Chapter 6
Ectomycorrhizal Fungal Lineages: Detection of Four New Groups and Notes on Consistent Recognition of Ectomycorrhizal Taxa in High-Throughput Sequencing Studies

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6.1 Introduction

Ectomycorrhizal (EcM) fungi represent a diverse group that forms mutualistic associations with plant roots. Due to different opinions and methods, there has been significant controversy in "separating the wheat from the chaff" when assigning mycorrhizal status to fungal species or Operational Taxonomic Units (OTUs) that are recovered from molecular identification studies (Rinaldi et al. 2008; Tedersoo et al. 2010; Tedersoo and Smith 2013). Based on phylogenetic information, the EcM fungal species and genera have been grouped into monophyletic "lineages" to reflect their independent evolution from non-mycorrhizal ancestors (Tedersoo et al. 2010). Accumulating evidence suggests that this is a unidirectional process by which mostly saprobic ancestors transition into a symbiotic lifestyle. These ectomycorrhizal biotrophic fungi subsequently lose the genes responsible for plant cell wall degradation (e.g., Kohler et al. 2015), and thus reversals to saprotrophy or other trophic lifestyles are rare, nonexistent, or transient.

Using sequence metadata as well as phylogenetic and statistical analyses, Tedersoo and Smith (2013) added additional lineages of previously unrecognized EcM fungi that were only known from sequence data obtained from plant roots and/or soil. These reports increased the number of EcM lineages to 78–82. Since 2013, a number of revealing molecular identification and phylogenetic studies have

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been published that motivated us to revise the EcM fungal lineages in order to match the most recent knowledge.

Molecular identification studies of fungi from soil typically rely on the best BLASTn matches or Naïve Bayesian Classifier (Porras-Alfaro et al. 2014) to assign representative sequences of OTUs to species, genera, families, and higher taxonomic ranks based on subjective similarity thresholds (Tedersoo and Nilsson 2016). Both traditional Sanger sequencing and high-throughput sequencing (HTS) studies usually fail to account for the fact that the ITS regions (as well as other molecular markers) differ in their rate of evolution and therefore in the level of separation between species and across lineages. For example, it is likely that an OTU with 95% full-length ITS sequence match to the taxon *Russula vinosa* represents an ectomycorrhizal species in the genus *Russula*, but the same is not necessarily true for *Cenococcum geophilum* or *Meliniomyces bicolor*. What can we conclude about the trophic mode of OTUs from soil or roots that match *R. vinosa* or any other EcM fungal taxon at 80%, 85%, or 90% similarity? Inclusion or exclusion of these taxa may strongly bias the view of the EcM to saprotroph ratio and the environmental effects on fungal guilds if these OTUs are highly abundant.

Although macroscopic EcM fungi are relatively well studied compared to some other fungal groups, molecular ecology studies in tropical ecosystems or in the Southern Hemisphere commonly encounter problems in identification due to a dearth of well-annotated reference sequences from identified specimens, axenic cultures, or EcM roots. If the studies are to compare overall fungal diversity, this is not a significant problem. However, trophic groups of fungi respond to different predictors and display different biogeographic patterns. Therefore, most studies attempt to separate EcM fungi, arbuscular mycorrhizal (AM) fungi, and putative plant pathogens from potential saprotrophs. So far, the assignment of a trophic status has been typically performed based on taxonomic assignments either manually or in a semiautomatic fashion (Nguyen et al. 2016). Although ecological traits should be clearly related to collections or at least reference OTUs or species hypotheses (SHs; Kõljalg et al. 2016) rather than genus or family names, there is currently no annotated system for rigorously incorporating additional information on important ecological, morphological, and physiological traits (e.g., EcM exploration type, fruit body type, enzymatic capacities, etc.). This means that most data sets require time-consuming manual trophic assignments based on expert knowledge in order to extract critical ecological details. The current system also renders the correctness of taxonomic labeling and specimen identification of great analytical importance. Hence, we aim to assign information about EcM fungal lineages to individual isolates (accessions) and SHs in UNITE and to establish group-specific ITS sequence similarity thresholds for delimiting EcM fungal lineages based on our previous experience with high-throughput sequencing.

6.2 Approaches

We critically evaluated recent studies about the phylogeny and molecular identification of EcM fungi published since 2013. We also rechecked the sequences and metadata accumulated in the International Nucleotide Sequence Databases consortium (INSDc) and UNITE over the same time period. Lastly, we ran simple maximum likelihood phylogenetic analyses as described in Tedersoo and Smith (2013) to establish the monophyly of putative EcM groups.

