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High diversity and widespread occurrence of mitotic spore mats in ectomycorrhizal *Pezizales*

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Running Head: Diversity of spore mats in the ectomycorrhizal *Pezizales*

Abstract

Fungal mitospores may function as dispersal units and/ or spermatia, and thus play a role in distribution and/or mating of species that produce them. Mitospore production in ectomycorrhizal (EcM) *Pezizales* is rarely reported, but here we document mitospore production by a high diversity of EcM *Pezizales* on three continents, in both hemispheres. We sequenced the internal transcribed spacer (ITS) and partial large subunit (LSU) nuclear rDNA from 292 spore

mats (visible mitospore clumps) collected in Argentina, Chile, China, Mexico, South America, and the USA between 2009-2012. We collated spore mat ITS sequences with 105 fruit body and 47 EcM root sequences to generate operational taxonomic units (OTUs). Phylogenetic inferences were made through analyses of both molecular datasets.

Forty-eight OTUs from spore mats represented ≥ six independent EcM *Pezizales* lineages and included truffles and cup fungi. Seven OTUs within three putative lineages have no known meiospore stage. Mitospores failed to germinate on sterile media, or form ectomycorrhizas on *Quercus, Pinus*, and *Populus* seedlings, consistent with a hypothesized role of spermatia. The broad geographic range, high frequency, and phylogenetic diversity of spore mats produced by EcM *Pezizales* suggests that a cryptic mitospore stage may be an important biological feature of this group in terms of mating, reproduction, and/or dispersal.

Introduction

Ectomycorrhizal (EcM) fungi are important plant symbionts that improve plant nutrient status (Baxter & Dighton, 2001), mediate drought effects (Warren *et al*., 2008), and enhance seedling establishment (Ashkannejhad & Horton, 2006; Nara, 2006). EcM fungi are diverse, and are comprised of an estimated 20,000–25,000 species from 66 lineages. Within the *Pezizales* (*Ascomycota*), the order that includes morels and truffles, EcM symbioses have evolved independently at least 16 times (Tedersoo *et al*., 2010). Although *Basidiomycota* often dominate EcM root communities, *Pezizales* are diverse and are dominant EcM symbionts in many ecosystems, particularly habitats subjected to drought (Gehring *et al*., 1998; Smith *et al*., 2007b) or frequent fires (Warcup, 1990; Fujimura *et al*., 2005). Some EcM *Pezizales* proliferate in response to disturbance and at forest edges (Dickie & Reich, 2005; Tedersoo *et al*., 2006b).

Many pezizalean EcM species show some degree of affinity for mineral soils or soils with high pH (Petersen, 1985; Tedersoo *et al*., 2006a; García -Montero *et al.*, 2008; Iotti *et al.*, 2010). Other pezizalean EcM taxa such as *Tuber* spp. are also frequently detected taxa in molecular studies of undisturbed forests (Walker *et al.*, 2005; Morris *et al.*, 2009) and managed tree plantations (Bonito *et al*., 2011).

Reproduction and dispersal in fungi is carried out through the production of mitospores (spores produced by mitosis) and/or meiospores. Previous research suggests that EcM fungi reproduce and disperse exclusively or primarily through meiospores produced inside or on the surface of fruit bodies (Hutchison, 1989). Types of fruit bodies produced by EcM fungi include above ground mushrooms, cup fungi, jelly fungi, and resupinate crusts from which meiospores are forcibly discharged to be dispersed in the wind; or below ground fruiting structures that in most cases are truffle-like (closed), lack forcible spore discharge, and disperse their meiospores passively or through animal mediation (e.g. earthballs, truffles) (Tedersoo *et al.*, 2010). Many saprotrophic and pathogenic relatives of EcM fungi produce mitospores (Nobles, 1958; Walther *et al.*, 2005), but it has been suggested that the EcM symbiosis may in some way be incompatible with mitospore production (Hutchison, 1989; Walther *et al.*, 2005). However, most research on sporogenesis and spore dispersal in EcM fungi has focused on species of *Basidiomycota* (Hutchison, 1989); *Ascomycota* have received considerably less attention.

Even though *Ascomycota* are noted for their ability to form mitospores, many of these forms have not yet been linked to a meiosporic species (Shenoy *et al*., 2007). This disconnect may be due to spatial and temporal differences in production of these two spore types and also to the

difficulty of stimulating spore production in pure culture. In addition, some fungi may have lost the ability to form meiospores (Taylor *et al.*, 1999).

The few reports of mitospore formation by EcM *Pezizales* in culture include. *Tarzetta catinus* (Dodge, 1937, as *Peziza pustulata*), *Tricharina hiemalis*, *T. ochroleuca*, *Wilcoxina mikolae* (Yang & Korf, 1985a) and *Muciturbo reticulatus* (Warcup & Talbot, 1989). Only a few EcM fungi have been unequivocally linked to mitosporic stages in nature. The first was *Muciturbo*, which forms a spore mat (clump of mitospore-bearing mycelium visible to the unaided eye) on the soil surface prior to fruit body formation (Warcup & Talbot, 1989). ITS sequences were used to link spore mats on soil to an unknown species in the /pachyphloeus-amylascus lineage (Norman & Egger, 1999), and two species of *Tuber* (Urban *et al.,* 2004). ITS sequences of asexual spore mats also matched *Fagus* and *Quercus* EcM root tip sequences (Urban *et al*., 2004; Tedersoo *et al*., 2006b; Palmer *et al*., 2008).

In this paper, lineage nomenclature is preceded by a forward slash, and follows Moncalvo *et al*. (2002), while *Pezizales* lineage circumscription follows Tedersoo *et al*. (2010).

During preliminary surveys of *Pezizales* spore mats in 2009, we found that mitospores of *Pachyphloeus* and *Tuber* are widespread and conspicuous in hardwood and mixed forests of the Eastern USA. These findings led us to ask the following: 1) What proportion of EcM *Pezizales* lineages produce spore mats? 2) What habitats are EcM *Pezizales* spore mats produced in? 3) What is the distribution of EcM *Pezizales* that produce spore mats? 4) Can EcM *Pezizales* mitospores form ectomycorrhizas on forest trees? We discovered that the majority of known lineages of EcM *Pezizales* commonly produce spore mats; spore mats are produced mainly on exposed soil or woodland debris; and they are distributed on four continents, in both

hemispheres. We encountered novel examples in the /fischerula, /hydnobolites, /hydnotrya, /pachyphloeus-amylascus, /terfezia-peziza depressa and /tuber-helvella lineages. Our results call for a reassessment of the life stages of EcM *Pezizales*.

Materials and Methods

Fungal material – During spring, summer, and fall of 2009-2011 spore mats were encountered in a variety of habitats with EcM trees, such as forested hiking trails, washes, creek edges, parks, and urban wooded areas. We opportunistically collected these spore mats across the Eastern USA during 2009-2011, in northeast Mexico and southeast China in August and September of 2010, and in Chile and Argentina in March and April of 2012. Surveyed forest types included broadleaf deciduous, oak-savanna, mixed broadleaf-*Pinaceae,* and pure *Pinaceae* forests. Spore mats were photographed in the field, placed in clean plastic containers or wrapped in aluminum foil. Collecting implements were cleaned between uses to prevent cross-contamination. For all collections we recorded the date, location, the EcM canopy plants, and basic habitat information. Specimens were dried in a forced air dryer or in a closed plastic container with silica gel drying beads (Henkel *et al.*, 2006). Each collection was glued to archival paper cards and stored in herbarium boxes for morphological examination, molecular study, and voucher accession. Specimens are deposited in the Duke University Herbarium (DUKE), the Farlow Herbarium at Harvard University (FH), the Herbarium Jose Castillo Tovar (ITCV) Mexico, Kunming Institute of Botany (KUN), and the University of Minnesota Herbarium (MIN).

In order to assess whether meio- and mitospores are produced concurrently, we also collected truffles and other *Pezizales* fruit bodies in the vicinity of spore mats. These were examined microscopically for identification and ca. 3 mm^3 of clean tissue was sampled for DNA. EcM root

tips were collected as described in Guevara *et al*. (2012 in press) in Mexico in Aug. 2008 and Eastern US in Jul. 2010. To obtain broader diversity and better phylogenetic placement of our samples, fruit body collections of EcM *Pezizales* were incorporated into this study. These included personal herbaria materials, and loans from the following institutions: the Farlow Herbarium at Harvard University (FH), Oregon State University (OSC), Cornell University Herbarium (CUP), University of Bergen (BG), and Real Jardín Botánico-CSIC (MA). Voucher information is listed in Table S1.

Molecular protocols - DNA was extracted from spore mats, fruit bodies, and EcM root tips using a modified CTAB protocol (Gardes & Bruns, 1993) or an Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's instructions, but with 20% of the recommended volume. For the remaining spore mats, we added small pieces of tissue to PCR reactions for direct amplification (Bonito, 2009).

PCR products were run on 1.5% agarose gels containing ethidium bromide or stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). Amplicons were digested with the EXO and AP enzymes (Glenn & Schable, 2005), or cleaned by standard ethanol precipitation. Amplicons were sequenced in both directions with an ABI Big Dye Terminator Sequencing Kit (v3.1) and run on an ABI 3730xl capillary sequencer (Applied Biosystems, Foster City, CA, USA) at the Duke University sequencing facility and the University of Minnesota Biomedical Genomics Facility. Sequences were trimmed, edited, and assembled in Sequencher v. 4.10.1 (Gene Codes Inc., Ann Arbor, MI, USA).

Species determination and phylogenetic analysis of ITS – The ITS region of rDNA, an official barcode for fungal species identification (Schoch *et al.*, 2012), has proven effective for

delimiting *Pezizales* at the species level (Smith *et al.*, 2007a; Bonito *et al.*, 2010). We used PCR to amplify the entire ITS rDNA repeat with combinations of primers ITS1, ITS1F, ITS5 (forward) and ITS2, ITS4, or LR3 (reverse) (White *et al.*, 1990; Gardes & Bruns, 1993). After sequences were obtained and assembled, we performed BLAST searches on all and downloaded similar sequences from GenBank for phylogenetic comparisons. Lastly, to find closely related EcM fungal sequences, we used the Emerencia "genus search" function to search for insufficiently identified sequences using queries for *Fischerula, Hydnobolites, Hydnocystis, Pachyphloeus, Peziza, Ruhlandiella, Scabropezia* and *Tuber* (Nilsson *et al.*, 2005; Ryberg *et al.*, 2009). We then trimmed all sequences to begin at the "CATTA" motif of ITS1 and end at the "CAATAAGC" motif of ITS2. We uploaded trimmed sequences into a Sequencher file, and sorted them into OTUs based on 96% sequence similarity using the "dirty data" algorithm. Phylogenetic relationships among closely related OTUs were inferred within the four most speciose genera. Sequences from each OTU were selected to represent unique geographic localities and isolation sources. Four sets of ITS sequences were aligned including 41 sequences of *Hydnobolites* from the /marcelleina-peziza gerardii lineage (from 14 fruit bodies, 10 EcM roots, and 17 spore mats); 94 sequences of /pachyphloeus-amylascus (from 36 fruit bodies, 25 EcM roots or environmental samples, and 33 spore mats); 45 sequences of *Tuber* from the /tuber-helvella lineage (from 19 fruit bodies, 16 EcM roots, and 11 spore mats); and 45 sequences of /terfezia-peziza depressa (from 12 fruit bodies, 16 EcM roots, and 17 spore mats). Sequences were aligned in MAFFT v 6.822 (Katoh and Toh, 2010), and alignments manually improved in Se-Al v 2.0a11 (Rambaut, 2007). Ambiguously aligned regions were excluded in GBlocks using the least stringent setting (Castresana, 2000; Talavera & Castresana, 2007). Phylogenetic

