (Herrera 1950; Guzmán and Herrera 1969; Guzmán and Herrera 1973; Urista et al. 1985; Esqueda et al. 2010; Esqueda et al. 2012). The reduction of names of all specimens in the MEXU fungarium to *P. pistillaris* has not been previously investigated in light of molecular data.

Despite the occurrence of *Podaxis* in arid regions of Mexico, the ethnomycological use of this mushroom in the country is undocumented. This is particularly important since *Podaxis* spp. have been widely utilized for its culinary value by indigenous people, particularly in the Tehuacán-Cuicatlán Biosphere Reserve (RBTC) in the south of Mexico. In this context, the goals of this study were: 1) to analyze via ITS sequencing newly collected and fungarium specimens of *Podaxis* from Mexico to better predict their molecular phylogenetic placement and thus establish if one or more phylogenetic species of *Podaxis* exist in Mexico; and 2) to describe the traditional use, handling and economic importance of *Podaxis* spp. in the RBTC by observations and interviews with the local people.

Methods

Fungal material

Eighteen fungarium and five fresh *Podaxis* specimens from different arid regions of Mexico were used for the phylogenetic study. The fresh fruiting bodies were obtained from four sites in three communities of the state of Oaxaca (Table 1); all collections were made during rainy season. Sampling, description, digitalization, and drying of mushrooms were performed as recommended by Cifuentes et al. (1986). We analyzed the specimens in the laboratory, and measured macro and microscopic characteristics

Table 1. Voucher specimens in the fungi collection of the Herbario Nacional de México (MEXU) at the Instituto de Biología, Universidad Nacional Autónoma de México.

Voucher (MEXU)	Clade	Locality	Collector and collection date (mm/dd/yyyy)	Location geography	Type of vegetation	Habitat	Native language	GenBank
1191	D	Oaxaca RBTC	T. Herrera, M. Ruiz-Oronoz (10/16/1948)	San Pedro Chicozapotes, San Juan Bautista Cuicatlán municipality, 633 masl, 17°46.232'N, 96°57.209'W	TrDF	Sandy soil	Cuicatec	KY034680
10805	D	Oaxaca	A. Solís-Magallanes (07/1976)	Presa Benito Juárez, Oaxaca-Tehuantepec	--	Limestone soil	--	KY034681
12808	D	Oaxaca	O. Téllez, M. Sousa, L. Rico (02/20/1978)	Salina Cruz-Pochutla, Salina Cruz, 20 masl	TrDF	--	--	KY034682
7023	D	Oaxaca	T. Herrera (08/04/1979)	Istmo of Tehuantepec	--	Sandy soil	--	KY034683
27558	D	Oaxaca RBTC	A. Medina-Ortiz (06/24/2009)	El Brujo, Santa María Tecomavaca municipality, 626 masl, 17°57.501'N, 97°1.266'W	TrDF	Sandy soil, sandy clay in stony, clayey silt, and cultivation soil	Mazatec and Mixtec	KY034684
27845	D	Oaxaca RBTC	A. Medina-Ortiz, A. de la Cruz-Martínez (10/07/2013)	Santiago Quiotepec, San Juan Bautista Cuicatlán municipality, 626 masl, 17°57.501'N, 97°1.266'W	TrDF and DS	Sandy clay	Cuicatec	KY034686
27557	D	Oaxaca RBTC	A. Medina-Ortiz, E. Pérez-Silva, A. García-Mendoza (07/12/2014)	Cuicatlán-Concepción Pápalo, San Juan Bautista Cuicatlán municipality. 630 masl, 17°47.727'N, 96°57.530'W	TrDF and DS	Sandy soil	Cuicatec	KY034687
5772	D	Durango	J. Sánchez (09/10/1966)	Estación Chocolate, Lerdo, Durango-Torreón	DS	--	--	KY034678
12338	D	Baja California Sur	E. Pérez-Silva 09/01/ 1978	Econhotel, La Paz	--	--	--	KY034673
5015	D	Tamaulipas	A. Gómez-Pompa, E. Nebling (09/03/1967)	Mante-Gonzáles City	ThDF	Clay soil	--	KY034689
7212	D	Tamaulipas	A. Marino (03/10/1970)	Abasolo municipality	AZ	Silty soil	--	KY034690
22610	E	Sonora	Romo (03/08/1990)	Estación Pesqueira, San Miguel de Horcasitas municipality	--	--	--	KY034688
8423	E	Coahuila	R. Hernández, R López, F. Medrano (09/29/1973)	Hidalgo municipality	AZ	Sandy clay	--	KY034674

Voucher (MEXU)	Clade	Locality	Collector and collection date (mm/dd/yyyy)	Location geography	Type of vegetation	Habitat	Native language	GenBank
8425	E	Coahuila	R. Hernández, R. López, F. Medrano (09/28/1973)	Hidalgo municipality, 150-200 masl	CV	Sandy clay with some grass	--	KY034675
8422	E	Coahuila	R. Hernández, R. López (09/28/1973)	Rancho Palo Blanco, Hidalgo municipality, 147 masl	CV	Sandy clay	--	KY034676
8424	E	Coahuila	R. Hernández, R. López, F. Medrano (09/28/1973)	Rancho Palo Blanco, Hidalgo municipality	CV	Sandy clay	--	KY034677
8426	E	Nuevo León	R. Hernández, R. López, F. Medrano (09/27/1973)	Rancho San José, Anáhuac municipality, 144 masl	CV	Sandy clay	--	KY034679
27843	E	Oaxaca RBTC	A. Medina-Ortiz (09/09/2011)	La Sabana, Santa María Tecomavaca municipality 626 masl, 17°57.501'N, 97°1.266'W	TrDF	Sandy soil, sandy clay, in stony, clayey silt, and cultivation soil	Mazatec and Mixtec	KY034685
21635	N/A	Oaxaca	A. Calderón (07/11/1988)	Zipolite, Puerto Ángel, San Pedro Pochutla municipality	S	Sandy soil	--	N/A
27844	N/A	Oaxaca RBTC	A. Medina-Ortiz, F. Medina-Ruiz (07/13/2013)	Jiotillo redondo, Santa María Tecomavaca municipality 626 masl, 17°57.501'N, 97°1.266'W	TrDF and DS	Sandy clay	Mazatec and Mixtec	N/A
11887	N/A	Oaxaca	O. Téllez (10/24/1977)	San Pedro Totolapam, Oaxaca-Tehuantepec	--	--	--	N/A
1148	N/A	Sonora	E. Matuda (11/22/1962)	Sonoyta, 150 masl	AZ	Sandy clay	--	N/A
22608	N/A	Sonora	M. Esqueda (08/29/ 1988)	Hermosillo	--	Sandy soil	--	N/A

AZ: Arid zone; CV: Chaparral vegetation; DS: Desert scrub; S: Seashore; ThDF: Thorny deciduous forest; TrDF: Tropical deciduous forest. N/A: No sequence data obtained.

(Herrera 1950; Guzmán and Herrera 1969). The collected specimens were deposited in the fungarium of the Herbario Nacional de Mexico (MEXU) of the Instituto de Biología at the Universidad Nacional Autónoma de México (UNAM). In addition, basidiospores we obtained from the center of the dried cap of each of fresh and fungarium fruiting bodies fixed with KOH 5% and photographs were taken (Figure 2 and Suppl. material 1).

DNA extraction, PCR amplification and Sanger sequencing

DNA was extracted from a powder of dried cap (pileus, fresh samples) and the center of the stipe (fungarium samples) from specimens indicated in Table 1. Approximately 5 mg of powdered fungal material was transferred to a bashing bead tube with DNA lysis buffer provided by Zymo research fungal/ bacterial DNA extraction kit. Next, DNA was extracted using the procedures indicated in the Zymo fungal/ bacterial DNA MiniPrep kit.

The entire ITS region was PCR-amplified on an Applied Biosystems Veriti thermal cycler using PuReTaq Ready-To-Go PCR Beads with ITS5 and ITS4 primers (Gardes et al. 1991; White et al. 1990). The PCR reaction was carried out in 25 µL containing 3–5 µL template DNA, 2.5 µL BSA, 2.5 µL 50% DMSO, and 1 µL of each 10 µM forward (ITS5) and reverse (ITS4) primers. Molecular biology grade water from Fisher scientific was added to reach 25 µL. The following thermocycling parameters were used for the amplification: initial denaturation at 95°C for 5 min followed by 39 cycles at 95°C for 30 s, 55°C for 15 s, and 72°C for 1 min, and a final extension step of 10 min at 72°C (Schoch et al. 2012). The PCR products were then examined on an ethidium bromide-stained 1% agarose gel (Fisher Scientific) along with a 1 kb DNA ladder (Promega) to estimate the size of the amplified band. PCR products were purified using a Wizard SV Gel and PCR Clean-up System.

Sanger sequencing of the purified PCR products was performed at Eurofins Genomics (http://www.operon.com/default.aspx) using BigDye Terminator v3.1 cycle sequencing. The sequencing was accomplished bidirectionally using both strands with a combination of ITS5 and ITS4 primers. Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer. For both sequencing reactions, approximately 15 µL of PCR template were used along with 2 µM sequencing primers.

Phylogenetic analysis

Sequences were assembled with Sequencher 5.3 (Gene Codes), optimized and then manually corrected when necessary; the latter step was to assure that the computer algorithm was properly assigning base calls. Each sequence fragment was subjected to an individual Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) search

in NCBI GenBank to verify its identity. Detailed BLAST search using ITS data were conducted utilizing only published sequences as outlined previously (Raja et al. 2017).

The newly obtained ITS sequences (Table 1) were aligned with ITS sequences of authenticated published sequences or from vouchered fungarium samples (Brock et al. 2009; Osmundson et al. 2013), such as those from a recent phylogenetic study on *Podaxis* spp. (Conlon et al. 2016) using the multiple sequence alignment program MUSCLE (Edgar 2004), with default parameters in operation. *Leucoprinus* was used as an outgroup taxon based on previous studies (Hopple and Vilgalys 1999; Conlon et al. 2016). MUSCLE was implemented using the program Seaview v. 4.3. (Galtier et al. 1996; Gouy et al. 2010). Maximum Likelihood (ML) analysis was perfomed using RAxML v. 7.0.4 (Stamatakis et al. 2008). The analysis was run on the CIPRES Portal v. 3.3 (Miller et al. 2010) with the default rapid hill-climbing algorithm and GTR model employing 1000 fast bootstrap searches. Clades with bootstrap values ≥ 70% were considered significant and strongly supported (Hillis and Bull 1993).

Spore morphology

All spores were measured using a Carl Zeiss Primo Star microscope (Carl Zeiss, Germany) with a Canon PowerShot G6 camera with a Zeiss universal digital camera adapter d30 M37/52´0.75. For each specimen, 25 spores were measured for spore length and width, and presence or absence of a germ pore (Table 2). The Mann-Whitney (U-test) was used to determine whether the mean values of the spore lengths and widths were significantly different between the MEXU specimens assigned to the phylogenetic clades.

Ethnomycological study

This study was conducted in the RBTC, which is located in the states of Puebla and Oaxaca in central Mexico (between 17°32'24" and -18°52'55"N, and 97°48'43" and -97°48'43"W; Figure 1), and its mainly characterized by arid and semiarid vegetation (Valiente-Banuet et al. 2009). This region comprises eight ethnic groups: two in Puebla, the Popolocas and Nahuas; and six in Oaxaca, the Mixtecs, Cuicatecs, Mazatecs, Chinantecs, Chocholtecs, and Ixcatecs (SEMARNAT and CONANP 2013). In the regions where this study was conducted, some people spoke an indigenous language but Spanish was the prevalent mean of communication among them (Table 1). Local people from the RBTC were randomly selected for the ethnomycological interview.

Inhabitants of the region, most 18 years and older, shared their knowledge through the following questionnaire: i) personal information (name, age, sex, ethnicity, place of birth, residence, occupation, and number of family members); ii) knowledge of mushrooms from the locality (traditional name, description of the fruiting body, myths, and uses); iii) how they collect the mushrooms (frequency of collection, if they eat it or buy it); iv) importance of mushrooms in their life; v) how many different mushrooms they

Table 2. Basidiospore measurements.

MEXU	Clade	State	Length ± SD	Ranges	Width ± SD	Ranges	L/W ± SD
1191	D	Oaxaca	11.56 ± 0.71	10 < L < 13	10.96 ± 0.20	10 < W < 11	1.05 ± 0.06
10805	D	Oaxaca	10.92 ± 0.76	10 < L < 13	9.24 ± 0.52	8 < W < 10	1.18 ± 0.06
12808	D	Oaxaca	12.52 ± 1.16	11 < L < 15	9.76 ± 0.93	8 < W < 11	1.29 ± 0.09
7023	D	Oaxaca	10.64 ± 0.70	10 < L < 12	9.68 ± 0.56	9 < W < 11	1.10 ± 0.06
27558	D	Oaxaca	11.28 ± 0.61	10 < L < 12	10.52 ± 0.51	10 < W < 11	1.07 ± 0.05
27845	D	Oaxaca	10.44 ± 0.51	10 < L < 11	9.84 ± 0.47	9 < W < 11	1.06 ± 0.06
27557	D	Oaxaca	10.04 ± 0.68	9 < L < 11	9.20 ± 0.71	8 < W < 10	1.09 ± 0.07
5772	D	Durango	10.80 ± 0.96	9 < L < 13	10.04 ± 0.73	9 < W < 12	1.08 ± 0.06
12338	D	Baja California Sur	11.28 ± 0.89	10 < L < 13	10.52 ± 0.65	10 < W < 12	1.07 ± 0.05
5015	D	Tamaulipas	10.72 ± 0.89	9 < L < 13	9.76 ± 0.60	9 < W < 11	1.10 ± 0.08
7212	D	Tamaulipas	10.32 ± 0.63	9 < L < 12	9.76 ± 0.66	9 < W < 11	1.06 ± 0.06
22610	E	Sonora	15.88 ± 0.97	14 < L < 18	14.00 ± 0.82	13 < W < 16	1.14 ± 0.05
8423	E	Coahuila	14.72 ± 0.79	13 < L < 16	13.88 ± 0.78	13 < W < 16	1.06 ± 0.05
8425	E	Coahuila	14.36 ± 1.22	12 < L < 17	13.28 ± 0.79	12 < W < 15	1.08 ± 0.05
8422	E	Coahuila	14.32 ± 1.03	12 < L < 16	12.76 ± 0.97	11 < W < 15	1.13 ± 0.08
8424	E	Coahuila	14.76 ± 0.60	14 < L < 16	13.68 ± 0.63	13 < W < 15	1.08 ± 0.04
8426	E	Nuevo León	15.36 ± 1.22	13 < L < 18	12.84 ± 0.85	12 < W < 15	1.20 ± 0.08
27843	E	Oaxaca	11.16 ± 0.75	10 < L < 13	10.48 ± 0.59	10 < W < 12	1.07 ± 0.05
21635	N/A	Oaxaca	10.08 ± 0.40	9 < L < 11	9.12 ± 0.73	8 < W < 10	1.11 ± 0.10
27844	N/A	Oaxaca	10.88 ± 0.60	10 < L < 12	10.36 ± 0.49	10 < W < 11	1.05 ± 0.06
10887	N/A	Oaxaca	12.32 ± 1.22	11 < L < 15	10.32 ± 0.63	10 < W < 12	1.19 ± 0.08
1148	N/A	Sonora	16.44 ± 1.04	15 < L < 19	14.20 ± 1.22	12 < W < 17	1.16 ± 0.06
22608	N/A	Sonora	12.28 ± 1.06	11 < L < 15	11.60 ± 1.00	10 < W < 15	1.06 ± 0.06

N/A = Sequence data not available.

see in their locality; vi) if they thought it is important to know the mushrooms; vii) what kind of problems they have when they collect mushrooms in the field; and viii) what information they need to identify the mushrooms.

