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Benoît Perez-Lamarque [1,2 *], Maarja Öpik [3], Odile Maliet [1], Ana C. Afonso Silva [1], Marc-André Selosse [2,4], Florent Martos [2], and Hélène Morlon [1]

[1] *Institut de biologie de l'École normale supérieure (IBENS), École normale supérieure, CNRS, INSERM, Université PSL, 46 rue d'Ulm, 75 005 Paris, France*

[2] *Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'histoire naturelle, CNRS, Sorbonne Université, EPHE, UA, CP39, 57 rue Cuvier 75 005 Paris, France*

[3] *University of Tartu, 40 Lai Street, 51 005 Tartu, Estonia*

[4] *Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland*

* *corresponding author: benoit.perez@ens.psl.eu; ORCID:* 0000-0001-7112-7197

Abstract:

Analyzing diversification dynamics is key to understanding the past evolutionary history of clades that led to present-day biodiversity patterns. While such analyses are widespread in well-characterized groups of species, they are much more challenging in groups which diversity is mostly known through molecular techniques. Here, we use the largest global database on the small subunit (SSU) rRNA gene of Glomeromycotina, a subphylum of microscopic arbuscular mycorrhizal fungi that provide mineral nutrients to most land plants by forming one of the oldest terrestrial symbioses, to analyze the diversification dynamics of this clade in the past 500 million years (Myr). We perform a range of sensitivity analyses and simulations to control for potential biases linked to the nature of the data. We find that Glomeromycotina tend to have low speciation rates compared to other eukaryotes. After a peak of speciations between 200 and 100 Myr ago, they experienced an important decline in speciation rates toward the present. Such a decline could be at least partially related to a shrinking of their mycorrhizal niches and to their limited ability to colonize new niches. Our analyses identify patterns of diversification in a group of obligate symbionts of major ecological and evolutionary importance and illustrate that short molecular markers combined with intensive sensitivity analyses can be useful for studying diversification dynamics in microbial groups.

Key words: microbial diversification, arbuscular mycorrhiza, obligate symbiosis, ecological niche, macroevolution, fungi.

Introduction:

Understanding past dynamics of speciation and extinction, as well as the abiotic and biotic factors that modulate the frequency of speciation and extinction events (i.e. diversification rates) is key to understanding the historical processes that shaped present-day biodiversity patterns (Barnosky, 2001; Condamine, Rolland, Höhna, Sperling, & Sanmartín, 2018; Morlon, 2014; Varga et al., 2019) (Barnosky, 2001; Benton, 2009; Chomicki, Kiers, & Renner, 2020; Clarke & Gaston, 2006). While phylogenetic analyses of diversification are widespread in well-characterized groups of species, such as animals and plants (Givnish et al., 2015; Magallón & Sanderson, 2001; Rolland, Condamine, Jiguet, & Morlon, 2014; Upham, Esselstyn, & Jetz, 2019), they are much more challenging in groups which diversity is mostly known through environmental DNA sequences and molecular techniques. In particular, the characterization of poorly cultivable microbial groups such as most bacteria and fungi is often limited to metabarcoding techniques, which consist in the specific amplification and sequencing of a short DNA region (Taberlet, Bonin, Zinger, & Coissac, 2018). One the one hand, these data often render species delineation, phylogenetic reconstruction, and the estimation of global scale diversity highly uncertain, which all affect the phylogenetic inference of diversification dynamics (Lekberg et al., 2018; Moen & Morlon, 2014). On the other hand, it is possible to assess the robustness of phylogenetic diversification analyses to data uncertainty. Given the current limitations of sequencing technologies and the nature of the molecular data available for most microbial groups, using metabarcoding data and performing thorough robustness analyses is one of the only (if not the only) possible approach to analyze their diversification dynamics (Davison et al., 2015; Lewitus, Bittner, Malviya, Bowler, & Morlon, 2018; Louca et al., 2018).

Here we analyze the diversification dynamics of arbuscular mycorrhizal fungi from the subphylum Glomeromycotina. These fungi are obligate symbionts that have been referred to as an "evolutionary cul-de-sac, albeit an enormously successful one" (Malloch, 1987; Morton, 1990). This alludes to their ecological success despite limited morphological

and species diversities: they associate with the roots of >80% of land plants, where they provide mineral resources in exchange for photosynthates (Smith & Read, 2008). Present in most terrestrial ecosystems, Glomeromycotina play key roles in plant protection, nutrient cycling, and ecosystem processes (van der Heijden, Martin, Selosse, & Sanders, 2015). Fossil evidence and molecular phylogenies suggest that Glomeromycotina contributed to the emergence of land plants (Feijen, Vos, Nuytinck, & Merckx, 2018; Field, Pressel, Duckett, Rimington, & Bidartondo, 2015; Selosse & Le Tacon, 1998; Strullu-Derrien, Selosse, Kenrick, & Martin, 2018) and coevolved with them for more than 400 million years (Myr)(Lutzoni et al., 2018; Simon, Bousquet, Lévesque, & Lalonde, 1993; Strullu-Derrien et al., 2018).

Glomeromycotina are microscopic soil- and root-dwelling fungi that are hard to differentiate based on morphology and difficult to cultivate without host plant. Although their classical taxonomy is mostly based on the characters of spores and root colonization (Smith & Read, 2008; Stürmer, 2012), Glomeromycotina species delineation has greatly benefited from DNA sequencing (Krüger, Krüger, Walker, Stockinger, & Schüßler, 2012). Experts have defined "virtual taxa" (VT) based on a minimal 97% similarity of a region of the 18S small subunit (SSU) rRNA gene and monophyly criteria (Öpik, Davison, Moora, & Zobel, 2014; Öpik et al., 2010). As for many other pragmatic species delineation criteria, VT have rarely been tested for their biological relevance (Powell, Monaghan, Öpik, & Rillig, 2011), and a consensual system of Glomeromycotina classification is still lacking (Bruns, Corradi, Redecker, Taylor, & Öpik, 2018). Besides the rDNA region, Glomeromycotina remain poorly known genetically: other gene sequences are available for only a few species (James et al., 2006; Lutzoni et al., 2018) and less than 30 complete genomes are currently available (Venice et al., 2020).

Hence, despite the ecological ubiquity and evolutionary importance of Glomeromycotina, large-scale patterns of their diversification dynamics, as well as the factors that correlate with these dynamics, remain poorly known. A previous dated

phylogenetic tree of VT found that many speciation events occurred after the last major continental reconfiguration around 100 Myr ago (Davison et al., 2015), suggesting the radiation of Glomeromycotina is not linked to vicariant speciation during this geological event. Indeed, vicariant speciation might only play a minor role in Glomeromycotina diversification, as these organisms have spores that disperse efficiently, promoting gene flow (Bueno & Moora, 2019; Correia, Heleno, da Silva, Costa, & Rodríguez-Echeverría, 2019; Egan, Li, & Klironomos, 2014). Based on the diversity and abundance of Glomeromycotina in tropical grasslands (Read, 1991), it has been suggested (but never tested) that these habitats are diversification hotspots for Glomeromycotina (Pärtel et al., 2017). In this case, the pace of Glomeromycotina diversification through time could be tightly linked to changes in the total area of tropical grasslands. Finally, Glomeromycotina are currently obligate symbionts and their evolutionary history could thus have been largely influenced by their interactions with their host plants (Lutzoni et al., 2018; Sauquet & Magallón, 2018; Zanne et al., 2014). Over the last 400 Myr, land plants have experienced massive extinctions and radiations (Cleal & Cascales-Miñana, 2014; Zanne et al., 2014), adaptations to various ecosystems (Bredenkamp, Spada, & Kazmierczak, 2002; Brundrett & Tedersoo, 2018), and associations with different soil microorganisms (Werner et al., 2018; Werner, Cornwell, Sprent, Kattge, & Kiers, 2014). All these events could have influenced the diversification dynamics of Glomeromycotina, although their relative generalism (Perez-Lamarque, Selosse, Öpik, Morlon, & Martos, 2020; Sanders, 2003; van der Heijden et al., 2015) could buffer this influence.

We aim to characterize the pace of Glomeromycotina diversification in the last 500 Myr and to test the association between diversification rates and a variety of biotic and abiotic factors. We begin by reconstructing several thoroughly sampled phylogenetic trees of Glomeromycotina, considering several criteria of species delineations and uncertainty in phylogenetic reconstructions. We combine this phylogenetic data with paleoenvironmental data and data of current Glomeromycotina geographic distributions, ecological traits, interaction with host plants, and genetic diversity. Finally, we apply a

series of birth-death models of cladogenesis to answer specific questions and test hypotheses related to Glomeromycotina diversification: (i) how often do speciation events occur? (ii) were speciation rates relatively constant, or were they higher during specific periods of evolutionary history? and do speciation rates decline through time, as observed for many macroorganisms (Moen & Morlon, 2014)? (iii) are speciation rates positively correlated with past temperature, CO_2 concentration, and/or land plant diversity? (iv) are present-day speciation rates correlated with geographic distribution, spore size (itself often inversely related to dispersal capacity, Nathan et al., 2008), degree of specialization toward plant species, and genetic diversity? For each of these questions, we thoroughly assess the robustness of our results to uncertainty in the data.

Material & methods:

Virtual taxa phylogenetic reconstruction:

We downloaded the Glomeromycotina SSU rRNA gene sequences from MaarjAM, the largest global database of Glomeromycotina gene sequences updated in June 2019 (Öpik et al., 2010). We reconstructed several Bayesian phylogenetic trees of the 384 *virtual taxa* (VT) from the corresponding representative sequences available in the MaarjAM database (Supplementary Methods 1). We used the full length (1,700 base pairs) SSU rRNA gene sequences from (Rimington et al., 2018) to better align the VT sequences using MAFFT (Katoh & Standley, 2013). We selected the 520 base pair central variable region of the VT aligned sequences and performed a Bayesian phylogenetic reconstruction using BEAST2 (Bouckaert et al., 2014). We set the crown root age at 505 Myr (Davison et al., 2015), which is coherent with fossil data and previous dated molecular phylogenies (Lutzoni et al., 2018; Strullu-Derrien et al., 2018). We also used the youngest (437 Myr) and oldest (530 Myr) crown age estimates from (Lutzoni et al., 2018) in diversification analyses that may be particularly sensitive to absolute dates.

