Systematics, diversity and evolution of the suborder Tricholomatineae (Agaricales)

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Marisol Sanchez Garcia
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ABSTRACT

The suborder Tricholomatineae is one of the several major groups of Agaricales, the largest order of mushroom-forming fungi. This suborder contains three families: Tricholomataceae, Entolomataceae and Lyophyllaceae, as well as many genera of *incertae sedis*. Members of the Tricholomatineae exhibit variation in nutritional mode, including mycoparasites, saprotrophs, termite-associates, bryophyte parasites, and ectomycorrhizal (ECM) symbionts, which makes the clade ideal for studying trophic evolution in fungi from a phylogenetic perspective.

This dissertation combines taxonomy and evolutionary analyses to contribute to the knowledge of fungal diversity and mycorrhizal evolution. First, I present a systematic revision of the family Tricholomataceae within a molecular context, including molecular annotations of generic types and other type collections, and reducing this family from 98 to seven genera. Then, I use a multi-gene phylogeny of the suborder Tricholomatineae to test the hypothesis that the ECM symbiosis is a key innovation that promoted species diversification. I find evidence that supports such increases in two of six ECM lineages of the Tricholomatineae. However, no increases in diversification are detected in the other four ECM clades of this suborder. This suggests that diversification of those two clades was not due to the evolution of the ECM lifestyle, and I hypothesize that it could be due to the expansion and dominance of its main hosts and ability to associate with a variety of hosts. Diversification in one of those two clades, the Rhodopolioid clade of the genus *Entoloma*, could be due to a unique combination of spore morphology and ECM habit. The spore morphology may represent an exaptation that aided spore dispersal and colonization. Finally, I describe a new genus with three new species from the Pakaraima Mountains in Guyana. These taxa are within the Catathelasma clade, which I recognize as the family Catathelasmataceae, that form an independent clade of ECM fungi and the first known case of a Neotropical endemic ECM lineage within the Agaricomycetes.

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INTRODUCTION

Taxonomy is fundamental to understanding biodiversity and has implications in ecology, conservation and evolutionary biology. Despite this essential role, it has been in decline during the last decade (Wilson, 2004; Schlick-Steiner et al., 2010). As new methodological tools to analyze molecular and morphological data to understand the evolutionary history of organisms are being developed, taxonomy is facing a big challenge. Most of these recently developed methods require complete phylogenies or knowledge of the degree of understanding (Alfaro et al., 2009; FitzJohn et al., 2009; Rabosky, 2014).

Mycologists face even a bigger challenge as fungal diversity is immense, with 720,000 – 5.1 million species estimated worldwide (Hawksworth, 2001; O'Brien et al., 2005; Blackwell, 2011), and only 98,000 species described to date (Kirk et al., 2008). This makes evident the need for more taxonomic research to discover, describe, and understand the vast fungal diversity.

In addition, fungi play vital roles in nutrient cycling processes in terrestrial ecosystems through decomposition of organic matter and the formation of symbiotic associations with different organisms. One such associations is the ectomycorrhizal (ECM) symbiosis, an association between fungi and the roots of plants, mainly dominant trees in forest ecosystems (Leake and Read, 1997), where plants obtain nutrients, increased water absorption, and protection against pathogens in exchange for photosynthates (Smith and Read, 2008).

The evolution of mycorrhizal associations had a remarkable impact on terrestrial ecosystems, facilitating the colonization of land by plants (Malloch, 1980; Wang et al., 2010). ECM fungi evolved independently among separate lineages of fungi from saprotrophic ancestors with no known reversals (Hibbett et al., 2000; Bruns and Shefferson, 2004; Matheny et al., 2006). It has been shown, however, that ECM fungi retain some ancestral capabilities for decomposition and are direct contributors to loss of soil carbon (Buée et al., 2007; Talbot et al., 2008; Martin and Nehls, 2009). These new findings have implications for understanding the carbon cycle and the role of ECM fungi in forest ecosystems,

and require new parameters in models of global climate change (Talbot et al., 2008; Todd-Brown et al., 2011).

Dissertation outline

This dissertation combines taxonomy and evolutionary analyses to contribute to knowledge of fungal diversity and mycorrhizal evolution. I used as a study system the suborder Tricholomatineae, which is one of the several major groups of Agaricales, the largest order of mushroom-forming fungi. This suborder contains three families: Tricholomataceae, Entolomataceae, and Lyophyllaceae, as well as many genera of *incertae sedis* (Matheny et al., 2006; Binder et al., 2010; Dentinger et al., 2015). Members of the Tricholomatineae exhibit variation in nutritional mode, including mycoparasites, saprotrophs, termite-associates, bryophyte parasites, and ECM symbionts (Matheny et al., 2006; Hofstetter et al., 2014), which makes it an ideal clade to study trophic evolution in fungi from a phylogenetic perspective.

In chapter I, I reconstruct a molecular phylogeny to provide information about the delimitation and relationships of taxonomic groups within the suborder Tricholomatineae and specifically within the family Tricholomataceae.

The main focus of this research is to systematically re-evaluate the family Tricholomataceae, including ribosomal RNA (nLSU, nSSU and ITS), and protein-coding *rpb2* sequences. The aims of this work are (i) to generate a multi-gene phylogeny on which to base a modern systematic revision of the Tricholomataceae s.str.; (ii) to circumscribe genera belonging to this family based on a well-supported phylogeny; and (iii) to provide a taxonomic key for their identification.

This work represents the first systematic revision of the Tricholomataceae within a molecular context, including molecular annotations of generic types and other type collections. The Tricholomataceae is substantially reduced in circumscription comprising seven genera and one species-poor grade composed of taxa that merit further study. In addition to the Tricholomataceae, at least twelve other lineages can now be placed in the Tricholomatoid clade:

Entolomataceae, Lyophyllaceae, *Singerocybe*, *Clitocybe-Lepista-Collybia*, *Clitocybe* p.p., the *Catathelasma* clade, *Giacomia*, *Pseudoclitopilus*, *Pseudoclitocybe-Musumecia-Pogonoloma*, *Notholepista*, *Aspropaxillus*, and *Infundibulicybe*.

In chapter II, I use a multi-gene phylogeny of the suborder Tricholomatineae to evaluate whether the ECM mode is a key innovation that promoted diversification within this suborder. I test this by inferring the nutritional mode of members of this suborder using stable isotope ratio techniques to predict the source of carbon and nitrogen of these taxa. I hypothesize that the ECM mode has evolved multiple times in the Tricholomatoid clade, and that these transitions represent evolutionary key innovations that promoted species to diversify at a higher rate than in non-ECM lineages. I find evidence that supports increases in diversification in two of six ECM lineages of the Tricholomatineae. One of these, *Tricholoma*, most likely began diversification by the late Eocene, which was probably favored by cooling temperatures and the expansion of their host communities, as shown in other groups of ECM fungi (Ryberg and Matheny, 2012; Looney et al., 2016). The second clade, the Rhodopolioid clade began diversification by the early Miocene. Spore morphology in this group could be a pre-adaptation or exaptation that favored spore dispersal and colonization. This is the first work that aims to test the hypothesis that the ECM lifestyle is a key innovation by comparing the net diversification rates of ECM lineages with those of saprotrophic clades.

In chapter III, I describe a new genus (*Guyanagarika*) and three new species (*G. aurantia, G. pakaraimensis*, and *G. anomala*) of ECM fungi from the Pakaraima Mountains of Guyana in the Guiana Shield. These taxa form associations with *Dicymbe* spp. Molecular phylogenetic analyses place these taxa within the Catathalasma clade, a lineage in the suborder Tricholomatineae. I recognize this clade as the family Catathelasmataceae. *Guyanagarika* represents the first known case of a neotropical endemic ECM lineage within the Agaricales.

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CHAPTER I

Deconstructing the Tricholomataceae and introduction of the new genera *Albomagister, Corneriella, Pogonoloma* and *Pseudotricholoma*

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MSG obtained and analyzed the data, and wrote the manuscript. PBM contributed substantially to manuscript revisions. GP provided type specimens. DJL provided sequence data, collections, and contributed to manuscript revisions.

Abstract

The family Tricholomataceae, contained within the suborder Tricholomatineae, has traditionally been one of the largest families of the Agaricales. However, in this sense it is highly polyphyletic and requires emendation. Here we present a phylogeny of the Tricholomatineae based on nucleotide sequence data from two nuclear ribosomal RNA genes (large subunit and small subunit) and the second largest subunit of RNA polymerase II (*rpb2*). Our aim is to delimit the Tricholomataceae and identify monophyletic groups within the suborder Tricholomatineae. We also infer a separate phylogeny, based on the three genes above, in addition to sequences of the nuclear ribosomal internal transcribed spacers (ITS), in order to evaluate generic-level boundaries within the Tricholomataceae s.str. Based on this analysis we recover seven monophyletic genera within the Tricholomataceae s.str. that correspond to *Leucopaxillus*, *Tricholoma*, *Pseudotricholoma* stat. nov., *Porpoloma* s.str., *Dennisiomyces*, *Corneriella* gen. nov., and *Albomagister* gen. nov. Of the 98 genera that have been traditionally assigned to the Tricholomataceae sensu Singer, only four can be placed within it (*Tricholoma*, *Porpoloma*, *Dennisiomyces*, and *Leucopaxillus*). The genus *Porpoloma* is highly polyphyletic and divided into four genera: *Porpoloma* s.str., *Corneriella* gen. nov., *Pseudotricholoma* stat. nov., and *Pogonoloma* stat. nov. In all, four new genera are proposed. Taxonomic

descriptions, and a key to genera of the Tricholomataceae as emended here are also presented.

Introduction

Fungal classification has become more stable at the ordinal level and above (Hibbett et al., 2007) although novelties and relationships among higher-level taxa of Basidiomycota continue to be discovered (Zalar et al., 2005; Binder et al., 2010; Hodkinson et al., 2014). The order Agaricales is the largest order of mushroom-forming fungi (Agaricomycotina) with some 13,500 species (Kirk et al., 2008). Attempts have been made to recognize monophyletic groups within the Agaricales (Moncalvo et al., 2002) and major groupings within it (Matheny et al., 2006). These works, and others (Bodensteiner et al., 2004; Gulden et al., 2005; Garnica et al., 2007) have contributed to recognition of additional subclades and family-level groupings such as now found in regional floras (Knudsen et Vesterholt, 2012).

One such grouping, the suborder Tricholomatineae, which includes three families, the Tricholomataceae, Entolomataceae, and Lyophyllaceae (Matheny et al., 2006; Binder et al., 2010), has received considerable attention of late as the highly polyphyletic genus *Clitocybe* (Fr.) Staude has undergone extensive revision (Harmaja, 2003; Ammirati et al., 2007; Vizzini et al., 2011b). The Entolomataceae (Co-David et al., 2009; Baroni et al., 2011; Morgado et al., 2013) and Lyophyllaceae (Hofstetter et al., 2002) have been revised as well. However, little attention has been given to the Tricholomataceae, which in a broad sense once included as many as 98 genera (Singer, 1986).

The Tricholomataceae was first referred to as the Tricholomées by Roze (1876), including *Tricholoma* (Fr.) Staude, *Entoloma* (Fr. ex Rabenh.) P. Kumm, and *Hebeloma* (Fr.) P. Kumm.; however, this name was not validly published since it contains a French termination instead of a Latin termination (McNeill, 2012: Art. 18.4). In 1927, van Overeem (1927) published the name Tricholomataceae as a *nomen nudum* (Art. 38.1). Later Heim (1934) published the name "Tricholomaceae" also as a *nomen nudum*. Many authors continued using the

name Tricholomataceae assuming it was validly published (e.g., Singer, 1975; Pegler, 1977). Pouzar (1983) provided a valid diagnosis and thus validly published the family name. To maintain the traditionally accepted broad application of the name Tricholomataceae, despite the fact that numerous valid family names for it were published prior to 1983, the name Tricholomataceae is conserved against 24 other family names (McNeill et al. 2006).

Before the advent of molecular phylogenetics, others treated the Tricholomataceae in somewhat different ways. Bon (1974), for instance, split the family into six subfamilies: Tricholomatoideae (Sing.) Bon, Lepistoideae Bon, Leucopaxilloideae (Sing.) Bon, Lyophylloideae Kühner ex Bon, Collybioideae (Konr. et Maubl.) Bon, and Mycenoideae Bon. Jülich (1981) was the first to split the Tricholomataceae into separate families – Leucopaxillaceae Jülich (e.g., *Leucopaxillus* Boursier, *Porpoloma* Singer, *Cantharellula* Singer), Lyophyllaceae Jülich (e.g., *Lyophyllum* P. Karst and *Calocybe* Kühner), and Mycenaceae Overeem (e.g., *Mycena* (Pers.) Roussel, *Baeospora* Singer, *Dennisiomyces* Singer). Pouzar (1983) excluded some genera considered by Singer (1986) as part of the Tricholomataceae such as *Rhodotus* R. Maire, *Termitomyces* Heim, *Macrocystidia* Joss., and *Catathelasma* Lovejoy. Singer (1986) presented a broadly circumscribed Tricholomataceae encompassing 98 genera with a pale spore print (white, cream color, light creamy pink, pale violet, light greenish, or pale sordid grayish), lamellae variously attached to the stipe (sometimes free, adnexed, adnate, sinuate, or decurrent); lamellar trama interwoven, irregular, subregular or regular; spores amyloid or inamyloid; and occurring in a wide range of environments, e.g., on the ground in woods, on living hosts, or in prairies.

The polyphyly of the Tricholomataceae was demonstrated in several molecular systematic works (Hofstetter et al., 2002; Moncalvo et al., 2000; 2002; Matheny et al., 2006; Garnica et al., 2007). Some taxa previously included in Tricholomataceae have been placed in other families such as Lyophyllaceae

(Hofstetter et al., 2002), Mycenaceae (Moncalvo et al., 2002), Physalacriaceae (Binder et al., 2006), and Hygrophoraceae (Lodge et al., 2014), vindicating some efforts of Jülich (1981) (see Table I.1[1] for an overview of the currently known phylogenetic distribution of Singer's 98 genera of Tricholomataceae). Although there have been some efforts to delimit fungal families, the limits and constituents of the Tricholomataceae in its strict sense have not been redefined or identified based on molecular evidence.

The main focus of this research is to systematically re-evaluate the family Tricholomataceae. We present phylogenetic analyses of 160 taxa of the Tricholomatineae (Matheny et al., 2006), including ribosomal RNA (nLSU, nSSU and ITS), and *rpb2* sequences. The aims of this work are (i) to generate a multi-gene phylogeny on which to base a modern systematic revision of the Tricholomataceae s.str.; (ii) to circumscribe genera belonging to this family based on a well-supported phylogeny; and (iii) to provide a taxonomic key for their identification.

Materials and Methods

Field collections and microscopy. — Notes of macroscopic features were taken from fresh collections in the field following protocols of Largent et al. (1977). Many were photographed *in situ* or in a laboratory setting. Colors of fresh specimens were documented with the Methuen handbook of Colour (Kornerup et Wanscher, 1984) or Munsell soil color charts (Munsell, 1975). Specimens were then air-dried on a food dehydrator for later microscopic examination and DNA extraction. Dried materials collected by us, as well as, materials borrowed from herbaria were examined to obtain morphological information on spores, cystidia, pileipellis, and other microscopic characters.

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[1] All tables and figures are located in the Appendix

Taxon sampling. — The selection of taxa for this study was based on previous works that included members of the Tricholomatineae (Moncalvo et al., 2000; 2002; Matheny et al., 2006; Binder et al., 2010; Vizzini et al., 2012). Sequences available in GenBank from previous studies were downloaded (Table I.2). Additionally, new sequences were produced to extend taxon and gene sampling (Table I.2). Type species were specifically targeted for sequencing in order to correctly assign generic names to clades. Specimens were borrowed from the following herbaria: CORT, FL, FH, GB, IBUG, K, MICH, NYBG, TENN, XAL, and WTU (herbarium abbreviations per Thiers [continuously updated]). Type specimens of *Dennisiomyces griseus* (Dennis) Singer, *Hygrophorus subaustralis* A.H. Sm. & Singer, *Leucopaxillus septentrionalis* Singer & A.H. Sm., *Porpoloma bambusarum* Desjardin & Hemmes, *Pr. sejunctum* Singer, *Pr. portensosum* Singer, *Pr. terreum* Singer, and *Pr. umbrosum* (A.H. Sm. & M.B. Walters) Singer were sampled for molecular annotation. Nomenclatural novelties have been registered in MycoBank (Crous et al., 2004).

DNA extraction, PCR, sequencing and cloning. — Genomic DNA was extracted using 10–30 mg of dried basidiome tissue. Samples were ground in liquid nitrogen with a mortar and pestle. DNA extractions were performed using the E.Z.N.A. Fungal DNA Kit (Omega Bio-Tek, Norcross, Georgia), for specimens younger than 30 years old and the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Norcross, Georgia) for specimens older than 30 years.

Primer pairs used were as follows: for the internal transcribed spacer (ITS): ITS1F-ITS4, ITS1F-ITS2 and 5.8S-ITS4 (White et al., 1990; Gardes et Bruns, 1993); for the nuclear large subunit ribosomal DNA (nLSU): LR0R-LR7, LR0R-LR5 and LR0R-LR16 (Vilgalys et Hester, 1990, http://www.biology.duke.edu/fungi/mycolab/primers/htm); for the nuclear small subunit ribosomal DNA (nSSU): PNS1-NS8, PNS1-NS41 and NS51-NS8 (White et al., 1990; Hibbett, 1996) and for the second largest subunit of RNA polymerase II (*rpb2*), b6f-b7.1R (Matheny, 2005). The same primers were used for sequencing with the addition of NS19b (http://nature.berkeley.edu/brunslab/tour/primers.html) for nSSU.

PCR conditions to amplify ITS, nLSU, and nSSU are outlined in White et al. (1990) and for *rpb2* in Matheny (2005). PCR products were visualized on a 1% agarose gel. Successfully amplified products were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, California) following the manufacturer's protocol. Sequence reactions were prepared with BigDye Terminator 3.1 (Applied Biosystems, Foster City, California), purified using Sephadex G-50 columns (General Electric Healthcare, Piscataway, New Jersey), and sequenced on an automated DNA sequencer (ABI 3730) at the Molecular Biology Resource Facility of the University of Tennessee. Raw sequences were edited and assembled using Sequencher 4.9 (Gene Codes, Ann Arbor, Michigan).

Some chromatograms showed sequences with several indels; therefore, the purified PCR products of those samples were inserted into a pCR 2.1-TOPO plasmid vector and cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, California) following the manufacture's protocol. The analysis of the positive transformants was done screening colonies by PCR using the primer pairs M13F-M13R. When the presence of the expected product was observed in the electrophoresis gel, PCR products were purified and sequenced following the methods detailed above.

Phylogenetic analyses. — Two datasets were assembled, one containing members of the suborder Tricholomatineae (Matheny et al., 2006; Binder et al., 2010) to show the placement and delimitation of the Tricholomataceae s.str. and the second containing only members of Tricholomataceae s.str. to delimit genera belonging to this family.

The Tricholomatineae dataset includes 123 taxa (120 nLSU, 62 nSSU, and 70 *rpb2* sequences). The Tricholomataceae dataset includes 110 taxa (65 nLSU, 26 nSSU, 38 *rpb2*, and 101 ITS sequences). For the latter dataset we included ITS sequences to increase resolution at the generic and infrageneric level and to increase statistical support, as it has been shown in previous works (Lawrey et al., 2009; Lodge et al. 2014). One taxon that was not represented in the Tricholomatineae dataset, but included in the Tricholomataceae dataset, was *Porpoloma pes-caprae* (Fr.) Singer. This taxon could not be included in the larger

and more inclusive analysis because it is represented by only an ITS sequence. However, the closest match in a BLASTn search was *Tricholoma myomyces* (Pers.) J.E. Lange (82%), which justifies its inclusion in the Tricholomataceae dataset.

Alignments were constructed separately for each rRNA region and *rpb2* using MAFFT 6.717 (Katoh et Toh, 2008), manually adjusted in MacClade 4.08 (Maddison et Maddison, 2005), and concatenated in SeaView 4.2.3 (Gouy et al., 2010). Regions of the ITS dataset with ambiguous alignments were excluded. Unsampled gene regions were coded as missing data. Simulation studies show that despite large amounts of missing data, adding taxa can improve phylogenetic resolution (Wiens, 2006; Wiens et Morrill, 2011). Partition strategies for each concatenated dataset and the best-fit models of molecular evolution were chosen using PartitionFinder 1.0.1 (Lanfear et al., 2012).

Maximum Likelihood (ML) was performed on both datasets using RAxML 7.9.1 (Stamatakis, 2006), implementing the GTR+GAMMA+I model, and executing 1000 rapid ML bootstraps replicates. Bayesian inference (BI) analyses were performed using MrBayes 3.2.2, also implementing the GTR+GAMMA+I model (Huelsenbeck et Ronquist, 2001; Ronquist et Huelsenbeck, 2003; Altekar et al., 2004) with two independent runs and four chains, sampling every 5000 generations. Output was evaluated using Tracer 1.5 (Rambaut et Drummond, 2007) and AWTY (Nylander et al., 2008) in order to determine whether the chains reached the stationary phase and the appropriate number of generations to discard as burn-in. In addition, the standard deviation of split frequencies was considered for this purpose following the MrBayes manual. The BI analyses were run for 50 million generations with a burn-in of 12.5 million generations, leaving 7501 trees per run to summarize into a maximum clade credibility tree using TreeAnnotator 1.7.5 distributed as part of the BEAST package (Drummond et al., 2012). We consider bootstrap values >70% and Bayesian posterior probabilities >0.95 as evidence for significant or strong support. *Infundibulicybe gibba* (Pers.) Harmaja was used to root the Tricholomatineae phylogeny following Matheny et al. (2006), Binder et al. (2010), and Lodge et al. (2014). The Tricholomataceae phylogeny was midpoint rooted as the sister group of this family is not known

with confidence. Rooting was done in FigTree v1.4.0. Individual datasets (ITS, nLSU, nSSU and *rpb2*) were examined separately under the ML criterion to inspect for intergene conflict, in which different gene regions strongly support different topologies. After this assessment, the gene datasets were concatenated and analyzed using ML and BI as described above.

Results

Delimitation of the family Tricholomataceae s.str. — The Tricholomatineae dataset contains 4372 included sites. The alignment was separated in five partitions: (1) nLSU; (2) nSSU; (3) *rpb2* first codon positions; (4) *rpb2* second codon positions; and (5) *rpb2* third codon positions. We did not detect cases of strongly supported intergene conflict.

The ML and Bayesian analyses of the concatenated datasets do not show major conflicts in any of the well-supported clades of the trees.

Phylogenetic analyses of the Tricholomatineae dataset (Fig. I.1) recover a robust grouping (99% MLBP and 1.00 BPP) composed of six major subgroups that share a most recent common ancestor with the genus *Tricholoma*. We recognize this group as the Tricholomataceae s.str. with the following genera: (1) *Dennisiomyces* (type: *D. glabrescentipes* Singer), (2) *Porpoloma* s.str. (type: *Pr. sejunctum*), (3) *Corneriella* gen. nov. (type: *Co. bambusarum*), (4) *Albomagister* gen. nov. (type: *A. subaustralis*), (5) *Tricholoma* (type: *T. flavovirens* (Pers.:Fr.) S. Lundell), and (6) *Pseudotricholoma* stat. nov. (type: *Ps. umbrosum*) (7) *Leucopaxillus* (type: *L. paradoxus* (Costantin et L.M. Dufour) Boursier). The species *Clitocybe adirondackensis* (Peck) Sacc., is recovered as sister to the Tricholomataceae s.str. but with weak support (<50% MLBP and <0.95 BPP).

The genera *Tricholoma*, *Dennisiomyces*, and *Leucopaxillus* were recovered as monophyletic. Each of these groups received high posterior probabilities and bootstrap values. We also recovered a monophyletic group containing *Hygrophorus subaustralis* and four undetermined specimens collected in the southern Appalachians that resemble small species of *Tricholoma.* However, their small inamyloid spores, prominent pleurocystidia and cheilocystidia, and

presence of clamp connections exclude them from this and any other tricholomatoid genera, thus *Albomagister* gen. nov. is proposed to accommodate *H. subaustralis*.

The genus *Porpoloma* is shown to be highly polyphyletic with species distributed in four different groups within the suborder Tricholomatineae, three of which are found in the Tricholomataceae s.str.: (1) *Porpoloma* s.str., (2) *Corneriella* gen. nov., and (3) *Pseudotricholoma* stat. nov. The fourth clade, which is sister to *Pseudoclitocybe* (Singer) Singer and *Musumecia* Vizzini and Contu with strong support, contains *Pr. spinulosum* (Kühner et Romagn.) Singer and *Pr. macrocephalum* (Schulzer) Bon. *Porpoloma* subg. *Pogonoloma* Singer is elevated to generic level to accommodate these two taxa.

Delimitation of seven genera in the Tricholomataceae s.str. — The Tricholomataceae dataset consists of 4729 characters after the exclusion of ambiguously aligned regions in the ITS (186–230, 244–273, 289–328, 808–823, 861–872). The alignment was separated in five partitions: (1) ITS; (2) nSSU and nLSU; (3) *rpb2* first codon positions; (4) *rpb2* second codon positions; and (5) *rpb2* third codon positions.

The ML and Bayesian analyses show consistent topologies recovering the same main clades with high support (>70% MLBP and >0.95 BPP). The phylogeny produced from this dataset (Fig. I.2) shows the same seven main clades as in Fig. I.1, and a single branch representing *Pr. pes-caprae* referred to as the "*pes-caprae*" lineage.

Leucopaxillus contains representatives from Europe, Australia, and North and South America, including samples from temperate and tropical regions. The genus is characterized by species with amyloid and verrucose spores and presence of clamp connections on basidiocarp tissues. The presence of clamp connections and absence of urticoid hymenial cystidia distinguish it from *Melanoleuca* Pat., species of which may outwardly resemble those of *Leucopaxillus*. *Melanoleuca*, however, is a member of the Pluteoid clade (Matheny et al., 2006; Garnica et al., 2007).

Sister to *Leucopaxillus* with strong support is *Pseudotricholoma* stat. nov., a clade that contains two temperate taxa from the Northern Hemisphere, *Ps. umbrosum* and *Ps. metapodium* (Fr.) Singer. The name *Pseudotricholoma* is elevated to genus level following the classification of Singer (1986) where he included *Po. umbrosum* as the type species of *Porpoloma* subg. *Pseudotricholoma*. The two species in this clade are united by their smooth amyloid spores and red bruising reaction to the context of the basidiocarps.

Tricholoma appears to be sister to *Pseudotricholoma* and *Leucopaxillus* but with weak support (Fig. I.2). The circumscription and monophyly of *Tricholoma* has been well established (Bon, 1984; Riva, 2003). The "*pes-caprae*" lineage (based on one sample and ITS data only) could also be the sister group to *Tricholoma*, but support for this relationship is not significant (<65% MLBP and <0.95 BPP) (Fig. I.2).

Porpoloma s.str. contains sequence data from three type specimens, *Pr. sejunctum*, *Pr. terreum*, and *Pr. portentosum*. The first one, *Pr. sejunctum*, is also the type of the genus, therefore, the generic name *Porpoloma* can readily be assigned to members of this clade. The other two species included in the group (*Porpoloma* sp. 1 and *Porpoloma* sp. 2) were collected in Tasmania. In this clade there are also four environmental samples from South American *Nothofagus* forests (Nouhra et al., 2013), three of which correspond to *Pr. terreum* and were isolated from *N. dombeyi* (Mirb.) Oerst. and *N. obliqua* (Mirb.) Oerst. roots. Another represents *Pr. sejunctum* isolated from *N. dombeyi* roots. *Porpoloma* s.str. is restricted to the Southern Hemisphere forming ECM associations with *Nothofagus* (Trappe, 1962; Griffith, 2004) and probably with *Eucalyptus* L'Her and/or *Acacia* Mill.

The genus *Dennisiomyces* comprises mainly neotropical species. This clade contains the type species of the genus, *D. glabrescentipes*, the type of *D. griseus*, and five unidentified species, four from the Caribbean and one from the southeast United States (at least seven species in all).

Sister to *Porpoloma* and *Dennisiomyces* is the new genus *Corneriella*. The grouping of these three genera receives significant support (89% MLBP and 0.99 BPP). *Corneriella* includes four tropical species, *Porpoloma bambusarum* described

from Hawaii, *Cantharellula humicola* Corner described from Malaysia, and two unidentified species from Puerto Rico and Brazil. The close relationship between *Pr. bambusarum* and *Ca. humicola* was previously suggested by Desjardin and Hemmes (2001). In the present work we confirm that these two taxa are closely related and belong to the new genus *Corneriella*. *Cantharellula* is not monophyletic as the type of the genus (*C. umbonata*) is now placed in the Hygrophoraceae (Lodge et al., 2014). One of the unidentified species of *Corneriella* was previously labeled as *Porpoloma* sp. (PR3995). Sequences from this collection have been included in previous phylogenies (Moncalvo et al., 2002; Vizzini et al., 2012), where it has been treated as *Porpoloma*.

The last clade includes *Hygrophorus subaustralis* and three unidentified specimens collected from the southeast United States. *Hygrophorus subaustraulis* superficially resembles some small white *Leucopaxillus* or *Tricholoma* species, however, its microscopic characters and phylogenetic placement suggest it belongs to a different genus. Therefore, the genus *Albomagister* gen. nov. is established to accommodate this and two other undescribed species. The latter two are morphologically similar to *H. subaustralis,* but they form separate phylogenetic lineages (Fig. I.2). ITS sequences of these two are 82–83% similar to those of *A. subaustralis*. The taxonomy of these is under investigation.

Two additional lineages, ECV4038 and MBP06VIII09, form a grade with respect to *Albomagister*. Presently, each is known only from a single collection and they are insufficiently known.

Taxa outside the Tricholomataceae s.str. in the Tricholomatineae clade. — Two families, the Entolomataceae and Lyophyllaceae, cluster outside the Tricholomataceae s.str. (Fig. I.1) as expected from prior works (Hofstetter et al., 2002; Moncalvo et al., 2002; Matheny et al., 2006). *Singerocybe*, as mentioned above, is recovered as sister to the Tricholomataceae s.str., but this result is weakly supported. An ensemble of additional clades was recovered in the Tricholomatineae consistent with several prior studies (Matheny et al., 2006; Ammirati et al., 2007; Vizzini et al., 2011a; 2012), but their positions within the suborder Tricholomatineae clade tend to be poorly supported and thus not well-

resolved: (1) a strongly supported group containing *Clitocybe* s.str., *Lepista* (Fr.) W.G. Sm., and *Collybia* (Fr.) Staude; (2) *Clitocybe* p.p.; (3) samples of the *Catathelasma* clade; (4) *Pseudoclitopilus* Vizzini et Contu; (5) *Giacomia* Vizzini et Contu; (6) a robust monophyletic grouping of *Pseudoclitocybe*, *Musumecia*, and *Pogonoloma* stat. nov.; (7) *Notholepista* Vizzini et Contu; (8) *Aspropaxillus*; and (9) *Infundibulicybe* Harmaja.

Taxonomy

Tricholomataceae R. Heim ex Pouzar, *Česká Mykol.* 37: 175. 1983.
Type genus: *Tricholoma* (Fr.) Staude, Schwämme Mitteldeutschl.: xxviii, 125. 1857.

Habit tricholomatoid, rarely tricholomatoid-collybioid. Pileus conical, convex, plano-convex to applanate, smooth, tomentose, or scaly, dry or viscid, rarely hygrophanous. Lamellae adnate, adnexed, sinuate-emarginate to decurrent. Spore deposit pure white, rarely pale cream. Spores subglobose, ellipsoid or ellipsoid-oblong, hyaline, thin-walled, without a germ pore, smooth or verrucose, amyloid or inamyloid, not dextrinoid. Species with inamyloid spores never with decurrent lamellae. Basidia without siderophylous granulation. Cystidia present or absent as cheilocystidia, pleurocystidia present in some groups. Hymenophoral trama regular. Pileipellis a cutis, ixocutis or trichoderm. Clamps present or absent. Mycorrhizal or saprotrophic on soil, humus and debris in forests and grasslands. Primarily north and south temperate in distribution, some tropical.

Genera included: *Albomagister* gen. nov., *Corneriella* gen. nov., *Dennisiomyces*, *Leucopaxillus*, *Porpoloma*, *Pseudotricholoma* stat. nov., and *Tricholoma* (Fig. I.3A–G).

Albomagister Sánchez-García, Birkebak & Matheny, **gen. nov.**
MycoBank MB807772
Type: ***Albomagister subaustralis*** (A.H. Sm. & Hesler) Sánchez-García, Birkebak & Matheny, **comb. nov.** ≡ *Hygrophorus subaustralis* A. H. Sm. & Hesler, Lloydia 5(1): 46. 1942 – Holotype: USA: NC, Great Smoky Mountains National Park,

Martin's gap, along the trail to Bryson Place, 2 Sept 1938, *A.H. Smith 10844* (MICH!). (Fig. I.3A).
MycoBank MB807777
Etymology: from the Latin *albus*=white; *magister*=mister, referring to the field name applied to this fungus "Mr. white".