inferences from alignments were estimated under Bayesian posterior probability (BP) and maximum likelihood (ML) analyses. ML was estimated using RAxML 7.2.8 (Stamatakis 2006) with a GTR + G model of nucleotide substitution. Rapid bootstrapping (Stamatakis *et al.*, 2008) was implemented with 1000 replicates. The best scoring ML tree and bootstrap (BS) values ≥ 70% are reported. For Bayesian analysis, a model of substitution and the priors were determined in JModelTest 0.1.1 (Posada, 2008) under the Akaike Information Criterion, and posterior probabilities were estimated using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Two million generations were run in two parallel searches on four chains, and trees sampled every 100 generations. The first 25% of samples in each set were discarded as burnin. Stationarity was evaluated based on the standard deviation of split frequency (less than 0.01) and mixing behavior of the chain was checked in Tracer (Rambaut & Drummond, 2007), to ensure that coverage was adequate. Posterior probability (PP) values > 95% were considered significant. ML and BP were run on XSEDE on the CIPRES web portal (Miller *et al*., 2010). Our ITS datasets included 171 newly generated sequences (supplementary Table S1) and 99 sequences downloaded from GenBank (supplementary Table S2).

Placement of OTUs within a phylogenetic context - After unique OTUs were determined, we examined diversity of mitospore producing *Pezizales* within a phylogenetic context based on domains D1 and D2 of the LSU. The LSU was selected because many representative *Pezizales* sequences are available in GenBank. The LSU has also been well-sampled in previous phylogenetic analyses of the *Pezizales*, providing a backbone of taxa representing known lineages within the order (Hansen & Pfister, 2006; Tedersoo *et al.*, 2006a; Perry *et al.*, 2007). From these previous studies, we chose representative sequences from each major clade to

provide a framework to place our newly generated sequences. The LSU was amplified and sequenced for representative spore mats from each OTU with combinations of primers ITS3, ITS5 or LROR (forward) and LR3, LR5 (Vilgalys & Hester, 1990; White *et al*., 1990) or LR5F (reverse) (Tedersoo *et al*., 2008). Our LSU dataset included 192 sequences: 66 newly generated for this study (supplementary Table S1) and 126 downloaded from GenBank (supplementary Table S2). In addition to taxa used to build the phylogenetic framework, downloaded sequences also included those from EcM root tips and nonmycorrhizal mitosporic *Pezizales*. Due to difficulty in aligning across the order, we aligned sequences in two subsets: subset one with the *Pezizaceae,* and subset two with the *Pezizales* exclusive of the *Pezizaceae*. Subset one had 135 sequences from 72 fruit bodies, 23 EcM roots, and 40 asexual spore mats with 816 basepairs (bp). Subset two had 76 sequences from 61 fruit bodies, 4 EcM roots, and 11 asexual spore mats with 761 bp. The LSU sequences were aligned by hand in SeAl. *Orbilia vinosa* served as the outgroup in phylogenetic analyses for both subsets. Ambiguous region exclusion, selection of model of substitution, and phylogenetic analyses of the LSU dataset were as described for the ITS region except that for BP the data sets were run for 20 million generations.

Culturing Protocol - Intact fruit bodies of *Pachyphloeus* and *Hydnobolites* were surface sterilized by submergence in 10% bleach for 10 minutes, rinsed three times in sterile water, broken open using sterile technique, and interior tissue removed and placed on Modified Melin Norkrans Agar, Malt Extract Agar (1/2 strength), and modified Woody Plant Medium (1/2 strength). These agar media were supplemented with 10 mg/L each of the antibiotics Streptomycin and Chloramphinicol. Direct culturing and dilution plating of asexual spore mats on these same media were carried out in order to germinate the spores and grow these fungi.

Direct culturing entailed sampling of spores and/or mycelia (*Hydnobolites*, *Pachyphloeus*, *Pezizaceae* 2, and *Tuber*) directly and plating with sterile technique either embedded in the media or on the surface. For dilution plating, a small clump of spores was homogenized in an eppendorf tube with 2ml of sterile water and left to sit for 1 hr. Three serial dilutions were made (10^{-3}) and 30 µl was plated and spread evenly with a sterile glass rod. Cultures were maintained in a growth chamber, and examined weekly over the following six months.

EcM root inoculation – *Quercus, Pinus,* and *Populus* species are dominant EcM hosts in Northern hemisphere forests and in many cases asexual spore mats were present near these hosts. Consequently, we chose *Quercus phellos*, *Pinus taeda*, and *Populus deltoides* for our inoculation experiments. One batch of inoculum was made with fresh spores harvested from spore mats the same day, and a second batch of inoculum was made with spores that had been air-dried at room temperature for 3-days. Plant roots were inoculated at Duke University following similar methods used by Bonito *et al.* (2011) for inoculating seedlings with truffle spores. Briefly, a given mass (0.20 – 1.20 g) of spores was mixed into an appropriate volume of double autoclaved soil-less potting mixture composed of vermiculite, perlite, peat, and kaolin clay (4:4:1:1). We used five OTUs from four different lineages, representing the /tuber-helvella, /pachyphloeus-amylascus, hydnotrya, /terfezia-peziza depressa lineages. We included five seedling replicates for each treatment. Spore inoculum level was calculated for a subsample of spores in a hemacytometer, with the addition of 0.1% tween 20 (to reduce spore clumping and surface tension). Spore inoculation densities ranged between 100 million to 1.0 billion spores per plant. Seedlings (oak & pine) and cuttings (poplar) were planted in “cone-tainers” containing a soil volume of ca. 250 ml^2 (Stuewe & Sons, Inc., Tangent, OR, USA). Plants were maintained in the

Duke greenhouses and were watered every 3 days. After 180 days of growth (18 hr days/8 hr nights) plants were harvested and the roots were washed clean. Root tips were then examined under a stereoscope for EcM colonization by pezizalean fungi, characterized by a smooth, thin, brown mantle and lack of rhizomorphs. Observed EcM root tips were collected and the ITS region of rDNA was sequenced.

Measurement of spores and spore mats - Spore mats were photographed *in-situ*. To measure and quantify mitospores, twenty spores from representative spore mats from each lineage were measured in 2.5% KOH and their size ranges and averages determined. Spore densities (spores/area) for representative OTUs of each of the major clades were quantified with a hemacytometer (Propper Manufacturing Co., Long Island City, NY), according to manufacturer instructions, by suspending 2.5 mm^2 cores into 100ml of a 0.1% solution of Tween20. Count averages are reported from three excised plugs per sample of three representative OTUs from the four most speciose clades (/marcelleina-peziza gerardii, /pachyphloeus-amylascus, /terfezia-peziza, and /tuber-helvella). The areas of imaged spore mats were found using Image J64 (Rasband, 2011).

Results

Species determination - A total of 245 spore mats, 83 sporocarps, and 10 EcM root tips from the North America, Europe, South America, and China, were sequenced for this study (Table S1). Sequences of ITS were sorted into 48 OTUs based on 96% similarity (Table 1). Independent phylogenetic analyses based on ITS placed them as follows: the cup fungus *Scabropezia* (1 OTU), the truffle genus *Pachyphloeus* (14 OTUs), close to *Pachyphloeus* or *Scabropezia* sequences, but not matching fruit body sequences (8 OTUs), all within the /pachyphloeus-

amylascus lineage (*Pezizaceae*); the truffle genus *Hydnobolites* (13 OTUs) in the /marcelleina-peziza gerardii lineage (*Pezizaceae*); the truffle genus *Tuber* (3 OTUs) in the /tuber-helvella lineage (*Tuberaceae*); the truffle genus *Fischerula* (1 OTU); the truffle genus *Hydnotrya* (1 OTU) in the /hydnotrya lineage (Discinaceae); a *Ruhlandiella*-like species (1 OTU) in the /terfezia-peziza depressa lineage; *Pezizaceae* taxa within the /terfezia-peziza depressa lineage that could not be placed in any known genus, and are henceforth referred to as *Pezizaceae* 2-1, -2, -3, and -4 (4 OTUs); and *Pezizaceae* taxa that could not be placed in any known lineages and are referred to as *Pezizaceae* 1-1 and -2, and *Pezizaceae* 3 (3 OTUs).

The /pachyphloeus-amylascus lineage (21 OTUs) accounted for 43% of species diversity of sequenced spore mats (Table 1). Among the /pachyphloeus-amylascus OTUs, fifteen spore mat sequences matched fruit bodies, fourteen matched EcM root tip sequences, and thirteen matched both (Fig. 1, Table 1). Four of the 21 /pachyphloeus-amylascus spore mat OTUs matched described species, while 17 represent unknown or undescribed species. The most frequently collected and widely distributed species of the /pachyphloeus-amylascus lineage was *P. thysellii*. Pink-colored spore mats (Fig. 8c) of this species were collected in the USA and China, and also detected on EcM roots or environmental samples from Canada and Europe. *Pachyphloeus citrinus* also has a broad geographic range that includes Europe, Mexico and the USA. Species in the /pachyphloeus-amylascus lineage were associated with several genera of angiosperm host plants (Table 1).

The *Pezizaceae* 1 and *Pezizaceae* 3 OTUs were not highly similar to any fruitbody sequences, and were not included in the ITS analyses because their sequences were too divergent to be aligned.

Twenty-five percent (13) of the OTUs were in the /marcelleina-peziza gerardii lineage, and highly similar to *Hydnobolites* sequences (*Pezizaceae*) (Table 1). *Hydnobolites* (Fig. 8i) is a truffle genus with only two accepted species (*H. californicus* and *H. cerebriformis*) and no previous reports of mitospore production. Sequences from the two described species did not match spore mats whereas five spore mat sequences matched fruit bodies of undescribed *Hydnobolites* species (Smith and Healy, unpublished data), and two matched European orchid mycorrhizae sequences (*Epipactis*, Table 1).

Three OTUs in the /tuber-helvella lineage were allied with the genus *Tuber* (*Tuberaceae*) but could not be assigned to any described species (Table 1). *Tuber* 1 was common and fruited in extensive patches, but did not match sequences from fruit bodies or EcM roots. Phylogenetic analyses placed this OTU close to *T. borchii* and *T. dryophilum*, for which spore mats were previously described (Urban *et al*., 2004). *Tuber* 2 and *Tuber* 3 matched fruit body sequences of undescribed *Tuber* species from MN that are nested within the /maculatum and the /puberulum lineages (Fig. 5) of Bonito *et al.* (2010). *Tuber* 2 matched German *Epipactis* orchid root tips, and *Tuber* 3 matched NA *Quercus* EcM root tip sequences (Table 1, Fig. 3). These results constitute the first report of spore mats in the /maculatum lineage and double the number of species with mitosporic states previously reported in the /puberulum lineage.

