Host Shift Speciation of the Ectomycorrhizal Genus *Suillus* (Suillineae, Boletales) and Biogeographic Comparison With Its Host Pinaceae

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*Suillus* is a genus of ectomycorrhizal fungi associated almost exclusively with Pinaceae. Lack of sample collections in East Asia and unresolved basal phylogenetic relationships of the genus are the major obstacles for better understanding the *Suillus* evolution. A resolved phylogeny of *Suillus* representing global diversity was achieved by sequencing multiple nuclear ribosomal and protein coding genes and extensive samples collected in East Asia. Fungal fossils are extremely rare, and the Eocene ectomycorrhizal symbiosis (ECM) fossil of *Pinus* root has been widely used for calibration. This study explored an alternative calibration scenario of the ECM fossil for controversy. Ancestral host associations of *Suillus* were estimated by maximum likelihood and Bayesian Markov chain Monte Carlo (MCMC) analyses, inferred from current host information from root tips and field observation. Host shift speciation explains the diversification of *Suillus* major clades. The three basal subgenera of *Suillus* were inferred to be associated with *Larix*, and diverged in early Eocene or Upper Cretaceous. In the early Oligocene or Paleocene, subgenus *Suillus* diverged and switched host to *Pinus* subgenus *Strobus*, and then switched to subgenus *Pinus* four times. *Suillus* subgenus *Douglasii* switched host from *Larix* to *Pseudotsuga* in Oligocene or Eocene. Increased species diversity occurred in subgenus *Suillus* after it switched host to *Pinus* but no associated speciation rate shifts were detected. Ancestral biogeographic distributions of *Suillus* and Pinaceae were estimated under the Dispersal Extinction Cladogenesis (DEC) model. Ancestral distribution patterns of *Suillus* and Pinaceae are related but generally discordant. Dispersals between Eurasia and North America explain the prevalence of disjunct *Suillus* taxa.

**Keywords:** ancestral range, diversification rate, fossil calibration, host specificity, multigene phylogeny
INTRODUCTION

The ectomycorrhizal symbiosis (ECM) is a common relationship between plants and fungi responsible for exchanging carbohydrates, mineral nutrients, and water (Landeweert et al., 2001; Nehls et al., 2010). Extant ectomycorrhizal fungi have evolved independently from over 80 saprotrophic ancestral lineages (Tedersoo and Smith, 2013, 2017; Martin et al., 2016).

The majority of ECM fungi are generalists, whereby one ECM fungal species can form an association with a wide variety of plant families (Bruns et al., 2002; Kennedy et al., 2003; Smith et al., 2009). However, a subset of ECM fungi is host-specific, i.e., they are associated with particular plant taxonomic groups. In contrast, their host plants are commonly generalists with the ability to recruit a broad spectrum of ECM fungal taxa (Ishida et al., 2007; Krpata et al., 2008; Deslippe et al., 2011; Leski and Rudawska, 2012). Species in the ECM genus Suillus have exceptionally high host fidelity to specific trees within the pine family (Pinaceae), with varying specificity from genera down to species (Thiers, 1975; Kretzer et al., 1996; Nguyen et al., 2016).

The evolutionary mechanisms explaining host specificity between symbiotic fungi and host plants include host shift speciation, coevolution, and cospeciation (Vienne et al., 2013). Host shift speciation has been confirmed in many ECM fungal lineages, including Stropholomycetes, Leccinum, Hebeloma, Pisolithus, and Laccaria (Aaen et al., 2000; Martin et al., 2002; Bakker et al., 2004; Sato et al., 2017; Wilson et al., 2017). Diversification after host switching has also been documented in Stropholomycetes, Leccinum, and Hebeloma, providing the ecological advantages of adapting to novel hosts and niches (Aaen et al., 2000; Bakker et al., 2004; Sato et al., 2017).

Being host-specific, most Suillus species are restricted to the Northern Hemisphere along with their Pinaceae hosts (Nguyen et al., 2016). Studies have shown that Suillloid taxa might have facilitated the invasion of Pinaceae into the Southern Hemisphere (Dickie et al., 2010; Hayward et al., 2015; Policelli et al., 2019). Diversity of Suillus species is severely underestimated in East Asia, with a significant lack of sampling hindering further biogeographic analyses (Wu et al., 2006; Mueller et al., 2001). Despite current efforts to discover novel species, the contemporary richness of the Suillus diversity in East Asia remains unevaluated (Verma and Reddy, 2015; Shi et al., 2016; Zhang et al., 2017).

