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Review

Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground



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ABSTRACT

In the fungal kingdom, the ectomycorrhizal (EcM) symbiosis has evolved independently in multiple groups that are referred to as lineages. A growing number of molecular studies in the fields of mycology, ecology, soil science, and microbiology generate vast amounts of sequence data from fungi in their natural habitats, particularly from soil and roots. However, as the number and diversity of sequences has increased, it has become increasingly difficult to accurately identify the fungal species in these samples and to determine their trophic modes. In particular, there has been significant controversy regarding which fungal groups form ectomycorrhizas, the morphological “exploration types” that these fungi form on roots, and the ecological strategies that they use to obtain nutrients. To address this problem, we have synthesized the phylogenetic and taxonomic breadth of EcM fungi by using the wealth of accumulated sequence data. We also compile available information about exploration types of 143 genera of EcM fungi (including 67 new reports) that can be tentatively used to help infer the ecological strategies of different fungal groups. Phylogenetic analyses of ribosomal DNA ITS and LSU sequences enabled us to recognize 20 novel lineages of EcM fungi. Most of these are rare and have a limited distribution. Five new lineages occur exclusively in tropical and subtropical habitats. Altogether 46 fungal genera were added to the list of EcM fungal taxa and we anticipate that this number will continue to grow rapidly as taxonomic works segregate species-rich genera into smaller, monophyletic units. Three genera were removed from the list of EcM groups due to refined taxonomic and phylogenetic information. In all, we suggest that EcM symbiosis has arisen independently in 78–82 fungal lineages that comprise 251–256 genera. The EcM fungal diversity of tropical and southern temperate ecosystems remains significantly understudied and we expect that these regions are most likely to reveal additional EcM taxa.

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1. Introduction

The ectomycorrhizal (EcM) symbiosis has evolved multiple times both in plants and fungi. The fungal kingdom includes at least 66 independent lineages of EcM fungi, mostly members of the Basidiomycota and Ascomycota (Tedersoo et al., 2010). In the past it has been challenging to unambiguously determine whether some fungal groups are ectomycorrhizal or not because of limited or ambiguous evidence and alternative interpretations (Rinaldi et al., 2008; Tedersoo et al., 2010; Comandini et al., 2012; Ryberg and Matheny, 2012). However, community studies of ectomycorrhizal fungi have become more sophisticated over the past two decades and a variety of techniques have been used to determine EcM status and to delimit groups of EcM taxa, including experimental synthesis trials, field observations combining anatomical and molecular techniques, stable nitrogen and carbon isotope signatures, and phylogenetic analyses. Unfortunately, many EcM fungal taxa detected as environmental sequences do not match sequences of fruit-body vouchers or pure cultures (Tedersoo et al., 2010). Although reference sequences from fruit-bodies are accumulating at an exponential rate, many sequences from EcM root tips remain unmatched to their sexual stages. This suggests that much of the EcM fungal diversity is indeed cryptic. Nevertheless, many described species and even genera lack publicly available sequence data (Tedersoo et al., 2010) and this underscores the need to produce DNA sequences from identified herbarium specimens (Brock et al., 2009).

The rapidly growing DNA sequence data in public repositories, in conjunction with recently developed sequence annotation tools (e.g. PlutoF workbench – Abarenkov et al., 2010; Tedersoo et al., 2011a), provide an invaluable source of metadata about the host plants, isolation sources, and geographic origin of EcM fungal isolates. By using the data in public sequence databases, Hynson et al. (2013), Tedersoo et al. (2013a) and Veldre et al. (2013) recently detected additional putative EcM lineages within the Serendipitaceae (Sebacinales group B), Pyronemataceae and Ceratobasidiaceae. These fungal lineages were previously considered root endophytes, saprotrophs or parasites. The limited number of sequences from these lineages suggests that they are uncommon in EcM fungal communities or were considered root contaminants in the original studies (e.g. Oberwinkler et al., 2013).

It is important to understand which fungal taxa are EcM and which are not. EcM fungi play fundamentally different roles in forest communities and in ecosystems compared to other functional guilds such as fungal root endophytes and decomposers. For example, EcM fungi are uniquely adapted to facilitate mineral nutrition of plants and to distribute recent photosynthates into the mycorrhizosphere soil (Buee et al., 2009). Mycorrhizal fungi have mostly lost the powerful enzymes used for attacking plant cell walls and degrading organic compounds such as lignin (Eastwood et al., 2011). Furthermore, since EcM fungi are often the most abundant organisms in forest soils, it is important to understand their ecology for the purposes of management and conservation. This is particularly relevant for taxa in the Ceratobasidiaceae, since this group includes beneficial orchid and

ectomycorrhizal symbionts as well as devastating fungal plant pathogens (Veldre et al., 2013). Several lineages within Pezizales are associated with tree roots in truffières where they compete for space and resources with the valuable truffle “crop” species (Bonito et al., 2011, 2012; Rubini et al., 2011). However, most species of Pezizales are saprotrophic and a few are pathogenic (Hansen and Pfister, 2006). The rapid shift from root tip-based studies to soil fungal community studies necessitates discriminating between mycorrhizal and non-mycorrhizal fungi. Molecular studies of soil increasingly use sophisticated second and third generation sequencing technologies to generate millions of reads. In contrast to EcM roots and fruit-bodies that can be stored as vouchers and morphologically examined in the future, soil-based studies cannot provide morphological or ultrastructural information to infer ecological interactions. These high-throughput sequencing methods are an easy and cost-effective way to study mycorrhizal ecology in situ but currently our ability to adequately identify fungal DNA sequences and interpret the ecological role of these species is lagging behind our ability to produce sequence data. The phylogenetic and functional breadth of fungi in soil and other complex substrates poses a great challenge for taxonomic identification as well as functional characterization. Because of the large volume of sequence data and the large number of fungal taxa involved in the EcM symbiosis, researchers would benefit from a well-annotated reference database from which they can automatically extract information on ecology and taxonomy of fungal taxa (Köljalg et al., 2013).

Within EcM fungi, there are major differences in ecological strategies of dispersal (Ishida et al., 2008), metabolic activity (Trocha et al., 2010) and in relative benefits to plant hosts (van der Heijden and Kuyper, 2003; Nara, 2006). This is at least partly ascribed to differences in the relative carbon cost to plants and efficiency in enzymatic access to organically bound nutrients, nutrient uptake and nutrient transfer (Courty et al., 2010). Species of EcM fungi differ strongly in their potential enzymatic capacity, which is a function of both the substrate and climatic conditions (Courty et al., 2010). Evidence suggests that key enzyme functions are highly variable between (and within) EcM lineages and are partly predictable based on phylogenetic relationships among the EcM fungi (Tedersoo et al., 2012b). Not surprisingly, the abundance and morphology of the extraradical mycelial system is the single most important variable in determining enzymatic capacity (Tedersoo et al., 2012b). The presence and characteristics of extraradical hyphae and rhizomorphs serve as proxies for foraging strategies referred to as “exploration types” (Agerer, 2001, 2006). Species of medium-distance and long-distance exploration types tend to exhibit similar responses to climatic gradients (Ostonen et al., 2011), N fertilization or pollution (Lilleskov et al., 2011; Kjoller et al., 2012) and carbon influx (Markkola et al., 2004). Most fungi with long-distance exploration strategies appear specialized in N uptake from organic sources and they apparently expend significant carbon resources on rhizomorphs so they appear to lose their relative benefits or competitive abilities in disturbed systems (Lilleskov et al., 2011). In contrast, smooth and short-distance exploration types are more frequently detected in

mineral soils and these types are more resilient in response to disturbance, apparently because they can easily regenerate their reduced system of extraradical hyphae. So far, in-depth studies of EcM morphology and examination of subterranean foraging strategies cover only the most common genera and species (reviewed in Agerer, 2006). Based on these studies it appears that EcM morphology and exploration types are mostly phylogenetically conserved (Eberhardt, 2002; Agerer, 2006), but that there are variations in foraging strategy within the largest and most well studied genera (e.g. *Russula*, *Lactarius* and *Tomentella*). This information is increasingly referred to in fruit body, root tip and soil mycelium-based community analyses (e.g. Deslippe et al., 2011; Ostonen et al., 2011), but most studies do not determine these types *in situ*.