Habit tricholomatoid. Lamellae sinuate to adnexed, white, edges concolorous. Spores inamyloid, smooth, thin-walled. Cheilocystidia and pleurocystidia present and conspicuous, thin-walled and hyaline. Pileipellis with ascending interwoven cylindrical elements. Clamp connections present. On soil, in forests beneath woody vascular angiosperms and coniferous plants. Probably ectomycorrhizal (Birkebak et al., 2013).

Corneriella Sánchez-García, **gen. nov.**
MycoBank MB808480
Type: ***Corneriella bambusarum*** (Desjardin & Hemmes) Sánchez-García, **comb. nov**. ≡ *Porpoloma bambusarum* Desjardin & Hemmes, Harvard Pap. Bot. 6: 97. 2001 – Holotype: USA: HI, O'ahu, Manoa Balley, Manoa Falls Trail, 8 Jan 1992, D.E. *Desjardin 5462*, (SFSU n.v.; isotype: BISH n.v., NYBG!). (Fig. I.3E).
MycoBank MB808481
Etymology: Named after Edred John Henry Corner, a British botanist and mycologist who described *Ca. humicola*, a species now transferred to this genus.
Habit tricholomatoid. Lamellae adnexed to sinuate or decurrent, sometimes forked, white when young, becoming darker. Spores amyloid, smooth, thin-walled. Cheilocystidia present, conspicuous, of various shapes, cylindric to flexuous, lageniform or fusoid, thin-walled. Pleurocystidia absent. Pileipellis a cutis with suberect to erect terminal cells. Clamp connections present. On soil and humus. Putatively saprotrophic.

Corneriella humicola (Corner) Sánchez-García, **comb. nov.** ≡ *Cantharellula humicola* Corner, Beih. Nova Hedwigia 109: 13. 1994 – Holotype: MALAYSIA: Peninsular Malaysia, Pahang, Pasoh forest, 30 Sept 1966, *E.J.H. Corner* (E n.v.).

MycoBank MB808482

Dennisiomyces Singer, Anais Soc. Biol. Pernambuco 13(1): 225. 1955. (Fig. I.3B).
Type: *Dennisiomyces glabrescentipes* Singer, Anais Soc. Biol. Pernambuco 13(1): 225. 1955.

Leucopaxillus Boursier, Bull. Trimest. Soc. Mycol. Fr. 41(3): 393. 1925. (Fig. I.3C).
Type : *Leucopaxillus paradoxus* (Costantin & L.M. Dufour) Boursier, Bull. Trimest. Soc. Mycol. Fr. 41(3): 393. 1925 ≡ *Clitocybe paradoxa* Costantin & L.M. Dufour, Nouv. Fl. Champ., Edn 1 (Paris): 262. 1896 ≡ *Lepista paradoxa* (Costantin & L.M. Dufour) Maire, Bull. Soc. Mycol. France 40 (3): 307. 1926.
Comments: The current definition of *Leucopaxillus* excludes species with smooth spores characterized by a weak amyloid reaction. These species are now placed within *Aspropaxillus* Kühner & Maire (type: *A. giganteus* (Sowerby) Kühner & Maire) (Vizzini & al., 2012).

Porpoloma Singer, Sydowia 6(1–4): 198. 1952. (Fig. I.3G).
Type: *Porpoloma sejunctum* Singer, Sydowia 6(1–4): 198. 1952.

Pseudotricholoma (Singer) Sánchez-García & Matheny, **stat. nov.** *Cantharellula* subg. *Pseudotricholoma* Singer, Sydowia 2: 30. 1948 ≡ *Porpoloma* sect. *Pseudotricholoma* (Singer) Bon, Doc. Mycol. 9(33): 25. 1978.
MycoBank MB807899
Type: ***Pseudotricholoma umbrosum*** (A.H. Sm. & M.B. Walters) Sánchez-García & Matheny, **comb. nov.** ≡ *Tricholoma umbrosum* A.H. Sm. & M.B. Walters, Mycologia 35(4): 477. 1943 ≡ *Cantharellula umbrosa* (A.H. Sm. & M.B. Walters) Singer, Lilloa 22: 238. 1951 ≡ *Porpoloma umbrosum* (A.H. Sm. & M.B. Walters) Singer, Sydowia 15(1–6): 53. 1962 [1961] ≡*Porpoloma elytroides* var. *umbrosum* (A.H. Sm. & M.B. Walters) Bon, Doc. Mycol. 20 (78): 38. 1990 – Holotype: CANADA: Nova Scotia, *prope* Truro, 16 Jul 1931, *Wehmeyer 640*, leg. A.H. Smith (MICH!). (Fig. I.3F).

MycoBank MB807901
Habit tricholomatoid, pileus dry, fibrillose. Lamellae adnate to emarginate, pale to brownish, edges concolorous, when bruised reddening then blackening. Spores amyloid, smooth, ellipsoid to ellipsoid-oblong, thin-walled. Cheilocystidia absent or rare. Pleurocystidia absent. Pileipellis a cutis of cylindrical hyphae. Clamp connections present. On soil, in woods and grasslands of the Northern Hemisphere. Probably biotrophic or ectomycorrhizal.

Pseudotricholoma metapodium (Fr.) Sánchez-García & Matheny, **comb. nov.** ≡ *Agaricus metapodius* Fr., Observ. Mycol. (Havniae) 2: 110. 1818 ≡ *Hygrophorus metapodius* (Fr.) Fr., Epicr. syst. Mycol. (Upsaliae): 328. 1838 (1836-1838) ≡ *Camarophyllus metapodius* (Fr.) Wünsche, Die Pilze. Eine Anleitung zur Kenntniss derselben: 115. 1877 ≡ *Neohygrocybe metapodia* (Fr.) Herink, Sb. severoceského Musea, Historia Naturalis 1: 71. 1958 ≡*Hygrocybe metapodia* (Fr.) M.M. Moser, Kl. Krypt.-Fl., Edn 3 (Stuttgart) 2b/2: 63. 1967 ≡ *Porpoloma metapodium* (Fr.) Singer Beihefte zur Sydowia 7: 19. 1973 ≡ *Tricholoma metapodium* (Fr.) Papetti, Boll. del Circulo Micologico 'Giovanni Carini' 37: 48. 1999 – **Neotype (here designated):** SWEDEN: Västergötland, Vänersborg, Vänersborg, Rudet SV, Gundlebosjön N, 8 Aug 2006, *Leif & Anita Stridvall 06/134* (GB!).
MycoBank MB807902
Comments: When Fries originally described this taxon, he did not indicate a typo for the name. No later authors, including those of the subsequent combinations, designated a type. Despite an exhaustive search, we were unable to find any original material. Therefore, we select Leif & Anita Stridvall 06/134 as the neotype. The specimen is from Sweden and it was collected relatively close from Femsjö, where Fries collected the original material.

Tricholoma (Fr.) Staude, Schwämme Mitteldeutschl.: xxviii, 125. 1857. (Fig. I.3D). Type : *Tricholoma flavovirens* (Pers.:Fr.) S. Lundell, Fungi Exsiccati Suecici Fasc. 23–24: no. 1102. 1942 ≡ *Agaricus flavovirens* Pers., in Hoffmann, Naturgetr. Abbild.

Beschr. Schwämme (Prague) 3: tab. 24. 1793 ≡ *Agaricus aureus* Schaeff., Fung. bavar. palat. nasc. (Ratisbonae) 4: 19, t. 41. 1774 ≡ *Agaricus luteus* Batsch, Elenchus fungorum: 45. 1783
≡ *Amanita punctata* var. *aurea* (Schaeff.) Lam., Encycl. Méth. Bot. 1-1: 106. 1783.
= ***Tricholoma equestre*** (L.) P. Kumm Führ. Pilzk. (Zerbst): 130. 1871 ≡ *Agaricus equestris* L., Species Plantarum: 1173. 1753 =*Agaricus auratus* Paulet, Traité sur les Champignons Comestibles (Paris) 2: 137. 1793 =*Agaricus sinuatus* var. *arenarius* Laterrade, Flore bordelaise Gironde, ed. 4: 534. 1846.
Comments: *Agaricus flavovirens* was sanctioned by Fries (1821) therefore, it has priority over its earlier synonyms *A. luteus* and *A. aureus* (Art. 13.1). For a detailed discussion about the synonymy of *T. flavovirens* and *T. equestre* see Deng et Yao, 2005 and Moukha et al., 2013.

Pogonoloma (Singer) Sánchez-García, **stat. nov.** *Porpoloma* subg. *Pogonoloma* Singer, Sydowia 15(1–6): 53. 1962 [1961] *Porpoloma* sect. *Pogonoloma* (Singer) Bon, Doc. Mycol. 9(33): 25. 1978.
MycoBank MB807897
Type : *Porpoloma spinulosum* (Kühner & Romagn.) Singer, Sydowia 15(1–6): 53. 1962 [1961] ≡*Tricholoma spinulosum* Kühner & Romagn., Bull. Soc. L. Lyon 16: 134. 1947.
Habit tricholomatoid, pileus with a pilose margin. Lamellae adnate to emarginate, white to cream sometimes turning pinkish or yellowish. Spores amyloid, smooth, ellipsoid, thin-walled. Cheilocystidia poorly differentiated or absent. Pleurocystidia absent. Pileipellis a cutis of cylindrical hyphae with intracellular pigments. Clamp connections present. On soil. Presumably saprotrophic.

Pogonoloma spinulosum (Kühner & Romagn.) Sánchez-García, **comb. nov.**
Tricholoma spinulosum Kühner & Romagn., Bull. Soc. L. Lyon 16: 134. 1947
Porpoloma spinulosum (Kühner & Romagn.) Singer, Sydowia 15(1–6): 53. 1962 [1961] – **Lectotype (here designated):** FRANCE: Seine et Marne, Ozoir la

Ferrière, near the intersection of Ferrandière, 7 Oct 1932, *R. Kühner* (G No. G00126725 n.v.).
Comments: We studied a photograph of a specimen named *T. spinulosum* that was collected and annotated by Kühner as "typus". Since this taxon has not been typified we designate this specimen as a lectotype.
MycoBank MB807900
Basionym: *Tricholoma spinulosum* Kühner & Romagn., Bull. Soc. L. Lyon 16: 134. 1947.

Pogonoloma macrocephalum (Schulzer) Sánchez-García, **comb. nov.** ≡ *Agaricus macrocephalus* Schulzer Icon. Sel. Hymenomyc. Hung. 11, tab. 3. 1874 ≡*Coolia macrocephala* (Schulzer) Huijsman, Medded. Nedl. Mycol. Ver. 28: 60. 1943≡ *Leucopaxillus macrocephalus* (Schulzer) Bohus, Fragm. Bot. Mus. Hist.-Nat. Hung. 4(1–4): 37. 1966 ≡ *Porpoloma macrocephalum* (Schulzer) Bon, Doc. Mycol. 9(3): 26. 1978 – **Lectotype (here designated):** illustration cited in Kalchbrenner, Icon. Sel. Hymenomyc. Hung. Tab. 3. Fig. 1. 1874
MycoBank MB807903

Singerocybe adirondackensis (Peck) Sánchez-García & Matheny, **comb. nov.** ≡ *Agaricus adirondackensis* Peck Ann. Rep. Reg. N.Y. St. Mus. 23: 77. 1872 ≡*Clitocybe adirondackensis* (Peck) Sacc., Syll. Fung. 5: 180. 1887 – Holotype: USA: NY, Essex Co., North Elba, Adirondack Mountains, Aug and Sept, *C.H. Peck*, watercolor by Peck (NYS n.v.).
MycoBank MB807764
Comments: We transfer this species to *Singerocybe* Harmaja on account of the inflated bladder-like intercalary cells in the pileipellis and a close ITS BLASTn match with European samples of *S. phaeophtalma* (Pers.) Harmaja (~97%).

Key to genera in the family Tricholomataceae s.str.

1a. Spores inamyloid …….. 2
1b. Spores amyloid ……..… 3

2a. Cheilocystidia and pleurocystidia conspicuous and long pedicelate arising below the hymenium; clamp connections present ……..…….... *Albomagister*
2b. Cheilocystidia present or absent; pleurocystidia rare, if present arising from the hymenium; clamp connections rarely present…… *Tricholoma*
3a. Spores verrucose…… *Leucopaxillus*
3b. Spores smooth…… 4
4a. Cheilocystidia and pleurocystidia present ……. *Dennisiomyces*
4b. Pleurocystidia absent; cheilocystidia present or absent……5
5a. Cheilocystidia present, edges of lamellae sterile; distributed in the southern hemisphere ……..……*Porpoloma*
5b. Cheilocystidia present or absent; distributed in temperate forests and grasslands of the northern hemisphere or in the tropics ……6
6a. Basidiocarps not changing color where cut or bruised; lamellae sometimes forked; cheilocystidia present and well differentiated; distributed in the tropics.……... *Corneriella*
6b. Basidiocarps changing red where cut or bruised; lamellae not forked; cheilocystidia absent or poorly differentiated; distributed in temperate forests and grasslands of the northern hemisphere…….. *Pseudotricholoma*

Discussion

Several attempts have been made to delimit the Tricholomataceae based on morphological features ranging from a broad and inclusive group (e.g., Singer 1986) to a more reduced entity (Jülich 1981). Floristic treatments (such as *Funga Nordica*) now take into consideration a much-reduced Tricholomataceae based on input from molecular phylogenetic studies (Moncalvo et al., 2002, Matheny et al., 2006, Garnica et al., 2007, Binder et al., 2010).

Some genera now recognized as Tricholomataceae s.str. have also been placed in other families. For example, *Dennisiomyces* was placed by Jülich (1981) in the Marasmiaceae, and *Porpoloma* and *Leucopaxillus* in the Leucopaxillaceae. Part of the taxonomic confusion stems from the fact that groups like *Porpoloma* and *Leucopaxillus* are not monophyletic.

The genus *Tricholoma* is one of the two genera of the family with inamyloid spores, and the only one that contains some species without clamp connections (Bessette et al., 2013; Bigelow, 1979; Christensen et Heilmann-Clausen, 2013; Ovrebo et Tylutki, 1975; Riva, 2003).

The genus *Porpoloma* was divided by Singer (1975) into three subgenera: *Porpoloma*, *Pseudotricholom*a (Singer) Singer, and *Pogonoloma* Singer. Here, we confirm that *Porpoloma* subgenus *Porpoloma* contains species known to date only from temperate regions of the Southern Hemisphere and that form ectomycorrhizal associations. Members of this genus have smooth, amyloid, and ellipsoid spores, cheilocystidia, and a pileipellis with intracellular brown pigments. This excludes *Pr. bambusarum* and several other species not sampled here (e.g., *Pr. boninense* (S. Ito & Imai) Hongo, and *Pr. aranzadii* Laskibar, Marrillaga et M. Bon). *Porpoloma bambusarum* now serves as the type of the new genus *Corneriella*, but additional species of *Porpoloma* s.l. are in need of revision to determine their clade placement. For instance, the presence of cheilocystidia in *Pr. boninense* and *Pr. aranzadii* (Hongo, 1980; Laskibar et al., 2001) suggests that these taxa could belong to *Porpoloma* s.str., however, there is no evidence that they form ECM associations as it occurs in *Porpoloma* s.str.

We elevate *Porpoloma* subgenus *Pseudotricholoma* to the generic level confirming within it two species: *Ps. umbrosum* (*Pr. umbrosum*) and *Ps. metapodium* (*Pr. metapodium*). *Pseudotricholoma umbrosum* and *Ps. metapodium* are morphologically similar. It has been suggested they are different forms or geographic variants of the same species (Singer, 1986). Our phylogeny (Fig. I.2) shows the separation of two clades, *Ps. umbrosum* representing specimens from North America and *Ps. metapodium* represented by collections from Europe, thus geographic separation is the main difference between these two species. Additionally, *Ps. metapodium* is usually found in meadows, while *Ps. umbrosum* is found in forests (Smith et Walters, 1943; Bon, 1978).

Porpoloma elytroides resembles *Pseudotricholoma* in having reddening flesh and a farinaceous odor, and could be placed in this genus following our taxonomic key. However, we have not studied collections of *Pr. elytroides*.

Singer (1956) described *Porpoloma* subg. *Pes-caprae* to accommodate *Pr. pes-caprae*, which was later transferred to *Porpoloma* subg. *Pseudotricholoma* (Singer, 1962). Here, we find that *Pr. pes-caprae* is an independent lineage apart from the rest of *Porpoloma* and may form a sister lineage to *Tricholoma* (Fig. I.2). Multiple gene sequence data from additional collections are necessary to confirm the autonomous position of *Pr. pes-caprae.*

Bon (1978) included two species in *Porpoloma* subg. *Pogonoloma*: *Pr. macrocephalum* and *Pr. macrorrhizum* (Sacc.) Bon. Our results (Fig. I.1) suggest that *Pr. macrocephalum* and *Pr. spinulosum* form an independent clade sister to *Pseudoclitocybe* and *Musumecia*. Based on this evidence we have elevated subgenus *Pogonoloma* to generic level including *Pr. spinulosum* (≡*Tricholoma spinulosum* Kühner & Romagn.) and *Pr. macrocephalum*.

Porpoloma bambusarum is the other species sampled that does not belong to *Porpoloma* s.str. This species is transferred to the new genus *Corneriella* together with *Cantharellula humicola*. The genus *Cantharellula* Singer was shown to be polyphyletic in a recent analysis by Lodge et al. (2014), where the type species *C. umbonata* (J.F. Gmel.) Singer was recovered as a sister group to *Pseudoarmillariella ectypoides* (Peck) Singer, both now included in the Hygrophoraceae. In that analysis *Ca. humicola* was recovered within the suborder Tricholomatineae and was excluded from *Cantharellula* s.str. based on the presence of cheilocystidia and regular rather than tridirectional lamellar trama. Members of *Corneriella* have smooth and amyloid spores and cheilocystidia, but lack pleurocystidia.

Dennisiomyces is a poorly known genus with only five species described to date: *D. glabrescentipes*, *D. fuscoalbus* Singer, *D. rionegrensis* Singer from Brazil, *D. griseus* from Trinidad, and *D. lanzonii* Robich from Italy (Dennis, 1951; Singer, 1955; 1989; Robich, 1989).

Dennisiomyces can be separated from other groups in the Tricholomataceae s.str. by the smooth and amyloid spores, the presence of cheilocystidia and pleurocystidia, presence of clamp connections, and erect to suberect terminal cells of the pileipellis.

This genus is more diverse than previously thought as we have detected what are probably five undescribed species from the neotropics and southeast U.S. Two additional species with affiliations to *Dennisiomyces* include *Gymnopus fulvidiscus* Murrill and *Pleurella ardesiaca* (Svenson & Taylor) E. Horak (Singer, 1986). The type of *G. fulvidiscus* fits the description of *Dennisiomyces* because of the amyloid and smooth spores, presence of cheilocystidia and pleurocystidia, and presence of clamp connections based on notes deposited with the specimen. Singer (1986) suggested the transfer of *G. fulvidiscus* to *Dennisiomyces*, and we predict it will indeed belong to the genus but have not studied the type.

Pleurella ardesiaca is a lignicolous species from New Zealand characterized by smooth, amyloid spores and presence of cheilocystidia and clamp connections, which suggest a close relationship with *Porpoloma* s.str. (Horak, 1971). However, the lignicolous habitat is not consistent with placement in *Dennisiomyces* or *Porpoloma*. An ITS sequence (JQ694106) is publically available from a collection (PDD 87446) referred to as *Pleurella ardesiaca* from New Zealand. BLASTn results suggest the nearest alliance of this specimen to *Baeospora* in the Marasmioid clade (Matheny et al., 2006) or to *Hypsizygus* (Gillet) Singer in the Lyophyllaceae. A thorough analysis of the type is required to confirm its taxonomic and phylogenetic position.

The genus *Albomagister* is macroscopically similar to *Leucopaxillus* or *Tricholoma*; however, its smooth inamyloid spores separate it from *Leucopaxillus*, while the presence of prominent pleuro- and cheilocystidia distinguish it from *Tricholoma*. The hyphae of the lamellar trama are parallel rather than divergent as in *Hygrophorus* Fr.

Albomagister subaustralis is a species now known from North Carolina and Tennessee. Dennis (1953) also reported it from Trinidad. However, this is doubtful since in his description he notes the presence of cheilocystidia and pleurocystidia with long tapering apices, a feature inconsistent with the type and other collections studied by us (Fig. I.3A). We consider Dennis' material of *H. subaustralis* to represent another species, possibly of *Albomagister*.

Within *Albomagister* we examined numerous collections of *A. subaustralis* studied by Hesler et Smith (1963), including the type (*Hygrophorus subaustralis*), and found one collection (AHS14872) identified as such that represents most likely an undescribed species of *Albomagister* (as *Albomagister* sp. 2 in Fig. I.2). This specimen was collected in the same locality as *A. subaustralis* showing that these two species co-exist in similar environments. In addition, we found one more collection that resembles *A. subaustralis*, but it seems to be a different phylogenetic species of *Albomagister* (as *Albomagister* sp. 3 in Fig. I.2); this species was also found co-existing with *A. subaustralis*.

The two undetermined specimens (ECV4038 and MBP06VIII09) are insufficiently known, and we cannot drive any conclusion regarding their taxonomy. The former possesses cheilocystidia, pleurocystidia and inamyloid spores, whereas the latter lacks pleurocystidia.

Conclusions

This work represents a significant systematic revision of the Tricholomataceae within a molecular context, including molecular annotations of generic types and other type collections. The Tricholomataceae is substantially reduced in circumscription, containing at least seven genera and one species-poor grade composed of taxa that merit further study. In addition to the Tricholomataceae, at least twelve other lineages can now be placed in the suborder Tricholomatineae: Entolomataceae, Lyophyllaceae, *Singerocybe*, *Clitocybe-Lepista-Collbyia*, *Clitocybe* p.p., the *Catathelasma* clade (represented here by *Cleistocybe)*, *Giacomia*, *Pseudoclitopilus*, *Pseudoclitocybe-Musumecia-Pogonoloma*, *Notholepista*, *Aspropaxillus*, and *Infundibulicybe*. Our multi-gene analyses are unable to resolve relationships among these latter groups, so additional gene and taxon sampling will likely be required to sort out their phylogenetic relationships.

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Appendix

Table I.1. Tricholomataceae sensu Singer (1986) and their current phylogenetic placement based on molecular sampling.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Agaricochaete	*Agaricochaete mirabilis* Eichelb.	None	Unknown
Amparoina	*Amparoina spinosissima* (Singer) Singer	None	Unknown
Amyloflagellula	*Amyloflagellula pulchra* (Berk. & Broome) Singer	Bodensteiner & al., 2004; Binder & al., 2006; Douanla-Meli & Langer, 2008	Marasmiaceae
Anthracophyllum	*Anthracophyllum beccarianum* Ces.	Moncalvo & al., 2002; Matheny & al., 2007	Omphalotaceae
Aphyllotus	*Aphyllotus campanelliformis* Singer	None	Marasmiaceae
Armillaria	*Armillaria straminea* (Krlz.) Kummer.	Moncalvo & al., 2002	Sister to *Cystoderma* (*Floccularia*)
Armillariella	*Armillariella mellea* (Vahl) P. Karst.	Moncalvo & al., 2002; Matheny & al., 2006	Physalacriaceae* (*Armillaria*)
Arrhenia	*Arrhenia auriscalpium* (Fr.) Fr.	Lutzoni & al., 1997; Moncalvo & al., 2002; Redhead & al., 2002; Garnica & al., 2007; Lawrey & al., 2009; Lodge & al., 2013	Hygrophoraceae*
Arthrosporella	*Arthrosporella ditopa* (Singer) Singer	None	Unknown
Asproinocybe	*Asproinocybe lactifera* R. Heim	None	Unknown

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Asterophora	*Asterophora lycoperdoides* (Bull.) Ditmar	Hofstetter & al., 2001; Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2010	Lyophyllaceae*
Baeospora	*Baeospora myosura* (Fr.) Singer	Moncalvo & al., 2002; Matheny & al., 2006	Cyphellaceae*
Callistodermatium	*Callistodermatium violascens* Singer	None	Unknown
Callistosporium	*Callistosporium palmarum* Singer	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2010	Catathelasma clade
Calocybe	*Calocybe gambosa* (Fr.) Donk	Hofstetter & al., 2002; Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007	Lyophyllaceae*
Calyptella	*Calyptella capula* (Holmsk.) Quél.	Moncalvo & al., 2002; Bodensteiner & al., 2004	Incertae sedis* Sister to resupinatus clade but with weak support
Campanella	*Campanella buettneri* Henn.	Moncalvo & al., 2002	Marasmiaceae
Cantharellula	*Cantharellula umbonata* (J.F. Gmel.) Singer	Moncalvo & al., 2002; Lawrey & al., 2009; Binder & al., 2010; Lodge & al., 2013	Hygrophoraceae* *C. foetida* LSU sequence 94% similarity with *Pseudoclitocybe cyathiformis* *C. humicola* – Tricholomataceae
Catathelasma	*Catathelasma evanescens* Lovejoy	Moncalvo & al., 2002; Matheny & al., 2006; Ammirati & al., 2007; Garnica & al., 2007; Saar & al., 2009; Binder & al., 2010	Catathelasma clade

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Cellypha	*Cellypha goldbachii* (Weinm.) Donk	None	Unknown
Chaetocalathus	*Chaetocalathus craterellus* (Durieu & Lév.) Singer	Moncalvo & al., 2002; Bodensteiner & al., 2004; Matheny & al., 2006	Marasmiaceae*
Cheimonophyllum	*Cheimonophyllum candidissimum* (Berk. & M.A. Curtis	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2006; 2010	Cyphellaceae*
Clitocybe	*Clitocybe nebularis* (Batsch) P. Kumm.	Hofstetter & al., 2002; Moncalvo & al., 2002; Walther & al., 2005; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Incertae sedis* Tricholomatoid clade
Clitocybula	*Clitocybula lacerata* (Scop.) Métrod	Moncalvo & al., 2002; Matheny & al., 2006; Malysheva & al., 2010	Incertae sedis* Hydropoid clade Tricholomatoid clade
Collybia	*Collybia tuberosa* (Bull.) P. Kumm.	Moncalvo & al., 2002; Bodensteiner & al., 2004; Walther & al., 2005; Matheny & al., 2006; Binder & al., 2010	Incertae sedis* Tricholomatoid clade
Crinipellis	*Crinipellis stipitaria* (Fr.) Pat.	Moncalvo & al., 2002; Bodensteiner & al., 2004; Walther & al., 2005; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Marasmiaceae*
Cymatella	*Cymatella minima* Pat.	None	Unknown

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Cyphella	*Cyphella digitalis* (Alb. & Schwein.) Fr.	Binder & al., 2006; Matheny & al., 2006	Cyphellaceae*
Cyphellostereum	*Cyphellostereum pusiolum* (Berk. & M.A. Curtis) D.A. Reid	Larsson & al., 2004; Matheny et al, 2006; Larsson, 2007; Lawrey & al., 2009; Lodge et al. 2013	Hygrophoraceae* *C. laeve* (=*Muscinupta laevis*) in the Hymenochaetales
Cyptotrama	*Cyptotrama macrobasidium* Singer	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2006	Physalacriaceae
Deigloria	*Deigloria pulchella* Agerer	None	Unknown
Delicatula	*Delicatula integrella* (Pers.) Fayod	Saar & al., 2009 (misidentified sequence of *Melanoleuca*)	Unknown
Dennisiomyces	*Dennisiomyces glabrescentipes* Singer	This study	Tricholomataceae*
Dermoloma	*Dermoloma cuneifolium* (Fr.) Singer	Kropp, 2008	Agaricaceae – *D. inconspicuum*
Dictyopanus	*Dictyopanus pusillus* (Pers. ex Lév.) Singer	Jiankang & al., 2001; Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007	Mycenaceae* (*Panellus*)
Epicnaphus	*Epicnaphus phalaropus* Singer	None	Unknown
Fayodia	*Fayodia bisphaerigera* (J.E. Lange) Singer	Moncalvo & al., 2002; Garnica & al., 2007	Fayodioid clade Incertae sedis
Filoboletus	*Filoboletus mycenoides* Henn.	Moncalvo & al., 2002	Mycenaceae
Fissolimbus	*Fissolimbus fallaciosus* E. Horak	None	Unknown
Flagelloscypha	*Flagelloscypha minutissima* (Burt) Donk	Bodensteiner & al., 2004; Binder & al., 2006	Lachnellaceae

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Flammulina	*Flammulina velutipes* (Curtis) Singer	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2006; Ripková & al., 2010	Physalacriaceae*
Gerronema	*Gerronema melanomphax* Singer	Moncalvo & al., 2002; Redhead & al., 2002	Hydropoid clade
Gloiocephala	*Gloiocephala epiphylla* Massee	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2006	Physalacriaceae*
Hemimycena	*Hemimycena lactea* (Pers.) Singer	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2010	Sister to Cyphellaceae*
Hohenbuehelia	*Hohenbuehelia petaloides* (Bull.) Schulzer	Moncalvo & al., 2002; Matheny & al., 2006	Pleurotaceae*
Hydropus	*Hydropus fuliginarius* (Batsch) Singer	Moncalvo & al., 2002; Bodensteiner & al., 2004; Matheny & al., 2006; Binder & al., 2010	Hydropoid clade*
Hymenogloea	*Hymenogloea riofrioi* (Pat.) Pat.	Moncalvo & al., 2002	Marasmiaceae
Hypsizygus	*Hypsizygus tessulatus* (Bull.) Singer	Moncalvo & al., 2002; Hofstetter & al., 2002	Lyophyllaceae*
Laccaria	*Laccaria laccata* (Scop.) Cooke	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007	Hydnangiaceae*
Lachnella	*Lachnella alboviolascens* (Alb. & Schwein.) Fr.	Moncalvo & al., 2002; Matheny & al., 2006	Lachnellaceae*
Lactocollybia	*Lactocollybia lacrimosa* (R. Heim) Singer	None	Unknown

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Lepista	*Lepista paneolus* (Fr.) P. Karst.	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Incertae sedis Tricholomatoid clade
Leptoglossum	*Leptoglossum muscigenum* (Bull.) P. Karst. (=*Arrhenia spathulata* (Fr.) Redhead)	Lutzoni & al., 1997; Moncalvo & al., 2002; Redhead & al., 2002; Garnica & al., 2007; Lawrey & al., 2009; Lodge & al., 2013	Hygrophoraceae* (*Arrhenia*)
Leucopaxillus	*Leucopaxillus paradoxus* (Constantin & L.M. Dufour) Boursier	Vizzini & al., 2012; this study	Tricholomataceae*
Lulesia	*Lulesia densifolia* Singer	None	Unknown
Lyophyllum	*Lyophyllum semitale* (Fr.) Kühner	Hofstetter & al., 2002; Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Lyophyllaceae*
Macrocystidia	*Macrocystidia cucumis* (Pers.) Joss.	Moncalvo & al., 2002; Walther & al., 2005; Matheny & al., 2006	Incertae sedis*
Manuripia	*Manuripia bifida* Singer	None	Unknown
Marasmiellus	*Marasmiellus juniperus* Murrill	Moncalvo & al., 2002; Mata & al., 2004; Walther & al., 2005; Garnica & al., 2007	Omphalotaceae*
Marasmius	*Marasmius rotula* (Scop.) Fr.	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Marasmiaceae*

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Melanoleuca	*Melanoleuca melaleuca* (Pers.) Murrill	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007	Pluteoid clade*
Micromphale	*Micromphale venosum* (Pers.) Gray	Moncalvo & al., 2002; Walther & al., 2005; Mata & al., 2004	Omphalotaceae
Mniopetalum	*Mniopetalum globisporum* Donk (=*Rimbachia arachnoidea* (Peck) Redhead)	Moncalvo & al., 2002	Incertae sedis Omphalina group (*Rimbachia*)
Mycena	*Mycena galericulata* (Scop.) Gray	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Mycenaceae* some species in the hydropoid clade
Mycenella	*Mycenella cyatheae* (Singer) Singer	Garnica & al., 2007	Incertae sedis
Mycoalvimia	*Mycoalvimia theobromicola* Singer	None	Unknown
Neoclitocybe	*Neoclitocybe byssiseda* (Bres.) Singer	None	Unknown
Omphalina	*Omphalina pyxidata* (Bull.) Quél.	Moncalvo & al., 2002; Vizzini & al., 2011	Tricholomatoid clade*
Oudemansiella	*Oudemansiella platensis* (Speg.) Speg.	Moncalvo & al., 2002; Binder & al., 2006; Petersen & Hughes, 2010	Physalacriaceae
Palaeocephala	*Palaeocephala cymatelloides* (Dennis & D.A. Reid) Singer	None	Unknown
Panellus	*Panellus stipticus* (Bull.) P. Karst.	Jiankang & al., 2001; Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007	Mycenaceae*

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Pegleromyces	*Pegleromyces collybioides* Singer	None	Unknown
Phaeodepas	*Phaeodepas dennisii* D.A. Reid	None	Unknown
Physalacria	*Physalacria inflata* (Schwein.) Peck	Moncalvo & al., 2002; Binder & al., 2005; 2006; Dentinger & McLaughlin, 2006; Matheny & al., 2006; Binder & al., 2010	Physalacriaceae* Note: Sequences of *P. inflata* used in Binder & al., 2005 are misidentified.
Physocystidium	*Physocystidium cinnamomeum* (Dennis) Singer	None	Unknown
Pleurocollybia	*Pleurocollybia praemultifolia* (Murrill) Singer	Moncalvo & al., 2002	Incertae sedis Callistosporoid clade; Catathelasma clade
Pleurocybella	*Pleurocybella porrigens* (Pers.) Singer	Moncalvo & al., 2002; Binder & al., 2010	Incertae sedis* Hygrophoroid clade
Pleuromycenula	*Pleuromycenula ellipsoidea* Singer	None	Unknown
Podabrella	*Podabrella microcarpa* (Berk. & Broome) Singer	Hofstetter & al., 2002; Moncalvo & al., 2002; Froslev & al., 2003; Matheny & al., 2006; Binder & al., 2010	Lyophyllaceae* (*Termitomyces*)
Porpoloma	*Porpoloma sejunctum* Singer	Vizzini & al., 2012, this study	*Porpoloma* sensu stricto Tricholomataceae* *P. macrocephalum and P. spinulosum* - incertae sedis, tricholomatoid clade (transferred to *Pogonoloma*)

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Pseudoarmillariella	*Pseudoarmillariella ectypoides* (Peck) Singer	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2010, Lodge & al., 2013	Hygrophoraceae*
Pseudoclitocybe	*Pseudoclitocybe cyathiformis* (Bull.) Singer	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Incertae sedis* Tricholomatoid clade
Pseudohiatula	*Pseudohiatula cyatheae* Singer	Petersen & Hughes, 2010	Physalacriaceae
Pseudoomphalina	*Pseudoomphalina kalchbrenneri* (Bres.) Singer	Malysheva & al., 2010	Tricholomatoid clade*
Resinomycena	*Resinomycena rhododendri* (Peck) Redhead & Singer	Moncalvo & al., 2002	Mycenaceae*
Resupinatus	*Resupinatus applicatus* (Batsch) Gray	Moncalvo & al., 2002; Bodensteiner & al., 2004; Matheny & al., 2006	Pleurotaceae*
Rhodotus	*Rhodotus palmatus* (Bull.) Maire	Moncalvo & al., 2002; Petersen & Hughes 2010	Physalacriaceae*
Rimbachia	*Rimbachia paradoxa* Pat.	Moncalvo & al., 2002	Incertae sedis Omphalina group
Skepperiella	*Skepperiella spathularia* (Berk. & M.A. Curtis) Pilát	None	Unknown
Stigmatolemma	*Stigmatolemma incanum* Kalchbr.	Moncalvo & al., 2002; Bodensteiner & al., 2004; Matheny & al., 2006	Pleurotaceae (*Resupinatus*)
Strobilurus	*Strobilurus conigenoides* (Ellis) Singer	Moncalvo & al., 2002; Garnica & al., 2007	Physalacriaceae*

Table I.1. Continued.

