



# Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletales, Basidiomycota)

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#### Summary

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**Key words:** ancestral reconstruction, biogeography, boreotropical hypothesis, divergence times, ectomycorrhizal evolution, Lagrange, mesophytic forests, supertrees. • This study uses phylogenetic analysis of the Sclerodermatineae to reconstruct the evolution of ectomycorrhizal host associations in the group using divergence dating, ancestral range and ancestral state reconstructions.

• Supermatrix and supertree analysis were used to create the most inclusive phylogeny for the Sclerodermatineae. Divergence dates were estimated in BEAST. Lagrange was used to reconstruct ancestral ranges. BAYESTRAITS was used to reconstruct ectomycorrhizal host associations using extant host associations with data derived from literature sources.

• The supermatrix data set was combined with internal transcribed spacer (ITS) data sets for *Astraeus*, *Calostoma*, and *Pisolithus* to produce a 168 operational taxonomic unit (OTU) supertree. The ensuing analysis estimated that basal Sclerodermatineae originated in the late Cretaceous while major genera diversified near the mid Cenozoic. Asia and North America are the most probable ancestral areas for all Sclerodermatineae, and angiosperms, primarily rosids, are the most probable ancestral hosts.

• Evolution in the Sclerodermatineae follows the biogeographic history of disjunct plant communities associated with early Cenozoic mesophytic forests and a boreotropical history. Broad geographic distributions are observed in the most promiscuous Sclerodermatineae (those with broad host ranges), while those with relatively limited distribution have fewer documented ectomycorrhizal associations. This suggests that ectomycorrhizal generalists have greater dispersal capabilities than specialists.

#### Introduction

The Sclerodermatineae is a monophyletic assemblage of hymenomycetes (mushroom-forming fungi) and gasteromycetes ('puff ball'-forming fungi) in the Boletales (Basidiomycota). The group presently includes 78 described species in nine genera, including six gasteromycete genera (Astraeus, Calostoma, Diplocystis, Pisolithus, Scleroderma and Tremellogaster) and three hymenomycete genera (Boletinellus, Gyroporus and Phlebopus)(Kirk et al., 2008). Since its description by Binder & Bresinsky (2002) there have been several phylogenetic studies that involve the Sclerodermatineae (Binder & Hibbett, 2006; Louzan et al., 2007; Wilson et al., 2011). Of these, Louzan et al. (2007) had the greatest taxonomic sampling, using 43 sequences of nuclear ribosomal large subunit (25S), representing 32 species. Binder & Hibbett (2006) carried out the most character-rich study, using five genes (16S, 25S, 5.8S, mitochondrial ATPase subunit 6 (atp6) and mitochondrial large subunit (mtLSU)), but from only seven species. Recent studies describe numerous cryptic species within the Sclerodermatineae and have contributed to the taxonomic expansion of

of hensive phylogenetic analysis of the Sclerodermatineae, using an inclusive sampling of taxa and molecular sequences, to evaluate taxonomic relationships and examine patterns of age, ancestral ranges, and ectomycorrhizal host associations within *ma*, the suborder. Most Sclerodermatineae are considered to be ectomycorrhizal (Binder & Hibbett, 2006; Wilson *et al.*, 2007). For example, der *Pisolithus* and *Scleroderma* are used in reforestation projects because they can form ectomycorrhizas with multiple species of

because they can form ectomycorrhizas with multiple species of host trees (Molina & Trappe, 1982b; Danielson, 1984; Sanon *et al.*, 2009). However, the ectomycorrhizal roles of *Phlebopus* and *Boletinellus* are suspect. These genera constitute the Boletinellaceae, which is an early-diverging lineage within the Sclerodermatineae. *Boletinellus* has been described as the 'ash bolete', but its association with *Fraxinus* spp. is poorly understood and may be a tripartite relationship involving an arthropod (aphid) intermediate (Tedersoo *et al.*, 2009). The ecology of *Phlebopus* is ambiguous; some studies have reported that species of *Phlebopus* can be cultivated as saprotrophs (Thoen & Ducousso, 1989; Ji

the group (Martín et al., 2002; Læssøe & Jalink, 2004; Phosri

et al., 2007; Binder et al., 2009). Here, we present a compre-

*et al.*, 2010), while others suggest they are ectomycorrhizal fungi (Sanmee *et al.*, 2010). Species of *Phlebopus* produce large fruiting bodies that are collected from a wide range of habitats, including grasslands in Africa and Australia (which lack ectomycorrhizal hosts), fragmented forests in Argentina and Bolivia, and Southeast Asian forests of Dipterocarpaceae.

Members of the Sclerodermatineae have been reported to form partnerships with diverse hosts. However, the methods used to identify ectomycorrhizal hosts vary widely among studies. *Astraeus, Calostoma, Pisolithus* and *Scleroderma* have been conclusively shown to form ectomycorrhizas with angiosperms and gymnosperms through synthesis studies (Molina, 1981; Molina & Trappe, 1982a; Danielson, 1984) and molecular analyses (Tedersoo *et al.*, 2007; Wilson *et al.*, 2007). Unfortunately, there are many cases where the determination of ectomycorrhizal host is based on the observed proximity of fungus and putative hosts. For example, the designation of *Gyroporus* as ectomycorrhizal with oak (*Quercus*) and pine (*Pinus*) appears to be based solely on field observations (Agerer, 1999; Raidl *et al.* 2006).

To reconstruct the evolution of ectomycorrhizal associations, several factors must be considered, including the relative ages and ancestral geographic ranges of the fungi and their plant hosts. The relative ages of Boletales and their prospective hosts were addressed by Hibbett & Matheny (2009), who suggested that the Boletales are younger than angiosperms and conifers, but slightly older than the rosids, which contain many ectomycorrhizal partners of extant Sclerodermatineae. The oldest fossil attributed to Boletales is an Eocene ectomycorrhiza on Pinus that was interpreted as a member of the Suillineae (LePage et al., 1997). The current range of the Sclerodermatineae is broad, with some genera (Pisolithus and Scleroderma) occurring on all major continents except Antarctica, while others have ranges limited to a few continents (Calostoma), or small geographic areas (Diplocystis and Tremellogaster). The only phylogeographic study in Sclerodermatineae to date is that of Martín et al. (2002), who studied distributions of Pisolithus and its hosts.

This study had four main goals: (1) to assemble a maximally inclusive phylogenetic tree for the Sclerodermatineae by combining trees from internal transcribed spacer (ITS) data sets with a multi-gene supermatrix data set using supertree analyses; (2) to compile host association data from the literature for Sclerodermatineae taxa, and classify the different methods used to determine host associations; (3) to use molecular dating analysis to estimate the ages of Sclerodermatineae groups and reconstruct their ancestral geographic ranges; and (4) to reconstruct the evolution of host associations in the Sclerodermatineae.

