doi:10.1111/evo.13099

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

Marisol Sánchez-García[1,2,3] and Patrick Brandon Matheny[1]

[1]*Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, Tennessee 37996-1610*

[2]*Current Address: Biology Department, Clark University, Worcester, Massachusetts 01610*

[3]*E-mail: MSanchezGarcia@clarku.edu*

Received January 4, 2016
Accepted October 10, 2016

Although fungi are one of the most diverse groups of organisms, little is known about the processes that shape their high taxonomic diversity. This study focuses on 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. Ancestral state reconstruction (ASR) supports the ancestor of the Tricholomatineae as non-ECM. 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 also 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.

KEY WORDS: **Basidiomycota, ecological opportunity, macroevolution, speciation and extinction.**

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 geographical opportunities such as opening of new environments, extinction of competitors, or the evolution of key innovations that permit entry into new niche spaces (Simpson 1953; Heard and Hauser 1995; Schluter 2000; Yoder et al. 2010). Estimating diversification rates can help to the study of patterns of species richness and to understand the role of biotic and abiotic factors in shaping 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 of some ectomycorrhizal Boletales is higher than that of nongasteroid 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*, a genus of ascomycetous molds, showed that host jumps are associated with an increase in speciation rate, indicating that a transition from saprotrophy to

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

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 diversity through different evolutionary pathways. Recently, Gaya et al. (2015) studied an order of lichenized fungi, the 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. Nagy et al. (2012) studied the family Psathyrellaceae to identify morphological traits that could represent key innovations. Their results suggest that the loss of a veil (a protective tissue layer found on the fruiting bodies of some mushroom-forming fungi) and the gain of hairs, which represent a transition to a new defense mechanism, might be correlated with an explosive radiation within this group.

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 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 evolution of ectomycorrhizal (ECM) symbiosis has evolved numerous times across the fungal and land plant tree of life (Tedersoo and Smith 2013). This association increases the efficiency in the acquisition of nitrogen and phosphorous in plants, while providing carbon to the fungi (Smith and Read 2008), 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), and as a result increasing diversification rates.

In this study, we investigate diversity patterns of ectomycorrhizal (ECM) fungi within the suborder Tricholomatineae of the Agaricales, the largest order of mushroom-forming fungi. Approximately 30 families of plants are involved in ECM 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 7750 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). Furthermore, 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).

Many ECM fungal clades have high species richness. The diversity of plants and fungi involved in this association, the potential ability to colonize new niches, and the ecological impact that it has on terrestrial ecosystems coupled with 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 trait-independent diversification rates 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 Tricholomatoid 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 represents a key innovation, then we would expect to see an increase in diversification rate in clades with 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 S1). 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 nuclear ribosomal large subunit sequences (nLSU) sequences were available; then we searched for more loci available (5.8s, the largest and second largest subunit of RNA polymerase II (*rpb1* and *rpb2*), and the nuclear ribosomal small subunit (nSSU)) 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, polymerase chain reaction (PCR), and sequencing protocols for the internal transcribed spacers (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.

ESTIMATION OF DIVERGENCE TIMES

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 *Clavaria 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 2014). Sixty million generations were excluded as burn-in. One of the chains was excluded from the analysis because the mean log-likelihood value was considerably lower than the others, which could be due to the chain being trapped on a local optimum. 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). Such reviews considered several lines of evidence to assign a trophic status (in vitro experiments, in situ identification, and stable isotopes). 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). Samples were analyzed at the University of New Hampshire Stable Isotope Laboratory.

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.

ASR OF ECM LINEAGES

To test the hypothesis that the non-ECM mode is the ancestral state of the Tricholomatineae, and the ECM mode is a derived character, we performed maximum likelihood (ML) and Bayesian ASR analyses 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 5000 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 four models using a ML approach: (1) full model ($\lambda 0$, $\lambda 1$, $\mu 0$, $\mu 1$, $q01$, and $q10$), (2) irreversible model and equal extinction rates ($q10 = 0$, $\mu 0 = \mu 1$), (3) irreversible model and equal speciation rates ($q10 = 0$, $\lambda 0 = \lambda 1$); and 4) irreversible model with equal speciation and extinction rates ($q10 = 0$, $\lambda 0 = \lambda 1$, and $\mu 0 = \mu 1$). We compared the ML outputs to test whether each constrained model was significantly different from the full model using the ANOVA function in R. In addition, we compared Akaike information criterion (AIC) scores to identify the best model. We conducted MCMC estimations of the best model for 10 thousand generations. To account for phylogenetic uncertainty, we ran the full model in BiSSE under a ML approach on 100 randomly sampled trees from the posterior distribution of the BEAST analysis.

A recent study by Wright et al. (2015) has highlighted the sensitivity of ASRs to model parameters and assumptions. To test

the robustness of the sampling correction function in our dataset, we ran a BiSSE analysis assuming a fully sample phylogeny.

TRAIT-INDEPENDENT DIVERSIFICATION RATE SHIFTS

Bayesian analysis of macroevolutionary mixtures (BAMM version 2.5; Rabosky 2014) was used to estimate diversification rates independently of character states. This method detects and estimates heterogeneity in evolutionary rates across a phylogeny; in this case, we used the MCC tree obtained from the BEAST analysis. We ran 10 million generations of reversible jump MCMC sampling. We used the package BAMMtools 2.1.4 (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 (Table S2) 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). We obtained a 95% credible set of diversification rate shifts with a threshold of 5. We estimated marginal odds ratios for each branch in our phylogeny to evaluate evidence for a rate shift on specific branches relative to the evidence of a null model of no shifts (Shi and Rabosky 2015). 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. 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 117.11 Mya (with a 95% node height posterior density [HPD] of 74.41–165.61 Mya), hereafter written as 117.11 (74.41–165.61) 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 78.04 (48.13–111.74) Mya, the Entolomataceae 86 (55–122) Mya, and the Lyophyllaceae 79.29 (48.25–114.11) Mya. Another major lineage is the *Catathelasma* clade with an estimated stem age at 97.22 (62.9–138.57) Mya, and crown age at 70.77 (40.57–105.93) Mya.

