Mycorrhizal symbiosis with Mucoromycotina facilitated the terrestrialization of land plants

# Authors

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# Abstract

The mycorrhizal symbiosis between soil fungi and land plants is one of the most widespread and ecologically important mutualisms on earth. It has long been hypothesized that the Glomeromycota, the mycorrhizal symbionts of the majority of plants, facilitated colonization of the terrestrial environment by plants in the Ordovician. This view was recently challenged by the discovery of mycorrhizal associations with Mucoromycotina in several early diverging lineages of land plants, suggesting involvement of these fungi in the terrestrialization process. However, competing, plausible hypotheses for the order of divergence of the land plants result in different reconstructed scenarios for the build up of repertoires of mycorrhizal symbiosis. Utilizing a large species-level database of mycorrhizal associations and a Bayesian approach to state transition dynamics we here show that the recruitment of Mucoromycotina is the primary transition from the initial state of no mycorrhizal association. We further found that transitions between different combinations of either or both of Mucoromycotina and Glomeromycota occur at high rates and found similar promiscuity among combinations that include either or both of Glomeromycota and Ascomycota with a nearly fixed association with Basidiomycota: gains and losses of association with the latter occur at exceedingly low rates. Our results demonstrate that the most likely scenario whereby symbiosis between land plants and mycorrhiza was built up involved terrestrialisation mediated by Mucoromycotina.

Land plants diverged from aquatic algae in the Neoproterozoic1 as a lineage that would eventually undergo the ecological transition to terrestrial life. This transition altered the global climate and the biosphere through biotic chemical weathering of rocks, carbon fixation, and an increase in atmospheric oxygen levels2,3. Terrestrial life requires plants to extract nutrients and moisture from the substrate, while photosynthesizing organs remain above the surface. As the evolution of roots lagged behind the transfer to the terrestrial environment4, these could not have facilitated the first stages of terrestrialization. A likely early adaptation to overcome this challenge would have been symbiosis with a fungal partner5,6. In these mycorrhizal associations, the fungal partner can absorb moisture and nutrients with long hyphal structures and pass these on to the plant in return for photoassimilates.

Mycorrhizal symbiosis is found in over 90% of extant land plant families7, which form associations with members of four different fungal phyla: Glomeromycota, Basidiomycota, Ascomycota and Zygomycota (the latter of which represented by Mucoromycotina8,9). The great majority of land plants associate with arbuscular mycorrhizal fungi from the phylum Glomeromycota, while other types of mycorrhizal associations, such as ectomycorrhiza, ericoid mycorrhiza and orchid mycorrhiza, belong to the Basidiomycota or Ascomycota8.

The Glomeromycota are a fungal phylum of mostly obligate mycorrhizal fungi10. Fossil evidence suggests that this group has coevolved with land plants for at least 407 Myr, as vesicles, spores, intracellular coils, and arbuscule-like structures resembling extant mycorrhizal infections were found in Rhynie Chert fossils of *Horneophyton* *lignierin*11. Further support for ancient origin of these interactions comes from genomics, as genes involved in the formation of arbuscular mycorrhizal infections are homologs and were acquired in a stepwise manner, with potentiation starting as early as the last common ancestor of Charophytes and Embryophytes12–14.

Within Ascomycota and Basidiomycota, inferring the origin of the mycorrhizal habit is complicated by the many independent origins of a wide variety of nutritional modes, such as saprophytes, parasites, insect symbionts, pathogens, mycorrhiza and lichens15–18. In Basidiomycota, mycorrhizal habit is found in 11-15 out of 20 Agaricomycete orders15, including the two most basal lineages Sebacinales and Cantharellales. Whether or not mycorrhizal habit in the approximately 246 Myr old1 Agaricomycete clade was ancestral or has multiple independent origins is a topic of discussion17–23. Within Ascomycota, over 40% of species are lichenized16,24, and mycorrhizal habit occurs in fewer genera than in Basidiomycota25. However, the age estimate for the mycorrhizal genus *Tuber* alone is between 271 and 140 Ma26. Potential hosts for mycorrhizal members of Ascomycota are found in liverworts (Metzgeridae and Jungermanniales), gymnosperms (Pinales), and several flowering plant lineages (Orchidaceae and several clades of Eudicots)7,27. Amongst these, the oldest potential hosts are members of the liverworts, in a clade whose age is estimated between 243 and 393 Mya28–30, thus suggesting that the onset of the mycorrhizal habit in Ascomycota and Basidiomycota was around this time27,31–36. However, the oldest fossil evidence consists of relatively young mycorrhizal root fossils dating 50 Mya37.