We have three main purposes in this review. First, we gather and interpret information about cryptic EcM lineages from environmental sequences in public databases and we give these lineages formal names and designated reference sequences. Second, we revise the lineage concept in several groups of EcM fungi based on newly available taxonomic and ecological information. Third, we compile existing published and unpublished information about the exploration types of all EcM fungal genera by lineages. Information about all of the EcM lineages detailed in this paper and by Tedersoo et al. (2010) are provided in Table S1 and regularly updated in the UNITE homepage (<http://unite.ut.ee/EcMlineages.php>).

2. Methods

We searched the fungal ITS sequence data deposited in International Nucleotide Sequence Database consortium (INSDC) and UNITE (as of 01.12.2012) using the PlutoF workbench, an online tool that allows users to permanently annotate sequence quality and metadata (Abarenkov et al., 2010). We updated the metadata on EcM fungi (source of isolation, host or substrate, and geographical origin) in PlutoF by searching in the published studies or directly consulting the authors of the studies. We primarily focused on sequences with the term “ectomycorrhiza” listed as the “isolation source” in PlutoF. All of the sequences with this designation were assigned to EcM fungal lineages or given a status “non-ectomycorrhizal” or “uncertain” based on sequence alignments and phylogenetic analyses following the protocols of Tedersoo et al. (2011a). We also consulted recent publications to evaluate the opinion of other authors on previously unrecognized EcM taxa. We downloaded sequences and associated metadata for all these EcM groups and their non-EcM relatives (outgroup sequences) as determined by blastN searches against INSDC and UNITE. We used multiple outgroup taxa when possible, because this improved our ability to evaluate the EcM status and monophyly of the ingroup taxa. All of the analyses used ITS rDNA alignments but in several cases we were also able to use the flanking LSU alignments in order to enhance the phylogenetic signal. In one group of EcM-associated Agaricomycetes (/agaricomycetes1), LSU sequences were used separately to determine their broad placement among Basidiomycota. Briefly, all downloaded sequences were aligned with MAFFT 7 (Katoh and Standley, 2013), trimmed and manually corrected in SeaView 4 (Gouy

et al., 2010), and subjected to RaXML Maximum Likelihood analyses with the GTR + G + I evolutionary model and 1000 fast bootstrap replicates (Stamatakis et al., 2008). We used a series of Fisher’s Exact tests to investigate the null hypothesis that sequences derived from EcM root tips are non-randomly distributed in the target lineage and its sister groups. We anticipate that *a posteriori* selection of the ingroup and outgroup, presence of redundant sequences (i.e. similar sequences from the same study) and multiple testing may introduce biases to these results.

We evaluated all EcM-associated sequences and mycorrhizal literature published since our previous review (Tedersoo et al., 2010) in order to determine EcM fungal lineages that may be present in the database but have not been formally recognized. In order to be considered EcM symbionts, a fungal lineage had to meet at least one of the three following criteria: 1) the EcM-derived sequences form a well-supported monophyletic clade that includes all or mostly EcM fungal sequences and does not include sequences from non-EcM habitats (e.g. sequences from roots of non-EcM plants, agricultural soil, etc.); 2) EcM root tip vouchers (obtained directly from the study authors) exhibited typical EcM morphology and the morphological features were both consistent among different vouchers and also consistent with the phylogenetic position of the fungal lineage (cf. Agerer, 2006); and 3) a phylogenetic test statistic indicating that there is a greater probability of association with EcM root tips in the ingroup taxa as compared to outgroup taxa.

To determine exploration types of fungi, we relied on an extensive online database about morphological and anatomical descriptions of EcM at www.deemy.de (see also Agerer, 2006). For groups for which no exploration type data are available, we studied the publications that included brief EcM descriptions or directly contacted study authors to obtain additional information on EcM morphology. Lastly, we used light microscopy to re-analyze the EcM morphology of vouchered EcM root tips from our own community studies.

3. Novel lineages of ectomycorrhizal fungi

Based on ITS sequence data, together with updated metadata and findings from previous studies, we identified 20 previously unrecognized EcM lineages (11 in Basidiomycota, 7 in Ascomycota and 2 in Zygomycota) (Table 1). Eight lineages are derived from separating previously described lineages into two groups in light of accumulated sequence and phylogenetic information. We also briefly describe what is known about the EcM exploration types of these new lineages. Information about the exploration types of all EcM lineages and genera are given in Table S1.

Basidiomycota

The /agaricales1 lineage is known from fruit body collections (representative specimen TH9235; INSDC accession KC155374) and associated root tips from *Dicymbe jenmanii* (under the same accession; Smith et al., 2013a) and *Dicymbe corymbosa* (M.E. Smith and T.W. Henkel, unpublished) from Guyana. The ITS region of this group is short compared with

Table 1 – Information about the newly described EcM lineages

Lineage	Number of sequences in UNITE (as of 01.05.2013)	Representative sequence in INSDc or UNITE	P-value from Fisher's exact test (total n)
/agaricales1	1	KC155374	nd
/agaricomycetes1	8	UDB008409	0.003 (16)
/atheliales1	10	UDB014265	nd
/atheliales2	10	JN168682	0.015 (12)
/boletopsis	23	UDB016630	nd
/hydropus	4	JN168774	0.071 (8)
/serendipita1	66	UDB002608	<0.001 (153)
/serendipita2	21	EU909214	<0.001 (43)
/sordariales1	41	UDB017246	<0.001 (50)
/sordariales2	33	UDB008982	<0.001 (42)
/tulasnella1	53	AY192445	nd
/tulasnella2	15	UDB002679	nd
/xenasmatella	10	JN168733	<0.001 (24)
/aleurina	33	KC905032	nd
/pustularia	52	EU649088	nd
/rhodoscypha	2	FJ236854	nd
/pyronemataceae1	11	JN569352	<0.001 (31)
/pyronemataceae2	3	AY702741	0.012 (9)
/endogone1	1	AY977045	nd
/endogone2	2	UDB002714	nd

most other species of Agaricales (ITS1: 111 bp; ITS2: 149 bp), which may partly explain why this taxon has no close matches in the INSDc and UNITE databases. Nuclear and mitochondrial LSU sequences and nuclear SSU sequences indicate that this fungus has evolutionary affinities with pink-spored or white-spored Agaricales, but it is distinct from any previously recognized EcM lineage. This fungus forms dense clusters of white ectomycorrhizas of the medium-distance fringe exploration type beneath fruit-bodies in sandy soils (see Fig 4 of Smith et al., 2013a).

The /agaricomycetes1 lineage comprises a monophyletic group of eight sequences (BS = 70) that were obtained from healthy EcM root tips. This lineage is split into two strongly supported clades (BS = 100; Fig 1a), a Southern temperate group (Horton et al., 2013; Nouhra et al., 2013) and a Northern temperate group (Bergemann and Garbelotto, 2006; Ishida et al., 2007). Both the ingroup and its sister clade form a deep branch among the Agaricomycetes and cannot be placed into any of the currently recognized orders (Fig 1b). We have examined four Argentinean EcM root tip vouchers that represent two distinct species. Both species exhibit short-distance exploration types with narrow, thin-walled hyphae and an EcM mantle anatomy that is intermediate between the pseudoparenchymatous and plectenchymatous types. One of the species has long, unicellular, needle-like cystidia, while the other species lacks cystidia.

