Biogeographic history of a large clade of ectomycorrhizal fungi, the Russulaceae, in the Neotropics and adjacent regions

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Received: 22 June 2022 Accepted: 23 June 2022

New Phytologist (2022) **236:** 698–713 **doi**: 10.1111/nph.18365

Key words: boreotropical migration, dispersal, diversification, ectomycorrhizal fungi, Neotropics, Patagonia, Russulaceae, vicariance.

Summary

• The biogeography of neotropical fungi remains poorly understood. Here, we reconstruct the origins and diversification of neotropical lineages in one of the largest clades of ectomycorrhizal fungi in the globally widespread family Russulaceae.

• We inferred a supertree of 3285 operational taxonomic units, representing worldwide internal transcribed spacer sequences. We reconstructed biogeographic history and diversification and identified lineages in the Neotropics and adjacent Patagonia.

• The ectomycorrhizal Russulaceae have a tropical African origin. The oldest lineages in tropical South America, most with African sister groups, date to the mid-Eocene, possibly coinciding with a boreotropical migration corridor. There were several transatlantic dispersal events from Africa more recently. Andean and Central American lineages mostly have north-temperate origins and are associated with North Andean uplift and the general north-south biotic interchange across the Panama isthmus, respectively. Patagonian lineages have Australasian affinities. Diversification rates in tropical South America and other tropical areas are lower than in temperate areas.

• Neotropical Russulaceae have multiple biogeographic origins since the mid-Eocene involving dispersal and co-migration. Discontinuous distributions of host plants may explain low diversification rates of tropical lowland ectomycorrhizal fungi. Deeply diverging neotropical fungal lineages need to be better documented.

Introduction

A fundamental challenge of evolutionary biology is to determine the drivers of the exceptional neotropical biodiversity (Antonelli & Sanmartín, 2011; Hughes *et al.*, 2013; Antonelli *et al.*, 2018; Palma-Silva *et al.*, 2022). Phylogenetic biogeography has shown how continental-scale events such as the rise of the Andes (Antonelli *et al.*, 2009; Hoorn *et al.*, 2010; Pérez-Escobar *et al.*, 2022), biotic interchange between North and South America (Cody *et al.*, 2010; Bacon *et al.*, 2015), and transoceanic dispersal, including across the Atlantic from Africa (Pennington & Dick, 2004; Renner, 2004), have shaped extant diversity of neotropical plants and animals. Though some historical biogeographic studies have included neotropical fungi (Matheny *et al.*, 2009; Leavitt *et al.*, 2012; Wilson *et al.*, 2012; Tedersoo *et al.*, 2014b; Harrower *et al.*, 2015; Amalfi, 2016), more are needed given the ecological importance of fungi as saprotrophs, pathogens, or mutualistic symbionts, which can influence the distribution of associated plants and vice versa (Peay *et al.*, 2016).

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Ectomycorrhizal (ECM) fungi are obligate mutualistic root symbionts, mainly of trees (Smith & Read, 2010; Tedersoo et al., 2020). They have global ecosystem importance by sustaining the biomass of the majority of trees worldwide (Soudzilovskaia et al., 2019; Steidinger et al., 2019). Unlike the majority of organisms, most ECM fungal groups peak in diversity and abundance in temperate latitudinal zones (Tedersoo & Nara, 2010; Tedersoo et al., 2014a; Steidinger et al., 2019). This suggests that they may have originated and have a longer history and/or have diversified faster in the temperate zone. Nonetheless, ECM trees are dominant in some tropical forests, where the symbiosis plays a key role in nutrient cycling and competitive abilities (Henkel, 2003; McGuire, 2007; Corrales et al., 2016, 2018; Carriconde et al., 2019; Henkel & Mayor, 2019; Hall et al., 2020). The importance of ECM fungi in the Neotropics is exemplified by the recent discoveries of high-diversity sites with many undescribed species (e.g. Henkel et al., 2012; Roy et al., 2016; Vasco-Palacios et al., 2018; Delgat et al., 2020; Corrales & Ovrebo, 2021). ECM fungi thus form an important component of many neotropical ecosystems.

The current geographic distribution of ECM fungi is the product of environment and host plant presence (van der Linde et al., 2018), as well as historical contingencies such as area of origin and dispersal limitation (Peay et al., 2016). The Neotropics contain phylogenetically diverse ECM host plants distributed across a variety of habitats and elevation zones, suggesting distinct biogeographic ECM domains. For example, ECM host plants in the neotropical lowlands are from predominantly tropical lineages in the Fabaceae, Cistaceae, Dipterocarpaceae, Polygonaceae, and Nyctaginaceae, whereas mainly north-temperate Betulaceae, Fagaceae, and Juglandaceae occur at higher elevations in the Andes or Central America (Tedersoo, 2017; Tedersoo & Brundrett, 2017; Corrales et al., 2018). Patagonia, in southern South America, has yet another dominant host lineage, the Nothofagaceae, whose associated macromycota is quite removed from that of northern South America (Singer, 1953; Trierveiler-Pereira et al., 2014), although Nothofagaceae were probably present at tropical latitudes in South America in the Eocene (Jaramillo et al., 2006).

Though many ECM fungal lineages have broad distributions (Tedersoo et al., 2014a), the drivers of their biogeographic spread have been difficult to identify. A major problem is distinguishing between continental vicariance, overland migration, or overseas dispersal. Moyersoen (2006) hypothesized that Africa-South American (i.e. Gondwanan) vicariance, between 120 and 90 million years ago (Ma) (Müller et al., 2016), explained the presence of Pakaraimaea, a phylogenetically distinct genus of ECM host plants, in South America. Africa-South America vicariance has, however, been refuted for most other groups of plants (Pennington & Dick, 2004), including the pantropical ECM gymnosperm Gnetum (Won & Renner, 2006). It was also rejected for the ECM fungal genus Inocybe (Matheny et al., 2009). In turn, vicariance is likely responsible for the Australian-Patagonian (i.e. southern Gondwanan) disjunction of Nothofagaceae (Cook & Crisp, 2005). An alternative explanation, boreotropical migration across land routes when Palaeocene/Eocene tropical climates

extended into high latitudes (Wolfe, 1975), was suggested for disjunctions in the north-temperate host lineage Juglandaceae (Zhang *et al.*, 2022) and the ECM fungal groups *Amanita* sect. *Caesarea* (Sánchez-Ramírez *et al.*, 2014) and Sclerodermatinae (Wilson *et al.*, 2012). Over the past 20 Myr, narrowing and closure of the Panama isthmus facilitated north–south dispersal (Bacon *et al.*, 2015; O'Dea *et al.*, 2016), and the rapid rise of the northern Andes 5–8 Ma created high-altitude environments for north-temperate arrivals (Pérez-Escobar *et al.*, 2022). The southward extensions of the north-temperate ECM host genera *Alnus* and *Quercus* into the Andes are examples of this (Tedersoo, 2017).

An important group of mushroom-forming ECM fungi is found in the Russulaceae (Russulales, Agaricomycetes, Basidiomycota), a speciose family that is globally distributed over low and high latitudes wherever ECM vegetation is found. Recent systematic work has clarified Russulaceae relationships (Buyck et al., 2008, 2018; Verbeken et al., 2014; Wisitrassameewong et al., 2016; De Crop et al., 2017; Wang et al., 2018): the four mushroom-forming genera - Lactarius, Lactifluus, Multifurca, and Russula, along with several nested sequestrate genera - have >4500 species (He et al., 2019) and form a monophyletic ECM lineage emerging from a small grade of saprotrophic genera (Looney et al., 2018, 2022). For simplicity, we refer to this ECM lineage (equivalent to '/russula-lactarius' sensu Tedersoo et al., 2010a) as 'Russulaceae' in the following. Russulaceae are routinely recovered in sporocarp and DNA-based surveys, including in the Neotropics (Tedersoo et al., 2010b; Tedersoo & Nara, 2010; Smith et al., 2011; Henkel et al., 2012; Roy et al., 2016; Vasco-Palacios et al., 2018; Corrales & Ovrebo, 2021). They associate with most known ECM plant lineages (Tedersoo & Brundrett, 2017), as well as orchids (Dearnaley, 2007).

Most species of Russulaceae are temperate, including boreal, in distribution. This includes the majority of the largest genus Russula, for which a temperate origin and a higher temperate diversification rate relative to the Tropics has been inferred (Looney et al., 2016). However, a tropical African origin has long been posited for Russulaceae based on endemism and morphological distinctiveness (Pirozynski, 1983; Buyck et al., 1996). In the wider Neotropics, Russulaceae occur in all highland and lowland regions where ECM vegetation is found, and modern work has revealed a plethora of new species (Buyck & Ovrebo, 2002; Miller et al., 2002, 2012; Wartchow & Cavalcanti, 2010; Cheype & Campo, 2012; Sà et al., 2013, 2019; Sà & Wartchow, 2013, 2016; Wartchow et al., 2013; Montoya et al., 2014; Trierveiler-Pereira et al., 2014; Crous et al., 2017; Delgat et al., 2020; Duque Barbosa et al., 2020; Silva-Filho et al., 2020; Manz et al., 2021; Vera et al., 2021). Several lowland neotropical Russulaceae have affinities with tropical African species (Buyck, 1990; Buyck & Ovrebo, 2002; De Crop et al., 2017). Molecular divergence time estimates vary, however. A recent phylogenomic study (Looney et al., 2022) estimated a crown age of c. 60 Myr (uncertainty range 57-64 Myr) for the ECM Russulaceae, which places its diversification firmly after Gondwanan breakup. Prior estimates of Looney et al.(2016) and Varga et al.(2019) (58 and 83 Myr, respectively) also supported a post-Gondwanan scenario.