Results obtained from BayesTraits (ML and BI) support the ancestral state of nutritional mode in the Tricholomatineae as non-ECM with a probability value of 0.99. 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. 1). *Albomagister* diverged from its sister clade that includes *Corneriella*, *Dennisiomyces*, and *Porpoloma* 63.40 (37.49–90.30) Mya. *Porpoloma*, an ECM group restricted to the Southern Hemisphere, diverged from *Corneriella* and *Dennisiomyces* 39.97 (21.98–58.51) Mya. *Tricholoma*, the largest genus within the family Tricholomataceae, diverged from its saprotrophic sister clades *Dermoloma* and *Pseudotricholoma* 62.19 (36.26–92.0) Mya. *Catathelasma*, an ECM genus restricted to coniferous forests of the Northern Hemisphere diverged from *Cleistocybe* 50.93 (27.27–76.57) 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 26.74 (12.15–42.85) 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 68.48 (42.30–96.54) Mya; its crown age was estimated at 42.28 (16.10–73.82) Mya.

EVOLUTION OF NUTRITIONAL MODE AND EFFECT ON DIVERSIFICATION RATES

The "full model" from BiSSE that allows all six parameters to be estimated shows a transition rate from ECM to non-ECM of zero probability (Table 1), 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 ASR of nutritional mode, the other models tested in the BiSSE framework (Table 1) 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 2). 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. 2). However, results from the ANOVA test show that this model is not significantly different from the "full model" and the "irreversible model with equal speciation rates" (Table 2). Both models also support higher net diversification rates in ECM clades compared to non-ECM clades. Overall, these three models support state dependent diversification, in which the ECM mode is correlated with higher net diversification rates. As stated earlier,

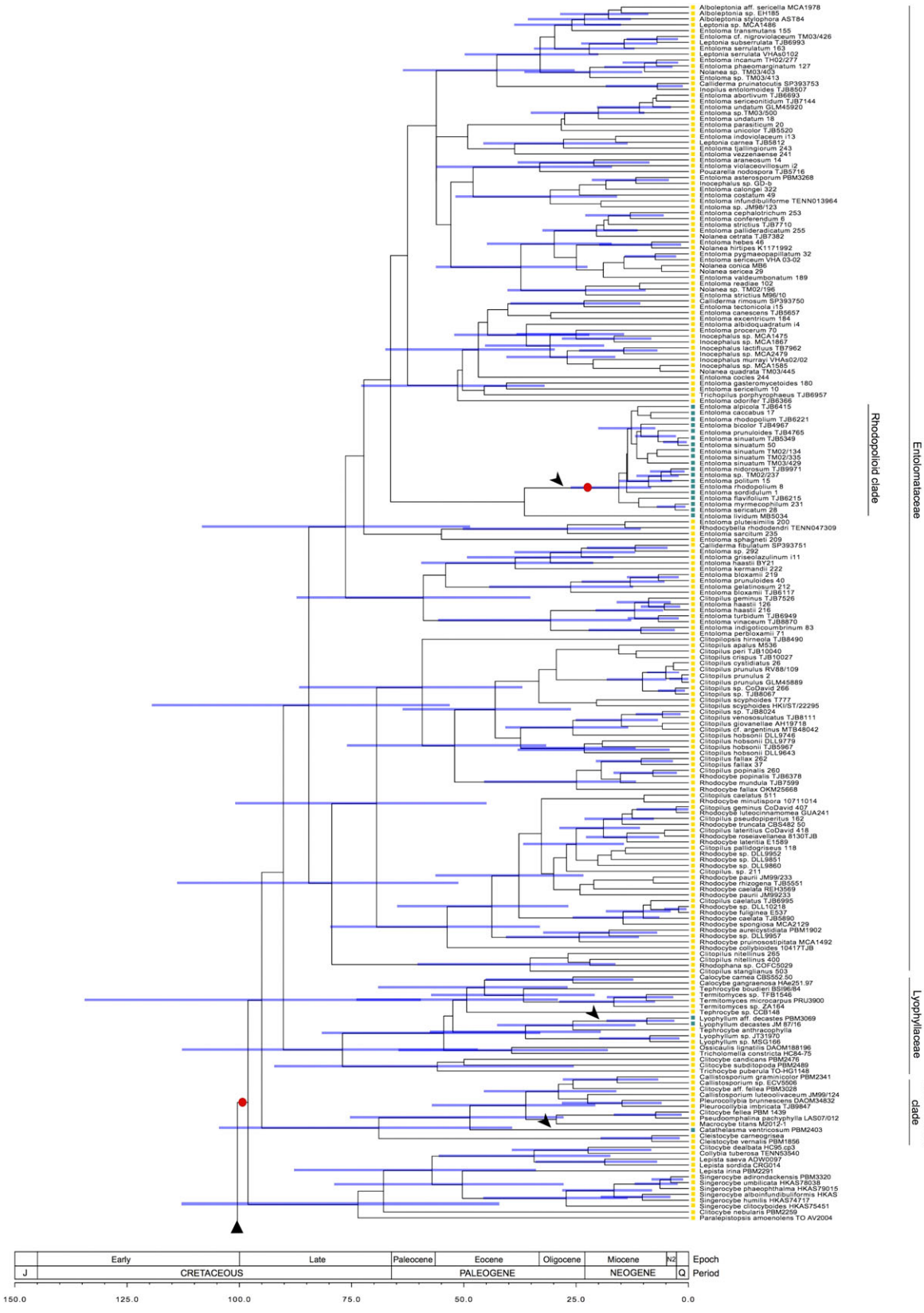

Figure 1. Maximum clade credibility tree of the suborder Tricholomatineae from the dating analysis in BEAST using a five-loci dataset (nLSU, nSSU, 5.8S, *rpb1* and *rpb2*); 95% confidence intervals for nodes with a posterior probability ≥ 0.75 are shown. Blue squares indicate ECM taxa and yellow squares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