The /atheliales1 lineage represents a monophyletic group (BS = 96) of tropical EcM root tip isolates and unidentified fruit body specimens. Phylogenetic analysis was unable to resolve a sister group (Fig 2). An unpublished sequence from a fruit body collected in a Cuban *Coccoloba uvifera* stand and an EcM isolate from dry dipterocarp forest in Thailand (Phosri et al., 2012) are relatively similar and they form a monophyletic clade (BP = 100) that is basal to the rest of the lineage. Other sequences in this group are derived from EcM roots of Dipterocarpaceae in Malaysia (Peay et al., 2010), India (L. Tedersoo

and M. Bahram, unpublished), Cameroon, Gabon and Madagascar (Tedersoo et al., 2011b). In Indian and African sites, members of the /atheliales1 EcM lineage were locally abundant. Species in this fungal lineage form EcM of a medium-distance mat exploration type characterized by abundant mycelium, rhizomorphs and binding of soil

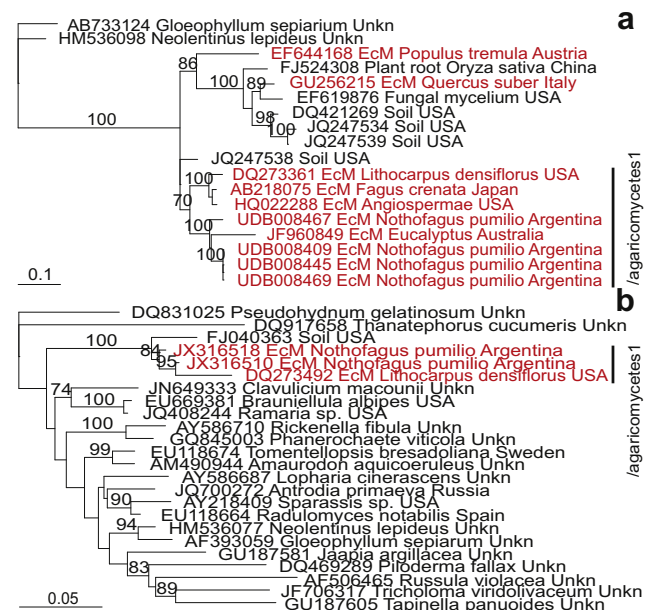


Fig 1 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of sequences in the /agaricomycetes1 lineage based on a) ITS sequences; and b) LSU sequences. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

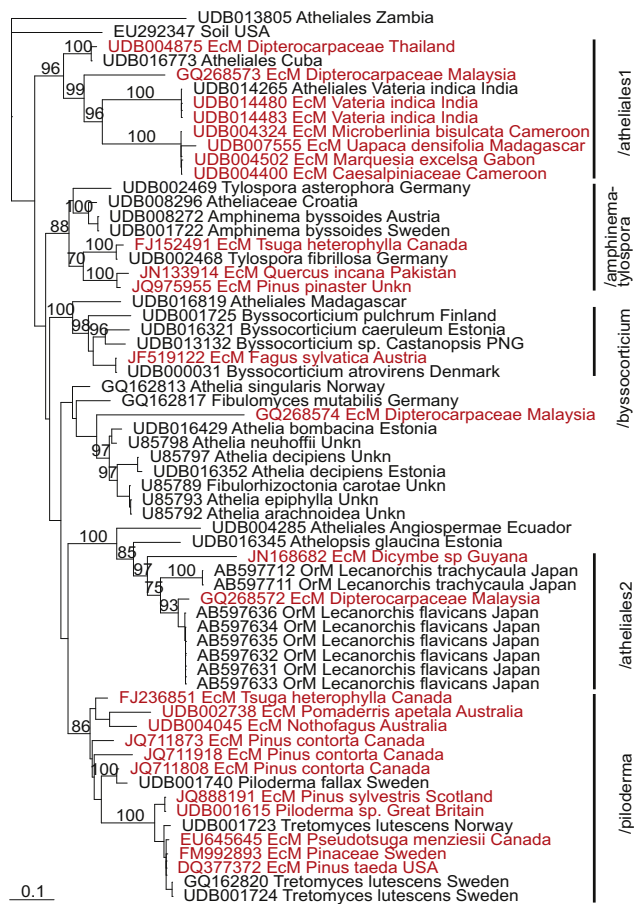


Fig 2 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of sequences in the Atheliales based on combined ITS and LSU sequences. The /piloderma, /amphinema-tylospora and /byssocorticium lineages are represented by a few divergent sequences for illustration purposes. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

particles. Taxa in this lineage were found to exhibit strong leucine aminopeptidase activity, whereas members of other EcM lineages from the same site did not (Tedersoo et al., 2012b).

The /atheliales2 lineage constitutes a monophyletic group (BS = 97) that has been recovered only from EcM root tips and the roots of orchids in tropical and subtropical ecosystems. An unidentified Atheliales sp. from Ecuador (BP = 100) and the non-EcM taxon *Athelopsis glaucina* from Estonia (BP = 85) form successive sister groups to this EcM lineage (Fig 2). An isolate from a *Dicycme* root tip (Smith et al., 2011) forms a basal group within the /atheliales2 lineage (BP = 97). Another EcM root tip from a Dipterocarpaceae host (Peay et al., 2010) is nested within uncultured isolates from non-photosynthetic orchids (*Lecanorchis* spp.) from Shikoku, a subtropical island of Japan (Okayama et al., 2012). We consider the orchid association to be strongly indicative of EcM habit since *Lecanorchis* spp. are mycoheterotrophic and their sister taxa

associate with well-known EcM fungi in *Quercus-Castanopsis* forests (Okayama et al., 2012). The /atheliales1 and /atheliales2 lineages are phylogenetically distinct from the previously known EcM lineages within Atheliales (e.g. /piloderma, /amphinema-tylospora and /byssocorticium), but the relative positions of these different groups within the order remain unclear.

The /boletopsis lineage is erected in this study based on sequences of *Boletopsis* fruit-bodies and mycorrhizas that form a strongly supported monophyletic group with no close relatives (Cooper and Leonard, 2012; U. Kõljalg et al., unpublished). *Boletopsis* is well-known for its conspicuous fruit-bodies with a poroid hymenium but this group has been recovered only twice in EcM community studies (Izzo et al., 2005; Bergemann and Garbelotto, 2006) suggesting that this lineage is rare on EcM roots. This lineage is distributed in the temperate and boreal zone of the Northern hemisphere as well as in *Nothofagus* forests in New Zealand. It is possible that *Boletopsis nothofagi* was introduced to New Zealand from the Northern Hemisphere, but more data are needed (Cooper and Leonard, 2012).

The putative /hydropus lineage represents a small monophyletic group of sequences derived from EcM root tips in Malaysia (Peay et al., 2010) and Guyana (Smith et al., 2011), and from leaf litter in a *Dicycme*-dominated forest in Guyana (K. McGuire, unpublished). This group is nested within the genus *Hydropus* (Agaricales) that comprises putative saprobes (Fig 3). Compared to the outgroup taxa, the association with EcM roots was not significant in the ingroup sequences (Fisher's Exact test: $P = 0.071$) leaving open the possibility that /hydropus lineage is a parasitic or saprotrophic taxon that invades EcM roots.