This study compares the evolutionary relationships and distribution patterns of Suillus and their hosts Pinaceae throughout history. Multiple nuclear protein-coding genes of Suillus were collected, given that previous phylogenies based solely on ribosomal rRNA sequences could not resolve basal relationships of the genus (Kretzer et al., 1996; Wu et al., 2000; Mueller et al., 2001). To fill the sampling gap, many Suillus specimens were collected from East Asia, covering the geographic distributions of Pinaceae hosts, including approximately 26 operation taxonomic units and for more information on host, distribution range and diversity.

or diversification rate shifts driven by Suillus host switches or geographic range variation?

MATERIALS AND METHODS

Specimens and Molecular Data Collection

Suillus specimens from North American and European herbaria were sampled for DNA extraction. To fill the geographic sampling gap, intensive field collections were conducted over 10 years in East Asia covering Pinaceae distribution ranges (Farjon, 1990, 2005). A list of 103 Suillus specimens representing approximately 86 species used in this study is given in Supplementary Table 1.

DNA extraction, PCR and cycle sequencing are as previously described (Zhang et al., 2017). For nuclear ribosomal rRNA internal transcribed spacers 1 and 2, the 5.8S rRNA gene and parts of the 28S rRNA genes, primers ITS-1F and ITS-4 were used (White et al., 1990; Gardes and Bruns, 1993). For some specimens with DNA degradation, amplification was carried out with internal primers ITS-2 and ITS-3 (White et al., 1990). For the nuclear partial 28S large subunit rRNA genes, primers LR0R and LR5 were used (Vilgalys and Hester, 1990). For amplification of the nuclear protein coding translation elongation factor EF1-alpha (TEFI) partial gene, the following primers were used: primers TEFI-983f and TEFI-2212r, with additional internal primer TEFI-1567r (Rehner and Buckley, 2005), and one newly designed Suillus specific internal primer TEFI-Sintf (5' - TYR CAC AGC ATG MCA TGG TA - 3'). For amplification of the nuclear protein coding RNA polymerase II largest subunit (RPB1) partial gene, the following primers were used: primers RBP1-Af and RPB1-Cr (Stiller and Hall, 1997; Matheny, 2005), with additional internal primer RPB1-Int2.2f (Binder et al., 2010) and RPB1-Int2.1r (Froslev et al., 2005). For amplification of the nuclear protein coding RNA polymerase II second largest subunit (RPB2) partial gene, the primers were: RPB2-6f and RPB2-7.1r (Matheny et al., 2007), and newly designed Suillus specific internal primers RPB2-SintR (5' - CTC CRT CNT CNT CGC GRT AA - 3') and RPB2-SintF (5' - CAC GAC CRG CRT CYG TGT AY - 3').

Phylogenetic Analyses

An ITS phylogeny of Suillus was generated for identifying operation taxonomic units and for more information on host, distribution range and diversity. Rhizopogon abietis, Rhizopogon ochraceisporus, and Truncocolumella citrina were chosen as outgroups for the ITS dataset (Binder and Hibbett, 2006). Initial alignment for the ITS sequences was obtained in Mesquite 2.75 with manual adjustments (Edgar, 2004; Maddison and Maddison, 2011).

Phylogenetic tree of ITS sequences was inferred from maximum likelihood (ML) and Bayesian methods performed on the CIPRES computing facility (Miller et al., 2015). Bootstrap analyses of ML were performed using RAxML 8.0.0 HPC2 on XSEDE with 1000 bootstrap replicates (Stamatakis, 2014). Bayesian phylogenetic analysis was performed using MrBayes.
3.2.6 (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012). Number of substitution types Nst was set at 6, with 2 runs, 4 chains per run, each run searching for 1,000,000 generations sampling every 1000th generation. The first 10% of the sampled Bayesian trees of the analysis was discarded as the burnin. For convergence diagnosis, the estimated sample size (ESS) was above 200.

A supermatrix dataset was assembled with four loci including 28S, TEF1, RPB1, and RPB2. For the supermatrix dataset, each sample was selected from the ITS phylogeny to represent a unique haplotype. ITS sequences and introns of protein coding genes were not included because of their high level of sequence variability and poorer resolution at deeper nodes. Before concatenation, significant topological incongruence was evaluated among single gene phylogenies of 28S, TEF1, RPBI, and RPB2 using a cutoff of ≥ 70% maximum likelihood bootstrap (MLB) support and ≥ 0.98 Bayesian posterior probabilities (BPP). No major conflicts were detected for the inter-species evaluation. Rhizophogon nigrescens and Gomphidius roseus were chosen as outgroups for the multigene dataset (Binder and Hibbett, 2006).