## **Materials and Methods**

#### DNA extraction, PCR, and cycle sequencing

The molecular techniques in this study, including primers and the method for cloning PCR product, that were used to obtain nucleotide sequence data from the sporocarps of Sclerodermatineae taxa are described in Wilson *et al.* (2011).

#### Data sets

We aligned sequences using CLUSTAL X 1.81 (Thompson *et al.*, 1997) with default settings, followed by manual alignment using MACCLADE v 4.03 (Maddison & Maddison, 2005). Taxa and sequence information for all data used in this analysis are listed in Supporting Information Notes S1.

The Sclerodermatineae supermatrix data set comprises 112 operational taxonomic units (OTUs) (106 ingroup OTUs) represented by nuclear ribosomal and protein-coding genes. We generated 217 sequences (68 25S, 41 ITS, 37 RNA polymerase II subunit 1 (RPB1) and 40 subunit 2 (RPB2) genes, and 31 translation elongation factor 1a (ef1a)) and acquired 41 sequences from GenBank (44 25S, seven ITS, seven RPB1, eight RPB2, and eight ef1 $\alpha$ ). Only 5.8S rRNA data from ITS sequences were used in the supermatrix because of the high level of sequence variability in the ITS1 and ITS2 regions. Both nuclear ribosomal DNA and protein-coding sequences are present in 49 OTUs. The remaining 63 OTUs are represented by 25S rRNA sequence data only. A total of 687 characters were removed from the 4947-character supermatrix because of the variability of intron regions.

We assembled three separate ITS data sets for Astraeus, Calostoma and Pisolithus. The Astraeus data set consists of 22 ITS sequences, of which we generated five; the rest were obtained from Phosri et al. (2007). The Pisolithus data set consists of 37 sequences. We produced six of these sequences and obtained the rest from Martín et al. (2002). The Calostoma data set consists of 24 ITS sequences that we generated (except Calostoma sp. EU543222). The ITS phylogenies generated for supertree analyses were rooted with the most basal taxon for the genus, as indicated in the supermatrix analyses. Gyroporus castanaeus (EU718099) and Gyroporus cyanescens (EU718102) were added to each of the ITS data sets to serve as the outgroup for the Bayesian phylogenies presented in Notes S3. Sequences in each data set were aligned across the entire ITS region (ITS1, 5.8S and ITS2). The following figures represent the numbers of characters excluded from phylogenetic analyses because of ambiguities in character alignment, over the number of characters in the ITS alignments: Astraeus = 99/843,*Calostoma* = 310/912, Pisolithus = 123/793.

#### Phylogenetic analysis

Phylogenetic analyses were performed on a Macintosh G5 and a Linux cluster in the Clark University Center for Scientific Computing. Bayesian and maximum likelihood methods were used to produce phylogenies from the Sclerodermatineae supermatrix and all three ITS data sets. The phylogenies from these data sets were incorporated into a supertree analysis using the technique of matrix representation using parsimony (MRP), which produced the supertree data matrix that was analyzed using parsimony.

Bayesian Metropolis-coupled Markov-chain Monte Carlo analyses were performed using the GTR + I + G model of evolution in MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck, 2003). The analyses used four chains, sampling every 100th tree for 10 million generations. The burn-in value for each analysis was determined by charting likelihoods of trees and removing those before the chains converged around a stable average likelihood. We reported posterior probabilities  $\geq 0.95$  and considered probabilities  $\geq 0.98$  to constitute strong support for clades.

Maximum likelihood tree generation and bootstrap analyses were performed using RAxML v. 2.2.3 (Stamatakis, 2006). Thousand bootstrap replicates were performed under the GTR + I + G model of evolution. Bootstrap support  $\geq$  80% was reported on branches, with  $\geq$  90% considered to be strong support.

For supertree analyses, the MRP data matrix was generated by CLANN (Creevey & McInerney, 2005). The data matrix from the four combined Bayesian phylogenies contained 163 characters representing 168 OTUs (112 OTUs from the Sclerodermatineae supermatrix, 18 new OTUs from *Astraeus*, five from *Calostoma*, and 33 from *Pisolithus*).

We analyzed the MRP matrix using parsimony implemented in PAUP\* v. 4.0 beta 10 (Swofford, 2003). The parsimony analysis used heuristic searches with 1000 random addition sequence replicates with TBR branch swapping and keeping 10 trees per replicate. In the initial stages of producing the supertree, some of the taxa 'misbehaved' in the sense that taxa from one group would migrate into an unrelated group (e.g. a Calostoma taxon would wind up in the Gyroporus clade). To control this 'misbehaving', a backbone constraint was enforced to maintain the integrity of known clades in supertree production. MRP characters were given a weight of 0.5-1 corresponding to RAxML likelihood bootstrap scores of 50-100% from the supermatrix and ITS phylogenies. Characters with  $\leq 50\%$  bootstrap support were given a score of 0.5. Parsimony bootstrap analyses were performed on the MRP matrix with 1000 replicates using heuristic search methods with random taxonomic addition analyses, character states sampled in proportion to their weights per bootstrap replicate, SPR branch swapping, and saving 10 trees per replicate.

#### Divergence time estimation

To estimate the ages of divergence events in the Sclerodermatineae, we used the secondary calibration procedure described by Renner (2005) and employed by Matheny et al. (2009), Skrede et al. (2011), and Ryberg & Matheny (2011) using BEAST v.1.6.1 (Drummond et al., 2006; Drummond & Rambaut, 2007). We used BEAUTI v.1.6.1 to create XML files with the following analytical settings: GTR model, uncorrelated relaxed clock with lognormal rate distribution; estimating separate rates for genes 5.8S, 25S, RPB1, RPB2 and ef1a while using two codon partitions ((1 + 2), 3) for RPB1, RPB2 and ef1 $\alpha$ ; Tree Prior set to Yule Process speciation; running 10 million generations, sampling every 1000th tree. Each analysis was run three times. The first 10% of the trees were removed as the burn-in and the remaining trees were combined using LOGCOMBINER v1.6.1. A summary tree was produced using TREE ANNOTATOR v1.6.1 (Drummond & Rambaut, 2007). Means and 95% highest posterior densities (HPDs) for nodes of interest were examined from BEAST logfiles using TRACER v1.5 (Drummond & Rambaut, 2007).

Part 1 of the BEAST analysis used an 18-taxon data set (Notes S7). Taxonomic groups to estimate time to most recent common ancestor (tMRCA) were defined in BEAUTI. These include the Agaricales, Boletales, Boletineae, Coniophoineae, Core Sclerodermatineae, Sclerodermatineae, Suillineae, marasmioid fungi and Tapinellineae. Two nodes were calibrated using fossil data. The marasmioid fungi (Marasmius rotula and Mycena amabillisima) were calibrated based on a 90-Ma fossil Archaeomarasmius legetti from mid-Cretaceous amber (Hibbett et al., 1997). In BEAUTI this was set as an exponential prior with an offset of 90 and mean of 10. The Suillineae (Suillus pictus and Gomphus roseus) were calibrated using a 50-Ma permineralized suilloid ectomycorrhiza fossil associated with Pinaceae roots (LePage et al., 1997). In BEAUTI this prior was set as an exponential distribution with an offset of 50 and a mean of 25. This mean was used to incorporate a 140-Ma date, the age of the oldest known fossil in the Pinaceae (LePage, 2003), within the 95% HPD. This prior sets up a likely age range for the Pinaceae ectomycorrhizal association and probable age of the Suillineae.