To reproducibly separate EcM fungi from non-mycorrhizal fungi in HTS studies, we compiled information about the BLASTn identification of soil fungi based on the ITS2 subregion in the 454 pyrosequencing (Tedersoo et al. 2014a, 2016a) and Illumina MiSeq (Tedersoo et al. 2015a, b) HTS data sets. We also added unpublished data targeting the full ITS region that was obtained by combining primers ITS9MUNngs and ITS4ngsUni (Tedersoo and Lindahl 2016) and Pacific Biosciences RS II platform for a subset of soil samples collected from Estonia and Australia. This approach is built on the inherent assumption that EcM fungi are monophyletic groups that are separated from non-mycorrhizal relatives and that EcM lineages display a "phylogenetic gap" compared with non-mycorrhizal sister taxa. There is ample evidence for this phenomenon in phylogenetic studies, where EcM lineages are usually separated from other non-EcM taxa with relatively strong statistical support and great phylogenetic distances (e.g., a long stem). The first author has used this approach in multiple studies published since 2014. Elaborating on this further and releasing this information was motivated by the urge to make interpretation of high-throughput sequencing data more reliable.

To be able to recognize these phylogenetic gaps and separate EcM groups from non-EcM taxa, we used both accumulated ITS Sanger sequence data and HTS data. Briefly, we compiled publicly available Sanger sequences from all EcM lineages and their putative sister groups (Tedersoo et al. 2011a; Tedersoo and Smith 2013) as references. Using the above HTS data sets, we established multiple statistical indices based on sequence length, sequence coverage, and BLASTn score. We studied the distribution of these metrics in different lineages and also in certain related groups *that matched best* to particular EcM lineages. Among multiple candidates, we selected "BLASTn score to query sequence length ratio (S/L ratio)" and "sequence identity (%)" as the most promising indices that display the most pronounced gap between EcM and non-EcM groups. We verified the results using additional BLASTn searches, retrieving the 100 best matches and/or via phylogenetic analyses (cf. Tedersoo and Smith 2013).

6.3 Additional EcM Fungal Lineages

The **/leotia lineage** is erected to accommodate the genus *Leotia* of Helotiales (Fig. 6.1). Kühdorf et al. (2015) demonstrated that certain species in the genus *Leotia* are common root symbionts that form arbutoid EcM of short-distance exploration type with *Comarostaphylis* in Costa Rica. Their description of a plectenchymatous mantle of narrow clampless hyphae and thick-walled emanating hyphae roughly matches the descriptions of EcM of various groups of Helotiales. The authors also showed that a disproportionate amount of environmental sequences affiliated with the EcM group originate from Sanger-sequenced ectomycorrhizal root tips. In our previous studies, we had not noticed this sequence grouping in *Leotia* and thus considered this group as non-EcM based on unconfirmed root tip data from Zambia and Australia (cf. Tedersoo et al. 2010). However, earlier assessments based on isotopic evidence had previously provided suggestive evidence for an EcM habit in *Leotia* (Zeller et al. 2007). So far, EcM associations have only been convincingly shown for a single clade that comprises the *L. lubrica* and *L. viscosa* species complexes (Kühdorf et al. 2015). We ran a maximum likelihood phylogeny by including all Sanger sequences from fruit bodies, EcM root tips, and soil affiliated to *Leotia* spp. and demonstrate that most species of *Leotia* are likely ectomycorrhizal (Fig. 6.1). This analysis indicates that the /leotia lineage is widely distributed in all continents except perhaps lowland South America. *Leotia* spp. associate with Pinaceae, Fagales, Arbutoideae, *Uapaca*, and putatively with the *Berlinia* group (Fabales) and Dipterocarpaceae in Africa and India (Tedersoo et al. 2014a).

The **/porpoloma lineage** is separated from /tricholoma based on the results of a multigene phylogenetic analysis by Sánchez-García et al. (2014). The /porpoloma lineage is comprised of a core group of *Porpoloma*, but excludes several species that have been transferred to other segregate genera: *P. umbrosum* and *P. metapodium* to *Pseudotricholoma*, *P. spinulosum* and *P. macrocephalum* to *Pogonoloma*, *P. bambusarium* to *Corneriella*, and *P. pes-caprae* to *Pseudoporpoloma* (Sánchez-García et al. 2014; Vizzini et al. 2016). As currently circumscribed, /porpoloma is a Southern Hemisphere lineage that is found in southern South America, Australia, and New Zealand. The root tip sequences originally assigned to /tricholoma (UDB002748 from *Pomaderris apetala*: Tedersoo et al. 2008; UDB007061 from *Nothofagus dombeyi* and UDB007096 and UDB007123 from *N. obliqua*: Nouhra et al. 2013) actually represent /porpoloma. Sequences belonging to /porpoloma have been also recovered from *Nothofagus nervosa* seedlings by Fernández et al. (2013) in Argentina (KJ701291). Microscopic studies of the Tasmanian and Patagonian material suggest that EcM of *Porpoloma* are similar to that of /tricholoma with a plectanchymatous mantle and hairy rhizomorphs that place it to the medium-distance fringe exploration type. *Pseudoporpoloma pes-caprae* represents a European grassland-inhabiting species that forms a sister group to the genus *Tricholoma* of the /tricholoma lineage (Sánchez-García et al. 2014; Vizzini et al. 2016). Following separation of *Porpoloma*, we treat the /tricholoma lineage as consisting only of *Tricholoma* species. The /tricholoma