Results

General morphology of *Podaxis*

All specimens studied (Table 1) share the typical morphological characteristics of the genus *Podaxis* (Figure 2 and Suppl. material 1): white or grayish-white fruit body when young and brown in old or dry specimens, with a long bulbous stem, traversing the gleba as a columella supporting the pileus at the apex. Pileus enveloping a large portion of the stipe, including most of the upper part, with a peridium of two layers and a well-developed capillitium. Exoperidium scaly, most of the scales deciduous at maturity. Endoperidium persistent, membranous, when dehiscing, becoming free

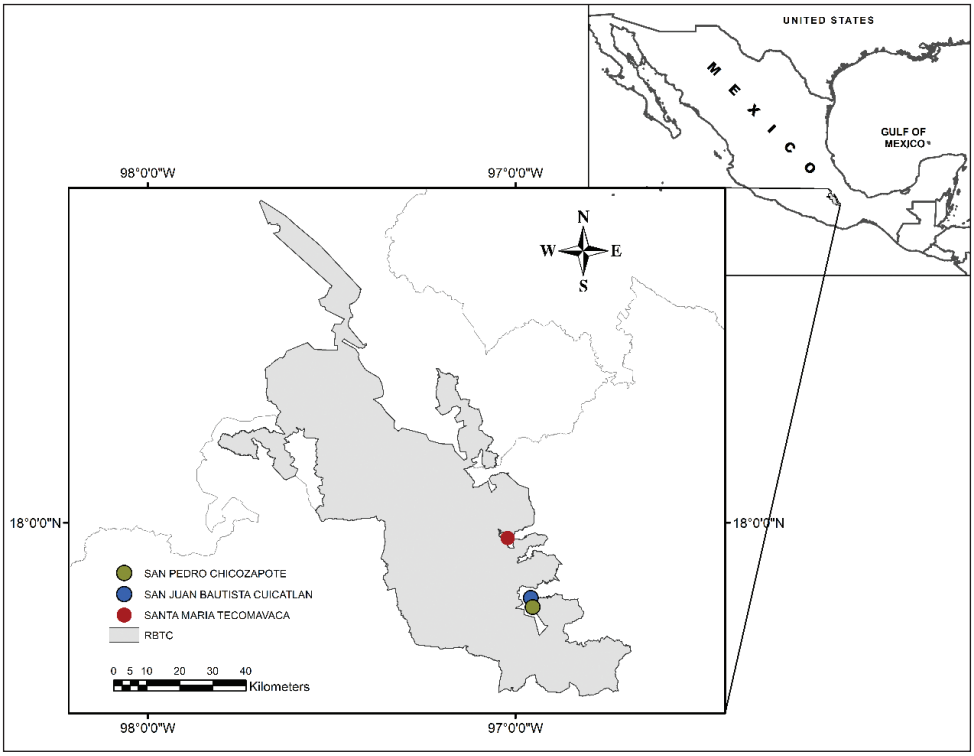

Figure 1. Localities studied in the RBTC. Map was elaborated with ArcGIS.

from the stipe at the base and by longitudinal fissures. Capillitium threads simple, eventually branched and septate, hyaline or pigmented, and flattened. Spores smooth, pigmented, apical pore present, wall of two layers. Basidia fasciculate with 1–4 spores on short sterigmata (Figure 2 and Suppl. material 1).

Variation in basidiospore size and morphology

The length and width, ranges and standard deviation (SD) of basidiospores are outlined in Table 2. MEXU specimens were grouped into two clades (see molecular phylogenetic analysis; Figure 4). Clade D: size of basidiospores in this clade ranged from 9–13 × 8–12 µm (mean = 11–12 × 9–10 µm); and clade E, size of basidiospores in this clade ranged from 9–17 × 9–16 µm (mean = 10–15 × 10–14 µm). Overall the basidiospores in clade D were smaller than basidiospores in clade E (Table 2). Based on the Mann-Whitney (U-test), we found that spore length ($p < 0.001$; Figure 3A) and width ($p < 0.001$; Figure 3B), were significantly different between clades D and E, which supports their molecular phylogenetic placements based on the ITS phylogeny (Figure 4). The color of spores in clade D was generally lighter when compared to those in clade E, which were dark reddish-brown (Figure 2 and Suppl. material 1).

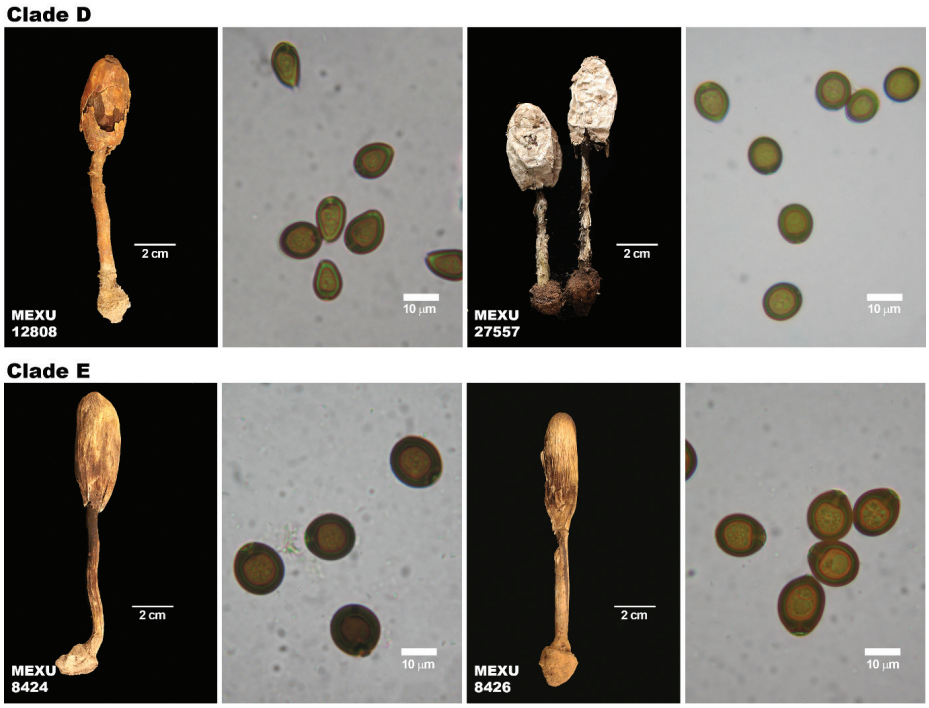

Figure 2. Fruit bodies and basidiospores of selected *Podaxis* specimens from clades D (MEXU 12808 and 27557) and E (MEXU 8424 and 8426).

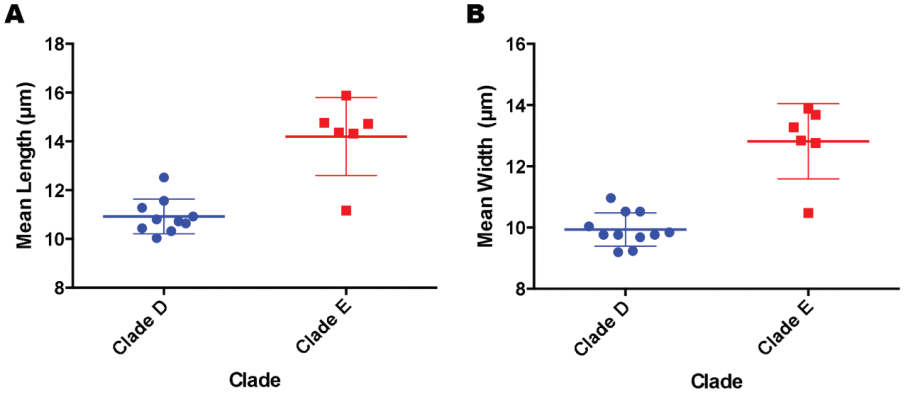

Figure 3. Mean ± SE spore (**A**) length and (**B**) width of MEXU *Podaxis* specimens from clades D and E. Results of Mann-Whitney U test: D–E, $p < 0.001$.

Phylogenetic analysis of molecular data

Eighteen new ITS sequences from different specimens of *Podaxis* from Mexico were obtained; these included four from freshly collected specimens, and 14 from samples in the MEXU fungarium (Table 1). High quality DNA and PCR products were obtained

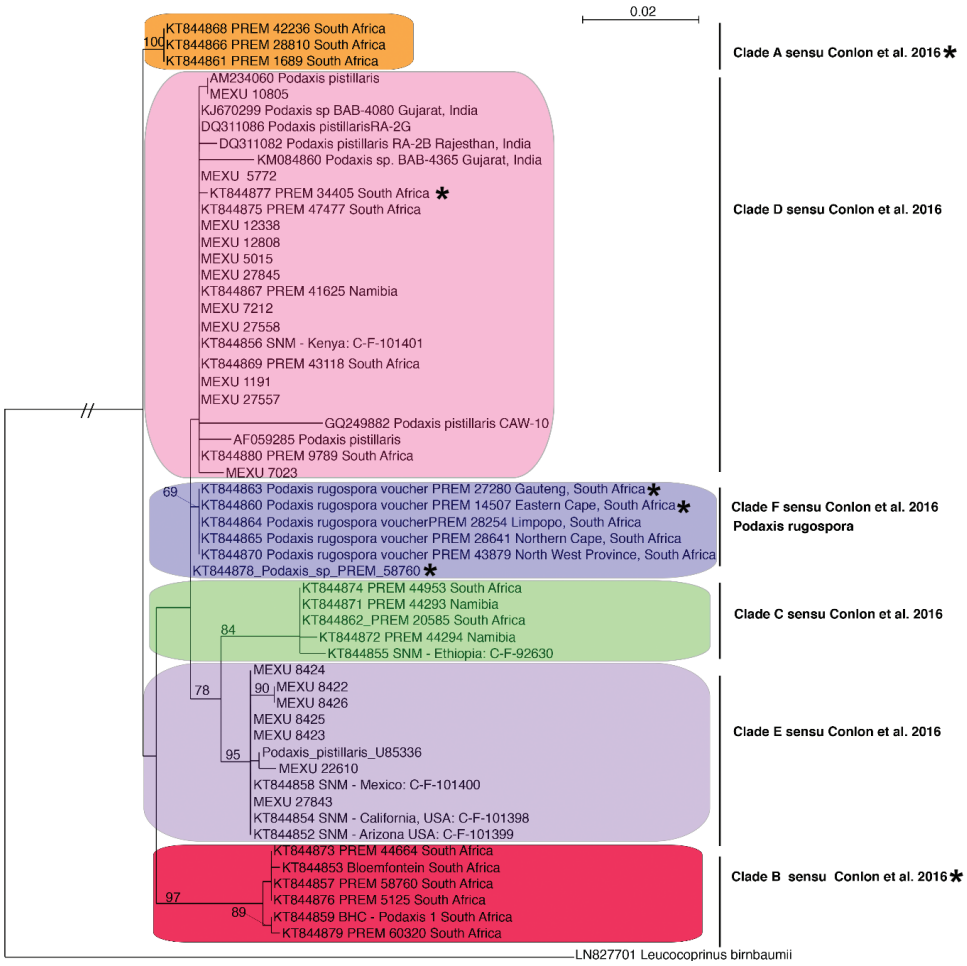

Figure 4. Phylogram of the most likely tree (-lnL = 1860.99) from a RAxML analysis of 56 taxa based on ITS rDNA (681 bp). Numbers above the nodes refer to RAxML bootstrap support values ≥ 70% based on 1000 replicates. Clades to the right (**A–F**) are labeled as per Conlon et al. 2016. The tree is rooted with Leucocoprinus birnbaumii. Symbol (*) next to collections indicates, it was reported from termite mounds. Bar indicates nucleotide substitutions per site.

for all specimens, including MEXU 1191, a collection made in 1948. We were unable to obtain DNA from MEXU 11887, 21635, and 5015 while MEXU 1148 and 27844 produced a PCR band, but resulted in mixed signals perhaps due to low or poor quality of DNA. The ITS alignment consisted of 55 taxa of *Podaxis* and one outgroup taxon (*Leucocoprinus birnbaumii*). The original ITS alignment consisted of 848 nucleotides, after ambiguous regions were limited and removed via GBlocks, the final ITS alignment contained 681 nucleotides.

RAxML analysis of the ITS dataset produced a single most likely tree (Figure 4). We recovered the same six clades (A, B, C, D, E, and F) that were revealed in Conlon

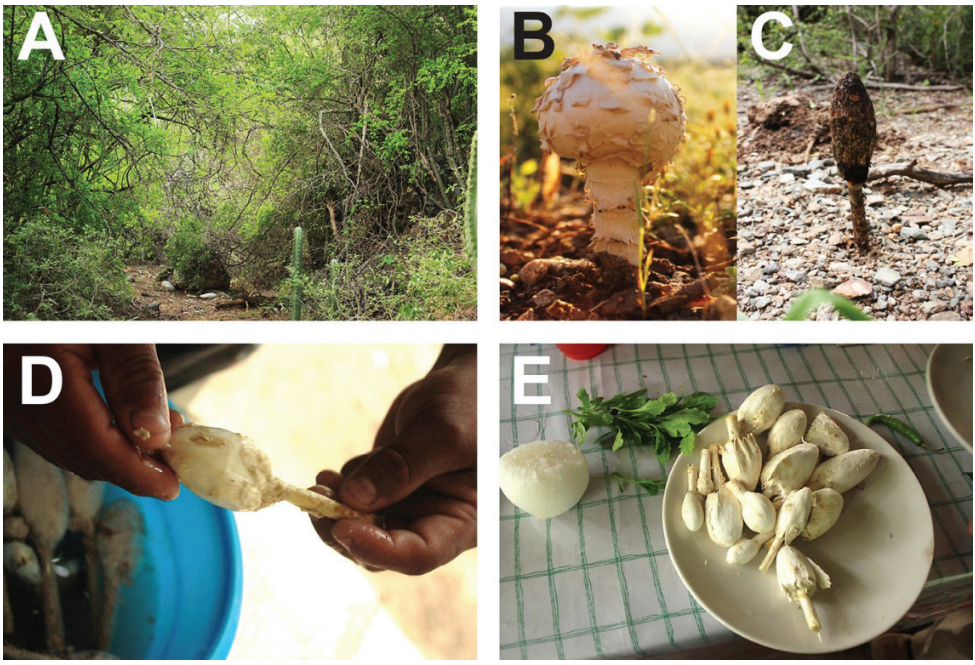

Figure 5. A Desert scrub **B** Immature state of *Podaxis* sp. (MEXU 27843) and **C** at maturity (the gleba changes from white to dark brown (MEXU 27844); Culinary preparation: **D** cleaning process of the fruiting body of *Podaxis* sp. and **E** mixing ingredients for the typical dish [onions, "epazote" (*Dysphania ambrosioides*) and green pepper (chile verde)].

et al. (2016). *Podaxis* spp. from Mexico are phylogenetically placed into two clades (D and E). Seven MEXU specimens (8424, 8422, 8426, 8425, 8423, 22610 and 27843) are placed in (clade E, *sensu* Conlon et al. 2016), with 95% RAxML boostrap support and grouped together with a sequence of *P. pistillaris* (GenBank: U85336), which has been reported in previous molecular phylogenetic studies of Agaricales fungi (Johnson 1999; Vellinga 2004), while 11 other MEXU specimens (10805, 5772, 12338, 12808, 5015, 27845, 7217, 27558, 1191, 27557 and 7023) were nested within clade D (*sensu* Conlon et al. 2016); however this clade did not receive significant RAxML bootstrap support. All ITS sequences generated from this study were deposited in the GenBank and accession numbers are provided in Table 1 (KY034673–KY034690).

Ethnomycology

Ethnomycological importance lies in the fact that people from this region eat the fruiting body of *Podaxis*, commonly known as "hongo" (mushroom), "hongo blanco comestible" (white edible mushroom), or "soldadito" (little soldier), almost daily during rainy season (Figure 5A–C). They cook the mushroom and mix it with green peppers, onions and "epazote" (*Dysphania ambrosioides*), and then make "empanadas" (stuffed

corn tortilla with the mix) (Figure 5D–E). It is considered a tasty mushroom, and according to the habitants of the region, as "one of the tastiest and most nourishing products the land gives us". The local people consider this fungus similar to a "piece of chicken" because of it taste. They also eat it raw, mixed with zucchini, or incorporated in chicken soup and the typical dishes "tesmole", "caldillo" and "mole".

Through the years, the local people have acquired the necessary knowledge to easily locate, harvest and select this mushroom from the land. Although this mushroom is mainly used for personal consumption, some people collect it and sell it in the community. They have also acquired the knowledge about the phenology and ecology of *Podaxis* spp., and they relate the "acidity of rain" with the germination of its spores. In addition, most of the people agree on the following: "when there are constant rains, the fungi starts to grow", "small mushrooms show up after it rains, the sun comes out and the sky is clear", "in order for it to grow, the mushroom needs sunlight for one or two days". Concerning the habitat and soil, they indicate that: "mushrooms grow mainly on the river bank or on sandy soil" but also "mushrooms are produced throughout the mountain slopes, even on agricultural production areas". They also say: "if you find one, you will find two" or "they are born in pairs". Finally, when a mushroom fruiting body has "aged", the local people spread the spores in places where they want the fungi to grow next rain season, and they say: "if they don't grow this season, they'll grow during the next one".

Discussion

Phylogenetic affiliations of MEXU specimens based on molecular and morphological comparison

Podaxis pistillaris sensu lato has been collected and reported from numerous semi-arid regions around the world, fruiting mainly in the rainy seasons. In Mexico, it has widely been collected from north to south (Herrera 1950; Dennis 1960; Guzmán and Herrera 1973; Urista et al. 1985; Aparicio-Navarro et al. 1991). Despite its wide geographical distribution, the identification of *P. pistillaris* remains confusing mainly because the type specimen of *P. pistillaris* collected and described from India has not been sequenced (Linnaeus 1771), making a true molecular phylogenetic assessment difficult. It is likely that cryptic speciation is rampant in this widely distributed species.