The preponderance of the evidence till recently has led to wide acceptance of the view that Glomeromycota were the ancestral mycorrhizal symbionts of land plants38,39. The ancestral symbiosis was assumed to have been replaced in several plant lineages by other types of mycorrhizal associations in multiple independent shifts7. However, the recent discovery that many members of early diverging lineages of land plants, including liverworts, hornworts, and basal vascular plants, engage in mycorrhizal symbiosis with Mucoromycotina (Zygomycota), challenged this notion and suggest that Mucoromycotina could also have facilitated terrestrialisation9,38,40,41. Subsequent to this discovery, Rhynie Chert fossils where re-evaluated, revealing mycorrhizal infections resembling both Glomeromycota and Mucoromycotina11. Furthermore, it is unknown whether the molecular pathways involved in the formation of mycorrhizal infections with Mucoromycotina are the same as those involved in the symbiosis with Glomeromycota.

Given that the four fungal phyla diverged prior to the divergence of land plants1, it is possible to treat mycorrhizal association with these phyla in different combinations as character states on the plant phylogeny and analyse transition dynamics between these states in a Bayesian phylogenetic comparative context. Database description. Ancestral state reconstruction-Rutger Taking into account the uncertainty of the evolutionary relationships of early embryophytes42,43, we assessed the probability of all possible combinations of mycorrhizal associations for the most recent common ancestor of land plants.

# Results

Ancestral state reconstruction indicates that the most recent common ancestor of all Embryophyta was mycorrhizal (Fig. 3). The different hypotheses for the evolution of plants42,43 had very little effect on the results. The probability of a non-mycorrhizal ancestral state was at most 0.011 (Fig. 3). The most likely state of mycorrhizal association was an association with Glomeromycota only, with a probability between 0.44 and 0.62, depending on the topology of the land plant phylogeny. The probability that the last common ancestor was associated with Mucoromycotina as well as Glomeromycota varied between 0.24 and 0.27. After these two most likely states, highest probabilities were assigned to Glomeromycota in combination with Ascomycota, Basidiomycota, or both Basidiomycota and Mucoromycotina (in order of probability), but all of these three states had a probability lower than 0.095 (Fig. 3). The Glomeromycota were the most likely associates in all nodes older than 300 Myr (Fig. 4) and Mucoromycotina and Glomeromycota as a combined state was the second most likely state in these nodes.

The oldest switches to Ascomycota or Basidiomycota were found in liverworts (Fig. 2; Fig. 5; Fig. 6). A shift between Glomeromycota and Basidiomycota happened along the branches leading to Metzgeriidae and Jungermanniidae, and could have involved two independent shifts or one shift (Fig. 5). The first node associated with Basidiomycota with a probability higher than 0.6 has age estimate of around 200 Million years (Fig. 5; Fig. 6). Other independent switches to Ascomycota and Basidiomycota are found in Pinales, Orchidaceae, Ericales, Malphigiales and Fagales, but more independent shifts to Ascomycota and Basidiomycota associations can be found (Fig. 2). The long branch leading to Pinales, makes it difficult to precisely estimate the time of the switch to the ectomycorrhizal Ascomycota and Basidiomycota in this group.

# Discussion

Ancestral state reconstruction supports the hypothesis that the terrestrialization of land by plants was facilitated by symbiotic interactions with fungi. This result is in accordance with evidence from the fossil record11 and genomics12–14. The recent discovery of mycorrhizal associations with Mucoromycotina in many early diverging lineages of Embryophyta38,40,41 is reflected by a substantial probability of an association with Mucoromycotina in the most basal nodes, but this state is always found in combination with Glomeromycota and the probability of a state with only Glomeromycota is still highest for these nodes (Figure 3). Around 407 Mya, Glomeromycota and Mucoromycotina were associated with *Horneophyton ligneri*11, suggesting that our current model underestimates the presence of Mucoromycotina in the older nodes. Future sequencing for Mucoromycotina would be the way find out how widespread these previously overlooked fungi are and help to resolve the question concerning their role in terrestrialization.