A single spore mat of a *Hydnotrya* sp. (/hydnotrya lineage, *Discinaceae*), and a single spore mat of *Fisherula* (/fischerula lineage, family uncertain) were discovered in Fall 2010 and 2011, respectively (Figs. 8l-m). The growth forms of both were similar to that of *Tuber* (Table S4). The /fischerula and /hydnotrya spore mat sequences did not match any fruit body or EcM root tip sequences, and were not included in the ITS analyses. The ITS from a single spore mat of the

truffle genus *Hydnocystis* (*Pyronemataceae*), discovered in Fall of 2011, matched a fruit body from the same woods. However, *Hydnocystis* is not known to be EcM, and so is not included in any further discussion of EcM *Pezizales*.

Two clades with spore mat sequences are in the /terfezia-peziza depressa lineage. One OTU from spore mats collected in Argentina and Chile was shared with a fruitbody of an undescribed *Ruhlandiella*-like species (/terfezia-peziza depressa lineage) collected previously in Chile (Smith & Pfister, unpublished data). Four spore mat OTUs (*Pezizaceae* 2-1 to 2-4) were similar or identical to sequences from EcM roots but not close to any fruit body sequences. The /terfezia-peziza depressa lineage (*Pezizaceae*) includes both truffles (*Terfezia, Mycoclelandia, Tirmania, Cazia, Peziza* in part) and epigeous cup fungi (*Peziza* in part spp.) (Fig. 4). *Pezizaceae* 2-1 and 2-2 are geographically widespread as spore mats in the Eastern USA (Table 1) and have been sequenced from EcM root tips in Europe and Argentina. *Pezizaceae* 2-1 and 2-2 also have a broad host range including woody broadleaf, and *Pinaceae* trees, as well as herbaceous species. The *Pezizaceae* 2 clade of spore mats did not share any well-supported nodes with available fruit body sequences (Fig. 4).

Phylogenetic analysis of LSU - Topologies of strongly supported nodes resulting from ML and BP analyses were similar. Except for the /leucangium clade, there was no major disagreement among strongly supported nodes in our analyses or with previous analyses by Læssøe & Hansen (2007), Perry *et al.* (2007), or Tedersoo *et al.* (2006a). The *Pezizaceae* ML tree is shown in Figs. 5 and 6. The ML tree of *Pezizales* excluding *Pezizaceae* is shown in Fig. 7. The /leucangium lineage identified in Tedersoo *et al.* (2010) included *Fischerula*, based on strong maximum parsimony (MP) bootstrap support in a study by Hansen & Pfister (2006). In agreement with

Tedersoo *et al.* (2010), our analyses (Fig. 7) lacked strong support for a monophyletic relationship between *Fischerula* and *Leucangium*. We refer *Fischerula* taxa to a putatively independent /fischerula lineage.

Here we report mitospore production by five defined EcM fungal lineages and three putative lineages that are yet to be defined. Mitospores from defined EcM lineages include: /pachyphloeus-amylascus (Fig. 5); /marcelleina-peziza gerardii, and /terfezia-peziza depressa, (Fig. 6); /hydnotrya, and /tuber-helvella (Fig. 7). Undefined lineages include /fischerula (Fig. 7), *Pezizaceae* 1 and *Pezizaceae* 3 (Fig. 5). While *Pezizaceae* 1 occurs in a strongly supported clade with EcM root tips, there is no evidence for the trophic status of *Pezizaceae* 3. Since phylogenetic analyses of the LSU places this OTU among EcM clades, we suspect an EcM status for *Pezizaceae* 3, and include it in our analyses. Spore mats were previously unknown in the /marcelleina-peziza gerardii, /hydnotrya, and /fischerula lineages. When these results are compiled with previous reports of mitospore production by EcM *Pezizales* species, (indicated by "+" in Figs 5-7), the LSU analyses suggest that at least nine of the sixteen EcM *Pezizales* lineages identified in Tedersoo *et al.* (2010) and three additional lineages preliminarily identified in this study can produce mitospores: /pachyphloeus-amylascus (Fig. 5), /marcelleina-peziza gerardii, /terfezia-peziza depressa (Fig. 6), /geopora, /hydnotrya, /fischerula, /sphaerosporella, /tarzetta, /tuber-helvella, and /wilcoxina (Fig. 7), *Pezizaceae* 1, and *Pezizaceae* 3 (Fig. 5). An additional 25 saprotrophic or biotrophic species and five pathogenic species that produce mitospores are included in the phylogeny to illuminate potential phylogenetic patterns of mitospore production. Among families with EcM lineages that produce spore mats, 43 of 48

328 OTUs were *Pezizaceae* (Figs. 5-6), three were *Tuberaceae*, one was *Discinaceae,* and one was of
329 uncertain family (Fig. 7).

330 **Biogeography, phenology, habitat, and spore mat size -** Spore mats of pezizalean EcM fungi
331 were diverse and common over a wide geographic area in the Northern Hemisphere, including
332 the Eastern USA (6 lineages, 40 OTUs), Mexico (1 lineage, 3 OTUs), China (3 lineages, 7
333 OTUs), Argentina (2 lineages, 2 OTUs) and Chile (1 lineage, 1 OTU) (supplementary Fig. S1).
334 There was a lag time in production of spore mats in MN compared to NC, by at least one month
335 (supplementary Fig. S2). Spore mat production roughly corresponded to above freezing
336 temperatures and moderate precipitation. Collections during 2011 expanded the fruiting dates
337 from April in NC to Oct. in MN and Dec. in NC (Table S1). Spore mats were not found under
338 drought conditions. At the other extreme, heavy rainfall tended to obliterate the mats, washing
339 away the spores. In general, spore mats were collected on bare soil, rocks or woodland debris on
340 the ground. They were most diverse and abundant in woodlands that included EcM hardwoods,
341 or a mixture of hardwoods and *Pinaceae*. They were not found under *Pinaceae* where heavy duff
342 layers were present (Table 1). The most ubiquitous OTUs were *Pezizaceae* 2-1, *Pezizaceae* 2-2,
343 and *P. thysellii*, found on multiple continents in woodlands protected from human disturbance
344 (although usually on bare soil due to natural disturbance), as well as human-disturbed areas
345 (Table 1, Fig. S1). Spore mats produced between 1.5 x 10^3 and 11 x 10^3 spores/mm^2, depending
346 on the lineage (supplementary Table S3). In general, *Pezizaceae* spore mats were dense with
347 sporogenous hyphae, and determinate in growth, forming cushion-like mounds on the soil (Fig.
348 8a,c,e,g,i,j), while /fischerula, *Discinaceae*, and *Tuberaceae* spore mats were single to sparsely-

layered, and grew indeterminately, and effusely in a dendroid pattern over the surfaces of soil, leaves, rocks, and twigs (Fig. 8k,m; Table S3).

Culturing of asexual spores and EcM root inoculation – Attempts to culture the mitospores from spore mats were unsuccessful, producing only bacteria, non-target fungi, or no growth. Ectomycorrhizae failed to establish from mitospore inoculation with any OTU.

Contaminating fungi - Multiple genera of spore mats from MN, NC, and Mexico collected during humid weather were contaminated by one of three species in a complex around *Paecilomyces penicillatus* (*Hypocreales*) (supplementary Table S4). These were not included in analyses of anamorph-producing EcM *Pezizales*.

Discussion

Contrary to previous suggestions that EcM fungi generally do not produce mitospores, our data demonstrate that a majority (nine) of the 16 EcM *Pezizales* lineages defined by Tedersoo *et al*. (2010), plus three putative lineages identified here, produce mitospores. We show that the production of spore mats is widespread geographically, includes a high diversity of cup fungi (including a preponderance of truffles), and includes known EcM lineages for which sporocarp records are lacking. Collections from Eastern USA, Mexico, China, and South America, along with previous reports from Europe indicate that mitospore-producing EcM *Pezizales* occur with EcM angiosperms in temperate zones on at least four continents, and in both hemispheres.

Our analyses suggest that mitospores are a common feature among *Pezizales* in general, regardless of lifestyle. The *Orbiliales*, which have many mitosporic species, are inferred as basal

to the *Pezizales* (James *et al*., 2006; Kumar *et al*., 2012), implying that the production of mitospores in the *Pezizales* is a plesiomorphic condition.

By including sequences derived from spore mats and EcM root tips in phylogenetic analyses we were able to improve resolution of fine scale phylogenies in /marcelleina-peziza gerardii, /pachyphloeus-amylascus, and /terfezia-peziza; and to match life cycle stages (i.e. ectomycorrhizae, fruit bodies, and mitosporic forms) in taxa of /marcelleina-peziza gerardii, /pachyphloeus-amylascus, /terfezia-peziza depressa, and /tuber-helvella. Spore mat data contributed to geographic distribution and habitat profiles for specific taxa, and also revealed a greater diversity of cryptic truffle-like species than was previously known in *Hydnobolites* (16 undescribed species), *Fischerula* (one undescribed species), *Hydnotrya* (one undescribed species), a *Ruhlandiella*-like taxon (one undescribed species), and species in the truffle-cup fungus lineage of /pachyphloeus-amylascus (21 undescribed species). Truffles are produced belowground, so they can be difficult to find, but spore mats are readily visible on the soil surface. Unlike fruit bodies, mitospores are apparently produced over a full season, given adequate moisture, thereby increasing their chances of detection. Among pezizalean families, the large, brightly colored *Pezizaceae* spore mats are the most obvious, which may be why they were the most commonly collected in this study (43 out of 48 OTUs). Spore mats of /tuber (*Tuberaceae*, 3 OTUs), /hydnotrya (*Discinaceae*, 1 OTU), and /fischerula (1 OTU) are less noticeable, and collected infrequently. Since our survey turned up such high diversity while being carried out over a relatively short time, it is possible that there are other lineages, (particularly in Europe, Asia, and in the Southern Hemisphere), that produce spore mats that

were either not encountered during this study, were not in the geographic areas we searched, or were overlooked.

Asexual spore mats allowed us to detect cryptic diversity in several well-known ECM lineages but also revealed a geographically widespread clade within the /terfezia-peziza depressa lineage that was previously known only from a single spore mat and numerous EcM root tips. Although the terfezia-peziza depressa lineage includes both truffles and cup fungi, our analyses gave no strong support for a sister lineage to the *Pezizaceae* 2 clade and therefore a putative fruiting body form cannot be predicted for these species. *Pezizaceae* 1 and *Pezizaceae* 3 cannot be confidently placed in any known lineages, and so a fruiting body form cannot be predicted for these OTUs either.