Delineation into Evolutionary Units (EUs):

We considered several ways to delineate Glomeromycotina species based on the SSU rRNA gene. In addition to the VT species proxy, we delineated Glomeromycotina *de novo* into evolutionary units (EUs) using a monophyly criterion and 5 different thresholds of sequence similarity ranging from 97 to 99%. We gathered Glomeromycotina sequences of the SSU rRNA gene from MaarjAM, mainly amplified by the primer pair NS31–AML2 (variable region) (Lee, Lee, & Young, 2008; Simon, Lalonde, & Bruns, 1992) (dataset 1, Supplementary Table 1). There were 36,411 sequences corresponding to 27,728 haplotypes. We first built a phylogenetic tree of these haplotypes and then applied to this tree our own algorithm of EU delineation (R-package RPANDA (Morlon et al., 2016; R Core Team, 2020))

that traverses the tree from the root to the tips, at every node computes the average similarity of all sequences descending from the node, and collapses the sequences into a single EU if their sequence dissimilarity is lower than a given threshold (Supplementary Methods 2). In other words, Glomeromycotina sequences are merged into the same EU if they form a monophyletic clade and if they are on average more similar than the sequence similarity threshold. Finally, we performed Bayesian phylogenetic reconstructions of the EUs using BEAST2, using the same crown ages as above (Supplementary Methods 1).

Coalescent-based species delineation analyses:

Finally, we considered the Generalized Mixed Yule Coalescent method (GMYC) (Fujisawa & Barraclough, 2013; Pons et al., 2006), a species delineation approach that does not require specifying an arbitrary similarity threshold. GMYC estimates the time *t* in a reconstructed calibrated tree that separates species diversification (Yule process – before *t*) and intraspecific differentiation (coalescent process – after *t*). GMYC is too computationally intensive to be applied to the 36,411 SSU sequences; we used it here on three clades of manageable size (the family Claroideoglomeraceae; the order Diversisporales; and an early-diverging clade composed of the orders Archaeosporales and Paraglomerales) to (i) investigate whether the SSU gene evolves fast enough to accumulate substitutions between Glomeromycotina speciation events (Bruns et al., 2018) and (ii) evaluate the biological relevance of the VT and various EUs delineations. For each clade, we reconstructed Bayesian phylogenetic trees of haplotypes (Supplementary Methods 1). We then ran GMYC analyses (splits R-package (Ezard, Fujisawa, & Barraclough, 2009)) on each of these trees and evaluated the support of the GMYC model compared to a null model in which all tips are assumed to be different species, using a likelihood ratio test (LRT). If the LRT supports the GMYC model, different SSU haplotypes belong to the same Glomeromycotina species, *i.e.* the SSU rRNA gene has time to accumulate substitutions between Glomeromycotina speciation events.

Total diversity estimates:

We evaluated how thoroughly sampled our species-level Glomeromycotina phylogenetic trees are by estimating the total number of VT and EUs using rarefaction curves and the Bayesian Diversity Estimation Software (BDES (Quince, Curtis, & Sloan, 2008)) (Supplementary Methods 3). The BDES estimates the total number of species by extrapolating a sampled taxa abundance distribution at global scale (Quince et al., 2008).

Additional molecular markers:

We explored the possibility to carry some of our analyses using two other molecular markers: the large subunit (LSU) rRNA gene and the ITS2 region. We downloaded the Glomeromycotina LSU database of Delavaux et al. (2020) as well as the LSU sequences available in MaarjAM. We obtained a total 2,044 sequences that we aligned using MAFFT and TrimAl. We retained the 1,760 unique haplotypes, reconstructed the phylogenetic tree of the LSU sequences using BEAST2 and used the resulting calibrated tree to delineate Glomeromycotina LSU units with the GMYC model (same pipeline as above). We similarly downloaded the Glomeromycotina ITS dataset of Lekberg et al. (2018). We tried to align them but confirmed that the ITS sequences of Glomeromycotina are very difficult to align, making them unsuitable for phylogenetic reconstruction and subsequent diversification analyses (Supplementary Fig. 1).

Diversification analyses:

Unless specified differently, our diversification analyses were performed using the SSU rRNA gene. In order to account for various sources of uncertainties in the SSU rRNA data, we replicated all our diversification analyses across different species delineations, phylogenetic reconstructions and dating, and total diversity estimates. For each species delineation criterion, we obtained a consensus tree and selected 12 trees equally spaced in

4 independent Bayesian chains, hereafter referred to as the replicate trees. When the 12 trees were not sufficient to conclude, we used 100 replicate trees.

We estimated lineage-specific speciation rates using ClaDS, a Bayesian diversification model that accounts for speciation rate heterogeneity by modeling small rate shifts at speciation events (Maliet, Hartig, & Morlon, 2019). At each speciation event, the descending lineages inherit new speciation rates sampled from a log-normal distribution with an expected value $\log[\alpha \times \lambda]$ (where λ represents the parental speciation rate and α is a trend parameter) and a standard deviation σ. We considered the model with constant turnover ε (*i.e.* constant ratio between extinction and speciation rates; *ClaDS2*) and ran a newly-developed ClaDS algorithm based on data augmentation techniques which enables us to estimate mean rates through time (Maliet & Morlon, 2022). We ran ClaDS with 3 independent chains, checked their convergence using a Gelman-Rubin diagnostic criterion (Gelman & Rubin, 1992), and recorded lineage-specific speciation rates. We also recorded the estimated hyperparameters (α, σ, ε) and the value $m=\alpha \times \exp(\sigma^2/2)$, which indicates the general trend of the rate through time (Maliet et al., 2019). We replicated these analyses using the LSU gene.

In addition, we applied CoMET (TESS R-package (Höhna, May, & Moore, 2016; May, Höhna, & Moore, 2016)), another diversification approach that does not consider rate variation across lineages, but models temporal shifts in speciation and extinction rates affecting all lineages simultaneously. CoMET is a piecewise-constant model in a Bayesian framework. We chose the Bayesian priors according to maximum likelihood estimates from TreePar (Stadler, 2011), disallowed or not mass extinction events, and ran the MCMC chains until convergence (minimum effective sample sizes of 500).

We also fitted a series of time-dependent and environment-dependent birth-death diversification models using RPANDA (Condamine, Rolland, & Morlon, 2013; Morlon et al., 2016) to confirm the observed temporal trends and test the influence of temperature,

254 pCO_2, and land plant fossil diversity on rates of Glomeromycotina speciation. For the time-
255 dependent models, we considered models with constant or exponential variation of
256 speciation rates through time and null or constant extinction rates (*fit_bd* function). As
257 extinction is notoriously hard to estimate from reconstructed phylogenies (Rabosky, 2016),
258 we tested the robustness of the inferred temporal trend in speciation when fixing
259 arbitrarily high levels of extinction (Supplementary Methods 4). For the environment-
260 dependent models, we considered an exponential dependency of the speciation rates with
261 the environmental variable (env), *i.e.* speciation rate=b*exp(a*env), where a and b are two
262 parameters estimated by maximum likelihood (*fit_env* function). Best-fit models were
263 selected based on the corrected Akaike information criterion (AICc), considering that a
264 difference of 2 in AICc indicates that the model with the lowest AICc is better. We
265 replicated these analyses using the LSU gene.
266
267 The influence of temperature was tested on the complete Glomeromycotina
268 phylogenetic trees, using estimates of past global temperature (Royer, Berner, Montañez,
269 Tabor, & Beerling, 2004). We also carried a series of simulation analyses to test the
270 robustness of our temperature-dependent results (Supplementary Methods 5). The
271 influence of pCO_2 (Foster, Royer, & Lunt, 2017) and of land plant fossil diversity was tested
272 starting from 400 Myr ago, as these environmental data are not available for more ancient
273 times. For these analyses we sliced the phylogenies at 400 and 200 Myr ago, and applied
274 the diversification models to the sliced sub-trees larger than 50 tips. Estimates of land plant
275 diversity were obtained using all available Embryophyta fossils from the Paleobiology
276 database (https://paleobiodb.org) and using the shareholder quorum subsampling method
277 (Supplementary Methods 6; (Alroy, 2010)).
278
279 We considered missing species in all our diversification analyses by imputing
280 sampling fractions, computed as the number of observed VT or EUs divided by the
281 corresponding BDES estimates of global Glomeromycotina diversity (Table 1). We used a
282 global sampling fraction for all Glomeromycotina, as the main Glomeromycotina clades

had a similar sampling fraction (Supplementary Table 2). To assess the robustness of our results to global diversity estimates, we replicated all diversification analyses using a range of lower sampling fractions (from 90% to 50%, *i.e.* assuming that only that percentage of the global Glomeromycotina species diversity is in fact represented in our dataset).

Testing for correlates of present-day Glomeromycotina speciation rates:

To further investigate the potential factors correlating with Glomeromycotina speciation rates, we assessed the relationship between lineage-specific estimates of present-day speciation rates (obtained with the ClaDS analyses) and characteristics of each Glomeromycotina taxonomic unit, *i.e.* VT or EUs.

First, to assess the effect of specialization on speciation rates, we characterized Glomeromycotina relative niche width using a set of 10 abiotic and biotic variables recorded in MaarjAM database for each Glomeromycotina unit. In short, among a curated dataset containing Glomeromycotina sequences occurring only in natural ecosystems (dataset 2; Supplementary Table 2; Perez-Lamarque et al., 2020), for each Glomeromycotina unit, we reported the number of continents, ecosystems, climatic zones, biogeographic realms, habitats, and biomes where it was sampled, as well as its number of plant partners, their phylogenetic diversity, and its centrality in the plant-fungus bipartite network, and performed a principal component analysis (PCA; Supplementary Methods 7). For Glomeromycotina units represented by at least 10 sequences, we tested whether these PCA coordinates reflecting Glomeromycotina niche widths were correlated with the present-day speciation rates using both linear mixed-models (not accounting for phylogeny) or MCMCglmm models (Hadfield, 2010). For MCMCglmm, we assumed a Gaussian residual distribution, included the fungal phylogenetic tree as a random effect, and ran the MCMC chains for 1,300,000 iterations with a burn-in of 300,000 and a thinning interval of 500.