Partitioning strategy and molecular models were searched using PartitionFinder V1.1.1 (Lanfear et al., 2012). The greedy algorithm was used to explore all nucleotide substitution models available under the Bayesian information criterion (BIC). Codon positions of protein coding genes were regarded for the partitioning analyses. Phylogenetic trees of the supermatrix were constructed with RAxML and Bayesian methods as described for the ITS phylogeny. Partitioning schemes and substitution models were set for the RAxML analysis under GTRGAMMA with 1000 bootstrap replicates. For the BI analysis, MrBayes 3.2.6 was implemented with the partitioned supermatrix and substitution models suggested by PartitionFinder (Ronquist et al., 2012).

Reconstructing Ancestral Host Associations

Current host associations of Suillus species were identified from global environmental samples, field observations and literature references. To be more conservative on host identifications, mycological references containing taxonomic uncertainties were not included. Host information for a certain Suillus species is documented if root tip samples were in the same OTUs defined by ≥ 70% MLB or ≥ 0.98 BPP in the ITS phylogeny. Field observations can provide reliable host information for Asian Suillus because almost each Pinus and Larix species grows in separate habitat (Farjon, 1990, 2005). Hosts of Suillus were classified to the generic level for Larix and Pseudotsuga, and to the subgeneric level for Pinus.

To reconstruct ancestral host associations for Suillus, ML and Bayesian Markov chain Monte Carlo (MCMC) analyses were conducted in BayesTraits v2.0 (Pagel and Meade, 2006). MCMC analyses were run for 1.01 × 10^6 iterations, sampling every 1,000, with the first 10,000 iterations discarded as a burn-in. The most probable host with a common ancestor was inferred if the acceptance rate is between 20–40% when the chain is at convergence.

Current Distributions of Suillus and Pinaceae

Collection site data were obtained from herbarium labels for Suillus taxa. Current ranges of Suillus species were also inferred from the global ITS phylogeny including environmental samples. Introduced taxa were excluded from the biogeographic analyses, e.g., Suillus luteus associated with introduced Pinus sylvestris in North America and S. lakei with introduced Pseudotsuga menziesii in Europe.

The current distributions of Pinaceae species were limited to their natural ranges (Farjon, 1990, 2005). Pinus, Larix, and Pseudotsuga distribution data across the world were also compiled from the global biodiversity information facility database (GBIF).

Ancestral Range Estimation

To estimate ancestral ranges we used the Dispersal Extinction Cladogenesis (DEC) model of Lagrange implemented in R package BioGeoBEARS (Ree and Smith, 2008; Matzke, 2014). The founder-event dispersal parameter “f” was introduced for DEC assuming a cladogenetic event (Matzke, 2014).

The extant distributions of Suillus and Pinaceae were divided into four biogeographic units: (1) ENA: Eastern North America, east of the Rocky mountains, encompassing Canada to Florida; (2) WNA, Western North America, west of the Rocky mountains, encompassing Alaska through Central America; (3) EUA, encompassing Europe, Northern China and Central Asia; (4) In, Indo-Pacific, encompassing subtropical Southern China, Southeast Asia and the western Himalayas. We also refer the combined EUA and Indo-Pacific as Eurasia. Events including cladogenesis, anagenesis, and vicariance were interpreted based on the most probable range for each ancestral species.

Time-Scaled Phylogenies of Suillus and Pinaceae

A two-step calibration procedure was conducted following previous examples (Renner, 2005; Wilson et al., 2012, 2017). Step one of the BEAST analysis used two fungal fossils to calibrate the phylogeny of 37 Agaricomycete taxa, including 19 Suillus taxa. Taxonomic groups were defined in BEAUti including Agaricales, Boletales, Boletineae, Boletinus, Sclerodermatineae, “Marasmioid” fungi and Suillineae. Suillus taxa from five subgenera represent the genus. Two fossils in Agaricomycetes were utilized for calibration in step one. Archaeoamarasmius leggettii from mid-Cretaceous amber of New Jersey resembles the extant genera Marasmius and Marasmiellus (Hibbett et al., 1997). Archaeoamarasmius leggettii was regarded as the most recent common ancestor (MRCA) of Marasmius rotula and Mycena amabilisims and was calibrated at 90 Ma (million year ago) with a mean of 10 using a lognormal distribution (Renner, 2005; Wilson et al., 2012, 2017). The second fossil used for calibration is an ECM root of Pinus preserved in the Princeton chert of British Columbia in the early Eocene (c. 47.8 Ma) (Lepage et al., 1997; Pigg and Devore, 2016). The ECM fossil was used to calibrate
the node for the Suillineae, including Suillus, Rhizopogon, and Gomphidius, and was calibrated using a lognormal distribution with an initial value 50 Ma and a log (mean) set 25. The same set of fossil calibrations was carried out in previous studies of Agaricomycetes (Floudas et al., 2012; Wilson et al., 2012, 2017). The Princeton chert fossil does not provide information on sporocarp morphology but the Pinus host is known. This study explores an alternative scenario: the fossil could be at clade(s) of Suillus associated with Pinus. Step two calibration inferred calibration points of major clades from step one to estimate divergence dates of the comprehensive Suillus phylogeny.