The /serendipita lineage as defined in Tedersoo et al. (2010) is re-considered here based on new information. In a series of publications, Warcup (1988, 1990b) suggested that certain strains of *Serendipita vermifera* (Sebacinales clade B sensu Weiss et al., 2004) isolated from orchid roots are able to form EcM in axenic conditions. However, subsequent field and experimental studies have failed to confirm these findings in Australia and elsewhere. Notably, Warcup (1988) reported on EcM formation in arbuscular mycorrhizal herbs and EcM Myrtales, but the mantle was usually fragmentary and only one or two cell layers deep. This suggests that he used an overly relaxed definition for ectomycorrhizas that probably included any root endophytes and saprobes that attach to roots (Brundrett, 2009). To the best of our knowledge, the isolates Warcup considered EcM have not been sequenced (M. Weiss and P. McGee, pers. comm March, 2013). More recently, however, molecular studies have identified species of *Serendipita* from EcM root tips in temperate, boreal, and subarctic communities throughout the Northern hemisphere (Ryberg et al., 2009; Stefani et al., 2009). In northern boreal and temperate forests, fungi identified as *Serendipita* spp. are dominant on roots of *Pyrola japonica* seedlings, indicating their substantial role in both carbon and mineral nutrition of the ericoid plant (Hashimoto et al., 2012). Although these fungi also occur on adult *Pyrola rotundifolia* (Vincenot et al., 2009), Hynson et al. (2013) were the first to suggest that several *Serendipita* spp. associating with *Pyroleae* are actually ectomycorrhizal based on their tight clustering in phylogenetic trees. Based on full-

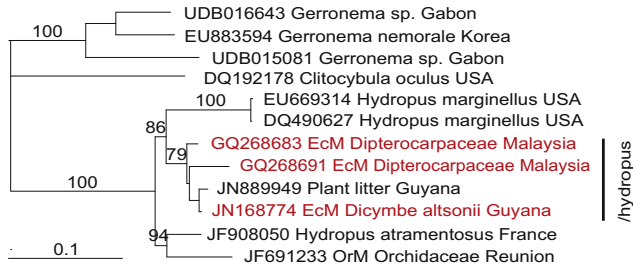


Fig 3 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of sequences in the /hydropus lineage based on ITS sequences. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

length ITS sequences of the Sebacinale clade B, it appears that EcM taxa fall into two major monophyletic clades (Figs 4, S1), hereafter referred to as the /serendipita1 and /serendipita2 EcM lineages. In both of these groups, the sebacinean taxa were also recovered from non-EcM roots by specifically targeting these taxa using lineage-specific primers and sometimes cloning (Weiss et al., 2011; Garnica et al., 2013). Use of specific primers may result in detection of species that are not necessarily prevalent; we suggest that this incidental detection of these taxa from non-EcM root systems does not exclude them as EcM symbionts.

The /serendipita1 lineage forms a monophyletic group (BS = 97). Species of this group associate with Salicaceae, Fagaceae, Betulaceae, Pinaceae, Tiliaceae and Pyroleae in temperate, boreal, subalpine and subarctic habitats of the Northern Hemisphere (Fig 4). We have been able to study the anatomy of only a single root tip isolate, UDB002608, that is characterized by a plectenchymatous mantle and smooth surface with no hyphae. In another isolate, JX316811, sparse extraradical hyphae were observed (C. Andrew, pers. comm. March, 2013). Thus, the examined species of the /serendipita1 lineage belong to either contact or short-distance exploration types.

The /serendipita2 lineage is phylogenetically distinct and it forms a monophyletic group (BS = 98; Fig 4). Members of this lineage associate predominantly with Pinaceae, but to a lesser extent with Fagaceae and Pyroleae in subtropical and temperate forest ecosystems. Although no root tips were available to us for study, species of this group probably fall into the contact or short-distance exploration types described for other Sebacinales (Urban et al., 2003; Agerer, 2006).

The /tulasnella lineage (sensu Tedersoo et al., 2010) is similarly split into two groups. These two lineages are monophyletic, but distantly related to one another within the Tulasnellaceae phylogeny (J. Oja, unpublished).

The /tulasnella1 lineage comprises a deeply diverging monophyletic clade of closely related fungal species that colonize both the rhizoids of hepatics (Bidartondo et al., 2003; Bidartondo and Duckett, 2010) and EcM root tips of *Betula pendula* and *Pinus sylvestris* (Bidartondo et al., 2003; I. Ostonen et al., unpublished) in temperate forests of Europe. In addition, sequences from *Betula nana*-dominated tundra soil in Alaska

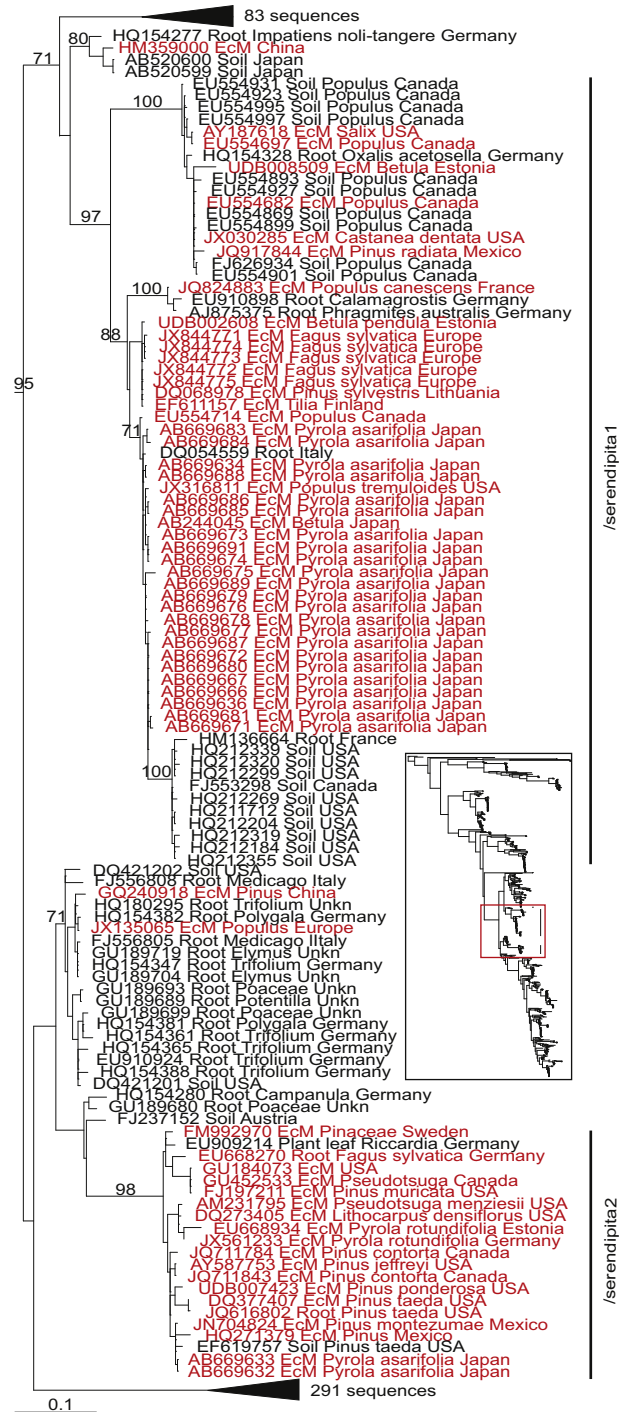


Fig 4 – Cut-out of an unrooted maximum likelihood phylogram demonstrating phylogenetic placement of EcM lineages in the Serendipitaceae family (Sebacinale clade B) based on ITS sequences. The inclusive tree (in the rectangle) is given in Fig S1. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

(Deslippe et al., 2011) fall into this group, but the sequences were recently withdrawn from INSDc. The EcM formed by *Tulasnella* are smooth with no extraradical mycelium and correspond to the contact exploration type.