311 Next, we tested the relationship between speciation rates and geographic
312 characteristics of Glomeromycotina units. To evaluate the effect of latitude on speciation
313 rates, we associated each Glomeromycotina unit with its set of latitudes and used similar
314 MCMCglmm with an additional random effect corresponding to the Glomeromycotina
315 unit. To account for inhomogeneous sampling along the latitudinal gradient, we re-ran the
316 model on jackknifed datasets (we re-sampled 1,000 interactions per slice of latitude of
317 twenty degrees). Similarly, we tested the effect of climatic zone and habitat on speciation
318 rates.
319
320 Then, to assess the effect of dispersal capacity on speciation rates, we evaluated the
321 relationship between spore size and speciation rate for the few (n=32) VT that contain
322 sequences of morphologically characterized Glomeromycotina isolates (Davison et al.,
323 2018). We gathered measures of their average spore length (Davison et al., 2018) and tested
324 their relationship with speciation rate by using a phylogenetic generalized least square
325 regression (PGLS).
326
327 Finally, as a first attempt at connecting Glomeromycotina macroevolutionary
328 diversification to microevolutionary processes, we measured intraspecific genetic
329 diversities across Glomeromycotina units. For each Glomeromycotina unit containing at
330 least 10 sequences, we computed genetic diversity using Tajima's estimator (Tajima,
331 1983)($\theta\pi$; Supplementary Methods 8). Using similar statistical tests as above, we
332 investigated the correlation of Glomeromycotina genetic diversity with speciation rate,
333 niche width, geographic characteristics, and spore size. We tested the robustness of the
334 results to the minimal number of sequences per Glomeromycotina unit (10, 15, or 20) used
335 to compute genetic diversity and to perform the PCA.
336
337 These statistical models were replicated on the different phylogenetic trees
338 (consensus or replicates) for each delineation and we reported p-values (P) corresponding
339 to two-sided tests.

Simulation analyses:

The use of a short and slowly evolving gene such as the central region of the SSU rRNA gene to delineate species may lead to an artificial lumping of species into the same unit that would reduce the number of phylogenetic branching events toward the present and result in a biased inference of temporal diversification dynamics, including an artifactual detection of a diversification slowdown (Moen & Morlon, 2014). We used simulations mimicking the evolution of the SSU rRNA gene as Glomeromycotina diversified to quantify this potential bias.

We simulated the diversification of a clade of species in the last 505 Myr, according to two scenarios: (i) constant speciation rate and no extinction and (ii) constant speciation and extinction rates (Supplementary Figure 2a). To model intraspecific differentiation, we added intraspecific splits on these simulated species trees by grafting coalescent events at each tip: for each species, we uniformly sampled between 2 and 15 individuals and we considered that all these individuals had to coalesce before the last speciation event; the age of the coalescent tree within each species was uniformly sampled between 0 and the age of the last speciation event (with a maximum of 30 Myr). We used the functions *pbtree* and *rcoal* from the R-packages phytools and ape (Paradis, Claude, & Strimmer, 2004; Revell, 2012) to simulate the species phylogenies and the intraspecific coalescences respectively. We used two net diversification rates (r=0.010 and r=0.015) for simulating the species phylogenies, in order to reach a total number of species similar to that obtained with our empirical data when using the VT and EU99 delineations, respectively. Next, we simulated the evolution of short 520 bp DNA sequences on the obtained trees, using the function *simulate_alignment* (R-package HOME; Perez-Lamarque & Morlon, 2019). We used a substitution rate of 0.001 event per Myr and only 25% of variable sites, which resulted in an alignment that mimicked the Glomeromycotina SSU rDNA alignment. We performed 10 simulations per scenario. For each of these simulations we kept the unique

haplotypes at present and applied the same pipelines as above, using the EU99 species delineation criteria: after delineating the EU99 units, we reconstructed the EU99 phylogenetic trees, ran the ClaDS analyses on these trees, and recorded mean estimated speciation rates at present and 50, 100, and 150 Myr ago.

Results:

Glomeromycotina species delineations & phylogenetic reconstructions:

We automatically delineated Glomeromycotina into evolutionary units (EU) using a monophyly criterion and several thresholds of SSU rRNA sequence similarity (from 97% to 99%). The EU97.5 and EU98 delineations (obtained using a threshold of 97.5% and 98% respectively) provided a number of Glomeromycotina units (340 and 641) relatively comparable to the 384 currently recognized *virtual taxa* (VT), while the EU97 delineation had much less units (182). Conversely, the EU98.5 and EU99 delineations yielded a much larger number of Glomeromycotina units (1,190 and 2,647). These numbers obtained with the EU98.5 and EU99 delineations were consistent with the numbers obtained using GMYC analyses, which delineate species-like units based on detecting when splitting events in the haplotype tree start to follow branching patterns consistent with intra-specific differentiations (*i.e.* coalescent patterns) instead of speciation events (i.e. birth-death patterns; Supplementary Tables 3, 4, & 5). The GMYC results therefore support the idea that some VT might lump together several cryptic species (Bruns et al., 2018)(Supplementary Note 1), and that a 98.5 or 99% similarity threshold is more relevant for Glomeromycotina species delineation. In addition, the GMYC model is significantly supported over the model where all SSU rRNA haplotypes correspond to a different species (GMYC LRT: $P<0.05$; Supplementary Fig. 3), with on average 10 SSU haplotypes per species-like unit, and a mean intraspecific sequence similarity of 99% (Supplementary Table 5 & Supplementary Fig. 3). This indicates that the region of the SSU marker used to characterized Glomeromycotina evolves fast enough to accumulate substitutions between Glomeromycotina speciation events, meaning that it is an informative (although not perfect) marker for delineating Glomeromycotina species-like units. In comparison, the same pipeline carried on the LSU database delineated only 181 GMYC units, suggesting that it was much less complete than the SSU database. We replicated the subsequent

diversification analyses using the LSU region, even though we put more trust in our results using the SSU database given the incompleteness of the LSU database.

Rarefaction curves as well as BDES (Bayesian Diversity Estimation Software) and Chao2 estimates of diversity suggested that more than 90% of the total Glomeromycotina diversity is represented in our SSU dataset regardless of the delineation threshold (Fig. 1, Table 1, Supplementary Tables 5 & 6), which is consistent with the proportion of new Glomeromycotina units detected in recent studies (Sepp et al., 2019).

The reconstructed Bayesian phylogenetic trees based on VT and EU delineations did not yield high support for the nodes separating the main Glomeromycotina orders; yet, the trees had no significantly-supported conflicts either, and similar branching times of the internal nodes overall (Fig. 2, Supplementary Fig. 4). As expected, finer delineations resulted in an increase in the number of nodes close to the present (Supplementary Fig. 5). However, we observed a slowdown in the accumulation of new lineages close to the present in all lineage through time plots (LTTs), including those with the finest delineations (EU98.5 and EU99; Supplementary Fig. 6).

Temporal diversification dynamics:

We found that speciation rates for Glomeromycotina ranged from 0.005 to 0.03 events per lineage per Myr, using both the VT and EU SSU rRNA delineations (Fig. 2; Supplementary Fig. 7). Speciation rates varied both within and among Glomeromycotina orders, with Glomerales and Diversisporales having the highest present-day speciation rates (Supplementary Fig. 8). As expected we observed higher present-day speciation rates for finer delineations, but at the haplotype level (i.e. at the level of the individual SSU rRNA sequences within each unit) we found a significant correlation of present-day speciation rates computed with ClaDS using different delineations (Supplementary Fig. 9). Whatever the delineations, Glomeromycotina experienced their highest speciation rates between 200

and 100 Myr ago according to estimates obtained with ClaDS (Fig. 2; Supplementary Fig. 10) and between 150 and 50 Myr ago according to CoMET (Fig. 2; Supplementary Fig. 11). ClaDS estimates of speciation rates at 150 Myr ago were 26% (± s.d. 17) higher than those at 300 Myr with the EU99 delineation. With the VT delineation, the increase was of 3% (±8). The peak was even stronger using CoMET: 30% ± 20 higher at 150 Myr in comparison to 300 Myr with the EU99 delineation (71% ± 40 with the VT delineation; Fig. 2).

The peak of speciation rates was followed by a decline in the recent past (Fig. 2; Supplementary Fig. 10), as suggested by the plateauing of the LTTs. A global decline of the speciation rates through time was independently supported by ClaDS and CoMET analyses, as well as time-dependent models in RPANDA (Morlon, Parsons, & Plotkin, 2011)(Supplementary Figs. 11, 12, & 13). This speciation rate decline was robust to all species delineations, the branching process prior (Supplementary Table 7), phylogenetic uncertainty, and assumed sampling fractions as low as 50%, except in ClaDS analyses where the trend disappeared in some EU99 trees and for sampling fractions lower than 70% (Supplementary Figs. 14 & 15). We also found a period of high speciation rates between 200 and 100 Myr ago followed by a decline in our analyses with the LSU region, for assumed sampling fractions as low as 60% (Supplementary Figure 16).

We did not find a strong signal of extinction in our analyses: the turnover rate estimated from ClaDS was generally close to zero (Supplementary Fig. 12b), and models including extinctions were never selected in RPANDA (Supplementary Fig. 13). Similarly, the extinction rates estimated in piecewise-constant models (CoMET) were not significantly different from 0 and we did not find significant support for mass extinction events (Supplementary Fig. 17). Yet, forcing the extinction rate to high positive values did not modify the general trend of speciation rate slowdown (Supplementary Figs. 18 & 19).