A much older estimate of 188 Myr (Sánchez-García *et al.*, 2020) appears to be an outlier (Fig. 1), pre-dating even the radiation of the oldest ECM host plant lineage, Pinaceae, in the Late Jurassic (Tedersoo, 2017). Here, we assume that the ECM Russulaceae diversified when South America was already separated from Africa, and we reconstruct their biogeographic history in the Neotropics under this scenario.

To accomplish this, we generated a time-calibrated phylogeny by supplementing a backbone tree of globally representative sequences with new data from a large, collaborative sampling effort focusing on the Neotropics. The resulting supertree was used to estimate range evolution and diversification. We tested the following hypotheses regarding neotropical Russulaceae:

(1) Tropical South American lowlands host old lineages that are related to tropical African taxa, whereas the Andes and the adjacent Patagonia have younger lineages unrelated to tropical low-land taxa.

(2) Dispersal into the Neotropics coincided with Palaeocene and Eocene boreotropical conditions and the closure of the Panama isthmus.

(3) Tropical South America and with other tropical areas have a lower diversification rate than temperate areas.

Materials and Methods

Sample collection and sequencing

We aimed to generate a phylogenetic tree of Russulaceae that is globally representative and well sampled for the Neotropics. We collected sporocarps and ECM root tips, and obtained samples from dried fungarium specimens, from several neotropical and neighbouring countries: Belize, Brazil, British Virgin Islands, Chile, Colombia, Costa Rica, Ecuador, French Guiana, Guadeloupe, Guyana, Martinique, Panama, and Puerto Rico. Tissue samples were preserved in $2\times$ cetyltrimethyl ammonium



Fig. 1 Overview of published crown age estimates for the ectomycorrhizal Russulaceae (*Lactarius*, *Lactifluus*, *Multifurca*, and *Russula*). The Looney *et al*. (2022) estimate (in bold) was used for calibration in this study. Dots are median values/point estimates, error bars represent 95% posterior density intervals (Looney *et al.*, 2016, 2022) and the range in 10 calibrated trees (Varga *et al.*, 2019).

bromide solution. DNA was extracted using a Wizard Genomic DNA Purification kit (Promega). We were able to amplify the internal transcribed spacer (ITS) region from 241 specimens, using standard primers and protocols (Gardes & Bruns, 1993). In addition, partial large ribosomal subunit (LSU; primers CTB6+TW14 (White *et al.*, 1990), or LR0R+LR7 (Vilgalys & Hester, 1990)) and partial RNA polymerase II gene, second largest subunit (*rpb2*; primers fRPB2-5F+bRPB2-7cR (Liu *et al.*, 1999)), were amplified for 222 and 43 samples, respectively. Amplicons were then Sanger-sequenced (GATC Biotech, Konstanz, Germany, or Macrogen, Lille, France) and chromatograms edited in GENEIOUS v.6 (Biomatters, Auckland, New Zealand).

Internal transcribed spacer dataset assembly

To produce a tree that would include as much diversity of Russulaceae as possible, including undescribed taxa and environmental samples, we used an approach based on operational taxonomic units (OTUs) similar to that of Looney *et al.*(2016).

We downloaded all 35 944 ITS sequence records annotated as Russulaceae (as of August 2021) on the International Nucleotide Sequence Database Collaboration or the fungal sequence database UNITE (Kõljalg et al., 2013) using the PLUTOF web platform (https://plutof.ut.ee). Sequences annotated as 'chimeric' were excluded, and we added our 241 new ITS sequences. The ITS1-5.8S-ITS2 region was extracted from this set using ITSx v.1.1.3 (Bengtsson-Palme et al., 2013), keeping only matches at least 50 bp long. Nineteen sequences associated with backbone tips (see later) that did not pass this filtering step were added back in. The resulting set of 29 479 sequences was then clustered into OTUs using VSEARCH v.2.9.1 (Rognes et al., 2016) with the cluster representative being the longest sequence (--cluster_fast option). We used a 97% identity threshold, which is probably conservative (i.e. lumping species) but appropriate for the macroevolutionary scale of our study. Sequences clustered into 3543 OTUs, and their representative sequences were used for subsequent analyses.

Phylogenetics

A 'supertree' approach was used, estimating first a backbone phylogeny using conserved markers and then inserting backboneconstrained, genus-level phylogenies estimated from the ITS sequences. For the backbone, we first assembled a set of nuclear LSU (nrLSU) (28S), *rpb1* (RNA polymerase II gene, largest subunit), and *rpb2* sequences from 437 accessions of Russulaceae and seven outgroup Russulales, based on previous studies (Buyck *et al.*, 2008, 2018, 2020; Verbeken *et al.*, 2014; Looney *et al.*, 2016; De Crop *et al.*, 2017; Wang *et al.*, 2018). We added 28 of the newly sequenced Russulaceae for which nrLSU and/or *rbp2* were amplified successfully. In the backbone accessions, 324 matched accessions in the ITS dataset and were used to constrain the genus phylogenies (see later). Sequences for each locus were aligned with MAFFT/E-INS-I v.7.407 (Katoh & Standley, 2013), end columns with >90% missing data were trimmed, and alignments then concatenated. A maximum likelihood phylogeny was estimated using RAxML v.8 (Stamatakis, 2014), a GTR+ Γ substitution model for each of the LSU, *rpb1*, and *rpb2* partitions, and 1000 rapid bootstrap searches. Transfer bootstrap expectation (TBE) was calculated in addition to traditional Felsenstein bootstrap support, which is sensitive to 'rogue taxa' in large datasets (Lemoine *et al.*, 2018).

We then estimated phylogenies from the ITS dataset of representative OTU sequences. To improve alignment and tree estimation, we divided them into three genus datasets (combining the small Multifurca with its sister Lactarius). The ITS sequences were searched via BLASTN (Altschul et al., 1990) against the ITS sequences associated with backbone tips to assign them to a genus dataset. Sequences with <70% similarity to a backbone ITS sequence and only a single OTU member were discarded as likely chimeric or mislabelled as Russulaceae. Rapid alignments and trees were made with MAFFT (automatic option) and FASTTREE v.2.1.10 (Price et al., 2010) for visual inspection. We removed a further three sequences that appeared as conspicuously long branches. The final set of ITS sequences per genus was then aligned (with suitable outgroup sequences from the other genera) using MAFFT/E-INS-I. Alignments were trimmed and RAxML trees inferred as described for the backbone, specifying ITS1, 5.8S, and ITS2 partitions; splits that had a TBE support of 0.7 or higher in the backbone tree were constrained in the tree search.

To generate a single Russulaceae supertree, we first timecalibrated the backbone tree, with outgroups removed, using penalized likelihood in TREEPL v.1.0 (Smith & O'Meara, 2012). The smoothing parameter value was selected through randomsubsample-and-replicate cross-validation for orders of magnitude between 10^{-1} and 10^{3} . In the absence of known Russulaceae fossils, we fixed the crown age to 1. Subclade trees were timecalibrated in the same manner (with a further 83 long-branch tips removed) and then inserted into the backbone tree using the 'bind.tree' function in R, scaling branch lengths relative to the backbone crown age of the clade replaced. To provide an absolute timescale, we report ages obtained when multiplying branch lengths with the age estimate of Looney et al. (2022); that is, 60 Myr with a range of 57-64 Myr (Fig. 1). The final supertree contained 3285 tips, corresponding to OTUs identified in the ITS dataset and representing 29 167 ITS sequences.

For phylogenetic tree manipulation and plotting, we used the packages APE v.5.3 (Paradis & Schliep, 2019), GEIGER v.2.0.6.1 (Harmon *et al.*, 2008), PHYTOOLS v.0.6.60 (Revell, 2011), and PLOTRIX v.3.7.5 (Lemon, 2006) in R v.3.6.1.

Biogeographic areas

We compared the biogeographic histories of Russulaceae among parts of the Neotropics and adjacent regions that feature different ECM host plant assemblages (Tedersoo, 2017; Nouhra *et al.*, 2019; Delgat *et al.*, 2020) (Fig. 1a): (1) Central America/ Caribbean, with both tropical-origin ECM host plant lineages such as *Coccoloba* or Nyctaginaceae tribe Pisonieae and temperate-origin Fagaceae, Juglandaceae, and Pinaceae. (2) The Andes, with the temperate-derived *Alnus acuminata*, widespread in the central part of the range, and *Quercus humboldtii*, restricted to northern Colombia, and some lowland Nyctaginaceae that reach the montane Yungas (Geml *et al.*, 2014). (3) Lowland tropical South America, with several distinct, tropical host plant groups in the Cistaceae, Fabaceae, Polygonaceae, Nyctaginaceae, Dipterocarpaceae (e.g. *Dicymbe, Aldina, Coccoloba, Guapira, Neea, Pakaraimaea, Pseudomonotes*). (4) Patagonia, with Nothofagaceae (Tedersoo, 2017). We defined these areas by merging the corresponding ecoregions of Morrone (2014, 2015), using the shapefiles of Löwenberg-Neto (2014, 2015). The rest of the global range of Russulaceae was divided into the five broad regions Afrotropics, Australasia with Oceania, Indomalaya, Nearctic, and Palaearctic (Fig. 1a; Dinerstein *et al.*, 2017).

Operational taxonomic units were assigned to one or more of these areas first by the country recorded for each ITS sequence clustering with the OTU, if this was unambiguous. We then used, in the following order, sampling coordinates, area descriptions geocoded with the 'geocode_OSM' R function, and information from the original literature associated to the record (for some of the remaining unassigned tips) to assign remaining sequences, using the R packages SF (Pebesma, 2018) and TMAP-TOOLS (Tennekes, 2020). A total of 25 937 sequences (88.9% of those represented in the supertree) and 3153 OTUs (96%) could be assigned in this way (Table 1). We plotted the tip areas against the tree for visual inspection. In five instances, the placement (isolated tropical South American or Patagonian tip inside a north-temperate clade) and the host and sampling metadata suggested introduced occurrences; these areas were ignored.