The function(s) of the EcM spore mats collected during this study remains unknown. One working hypothesis is that spore mats are an ecologically adaptive mechanism for contacting and colonizing new flushes of fine roots. It is known that pezizalean fungi are adapted to disturbed, or edge habitats (Petersen, 1985; Egger, 1986). One possible advantage of mitospore production is the ability to reproduce quickly following rainfall. If the soil with extramatrical mycelium is bare, the mycelium in upper soil horizons would have a greater chance of capturing incident rain water necessary for mitospore production. High numbers of mitotic propagules could serve as a quick means for colonizing roots, an idea that is compatible with the ruderal strategy previously hypothesized for *Pachyphloeus* (Dickie & Reich, 2005; Tedersoo *et al*., 2006a). Woodlands that experience litter-clearing disturbances, such as fire, may provide similar conditions favorable for EcM fungi that produce spore mats.

411 Testing of such hypotheses should be possible for EcM *Pyronemataceae*. Mitospores from
412 *Tricharina hiemalis* and *Wilcoxina mikolae* germinated and produced fruit bodies in culture
413 (Yang & Korf 1985a, 1985b). Only polyspore isolates produced fertile fruit bodies of *W. mikolae*
414 (Yang & Korf, 1985a), consistent with heterothallism (obligate outcrossing). Two conidia of
415 *Tarzetta* germinated in culture after heat shock, but only one, an unusually large mitospore,
416 developed into normal mycelium (Dodge, 1937). These reports suggest that mitospores in the
417 *Pyronemataceae* may serve as propagules in some cases, but may be involved as spermatia in
418 other cases. It should be noted that the mitospores of *Tricharina* and *Wilcoxina* are intercalary in
419 the filaments, and these species do not form obvious spore mats. We did not find any EcM
420 *Pyronemataceae* spore mats in our surveys.
421 *Muciturbo reticulatus* is apparently the only EcM *Pezizaceae* species reported to produce
422 mitospores in culture, although the spores did not germinate (Warcup & Talbot, 1989). Attempts
423 to germinate mitospores of other EcM *Pezizales* have likewise been unsuccessful (Table S5). To
424 understand the role of mitospores in EcM *Pezizales*, it may be useful to ascertain the role of
425 mitospores in close relatives that are saprobic or plant-pathogenic. Mitospores of at least thirteen
426 *Pezizaceae* species have been produced in culture, mitospores of five of these germinated (Table
427 S5), and *Cleistoiodophanus* formed fruit bodies in culture. Although mitotic spores were
428 produced abundantly in the same culture as fruiting bodies were formed, there was no male
429 structure observed in the formation of fertile tissue (Bezerra & Kimbrough, 1976). Since the
430 mitospores could germinate, and eventually give rise to fruiting bodies, they could act as
431 dispersal units. The lack of observation of a male structure participating in the formation of
432 fertile tissue does not preclude its participation in a less obvious manner. Thus, as in the

433 *Pyronemataceae,* there are at least two possible roles that mitospores may play in
434 *Cleistoiodophanus*. The requirements to axenically manipulate mitospores of most other
435 *Pezizaceae* are elusive (see Table S5 for unsuccessful attempts). The failure to germinate EcM
436 *Pezizales* mitospores in culture in previous studies and in our study, and the failure to form
437 mycorrhizae in the presence of fine roots suggests an alternative function to a propagative unit. A
438 hypothesis posed by Urban *et al*. (2004, for *Tuberaceae* spore mats) is that these spores serve as
439 spermatia, necessary for fertilization in sexual reproduction.
440 Only recently was it verified with molecular evidence that *Tuber* species outcross, but how
441 this occurs is still a mystery (Riccioni *et al*., 2008). It is possible that for heterothallic species,
442 establishment of the dikaryotic phase in truffles such as *Tuber* may be impeded by subterranean
443 location. We propose that mitospores produced on the soil surface, and subsequently carried by
444 rainwater, arthropods or other animals to EcM hyphae in the soil, facilitate the coming together
445 of compatible nuclei. A function of spermatia for outcrossing, has been suggested for mitospores
446 in other ascomycetes (Kohn, 1993).
447 Either function, to provide for genetic exchange or to disperse propagules to infect new root
448 tips, may help to explain why spore mats were rarely found in *Pinaceae* forests, and then only on
449 bare soil. A thick duff layer may prevent the dissemination of nuclear donors or propagules or
450 perhaps prevent spore mat formation all together.
451 Morphologies of most *Pezizaceae* spore mats reported here fit previously described
452 mitosporic forms (reviewed in Hennebert, 1973). Mitosporic forms were previously classified as
453 form genera, thus the saprobic cup fungus *Peziza ostracoderma* has a mitosporic state that was
454 named *Chromelosporium fulvum* (Hennebert & Korf, 1975). Woodland terricolous species

455 described in Hennebert (1973) are morphologically similar to some of the mitosporic forms
456 sequenced here. Spore mats of both the /terfezia-peziza depressa and /pachyphloeus-amylascus
457 lineages have previously been classified under *Chromelosporium* (Palmer *et al.,* 2008).
458 *Glischroderma*, another form genus, has also been tied to *Pachyphloeus* (Norman & Egger,
459 1999). *Glischroderma* spore mats were described as having a covering (Malençon, 1964), which
460 was not detected on *Pachyphloeus* spore mats in this study, although the long hyphal projections
461 can sometimes cause the spore mat to appear covered when the projections are matted down.
462 Although the role(s) of mitospores of EcM *Pezizales* was not fully established in this study,
463 the discovery of spore mats for *Pachyphloeus* and *Tuber*, and for four additional hypogeous
464 lineages (/hydnobolites, /hydnotrya, /fischerula, and a *Ruhlandiella*-like taxon in /terfezia-peziza
465 depressa) signals that the lifecycle of these truffles is more complex than previously known. The
466 high diversity and broad geographic distribution of EcM *Pezizales* that produce spore mats
467 suggests that production of mitospores is more important in the life history of this ecological
468 guild of fungi than has previously been appreciated.

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References

Ashkannejhad S, Horton T (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist,* 169, 345-354.

Baxter JW, Dighton J (2001) Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host-symbiont culture conditions. *New Phytologist,* 152, 139-149.

Bezerra JL, Kimbrough JW (1976) Structure and development of *Cleistoiodophanus conglutinatus* gen. & sp. N. (*Ascobolaceae*). *American Journal of Botany,* 63, 838-844.

Bonito G (2009) Fast DNA- based identification of the black truffle *Tuber melanosporum* with

direct PCR and species–specific primers. *FEMS Microbiology Letters.* 301, 171–175.

Bonito G, Brenneman T, Vilgalys R (2011) Ectomycorrhizal fungal diversity in orchards of cultivated pecan (*Carya illinoinensis*; *Juglandaceae*). *Mycorrhiza,* 21, 601-612.

Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology,* 19, 4994-5008.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution,* 17, 540-552.

Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology,* 93, 244-255.

Dodge, C. (1937) The conidial stage of *Peziza pustulata*. *Mycologia,* 24,651-655.

Fujimura K, Smith J, Horton T, Weber N (2005) Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*).after prescribed fires in eastern Oregon, USA. *Mycorrhiza,* 15,79-86.

García-Montero LG, Díaz P, Martín-Fernández S, Casermeiro MA (2008) Soil factors that favour the production of *Tuber melanosporum* carpophores over other truffle species: a multivariate statistical approach. *Acta Agriculturae Scandinavica* Section B *Soil and Plant Science,* 58, 322-329.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology,* 2,113-118.

Gehring C, Theimer T, Whitham T, Keim P (1998) Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. *Ecology,* 79, 1562-1572.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology,* 395,202-222.

Guevara G, Bonito G, Cazares E *et al.* (2012) New North American truffles (*Tuber* spp.) and their ectomycorrhizal associations. *Mycologia* in press.

Hansen K, Pfister D (2006) Systematics of the Pezizomycetes—the operculate discomycetes. *Mycologia,* 98,1029-1040.

Hennebert GL (1973) *Botrytis* and *Botrytis*-like genera. *Persoonia,* 7,183-204.

Hennebert GL, Korf RP (1975) The peat mould, *Chromelosporium ollare*, conidial state of *Peziza ostracoderma*, and its misapplied names, *Botrytis crystallina*, *Botrytis spectabilis, Ostracoderma epigaeum* and *Peziza atrovinosa*. *Mycologia* 67, 214-240.

Huelsenbeck JP, Ronquist FR (2001) Mr. Bayes: Baysian inference of phylogenetic trees. *Biometrics,* 17, 754-755.

Hutchison L (1989) Absence of conidia as a morphological character in ectomycorrhizal fungi. *Mycologia,* 81, 587-594.

Iotti M, Lancellotti E, Hall I, Zambonelli A (2010) The ectomycorrhizal community in natural *Tuber borchii* grounds. *FEMS Microbiology Ecology,* 72, 153-310.

James TY, Kauff F, Schoch CL *et al*. (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature,* 443, 818–822.

Katoh K, Toh H (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics,* 26,1899-1900.

Kohn LM (1993) What do we need to know about discomycetous anamorphs? In Reynolds, DR, Taylor, JW (eds). *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*. CAB International, Wallingford, pp 129-139.

Kumar TKA, Healy R, Spatafora JW *et al*. (2012) *Orbilia* ultrastructure, character evolution, and phylogeny of *Pezizomycotina*. *Mycologia,* 104, 462-476.

Læssøe T, Hansen K (2007) Truffle trouble: what happened to the *Tuberales*? *Mycological Research,* 111, 1075-1099.

Malençon G (1964) Le *Glischroderma cinctum* Fuck., sa structure et ses affinitiés. *Bulletin Trimestriel de la Societe Mycologique de France*, 80,197-211.

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).* 14 Nov 2010, New Orleans, pp 1-8.

Moncalvo J M, Vilgalys R, Redhead SA, *et al*. (2002) One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution,* 23,357-400.

Morris MH, Pérez-Pérez MA, Smith ME, Bledsoe CS (2009) Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks (*Quercus* spp.) in a tropical cloud forest. *Fems Microbiology Ecology,* 69, 274-287.

Nara K (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist,* 169, 169-178.

Nilsson RH, Kristiansson E, Ryberg M, Larsson K-H (2005) Approaching the taxonomic affiliation of unidentified sequences in public databases - an examle from the mycorrhizal fungi. *BMC Bioinformatics,* 6, 178.

Nobles MK (1958) Cultural Characters as a guide to the Taxonomy and Phylogeny of the *Polyporaceae. Canadian Journal of Botany,* 36, 883–926.

Norman JE, Egger KN (1999) Phylogenetic analysis of *Peziza* and related genera. *Mycologia,* 91, 820-829.

Palmer JM, Lindner DL, Volk TJ (2008) Ectomycorrhizal characterization of an American chestnut (*Castanea dentata*)-dominated community in western Wisconsin. *Mycorrhiza,* 19, 27-36.

Perry BA, Hansen K, Pfister DH (2007) A phylogenetic overview of the family *Pyronemataceae* (*Ascomycota, Pezizales*).*Mycological Research,* 111, 549-571.

Petersen PM (1985) The ecology of Danish soil inhabiting *Pezizales* with emphasis on edaphic conditions. *Opera Bot,* 77,1-38.

Posada D (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution,* 25, 1253-1256.

Rambaut A (2007) Se-Al: Sequence Alignment Editor, http://tree.bio.ed.ac.uk/software/seal/.