Genus	Type species	Reference of molecular sampling	Current family or phylogenetic placement (current name)
Stromatocyphella	*Stromatocyphella conglobata* (Burt) W.B. Cooke	None	Unknown
Tectella	*Tectella operculata* (Berk. & M.A. Curtis) Murrill	Moncalvo & al., 2002	Incertae sedis
Termitomyces	*Termitomyces striatus* (Beeli) R. Heim	Hofstetter & al., 2002; Moncalvo & al., 2002; Froslev & al., 2003; Matheny & al., 2006; Binder & al., 2010	Lyophyllaceae*
Tricholoma	*Tricholoma flavovirens* (Pers.:Fr.) S. Lundell	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Tricholomataceae*
Tricholomopsis	*Tricholomopsis rutilans* (Schaeff.) Singer	Moncalvo & al., 2002; Matheny & al., 2006; Garnica & al., 2007; Binder & al., 2010	Incertae sedis* Hygrophoroid clade
Trogia	*Trogia aplorutis* (Mont.) Fr.	Wilson & Desjardin, 2005	Incertae sedis Sister of the marasmioid clade
Xeromphalina	*Xeromphalina campanella* (Batsch) Maire	Moncalvo & al., 2002; Matheny & al., 2006; Binder & al., 2010	Hygrophoroid clade*

* The type species has been sampled

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Table I.2. Species sampled and GenBank accession numbers.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
"Inocephalus" sp.			USA		DQ457683	DQ457622	DQ472728
***Albomagister* sp.2**	*Hygrophorus subaustralis* A.H. Sm. & Hesler	MSG137 (TENN:068776)	USA	KJ417247	KJ417178		KJ424363
***Albomagister* sp.2**	*Hygrophorus subaustralis*	MSG136 (TENN:068775)	USA	KJ417248			KJ424364
***Albomagister* sp.2**	*Hygrophorus subaustralis*	AHS14872 (MICH:058090)	USA	KJ417249			
***Albomagister* sp.3**	*Hygrophorus subaustralis*	ECV4202 (TENN:065323)	USA	KJ417250	KJ417179		KJ424365
***Albomagister subaustralis* (A.H. Sm. & Hesler) Sánchez-García, Birkebak & Matheny**	*Hygrophorus subaustralis*	ECV4049 (TENN:064621)	USA	KJ417251	KJ417180		KJ424366
Albomagister subaustralis	*Hygrophorus subaustralis*	MGW676 (TENN:064620)	USA	KJ417252	KJ417181	KJ417255	KJ424367
Albomagister subaustralis	*Hygrophorus subaustralis*	WBU11 (TENN:066902)	USA	KJ417253			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH14423 (TENN:014423)	USA	KJ417254			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH23221 (TENN:023221)	USA	KJ417255			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH39069 (TENN:039069)	USA	KJ417256			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH20463 (TENN:020463)	USA	KJ417257			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH18628 (TENN:8018628)	USA	KJ417258			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH22663 (TENN:022663)	USA	KJ417259			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH23195 (TENN:023195)	USA	KJ417260			
Albomagister subaustralis	*Hygrophorus subaustralis*	LRH17951 (TENN:017951)	USA	KJ417261			
Albomagister subaustralis	*Hygrophorus subaustralis*	AHS10844 (MICH:010951)*	USA	KJ417262			
Albomagister subaustralis	*Hygrophorus subaustralis*	SAT1221702 (TENN:067343)	USA	KJ417263			
***Aspropaxillus candidus* (Bres.) Raithelh.**		MOS71116 (TENN:037113)	AUSTRIA		KJ417182		
***Aspropaxillus giganteus* (Quél.) Kühner & Maire**		RHP12192 (TENN:060129)	RUSSIA		KJ417183	KJ417256	KJ424368
Aspropaxillus giganteus		JJ021011 (GB)			KJ417240		
Aspropaxillus giganteus		GC-98046	ITALY		JQ639152		
***Aspropaxillus septentrionalis* (Singer & A.H. Sm.) Vizzini**		AHS24982 (MICH:005826)*	USA		KJ417184		
***Callistosporium graminicolor* Lennox**		PBM2341 (WTU)	USA		AY745702	AY752974	KJ424369
***Calocybe carnea* (Bull.) Donk**		(CBS:552.50)			AF223178	DQ367418	DQ367432

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
***Calocybe gangraenosa* (Fr.) Hofstetter, Moncalvo, Redhead & Vilgalys**		HAe251.97			AF223202	DQ367420	DQ367434
***Catathelasma ventricosum* (Peck) Singer**		PBM2403	USA		DQ089012	DQ435811	DQ470830
***Cleistocybe carneogrisea* (Malençon) Vizzini**		S.Poumarat s.n. (TENN:063842)	FRANCE		HQ728527	HQ728528	
***Cleistocybe vernalis* Ammirati, A.D. Parker & Matheny**		PBM1856 (WTU)	USA		AY647208	DQ092913	
***Clitocybe* aff. *fellea* Peck**		PBM3028 (TENN:062782)	USA		HQ728534	HQ728535	HQ728536
***Clitocybe candicans* (Pers.) P. Kumm.**		PBM2476	USA		AY645055	AY771609	DQ385881
***Clitocybe dealbata* (Sowerby) P. Kumm.**		HC95.cp3			AF223175	DQ825431	DQ825407
***Clitocybe nebularis* (Batsch) P. Kumm.**		PBM2259	USA		DQ457658	DQ437681	DQ470833
***Clitocybe subditopoda* Peck**		PBM2489	USA		AY691889	AY771608	AY780942
***Clitopilus prunulus* (Scop.) P. Kumm.**		TJB6838	USA		AY700181	AY771607	DQ825408
***Collybia tuberosa* (Bull.) P. Kumm.**		RHP7300 (TENN:053540)	SWEDEN		AY639884	AY771606	AY787219
***Corneriella bambusarum* (Desjardin & Hemmes) Sánchez-García**	*Porpoloma bambusarum* Desjardin & Hemmes	DED5462 (NYBG)*	USA	KJ417264	KJ417185		KJ424370
Corneriella bambusarum	*Porpoloma bambusarum*	DED6572 (NYBG:0775752)	USA	KJ417265			
***Corneriella humicola* (Corner) Sánchez-García**	*Cantharellula humicola* Corner	DED7871	THAILAND	KF291051	KF291052	KF291053	
***Corneriella* sp.**	*Dennisiomyces* sp.	PR-4733 (CFMR)	PUERTO RICO	KF306336	KF306337		KF306338
***Corneriella* sp.**	*Porpoloma* sp.	PR-3995 (CFMR)	PUERTO RICO	EF421106	AF261395		AF421013
***Corneriella* sp.**	*Dennisiomyces griseus* (Dennis) Singer	JPF943A (K(M):188911)	MARTINIQUE	KJ417324	KJ417234		
***Dennisiomyces glabrescentipes* Singer**		JPF1000A (K(M):188910)	MARTINIQUE	KJ417326	KJ417235		
Dennisiomyces griseus		Dennis-293 (K(M):160882)*	TRINIDAD & TOBAGO	KJ417325			

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
***Dennisiomyces* sp. 1**		BZ-916 (CFMR)	BELIZE	KF291063	KF291064		KF291066
***Dennisiomyces* sp. 2**	*Porpoloma* sp.	PR-4763 (CFMR)	PUERTO RICO	KJ417269	KJ417191		
***Dennisiomyces* sp. 3**	*Porpoloma* sp.	PR-4764 (CFMR)	PUERTO RICO	KJ417270			
***Dennisiomyces* sp. 4**	*Porpoloma* sp.	PR-6334 (CFMR)	PUERTO RICO	KJ417271	KJ417192	KJ417258	
***Dennisiomyces* sp. 4**	*Porpoloma* sp.	PR-6613 (CFMR)	PUERTO RICO	KJ417268	KJ417190	KJ417257	
***Dennisiomyces* sp. 5**		BPL244 (TENN:067232)	USA		KJ417186		KJ424371
***Dennisiomyces* sp. 5**		PBM3862 (TENN:067469)	USA		KJ417187		KJ424372
***Dennisiomyces* sp. 5**		BPL254 (TENN:067388)	USA	KJ417266	KJ417188		KJ424373
***Dennisiomyces* sp. 5**		PBM3861 (TENN:067462)	USA	KJ417267	KJ417189		KJ424374
***Dennisiomyces* sp. 5**		DLNC15-05 (TENN:061833)	USA	KJ417327	KJ417239		
***Dennisiomyces* sp. 5**		DLTN34-05 (TENN:061851)	USA	KJ417328			
***Entoloma asterosporum* (Coker & Couch) T.J. Baroni & Matheny**		PBM3268 (TENN:064538)	USA		JF706310	JF706311	JF706312
***Entoloma sericeum* Quél.**		VHA 03-02			DQ367423	DQ367421	DQ367435
***Entoloma sinuatum* (Bull.) P. Kumm.**		TJB5349	USA		AY691891	AY657007	KJ424375
***Entoloma* sp.**		TJB4765	USA		AY700180	AY665784	DQ385883
***Entoloma strictius* (Peck) Sacc.**		M96/10			AF042620	AF287832	AY218483
***Giacomia mirabilis* (Bres.) Vizzini & Contu**		GC-94141	ITALY		JQ639154		
***Infundibulicybe gibba* (Pers.) Harmaja**		JCS0704B	USA		DQ457682	DQ115780	DQ472727
***Lepista irina* (Fr.) H.E. Bigelow**		PBM2291 (WTU)	USA		DQ234538	AY705948	DQ385885
***Lepista personata* (Fr.) Cooke**		ADW0097 (TENN:066100)	USA		KJ417193	KJ417259	KJ424376
***Lepista sordida* (Schumach.) Singer**		CRG014 (GLM:45949)	USA		AY207225	KJ417260	KJ137273
***Leucopaxillus albissimus* (Peck) Singer**		Landeros 7/8/A (XAL)	MEXICO	KJ417272	KJ417194		
Leucopaxillus albissimus		DAOM182713			AF261393		
Leucopaxillus albissimus		F9693 (FH:00301850)	CANADA	KJ417273			
Leucopaxillus albissimus		Singer-301900 (FH:00301900)	USA	KJ417274			

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
Leucopaxillus alboalutaceus (F.H. Møller & Jul. Schäff.) F.H. Møller		LAS00/082 (GB:0065210)	SWEDEN	KJ417275	KJ417195	KJ417261	KJ424377
Leucopaxillus alboalutaceus		LAS88/79 (GB:0065215)	SWEDEN	KJ417245			
Leucopaxillus alboalutaceus		GC-97076	ITALY	JQ639147			
Leucopaxillus amarus (Alb. & Schwein.) Kühner		TFB54925 (TENN:040325)	USA	KJ417276			
Leucopaxillus amarus		Mos63/271 (TENN:037007)	SWITZERLAND		KJ417196		
Leucopaxillus amarus		LGD5690 (IBUG)	MEXICO	KJ417277			
Leucopaxillus amarus		BW122201 (WTU:F-8827)	USA	KJ417278	KJ417197		
Leucopaxillus amarus		Smith-15870 (MICH:0073741)	USA	KJ417279			
Leucopaxillus amarus		Cooke 22708 (FH:00301878)	USA	KJ417280			
Leucopaxillus cerealis (Lasch) Singer		LAS01/031 (GB:0110964)	SWEDEN	KJ417281			KJ424378
Leucopaxillus cerealis		(GB:0068895)	SWEDEN	KJ417282	KJ417198	KJ417262	KJ424379
Leucopaxillus cerealis		LAS04/012 (GB:065211)	SWEDEN	KJ417283			KJ424380
Leucopaxillus cerealis		LAS01/147 (GB)	SWEDEN	KJ417246	KJ417241		
Leucopaxillus cerealis		TO AVL20112	ITALY	JQ639148	JQ639149		
Leucopaxillus cf. *masakanus* Pegler		TJB8321 (CORT)	US VIRGIN ISLANDS	KJ417284			
Leucopaxillus eucalyptorum (Cleland) Grgur.		REH9110 (NYBG:01115433)	AUSTRALIA	KJ417285	KJ417199		
Leucopaxillus eucalyptorum		RHP8368 (TENN:055040)	ARGENTINA	KJ417286	KJ417200		
Leucopaxillus gentianeus (Quél.) Kotl		EL291/11 (GB)	SWEDEN	KJ417287	KJ417201		KJ424381
Leucopaxillus gentianeus		(TENN:05616)	USA		AF261394		
Leucopaxillus gracillimus Singer & A.H. Sm		Guzman-34476 (XAL)	MEXICO	KJ417288			
Leucopaxillus gracillimus		TJB8315 (CORT)	US VIRGIN ISLANDS	KJ417289			
Leucopaxillus laterarius (Peck) Singer & A.H. Sm.		PBM3060 (TENN:063507)	USA	KJ417290	KJ417202	KJ417263	
Leucopaxillus laterarius		TFB56010 (TENN:044433)	USA	KJ417291	KJ417203		
Leucopaxillus laterarius		RHP29877 (TENN:029877)	USA	KJ417292	KJ417204		
Leucopaxillus laterarius		ALC178 (IBUG)	MEXICO	KJ417293			

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
Leucopaxillus laterarius		VR1717 (IBUG)	MEXICO	KJ417294			
Leucopaxillus lilacinus **Bougher**		PBM3584 (TENN:066653)	AUSTRALIA	KJ417295	KJ417205	KJ417264	KJ424382
Leucopaxillus monticola **(Singer & A.H. Sm.) Bon**		TO AVL20111	ITALY	JQ639156			
Leucopaxillus paradoxus **(Costantin & L.M. Dufour) Boursier**		TK04/091 (GB:0110968)	HUNGARY	KJ417296	KJ417206	KJ417265	KJ424383
Leucopaxillus tricolor **(Peck) Kühner**		TFB13462 (TENN:061725)	USA	KJ417323	KJ417207	KJ417266	KJ424384
Lyophyllum decastes **(Fr.) Singer**		JM 87/16			AF042583	DQ367419	DQ367433
Lyophyllum **aff.** *decastes*		PBM3069 (TENN:063624)	USA		HQ832459	HQ832426	HQ832437
Lyophyllum **sp.**		PBM 2688	USA		DQ094785	DQ457628	
Musumecia bettlachensis **Vizzini & Contu**		TO HG2284	ITALY		JF926521		
Notholepista subzonalis **(Peck) Vizzini & Contu**		(GB:0087013)	SWEDEN		KJ417208	KJ417267	KJ424385
Notholepista subzonalis		TK98/279 (GB)			KJ417242		
Ossicaulis lignatilis **(Pers.) Redhead & Ginns**		DAOM188196			AF261396	AF334923	DQ825410
Pogonoloma macrocephalum **(Schulzer) Sánchez-García**	*Porpoloma macrocephalum* (Schulzer) Bon	MOS69/85 (TENN:037026)	AUSTRIA		KJ417209	KJ417268	
Pogonoloma macrocephalum	*Porpoloma macrocephalum*	GC-96016	ITALY		JQ639163		
Pogonoloma spinulosum **(Kühner & Romagn.) Sánchez-García**	*Porpoloma spinulosum* (Kühner & Romagn.) Singer	Reid S346 (K(M):005346)	UNITED KINGDOM		KJ417236		
Pogonoloma spinulosum	*Porpoloma spinulosum*	(K(M):089387)	UNITED KINGDOM		KJ417237		
Pogonoloma spinulosum	*Porpoloma spinulosum*	(K(M):107286)	UNITED KINGDOM		KJ417238		KJ424401
Porpoloma pes-caprae **(Fr.) Singer**		MOS67/265 (TENN:037121)	POLAND	KJ417297			

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
***Porpoloma portentosum* Singer**		MES531 (FL)	CHILE	KJ417298	KJ417210		KJ424386
Porpoloma portentosum		REH5788 (NYBG)	ARGENTINA	KJ417299	KJ417211		KJ424387
Porpoloma portentosum		Singer-M149 (MICH: 011834)*	ARGENTINA	KJ417300			
***Porpoloma sejunctum* Singer**		(CONC:F0416)	CHILE	KJ417301	KJ417212		KJ424388
Porpoloma sejunctum		Singer-M256 (MICH:01183)*	ARGENTINA	KJ417302			
Porpoloma sejunctum		Environmental sample	ARGENTINA	JX316261			
***Porpoloma* sp. 2**		PBM3441 (TENN:065358)	AUSTRALIA	KJ417303	KJ417213	KJ417269	KJ424389
***Porpoloma* sp. 1**		PBM3238 (TENN:065473)	AUSTRALIA	KJ417304	KJ417214	KJ417270	KJ424390
***Porpoloma terreum* Singer**		REH5830 (NYBG)	CHILE	KJ417305	KJ417215		KJ424391
Porpoloma terreum		(CONC:F0030)	CHILE	KJ417306	KJ417216		
Porpoloma terreum		Singer-M372 (MICH:011746)*	ARGENTINA	KJ417307			
Porpoloma terreum		Environmental sample	ARGENTINA	JX316283			
Porpoloma terreum		Environmental sample	ARGENTINA	JX316318			
Porpoloma terreum		Environmental sample	ARGENTINA	JX316344			
***Pseudoclitocybe cyathiformis* (Bull.) Singer**		JFA12811	USA		EF551313	GU187659	GU187515
Pseudoclitocybe cyathiformis		TO HG2284	ITALY		JF926523		
***Pseudoclitocybe expallens* (Pers.) M. Moser**		TO HG2286	ITALY		JF926525		
***Pseudoclitocybe* sp.**		RHP12643 (TENN:061314)	USA		KJ417217		KJ424392
***Pseudoclitopilus rhodoleucus* (Sacc.) Vizzini & Contu**		TK03/203 (GB:0110967)	SWEDEN		KJ417218		KJ424393
Pseudoclitopilus rhodoleucus		TK02/061 (GB)			KJ417243		
***Pseudotricholoma metapodium* (Fr.) Sánchez-García & Matheny**	*Porpoloma metapodium* (Fr.) Singer	AH22102006 (K)	UNITED KINGDOM	KJ417308	KJ417219	KJ417271	KJ424394
Pseudotricholoma metapodium	*Porpoloma metapodium*	LAS06/134 (GB:066422)*	SWEDEN	KJ417309	KJ417220		KJ424395
Pseudotricholoma metapodium	*Porpoloma metapodium*	EL155/09 (GB)	NORWAY	KJ417310			KJ424396

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
***Pseudotricholoma umbrosum* (A.H. Sm. & M.B. Walters) Sánchez-García & Matheny**	*Porpoloma umbrosum* (A.H. Sm. & M.B. Walters) Singer	TFB5481 (TENN:052643)	USA	KJ417311	KJ417221		
Pseudotricholoma umbrosum	*Porpoloma umbrosum*	REH4796 (NYBG:505218)	USA	KJ417312	KJ417222		KJ424397
Pseudotricholoma umbrosum	*Porpoloma umbrosum*	TJB7179 (CORT)	USA	KJ417313	KJ417223		
Pseudotricholoma umbrosum	*Porpoloma umbrosum*	Wehmeyer-640 (MICH:012331)*	CANADA	KJ417314			
Pseudotricholoma umbrosum	*Porpoloma umbrosum*	PBM3267 (TENN:064489)	USA	KJ417315	KJ417224		KJ424398
***Rhodocybe mundula* (Lasch) Singer**		TJB7599			AY700182	DQ089017	
***Singerocybe adirondackensis* (Peck) Sánchez-García & Matheny**	*Clitocybe adirondackensis* (Peck) Sacc.	PBM3320 (TENN:064660)	USA		HQ728530	HQ728531	HQ728532
***Tephrocybe anthracophila* (Lasch) P.D. Orton**		JT31970	AUSTRALIA		KJ417225		
***Tephrocybe boudieri* (Kühner & Romagn.) Derbsch**		BSI96/84			DQ825430	DQ825433	DQ825411
***Termitomyces microcarpus* (Berk. & Broome) R. Heim**		PRU3900			AF042587	DQ851581	
***Termitomyces* sp.**		ZA164	SOUTH AFRICA		DQ110875	DQ092922	DQ437070
***Tricholoma elegans* G. Stev.**		PBM3142 (TENN:063711)	NEW ZEALAND	KJ417316	KJ417226		
***Tricholoma equestre* (L.) P. Kumm**				HM590872			
***Tricholoma flavovirens* (Alb. & Schwein.) S. Lundell**			PUERTO RICO	EU186310			
***Tricholoma inamoenum* (Fr.) Gillet**					AY293215	AY293161	
***Tricholoma matsutake* (S. Ito & S. Imai) Singer**				AB188557	U62964	U62538	

Table I.2. Continued.

Species name in this study	Previous name	Voucher number[1]	Country	ITS	nLSU	nSSU	RPB2
***Tricholoma myomyces* (Pers.) J.E. Lange**		KMS589		DQ825428	U76459	DQ367422	DQ367436
***Tricholoma palustre* A.H. Sm.**		PBM2494	USA		AY700197	AY757267	DQ484055
***Tricholoma saponaceum* (Fr.) P. Kumm.**		PBM2514 (CUW)	USA	DQ494700	AY647209	AY654883	
***Tricholoma* sp.**		PBM3141 (TENN:063710)	NEW ZEALAND	KJ417317	KJ417227	KJ417272	
***Tricholoma* sp.**		PBM3085 (TENN:063664)	NEW ZEALAND	KJ417318	KJ417228	KJ417273	
***Tricholoma* sp.**		RHP13062 (TENN:061065)	NEW ZEALAND		KJ417229	KJ417274	
***Tricholoma* sp.**		SAR1290			AF042592	AF287839	
***Tricholoma subresplendens* (Murrill) Murrill**		SAT1027901 (TENN:065679)	USA	KJ417319	KJ417230	KJ417275	
***Tricholoma viridiolivaceum* G. Stev.**		PBM3093 (TENN:063670)	NEW ZEALAND	JF706316	JF706317	JF706318	JF706319
***Tricholomella constricta* (Fr.) Zerova ex Kalamees**		HC84-75			AF223188	DQ825434	DQ825412
Undet. gray scaly		ECV4038 (TENN:064609)	USA	KJ417320	KJ417231	KJ417276	KJ424399
Undet. small white		MBP080609 (TENN:064359)	USA	KJ417321	KJ417232	KJ417277	KJ424400
Undet. small white		MSG144 (TENN:068777)	USA	KJ417322	KJ417233		KJ424402

[1] Collector numbers followed by herbarium accession numbers in parentheses (Collector's abbreviations are as follows: ADW – A.D.Wolfenbarger; AH – A.Henrici; AHS – A.H.Smith; ALC – A.Loeza; BPL – B.P.Looney; DED – D.E.Desjardin; ECV – E.C.Vellinga; EL – E.Larsson; FL – F.Landeros; GC – G.Consiglio; GD – G.Daniele; GG – G.Guzmán: JCS – J.C.Slot; JFA – J.F.Ammirati; JPF – J.P.Fiard ; JT – J.Trappe; LAS – L. & A. Stridvall; LGD – L.Guzmán-Dávalos; LRH – L.R.Hesler; MBP – M.B.Pilkington; MES – M.E.Smith; MGW – M.G.Wood; MOS – M.M.Moser; MSG – M.Sánchez-Garcia; PBM – P.B.Matheny; REH – R.E.Halling; RHP – R.H.Petersen; SAR – S.A.Rehner; SAT – S.A.Trudell; TJB – T.J.Baroni; TK – T.Knutsson; VRC – V.Ramírez-Cruz; WBU – W.Burnette)
* Type specimen

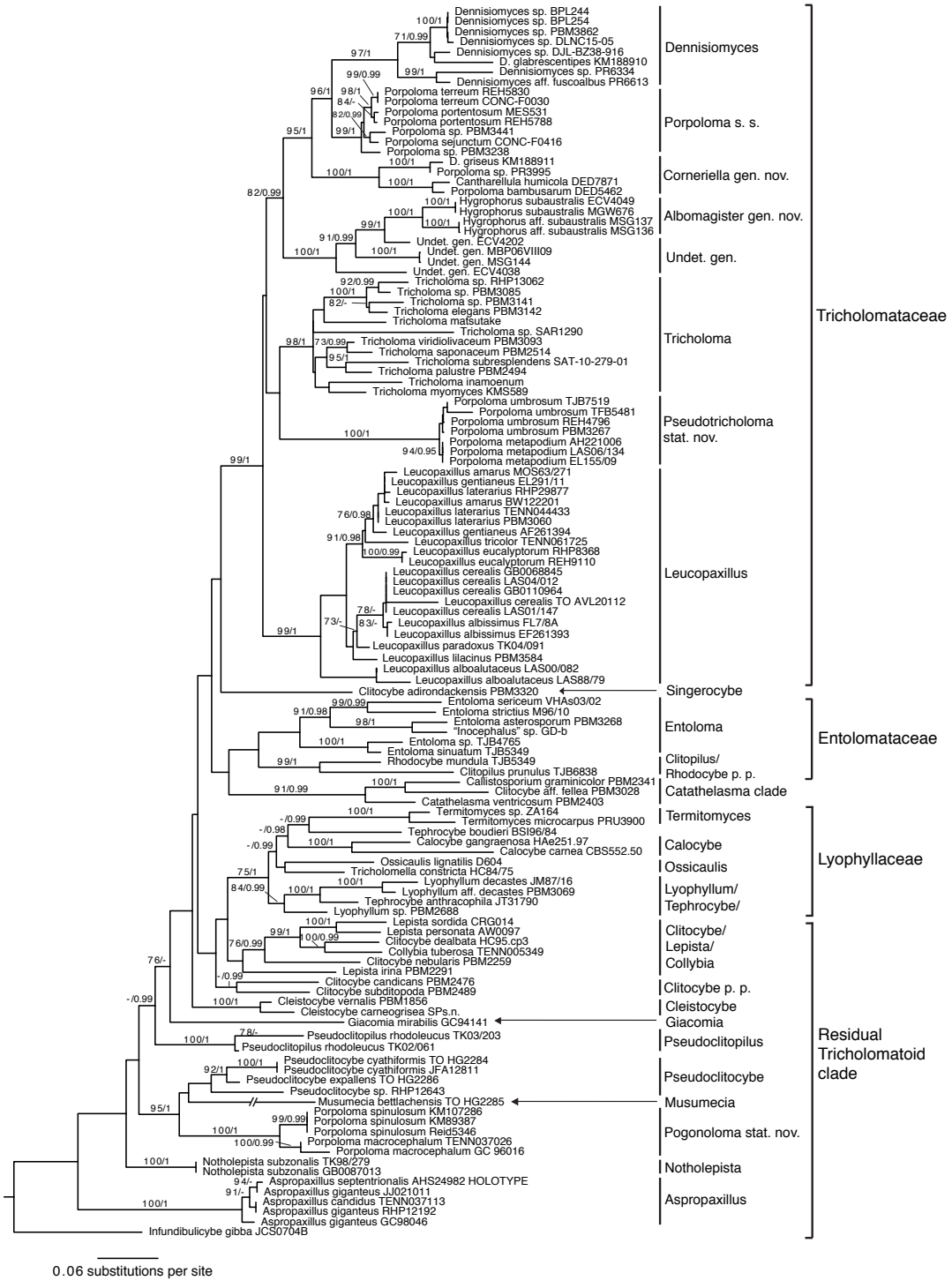

Figure I.1. Maximum Likelihood phylogeny (nLSU, nSSU, *rpb2*) of the suborder Tricholomatineae. Bootstrap values ≥70 and Bayesian posterior probabilities ≥0.95 are shown above branches. Taxa labels follow former nomenclature.

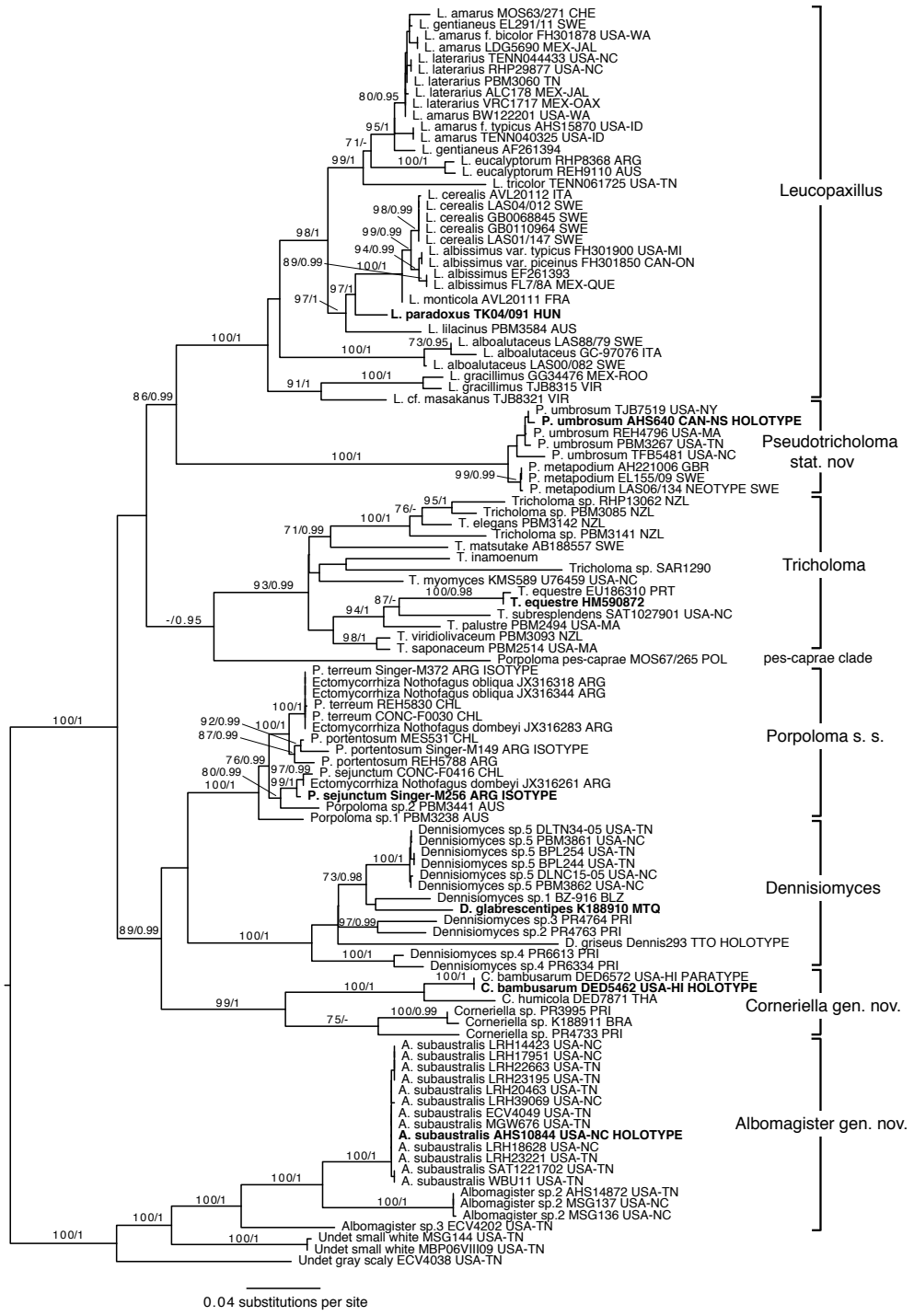

Figure I.2. Mid-point rooted Maximum Likelihood phylogeny (ITS, nLSU, nSSU, *rpb2*) of the Tricholomataceae s.str. Bootstrap values ≥70 and Bayesian posterior probabilities ≥0.95 are shown above branches. Types of generic names are indicated in bold. Taxa labels follow updated nomenclature presented in the Taxonomy section.