We used BEAUti v.1.8.2 to create XML files that incorporated the calibration priors, partitioning schemes and Bayesian parameters for analysis in BEAST v1.8.2 (Drummond et al., 2012). Bayesian parameters included a GTR + I + G model, Yule process speciation, and an uncorrelated lognormal relaxed clock model. The Bayesian Markov-chain Monte Carlo (MC2) analysis was run for 30 million generations, sampling every 1000th tree. Each analysis was run two times. The first 10% of the trees were removed as the burn-in and the remaining trees were combined using LogCombiner v1.8.2. A summary tree was produced using TreeAnnotator v1.8.2 (Drummond et al., 2012). Convergence, burn-in, means, medians and 95% highest posterior densities (HPDs) for nodes of interest were examined from BEAST logfiles using Tracer v1.6.0. Maximum clade credibility trees from the two steps were created using TreeAnnotator (BEAST package) and to summarize the posterior samples of trees produced by BEAST.

The calibrated phylogeny of Pinus was pruned from a published phylogeny of conifers (Leslie et al., 2012), which was generated from two nuclear genes (18S and a phytochrome gene, PHYP) and two chloroplast genes (matK and rbcL). It included 84% of Pinus global diversity. Fossils Larix altoborealis, Picea burtonii, and Tsuga swedaea within Pinaceae were used to calibrate the phylogeny (Leslie et al., 2012).

**Diversification Rate Shifts**

Lineage-through-time (LTT) plots were conducted in the R package APE for Suillus. For better comparison, Suillus/Pinus lineages and genus Pinus were plated on the same LTT plot (Paradis et al., 2004; Leslie et al., 2012).

To reveal speciation rate variations in the phylogeny of Suillus, Bayesian analysis of Macroevolutionary Mixtures (BAMM) v2.0 was utilized (Rabosky, 2014; Rabosky et al., 2014). BAMM reconstructs branch-specific evolutionary rates and allows rates to vary through time and among lineages. Without prior knowledge of the number and location of distinct regimes of diversification rates, BAMM simulates a posterior distribution of shift configurations on phylogenetic trees. The priors for the BAMM run was simulated by BAMMtools (Rabosky et al., 2014). Our sampled 66 OTUs are about 72% of the c. 92 known global Suillus OTUs. The employed incomplete sampling of 60% estimated unknown Suillus diversity from unsampled Pinaceae hosts. BAMM was run under the reversible-jump MCMC method with 10 million generations for the calibrated phylogenies of Pinaceae and Suillus. The first 10% was discarded as burn-in and the convergence was checked in coda (Plummer et al., 2008). BAMMtools visualized the output of BAMM to generate the mean phylotrate plot and the 95% credible set of macroevolutionary rate configurations (credible shift sets). If zero rate shift was detected in the phylogeny, the expected number of shifts was not adjusted in BAMMtools to avoid type I error (Rabosky et al., 2014).

**RESULTS**

**Phylogenetic Analyses**

A total of 393 sequences were generated (93 ITS, 89 28S, 90 TEF1, 61 RPB1, 60 RPB2), and 98 sequences were acquired from GenBank (94 ITS, 1 28S, 1 TEF1, 1 RPB1, 1 RPB2) (Supplementary Table 1). The ITS dataset was 834 bp in total length with 313 parsimony informative sites. We added 26 new OTUs representing new species from China and other studies (Verma and Reddy, 2015; Shi et al., 2016). RAxML and Bayesian analyses provided congruent results for the ITS phylogeny (Supplementary Figure 1). Deep relationships of the ITS phylogeny remain unresolved (Supplementary Figure 1). Host and geographic information was inferred from the ITS phylogeny and was applied to the Suillus species level (Supplementary Figure 1 and Supplementary Table 1). Matrices and phylogenetic trees are available in Treebase (number S210962).