Fig 7 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of EcM lineages in the *Scutellinia*-*Trichophaea* clade of Pyronemataceae (sensu Hansen et al., 2013) based on combined ITS and LSU sequences. The /wilcoxina, /geopora and /sphaerosporella lineages are represented by a few divergent sequences for illustration purposes. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

records in INSDc. However, these are not concentrated in specific clades nor do they form monophyletic groups of EcM isolates, leading us to treat them as parasitic, endophytic or saprotrophic root colonizers rather than true EcM symbionts.

Ascomycota

The /aleurina lineage is comprised of species of *Aleurina*, *Gelinipes nom. prov.* and *Unicava nom. prov.* (J.M. Trappe et al.,

unpublished; Perry et al., 2007; Tedersoo et al., 2013a). Based on LSU, the /aleurina lineage is nested within the Pyronemataceae family and it has Southern Hemisphere distribution (Tedersoo et al., 2013a). However, the more inclusive ITS dataset of this EcM lineage suggests that the distribution involves both the Southern Hemisphere and Eastern Asia. In particular, this group has been found in natural forests in Japan (Ishida et al., 2007) and Vietnam (L. Tedersoo and co-workers, unpublished). In Argentina, the /aleurina lineage is divided into five

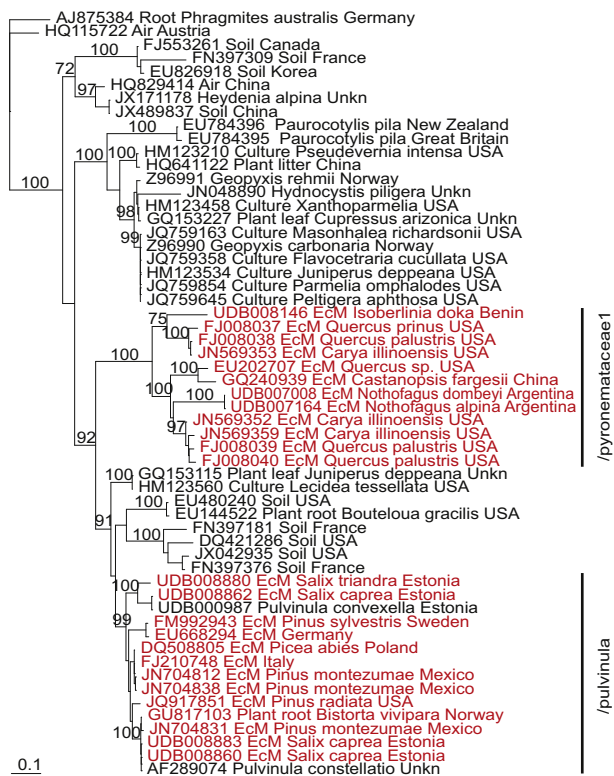


Fig 8 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of the /pulvinula and /pyronemataceae1 lineages based on combined ITS and LSU sequences. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

species based on ITS sequences from EcM roots (Nouhra et al., 2013) and at least two of these taxa correspond to named species (*Aleurina echinata* KC905033 and *Aleurina argentina* KC905032; Pfister & Smith, unpublished). In Australia, this group is thus far represented by a single species (Tedersoo et al., 2008a; Horton et al., 2013; Fig 6). We studied several EcM root tips from this group and all had a typical coarse pseudoparenchymatous mantle and either thick-walled hyphae or no hyphae. Thus, species of this lineage belong to either contact or short-distance exploration types.

The /pustularia lineage was recovered based on an LSU match between a *Pustularia patavina* fruit body and sequences of EcM root tips (Tedersoo et al., 2013a). Based on ITS sequences, this is a monophyletic group (BS = 100) of closely related isolates that have been found throughout the boreal and temperate forests of the Northern Hemisphere. This lineage is most commonly recovered from EcM roots of Pinaceae, but they are occasionally identified from Fagaceae, Tiliaceae, Salicaceae and Rosaceae (Fig 7). Members of this group exhibit mantle structure similar to that of *Geopora* (Tedersoo et al., 2006) and sparse extraradical hyphae that correspond to the short-distance exploration type. The genus name *Pustularia* had a very wide use and its type species, *Pustularia cupularis*, has been

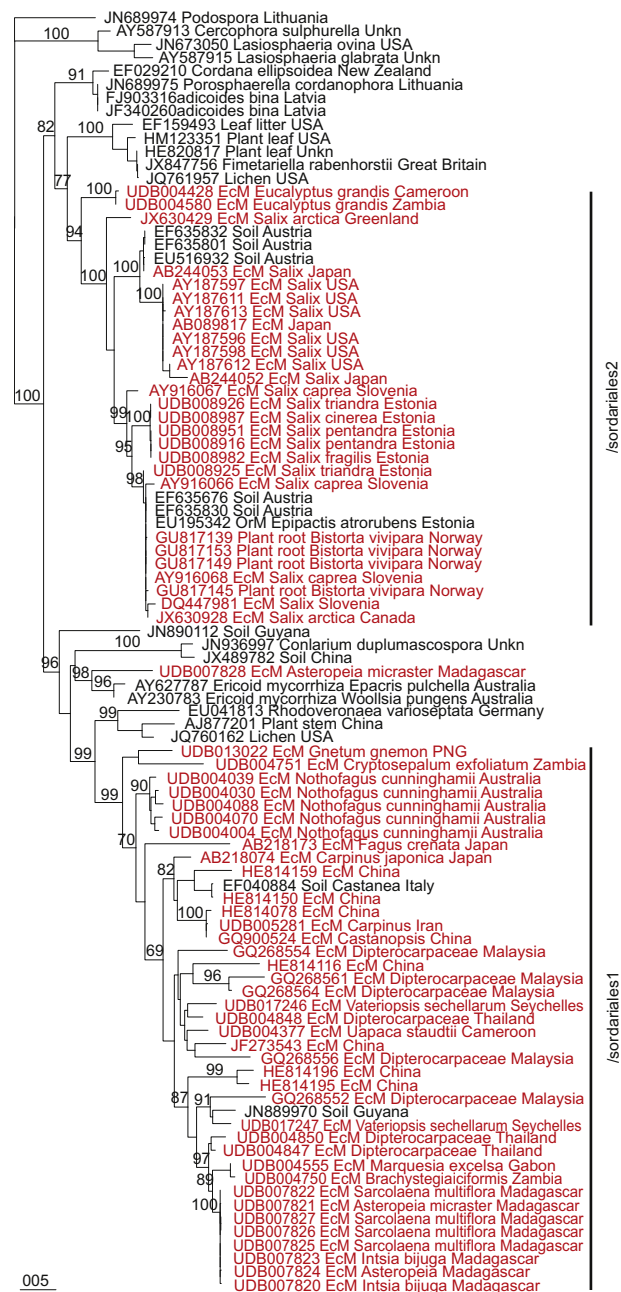


Fig 9 – Unrooted maximum likelihood phylogram demonstrating phylogenetic placement of EcM lineages within Sordariales based on combined ITS and LSU sequences. Significant bootstrap support (>70) is indicated above branches. Abbreviations: EcM, ectomycorrhiza; ErM, ericoid mycorrhiza; OrM, orchid mycorrhiza; Unkn, unknown country.

transferred to *Tarzettia*; therefore the genus name of *P. patavina* may change.