We summarized the overlap in OTUs between areas to assess recent dispersal and compared it with estimates of more ancient dispersal (see later).

Biogeographic modelling

We estimated ancestral areas using a simple Markov model of trait evolution. The frequently used dispersal-extinction-

Table 1 Overview of internal transcribed spacer (ITS) sequences andoperational taxonomic units (OTUs) by geographic area.

Area	ITS sequences		OTUs						
Focal									
Andes	46	0.2%	22	0.7%					
Central America/Caribbean	514	1.8%	213	6.5%					
Patagonia	16	0.1%	9	0.3%					
Tropical S. America	303	1%	109	3.3%					
Nonfocal									
Afrotropic	1055	3.6%	417	12.7%					
Australasia and Oceania	445	1.5%	226	6.9%					
Indomalayan	1585	5.4%	659	20.1%					
Nearctic	4550	15.6%	737	22.4%					
Palearctic	17 423	59.7%	1293	39.4%					
Unassigned	3230	11.1%	132	4%					
Total	29 167		3285						

The numbers shown are for sequences represented in the final Russulaceae supertree, after various filtering steps. Note that OTU numbers and percentages do not sum to 100% as one OTU can occur in several areas.

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cladogenesis model (Ree *et al.*, 2005) proved too computationally expensive for our phylogeny and also has various issues (Ree & Sanmartín, 2018). We fitted a one-parameter trait evolution model to the supertree and the tip areas assigned in the CORHMM R package v.2.7 (Beaulieu *et al.*, 2021), which can handle polymorphic tips and missing values.

With the estimated ancestral areas, we summarized lineages that have a relative likelihood of > 0.5 to occur in one of the four focal areas. We recovered their stem ages (divergence from groups outside the focal areas) and, if applicable, crown ages (first divergence within the focal areas). We then assessed, under the two ages scenarios, whether these ages coincide with the following biogeographic events: the split between South America and Antarctica, 50 Ma (van de Lagemaat *et al.*, 2021); boreotropical conditions during the Palaeocene and Eocene (Wolfe, 1975); rapid north Andean uplift, 8–5 Ma (Pérez-Escobar *et al.*, 2022); and the Panama isthmus biotic interchange, beginning 20 Ma (O'Dea *et al.*, 2016).

To count the number of dispersal events (i.e. area state changes) and infer dispersal rates *post hoc*, we stochastically mapped area evolution histories on the supertree 100 times using the 'makeSimmap' function in CORHMM. From these stochastic maps, we obtained median values and 95% quantile ranges of dispersal counts between areas. We also summarized dispersal counts to and from each area over time. For this, we used 10 equal-sized time windows since the origin of Russulaceae (representing 6 Myr windows under our calibration), which proved a reasonable compromise between detecting patterns in time vs uncertainty (i.e. the smaller the windows, the more uncertainty there is around the event counts).

Diversification analysis

Diversification rates were estimated for the full tree using BAMM v.2.5 (Rabosky, 2014). Critique, especially of earlier versions of BAMM (Moore *et al.*, 2016), has been addressed (Rabosky *et al.*, 2017); BAMM was best suited to our purpose as we needed specific per-branch rates for comparing diversification rates per area (see later). We ran BAMM for 200 million Markov chain Monte Carlo generations and sampling every 10 000th generation. Effective sample sizes were ensured to be > 200 using the R package CODA (Plummer *et al.*, 2006), and the first 20% of posterior samples were discarded as burn-in. Note that the conservative ITS clustering cut-off likely underestimated the number of species, and thus the most recent diversification rates.

In the absence of a feasible method to jointly model diversification and range evolution (Goldberg *et al.*, 2011) for such a large phylogeny and multiple areas, we partitioned per-branch diversification rates by area in an approach similar to Chazot *et al.*(2021). We randomly paired the BAMM posterior samples with the area evolution stochastic maps. For each pair, the tree branches were divided into segments with the same range and diversification regime and no longer than 2% of the root height. We then calculated the mean and 95% credible interval of diversification rate per area, both overall and for each of 20 equal-sized time windows. The R code for this is available in the online repository.

Results

Internal transcribed spacer sequences and occurrence in biogeographic areas

We compiled a dataset of 29 167 global ITS sequences of the ectomycorrhizal Russulaceae clade. These were obtained through a series of filtering steps applied to 35 944 sequences retrieved from public databases, combined with 241 new sequences generated for neotropical taxa. These sequences clustered into 3285 OTUs at a 97% similarity threshold, represented as tips in the Russulaceae supertree (Table 1), with 2234 belonging to *Russula*, 565 to *Lactarius*, 472 to *Lactifluus*, and 14 to *Multifurca*. Of these, 879 sequences and 353 OTUs could be assigned to one of four focal areas of the Neotropics and adjacent regions, with Central America/Caribbean having the highest numbers. Most sequences and OTUs in the dataset were assigned to the Palaearctic and Nearctic areas, respectively. The Palaearctic was overrepresented among sequences compared with OTUs (59.7% vs 39.4%), indicating a higher sampling depth in this area than in the others.

Russulaceae phylogeny and divergence times

We inferred a 3285-OTU Russulaceae supertree (Fig. 2b) based on ITS phylogenies constrained with a backbone phylogeny. The 444-tip backbone phylogeny we inferred from LSU, rpb1, and rpb2 data represents all named subgenera of the four Russulaceae genera (Supporting Information Fig. S1). Relationships among Russula subgenera were difficult to resolve in previous studies (Looney et al., 2016; Bazzicalupo et al., 2017; Buyck et al., 2018, 2020). We recovered low Felsenstein bootstrap, but high TBE, support among Russula subgenera and subg. Heterophyllidia as sister to the other subgenera. The backbone tree also suggests - based on a single LSU sequence - that the tropical South American Russula campinensis is sister to the rest of the genus and does not fall in any of the subgenera described. In Lactarius, the poorly defined subgenus Russularia is paraphyletic, and several unclassified lineages diverged earlier than the named subgenera, as found previously (Verbeken et al., 2014; Wisitrassameewong et al., 2016). The backbone notably supported an undescribed tropical South American species from Guyana (OTU KC155399) as sister to the remainder of Lactarius. Within Lactifluus, our backbone differed from relationships found previously (De Crop et al., 2017; Delgat et al., 2020) in finding TBE support for sister relationships between both subg. Lactariopsis/subg. Pseudogymnocarpi and subg. Lactifluus/subg. Gymnocarpi.

Under the age scenario of Looney *et al.*(2022), the estimated crown age of the largest genus *Russula* was *c*. 56 Myr (uncertainty range 53–59 Myr), followed by *Lactifluus* at 50 Myr (47–53 Myr), *Lactarius* at 42 Myr (40–45 Myr), and *Multifurca* at 34 Myr (32–36 Myr) (Fig. 2; see also the calibrated backbone in Fig. S1).



Fig. 2 Ancestral range estimation and neotropical lineages of ectomycorrhizal Russulaceae (*Lactarius*, *Lactifluus*, *Multifurca* and *Russula*). (a) Defined areas, including the four focal areas (enlarged inset): Afr, Afrotropics; And, Andes; Aus, Australasia; CAm, Central America/Caribbean; Ind, Indomalaya; Nea, Nearctic; Pal, Palaearctic. (b) Dated supertree of the ectomycorrhizal Russulaceae with inferred ancestral ranges. The 3285 tips represent 29 167 internal transcribed spacer sequences clustered into operational taxonomic units. Ages are based on the previous estimate of 60 Myr (uncertainty range 47–64 Myr; Looney *et al.*, 2022) for the crown age of the ectomycorrhizal Russulaceae. Ancestral ranges were estimated under a one-parameter trait evolution model integrating over multiple-state tips. Branches are coloured by the area with the highest relative likelihood as part of the inferred ancestral range at the child node. Relative likelihoods for ancestral areas are given as pie charts for genera, subgenera, and the Russulaceae crown node. Coloured dots indicate the stem branches of 110 lineages with an estimated origin in one of the focal areas (grey indicates that none of two or more focal areas has a relative likelihood of >0.5). (c) Stem ages and crown ages (where applicable) of 110 lineages with an estimated origin in one of the focal areas. Grey polygons and horizontal lines indicate major biogeographic events in the region.

Biogeographic origins and neotropical lineages

The Afrotropics were supported as the most likely ancestral area of Russulaceae in our biogeographic analysis (Fig. 2; see also all tips/OTUs detailed in Fig. S3). This was also the case for each of the three largest genera, *Lactarius, Lactifluus,* and *Russula,* with relative likelihoods of > 0.95 for the Afrotropics at these nodes. The earliest diverging clades at subgenus level in these three genera were also each estimated as Afrotropic in origin, except *Russula* subgenera *Russula* and *Crassotunicata* (Fig. S1a).

We found 110 distinct Russulaceae lineages occurring in the wider Neotropics, of which 76 are single-OTU lineages and 34 clades with more than one OTU (Fig. 2b,c; Table 2; see also lineages numbered in Fig. S3). The oldest lineages are found in tropical South America, with the oldest stem age at *c*. 42 Myr (40–45 Myr), indicating arrival in the mid-Eocene at the earliest. Some of these clades have spread to other areas: some Central American/Caribbean clades included tropical South America OTUs, and vice versa (e.g. nos. 53, 60, 66 and 73 in *Russula*, and nos. 106, 107 and 110 in *Lactifluus*; Fig. S3), whereas one tropical South American clade of *Lactifluus* (no. 106) included Australasian taxa. Estimated ages of these lineages allow us to assess whether they overlap with major biogeographic events that have shaped the regional biota (Fig. 2c).