All the studied specimens from the MEXU fungarium were named as *P. pistillaris* based on its morphological characteristics (Figure 2 and Suppl. material 1); however, molecular phylogenetic analysis of the ITS region of these specimens, along with ITS sequences from a recent study of *Podaxis* spp. from South Africa (Conlon et al. 2016), placed the MEXU specimens into two clades: D and E (Figure 4). Therefore, our analysis indicate there are at least two phylogenetic species of *Podaxis* in Mexico, and not all species of *Podaxis* collected from Mexico should be identified as *P. pistillaris.*

Interestingly, all specimens in clade E (Figure 4) have been reported from North America, including Mexico. In our ITS phylogeny seven MEXU specimens (8424, 8422, 8426, 8425, 8423, 22610 and 27843) were grouped with an ITS sequence of *P. pistillaris* (GenBank: U85336; Johnson 1999; Vellinga 2004) with significant bootstrap support (95%). However, at this time it is not possible to name this clade. This is because there are other species from the new world, including southwestern US, Mexico and Argentina such as *P. argentinus* Speg., *P. longii* McKnight, *P. microporus* McKnight (McKnight, 1985), *P. farlowii* Massee (Morse 1933), and *P. mexicanus* (Ellis 1893), which need to be examined in light of molecular phylogenetic analysis.

Clade E (Figure 4) is entirely comprised of specimens from the new world and all of these occur as free-living in desert-like semi-arid regions (Table 1). There have been reports of symbiotic association of *Podaxis* spp. with termites in Australia (Priest and Lenz 1999; Young et al. 2002), Nigeria (Alasoadura 1966), South Africa (Bottomley 1948; Conlon et al. 2016), and Bolivia (Rocabado et al. 2007). In this context, it is worth to mentioning that in the RBTC (Oaxaca, Mexico) such a symbiotic association with termites has not yet been reported. Further molecular studies of *Podaxis* specimens collected from the new world are required to test the hypothesis of loss or gain of termite symbiosis in this clade.

We examined the spore sizes and morphology of MEXU specimens from clade E and compared them to the measurements obtained from the type material of *P. pistillaris* in the LINN fungarium (Priest and Lenz 1999). The spore size of 10–14 × 9–12 μm from the type material fits the average measurements (10–15 × 10–14 μm) obtained from the MEXU specimens in clade E (Table 2 and Figure 3). The spore color of most specimens in clade E is also reddish-brown with thick-walls (Figure 2 and Suppl. material 1). These attributes are in agreement with the type specimen examined by Priest and Lenz (1999). However, the type specimen from the LINN herbarium needs to be sequenced to corroborate the morphological data.

Eleven of the eighteen specimens (10805, 5772, 12338, 12808, 5015, 27845, 7217, 27558, 1191, 27557, and 7023) were nested within clade D (*sensu* Conlon et al. 2016), but without significant RAxML bootstrap support (Figure 4). Other members in clade D include seven sequenced specimens from GenBank both labeled as *Podaxis* sp. and/or *Podaxis pistillaris* and mostly included specimens collected from desert-like arid regions in western India (Singh et al. 2006). When we removed all other GenBank data from our analysis and only included sequences from our study and those of Conlon et al. (2016), we found that clade D had significantly high bootstrap support (82%; data not shown). All specimens from clade D were reported to be free-living with the exception of PREM 34405 from South Africa (Conlon et al. 2016). The average spore measurements of MEXU specimens in clade D were 11–12 × 9–10 μm (Table 2 and Figure 3), which was well within the range of those reported in clade D by Conlon et al. (2016). Specimens in clade D were reported from both the New World (MEXU) and the Old World (South Africa and India), suggesting that species in this clade are widely distributed geographically.

Based on the fruiting body morphology, it was difficult to separate MEXU specimens in clade D and E (Figure 2 and Suppl. material 1). This observation agrees with the results from Conlon et al. (2016) as they reported that fruiting body morphology of *Podaxis* spp. does not significantly differ between the termite associated and free-living clades. The spores in clade D (free-living and termite associated) and E (free-living only) were both thick-walled (Figure 2 and Suppl. material 1); this result agrees with the observations made by Conlon et al. (2016), who reason that free-living, desert dwelling species have thick-walled spores as it may help prevent desiccation in desert-like dry environments. Due to the lack of molecular data from type specimens except for *P. rugospora* (Conlon et al. 2016), currently it is not possible to name specimens in either clade D or E. Based on our preliminary molecular phylogenetic analysis of MEXU specimens, it seems highly unlikely that all MEXU specimens represent *P. pistillaris.*

Habitat and geographical distribution

Podaxis species in Mexico are found predominately in open areas, growing solitary in sandy or clay soils of arid and tropical zones (Table 1). They have been found in the states of Baja California, Durango, Nuevo León, Tamaulipas, Oaxaca (Ruiz-Oronoz and Herrera 1948; Herrera 1950; Guzmán and Herrera 1973), Mexico City (Dennis 1960), Coahuila (Urista et al. 1985), Chihuahua (Moreno et al. 2010) and Sonora (Ellis 1893; Aparicio-Navarro et al. 1991). They have also been reported from the USA (Oregon, California, Arizona, Nevada, New Mexico, Texas, and Hawaiian) (Brasfield 1937; Keirle et al. 2004), Jamaica (Dennis 1953), Galapagos islands (Reid et al. 1980), Argentina (Martínez 1971), Brazil (Baseia and Galvão 2002), Bolivia (Rocabado et al. 2007), Asia (Sinai Peninsula, Israel, Saudi Arabia, Afghanistan, Iran, Pakistan, Kuwait, Qatar, India, China) (Morse 1941; Dring and Rayss 1963; Binyamini 1973; Watling and Gregory 1977; Patel and Tiwari 2012; Muhsin et al. 2012; Mahmoud and Al-Ghamdi 2014), Africa (Madagascar South, Congo, Nigeria, South Africa) (Dissing and Lange 1962; Bottomley 1948; Dring 1964; Alasoadura 1966; Conlon et al. 2016), and Australia (Hilton and Kenneally 1981; Priest and Lenz 1999; Young et al. 2002).

Ethnomycology

In Mexico, the use of *Podaxis* species for food consumption has not been reported. Our study includes data from interviews that state the consumption and farming of this mushroom within the RBTC. In this area, the species is greatly valued by the local people, who sell the fungus for 1–1.5 USD per kilogram or consume young fruiting bodies of *Podaxis* in typical dishes from the region, particularly as "empanadas" (Figure 5), a favorite among the people of the region. They also have developed the ability to locate and harvest the mushrooms, as well as farming (proto-cultivation) is considered very important during rainy/wet season. To consistently obtain more fruiting bodies,

the locals scatter the spores in the soil where they want the fungus to grow and emerge in the following wet season. This method of spore spreading helps them to locate and collect the mature fungus more quickly.

On the other hand, *Podaxis* has also been catalogued as a non-edible mushroom (Guzmán 1977) and has been referred as being toxic in Nigeria and South Africa (Alasoadura 1966); contrastingly, it has been reported as edible in Afghanistan, Pakistan, India, and Australia (Batra 1983). People from the Sind Province of Pakistan are familiar with the commerce of "Khumb" or "Khumbi" (fungus *P. pistillaris*). Khumbi is also a term used by rural communities in Haryana, India, who also refer to this fungus as "Saanp ki chhatri" (umbrella of a snake or snake's cap) (Mridu and Atri 2015). In this region, the mushroom is much appreciated as it is considered a delicacy with medicinal properties for the "Hakims", the dispensers of folk medicine (Khan et al. 1979).

The names attributed to this species in the three communities of the RBTC are "hongo" (mushroom), "hongo blanco comestible" (white edible mushroom) and "soldadito" (little soldier) (Table 1). This fungus is known as "black powderpuff" in Australia (Grey and Grey 2005), "desert shaggy mane" in Pakistan and the USA (Yousaf et al. 2013; Hopple and Vilgalys 1999), "Khumbi" in the India, "Al-Arjoon" in Saudi Arabia, Kuwait and Qatar; "Kama" in Iraq (Muhsin et al. 2012; Mahmound and Al-Ghamdi 2014), and as "Faswat imgaar", "Faswat al-awzaiq", and "Faswat al-dheib" in Yemen (Kreisel and Al-Fatimi 2004).

In Yemen and South Africa, *Podaxis* is used for its medicinal properties and antibacterial activity against *Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus subtilis*, *Serratia marcescens*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (Al-Fatimi et al. 2006; Panwar and Purohit 2002). In Australia, it has been used as hair dye (Batra 1983), while in West Africa, *P. pistillaris* is used to produce baby-powder (desiccative) as an anti-abortive (Gérault and Thoen 1992). Such medicinal properties arise from the chemical constituents of the fruiting body, which include nitrogen, crude protein, true protein, carbohydrates, lipids, and ash content (Gupta and Kapoor 1990; Gupta and Singh 1991; Khaliel et al. 1989 and 1991).

Conclusions

Podaxis is considered a very important mushroom in arid regions of the world due to its culinary and medicinal values. Further taxonomic and molecular phylogenetic studies of this genus are urgently required to better understand species boundaries and provide accurate names on specimens of *Podaxis,* particularly the ones used as food in Mexico and worldwide. Better understanding of *Podaxis* spp. might be possible when mycologists work closely with local communities in different parts of both the Old and New World. Our study provides preliminary morphological and molecular data from *Podaxis* specimens collected in Mexico along with its ethnomycolgy use. We anticipate our study will encourage future phylogenetic diversity analyses on this widely distributed yet taxonomically poorly studied genus of Agaricomycetes.

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References

Alasoadura SO (1966) Studies of the higher fungi of Nigeria II. Macrofungi associated with termite nests. Nova Hedwigia 11(1–4): 387–393.

Al-Fatimi MA, Jülich WD, Jansen R, Lindequist U (2006) Bioactive components of the traditionally used mushroom *Podaxis pistillaris*. Evidence-Based Complementary and Alternative Medicine 3(1): 87–92. https://doi.org/10.1093/ecam/nek008

Altschul SF, Gish W, Miller W, Myers EW, Lipman D J (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Aparicio-Navarro A, Quintero T, Esqueda M (1991) Distribución y datos ecologicos de *Podaxis pistillaris* Fr. In Sonora. IV Congreso Nacional de Micología. Universidad Autónoma de Tlaxcala. Tlaxacala, Tlax., 14–18 de octubre, 65 pp.

Baseia IG, Galvão TC (2002) Some interesting gasteromycetes (Basidiomycota) in dry areas from northeastern Brazil. Acta Botanica Brasilica 16(1): 1–8. https://doi.org/10.1590/S0102-33062002000100002

Batra LR (1983) Edible Discomycetes and Gasteromycetes of Afghanistan, Pakistan and Northwestern India. Biologia 29: 293–304.

Binyamini N (1973) Gasteromycetes of Sinai desert. Israel Journal of Botany 22: 33–37.

Bottomley AM (1948) Gasteromycetes of South Africa. Bothalia 4: 473–810. https://doi.org/10.4102/abc.v4i3.1859

Brasfield TW (1937) The morphology of *Podaxis pistillaris*. University of Iowa Studies in Natural History 17: 100–121.

Brock PM, Döring H, Bidartondo MI (2009) How to know unknown fungi: the role of a herbarium. New Phytologist 181: 719–724. https://doi.org/10.1111/j.1469-8137.2008.02703.x

Cifuentes J, Villegas M, Pérez-Ramirez L (1986) Hongos. In: Lot A, Chiang F (Eds) Manual de Herbario. Consejo Nacional de Flora de Mexico A.C., México, 55–64.

Conlon BH, De Beer ZW, Henrik H, Aanen DK, Poulsen M (2016) Phylogenetic analyses of diverse *Podaxis* specimens from Southern Africa reveal hidden diversity and new insights into associations with termites. Fungal Biology 120: 1065–1076. https://doi.org/10.1016/j.funbio.2016.05.011

Dennis RWG (1953) Some West Indian Gasteromycetes. Kew Bulletin 8(3): 307–328. https://doi.org/10.2307/4115517

Dennis RWG (1960) Fungi venezuelani: III. Kew Bulletin 14(3): 418–458. https://doi.org/10.2307/4114758

Dissing H, Lange M (1962) Gasteromycetes of Congo. Bulletin du Jardin botanique de l'État a Bruxelles 32(4): 325–416. https://doi.org/10.2307/3667249

Dring DM (1964) Gasteromycetes of West Tropical Africa. Mycological Papers 98: 1–60.

Dring DM, Rayss T (1963) The Gasteromycete fungi of Israel. Israel Journal of Botany 12: 147–178.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Ellis JB (1893) Descriptions of some new species of fungi. The Journal of Mycology 7(3): 274–278. https://doi.org/10.2307/3752450

Esqueda M, Coronado M, Gutierrez A, Valenzuela R, Chacón S, Gilbertson RL, Herrera T, Lizárraga M, Moreno G, Pérez-Silva E, Van Devender TR (2010). Hongos. In: Molina-Freaner FE, Van Devender TR (Eds) Diversidad biológica de Sonora. UNAM, México, 189–205.

Esqueda M, Gutierrez A, Coronado ML, Lizarraga M, Raymundo T, Valenzuela R (2012) Distribución de algunos hongos gasteroides (Agaricomycetes) en la planicie central del Desierto Sonorense. Revista Mexicana de Micología 36:1–8.

Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. Computer Applications in the Biosciences 12: 543–548. doi:10.1093/bioinformatics/12.6.543

Gardes M, White TJ, Fortin JA, Bruns TD, Taylor JW (1991) Identification of Indigenous and Introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Canadian Journal of Botany 69: 180–190. https://doi.org/10.1139/b91-026

Gérault A, Thoen D (1992) Les champignons dans les pharmacopees traditionelles de l'Afrique de L'Ouest. Revue de Médecine et de Pharmacie Africa 1(1): 45–53.

Gouy M, Guindon S, Gascuel O (2010) SeaView Version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27: 221–224. https://doi.org/10.1093/molbev/msp259

Grey P, Grey E (2005) Fungi down under the fungimap guide to Australian Fungi. Fungimap, 146.

Gupta A, Kapoor VP (1990) Carbohydrate analysis of some edible mushrooms. Vegetable Scienice 17(2): 227–229.

Gupta S, Singh SP (1991) Nutritive value of mushroom *Podaxis pistillaris*. Indian Journal of Mycology and Plant Pathology 21(3): 273–276.

Guzmán G (1977) Identificación de los hongos comestibles, venenosos, alucinantes y destructores de la madera. Limusa. México, 1–452.

Guzmán G (2011) El uso tradicional de los hongos sagrados: Pasado y presente. Etnobiologia 9: 1–21.

Guzmán G, Herrera T (1969) Macromicetos de las zonas áridas de México, II Gastermicetos. Anales del Instituto de Biología UNAM Serie Botánica 40(1): 1–92.

Guzmán G, Herrera T (1973) Especies de macromicetos citados de México. IV. Gasteromicetos. Boletín de la Sociedad Mexicana de Micología 7:105–119.

Herrera T (1950) Un hongo interesante en la región Cuicatlán, Oaxaca. Anales del Instituto de Biología UNAM Serie Botánica 21(1): 17–21.

Hillis DM, Bull JJ (1993) An empirical-test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192. https://doi.org/10.1093/sysbio/42.2.182

Hilton RN, Kenneally KF (1981) The desert *Coprinus* fungus *Podaxis pistillaris* in Western Australia. Western Australian Naturalist 15: 21–26.

Hopple JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups and monophyly. Molecular Phylogenetics and Evolution 13: 1–19. https://doi.org/10.1006/mpev.1999.0634

Jandaik CL, Kapoor CN (1976) Studies on vitamin requirements of some edible fungi. Indian Phytopathology 29: 259–261.

Johnson J (1999) Phylogenetic relationships within *Lepiota sensu lato* based on morphological and molecular data. Mycologia 91: 443–458. https://doi.org/10.2307/3761345

Keirle MR, Hemmes DE, Desjardin DE (2004) Agaricales of the Hawaiian Islands. 8. Agaricaceae: *Coprinus* and *Podaxis*; Psathyrellaceae: *Coprinopsis*, *Coprinellus* and *Parasola*. Fungal Diversity 15: 33–124.

Khaliel AS, Abou-Heilah AN, Kassim MY (1989) The Nutrient Composition of *Podaxis pistillaris.* Arab Gulf Journal of Scientific Research 7(3): 121–128.

Khaliel AS, Abou-Heilah AN, Kassim MY (1991) The main constituents and nutritive value of *Podaxis pistillaris*. Acta Botanica Hungarica 36:173–179.

Khan SM, Khan DA, Kurtzman RH (1979) Temperature studies on *Podaxis pistillaris*. Mycologia 71(4): 861–867. https://doi.org/10.2307/3759203

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the Fungi (10th edn). CAB International, 771 pp.

Kreisel H, Al-Fatimi M (2004) Basidiomycetes and larger Ascomycetes from Yemen. Feddes Repertorium 115(7-8): 547–561. https://doi.org/10.1002/fedr.200411053

Linnaeus CV (1771) Mantissa Plantarum. Generum Editionis VI et Specierum Editionis II. Laurentius Salvius, Stockholm.