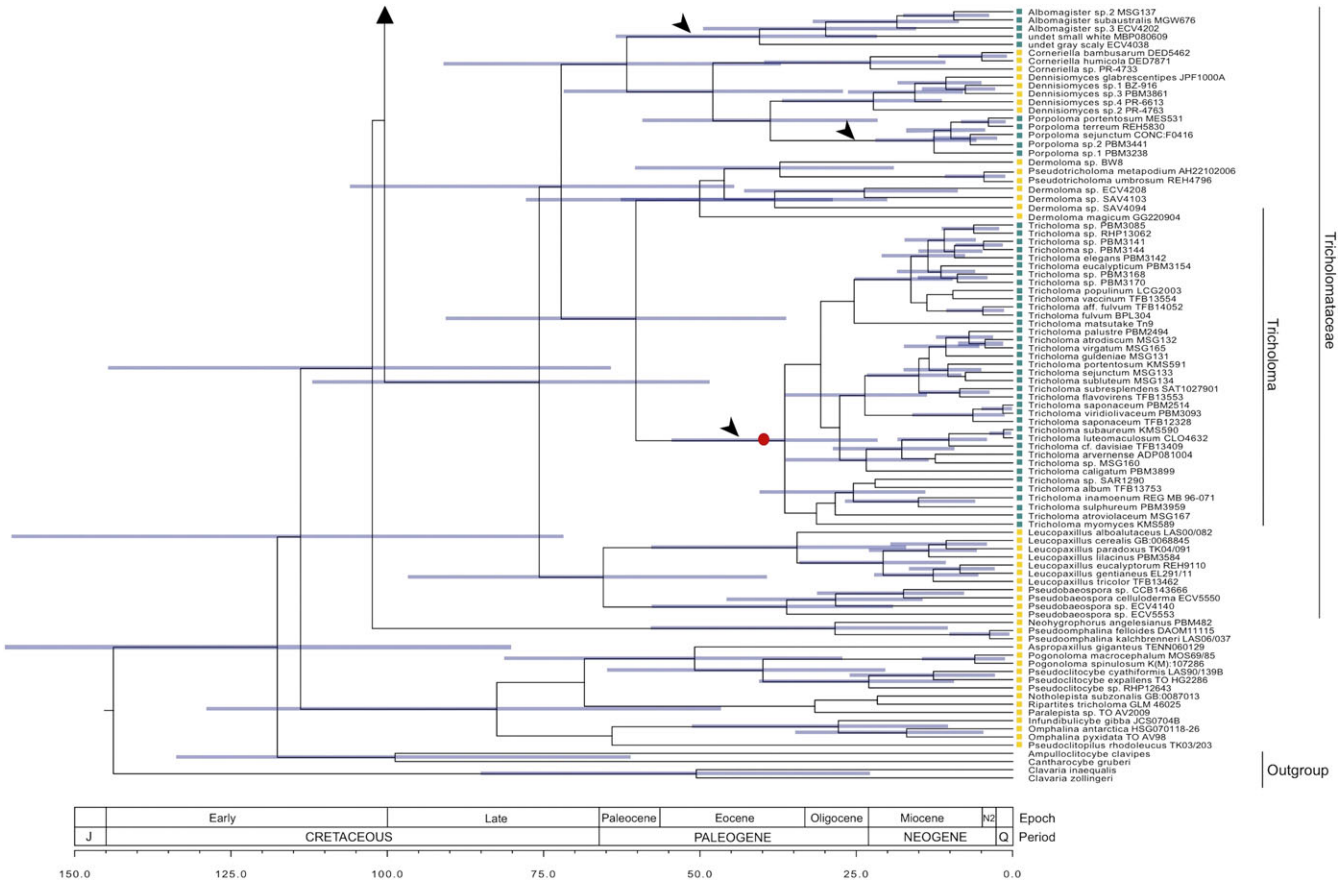

Figure 1. Continued.

Table 1. Parameter estimates from the BiSSE analyses (q = transition rate; λ = speciation rate; μ = extinction rate; r = net diversification rate [$\lambda - \mu$]; 0 = non-ECM; 1 = ECM).

Models	$\lambda 0$	$\lambda 1$	$\mu 0$	$\mu 1$	$r0$	$r1$	$q01$	$q10$
Full	0.146	0.276	0.090	0.166	0.056	0.110	0.0007	0.000
Irreversible, equal extinction rates ($q10 = 0$, $\mu 0 = \mu 1$)	0.153	0.223	0.099	0.099	0.054	0.123	0.0005	–
Irreversible, equal speciation rates ($q10 = 0$, $\lambda 0 = \lambda 1$)	0.168	0.168	0.116	0.033	0.052	0.135	0.0003	–
Irreversible, equal speciation, and extinction rates ($q10 = 0$, $\lambda 0 = \lambda 1$, $\mu 0 = \mu 1$)	0.192	0.192	0.136	0.136	0.056	0.056	0.0009	–
Full (complete phylogeny)	0.033	0.059	0.000	0.000	0.033	0.059	0.0009	0.000

All models accounted for incomplete sampling except when noted.

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.

The analyses that were run to account for phylogenetic uncertainty showed a consistent result in which the ECM clades show higher diversification rates than the non-ECM clades. The full model assuming a fully sampled phylogeny indicates that the net diversification rate of ECM clades is 1.78 times higher than that of non-ECM clades (Table 1), this result is consistent with the results obtained when accounting for incomplete sampling, which suggests that the sampling correction function is not biasing the BiSSE results.

TRAIT INDEPENDENT DIVERSIFICATION RATE SHIFTS

In the BAMM analysis, the most frequently sampled shift configuration ($f = 0.29$) shows three increases in diversification rate across the suborder Tricholomatineae (Figs. 3 and S1). One

Table 2. BiSSE model selection.

Models	Degrees of freedom (df)	Ln-likelihood	AIC	χ^2	P (>|Chi|)
Full	6	−1266.2	2544.4	NA	NA
Irreversible, equal extinction rates ($q10 = 0$, $\mu 0 = \mu 1$)	4	−1266.6	2541.1	0.6867	0.7094
Irreversible, equal speciation rates ($q10 = 0$, $\lambda 0 = \lambda 1$)	4	−1268.3	2544.7	4.2512	0.1194
Irreversible, equal speciation, and extinction rates ($q10 = 0$, $\lambda 0 = \lambda 1$, $\mu 0 = \mu 1$)	3	−1280.4	2566.8	28.3882	$3.011 \times 10^{-6***}$

$^{***}P < 0.001$.

AIC = Akaike information criterion.

All models were compared against the full model (q = transition rate; λ = speciation rate; μ = extinction rate; 0 = non-ECM; 1 = ECM).