The seven Andean lineages in *Russula, Lactarius*, and *Lactifluus* all appeared to have immigrated relatively recently, with no diversification *in situ*. The stem ages of all but one lineage (which appears as an old outlier) coincide with or are younger than the rapid north Andean uplift 5–8 Ma. These Andean lineages mainly emerge from Nearctic and/or Palaearctic clades, consistent with north-to-south migration. Only two Nyctaginaceae-associated OTUs from the Yungas emerge from lowland tropical South American clades (*Lactifluus* UDB004277¦L6094 and *Russula* UDB004278|L6090c; Fig. S3). There were also 12 Andean OTUs shared with other areas, mainly Central America/Caribbean and the Nearctic (Fig. 3a), suggesting very recent immigration into the Andes.

The four Patagonian lineages (*Russula*: nos. 7, 8 and 61; *Lactarius*: no. 94; Fig. S3c,r,ae) diverged at different times since *c*. 18 Ma and much more recently than the South America–Antarctica split. All four emerge from within or are sister to Australasian clades. Note that ITS sequences appear to be the only record of *Lactarius* associated with *Nothofagus* – based on sequence metadata – in the Patagonian region, as the genus was not listed in a previous overview (Barroetaveña *et al.*, 2019). These and their closest relatives from Australasia represent a yet undescribed clade of *Lactarius*.

Table 2 Overview of Russulaceae lineages in the Neotr	opics and adjacent regions.
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Area	No. of lineages	Of which clades	Oldest crown ages (Myr)	Oldest stem age (Myr)
Andes	7	0	_	16.3 (15.4–17.3)
Central America/Caribbean	80	16	13.1 (12.5–14)	26.3 (25–28.1)
Patagonia	4	3	11.8 (11.2–12.5)	17.8 (16.9–18.9)
Tropical South America	19	15	29.9 (28.4–31.9)	42.4 (40.3–45.2)

These represent lineages that have an ancestral relative likelihood of > 0.5 to occur in the area but may also occur in other areas. Ages are based on the previous estimate of 60 Myr (uncertainty range 47–64 Myr; Looney *et al.*, 2022) for the crown age of the ectomycorrhizal Russulaceae. Values in parentheses are age uncertainty ranges based on the 95% highest posterior density interval (57–64 Myr) of Looney *et al.* (2022).



Fig. 3 Connectivity between biogeographic areas in the ectomycorrhizal Russulaceae (*Lactarius, Lactifluus, Multifurca,* and *Russula*). (a) Overlap in operational taxonomic units (OTUs) between areas. Edge thickness is proportional to the number of shared OTUs. (b) Inferred numbers of dispersal events between areas. Edge thickness is proportional to the number of dispersals. Edges were only drawn for rates whose 95% quantile range excludes zero. The counts are mean numbers of dispersals inferred from stochastic mapping under the estimated dispersal/area transition rate.

The 80 lineages from Central America/Caribbean occurred in *Russula, Lactarius*, and *Lactifluus*. Whereas *Multifurca* occurs in Central American oak forests (Montoya *et al.*, 2003; Wang *et al.*, 2018), no ITS sequence assignable to that area was available. None of the lineages is old enough to have diverged under Palaeocene boreotropical conditions. All but three ages are coincident with the increased biotic interchange across the Panama isthmus. The large majority of Central American/Caribbean lineages emerged from north-temperate clades, whereas some occurred in clades with South American relatives.

Of the 19 lowland tropical South American lineages, several branched deeply at subgenus level. In *Russula*, a clade composed of *R. campinensis* (an unusual pleurotoid species from lowland tropical South America; Henkel *et al.*, 2000) and three related OTUs branched at *c.* 33 Ma (no. 82, Fig. S3x). Together with OTUs from several other areas, this group is sister to the remainder of *Russula*. From Guyana, KC155399 with two sequences (no. 95) represents an undescribed lineage of *Lactarius* sister to the rest of the genus (Fig. S3a). Another well-supported ancient lowland tropical South American clade of *Lactarius*, our tree places a lowland tropical South American clade with *Lactifluus*, *ceraceus* and related OTUs (no. 105, Fig. S3a) sister to subg. *Lactifluus* and not in subg. *Pseudogymnocarpi* (Crous *et al.*, 2017).

All lowland tropical South American lineages diverged well after the Africa-South America split. However, several of these

old lineages were sister to clades with an estimated African origin (e.g. nos. 42, 62 and 82 in *Russula*; no. 95 in *Lactarius*; nos. 105, 106 and 101 in *Lactifluus*). Boreotropical migration can potentially account for these relationships in seven lineages that diverged in the Eocene. The other 12 lineages were too young for boreotropical migration. The youngest of these is a South American lineage clearly emerging from within an African clade (no. 63, *Russula puiggarii* and relatives) at *c.* 9 Ma, strongly suggesting direct dispersal across the Atlantic.

Dispersal to and from the Neotropics

We summarized overlap in OTUs between areas, representing recent dispersal, and also estimated past dispersal events between them (Fig. 3). Two higher latitude areas, Nearctic and Palaearctic, have the highest OTU overlap (Fig. 3a) and the highest estimated dispersal count (Fig. 3b), followed by the Palaearctic and Indomalaya. Patagonia shared no OTU with any of the focal areas of the Neotropics. It, however, had possible ancient dispersal links with several other areas, which may reflect the uncertainty in simulating dispersal routes along branches, as plotting of ancestral areas strongly suggested the Patagonian lineages are related to Australasian lineages, as already noted herein. The Andes were most strongly linked with the Nearctic and Central America/Caribbean. Central America/Caribbean was most strongly linked with the Nearctic and Palaearctic. Lowland tropical South America only shared 25

(a) Andes

Lf

La

Ru







A tropical African origin of ectomycorrhizal Russulaceae but fastest diversification in the temperate zone

We estimated that the speciose, globally distributed ECM clade in Russulaceae, as well its three largest genera, likely originated in tropical Africa (Fig. 1). An Afrotropical origin for Russulaceae had long been postulated (Pirozynski, 1983; Buyck et al., 1996) but a biogeographic analysis of the largest genus, Russula (Looney et al., 2016), suggested a temperate origin for that genus. This discrepancy for Russula may be explained by increased collection and sequencing having led to tropical taxa now being better represented in phylogenetic analyses (Buyck et al., 2018). Early diverging tropical lineages in Russula were revealed in our study, possibly resulting from our larger sampling of ITS sequences - 18778 ITS sequences compared with 3348 in Looney et al.(2016). Unstable relationships among the major lineages of Russula were found in previous studies (Looney et al., 2016; Bazzicalupo et al., 2017; Buyck et al., 2018, 2020). The fact that we inferred African origins for most subgenera within Russula, however, supports an overall Africa origin of Russula even if the true relationships were different. Thus, results for both the largest genus, Russula, and



Fig. 4 Dispersal to and from the Neotropics through time in the ectomycorrhizal Russulaceae (*Lactarius, Lactifluus, Multifurca* and *Russula*). The counts are median numbers of dispersals inferred from stochastic mapping for 10 equally spaced time periods between the root of Russulaceae and the present. Values are given for (a–d) the four focal areas and (e) specifically for dispersal between the Afrotropics and tropical South America. Shaded areas are 95% quantile ranges. Ages are based on the previous estimate of 60 Myr (uncertainty range 47–64 Myr; Looney *et al.*, 2022) for the crown age of the ectomycorrhizal Russulaceae. Crown ages of the four genera are indicated for reference (Ru, *Russula*; Lf, *Lactifluus*; La, *Lactarius*; Mu, *Multifurca*). Ma, million years ago.

OTUs with Central America/Caribbean but had most dispersal links with the Afrotropics.

We also summarized Russulaceae immigration and emigration for the neotropical focal areas (Fig. 4). All areas showed an



the earliest diverging genus, *Lactifluus*, further support the overall African origin of Russulaceae.

Tropical origins have been inferred for several ECM fungal lineages (Matheny *et al.*, 2009; Dentinger *et al.*, 2010; Kennedy *et al.*, 2012; Sánchez-Ramírez *et al.*, 2014; Han *et al.*, 2018). In many of these groups, including Russulaceae, the greater species diversity in higher latitude regions therefore likely results from more recent, faster diversification relative to the Tropics. This is supported by our results of highest rates in the Nearctic and Palaearctic and the lowest in the Afrotropics and tropical South America (Fig. 5b), where it declined over time (Fig. 6). Most likely, the vast temperate and boreal forests dominated by ECM host lineages, such as Fagaceae and Pinaceae, offered opportunities for rapid diversification of lineages, as suggested previously (Bruns *et al.*, 1998; Ryberg & Matheny, 2012; Looney *et al.*, 2016). By contrast, the discontinuous, Fig. 5 Net diversification across clades and areas in the ectomycorrhizal (ECM) Russulaceae (Lactarius, Lactifluus, Multifurca, and Russula). Diversification rates were inferred with BAMM v.2.5 (Rabosky, 2014). (a) Per-branch net diversification rates averaged across posterior samples. The full 3385-tip supertree is shown with the distribution of tips. Ages and rates are based on the previous estimate of 60 Myr (uncertainty range 47-64 Myr; Looney et al., 2022) for the crown age of the ECM Russulaceae. Subgenera containing clades with increased diversification rates are labelled. Ma, million years ago. (b) Net diversification rates per area. Violin plots are coloured by area; the white dots represent median values, and thick and thin vertical lines show $1 \times$ and $1.5 \times$ interguartile ranges, respectively. Diversification rates were averaged for branch segments in random pairs of BAMM posterior samples with stochastic ancestral area maps.

clustered host distribution in the Tropics would have offered less niche space. Our dispersal analyses corroborated this scenario, with both a high number of shared OTUs and dispersals between the north-temperate regions (Fig. 3). The rapid increases of diversification rates we observed for Russulaceae in the Nearctic and Palaearctic indeed coincide with the spread of temperate vegetation after the Eocene thermal optimum (Zachos *et al.*, 2001). Some of these north-temperate lineages then migrated southward again into tropical latitudes, including Central America and the Andes (see later).