For the multigene phylogeny, we sampled 66 OTUs (72% of known Suillus OTUs). Four OTUs contained multiple geographic representatives: Suillus brevispes, Suillus ampliporus, Suillus flavidus, and Suillus spectabilis. For the subsequent multigene analysis, taxa were selected from the ITS phylogeny to evenly represent the taxonomic and geographic diversity within Suillus (Supplementary Figure 1). The total length of the supermatrix was 3914 bp with 858 informative sites. The supermatrix excluding introns was 3138 bp with 626 informative sites and was partitioned as: (1) 285 and the first and second codon positions of TEF1, RPB1, and RPB2, with GTR + I + G as the best model; (2) the third codon positions of TEF1, RPB1, and RPB2 with GTR + G as the best model.

Phylogenies based on the supermatrices resolved the basal relationships of Suillus (Supplementary Figure 2), with five subgenera resolved and supported (Figure 1 and Supplementary Figure 2). The subgenus Boletinus (node D) was basal and sister to all other subgenera. In contrast, the subgenus Spectabilis (node F) was sister to a monophyletic clade (node G) containing all remaining subgenera. The subgenus Larigini was sister to the subgenus Douglasii, and the common ancestor of the two was sister to the subgenus Suillus. We recommend that two new monophyletic sections be recognized for the subgenus Suillus—I and II (Supplementary Figure 4; node M and L).

**Evolutionary History and Host Associations of Suillus**

Step one calibration under the Suillineae scenario at node A (Supplementary Figure 3A) indicated that the crown age of Suillus was c. 40.2 (31.9–50.8) Ma. Subgenera Boletinus,
Spectabilis, and Larigini are all associated with Larix. Subgenus Douglasii switched host to Pseudotsuga at a stem age of c. 28.2 (median, 22.4–34.5) Ma in the mid-Oligocene (Supplementary Figure 4; node H). Another independent host switch, from Larix to Pinus, occurred in the late Eocene to early Oligocene, with a stem age of 33.7 (29.0–39.7) Ma (Supplementary Figure 4; node G). Ancestral host reconstruction revealed that the Pinus association initially occurred in Pinus subgenus Strobus, and later the subgenus Suillus independently switched to subgenus Pinus four separate times. The earliest switch to the subgenus Pinus was in section I at a stem age of 23.1 (18.6–27.8) Ma (Supplementary Figure 4; node N), leading to a relatively prosperous clade associated with subgenus Pinus. All other host switches to subgenus Pinus were in section II, among which the Suillus pinetorum and S. bovinus clade switched first in 18.8 (14.4–23.8) Ma (Supplementary Figure 4; node O). At node Q, a clade consisting of S. tomentosus, S. fuscotomentosus, and S. hirtellus switched hosts in the late Miocene at 6.6 (4.3–9.5) Ma. Lastly, at node R, the Suillus flavidus and S. megaporinus clade switched hosts in the late Miocene at 8.1 (5.0–11.6) Ma. Suillus species switched to Pseudotsuga, while Pinus did not revert to the ancestral Larix association. Host associations with both subgenera of Pinus are reported for S. flavidus and S. acidus but should be further verified. For the North American S. subaureus, an initial association with Pinus subgenus Strobos is required for transferring into Quercus in later developmental stage, therefore Quercus was not included in the BayesTraits analysis (Loftgren et al., 2018). Further, Suillus sinuspaullianus was also excluded from the host reconstruction due to the uncertainty of its host association (Pomerleau and Smith, 1962; Kretzer et al., 1996).

Alternative Calibration Scenario of the Ectomycorrhizal Symbiosis Fungal Fossil

Ancestral host association supported the Suillus-Pinus association in the subgenus Suillus (Clade I, Supplementary Figure 4). The Pinus root ECM fossil can be regarded as the subgenus Suillus, thus provide an alternative calibration scenario (Lepage et al., 1997). The ECM fossil under the Suillus-Pinus scenario was calibrated at the stem of subgenus Suillus (Supplementary Figure 3B, node G). The new calibration revealed an earlier origin of Agaricomycetes in 299.2 (237.7–360.7) Ma versus 159.6 (112.8–262.9) Ma in calibration revealed an earlier origin of Agaricomycetes in 299.2 (237.7–360.7) Ma versus 159.6 (112.8–262.9) Ma in Supplementary Table 2). Further, it found that Suillus diverged from Rhizopogon in the Upper Cretaceous, and the crown age of Suillus was 71.1 (60.6–85.7) Ma (Supplementary Figure 3B and Supplementary Table 2). Suillus switched from Larix to Pinus in Paleocene 54.3 (50.4–62.2) Ma at node G; from Larix to Pseudotsuga in Eocene 46.5 (36.2–56.3) Ma at node H (Supplementary Figure 3B and Supplementary Table 2). Inclusive Suillus tree and ancestral host reconstruction under the Suillus-Pinus scenario are provided in Supplementary Figures 3, 5.