Part 2 of the BEAST analysis used a 58-taxon data set (a subset of the 112-taxon supermatrix data set) with taxa selected for molecular dating and phylogeographic analysis (Notes S1). Groups used to evaluate tMRCAs include: Suillineae, Sclerodermatineae, Core Sclerodermatineae, *Astraeus*, Bolletinellaceae (*Boletus* and *Phlebopus*), *Calostoma*, Diplocystidiaceae, *Gyroporus*, *Pisolithus* and *Scleroderma*. One node, the Suillineae, was calibrated with Pinaceae fossils using the priors described in the preceding paragraph. Two nodes, the Sclerodermatineae and Core Sclerodermatineae, were calibrated using a lognormal distribution with offset, mean and stdev set to approximate the age and HPD of these nodes as estimated in the first part of this analysis.

#### Ancestral range reconstruction

To reconstruct the ancestral ranges for major groups of Sclerodermatineae, we used dispersal-extinction-cladogenesis (DEC) analysis developed by Ree & Smith (2008). These analyses were performed using the consensus phylogeny produced in the divergence time analysis. Scripts for analysis were produced using the Lagrange configurator (www.reelab.net/lagrange). Definitions of areas, range constraints and the dispersal rates for models are presented in Fig. 4(e). Seven areas were defined: North America, Central America, Asia, Southeast Asia, Europe, Africa and Australasia. Each taxon in our data set was assigned to one area based on the origin of the collection representing that taxon (Fig. 3). We tested the effect of range limitations on Sclerodermatineae ancestors by constraining ancestral ranges to either two or three areas (Fig. 4e).

To evaluate variation in dispersal rates, area matrices were assembled under two model criteria. First, the restricted dispersal model allows for a dispersal rate of 1.0 between adjacent areas, but nonadjacent areas are given a dispersal rate of 0. Secondly, the relaxed dispersal model uses the same rate of dispersal between adjacent areas, but allows for dispersal between non-adjacent areas using a reduced rate of 0.5 and 0.25 for species dispersal to two and three areas, respectively, away from the original range (see Fig. 3 and 4e). Because land masses have changed over geological history, organismal rates of dispersal between continents are affected by the availability of land bridges and migration routes that existed at different times. For both models, the area matrices shown in Fig. 3 define rates of dispersal for different geological time frames used in this analysis. These are divided among five time frames which are displayed in Fig. 3 and defined in Table 1.

#### Extant host associations in Calostoma

Host associations in Calostoma sarasini and Calostoma retisporum were studied in the Malaysian Provinces of Selangor, Negeri Sembilan and Sabah in January 2006, and May and December 2007. Fruiting bodies were collected and dried with a portion of the fruiting bodies stored in 1× CTAB or silica gel for future DNA extraction. Soil cores from directly beneath the fruiting bodies were extracted and sifted for ectomycorrhizal root tips, which were stored individually in 1× CTAB buffer. Fungal and plant DNA were isolated from ectomycorrhizal roots. PCR and cycle sequencing of fungal DNA from ectomycorrhizal root tips followed protocols described in Wilson et al. (2007), while analyses of plant DNA used primers rbcL-F1 and rbcL-R1 and protocols described in Sato et al. (2007) to amplify the ribulose biphosphate carboxylaze chloroplast gene (rbcL). Fungal ITS and plant rbcL sequences were used as queries in BLAST searches of GenBank.

#### Extant host associations in the Sclerodermatineae

We performed a literature search to survey the documented symbiotic partners for Sclerodermatineae taxa. We classified the data source as either (A) field collections/observations, or (B) data generated from laboratory/synthesis experiments. Four categories were created to classify the method used to identify the taxa

 Table 1
 Geological events used to define age ranges for

 dispersal-extinction-cladogenesis (DEC) analysis priors

DEC prior (Ma)	Event	Event marker in Fig. 3
90–50	Global land-masses leading to the break-up of Laurasia (North America-Europe) and Gondwana	А, В
50.20	(Australia–South America via Antarctica)	A Rto carby C
50-20	and Wallacia. Time of the fewest	A, B to early C
20–5	Miocene area. Establishment of migratory routes between Asia and North America (AKA Beringia), Australasia and Southeast Asia (AKA Wallacia)	С
5–2.5	Pliocene epoch. Africa and Europe collide	D
2.5–0	End of Beringia: when North America separates from Asia. Central America is formed, joining North and South America	E, F

involved in ectomycorrhizal associations: (1) molecular analysis, (2) rhizomorph tracing from fruit body to ectomycorrhizas, (3) morphological identification of ectomycorrhizas, and (4) association of fruiting bodies with nearby hosts.

We used the classifications described above to define host identifications as either stringent or liberal. Stringent definitions of host association use methods that demonstrate physical or molecular evidence for an association, including molecular analyses, synthesis studies, or, in some rare cases, tracing of fruiting body rhizomorphs to root tips occurring in mono-dominant stands. The remaining host determination methods were defined as liberal host identifications. Unpublished observations of host association are listed as pers. comm. or 'this study'. All host association data described here are given in Notes S2.

#### Ancestral host reconstruction

We performed ancestral state reconstructions (ASRs) of host associations in the supermatrix and supertree phylogenies. Parsimony and maximum likelihood methods were used to reconstruct the ancestral host for 10 nodes including the Sclerodermatineae, Core Sclerodermatineae, *Astraeus*, Diplocystidiaceae (Astraeus, Diplocystis and Tremellogaster, sensu Kreisel, 1974), *Boletinellus, Calostoma, Gyroporus, Pisolithus, Phlebopus*, and *Scleroderma.* States assigned to Sclerodermatineae taxa for all ASR analyses as well as a detailed description of coding strategies are given in Notes S1.

Two coding criteria were created to address ambiguity in host determinations. Under the liberal criterion, analyses used all host association data for ASR character coding assuming that fungal species were correctly identified. Analyses under the stringent criterion used only stringent definitions of host association for character coding. Under this criterion, the taxonomic identities of fungi were corroborated through DNA sequences or were justified using other evidence (e.g. geographic distribution limited to species).