The /rhodoscypa lineage comprises a small monophyletic group of sequences that was discerned based on LSU (Tedersoo et al., 2013a). There are only two EcM-derived ITS sequences available in INSDc and both originated from Pinaceae in SW Canada (Jones et al., 2008; S. Lim, unpublished).

The monotypic genus *Rhodoscypha* is distributed in the boreal and temperate forests of the Northern Hemisphere and is rare throughout its range. Unfortunately no root tips were available from the authors for microscopy.

The /pyronemataceae1 lineage comprises a heterogeneous group of EcM root-derived sequences that have no fruit body representatives. This group displays affinities to the *Pulvinula-Lazuardia* group, but is distinct from the /pulvinula EcM fungal lineage (Tedersoo et al., 2013a; Fig 8). ITS sequences of this group were derived from EcM roots of Fagaceae and Juglandaceae in the USA (Cavender-Bares et al., 2009; Bonito et al., 2012), Nothofagaceae in Argentina (Nouhra et al., 2013) and from Caesalpinioideae in Benin (L. Tedersoo and N.S. Yorou, unpublished). The Argentinean root tip vouchers have sparse hyphae and have a mantle structure that is intermediate between the pseudoparenchymatous and plectenchymatous types. We believe that this lineage is best characterized by short-distance exploration type.

The /pyronemataceae2 lineage is a monophyletic group (BS = 100) of three sequences recovered exclusively from EcM root tips of Pinaceae in California (Izzo et al., 2005; Smith et al., 2009) and Northern Mexico (Hoeksema et al., 2012; Fig 7). This small group is closely related to but clearly distinct from the /geopora lineage. Based on 97 % ITS similarity, each sequence could be recognized as a distinct taxon. No root tips were available for microscopy.

The /sordariales lineage of Tedersoo et al. (2010) is divided into two groups based on additional sequence data and phylogenetic analyses of combined ITS and LSU sequences (Fig 9).

The /sordariales1 lineage encompasses sequences from various angiosperm hosts in tropical (Tedersoo et al., 2007, 2011b; Peay et al., 2010; Phosri et al., 2012), southern temperate (Tedersoo et al., 2009a) and warm northern temperate (Ishida et al., 2007; Bahram et al., 2012) biomes, and forms a well-supported monophyletic group (BS = 99; Fig 9). Based on descriptions of EcM roots from the Seychelles (Tedersoo et al., 2007) as well as material from Australia and Africa, members of the /sordariales1 lineage form short-distance exploration type.

The /sordariales2 lineage represents a monophyletic group of sequences (BS = 94; Fig 9). While the bulk of the sequences originates from arctic, alpine and temperate habitats in association with *Salix* (Trowbridge and Jumpponen, 2004; Nara, 2006b; Timling et al., 2012; Tedersoo et al., 2013b) and *Bistorta vivipara* (Brevik et al., 2010), species in the basal clade of this lineage are EcM symbionts of *Eucalyptus* introduced to Africa. This group was common on eucalypts but absent on indigenous trees, suggesting their co-introduction to Africa (Jairus et al., 2011). Root tip vouchers from Estonia and Africa have a thin mantle and abundant hyphae, pointing to a short-distance exploration type.

In Ascomycota, there are many EcM fungal lineages that are comprised of only a few species (e.g. /cenococcum and several lineages in the Helotiales). Many of these species-poor lineages apparently are either limited in their distribution or are infrequently detected in EcM community studies, perhaps due to their specific ecological requirements. The lineages within Helotiales have remained challenging to accurately detect and identify, because they are closely related to

common root endophytes that are frequently detected by molecular (Tedersoo et al., 2009a,c) and culture-based surveys (Vrålstad et al., 2002; Kernaghan and Patriquin, 2011; Menkis and Vasaitis, 2011) of EcM tissues. Phylogenetic analyses of subgroups of Helotiales indicate that endophytic, ericoid mycorrhizal, soil-derived and EcM-associated isolates occur throughout the phylogeny (Vrålstad et al., 2002; Hambleton and Sigler, 2005). Without statistical approaches or information about EcM morphology and behavior in culture, it is difficult to assign a nutritional mode to an individual species in this group. An additional problem is the thin mantle and sometimes poorly developed Hartig net in these helotialean EcM (Yu et al., 2001; Tedersoo et al., 2008b; Münzenberger et al., 2009). These anatomical features have led to many ambiguous reports on EcM formation between Helotiales and various plants (Kohn et al., 1986; Haug et al., 2004; Peterson et al., 2008; Comandini et al., 2012). In Pezizales, where root endophytes have rarely been recovered in molecular and culture-based surveys, assignment of nutritional mode to particular taxa is more straightforward (Tedersoo et al., 2013a). In only one of the pezizalean families, the Pyronemataceae, we documented five novel lineages. Previous recognition of these groups was hampered by the lack of ITS sequences from sporocarps, because molecular taxonomic work has mostly utilized LSU and protein-encoding genes (Hansen et al., 2013). However, recent ecological studies have started to routinely sequence both ITS and partial LSU to improve biogeographic and taxonomic resolution (e.g. Smith et al., 2007). Matching LSU genes from fruit-bodies and EcM root tips can prove useful to assign nutritional mode to pezizalean groups (Tedersoo et al., 2006, 2013a; Smith et al., 2007).

Zygomycota

There is only limited phylogenetic information available for EcM fungal lineages in Zygomycota (Desiro et al., 2013). The /endogone lineage has been separated into two parts based on phylogenetic information from SSU, ITS and LSU genes (L. Tedersoo et al., unpublished data). Of species studied by Warcup (1990a), the Pinaceae-associated *Endogone flammicorona* and *Endogone lactiflua* constitute the /endogone1 lineage, whereas the Australian *Endogone aggregata*, *Endogone tuberculosa* and *Sclerogone eucalypti* form the /endogone2 lineage. Probably numerous sequenced EcM root tips from the Australian Myrtaceae, Nothofagaceae and Rhamnaceae (Tedersoo et al., 2008a, 2009a) also fall into the latter group. Notably, the type species of *Endogone*, *Endogone pisiformis*, is non-EcM (e.g. Berch, 1983). Sequences from old collections of *Densospora* spp., associated with Myrtaceae (McGee 1996), are phylogenetically distinct from the /endogone1 and /endogone2 lineages (L. Tedersoo et al., unpublished data).

Doubtful groups

Several EcM lineages such as /catathelasma, /sowerbyella, /endogone1 and /densospora have never been recovered in EcM community studies. Root tips of the /catathelasma lineage have been documented only once, when specifically searching for them under a fruit body of *Catathelasma*

imperiale (L. Tedersoo, unpublished). Although *Sowerbyella* is suggested to form EcM based on its habitat and the ^{15}N isotopic signature (Hobbie et al., 2001), there are still no direct observations on EcM in this group. Of novel lineages, /hydroplus and /xenasmatella require further support for their EcM status (see above).

4. New EcM fungal genera

Since taxonomists continually lump, split, and create new genera, the list of EcM groups is subject to change. There is still substantial controversy as to whether monophyletic lineages that contain different fruit body types should be treated as monogeneric or multigeneric groups. In particular, sequestrate fungi have traditionally been named in separate genera, but since these truffle-like taxa are often nested within groups of epigeous species, the nomenclature has become problematic. For example, the polyphyletic sequestrate fungi within the /cortinarius lineage were previously treated within the genus *Thaxterogaster*, but have now all been synonymized within the genus *Cortinarius*. In contrast, the sequestrate fungi within the /boletus and /rusula-lactarius lineage have mostly been retained as separate genera (but see Desjardin (2003) and Lebel and Tonkin (2007) for exceptions). Within the /boletus lineage, many new genera of both epigeous and hypogeous taxa have been erected in recent years. *Peziza sensu lato* represents the opposite scenario and this genus is still treated as a large, paraphyletic group even though it includes several well-defined lineages that include sequestrate and epigeous species as well as both EcM and non-EcM taxa.