Correlates of Glomeromycotina diversification:

When fitting environment-dependent models of diversification, we found that temperature-dependent models better fit Glomeromycotina diversification than time-dependent models, with higher speciation rates during warm climatic periods (Fig. 3; Supplementary Fig. 20). This was true for all Glomeromycotina delineations, sampling fractions, and crown ages (Supplementary Figs. 21, 22, 23, & 24), with the exception of some EU99 trees with a 50% sampling fraction (Supplementary Fig. 24). It was also true in our analyses using the LSU region, for sampling fractions down to 50% (Supplementary Figure 29). This signal of temperature dependency was not due to a temporal trend (Supplementary Figs. 25 & 26) nor to an artefact caused by rate heterogeneities (Supplementary Fig. 27). Evidence for temperature dependency, however, decreased in some clades closer to the present, as small trees tend to be best fit by constant or time-depend models (Supplementary Fig. 28). We detected a significant positive dependency of the speciation rates on CO_2 concentrations in some sub-trees, but rarely found a significant effect of plant fossil diversity (Supplementary Fig. 28).

The PCA of Glomeromycotina relative niche width characteristics had a first principal component (PC1) that indicated the propensity of each Glomeromycotina unit (VT or EUs) to be vastly distributed among continents, ecosystems and/or associated with many plant species and lineages (*i.e.* high generalism), whereas the second principal component (PC2) indicated the propensity of a given Glomeromycotina unit to associate with few plant species on many continents (*i.e.* high specialism toward plants; Supplementary Figs. 30, 31, & 32). Hence, PC1 reflects Glomeromycotina niche width, whereas PC2 discriminates the width of the abiotic relatively to the biotic niche (Fig. 4a-b). We found a positive correlation between lineage-specific speciation rates and PC1 in the majority of the VT and EU99 trees, but no significant correlation with PC2 (Fig. 4c-d; Supplementary Fig. 33a). However, these results were no longer significant when controlling for phylogenetic non-independence between Glomeromycotina units (Supplementary Fig. 33b), likely because a single *Glomeraceae* clade, including the abundant

and widespread morphospecies *Rhizophagus irregularis* and *R. clarus* (high PC1 values), had both the highest speciation rates and the largest niche widths among Glomeromycotina (Supplementary Fig. 34).

Although Glomeromycotina diversity is currently higher in the (sub)tropics (Supplementary Fig. 35), we found no effect of latitude on speciation rates, regardless of the Glomeromycotina delineation or the minimum number of sequences per Glomeromycotina unit (MCMCglmm: $P>0.05$). In addition, we actually did not detect a higher total number of Glomeromycotina species in grasslands compared to forests (Supplementary Figure 36; confirming the results of Davison et al. 2015), and it is thus not surprising that we reported no effect of habitat or climatic zone on speciation rates (Supplementary Fig. 37), suggesting that tropical grasslands are not particular diversification hotspots for Glomeromycotina. Similarly, we recovered no significant correlation between spore size and speciation rate (Supplementary Fig. 38), nor between spore size and level of endemism (Supplementary Fig. 39).

Finally, Tajima's estimator of Glomeromycotina genetic diversity was significantly and positively correlated with niche width (PC1) for all Glomeromycotina delineations and minimal number of sequences per Glomeromycotina unit considered, and in particular with abiotic aspects of the niche (PC2) in many cases (Fig. 4e-h; Supplementary Fig. 33). Genetic diversity was not correlated with speciation rate (Supplementary Fig. 33), latitude, habitat, climatic zone (MCMCglmm: $P>0.05$), or spore size (PGLS: $P>0.05$).

Simulation results:

When we simulated the evolution of a short DNA gene mimicking the SSU rRNA marker and used it to delineate species, we found that the number of EU99 delineated units was generally lower than the number of simulated species (~10% to 20% lower; Supplementary Figure 2b). Hence, even the EU99 delineation tends to lump together some

closely related species. As expected, this lumping resulted in an artefactual inference of a decline of speciation rates toward the present, but this artifactual decline was significantly smaller in magnitude than that observed in Glomeromycotina (Figure 5). Hence, these analyses suggest that the lumping of species resulting from the use of a small, slowly evolving marker is unlikely to fully explain the strong temporal decline in speciation rate we found in Glomeromycotina.

Discussion

Glomeromycotina species delineations, diversity, and phylogeny:

It is difficult to delineate species in Glomeromycotina, which are poorly differentiated morphologically and mainly characterized by environmental sequences (Bruns et al., 2018). Our GMYC analyses suggest that Glomeromycotina species-like units correspond to SSU rRNA haplotypes with a sequence similarity between 98.5 and 99%. With this criterion of species delineation, we estimate that there are between 1,300 and 2,900 Glomeromycotina 'species'. These estimates are largely above the number of currently described morphospecies or VT (Supplementary Note 1) but remain low in comparison with other fungal groups, like the Agaricomycetes that include taxa forming ectomycorrhiza (Varga et al., 2019).

Our phylogenies based on the SSU rRNA gene did not resolve the branching of the Glomeromycotina orders, with node supports similar to those of previous studies (Davison et al., 2015; Krüger et al., 2012; Rimington et al., 2018)(Supplementary Note 2). These findings confirm that additional genomic evidence is required to reach consensus. We considered this uncertainty in species delineation and phylogenetic reconstruction by repeating our diversification analyses across species delineation criteria and on a set of trees spanning the likely tree space. We found effects of species delineation consistent with *a priori* expectations: criteria that lump together more dissimilar sequences (e.g. those that use a lower percentage of similarity cut-off) result in lower diversity estimates, lower estimates of speciation rates, and patterns of diversification through time that reflect longer terminal branch-lengths, such as peaks of diversification that occur earlier. Despite this variability, we found that general patterns, such as the observed temporal decline in speciation rates and the significant association between temperature and speciation rates, were consistent across species delineations and trees. Therefore, our study based on a short SSU (or LSU) rRNA region should encourage both efforts to obtain more genetic data,

including longer reads (Krehenwinkel et al., 2019; Tedersoo, Albertsen, Anslan, & Callahan, 2021) and additional genomic information, with the aim of reconstructing better supported, comprehensive phylogenies and efforts to conduct diversification analyses despite uncertainty in the data for groups where better data is not yet available.

Glomeromycotina diversify slowly:

We found speciation rates for Glomeromycotina an order of magnitude lower than rates typically found for macro-eukaryotes (Maliet et al., 2019; Upham et al., 2019), like plants (Zanne et al., 2014), or Agaricomycetes (Varga et al., 2019). Low speciation rates in Glomeromycotina may be linked to their multinucleate hyphal state (Yildirir, Malar, Kokkoris, & Corradi, 2020), to their occasional long-distance dispersal that homogenizes populations globally over evolutionary timescales (Savary et al., 2018), and/or to the fact that they are generalist obligate symbionts (Morlon, Kemps, Plotkin, & Brisson, 2012). Regardless of the proximal cause, and contrary to Agaricomycetes for example, which present a large diversity of species, morphologies, and ecologies, Glomeromycotina have poorly diversified in the last 500 Myr despite their ubiquity; their niche space is restricted to plant roots and the surrounding soil because of their obligate dependence on plants for more than 400 Myr (Rich, Nouri, Courty, & Reinhardt, 2017; Tisserant et al., 2013).

Our estimates of speciation rates were highly variable across lineages. We reported the highest speciation rates in Glomeraceae and Diversisporaceae. Speciation rates in Paraglomeraceae and Archaeosporaceae, which are thought to be less beneficial for the plants than the fast diversifying Glomeraceae and Diversisporaceae (Säle et al., 2021), were an order of magnitude lower. We can therefore speculate that good symbiotic abilities may favor diversification, although this remains to be tested in further investigations.

We found little evidence for species extinction in Glomeromycotina, including at mass extinction events. Because Glomeromycotina are relatively widespread and have a

581 an ancient tendency toward generalism, they might therefore be quite resilient to land
582 plant mass extinctions and low extinction rates have been predicted before based on their
583 ecology (Morton, 1990). Yet, these low extinction rate estimates could also come from the
584 difficulty of estimating extinction from molecular phylogenies (Rabosky, 2016), one of the
585 limitations of phylogeny-based diversification analyses (Supplementary Note 3). Fossils of
586 Glomeromycotina that can be ascribed to species or genera are too scarce to support or
587 conflict with this finding.
588
589 **Glomeromycotina diversification through time:**
590
591 The observed peak of Glomeromycotina speciations detected between 200 and 100
592 Myr (or 150-50 Myr depending on the models) was mainly linked to the frequent
593 speciations in the largest family Glomeraceae. This peak was concomitant with the
594 radiation of flowering plants (Sauquet & Magallón, 2018), but also with a major continental
595 reconfiguration, including the breakdown of Pangea and the formation of climatically
596 contrasted landmasses (Davison et al., 2015). This period was also characterized by a warm
597 climate potentially directly or indirectly favorable to Glomeromycotina diversification,
598 such that disentangling the impact of these various factors on Glomeromycotina
599 diversification rates is not straightforward. Interestingly, a peak of speciations at this
600 period was also found in the Agaricomycetes, a clade of fungi including lineages forming
601 ectomycorrhizae (Varga et al., 2019).
602
603 This peak in the occurrence of speciation events was followed by a decline in
604 speciation rates. The detection of temporal declines in speciation rates in phylogenetic
605 diversification analyses can sometimes be artifactual, for example if some species are
606 incorrectly lumped together during species delineation or if the proportion of species not
607 represented in the phylogeny is under-estimated (Moen & Morlon, 2014). We considered
608 these potential biases, conducted sensitivity analyses, and found that the observed
609 slowdown was robust, and even amplified under scenarios of high extinction. Some