We used MACCLADE v. 4.07 (Maddison & Maddison, 2005) for parsimony ASR analyses of ancestral hosts. Parsimony ASR was performed using the phylogenetic supertree because it contained the most taxonomically inclusive data set. Liberal and stringent coding for host character states used binary and multi-state methods. Binary state coding defined host states as either angiosperms or gymnosperms. Multi-state coding defined host states by host family association. The host families for analyses include: Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae (Mimosoideae and 'Caesalpinioideae'), Myrtaceae, Nyctaginaceae, Pinaceae, Oleaceae, Polygonaceae, Salicaceae, and Sapindaceae (Notes S2). Both binary and multi-state coding allowed taxa to be polymorphic in their host associations.

Maximum likelihood ASR analysis was implemented in BAYESTRAITS (Pagel *et al.*, 2004). Using the 112-taxon supermatrix data set, we coded host character states using similar binary and multi-state methods, described in the preceding paragraph, under the stringent criterion. Under the liberal criterion, host association for a single species was assigned to each member of that genus. This was to maximize usage of host

association data from the literature for species that were not represented in this smaller data set. Multi-state coding required a more inclusive taxonomic ranking than that of the host family in order to reduce the number of host states for maximum likelihood analyses. These character states are gymnosperms (Pinaceae and Gnetaceae), Caryophyllales (Nyctaginaceae and Polygonaceae), eurosids I (Betulaceae, Fagaceae, Fabaceae, Nothofagaceae and Salicaceae), eurosids II (Cistaceae, Dipterocarpaceae and Sapindaceae), Myrtales (Myrtaceae) and asterids (Ericaceae and Oleaceae). The classifications for higher plants are provided by the Angiosperm Phylogeny Website (Stevens, 2001 http://www.mobot.org/MOBOT/research/APweb/). onwards; Maximum likelihood binary and multi-state analyses were performed on 100 posterior sampled phylogenies produced from Bayesian analyses. The average probabilities from all 100 analyses were calculated for each character state occurring at a node.

### **Results and Discussion**

#### Phylogenetic analyses

Fig. 1 is one of 100 RAxML phylograms from the multi-gene supermatrix analyses with 106 Sclerodermatineae OTUs comprising c. 64 species. This phylogenetic tree is congruent with the Bayesian consensus tree, though slightly more resolved. The Sclerodermatineae (1.0 posterior probability) was resolved with multiple clades strongly supported by both maximum likelihood bootstrap (MLB) percentages and posterior probabilities (PPs). Phlebopus and Boletinellus were resolved as sister taxa with 98% MLB and 1.0 PP. These genera were resolved sister to the Core Sclerodermatineae (100% MLB; 1.0 PP), which represents six gasteromycete genera, Astraeus (97% MLB; 1.0 PP), Calostoma (100% MLB; 1.0 PP), Scleroderma (90% MLB; 1.0 PP), Pisolithus (99% MLB; 1.0 PP), Diplocystis and Tremellogaster, along with the hymenomycete genus Gyroporus (100% MLB; 1.0 PP). Both Tremellogaster and Diplocystis are monotypic genera that are consistently resolved as closely related to Astraeus (Louzan et al., 2007), and in our study receive 82% MLB support (Fig. 1). This clade corresponds to the Diplocystidiaceae described by Kreisel (1974).

The 24 Gyroporus isolates in the data set are represented by approximately eight names, but these are distributed over 16 nonmonophyletic terminals (Fig. 1). Only 10 species are described for the genus, suggesting that there is much cryptic diversity (Kirk *et al.*, 2008). This result is similar for *Scleroderma*, where multiple OTUs of species *Scleroderma citrinum* and *Scleroderma areolatum* do not form monophyletic species groups. While this could be a result of misidentification, cryptic species have been reported in other Sclerodermatineae genera, including *Pisolithus* and *Astraeus* (Martín *et al.*, 2002; Phosri *et al.*, 2007). The results for Bayesian analysis of ITS data sets in *Astraeus, Calostoma* and *Phlebopus* are presented and discussed in Notes S3.

The MRP supertree (Fig. 2) resolves each of the strongly supported monophyletic genera from the Bayesian supermatrix tree and resolves additional species within *Astraeus, Pisolithus*, and

*Calostoma* (Notes S3). Several taxonomic relationships within *Astraeus* and *Pisolithus* were not resolved in the strict consensus of all 8480 most parsimonious trees. This may be a consequence of combining numerous taxa from the ITS trees with the relatively small clades in the supermatrix tree. By contrast, *Calostoma* has greater taxonomic representation in the supermatrix tree (Fig. 1), and most of the relationships are resolved in the strict consensus supertree (Fig. 2).

#### Divergence times in the Sclerodermatineae

The XML files for the BEAST analysis parts 1 and 2 are provided in Notes S9 and S10, respectively. The consensus tree result for part 1 and tMRCAs for parts 1 and 2 of this analysis are presented in Notes S4. The resulting tMRCAs for clades representing marasmioid fungi and the Suillineae fall within the expected ages given the fossil record. The median age for the Sclerodermatineae was 82.47 Ma with a 95% HPD of 54.74–115.43 Ma, while the Core Sclerodermatineae was *c*. 58.24 Ma with an HPD of 34.48–84.95 Ma. These values were used to establish tMRCAs for the Sclerodermatineae and Core Sclerodermatineae in part 2 of the analysis.

The results of divergence dating in the Sclerodermatineae (part 2) are displayed in Fig. 3 with the tMRCAs and HPDs summarized in Table 3 and Notes S4. The median age esti-Sclerodermatineae mated for the (80.46 Ma; HPD 59.81-109.64 Ma) is only a couple of million years younger than estimates from part 1 (82.47 Ma; HPD 54.74-115.43 Ma), whereas the Core Sclerodermatineae (66.02 Ma; HPD 49.27-90.28 Ma) is nearly 8 million years older than the estimates of part 1 (58.24 Ma; 34.48-84.95 Ma) (Notes S4). This places the crown ages for the Sclerodermatineae and Core Sclerodermatineae in the late Cretaceous. Diversification of the Core Sclerodermatineae took place in the early Cenozoic era (Fig. 3). The crown ages for the groups within the Core Sclerodermatineae are younger than the Core Sclerodermatineae crown age by a minimum of 23 Ma. The tMRCA for Calostoma (42.73 Ma; HPD 28.78-61.69) makes it the oldest Sclerodermatineae group, followed by Scleroderma (38.37 Ma; HPD 26.26-53.71 Ma), Diplocystidiaceae (38.22 Ma; HPD 21.76-57.15 Ma), Bolletinellaceae (36 Ma; HPD 18.7-54.48 Ma), Gyroporus (34.58 Ma; HPD 22.66-48.9 Ma), Pisolithus (28.90 Ma; HPD 16.75-43.02 Ma), and Astraeus (15.62 Ma; HPD 7.63-24.13 Ma), which represents the youngest member in the Sclerodermatineae (Table 3). The results of divergence dating analysis suggest that Sclerodermatineae ancestors are young enough to have initially associated with each of its current host families. As a result it is possible for ancestral Sclerodermatineae to have associated with the ancestors of current Sclerodermatineae ectomycorrhizal associates.