Rambaut A, Drummond AJ (2007) Tracer v1.4: http://beast.bio.ed.ac.uk/Tracer

Rasband, WS (2011) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/

Riccioni C, Belfiori B, Rubini A, *et al*. (2008) *Tuber melanosporum* outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. *New Phytologist,* 180, 466-478.

583 Ryberg M, Kristiansson E, Sjökvist E, Nilsson RH (2009) An outlook on the fungal internal
584 transcribed spacer sequences in GenBank and the introduction of a web-based tool for the
585 exploration of fungal diversity. *New Phytologist,* 181, 471-477.
586 Schoch CL, Seifert KA, Huhndorf S *et al*. Fungal Barcoding Consortium (2012) Nuclear
587 ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker
588 for Fungi. *Proceedings of the National Academy of Science,* 109, 6241-6246.
589 Shenoy BD, Jeewon R, Hyde KD (2007) Impact of DNA sequence-data on the taxonomy of
590 anamorphic fungi. *Fungal Diversity,* 26,1-54.
591 Smith M, Douhan G, Rizzo D (2007a) Intra-specific and intra-sporocarp ITS variation of
592 ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled
593 ectomycorrhizal roots from a *Quercus* woodland. *Mycorrhiza,* 18,15-22.
594 Smith M, Douhan GW, Rizzo DM, (2007b) Ectomycorrhizal community structure in a xeric
595 *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots.
596 *New Phytologist,* 174, 847-863.
597 Stamatakis A, (2006) RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with
598 thousands of taxa and mixed models. *Bioinformatics,* 22,2688–2690.
599 Stamatakis A, Hoover P, Rougemont J, (2008) A rapid bootstrap algorithm for the RAxML web
600 servers. *Systematic Biology,* 57,758–771.
601 Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and
602 ambiguously aligned blocks from protein sequence alignments. *Systematic Biology,* 56,
603 564-577.
604 Taylor JW, Jacobson DJ, Fisher MC (1999) The evolution of asexual fungi: reproduction,

speciation and classification. *Annual Review of Phytopathology,* 37, 197-246.

Tedersoo L, Hansen K, Perry B, Kjøller R (2006a) Molecular and morphological diversity of pezizalean ectomycorrhiza. *New Phytologist*, 170, 581-596.

Tedersoo L, Jairus T, Horton BM *et al.* (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist,* 180, 479-490.

Tedersoo L, May T, Smith M (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza,* 20, 217-263.

Tedersoo L, Suvi T, Larsson E, Koljalg U (2006b) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research,* 110, 734-748.

Urban A, Neuner-Plattner I, Krisai-Greilhuber I, Haselwandter K (2004) Molecular studies on terricolous microfungi reveal novel anamorphs of two *Tuber* species. *Mycological Research,* 108, 749-758.

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.

Walker J, Miller O, Horton J (2005) Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. *Molecular Ecology,* 14, 829-838.

Walther G, Garnica S, Weiss M (2005) The systematic relevance of conidiogenesis modes in the gilled *Agaricales*. *Mycological Research,* 109, 525-544.

Warcup JH, Talbot PHB (1989) *Muciturbo*:a new genus of hypogeous ectomycorrhizal Ascomycetes. *Mycological Research,* 92, 95-100.

Warren JM, Brooks JR, Meinzer FC, Eberhart JL (2008) Hydraulic redistribution of water from *Pinus ponderosa* trees to seedlings: evidence for an ectomycorrhizal pathway. *New Phytologist,* 178, 382-394.

White TM, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp 315-321. Academic Press, San Diego.

Yang CS, Korf RP (1985a). A monograph of the genus *Tricharina* and of a new, segregate genus, *Wilcoxina* (*Pezizales*). *Mycotaxon,* 24, 467-531.

Yang CS, Korf RP (1985b) *Ascorhizoctonia* gen. nov. and *Complexipes* emend., two genera for anamorphs of species assigned to *Tricharina* (discomycetes). *Mycotaxon,* 23, 457-481.

Data Accessibility

-DNA sequences: GenBank accessions JN102363 - JN102492, JN409337- JN409345, JN121300 JN121377, JF419257, JX414173-JX414224.

-Phylogenetic data: Five alignments to be deposited in Dryad.

Author Contributions

RH carried out all work (except for EcM synthesis and sequencing) that was done in the Midwest US with field and lab assistance from LK and TL, and wrote the paper with MES and GB. MES and GB carried out all the work that was done in the SE US with field and lab assistance from

647 KS. GB did the culture work on truffles and anamorphs and synthesized ectomycorrhizae. GW
648 did the root sampling and sequencing of Mexican oak ectomycorrhizae. Diversity of mitospore
649 producing EcM *Pezizales* in countries outside the US was enabled by the following: MES and
650 DHP in Chile and Argentina; MES and ZWG in China; GGG in Mexico; CH in England. DHP
651 shared his considerable knowledge of *Pezizales* anamorphs, JT shared world-wide collections of
652 truffles, RV hosted DNA extraction and sequencing by RH, MES and GB. DJM supervised RAH
653 and hosted DNA extraction and sequencing.

654 **Supporting Information**

655 Table S2 Downloaded sequences used in phylogenetic anaylses in this study. Table S3
656 Morphological comparisons of asexual spore mats in six lineages of ectomycorrhizal *Pezizales*.
657 Table S4 Spore mat contaminant OTUs, based on 96% similarity of ITS sequenced from spore
658 mats of diverse EcM *Pezizales* lineages. Table S5 Reports on *Pezizales* that have produced
659 mitospores under axenic conditions; and results of attempts to germinate mitospores, and
660 to produce fruiting bodies from mito- or meiospores. Fig. 1 Geographic distribution of OTUs
661 of EcM pezizalean spore mats and fruit bodies collected in the Eastern USA, Northeastern
662 Mexico, and Southeastern China. Fig. 2 Monthly spore mat diversity as measured by number of
663 OTUs, juxtaposed with monthly precipitation (in inches) in North Carolina and Minnesota in
664 2011.

665 **Figure Legends**

666 **Figs. 1-7** Best ML trees calculated with 1000 boostrap replicates. All ML analyses were based
667 on the GTR+G model of nucleotide substitution. Support values on branches indicated on the left

668 side for MB posterior probablilities > 95%, and on the right side for ML bootstrap proportions ≥
669 70%. 100% support indicated by "*". Sequences derived from fruit bodies are italicized, spore
670 mats are bolded, and ectomycorrhizal or *Epipactis* orchid mycorrhizal root tips are preceded by
671 "EcM", or "EpM" respectively. Sequences from previously reported asexual spore mats are
672 indicated by "+". Countries of origin, in parentheses, are abbreviated as follows: AR (Argentina),
673 AT (Austria), AU (Australia), CA (Canada), CH (Chile), CI (Canary Islands), CN (China), DK
674 (Denmark), DR (Dominican Republic), EE (Estonia), FR (France), GL (Greenland), GR
675 (Germany), HU (Hungary), IL (Israel), IT (Italy), LY (Libya), JP (Japan), KW (Kuwait), MX
676 (Mexico), NO (Norway), NZ (New Zealand), PG (Papua New Guinea), PL (Poland), PR (Puerto
677 Rico), PT (Portugal), SAf (South Africa), SP (Spain), UK (United Kingdom), US (United
678 States).

679 **Fig. 1** Best ML (-ln 6351.238547) phylogram of 102 taxa, 579 bp of the ITS rDNA in the
680 /pachyphloeus-amylascus lineage rooted with *Amylascus*. Model of evolution selected for
681 Bayesian analysis was TVM + I + G. Numbers to right of phylograms refer to OTUs listed in
682 Table 1.

683 **Fig. 2** Best ML (-ln -4486.473) phylogram of 43 taxa, 625 bp of the ITS rDNA in the
684 *Hydnobolites* clade of the /marcelliena-peziza gerardii lineage. Model of evolution selected for
685 Bayesian analysis was HKY + I + G. Numbers to right of phylograms refer to OTUs listed in
686 Table 1.

687 **Fig. 3** Best ML (-ln 3127.582) phylogram of 46 taxa, 727 bp of ITS rDNA in the *Tuber* clade in
688 the /tuber-helvella lineage. Model of evolution selected for Bayesian analysis was TIM2 + I + G.
689 Numbers to right of phylograms refer to OTUs listed in Table 1.

690 **Fig. 4** Best ML (-ln 4808.387664) phylogram of 54 taxa, 572 bp of the ITS rDNA in the
691 /terfezia-peziza lineage. GTR + G selected as model of evolution for Bayesian analysis.
692 Phylogram includes sequences from *Peziza* collected in the vicinity of spore mats during this
693 study.

694 **Figs. 5 - 6** The best ML phylogram from 135 taxa, 816 bp of the LSU rDNA from *Pezizaceae* (–
695 lnL=10873.195389). Model of evolution selected for Bayesian analysis was TIM3ef + G (Fig. 5
696 *Pezizaceae* part 1, Fig. 6 *Pezizaceae* part 2). The outgroup was *Orbilia vinosa*.

697 **Fig. 7** The best ML phylogram from 77 taxa, 884 bp of the *Pezizales* exclusive of *Pezizaceae* (-
698 lnL=12207.201668). Model of evolution selected for Bayesian analysis was GTR + I + G. The
699 outgroup was *Orbilia vinosa*. Taxa where asexual forms are known are in bold type, and their
700 lineages indicated at their phylogram nodes. Taxa where asexual states were reported in previous
701 studies are indicated by "+". "?" indicates discrepancy in the literature regarding mitospore
702 production. Sporocarp forms from which sequences were derived are indicated by filled circles
703 for hypogeous (truffle) fruit bodies and open circles for above ground fruit bodies. The trophic
704 status for each taxon, as designated by shade in the key at the top left, is displayed on the bar to
705 the right of the phylogram.

706 **Fig. 8a-h** Spore mats and corresponding fruit bodies of representative OTUs of EcM *Pezizales*.
707 **8a** Spore mat of /pachyphloeus-amylascus 21 (RHAM15), bar = 0.5 cm. **8b** *Pachyphloeus* fruit

708 body of /pachyphloeus-amylascus 21 (MX32624), bar = 1 cm. **8c** Spore mat of *P. thysellii*
709 (RHAM116), bar = 0.5 cm. **8d** Fruit body of *P. thysellii* (RH1180), bar = 1 cm **8e** Spore mat of
710 /pachyphloeus-amylascus 22 (RHAM126), bar = 1 cm**. 8f** *Pachyphloeus* fruit body of
711 /pachyphloeus-amylascus 22 (RH735), bar = 1 cm. **8g** Spore mat of /pachyphloeus-amylascus 4
712 (RHAM102), bar = 1 cm. **8h** *Scabropezia flavovirens* (RH1209), bar = 1 cm. **8i** Spore mats of
713 *Hydnobolites* 12 (RHAM483) with fruit body of matching ITS sequence (RH1358), bar = 0.5
714 cm. **8j** Spore mat of *Tuber* sp. 3 (RHAM226), bar = 1 cm. **8k** Fruit body of *Tuber* sp. 3
715 (RH1279), bar = 1 cm. **8l** Spore mat of /terfezia-peziza depressa 2-1 (RHAM371), bar = 1 cm.
716 **8m** Spore mat of *Fischerula* (RHAM489). **8n** Close up image of 8L taken through a dissecting
717 microscope, bar = 1 mm.