Evidence for mycorrhizal habit found in our ancestral state reconstruction predates evidence from the fossil record by 46-112 million years, depending on the phylogenetic hypothesis for early land plants. However, no conflict is expected for Glomeromycota fungal ecology, as most members of this phylum are obligate mycorrhizal symbionts23. For Mucoromycotina more research will be needed on the history of mycorrhizal interactions. For Ascomycete and Basidiomycete associations, the ecological history is a challenging subject16–19,48. The switch to Basidiomycota associations in liverworts was likely to have happened between 300 and 200 Mya while age estimates for the oldest Ascomycete association vary between 190 and 210 Mya (Figure 5). Many mycorrhizal associations in Ascomycota are found within Pezizomycetes. Age estimates for this group predate the age of the first associations1,25. The age estimate for the mycorrhizal genus *Tuber* alone is 271 to 140 Ma26, suggesting that Ascomycota associations could have been present in Embryophyta lineages before the reconstructed associations. For Basidiomycota, the age estimates for the first mycorrhizal associations coincide with the radiation of Agaricomycetes in the Triassic1.

This result challenges the notion that the last common ancestor of Agaricomycetes was a saprophyte and that the mycorrhizal habit evolved independently in many Agaricomycete orders17,18. On the other hand, there is a possibility of extinct mycorrhizal lineages co-occurring with the ancestor of Agaricomycetes. Habitat preferences same in liverworts-leading to similar mycorrhiza. These conflicting results could also be caused by overestimation of the age of liverworts. However, an extensive study on divergence dates in liverworts, based on nine liverwort fossils reveals even older estimates: 361 Mya for the divergence of Metzgeriidae and Jungermanniales28. Other estimates for this split are 272 Mya (CI: 243–310)29 and 308.7 ± 7.8 Mya30, suggesting that the age of the first Basidiomycota associations in this study might even be conservative. Further support for the reliability of the ancestral state reconstruction is found in the high degree of host specificity between liverworts and their Basidiomycota associates27 and the fact that this very basal embryophyte lineage is associated with the two most basal lineages of Agaricomycetes: *Sebacina* and *Tulasnella*15. Nevertheless, a mycorrhizal ancestral state in Agaricomycetes does not disagree with the expansion of lignin peroxidases in the branches leading to Auriculariales19, as the first divergence events in Agaricomycetes are older than the acquisition of lignin decomposing enzymes18,19. In order to resolve the question whether or not the Mycorrhizal habit in Agaricomycetes existed around the divergence time of Metzgeriidae and Jungermanniales, further research genomic research on the evolution of these enzymes and the genes involved in the mycorrhizal symbiosis is needed. Combined with evidence from the fossil record, this could help to understand the evolution of the mycorrhizal habit in this group of fungi.

Tracing the evolution of mycorrhizal associations on the plant phylogeny provides us with the probability of the ancestral symbionts, and provide evidence for the time and taxa in which shifts to other types of association occurred. Further applications of the model could include mycorrhizal types, independent rates for different transitions. Futhermore, using this model we could look for correlation of plant traits

The main reason for the absence of an effect of tree topology is the length of the branches leading to crown Bryophyta and crown Anthocerotophyta. Although the most basal splits of these groups are included in the phylogeny (*Takakia* and *Sphagnum* in Bryophyta and *Leiosporoceros* in Anthocerotophyta), the branches leading to the crown nodes of these groups are much longer than those leading to Marchantiophyta and Tracheophyta. The result of ancestral state thus depends more strongly on the latter two groups.