610 Glomeromycotina species are likely lumped together into the same SSU haplotypes
611 (Krüger et al., 2012), but both our use of an overly small assumed sampling intensity (50%)
612 and our simulation analyses demonstrated that this lumping is not sufficient to explain the
613 observed slowdown. In addition, we also detected a temporal decline in speciation rates
614 when using another marker (the LSU rRNA gene).
615
616 Temporal declines in speciation rates have been observed in many clades, including
617 microorganisms (Condamine, Rolland, & Morlon, 2019; Morlon et al., 2012; Rabosky &
618 Lovette, 2008). They have often been interpreted as a progressive reduction of the number
619 of available niches as species diversify and accumulate (Moen & Morlon, 2014; Rabosky,
620 2009). In Glomeromycotina, this potential effect of niche saturation could be exacerbated
621 by a reduction of their niches linked to both repetitive breakdowns of their symbiosis with
622 plants and climatic changes. Indeed, since the Cretaceous, many plant lineages evolved
623 alternative root symbioses or became non-symbiotic (Brundrett & Tedersoo, 2018;
624 Maherali, Oberle, Stevens, Cornwell, & McGlinn, 2016; Selosse & Le Tacon, 1998; Werner
625 et al., 2018): approximately 20% of extant plants do not interact with Glomeromycotina
626 anymore (van der Heijden et al., 2015). Additionally, the cooling of the Earth during the
627 Cenozoic reduced the surface of tropical regions (Meseguer & Condamine, 2020; Ziegler et
628 al., 2003), which tend to be a reservoir of ecological niches for Glomeromycotina (Brundrett
629 & Tedersoo, 2018; Davison et al., 2015; Read, 1991).
630
631 The difficulty of reconstructing past symbiotic associations prevents direct testing
632 the hypothesis that the emergence of new root symbioses in plants led to a decline in
633 speciation rates in Glomeromycotina. However, we tested the hypothesis that global
634 temperature changes affected speciation rates and found a strong relationship. Such
635 associations between temperature and speciation rates have been observed before in
636 eukaryotes and have several potential causes (Condamine et al., 2019). In particular, the
637 productivity hypothesis states that resources and associated ecological niches are more
638 numerous in warm and productive environments, especially when the tropics are large,

639 which entail higher speciation rates (Clarke & Gaston, 2006). This hypothesis is particularly
640 relevant for Glomeromycotina, which have many host plant niches in the tropics, as shown
641 by their latitudinal diversity gradient, and potentially relatively less in temperate and polar
642 regions (Toussaint et al., 2020), where a higher proportion of plants are non-mycorrhizal
643 (Bueno et al., 2017) or ectomycorrhizal (Brundrett & Tedersoo, 2018; Varga et al., 2019).
644 Hence, the observed effect of past global temperatures could reflect the shrinkage of
645 tropical areas and the associated decrease of the relative proportion of arbuscular
646 mycorrhizal plants. Future developments of diversification models incorporating
647 interspecific interactions would allow us to better test these hypotheses.
648
649 A few Glomeromycotina clades displayed a significant support for diversification
650 models with a positive dependency of speciation rates on CO_2 concentrations, which
651 reinforces the idea that for the corresponding Glomeromycotina, benefits retrieved from
652 plants could have been amplified by high CO_2 concentrations and fostered diversification
653 (Field et al., 2016; Humphreys et al., 2010). Conversely, we found a limited effect of land
654 plant fossil diversity, which indicates that variations in the tempo of Glomeromycotina
655 diversification did not systematically follow those of land plants. Still, the possible
656 concordance of the peak of Glomeromycotina speciations with the radiation of the
657 Angiosperms is noteworthy, in particular in Glomeraceae that frequently interact with
658 present-day Angiosperms (Rimington et al., 2018). Plant diversification might have
659 fostered the diversification of Glomeromycotina from the emergence of land plants until
660 the Mesozoic (Lutzoni et al., 2018; Morton, 1990), but less so thereafter, when
661 Glomeromycotina diversification declined while some flowering plants radiated,
662 including Glomeromycotina-associated groups, like the Poaceae, but also
663 Glomeromycotina-free groups such as the extraordinary radiation of Orchidaceae (Givnish
664 et al., 2015), blurring co-diversification patterns (Supplementary Fig. 40)(Cleal & Cascales-
665 Miñana, 2014; Ramírez-Barahona, Sauquet, & Magallón, 2020).
666
667 **Correlates of Glomeromycotina recent speciation rates:**

668

669 Looking at the correlates of Glomeromycotina present-day speciation rates, we found
670 no effect of habitat or climatic zone, even though Glomeromycotina are more frequent and
671 diverse in the tropics (Davison et al., 2015; Pärtel et al., 2017; Toussaint et al., 2020) and a
672 positive correlation with global temperature. Further work, including a more thorough
673 sampling of the distribution of Glomeromycotina species across latitudes and habitats,
674 would be required to confirm these patterns and to distinguish whether speciation events
675 are indeed no more frequent in the tropics or, if they are, whether long-distance dispersal
676 redistributes the new lineages at different latitudes over long time scales. Contrary to
677 previous predictions (Pärtel et al., 2017), we did not find that tropical grasslands are
678 diversification hotspots for Glomeromycotina; we actually did not even find higher
679 Glomeromycotina species richness in (tropical) grasslands *versus* forests at global scale, in
680 agreement with Davison *et al.* (2015).
681

682 Similarly, although the temporal changes in the availability of Glomeromycotina
683 niches likely influenced the diversification of the group, we found little support for
684 Glomeromycotina species with larger niche width having higher lineage-specific
685 speciation rates. We also note that there are important aspects of the niche that we do not
686 (and yet cannot) account for in our characterization of Glomeromycotina niche width: it is
687 thought that some Glomeromycotina species may mainly provide mineral nutrients
688 extracted from the soil, whereas others may be more specialized in protecting plants from
689 biotic or abiotic stresses (Chagnon, Bradley, Maherali, & Klironomos, 2013) and such (inter-
690 or intra-specific) functional variations may have evolutionary significance. Finally,
691 although spore size is often inversely related to dispersal capacity (Nathan et al., 2008),
692 which can limit speciation by increasing gene flow, we found no significant correlation
693 between spore size and speciation rates, which may be explained either by a weak or absent
694 effect or by the low number of species for which this data is available. In addition, the
695 absence of correlation between spore size and level of endemism suggests that even
696 Glomeromycotina with large spores experience long-distance dispersal (Davison et al.,

2018; Kivlin, 2020). Thus, if large spores might limit dispersal at smaller (*e.g.* intra-continental) scales in Glomeromycotina (Bueno & Moora, 2019; Chaudhary, Nolimal, Sosa-Hernández, Egan, & Kastens, 2020), this does not seem to affect speciation rates.

In Glomeromycotina, intraspecific variability is an important source of functional diversity (Munkvold, Kjøller, Vestberg, Rosendahl, & Jakobsen, 2004; Savary et al., 2018) and their genetic diversity may indicate the intraspecific variability on which selection can act, potentially leading to speciation. Here, geographically widespread Glomeromycotina species appear to be more genetically diverse, as previously suggested by population genomics (Savary et al., 2018), but do not necessarily speciate more frequently. Along with a decoupling between genetic diversity and lineage-specific speciation rate, this suggests that the accumulation of genetic diversity in the SSU region among distant subpopulations is not enough to spur Glomeromycotina speciation.

Analyzing diversification dynamics using a short marker gene:

Short DNA regions, like those used in metabarcoding surveys, typically do not allow to robustly delineate species, estimate global-scale diversity, and reconstruct phylogenetic trees. As these three aspects can all affect results of diversification analyses (Moen & Morlon, 2014), such analyses are rarely performed with these types of data. Yet, for many species-rich groups of organisms, in particular microorganisms, no other data currently provide a thorough representation of diversity at the "species" level. Hence, these data, although far from ideal, are the only one that can be used to study the past diversification of such groups (see Lewitus et al., 2018; Louca et al., 2018 for antecedents). The approach we took here is to recognize all these potential sources of uncertainty and biases and to test the robustness of our results. We demonstrated the usefulness of this approach: while some results inevitably depend on the choices made for species delineation, phylogenetic reconstruction, and the estimation of global scale diversity, others are sufficiently strong to hold despite uncertainty in the data. Our results therefore

illustrate that using a short DNA marker (e.g. a metabarcode) combined with intensive sensitivity analyses can be useful for studying the diversification dynamics of poorly-known groups.

Conclusion:

Our findings that Glomeromycotina have low speciation rates, likely constrained by the availability of suitable niches, reinforce the vision of Glomeromycotina as an "evolutionary cul-de-sac" (Malloch, 1987). We interpret the significant decline in speciation rates toward the present as the conjunction of the emergence of plant lineages not associated with Glomeromycotina and the reduction of tropical areas induced by climate cooling, in the context of obligate dependence of Glomeromycotina on plants. Temporal declines in speciation rates have often been interpreted as the signal of adaptive radiations (Harmon, Schulte, Larson, & Losos, 2003; Moen & Morlon, 2014), that is clades that experienced a rapid accumulation of morphological, ecological, and species diversity (Simpson, 1953). Conversely, Glomeromycotina provide here a striking example of a clade with slow morphological, ecological, and species diversification that features a pattern of temporal decline in speciation rates, that might reflect the reduction of the global availability of their mycorrhizal niches.

Acknowledgment:

The authors acknowledge C. Strullu-Derrien, M. Elias, D. de Vienne, A. Vogler, J.-Y. Dubuisson, C. Quince, S.-K. Sepp, and M. Chase for helpful discussions. They also thank L. Aristide, S. Lambert, J. Clavel, I. Quintero, I. Overcast, and G. Sommeria for comments on the manuscript, D. Marsh for English editing, and the Editor and three reviewers for improvements of an earlier version of this manuscript. BPL acknowledges B. Robira, F. Foutel-Rodier, F. Duchenne, E. Faure, E. Kerdoncuff, R. Petrolli, and G. Collobert for useful

755 discussions and C. Fruciano and E. Lewitus for providing codes. This work was supported
756 by a doctoral fellowship from the École Normale Supérieure de Paris attributed to BPL and
757 the École Doctorale FIRE – Programme Bettencourt. MÖ was supported by the European
758 Regional Development Fund (Centre of Excellence EcolChange) and University of Tartu
759 (PLTOM20903). Funding of the research of FM was from the Agence Nationale de la
760 Recherche (ANR-19-CE02-0002). HM acknowledges support from the European Research
761 Council (grant CoG-PANDA).