We suggest that ECM fungal diversity in tropical areas accumulated over a longer time period and more gradually than at higher latitudes. There are, however, also young clades of Russulaceae both in the Neotropics and Palaeotropics, and a simplistic terminology of 'cradles' vs 'museums' of biodiversity should probably be avoided (Vasconcelos *et al.*, 2022). Further discoveries of fossil fungi, as well

Fig. 6 Diversification rates within areas through time in the ectomycorrhizal (ECM) Russulaceae (Lactarius, Lactifluus, Multifurca and Russula). Diversification rates were averaged for branch segments in random pairs of BAMM posterior samples with stochastic ancestral area maps, for 20 equally spaced time periods between the root of Russulaceae and the present. (a-i) Rates for the nine biogeographic areas defined, with the focal areas at the top (a-d). Shaded areas are 95% quantile ranges. Ages and rates are based on the previous estimate of 60 Myr (uncertainty range 47-64 Myr; Looney et al., 2022) for the crown age of the ECM Russulaceae. Crown ages of the four genera are indicated for reference (Ru, Russula; Lf, Lactifluus; La, Lactarius; Mu, Multifurca). Afr, Afrotropics; And, Andes; Aus, Australasia; CAm, Central America/ Caribbean; Ind, Indomalaya; Nea, Nearctic; Pal, Palaearctic; Ma, million years ago.



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as ECM host plants, will hopefully refine the timeline of ECM fungal biogeography, especially in the Tropics.

Lowland neotropical Russulaceae: ancient lineages, potential boreotropical migration, and evidence for transatlantic dispersal

Lowland tropical South America harbours several deepbranching lineages within *Russula, Lactifluus*, and *Lactarius*, and most have affinities with tropical African lineages (Fig. 3b). Gondwanan vicariance is ruled out under the age scenario we considered. Splits between African and neotropical lineages are moreover asynchronous in time, making vicariance unlikely even if the very old age estimate of Sánchez-García *et al.*(2020) were accepted. Migration across an Eocene boreotropical vegetation corridor, supposedly together with host plants, could explain some of the oldest Africa–South America divergences in Russulaceae. The ECM dipterocarp genus *Pseudomonotes* from northern South America diverged from its tropical African sister genus in the Eocene, so it could have co-migrated during that time (Bansal *et al.*, 2022). The monotypic *Pakaraimaea* is probably older, but its precise affinities and age are yet to be confirmed (Ashton *et al.*, 2021). Both these ECM hosts are dominated by Russulaceae mycobionts in northern South America (Smith *et al.*, 2013; Vasco-Palacios *et al.*, 2018).

Several of the lowland South American lineages with African ancestors must have dispersed across the Atlantic, as they diverged after boreotropical conditions. The peak of dispersal from Africa to South America occurred after *c*. 30 Ma (Fig. 4). Tropical transatlantic dispersals are well evidenced in some tropical plant lineages (Pennington & Dick, 2004; Hughes *et al.*, 2013). The successful dispersal of ECM fungi across oceans seems unlikely, as spores must survive the long journey and encounter a suitable habitat with a compatible host tree (Kropp & Albee-Scott, 2010; Horton *et al.*,

2013). Various mechanisms of dispersal are debated, including by wind or birds (Caiafa *et al.*, 2021), but also co-dispersal of fungi and host plants (Golan & Pringle, 2017). Spore attachment to floating fruits has been suggested as a means of fungal co-dispersal with propagules of ECM *Pisonia* (Kropp & Albee-Scott, 2010) and *Coccoloba* (Séne *et al.*, 2018). Rafting of entire trees with roots and soil could theoretically vector plants and their fungal symbionts (Golan & Pringle, 2017). Possible ECM host candidates for co-dispersal from Africa to the Neotropics are *Aldina* and *Dicymbe*, which diverged from African ancestors in the Oligocene to Miocene, and thus after boreotropical conditions (Tedersoo, 2017). Extant species of these genera are major ECM host plants throughout the Guiana Shield and host a plethora of ECM fungi, including Russulaceae (Singer *et al.*, 1983; Moyersoen, 1993; Smith *et al.*, 2011; Vasco-Palacios *et al.*, 2018).

North-to-south migration into the Neotropics

Most Russulaceae in the Andes have biogeographic affinities with north-temperate lineages, distinct from those of lowland tropical South America (with the exception of Nyctaginaceae-associated species in the lower Yungas). We also found more OTUs shared between the Andes and northern regions than OTUs unique to the Andes, indicating recent range expansion. This suggests comigration with *Alnus* and *Quercus* as they moved southward in the Pleistocene (Tedersoo, 2017). *Alnus acuminat*a is a widespread pioneer species in middle elevation zones from Mexico to the Andes (Wicaksono *et al.*, 2017), and southward comigration of the ECM host species and its mycobionts has been suggested previously (Kennedy *et al.*, 2011).

In Central America and the Caribbean, immigration from the north, at a time of generally increased biotic exchange, appears to be the dominant dispersal direction. This is coherent with the importance of north-temperate hosts such as Quercus and Pinus in the region (Halling & Mueller, 2005; Tedersoo, 2017). Our data indeed show a strong recent increase in Russulaceae immigration vs emigration (Fig. 4b). However, South American Russulaceae lineages have also migrated northwards into the region. Delgat et al. (2020) previously showed that most Lactifluus species on Caribbean islands have South American affinities as opposed to north-temperate-derived Central American species (Delgat et al., 2020). The neotropical host lineages Coccoloba or Pisonieae probably moved northwards with their mycobionts before the Panama isthmus closure (Tedersoo, 2017). Clearly, the Central American/Caribbean region, which we defined very broadly here, has been a zone of exchange between north-temperate and neotropical lineages, which must have involved dispersal pathways across the Caribbean islands.

A distinct Patagonian ectomycorrhizal macromycota

The Patagonian macromycota has long been recognized as distinct (Singer, 1953; Trierveiler-Pereira *et al.*, 2014). Our results demonstrated that Patagonian Russulaceae have no affinity with either Andean or tropical lowland South American Russulaceae, despite the fact that the sole extant Patagonian host plant group

Nothofagaceae extended considerably northward in Eocene South America (Jaramillo *et al.*, 2006). Divergence times of Patagonian Russulaceae do not indicate a vicariant association with the South America–Antarctica split, unlike in the *Nothofagus*-specific fungal parasite *Cyttaria* (Peterson *et al.*, 2010). Even during the increasing glaciation of Antarctica, *Nothofagus* likely persisted in coastal areas until the Pleistocene (Poole & Cantrill, 2006) and provided geographic 'stepping stones' for bird-vectored ECM fungal dispersal between South America and Australasia (Caiafa *et al.*, 2021).

Conclusions

Most biogeographic studies of the Neotropics have focused on plants or animals. Here, we explored the neotropical biogeography of one of the largest families of ECM fungi, the Russulaceae, drawing the following conclusions:

(1) Tropical South American lowlands host several old lineages related to tropical African taxa, whereas the Andes and the adjacent Patagonia have younger, unrelated lineages.

(2) Some lineages may have dispersed into lowland tropical South America during Palaeocene/Eocene boreotropical conditions. Several, however, immigrated more recently via transatlantic dispersal from tropical Africa. Most origins of Central American/Caribbean taxa coincide with general increased biotic interchange across the closing Panama isthmus.

(3) Tropical South America and the Afrotropics have lower diversification rates than temperate areas, probably due to the discontinuous distribution of ECM hosts.

Our analysis demonstrates the need for more taxonomic and ecological work on neotropical ECM fungi. In the Russulaceae, several deep-branching neotropical lineages remain to be described. Our coarse-scale analysis needs to be followed by detailed regional work on host and environmental drivers of neotropical ECM fungal biogeography. Russulaceae have adapted to environments as contrasting as wet Amazonia (Singer & Araujo, 1979; Singer & Aguiar, 1986; Vasco-Palacios *et al.*, 2018) and the semi-arid Caatinga (Sà *et al.*, 2019), which we here all lumped under one geographic unit. Neotropical ECM fungi are phylogenetically diverse and may well perform unique and potentially irreplaceable ecological functions that need to be documented. This is particularly urgent in a time when neotropical forests are being destroyed at unprecedented levels (Gomes *et al.*, 2019).

Acknowledgements

JH was funded by a 'Future Leaders in Plant and Fungal Science' fellowship by the Royal Botanic Gardens, Kew. JH and MR received funding from the CNRS Nouragues Research Station (Mycotin project) and from the Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01, DIADEMA and DIAMOND projects). TWH was supported by National Science Foundation grants DEB-0918591, DEB-1556338, and National Geographic Society's Committee for Research and Exploration grants 6679-99 and 7435-03. EDC was supported by the 'Special Research Fund Ghent University' (BOF), grants B/13485/01 and BOF-PDO-2017-001201. Computational resources used at the UK's Crop

Diversity Bioinformatics HPC (James Hutton Institute and National Institute of Agricultural Botany (NIAB)) were funded by BBSRC grant BB/S019669/1. Curators of the fungal collections at the Center for Forestry Research (Madison, WI, USA), and the Field Museum of Natural History (Chicago, IL, USA) are gratefully acknowledged for specimen loans and sampling permission. Melissa C. W. Jaeger and Jaime Duque helped collect specimens in Brazil, and Jean-Louis Cheype kindly provided specimens from French Guiana. In Guyana, Mei Lin Chin, M. Catherine Aime, and numerous Patamona indigenous field assistants participated in field work, and the Guyana Evironmental Protection Agency provided research permits. The Société Mycologique de Nouvelle-Calédonie also participated in fieldwork. Laura Martínez-Suz, Oscar A. Pérez-Escobar, and Jérôme Chave provided valuable feedback on earlier versions of this manuscript. Kessy Abarenkov provided support for data download from the UNITE database, and the scientific computing teams at the James Hutton Institute and the NIAB provided technical support. Special thanks go out to Steven L. Miller and Jorinde Nuytinck for their advice during earlier stages of the study. Comments by three anonymous referees were invaluable for improving this paper.