Figure I.3. Genera of the Tricholomataceae s.str. A, *Hygrophorus subaustralis*, type of *Albomagister* gen. nov. (photo: Mike Wood); B, *Dennisiomyces* sp. 5; C, *Leucopaxillus laterarius*; D, *Tricholoma myomyces*; E, *Porpoloma bambusarum*, type of *Corneriella* gen. nov. (photo: Dennis Desjardin); F, *Porpoloma umbrosum*, now transferred to *Pseudotricholoma* (photo: Renée Lebeuf); G, *Porpoloma* s.str. sp. 1 from Australia. Scale bars equal 1 cm.

CHAPTER II

Is the switch to an ectomycorrhizal state an evolutionary key innovation in mushroom-forming fungi? A case study in the Tricholomatineae (Agaricales)

A version of this chapter has been submitted for publication by M. Sánchez-García and P. Brandon Matheny

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MSG designed the study, obtained and analyzed the data, and wrote the manuscript. PBM contributed to manuscript revisions.

Abstract

Although fungi are one of the most diverse groups of organisms, little is known about the processes that shape such an outstanding diversity. This study focuses on the evolution of ectomycorrhizal (ECM) mushroom-forming fungi, symbiotic associates of many trees and shrubs, in the suborder Tricholomatineae of the Agaricales. We used the BiSSE model and BAMM to test the hypothesis that the ECM habit represents an evolutionary key innovation that allowed the colonization of new niches followed by an increase in diversification rate. We detected two diversification rate increases in the genus *Tricholoma* and the Rhodopolioid clade of the genus *Entoloma*. However, no increases in diversification were detected in the four other ECM clades of Tricholomatineae. We suggest that diversification of *Tricholoma* was not only due to the evolution of the ECM lifestyle, but to the expansion and dominance of its main hosts and ability to associate with a variety of hosts. Diversification in the Rhodopolioid clade could be due to the unique combination of spore morphology and ECM habit. The spore morphology may represent an exaptation that aided spore dispersal and colonization. This is the first study to investigate rate shifts across a phylogeny that contains both non-ECM and ECM lineages.

Introduction

Species diversity is unevenly distributed across the tree of life (Gould et al., 1987). One explanation for this heterogeneous diversity pattern is clade age, in

which older clades have had more time to diversify and accumulate species than younger clades (McPeek and Brown, 2007; Wiens, 2011; Bloom et al., 2014). An alternative explanation is that species-rich clades have higher net diversification rates (speciation minus extinction) than species-poor clades (Mooers and Heard, 1997; Rabosky et al., 2007; Mullen et al., 2011; Wiens, 2011; Bloom et al., 2014). Diversification rates can be affected by ecological and geographic opportunities such as opening of new environments, extinction of competitors, or the evolution of key innovations (novel traits) that permit entry into new niche spaces (Simpson, 1953; Heard and Hauser, 1995; Schluter, 2000; Yoder et al., 2010). Estimating diversification rates contribute to the study of patterns of species richness and to understanding the role of biotic and abiotic factors that shape extant diversity (Wiens, 2011).

Novel analytical methods have provided insights into the evolution of fungi, and some patterns of diversification have begun to be explored. Wilson et al. (2011) investigated the effects of gasteromycetation on rates of diversification and found that the net diversification rate of gasteroid forms is higher than that of non-gasteroid forms, suggesting that if such rates remain constant, the gasteroid forms will become dominant in the clades where they have arisen. A study of the genus *Trichoderma* showed that host jumps are associated with an increase in speciation rates, indicating that a transition from saprotrophy to mycoparasitism may be acting as a driver of species diversification (Chaverri and Samuels, 2013). Kraichak et al. (2015) studied two of the largest families of lichenized fungi and showed that while these two families have evolved similar ecological strategies, they have reached such hyper-diversity through different evolutionary pathways. Recently, Gaya et al. (2015) studied the lichenized fungi Teloschistales and found that the presence of anthraquinones, a pigment that protects against ultraviolet light, promoted diversification of this group by providing the opportunity to colonize habitats with increased sun exposure.

In this study, we investigate diversity patterns of ectomycorrhizal (ECM) fungi within the suborder Tricholomatineae of the Agaricales (Dentinger et al., 2015). ECM fungi form symbiotic relationships with the roots of plants, in which

plants obtain nutrients, increased water absorption, and protection against pathogens in exchange for providing photosynthates to their fungal symbionts (Smith and Read, 2008). Approximately 30 families of plants are involved in this symbiosis, which include boreal, temperate, and tropical tree species in families that dominate terrestrial landscapes such as Pinaceae, Fagaceae, Betulaceae, Salicaceae, Myrtaceae, and Dipterocarpaceae (Malloch, 1980; Wang and Qiu, 2006; Brundrett, 2009). At least 7,750 species of ECM fungi have been described, but it has been estimated that there are as many as 25,000 ECM fungal species (Rinaldi et al., 2008; Brundrett, 2009), and that the ECM mode has evolved from saprotrophic ancestors as many as 78–82 times with no known reversals to their ancestral state (Tedersoo and Smith, 2013).

The diversity of plants and fungi involved in this association, the ecological impact that it has on terrestrial ecosystems, and the multiple and asynchronous origins of ECM fungi over time suggest that the ECM habit represents an evolutionary key innovation that provides access to new ecological resources followed by an increase in diversification rate (Pirozynski and Malloch, 1975; Malloch, 1980; Brundrett, 2009; Ryberg and Matheny, 2012; Tedersoo and Smith, 2013). However, this hypothesis has not been explicitly tested. Here, we used two different methods to estimate trait dependent and independent diversification rates in order to investigate whether transitions in nutritional mode (non-ECM to ECM) are associated with an increase in diversification rate. We focus our study on the suborder Tricholomatineae (formerly the Trichlomatoid clade; Matheny et al. 2006), which contains roughly 30 genera including ECM and non-ECM groups (Sánchez-García et al., 2014). If the ECM mode is a trait that promotes lineage diversification, then we would expect to see an increase in diversification rate in all clades that present this trait compared to those that are non-ECM.

Materials and Methods

Data assembly. — A dataset of the Tricholomatineae was assembled using sequences from Sánchez-García et al. (2014), and expanding gene sampling by

incorporating newly produced *rpb1* sequences as well as sequences from GenBank (Table II.1). To expand taxon sampling, we searched for sequences of other members of the Tricholomatineae that were available in GenBank. To avoid species redundancy, we only included taxa that were not represented in our dataset and for which nLSU-rRNA sequences were available; then we searched for more loci available (5.8s, *rpb1*, *rpb2*, and nSSU-rRNA) and included them only if we could confirm that such sequences were from the same collections (i.e. comparing definition lines and modifiers). In addition, we obtained sequences from collections of Tricholomatineae accessioned at the University of Tennessee Herbarium (TENN), targeting taxa that were not already represented in our dataset.

DNA extraction, PCR, and sequencing protocols for ITS, nLSU, nSSU, and *rpb2* are described in Sánchez-García et al. (2014). Conserved domains A–C from the *rpb1* gene region was amplified and sequenced using the same protocols used for *rpb2* but using primers gAf, fCr, int2f, int2.1f, and int2.1r (Stiller and Hall, 1997; Matheny et al., 2002; Frøslev et al., 2005). Individual loci were aligned using MAFFT 7.244 (Katoh and Standley, 2013) and manually adjusted using Aliview 1.17.1 (Larsson, 2014). Gblocks 0.91b (Castresana, 2000) was used to remove ambiguously aligned regions. The ITS1 and ITS2 regions were excluded due to the great variability they present across the suborder Tricholomatineae, so the only region used for the analyses from ITS data was the 5.8S. Individual gene datasets were concatenated using SeaView 4.5.4 (Gouy et al., 2010) after inspection of intergene conflict. PartitionFinder 1.0.1 (Lanfear et al., 2012) was used to find the best partition strategy and the best models of molecular evolution.

Field collections and microscopy. — A time calibrated phylogeny was reconstructed in BEAST 2.2.1 (Bouckaert et al., 2014) using an uncorrelated log-normal distributed clock. Sequences of *Ampulloclitocybe clavipes*, *Cantharocybe gruberi*, *Clavaria inaequalis*, and *C. zollingeri* were used for outgroup purposes and excluded for subsequent analyses. Calibration points for the Agaricales and the *Ampulloclitocybe-Cantharocybe* clades were obtained from Ryberg and Matheny

(2011). The dataset was divided into eight partitions as suggested by PartitionFinder, and the substitution model GTR+I+G was used for each partition with parameters unlinked across partitions. Four independent Markov chain Monte Carlo (MCMC) were run for 120 million generations, sampling every 12 000 generations. Chain convergence was assessed using Tracer 1.6 (Rambaut and Drummond, 2007). Sixty million generations were excluded as burn-in. One of the chains was excluded from the analysis since the mean log-likelihood value was considerably lower than the others. The remaining three chains were combined using LogCombiner 2.2.1. A maximum clade credibility (MCC) tree with median ages was obtained with TreeAnnotator 2.2.1.

Trophic status assignment. — Nutritional mode was coded as a binary state (0 for non-ECM and 1 for ECM). We based this assignment on recent reviews of ECM associations (Rinaldi et al., 2008; Tedersoo et al., 2010; Tedersoo and Smith, 2013). In addition we obtained stable isotope ratios (^{15}N:^{14}N and ^{13}C:^{12}C) from fungal sporocarps of 45 taxa in the Tricholomatineae with unknown or unclear trophic status. Stable isotope ratios can provide information about the acquisition, transformation, and export of C and N by fungi. Relative to saprotrophs, ECM fungi are more enriched in ^{15}N and depleted in ^{13}C (Hobbie et al., 1999; Griffith, 2004; Mayor et al., 2009). Given that we used herbarium specimens collected during different years to obtain information on stable isotopes, we corrected ^{13}C values for the Suess effect, which is a change in the ratio of atmospheric concentrations of carbon due to the lack of ^{14}C in fossil fuel derived CO_2 (Tans et al., 1979).

It is not usual that a genus shows a mixed trophic status. Regularly, all species within a genus considered to be ECM will have the ability to form mycorrhizae, or at least the species that are ECM will be defined in a monophyletic group (Taylor and Alexander, 2005; Tedersoo et al., 2010). Therefore, in cases where no information was available for taxa that were resolved within an ECM lineage, these taxa were scored as ECM.

Ancestral state reconstruction of ECM lineages. — Maximum likelihood (ML) and Bayesian ancestral state reconstruction (ASR) analyses were performed

using BayesTraits V2 (Pagel et al. 2004, available from www.evolution.reading.ac.uk/BayesTraits.html) on 100 randomly sampled trees from the posterior distribution of the BEAST analysis. For the ML ASR 100 attempts of maximizing the likelihood were executed. The Bayesian ASR was run for 50 million generations, sampling every 5 000 generations, and with a burn-in of 5 million generations. Transition rate priors were adjusted to uniform [0,1] based on ML values for these parameters.

Trait-dependent diversification rates. — To assess the effect of switches between non-ECM and ECM states, we used the binary state speciation and extinction model (BiSSE; Maddison et al. 2007) implemented in the Diversitree package in R (Fitzjohn, 2012) and corrected for incomplete phylogenies (FitzJohn et al., 2009). We estimated the total number of species missing for each character state according to data from Singer (1986), Kirk et al. (2008), Co-David et al. (2009), Morgado et al. (2013), and Kluting et al. (2014).

The BiSSE model estimates speciation (λ), extinction (μ), and transition rates (q) of each binary state (0 and 1). In this case 0 represents non-ECM and 1 represents ECM. We evaluated five models using a maximum likelihood approach and compared their AIC scores: 1) full model ($\lambda 0$, $\lambda 1$, $\mu 0$, $\mu 1$, q01, and q10); 2) irreversible model (q10 = 0); 3) irreversible model and equal extinction rates (q10 = 0, $\mu 0 = \mu 1$); 4) irreversible model and equal speciation rates (q10 = 0, $\lambda 0 = \lambda 1$); and 5) irreversible model with equal speciation and extinction rates (q10 = 0, $\lambda 0 = \lambda 1$, and $\mu 0 = \mu 1$). We performed a likelihood ratio test and compared AIC scores to identify the best model. In addition, we conducted MCMC estimations of each model for 10 thousand generations.

Trait-independent diversification rate shifts. — Bayesian Analysis of Macroevolutionary Mixtures (BAMM version 2.3; Rabosky 2014) was used to estimate diversification rates independently of character states. This method detects and estimates heterogeneity in evolutionary rates across a phylogeny. We ran 10 million generations of reversible jump MCMC sampling. We used the package BAMMtools (Rabosky et al., 2014) in R to estimate speciation and extinction priors and to evaluate the outputs. We assumed incomplete sampling

and assigned clade-specific sampling probabilities based on the estimated number of species for each of the genera represented in our phylogeny according to Kirk et al. (2008).

When available we followed estimates published by experts on specific groups (Singer, 1986; Co-David et al., 2009; Morgado et al., 2013; Kluting et al., 2014). However, it is widely known that fungal taxonomic diversity is often underestimated (Hawksworth, 2001; O'Brien et al., 2005; Blackwell, 2011). To account for the effects of phylogenetic uncertainty, we performed BAMM analyses on 100 randomly sampled trees from the posterior distribution of the BEAST analysis. These analyses were run for five million generations indicating specific priors obtained in BAMM for each phylogenetic tree.

Results

Divergence times and ECM lineages in the suborder Tricholomatineae. — The time-calibrated phylogeny consisted of 294 taxa including four outgroups (Fig. II.1). Fifty three percent of the nodes were recovered with a posterior probability >0.9. The median crown age of the Tricholomatineae is estimated at 117 Mya (with a 95% node height posterior density (HPD) of 74–166 Mya), hereafter written as 117 (74–166) Mya. Three main families (Tricholomataceae, Entolomataceae, and Lyophyllaceae) diverged during the late Cretaceous (78–93) Mya. The crown age of the family Tricholomataceae was estimated at 78 (48–111) Mya, the Entolomataceae 86 (55–122) Mya, and the Lyophyllaceae 79 (48–111) Mya. Another major lineage is the *Catathelasma* clade with an estimated stem age at 97 (63–139) Mya, and crown age at 71 (40–105) Mya.

The ancestral state of nutritional model in the Tricholomatineae was reconstructed as non-ECM. This state was also recovered as ancestral for the Tricholomateaceae, Entolomataceae, Lyophyllaceae, and the *Catathelasma* clade, which are the main monophyletic groups recognized within this suborder. We found six independent origins of the ECM mode in the Trichlomatineae: *Albomagister*, *Catathelasma*, *Entoloma* p. parte (Rhodopolioid clade), *Lyophyllum* p. parte, *Porpoloma*, and *Tricholoma* (Fig. II.1). *Albomagister* diverged from its sister

clade that includes *Corneriella*, *Dennisiomyces*, and *Porpoloma* 62 (37–90) Mya. *Porpoloma*, an ECM group restricted to the Southern Hemisphere, diverged from *Corneriella* and *Dennisiomyces* 39 (22–59) Mya. *Tricholoma*, the largest genus within the family Tricholomataceae, diverged from its saprotrophic sister clades *Dermoloma* and *Pseudotricholoma* 61 (36–92) Mya. *Catathelasma*, an ECM genus restricted to coniferous forests of the Northern Hemisphere diverged from *Cleistocybe* 50 (27–77) Mya. The genus *Lyophyllum* contains some species that are ECM (Tedersoo and Smith, 2013; Hofstetter et al., 2014); in our phylogeny we identified the clade containing *Lyophyllum decastes* as ECM. This clade diverged from its sister group 26 (12–43) Mya. The sixth ECM lineage within this suborder is a clade within *Entoloma*, the Rhodopolioid clade sensu Co-David et al. (2009), which split from its sister group 67 (42–97) Mya; its crown age was estimated at 40 (16–74) My.

Evolution of nutritional mode and effect on diversification rates. — The 'full model' that allows all six parameters to be estimated shows a transition rate from ECM to non-ECM of zero probability (Table II.2), which is consistent with previous works that support the hypothesis of no reversals from ECM to a saprotrophic state (Bruns and Shefferson 2004; Tedersoo and Smith 2013). Based on this hypothesis, our results from the BiSSE analysis, and ancestral state reconstruction of nutritional mode, the remaining models were constrained to a transition rate from ECM to non-ECM of zero (q10=0).

The 'irreversible model with equal extinction rates' was supported as the best model based on AIC scores (Table II.3). This model does not allow for reversals from ECM to non-ECM modes, constrains extinction rates to be equal in both non-ECM and ECM lineages, and allows for speciation rates to be independently estimated. Based on this model the net diversification rate of ECM lineages is 2.27 times higher than that of non-ECM lineages (Fig. II.2). However, a likelihood ratio test shows that this model is not significantly different from the 'full model' and the 'irreversible model' (Table II.3). Both of these latter models also support higher net diversification rates in ECM clades compared to non-ECM clades. These three models support state dependent diversification, in which the ECM

mode is correlated with higher net diversification rates. As stated earlier the model that constrains extinction rates to be equal ($\mu 0=\mu 1$) was better supported over the model that constrains speciation rates to be equal ($\lambda 0=\lambda 1$). This implies that speciation has played a major role in the diversification of ECM lineages.

Diversification rate shifts. — The most frequently sampled shift configuration (f=0.4) shows three increases in diversification rate across the suborder Tricholomatinaea (Fig. II.3A and Fig. II.4). One occurs at the base of *Tricholoma*, a second shift at the base of the clade that includes the Entolomataceae, Lyophyllaceae, and the *Catathelasma* clade, and a third shift in the Rhodopolioid clade of *Entoloma*. Furthermore, these shifts are supported by branch specific Bayes factors. The second most frequently sampled configuration (f=0.26) also supports three shifts, with the only difference that the shift in *Tricholoma* corresponds to a subclade within this genus (Fig. II.4).

When accounting for phylogenetic uncertainty in which the BAMM analyses were executed on 100 randomly sampled trees from the posterior distribution of the BEAST analyses we found support for one to four increases in diversification rates across the Tricholomatineae; 78% of the replicates recovered an increase in *Tricholoma*, while 94% recovered an increase in the Rhodopolioid clade.

Discussion

The ECM mode as an evolutionary key innovation is not fully supported. — Symbiotic relationships are widespread and ecologically important, and they may be considered evolutionarily advantageous, as they provide access to newly unexplored ecological resources (Pirozynski and Malloch, 1975; Margulis and Fester, 1991; Leigh Jr, 2010). Evolutionary key innovations are defined as evolutionary changes that promote an increase in net diversification rate (Heard and Hauser, 1995). If these evolutionary innovations evolve in multiple lineages, they can be recognized as being of adaptive significance (Hunter, 1998). The ECM symbiosis has evolved numerous times across the fungal and land plant tree of life. This association increases the efficiency in the acquisition of nitrogen and phosphorous in plants, while providing carbon to the fungi, which can

consequently allow both partners to allocate more resources into growth and improve fitness, resulting in the exploitation of new habitats (Pirozynski and Malloch, 1975). Many ECM fungal clades are particularly diverse, which suggests that the evolution of this trophic mode might be an evolutionary key innovation.

Results from the BiSSE analyses support this hypothesis since they show that the net diversification rates of ECM lineages are on average two times higher than the net diversification rates of non-ECM lineages (Fig. II.2). In contrast, the BAMM analyses do not support this hypothesis, since only two out of six ECM clades show and increase in diversification rates: *Tricholoma* and the Rhodopolioid clade of *Entoloma* (Fig. II.3B). If the ECM mode were linked to an increase in diversification rates, we should expect BAMM to detect increase rate shifts in all ECM clades of the Tricholomatineae.

Recent criticisms of the BiSSE approach have been discussed. Davis et al. (2013) showed that the power of the BiSSE method is affected by a low sample size and a high character tip bias. They indicated that a tree size of 300 tips has a higher statistical power than trees with 50 or 100 tips, and also recommended to exclude traits that represent less than 10% of tip sampling. Our phylogeny contains 295 taxa, of which 17% are ECM and 83% are non-ECM. Another criticism raised by Fitzjohn (2012) and Rabosky and Goldberg (2015) is the risk of false positives when a single shift in diversification rate occurs in a highly diverse clade, which can show a correlation between a trait and diversification rate even when such trait is neutral. In order to account for this problem, we used BAMM, which does not consider trait dependent diversification, but identifies shifts in diversification rates across the phylogeny. In our analyses we see that shifts in diversification in two ECM clades may be dominating the results from BiSSE, which could lead us to erroneously conclude that all the clades that present this trait have increased diversification rates. However, no increases were detected in the other four ECM clades of the Tricholomatineae when performing the BAMM analyses.

Diversification of *Tricholoma*. — *Tricholoma* forms ECM associations with various angiosperm families and the conifer family Pinaceae. It contains

approximately 200 species (Kirk et al., 2008). Some species (~20%) have a narrow host range and may be restricted to a single host genus (e.g. *T. diemii, T. populinum, T. robustum*), while the rest have an intermediate to wide host range. The latter species associate with several members of the same family, across families, or orders (e.g. *T. focale, T. magnivelare, T. focale, T. caligatum, T. sulphureum*) (Molina et al., 1998). Our analyses suggest that *Tricholoma* began to diversify during the late Eocene. After this time the Earth transitioned from a warm to a temperate climate, tropical forests contracted from temperate regions of today, and members of the Pinaceae and Fagales began to dominate temperate latitudes (Prothero and Berggren, 1992; Lear et al., 2008). *Tricholoma* is more prevalent in temperate forests than tropical ecosystems occurring with dominant trees such as *Quercus*, *Betula, Picea, Pinus, Nothofagus,* and *Tsuga* among others (Trappe, 1962). Climatic and biotic events following the Eocene could have promoted or triggered diversification of this lineage. Considering that the majority of species of *Tricholoma* have the ability to form ECM associations with a wide range of hosts, we cannot rule out the possibility that being a generalist has provided these taxa with the opportunity to expand their ranges to explore and adapt to new environmental niches, increasing in this way their rate of diversification. An extensive sampling of this clade including host information is needed to further test this hypothesis.

Bruns et al. (1998) suggested simultaneous radiations of ECM lineages during the Eocene-Oligocene transition when their hosts expanded due to the cooling of climates. Ryberg and Matheny (2012) tested this hypothesis and found that ECM lineages of Agaricales originated in two different periods, the late Cretaceous and the early Cenozoic. Their divergence time estimates suggest that the crown group of *Tricholoma* originated during the Cretaceous. We estimated a younger origin for both the stem and crown groups, but a late Cretaceous origin for the crown group of Tricholoma cannot be rejected. The stem age of Ryberg and Matheny (2012) may have been overestimated as they failed to include the sister group of *Tricholoma*, the clade containing *Dermoloma* and *Pseudotricholoma*. On the other hand, since our analyses were done at a suborder level we could not

include those taxa to which only ITS sequences were available; therefore, due to the small sampling size of *Tricholoma* (38 spp.), the age of the crown group in this study could be underestimated.

Diversification of the Rhodopolioid clade. — The Rhodopoliod clade is part of the highly diverse genus *Entoloma*, which contains approximately 1200 species (Noordeloos, 1992; Co-David et al., 2009; Morgado et al., 2013; Kluting et al., 2014). This cosmopolitan genus is characterized by having angular basidiospores (meiospores) in all views (Co-David et al., 2009). It contains species that are ECM, saprotrophs, or parasites. The ECM taxa are restricted to the Rhodopoliod clade and associate with *Quercus, Salix*, *Alnus,* and *Populus* (Antibus et al., 1981; Loree et al., 1989; Noordeloos, 1992; Agerer, 1997; Smith et al., 2007; Avis et al., 2008; Walker et al., 2008). This group originated during the late Eocene and began to diversify by the early Miocene, a period that was characterized by a transition from warmer to colder conditions with several reversals to warmer conditions (Zachos et al., 2001, 2008). During the early Miocene there was an expansion of biomes such as grasslands, deserts, taiga, and tundra (Wolfe, 1985; Jacobs et al., 1999; Strömberg, 2005), which could have favored the expansion of ECM hosts like *Salix* and *Populus*. Additionally, some species in this clade are common in grasslands (Noordeloos, 1992). These factors could have allowed members of the Rhodopoliod clade to expand their range and encounter new ecological opportunities for diversification.

Another factor that could have promoted an increase in diversification rates was the evolution of angular spores (angular in face and/or polar views). The entire genus *Entoloma* possesses this character, but in the case of the Rhodopolioid clade, it could be a pre-adaptation or exaptation that, if combined with the ECM mode, may have provided additional advantage to colonize new niches. ECM spores need to disperse near to their host roots in order to germinate and colonize them. Therefore, soil arthropods may be important vectors for their dispersal, and spore ornamentation can aid in such dispersal (Lilleskov and Bruns 2005). While the spores of Entolomataceae are not ornamented, their ultrastructure and shape are unique in that they gradually

develop bumps and ridges by the thickening of the epicorium, a layer between the tunica and the coriotunica, which gives them the polyhedral or angular shape (Pegler and Young, 1978; Clémençon, 2004). Surface topography affects wetting behavior, and surface roughness or irregularity can provide the spores with hydrophobic properties that favor their transport by arthropods or water (Davies, 1961; Lilleskov and Bruns, 2005; Spori et al., 2008). The latter dispersal mechanism could be favored by surface irregularity of the spores, which creates a high contact angle, reduces contact surface, and increases hydrophobicity. Particles with these properties can be captured by water droplets and then carried away, allowing for dispersal (Davies, 1961; Spori et al., 2008). Fungal spores are extremely important for reproduction and dispersal, and therefore important for colonization. Thus, their traits may be linked to critical ecological functions involved in the organisms' fitness. Information on this topic is scarce, but it is potentially an interesting area to investigate further.

At least 32 species have been confirmed in the Rhodopolioid clade (Kokkonen, 2015), and the entire group stems from a long branch with shallow tips consistent with our analytical observations of an increase in diversification rate in the group. Phylogenetic placement of other biotrophic taxa (e.g. *E. aprile*, *E. clypeatum*, *E. sepium*, and *E. saundersii*) is unclear (Kobayashi and Hatano, 2001), but a relationship with the Rhodopolioid clade has not been rejected (Kasuya et al., 2010; Kinoshita et al., 2012). Hesler (1967) suggested *E. clypeatum* is closely related to *E. rhodopolium*. While such biotrophic taxa have been considered mycorrhizal (Kinoshita et al., 2012), some studies show that the fungal cells infect and destroy the root cells forming a parasitic relationship rather than symbiotic (Agerer and Waller, 1993; Kobayashi and Hatano, 2001; Kasuya et al., 2010).

At least some members of the Rhodopolioid clade (e.g., *Entoloma sinuatum*) also possess unknown toxic secondary metabolites that produce a suite of gastrointestinal symptoms in humans (Benjamin, 1995). Little is known about their pharmacology (Bresinsky and Besl, 1990). Indeed, *E. sinuatum* is one of the

more common causes of mushroom poisoning in Europe (Alder, 1961; Benjamin, 1995).

Species poor ECM lineages within the Tricholomatineae. — While *Tricholoma* and the Rhodopolioid clade have elevated rates of diversification, the other four ECM lineages in the Tricholomatineae do not show this pattern. Their diversification rates are similar to background rates. A possible scenario for this could be that increased diversification rates in ECM lineages are limited to those that have the ability to associate with a wide range of hosts. *Porpoloma* and *Catathelasma* have a narrow to intermediate host range. *Porpoloma* is restricted to the Southern Hemisphere, forming ECM associations with *Nothofagus* (Trappe, 1962; Garrido, 1988), and probably with *Eucalyptus* and/or *Acacia* (P.B. Matheny pers. obs.), and *Catathelasma* only associates with members of the Pinaceae (Hutchison, 1992). As ECM hosts can restrict the distribution of their symbionts, this could also limit niche availability and, therefore, fail to provide the necessary scenario for an accelerated diversification. In addition, *Catathelasma* has been suggested to be a late colonizer in coniferous forests (Hutchison, 1992). This could be due to a slow growth rate or poor spore germination (Bowen, 1994), which can contribute to its niche limitation.

The case of *Lyophyllum* may be different since it has an intermediate to wide range, whereas it has only been reported *in situ* forming ECM associations with members of the Fagaceae (Agerer and Beenken, 1998; Bergemann and Garbelotto, 2006). ECM *Lyophyllum* species have been found in pine forests where members of Fagaceae are not reported (Larsson and Sundberg, 2011). This and the ability of some species to form associations with *Pinus* spp. in laboratory conditions (Pera and Alvarez, 1995; Kawai, 1997; Yamada et al., 2001; Visnovsky et al., 2014) suggest that *Lyophyllum* can associate with Fagaceae and Pinaceae. In vitro culturing studies show that *Ly. decastes* has a poor development of ECM structures (Yamada et al. 2001), and *Ly. shimeji* forms a thin mantle (Visnovsky et al. 2014), which could indicate a weak ECM association that limits its ability to colonize new niches. Structural differences in the mycorrhizae are functionally relevant; for example, mycorrhizae with a long distance exploration type may

acquire and transport nutrients more efficiently than medium and short distance types, while mycorrhizae with a contact or short distance exploration type usually have a more developed mantle that increases efficiency of storing and exchanging nutrients (Agerer, 2001; Smith and Read, 2008). *Lyophyllum* is the youngest of the ECM clades in the Tricholomatineae (26 (12–43) Mya), and it could be possible that it is transitioning to an ECM state and not yet had the time to diversify unlike *Tricholoma* and the Rhodopoloid clade.

Albomagister is a recently described lineage growing in plant communities dominated by *Quercus* spp. and *Tsuga heterophylla* (Sánchez-García et al., 2014), as well as *Pinus*, *Quercus*, and *Eucalyptus* (Moreau et al., 2015). Stable carbon and nitrogen isotope signatures support the ECM status of the group (Table II.4). However, the identity of its hosts and whether it can associate with more than one host remain unknown. Future studies, such as in vitro culturing and *in situ* identification (i.e. ECM roots samples), will be important to understand the ecology of this ECM group.

Factors promoting diversification of ECM fungi. — The effect of a trait on diversification rate may be dependent on several factors such as interactions with other taxa, additional traits from the same organisms, and physical environmental conditions (de Queiroz, 2002). The ECM mode is not fully supported as a key innovation in the Tricholomatineae, but diversification of some ECM lineages can be favored by physical factors such as historical climate change (e.g. cooling temperatures that allow the expansion of host plant communities). ECM structural features can affect ecological function (Agerer et al., 2000; Agerer, 2001): spore morphology can aid in dispersion and colonization of new areas. Exploration types (categories for describing the distribution of extraradical mycelium) may also play an important role in diversification, as they may alter the rate of acquisition and transport of nutrients, which can also depend on the type of environment. Medium-distance fringe and long-distance exploration types are particularly efficient at N uptake, which they obtain from complex organic molecules, but they have a negative response to N deposition. In contrast, short-distance and medium-distance smooth exploration types obtain

labile N such as amino acids and are more common in mineral soils (Lilleskov et al., 2011). Both *Tricholoma* and the Rhodopoliod clade form medium-distance exploration types with uniformly shaped rhizomorphs, but *Tricholoma* can also develop differentiated rhizomorphs. The ECM of the Rhodopolioid clade are hydrophilic, which allows for successful exploration of the substrate in the surrounding area (Agerer, 2001). As exploration types and ECM morphology are phylogenetically conserved traits and have a correlation with the production of enzymes (e.g. phenoloxidases) (Agerer et al., 2000; Eberhardt, 2002; Agerer, 2006; Tedersoo and Smith, 2013), testing the effects of these traits on diversification rates is a promising topic for further research.