Mahmound YAG, Al-Ghamdi AY (2014) *Podaxis pistillaris* (L. ex Pers) Fr. recorded from Al Mekwah City, Albaha, Saudi Arabia. Research Journal of Microbiology 9(2): 111–114. https://doi.org/10.3923/jm.2014.111.114

Martínez A (1971) Notas sobre el género *Podaxis* (Gasteromycetes) en Argentina. Boletín de la Sociedad Argentina de Botánica 14: 73–87.

McKnight HK (1985) The small-spored species of *Podaxis*. Mycologia 77(1): 24–35. https://doi.org/10.2307/3793245

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for Inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 1–8. https://doi.org/10.1109/GCE.2010.5676129

Moreno G, Lizárraga M, Esqueda M, Coronado LM (2010) Contribution to the study of gasteroid and secotioid fungi of Chihuahua, Mexico. Mycotaxon 112: 291–315. https://doi.org/10.5248/112.291

Morse EE (1933) A Study of the Genus *Podaxis.* Mycologia 25(1): 1–33. https://doi.org/10.2307/3754126

Morse EE (1941) *Podaxis pistillaris*. II. Mycologia 33(6): 609–610. https://doi.org/10.2307/3754778

Mridu, Atri NS (2015) *Podaxis pistillaris*- A common wild edible mushroom from Haryana (India) and its sociobiology. Kavaka 44: 34–37.

Muhsin TM, Abass AF, Al-Habeeb EK (2012) *Podaxis pistillaris* (Gasteromycetes) from the desert of southern Iraq, an addition to the known mycota of Iraq. Journal of Basrah Researches (Sciences) 38(3): 29–35.

Osmundson TW, Robert VA, Schoch CL, Baker LJ, Smith A, Robich G, Mizzan L, Garbelotto MM (2013) Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. PLoS One 8(4): e62419. doi:10.1371/journal.pone.0062419

Panwar CH, Purohi DK (2002) Antimicrobial activities of *Podaxis pistillaris* and *Phellorinia inquinans* against *Pseudomonas aeruginosa* and *Proteus mirabilis*. Mushroom Research 11: 43–44.

Patel US, Tiwari AK (2012) *Podaxis pistillaris* reported from Madhya Pradesh, India. Indian Journal of Fundamental and Applied Life Sciences 2(1): 233–239.

Phutela RP, Kaur H, Sodhi HS (1998) Physiology of an edible gasteromycete, *Podaxis pistillaris* (Lin. Ex Pers.) Fr. Journal of Mycology and Plant Pathology 28: 31–37.

Priest MJ, Lenz M (1999) The Genus *Podaxis* (Gasteromycetes) in Australia with a description of a new species from termite mounds. Australian Systematic Botany 12(1): 109–116. https://doi.org/10.1071/SB95043

Raja HA, Baker TR, Little JG, Oberlies NH (2017) DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification? Food Chemistry 214: 383–392. https://doi.org/10.1016/j.foodchem.2016.07.052

Reid DA, Pegler DN, Spooner BM (1980) An annotated list of the fungi of the Galapagos Islands. Kew Bulletin 35(4): 847–892. https://doi.org/10.2307/4110185

Rocabado D, Wright JE, Muchenik NF (2007) Catálogo de los Gasteromycetes (Fungi: Basidiomycotina) de Bolivia. Kempffiana 3(1): 3–13.

Ruiz-Oronoz M, Herrera T (1948) Levaduras, hongos macroscópicos, líquenes y hepáticas colectadas en Cuicatlán, Oax. Anales del Instituto de Biología UNAM Serie Botánica 19(2): 299–316.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America (PNAS) 109: 6241–6246. https://doi.org/10.1073/pnas.1117018109

SEMARNAT (2013) Secretaría de Medio Ambiente y Recursos Naturales y CONANP, Comisión Nacional de Áreas Naturales Protegidas. Programa de Manejo Reserva de la Biosfera Tehuacán-Cuicatlán. 1a edición. México, D.F., 329 pp. http://www.conanp.gob.mx/que_hacemos/pdf/programas_manejo/tehuacan_2013.pdf

Singh SK, Doshi A, Yadav MC, Kamal S (2006) Molecular characterization of specialty mushrooms of western Rajasthan, India. Current Science 91: 1225–1230.

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758–771. https://doi.org/10.1080/10635150802429642

The Mycology Collections data Portal (MyCoPortal) (2016) Harvard University, Farlow Herbarium. http://mycoportal.org/portal/collections/individual/index.php?occid=1438167

Urista E, Garcia J, Castillo J (1985) Algunas especies de Gasteromicetos del Norte de México. Revista Mexicana de Micología 1: 471–523.

Valiente-Banuet A, Solís L, Dávila P, Coro-Arizmendi M, Silva-Pereyra C, Ortega-Ramírez J, Treviño-Carreón J, Rangel-Landa S, Casas A (2009) Guía de la vegetación del Valle de Tehuacán-Cuicatlán. Universidad Nacional Autónoma de México, 1–206.

Vellinga EC (2004) Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. Mycological Research 108: 354–377. https://doi.org/10.1017/S0953756204009700

Watling R, Gregory NM (1977) Larger fungi from Turkey, Iran and neighboring countries. Karstenia 17: 70.

White TJ, Bruns TD, Lee SH, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gefland DH, Sninsky JJ, White TJ (Eds) PCR Protocols: A Guide to Methods and Application. Academic Press, San Diego, 315–322.

Young AM, Forster PI, and Booth R (2002) Notes on *Podaxis* Desv. in the 'wet tropics' & Einesleigh uplands bioregions of northern Queensland. 2002. Australasian Mycologist 21(1): 21–23.

Yousaf N, Khalid AN, Niazi AR (2013) Taxonomy of gasteroid fungi from some arid regions of Punjab, Pakistan. Journal of Biodiversity and Environmental Sciences 3(12): 253–263.

Supplementary material 1

Fruit bodies and basidiospores of *Podaxis* specimens

Authors: Abraham J. Medina-Ortiz, Teófilo Herrera, Marco A. Vásquez-Dávila, Huzefa A. Raja, Mario Figueroa

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Three new species of *Megasporia* (Polyporales, Basidiomycota) from China

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Abstract

Megasporia is a newly established polypore genus characterized by resupinate fruiting bodies with big pores, a dimitic hyphal structure with generative hyphae bearing clamp connections, more or less dextrinoid and cyanophilous skeletal hyphae, cylindrical, hyaline, thin-walled, smooth basidiospores, and growth mostly on fallen angiosperm branches. Species number is extremely rich in subtropical and tropical Asia. Three new species, namely *M. rimosa*, *M. tropica* and *M. yunnanensis* are described from China, and their illustrated descriptions are given. Differences between these new species and phylogenetically related and morphologically similar species are discussed. A key to the known species of *Megasporia* is provided.

Key words

Phylogeny, Polyporaceae, taxonomy, white-rot fungi

Introduction

Megasporia B.K. Cui et al. was recently derived from *Megasporoporia* Ryvarden & J.E. Wright, nested within the core polyporoid clade (Li and Cui 2013), both genera being members of Polyporaceae (Binder et al. 2005). *Megasporia* is morphologically similar to *Megasporoporia*, but they were phylogenetically distinct based on ITS and nLSU sequences (Li and Cui 2013). *Megasporia* is characterized by annual and resupinate basidiocarps, large pores, big basidiospores (mostly longer than 10 μm in length) which are mostly cylindrical, thin-walled, hyaline and negative in Cotton Blue and Melzer's reagent, a dimitic hyphal system with clamped generative hyphae and more or less dextrinoid skeletal hyphae, the presence of tetrahedric or polyhedric crystals among

hymenial elements (Li and Cui 2013). The genus mainly grows on fallen or dead angiosperm branches which have not decayed much and causes a white rot (Li and Cui 2013). It used to be considered that only a few species were in *Megasporoporia* s.l. (Ryvarden et al. 1982), but the species diversity in the genus is very rich in subtropical and tropical Asia, with 12 species having been described from the region (Li and Cui 2013). In addition, four species, *Megasporoporia cavernulosa* (Berk.) Ryvarden, *M. hexagonoides* (Speg.) J.E. Wright & Rajchenb., *M. mexicana* Ryvarden and *M. setulosa* (Henn.) Rajchenb., have been found in subtropical and tropical America, but none has been recorded from Europe (Ryvarden et al. 1982, Ryvarden and Melo 2014).

During the study of polypores from southern China, six specimens collected on fallen angiosperm branches were examined, phylogenetic relationships were analyzed based on ITS and nLSU rDNA sequences data, and three new species of *Megasporia* were discovered. The aim of this work demonstrates the diversity of *Megasporia* in China. Illustrated descriptions of these species and a key to known species in the genus are provided in the present paper.

Materials and methods

Morphological studies

Specimens examined were deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions were based on field notes and herbarium specimens. Color terms follow Petersen (1996). Micro-morphological data were obtained from dried specimens, as observed under a light microscope. Sections were studied at a magnification of up to ×1000 using a Nikon E 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic characters, measurements and drawings were made from slide preparations stained with Cotton Blue (CB) and Melzer's reagent (IKI). Spores were measured from sections cut from the tubes. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide, IKI– = both non-amyloid and non-dextrinoid, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from a given number (b) of specimens.

Molecular study and phylogenetic analysis

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain total genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications (Chen et al. 2016). The DNA was

amplified with the primers: ITS5 and ITS4 for ITS (White et al. 1990), and LR0R and LR7 for nLSU (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers.

Sequences generated in this study were aligned with additional sequences downloaded from GenBank (Table 1) using ClustalX (Thompson et al. 1997) and manually adjusted in BioEdit (Hall 1999). Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:20432).

Maximum parsimony phylogenetic analysis followed Li and Cui (2013). It was applied to the combined dataset of ITS and nLSU sequences using PAUP* version 4.0b10 (Swofford 2002). Sequences of *Cinereomyces lindbladii* (Berk.) Jülich and *Sebipora aquosa* Miettinen were used as outgroups to root trees following Li and Cui (2013). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using bootstrap analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree generated.

MrModeltest2.3 (Nylander 2004) was used to determine the best-fit evolution model for the combined dataset of ITS and nLSU sequences for estimating Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 1 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. Majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP) and 0.95 (BPP), respectively, were considered as significantly supported.

Results

Phylogenetic analysis

The combined ITS and nLSU dataset included 45 sequences of ITS and 44 sequences of nLSU regions from 45 fungal samples representing 37 species. The dataset had an aligned length of 1919 characters in the dataset, of which 1330 characters are constant, 178 are variable and parsimony-uninformative, and 411 are parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (TL = 2082, CI = 0. 451, RI =

Table 1. A list of species, specimens and GenBank accession numbers of sequences used in this study.

Species	Sample number	ITS	nLSU
Abundisporus violaceus	MUCL 38617	FJ411100	FJ393867
Cinereomyces lindbladii	Larsson 12078	FN907906	FN907906
Datronia mollis	RLG 6304sp	JN165002	JN164791
Dichomitus eucalypti	Oslo 910674	JQ780412	JQ780441
D. squalens	Cui 9639	JQ780407	JQ780426
Donkioporia expansa	MUCL 35116	FJ411104	FJ393872
Grammothele quercina	Dai 11768	JQ314364	JQ780423
Grammothelopsis subtropica	Cui 9035	JQ845094	JQ845097
Lentinus tigrinus	DSH 93-181	AY218419	AF518627
Megasporia cystidiolophora	Cui 2688	JQ780389	JQ780431
M. ellipsoidea	Cui 5222	JQ314367	JQ314390
M. ellipsoidea	**Cui 13685**	**KY449433**	**KY449444**
M. guangdongensis	Cui 9130	JQ314373	JQ780428
M. hengduanensis	Cui 8076	JQ780392	JQ780433
M. hexagonoides	**Dai 16449**	**KY449434**	**KY449445**
M. *hexagonoides*	He 2608	JQ314368	JQ314388
M. hexagonoides	**Yuan 4233**	**KY449435**	**KY449446**
M. major	Cui 10253	JQ314366	JQ780437
M. rimosa	**Dai 15357**	**KY449436**	**KY449447**
M. tropica	**Cui 13660**	**KY449437**	**KY449448**
M. tropica	**Cui 13740**	**KY449438**	**KY449449**
M. violacea	Cui 6601b	JQ780395	JQ780434
M. violacea	**Cui 13607**	**KY449439**	**KY449450**
M. violacea	**Cui 13648**	**KY449440**	**KY449451**
M. yunnanensis	**Cui 12594**	**KY449441**	**KY449452**
M. yunnanensis	**Cui 12614**	**KY449442**	**KY449453**
M. yunnanensis	**Dai 13870**	**KY449443**	**KY449454**
Megasporoporia bannaensis	Dai 12306	JQ314362	JQ314379
M. minor	Dai 12170	JQ314363	JQ314380
M. setulosa	JV1008/102J	JF894110	.
Megasporoporiella lacerata	Yuan 3880	JQ314377	JQ314395
M. pseudocavernulosa	Yuan 1270	JQ314360	JQ314394
M. rhododendri	Dai 4226	JQ314356	JQ314392
M. subcavernulosa	Cui 9252	JQ780378	JQ780416
Melanoderma microcarpum	Dai 9811	HQ678173	HQ678175
Perenniporia medulla-panis	Cui 3274	JN112792	JN112793
P. tephropora	Cui 8040	JN048763	HQ654118
Perenniporiella chaquenia	MUCL 49758	FJ411085	FJ393857
Polyporus brumalis	KHL 8558	AF347108	AF347108
Porogramme albocincta	TL 9894/03	JX109854	JX109854
Pseudofavolus cucullatus	WD 2157	AB587637	AB368114
Pycnoporus cinnabarinus	AFTOL-ID 772	DQ411525	AY586703
Pyrofomes demidoffii	MUCL 41034	FJ411105	FJ393873
Sebipora aquosa	Miettinen 8680	HQ659240	HQ659240
Trametes hirsuta	RLG5133T	JN164941	JN164801

New sequences are shown in bold.

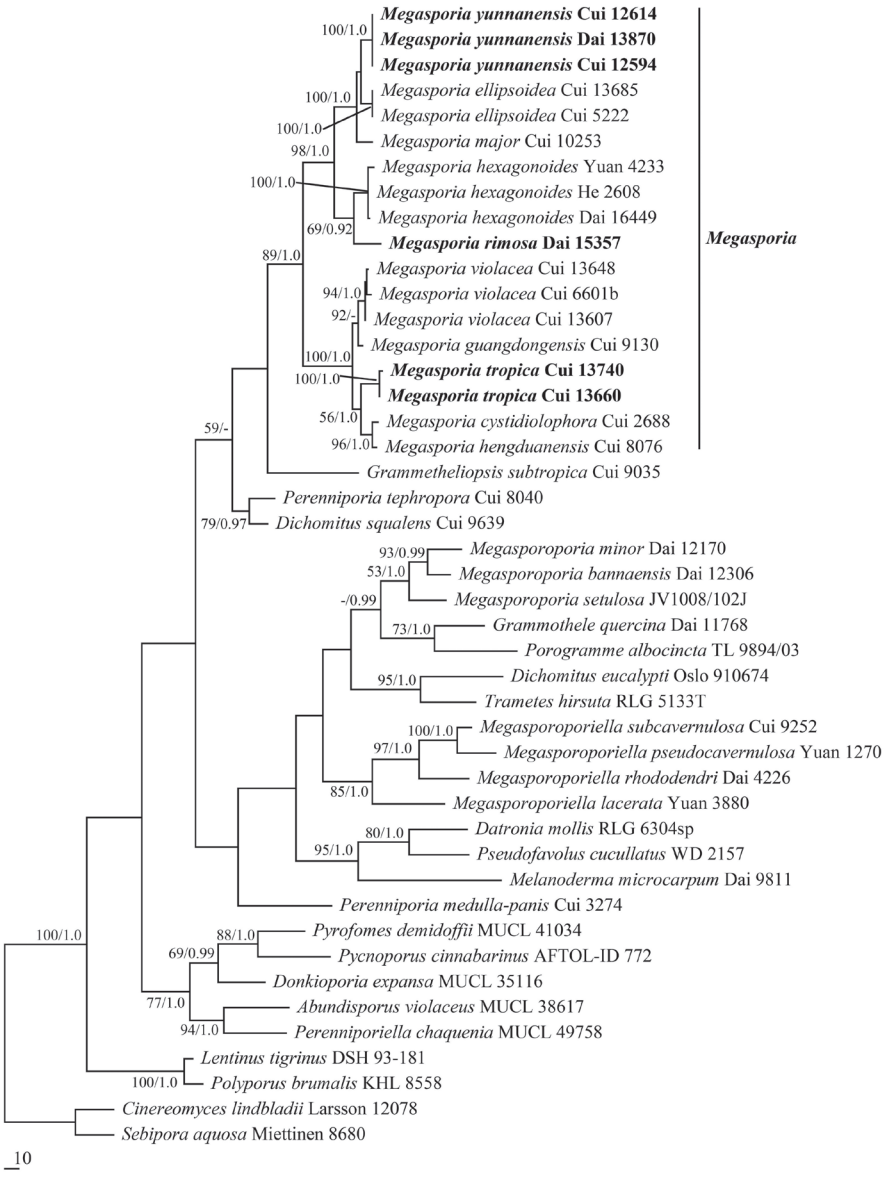

Figure 1. Strict consensus tree illustrating the phylogeny of *Megasporia* and its related species generated by maximum parsimony based on ITS+nLSU sequences. Branches are labeled with parsimony bootstrap proportions (before slanting line) high than 50% and bayesian posterior probabilities (after slanting line) more than 0.90.