#### Ancestral range reconstruction in the Sclerodermatineae

The Lagrange scripts used to evaluate the ancestral geographic ranges for the Sclerodermatineae, representing both range constraints and both dispersal rate models, are provided in



Fig. 1 One of 100 RAxML trees from the Sclerodermatineae supermatrix composed of 112 25S, 41 5.8S, 37 RPB1, 40 RPB2 and 31 ef1α sequences. Numbers adjacent to branches represent maximum likelihood bootstrap percentages (in bold) and Bayesian posterior probabilities. Images: (a) *Astraeus pteridis*; (b) *Astraeus* sp.; (c) *Pisolithus tinctorius*; (d) *Scleroderma citrinum*; (e, f) *Scleroderma* sp.; (g, h) *Gyroporus castaneus*; (i) *Calostoma cinnabarinum*; (j) *Calostoma rodwayi*; (k, l) *Boletinellus merulioides*; (m) *Phelbopus marginatus*. (Photo credits: (a), (b), (f) A. Wilson; (c) Darvin DeShazer; (d) Herbert Baker; (e) Tim Sage; (g) Eric Smith; (h) Michael Waisberg; (i) Mike Wood; (j) Christopher Dunk; (k) Eva Skific; (l) Dan Molter; (m) Ian Dodd).



**Fig. 2** Sclerodermatineae matrix representation using parsimony (MRP) supertree consisting of 168 taxa and assembled from phylogentic analyses of supermatrix and internal transcribed spacer (ITS) molecular data sets. One of 8480 most-parsimonious trees is shown. Bold branches indicate relationships that were resolved in the strict consensus analysis. Support for branches is indicated as circles or squares and was obtained from the supermatrix analyses or individual phylogenetic analyses of ITS data sets, respectively. Asterisks represent nodes constrained in MRP analyses.





**Fig. 3** Divergence time estimations in the Sclerodermatineae. Numbers next to nodes represent mean ages in millions of years ago (Ma) while bars represent 95% highest posterior densities (HPDs). Dark blue and orange bars identify time to most recent common ancestor (tMRCA) nodes. Orange and red bars identify calibrated nodes. Dispersal-extinction-cladogenesis (DEC) analysis priors for models and their corresponding area-dispersal rate matrices applied during defined age ranges are illustrated at the top of the figure. Geographical areas for DEC analysis are indicated at the terminal branches for Sclerodermatineae taxa. Letters A–F at the top of the figure identify geological events defined in Table 1.

Notes S11. The results of DEC analysis, reconstructing the ancestral ranges for major Sclerodermatineae groups, are displayed in Fig. 4(b) and summarized in Table 3. DEC range probabilities are provided in Notes S6.

The majority of reconstructions across the Sclerodermatineae favored an ancestral range centering on Asia, Southeast Asia, and North America. The result that was given the greatest probability, under the range constraint of  $\leq 3$  and both restricted and relaxed dispersal models, was a North and Central American ancestral range for the Sclerodermatineae and Core Sclerodermatineae (Fig. 4b). However, under this constraint, the probability for this ancestral range becomes progressively weaker in other groups of Sclerodermatineae, with the exception of the Diplocystidiaceae. This exception is probably attributable to the origins of *Diplocystis* and *Tremellogaster*, which are located in Central America, more specifically in the Caribbean and northern South America, respectively.

Under a range constraint of  $\leq 2$ , almost all Sclerodermatineae groups have the greatest probability of an ancestral range in Asia and/or Southeast Asia under both dispersal models (Fig. 4b). Almost no species of Sclerodermatineae represented in this study are found in more than two areas defined in our study. However, collections of Calostoma cinnabarinum used in this study were also found in North America, Central America, and Asia (China) (Notes S1). Under this constraint Asia is the ancestral area with the greatest probability for Sclerodermatineae, Core Sclerodermatineae, Astraeus and Boletinellaceae. Southeast Asia is the ancestral area with the greatest probability for Calostoma, Gyroporus and the Diplocystidiaceae. The results for this last group conflict with the results found using the  $\leq 3$  range constraint. This could be because Astraeus taxa are lending more weight to an Asian origin for the Diplocystidiaceae when ancestral ranges are constrained to  $\leq 2$  areas. The ancestral ranges for *Pisolithus* and Scleroderma are nearly equivocal between Asia and Southeast Asia, giving a slightly higher probability to the latter area. Ancestral ranges with the greatest probabilities are summarized in Table 3.

#### Extant host associations in Calostoma

We confirmed the ectomycorrhizal status of *Calostoma sarasini* (FJ807559, FJ807561 and FJ807562) and *Calostoma retisporum* (FJ807564 and FJ807565) by matching fungal ITS sequences between ectomycorrhizal root tips and fruiting bodies. The ectomycorrhizal root tips of *C. sarasini* even have the gelatinous cuticle observed in *C. cinnabarinum* (Wilson *et al.*, 2007). BLAST searches using rbcL sequences (FJ807566, FJ807567 and FJ807568) from *C. sarasini* ectomycorrhizas as queries identified *Lithocarpus* sp. as the host/partner in the top three hits (AB125015, AB125013 and AB125014). This is the first report of a *Calostoma* species with *Lithocarpus*, and supports the *Calostoma*–Fagaceae relationship described by Wilson *et al.* (2007).

The BLAST search with rbcL sequences from *C. retisporum* ectomycorrhizas (FJ807569 and FJ807570) revealed the top three hits of *Tristaniopsis* sp. (AM235660), *Eugenia uniflora* 

(AM235654), and *Myrtus communis* (AF294254) as potential hosts. The identification of an ectomycorrhizal partner from the Myrtaceae is a first for *Calostoma*. However, *Calostoma fuscum* has been observed growing predominantly in *Eucalyptus* forests in Australia (C. Dunk, pers. comm.), suggesting that this association with the Myrtaceae extends to other *Calostoma* taxa. *Calostoma* in the southern hemisphere is also associated with Nothofagus in Australia and New Zealand. Although direct molecular evidence of this association has yet to be obtained, gelatinous ectomycorrhizal root tips similar in morphology to those of *C. cinnabarinum* have been observed growing on Nothofagus (C. Dunk, pers. comm.).

#### Extant host associations in other Sclerodermatineae

Sclerodermatineae taxa and their host associations are reported in Notes S2 along with literature references and the classification of host determination method. Forty-one studies describe hosts for 36 Sclerodermatineae species (Table 2), which are reported to form ectomycorrhizal symbioses with as many as 68 species in 15 plant families. Most gymnosperm references indicate associations with the Pinaceae, but Ingleby (1999) identifies *Scleroderma sinnamariense* as ectomycorrhizal with *Gnetum* using morphological identification of root mantle hyphae.