Author contributions

JH and MR conceived the study. TWH, EDC, AV, P-AM, BB, HS and MG helped refine the study design. JH, P-AM, TWH, M-AN, FW, RC, MS, AV-P, SG, FC and MG collected material in the field. JH, MS, AV-P, EL, FC and SM performed laboratory work. JH, MR and TWH wrote the manuscript. All authors commented and agreed on the manuscript.

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Data availability

Data and intermediate results, as well as R scripts for dispersal and diversification analyses, are available in an open Zenodo repository (doi: 10.5281/zenodo.4727866).

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- Amalfi M. 2016. Fomitiporia (Basidiomycota) revisited: species concept, phylogenetic structure and biogeographical patterns. PhD thesis, Université Catholique de Louvain, Ottignies-Louvain-la-Neuve, Belgium.
- Antonelli A, Ariza M, Albert J, Andermann T, Azevedo J, Bacon C, Faurby S, Guedes T, Hoorn C, Lohmann LG *et al.* 2018. Conceptual and empirical advances in Neotropical biodiversity research. *PeerJ* 6: e5644.
- Antonelli A, Nylander JAA, Persson C, Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences, USA* 106: 9749–9754.
- Antonelli A, Sanmartín I. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60: 403–414.
- Ashton PS, Morley RJ, Heckenhauer J, Prasad V. 2021. The magnificent Dipterocarps: précis for an Epitaph? *Kew Bulletin* 76: 87–125.
- Bacon CD, Silvestro D, Jaramillo C, Smith BT, Chakrabarty P, Antonelli A. 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences, USA* 112: 6110–6115.
- Bansal M, Morley RJ, Nagaraju SK, Dutta S, Mishra AK, Selveraj J, Kumar S, Niyolia D, Harish SM, Abdelrahim OB *et al.* 2022. Southeast Asian Dipterocarp origin and diversification driven by Africa-India floristic interchange. *Science* 375: 455–460.
- Barroetaveña C, Salomón MES, Bassani V. 2019. Rescuing the ectomycorrhizal biodiversity associated with South American Nothofagaceae forest, from the 19th century naturalists up to molecular biogeography. *Forestry: An International Journal of Forest Research* 92: 500–511.
- Bazzicalupo AL, Buyck B, Saar I, Vauras J, Carmean D, Berbee ML. 2017. Troubles with mycorrhizal mushroom identification where morphological differentiation lags behind barcode sequence divergence. *Taxon* 66: 791–810.
- Beaulieu J, O'Meara B, Oliver J, Boyko J. 2021. CORHMM: hidden Markov models of character evolution. R package v.2.7. [WWW document] URL https://cran.r-project.org/web/packages/corHMM/ [accessed 10 February 2022].
- Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F *et al.* 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* 4: 914–919.
- Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. *Molecular Ecology* 7: 257–272.
- Buyck B. 1990. New taxa of tropical Russulae: *Pseudoepitheliosinae* subsect. nov. *Mycotaxon* 39: 317–327.
- **Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F. 2008.** Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect. *Ochricompactae. Fungal Diversity* **28**: 15–40.
- Buyck B, Ovrebo CL. 2002. New and interesting *Russula* species from Panamá. *Mycologia* 94: 888–901.
- Buyck B, Thoen D, Watling R. 1996. Ectomycorrhizal fungi of the Guinea– Congo Region. *Proceedings of the Royal Society of Edinburgh Section B: Biological Sciences* 104: 313–333.
- Buyck B, Wang X-H, Adamčíková K, Caboň M, Jančovičová S, Hofstetter V, Adamčík S. 2020. One step closer to unravelling the origin of *Russula*: subgenus *Glutinosae* subg. nov. *Mycosphere* 11: 285–304.
- **Buyck B, Zoller S, Hofstetter V. 2018.** Walking the thin line... ten years later: the dilemma of above- versus below-ground features to support phylogenies in the Russulaceae (Basidiomycota). *Fungal Diversity* **89**: 267–292.
- Caiafa MV, Jusino MA, Wilkie AC, Díaz IA, Sieving KE, Smith ME. 2021. Discovering the role of Patagonian birds in the dispersal of truffles and other mycorrhizal fungi. *Current Biology* 31: 5558–5570.e3.

Carriconde F, Gardes M, Bellanger J-M, Letellier K, Gigante S, Gourmelon V, Ibanez T, McCoy S, Goxe J, Read J *et al.* 2019. Host effects in high ectomycorrhizal diversity tropical rainforests on ultramafic soils in New Caledonia. *Fungal Ecology* 39: 201–212.

Chazot N, Condamine FL, Dudas G, Peña C, Kodandaramaiah U, Matos-Maraví P, Aduse-Poku K, Elias M, Warren AD, Lohman DJ et al. 2021. Conserved ancestral tropical niche but different continental histories explain the latitudinal diversity gradient in brush-footed butterflies. Nature Communications 12: 5717.

Cheype J-L, Campo E. 2012. Russula rubropunctatissima Cheype & E. Campo – une nouvelle russule découverte en Guyane Française. Bulletin de la Société Mycologique Française 128: 127–135.

Cody S, Richardson JE, Rull V, Ellis C, Pennington RT. 2010. The Great American biotic interchange revisited. *Ecography* 33: 326–332.

Cook LG, Crisp MD. 2005. Not so ancient: the extant crown group of Nothofagus represents a post-Gondwanan radiation. Proceedings of the Royal Society B: Biological Sciences 272: 2535–2544.

Corrales A, Henkel TW, Smith ME. 2018. Ectomycorrhizal associations in the tropics – biogeography, diversity patterns and ecosystem roles. *New Phytologist* 220: 1076–1091.

Corrales A, Mangan SA, Turner BL, Dalling JW. 2016. An ectomycorrhizal nitrogen economy facilitates monodominance in a neotropical forest. *Ecology Letters* 19: 383–392.

Corrales A, Ovrebo CL. 2021. Fungi of the Fortuna Forest Reserve: taxonomy and ecology with emphasis on ectomycorrhizal communities. In: Dalling J, Turner B, eds. *Smithsonian contributions to botany. Fortuna Forest Reserve, Panama: interacting effects of climate and soils on the biota of a wet premontane tropical forest*, 137–153. Washington, DC, USA: Smithsonian Institution.

Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, Hardy GESJ, Smith D, Summerell BA, Cano-Lira JF, Guarro J, Houbraken J et al. 2017. Fungal Planet description sheets: 625–715. Persoonia – Molecular Phylogeny and Evolution of Fungi 39: 270–467.

De Crop E, Nuytinck J, Van de Putte K, Wisitrassameewong K, Hackel J, Stubbe D, Hyde KD, Roy M, Halling RE, Moreau P-A *et al.* 2017. A multigene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. *Persoonia* 38: 58–80.

Dearnaley JDW. 2007. Further advances in orchid mycorrhizal research. *Mycorrhiza* 17: 475–486.

Delgat L, Courtecuisse R, De Crop E, Hampe F, Hofmann TA, Manz C, Piepenbring M, Roy M, Verbeken A. 2020. *Lactifluus* (Russulaceae) diversity in Central America and the Caribbean: melting pot between realms. *Persoonia* 44: 278–300.

Dentinger BTM, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau P-A, Nagasawa E, Soytong K, Taylor AF et al. 2010. Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). *Molecular Phylogenetics and Evolution* 57: 1276–1292.

Dinerstein E, Olson D, Joshi A, Vynne C, Burgess ND, Wikramanayake E, Hahn N, Palminteri S, Hedao P, Noss R *et al.* 2017. An ecoregion-based approach to protecting half the terrestrial realm. *BioScience* **67**: 534–545.

Duque Barbosa JA, Delgat L, Galváo Elias S, Verbeken A, Neves MA, de Carvalho A. 2020. A new section, *Lactifluus* section *Neotropicus* (Russulaceae), and two new *Lactifluus* species from the Atlantic Forest, Brazil. *Systematics and Biodiversity* 18: 347–361.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Geml J, Pastor N, Fernandez L, Pacheco S, Semenova TA, Becerra AG, Wicaksono CY, Nouhra ER. 2014. Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. *Molecular Ecology* 23: 2452– 2472.

Golan JJ, Pringle A. 2017. Long-distance dispersal of fungi. In: *The fungal kingdom*. Hoboken, NJ, USA: John Wiley & Sons, 309–333.

Goldberg EE, Lancaster LT, Ree RH. 2011. Phylogenetic inference of reciprocal effects between geographic range evolution and diversification. *Systematic Biology* 60: 451–465.

Gomes VHF, Vieira ICG, Salomão RP, ter Steege H. 2019. Amazonian tree species threatened by deforestation and climate change. *Nature Climate Change* 9: 547–553.

Hall JS, Harris DJ, Saltonstall K, Medjibe VP, Ashton MS, Turner BL. 2020. Resource acquisition strategies facilitate *Gilbertiodendron dewevrei* monodominance in African lowland forests. *Journal of Ecology* 108: 433–448.

Halling RE, Mueller GM. 2005. Common mushrooms of the Talamanca Mountains, Costa Rica. Bronx, NY, USA: New York Botanical Garden Press.
Han LH, Feng B, Wu G, Halling RE, Buyck B, Yorou NS, Ebika STN, Yang ZL. 2018. African origin and global distribution patterns: evidence inferred from phylogenetic and biogeographical analyses of ectomycorrhizal fungal genus Strobilomyces. Journal of Biogeography 45: 201–212.

Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24: 129–131.

Harrower E, Bougher NL, Henkel TW, Horak E, Matheny PB. 2015. Longdistance dispersal and speciation of Australasian and American species of *Cortinarius sect. Cortinarius. Mycologia* 107: 697–709.

He M-Q, Zhao R-L, Hyde KD, Begerow D, Kemler M, Yurkov A, McKenzie EHC, Raspé O, Kakishima M, Sánchez-Ramírez S et al. 2019. Notes, outline and divergence times of Basidiomycota. *Fungal Diversity* 99: 105–367.