Biogeographic History of Suillus and Pinaceae

The ancestors of Larix and Suillus-Larix were circumboreally distributed (Figure 1 and Supplementary Figure 6). Extant Larix species have distinct ranges within each continent. Suillus has dispersed across the North American and Eurasian continents more frequently than Larix. Disjunct pairs of Suillus were discovered from Oligocene to as recent as the Suillus paluster and S. ampliporus species complexes (Figure 1). The ancestor of Pseudotsuga was circumboreally distributed and diverged into current species with disjunct distributions (Supplementary Figure 6). Three Suillus species were associated with Pseudotsuga menziesii in Western North America (WNA, Figure 1, clade K).

Distinct biogeographic patterns were found in Pinus but not in Suillus associated with Pinus. For the subgenus Strobos, Pinus section Parrya was limited to WNA (Supplementary Figure 6, clade H). In contrast, no Suillus specimens were found in association with Pinus section Parrya. The Pinus section Quinquefoliae originated in Eurasia and dispersed to the New World, with the most recent lineages returning to Eurasia (Supplementary Figure 6, clade I). Suillus that switched host from Larix to sect. Quinquefoliae were reconstructed in the circumboreal region (Figure 1, clade I), with their current distributions influenced by both vicariance and inter-continental dispersal events. Prevalent disjunct Eurasian and North American taxa include S. placidus, S. kwantungensis-S. spraguei, S. decipience-S. phyllophitus, S. subaureus, and S. americanaus. For the subgenus Pinus, section Pinus was distributed in Eurasia, with exception of Pinus resinosa in Eastern North America (ENA), and P. tropicalis in WNA. While the section Trifoliae was mainly distributed in WNA before dispersing to ENA three times for a quarter of its species. For Suillus associated with subgenus Pinus, section Suillus was originally located in the circumboreal region. Clade S retained a basal Eurasian species, dispersed to North America, and then dispersed back to Eurasia. Compared with Pinus, North American lineages in clade S shifted host from section Pinus to section Trifoliae and then to Pinus concomitant with inter-continental dispersal. Originating from a circumboreal ancestor associated with five-needle pines, clade O diverged into a few Eurasian species associated with Pinus section Pinus, and another clade T associated with Pinus section Trifoliae. Finally, clade U includes two disjunct species: Suillus megaporinus from WNA and S. flavidus from North Eurasia.

Speciation Rate Shifts in Suillus and Pinaceae

The LTT plot reveals the constant accumulation of Suillus lineages through time (Supplementary Figure 7). In the phylorate plot, diversification rate shifts were not detected for Suillus (Supplementary Figure 8). The second calibration scenario does not influence this result. Diversification rates (average range 0.12–0.14) were homogenous across Suillus lineages but varied through geological time along the phylogenetic tree branches.
FIGURE 1 | Historical biogeography of Suillus estimated by DEC + J model in BioGeoBEARS. Pie diagrams at each node denote geographical units or combination of units occupied by ancestral taxa. Geographic units are represented by different colors. Combined units are shown by hatching colors or by lettering. Width of pie wedges refers to the probability of that geographic unit or combination of units. White wedges indicate the sum of the units (or combined units) with individual probabilities < 15%. Triangles on branches denote “jump” dispersal events (founder effect cladogenesis), solid triangles indicate range expansion dispersal events (anagenesis), and solid circles by pie diagrams represent vicariant speciation events. Ancestral host associations were annotated at the nodes. Letters by key nodes are discussed in the text. Current geographic ranges of terminal taxa are indicated by colored boxes and lettering. Parentheses indicate the subgenera and section as annotated.
years before switching to the subgenus Pinus calibration scenario. Periods were significantly longer under the second ECM fossil Strobus was associated with the subgenus Strobus lineages were associated with Larix trigger plant defensive responses (Liao et al., 2016). Suillus involves plant pathogenic pathways, and host switching might variations, and experiment over sufficient time to successfully Suillus to a novel host, Suillus should mirror that of Pinaceae in both topology and evolutionary time.