0.625, RC = 0.282, HI = 0.549), and one of the maximum parsimonious trees is shown in Figure 1. The best model for the combined ITS and nLSU sequences dataset estimated and applied in the BI was GTR+I+G. BI resulted in a similar topology with an average standard deviation of split frequencies = 0.007691 to MP analysis, and thus only the MP tree is provided. Both bootstrap values (≥50%) and BPPs (>0.90) are shown at the nodes (Figure 1).

Samples of *Megasporia* clustered together (89% ML and 1 BPPs, Figure 1), then grouped with the sample of *Grammothelopsis subtropica* B.K. Cui & C.L. Zhao with low support (43% ML and 0.90 BPPs). Sampled specimens of the two new species *M. tropica* and *M. yunnanensis* formed well-supported lineages (100% MP and 1.0 BPPs, Figure 1), while *Megasporia rimosa* sp. nov. clustered with *M. hexagonoides* with low support (69% MP, 0.92 BPPs).

Taxonomy

Megasporia rimosa **Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.**
MycoBank: MB819610
Figure 2

Diagnosis. Differs from other *Megasporia* species by its extremely thin and cracked basidiocarp (less than 0.5 mm thick) when dry.

Holotype. CHINA. Guangxi Auto. Reg., Shangsi County, Shiwandashan Nature Reserve, on fallen angiosperm branch, 06 June 2015, Y.C. Dai 15357 (BJFC019468).

Basidiocarps. Annual, resupinate, corky, without odor or taste when fresh, becoming hard corky and cracked upon drying, up to 17 cm long, 4 cm wide, and 0.4 mm thick at centre. Sterile margin thinning out, white when fresh, cream when dry, very narrow to almost lacking. Pore surface white to cream when fresh, cream when dry; pores angular, 3–4 per mm; dissepiments thick, entire. Subiculum pale buff, corky, up to 0.1 mm thick. Tubes cream, paler than subiculum, corky, up to 0.3 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae weakly dextrinoid, CB+; tissues unchanged in KOH.

Subiculum. Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, sometimes encrusted by crystals, 1.5–2.5 μm in diam; skeletal hyphae dominant, thick-walled with a narrow to medium lumen, moderately branched, mostly flexuous, interwoven, slightly gelatinized, sometimes encrusted by crystals, 2.5–3.5 μm in diam.

Tubes. Generative hyphae hyaline, thin-walled, occasionally branched, 1.5–2.5 μm in diam; skeletal hyphae dominant, thick-walled with a narrow lumen, unbranched, more or less straight, subparallel along the tubes, 2–3 μm in diam. Hyphal pegs absent, dendrohyphidia present along hymenium, cystidia absent; cystidioles present, mostly ventricose, thin-walled, smooth. Basidia broadly clavate to pear-shaped, with four sterigmata and a basal clamp connection, 20–28 × 5–7.5 μm; basidioles in shape similar to basidia, but smaller. Small tetrahedric or polyhedric crystals frequently present among subhymenium and hymenium.

Spores. Basidiospores cylindrical, hyaline, thin-walled, smooth, sometimes with one or two guttula, IKI–, CB–, (16.5–)16.8–20.2(–21) × (4.1–)4.3–5.5(–5.9) μm, L = 18.49 μm, W = 4.88 μm, Q = 3.97 (n = 30/1).

Etymology. *Rimosa* (Lat.): referring to the cracked hymenophore when dry.

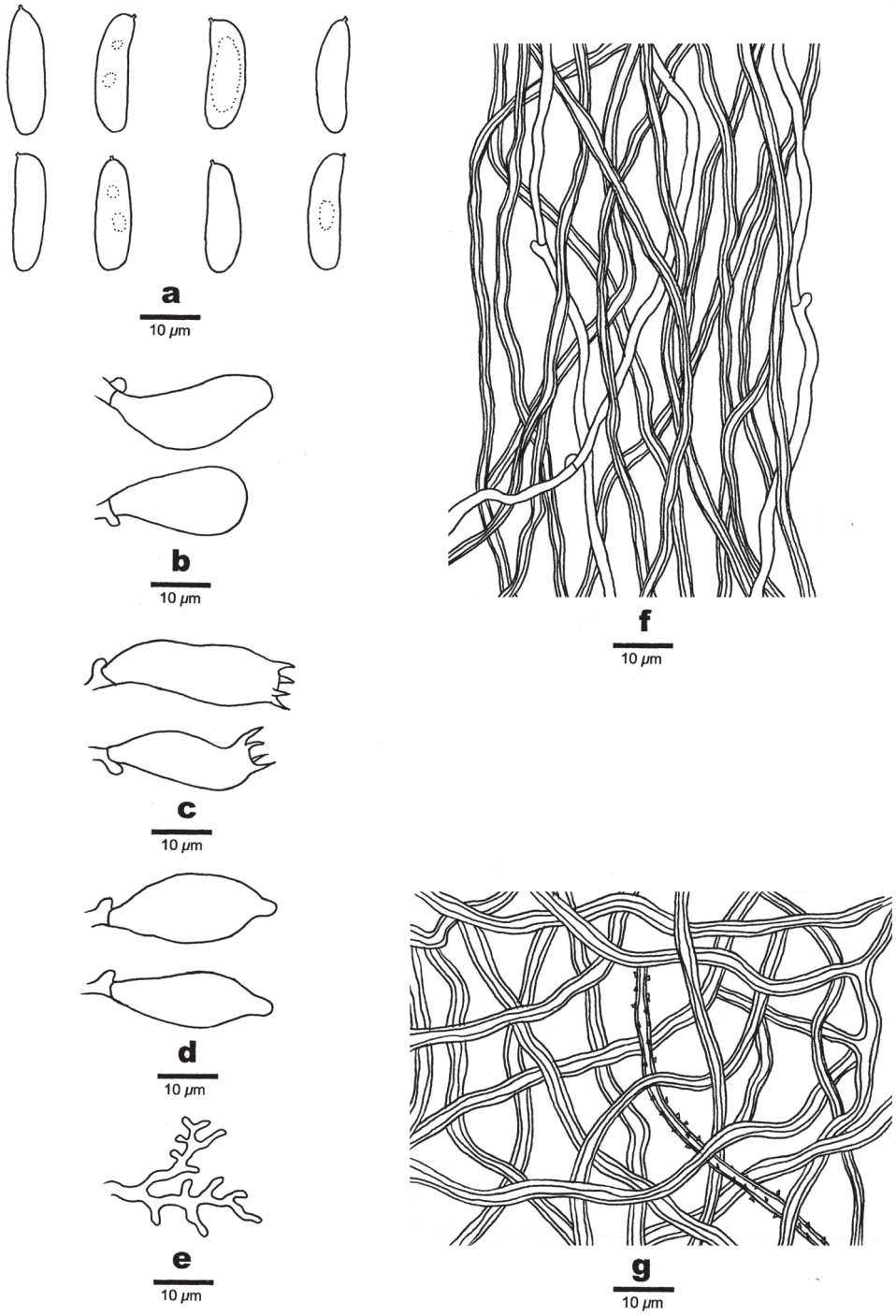

Figure 2. Microscopic structures of *Megasporia rimosa* sp. nov. (drawn from the holotype). **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Dendrohyphidia **f** Hyphae from trama. g. Hyphae from subiculum.

Megasporia tropica **Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.**
MycoBank: MB819611
Figure 3

Diagnosis. Differs from other *Megasporia* species by strongly dextrinoid skeletal hyphae, and by lacking dendrohyphidia, cystidioles and hyphal pegs.

Holotype. CHINA. Hainan Prov., Wuzhishan County, Wuzhishan Nature Reserve, on fallen angiosperm branch, 10 Nov 2015, B.K. Cui 13660 (BJFC022532).

Basidiocarps. Annual, resupinate, corky, without odor or taste when fresh, becoming hard corky to leathery upon drying, up to 5 cm long, 3 cm wide, and 1.5 mm thick at centre. Sterile margin thinning out, cream when dry, up to 1 mm wide. Pore surface clay-pink to fawn when dry; pores round, 2–3 per mm; dissepiments thin, entire to lacerate. Subiculum cream, corky, up to 0.5 mm thick. Tubes clay-pink, slightly darker than subiculum, corky, up to 1 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae strongly dextrinoid, CB+; tissues unchanged in KOH.

Subiculum. Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 2.5–3 μm in diam; skeletal hyphae dominant, thick-walled with a narrow to medium lumen, unbranched, more or less flexuous, loosely interwoven, 3–4 μm in diam.

Tubes. Generative hyphae hyaline, thin-walled, occasionally branched, 1.5–2 μm in diam; skeletal hyphae dominant, thick-walled with a narrow lumen, occasionally branched, more or less straight, subparallel along the tubes, 2–3 μm in diam. Hyphal pegs, dendrohyphidia and cystidia absent; cystidioles present, subulate, thin-walled, smooth. Basidia broadly clavate, with four sterigmata and a basal clamp connection, sometimes with a big guttule, 20–25 × 7–9.5 μm; basidioles pear-shaped, slightly smaller than basidia. Small tetrahedric or polyhedric crystals frequently present among subhymenium and hymenium.

Spores. Basidiospores cylindrical, hyaline, thin-walled, smooth, mostly with a big guttula, IKI–, CB–, (14.2–)14.7–18.8(–19.7) × (4.9–)5–6.5(–7.1) μm, L = 16.55 μm, W = 5.65 μm, Q = 2.83–3.04 (n = 60/2).

Additional specimen (paratype) examined. CHINA. Hainan Prov., Ledong County, Jianfengling Forest Park, on fallen angiosperm branch, 21 Nov 2015, B.K. Cui 13740 (BJFC022533).

Etymology. *Tropica* (Lat.): referring to the species occurring in the tropics.

Megasporia yunnanensis **Y. Yuan, X.H. Ji & Y.C. Dai, sp. nov.**
MycoBank: MB819612
Figure 4

Diagnosis. Differs from other *Megasporia* species by brownish tints on pore surface and lacking tetrahedric or polyhedric crystals.

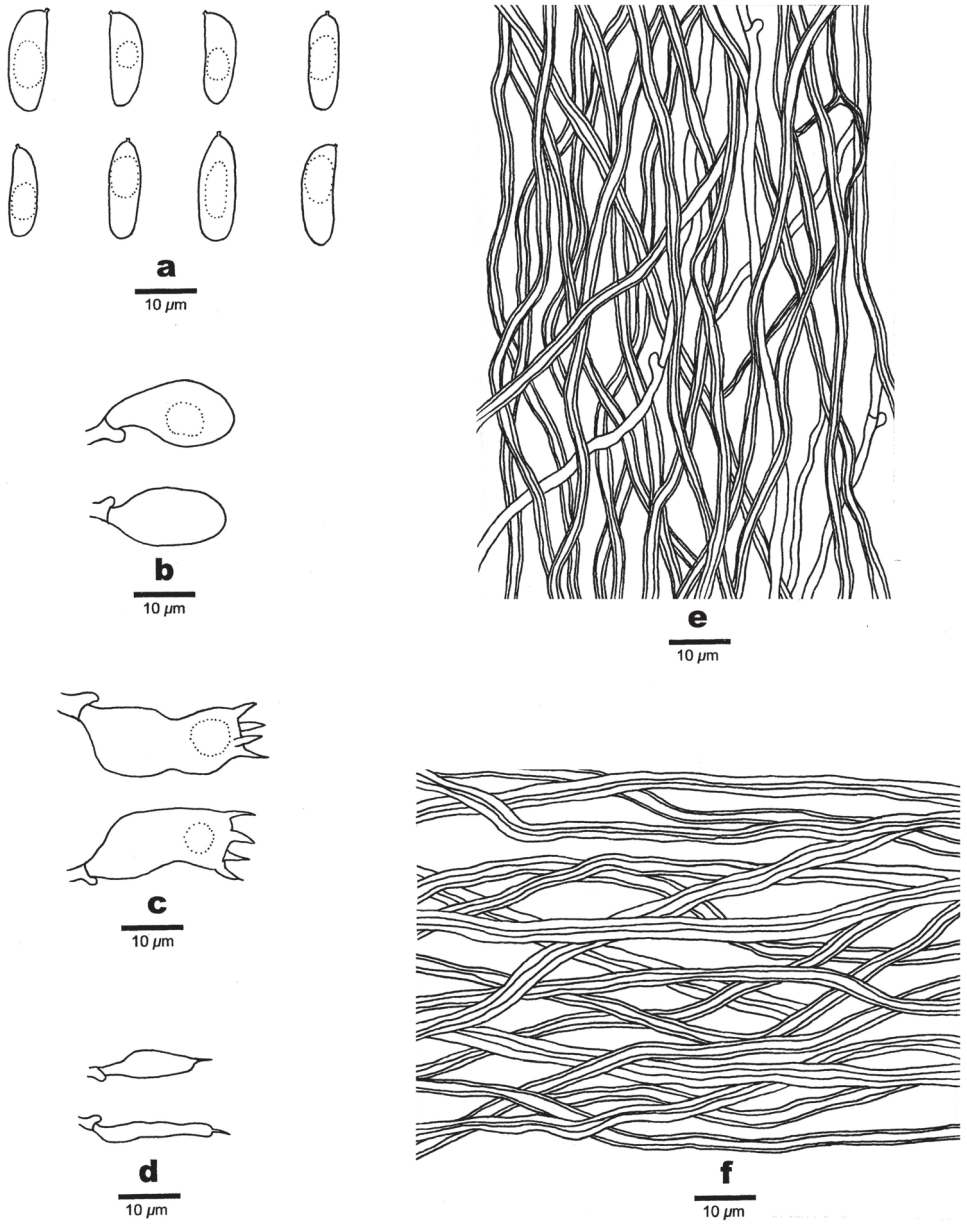

Figure 3. Microscopic structures of *Megasporia tropica* sp. nov. (drawn from the holotype). **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Hyphae from trama **f** Hyphae from subiculum.

Holotype. CHINA. Yunnan Province, Kunming, Wild Duck Lake Park, on fallen angiosperm branch, 28 July 2014, Y.C. Dai 13870 (BJFC017600).

Basidiocarps. Annual, resupinate, corky, without odor or taste when fresh, becoming hard corky upon drying, up to 3 cm long, 2 cm wide, and 2 mm thick at centre. Sterile margin thinning out, white when dry, up to 1 mm wide. Pore surface

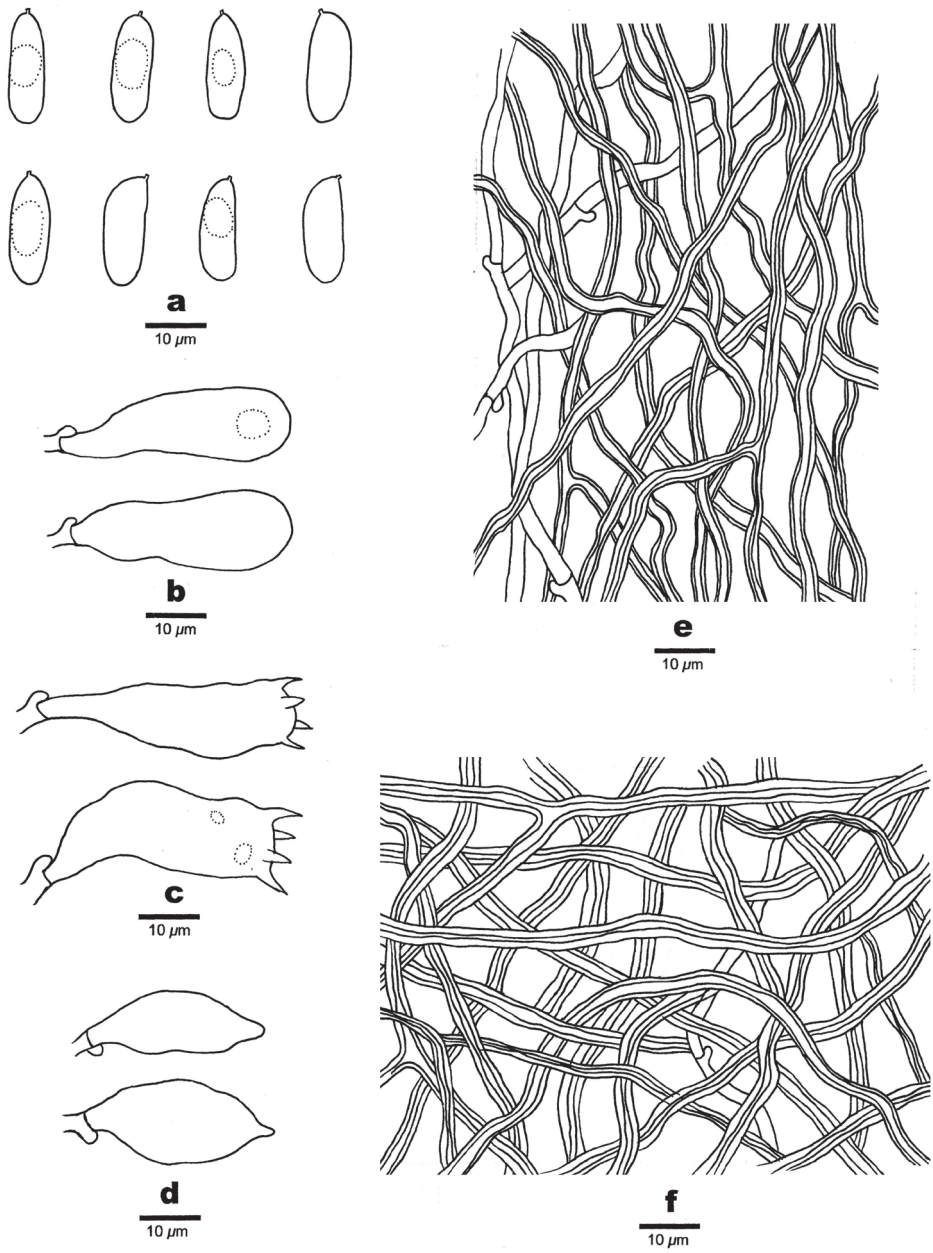

Figure 4. Microscopic structures of *Megasporia yunnanensis* sp. nov. (drawn from the holotype). **a** Basidiospores **b** Basidioles **c** Basidia **d** Cystidioles **e** Hyphae from trama **f** Hyphae from subiculum.

white to cream but with brownish tints when dry; pores round, 2–3 per mm; dissepiments thin, lacerate. Subiculum white, corky, up to 1 mm thick. Tubes cream, corky, up to 1 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae weakly dextrinoid, CB+; tissues unchanged in KOH.