For 14 previously recognized lineages, we provide updated information about the genera that are currently included. In total, we add 47 genera to the list of EcM fungi, most of which have been described very recently. We also remove three genus-level taxa (*Hydnocystis piligera*, *Tricharina ochroleuca*, *Rubinoletus rubinus*; see below). The updated list of EcM fungal genera is found in Table S1.

From matching fruit body and EcM root tip LSU sequences, there is good evidence that the Pezizalean genus *Parascutellinia* is ectomycorrhizal with *Salix* spp. in various habitats in Estonia (Tedersoo et al., 2013b). Unfortunately, no ITS sequences of *Parascutellinia* fruit-bodies are available in INSDC. Although *Parascutellinia* was considered a saprotrophic sister group to the /genea-humaria lineage in Tedersoo et al. (2010), recent phylogenetic analyses support its placement within this group (Hansen et al., 2013; Tedersoo et al., 2013b).

In previous reports by Tedersoo et al. (2010) and Comandini et al. (2012), the fungus *T. ochroleuca* was reported as an EcM-forming member of the /geopora lineage. However, all reports of EcM formation by *T. ochroleuca* are apparently based on partial ITS matches to sequences from cultures that are phylogenetically placed outside the /geopora lineage (Fig 7; see also Stielow et al., 2013). We find no credible evidence that any *Tricharina* species form EcM. Similarly, *H. piligera* (type species) forms a sister group to the non-EcM genus *Stephensia* and therefore we consider the genus *Hydnocystis* to be non-EcM. However, at least one species of *Hydnocystis s. lato*, *Hydnocystis clausa*, is an EcM member of the /geopora lineage. However, we

suggest that it should be treated as *Geopora clausa* (Tul. and C. Tul.) Burds. (Burdsall 1968; M.E. Smith, unpublished).

The /leucangium lineage is appended with the recently erected, monophyletic genus *Kalapuya* that is nested within this group (Trappe et al., 2010). While the genus *Fischerula* is distantly placed in some phylogenetic studies (Healy et al., 2013), most studies with more focused taxonomic sampling or inclusive ingroup sampling support placement of *Fischerula* within the /leucangium lineage (Trappe et al., 2010; Alvarado et al., 2011).

Several recently sequenced and/or newly described genera are accommodated in the /marcelleina-peziza gerardii lineage. *Delastria rosea*, a rare sequestrate species was recently placed as a sister group to species of *Hydnobolites* (Alvarado et al., 2011; Healy et al., 2013). *D. rosea* isolate JN102449 exhibits 99.5 % ITS sequence similarity with an EcM root tip isolate FJ013057 from Portugal (Rincon and Pueyo, 2010) and slightly less to many other root tip isolates, indicating that *Delastria* spp. are ectomycorrhizal. Kovacs et al. (2011) described the new sequestrate fungal genera *Temperantia* and *Stouffera* that are nested within the /marcelleina-peziza gerardii lineage (Kovacs et al., 2011; Healy et al., 2013), suggesting that these genera are ectomycorrhizal.

The /tuber-helvella lineage includes the Southern Hemisphere genera *Gymnohydnotrya* and *Nothojafnea* in addition to previously reported taxa (Bonito et al., 2013). The genus *Underwoodia* appears to be split into two distinct groups that likely constitute one genus for the Northern Hemisphere and one for the Southern Hemisphere (Bonito et al., 2013, M.E. Smith, unpublished). *Loculotuber* and *Paradoxa* also belong to this group, but these taxa will probably be synonymized with *Tuber* (Kinoshita et al., 2011; Alvarado et al., 2012; Bonito et al., 2013). In contrast to most *Tuber* species, the basal Southern Hemisphere species in *Gymnohydnotrya* and *Underwoodia sensu lato* exhibit a contact exploration type that is similar to species in the Helvellaceae.

A recently sequenced isolate of *Discinella terrestris* from New Zealand (GU222294) has 96–97 % ITS match to multiple species within the /helotiales4 lineage that has so far only documented from Australia (Tedersoo et al., 2008a; Horton et al., 2013). The core group of *Discinella sensu stricto* is probably non-ectomycorrhizal, because the type species *Discinella bou-dieri* has a Northern Hemisphere distribution and it has not been sequenced thus far.

The /hebeloma-alnicola lineage is widened by the addition of the genus *Psathyroma* (nom. prov.; P.B. Matheny, pers. comm. January 2013) that has been found mostly in New Zealand as fruit-bodies, but also as EcM root tips in Tasmania (Tedersoo et al., 2009a; Horton et al., 2013) and Argentina (Nouhra et al., 2013). In contrast to other members of this lineage, EcM of *Psathyroma* spp. exhibit a short-distance exploration type with abundant dark brown hyphae but no rhizomorphs. At least one species from the genus *Wakefieldia* (*Wakefieldia macrospora*) also belongs to the /hebeloma-alnicola lineage, because it is phylogenetically placed among species of *Alnicola*, *Hebeloma* and *Hymenogaster* (Kaounas et al., 2011). Sequences from EcM root tips (isolates HQ204662, HQ204659) of *Quercus ilex* in France (Richard et al., 2011) are 99.8 % similar to *W. macrospora*. However, the type species, *Wakefieldia striaespora* was described from SE Asian

dipterocarp forests and it may belong to a different group based on the combination of morphology and habitat (M.E. Smith, unpublished).

In the /inocybe lineage, *Tubariomyces* has been erected from *Inocybe* (Alvarado et al., 2010). *Tubariomyces* is inferred to associate with Cistaceae. However, since relatively few studies have focused on EcM communities of Cistaceae there are currently no available root-derived sequences that correspond to *Tubariomyces*.

The /piloderma lineage includes a recently erected genus *Tretomyces* that has been recorded only from boreal and temperate Pinaceae forests (Kotiranta et al., 2011; Fig 2).

Destuntzia fusca was considered putatively EcM (Tedersoo et al., 2010) and is now a confirmed member of the /ramaria-gautieria lineage based on ITS sequence data. The isolate EU697269 matches a group of ectomycorrhizal *Ramaria* species (not shown).

DNA sequences of ITS and LSU from the genus *Fevansia* indicate that this genus belongs to the EcM/albatrellus lineage (Smith et al., 2013b). Although *Fevansia* does not match closely with any EcM root tip sequences, this species is consistently found among EcM roots of Pinaceae and all of its closest relatives form EcM.