Confidence in assignment of trophic status. — *Tricholoma* and *Porpoloma* are widely accepted as ECM taxa (Trappe, 1962; Garrido, 1988; Molina et al., 1998; De Roman et al., 2005; Agerer, 2006; Zeller et al., 2007; Rinaldi et al., 2008; Tedersoo et al., 2010). On the contrary, there is inconsistent evidence regarding the mycorrhizal status of *Leucopaxillus*, which explains why we scored this genus as non-ECM in the present work. Synthesis experiments suggest it is saprotrophic (Hart et al., 2006; Tedersoo et al., 2010), while isotopic evidence indicates it is ECM (Trappe, 1962; Lu et al., 1998; Agerer, 2006; Rinaldi et al., 2008). Singer et al. (1983) discuss that there is no evidence that tropical species of *Leucopaxillus* are ECM. It has been suggested that *Le. lilacinus* forms ECM with *Eucalyptus* (Lu et al., 1998). Yamada et al. (2001) and Kohzu (1999) support *Leucopaxillus* as non-mycorrhizal, however, their conclusion is based on specimens of *Aspropaxillus giganteus*, a species traditionally considered as *Leucopaxillus* but has recently been shown to be phylogenetically distant from *Leucopaxillus* sensu stricto (Sánchez-García et al., 2014). Recent analysis of stable isotopes revealed that two species, *Le. laterarius* and *Le. tricolor,* are highly ^{15}N enriched suggesting a biotrophic status (Mayor et al., 2009; Birkebak et al., 2013). We obtained stable isotope data for several species of *Leucopaxillus* and did not find a clear pattern that defines this group as ECM or non-ECM (Fig. II.5). Further analyses such as synthesis experiments including several species of *Leucopaxillus*, use of radiocarbon (^{14}C) (Hobbie et al., 2012), and identification of

ECM roots *in situ* should be performed in order to confidently assign a trophic status to this group. In this work, we used a conservative approach, and we only considered a clade to be ECM if there were sufficient and consistent sources of evidence to support it as ECM.

In a review of the ectomycorrhizal lifestyle, Tedersoo et al. (2010) treated a clade of *Lyophyllum* (/paralyophyllum) as ECM based on the evidence that *Ly. decastes*, *Ly. shimeji* and *Ly. semitale* form ECM structures when growing in culture with *Pinus* spp. (Pera and Alvarez, 1995; Kawai, 1997; Yamada et al., 2001; Visnovsky et al., 2014), *in situ* identification forming ECM with *Quercus robur* and *Notholithocarpus densiflorus* (Agerer and Beenken, 1998; Bergemann and Garbelotto, 2006), and stable isotope signatures (Kohzu et al., 1999). In addition, we obtained stable isotope data for one taxon used in these analyses, which is consistent with an ECM signature (Fig. II.5; Table II.4). In the same review, the genus *Catathelasma* was also considered as ECM since it can form ECM structures when cultured with *P. banksiana* (Hutchison, 1992), and it shows a stable isotope signature consistent with an ECM lifestyle (Kohzu et al., 1999).

The Rhodopolioid clade is the only lineage within the genus *Entoloma* that is considered to be ECM. Evidence to support this includes synthesis experiments (Agerer, 1997; Montecchio et al., 2006), *in situ* identification (Walker et al., 2005; Smith et al., 2007, 2013; Geml et al., 2012), and stable isotope information (Kohzu et al., 1999; Trudell et al., 2004). This group also holds as monophyletic in recent taxonomic revisions of the family Entolomataceae (Co-David et al., 2009; Baroni and Matheny, 2011; Kinoshita et al., 2012). Within this group we exclude those taxa that associate with members of the Rosaceae, which seem to be a parasitic association rather than ECM (Agerer and Waller, 1993; Kobayashi and Hatano, 2001).

We considered *Albomagister* as ECM based on stable isotope evidence (Birkebak et al. 2013 and Table II.4). We recognize that stable isotope data should be considered as supplementary and not as the only source of evidence to assign a trophic status, as both $\delta^{15}N$ and $\delta^{13}C$ values may be affected by site and environmental factors (Henn and Chapela, 2000; Taylor et al., 2003; Mayor et al.,

2015). However, all the samples that were analyzed showed a consistent ECM pattern after normalization of the data. Therefore, our decision to consider *Albomagister* as ECM is justifiable. Further analyses including identification of colonized roots and laboratory syntheses experiments are necessary in order to confirm its ECM status and host identity.

Potential biases in this study. — Incomplete and non-random sampling can bias diversification analyses (Heath et al., 2008; Brock et al., 2011; Ryberg and Matheny, 2011). The methods used for this work account for these biases, however, they require the user to have enough information to assign number of missing taxa to specific clades. This can be challenging working with fungi since only a small percentage of the estimated fungal diversity is known, and some groups lack current taxonomic revisions to provide confident estimates of their total diversity. An example of this can be seen in the genus *Clitocybe*. This genus is estimated to have 300 species (Kirk et al., 2008), however, it is highly polyphyletic. Several taxa have been segregated (Harmaja, 2003; Vizzini and Ercole, 2012; Alvarado et al., 2014), but not all species have been revised. Therefore, we could not assign estimates of number of species for each clade. Instead, we assigned a global estimate for all the clades that are currently considered within the genus *Clitocybe* including some that have been just recently revised but lack information on their estimated number of species (e.g. *Paralepista*). A similar problem occurs with *Entoloma*. Approximately 1200 species have been estimated within this genus, but estimates for specific clades are scarce. Hence, we assigned global estimates for the entire genus. We acknowledge that some clades within the genus may be more diverse than others and that sampling may not be random, but increased efforts in taxonomic sampling will provide more information to carefully address this issue.

Conclusions

We found evidence that supports increases in diversification in two of six ECM lineages of Tricholomatineae. One of these, *Tricholoma*, most likely began diversification by the late Eocene, which was probably favored by cooling

temperatures and the expansion of their host communities, as shown in other groups of ECM fungi (Ryberg and Matheny, 2012; Looney et al., 2016). An increased sampling of *Tricholoma* including information regarding host association is needed in order to test the hypothesis that wide host potential and range is correlated with high diversification rates. The Rhodopolioid clade began diversification by the early Miocene. Spore morphology in this group could be a pre-adaptation or exaptation that favored spore dispersal and colonization. The investigation of secondary metabolites involved in human poisoning is needed for all taxa after their taxonomy is clarified.

This is the first work that aims to test the hypothesis that the ECM lifestyle is a key innovation by comparing the net diversification rates of ECM lineages with those of saprotrophic clades. While this hypothesis is not fully supported, we can address new questions: Why are some ECM clades so diverse? Is there a set of traits that when combined with the ability to form ECM associations that promotes their diversification? What abiotic conditions mediate the evolutionary outcome of this symbiosis? The integration of fields such as functional ecology, genomics, systematics, and evolutionary biology will enable us to answer these questions, to better understand how evolutionary forces operate in ECM fungi, and to recognize the mechanisms that shape ECM fungal diversity. Finding a unique trait that can be attributed to promote diversification of a group may not be possible (Donoghue and Sanderson, 2015). The ECM lifestyle by itself did not promote diversification, but a combination of fungal and plant traits, physical environment, including climate and geography, may have allowed certain ECM clades to reach their high taxonomic diversity.

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Appendix

Table II.1. GenBank accession numbers of the Tricholomatineae dataset used in the present study.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Alboleptonia* aff. *sericella	MCA1978		GU384609		GU384632	
***Alboleptonia* sp.**	EH185	KU058498	KU058535		KU138998	KU139031
Alboleptonia stylophora	AST84		GU384610		GU384633	
***Albomagister* sp. 2**	MSG137	KJ417247	KJ417178		KJ424363	KU139018
***Albomagister* sp. 3**	ECV4202	KJ417250	KJ417179		KJ424365	KU139019
Albomagister subaustralis	MGW676	KJ417252	KJ417181	KJ417255	KJ424367	KU139020
Ampulloclitocybe clavipes	PBM2474		AY639881	AY771612	AY780937	
Aspropaxillus giganteus	TENN060129	KP453698	KJ417183	KJ417256	KJ424368	KU139021
Calliderma fibulatum	SP393751		FJ973677			
Calliderma pruinatocutis	SP393753		FJ973680			
Calliderma rimosum	SP393750		FJ973678			
Callistosporium graminicolor	PBM2341	DQ484065	AY745702	AY752974	KJ424369	GU187493
Callistosporium luteoolivaceum	JM99/124		AF261405			
***Callistosporium* sp.**	ECV5506	KU058492	KU058529		KU138992	KU139022
Calocybe carnea	CBS552.50	AF357028	AF223178	DQ367418	DQ367432	
Calocybe gangraenosa	HAe251.97	AF357032	AF223202	DQ367420	DQ367434	
Cantharocybe gruberi	PBM510	DQ200927	DQ234540	DQ234546	DQ385879	DQ200927
Catathelasma ventricosum	PBM2403	DQ486686	DQ089012	DQ435811	DQ470830	
Clavaria inaequalis	MB04-016	DQ202267	AY745693	DQ437680	DQ385880	DQ447890
Clavaria zollingeri	TENN58652	AY854071	AY639882	AY657008	AY780940	AY857987
Cleistocybe carneogrisea	S.Poumarat s.n.	HQ728526	HQ728527	HQ728528		

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Cleistocybe vernalis	PBM1856	DQ486692	AY647208	DQ092913		
Clitocybe* aff. *fellea	PBM3028	HQ728533	HQ728534	HQ728535	HQ728536	GB
Clitocybe candicans	PBM2476	DQ202268	AY645055	AY771609	DQ385881	DQ447891
Clitocybe dealbata	HC95.cp3	AF357061	AF223175	DQ825431	DQ825407	DQ825414
Clitocybe felea	PBM1439		EF561629			
Clitocybe nebularis	PBM2259	DQ486691	DQ457658	DQ437681	DQ470833	DQ825415
Clitocybe subdipoda	PBM2489	DQ202269	AY691889	AY771608	AY780942	DQ447892
Clitopilopsis hirneola	TB8490		GU384611		GU384646	
Clitopilus apalus	M536		AF261287			
Clitopilus caelatus	511		GQ289208			
Clitopilus caelatus	TB6995		GU384625		GU384652	
Clitopilus* cf. *argentinus	MTB48042				KC816907	
Clitopilus crispus	10027TJB				KC816911	
Clitopilus cystidiatus	26		GQ289147		GQ289220	
Clitopilus fallax	262		GQ289209		GQ289275	
Clitopilus fallax	37		GQ289210		GQ289276	
Clitopilus geminus	Co-David 407		HM164411			
Clitopilus geminus	TB7526		GU384626		GU384655	
Clitopilus giovanellae	AH 19718		EF413028			
Clitopilus hobsonii	TJB5967				KC816917	
Clitopilus hobsonii	DLL9643				KC816914	
Clitopilus hobsonii	DLL9746				KC816915	
Clitopilus hobsonii	DLL9779				KC816916	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Clitopilus lateritius	Co-David 418		HM164410			
Clitopilus nitellinus	265		GQ289214		GQ289281	
Clitopilus nitellinus	400		GQ289215		GQ289282	
Clitopilus pallidogriseus	118		GQ289216		GQ289283	
Clitopilus peri	TB10040				KC816921	
Clitopilus popinalis	260		GQ289213		GQ289280	
Clitopilus prunulus	2		GQ289149		GQ289221	
Clitopilus prunulus	GLM45889		AY207161			
Clitopilus prunulus	RV88/109		AF042645			
Clitopilus pseudopiperitus	162		GQ289217		GQ289284	
Clitopilus scyphoides	T777		AF261288			
Clitopilus scyphoides	HKI ST 22295		AY207162			
***Clitopilus* sp.**	211		GQ289212		GQ289279	
***Clitopilus* sp.**	CoDavid 266		HM164412		HM164416	
***Clitopilus* sp.**	TB8024		GU384613		GU384647	
***Clitopilus* sp.**	TB8067		GU384612		GU384649	
Clitopilus stanglianus	503		GQ289218		GQ289285	
Clitopilus venosulcatus	TB8111				KC816930	
Collybia tuberosa	TENN 53540	AY854072	AY639884	AY771606	AY787219	AY857982
Corneriella bambusarum	DED5462	KJ417264	KJ417185		KJ424370	KU139023
Corneriella humicola	DED7871	KF291051	KF291052	KF291053		
***Corneriella* sp.**	PR-4733	KF306336	KF306337		KF306338	
Dennisiomyces glabrescentipes	JPF1000A	KJ417326	KJ417235			

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
***Dennisiomyces* sp. 1**	BZ-916	KF291063	KF291064		KF291066	
***Dennisiomyces* sp. 2**	PR-4763	KJ417269	KJ417191			
***Dennisiomyces* sp. 3**	PBM3861	KJ417267	KJ417189	KU058567	KJ424374	KU139024
***Dennisiomyces* sp. 4**	PR-6613	KJ417268	KJ417190	KJ417257		KU139025
Dermoloma magicum	GG220904	KU058495	KU058532		KU138995	
***Dermoloma* sp.**	BW8	KU058493	KU058530	KU058568	KU138993	KU139026
***Dermoloma* sp.**	ECV4208	KU058494	KU058531	KU058569	KU138994	KU139027
***Dermoloma* sp.**	SAV4094	KU058496	KU058533		KU138996	KU139028
***Dermoloma* sp.**	SAV4103	KU058497	KU058534		KU138997	KU139029
Entoloma abortivum	TB6693		GU384616		GU384642	
Entoloma albidoquadratum	i4		GQ289151		GQ289223	
Entoloma alpicola	TB6415		AF261302			
Entoloma araneosum	14		GQ289153		GQ289225	
Entoloma asterosporum	PBM3268	JF706309	JF706310	JF706311	JF706312	KU139030
Entoloma bicolor	TB4967		AF261298			
Entoloma bloxamii	219		GQ289154		GQ289226	
Entoloma bloxamii	TB6117		AF261289			
Entoloma caccabus	17		GQ289155		GQ289227	
Entoloma calongei	322		GQ289158			
Entoloma canescens	TB5657		DQ851575			
Entoloma cephalotrichum	253		GQ289157		GQ289229	
Entoloma* cf. *nigroviolaceum	TM03/426		EU522809			
Entoloma cocles	244		GQ289159		GQ289230	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Entoloma conferendum	6		GQ289160		GQ289231	
Entoloma costatum	49		GQ289232		GQ289161	
Entoloma excentricum	184		GQ289163		GQ289234	
Entoloma flavifolium	TB6215		AF261301		GU384644	
Entoloma gasteromycetoides	180		GQ289164		GQ289235	
Entoloma gelatinosum	212		GQ289165		GQ289236.	
Entoloma griseolazulinum	i11		GQ289166		GQ289237	
Entoloma haastii	126		GQ289167		GQ289238	
Entoloma haastii	216		GQ289168		GQ289239	
Entoloma haastii	BY21		AF261309			
Entoloma hebes	46		GQ289170		GQ289241	
Entoloma incanum	TM02/277		EU522763			
Entoloma indigoticoumbrinum	83		GQ289242		GQ289171	
Entoloma indoviolaceum	i13		GQ289172		GQ289243	
Entoloma infundibuliforme	TENN013964		HQ179672			
Entoloma kermandii	222		GQ289173		GQ289244	
Entoloma lividum	MB5034		AY571001			
Entoloma myrmecophilum	231		GQ289174		GQ289245	
Entoloma nidorosum	TB9971		GU384617		GU384643	
Entoloma odorifer	TB6366		AF261310			
Entoloma pallideradicatum	255		GQ289176		GQ289247	
Entoloma parasiticum	20		GQ289177		GQ289248	
Entoloma perbloxamii	71		GQ289178		GQ289249	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Entoloma phaeomarginatum	127		GQ289179		GQ289250	
Entoloma pluteisimilis	200		GQ289180		GQ289251	
Entoloma politum	15		GQ289181		GQ289252	
Entoloma procerum	70		GQ289183		GQ289254	
Entoloma prunuloides	40		GQ289184		GQ289255	
Entoloma prunuloides	TJB4765		AY700180		DQ385883	
Entoloma pygmaeopapillatum	32		GQ289185		GQ289256	
Entoloma readiae	102		GQ289186		GQ289257	
Entoloma rhodopolium	8		GQ289187		GQ289258	
Entoloma rhodopolium	TB6221		AF261300			
Entoloma sarcitum	235		GQ289188		GQ289259	
Entoloma sericatum	28		GQ289189		GQ289260	
Entoloma sericellum	10		GQ289190		GQ289261	
Entoloma sericeonitidum	TB7144		AF261315		EF421016	
Entoloma sericeum	VHA 03-02	DQ367430	DQ367423	DQ367421	DQ367435	
Entoloma serrulatum	163		GQ289192		GQ289263	
Entoloma sinuatum	TJB5349	DQ486700	AY691891	AY657007		DQ516074
Entoloma sinuatum	50		GQ289193		GQ289264	
Entoloma sinuatum	TM02/134		EU522735			
Entoloma sinuatum	TM02/335		EU522771			
Entoloma sinuatum	TM03/429		EU522811			
Entoloma sordidulum	1		GQ289194		GQ289265	
***Entoloma* sp.**	292		GQ289156		GQ289228	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
***Entoloma* sp.**	JM98/123		AF261322			
***Entoloma* sp.**	TM02/237		EU522752			
***Entoloma* sp.**	TM03/413		EU522803			
***Entoloma* sp.**	TM03/500		EU522847			
Entoloma sphagneti	209		GQ289195			
Entoloma strictius	M96/10	DQ494680	AF042620	AF287832	AY218483	
Entoloma strictius	TB7710		GU384618		GU384641	
Entoloma tectonicola	i15		GQ289196		GQ289266	
Entoloma tjallingiorum	243		GQ289197		GQ289267	
Entoloma transmutans	155		GQ289200		GQ289268	
Entoloma turbidum	TB6949		GU384630		GU384656	
Entoloma undatum	18		GQ289202		GQ289270	
Entoloma undatum	GLM 45920		AY207199			
Entoloma unicolor	TB5520		AF261312			
Entoloma valdeumbonatum	189		GQ289203		GQ289271	
Entoloma vezzenaense	241		GQ289204		GQ289272	
Entoloma vinaceum	TB8870		GU384631		GU384651	
Entoloma violaceovillosum	i2		GQ289205		GQ289273	
Infundibulicybe gibba	JCS0704B	DQ490635	DQ457682	DQ115780	DQ472727	DQ447913.
Inocephalus lactifluus	TB7962		AF261304			
Inocephalus murrayi	VHAs02/02		GU384590		GU384637	
***Inocephalus* sp.**	GD-b	DQ490636	DQ457683	DQ457622	DQ472728	
***Inocephalus* sp.**	MCA1475		GU384619		GU384636	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Inocephalus **sp.**	MCA1585				GU384639	
Inocephalus **sp.**	MCA1867		GU384621		GU384638	
Inocephalus **sp.**	MCA2479		GU384622		GU384640	
Inopilus entolomoides	TB8507		AF261293			
Lepiota serrulata	VHAs0102		GU384624		GU384634	
Lepista irina	PBM2291	DQ221109	DQ234538	AY705948	DQ385885	
Lepista saeva	ADW0097	KJ137270	KJ417193	KJ417259	KJ424376	KU139032
Lepista sordida	CRG014	KJ137272	AY207225	KJ417260	KJ137273	KU139033
Leptonia carnea	TB5812		AF261313			
Leptonia **sp.**	MCA1486		GU384623		GU384635	
Leptonia subserrulata	TB6993		AF261291			
Leucopaxillus alboalutaceus	LAS00/082	KJ417275	KJ417195	KJ417261	KJ424377	
Leucopaxillus cerealis	GB:0068845	KJ417282	KJ417198	KJ417262	KJ424379	KU139034
Leucopaxillus eucalyptorum	REH9110	KJ417285	KJ417199			
Leucopaxillus gentianeus	EL291/11	KJ417287	KJ417201		KJ424381	
Leucopaxillus lilacinus	PBM3584	KJ417295	KJ417205	KJ417264	KJ424382	
Leucopaxillus paradoxus	TK04/091	KJ417296	KJ417206	KJ417265	KJ424383	
Leucopaxillus tricolor	TFB13462	KJ417323	KJ417207	KJ417266	KJ424384	
Lyophyllum decastes	JM 87/16	AF357059	AF042583	DQ367419	DQ367433	
Lyophyllum **sp.**	PBM 2688	DQ182502	DQ094785	DQ457628		
Lyophyllum **sp.**	MSG166	KU058499	KU058536	KU058570	KU138999	KU139036
Lyphyllum **aff.** *decastes*	PBM3069	HQ832451	HQ832459	HQ832426	HQ832437	KU139035
Macrocybe titans	M2012-1		KC913200			

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Neohygrophurus angelesianus	PBM482	DQ494678	DQ470814	DQ457698		
Nolanea cetrata	TB7382		AF261319			
Nolanea conica	MB6		AF261317			
Nolanea hirtipes	K1171992		AF261320			
Nolanea quadrata	TM03/445		EU522821			
Nolanea sericea	29		GQ289191		GQ289262	
Nolanea **sp.**	TM02/196		EU522740			
Nolanea **sp.**	TM03/403		EU522800			
Notholepista subzonalis	GB:0087013	KP453695	KJ417208	KJ417267	KJ424385	
Omphalina antarctica	HSG070118-26		GQ483368			
Omphalina pyxidata	TO AV98	JN944402	JN944403			
Ossicaulis lignatilis	DAOM188196	DQ825426	AF261396	AF334923	DQ825410	
Paralepista **sp.**	TO AV2009	JQ585658	JQ585659			
Paralepistopsis amoenolens	TO AV2004	JQ585653	JQ585654			
Pleurocollybia brunnescens	DAOM34832		AF261407			
Pleurocollybia imbricata	TJB9847	HM105568	HM105568		HM105567	
Pogonoloma macrocephalum	MOS69/85	KP453700	KJ417209	KJ417268		
Pogonoloma spinulosum	K(M):107286	KP453705	KJ417238	KU058571	KJ424401	KU139037
Porpoloma portentosum	MES531	KJ417298	KJ417210	KU058572	KJ424386	KU139038
Porpoloma sejunctum	CONC:F0416	KJ417301	KJ417212	KU058573	KJ424388	
Porpoloma **sp. 1**	PBM3238	KJ417304		KJ417270	KJ424390	KU139039
Porpoloma **sp. 2**	PBM3441		KJ417213	KJ417269	KJ424389	KU139040
Porpoloma terreum	REH5830	KJ417305	KJ417215		KJ424391	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Pouzarella nodospora	TB5716		AF261308			
Pseudobaeospora celluloderma	ECV5550	KU058501	KU058538	KU058574	KU139001	KU139042
***Pseudobaeospora* sp.**	CCB143666	KU058500	KU058537		KU139000	KU139041
***Pseudobaeospora* sp.**	ECV4140				KU139002	KU139043
***Pseudobaeospora* sp.**	ECV5553	KU058502	KU058539	KU058575	KU139003	
Pseudoclitocybe cyathiformis	LAS90/139B	KP453710	KU058540	KU058576	KU139004	KU139044
Pseudoclitocybe expallens	TO HG2286	JF926524	JF926525			
***Pseudoclitocybe* sp.**	RHP12643	KP453697	KJ417217		KJ424392	KU139045
Pseudoclitopilus rhodoleucus	TK03/203	KP453696	KJ417218	KU058577	KJ424393	KU139046
Pseudoomphalina felloides	DAOM11115		AF261442			
Pseudoomphalina pachyphylla	LAS07/012	KU058504	KU058542	KU058579	KU139006	KU139048
Pseudoomphaline kalchbrenneri	LAS06/037	KU058503	KU058541	KU058578	KU139005	KU139047
Pseudotricholoma metapodium	AH22102006	KJ417308	KJ417219	KJ417271	KJ424394	KU139049
Pseudotricholoma umbrosum	REH4796	KJ417312	KJ417222	KU058580	KJ424397	KU139050
Rhodocybe aureicystidiata	PBM1902		AY380407		AY337412	
Rhodocybe caelata	REH3569				KC816932	
Rhodocybe caelata	TB5890		AF261282			
Rhodocybe collybioides	TB10417				KC816935	
Rhodocybe fallax	OKM25668		AF261283			
Rhodocybe fulginea	E537				KC816940	
Rhodocybe lateritia	E1589				KC816942	
Rhodocybe luteocinnamomea	GUA241				KC816943	
Rhodocybe minutispora	10711014				KC816947	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Rhodocybe mundula	TJB7599		AY700182		DQ474128	
Rhodocybe paurii	JM99/233		AY286004		KC816969	
Rhodocybe popinalis	TB6378		AF261285		GU384654	
Rhodocybe pruinosostipitata	MCA1492		GU384627		GU384653	
Rhodocybe rhizogena	TJB5551				KC816981	
Rhodocybe roseaiavellanea	TJB8130				KC816982	
***Rhodocybe* sp.**	DLL10218				KC816990	
***Rhodocybe* sp.**	DLL9851				KC816986	
***Rhodocybe* sp.**	DLL9860				KC816987	
***Rhodocybe* sp.**	DLL9952				KC816988	
***Rhodocybe* sp.**	DLL9957				KC816989	
Rhodocybe spongiosa	MCA2129		GU384628		GU384657	
Rhodocybe truncata	CBS482/50		AF223167		EF421019	
Rhodocybella rhododendri	TENN047309		HQ222034			
***Rhodophana* sp.**	COFC 5029				KC816984	
Ripartites tricholoma	GLM 46025		AY207297			DQ067936
Singerocybe adirondackensis	PBM3320	HQ728529	HQ728530	HQ728531	HQ728532	KU139051
Singerocybe alboinfundibuliformis	HKAS74716		JX514106		JX514138	
Singerocybe clitocyboides	HKAS75451		JX514099			
Singerocybe humilis	HKAS74717		JX514114		JX514150	
Singerocybe phaeophthalma	HKAS79015		KF208453		KF208465	
Singerocybe umbilicata	HKAS78038		KF208455		KF208461	
Tephrocybe anthracophylla	JT31970	KU058523	KJ417225			KU139064

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Tephrocybe boudieri	BSI96/84	AF357047	DQ825430	DQ825433	DQ825411	DQ825421
Tephrocybe **sp.**	CCB148	KU058524	KU058561		KU139014	KU139065
Termitomyces microcarpus	PRU3900		AF042587	DQ851581		
Termitomyces **sp.**	TFB1546		KU058562		KU139015	
Termitomyces **sp.**	ZA164	DQ494698	DQ110875	DQ092922	DQ437070	DQ447942
Trichocybe puberula	TO-HG1148	FM877683	FM877680			
Tricholoma aff. fulvum	TFB14052	KU058505	KU058543	KU058581	KU139007	KU139052
Tricholoma album	TFB13753	KU058506	KU058544	KU058582		
Tricholoma arvernense	DP081004-01	KU058507	KU058545			
Tricholoma atrodiscum	MSG132	KU058508	KU058546	KU058583	KU139008	KU139053
Tricholoma atroviolaceum	MSG167	KU058509	KU058547	KU058584	KU139009	KU139054
Tricholoma caligatum	PBM3899	KU058510	KU058548		KU139010	KU139055
Tricholoma elegans	PBM3142	KJ417316	KJ417226			KU139066
Tricholoma eucalypticum	PBM3154	KU058512	KU058550	KU058586		
Tricholoma **f.** *davisiae*	TFB13409	KU058511	KU058549	KU058585		KU139056
Tricholoma flavovirens	TFB13553	KU058513	KU058551	KU058587		KU139057
Tricholoma fulvum	BPL304	KU058514	KU058552	KU058588	KU139011	KU139058
Tricholoma guldeniae	MSG131	KU058515	KU058553	KU058589		KU139059
Tricholoma inamoenum	REG MB 96-071		AY293215	AY293161		
Tricholoma luteomaculosum	CLO4632	KU058516	KU058554	KU058590		KU139060
Tricholoma matsutake	Tn9	AB188557	U62964	U62538		
Tricholoma myomyces	KMS589	DQ825428	U76459	DQ367422	DQ367436	
Tricholoma palustre	PBM2494	DQ494699	AY700197	AY757267	DQ484055	

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Tricholoma populinum	LCG2003	JN019602	JN019649		JN019720	JN019668
Tricholoma portentosum	KMS591	AF357015			EF421014	EF421047
Tricholoma saponaceum	TFB12328	KU058517	KU058555	KU058591		
Tricholoma saponaceum	PBM2514	DQ494700	AY647209	AY654883		
Tricholoma sejunctum	MSG133	KU058518	KU058556	KU058592		
***Tricholoma* sp.**	PBM3085	KJ417318	KJ417228	KJ417273		KU139067
***Tricholoma* sp.**	PBM3141	KJ417317	KJ417227	KJ417272		KU139068
***Tricholoma* sp.**	PBM3168	KU058525	KU058563	KU058596	KU139016	KU139069
***Tricholoma* sp.**	PBM3170	KU058526	KU058564	KU058597		
***Tricholoma* sp.**	RHP13062		KJ417229	KJ417274		
***Tricholoma* sp.**	SAR1290		AF042592	AF287839		
***Tricholoma* sp.**	MSG160	KU058527	KU058565	KU058598	KU139017	KU139070
***Tricholoma* sp.**	PBM3144	KU058528	KU058566			
Tricholoma subaureum	KMS590	AF357016			EF421015	EF421048
Tricholoma subluteum	MSG134	KU058519	KU058557	KU058593	KU139012	KU139061
Tricholoma subresplendens	SAT1027901	KJ417319	KJ417230	KJ417275		KU139071
Tricholoma sulphureum	PBM3959	KU058520	KU058558	KU058594		KU139062
Tricholoma vaccinum	TFB13554	KU058521	KU058559			
Tricholoma virgatum	MSG165	KU058522	KU058560	KU058595	KU139013	KU139063
Tricholoma viridiolivaceum	PBM3093	JF706316	JF706317	JF706318	JF706319	KU139072
Tricholomella constricta	HC84-75	AF357036	AF223188	DQ825434	DQ825412	DQ825422
Tricopilus porphyrophaeus	TB6957		AF261290		EF421020	
Undet gray scaly	ECV4038	KJ417320	KJ417231	KJ417276	KJ424399	KU139073

Table II.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Undet small white	MBP080609	KJ417321	KJ417232	KJ417277	KJ424400	KU139074

Table II.2. Parameter estimates from the BiSSE analyses (q, transition rate; λ, speciation rate; μ, extinction rate; r, net diversification rate (λ -μ); 0, non-ECM; 1, ECM).

Models	λ0	λ1	μ0	μ1	r0	r1	q01	q10
Full	0.146	0.276	0.090	0.166	0.056	0.110	0.0007	0.000
Irreversible (q10 = 0)	0.148	0.277	0.092	0.168	0.055	0.110	0.0007	-
Irreversible, equal extinction rates (q10 = 0, μ0 = μ1)	0.153	0.223	0.099	0.099	0.054	0.123	0.0005	-
Irreversible, equal speciation rates (q10 = 0, λ0 = λ1)	0.168	0.168	0.116	0.033	0.052	0.135	0.0003	-
Irreversible, equal speciation and extinction rates (q10 = 0, λ0 = λ1, μ0 = μ1)	0.192	0.192	0.136	0.136	0.056	0.056	0.0009	-

Table II.2. Comparison of the BiSSE models based on a likelihood ratio test (q, transition rate; λ, speciation rate; μ, extinction rate; 0, non-ECM; 1, ECM).

Models	d.f.	ln-likelihood	AIC	X^2	*P*
Full	6	-1266.2	2544.4	-	-
Irreversible (q10 = 0)	5	-1266.2	2542.3	0.1233	0.7255
Irreversible, equal extinction rates (q10 = 0, μ0 = μ1)	4	-1266.6	2541.1	0.8100	0.3681
Irreversible, equal speciation rates (q10 = 0, λ0 = λ1)	4	-1268.3	2544.7	3.5645	0.022×10^{-18} ***
Irreversible, equal speciation and extinction rates (q10 = 0, λ0 = λ1, μ0 = μ1)	3	-1280.4	2566.8	24.1370	0.089×10^{-5} ***

*** $P < 0.001$

AIC – Akaike information criterion

Table II.4. Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of taxa of the Tricholomatineae.