Subiculum. Generative hyphae frequent, hyaline, thin-walled, occasionally branched, 2–3 μm in diam; skeletal hyphae dominant, thick-walled with a wide to narrow lumen, occasionally branched, mostly flexuous, interwoven, 3–4 μm in diam.

Tubes. Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 2–3 μm in diam; skeletal hyphae dominant, thick-walled with a wide to medium lumen, occasionally branched, flexuous, interwoven, 2.5–3.5 μm in diam. Hyphal pegs and cystidia absent, dendrohyphidia present; cystidioles present, mostly ventricose, thin-walled, smooth. Basidia broadly clavate, with four sterigmata and a basal clamp connection, 30–35 × 9–11 μm; basidioles in shape similar to basidia, but smaller. Tetrahedric or polyhedric crystals absent.

Spores. Basidiospores cylindrical, hyaline, thin-walled, smooth, IKI–, CB–, (15.1–)16.5–20.8(–21.5) × (5.1–)5.5–7.1(–7.5) μm, L = 18.38 μm, W = 6.19 μm, Q = 2.88–3.02 (n = 90/3).

Additional specimens (paratypes) examined. CHINA. Yunnan Province, Nanhua County, Dazhongshan Nature Reserve, on fallen angiosperm branch, 11 Sept 2015, B.K. Cui 12594 (BJFC022530). Chuxiong, Zixishan Nature Reserve, on fallen branch of *Rhododendron*, 12 Sept 2015, B.K. Cui 12614A (BJFC022531).

Etymology. *Yunnanensis* (Lat.): referring to the locality (Yunnan Province, China) where the species was found.

Discussion

In this study, seven previously accepted species of *Megasporia* (*M. cystidiolophora* (B.K. Cui & Y.C. Dai) B.K. Cui & Hai J. Li, *M. ellipsoidea* (B.K. Cui & P. Du) B.K. Cui & Hai J. Li, *M. guangdongensis* B.K. Cui & Hai J. Li, *M. hengduanensis* B.K. Cui & Hai J. Li, *M. hexagonoides* (Speg.) B.K. Cui, Y.C. Dai & Hai J. Li, *M. major* (G.Y. Zheng & Z.S. Bi) B.K. Cui, Y.C. Dai & Hai J. Li and *M. violacea* (B.K. Cui & P. Du) B.K. Cui, Y.C. Dai & Hai J. Li) were referred to morphological examination and phylogenetic analysis. Three new *Megasporia* species, *M. rimosa*, *M. tropica* and *M. yunnanensis*, are described based on morphological differences and molecular phylogenetic analysis. Sampled specimens of *Megasporia* formed a well-supported lineage (89% ML and 1.0 BPPs), indicating that all are phylogenetically distinct from other genera, as suggested by the combined ITS and nLSU dataset (Figure 1).

Among the accepted *Megasporia* species, *M. hexagonoides* and *M. major* have big basidiospores (16.6–21.8 × 5.2–6.8 μm, 15.2–20 × 5.5–7.1 μm, Li and Cui 2013). The three new species described in the current paper have similar basidiospores as *M. hexagonoides* and *M. major*, but they have distinct smaller pores than those in *M. hexagonoides* and *M. major* (2–4 per mm *vs.* 0.5–1.5 per mm). Furthermore, hyphal pegs are present in *M. hexagonoides* and *M. major*, while they are absent in the three new species. In addition, *M. rimosa* differs from other species in *Megasporia* by its extremely

thin basidiocarp (less than 0.5 mm thick) and cracked when dry, while fruiting bodies in other species are more than 1 mm thick, and not cracked when dry (Dai and Wu 2004, Du and Cui 2009, Li and Cui 2013). *Megasporia tropica* is distinguished from other species in the genus by lacking dendrohyphidia, cystidioles and hyphal pegs. *Megasporia yunnanensis* has brownish tints on its pore surface and lacks tetrahedric or polyhedric crystals, while other species in *Megasporia* have abundant tetrahedric or polyhedric crystals but lack brownish tints on their pore surfaces.

It seems that species of *Megasporia* prefer small branches rather than big logs; all specimens of the genus having been collected mostly on fallen branches and dead branches on living trees, and such branches being not strongly decayed. The basidiocarps of the genus are usually not very big and usually form small patches, although some patches may be merged finally. All the species of *Megasporia* have been found on angiosperm wood (never on gymnosperms), and they have a distribution in subtropical and tropical forests, especially in open environments, e.g. fallen branches along roads or paths. In addition, the species diversity of the genus is very rich in subtropical and tropical Asia, many more undescribed taxa are found from our samples based on phylogenetic analyses, but all these samples are sterile as a common feature of the genus, and the best season for producing basidiospores on these taxa are unknown.

Although *Megasporoporiella, Megasporoporia* and *Megasporia* are very similar, we found some difference among these genera both in morphology and ecology. The main difference is that *Megasporoporia* has di-trimitic hyphal structure and strongly dextrinoid skeletal hyphae, while dimitic hyphal structure and weakly to moderately dextrinoid skeletal hyphae are in *Megasporoporiella* and *Megasporia*. In addition, *Megasporoporiella* has a distribution in temperate region, while *Megasporia* in subtropical to tropics.

Key to known species of *Megasporia*

1	Pores 0.5–1.5 per mm	**2**
–	Pores 2–7 per mm	**4**
2	Basidiospores ellipsoid, gloeocystidia present	***M. ellipsoidea***
–	Basidiospores cylindrical, gloeocystidia absent	**3**
3	Pores 0.5–1 per mm, pore surface ash gray	***M. hexagonoides***
–	Pores 1–1.5 per mm, pore surface cream	***M. major***
4	Basidiospores < 15 μm in length, hyphal pegs present	**5**
–	Basidiospores > 15 μm in length, hyphal pegs absent	**8**
5	Pores 5–7 per mm, pores violet when fresh; dendrohyphidia present	***M. violacea***
–	Pores 2–5 per mm, pores cream to buff when fresh; dendrohyphidia absent	**6**
6	Pores 2–3 per mm, skeletal hyphae moderately dextrinoid	***M. hengduanensis***
–	Pores 3–5 per mm, skeletal hyphae strongly dextrinoid	**7**
7	Basidiospores 3.4–4.5 μm in width, cystidioles collapsed	***M. guangdongensis***
–	Basidiospores 4.1–5.6 μm in width, cystidioles not collapsed	***M. cystidiolophora***

8 Basidiocarp < 0.5 mm thick and cracked when dry.....***M. rimosa* sp. nov.**
– Basidiocarp > 1 mm thick and not cracked when dry.....**9**
9 Tetrahedric or polyhedric crystals present, dendrohyphidia absent.....
.....***M. tropica* sp. nov.**
– Tetrahedric or polyhedric crystals absent, dendrohyphidia present.....
.....***M. yunnanensis* sp. nov.**

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References

Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). System Biodiversity 3: 113–157. https://doi.org/10.1017/S1477200005001623

Chen JJ, Cui BK, Dai YC (2016) Global diversity and phylogeny of *Wrightoporia* (Russulales, Basidiomycota). Persoonia 37: 21–36. https://doi.org/10.3767/003158516X689666

Dai YC, Wu SH (2004) *Megasporoporia* (Aphyllophorales, Basidiomycota) in China. Mycotaxon 89: 379–388.

Du P, Cui BK (2009) Two new species of *Megasporoporia* (Polyporales, Basidiomycota) from tropical China. Mycotaxon 110: 131–138. https://doi.org/10.5248/110.131

Felsenstein J (1985) Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39: 783–791. https://doi.org/10.2307/2408678

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Li HJ, Cui BK (2013) Taxonomy and phylogeny of the genus *Megasporoporia* and its related genera. Mycologia 105: 368–383. https://doi.org/10.3852/12-114

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Petersen JH (1996) Farvekort. The Danish Mycological Society´s colour-chart. Foreningen til Svampekundskabens Fremme, Greve, 6.

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ryvarden L, Melo I (2014) Poroid fungi of Europe. Synopsis Fungorum 31: 1–455.

Ryvarden L, Wright JE, Rachenberg M (1982) *Megasporoporia* a new genus of resupinate polypores. Mycotaxon 16: 172–182.

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols, a guide to methods and applications. Academic Press, New York, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

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RESEARCH ARTICLE

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Species of *Hymenochaete* (Hymenochaetales, Basidiomycota) on bamboos from East Asia, with descriptions of two new species

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Abstract

Six species of *Hymenochaete* are found on bamboos in East Asia. Among them, *H. bambusicola* and *H. orientalis* are described and illustrated as new to science. *Hymenochaete bambusicola* is found exclusively on dead bamboos in northern Thailand and southwestern China, and characterized by the presence of dendrohyphidia and skeletal hyphae. It is phylogenetically and morphologically closely related to *H. innexa*, *H. koeljalgii* and *H. tropica*. *Hymenochaete orientalis* is found on bamboos in Taiwan and on angiosperm branches in southern China. It is distinguished by the absence of a hyphal layer and by having relatively large, oblong-ellipsoid to cylindrical basidiospores. *Hymenochaete orientalis* is morphologically similar to *H. longispora* and *H. cinnamomea*, and forms a distinct lineage close to *H. cinnamomea* in the ITS+nrLSU based phylogenetic analyses. An identification key to the six species on bamboos is given.

Key words

Bambusicolous fungi, Hymenochaetaceae, hymenochaetoid fungi, taxonomy

Introduction

Species of *Hymenochaete* Lév. are readily recognized by having brown basidiomes turning black in contact with potassium hydroxide, characteristic setae, generative hyphae without clamp connections, and smooth, thin-walled basidiospores. *Hymenochaete* is a

* These authors contributed equally to the paper.

large, morphologically heterogenous genus that includes more than one hundred and twenty species (Léger 1998, Parmasto 2004, Parmasto and Gilbertson 2005, He and Li 2011a, b, He and Dai 2012, Parmasto 2012). Wagner and Fisher (2002) separated the genus *Pseudochaete* T. Wagner & M. Fisch. from *Hymenochaete* according to phylogenetic analyses of nuclear large subunit ribosomal DNA sequences. Subsequently, this separation was supported by several molecular studies, and more species were described in or transferred to *Pseudochaete* (Larsson et al. 2006, He and Dai 2012, He and Li 2013, Parmasto et al. 2014). Yang et al. (2016) proposed *Hymenochaetopsis* S.H. He & Jiao Yang to replace *Pseudochaete*, since the latter has been used for algae since 1903. On the other hand, species in *Cyclomyces* Kunze ex Fr. and *Hydnochaete* Bres., with poroid/cyclolamellate and hydnoid hymenophores, were nested within the clades *Hymenochaetopsis* and *Hymenochaete* in the phylogenetic studies (Wagner and Fisher 2002, He and Dai 2012, Baltazar et al. 2014, Parmasto et al. 2014). Now, the former two genera are treated as synonyms of *Hymenochaete* (Fischer and Wagner 2001, Baltazar et al. 2014).

Although *Hymenochaete* s.l. is widely distributed in subtropical and tropical areas on angiosperm substrates, only one species, *H. muroiana* I. Hino & Katum., has been reported on bamboos in eastern Asia to date (Léger 1998, Parmasto 2005, Parmasto and Gilbertson 2005). In 2015 and 2016, several field trips were carried out in southern China, northern Thailand and central Taiwan, and many corticioid fungal specimens including those of *Hymenochaete* on bamboos were collected. Morphological and molecular studies of the specimens revealed six species of *Hymenochaete* on bamboos, two of which, *H. bambusicola* and *H. orientalis*, are described here as new.

Materials and methods

Morphological studies. Voucher specimens are deposited in the herbaria of Beijing Forestry University, Beijing, China (BJFC), the Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand (MFLU), and the National Museum of Natural Science, Taichung, Taiwan (TNM). Samples for microscopic examination were mounted in cotton blue and 2% potassium hydroxide (KOH). The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from given number of specimens (b). Color codes and names follow Kornerup and Wanscher (1978).

DNA extraction and sequencing. A CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd) was employed for DNA extraction and PCR amplification from dried specimens. The ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990) using the following procedure: initial denaturation at 95 °C for 4 min, followed by 34 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and final extension at 72 °C for 10 min. The nrLSU gene region was amplified

with the primer pair LR0R and LR7 (Vilgalys and Hester 1990, Lapeyre et al. 1993) using the following procedure: initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min, and final extension at 72 °C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and the sequences were deposited in GenBank (Table 1).

Phylogenetic analyses. The molecular phylogeny was inferred from a combined dataset of ITS and nrLSU sequences. The sequences retrieved from open databases originated from He and Dai (2012), He and Li (2013) and Parmasto et al. (2014, Table 1). *Fomitiporia banaennsis* Y.C. Dai and *F. punctata* (P. Karst.) Murrill were selected as outgroup taxa (He and Li 2013). Sequences were aligned using the ClustalX 1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit 7.0.5.3 (Hall 1999). Trees were shown in TreeView 1.6.6 (Page 1996).

Maximum likelihood (ML) and maximum parsimony (MP) analyses were conducted for the dataset. MP analysis were performed using PAUP* 4.0b10 (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analyses were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each generated tree. RAxML v.7.2.6 (Stamatakis 2006) was used for ML analysis. Default setting were used for all parameters in the ML analysis, and statistical support values were obtained using nonparametric bootstrapping with 1000 replicates (Hillis and Bull 1993).

Phylogeny results

The ITS+nrLSU sequences dataset contained 66 ITS and 69 nrLSU sequences from 69 samples representing 59 ingroup taxa and two outgroup taxa (Table 1). Eight ITS and 21 nrLSU sequences were newly generated. The dataset had an aligned length of 2226 characters, of which 611 were parsimony informative. MP analysis yielded 13 equally parsimonious trees (TL = 3172, CI = 0.397, RI = 0.734, RC = 0.291, HI = 0.603). ML analysis resulted in a topology similar to that of MP analysis. Only the MP tree is provided with both parsimony and likelihood bootstrap ≥ 70% labeled along the branches (Fig. 1). Alignments and trees are deposited at TreeBASE (submission ID: 20657). In the tree, samples of *H. bambusicola* and *H. orientalis* formed two distinct lineages. For *H. innexa* G. Cunn., *H. orientalis* and *H. rhabarbarina* (Berk.) Cooke, samples collected from bamboos and wood clustered together with high bootstrap values (Fig. 1).

Table 1. Taxa with locality and GenBank accession numbers for ITS and nrLSU sequences used in the phylogenetic analysis.