DISCUSSION

Lacking Cospeciation Patterns Between Suillus and Pinaceae

Cospeciation patterns were not identified between the Suillus subgenera and Pinaceae genera (Figure 2). Phylogenetic topologies were distinct between Suillus and Pinaceae. Basal subgenera of Suillus were all associated with Larix. Subgenera Larigni and Douglasii were sister clades, as were their hosts Larix and Pseudotsuga. Yet, given that only one species of Pseudotsuga was the host for subgenus Douglasii, this pattern was not congruent (Murata et al., 2013; Wen et al., 2014). Picea and Cathaya are not documented hosts for Suillus. Picea has been reported as one of the putative host genera for a species within the subgenus Spectabilis, but further studies are required to confirm this (Pomerleau and Smith, 1962). Pinus diverged into two subgenera, while subgenus Suillus diverged into two clades (I and II), which are not congruent with the host subgenera. If cospeciation had occurred between Suillus and Pinaceae, the phylogeny of Suillus should mirror that of Pinaceae in both topology and evolutionary time.

Host Shift Speciation of Suillus

Host shift speciation explained the evolutionary history of Suillus (Figure 1 and Supplementary Figure 4). To effectively adapt to a novel host, Suillus populations need to expand, accumulate variations, and experiment over sufficient time to successfully inoculate (Vienne et al., 2013). Host recognition of Suillus involves plant pathogenic pathways, and host switching might trigger plant defensive responses (Liao et al., 2016). Suillus basal lineages were associated with Larix for c. 20 million years before switching to the subgenus Strobus. Further, the subgenus Suillus was associated with the subgenus Strobus for c. 10 million years before switching to the subgenus Pinus. All these time periods were significantly longer under the second ECM fossil calibration scenario.

Suillus phylogeny did not mirror the phylogeny of Pinaceae, otherwise, host switching from Larix to a more closely related Pseudotsuga would have occurred before the switching to the Pinus subgenus Strobus, i.e., Picea and Cathaya would not have been skipped (Figure 2). The possibility cannot be excluded that extinction may help to explain the current host associations of Suillus, as Suillus could have switched to a now-extinct ancestor of Pseudotsuga, remaining only with Pseudotsuga menziesii (Murata et al., 2013; Wen et al., 2014).

A one-way direction of ECM fungal host subgenera level switching was first observed in Suillus, and this could be a unique trait in host-specific ECM fungi. After Suillus switched hosts from Larix to Pseudotsuga and the subgenus Strobus, they did not reverse to Larix. The same phenomenon is observed in the subgenus Suillus, whereby its host switched from the Pinus subgenus Strobus to the subgenus Pinus. It is plausible that the directional host switches reduce interspecific competition for ecological niches. Suillus species are pioneer ECM fungi for establishing plant seedlings and are generally not dominant in these underground communities (Gardes and Bruns, 1996; Zhou and Hogetsu, 2002; Policelli et al., 2019). Due to this ecological limitation, reducing interspecific competition is evolutionarily advantageous. Interspecific competition could also originate from another source: if different Suillus species inoculate the same host plant, they might compete for the same molecular pathways for symbiotic recognition and establishment (Liao et al., 2016).

Another question worth exploring is how Larix became the ancestral host for the genus Suillus. Truncoolumella, the sister genus of Suillus, is associated with Pseudotsuga and Tsuga (Zeller, 1940; Smith and Singer, 1959; Binder and Hibbett, 2006). The next closely related genus Rhizopogon, which is associated with Pinus, Picea, Pseudotsuga, Larix, Tsuga, Abies, and Picea of Pinaceae, as well as several Angiosperm genera (Molina and Trappe, 1994; Binder and Hibbett, 2006). Both host shift speciation and extinction may explain the basal association with Larix in the genus Suillus.
Uncertainty of the Ectomycorrhizal Symbiosis Fossil for Calibration

As a result of the ephemeral existence and soft tissue of fungal sporocarps, fungal fossils are extremely rare (Berbee and Taylor, 2010). The Eocene ECM fossil of Princeton chert has been widely used in mycological calibrations under the Suillinea scenario (Floudas et al., 2012; Wilson et al., 2012, 2017; Aime et al., 2018; Kraichak et al., 2018). But the application of this fossil for fungal calibration is controversial for the following reasons. The fossil was identified as a *Pinus* root tip with Suillineaee ectomycorrhizae (Lepage et al., 1997). The fossil provided no sporocarp characters so its identity was uncertain. If host association of the ECM fossil is regarded as a prominent character, cautions must be made as other alternative calibration scenarios exist. We tried an alternative calibration scenario of the fossil in the *Suillus-Pinus* lineage. Yet different scenarios of the ECM fossil in the Rhizopogon-*Pinus* lineages have not yet been explored. As *Suillus* does not strictly cospeciate with *Pinus*, it is not applicable to compare the evolutionary history of *Pinus* and *Suillus* to find the preferred scenario.