Name	Voucher number	$\delta^{13}C$	$\delta^{15}N$	Inferred trophic status
***Albomagister* sp. 2**	MSG137	-27.05	1.7	MIC
Albomagister subaustralis	ECV4049	-25.86	9.08	MIC
Albomagister subaustralis	ECV4142	-24.53	7.33	MIC
Albomagister subaustralis	MGW676	-25.7	10.28	MIC
Aspropxillus septentrionalis	AHS24982	-20.85	4.34	SAP
Callistosporium luteo-olivaceum	ECV5506	-21.92	0.45	SAP
Calocybe naucoria	TENN29042	-23.44	-2.6	SAP
Cleistocybe vernalis	PBM1856	-18.65	0.84	SAP
Clitocybe candicans	RP422	-22.08	1.01	SAP
Clitocybe dealbata	DAOM114003	-21.79	2.67	SAP
Clitocybe nebularis	TFB14041	-22.96	1.56	SAP
***Clitocybe* sp.**	RHP25127	-23.31	-1.08	SAP
Clitopilus prunulus	RHP13764	-20.97	2.42	SAP
Clitopilus scyphoides	TFB13536	-24.02	1.05	SAP
***Dennisiomyces* sp. 3**	ECV4175	-24.52	0.95	SAP
Infundibulicybe gibba	PBM2788	-21.4	0.1	SAP
Lepista irina	EHUBBS8	-23.03	4.98	SAP
Lepista personata	ADW0097	-21.91	6.64	SAP
Lepista sordida	CRG014	-15.79	4.53	SAP
Leucopaxillus alboalutaceus	LAS00/082	-21.74	3.44	SAP
Leucopaxillus amarus	Mos63/271	-22.74	9.27	MIC
Leucopaxillus cerealis	GB0068845	-21.01	2.58	SAP
Leucopaxillus eucalyptorum	REH9110	-22.32	2.51	SAP
Leucopaxillus eucalyptorum	REHP8368	-21.71	3.96	SAP
Leucopaxillus gracillimus	AFM752	-23.31	7.71	MIC
Leucopaxillus gracillimus	Guzman34476	-24.19	6.61	MIC
Leucopaxillus gracillimus	TJB8315	-23.49	9.8	MIC
Leucopaxillus laterarius	VR1717	-22.81	4.26	SAP
Leucopaxillus laterarius	PBM3060	-20.11	12.64	MIC
Leucopaxillus lilacinus	PBM3584	-26.66	8.37	MIC
Leucopaxillus masakanus	TJB8321	-23.84	10.65	MIC
Leucopaxillus paradoxus	NR2004-091	-21.02	3.21	SAP
Leucopaxillus tricolor	DJLNC 15-05	-22.9	10.47	MIC

Table II.4. Continued.

Table II.4. Continued.

Name	Voucher number	$\delta^{13}C$	$\delta^{15}N$	Inferred trophic status
Lyophyllum aff. decastes	PBM3069	-25.24	14.64	MIC
Notholepista subzonalis	GB0087013	-23.98	-0.41	SAP
Porpoloma portentosum	MES531	-26.99	8.02	MIC
Porpoloma sejunctum	CONC-F0416	-21.82	9.84	MIC
***Porpoloma* sp. 1**	PBM3238	-23.25	7.78	MIC
***Porpoloma* sp. 2**	PBM3441	-30	6.53	MIC
Porpoloma terreum	REH5830	-24.35	6.69	MIC
Pseudoclitocybe sp.	RHP12643	-23.84	1.76	SAP
Pseudoomphalina kalchbrenneri	LAS06/037	-22.24	-0.94	SAP
Pseudoomphalina pachyphylla	LAS07/012	-23.06	-0.41	SAP
Rhodocybe mundula	TFB14074	-22.63	1.88	SAP
Singerocybe adirondackensis	PBM3320	-22.7	1.19	SAP
***Termitomyces* sp.**	TFB1546	-23.67	1.86	SAP
Undet. gray scaly	ECV4038	-24.03	13.1	MIC

MIC = ectomycorrhizal
SAP = saprotroph

Figure II.1. Maximum clade credibility tree of the suborder Tricholomatineae from the dating analysis in BEAST using a five-loci dataset (LSU, SSU, 5.8S, *rpb1* and *rpb2*). 95% confidence intervals for nodes with a posterior probability ≥ 0.75 are shown. Blue squares indicate ECM taxa and yellow squares denote non-ECM taxa. Red circles represent the locations of diversification rate increases identified from the BAMM analyses.

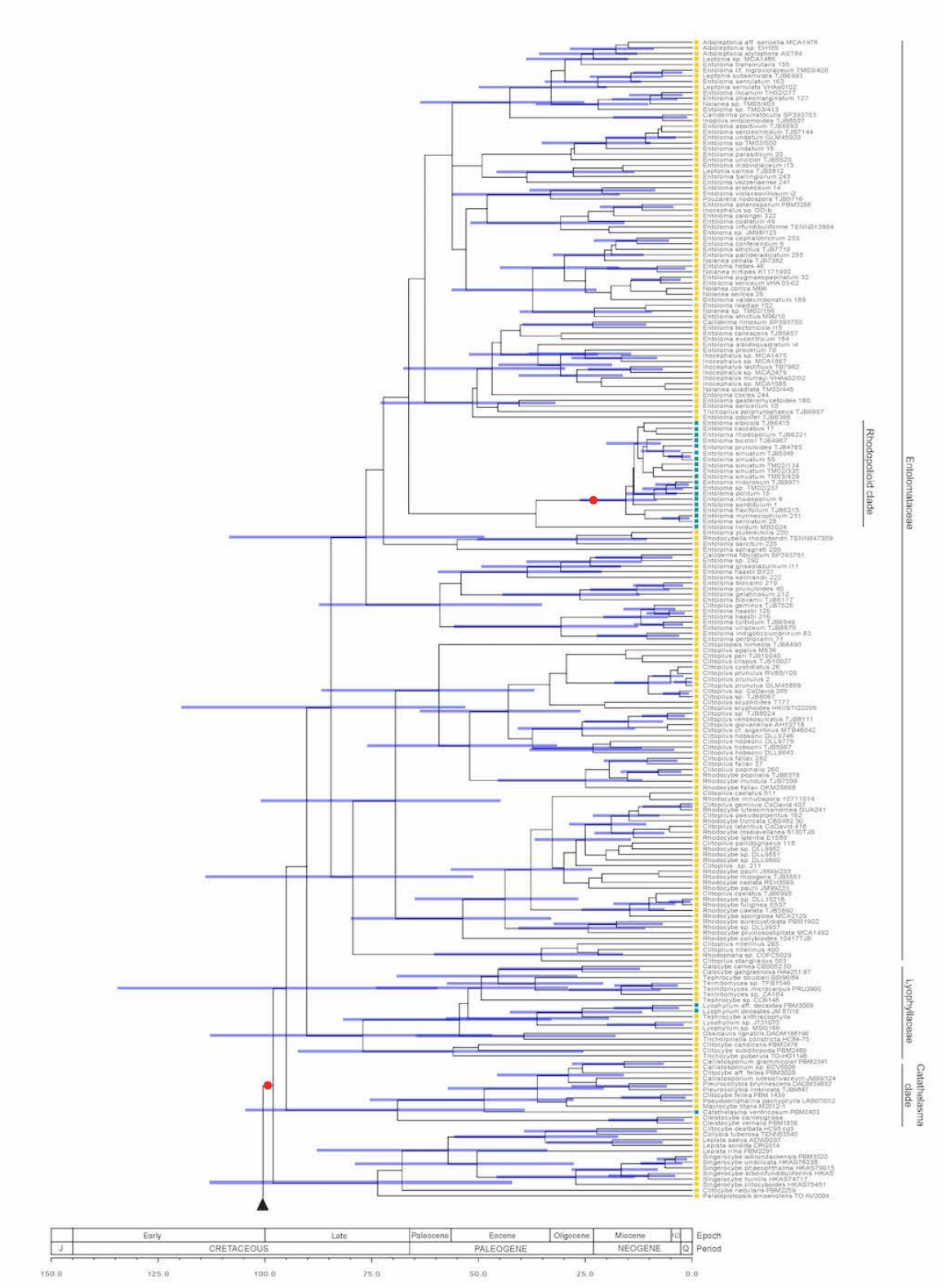

Figure II.1. Continued

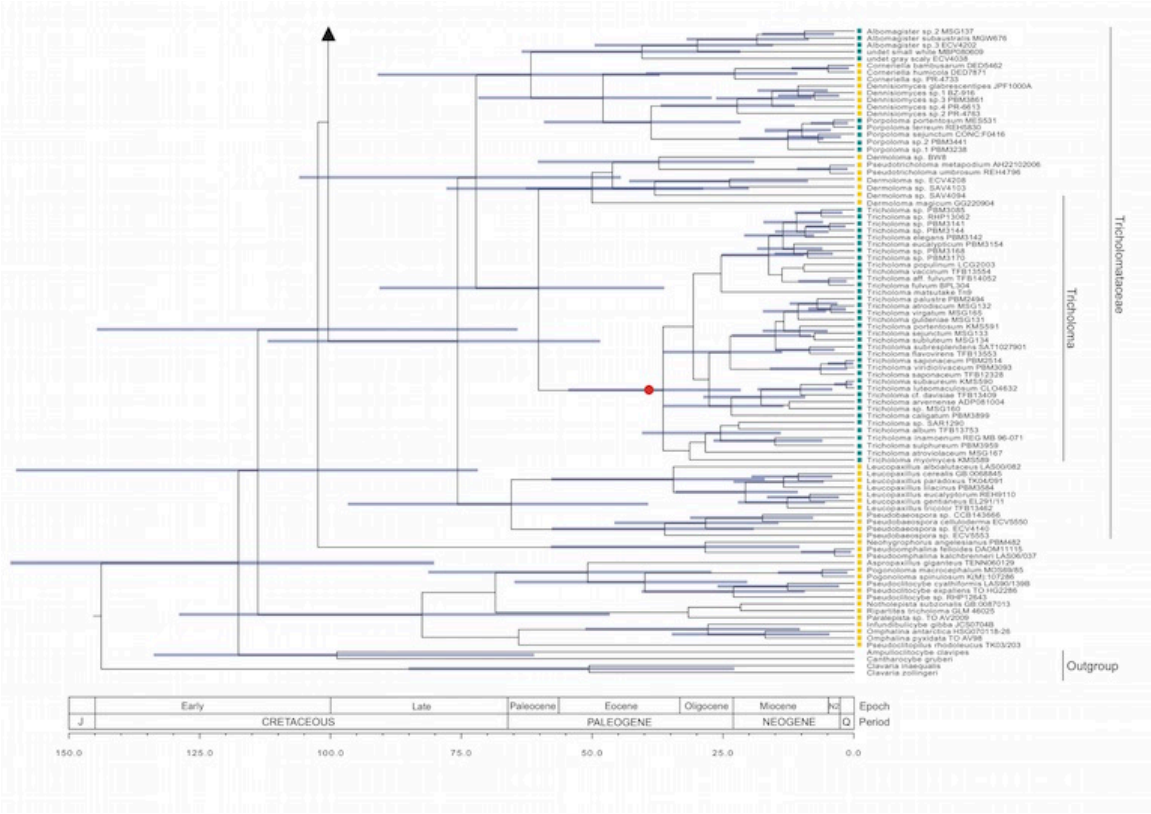

Figure II.1. Continued

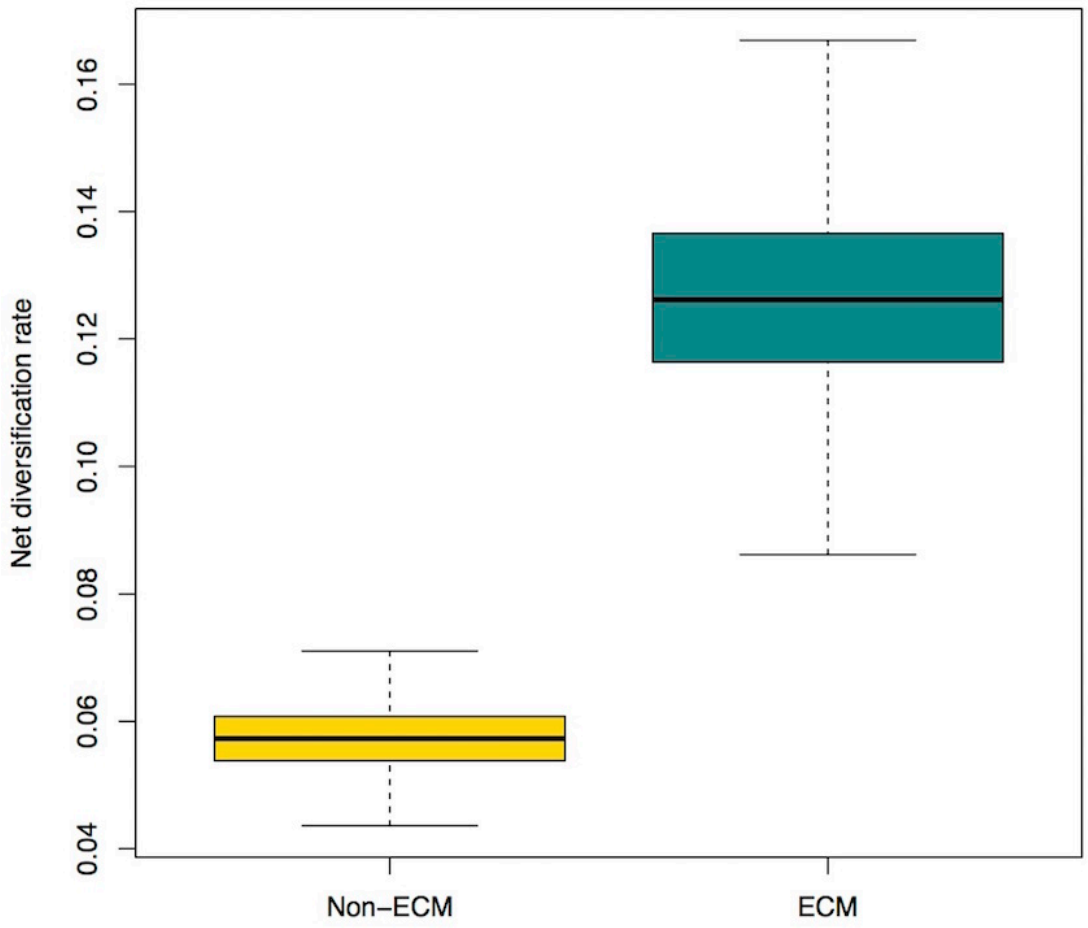

Figure II.2. Box plot of MCMC estimates of state-dependent net diversification rate (λ - μ) of non-ECM and ECM clades in the suborder Tricholomatineae obtained from the best BiSSE model.

Figure II.3. A. Phylorate plot for the best shift configuration of the Tricholomatineae (f = 0.4). Colors indicate diversification rates on each branch of the maximum clade credibility tree. Red circles indicate the locations of shifts to increased diversification (see Fig. II.4 for the 95% credible set of distinct shift configurations). B. Rate through time plot obtained from the BAMM analyses. Numbers correspond to the rate shifts shown in the phylorate plot. Gray line represents the suborder Tricholomatineae. Color density shading indicates confidence on evolutionary rate reconstructions.

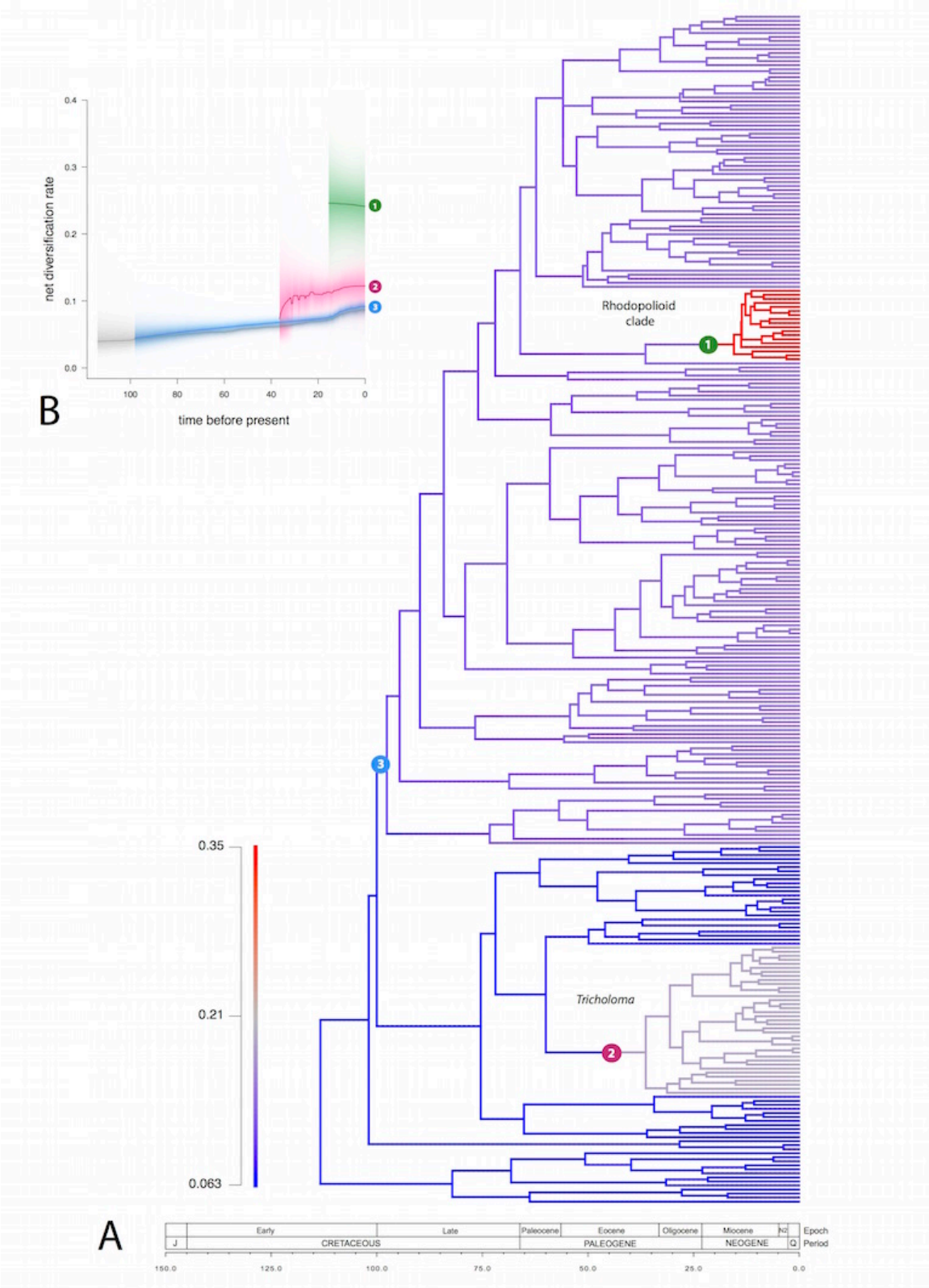

Figure II.3 Continued

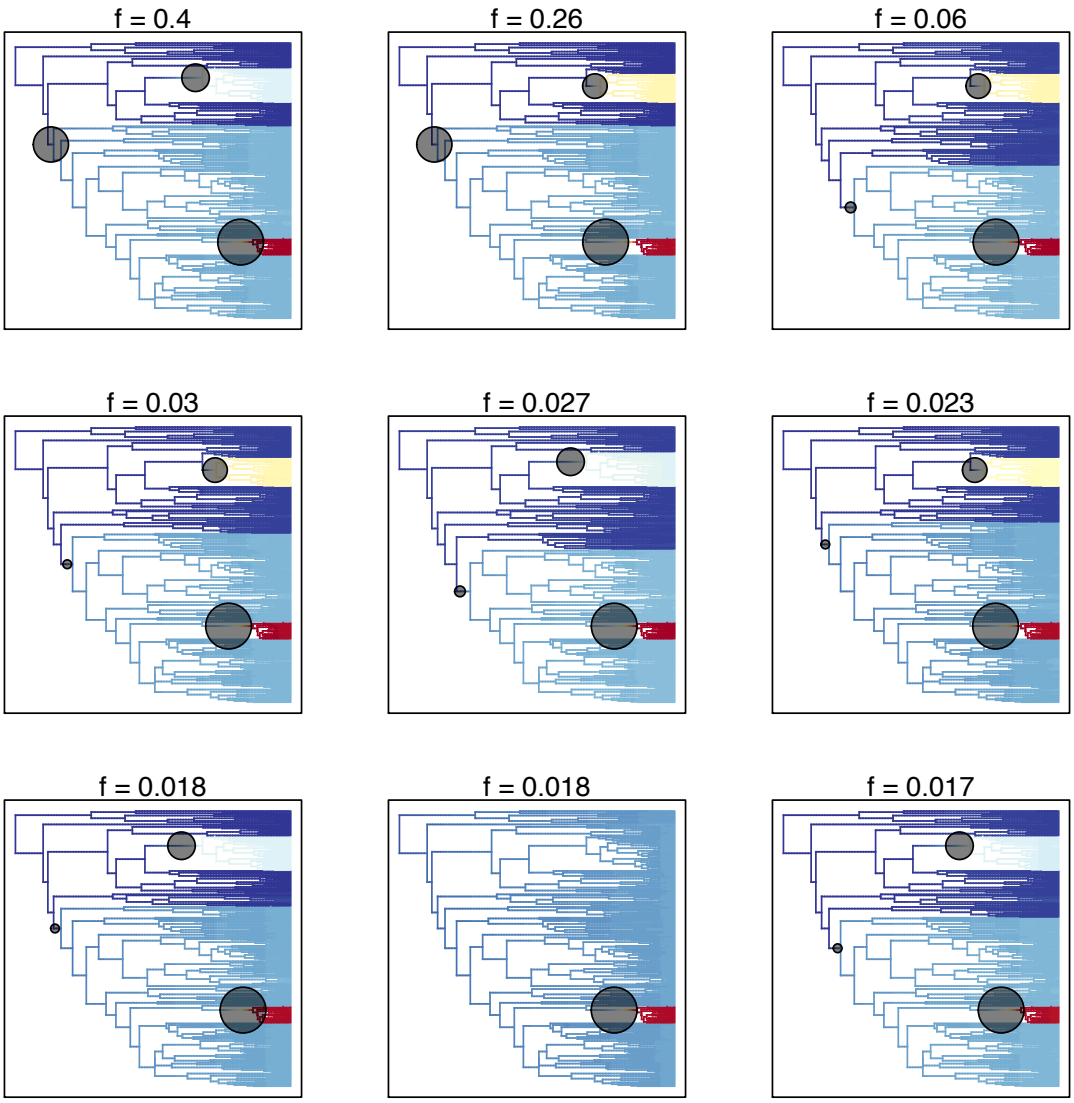

Figure II.4. 95% credibility set of shift configurations based on a Bayes factor criterion of 5. Circles represent the locations of rate shifts. The f values correspond to the proportion of the posterior distribution than can be assigned to that shift configuration. Warm colors denote increases in diversification rate, and cool colors indicate decreases in diversification rates.

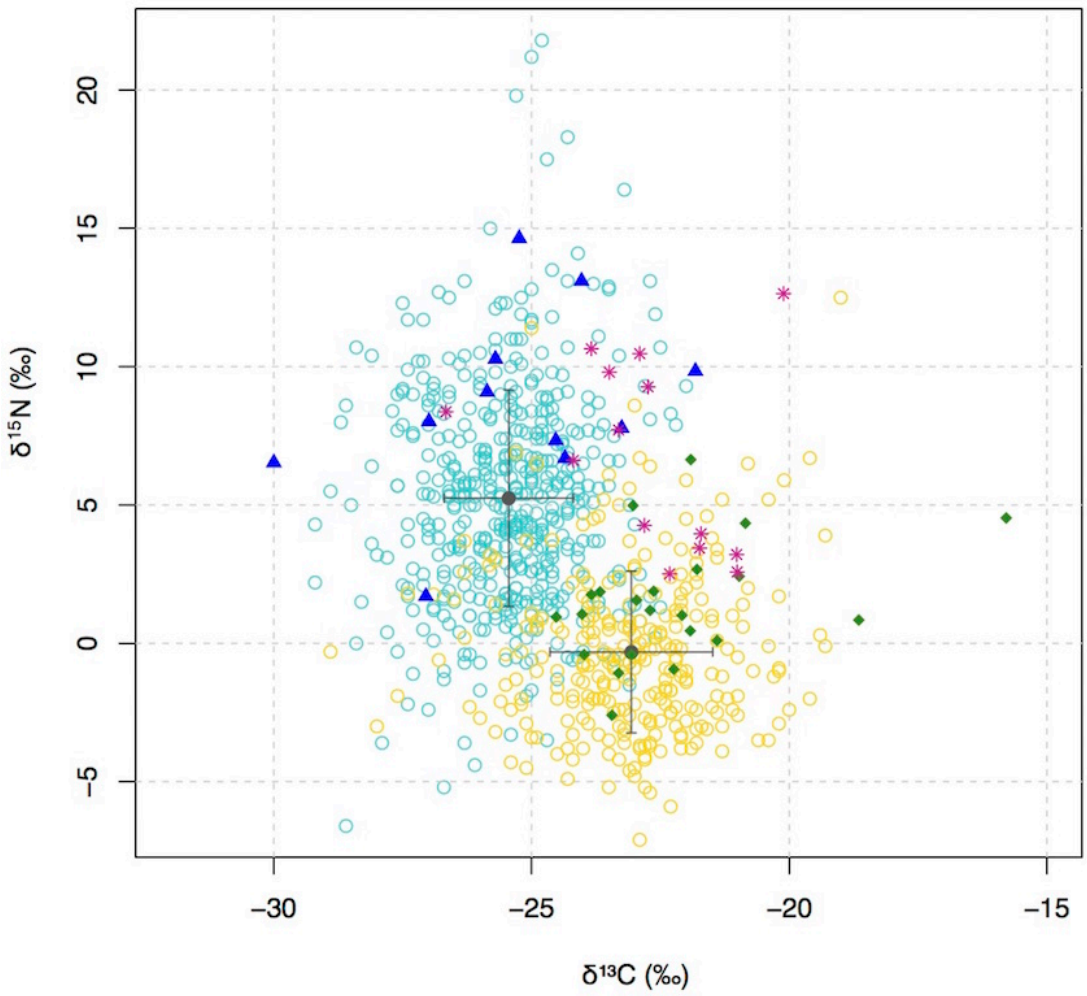

Figure II.5. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of 47 taxa of the Tricholomatineae (blue triangles, green diamonds and pink asterisks) compared to a global dataset from Mayor et al. (2009) with known ECM (blue circles) and saprotrophic (yellow circles) isotopic signatures. Gray circles represent mean values ± SD. Taxa within the Tricholomatineae are represented as follows: blue triangles correspond to ECM fungi, green diamonds correspond to saprotrophic fungi. Pink asterisks represent *Leucopaxillus* species. See Table II.4 for the $\delta^{13}C$ and $\delta^{15}N$ values and taxon names.

CHAPTER III

A new lineage of ectomycorrhizal fungi endemic to the Neotropics

A version of this chapter will be submitted for publication by M. Sánchez-García, Terry Henkel, M. Catherine Aime, Matthew E. Smith, and P. Brandon Matheny

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MSG obtained and analyzed morphological and molecular data, and wrote the manuscript. TH, MCA, and MES collected the specimens. PBM contributed to manuscript revisions.

Abstract

A new genus and three new species of Agaricomycetes are described from the Pakaraima Mountains of Guyana in the central Guiana Shield. These taxa have been found to form ectomycorrhizal (ECM) associations with *Dicymbe* spp. Molecular phylogenetic analyses place *Guyanagarika* gen. nov. within the *Catathelasma* clade, a lineage in the suborder Tricholomatineae. We recognize the so-called *Catathelasma* clade as the family Catathelasmataceae to include the genera *Callistosporium, Catathelasma, Guyanagarika, Pleurocollybia, Pseudolaccaria,* and *Macrocybe*. The genera *Catathelasma* and *Guyanagarika* represent two independent origins of ECM fungi in this family, and *Guyanagarika* is the first known case of an endemic neotropical ECM genus within the Agaricales.

Introduction

Fungi represent one of the most diverse groups of organisms with an estimated 5.1 million species (O'Brien et al., 2005; Blackwell, 2011). In addition to their great taxonomic diversity, they play vital roles in nutrient cycling processes in terrestrial ecosystems through decomposition of organic matter, forming mycorrhizal symbioses, and as parasites (Leake and Read, 1997). One important group, the ectomycorrhizal (ECM) fungi, form symbiotic relationships with the roots of plants, in which the fungi provide nutrients, water, and protection against pathogens, while obtaining carbohydrates from their photosynthetic

partners (Smith and Read, 2008). This symbiosis has evolved independently about 80 times across the fungal tree of life (Tedersoo and Smith, 2013). ECM fungi comprise about 7 750 described species, but the actual magnitude of this diversity is estimated at 25 000 species (Rinaldi et al., 2008).

Recent studies have demonstrated that ECM fungal diversity is higher in temperate and boreal ecosystems than in tropical ecosystems (Tedersoo et al., 2012, 2014), but the importance of these fungi in tropical ecosystems has also been highlighted, i.e. they alleviate plant stress (Bandou et al., 2006) and contribute to plant establishment and community structure (Newbery et al., 2002; Peay et al., 2010). Some studies have documented high ECM fungal diversity in tropical ecosystems (Peay et al., 2010; Tedersoo et al., 2010; Bâ et al., 2012). ECM diversity in *Dicymbe*- and *Pakaraimaea*-dominated forests in Guyana has been shown to be remarkably high (Smith et al., 2011, 2013; Henkel et al., 2012). In recent years, several new genera and species have been described from this area (i.e. Henkel et al., 2011, 2016; Husbands et al., 2013; Grupe et al., 2015; Smith et al., 2015).

Smith et al. (2013) reported the discovery of a conspicuous yet enigmatic orange-colored agaric species from the Pakaraima Mountains of Guyana. This fungus morphologically resembles species that belong to the genus *Tricholoma* (Fr.) Staude; however, nuclear ribosomal large subunit (LSU) sequences show closer affinities to members of *Entoloma* P. Kumm. and *Clitocybe* (Fr.) Staude, but with low statistical support. Internal transcribed spacers (ITS) sequences obtained from *Dicymbe* ECM root tips collected beneath the fruitbodies matched those sequences obtained directly from the fruitbodies, supporting an ECM relationship between this taxon and *Dicymbe* spp. Here, we re-examined those and additional materials to elucidate the phylogenetic position and taxonomy of this enigmatic mushroom.

Materials and Methods

Collections and morphological analyses. — Collections were made during May–July of 1998–2002, 2008, 2010, 2012, 2013, and 2015 from the Upper Potaro

and Upper Mazaruni River Basins in the Pakaraima Mountains of Guyana in the central Guiana Shield. Collecting sites in the Upper Potaro River Basin were dominated by *Dicymbe corymbosa* Spruce ex. Benth. and/or *D. altsonii* Sandw. (Henkel et al., 2012), and collecting sites in the Upper Mazaruni River Basin were dominated by *Pakaraimaea dipterocarpaceae* Maguire & P.S. Ashton and *D. jenmanii* Sandwith (Smith et al., 2013).

Descriptions of macromorphological characters were made from fresh collections in the field. Colors were documented with the Methuen handbook of Colour (Kornerup and Wanscher, 1984). Sporocarps were dried in silica gel for future microscopic examination and molecular analyses. Dried collections were later examined using a Nikon Eclipse 80i microscope. Specimens were sectioned by hand and mounted in water, 5% KOH, Melzer's reagent (to test for an amyloid reaction), or stained with congo red, sudan IV or cotton blue (to test for a cyanophilous reaction) following recommendations of Clémençon (2009). Collections were deposited in the following herbaria: BRG, HSU, PUL, and TENN (herbarium abbreviations per Thiers [continuously updated]). ECM roots attached to hyphal cords extending from the base of some specimens were collected for future morphological and molecular studies.

DNA extraction, PCR amplification, and sequencing. — The protocols of Smith et al. (2011) and Sánchez-García et al. (2014) were followed for DNA extraction, PCR amplification, and sequencing of the internal transcribed spacers (ITS), LSU, nuclear ribosomal small subunit (SSU), the largest subunit of RNA polymerase II (*rpb1*), and the second largest subunit of RNA polymerase II (*rpb2*). The conserved domains A–C from the *rpb1* gene region were amplified and sequenced using the same protocols used for *rpb2* in Sánchez-García et al. (2014) but with the primers gAf, fCr, int2f, int2.1f, and int2.1r (Stiller and Hall, 1997; Matheny et al., 2002; Frøslev et al., 2005). GenBank accession numbers are shown in Table III.1.

Sequence alignment and phylogenetic analyses. — Phylogenetic analysis of LSU sequences in Smith et al. (2013) suggested that this mushroom was closely related to *Entoloma* and *Clitocybe*, genera of the suborder Tricholomatineae. To

evaluate this phylogenetic placement, we incorporated the LSU, SSU, *rpb1*, and *rpb2* sequences into a dataset of the Tricholomatineae that consisted of 255 taxa including *Ampulloclitocybe clavipes* (Pers.) Redhead, Lutzoni, Moncalvo & Vilgalys as an outgroup, hereafter referred to as the Tricholomatineae dataset. Alignments for individual gene regions were done with MAFFT 7.244 (Katoh and Standley, 2013) and manually adjusted with Aliview 1.17.1 (Larsson, 2014). Individual gene alignments were concatenated using SeaView 4.5.4 (Gouy et al., 2010), after inspection for intergene conflict. PartitionFinder 1.0.1 (Lanfear et al., 2012) was used to find the best partition strategy and the best models of molecular evolution.