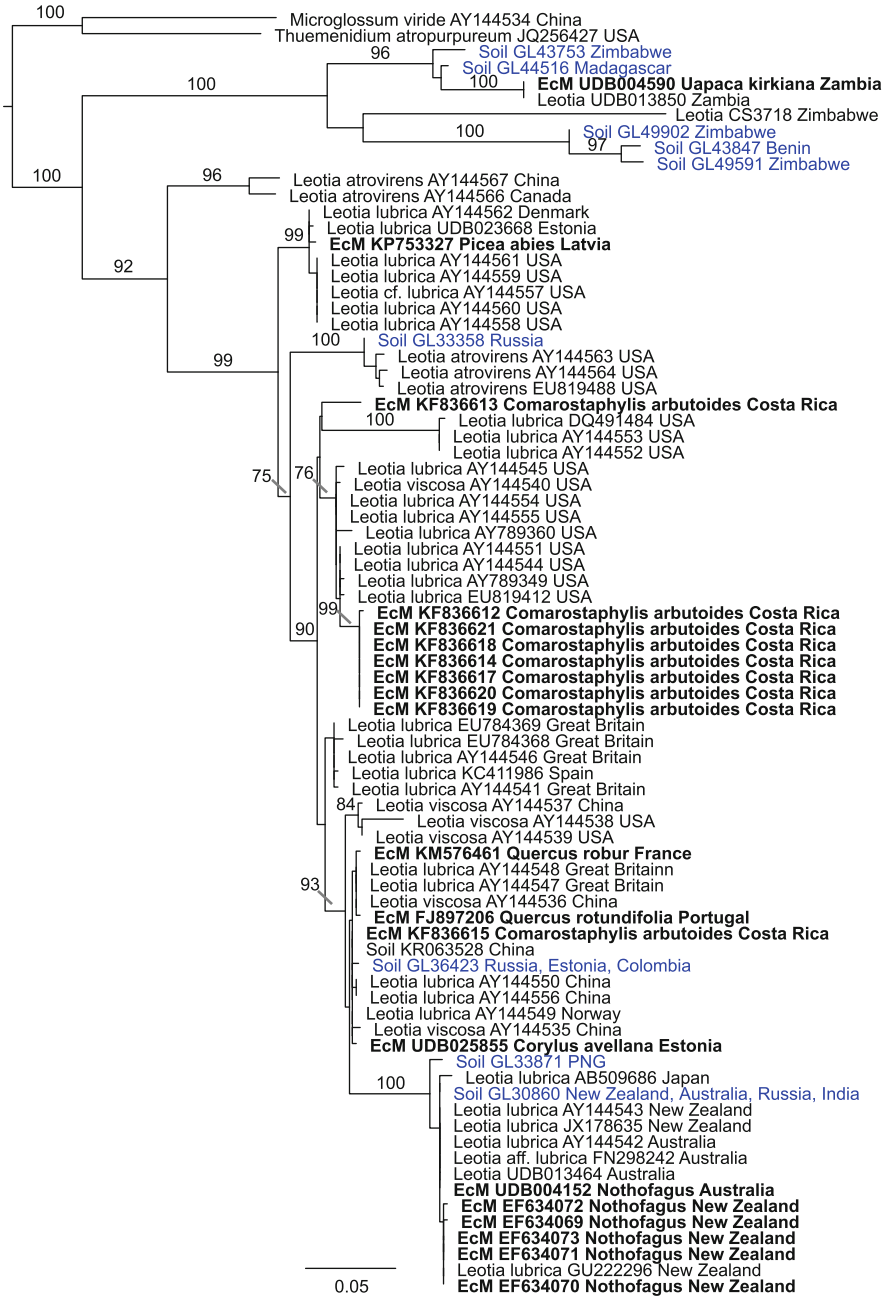

Fig. 6.1 Phylogenetic placement of sequences from ectomycorrhizal root tips (EcM; in *bold*) and soil (data from Tedersoo et al. 2014a highlighted) among *Leotia* species as based on fruit bodies. Bar, 0.05 changes per position. The ITS phylogram consists of 80 terminal taxa and 603 aligned positions, with *Thuemenidium atropurpureum* and *Microglossum viride* representing an outgroup based on closest BLASTn matches to *Leotia* spp. Note the unexpectedly high taxonomic diversity in the earliest diverging African clade

lineage is distributed in both the Northern and Southern hemispheres as well as tropical mountain regions with Fagales and Pinales and putatively with *Dicymbe* in the Guiana Shield region (M. E. Smith, personal observation).