Henkel TW. 2003. Monodominance in the ectomycorrhizal *Dicymbe corymbosa* (Caesalpiniaceae) from Guyana. *Journal of Tropical Ecology* **19**: 417–437.

Henkel TW, Aime MC, Chin MML, Miller SL, Vilgalys R, Smith ME. 2012. Ectomycorrhizal fungal sporocarp diversity and discovery of new taxa in *Dicymbe* monodominant forests of the Guiana Shield. *Biodiversity and Conservation* 21: 2195–2220.

Henkel TW, Aime MC, Miller SL. 2000. Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status. *Mycologia* 92: 1119–1132.

Henkel TW, Mayor JR. 2019. Implications of a long-term mast seeding cycle for climatic entrainment, seedling establishment and persistent monodominance in a Neotropical, ectomycorrhizal canopy tree. *Ecological Research* 34: 472–484.

Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP *et al.* 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.

Horton TR, Swaney DP, Galante TE. 2013. Dispersal of ectomycorrhizal basidiospores: the long and short of it. *Mycologia* 105: 1623–1626.

Hughes CE, Pennington RT, Antonelli A. 2013. Neotropical plant evolution: assembling the big picture. *Botanical Journal of the Linnean Society* 171: 1–18.

Jaramillo C, Rueda MJ, Mora G. 2006. Cenozoic plant diversity in the Neotropics. Science 311: 1893–1896.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software v.7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kennedy PG, Garibay-Orijel R, Higgins LM, Angeles-Arguiz R. 2011. Ectomycorrhizal fungi in Mexican *Alnus* forests support the host co-migration hypothesis and continental-scale patterns in phylogeography. *Mycorrhiza* 21: 559–568.

Kennedy PG, Matheny PB, Ryberg KM, Henkel TW, Uehling JK, Smith ME. 2012. Scaling up: examining the macroecology of ectomycorrhizal fungi. *Molecular Ecology* 21: 4151–4154.

Köljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM *et al.* 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22: 5271–5277.

Kropp BR, Albee-Scott S. 2010. *Inocybe tauensis*, a new species from the Samoan Archipelago with biogeographic evidence for a Paleotropical origin. *Fungal Biology* 114: 790–796.

van de Lagemaat SHA, Swart MLA, Vaes B, Kosters ME, Boschman LM, Burton-Johnson A, Bijl PK, Spakman W, van Hinsbergen DJJ. 2021. Subduction initiation in the Scotia Sea region and opening of the Drake Passage: when and why? *Earth-Science Reviews* 215: 103551.

Leavitt SD, Esslinger TL, Lumbsch HT. 2012. Neogene-dominated diversification in neotropical montane lichens: dating divergence events in the lichen-forming fungal genus Oropogon (Parmeliaceae). American Journal of Botany 99: 1764–1777.

710 Research

Lemoine F, Entfellner J-BD, Wilkinson E, Correia D, Felipe MD, Oliveira T, Gascuel O. 2018. Renewing Felsenstein's phylogenetic bootstrap in the era of big data. *Nature* 556: 452–456.

Lemon J. 2006. PLOTRIX: a package in the red light district of R. R News 6(4): 8-12.

van der Linde S, Suz LM, Orme CDL, Cox F, Andreae H, Asi E, Atkinson B, Benham S, Carroll C, Cools N *et al.* 2018. Environment and host as largescale controls of ectomycorrhizal fungi. *Nature* 558: 243–248.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology* and Evolution 16: 1799–1808.

- Looney B, Miyauchi S, Morin E, Drula E, Courty PE, Kohler A, Kuo A, LaButti K, Pangilinan J, Lipzen A *et al.* 2022. Evolutionary transition to the ectomycorrhizal habit in the genomes of a hyper-diverse lineage of mushroomforming fungi. *New Phytologist* 233: 2294–2309.
- Looney BP, Meidl P, Piatek MJ, Miettinen O, Martin FM, Matheny PB, Labbé JL. 2018. Russulaceae: a new genomic dataset to study ecosystem function and evolutionary diversification of ectomycorrhizal fungi with their tree associates. *New Phytologist* 218: 54–65.
- Looney BP, Ryberg M, Hampe F, Sánchez-García M, Matheny PB. 2016. Into and out of the tropics: global diversification patterns in a hyperdiverse clade of ectomycorrhizal fungi. *Molecular Ecology* 25: 630–647.
- Löwenberg-Neto P. 2014. Neotropical region: a shapefile of Morrone's (2014) biogeographical regionalisation. *Zootaxa* 3802: 300.

Löwenberg-Neto P. 2015. Andean region: a shapefile of Morrone's (2015) biogeographical regionalisation. *Zootaxa* 3985: 600.

- Manz C, Adamčík S, Looney BP, Corrales A, Ovrebo C, Adamčíková K, Hofmann TA, Hampe F, Piepenbring M. 2021. Four new species of *Russula* subsection *Roseinae* from tropical montane forests in western Panama. *PLoS ONE* 16: e0257616.
- Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin DE, Horak E, Kropp BR, Lodge DJ, Soytong K, Trappe JM *et al.* 2009. Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography* 36: 577–592.
- McGuire KL. 2007. Common ectomycorrhizal networks may maintain monodominance in a tropical rainforest. *Ecology* 88: 567–574.

Miller SL, Aime MC, Henkel TW. 2002. Russulaceae of the Pakaraima Mountains of Guyana. I. New species of pleurotoid *Lactarius. Mycologia* 94: 545–553.

- Miller SL, Aime MC, Henkel TW. 2012. Russulaceae of the Pakaraima Mountains of Guyana 2. New species of *Russula* and *Lactifluus. Mycotaxon* 121: 233–253.
- Montoya L, Bandala VM, Garay E. 2014. Two new species of *Lactarius* associated with *Alnus acuminata* subsp. *arguta* in Mexico. *Mycologia* 106: 949–962.
- Montoya L, Bandala VM, Halling R. 2003. Lactarius furcatus in Mexico and Costa Rica. Mycotaxon 87: 311–316.
- Moore BR, Höhna S, May MR, Rannala B, Huelsenbeck JP. 2016. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. *Proceedings of the National Academy of Sciences*, *USA* 113: 9569–9574.
- Morrone JJ. 2014. Biogeographical regionalisation of the Neotropical region. *Zootaxa* 3782: 1–110.
- Morrone JJ. 2015. Biogeographical regionalisation of the Andean region. *Zootaxa* 3936: 207–236.
- Moyersoen B. 1993. Ectomicorrizas y micorrizas vesiculo-arbusculares en Caatinga Amazonica. Caracas, Venezuela: O Huber.

Moyersoen B. 2006. *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist* **172**: 753–762.

Müller RD, Seton M, Zahirovic S, Williams SE, Matthews KJ, Wright NM, Shephard GE, Maloney KT, Barnett-Moore N, Hosseinpour M et al. 2016. Ocean basin evolution and global-scale plate reorganization events since Pangea breakup. Annual Review of Earth and Planetary Sciences 44: 107–138.

Nouhra ER, Palfner G, Kuhar F, Pastor N, Smith ME. 2019. Ectomycorrhizal fungi in South America: their diversity in past, present and future research. In: Pagano MC, Lugo MA, eds. *Fungal biology. Mycorrhizal fungi in South America.* Cham, Switzerland: Springer International, 73–95.

- O'Dea A, Lessios HA, Coates AG, Eytan RI, Restrepo-Moreno SA, Cione AL, Collins LS, de Queiroz A, Farris DW, Norris RD *et al.* 2016. Formation of the Isthmus of Panama. *Science Advances* 2: e1600883.
- Palma-Silva C, Turchetto-Zolet AC, Fay MF, Vasconcelos T. 2022. Drivers of exceptional Neotropical biodiversity: an updated view. *Botanical Journal of the Linnean Society* 199: 1–7.
- Paradis E, Schliep K. 2019. APE 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528.
- Peay KG, Kennedy PG, Talbot JM. 2016. Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology* 14: 434–447.
- Pebesma E. 2018. Simple features for R: standardized support for spatial vector data. *The R Journal* 10: 439–446.
- Pennington RT, Dick CW. 2004. The role of immigrants in the assembly of the South American rainforest tree flora. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 1611–1622.
- Pérez-Escobar OA, Zizka A, Bermúdez MA, Meseguer AS, Condamine FL, Hoorn C, Hooghiemstra H, Pu Y, Bogarín D, Boschman LM *et al.* 2022. The Andes through time: evolution and distribution of Andean floras. *Trends in Plant Science* 27: 364–378.
- Peterson KR, Pfister DH, Bell CD. 2010. Cophylogeny and biogeography of the fungal parasite *Cyttaria* and its host *Nothofagus*, southern beech. *Mycologia* 102: 1417–1425.
- Pirozynski KA. 1983. Pacific mycogeography: an appraisal. Australian Journal of Botany Supplementary Series 13: 137–160.
- Plummer M, Best N, Cowles K, Vines K. 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6: 7–11.
- Poole I, Cantrill DJ. 2006. Cretaceous and Cenozoic vegetation of Antarctica integrating the fossil wood record. *Geological Society, London, Special Publications* 258: 63–81.
- Price MN, Dehal PS, Arkin AP. 2010. FAST TREE 2 approximately maximumlikelihood trees for large alignments. *PLoS ONE* 5: e9490.
- Rabosky DL. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* 9: e89543.
- Rabosky DL, Mitchell JS, Chang J. 2017. Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* 66: 477–498.
- Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59: 2299–2311.
- Ree RH, Sanmartín I. 2018. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* 45: 741–749.
- Renner S. 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences* 165: S23–S33.
- Revell LJ. 2011. PHYTOOLS: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.
- Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584.
- Roy M, Schimann H, Braga-Neto R, Silva RAED, Duque J, Frame D, Wartchow F, Neves MA. 2016. Diversity and distribution of ectomycorrhizal fungi from Amazonian lowland white-sand forests in Brazil and French Guiana. *Biotropica* 48: 90–100.
- Ryberg M, Matheny PB. 2012. Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proceedings of the Royal Society B: Biological Sciences* 279: 2003– 2011.
- Sà MCA, Baseia IG, Wartchow F. 2013. *Lactifluus dunensis*, a new species from Rio Grande do Norte, Brazil. *Mycosphere* 4: 261–265.
- Sà MCA, Delgat L, Verbeken A, Sulzbacher MA, Baseia IG, Wartchow F. 2019. A new species of *Lactifluus* (Russulales, Agaricomycetes) from the Brazilian caatinga semiarid region. *New Zealand Journal of Botany* 57: 169–178.
- Sà MCA, Wartchow F. 2013. Lactifluus aurantiorugosus (Russulaceae), a new species from Southern Brazil. Darwiniana, Nueva Serie 1: 54–60.
- Sà MCA, Wartchow F. 2016. Russula omnileuca, a new species from Pernambuco, Brazil. Sydowia 68: 63–68.
- Sánchez-García M, Ryberg M, Khan FK, Varga T, Nagy LG, Hibbett DS. 2020. Fruiting body form, not nutritional mode, is the major driver of diversification