# Material & Method

*Database*

A database of 804 plant species and their mycorrhizal status was compiled, distributed over 86 plant orders. The database includes 25 hornworts, 7 mosses, 100 liverworts, 543 angiosperms, 79 gymnosperms, 21 lycopods, and 29 polypods species. For these plants species, 144 Ascomycota, 306 Basidiomycota, 450 Glomeromycota, and 27 Mucoromycotina associations were found (Table S1). DNA sequences for host plants were downloaded from Genbank via Geneious version 8.1.649. For liverworts, hornworts, polypods and lycopods a number of additional species were added to the dataset to reduce the amount of missing markers per group, resulting in a total of 825 species for phylogenetic analysis. For 129 species, full or partial chloroplast genomes were available and these were used to extract *psbA*, *rbcL* and *rps4* sequences. Full or partial rbcL sequences were available for all other plant species, and any available sequences from the other two markers were downloaded as well. Accession numbers are listed in the supplementary data (Table S2).

*Phylogenetic analysis and divergence dating*

Sequences were aligned with MAFFT v.750, using FFT-NS-i Iterative refinement method. The best partitioning scheme was selected with Partitionfinder 251,52. All codon positions of the three markers were assigned to separate partitions, except for the first and second codon position of *psbA*, which were placed in a single partition. The GTR+I+G model was selected for all partitions using the aicc. A search for the best tree with multiparametric bootstrap supports was conducted with RAxML53. Bootstrapping was stopped automatically after reaching the extended majority rule criterium (MRE). The molecular dataset was reduced to first and second codon positions of rbcL for divergence dating with BEAST2 v2.3.254. The maximum likelihood tree was used to fix the tree topology. To test the effect of different phylogenetic hypotheses42,43,55 for the deep-time relationships of land plants on ancestral state reconstruction, the likelihood tree was rooted according to the different hypotheses and each topology was submitted to divergence dating (Fig). To date the phylogenetic tree, six fossil calibration points were used (Table S3). A lognormal distribution was chosen for each of the calibration points, in order to have a soft maximum constraint. The standard deviation of log transformed distribution was 1.25 for each calibration point and the M parameter was modified to ensure that the 97.5% quantile was below the soft maximum limit of the fossil constraint. A Yule prior was chosen for the analysis, with a lognormal relaxed clock model. The MCMC analyses for of 150 million generations. Trace files were updated every 500 generations, and trees sampled every 10,000 generations.

Log Combiner was used to resample the trees every … generations with a burnin of … percent, to a total of … trees. The maximum clade credibility tree was constructed with Tree annotator v2.2.156.

ATxMB burnin 5%; 14251 trees processed. Mean heights

*Ancestral state reconstruction*

Before ancestral state reconstruction, species that were solely added to strengthen the coverage of markers for some taxa were dropped from the phylogeny. Rutger

# References max 70

1. Hedges, S. B., Marin, J., Suleski, M., Paymer, M. & Kumar, S. Tree of Life Reveals Clock-Like Speciation and Diversification. *Mol. Biol. Evol.* **32,** 835–845 (2015).

2. Lenton, T. M., Crouch, M., Johnson, M., Pires, N. & Dolan, L. First plants cooled the Ordovician. *Nat. Geosci.* **5,** 86–89 (2012).

3. Selosse, M.-A., Strullu-Derrien, C., Martin, F. M., Kamoun, S. & Kenrick, P. Plants, fungi and oomycetes: a 400-million year affair that shapes the biosphere. *New Phytol.* **206,** 501–506 (2015).

4. Brundrett, M. C. Coevolution of roots and mycorrhizas of land plants. *New Phytol.* **154,** 275–304 (2002).

5. Selosse, M.-A. & Strullu-Derrien, C. Origins of the terrestrial flora: A symbiosis with fungi? in *BIO Web of Conferences* **4,** 1–12 (EDP Sciences, 2015).

6. Pirozynski, K. A. & Malloch, D. W. The origin of land plants: A matter of mycotrophism. *BioSystems* **6,** 153–164 (1975).

7. Wang, B. & Qiu, Y. L. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16,** 299–363 (2006).

8. van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A. & Sanders, I. R. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.* **205,** 1406–1423 (2015).

9. Field, K. J. *et al.* First evidence of mutualism between ancient plant lineages (Haplomitriopsida liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO 2. *New Phytol.* **205,** 743–756 (2015).