Taxa	Voucher	Locality	ITS	28S
Hymenochaete acerosa	He 338	China: Xizang	JQ279543	JQ279657
Hymenochaete adusta	He 207	China: Guangdong	JQ279523	KU975497[a]
Hymenochaete anomala	He 592	China: Hainan	JQ279566	JQ279650
Hymenochaete asetosa	Dai 10756	China: Hainan	JQ279559	JQ279642
Hymenochaete attenuata	He 28	China: Hainan	JQ279526	JQ279633
Hymenochaete bambusicola	He 4116	Thailand: Chiang Mai	KY425674[a]	KY425681[a]
Hymenochaete bambusicola	He 4121	Thailand: Chiang Mai	KY425675[a]	KY425682[a]
Hymenochaete biformisetosa	He 1445	China: Yunnan	KF908247	KU975499[a]
Hymenochaete cana	He 1305	China: Guangxi	KF438169	KF438172
Hymenochaete cinnamomea	He 755	China: Heilongjiang	JQ279548	JQ279658
Hymenochaete cinnamomea	He 2074	USA: Minnesota	KU975460[a]	KU975500[a]
Hymenochaete cruenta	He 766	China: Heilongjiang	JQ279595	JQ279681
Hymenochaete cyclolamellata	Cui 7393	China: Guangdong	JQ279513	JQ279629
Hymenochaete denticulata	He 1271	China: Guangxi	KF438171	KF438174
Hymenochaete epichlora	He 525	China: Hainan	JQ279549	JQ279659
Hymenochaete floridea	He 536	China: Hainan	JQ279597	JQ279683
Hymenochaete fulva	He 640	China: Yunnan	JQ279565	JQ279648
Hymenochaete huangshanensis	He 432	China: Anhui	JQ279533	JQ279671
Hymenochaete hydnoides	He 245	China: Hunnan	JQ279590	JQ279680
Hymenochaete innexa	He 446	China: Anhui	JQ279585	JQ279673
Hymenochaete innexa	He 4640	China: Taiwan	—	KY425683[a]
Hymenochaete koeljalgii	TFC 1996-007	Tanzania: Tanga	—	HE651003
Hymenochaete longispora	He 217	China: Guangdong	JQ279537	KU975514[a]
Hymenochaete luteobadia	He 8	China: Hainan	JQ279569	KU975515[a]
Hymenochaete megaspora	He 302	China: Xizang	JQ279553	JQ279660
Hymenochaete minor	He 933	China: Guangxi	JQ279555	JQ279654
Hymenochaete minuscula	He 253	China: Guizhou	JQ279546	KU975516[a]
Hymenochaete murina	He 569	China: Hainan	JQ716406	JQ716412
Hymenochaete muroiana	He 405	China: Xizang	JQ279542	KU975517[a]
Hymenochaete muroiana	He 4044	Thailand: Chiang Rai	KY425676[a]	KY425684[a]
Hymenochaete nanospora	He 475	China: Anhui	JQ279531	JQ279672
Hymenochaete ochromarginata	He 47	China: Hainan	JQ279579	JQ279666
Hymenochaete odontoides	Dai 11635	China: Beijing	JQ279563	JQ279647
Hymenochaete orientalis	He 4601	China: Taiwan	KY425677[a]	KY425685[a]
Hymenochaete orientalis	He 1057	China: Guangxi	KY425678[a]	KY425686[a]
Hymenochaete orientalis	He 1230	China: Guangxi	KY425679[a]	KY425687[a]
Hymenochaete parmastoi	He 867	China: Guangxi	JQ780063	KU975518[a]
Hymenochaete paucisetigera	Cui 7845	China: Jiangxi	JQ279560	JQ279644
Hymenochaete rhabarbarina	He 280	China: Yunnan	JQ279574	KY425688[a]
Hymenochaete rhabarbarina	He 4636	China: Taiwan	KY425680[a]	KY425689[a]
Hymenochaete rhabarbarina	TFC 1995-028	France: La Réunion	—	HE651007
Hymenochaete rhododendricola	He 389	China: Xizang	JQ279577	JQ279653
Hymenochaete rubiginosa	He 1049	China: Guangxi	JQ716407	JQ279667
Hymenochaete separabilis	He 460	China: Anhui	JQ279572	JQ279655

Taxa	Voucher	Locality	ITS	28S
Hymenochaete setipora	Cui 6301	China: Hainan	JQ279515	JQ279639
Hymenochaete spathulata	He 685	China: Yunnan	JQ279591	KU975529[a]
Hymenochaete sphaericola	He 303	China: Xizang	JQ279599	JQ279684
Hymenochaete sphaerospora	He 715	China: Yunnan	JQ279594	KU975531[a]
Hymenochaete tasmanica	He 449	China: Anhui	JQ279582	JQ279663
Hymenochaete tongbiguanensis	He 1552	China: Hainan	KF908248	KU975532[a]
Hymenochaete tropica	He 574	China: Hainan	JQ279587	JQ279675
Hymenochaete ulmicola	He 864	China: Jilin	JQ780065	KU975534[a]
Hymenochaete unicolor	He 468a	China: Anhui	JQ279551	JQ279662
Hymenochaete villosa	He 537	China: Hainan	JQ279528	JQ279634
Hymenochaete xerantica	Cui 9209	China: Yunnan	JQ279519	JQ279635
Hymenochaetopsis corrugata	He 761	China: Heilongjiang	JQ279606	JQ279621
Hymenochaetopsis gigasetosa	He1442	China: Yunnan	KT828670	KT828674
Hymenochaetopsis intricata	He 412	China: Xizang	JQ279608	JQ279624
Hymenochaetopsis lamellata	Cui 7629	China: Guangdong	JQ279603	JQ279617
Hymenochaetopsis laricicola	Dai 13458	China: Heilongjiang	KT828672	KT828676
Hymenochaetopsis latesetosa	He 502	China: Hainan	JQ716405	JQ716410
Hymenochaetopsis olivacea	Dai 12789	USA: Connecticut	KT828678	KT828679
Hymenochaetopsis rigidula	He 379	China: Xizang	JQ279613	JQ279620
Hymenochaetopsis subrigidula	He 1157	China: Yunnan	JQ716403	JQ716409
Hymenochaetopsis tabacina	He 810	China: Jilin	JQ279611	JQ279626
Hymenochaetopsis tabacinoides	Cui 10428	China: Yunnan	JQ279604	JQ279618
Hymenochaetopsis yasudae	He 375	China: Xizang	JQ279615	JQ279627
Fomitiporia bannaensis	MUCL 46950	China: Yunnan	GU461943	EF429218
Fomitiporia punctata	MUCL 47629	Japan	GU461950	GU461982

[a] Sequences newly generated in this study.

Taxonomy

Hymenochaete bambusicola **S.H. He, sp. nov.**
MycoBank: MB 819604
Figs 2, 3

Diagnosis. This species is distinguished by the presence of dendrohyphidia and skeletal hyphae and the preferred substrate of bamboo tissues.

Holotype. THAILAND. Chiang Mai Province: Mork Fa, on fallen bamboo, 25 Jul 2016, He 4116 (holotype: BJFC; isotype: MFLU).

Etymology. "*Bambusicola*" (Lat.) refers to growing on bamboo.

Fruiting body. Basidiomes annual, resupinate, effused, closely adnate, coriaceous, at first as small irregular patches, later confluent up to 50 cm long, up to 200 μm thick. Hymenophore smooth, greyish red [7B(3–6)], brownish orange [7C(3–6)], greyish brown (7D3) to light brown [7D(4–6)], not cracked; margin thinning out, light brown [7D(4–8)] to brown [7E(4–8)], usually darker than the hymenophore surface, velvety, up to 1 mm wide. Tissues darkening in KOH.

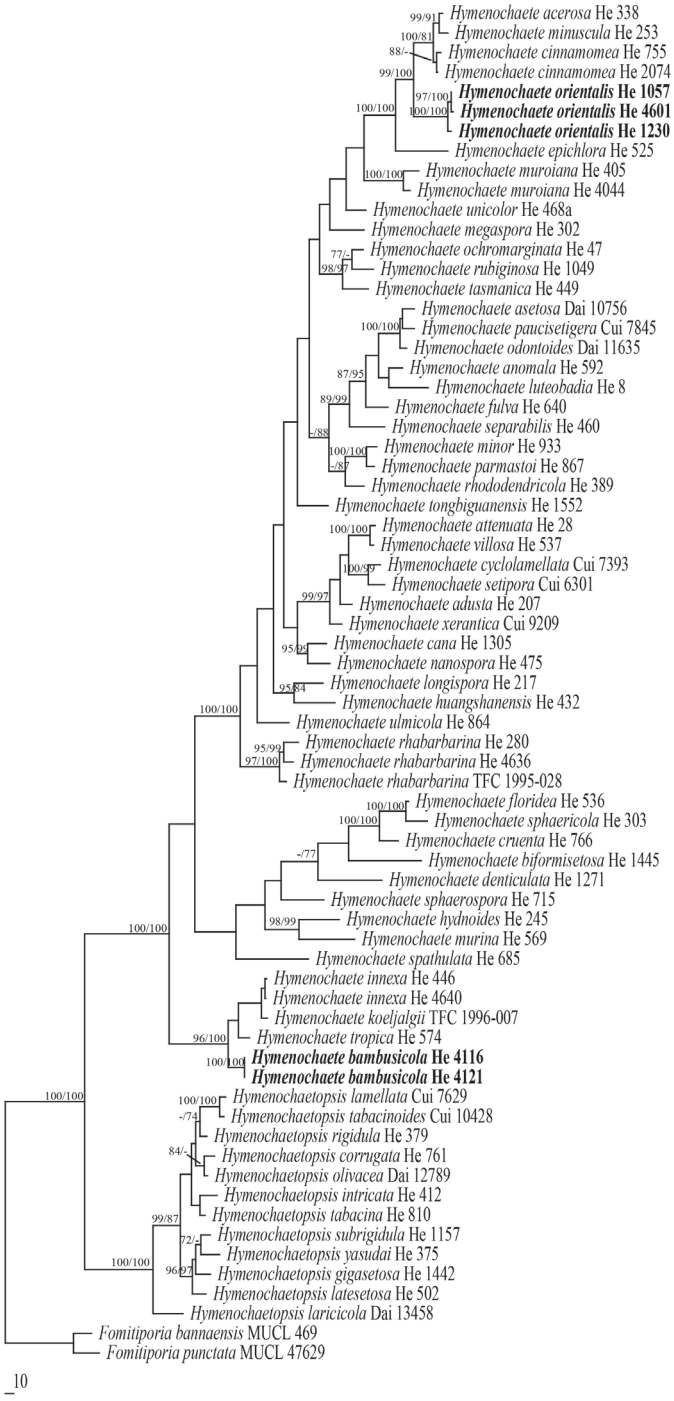

Figure 1. Strict consensus tree obtained from maximum parsimony analysis of combined ITS and nrLSU sequence data of taxa of *Hymenochaete* and *Hymenochaetopsis*. Branches are labeled with parsimony bootstrap (before slash) ≥ 70% and likelihood bootstrap (after slash) ≥ 70%.

Figure 2. Basidiomes of *Hymenochaete bambusicola*. **A** He 4116 (holotype) **B** He 4121. Scale bars : 1 cm.

Microscopic structures. Hyphal system dimitic. Tomentum absent, cortex and hyphal layer present. Cortex up to 10 µm thick, composed of densely interwoven and agglutinated hyphae, sometimes indistinct. Hyphal layer composed of loosely interwoven skeletal and generative hyphae. Skeletal hyphae dominant, golden yellow to yellowish brown, distinctly thick-walled to subsolid, frequently branched, non-septate, 1–3 µm in diam. Generative hyphae scattered, simple-septate, colorless to pale yellow, thin- to thick-walled, moderately branched, 2–5 µm in diam. Setae scattered to abundant, subulate, yellowish to reddish brown, bearing a thick hyphal sheath and an acute tip, without encrustations or sometime slightly encrusted, originating from the subhymenium or the hyphal layer, (55–)70–150(–170) × 7–11 µm, projecting above the hymenium up to 60 µm. Dendrohyphidia numerous, yellowish brown, bearing a thick-walled stem up to 5 µm wide, with branches up to 10 µm long. Basidia clavate to subcylindrical, colorless, with 4 sterigmata and a basal simple septum, 15–18 × 3–4 µm;

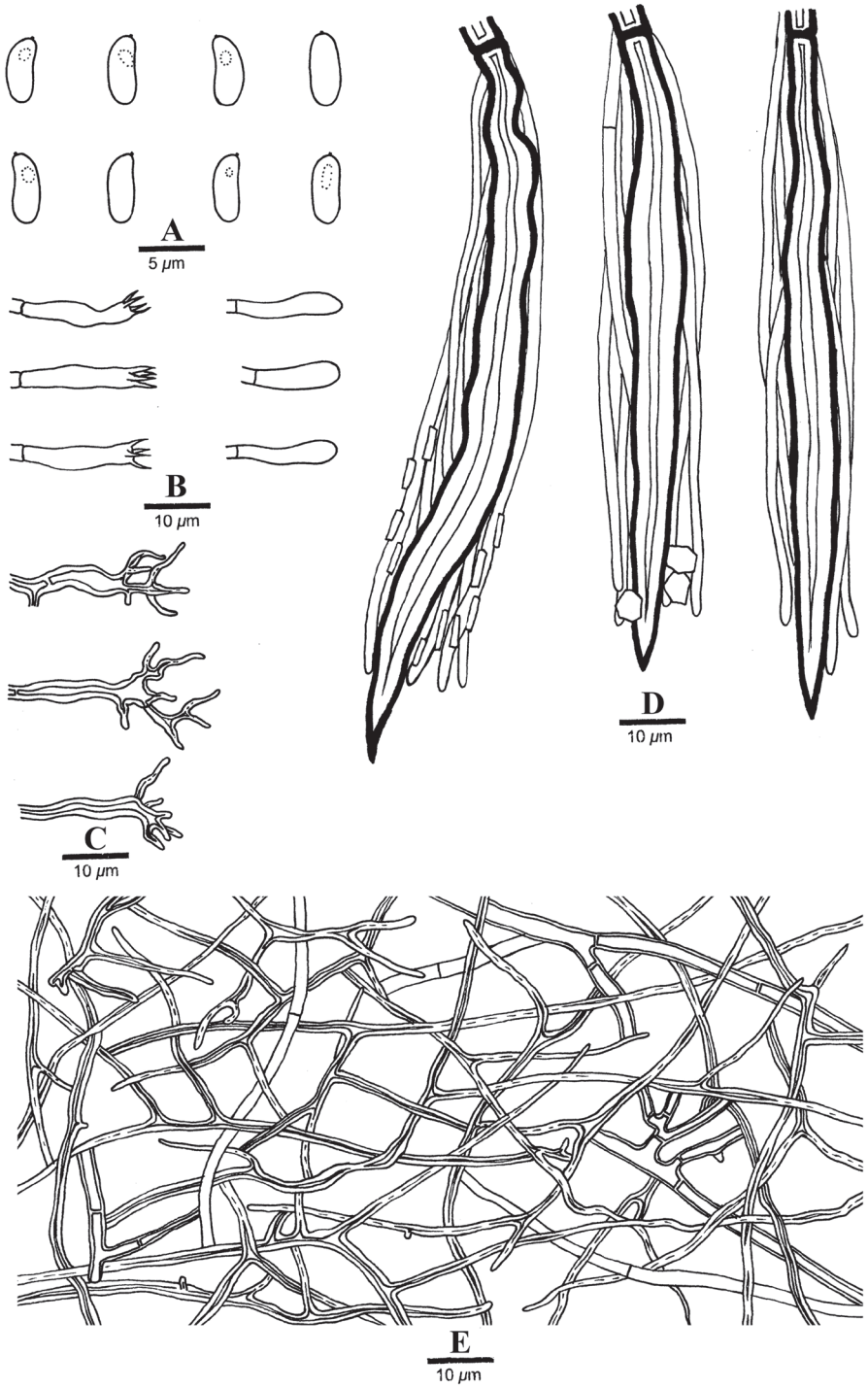

Figure 3. Microscopic structures of *Hymenochaete bambusicola* (drawn from holotype). **A** Basidiospores **B** Basidia and basidioles **C** Dendrohyphidia **D** Setae **E** Hyphae from hyphal layer.

basidioles similar to basidia but smaller. Basidiospores short cylindrical, slightly curved, colorless, thin-walled, smooth, usually with a small guttula, (4–)4.5–6 × 2–2.5(–2.8) μm, L = 5.1 μm, W = 2.2 μm, Q = 2.3 (n = 60/2).

Additional specimens examined (paratypes: BJFC & MFLU). THAILAND. Chiang Mai Province: Mork Fa, on fallen bamboo, 25 Jul 2016, He 4121 & 4131. CHINA. Yunnan Province: Jinghong, Virgin Forest Park, on fallen bamboo, 7 Jun 2011, He 652.