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Data Accessibility:

All of the data used in this study are available in the open-access MaarjAM database (https://maarjam.botany.ut.ee). Spore lengths of Glomeromycotina were collected in the supplementary data of Davison et al. (2018). New scripts for delineating evolutionary units (EU) are available in the R-package RPANDA (through this GitHub branch for the moment: https://github.com/hmorlon/PANDA/tree/Benoit_phylosignal). The alignment of all the SSU rRNA sequences and their associated metadata publicly accessible through the Open Science Framework (osf) portal: osf.io/y2ts5.

Author contributions:

All the authors designed the study. MÖ gathered the data and BPL performed the analyses. OM and ACAS provided some codes. BPL and HM wrote the first version of the manuscript and all authors contributed substantially to the revisions.

Competing Interests statement:

The authors declare that there is no conflict of interest.

Tables:

Table 1: Estimation of the total diversity of Glomeromycotina:

Estimated sampling fraction using the Bayesian Diversity Estimation Software (BDES; Quince et al., 2008) for the different species delineations (VT or EU) assuming a Sichel species abundance distribution. The estimated number of units corresponds to the median value and we indicated the 95% confidence interval. We indicated the sampling fractions for each delineation, computed as the number of observed VT or EUs divided by the corresponding BDES estimates of global Glomeromycotina diversity.

The Sichel distribution was selected compared to other distributions (log-normal, log-Student, and inverse gaussian) based on lowest deviance information criterion (DIC).

Species delineation	Observed number of units	Estimated number of units	Sampling fraction	95% confidence interval	
				Lower boundary	Upper boundary
VT	384	403	95%	391 (98%)	422 (91%)
EU97	182	187	97%	183 (99%)	194 (94%)
EU97.5	340	357	95%	348 (98%)	370 (92%)
EU98	641	677	95%	663 (97%)	695 (92%)
EU98.5	1,190	1,268	94%	1,247 (95%)	1,293 (92%)
EU99	2,647	2,852	93%	2,817 (94%)	2,890 (92%)

Figures

Figure 1: Molecular-based species delineations of Glomeromycotina (arbuscular mycorrhizal fungi) give consistent results and indicate a nearly complete sampling.

We compared the *virtual taxa* (VT) delineation from (Öpik et al., 2010) with newly-developed automatic delineations into *evolutionary units* (EUs) based on an average threshold of similarity and a criterion of monophyly.

(a) The proportion of Glomeromycotina units (VT or EUs) in each Glomeromycotina family reveals constant proportions across delineations, although Glomeraceae tend to be relatively less abundant compared with the other Glomeromycotina family in the VT delineation. The main Glomeromycotina orders are indicated on the right of the charts: Paraglomerales + Archaeosporales, Diversisporales, and Glomerales (Glomeraceae + Claroideoglomeraceae).

(b) Rarefaction curves indicating the number of Glomeromycotina units as a function of the percentage of sampled Glomeromycotina accession revealed that the Glomeromycotina sampling in MaarjAM is close to saturation for all delineations (VT or EUs). Rarefactions were performed 100 times every 5 percent and the median of the 100 replicates is represented here.

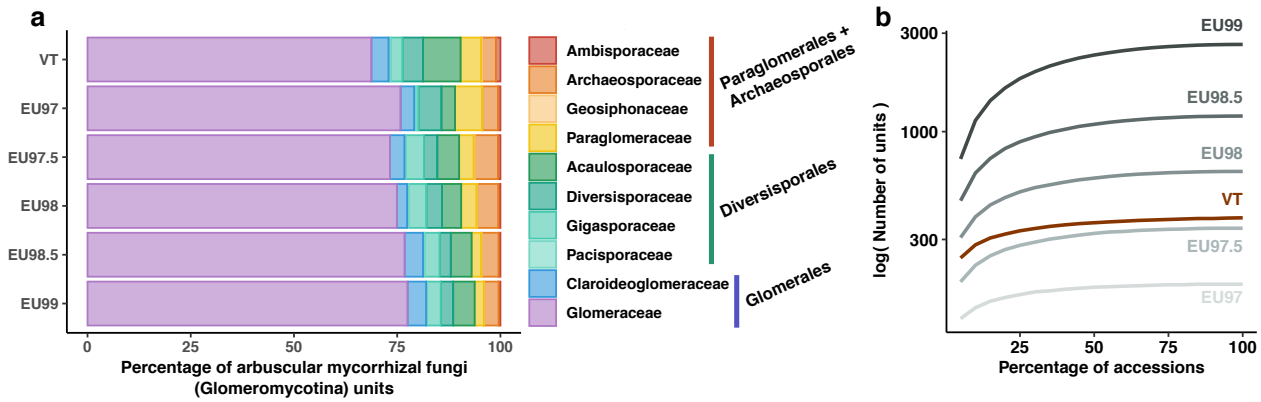
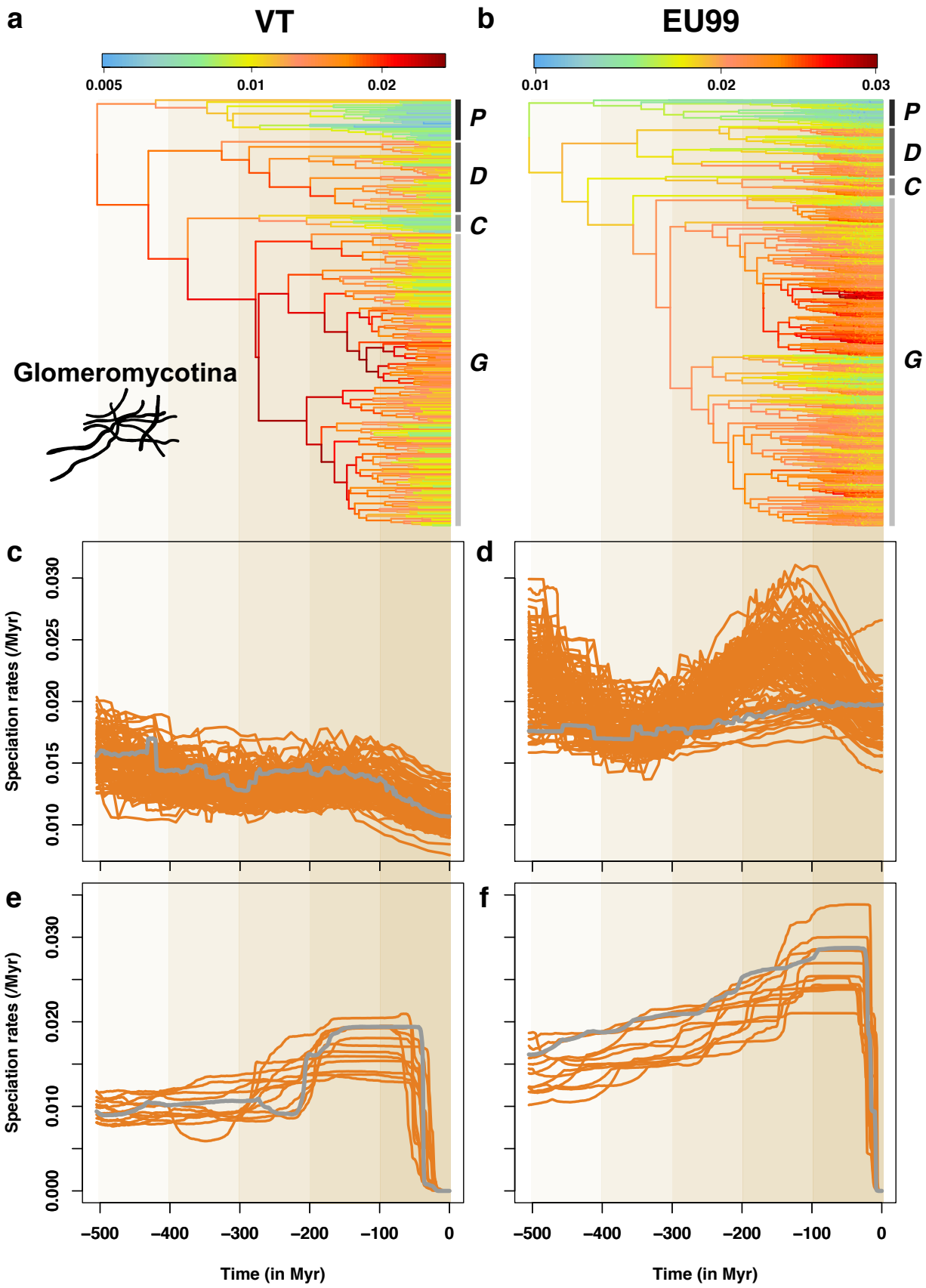


Figure 2: The speciation dynamic of Glomeromycotina (arbuscular mycorrhizal fungi) varies significantly through time and between lineages.

(a-b): Glomeromycotina consensus phylogenetic trees corresponding to the VT (a) and EU99 (b) species delineations. Branches are colored according to the lineage-specific speciation rates estimated by ClaDS using the BDES estimated sampling fraction: lineages with low and high speciation rates are represented in blue and red, respectively.
The main Glomeromycotina clades are indicated with the following letters: *P* = Paraglomerales + Archaeosporales, *D* = Diversisporales, *C* = Claroideoglomeraceae, and *G* = Glomeraceae.

(c-d): Mean speciation rates through time estimated by ClaDS, for the VT (c) and EU99 (d) delineations and using the BDES estimated sampling fraction. The mean speciation rate corresponds to the maximum *a posteriori* (MAP) of the mean speciation rate across all fungal lineages back in time (including extinct and unsampled lineages). Orange and grey lines represent the independent replicate trees and the consensus tree, respectively: because some of the 12 replicate trees showed different trends, we replicated ClaDS inferences using 100 replicate trees. Unlike most replicate trees, the EU99 consensus tree tends to present a limited decline in speciation rates, which reinforces the idea that consensus trees can be misleading (Janzen & Etienne, 2017).