in mushroom-forming fungi. Proceedings of the National Academy of Sciences,

- USA 117: 32528–32534. Sánchez-Ramírez S, Tulloss RE, Amalfi M, Moncalvo J-M. 2014. Palaeotropical origins, boreotropical distribution and increased rates of diversification in a clade of edible ectomycorrhizal mushrooms (*Amanita* section *Caesareae*). *Journal of Biogeography* 42: 351–363.
- Séne S, Selosse M-A, Forget M, Lambourdière J, Cissé K, Diédhiou AG, Rivera-Ocasio E, Kodja H, Kameyama N, Nara K et al. 2018. A pantropically introduced tree is followed by specific ectomycorrhizal symbionts due to pseudo-vertical transmission. *The ISME Journal* 12: 1806–1816.

Silva-Filho AGS, Sá MCA, Komura DL, Moncalvo J-M, Margaritescu S, Roy M, Wartchow F. 2020. Two novel species of *Lactifluus* subg. *Pseudogymnocarpi* (Russulaceae) from Brazil. *Phytotaxa* 436: 222–236.

Singer R. 1953. Four years of mycological work in southern South America. *Mycologia* 45: 865–891.

Singer R, Aguiar IA. 1986. Litter decomposing and ectomycorrhizal Basidiomycetes in an igapó forest. *Plant Systematics and Evolution* 153: 107– 117.

Singer R, Araujo I, Ivory M. 1983. The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia (Litter decomposition and ectomycorrhiza in Amazonian forests 2.). *Beihefte zur Nova Hedwigia* 77: 1– 352.

Singer R, Araujo IJS. 1979. Litter decomposition and Ectomycorrhiza in Amazonian forests. 1. A comparison of litter decomposing and ectomycorrhizal Basidiomycetes in latosol-terra-firme rain forest and white podzol campinarana. *Acta Amazonica* 9: 25–42.

Smith ME, Henkel TW, Catherine Aime M, Fremier AK, Vilgalys R. 2011. Ectomycorrhizal fungal diversity and community structure on three cooccurring leguminous canopy tree species in a Neotropical rainforest. *New Phytologist* **192**: 699–712.

Smith ME, Henkel TW, Uehling JK, Fremier AK, Clarke HD, Vilgalys R. 2013. The ectomycorrhizal fungal community in a neotropical forest dominated by the endemic dipterocarp *Pakaraimaea dipterocarpacea*. *PLoS ONE* 8: e55160.

Smith SA, O'Meara BC. 2012. TREEPL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* 28: 2689–2690.

Smith SE, Read DJ. 2010. Mycorrhizal symbiosis. New York, NY, USA: Academic Press.

Soudzilovskaia NA, van Bodegom PM, Terrer C, van't Zelfde M, McCallum I, Luke McCormack M, Fisher JB, Brundrett MC, de Sá NC, Tedersoo L. 2019. Global mycorrhizal plant distribution linked to terrestrial carbon stocks. *Nature Communications* 10: 5077.

Stamatakis A. 2014. RAXML v.8: a tool for phylogenetic analysis and postanalysis of large phylogeneis. *Bioinformatics* 30: 1312–1313.

Steidinger BS, Crowther TW, Liang J, Nuland MEV, Werner GDA, Reich PB, Nabuurs GJ, de Miguel S, Zhou M, Picard N et al. 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* 569: 404–408.

Tedersoo L. 2017. Global biogeography and invasions of ectomycorrhizal plants: past, present and future. In: Tedersoo L, ed. *Ecological studies. Biogeography of* mycorrhizal symbiosis. Cham, Switzerland: Springer International, 469–531.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A et al. 2014a. Global diversity and geography of soil fungi. *Science* 346: 1256688.

Tedersoo L, Bahram M, Ryberg M, Otsing E, Kõljalg U, Abarenkov K. 2014b. Global biogeography of the ectomycorrhizal/sebacina lineage (Fungi, Sebacinales) as revealed from comparative phylogenetic analyses. *Molecular Ecology* 23: 4168–4183.

Tedersoo L, Bahram M, Zobel M. 2020. How mycorrhizal associations drive plant population and community biology. *Science* 367: eaba1223.

Tedersoo L, Brundrett MC. 2017. Evolution of ectomycorrhizal symbiosis in plants. In: Tedersoo L, ed. *Ecological studies. Biogeography of mycorrhizal symbiosis.* Cham, Switzerland: Springer International, 407–467.

Tedersoo L, May TW, Smith ME. 2010a. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20: 217–263. Tedersoo L, Nara K. 2010. General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. *New Phytologist* 185: 351–354.

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M. 2010b. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. *The ISME Journal* 4: 465–471.

Tennekes M. 2020. *TMAPTOOLS: thematic map tools.* R package v.3.0. [WWW document] URL https://cran.r-project.org/web/packages/tmaptools/index.html [accessed 3 July 2020].

Trierveiler-Pereira L, Smith ME, Trappe JM, Nouhra E. 2014. Sequestrate fungi from Patagonian *Nothofagus* forests: *Cystangium* (Russulaceae, Basidiomycota). *Mycologia* 107: 13–302.

Varga T, Krizsán K, Földi C, Dima B, Sánchez-García M, Sánchez-Ramírez S, Szöllősi GJ, Szarkándi JG, Papp V, Albert L et al. 2019. Megaphylogeny resolves global patterns of mushroom evolution. Nature Ecology & Evolution 3: 668.

Vasconcelos T, O'Meara BC, Beaulieu JM. 2022. Retiring "cradles" and "museums" of biodiversity. *The American Naturalist* **199**: 194–205.

Vasco-Palacios AM, Hernandez J, Peñuela-Mora MC, Franco-Molano AE, Boekhout T. 2018. Ectomycorrhizal fungi diversity in a white sand forest in western Amazonia. *Fungal Ecology* 31: 9–18.

Vera M, Adamčík S, Adamčíková K, Hampe F, Caboň M, Manz C, Ovrebo C, Piepenbring M, Corrales A. 2021. Morphological and genetic diversification of *Russula floriformis*, sp. nov., along the Isthmus of Panama. *Mycologia* 113: 807– 827.

Verbeken A, Stubbe D, van de Putte K, Eberhardt U, Nuytinck J. 2014. Tales of the unexpected: angiocarpous representatives of the Russulaceae in tropical South East Asia. *Persoonia* 32: 13–24.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.

Wang X-H, Halling RE, Hofstetter V, Lebel T, Buyck B. 2018. Phylogeny, biogeography and taxonomic re-assessment of *Multifurca* (Russulaceae, Russulales) using three-locus data. *PLoS ONE* 13: e0205840.

Wartchow F, Bezerra JL, Cavalcanti MAQ. 2013. Lactifluus batistae (Russulaceae), a new species from Bahia, Brazil. Agrotrópica 25: 103–108.

Wartchow F, Cavalcanti MAQ. 2010. Lactarius rupestris—a new species from the Brazilian semi-arid region. Mycotaxon 112: 55–63.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JN, White TJ, eds. *PCR protocols: a guide to methods and applications.* London, UK: Academic Press, 315–322.

Wicaksono CY, Aguirre-Guiterrez J, Nouhra E, Pastor N, Raes N, Pacheco S, Geml J. 2017. Contracting montane cloud forests: a case study of the Andean alder (*Alnus acuminata*) and associated fungi in the Yungas. *Biotropica* 49: 141–152.

Wilson AW, Binder M, Hibbett DS. 2012. Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletales, Basidiomycota). *New Phytologist* 194: 1079–1095.

Wisitrassameewong K, Looney BP, Le HT, De Crop E, Das K, Van de Putte K, Eberhardt U, Jiayu G, Stubbe D, Hyde KD et al. 2016. Lactarius subgenus Russularia (Basidiomycota, Russulales): novel Asian species, worldwide phylogeny and evolutionary relationships. Fungal Biology 120: 1554–1581.

Wolfe JA. 1975. Some aspects of plant geography of the northern hemisphere during the late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62: 264–279.

Won H, Renner SS. 2006. Dating dispersal and radiation in the gymnosperm Gnetum (Gnetales)—clock calibration when outgroup relationships are uncertain. Systematic Biology 55: 610–622.

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

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Zhang Q, Ree RH, Salamin N, Xing Y, Silvestro D. 2022. Fossil-informed models reveal a boreotropical origin and divergent evolutionary trajectories in the walnut family (Juglandaceae). *Systematic Biology* 71: 242–258.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Russulaceae backbone phylogeny.

Fig. S2 Russulaceae backbone phylogeny, time calibrated.

Fig. S3 Detailed Russulaceae supertree.

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