10. Latgé, J. P. & Calderón, F. *The Mycota. A comprehensive Treatise on Fungi as Experimental Systems for Basis and Applied Research.* *Growth, Differentiation and sexuality, 2nd Edition. Springer Berlin Heidelberg* **1,** (2006).

11. Strullu-Derrien, C. *et al.* Fungal associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: Novel insights into ancestral plant-fungus symbioses. *New Phytol.* **203,** 964–979 (2014).

12. Karandashov, V., Nagy, R., Wegmüller, S., Amrhein, N. & Bucher, M. Evolutionary conservation of a phosphate transporter in the arbuscular mycorrhizal symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 6285–90 (2004).

13. Wang, B. *et al.* Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytol.* **186,** 514–525 (2010).

14. Delaux, P.-M. *et al.* Algal ancestor of land plants was preadapted for symbiosis. *Proc. Natl. Acad. Sci.* **112,** 13390–13395 (2015).

15. Hibbett, D. S. *et al.* in *Systematics and Evolution, The Mycota Part A* (eds. McLaughlin, D. J. & Spatafora, J. W.) **7A,** 373–429 (Springer-Verlag Berlin Heidelberg, 2014).

16. Schoch, C. L. *et al.* The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. *Syst. Biol.* **58,** 224–239 (2009).

17. Hibbett, D. S. & Matheny, P. B. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biol.* **7,** 1–13 (2009).

18. Floudas, D. *et al.* The Paleozoic Origin of Enzymatic Lignin Decomposition Reconstructed from 31 Fungal Genomes. *Science (80-. ).* **336,** 1715–1719 (2012).

19. Kohler, A. *et al.* Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat. Genet.* **47,** 410–5 (2015).

20. Hibbett, D. S., Gilbert, L. B. & Donoghue, M. J. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407,** 506–508 (2000).

21. Matheny, P. B. *et al.* Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia* **98,** 982–995 (2006).

22. Weiß, M., Selosse, M.-A., Rexer, K., Urban, A. & Oberwinkler, F. Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycol. Res.* **108,** 1003–1010 (2004).

23. James, T. Y. *et al.* Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443,** 818–822 (2006).

24. Ekman, S., Andersen, H. L. & Wedin, M. The Limitations of Ancestral State Reconstruction and the Evolution of the Ascus in the Lecanorales (Lichenized Ascomycota). *Syst. Biol.* **57,** 141–156 (2008).

25. Rinaldi, A. ., Comandini, O. & Kuyper, T. . Ectomycorrhizal fungal diversity : separating the wheat from the chaff. *Fungal Divers.* **33,** 1–45 (2008).

26. Jeandroz, S., Murat, C., Wang, Y., Bonfante, P. & Tacon, F. Le. Molecular phylogeny and historical biogeography of the genus Tuber, the ‘true truffles’. *J. Biogeogr.* **35,** 815–829 (2008).

27. Pressel, S., Bidartondo, M. I., Ligrone, R. & Duckett, J. G. Fungal symbioses in bryophytes : New insights in the Twenty First Century. *Nat. Hist.* **253,** 238–253 (2010).

28. Cooper, E. D., Henwood, M. J. & Brown, E. A. Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution. *Biol. J. Linn. Soc.* **107,** 425–441 (2012).

29. Newton, A. E., Wikström, N., Bell, N., Forrest, L. L. & Ignatov, M. S. in *Pleurocarpous Mosses: Systematics and Evolution.* (eds. Newton, A. E. & Tangney, R. S.) 329–358 (CRC Press, 2006).

30. Heinrichs, J., Hentschel, J., Wilson, R., Feldberg, K. & Schneider, H. Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): Estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon* **56,** 31–44 (2007).

31. Kottke, I. *et al.* Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while Aneura pinguis (Metzgeriales) is associated with a Tulasnella species. *Mycol. Res.* **107,** 957–968 (2003).

32. Bidartondo, M. I. & Duckett, J. G. Conservative ecological and evolutionary patterns in liverwort-fungal symbioses. *Proc. Biol. Sci.* **277,** 485–492 (2010).

33. Read, D. J., Ducket, J. G., Francis, R., Ligron, R. & Russell, A. Symbiotic fungal associations in ‘lower’ land plants. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **355,** 815-830-831 (2000).