(e-f): Mean speciation rates through time estimated by CoMET, for the VT (c) and EU99 (d) delineations and using the BDES estimated sampling fraction. Orange and grey lines represent the 12 independent replicate trees and the consensus tree, respectively.


a
VT
b
EU99
0.005
0.01
0.02
0.01
0.02
0.03
P
D
C
G
Glomeromycotina
c
d
e
f
Speciation rates (/Myr)
0.010
0.015
0.020
0.025
0.030
0.000
−500
−400
−300
−200
−100
0
Time (in Myr)

1161

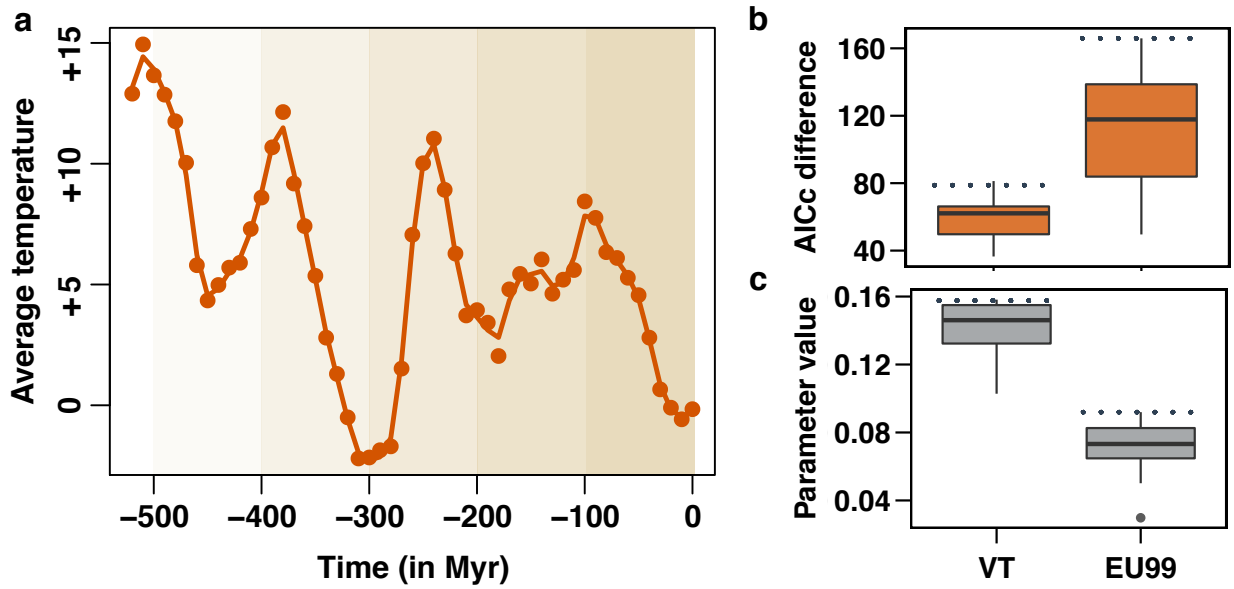
Figure 3: Temperature-dependent diversification models reveal that global temperature positively associates with the speciation rates of Glomeromycotina (arbuscular mycorrhizal fungi) in the last 500 million years.

(a): Average global temperature in the last 500 million years (Myr) relative to the average temperature of the period 1960-1990. The smoothed orange line represents cubic splines with 33 degrees of freedom used to fit temperature-dependent models of Glomeromycotina diversification with RPANDA. This default smoothing was estimated using the R function *smooth.spline*.

(b): AICc difference between the best-supported time-dependent model and the temperature-dependent model in RPANDA, for the VT (left) and EU99 (right) delineations, using the BDES estimated sampling fraction. An AICc difference greater than 2 indicates that there is significant support for the temperature-dependent model.

(c): Parameter estimations of the temperature-dependent models (speciation rate ~ exp(parameter * temperature)). A positive parameter value indicates a positive effect of temperature on speciation rates.

For both delineations, the boxplots represent the results obtained for the consensus tree and the 12 independent replicate trees. Boxplots indicate the median surrounded by the first and third quartiles, and whiskers extend to the extreme values but no further than 1.5 of the inter-quartile range. The horizontal dotted lines highlighted the values estimated for the consensus trees. Compared to the replicate trees, the consensus trees tends to present extreme values (stronger support for temperature-dependent model), which reinforces the idea that consensus trees can be a misleading representation (Janzen & Etienne, 2017).


a
Average temperature
+15
+10
+5
0
−500
−400
−300
−200
−100
0
Time (in Myr)
b
AICc difference
160
120
80
40
c
Parameter value
0.16
0.12
0.08
0.04
VT
EU99

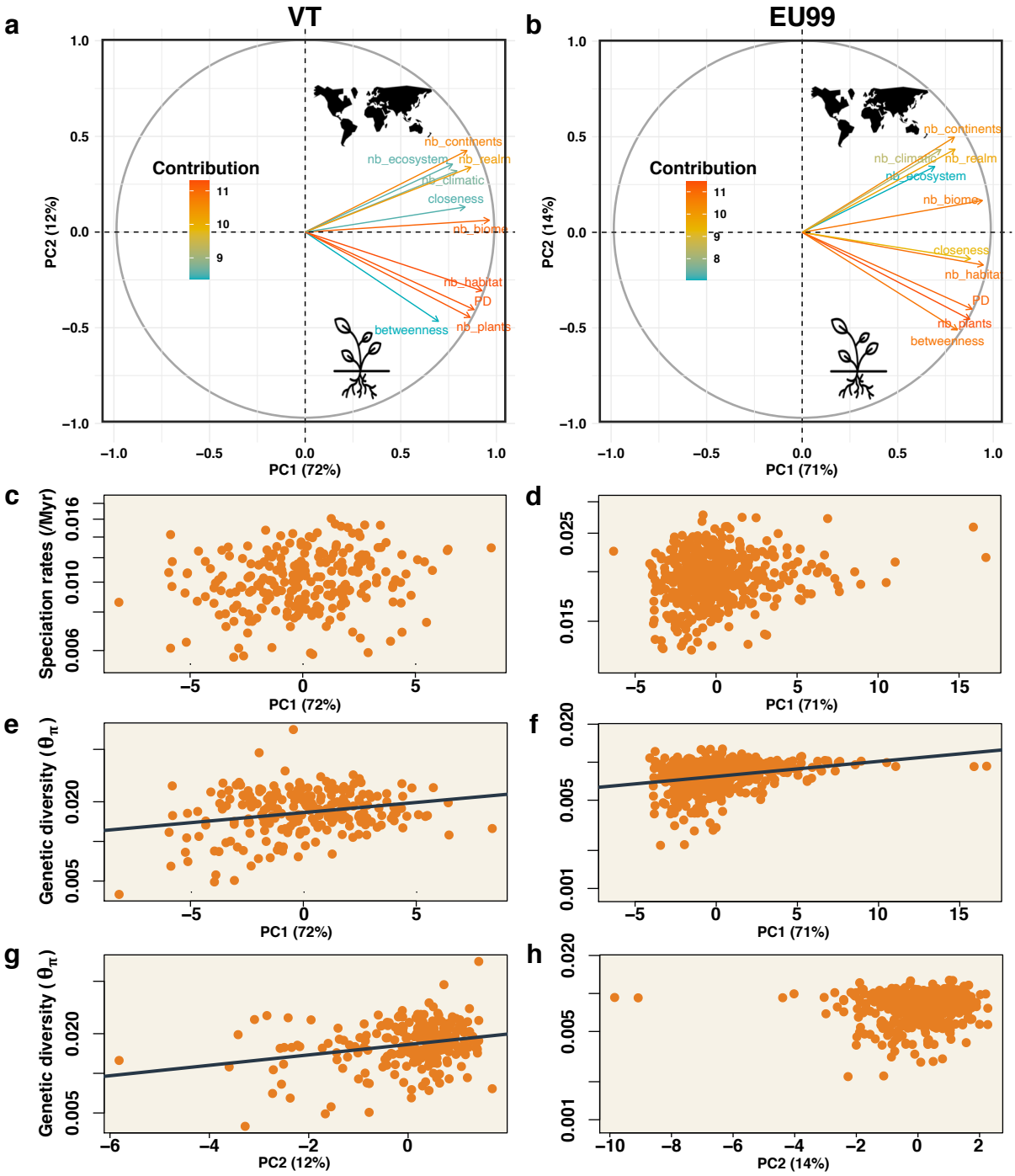
1188

Figure 4: Abiotic and biotic correlates of speciation rates and genetic differentiation in Glomeromycotina (arbuscular mycorrhizal fungi)

(a-b): Projection of 10 abiotic and biotic variables on the two principal coordinates according to the VT (a) or EU99 (b) delineations. Principal coordinate analysis (PCA) was performed for the Glomeromycotina units represented by at least 10 sequences. Colors represent the contribution of the variable to the principal coordinates. The percentage for each principal coordinate (PC) indicates its amount of explained variance.
Tested variables were: the numbers of continents on which the Glomeromycotina unit occurs (nb_continent), of realms (nb_realm), of ecosystems (nb_ecosystems), of habitats (nb_habitats), of biomes (nb_biomes), and climatic zones (nb_climatic) (Öpik et al., 2010), as well as information about the associated plant species of each unit, such as the number of plant partners (nb_plants), the phylogenetic diversity of these plants (PD), and the betweenness and closeness measurement of each fungal unit in the plant-fungus interaction network (see Methods).

(c-d): Speciation rates as a function of the PC1 coordinates for each VT (c) or EU99 (d) unit. Only the Glomeromycotina consensus tree is represented here (other replicate trees are presented in Supplementary Fig. 33).

