Global drivers of obligate mycorrhizal symbionts diversification

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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.

194 **Total diversity estimates:**

195

196 We evaluated how thoroughly sampled our species-level Glomeromycotina
197 phylogenetic trees are by estimating the total number of VT and EUs using rarefaction
198 curves and the Bayesian Diversity Estimation Software (BDES (Quince, Curtis, & Sloan,
199 2008)) (Supplementary Methods 3). The BDES estimates the total number of species by
200 extrapolating a sampled taxa abundance distribution at global scale (Quince et al., 2008).

201

202 **Additional molecular markers:**

203

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

215

216 **Diversification analyses:**

217

218 Unless specified differently, our diversification analyses were performed using the
219 SSU rRNA gene. In order to account for various sources of uncertainties in the SSU rRNA
220 data, we replicated all our diversification analyses across different species delineations,
221 phylogenetic reconstructions and dating, and total diversity estimates. For each species
222 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,

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

281 had a similar sampling fraction (Supplementary Table 2). To assess the robustness of our
282 results to global diversity estimates, we replicated all diversification analyses using a range
283 of lower sampling fractions (from 90% to 50%, *i.e.* assuming that only that percentage of
284 the global Glomeromycotina species diversity is in fact represented in our dataset).
285
286 **Testing for correlates of present-day Glomeromycotina speciation rates:**
287
288 To further investigate the potential factors correlating with Glomeromycotina
289 speciation rates, we assessed the relationship between lineage-specific estimates of
290 present-day speciation rates (obtained with the ClaDS analyses) and characteristics of each
291 Glomeromycotina taxonomic unit, *i.e.* VT or EUs.
292
293 First, to assess the effect of specialization on speciation rates, we characterized
294 Glomeromycotina relative niche width using a set of 10 abiotic and biotic variables
295 recorded in MaarjAM database for each Glomeromycotina unit. In short, among a curated
296 dataset containing Glomeromycotina sequences occurring only in natural ecosystems
297 (dataset 2; Supplementary Table 2; Perez-Lamarque et al., 2020), for each Glomeromycotina
298 unit, we reported the number of continents, ecosystems, climatic zones, biogeographic
299 realms, habitats, and biomes where it was sampled, as well as its number of plant partners,
300 their phylogenetic diversity, and its centrality in the plant-fungus bipartite network, and
301 performed a principal component analysis (PCA; Supplementary Methods 7). For
302 Glomeromycotina units represented by at least 10 sequences, we tested whether these PCA
303 coordinates reflecting Glomeromycotina niche widths were correlated with the present-
304 day speciation rates using both linear mixed-models (not accounting for phylogeny) or
305 MCMCglmm models (Hadfield, 2010). For MCMCglmm, we assumed a Gaussian residual
306 distribution, included the fungal phylogenetic tree as a random effect, and ran the MCMC
307 chains for 1,300,000 iterations with a burn-in of 300,000 and a thinning interval of 500.
308

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

338 **Simulation analyses:**

339

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

347

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

367 delineation criteria: after delineating the EU99 units, we reconstructed the EU99
368 phylogenetic trees, ran the ClaDS analyses on these trees, and recorded mean estimated
369 speciation rates at present and 50, 100, and 150 Myr ago.

370 ***Results:***

371

372 **Glomeromycotina species delineations & phylogenetic reconstructions:**

373

374 We automatically delineated Glomeromycotina into evolutionary units (EU) using
375 a monophyly criterion and several thresholds of SSU rRNA sequence similarity (from 97%
376 to 99%). The EU97.5 and EU98 delineations (obtained using a threshold of 97.5% and 98%
377 respectively) provided a number of Glomeromycotina units (340 and 641) relatively
378 comparable to the 384 currently recognized *virtual taxa* (VT), while the EU97 delineation
379 had much less units (182). Conversely, the EU98.5 and EU99 delineations yielded a much
380 larger number of Glomeromycotina units (1,190 and 2,647). These numbers obtained with
381 the EU98.5 and EU99 delineations were consistent with the numbers obtained using GMYC
382 analyses, which delineate species-like units based on detecting when splitting events in the
383 haplotype tree start to follow branching patterns consistent with intra-specific
384 differentiations (*i.e.* coalescent patterns) instead of speciation events (i.e. birth-death
385 patterns; Supplementary Tables 3, 4, & 5). The GMYC results therefore support the idea
386 that some VT might lump together several cryptic species (Bruns et al.,
387 2018)(Supplementary Note 1), and that a 98.5 or 99% similarity threshold is more relevant
388 for Glomeromycotina species delineation. In addition, the GMYC model is significantly
389 supported over the model where all SSU rRNA haplotypes correspond to a different
390 species (GMYC LRT: $P<0.05$; Supplementary Fig. 3), with on average 10 SSU haplotypes
391 per species-like unit, and a mean intraspecific sequence similarity of 99% (Supplementary
392 Table 5 & Supplementary Fig. 3). This indicates that the region of the SSU marker used to
393 characterized Glomeromycotina evolves fast enough to accumulate substitutions between
394 Glomeromycotina speciation events, meaning that it is an informative (although not
395 perfect) marker for delineating Glomeromycotina species-like units. In comparison, the
396 same pipeline carried on the LSU database delineated only 181 GMYC units, suggesting
397 that it was much less complete than the SSU database. We replicated the subsequent