The **/phaeocollybia lineage** is erected to accommodate species of *Phaeocollybia*. Three species of *Phaeocollybia* were reported from the roots of *Abies religiosa* in Mexico (Argüelles-Moyao et al. 2017), although the morphology of the ectomycorrhizas was not described. Many previous studies performed in the habitats of *Phaeocollybia* in North America have not detected this group on root tips. Although stable isotopes suggested the potential EcM or other biotrophic habit for *Phaeocollybia* spp., we previously considered this group non-mycorrhizal, because of long pseudorhizas being attached to long roots deep in soil (Redhead and Malloch 1985). The highest species diversity of *Phaeocollybia* (approximately 90 spp.) occurs in the temperate rainforests of western North America (Pacific Northwest), but individual endemic species are known from many regions, including Turkey, China, and northern South America (Brazil, Columbia; Coimbra et al. 2012). More work is needed to confirm that *Phaeocollybia* is a monophyletic group, to ensure that all species form EcM, and to document the morphology and exploration types of the symbiotic association.

We propose a novel **lineage /endogone3** within Mucoromycotina based on molecular identification of *Endogone* sp. (accessions LC159474-LC159479) from *Quercus* spp. root tips and anatomical descriptions of the association (Yamamoto et al. 2017). These samples comprise a novel lineage because they represent a sister group to the saprotrophic *Endogone pisiformis* (Berch and Fortin 1983a, b; Berch and Castellano 1986). They are also distantly related to the /endogone1 lineage (represented by *E. flammicorona* and *E. lactiflua*), /endogone2 (*E. aggregata*, *E. tuberculosa*, *Sclerogone eucalypti*) and /densospora (*Densospora* spp.) in a multigene phylogeny of Yamamoto et al. (2017). Unfortunately, the ITS sequences were not produced, which renders DNA barcoding-based identification of this group problematic. There are also no fruit bodies matching the sequences of these collections, and, therefore, the taxonomic identity and distribution of the /endogone3 lineage remain unknown.

6.4 New Names for Previously Known EcM Lineages

The **/guyanagarika lineage** is created here to accommodate the lineage previously referred to by Tedersoo and Smith (2013) as /agaricales1. The genus *Guyanagarika* was recently erected by Sánchez-García et al. (2016) and includes only three closely related species that all occur in the Guiana Shield region of northern South America. No sequences or sporocarps from these taxa have been collected or detected outside of this region, suggesting that this may be a narrowly endemic lineage that has evolved in the Neotropics and is restricted to endemic EcM host trees such as species of *Dicymbe* and *Pakaraimaea*. The robust multi-locus phylogenetic analysis by Sánchez-García

et al. (2016) placed this lineage within an expanded Catathelasmataceae but clearly separated from the members of the /catathelasma EcM lineage.

The /**phaeohelotium lineage** is erected to accommodate the /helotiales2 lineage that is naturally found only in the Southern Hemisphere. Dr. P. Johnston (unpubl.) first released sequences from fruit bodies of *Discinella terrestris* in New Zealand that matched closely to sequences from EcM root tips in Tasmania. The type species *D. boudieri* is only distantly related to the *D. terrestris* species complex, so *D. terrestris* was transferred to the new genus *Phaeohelotium* (Baral et al. 2013). The four described *Phaeohelotium* species are known from New Zealand and Australia and have also been documented in eucalypt plantations in Spain. Baral et al. (2013) also pointed to the observations of Warcup (1990a) that fruit bodies of *D. terrestris* sensu lato commonly co-occurred with other pyrophilic EcM and saprotrophic fungi after wildfire in Australia.

The /**tremellodendropsis lineage** is generated to accommodate the previously described /agaricomycetes1 lineage. This EcM lineage was initially erected to cover a cohesive group of Basidiomycota detected from EcM root tips especially from the Southern Hemisphere (Tedersoo and Smith 2013). A very recent fungal DNA barcoding initiative enabled to match these sequences to undescribed species of *Tremellodendropsis* from the formally monotypic order Tremellodendropsidales (Truong et al. 2017). This order forms a successive sister to Phallomycetidae, Stereopsidales, and *Clavulicium macounii* (Berbee et al. 2016). As discussed in Tedersoo and Smith (2013), not all putative species of Tremellodendropsidales are ectomycorrhizal.