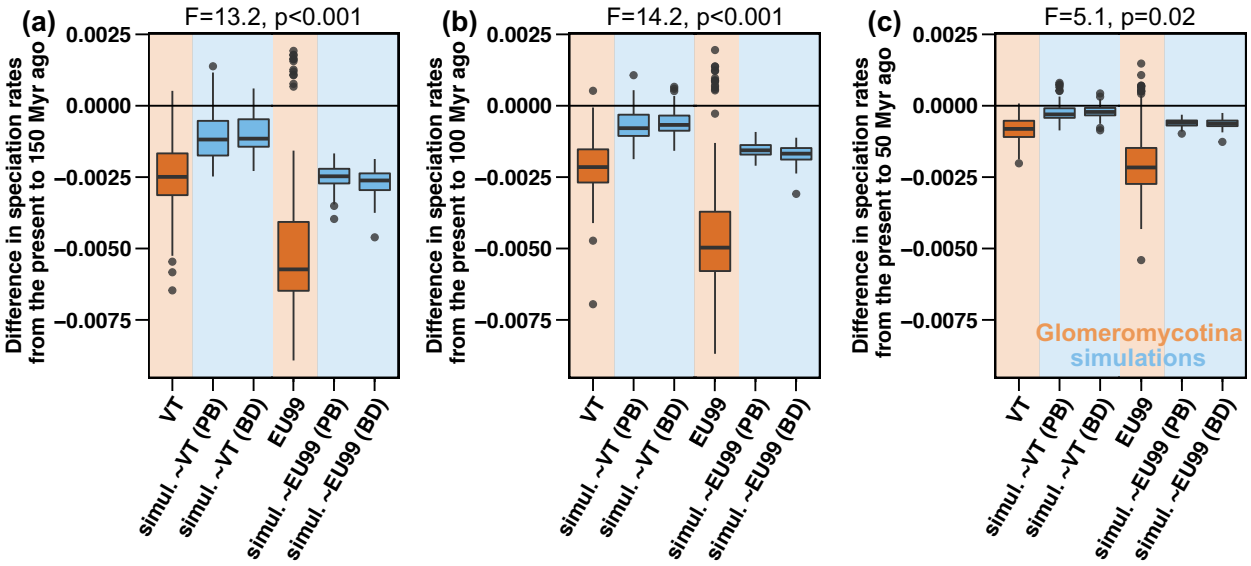
(e-h): Genetic diversity (Tajima's $\theta\pi$ estimator) as a function of the PC1 (e-f) or PC2 (g-h) coordinates for each VT (e-g) or EU99 (f-h) unit. Only the Glomeromycotina consensus tree is represented here (other replicate trees are presented in Supplementary Fig. 33). The grey lines indicate the statistically significant linear regression between the two variables inferred using MCMCglmm.


a
VT
b
EU99
Contribution
nb_continents
nb_ecosystem
nb_realm
nb_climatic
closeness
nb_biome
nb_habitat
PD
nb_plants
betweenness
PC1 (72%)
PC2 (12%)
PC1 (71%)
PC2 (14%)
c
d
e
f
g
h
Speciation rates (/Myr)
Genetic diversity (θπ)

1213

Figure 5: Artefactual species lumping and lack of phylogenetic resolution in the SSU rRNA region are not enough to explain the temporal decline in speciation rates detected in Glomeromycotina.

Comparison of the magnitude of the decline in speciation rates observed in Glomeromycotina (in orange) and on simulated data (in blue). The intensity of the slowdown is measured as the difference between the mean speciation rate estimated at present and the mean speciation rate estimated 150 Myr ago (a), 100 Myr ago (b), or 50 Myr ago (c). Negative differences indicate a speciation rate decline. Sequence alignments were simulated on phylogenetic trees obtained under a scenario of constant speciation rate and no extinction (*i.e.* pure birth "PB") and constant speciation and extinction rates (*i.e.* birth death "BD"), with characteristics mimicking the slow evolution of the SSU rRNA marker. We simulated phylogenies with two different net diversification rates, such that we obtained simulations with total numbers of species similar to the total numbers of VT or EU99 units (Supplementary Fig. 2). Boxplots indicate the median surrounded by the first and third quartiles, and whiskers extend to the extreme values but no further than 1.5 of the inter-quartile range. Each boxplot represents results for the consensus trees and the 12 independent replicate trees for each of the 10 simulations, and for the consensus trees and the 100 independent replicate trees for the Glomeromycotina. Differences between the magnitude of the decline measured in Glomeromycotina (VT or EU99) and in the corresponding simulations were tested using linear models (reported at the top of the plots).


(a)
F=13.2, p<0.001
(b)
F=14.2, p<0.001
(c)
F=5.1, p=0.02
Difference in speciation rates from the present to 150 Myr ago
Difference in speciation rates from the present to 100 Myr ago
Difference in speciation rates from the present to 50 Myr ago
0.0025
0.0000
−0.0025
−0.0050
−0.0075
Glomeromycotina simulations
VT
simul. ~VT (PB)
simul. ~VT (BD)
EU99
simul. ~EU99 (PB)
simul. ~EU99 (BD)

1235

Supplementary Tables Legends:

Supplementary Table 1: Data selection in the MaarjAM database.

Supplementary Table 2: Documented plant-Glomeromycotina interactions.

Supplementary Table 3: Characteristics of the fungal units (VT or EU) in the database.

Supplementary Table 4: Number of units (VT, EU, or GMYC) per fungal clades.

Supplementary Table 5: GMYC delineation and corresponding sampling fraction.

Supplementary Table 6: The estimated sampling fraction using Chao2 index suggested a sampling fraction >90%.

Supplementary Table 7: The prior selection for the VT Bayesian phylogenetic reconstructions (BEAST) using nested sampling (NS) favored a log-normal and Pure-birth prior.

Supplementary Figures Legends:

Supplementary Figure 1: Visualization of Glomeromycotina sequence alignments: The ITS marker is not a good marker for Glomeromycotina phylogenetic reconstruction compared to the SSU rRNA region.

Supplementary Figure 2: Simulated diversification scenarios.

Supplementary Figure 3: GMYC species delineations in Glomeromycotina clades significantly support the existence of intraspecific haplotypes in the SSU rRNA gene.

Supplementary Figure 4: Consensus Glomeromycotina phylogenetic trees for the different species delineations.

Supplementary Figure 5: Node depth distribution of the consensus Glomeromycotina phylogenetic trees for the different delineations.

Supplementary Figure 6: The accumulation of fungal lineages through time present a slowdown toward the present in the reconstructed Glomeromycotina phylogenetic trees.

Supplementary Figure 7: Speciation rates per lineage estimated by ClaDS show that Glomeromycotina experienced heterogeneous diversification rates across clades and time.

Supplementary Figure 8: Present-day speciation rates at the tips estimated by ClaDS show that Glomeromycotina have heterogeneous diversification rates across clades.

Supplementary Figure 9: Speciation rates at the tips estimated by ClaDS for VT and EU delineations are significantly correlated.

Supplementary Figure 10: The average speciation rates through time estimated by ClaDS show that Glomeromycotina experienced a decline in speciation rates toward the present after a period of high speciation rates.

Supplementary Figure 11: The speciation rates through time estimated by CoMET show that Glomeromycotina experienced a decline in speciation rates toward the present after a period of high speciation rates.

Supplementary Figure 12: Estimated hyperparameters of ClaDS2 runs, for the different Glomeromycotina delineations, using the BDES estimated sampling fraction.

Supplementary Figure 13: The speciation rates through time estimated by RPANDA show that Glomeromycotina experienced a decline in speciation rates toward the present.

Supplementary Figure 14: The speciation rates through time estimated by ClaDS also show that Glomeromycotina experienced a decline in speciation rates toward the present even when using sampling fractions <90%.

Supplementary Figure 15: The speciation rates through time estimated by CoMET also show that Glomeromycotina experienced a decline in speciation rates toward the present even when using sampling fractions <90%.

Supplementary Figure 16: Speciation rates through time estimated by ClaDS using the 28S large sub-unit of the rRNA gene (LSU rRNA gene).

Supplementary Figure 17: Low support for extinction according to CoMET.

Supplementary Figure 18: Inferred diversification rates decline faster in time-dependent models with fixed extinction rates.

Supplementary Figure 19: Inferred diversification rates decline faster in congruent models with fixed extinction rates.

Supplementary Figure 20: Variation of the environmental variables through time tested with RPANDA.

Supplementary Figure 21: Speciation rates through time according to the temperature-dependent diversification model in RPANDA.

Supplementary Figure 22: Temperature-dependent models are significantly supported in Glomeromycotina.

Supplementary Figure 23: Temperature-dependent models are significantly supported in Glomeromycotina, even when using sampling fractions <90%.

Supplementary Figure 24: Effect of the Glomeromycotina crown age on the RPANDA models.

Supplementary Figure 25: Temperature-dependent models are not artifactually supported when time-dependency is simulated.

Supplementary Figure 26: Temperature-dependent models are not supported because of a global temporal trend in temperature variation.

Supplementary Figure 27: The support for temperature-dependent models is not linked to the heterogeneity of rates across lineages.

Supplementary Figure 28: The different Glomeromycotina sub-clades present significant support for temperature-dependence diversification, but also for dependences with CO_2 and land plants.

Supplementary Figure 29: Diversification models estimated with RPANDA when using the 28S large sub-unit of the rRNA gene (LSU rRNA gene).

Supplementary Figure 30: Characterizing Glomeromycotina niche width using principal coordinate analysis (PCA).

Supplementary Figure 31: Characterizing Glomeromycotina niche width using principal coordinate analysis (PCA): Individual projection on the two principal coordinates according to the different Glomeromycotina delineations.

Supplementary Figure 32: Characterizing Glomeromycotina niche width using principal coordinate analysis (PCA): Projection of the 10 abiotic and biotic variables on the two principal coordinates according to the different Glomeromycotina delineations.

Supplementary Figure 33: Correlations between speciation rates at the tips, estimates of genetic diversity (Tajima's $\theta\pi$ estimator - referred to as "Nei diversity") and PC1 and PC2 coordinates.

Supplementary Figure 34: The *Rhizophagus* clade with large niche width present the highest speciation rates.

Supplementary Figure 35: Significant latitudinal gradient of Glomeromycotina diversity.

Supplementary Figure 36: The total number of Glomeromycotina species is not higher in (tropical) grasslands.

Supplementary Figure 37: No effect of ecosystem types or climatic zones on Glomeromycotina speciation rates.

Supplementary Figure 38: No significant effect of mean spore length on VT speciation rates.

Supplementary Figure 39: No significant correlation between mean spore length and endemism.

Supplementary Figure 40: Average Glomeromycotina speciation rates and land plant diversity are decoupled for ~130 Myr.