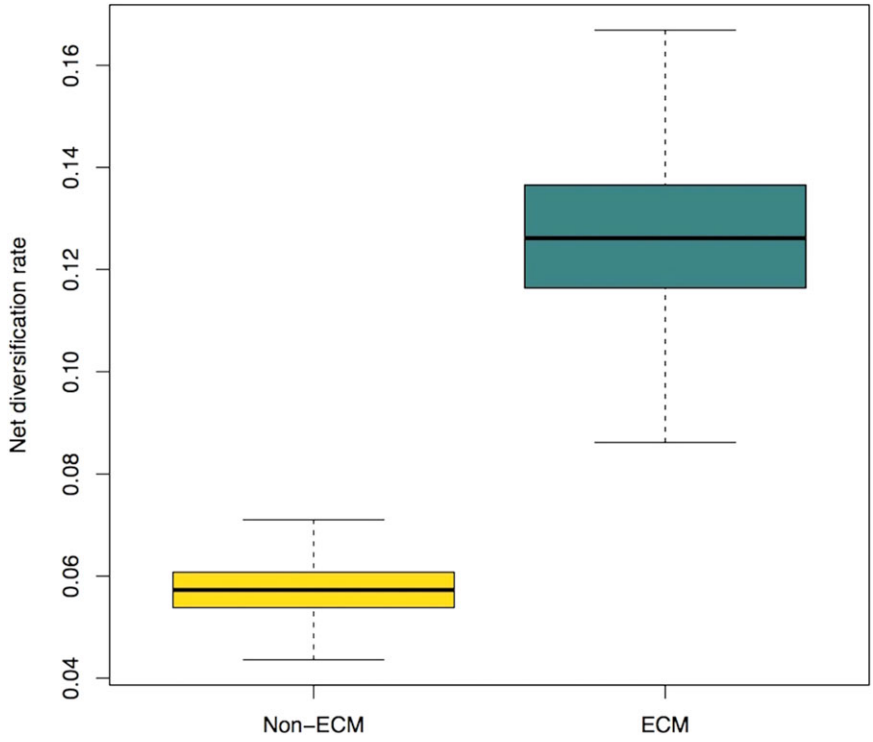

Figure 2. Box plot of MCMC estimates of state-dependent net diversification rate ($\lambda - \mu$) of non-ECM and ECM clades in the suborder Tricholomatineae obtained from the best BiSSE model.

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*. Each shift is strongly supported by marginal odds ratios. The second most frequently sampled configuration ($f = 0.19$) also supports three shifts, with the only difference that the shift in *Tricholoma* corresponds to a subclade within this genus (Fig. S1). 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; 92% of the replicates recovered an increase in *Tricholoma*, whereas 98% recovered an increase in the Rhodopolioid clade.

Discussion

ECM MODE IS NOT FULLY SUPPORTED AS A KEY INNOVATION

Results from the BiSSE analyses support the hypothesis that the ECM mode is a key innovation, in contrast to the BAMM analyses, which do not support this hypothesis. However, recent criticisms

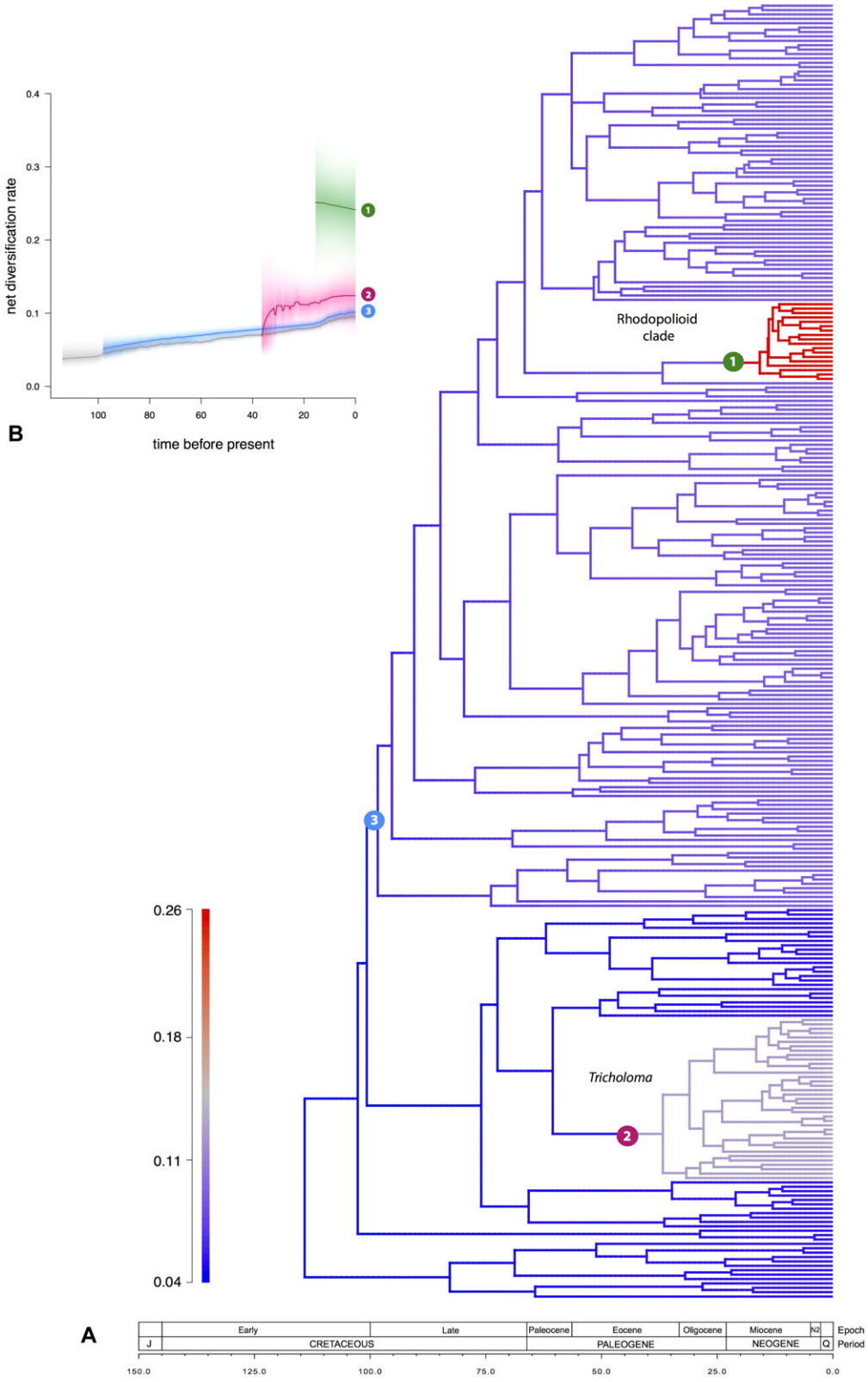

Figure 3. (A) Phylorate plot for the best shift configuration of the Tricholomatineae ($f = 0.29$). Colors indicate net diversification rates on each branch of the maximum clade credibility tree. Circles indicate the locations of shifts to increased diversification (see Fig. S1 for the 95% credible set 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.

of the BiSSE approach have been presented. 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 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, features that meet the recommendations of Davis et al. (2013). 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. 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 to an erroneous conclusion 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 as many as nine different 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*), whereas the rest have an intermediate to wide host range (e.g., *T. focale*, *T. magnivelare*, *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 adapting to new environmental niches, increasing in this way their rate of diversification.