6.5 Recently Revised Ectomycorrhizal Fungal Lineages

The /**cenococcum lineage** was discussed by Tedersoo et al. (2010) and Tedersoo and Smith (2013) as likely a group with only a few species and for which the sister taxon was poorly resolved. However, Spatafora et al. (2012) resolved several major lineages within /cenococcum and identified this lineage as belonging to Gloniaceae (with species of *Glonium* as the closest relatives). More recently, Obase et al. (2016) described the non-EcM *Pseudocenococcum floridanum* as a sister taxon to *Cenococcum* (see also Chap. 14).

In **Mucoromycota**, Tedersoo and Smith (2013) considered three EcM fungal lineages, viz., /endogone1, /endogone2, and /densospora. Because the two latter lineages had no fruit body sequences available, there was no information about their true taxonomic affinities. Our sequencing of Australian-type material (Tedersoo et al. 2016b) and recent phylogenetic analysis by Yamamoto et al. (2015) revealed that the EcM root tip sequences putatively assigned to the /endogone2 lineage are actually affiliated with *Densospora* in the /densospora lineage. Probably not all species of the genus *Densospora* form EcM (Warcup 1985; McGee 1996). The genus *Sphaerocreas* is also closely related to *Densospora* and affiliated EcM sequences, but its ecology is not well understood (Hirose et al. 2014). The /endogone2 lineage is comprised of the

Australian species *Endogone tuberculosa*, *E. aggregata*, and potentially *Sclerogone eucalypti* (Tedersoo and Smith 2013). Specimens of EcM species *Endogone aggregata* and *E. magnospora nom. nud.* (a putative member of this group) were recently sequenced, but these do not match closely to any sequences from EcM root tips. Specimens of *E. tuberculosa* and *S. eucalypti* have not yet been sequenced due to the age and paucity of herbarium materials. Furthermore, fruit body specimens and root tips of *Endogone* and *Densospora* are problematic to amplify and sequence because of multiple divergent ITS copies and long homopolymers (Tedersoo et al. 2016b). Endogonales resemble Glomeromycota (recently proposed as Glomeromycotina within Mucoromycota; Spatafora et al. 2016) in that they form nonseptate, multinucleate hyphae. This has been best demonstrated in pure cultures of *E. pisiformis* (Jabaji-Hare and Charest 1987). In the /endogone1 lineage, only members of the *E. flammicorona* and *E. lactiflua* species complexes (*Endogone* group B sensu Yamamoto et al. 2015) have been shown to form EcM (Warcup 1990b). Unfortunately, direct molecular evidence of EcM colonization by species in the /endogone1 and /endogone2 lineages is still lacking. The trophic status and ecophysiology of Endogonales requires urgent attention, because multiple distant clades of this group are likely recognized in the morphological species "*Glomus tenue*" *s. lat*. These have been referred to as "fine endophytes" that routinely colonize roots and form arbuscule-like structures in AM vascular plants (Orchard et al. 2017) and coils of hyphae in liverworts (Field et al. 2015).

6.6 Potential EcM Lineages that Require More Data

Several EcM lineages or putative EcM lineages still require more sampling effort to elucidate their interactions with host plants or clarify their putative trophic modes. For some of these taxa, their EcM status is suggested by the fruiting habit, associations with host plants, and/or isotopic data. However, for several groups we still lack solid data on EcM morphology and/or molecular confirmation on an EcM association.

The **/sowerbyella lineage** comprises the genus *Sowerbyella* that consists of 14 species (Yao and Spooner 2006). Members of the genus are typically found on the forest floor with EcM hosts. The rooting habit of the fruiting bodies, the fact that no member of the genus has been grown in axenic culture, and the isotopic signatures of some species (Hobbie et al. 2001) suggest that this genus may be EcM. However, there is still is no good molecular or anatomical data to show the EcM habit in this group. Hansen et al. (2013) resolved *Sowerbyella* on a branch among non-EcM relatives (e.g., *Aleuria*, *Lasiobolidium*) and suggested that *Sowerbyella* may not be EcM.

The multi-locus phylogenetic analysis of Sánchez-García et al. (2014) that separated /porpoloma from /tricholoma also proposed ***Albomagister*** as a segregate genus that is phylogenetically distinct from other Tricholomataceae. *Albomagister* was hypothesized to be EcM, because species in this genus fruit on the forest floor in association

with EcM Fagales and Pinaceae and have $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures that are similar to some other EcM fungi (e.g., members of the /catathelasma lineage) (Birkebak et al. 2013). It is possible that *Albomagister* represents another independent EcM lineage but root tips have never been sampled to test this hypothesis further.