A maximum likelihood (ML) analysis was performed with RAxML 8.1.17 (Stamatakis, 2014) executing 1000 rapid ML bootstrap searches. Bayesian inference (BI) analyses were performed using MrBayes 3.2.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Altekar & al., 2004) with two independent runs and four Markov chain Monte Carlo (MCMC) for 50 million generations, and sampling trees every 5000 generations. To determine the appropriate number of generations to discard as burn-in, we evaluated the output using Tracer 1.5 (Rambaut and Drummond, 2007), after which 25 % of the trees were discarded. Trees sampled from the posterior distribution were summarized into a maximum clade credibility (MCC) tree using TreeAnnotator 1.7.5 (Drummond & al., 2012). Bootstrap values (BS) ≥70 and posterior probabilities ≥0.90 were considered evidence for strong support.

Preliminary analyses from the Tricholomatineae dataset suggested that this taxon could be closely related to the *Catathelasma* clade. We assembled a second dataset that included members of this clade, the orange-colored mushroom, and the Tricholomataceae as outgroup, hereafter referred as the *Catathelasma* clade dataset. We incorporated ITS sequences. ML and BI analyses were run as described above.

Species delimitation. — Preliminary phylogenetic analyses have suggested the presence of three cryptic species within the clade under study. We used the reversible-jump (rj)MCMC algorithm in BP&P version 3 (Yang and Rannala,

2010, 2014) to delimit species under the species coalescent model; this method uses multiple loci data to generate posterior probabilities of assigned species. A gamma prior G (2,2000) was used on the population size parameters, and G (2,1000) for the age of the root in the species tree, while the other divergence time parameters were assigned the Dirichlet prior (Yang and Rannala, 2014). The analysis was run for 500 000 generations and a burn-in of 50 000; the analysis was run twice to confirm consistency between runs.

Results

Phylogenetic analyses. — The Tricholomatineae dataset consisted of 269 taxa and 4917 nucleotide positions. The alignment was separated into nine partitions: (1) nLSU; (2) nSSU; (3) *rpb2* first codon positions; (4) *rpb2* second codon positions; (5) *rpb2* third codon positions; (6) *rpb1* first codon positions; (7) *rpb1* second codon positions; (8) *rpb1* third codon positions; and (9) *rpb1* intron 2, implementing the GTR+GAMMA+I model for both the ML and BI analyses as suggested by PartitionFinder.

The *Catathelasma* clade dataset consisted of 50 taxa and 5921 nucleotide positions. The alignment was separated in seven partitions as suggested by PartitionFinder: (1) ITS; (2) nLSU; (3) nSSU; (4) *rpb2* all codon positions; (5) *rpb1* first and second codon positions; (6) *rpb1* third codon positions; and (7) *rpb1* intron 2, implementing the GTR+GAMMA+I model for both the ML and BI analyses as suggested by PartitionFinder.

The orange-colored mushroom here named *Guyanagarika* gen. nov. was recovered with high support (BS-96/PP-0.99) as part of the *Catathelasma* clade (Matheny et al., 2006), a lineage that includes the genera *Callistosporium* Singer, *Catathelasma* Lovejoy, *Macrocybe* Pegler & Lodge, *Pleurocollybia* Singer, *Pseudolaccaria* (Fr.) Vizzini & Contu, *Clitocybe fellea*, and *Clitocybe* aff. *fellea* (Fig. III.1).

Based on these analyses we recognize the *Catathelasma* clade as the family Catathelasmataceae Wasser. This family was established in 1985 to include only one member, *Catathelasma*. Jülich (1981) recognizes it as the family

Biannulariaceae Jülich, and includes the genus *Biannularia* Beck; however, this was synonymized with *Catathelasma*, keeping the latter name due to principle of priority. Singer (1986) recognizes this group as the tribe Biannularia Singer, in which he also includes *Armillaria* (Fr.) Staude, which now is known to belong to the family Physalacriaceae.

In the *Catathelasma* dataset, *Guyanagarika* gen. nov. was recovered as sister to *Callistosporium*, *Pleurocollybia, Macrocybe*, *Pseudolaccaria*, and *Clitocybe* aff. *fellea*. Three main clades were recovered within *Guyanagarika* (Fig. III.2), these three clades were supported as separate species by BP&P, with a posterior probability of 1.0. We describe them as: 1) *Guyanagarika aurantia* sp. nov., 2) *G. pakaraimensis* sp. nov., and 3) *G. anomala* sp. nov..

Taxonomy

Catathelasmataceae Wasser in Agarikovye griby SSSR (Kiev): 29. 1985.
Type genus: *Catathelasma* Lovejoy, Bot. Gaz. 50: 383. 1910.

Habit tricholomatoid, collybioid or pleurotoid. Lamellae adnate, adnexed, sinuate, emarginated to decurrent. Basidiospore deposit white. Basidiospores ellipsoid, hyaline, smooth, inamyloid or amyloid, acyanophilic or cyanophilic. Cheilocystidia present or absent, pleurocystidia absent, if present then only as pseudocystidia. Hymenophoral trama regular to bilateral becoming regular. Pileipellis a cutis, ixocutis or cutis becoming a trichoderm. Clamp connections present or absent. On soil or rotten wood. Saprotrophic or ectomycorrhizal.

Genera included: *Guyanagarika* gen. nov., *Callistosporium, Catathelasma, Macrocybe, Pleurocollybia, Pseudolaccaria*,

Guyanagarika Sánchez-García, T.W. Henkel & Aime, **gen. nov.**
MycoBank MBXXXXXX

Type**: *Guyanagarika aurantia*** Sánchez-García, T.W. Henkel & Aime, **sp. nov.**
Etymology: Guyana, in reference to the country of origin; and from the Greek agariko = agaric.

Habit tricholomatoid. Pileus dark orange becoming lighter towards the margin, broadly convex to broadly sub-conic to plano-convex with prominent broad umbo with age. Surface glabrous to minutely tomentose. Lamellae sub-thick to thick, adnate to adnexed when young to sinuate, brittle. Stipe equal, tapering from apex to base, solid. Basidiospores hyaline, smooth, inamyloid, acyanophilic. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis transitioning to a trichoderm. Clamp connections present in all tissues. Thromboplerous hyphae present in all tissues.

Guyanagarika aurantia Sánchez-García, T.W. Henkel & Aime, **sp. nov.**
(Fig. III.3)
MycoBank MBXXXXXX
Etymology: from the Latin *aurantia* = orange-colored

Diagnosis: Morphologically similar to *G. pakaramensis* and *G. anomala*, but forming a distinctive clade supported by multiple loci data (ITS, nLSU, *rpb1*, and *rpb2*).

Holotype: GUYANA. REGION 7 CUYUNI-MAZARUNI: Pakaraima Mountains, Upper Mazaruni River Basin, ~6 km west of Mt. Ayanganna in vicinity of Pegaima savanna camp site at 5°26'21.3"N; 60°04'43.1"W, 300 m south of base camp. On white sand soils of forest dominated by *Pakaraimaea dipterocarpaceae* and *Dicymbe jenmanii*. 6 June 2012. Henkel 9693 (BRG; isotypes HSU, TENN 070902).

Pileus 30–60 mm broad, 15–32 mm tall, convex to sub-conic to plano-convex with prominent umbo 6–13 mm tall with age, orange throughout (5A8–6B3), darker orange (6B8–6C6) over disc when young, hygrophanous somewhat to lighter yellowish orange (4A6–4A7) with age, otherwise uniformly concolorous, margin incurved and sub-crenulate to crenulate when young, and broadly undulate at maturity, edge sub-crenate; surface glabrous to minutely tomentose especially over disc, under hand lens a regular, low, sub-erect to erect pile of fibrillose elements more concentrated over disc, on mature specimens near

margin erect elements reduced, nearly minutely granulose-pruinose; sub-dry to moist; trama off-white to pale cream (4A3–4A4), orange-concolorous under pileipellis, 0.5 mm at margin, 2 mm over lamellae, and 9 mm over stipe/umbo, unchanging. **Lamellae** sub-thick to thick, sub-distant, adnate-adnexed when young, sinuate at maturity, cream to light orange (4A5–4A6–4A7), brittle, unchanging, with small hygrophanous light streaks on faces; edges concolorous, smooth, irregularly slightly eroded in places, lamellulae 1–7 (–10) mm long, occasionally up to three. **Stipe** 45–105 (–180) x 6–11 (–15) mm, equal to sub-equal or tapering evenly and slightly from apex to base, usually light orange (4A5–4A6), rarely creamish orange (4A3–4A4), sometimes lightening to nearly white over basal one-fifth, glabrous to finely longitudinally striate macroscopically, under hand lens with a fine, dense, and longitudinal mat with occasional minute projecting elements; trama off-white, faintly cream near stipitipellis, fibrous, solid, snapping easily and cleanly, cartilaginous; basal mycelium and off-white mat subtended by thin to thick white hyphal chords. Odor none to fungoid when cut to slightly soapy-chemical. Taste none to slightly farinaceous.

Basidiospores 7.2–8.5 x 4.4–5.5 μm, hyaline, smooth, ellipsoid, Q range = 1.4–1.8, Q mean = 1.59, inamyloid, acyanophilic, usually containing 2–4 guttules. **Basidia** 38–52 x 4.4–7 μm, clavate, 4-sterigmate, occasionally 2-sterigmate, hyaline. Edge of the lamellae sterile due to the presence of basidioles and rarely protruding elements. **Hymenophoral** trama regular to subregular. **Pileipellis** a cutis with transitions to trichoderm, made up of interwoven hyphae, orange-ochraceous in mass with intracellular pigments, suberected cylindrical terminal elements; subpellis consisting of interwoven hyphae. **Stipitipellis** a cutis with ellipsoid to cylindrical terminal elements. **Clamp connections** and **thromboplerous hyphae** present in all tissues.

Habit, habitat and distribution: Solitary to scattered on humic mat under *Pakaraimeae dipterocarpaceae*, also found under *Dicymbe corymbosa* on white grey sand soils, known from the Upper Mazaruni River Basin, ~6 km west of Mt. Ayanganna, Upper Potaro River, and the Upper Ireng River.

Other specimens examined: GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River, within 5 km radius of Potaro base camp at 5°18'04.8"N; 59°54'40.4"W, 710 m, vicinity of base camp. 16 June 2000. Henkel 7505 (BRG, HSU, TENN 070899); 1 June 2001. Aime 1741 (BRG, PUL, TENN 070895); ~ 4 km southeast of base camp near mixed forest plot 3. 12 June 2001. Henkel 8269 (BRG, HSU, TENN 070900); 1.5 km northeast base camp. 28 May 2010. Henkel 9235 (BRG, HSU, TENN 070901); 50 m northeast of base camp. 19 June 2013. Henkel 9835 (BRG, HSU, TENN 070903); ~ 300 m northeast of base camp behind Leon camp. Henkel 9836 (BRG, HSU, TENN 070904); ~ 0.5 km east of base camp between North Benny's Ridge and Leon camp. 11 June 2015. Henkel 10068 (BRG, HSU, TENN 070896); Upper Ireng River, Suruwubaru Creek, 1.5 km upstream from juncture with Yuarka River, immediate riverside. 2 March 1997. Henkel 6270. (BRG, HSU, TENN 070897); 0.5 km East from riverside base camp. 27 May 2009. Henkel 7062 (BRG, HSU, TENN 070898).

Guyanagarika pakaraimensis Sánchez-García, T.W. Henkel & Aime, **sp. nov.** (Fig. III.4)

MycoBank MBXXXXXX

Etymology: pakaraimensis (-ensis Latin) indicating place of origin, referring to the locality where this species is found, the Pakaraima Mountains of Guyana.

Diagnosis: Morphologically similar to *G. aurantia* and *G. anomala*, but forming a distinctive clade supported by multiple loci data (ITS, nLSU, *rpb1*, and *rpb2*).

Holotype: GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River, within 5 km radius of Potaro base camp at 5°18'04.8"N; 59°54'40.4"W, 710 m, vicinity of base camp. 15 July 2008. Henkel 8941 (BRG; isotypes HSU, TENN 070918).

Pileus (17–) 38–85 mm broad, 10–16 mm tall, broadly convex to plano-convex to uplifted, with a low, broad umbo 9–14 mm tall; color deep orange (6A8–6B8) at center, peach-orange (5F4–5F5) to rich orange (5A8–5B8) towards the margin; margin entire, incurved when young to sub-crenulate with age, sometimes crisp

to eroded, irregularly split towards disc; surface appearing glabrous when fresh, but with age composed of fibrillose hairs upturned above disc and becoming repent towards margin with maturity, outer one-third becoming crenate above gills with maturity; sub-moist to moist; trama <0.5 mm at margin, color off-white, 2 mm centrally, 8 mm above stipe, unchanging.. **Lamellae** sub-thick to thick, sub-distant to distant, adnate-adnexed to subsinuate and slightly decurrent, color pale yellow to pale orange (4A4–5A4), brittle, unchanging, edges concolorous, finely eroded, but otherwise smooth, lamellulae 1–3 mm long, usually one to three. **Stipe** (40–) 55–100 (–150) x (6–) 10–22 mm, equal, occasionally tapering from apex towards base centrally, apex central to eccentric, color pale orange to orange cream cream (5A3–5A6) at apex and paler at base, fibrillose hairs creating granulose appearance, striate lines running down throughout, solid; trama color off-white, solid, stipitipellis 0.5 mm thick; basal mycelium white grading into thin white rhizomorphs connected to concolorous ectomycorrhizae. Odor mild to faintly farinaceous to acrid. Taste mild to faintly farinaceous.

Basidiospores 6.7–8.7(–9.2) x 4.1–5.7 μm, hyaline, smooth, ellipsoid, Q range =1.3–1.7, Q mean = 1.52, inamyloid, acyanophilic, usually 2–4 guttules. **Basidia** 40–48 x 5–8 μm, clavate, 4-stererigmate, occasionally 2-sterigmate, hyaline. Edge of the lamellae sterile due to the presence of basidioles and sometimes protruding elements. **Hymenophoral** trama regular to subregular. **Pileipellis** a cutis with transitions to trichoderm, of interwoven hyphae, orange-ochraceous in mass with intracellular pigments, suberected cylindrical terminal elements; subpellis consisting of interwoven hyphae. **Stipitipellis** a cutis with ellipsoid to cylindrical terminal elements. **Clamp connections** and **thromboplerous hyphae** present in all tissues.

Habit, habitat and distribution: solitary to scattered on humic mat under *Dicymbe corymbosa*, known from the Upper Potaro River and the Upper Ireng River.

Other specimens examined: GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River. Henkel 6995 (BRG, HSU, TENN 070916); Benny's Ridge. 29 May 2012. Aime 4776 (BRG, PUL, TENN 070908); across the River, vicinity of plot 3. 3 June 2012. Aime 4820 (BRG, PUL, TENN 070909); within 5 km radius of Potaro base camp at 5°18'04.8"N; 59°54'40.4"W, 710 m, 1.5 km southeast of base camp. 2 July 2002. Henkel 8512 (BRG, HSU, TENN 070917); 17 May 2010. Aime 3941 (BRG, PUL, TENN 070905); 27 May 2012. Aime 4749 (BRG, PUL, TENN 070906); 29 May 2012. Aime 4775 (BRG, PUL, TENN 070907); 8 June 2015. Henkel 10031 27 May 2012. Aime 4749 (BRG, HSU, TENN 070906); ~0.4 km northeast of base camp behind Leon camp. 10 June 2015. Henkel 10051 (BRG, HSU, TENN 070911); ~ 0.5 km northeast of base camp, 18 June 2015. Henkel 10114 (BRG, HSU, TENN 070912); ~ 1 km northeast of base camp in vicinity of old Ayanganna airstrip. Henkel 10123 (BRG, HSU, TENN 070913).

Guyanagarika anomala Sánchez-García, T.W. Henkel & Aime, **sp. nov.**
(Fig. III.5)
MycoBank MBXXXXXX
Etymology: from the Latin *anomala* = abnormal, anomalous

Diagnosis: Morphologically similar to *G. aurantia* and *G. pakaramensis*, but forming a distinctive clade supported by multiple loci data (ITS, nLSU, *rpb1*, and *rpb2*).

Holotype: GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River, within 5 km radius of Potaro base camp at 5°18'04.8"N; 59°54'40.4"W, 710 m, vicinity of base camp. 26 May 2010. Henkel 7419 (BRG; isotypes HSU, TENN 070920). Paratype: 18 May 2001. Aime 1519 (BRG, PUL, TENN 070919).

Pileus 40–130 mm broad, convex to plane to slightly uplifted, deep orange (5A8–5B8–5C8) at disc, paler (4A5) towards margin; margin broadly scalloped and wavy; surface glabrous to coarsely rugose, whitish canescent over disc,

trama off-white. **Lamellae** sub-thick to thick, close to sub-distant, adnate to adnexed, broad, pale yellow-orange (4A4), brittle, edges coarsely and irregularly serrate with age, margin down curved, up to 6 lamellulae. **Stipe** 35-140 mm equal or tapering towards the base, pale yellow orange (4A2–4A3), mostly glabrous, longitudinally striate. Odor none. Taste mild. Trama off-white. Fibrous.

Basidiospores 7–9(–9.5) x 4.1–5.5 μm, hyaline, smooth, ellipsoid, Q range =1.4–1.8, Q mean = 1.63, inamyloid, acyanophilic, usually 2–4 guttules. **Basidia** 48–55 x 5–8.8 μm, clavate, 4–stererigmate, occasionally 2–sterigmate, hyaline. Edge of the lamellae sterile due to the presence of basidioles. **Hymenophoral** trama regular to subregular. **Pileipellis** a cutis with transitions to trichoderm, of interwoven hyphae, orange-ochraceous in mass with intracellular pigments, suberected cylindrical terminal elements. Subpellis consisting of interwoven hyphae. Stipitipellis a cutis with ellipsoid to cylindrical terminal elements. **Clamp connections** and **thromboplerous hyphae** present in all tissues.

Habit, habitat and distribution: solitary on humic mat under *D. corymbosa*, known from the Upper Potaro River.

Discussion

Guyanagarika is morphologically similar to the ECM genus *Tricholoma*; it has a white spore deposit, tricholomatoid habit, inamyloid spores, and shares with some species of *Tricholoma* the presence of clamp connections (Bigelow, 1979; Riva, 2003). The thickness of the lamellae gives some resemblance to the genus *Hygrophorus*, but the lack of divergent hymenophoral trama excludes it from this group. *Guyanagarika* is easily recognized in the field by its bright orange color, prominent umbo, and sub-thick to thick lamellae.

It is widely accepted that some fungal species are difficult to distinguished based on morphological characters only, and cryptic diversity is rather common (Geml et al., 2006; Hallenberg et al., 2007; Gazis et al., 2011; Sanchez-Ramirez et al., 2015; Singh et al., 2015). It has also been shown that the application of species recognition criteria can affect not just diversity estimates, but also ecological and evolutionary hypotheses, and conservation strategies (Agapow et al., 2004;

Frankham et al., 2012). We recognize the three independent evolutionary lineages within *Guyanagarika* as different species. The recognition of *G. aurantia, G. pakaraimensis*, and *G. anomala* is only possible through molecular markers.

The presence of cryptic species in this area has been previously documented within *Clavulina sprucei* and *Singerocomus rubriflavus* (Henkel et al., 2011, 2016), but the lack of material has impeded the recognition and formal description of such species. Henkel et al. (2016) found that the possible cryptic species of *S. rubriflavus* associate with different host plants, which can be driving speciation within this group. We do not see this occurring within the three species of *Guyanagarika*, as these taxa are sympatric and have overlapping hosts.

Initially, the Catathelasmataceae consisted of only one genus, *Catathelasma*. Singer (1986) included this genus in his broad concept of the Tricholomataceae, and the two families were synonymized. Moncalvo et al. (2002) and Matheny et al. (2006) recognized the *Catathelasma* clade as one of the major groupings within the Tricholomatoid clade (suborder Tricholomatineae). At that time only three taxa were recognized as part of this clade, *Callistosporium*, *Catathelasma*, and *Clitocybe subvelosa*, which was later transferred to the genus *Cleistocybe* Ammirati, A.D. Parker & Matheny (Ammirati et al., 2007). Our analyses failed to recover the latter as part of the *Catathelasma* clade, but recovered the genera *Callistosporium*, *Catathelasma*, *Pleurocollybia*, *Pseudolaccaria*, *Macrocybe*, and *Clitocybe* aff. *fellea*. While there are no obvious morphological and ecological synapomorphies that unite the Cathathelasmataceae (Table III.2), these taxa consistently form a monophyletic group within the suborder Tricholomatineae (Matheny et al., 2006; Ammirati et al., 2007; Sánchez-García et al., 2014).

The relationship between *Cleistocybe* and *Catathelasma* has been examined in detailed by Ammirati et al. (2007); both genera are characterized by having a partial veil, divergent hymenophoral trama, and acyanophilic spores. Based on these morphological similarities, and the fact that the phylogenetic position of *Cleistocybe* within the Tricholomatineae is not well supported (Fig. III.1), we consider that this taxon may certainly be part of the Catathelasmataceae, and that failure to recover *Cleistocybe* as part of this family could be due to the lack of *rpb1*

and *rpb2* sequences in our phylogenetic analyses. These two loci have been shown to increase resolution and have higher proportion of informative characters than ribosomal DNA sequences (Matheny et al., 2002; Frøslev et al., 2005; Schoch et al., 2009); however, to date only ribosomal sequences are available for this genus.

Phylogenetic analyses of the *Catathelasma* dataset (Fig. III.2) show a collection of *Pleurocollybia* sp. as part of *Callistosporium*. These sequences were retrieved from GenBank, and we cannot confirm its taxonomic identity. We suggest that it could be a misidentified specimen of *Callistosporium* since both genera are lignicolous and morphologically similar (Table III.2).

Based on our phylogenetic analyses, *Clitocybe fellea* seems to be part of *Pseudolaccaria*. Future work may synonymize this taxon with *Pseudolaccaria pachyphylla*, or describe it as a new species within *Pseudolaccaria*, as it has been previously discussed by Lavorato et al. (2015).

Guyanagarika represents an independent evolutionary origin of the ECM lifestyle. Some putatively endemic lineages have been recognized from ECM root sequences (Tedersoo and Smith, 2013), but those taxa have not been formally described. While other endemic ECM neotropical genera that belong to the family Boletaceae have been recently described (Smith et al., 2015; Henkel et al., 2016), *Guyanagarika* represents the first endemic ECM neotropical genus discovered among the Agaricales.

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Appendix

Table III.1. Taxa and GenBank accession numbers for sequences used in the phylogenetic analyses.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Alboleptonia* aff. *sericella	MCA1978		GU384609		GU384632	
***Alboleptonia* sp.**	EH185		KU058535		KU138998	KU139031
Alboleptonia stylophora	AST84		GU384610		GU384633	
***Albomagister* sp. 2**	MSG137		KJ417178		KJ424363	KU139018
***Albomagister* sp. 3**	ECV4202		KJ417179		KJ424365	KU139019
Albomagister subaustralis	MGW676	KJ417252	KJ417181	KJ417255	KJ424367	KU139020
Ampulloclitocybe clavipes	PBM2474		AY639881	AY771612	AY780937	
Aspropaxillus giganteus	TENN060129		KJ417183	KJ417256	KJ424368	KU139021
Callistosporium graminicolor	PBM2341	DQ484065	AY745702	AY752974	KJ424369	GU187493
Callistosporium luteoolivaceum	JM99/124		AF261405			
Callistosporium luteoolivaceum	MSM#004	KJ101607				
Callistosporium xanthophyllum	14096	JF907781				
Callistosporium xanthophyllum	IB19770276	AF325667	AF261406			
Calocybe carnea	CBS552.50		AF223178	DQ367418	DQ367432	
Calocybe gangraenosa	HAe251.97		AF223202	DQ367420	DQ367434	
Catathelasma imperiale	DAOM225247	KP255468	AF261402			
Catathelasma ventricosum	DAOM221514	KP255469	AF261401		KP255482	
Catathelasma ventricosum	PBM2403		DQ089012	DQ435811	DQ470830	
Cleistocybe carneogrisea	S.Poumarat s.n.		HQ728527	HQ728528		
Cleistocybe vernalis	PBM1856		AY647208	DQ092913		
Clitocybe* aff. *fellea	PBM3028	HQ728533	HQ728534	HQ728535	HQ728536	GB

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Clitocybe candicans	PBM2476		AY645055	AY771609	DQ385881	DQ447891
Clitocybe dealbata	HC95.cp3		AF223175	DQ825431	DQ825407	DQ825414
Clitocybe fellea	PBM1439		EF561629			
Clitocybe nebularis	PBM2259		DQ457658	DQ437681	DQ470833	DQ825415
Clitocybe subdipoda	PBM2489		AY691889	AY771608	AY780942	DQ447892
Clitopilopsis hirneola	TB8490		GU384611		GU384646	
Clitopilus apalus	M536		AF261287			
Clitopilus caelatus	511		GQ289208			
Clitopilus caelatus	TB6995		GU384625		GU384652	
Clitopilus cystidiatus	26		GQ289147		GQ289220	
Clitopilus fallax	262		GQ289209		GQ289275	
Clitopilus fallax	37		GQ289210		GQ289276	
Clitopilus geminus	Co-David 407		HM164411			
Clitopilus geminus	TB7526		GU384626		GU384655	
Clitopilus giovanellae	AH 19718		EF413028			
Clitopilus lateritius	Co-David 418		HM164410			
Clitopilus pallidogriseus	118		GQ289216		GQ289283	
Clitopilus popinalis	260		GQ289213		GQ289280	
Clitopilus prunulus	2		GQ289149		GQ289221	
Clitopilus prunulus	GLM45889		AY207161			
Clitopilus prunulus	RV88/109		AF042645			
Clitopilus pseudopiperitus	162		GQ289217		GQ289284	
Clitopilus scyphoides	T777		AF261288			

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Clitopilus scyphoides	HKI ST 22295		AY207162			
***Clitopilus* sp.**	211		GQ289212		GQ289279	
***Clitopilus* sp.**	CoDavid 266		HM164412		HM164416	
***Clitopilus* sp.**	TB8024		GU384613		GU384647	
***Clitopilus* sp.**	TB8067		GU384612		GU384649	
Collybia tuberosa	TENN 53540		AY639884	AY771606	AY787219	AY857982
Corneriella bambusarum	DED5462		KJ417185		KJ424370	KU139023
Corneriella humicola	DED7871		KF291052	KF291053		
***Corneriella* sp.**	PR-4733		KF306337		KF306338	
Dennisiomyces glabrescentipes	JPF1000A		KJ417235			
***Dennisiomyces* sp. 1**	BZ-916	KF291063	KF291064		KF291066	
***Dennisiomyces* sp. 2**	PR-4763		KJ417191			
***Dennisiomyces* sp. 3**	PBM3861	KJ417267	KJ417189	KU058567	KJ424374	KU139024
***Dennisiomyces* sp. 4**	PR-6613		KJ417190	KJ417257		KU139025
Entoloma abortivum	TB6693		GU384616		GU384642	
Entoloma albidoquadratum	i4		GQ289151		GQ289223	
Entoloma alpicola	TB6415		AF261302			
Entoloma araneosum	14		GQ289153		GQ289225	
Entoloma asterosporum	PBM3268		JF706310	JF706311	JF706312	KU139030
Entoloma bicolor	TB4967		AF261298			
Entoloma bloxamii	219		GQ289154		GQ289226	
Entoloma bloxamii	TB6117		AF261289			
Entoloma caccabus	17		GQ289155		GQ289227	

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Entoloma calongei	322		GQ289158			
Entoloma canescens	TB5657		DQ851575			
Entoloma cephalotrichum	253		GQ289157		GQ289229	
Entoloma* cf. *nigroviolaceum	TM03/426		EU522809			
Entoloma cocles	244		GQ289159		GQ289230	
Entoloma conferendum	6		GQ289160		GQ289231	
Entoloma costatum	49		GQ289232		GQ289161	
Entoloma excentricum	184		GQ289163		GQ289234	
Entoloma flavifolium	TB6215		AF261301		GU384644	
Entoloma gasteromycetoides	180		GQ289164		GQ289235	
Entoloma gelatinosum	212		GQ289165		GQ289236.	
Entoloma griseolazulinum	i11		GQ289166		GQ289237	
Entoloma haastii	126		GQ289167		GQ289238	
Entoloma haastii	216		GQ289168		GQ289239	
Entoloma haastii	BY21		AF261309			
Entoloma hebes	46		GQ289170		GQ289241	
Entoloma incanum	TM02/277		EU522763			
Entoloma indigoticoumbrinum	83		GQ289242		GQ289171	
Entoloma indoviolaceum	i13		GQ289172		GQ289243	
Entoloma infundibuliforme	TENN013964		HQ179672			
Entoloma kermandii	222		GQ289173		GQ289244	
Entoloma myrmecophilum	231		GQ289174		GQ289245	
Entoloma nidorosum	TB9971		GU384617		GU384643	

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Entoloma odorifer	TB6366		AF261310			
Entoloma pallideradicatum	255		GQ289176		GQ289247	
Entoloma parasiticum	20		GQ289177		GQ289248	
Entoloma perbloxamii	71		GQ289178		GQ289249	
Entoloma phaeomarginatum	127		GQ289179		GQ289250	
Entoloma pluteisimilis	200		GQ289180		GQ289251	
Entoloma politum	15		GQ289181		GQ289252	
Entoloma procerum	70		GQ289183		GQ289254	
Entoloma prunuloides	40		GQ289184		GQ289255	
Entoloma prunuloides	TJB4765		AY700180		DQ385883	
Entoloma pygmaeopapillatum	32		GQ289185		GQ289256	
Entoloma readiae	102		GQ289186		GQ289257	
Entoloma rhodopolium	8		GQ289187		GQ289258	
Entoloma rhodopolium	TB6221		AF261300			
Entoloma sarcitum	235		GQ289188		GQ289259	
Entoloma sericatum	28		GQ289189		GQ289260	
Entoloma sericellum	10		GQ289190		GQ289261	
Entoloma sericeonitidum	TB7144		AF261315		EF421016	
Entoloma sericeum	VHA 03-02		DQ367423	DQ367421	DQ367435	
Entoloma serrulatum	163		GQ289192		GQ289263	
Entoloma sinuatum	TJB5349		AY691891	AY657007		DQ516074
Entoloma sinuatum	50		GQ289193		GQ289264	
Entoloma sinuatum	TM02/134		EU522735			

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Entoloma sinuatum	TM02/335		EU522771			
Entoloma sinuatum	TM03/429		EU522811			
Entoloma sordidulum	1		GQ289194		GQ289265	
***Entoloma* sp.**	292		GQ289156		GQ289228	
***Entoloma* sp.**	JM98/123		AF261322			
***Entoloma* sp.**	TM02/237		EU522752			
***Entoloma* sp.**	TM03/413		EU522803			
***Entoloma* sp.**	TM03/500		EU522847			
Entoloma sphagneti	209		GQ289195			
Entoloma strictius	M96/10		AF042620	AF287832	AY218483	
Entoloma strictius	TB7710		GU384618		GU384641	
Entoloma tectonicola	i15		GQ289196		GQ289266	
Entoloma tjallingiorum	243		GQ289197		GQ289267	
Entoloma transmutans	155		GQ289200		GQ289268	
Entoloma turbidum	TB6949		GU384630		GU384656	
Entoloma undatum	18		GQ289202		GQ289270	
Entoloma undatum	GLM 45920		AY207199			
Entoloma unicolor	TB5520		AF261312			
Entoloma valdeumbonatum	189		GQ289203		GQ289271	
Entoloma vezzenaense	241		GQ289204		GQ289272	
Entoloma vinaceum	TB8870		GU384631		GU384651	
Entoloma violaceovillosum	i2		GQ289205		GQ289273	
Guyanagarika aurantia	ECM root1	KX092071				