Only 37% of the host association reports can be considered 'stringent' (Table 2) based on our assessment of host association methods. These represent a little more than half of the plant families (n = 8) associated with the Sclerodermatineae. The literature describes more host associations with the angiosperms (n = 73) relative to the gymnosperms (n = 22). However, stringent methods were used to determine host associations in a little more than half of the gymnosperms (n = 12) compared with about a third of the angiosperms (n = 23). The greatest diversity of associations is with the angiosperms, where 13 families form ectomycorrhizal relationships with up to 52 Sclerodermatineae taxa. The host associations in *Pisolithus* and *Scleroderma* have been the most frequently studied, with 46 and 32 citations, respectively, while no more than six references were found for any of the remaining Sclerodermatineae genera.

The age and extant distribution for 12 of the 15 host families identified above are given in Fig. 4(a) and (c), respectively. Citations and references for the ages of Sclerodermatineae host families are provided in Notes S5. The Nyctaginaceae, Polygonaceae, and Cistaceae were not included because of a lack of information pertaining to their age, distribution and data describing ectomycorrhizal associations with the Sclerodermatineae (Notes S2).

#### Ancestral host reconstruction

ASRs are reported in Fig. 4(d). The MACCLADE file for parsimony ASR is presented in Notes S12. The results of reconstructions vary across analyses for the nodes. Binary and multi-state parsimony, and binary maximum likelihood ASRs gave the highest probability to a gymnosperm as the ancestral host at the root of the phylogeny (data not shown). For ancestral host states above the root, the reconstruction of either an angiosperm or a







#### (e) DEC Analysis Areas, Constraints and Models



**Fig. 4 (previous and current page)** Relative ages, dispersal-extinction-cladogenesis (DEC) analysis ancestral range reconstruction, and ancestral ectomycorrhizal host reconstruction. (a) Relative ages of Sclerodermatineae groups and their putative ectomycorrhizal host families. Ages are expressed in millions of years ago (Ma). (b) DEC ancestral range results under restricted dispersal and relaxed dispersal models. Maps in the left column of each model represent constrained analyses limited to reconstructions of  $\leq 2$  areas, while the column on the right represent  $\leq 3$  areas constraint. Darker shades of gray indicate higher probabilities for the ancestral range. (c) Extant and ancestral ranges for putative ectomycorrhizal host families. Host ranges indicated with a '!' are reconstructions based on information gathered from online searches. (d) Ancestral ectomycorrhizal host reconstruction using binary-state and multi-state analyses, parsimony and maximum likelihood methods, under stringent and liberal coding criteria. Parsimony results indicate tree length (in steps) for ancestral host reconstructions, and the hosts reconstructed for the clade indicated. Maximum likelihood results are given as bars representing probabilities for different host groups. Bars indicate 95% highest posterior density (HPD). (e) Illustrated description of DEC parameters used in this analysis.

 Table 2
 Summary of Sclerodermatineae host association information

 described in the literature
 Interview

References41Total Sclerodermatineae species37Number of Sclerodermatineae associated host taxa described from literatureTotalPlant host families158Plant host families15813713Total angiosperm2213713Number of Sclerodermatineae ECM associations described from literatureTotalNumber of Sclerodermatineae ECM associations described from literatureTotalCited ECM associations10636Astraeus75202Boletinellus10101Calostoma33Diplocystis404Gyroporus25121396Prisolithus101Scleroderma381622133Angiosperms5815401330Diplocystis40413Scleroderma381622212Tremellogaster101396Pinaceae129513214321510116131713318196191331910119 <td< th=""><th></th><th>Total</th><th></th><th></th></td<>		Total		
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Sapindaceae101Cistaceae201Oleaceae101	Salicaceae	2	0	2
Cistaceae 2 0 1 Oleaceae 1 0 1	Sapindaceae	1	0	1
Oleaceae 1 0 1	Cistaceae	2	0	1
	Oleaceae	1	0	1

gymnosperm ancestral host for a particular node varied across analyses (e.g. *Phlebopus* under stringent and liberal binary analyses, or Core Sclerodermatineae under liberal binary and multi-state analyses). However, in the case of *Calostoma* and *Pisolithus*, reconstructions unambiguously favor an angiosperm as the ancestral host (Fig. 4d).

Reconstructions in the Sclerodermatineae and Core Sclerodermatineae across all multi-state analysis support ancestral hosts from the Pinaceae and eurosids I. These results largely suggest that an angiosperm ancestor is the most probable ancestral host; a result found in the study of other ectomycorrhizal groups (Hosaka et al., 2008; Matheny et al., 2009). However, the Pinaceae were resolved as the most probable ancestral host in parsimony multi-state ASR. This difference may be attributable to the coding sensitivity of parsimony when the overall weight of angiosperms from the binary-state analysis was reduced in the multi-state analysis as a result of it being fragmented into smaller constituents. The challenge of reconstructing ectomycorrhizal host associations is discussed in recent works by Ryberg et al. (2010) and Ryberg & Matheny (2011). The ambiguity between ASR results could also stem from sensitivities in interpreting ectomycorrhizal associations from the literature in coding (liberal vs stringent), different methods of data usage (binary vs multi-state), and/or different methods of analysis (parsimony vs maximum likelihood). The development of new stringent host association data could help resolve some of these ambiguities. Ultimately, our analyses suggest that the Pinaceae and rosid angiosperms played an important role as host to ancestral Sclerodermatineae and Core Sclerodermatineae.

The ancestral association with the asterids was produced in the multi-state, liberal coding analysis (Fig. 4d). This is potentially another example of coding sensitivity with the signal originating from *Fraxinus* (Oleaceae) in the Boletinellaceae. This association is dismissed as a possible ancestral host because the ectomyocorrhizal association between *Boletinellus* and *Fraxinus* is seen as dubious (Wang & Qiu, 2006; Tedersoo *et al.*, 2009). The ectomycorrhizal nature of *Phlebopus* is also considered dubious because, even though it has been shown to produce ectomycorrhizas *in vitro* (Sanmee *et al.*, 2010), it was not able to do so with *Fagara coco*, despite being associated *in silva* (Nouhra *et al.*, 2008). Ultimately, the Boletinellaceae association with plants is not disputed in this study, but the nature of this association needs further investigation.

A summary of age, range and host reconstruction information is presented in Table 3, with the last column identifying the probable ectomycorrhizal hosts based on a consideration of these data. Reconstructions of Core Sclerodermatineae lineages describe a number of host gains and losses that include new associations with the Dipterocarpaceae, Ericaceae, Myrtaceae, and Sapindales (Table 3). The present study used the parsimony ASR data set to evaluate host switching with MACCLADE (Notes S8). Overall, most host switching across binary/multi-state analysis and liberal/stringent coding occurs from Pinaceae hosts to angiosperm hosts. Within the angiosperms, host switching activity is largely centered around the Myrtaceae and the Fagaceae. These results are consistent with an ancestral association with the Pinaceae and numerous transitions to angiosperms, probably involving the Fagaceae and Myrtaceae.