718 **Table 1** Asexual spore mats, fruit bodies and ectomycorrhizal root tip matches based on ≥ 96% similarity in ITS region of
719 nuclear ribosomal DNA
720

Lineage/ OTU	Rep. seq.	Seq Nos[a].	Habitat[b]	Geographic range of sequence source[c] and EcM hosts[d]			
				spore mat	fruitbody	EcM	host
/fischerula	JX414173	1/0/0	A	US			
/hydnotrya	JN102492	1/0/0	A	US			
/marcelleina-peziza gerardii 1	JN102392	1/0/0	P	US			
/marcelleina-peziza gerardii 2	JN102436	2/0/0	M	CN			
/marcelleina-peziza gerardii 3	JN102390	4/2/0	A, M, P	US	US		
/marcelleina-peziza gerardii 4	JN102425	1/0/0	M	US			
/marcelleina-peziza gerardii 5	JN102440	3/1/0	M	CN	CN		
/marcelleina-peziza gerardii 6	JN102384	1/3/0	A	US	US		
/marcelleina-peziza gerardii 7	JN102388	2/0/0	A	US			
/marcelleina-peziza gerardii 8	JN102372	1/0/0	A	US			
/marcelleina-peziza gerardii 9	JN102394	1/0/0	A	US			
/marcelleina-peziza gerardii 10	JN102377	4/0/0	A	US			
/marcelleina-peziza gerardii 11	JN102393	6/1/0	A, S	US	MX		
/marcelleina-peziza gerardii 12	JX414187	2/5/0	A	US	US		
/marcelleina-peziza gerardii 13	JX414188	1/0/2	A	US		G, IT	EP
Pachyphloeus citrinus	JN102363	8/9/1	A, D	MX, US	IT, MX, UK, US	G	CP, FG, TL
Pachyphloeus marroninus	JN102364	5/4/2	A, S	US	MX, US	MX	QC
Pachyphloeus thysellii	JN102370	24/7/4	All	CN, US	US	CA (env), CN, EE	Al, QC
/pachyphloeus-amylascus 5	JN102389	2/0/0	A	US			

/pachyphloeus-amylascus 6	JN102414	1/0/0	A, M	US			
/pachyphloeus-amylascus 7	JN102432	1/0/0	D	US			
/pachyphloeus-amylascus 8	JN102431	1/1/2	M	US	US	MX, US	QC
/pachyphloeus-amylascus 9	JN102430	6/3/3	M	MX, US	SP, UK	DK, EE, IT	FG
/pachyphloeus-amylascus 10	JN102368	6/0/0	M	US			
/pachyphloeus-amylascus 11	JN102439	1/0/1	A, M, S	CN		MX	QC
/pachyphloeus-amylascus 13	JN102395	3/4/2	D	US	US	US	QC
/pachyphloeus-amylascus 14	JN102435	1/0/1	A, D, S	CN			
/pachyphloeus-amylascus 15	JN102367	5/1/0	M	US	US		
/pachyphloeus-amylascus 16	JN102433	11/0/2	S	US		US	
/pachyphloeus-amylascus 17	JN102421	11/16/2	M	MX, US	MX, US	MX	QC
/pachyphloeus-amylascus 18	JN102404	6/1/0	A	US			
/pachyphloeus-amylascus 20	JN102409	11/16/0	A	MX, US	MX, US		
/pachyphloeus-amylascus 21	JN102380	5/14/2	A, D, M	US	MX, US		
/pachyphloeus-amylascus 22	JN102375	13/4/1	A, D	US	US	US	
/pachyphloeus-amylascus 23	JN102434	1/4/1	A, M	CN	EU	JP	CP
Pezizaceae 1-1	JN102379	1/0/0	A	US		US (env)	
Pezizaceae 1-2	JN102406	2/0/0	M	US		US (env)	
Pezizaceae 2-1	JN102366	49/0/10	M	US		EE, G, NZ, PL, US	LX, PN, QC, SX
Pezizaceae 2-2	JN102422	33/0/5	A, D, M, S	US		G, PL, US	BT, PN, HM, QC
Pezizaceae 2-3	JN102438	5/0/0	A, D, M, S	CN			

Pezizaceae 2-4	JN102426	2/0/1	M	US		US (env)	
Pezizaceae 3	JX414201	3/0/0	A	AR			
Ruhlandiella sp. nov.	JX415205	16/1/0	A	AR, CH			
Scabropezia flavovirens	JN102402	4/3/1	A	US		EE	Al
Scabropezia sp.	JN121319	3/0/0	A	US	FR, US		
/tuber helvella 1	JN102420	22/0/0	A	US			
/tuber helvella 2	JN102385	1/2/1	A, M	US	US	G	EP
/tuber helvella 3	JN102387	4/5/3	A	US	US	G, MX, US	EP, CY, QC

a Sequence sources for OTU are listed in the order: asexual spore mat/ fruit body/ ecomycorrhizal root tip.

b Habitats are listed for asexual spore mat collections only. Abbreviations: A (angiosperm dominated woods); D (disturbed angiosperm wooded lot such as campus lawn, and picnic ground in park); M (mixed *Pinaceae* and angiosperm); P (*Pinaceae* woods); S (oak savanna).

c Countries: AR (Argentina), CA (Canada), CH (Chili), CN (China), DK (Denmark), EE (Estonia), FR (France), GR (Germany), IT (Italy), JP (Japan), MX (Mexico), NZ (New Zealand), PL (Poland), SP (Spain), UK (United Kingdom),.

d Hosts: Al (*Alnus*), BT (*Betula*), CP (*Carpinus*), CY (*Carya*), EP (*Epipactis*), FG (*Fagus*), HM (*Helianthemum*), LX (*Larix*), PN (*Pinus*), QC (*Quercus*), SX (*Salix*), TL (*Tilia*).