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Guyanagarika aurantia	ECM root2	KX092072				
Guyanagarika aurantia	MCA1741	KX092073	KX092097		KX092129	
Guyanagarika aurantia	TH10068	KX092074	KX092098		KX092130	KX092111
Guyanagarika aurantia	TH6270	KX092075				
Guyanagarika aurantia	TH7062	KX092076				
Guyanagarika aurantia	TH7505	KX092077			KX092131	KX092112
Guyanagarika aurantia	TH8269	KX092078				
Guyanagarika aurantia	TH9235	KC162210	KC162210	KC162209	KX092132	KX092113
Guyanagarika aurantia	TH9693	KC162210	KX092099	KX092111	KX092133	KX092114
Guyanagarika aurantia	TH9835	KC162210	KX092100	KX092112	KX092134	KX092115
Guyanagarika aurantia	TH9836	KX092079	KX092101	KX092113	KX092135	KX092116
Guyanagarika pakaraimensis	MCA3941	KX092080			KX092136	
Guyanagarika pakaraimensis	MCA4749	KX092081	KX092102		KX092137	KX092117
Guyanagarika pakaraimensis	MCA4775	KX092082	KX092103		KX092138	KX092118
Guyanagarika pakaraimensis	MCA4776	KX092083	KX092104		KX092139	
Guyanagarika pakaraimensis	MCA4820	KX092084			KX092140	
Guyanagarika pakaraimensis	TH10031	KX092085	KX092105		KX092141	KX092119
Guyanagarika pakaraimensis	TH10051	KX092086	KX092106		KX092142	KX092120
Guyanagarika pakaraimensis	TH10114	KX092087	KX092107		KX092143	KX092121
Guyanagarika pakaraimensis	TH10123	KX092088	KX092108		KX092144	KX092122
Guyanagarika pakaraimensis	TH6595	KX092089				
Guyanagarika pakaraimensis	TH6618	KX092090				
Guyanagarika pakaraimensis	TH6995	KX092091				

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Guyanagarika pakaraimensis	TH8512	KX092092				
Guyanagarika pakaraimensis	TH8941	KT339200	KT339200	KX092114	KX092145	KX092123
Guyanagarika anomala	MCA1519	KX092093	KX092109	KX092115	KX092146	
Guyanagarika anomala	TH7419	KX092094	KX092110		KX092147	
Infundibulicybe gibba	JCS0704B		DQ457682	DQ115780	DQ472727	DQ447913.
Inocephalus lactifluus	TB7962		AF261304			
Inocephalus murrayi	VHAs02/02		GU384590		GU384637	
***Inocephalus* sp.**	GD-b		DQ457683	DQ457622	DQ472728	
***Inocephalus* sp.**	MCA1475		GU384619		GU384636	
***Inocephalus* sp.**	MCA1867		GU384621		GU384638	
***Inocephalus* sp.**	MCA2479		GU384622		GU384640	
Inopilus entolomoides	TB8507		AF261293			
Leptonia serrulata	VHAs0102		GU384624		GU384634	
Lepista irina	PBM2291		DQ234538	AY705948	DQ385885	
Lepista saeva	ADW0097		KJ417193	KJ417259	KJ424376	KU139032
Lepista sordida	CRG014		AY207225	KJ417260	KJ137273	KU139033
Leptonia carnea	TB5812		AF261313			
***Leptonia* sp.**	MCA1486		GU384623		GU384635	
Leptonia subserrulata	TB6993		AF261291			
Leucopaxillus alboalutaceus	LAS00/082		KJ417195	KJ417261	KJ424377	
Leucopaxillus cerealis	GB:0068845	KJ417282	KJ417198	KJ417262	KJ424379	KU139034
Leucopaxillus eucalyptorum	REH9110		KJ417199			
Leucopaxillus gentianeus	EL291/11		KJ417201		KJ424381	

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Leucopaxillus lilacinus	PBM3584		KJ417205	KJ417264	KJ424382	
Leucopaxillus paradoxus	TK04/091	KJ417296	KJ417206	KJ417265	KJ424383	
Leucopaxillus tricolor	TFB13462		KJ417207	KJ417266	KJ424384	
Lyophyllum decastes	JM 87/16		AF042583	DQ367419	DQ367433	
Lyophyllum **sp.**	PBM 2688		DQ094785	DQ457628		
Lyophyllum **sp.**	MSG166		KU058536	KU058570	KU138999	KU139036
Lyphyllum **aff.** *decastes*	PBM3069		HQ832459	HQ832426	HQ832437	KU139035
Macrocybe titans	M2012-1		KC913200			
Neohygrophurus angelesianus	PBM482		DQ470814	DQ457698		
Nolanea cetrata	TB7382		AF261319			
Nolanea conica	MB6		AF261317			
Nolanea hirtipes	K1171992		AF261320			
Nolanea quadrata	TM03/445		EU522821			
Nolanea sericea	29		GQ289191		GQ289262	
Nolanea **sp.**	TM02/196		EU522740			
Nolanea **sp.**	TM03/403		EU522800			
Notholepista subzonalis	GB:0087013		KJ417208	KJ417267	KJ424385	
Omphalina antarctica	HSG070118-26		GQ483368			
Omphalina pyxidata	TO AV98		JN944403			
Ossicaulis lignatilis	DAOM188196		AF261396	AF334923	DQ825410	
Paralepista **sp.**	TO AV2009		JQ585659			
Paralepistopsis amoenolens	TO AV2004		JQ585654			
Pleurocollybia brunnescens	DAOM34832		AF261407			

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Pleurocollybia imbricata	TJB9847	HM105568	HM105568		HM105567	
***Pleurocollybia* sp.**	MEL2363162	KP311477	KP311402			
Pogonoloma macrocephalum	MOS69/85		KJ417209	KJ417268		
Pogonoloma spinulosum	K(M):107286		KJ417238	KU058571	KJ424401	KU139037
Porpoloma portentosum	MES531	KJ417298	KJ417210	KU058572	KJ424386	KU139038
Porpoloma sejunctum	CONC:F0416	KJ417301	KJ417212	KU058573	KJ424388	
***Porpoloma* sp. 2**	PBM3441		KJ417213	KJ417269	KJ424389	KU139040
Porpoloma terreum	REH5830		KJ417215		KJ424391	
Pouzarella nodospora	TB5716		AF261308			
Pseudoclitocybe cyathiformis	LAS90/139B		KU058540	KU058576	KU139004	KU139044
Pseudoclitocybe expallens	TO HG2286		JF926525			
***Pseudoclitocybe* sp.**	RHP12643		KJ417217		KJ424392	KU139045
Pseudoclitopilus rhodoleucus	TK03/203		KJ417218	KU058577	KJ424393	KU139046
Pseudoomphalina felloides	DAOM11115		AF261442			
Pseudoomphalina pachyphylla	LAS07/012	KU058504	KU058542	KU058579	KU139006	KU139048
Pseudoomphaline kalchbrenneri	LAS06/037		KU058541	KU058578	KU139005	KU139047
Pseudotricholoma metapodium	AH22102006		KJ417219	KJ417271	KJ424394	KU139049
Pseudotricholoma umbrosum	REH4796		KJ417222	KU058580	KJ424397	KU139050
Rhodocybe aureicystidiata	PBM1902		AY380407		AY337412	
Rhodocybe fallax	OKM25668		AF261283			
Rhodocybe mundula	TJB7599		AY700182		DQ474128	
Rhodocybe paurii	JM99/233		AY286004		KC816969	
Rhodocybe popinalis	TB6378		AF261285		GU384654	

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Rhodocybe pruinosostipitata	MCA1492		GU384627		GU384653	
Rhodocybe spongiosa	MCA2129		GU384628		GU384657	
Rhodocybe truncata	CBS482/50		AF223167		EF421019	
Rhodocybella rhododendri	TENN047309		HQ222034			
Ripartites tricholoma	GLM 46025		AY207297			DQ067936
Singerocybe adirondackensis	PBM3320		HQ728530	HQ728531	HQ728532	KU139051
Singerocybe alboinfundibuliformis	HKAS74716		JX514106		JX514138	
Singerocybe clitocyboides	HKAS75451		JX514099			
Singerocybe humilis	HKAS74717		JX514114		JX514150	
Singerocybe phaeophthalma	HKAS79015		KF208453		KF208465	
Singerocybe umbilicata	HKAS78038		KF208455		KF208461	
Tephrocybe anthracophylla	JT31970		KJ417225			KU139064
Tephrocybe boudieri	BSI96/84		DQ825430	DQ825433	DQ825411	DQ825421
***Tephrocybe* sp.**	CCB148		KU058561		KU139014	KU139065
Termitomyces microcarpus	PRU3900		AF042587	DQ851581		
***Termitomyces* sp.**	TFB1546		KU058562		KU139015	
***Termitomyces* sp.**	ZA164		DQ110875	DQ092922	DQ437070	DQ447942
Trichocybe puberula	TO-HG1148		FM877680			
Tricholoma* aff. *fulvum	TFB14052		KU058543	KU058581	KU139007	KU139052
Tricholoma album	TFB13753		KU058544	KU058582		
Tricholoma arvernense	DP081004-01		KU058545			
Tricholoma atrodiscum	MSG132		KU058546	KU058583	KU139008	KU139053
Tricholoma atroviolaceum	MSG167		KU058547	KU058584	KU139009	KU139054

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
Tricholoma caligatum	PBM3899		KU058548		KU139010	KU139055
Tricholoma elegans	PBM3142		KJ417226			KU139066
Tricholoma eucalypticum	PBM3154		KU058550	KU058586		
Tricholoma* f. *davisiae	TFB13409		KU058549	KU058585		KU139056
Tricholoma flavovirens	TFB13553		KU058551	KU058587		KU139057
Tricholoma fulvum	BPL304		KU058552	KU058588	KU139011	KU139058
Tricholoma guldeniae	MSG131		KU058553	KU058589		KU139059
Tricholoma inamoenum	REG MB 96-071		AY293215	AY293161		
Tricholoma luteomaculosum	CLO4632		KU058554	KU058590		KU139060
Tricholoma matsutake	Tn9		U62964	U62538		
Tricholoma myomyces	KMS589		U76459	DQ367422	DQ367436	
Tricholoma palustre	PBM2494		AY700197	AY757267	DQ484055	
Tricholoma populinum	LCG2003		JN019649		JN019720	JN019668
Tricholoma saponaceum	TFB12328		KU058555	KU058591		
Tricholoma saponaceum	PBM2514		AY647209	AY654883		
Tricholoma sejunctum	MSG133		KU058556	KU058592		
***Tricholoma* sp.**	PBM3085		KJ417228	KJ417273		KU139067
***Tricholoma* sp.**	PBM3141		KJ417227	KJ417272		KU139068
***Tricholoma* sp.**	PBM3168		KU058563	KU058596	KU139016	KU139069
***Tricholoma* sp.**	PBM3170		KU058564	KU058597		
***Tricholoma* sp.**	RHP13062		KJ417229	KJ417274		
***Tricholoma* sp.**	SAR1290		AF042592	AF287839		
***Tricholoma* sp.**	MSG160		KU058565	KU058598	KU139017	KU139070

Table III.1. Continued.

Name	Voucher or field number	ITS	LSU	SSU	RPB2	RPB1
***Tricholoma* sp.**	PBM3144		KU058566			
Tricholoma subluteum	MSG134	KU058519	KU058557	KU058593	KU139012	KU139061
Tricholoma subresplendens	SAT1027901		KJ417230	KJ417275		KU139071
Tricholoma sulphureum	PBM3959		KU058558	KU058594		KU139062
Tricholoma vaccinum	TFB13554		KU058559			
Tricholoma virgatum	MSG165	KU058522	KU058560	KU058595	KU139013	KU139063
Tricholoma viridiolivaceum	PBM3093		JF706317	JF706318	JF706319	KU139072
Tricholomella constricta	HC84-75		AF223188	DQ825434	DQ825412	DQ825422
Tricopilus porphyrophaeus	TB6957		AF261290		EF421020	
Undet gray scaly	ECV4038		KJ417231	KJ417276	KJ424399	KU139073
Undet small white	MBP080609		KJ417232	KJ417277	KJ424400	KU139074

Table III.2. Comparison of the key characters of the members of the Catathelasmataceae recognized in this study and the genus *Cleistocybe*.

	Callistosporium	*Catathelasma*	*Macrocybe*	*Pseudolaccaria*	*Pleurocollybia*	*Guyanagarika*	*Cleistocybe*
Habit	Collybioid	Tricholomatoid, veil-double	Tricholomatoid	Tricholomatoid	Pleurotoid, collybioid	Tricholomatoid	Clitocyboid, with veil
Lamellae	Subdecurrent, adnexed or emarginated	Decurrent or adnate to sinuate-adnexed	Attached	Emarginate	Adnexed, sinuate, adnate or slightly decurrent	Adnexed to sub-sinuate	Decurrent to long decurrent
Hymenophoral trama	Regular	Bilateral becoming regular	Regular	Regular	Regular	Regular	Interwoven to subparallel, more or less divergent when young
Spores	Ellipsoid, smooth, inamyloid, scarcely or weakly cyanophilic	Oblong, amyloid, smooth, acyanophilic	Ellipsoid, smooth, inamyloid, cyanophilic	Ellipsoid, smooth, amyloid, cyanophilic	Ellipsoid, smooth, inamyloid, weakly to distinctive cyanophilic	Ellipsoid, smooth, inamyloid, with guttules, acyanophilic	Ellipsoid to oblong to ovate or subfusoid, smooth, inamyloid, acyanophilic
Cheilocystidia	None	Cylindrical	None	None, basidioles	Sometimes present	None	None
Pleurocystidia	None	None	Pseudocystidia	None	None	None	None
Clamps	Absent	Present	Present	Present	Absent	Present	Present
Pileipellis	Cutis, hyphae with encrusting and intracellular pigments	Ixocutis	Cutis	Cutis of encrusted hyphae	Cutis-like, filamentous hyphae multiseptate	Cutis becoming a trichoderm with intracellular pigments	Interwoven, non-gelatinous or gelatinous
Habitat	On the base of palm trees, wood, *Sphagnum*, earth	Terrestrial under conifers, ectomycorrhizal	On Soil, grasslands, sand, Costa Rica ants nests	Sandy soil, conifereous forests, grasslands	On rotten wood	On soil, ectomycorrhizal	Terrestrial
Distribution	Asia (tropics), America, Canada to Argentina, Europe	Europe, North America, Japan	Tropics	Europe	America, North to South	Tropics, Guyana	North America

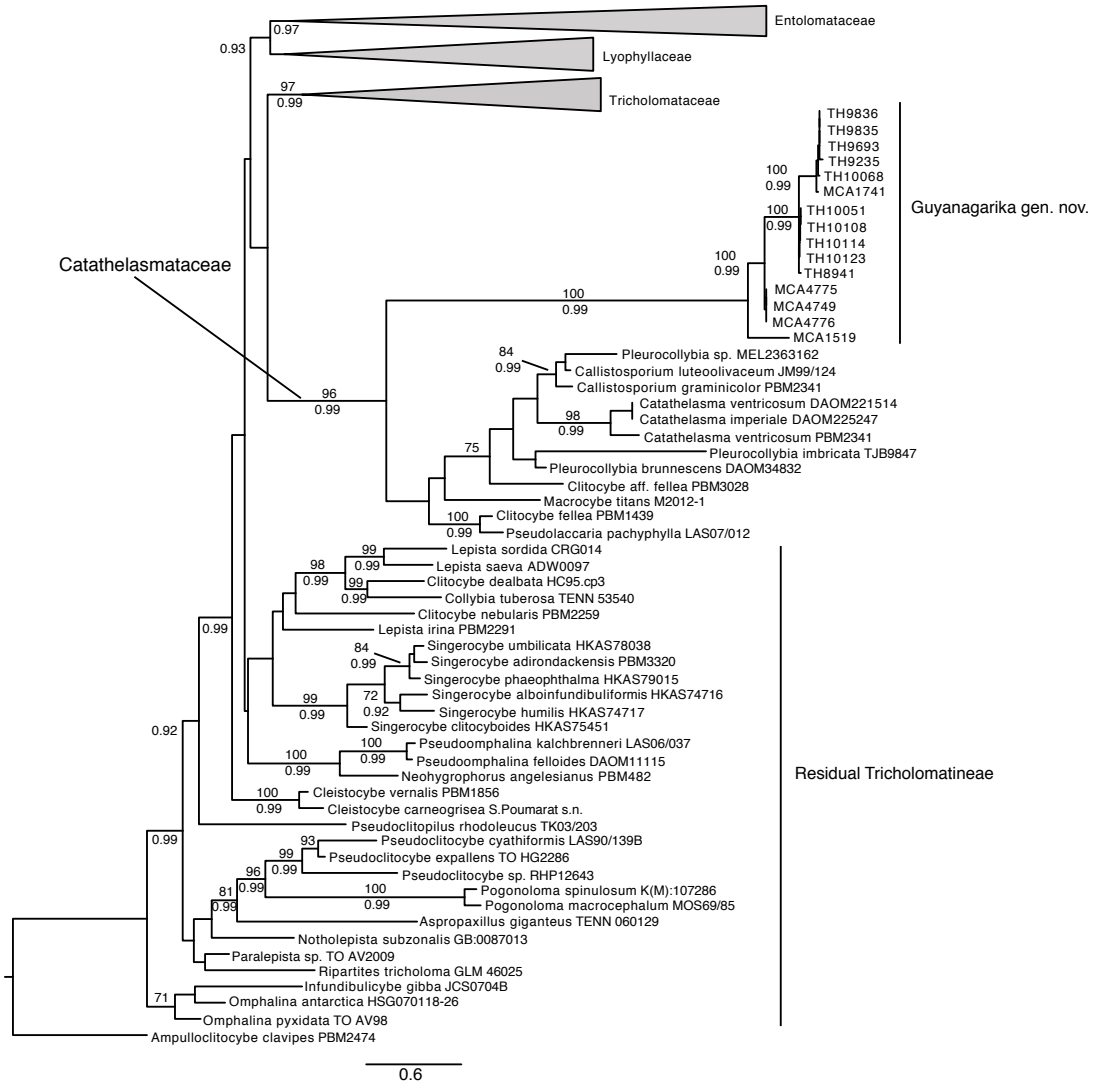

Figure III.1. Maximum likelihood phylogeny (nLSU, nSSU, rpb1, rpb2) of the suborder Tricholomatineae. Bootstrap values ≥70 are shown above branches and Bayesian posterior probabilities ≥0.90 are shown below branches.

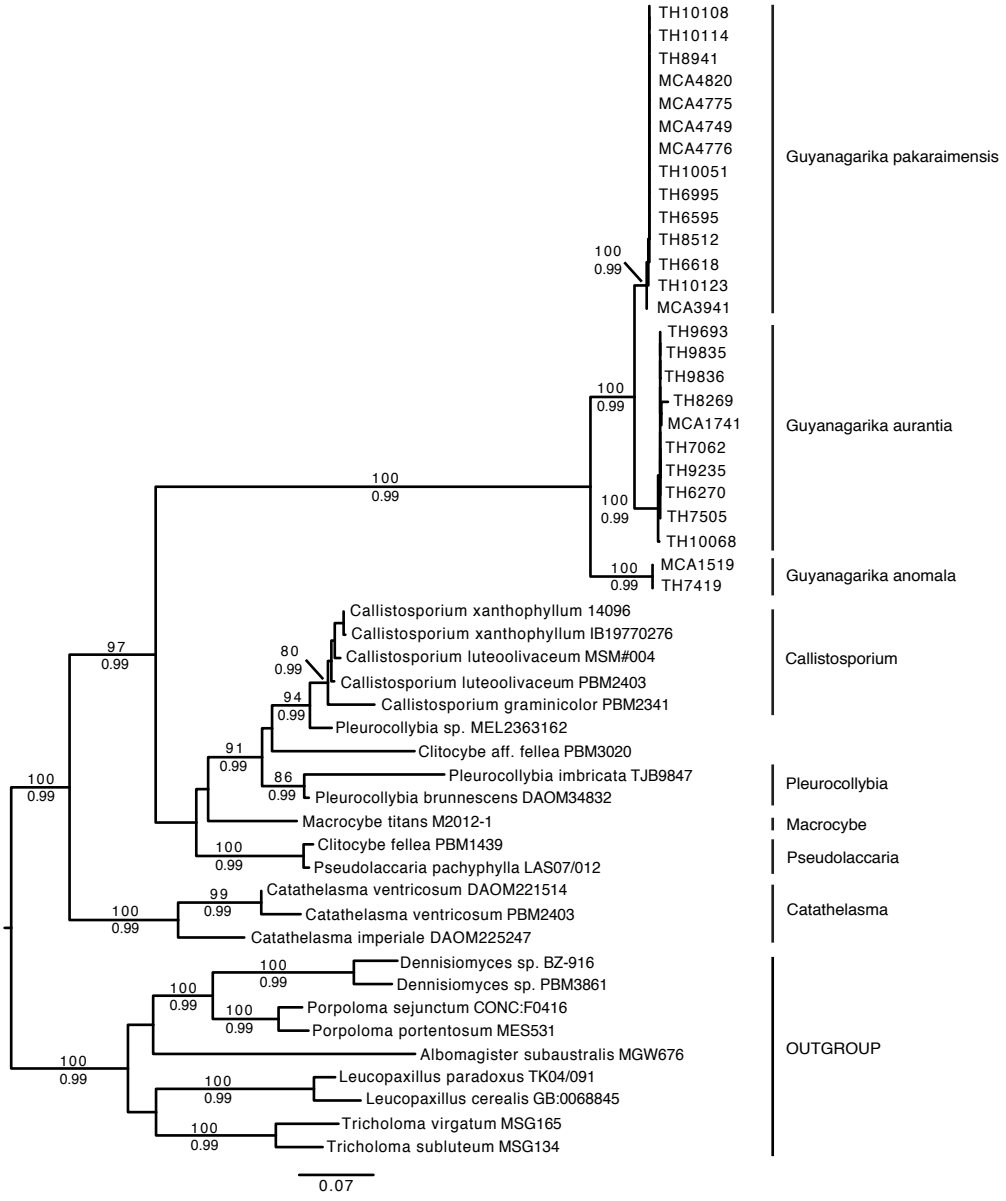

Figure III.2. Maximum likelihood phylogeny (nLSU, nSSU, *rpb1*, *rpb2*, ITS) of the family Catathelasmataceae. Bootstrap values ≥70 are shown above branches and Bayesian posterior probablilities >0.90 are shown below branches.

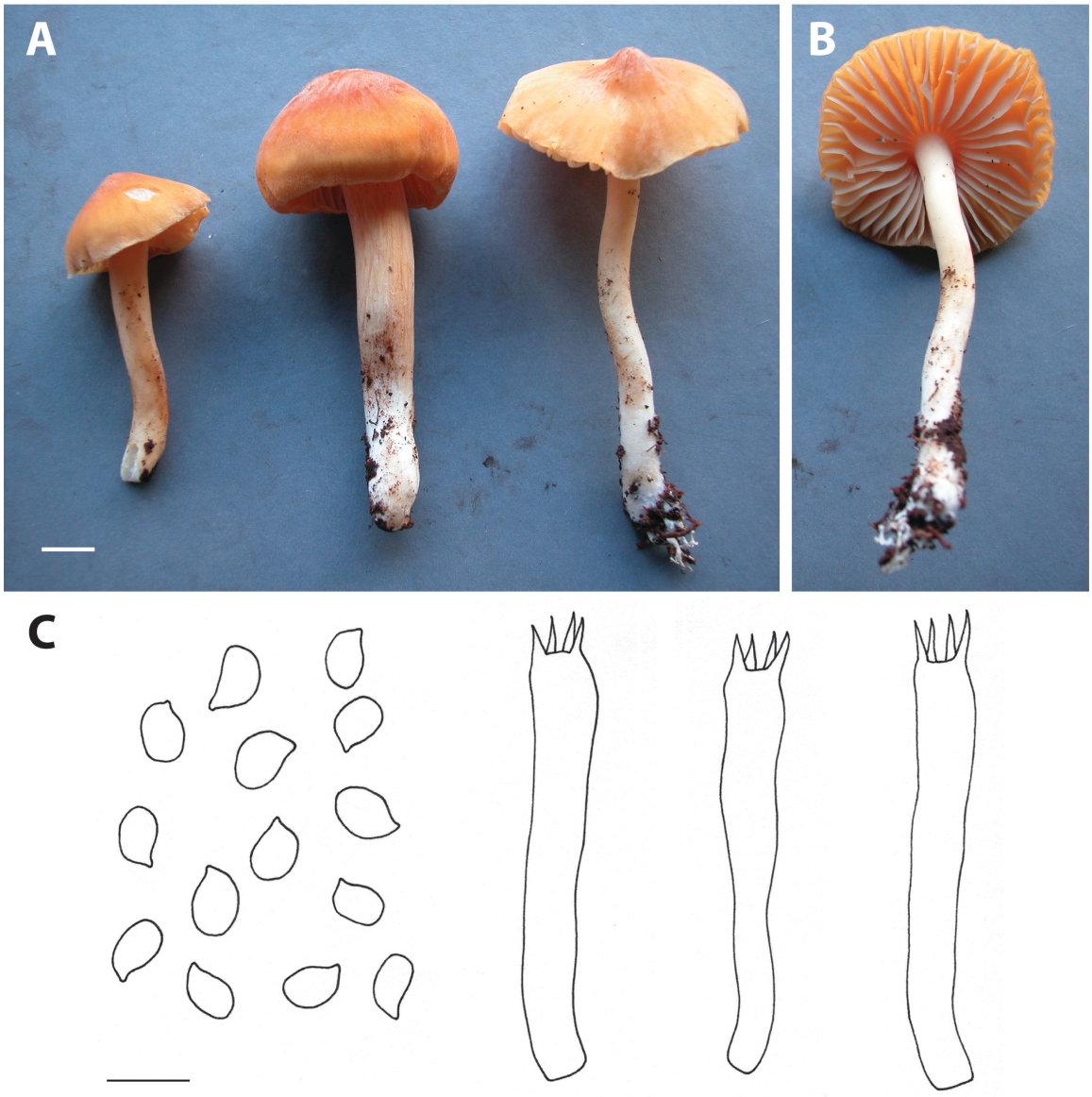

Figure III.3. *Guayanagarica aurantia* **A.** Basidiomata (holotype). Bar = 10 mm. **B.** Basidioma ventral view. **C.** Basidiospores and basidia. Bar = 10 μm

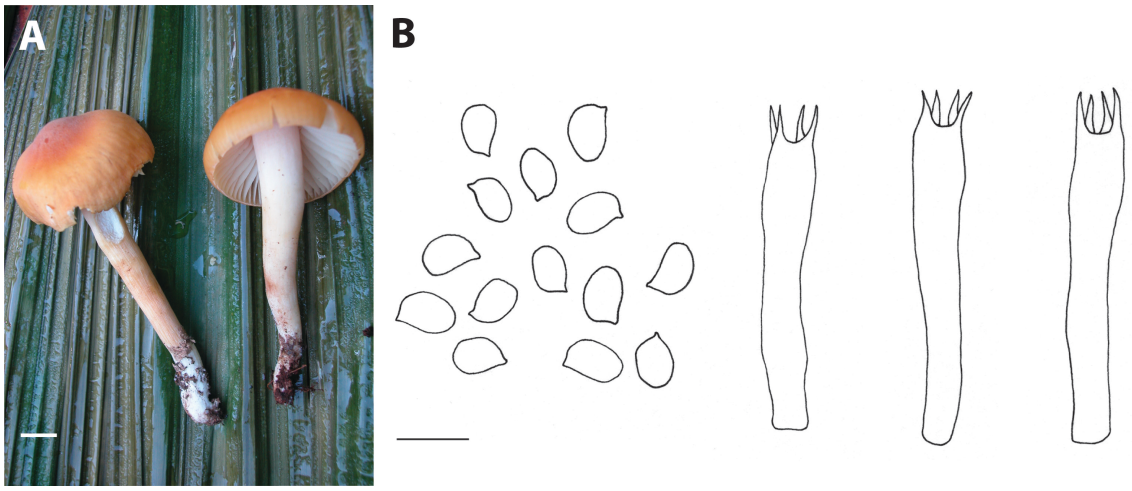

Figure III.4. *Guayanagarica pakaraimensis* **A.** Basidiomata (holotype). Bar = 10 mm. **B.** Basidiospores and basidia. Bar = 10 μm

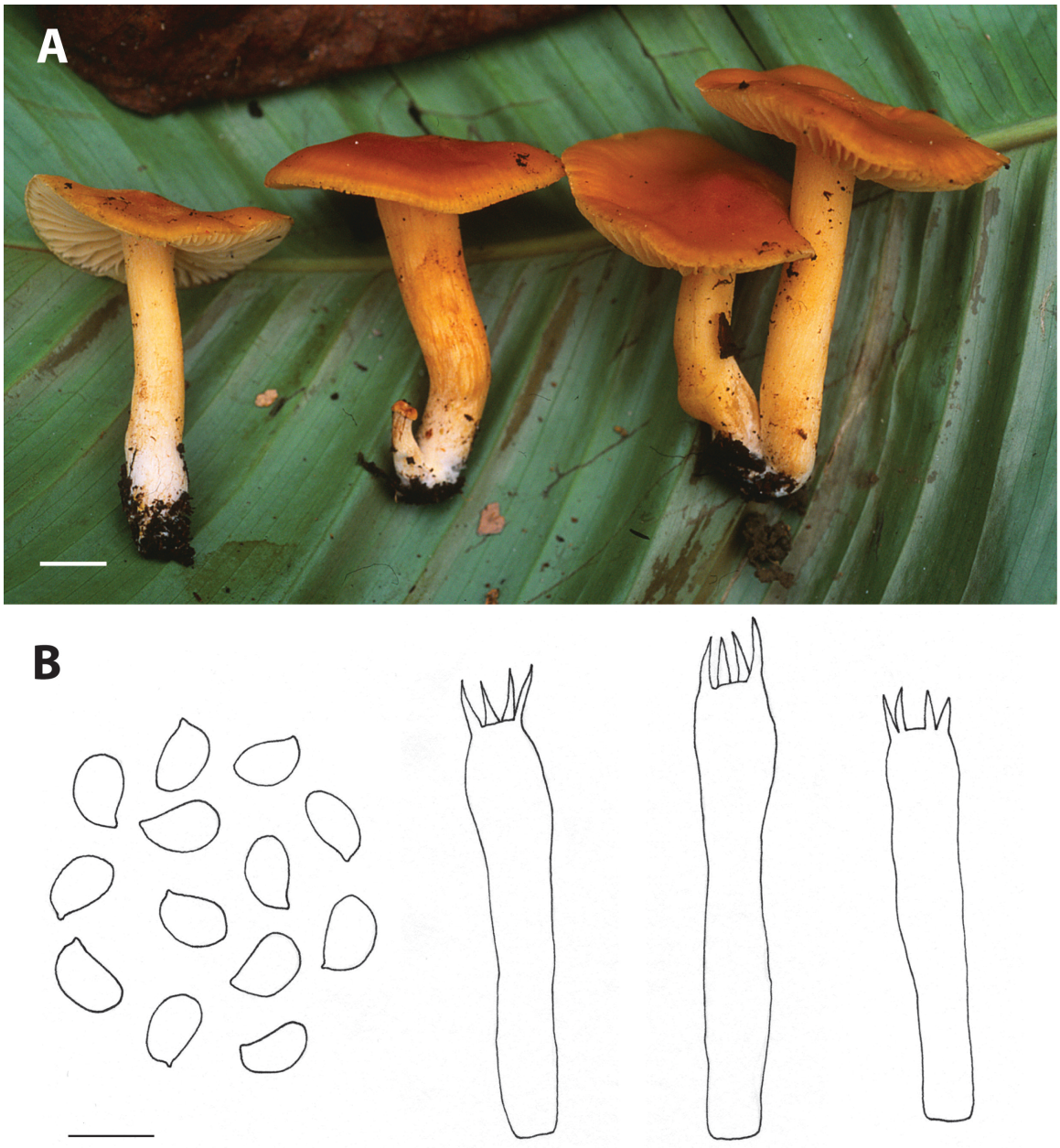

Figure III.5. *Guayanagarica anomala* **A.** Basidiomata (holotype). Bar = 10 mm. **B.** Basidiospores and basidia. Bar = 10 μm

CONCLUSIONS

This dissertation represents an important contribution to the systematics, diversity, and evolution of the Agaricomycetes, specifically the suborder Tricholomatineae. I present the emendation of the families Tricholomataceae and Catathelasmataceae, including the description of five new genera: *Albomagister, Corneriella, Guyanagarika, Pseudotricholoma,* and *Pogonoloma,* and three new species: *G. aurantia, G. pakaraimensis, and G. anomala*. I also test the hypothesis that the ECM lifestyle represents a key innovation by comparing the net diversification rates of ECM lineages with those of saprotrophic clades. While this hypothesis is not fully supported, we can address new questions: Is there a set of traits that when combined with the ability to form ECM associations promotes their diversification? What abiotic conditions mediate the evolutionary outcome of this symbiosis? The integration of fields such as functional ecology, genomics, systematics, and evolutionary biology will enable us to answer these questions, to better understand how evolutionary forces operate in ECM fungi, and to recognize the mechanisms that shape ECM fungal diversity.

VITA

Marisol Sanchez Garcia was borned in Mexico City. In 2008 she earned a Biology degree from the Universidad Nacional Autónoma de México (UNAM). In 2010 she completed a Masters of Science in Systematics at the same university. In 2011, Marisol moved to Knoxville, TN to pursue a PhD degree in the Department of Ecology and Evolutionary Biology at the University of Tennessee.