34. Upson, R., Read, D. J. & Newsham, K. K. Widespread association between the ericoid mycorrhizal fungus Rhizoscyphus ericae and a leafy liverwort in the maritime and sub-Antarctic. *New Phytol.* **176,** 460–471 (2007).

35. Pressel, S., Ligrone, R., Duckett, J. G. & Davis, E. C. A novel ascomycetous endophytic association in the rhizoids of the leafy liverwort family, Schistochilaceae (Jungermanniidae, Hepaticopsida). *Am. J. Bot.* **95,** 531–541 (2008).

36. Pressel, S., Ligrone, R. & Duckett, J. G. Chapter Six: The Ascomycete Rhizoscyphus ericae Elicits a Range of Host Responses in the Rhizoids of Leafy Liverworts: An Experimental and Cytological Analysis. *Fieldiana Bot.* **47,** 59 (2008).

37. Lepage, B. a., Currah, R. S., Stockey, R. a. & Rothwell, G. W. Fossil ectomycorrhizae from the middle Eocene. *Am. J. Bot.* **84,** 410–412 (1997).

38. Bidartondo, M. I. *et al.* The dawn of symbiosis between plants and fungi. *Biol. Lett.* **7,** 574–577 (2011).

39. Ligrone, R. *et al.* Glomeromycotean associations in liverworts: A molecular, cellular, and taxonomic analysis. *Am. J. Bot.* **94,** 1756–1777 (2007).

40. Desirò, A., Duckett, J. G., Pressel, S., Villarreal, J. C. & Bidartondo, M. I. Fungal symbioses in hornworts: a chequered history. *Proc. R. Soc. London B Biol. Sci.* **280,** 20130207 (2013).

41. Rimington, W., Pressel, S., Duckett, J. & Bidartondo, M. I. Fungal associations of basal vascular plants: reopening a closed book? *New Phytol.* **205,** 1394–1398 (2015).

42. Cox, C. J., Li, B., Foster, P. G., Embley, T. M. & Civan, P. Conflicting Phylogenies for Early Land Plants are Caused by Composition Biases among Synonymous Substitutions. *Syst. Biol.* **63,** 272–279 (2014).

43. Wickett, N. J. *et al.* Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proc. Natl. Acad. Sci.* **111,** E4859–E4868 (2014).

44. Selosse, M. A. *et al.* Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytol.* **174,** 864–878 (2007).

45. Field, K. J. *et al.* Functional analysis of liverworts in dual symbiosis with Glomeromycota and Mucoromycotina fungi under a simulated Palaeozoic CO2 decline. *ISME J.* **10,** 1–13 (2015).

46. Egerton-Warburton, L. & Allen, M. F. Endo- and ectomycorrhizas in Quercus agrifolia Nee. (Fagaceae): Patterns of root colonization and effects on seedling growth. *Mycorrhiza* **11,** 283–290 (2001).

47. Dickie, I. A., Koide, R. T. & Fayish, A. C. Vesicular-arbuscular mycorrhizal infection of *Quercus rubra* seedlings. *New Phytol.* **151,** 257–264 (2001).

48. Lutzoni, F., Pagel, M. & Reeb, V. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411,** 937–940 (2001).

49. Kearse, M. *et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28,** 1647–1649 (2012).

50. Katoh, K. & Standley, D. M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **30,** 772–780 (2013).

51. Lanfear, R., Calcott, B., Kainer, D., Mayer, C. & Stamatakis, A. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evol. Biol.* **14,** 82 (2014).

52. Lanfear, R., Calcott, B. & Frandsen, P. PartitionFinder 2: new methods for selecting partitioning schemes and models of molecular evolution for large datasets. *Forthcoming*

53. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30,** 1312–1313 (2014).

54. Bouckaert, R. *et al.* BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* **10,** e1003537 (2014).

55. Clarke, J. T., Warnock, R. C. M. & Donoghue, P. C. J. Establishing a time-scale for plant evolution. *New Phytol.* **192,** 266–301 (2011).

56. Rambaut, A. & Drummond, A. J. TreeAnnotator v2.2.1. (2015).

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# Author contributions