Remarks. *Hymenochaete bambusicola* belongs to sect. *Hymenochaete* sensu Léger (1998) and is morphologically similar to *H. tropica* S.H. He & Y.C. Dai. However, *H. tropica* has a monomitic hyphal system, shorter setae (50–90 × 7–11 μm), and dendrohyphidia with shorter branches (He and Dai 2012). *Hymenochaete innexa* and *H. koeljalgii* Parmasto also resemble *H. bambusicola*, but differ from the latter species by having simple hyphidia (not or rarely branched) and absence of skeletal hyphae (Dai 2010, Parmasto et al. 2014). *Hymenochaete ceratophora* Job [= *H. alabastrina* G.A. Escobar ex J.C. Léger or *Dichochaete ceratophora* (Job) Parmasto] is similar to *H. bambusicola* by having a dimitic hyphal system with thick-walled, branched dichohyphae, numerous dendrohyphidia and short cylindrical basidiospores; however, the former species can be distinguished from the latter by having shorter setae (60–110 × 6–10 μm), crystals in hymenium and subhymenium and a distribution in Mesoamerica and South America on unknown substrates (Léger 1998, Parmasto 2000). *Hymenochaete tasmanica* Massee (sect. *Hymenochaete*) is somewhat similar to *H. bambusicola* by having dendrohyphidia; however, *H. tasmanica* lacks skeletal hyphae, has a stratified subhymenium and grows on angiosperm substrates (Léger 1998). In the phylogenetic tree, *H. bambusicola* formed a fully supported clade with *H. innexa*, *H. koeljalgii* and *H. tropica* (Fig. 1).

***Hymenochaete orientalis* S.H. He, sp. nov.**
MycoBank: MB 819605
Figs 4, 5

Diagnosis. This species is distinguished by lacking a hyphal layer and having relatively large, oblong-ellipsoid to cylindrical basidiospores.

Holotype. CHINA. Taiwan: Nantou County, Ren'ai Township, Nandongyan Mountains, on fallen bamboo, 7 Dec 2016, He 4601 (holotype: BJFC; isotype: TNM).

Etymology. "*Orientalis*" (Lat.) refers to the known distribution in East Asia.

Fruiting body. Basidiomes annual, resupinate, effused, closely adnate, crustaceous to coriaceous, at first as small irregular patches, later confluent up to 20 cm long, up to 100 μm thick. Hymenophore smooth, brownish orange [7C(5–8)], light brown [7D (5–8)], brown [7E (5–8)] to reddish brown [8E (4–8)], not cracked; margin indeterminate, concolorous with hymenophore surface. Tissues darkening in KOH.

Microscopic structures. Tomentum, cortex and hyphal layer absent. Hyphal system monomitic. Generative hyphae simple-septate, colorless to pale yellow, moderately

Figure 4. Basidiomes of *Hymenochaete orientalis*. **A** He 4601 (holotype) **B** He 1230. Scale bars: 1 cm.

thick-walled, frequently branched at right angles, densely interwoven, agglutinated, 2.5–4 µm in diam. Setae abundant, subulate, bearing an acute tip, yellowish to reddish brown, arranged in 1–3 overlapping rows, usually with a hyphal sheath and a basal hyphal node composed of densely interwoven hyphae, without encrustations or slightly encrusted with age, 70–120 × 7–10 µm, projecting out of the hymenium up to 85 µm. Basidia clavate to subcylindrical, usually with a constriction in the middle part, some with walls thickening towards the base, colorless, with 4 sterigmata and a basal simple septum, 15–23 × 4–5.5 µm; basidioles similar to basidia but smaller. Basidiospores oblong-ellipsoid to cylindrical, with a small apiculus, colorless, thin-walled, smooth, sometimes with one or two small guttulae, 6–8(–8.5) × 3–3.8(–4) µm, L = 6.7 µm, W = 3.3 µm, Q = 2–2.1 (n = 60/3).

Additional specimens examined (paratypes: BJFC). CHINA. Guangxi Autonomous Region: Jinxiu County, Dayaoshan Nature Reserve, on fallen angiosperm twig, 25 Aug 2011, He 1000; Nanning, Damingshan Nature Reserve, on fallen angiosperm twig, 29 Aug 2011, He 1057; Qingxiushan Park, on fallen angiosperm twig, 15 Jul 2012, He 1230. Guangdong Province: Guangzhou, South China Botanical Garden, on fallen angiosperm twig, 5 Jul 2010, He 235; Heyuan County, Daguishan Forest Park, on fallen angiosperm twig, 18 Aug 2011, He 1212.

Remarks. *Hymenochaete orientalis* belongs to sect. *Gymnochaete* G.A. Escobar ex J.C. Léger sensu Léger (1998), and is morphologically very similar to *Hymenochaete*

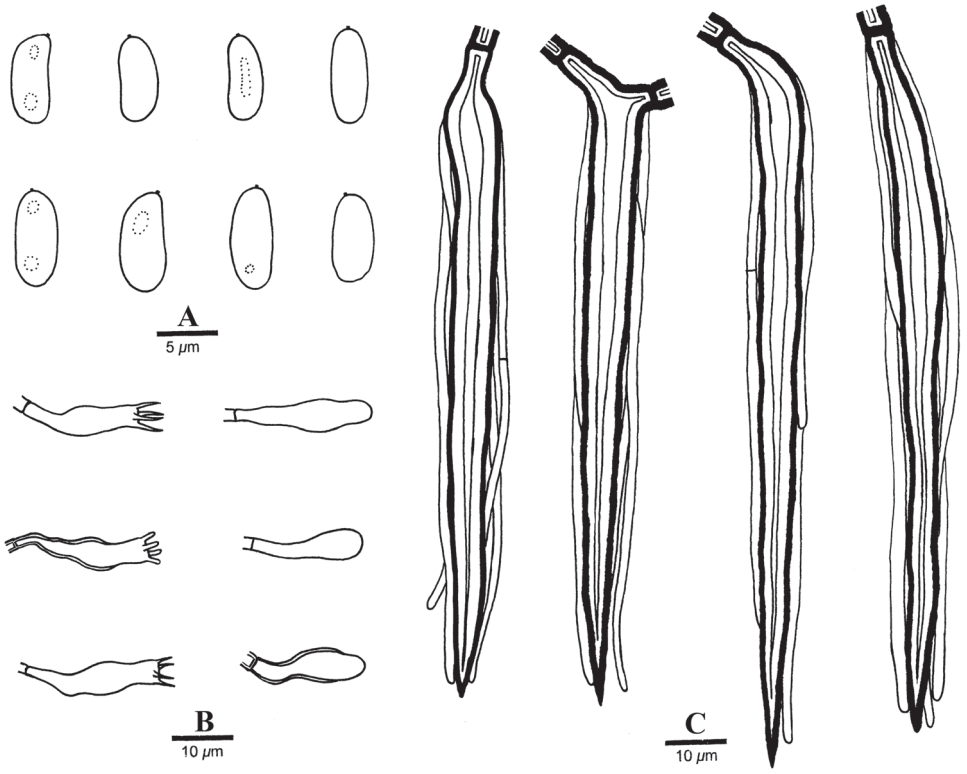

Figure 5. Microscopic structures of *Hymenochaete orientalis* (drawn from holotype). **A** Basidiospores **B** Basidia and basidioles **C** Setae.

longispora Parmasto. However, *H. longispora* has hyphidia in hymenium and longer basidiospores, 8–10 μm according to Léger (1998). *Hymenochaete cinnamomea* (Pers.) Bres. also resembles *H. orientalis*, but differs by the presence of a hyphal layer composed of loosely interwoven hyphae and smaller basidiospores, 5–6.5 × 2.5–3 μm according to Léger (1998). *Hymenochaete minuscula* G. Cunn. in sect. *Gymnochaete* can be distinguished from *H. orientalis* by its smaller setae (40–56 × 5–6 μm) and basidiospores (4–5 × 1.8–2.2 μm) according to Léger (1998). In the phylogenetic tree, *H. orientalis* formed a lineage close to *H. cinnamomea* and *H. minuscula*, but is distant from *H. longispora* (Fig. 1).

Other specimens examined (BJFC)

Hymenochaete innexa: CHINA. Taiwan: Nantou County, Xinyi Township, Xitou Research Center, on fallen bamboo, 11 Dec 2016, He 4640. ***Hymenochaete muroiana***: CHINA. Xizang Autonomous Region: Linzhi County, Gadinggou Forest Park, on dead bamboo, 25 Sep 2010, He 405. Hunan Province: Dong'an County, Shunhuangshan Nature Reserve, on bamboo stump, 13 Jul 2015, He 2379. Hainan Province:

Wuzhishan County, Wuzhishan Nature Reserve, on dead bamboo, 10 Jun 2016, He 3953. Guangdong Province: Zhaoqing County, Dinghushan Nature Reserve, on dead bamboo, 30 Jun 2010, He 172. Guangxi Autonomous Region: Jinxiu County, Dayaoshan Nature Reserve, 23 Aug 2011, He 947. Taiwan: Nantou County, Ren'ai Township, Nandongyan Mountains, on dead bamboo, 7 Dec 2016, He 4608. THAILAND. Chiang Rai Province: Campus of Mae Fah Luang University, on dead bamboo, 21 Jul 2016, He 4044. ***Hymenochaete rhabarbarina***: CHINA. Taiwan: Nantou County, Xinyi Township, Xitou Research Center, on fallen bamboo, 11 Dec 2016, He 4636. ***Hymenochaete tropica***: CHINA. Hainan Province: Wuzhishan County, Wuzhishan Nature Reserve, on fallen bamboo, 10 Jun 2016, He 3959.

Discussion

As shown in previous studies (Larsson et al. 2006, He and Dai 2012, He and Li 2013, Parmasto et al. 2014), species of *Hymenochaetopsis* formed a highly supported clade in our phylogenetic tree (Fig. 1). Four species, *Hymenochaete bambusicola*, *H. innexa*, *H. koeljalgii* and *H. tropica* clustered in a fully supported clade. Morphologically, these species have some similar features, such as strictly resupinate basidiomes, presence of abundant hyphidia, and oblong-ellipsoid to short cylindrical basidiospores. In the phylogenetic tree of Parmasto et al. (2014), *H. koeljalgii* clustered with *H. floridea* Berk. & Broome, but in present analyses the latter species grouped with *H. sphaericola* Lloyd and *H. cruenta* (Pers.) Donk. The topology of these and other closely related species is still not completely resolved. *Hymenochaete orientalis* nested within a highly supported clade with *H. cinnamomea*, *H. minuscula*, *H. acerosa* S.H. He & Hai J. Li, and *H. epichlora* (Berk. & M.A. Curtis) Cooke. This clade includes species of the *H. cinnamomea* group (Parmasto 2001, He and Li 2011a). In Parmasto et al. (2014), *H. cinnamomea* formed a fully supported clade with the generic type *H. rubiginosa* (Dicks.) Lév. and *H. ochromarginata* P.H.B. Talbot; however, in our tree, *H. cinnamomea* group and *H. rubiginosa* group are in a large clade that is not supported. This may be because our phylogenetic analysis includes more taxa related to *H. cinnamomea* and *H. rubiginosa*.

Until now, six species, *H. bambusicola*, *H. innexa* (Fig. 6A), *H. muroiana* (Fig. 6B), *H. orientalis*, *H. rhabarbarina* (Fig. 6C) and *H. tropica* (Fig. 6D) have been found on bamboos in East Asia. Among these species, *H. muroiana*, originally reported from Japan is a common species in East Asia. Parmasto (2012) described *H. muroiana* subsp. *africana* Parmasto on bamboo from Kenya, which is very similar to *H. muroiana* subsp. *muroiana* in morphology. However, it is not clear whether they are conspecific in phylogeny. In addition to *H. muroiana*, *H. bambusicola* is also found exclusively on dead bamboos based on present materials. *Hymenochaete innexa*, *H. rhabarbarina* and *H. tropica* are here reported on bamboos for the first time.

Figure 6. Basidiomes of previously known *Hymenochaete* species on bamboos from East Asia. **A** *H. innexa* (He 4640) **B** *H. muroiana* (He 4608) **C** *H. rhabarbarina* (He 4636) **D** *H. tropica* (He 3959).

Key to species of *Hymenochaete* on bamboos in East Asia

1 Hyphidia present **2**
– Hyphidia absent **4**
2 Hyphal layer absent, hyphidia unbranched ..... ***H. innexa***
– Hyphal layer present, hyphidia branched **3**
3 Skeletal hyphae present, hyphidia dendroid with long branches..... ***H. bambusicola***
– Skeletal hyphae absent, hyphidia bifurcated to dendroid with short branches... ***H. tropica***
4 Hyphal layer present, hyphae encrusted with yellow resinous granules..... ***H. rhabarbarina***
– Hyphal layer absent, hyphae smooth **5**
5 Setae 40–50 µm long, basidiospores 4–6 µm long..... ***H. muroiana***
– Setae 70–120 µm long, basidiospores 6–8 µm long..... ***H. orientalis***

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References

Baltazar JM, Pildain MB, Gorjón SP, da Silveira RMB, Rajchenberg M (2014) Phylogenetic relationships of *Hydnum peroxydatum* support the synonymy of *Hydnochaete* with *Hymenochaete* (Hymenochaetaceae, Agaricomycetes). Mycologia 106: 323–327. https://doi.org/10.3852/13-154

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. https://doi.org/10.1093/nar/gkg500

Dai YC (2010) Hymenochaetaceae (Basidiomycota) in China. Fungal Diversity 45: 131–343. https://doi.org/10.1007/s13225-010-0066-9

Felsenstein J (1985) Confidence intervals on phylogenetics: an approach using bootstrap. Evolution 39: 783–791. https://doi.org/10.2307/2408678

Fischer M, Wagner T (2001) Proposal to conserve *Hymenochaete* Lév. nom cons. (Hymenochaetales, Basidiomycetes), against an additional name, *Cyclomyces* Fr. Taxon 50: 1185–1186. https://doi.org/10.2307/1224741

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98

He SH, Dai YC (2012) Taxonomy and phylogeny of *Hymenochaete* and allied genera of Hymenochaetaceae (Basidiomycota) in China. Fungal Diversity 56: 77–93. https://doi.org/10.1007/s13225-012-0174-9

He SH, Li HJ (2011a) Two new species of *Hymenochaete* (Hymenochaetales) from China. Mycotaxon 115: 375–382. https://doi.org/10.5248/115.375

He SH, Li HJ (2011b) *Hymenochaete rhododendricola* and *H. quercicola* spp. nov. (Basidiomycota, Hymenochaetales) from Tibet, southwestern China. Nordic Journal of Botany 29: 484–487. https://doi.org/10.1111/j.1756-1051.2011.01217.x

He SH, Li HJ (2013) *Pseudochaete latesetosa* and *P. subrigidula* spp. nov. (Hymenochaetales, Basidiomycota) from China based on morphological and molecular characters. Mycological Progress 12: 331–339. https://doi.org/10.1007/s11557-012-0838-6

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192. https://doi.org/10.1093/sysbio/42.2.182

Kornerup A, Wanscher JH (1978) Methuen handbook of colour. 3rd Ed. Eyre Methuen, London, 1–252.

Lapeyre B, Michot B, Feliu J, Bachellerie JP (1993) Nucleotide sequence of the Schizosaccharomyces pombe 25S ribosomal RNA and its phylogenetic implications. Nucleic Acids Research 21: 3322–3322. https://doi.org/10.1093/nar/21.14.3322

Larsson KH, Parmasto E, Fischer M, Langer E, Nakasone K, Redhead S (2006) Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. Mycologia 98: 926–936. https://doi.org/10.3852/mycologia.98.6.926

Léger JC (1998) Le genre *Hymenochaete* Léveillé. Bibliotheca Mycologica 171: 1–319.

Page RMD (1996) Treeview: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences Cabios 12: 357–358.

Parmasto E (2000) New taxa and new combinations in hymenochaetoid fungi (Hymenomycetes). Folia Cryptogamica Estonica 37: 55–66.

Parmasto E (2001) Hymenochaetoid fungi (Basidiomycota) of North America. Mycotaxon 79: 107–176.

Parmasto E (2004) Two new species of *Hymenochaete* (Hymenomycetes, Basidiomycota) from India. Folia Cryptogamica Estonica 40: 41–42.

Parmasto E (2005) New data on rare species of *Hydnochaete* and *Hymenochaete* (Hymenochaetales). Mycotaxon 91: 137–163.

Parmasto E, Gilbertson RL (2005) The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands. Mycotaxon 94: 189–214.

Parmasto E (2012) New taxa of *Hymenochaete* (Agaricomycetes, Hymenochaetales) with a note on *H. caucasica*. Mycotaxon 121: 477–484. https://doi.org/10.5248/121.477

Parmasto E, Saar I, Larsson E, Rummo S (2014) Phylogenetic taxonomy of *Hymenochaete* and related genera (Hymenochaetales). Mycological Progress 13: 55–64. https://doi.org/10.1007/s11557-013-0891-9

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wagner T, Fischer M (2002) Classification and phylogenetic relationships of *Hymenochaete* and allied genera of the Hymenochaetales, inferred from rDNA sequence data and nuclear behaviour of vegetative mycelium. Mycological Progress 1: 93–104. https://doi.org/10.1007/s11557-006-0008-9

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols, a guide to methods and applications. Academic, San Diego, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Yang J, Dai LD, He SH (2016) *Hymenochaetopsis* nom. nov. proposed to replace *Pseudochaete* (Hymenochaetales, Basidiomycota) with descriptions of *H. laricicola* sp. nov and *H. gigasetosa* new to China. Mycological Progress 15: 13. https://doi.org/10.1007/s11557-015-1153-9