721

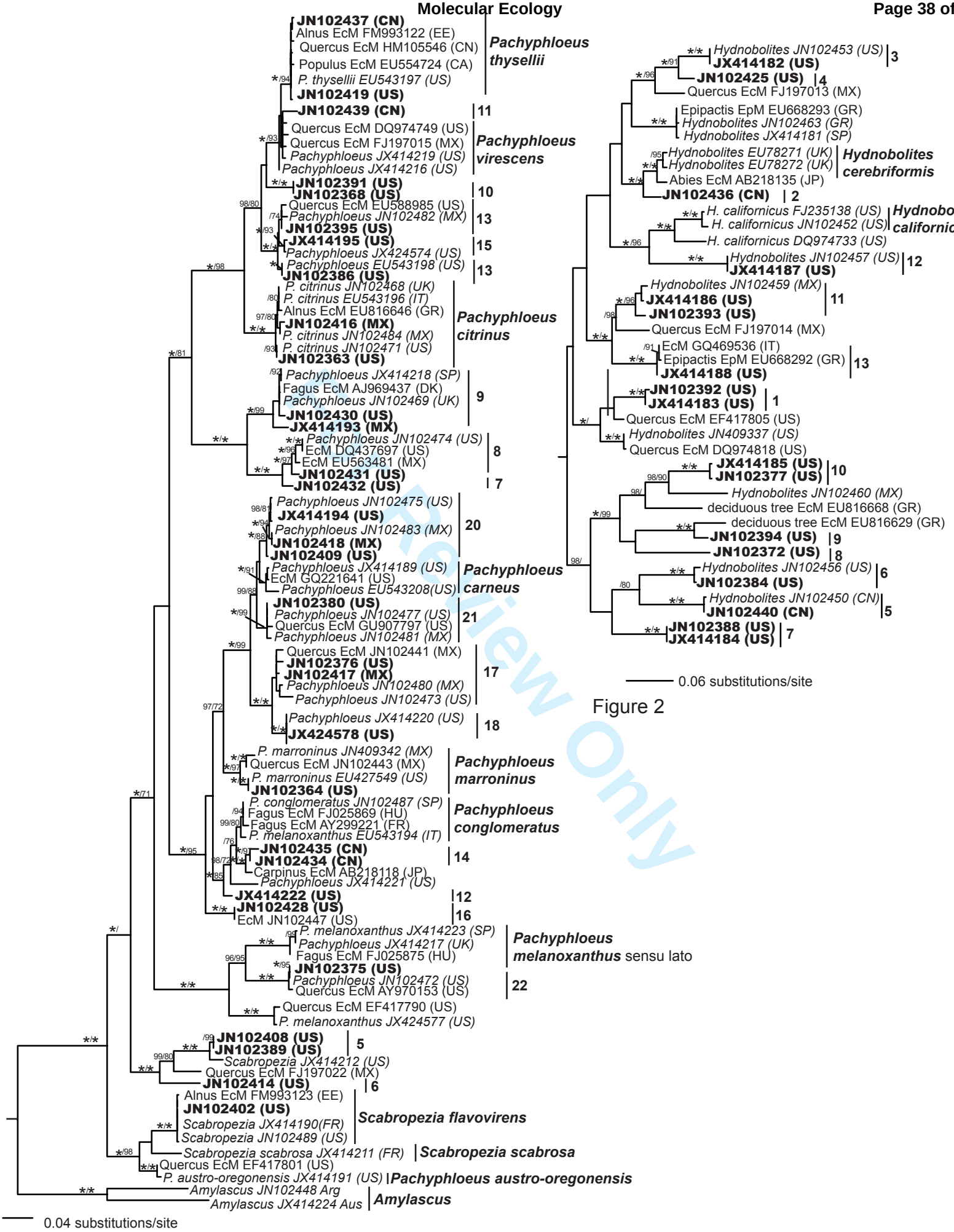


Figure 1

Figure 2

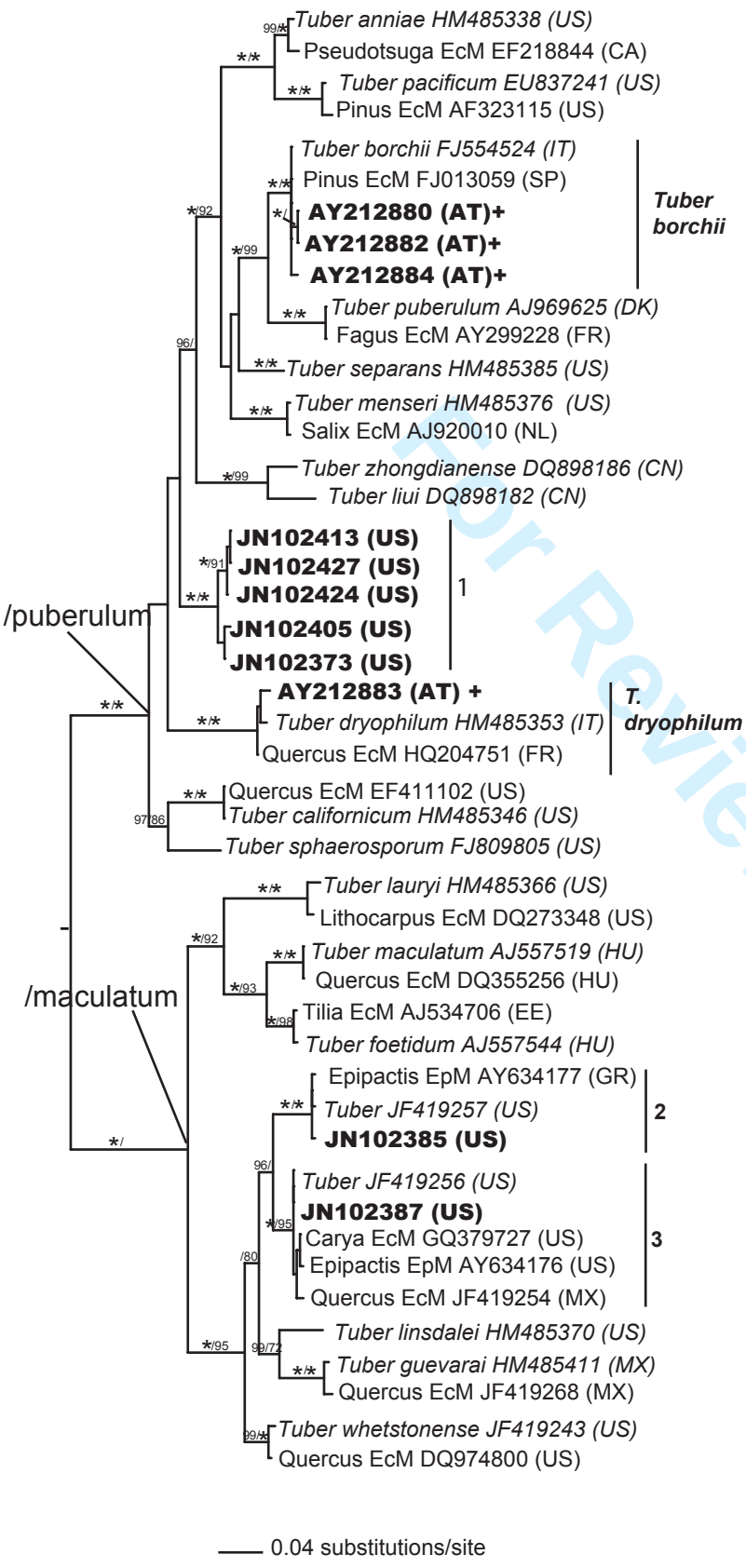
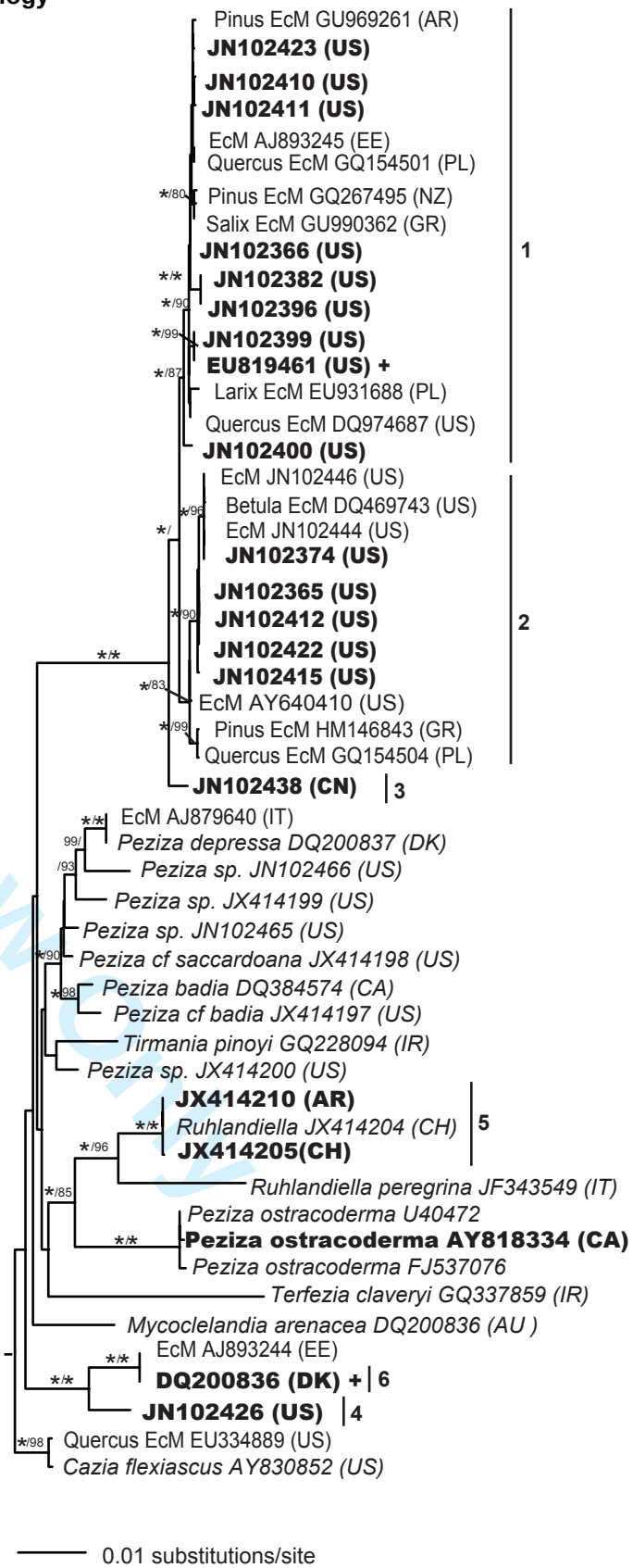


Figure 3



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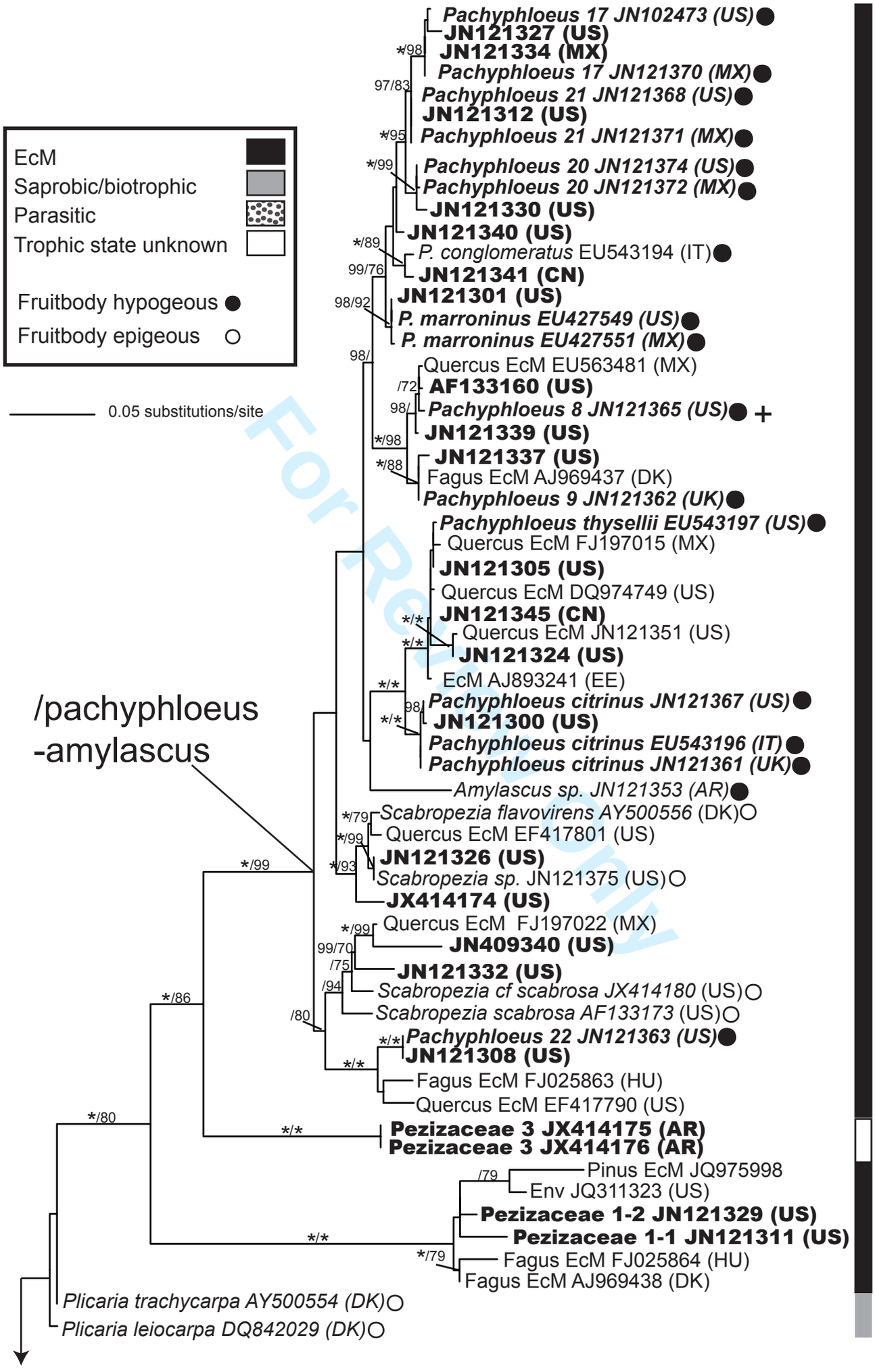