6.7 New Additions of Genera Confirmed as Ectomycorrhizal

The genera of several EcM fungal lineages have been recently revised, resulting mostly in the splitting of large and heterogeneous genera into smaller groups. In the **/sebacina lineage**, the early diverging genus *Helvellosebacina* was separated from the rest of *Sebacina* whereas *Tremellodendron* was merged into *Sebacina* (Oberwinkler et al. 2014). The genus *Tremelloscypha* was resolved as the sister lineage to *Sebacina* and *Helvellosebacina* (but see Tedersoo et al. (2014b)), and all members of all three genera form a monophyletic group of EcM taxa. Oberwinkler et al. (2014) placed all of the non-mycorrhizal taxa in segregate genera, including *Chaetospermum*, *Craterocolla*, *Globulisebacina* (comprising *Efibulobasidium rolleyi*), and *Paulisebacina* (comprising *Sebacina allantoidea*).

The genus *Psathyloma* was provisionally included in the **/hebeloma-alnicola lineage** by Tedersoo and Smith (2013), but it was officially described only recently (Soop et al. 2016). It comprised three species, viz., *P. leucocarpum* and *P. catervatim* in New Zealand and Tasmania, and a third undescribed species from Argentina (root tip: JX316416). All known sequences and specimens of *Psathyloma* are known from Southern Hemisphere *Nothofagus* forests. In the analysis of Soop et al. (2016), the genus *Psathyloma* was resolved as the sister lineage to other taxa in the /hebeloma-alnicola lineage, suggesting the possibility of an ancient divergence between *Psathyloma* and all other genera in this group.

The **/boletus lineage** has been a subject to explosive radiation of descriptions of novel genera, several of which turn out to be non-monophyletic after addition of new taxa or information from other genes. The recent additions include *Alessioporus*, *Pulchroboletus* (Gelardi et al. 2014a), *Baorangia*, *Lanmaoa*, *Parvixerocomus*, *Rugiboletus* (Wu et al. 2015), *Binderoboletus*, *Guyanaboletus*, *Singerocomus* (Henkel et al. 2016), *Butyriboletus* (Arora and Frank 2014), *Caloboletus* (Vizzini 2014a), *Castellanea*, *Costatisporus*, *Jimtrappea* (Smith et al. 2015), *Cupreoboletus* (Gelardi et al. 2015a), *Crocinoboletus* (Zeng et al. 2014), *Cyanoboletus* (Gelardi et al. 2014b), *Exsudoporus* (Vizzini 2014b), *Imleria* (Vizzini 2014d), *Hourangia* (Zhu et al. 2015), *Neoboletus* (Vizzini 2014c), *Nigroboletus* (Gelardi et al. 2015b), *Pseudoaustroboletus* (Li et al. 2014), and *Rubroboletus* (Zhao et al. 2014).

6.8 Notes on the /Elaphomyces Lineage

The monophyly of the /elaphomyces lineage and the genus *Elaphomyces* was recently questioned by Buyck et al. (2016). Although we agree that this group warrants further taxonomic and phylogenetic research, we disagree with the suggestion that the African sequences published in Tedersoo et al. (2011b) are erroneous. We also disagree with the weak evidence that was presented for the polyphyly of this group of sequestrate hypogeous fungi. Re-evaluating the sequence data revealed that BLASTn results were meaningful only when conservative parameters (word size = 7, match score = 1, mismatch score = 3, gap opening cost = 5, gap extension cost = 2) but not MegaBLAST parameters were chosen. Buyck et al. (2016) also used only the ITS region for analysis and selected a specimen from another subclass (Chaetothyriomycetidae) as outgroup. Since the sequences of multiple clades within the /elaphomyces lineage are not alignable due to extremely high ITS sequence divergence (particularly among some undescribed tropical taxa), any phylogenetic analyses are likely to generate spurious results. We consider this study to be misleading and insufficient to suggest the polyphyly of Elaphomycetaceae or the /elaphomyces lineage. Here we do not make any changes in regard to the /elaphomyces lineage, but we do recommend caution when assigning sequences to the /elaphomyces lineage based on BLASTn searches.

6.9 Saprotrophic, Facultatively Biotrophic *Phlebopus*

In Tedersoo et al. (2010, p. 243), we discussed the mycorrhizal status of *Phlebopus* and considered this genus to be non-EcM but biotrophic. *Phlebopus* spp. readily form fruit bodies without any EcM host plants in sterile and nonsterile media and in natural conditions (Ji et al. 2011; Zhang et al. 2015; Kumla et al. 2016). In nature, *Phlebopus* spp. grow superficially and colonize the epidermal cells of AM and rarely EcM plants and associate with scale insects that form root galls in these roots (Zhang et al. 2015 and references therein). In axenic and synthesis trials in sterile and nonsterile substrate, *Phlebopus* spp. are reported to form ectomycorrhizal structures with EcM Australian *Acacia* spp. (Thoen and Ducouso 1989) and *Pinus kesiya* (Kumla et al. 2016). Although the illustrations of synthesized EcM structures are convincing in the latter study, we cannot accept *Phlebopus* as ectomycorrhizal because these associations are lacking or extremely rare in natural conditions (Zhang et al. 2015). We interpret the root-associated habit of *Phlebopus* as biotrophic but both non-mycorrhizal and non-parasitic, because the inoculated plants show no signs of decline (Kumla et al. 2016). The biotrophic associations with both roots and scale insects are likely facultative, because *Phlebopus* spp. are able to complete their life cycle saprotrophically without any of these interactions.