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*. However, because 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 Rhodopolioid clade is part of the highly diverse genus *Entoloma*, which contains approximately 1200 species (Noordeloos 1992; Morgado et al. 2013). 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 Rhodopolioid clade and associate with *Quercus*, *Salix*, *Alnus*, and *Populus* (Loree et al. 1989; Noordeloos 1992; Smith et al. 2007). This group originated during the late Eocene and began to diversify by the early Miocene, a period 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 favor the expansion of ECM hosts such as *Salix* and *Populus*. Additionally, some species in this clade are common in grasslands (Noordeloos 1992). These factors could have allowed members of the Rhodopolioid clade to expand their range and encounter new ecological opportunities for diversification.

Another factor that could have promoted an increase in diversification rates in the Rhodopolioid clade was the evolution of angular spores (angular in face and/or polar views), in combination with the evolution of the ECM mode. The entire genus *Entoloma* possesses angular spores, but in the case of the Rhodopolioid clade, it could be a preadaptation or exaptation that, when combined with the ECM mode, may have provided additional advantage to colonize new niches. ECM spores need to be dispersed near their plant host roots 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). Although 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). The surface irregularity of the spores creates a high contact angle, reduces contact surface, and increases hydrophobicity that favor their transport by arthropods or water (Davies 1961; Lilleskov and Bruns 2005; Spori et al. 2008).

SPECIES-POOR ECM LINEAGES WITHIN THE TRICHOLOMATINEAE

The remaining ECM lineages in the Tricholomatineae showed no elevated rates of diversification and, unlike *Tricholoma*, associated with a narrower range of hosts. *Porpoloma* is restricted to the Southern Hemisphere, forming ECM associations with *Nothofagus* (Trappe 1962; Garrido 1988), and probably with *Eucalyptus* and/or *Acacia*. *Catathelasma* 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 accelerated diversification. In addition, *Catathelasma* has been suggested to be a late colonizer in coniferous forests, which could be due to a slow growth rate or poor spore germination (Hutchison 1992; Bowen 1994).

Lyophyllum has an intermediate to wide host 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 *L. decastes* has a poor development of ECM structures (Yamada et al. 2001), and *L. shimeji* forms a thin mantle of hyphae on root tips (Visnovsky et al. 2014), which could indicate a weak ECM association that limits its ability to colonize new niches. *Lyophyllum* is the youngest of the ECM clades in the Tricholomatineae, 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 Rhodopolioid clade.

Albomagister occurs in plant communities dominated by *Quercus* and *Tsuga* (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 S3). However, the identity of its hosts and whether it can associate with more than one host remain unknown.

FACTORS PROMOTING DIVERSIFICATION OF ECM FUNGI

The effect of a trait on diversification rate may be dependent on factors such as interactions with other taxa, additional traits from the same organisms, and 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 may be favored by extrinsic 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). 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- 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 Rhodopolioid clade form medium-distance exploration types with uniformly shaped rhizomorphs, but *Tricholoma* can also develop differentiated rhizomorphs. The ECM of the Rhodopolioid clade is 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 (Garrido 1988; Tedersoo et al. 2010). On the contrary, there is inconsistent evidence regarding the mycorrhizal status of *Leucopaxillus*, which we scored as non-ECM in the present work. Synthesis experiments suggest it is saprotrophic (Hart et al. 2006), whereas isotopic evidence indicates it is ECM (Lu et al. 1998; Agerer 2006; Rinaldi et al. 2008). 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 been shown to be phylogenetically distant from *Leucopaxillus* sensu stricto (Sánchez-García et al. 2014). Recent analysis of stable isotopes revealed that two species, *L. laterarius* and *L. 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. 4). 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 within *Lyophyllum* (paralyophyllum) as

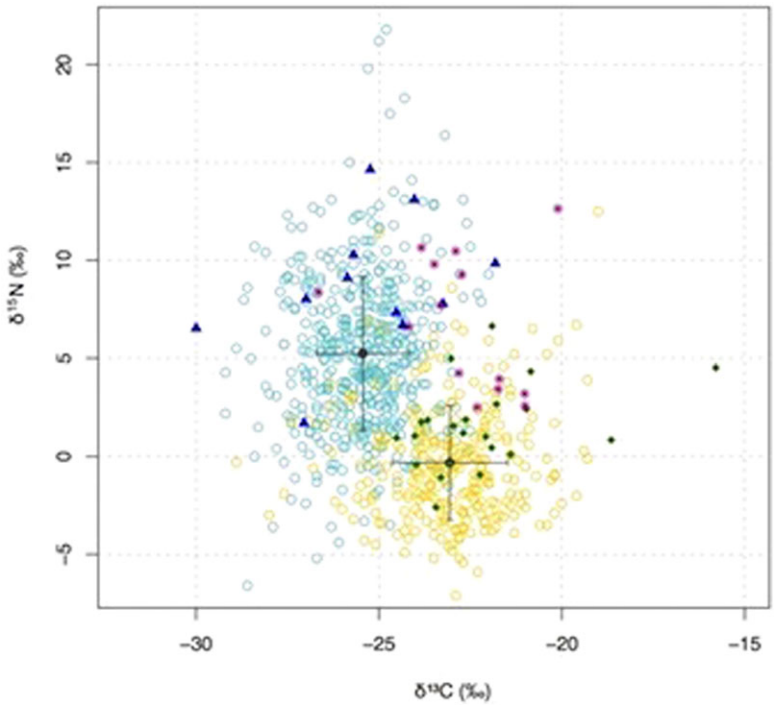

Figure 4. **Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of 47 taxa of the Tricholomatineae (blue triangles, green diamonds, and pink asterisks) compared to a global dataset from Mayor et al. (2009) with known ECM (blue circles) and saprotrophic (yellow circles) isotopic signatures. 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 S3 for the $\delta^{13}C$ and $\delta^{15}N$ values and taxon names.**

ECM based on the evidence that *L. decastes*, *L. shimeji* and *L. 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* and *Notholithocarpus* (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. 4; Table S3). In the same review, the genus *Cathatelasma* was also considered as ECM because it can form ECM structures when cultured with *Pinus 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; Geml et al. 2012), and stable isotope information (Kohzu et al. 1999; Trudell et al. 2004). This group is also 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 consider *Albomagister* as ECM based on stable isotope evidence (Birkebak et al. 2013 and Table S3), and our observations that species in this genus fruit on soil and at similar times of multiple years. 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). Some non-ECM taxa may also fruit on soil. However, all the samples that were analyzed showed a consistent ECM pattern after normalization of stable isotope data.