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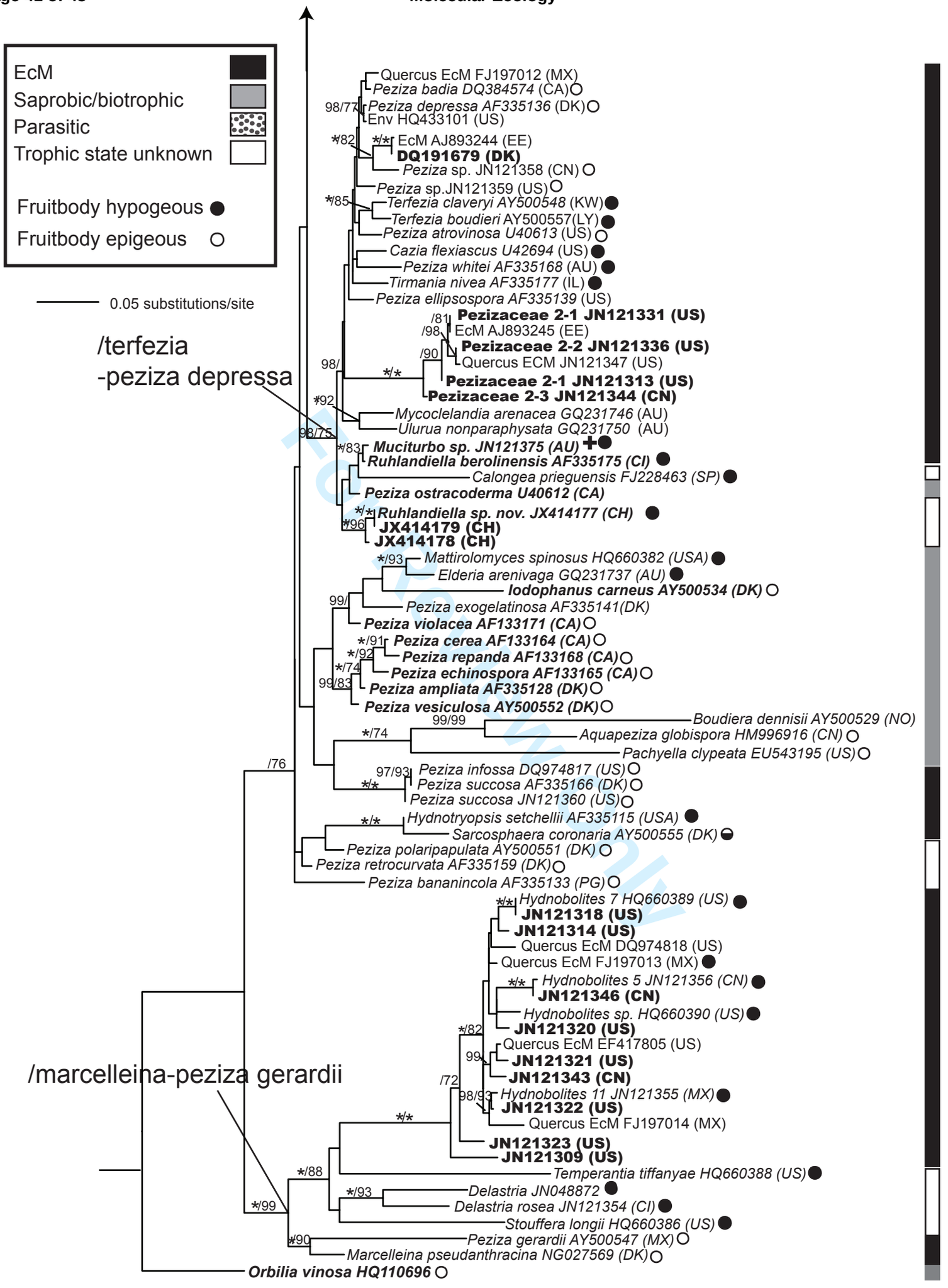
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Saprobic/biotrophic
Parasitic
Trophic state unknown
Fruitbody hypogeous
Fruitbody epigeous
0.05 substitutions/site
/terfezia
-peziza depressa
/marcelleina-peziza gerardii
Quercus EcM FJ197012 (MX)
Peziza badia DQ384574 (CA)
Peziza depressa AF335136 (DK)
Env HQ433101 (US)
EcM AJ893244 (EE)
DQ191679 (DK)
Peziza sp. JN121358 (CN)
Peziza sp.JN121359 (US)
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Terfezia boudieri AY500557(LY)
Peziza atrovinosa U40613 (US)
Cazia flexiascus U42694 (US)
Peziza whitei AF335168 (AU)
Tirmania nivea AF335177 (IL)
Peziza ellipsospora AF335139 (US)
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EcM AJ893245 (EE)
Pezizaceae 2-2 JN121336 (US)
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Pezizaceae 2-3 JN121344 (CN)
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Calongea prieguensis FJ228463 (SP)
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JX414179 (CH)
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Sarcosphaera coronaria AY500555 (DK)
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Peziza bananincola AF335133 (PG)
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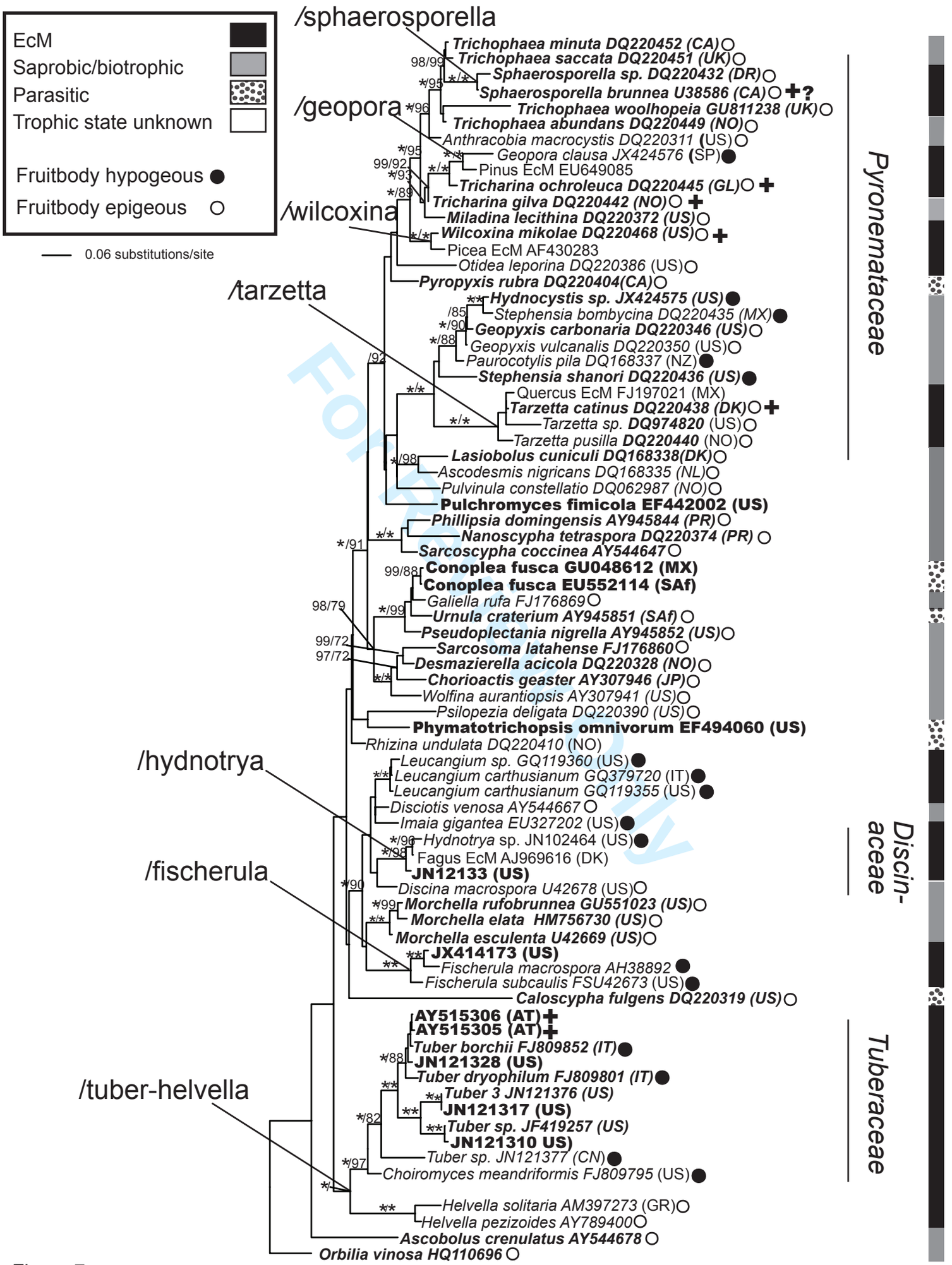
Orbilia vinosa HQ110696

Figure 6


EcM
Saprobic/biotrophic
Parasitic
Trophic state unknown
Fruitbody hypogeous
Fruitbody epigeous
0.06 substitutions/site
/sphaerosporella
/geopora
/wilcoxina
/tarzetta
/hydnotrya
/fischerula
/tuber-helvella
Trichophaea minuta DQ220452 (CA)
Trichophaea saccata DQ220451 (UK)
Sphaerosporella sp. DQ220432 (DR)
Sphaerosporella brunnea U38586 (CA)
Trichophaea woolhopeia GU811238 (UK)
Trichophaea abundans DQ220449 (NO)
Anthracobia macrocystis DQ220311 (US)
Geopora clausa JX424576 (SP)
Pinus EcM EU649085
Tricharina ochroleuca DQ220445 (GL)
Tricharina gilva DQ220442 (NO)
Miladina lecithina DQ220372 (US)
Wilcoxina mikolae DQ220468 (US)
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Otidea leporina DQ220386 (US)
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Geopyxis carbonaria DQ220346 (US)
Geopyxis vulcanalis DQ220350 (US)
Paurocotylis pila DQ168337 (NZ)
Stephensia shanori DQ220436 (US)
Quercus EcM FJ197021 (MX)
Tarzetta catinus DQ220438 (DK)
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Tarzetta pusilla DQ220440 (NO)
Lasiobolus cuniculi DQ168338(DK)
Ascodesmis nigricans DQ168335 (NL)
Pulvinula constellatio DQ062987 (NO)
Pulchromyces fimicola EF442002 (US)
Phillipsia domingensis AY945844 (PR)
Nanoscypha tetraspora DQ220374 (PR)
Sarcoscypha coccinea AY544647
Conoplea fusca GU048612 (MX)
Conoplea fusca EU552114 (SAf)
Galiella rufa FJ176869
Urnula craterium AY945851 (SAf)
Pseudoplectania nigrella AY945852 (US)
Sarcosoma latahense FJ176860
Desmazierella acicola DQ220328 (NO)
Chorioactis geaster AY307946 (JP)
Wolfina aurantiopsis AY307941 (US)
Psilopezia deligata DQ220390 (US)
Phymatotrichopsis omnivorum EF494060 (US)
Rhizina undulata DQ220410 (NO)
Leucangium sp. GQ119360 (US)
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JN121310 US)
Tuber sp. JN121377 (CN)
Choiromyces meandriformis FJ809795 (US)
Helvella solitaria AM397273 (GR)
Helvella pezizoides AY789400
Ascobolus crenulatus AY544678
Orbilia vinosa HQ110696
Pyronemataceae
Discin-aceae
Tuberaceae

Figure 7

Figure 8

Fig. 8a-h Spore mats and corresponding fruit bodies of representative OTUs of EcM Pezizales. 8a Spore mat of /pachyphloeus-amylascus 21 (RHAM15), bar = 0.5 cm. 8b Pachyphloeus fruit body of /pachyphloeus-amylascus 21 (MX32624), bar = 1 cm. 8c Spore mat of P. thysellii (RHAM116), bar = 0.5 cm. 8d Fruit body of P. thysellii (RH1180), bar = 1 cm 8e Spore mat of /pachyphloeus-amylascus 22 (RHAM126), bar = 1 cm. 8f Pachyphloeus fruit body of /pachyphloeus-amylascus 22 (RH735), bar = 1 cm. 8g Spore mat of /pachyphloeus-amylascus 4 (RHAM102), bar = 1 cm. 8h Scabropezia flavovirens (RH1209), bar = 1 cm. 8i Spore mats of Hydnobolites 12 (RHAM483) with fruit body of matching ITS sequence (RH1358), bar = 0.5 cm. 8j Spore mat of Tuber sp. 3 (RHAM226), bar = 1 cm. 8k Fruit body of Tuber sp. 3 (RH1279), bar = 1 cm. 8l Spore mat of /terfezia-peziza depressa 2-1 (RHAM371), bar = 1 cm. 8m Spore mat of Fischerula (RHAM489). 8n Close up image of 8L taken through a dissecting microscope, bar = 1 mm.
203x254mm (300 x 300 DPI)