#### Evolution of host associations in the Sclerodermatineae

Synthesizing the results from divergence dating, ancestral range, and ancestral host reconstruction analysis, it appears that the

#### Table 3 Summary of ancestral host reconstruction analysis in the Sclerodermatineae

Clade	Extant host families accord- ing to literature	Results from da			
		tMRCA (Ma)*	Ancestral range	Ancestral host	Likely ancestral hosts**
Sclerodermatineae	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Nothofagaceae Nyctaginaceae, Pinaceae, Oleaceae, Polygonaceae, Salicaceae, Sapindaceae	(59.81–) 80.46 (–109.64)	Asia, North America, Asia + North America	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Fagaceae, Fabaceae, Salicaceae, Oleaceae	Pinaceae, Betulaceae, Fagaceae, Fabaceae, Salicaceae
Core Sclerodermatineae	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Nothofagaceae, Nyctaginaceae, Pinaceae, Polygonaceae, Salicaceae, Sapindaceae	(49.27–) 66.02 (–90.28)	Asia, North America, Asia + North America	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fabaceae, Fagaceae, Salicaceae
Astraeus	Betulaceae, Ericaceae, Pinaceae	(7.63–) 15.1 (–24.13)	Asia, North America, Southeast Asia, Asia + Southeast Asia, Asia + North America	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Ericaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Ericaceae, Fagaceae, Salicaceae
Boletinellaceae <sup>1</sup>	Dipterocarpaceae, Fabaceae, Myrtaceae, Pinaceae, Oleaceae	(18.70–) 34.96 (–54.48)	Asia, Southeast Asia, Asia + Southeast Asia	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Myrtaceae, Oleaceae, Salicaceae	Pinaceae, Dipterocarpaceae, Fabaceae, Myrtaceae, Oleaceae
Calostoma	Fagaceae, Myrtaceae	(28.78–) 42.73 (–61.69)	Southeast Asia, Asia, Asia + Southeast Asia	Angiosperms: Fagaceae, Myrtaceae	Fagaceae, Myrtaceae
Diplocystidiaceae	Betulaceae, Ericaceae, Fabaceae, Nyctaginaceae, Pinaceae, Polygonaceae	(21.76–) 38.22 (– 57.15)	Southeast Asia, North America, Central America, North + Central America,	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fagaceae, Salicaceae
Gyroporus	Fagaceae, Pinaceae	(22.66–) 33.52 (–48.90)	Southeast Asia, Asia, Asia + Southeast Asia	Gymnosperms: Pinaceae; Angiosperms: Fagaceae	Pinaceae, Fagaceae
Pisolithus	Betulaceae, Cistaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Myrtaceae, Pinaceae	(16.75–) 28.02 (–43.02)	Southeast Asia, Asia	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Dipterocarpaceae, Ericaceae, Myrtaceae	Pinaceae, Dipterocarpaceae, Ericaceae, Myrtaceae
Scleroderma	Betulaceae, Dipterocarpaceae, Ericaceae, Fagaceae, Fabaceae, Gnetaceae, Myrtaceae, Pinaceae, Salicaceae, Sapindaceae	(26.26–) 38.37 (–53.41)	Asia + Southeast Asia, Southeast Asia, Asia, North America	<i>Gymnosperms</i> : Pinaceae; <i>Angiosperms</i> : Betulaceae, Ericaceae, Fagaceae, Fabaceae, Salicaceae	Pinaceae, Betulaceae, Fabaceae, Fagaceae, Salicaceae

\*(2.5% HPD-) Median age (-97.5% HPD).

\*\*Likely ancestral hosts are determined through a combined assessment of time to most recent common ancestor (tMRCA), ancestral range and ancestral host reconstructions.

<sup>1</sup>Although ectomycorrhizal relationships have been established in many Boletinellaceae species, it is likely that species of this group are only facultatively mycorrhizal. HPD, highest posterior density.

Sclerodermatineae originated in Asia and North America during the late Cretaceous and diversified in the early Cenozoic, predominantly with the Pinaceae and rosids (Table 3). The modern consensus of angiosperm biogeography suggests that mixed mesophytic forests dominated the northern hemisphere during the early Cenozoic (Wolfe, 1975; Wen, 1999; Xiang & Soltis, 2001). These forests play an important role in the 'boreotropical hypothesis' which describes how disjunct distributions of extant neo- and paleotropical angiosperms were established via intercontinental land bridges (Wolfe, 1975; Tiffney, 1985). These forests were later fragmented during the Oligocene and Miocene as a result of the formation of intercontinental glaciers, disrupting dispersal routes between New World and Old World populations (Zachos et al., 2001). The Fagaceae is an example of a boreotropical group, and an important Sclerodermatineae host, whose disjunct North American and Asian distribution is the result of vicariance in the Northern Hemisphere (Manos & Stanford, 2001).

The results of this study suggest that Sclerodermatineae ancestors dispersed with their ectomycorrhizal hosts in the early Cenozoic mesophytic forests (Wolfe, 1975, 1978; Gentry, 1988; White *et al.*, 1997; Buerki *et al.*, 2011). Around the time of the late Eocene/early Oligocene, the Core Sclerodermatineae began to diversify, but populations soon became fragmented as a result of climatic changes in the Oligocene and the break-up of their hosts' ranges. This study suggests that ectomycorrhizal associations with the Dipterocarpaceae, Ericaceae, Myrtaceae, Nothofagaceae, Sapindaceae, and potentially the Fabaceae were derived in later Sclerodermatineae lineages. This is based on the results of our ASR analyses (Fig. 4d; Table 3) and the different biogeographic histories for these hosts (Gentry, 1988; Sytsma *et al.*, 2004).

The phylogenetic assessment of the boreotropical hypothesis developed by Lavin & Luckow (1993) was applied to the Sclerodermatineae using area ASR parsimony analysis on the supertree data set (Notes S8, S13). Although the results are not entirely conclusive, some Sclerodermatineae clades do demonstrate a pantropical distribution that is consistent with the boreotropical hypothesis. This is interesting because it corroborates the suggestion of Matheny *et al.* (2009) that ectomycorrhizal fungi in the Northern Hemisphere dispersed according to hypotheses used to describe plant distributions. Future studies using expanded data sets should be able to demonstrate the importance of host associations to the dispersal of Northern Hemisphere ectomycorrhizal fungi.