POTENTIAL BIASES IN THIS STUDY

Incomplete and nonrandom 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 the number of missing taxa to specific clades. This can be challenging working with fungi because 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. 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.

ACKNOWLEDGMENTS

This work was supported by graduate research awards from the Botanical Society of America, the Department of Ecology and Evolutionary Biology at the University of Tennessee, the North American Mycological Association Memorial Fellowship to MSG, and NSF award DEB-1354802 to Matthew E. Smith and PBM. We thank Erik Hobbie for helpful information about the normalization of stable isotopes. The authors declare no conflict of interest. The authors appreciate constructive criticism from prior reviews by two anonymous reviewers and the associate editor, Dr. Gavin Thomas.

DATA ARCHIVING

The Dryad doi for our data is 10.5061/dryad.53975.

LITERATURE CITED

Agerer, R. 1997. *Entoloma sinuatum* (Bull.: Fr.) Kummer + *Salix* spec. Descr. Ectomyc. 2:13–18.

———. 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114.

———. 2006. Fungal relationships and structural identity of their ectomycorrhizae. Mycol. Prog. 5:67–107.

Agerer, R., and L. Beenken. 1998. *Lyophyllum decastes* (Fr.) Sing. + *Quercus robur* L. Descr. Ectomyc. 3:43–47.

Agerer, R., and K. Waller. 1993. Mycorrhizae of *Entoloma saepium*: parasitism or symbiosis? Mycorrhiza 3:145–154.

Agerer, R., M. Schloter, and C. Hahn. 2000. Fungal enzymatic activity in fruitbodies. Nova Hedwigia 71:315–336.

Baroni, T. J., and P. B. Matheny. 2011. A re-evaluation of gasteroid and cyphelloid species of Entolomataceae from eastern North America. Harvard Pap. Bot. 16:293–310.

Bergemann, S. E., and M. Garbelotto. 2006. High diversity of fungi recovered from the roots of mature tanoak (*Lithocarpus densiflorus*) in northern California. Can. J. Bot. 84:1380–1394.

Birkebak, J., J. R. Mayor, M. Ryberg, and P. B. Matheny. 2013. A systematic, morphological, and ecological overview of the Clavariaceae (Agaricales). Mycologia 105:896–911.

Blackwell, M. 2011. The fungi: 1, 2, 3 ... 5.1 million species? Am. J. Bot. 98:426–438.

Bloom, D. D., M. Fikáček, and A. E. Z. Short. 2014. Clade age and diversification rate variation explain disparity in species richness among water scavenger beetle (Hydrophilidae) lineages. PLoS One 9:e98430.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10:e1003537.

Bowen, G. D. 1994. The ecology of ectomycorrhiza formation and functioning. Plant Soil 159:61–67.

Brock, C. D., L. J. Harmon, and M. E. Alfaro. 2011. Testing for temporal variation in diversification rates when sampling is incomplete and nonrandom. Syst. Biol. 60:410–419.

Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77.

Bruns, T. D., and R. P. Shefferson. 2004. Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. Can. J. Bot. 1132:1122–1132.

Bruns, T. D., T. M. Szaro, M. Gardes, K. W. Cullings, J. J. Pan, D. L. Taylor, T. R. Horton, A. Kretzer, M. Garbelotto, and Y. Li. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol. Ecol. 7:257–272.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17:540–552.

Chaverri, P., and G. J. Samuels. 2013. Evolution of habitat preference and nutrition mode in a cosmopolitan fungal genus with evidence of interkingdom host jumps and major shifts in ecology. Evolution 67:2823–2837.

Clémençon, H. 2004. Cytology and plectology of the Hymenomycetes. J. Cramer, Stuttgart and Berlin.

Co-David, D., D. Langeveld, and M. E. Noordeloos. 2009. Molecular phylogeny and spore evolution of Entolomataceae. Persoonia 23:147–76.

Davies, R. R. 1961. Wettability and the capture, carriage and deposition of particles by raindrops. Nature 191:616–617.

Davis, M. P., P. E. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol. Biol. 13:38.

de Queiroz, A. 2002. Contingent predictability in evolution: key traits and diversification. Syst. Biol. 51:917–929.

Eberhardt, U. 2002. Molecular kinship analyses of the agaricoid Russulaceae: correspondence with mycorrhizal anatomy and sporocarp features in the genus *Russula*. Mycol. Prog. 1:201–223.

FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

FitzJohn, R. G., W. P. Maddison, and S. P. Otto. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Frøslev, T. G., P. B. Matheny, and D. S. Hibbett. 2005. Lower level relationships in the mushroom genus *Cortinarius* (Basidiomycota, Agaricales): a comparison of RPB1, RPB2, and ITS phylogenies. Mol. Phylogenet. Evol. 37:602–618.

Garrido, N. 1988. Agaricales s.l. und ihre Mykorrhizen in den *Nothofagus*-Wäldern Mittelchiles. Bibl. Mycol. 120:1–528.

Gaya, E., S. Fernández-Brime, R. Vargas, R. F. Lachlan, C. Gueidan, M. Ramírez-Mejía, and F. Lutzoni. 2015. The adaptive radiation of lichen-forming Teloschistaceae is associated with sunscreening pigments and a bark-to-rock substrate shift. Proc. Natl. Acad. Sci. 112:11600–11605.

Geml, J., I. Timling, C. H. Robinson, N. Lennon, H. C. Nusbaum, C. Brochmann, M. E. Noordeloos, and D. L. Taylor. 2012. An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. J. Biogeogr. 39:74–88.

Gould, S. J., N. L. Gilinsky, and R. Z. German. 1987. Asymmetry of lineages and the direction of evolutionary time. Science 236:1437–1441.

Gouy, M., S. Guindon, and O. Gascuel. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27:221–224.

Griffith, G. W. 2004. The use of stable isotopes in fungal ecology. Mycologist 18:177–183.

Hart, S. C., C. A. Gehring, P. C. Selmants, and R. J. Deckert. 2006. Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forests of the south-western USA. Funct. Ecol. 20:42–51.

Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105:1422–1432.

Heard, S. B., and D. L. Hauser. 1995. Key evolutionary innovations and their ecological mechanisms. Hist. Biol. 10:151–173.

Heath, T. A., D. J. Zwickl, J. Kim, and D. M. Hillis. 2008. Taxon sampling affects inferences of macroevolutionary processes from phylogenetic trees. Syst. Biol. 57:160–166.