6.10 Recognition of EcM Fungal Lineages

Based on the criteria in Sect. 6.2, we propose specific criteria for separation of EcM fungal lineages from related non-EcM groups (Table 6.1) using the ITS2 subregion and full ITS. For ITS2 and full ITS, respectively, 45 (53%) and 60 (70%) lineages could be reliably delimited based on the BLASTn score to query sequence length (S/L) ratio alone because of a significant phylogenetic gap between EcM and closely related non-EcM groups. One quarter of lineages exhibited a small range of S/L values, where trophic assignment is unambiguous. In these cases, assignment of individual lineages should be sought for support by manual BLASTn queries and/or phylogenetic analyses for greater reliability. In general, placement tended to be relatively more ambiguous for the most diverse EcM groups such as the /russula-lactarius, /inocybe, /clavulina, and /boletus lineages but not in the /tomentella-thelephora and /cortinarius lineages. Phylogenetic analyses suggested ambiguity in cases where the non-EcM outgroup(s) was separated by a relatively short stem (e.g., /tricholoma: Sánchez-García et al. 2014), or the outgroup had a low rate of ITS evolution (e.g., /inocybe: Ryberg et al. 2010), or there in rapidly evolving clades within the EcM lineages (e.g., /clavulina: Kennedy et al. 2012; /boletus: Nuhn et al. 2013). Except for /hysterangium, /inocybe, and /clavulina, <2% of OTUs across the lineages of ambiguously delimited groups fell into the uncertain range of S/L values, indicating the overall rate for correct placement at 97–98%.

6.11 Conclusions

With the addition of the /leotia, /porpoloma, /endogone3, and /phaeocollybia lineages to information from a previous review (Tedersoo and Smith 2013), the number of EcM fungal lineages has now grown to 82–86 separate groups comprising 279–284 genera. The rate of discovery of novel EcM lineages is notably declining because the most common groups have been already described. This is due to a huge increase in the number of in situ molecular identification studies of EcM fungal communities on roots as compared to a decade ago. However, the number of profound EcM community studies tends to decline in recent years, because most laboratories have switched to HTS-based identification of EcM fungi directly from bulked root and soil samples. Since EcM fungi naturally co-occur with many other fungal and eukaryote groups, it is impossible to verify the EcM habit from these types of studies. Our overview about the parameters of semiautomatic EcM lineage recognition should enable accurate trophic assignment of 95–99% of fungal OTUs to EcM and non-EcM categories. Further developments in this field should include development and automatized application of taxon-specific sequence similarity thresholds for taxa by using expert molecular taxonomic knowledge. In the future, it will also be important to use additional, phylogenetically or functionally informative loci for HTS-based

Table 6.1 Critical values for ITS2-based separation of EcM fungal lineages from non-mycorrhizal groups

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/acephala macrosclerotiorum	0.30	0.46	98	nd	nd	98
/albatrellus	0.47	0.40	80	0.34	0.60	85
/aleurina	0.37	0.46	82	0.34	0.87	85
/amanita	0.39	0.40	80	0.30	0.38	77
/amphinema-tylospora	0.60	0.56	85	0.50	0.70	90
/atheliales1	0.43	0.50	80	nd	0.50	80
/atheliales2	0.50	0.65	85	nd	nd	79
/austropaxillus	0.40	0.60	90	nd	0.70	90
/boletopsis	0.55	0.55	84	nd	nd	92
/boletus	0.40	0.12	75	0.30	0.50	80
/byssocorticium	0.57	0.50	84	nd	0.70	88
/cantharellus	0.30	0.15	76	nd	0.35	75
/catathelasma	nd	nd	95	nd	nd	95
/cenococcum	0.65	0.70	95	nd	nd	95
/ceratobasidium1	0.65	0.68	90	nd	nd	90
/ceratobasidium2	0.65	0.70	90	nd	nd	90
/clavariadelphus	0.40	0.50	90	nd	nd	90
/clavulina	0.40	0.28	80	0.35	0.45	80
/coltricia	0.10	0.25	75	0.25	0.33	75
/cortinarius	0.49	0.51	85	0.55	0.60	84
/densospora	nd	nd	nd	nd	nd	80
/descolea	0.56	0.50	83	nd	nd	85
/elaphomyces	0.15	0.27	72	nd	0.50	80
/endogone1	nd	nd	nd	nd	nd	90
/endogone2	0.35	0.40	nd	0.35	0.60	85
/endogone3	nd	nd	nd	nd	nd	nd
/entoloma	0.61	0.57	86	nd	nd	88
/galactinia	0.20	0.60	84	nd	nd	85
/genea-humaria	0.30	0.25	76	0.30	0.50	80
/geopora	0.33	0.66	88	nd	0.80	90
/guyanagarica (/agaricales1)	0.31	0.41	75	0.61	0.75	85
/hebeloma-alnicola	0.56	0.52	85	0.60	0.50	86
/helotiales1	0.77	0.79	96	0.70	0.95	96
/helotiales3	nd	nd	nd	nd	nd	95
/helotiales4	nd	nd	90	nd	0.80	90