The /boletus lineage has been enriched with several recently described genera or genera with newly generated molecular data (Nuhn et al., 2013). The LSU sequences of the sequestrate *Gymnogaster boletoides* falls into the /boletus lineage (Halling et al., 2012b). The monotypic *Heliogaster* was segregated from *Octaviania* and it represents a sequestrate *Xerocomus* species (Orihara et al., 2010). No EcM root tips are matched to *Heliogaster columellifera*, but the phylogenetic position indicates that it is ectomycorrhizal. *Tubosaeta* belongs to the /boletus lineage based on ITS sequences (Brock et al., 2009). However, none of the sequenced specimens match ITS sequences from EcM root tips. *Rossbeevera* was erected to accommodate a monophyletic group of sequestrate fungi from Australia and Japan (Lebel et al., 2012). Sequences corresponding to this group have been found from EcM root tips in Australia (Tedersoo et al., 2009a; Horton et al., 2013). *Turmalinea* is a recently described sequestrate genus that is sister to *Rossbeevera*; this group is phylogenetically related to other EcM-forming members of the /boletus lineage and *Turmalinea* species are consistently found with EcM trees (Orihara et al., 2013). *Spongiforma* represents a recently described sequestrate genus that is related to *Porphyrellus* (Desjardin et al., 2009). The pileate genus *Borofutus* has been described to accommodate a sister species to *Spongiforma* (Hosen et al., 2013). No EcM isolates correspond to these two genera but both groups are inferred as EcM. *Zangia* has been erected from *Tylopilus* and this group is currently represented only by Chinese species (Li et al., 2011). No EcM root tip sequences correspond to *Zangia roseola*, the only species for which an ITS sequence is available. The recently described sequestrate genus *Soliococcus* is phylogenetically allied with *Bothia* and is inferred as EcM as it is always encountered among ECM Myrtaceae and *Allocasuarina* in Northern Queensland and Papua New Guinea (Trappe et al., 2013). *Corneroboletus* has been described to accommodate *Boletus indecorus* (Zeng et al., 2012). No EcM root isolate corresponds to *Corneroboletus*. *Hemileccinum* was described to accommodate *Boletus impolitus* and *Boletus depilatus*

(Sutara, 2008). *Australopilus* and *Harrya* are Australian genera that have been erected as segregates of the Northern Hemisphere genus *Tylopilus* (Halling et al., 2012b). The genus *Sutorius* has been erected from *Boletus* (Halling et al., 2012a). For some reason, no ITS sequences exist for *Australopilus*, *Gymnogaster*, *Harrya*, *Phylloboletellus*, *Royoungia* and *Sutorius*, and therefore root tip matches to these genera cannot be evaluated. The type species of *Rubinoboletus*, *R. rubinus* is nested within the non-EcM *Chalciporus*, but a few other species are nested among EcM taxa within the /boletus lineage (Nuhn et al., 2013). Nuhn et al. (2013) listed a number of genera that belong to the Boletaceae (*Boletochaete*, *Gastroleccinum*, *Paxillogaster*, *Sinoboletus*) or Paxillaceae (*Austrogaster*, *Hoehnelogaster*, *Meiorganum*) based on morphological characters, but they lack ITS or LSU sequence data to evaluate their phylogenetic position and their EcM status.

Rhopalogaster belongs to the /suillus-rhizopogon lineage based on LSU sequence data (Hosaka et al., 2006). *Rhopalogaster transversarium* isolate DQ218599 has 96.6 % LSU sequence identity with *Suillus hirtellus* fruit body AY612828. ITS sequences of this genus are lacking from sequence databases.

5. Confirmed non-EcM genera

The genus *Amogaster* that produces sequestrate fruit-bodies was originally considered a member of Boletales. However, this species is nested within the saprotrophic genus *Lepiota* and does not form EcM (Ge and Smith, 2012). Similarly, American species of *Gigasperma* are nested within *Lepiota* and are currently treated in the sequestrate, non-EcM genus *Cryptolepiota* (Kropp et al., 2012). Sequestrate forms have evolved multiple times in Lepiotaceae (Ge and Smith, 2012), which is a common phenomenon in Basidiomycota. The type species of *Gigasperma*, *Gigasperma cryptica* is found exclusively in New Zealand and is nested within the /cortinarius EcM lineage (Kropp et al., 2012). The fruit body sequence generated by Kropp et al. (2012) has 93.7 % ITS sequence match with *Cortinarius elaiops* (JX000369). *Neopaxillus* forms a monophyletic sister group to *Crepidotus* and *Simocybe* and no EcM-derived ITS sequences fall into this group so *Neopaxillus* is therefore considered non-ectomycorrhizal (Vizzini et al., 2012).

6. Biodiversity and biogeography

The lack of randomly obtained sequences from certain lineages suggests that, despite our good overall understanding of EcM fungal communities, several EcM groups still await discovery due to their natural rarity. Based on fruit body records, we previously suggested that tropical-endemic EcM lineages were either rare or absent (Tedersoo et al., 2010). However, here we report four putatively EcM groups that are hitherto known only from tropical habitats (/agaricales1, /atheliales1, /hydropus, /xenasmatella) as well as one lineage that is found in both tropical and subtropical ecosystems (/atheliales2). All these lineages are relatively rare and species-poor except for the /atheliales1 lineage (see above). Given that four of these groups are distributed on multiple continents, it is reasonable to assume that these lineages are either relatively old or have excellent capacity for dispersal. However, given the rarity of

these taxa, we hypothesize that vicariance is probably more important than long-distance dispersal in explaining the distribution of these EcM groups. Nonetheless, we acknowledge that the present data are too scanty to generate any realistic biogeographic scenarios to explain their origins. In addition, several common groups of EcM fungi such as the /*inocybe* and /*clavulina* lineages may have evolved in tropical regions based on molecular data (Matheny et al., 2009; Smith et al., 2011; Kennedy et al., 2012). These groups have effectively spread to both temperate and arctic ecosystems, suggesting that long-term migration from tropical to temperate climates is possible for some EcM groups with tropical origins. We deduce that the rare tropical lineages described here may not have been able to expand beyond the tropics because of inefficient dispersal capacities or due to an inability to withstand cold temperatures. Based on large-scale biogeographic distribution patterns of EcM taxa (Geml et al., 2012; Tedersoo et al., 2012a; Timling et al., 2012; Bahram et al., 2013), we suggest that intolerance of low temperature may have limited migration of many lineages to subarctic and arctic ecosystems.

7. Concluding remarks

Based on accumulated ITS sequences and associated meta-data, we describe 20 new lineages of EcM fungi. Thus, the number of distinct EcM lineages is elevated to 78–82 and the number of EcM fungal genus-level taxa is elevated to 251–256. However, several putative lineages require further morphological or ultrastructural proof, because saprotrophic Ascomycota, Basidiomycota and Zygomycota are all commonly detected as saprotrophs or endophytes with EcM root tips (Morris et al., 2008; Lindner and Banik, 2009; Tedersoo et al., 2009c; Nouhra et al., 2013). Given the rarity of many lineages in EcM community studies, we suggest that continued research will reveal new EcM lineages, especially in tropical and Southern Hemisphere ecosystems where fewer EcM community studies have been conducted. This synthesis of new data from our own studies and from the INSDC indicates that several uncommon, species-poor lineages may indeed be limited to tropical and subtropical ecosystems.

Information about EcM morphology allowed us to determine the main exploration types for both the novel groups and poorly studied taxa that have not been addressed in previous in-depth studies. Although most EcM fungal lineages (and genera therein) possess a single exploration type, the most common and species-rich genera exhibit multiple exploration types (Agerer, 2006). Based on these findings, we caution that exploration type cannot be consistently extrapolated from a few species to the entire genus or lineage. Therefore, we strongly encourage researchers to determine foraging strategies based on original experimental material. To be able to seek further molecular or morphological proof and to study the morphology in more detail in future, researchers should keep voucher root tips. A large number of in-depth morphological and anatomical descriptions of unidentified EcM is also available online (www.deemy.de). Molecular identification of these well-described EcM samples would further our understanding of EcM fungal community ecology.

Nevertheless, we believe that our summary of current knowledge on exploration types will be useful for analysis and interpretation of results from molecular studies that rely on fungal DNA from hyphal mesh bags and soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.fbr.2013.09.001>.

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