Henn, M. R., and I. H. Chapela. 2000. Differential C isotope discrimination by fungi during decomposition of C_3- and C_4-derived sucrose. Appl. Environ. Microbiol. 66:4180–4186.

Hobbie, E. A., S. A. Macko, and H. H. Shugart. 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118:353–360.

Hofstetter, V., S. A. Redhead, F. Kauff, J.-M. Moncalvo, P. B. Matheny, and R. Vilgalys. 2014. Taxonomic revision and examination of ecological transitions of the Lyophyllaceae (Basidiomycota, Agaricales) based on a multigene phylogeny. Cryptogam. Mycol. 35:399–425.

Hunter, J. P. 1998. Key innovations and the ecology of macroevolution. Trends Ecol. Evol. 13:31–36.

Hutchison, L. J. 1992. Host range, geographical distribution and probable ecological status of *Catathelasma imperiale* in North America. Mycologia 84:472–475.

Jacobs, B. F., J. D. Kingston, and L. L. Jacobs. 1999. The origin of grass-dominated ecosystems. Ann. MO Bot. Gard. 86:590–643.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772–780.

Kawai, M. 1997. Artificial ectomycorrhiza formation on roots of air-layered *Pinus densiflora* saplings by inoculation with *Lyophyllum shimeji*. Mycologia 89:228–232.

Kinoshita, A., H. Sasaki, and K. Nara. 2012. Multiple origins of sequestrate basidiomes within *Entoloma* inferred from molecular phylogenetic analyses. Fungal Biol. 116:1250–1262.

Kirk, P., P. F. Cannon, D. W. Minter, and J. A. Stalpers. 2008. The dictionary of fungi. CAB International, Wallingford, U.K.

Kluting, K. L., T. J. Baroni, and S. E. Bergemann. 2014. Toward a stable classification of genera within the Entolomataceae: A phylogenetic re-evaluation of the Rhodocybe-Clitopilus clade. Mycologia 106:1127–1142.

Kobayashi, H., and K. Hatano. 2001. A morphological study of the mycorrhiza of *Entoloma clypeatum* f. *hybridum* on *Rosa multiflora*. Mycoscience 42:83–90.

Kohzu, A., T. Yoshioka, T. Ando, M. Takahashi, K. Koba, and E. Wada. 1999. Natural ^{13}C and ^{15}N abundance of field-collected fungi and their ecological implications. New Phytol. 144:323–330.

Kraichak, E., P. K. Divakar, A. Crespo, S. D. Leavitt, M. P. Nelsen, R. Lücking, and H. T. Lumbsch. 2015. A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. Sci. Rep. 5:10028

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695–1701.

Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 22:3276–3278.

Larsson, E., and H. Sundberg. 2011. *Lyophyllum shimeji*, a species associated with lichen pine forest in northern Fennoscandia. Mycoscience 52:289–295.

Lear, C. H., T. R. Bailey, P. N. Pearson, H. K. Coxall, and Y. Rosenthal. 2008. Cooling and ice growth across the Eocene–Oligocene transition. Geology 36:251–254.

Leigh, E. G. 2010. The evolution of mutualism. J. Evol. Biol. 23:2507–2528.

Lilleskov, E. A, and T. D. Bruns. 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97:762–769.

Lilleskov, E. A., E. A. Hobbie, and T. R. Horton. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecol. 4:174–183.

Loree, M. A. J., I. Lumme, M. Niemi, and T. Tormala. 1989. Inoculation of willows (*Salix* spp.) with ectomycorrhizal fungi on mined boreal peatland. Plant Soil 116:229–238.

Lu, X., N. Malajczuk, and B. Dell. 1998. Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhiza 8:81–86.

Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. Syst. Biol. 56:701–710.

Malloch, D. W. 1980. Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants. Proc. Natl. Acad. Sci. 77:2113–2118.

Margulis, L., and R. Fester. 1991. Symbiosis as a source of evolutionary innovation: speciation and morphogenesis. MIT Press, Cambridge, MA.

Matheny, P. B., Y. J. Liu, J. F. Ammirati, and B. Hall. 2002. Using rpb1 sequences to improve phylogenetic inference among mushrooms. Am. J. Bot. 89:688–698.

Matheny, P. B., J. M. Curtis, V. Hofstetter, M. C. Aime, J.-M. Moncalvo, Z.-W. Ge, J. C. Slot, J. F. Ammirati, T. J. Baroni, N. L. Bougher, et al. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995.

Mayor, J., M. Bahram, T. Henkel, F. Buegger, K. Pritsch, and L. Tedersoo. 2015. Ectomycorrhizal impacts on plant nitrogen nutrition: emerging isotopic patterns, latitudinal variation and hidden mechanisms. Ecol. Lett. 18:96–107.

Mayor, J. R., E. A. G. Schuur, and T. W. Henkel. 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol. Lett. 12:171–183.

McPeek, M. A, and J. M. Brown. 2007. Clade age and not diversification rate explains species richness among animal taxa. Am. Nat. 169:E97–E106.

Molina, R., H. B. Massicotte, and J. M. Trappe. 1998. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. Pp. 357–423 *in* M. F. Allen, ed. Mycorrhizal functioning. Chapman & Hall, London.

Montecchio, L., S. Roca, P.-E. Courty, and J. Garbaye. 2006. *Entoloma nitidum* Quél. + *Carpinus betulus* L. Descr. Ectomycorrhizae 9/10:33–38.

Mooers, A. Ø., and S. B. Heard. 1997. Inferring evolutionary process from phylogenetic tree shape. Q. Rev. Biol. 72:31–54.

Moreau, P.-A., J.-M. Bellanger, S. Biancardini, and F. Richard. 2015. *Albomagister alesandrii* sp. nov., un nouvel élément du patrimoine naturel de la corse. Bull. Semest. la Fédération des Assoc. Mycol. Méditerranéennes 48:7–14.

Morgado, L. N., M. E. Noordeloos, Y. Lamoureux, and J. Geml. 2013. Multigene phylogenetic analyses reveal species limits, phylogeographic patterns, and evolutionary histories of key morphological traits in *Entoloma* (Agaricales, Basidiomycota). Persoonia 31:159–178.

Mullen, S. P., W. K. Savage, N. Wahlberg, and K. R. Willmott. 2011. Rapid diversification and not clade age explains high diversity in neotropical *Adelpha* butterflies. Proc. Biol. Sci. 278:1777–1785.

Nagy, L. G., J. Házi, B. Szappanos, S. Kocsubé, B. Bálint, G. Rákhely, C. Vágvögyi, and T. Papp. 2012. The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (Agaricales, Fungi). Syst. Biol. 61:595–607.