Comparing Biogeographic Histories of *Suillus* and Pinaceae

The crown age of Pinaceae remains controversial, even using different fossils to calibrate (Wang et al., 2000; Lin et al., 2010; Leslie et al., 2012; Lu et al., 2014; Gernandt et al., 2016; Ran et al., 2018). As the original date of *Suillus* is still debated, rigid comparison between *Suillus* and Pinaceae referring to paleoclimate and geographic histories was refrained. Instead, general biogeographic patterns were compared with emphasis on the disjunct taxa of Eurasia-ENA and Eurasia-WNA. The biogeographic histories of *Suillus* and Pinaceae are generally discordant, as discussed below.

Consistent with other studies, the ancestor of *Larix* and *Pseudotsuga* was reconstructed to be within the circumboreal region (Supplementary Figure 6; Semerikov et al., 2003; Wei and Wang, 2003, 2004). However, other studies have recognized three biogeographic clades of *Larix* with phylogenetic supports: two Eurasian clades and one North American clade (Semerikov et al., 2003; Wei and Wang, 2003, 2004). No distinct geographic clades of *Suillus* associated with *Larix* were found in our study (Figure 1). Dispersions of *Larix* and *Suillus* were likely through the Bering land bridge (BLB) and the North Atlantic land bridge (NALB) (Wang and Ran, 2014; Jiang et al., 2019). North American and East Asian clades of *Pseudotsuga* are supported with phylogenetic data (Wei et al., 2010). Three *Suillus* species were in association with *Pseudotsuga menziesii* in WNA. With no ancient *Suillus* lineages found in the WNA, the host switching to *Pseudotsuga* could have assisted the *Suillus* dispersal to WNA. *Suillus* taxa were anticipated in Asian *Pseudotsuga* trees but were not found after extensive environmental sampling (Murata et al., 2013; Wen et al., 2014).

Vicariance plays a major role in shaping the *Pinus* biogeography, as all major clades of *Pinus* have distinct ranges (Eckert and Hall, 2006; Hao et al., 2015; Gallien et al., 2016). Long-distance dispersal across North America and Eurasia occurred approximately 3–5 times in *Pinus*. In contrast, inter-continental dispersal events influenced the evolution of the *Suillus* subgenus *Suillus*. North American and Eurasian disjunct taxa, arising in different geological epochs, were prevalent in subgenus *Suillus*. WNA is enriched with two *Pinus* sections and half of the *Pinus* diversity. The radiation of *Pinus* in WNA was shaped by the complex climatic history and orogeny of the Rocky Mountains and the Mexican highlands (Mastretta-Yanes et al., 2015; Antonelli et al., 2018; Hagen et al., 2019). In contrast, major clades of subgenus *Suillus* were not limited within biogeographic divisions, and the diversity of *Suillus* in WNA was relatively low. In addition, *Suillus* sporocarps were not yet identified from *Pinus* sections *Parrya*, though *Suillus* was putatively reported from *Pinus edulis* root tip samples (Patterson et al., 2019). *Suillus* might have shifted to the WNA *Pinus* relatively recent in geological time; thus, climatic history and orogeny did not have the same effects on *Suillus*. Alternatively, the ability for *Suillus* to frequently disperse over long distances could have blurred their geographic boundaries.

Diversification Rate Shifts of *Suillus*

The BAMM analysis detected no speciation rate shifts within *Suillus*. Reported diversification rate shifts in fungi usually involve hyper diversified lineages initiated with a key innovation or migration into a significantly different environment (Kraichak et al., 2015; Sánchez-Ramírez et al., 2015; Wilson et al., 2017). Yet, *Suillus* host switches do not involve fundamental changes in living habit or adapting to very different environments. Host *Pinus* and the *Suillus* subgenus *Suillus* follow independent evolutionary trajectories. Overall, *Suillus* are almost absent from the Pinyon pines. Establishing associations with the subgenus *Pinus* lagged until the early Miocene or Eocene; therefore, *Suillus* likely missed the opportunity to diversify extensively in concert with subgenus *Pinus*.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

GM, P-GL, and RZ designed the study. RZ and XF-S performed the experiments. RZ and AW analyzed the data. RZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.831450/full#supplementary-material
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