(continued)

Table 6.1 (continued)

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/helotiales5	nd	nd	96	nd	nd	96
/helotiales6	nd	nd	nd	nd	nd	95
/hydnellum-sarcodon	0.31	0.40	84	0.30	nd	85
/hydnotrya	nd	nd	80	nd	nd	85
/hydropus	0.75	0.85	92	nd	nd	92
/hygrophorus	0.43	0.38	80	nd	0.50	82
/hysterangium	0.20	0.09	70	nd	0.15	75
/inocybe	0.42	0.35	80	0.48	0.50	80
/laccaria	0.74	0.64	88	nd	0.85	90
/leotia	nd	0.50	85	nd	0.50	80
/leucangium	nd	nd	75	nd	nd	78
/marcelleina-peziza gerardii	0.23	0.23	65	nd	0.50	80
/meliniomyces	nd	nd	97	0.95	0.97	98
/otidea	0.34	0.38	86	0.30	0.70	88
/pachyphloeus-amylascus	0.13	0.38	75	nd	0.55	82
/paralyophyllum	0.72	0.73	90	nd	nd	93
/paxillus-gyrodon	0.40	0.60	87	nd	0.75	88
/phaeocollybia	nd	0.50	82	nd	nd	85
/phaeohelotium (/helotiales2)	nd	nd	96	nd	nd	96
/phellodon-bankera	0.30	0.56	88	nd	0.70	87
/piloderma	0.51	0.49	85	0.40	0.55	82
/pisolithus-scleroderma	0.27	0.29	79	nd	0.50	82
/porpoloma	nd	nd	92	nd	nd	89
/pseudotomentella	0.42	0.51	85	nd	0.65	86
/pulvinula	0.55	0.48	77	0.60	0.70	85
/pustularia	0.60	0.75	91	nd	0.90	93
/pyronemataceae1	0.44	0.41	78	nd	0.60	82
/pyronemataceae2	nd	nd	90	nd	nd	90
/ramaria-gautieria	0.35	0.30	70	nd	0.40	78
/rhodoscypha	0.52	0.65	88	0.30	0.70	88
/russula-lactarius	0.30	0.22	78	0.40	0.60	83
/sarcosphaera-hydnotryopsis	nd	nd	85	nd	nd	80
/sebacina	0.48	0.51	80	nd	0.60	85
/serendipita1	0.68	0.75	93	nd	nd	92

(continued)

Table 6.1 (continued)

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/serendipita2	0.70	0.77	93	nd	nd	93
/sordariales1	0.55	0.49	85	0.45	0.60	83
/sordariales2	nd	0.70	90	nd	0.74	89
/sowerbyella	nd	nd	86	nd	nd	88
/sphaerosporella	0.49	0.56	85	nd	nd	86
/suillus-rhizopogon	0.12	0.63	82	nd	nd	80
/tarzetta	0.17	0.40	76	nd	0.70	88
/terfezia-peziza depressa	0.24	0.60	85	nd	0.60	85
/tomentella-thelephora	0.33	0.45	82	nd	0.70	88
/tomentellopsis	0.50	0.72	90	nd	0.80	90
/tremellodendropsis (agaricomycetes1)	0.65	0.63	89	nd	nd	90
/tricholoma	0.55	0.52	85	0.50	0.60	83
/tuber-helvella	0.30	0.50	80	nd	0.50	80
/tulasnella1	0.79	0.82	93	nd	nd	90
/tulasnella2	nd	nd	80	nd	nd	85
/wilcoxina	0.45	0.65	86	nd	nd	90
/xenasmatella	0.62	0.65	95	nd	nd	90

approaches beyond ITS sequencing. It should also be possible in the future to automatically assign traits and functions to the EcM fungi based on a combination of the taxonomy and what is known about reference taxa. Much has yet to be done to incorporate information about functional genes of taxa obtained from genomics studies and using probabilistic approaches rather than binary (presence/absence) functional assignments.

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