Noordeloos, M. E. 1992. Fungi Europeai: *Entoloma* s.l. Giovanna Biella, Saronno, Italy.

O'Brien, H. E., J. L. Parrent, J. A. Jackson, and J.-M. Moncalvo. 2005. Fungal community analysis by large-scale sequencing of environmental samples fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. 71:5544–5550.

Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53:673–684.

Pegler, D. N., and T. W. K. Young. 1978. Entolomataceae Kotl. & Pouz. World pollen and spore flora. Pp. 1–24, Vol. 7. Almqvist & Wiksell, Stockholm.

Pera, J., and I. F. Alvarez. 1995. Ectomycorrhizal fungi of *Pinus pinaster*. Mycorrhiza 5:193–200.

Pirozynski, A. K., and D. W. Malloch. 1975. The origin of land plants: a matter of mycotrophism. Biosystems 6:153–164.

Prothero, D. R., and W. A. Berggren. 1992. Eocene–Oligocene climatic and biotic evolution. Princeton University Press, New Jersey.

Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS One 9:e89543.

Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst. Biol. 64:340–355.

Rabosky, D. L., S. C. Donnellan, A. L. Talaba, and I. J. Lovette. 2007. Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. Proc. Biol. Sci. 274:2915–2923.

Rabosky, D. L., M. Grundler, C. Anderson, P. Title, J. J. Shi, J. W. Brown, H. Huang, and J. G. Larson. 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol. Evol. 5:701–707.

Rambaut, A., and A. J. Drummond. 2014. Tracer version 1.6. Available at http://beast.bio.ed.ac.uk/Tracer (accessed March 2015).

Rinaldi, A. C., O. Comandini, and T. W. Kuyper. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45.

Ryberg, M., and P. B. Matheny. 2011. Dealing with incomplete taxon sampling and diversification of a large clade of mushroom-forming fungi. Evolution 65:1862–1878.

———. 2012. Asynchronous origins of ectomycorrhizal clades of Agaricales. Proc. R. Soc. B 279:2003–2011.

Sánchez-García, M., P. B. Matheny, G. Palfner, and D. J. Lodge. 2014. Deconstructing the Tricholomataceae (Agaricales) and introduction of the new genera *Albomagister*, *Corneriella, Pogonoloma* and *Pseudotricholoma*. Taxon 63:993–1007.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, New York.

Shi, J. J., and D. L. Rabosky. 2015. Speciation dynamics during the global radiation of extant bats. Evolution 69:1528–1545.

Simpson, G. G. 1953. The major features of evolution. Columbia Univ. Press, New York.

Singer, R. 1986. The Agaricales in modern taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein.

Smith, M. E., G. W. Douhan, and D. M. Rizzo. 2007. Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol. 174:847–63.

Smith, S. E., and D. Read. 2008. Mycorrhizal symbiosis. 3rd. ed. Academic Press, London.

Spori, D. M., T. Drobek, S. Zürcher, M. Ochsner, C. Sprecher, A. Mühlebach, and N. D. Spencer. 2008. Beyond the lotus effect: roughness influences on wetting over a wide surface-energy range. Langmuir 24:5411–5417.

Stiller, J. W., and B. D. Hall. 1997. The origin of red algae: implications for plastid evolution. Proc. Natl. Acad. Sci. USA 94:4520–4525.

Strömberg, C. A. E. 2005. Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. Proc. Natl. Acad. Sci. USA 102:11980–11984.

Tans, P. P., F. M. De Jong, and W. G. Mook. 1979. Natural atmospheric ^{14}C variation and the Suess effect. Nature 280: 826–828.

Taylor, A. F. S., and I. Alexander. 2005. The ectomycorrhizal symbiosis: life in the real world. Mycologist 19:102–112.

Taylor, A. F. S., P. M. Fransson, P. Högberg, M. N. Högberg, and A. H. Plamboeck. 2003. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. New Phytol. 159:757–774.

Tedersoo, L., and M. E. Smith. 2013. Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol. Rev. 27:83–99.

Tedersoo, L., T. W. May, and M. E. Smith. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263.

Trappe, J. M. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28:538–606.

Trudell, S., P. T. Rygiewicz, and R. L. Edmonds. 2004. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth plants and soils conifer forests. New Phytol. 164:317–335.

Visnovsky, S. B., N. Cummings, A. Guerin-Laguette, Y. Wang, A. Yamada, H. Kobayashi, M. Kawai, and A. R. Pitman. 2014. Detection of the edible ectomycorrhizal fungus *Lyophyllum shimeji* colonising seedlings of cultivated conifer species in New Zealand. Mycorrhiza 24:453–463.

Walker, J. F., O. K. Miller, and J. L. Horton. 2005. Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Mol. Ecol. 14:829–838.

Wang, B., and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Wiens, J. J. 2011. The causes of species richness patterns across space, time, and clades and the role of "ecological limits." Q. Rev. Biol. 86:75–96.

Wilson, A. W., M. Binder, and D. S. Hibbett. 2011. Effects of gasteroid fruiting body morphology on diversification rates in three independent clades of fungi estimated using binary state speciation and extinction analysis. Evolution 65:1305–1322.

Wolfe, J. A. 1985. Distribution of major vegetational types during the tertiary. Pp. 357–375 *in* E. T. Sundquist and W. S. Broecker, eds. The carbon cycle and atmospheric CO_2: natural variations Archean to present. American Geophysical Union, Washington, D.C.

Wright, A. M., K. M. Lyons, M. C. Brandley, and D. M. Hillis. 2015. Which came first: the lizard or the egg? Robustness in phylogenetic reconstruction of ancestral states. J. Exp. Zool. B. Mol. Dev. Evol. 324:504–16.

Yamada, A., T. Ogura, and M. Ohmasa. 2001. Cultivation of mushrooms of edible ectomycorrhizal fungi associated with *Pinus densiflora* by in vitro mycorrhizal synthesis. Mycorrhiza 11:59–66.

Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, et al. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. 23:1581–1596.

Zachos, J. C., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.

Zachos, J. C., G. R. Dickens, and R. E. Zeebe. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature 451:279–283.

Associate Editor: G. Thomas
Handling Editor: R. Shaw

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. GenBank accession numbers of the Tricholomatineae dataset used in the present study.
Table S2. Clade-specific sampling probabilities used in the BAMM analyses.
Table S3. Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of taxa of the Tricholomatineae.
Figure S1. 95% credibility set of shift configurations based on a Bayes factor criterion of 5.