Distributions of Sclerodermatineae outside of their ancestral host range can be explained by long-distance dispersal events, which requires host switching if dispersing to exotic habitats. The Sclerodermatineae have been shown to disperse long distances in a study of *Pisolithus* by Moyersoen *et al.* (2003). The New Zealand and Australian disjunct observed in *Pisolithus* species is also observed in other groups of fungi (Hosaka *et al.*, 2006; Moncalvo & Buchanan, 2008) and plants (Pole, 1994; Knapp *et al.*, 2005). Each of these studies rules out the possibility of ancient Gondwanan vicariance because of the age of the organismal groups involved. A long-distance dispersal capacity is demonstrated in other Sclerodermatineae as *Calostoma* also has this Australasian distribution. In addition, long-distance dispersal between North America and China appears to be possible in *C. cinnabarinum* according to the results of our ITS analysis (Notes S3). Vacariance probably has a role in the divergence of New World from Asian Sclerodermatineae. However, a more detailed sampling of Sclerodermatineae populations is needed to identify any significant effects of isolation in these fungi.

A generalist ectomycorrhizal habit probably facilitated the wide distribution of many Sclerodermatineae groups. This study supports Martín et al.'s (2002) suggestion that the ancestral Pisolithus was an ectomycorrhizal generalist. They also demonstrated the association between a broad geographic distribution and ectomycorrhizal promiscuity through Pisolithus associations with Afzelia (Fabaceae) in Africa (Pisolithus sp. 1), Acacia (Fabaceae) and Eucalyptus (Myrtaceae) in Australia (Pislolithus albus), Cistus in Spain (Pislolithus sp. 3), and Pinus (Pinaceae) and Quercus (Fagaceae) in Europe and North America (Pislolithus sp. 4 and Pislolithus tinctorius). The genus Scleroderma shares many of the same ectomycorrhizal associations as other broadly distributed Sclerodermatineae (Table 3), as demonstrated in recent studies describing Scleroderma species outside its ancestral range (Sanon et al., 2009; Nouhra et al., 2012). The current ASR results for Gyroporus are limited to the Pinaceae and Fagaceae (Table 3). This is probably a result of a paucity of information regarding the ectomycorrhizal host association for the genus (Table 2). Collections of Gyroporus from Australasia and Africa (Fig. 3) suggest an ability to form broader ectomycorrhizal associations that have yet to be defined. However, Calostoma's host associations are similarly limited in ASR results and species have not been observed outside the distribution of hosts in the Fagaceae, Myrtaceae and Nothofagaceae. Overall, this suggests that the ability to form diverse host associations enables ectomycorrhizal fungi to distribute broadly.

The Diplocystidiaceae have North and Central America as their most probable ancestral range under the relaxed dispersal model and three-area constraint (Fig. 4b). Two Diplocystidiaceae species are limited to the neotropics. Tremellogaster surinamensis is found only in northern South America under *Dicymbe* ('Caesalpinioide') (Linder, 1930), while Diplocystus wrightii is found in the Caribbean with potential ectomycorrhizal hosts Neea buxifolia (Nyctaginaceae), Coccoloba uviferea (Polygonaceae), and Pinus cubensis (Louzan et al., 2007). The ancestral range of Astraeus is similar to that of the Diplocystidiaceae (Fig. 4b) despite its current disjunct distribution (Fig. 3). It also shares several of the same ectomycorrhizal hosts as other Sclerodermatineae (Table 3). However, the biogeographic history of Astraeus requires further investigation as its age (c. 15 Ma) is too young for it to have evolved in the Eocene mesophytic forests. As a result the vicariance of the Oligocene could not have shaped the evolution of this group, as is suggested for other Sclerodermatineae.

The evolutionary history of the Sclerodermatineae is similar to that of other Boletales groups. The distributions of *Pulveroboletus* and *Tylopilus* are described by Halling (2001) as being 'relictually disjunct'. He suggests that *Pulveroboletus* is a relatively old genus found with Pinaceae and *Quercus* in North and Central America, with Myrtaceae and Casurinaceae in Australia, and with Fagaceae in Southeast Asian forests. This distribution is strikingly similar to that of *Calostoma*, and both are also absent in Europe. In addition, Wolfe & Bougher (1993) describe *Tylopilus* as originating from Laurasia before migrating to Australasia over Pliestoscene land bridges and diversifying. The subgenus *Roseoscabra* is associated with Pinaceae, Fagaceae, Salicaceae, Myrtaceae, Mimosacaceae and Casurinaceae and distributed in the eastern USA, Costa Rica, Japan, China and northeastern Australia, but again is absent in Europe (Halling, 2001).

#### Conclusion

This study produced the most inclusive phylogenetic analyses for the Sclerodermatineae to date, to evaluate the evolution of ectomycorrhizal host associations using divergence dating, ancestral range and ancestral host reconstruction analysis. The results describe the Sclerodermatineae originating at the end of the Cretaceous with an ancestral range of North America, Asia and Southeast Asia, but many of the extant lineages did not diversify until the middle of the Cenozoic. During this time the Sclerodermatineae was ectomycorrhizal with as many as four host families (i.e. Pinaceae, Betulaceae, Fagaceae, and Salicaceae) that were associated with the Northern Hemisphere mesophytic forests of the early Cenozoic. Early Sclerodermatineae were distributed over a broad geographical area associated with its ancestral ectomycorrhizal hosts. The current distribution patterns seen in Sclerodermatineae are consistent with the boreotropical hypothesis proposed to describe the disjunct distribution of pantropical flora. Additional data and analysis may yield better insight into the importance of boreotropical flora in the evolution of ectomycorrhizal fungi. Extant distributions outside the Sclerodermatineae ancestral range are potentially attributable to a combination of long-distance dispersal capabilities and the ability to form diverse ectomycorrhizal associations.

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## **Supporting Information**

Additional supporting information may be found in the online version of this article.

**Notes S1** Sclerodermatineae taxa, GenBank IDs and character state assignments for ancestral host analysis. Detailed methodology for the assignment of host character states.

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**Notes S2** Sclerodermatineae host association references and methods of host association determination.

**Notes S3** Bayesian ITS phylogenies for *Astraeus, Calostoma*, and *Pisolithus* and discussion of these results.

Notes S4 Molecular dating analysis part 1 results.

Notes S5 Sclerodermatineae host families, their ages, and references.

**Notes S6** Dispersal-extinction-cladogenesis (DEC) analysis ancestral range probabilities for Sclerodermatineae groups.

Notes S7 Data set for molecular dating analysis part 1.

Notes S8 Sclerodermatineae data set for host switching analysis performed using parsimony.

Notes S9 .xml file for molecular dating analysis part 1.

Notes S10 .xml file for molecular dating analysis part 2.

**Notes S11** Concatenated python scripts for dispersal-extinctioncladogenesis (DEC) analysis. Four scripts representing: (1) restricted model/two-area constraint, (2) restricted model/ three-area constraint, (3) relaxed model/two-area constraint, and (4) relaxed model/three-area constraint.

**Notes S12** NEXUS file for analyzing character state evolution and host switching in the Sclerodermatineae using parsimony.

**Notes S13** Results of the boreotropical hypothesis test in the Sclerodermatineae using parsimony and the supertree data set.

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