398 diversification analyses using the LSU region, even though we put more trust in our results
399 using the SSU database given the incompleteness of the LSU database.
400
401 Rarefaction curves as well as BDES (Bayesian Diversity Estimation Software) and
402 Chao2 estimates of diversity suggested that more than 90% of the total Glomeromycotina
403 diversity is represented in our SSU dataset regardless of the delineation threshold (Fig. 1,
404 Table 1, Supplementary Tables 5 & 6), which is consistent with the proportion of new
405 Glomeromycotina units detected in recent studies (Sepp et al., 2019).
406
407 The reconstructed Bayesian phylogenetic trees based on VT and EU delineations did
408 not yield high support for the nodes separating the main Glomeromycotina orders; yet, the
409 trees had no significantly-supported conflicts either, and similar branching times of the
410 internal nodes overall (Fig. 2, Supplementary Fig. 4). As expected, finer delineations
411 resulted in an increase in the number of nodes close to the present (Supplementary Fig. 5).
412 However, we observed a slowdown in the accumulation of new lineages close to the
413 present in all lineage through time plots (LTTs), including those with the finest delineations
414 (EU98.5 and EU99; Supplementary Fig. 6).
415
416 **Temporal diversification dynamics:**
417
418 We found that speciation rates for Glomeromycotina ranged from 0.005 to 0.03
419 events per lineage per Myr, using both the VT and EU SSU rRNA delineations (Fig. 2;
420 Supplementary Fig. 7). Speciation rates varied both within and among Glomeromycotina
421 orders, with Glomerales and Diversisporales having the highest present-day speciation
422 rates (Supplementary Fig. 8). As expected we observed higher present-day speciation rates
423 for finer delineations, but at the haplotype level (i.e. at the level of the individual SSU rRNA
424 sequences within each unit) we found a significant correlation of present-day speciation
425 rates computed with ClaDS using different delineations (Supplementary Fig. 9). Whatever
426 the delineations, Glomeromycotina experienced their highest speciation rates between 200

427 and 100 Myr ago according to estimates obtained with ClaDS (Fig. 2; Supplementary Fig.
428 10) and between 150 and 50 Myr ago according to CoMET (Fig. 2; Supplementary Fig. 11).
429 ClaDS estimates of speciation rates at 150 Myr ago were 26% (± s.d. 17) higher than those
430 at 300 Myr with the EU99 delineation. With the VT delineation, the increase was of 3% (±8).
431 The peak was even stronger using CoMET: 30% ± 20 higher at 150 Myr in comparison to
432 300 Myr with the EU99 delineation (71% ± 40 with the VT delineation; Fig. 2).
433
434 The peak of speciation rates was followed by a decline in the recent past (Fig. 2;
435 Supplementary Fig. 10), as suggested by the plateauing of the LTTs. A global decline of the
436 speciation rates through time was independently supported by ClaDS and CoMET
437 analyses, as well as time-dependent models in RPANDA (Morlon, Parsons, & Plotkin,
438 2011)(Supplementary Figs. 11, 12, & 13). This speciation rate decline was robust to all
439 species delineations, the branching process prior (Supplementary Table 7), phylogenetic
440 uncertainty, and assumed sampling fractions as low as 50%, except in ClaDS analyses
441 where the trend disappeared in some EU99 trees and for sampling fractions lower than
442 70% (Supplementary Figs. 14 & 15). We also found a period of high speciation rates
443 between 200 and 100 Myr ago followed by a decline in our analyses with the LSU region,
444 for assumed sampling fractions as low as 60% (Supplementary Figure 16).
445
446 We did not find a strong signal of extinction in our analyses: the turnover rate
447 estimated from ClaDS was generally close to zero (Supplementary Fig. 12b), and models
448 including extinctions were never selected in RPANDA (Supplementary Fig. 13). Similarly,
449 the extinction rates estimated in piecewise-constant models (CoMET) were not
450 significantly different from 0 and we did not find significant support for mass extinction
451 events (Supplementary Fig. 17). Yet, forcing the extinction rate to high positive values did
452 not modify the general trend of speciation rate slowdown (Supplementary Figs. 18 & 19).

453 **Correlates of Glomeromycotina diversification:**

454

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

469

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

482 (Supplementary Fig. 33b), likely because a single *Glomeraceae* clade, including the abundant
483 and widespread morphospecies *Rhizophagus irregularis* and *R. clarus* (high PC1 values), had
484 both the highest speciation rates and the largest niche widths among Glomeromycotina
485 (Supplementary Fig. 34).
486
487 Although Glomeromycotina diversity is currently higher in the (sub)tropics
488 (Supplementary Fig. 35), we found no effect of latitude on speciation rates, regardless of
489 the Glomeromycotina delineation or the minimum number of sequences per
490 Glomeromycotina unit (MCMCglmm: P>0.05). In addition, we actually did not detect a
491 higher total number of Glomeromycotina species in grasslands compared to forests
492 (Supplementary Figure 36; confirming the results of Davison et al. 2015), and it is thus not
493 surprising that we reported no effect of habitat or climatic zone on speciation rates
494 (Supplementary Fig. 37), suggesting that tropical grasslands are not particular
495 diversification hotspots for Glomeromycotina. Similarly, we recovered no significant
496 correlation between spore size and speciation rate (Supplementary Fig. 38), nor between
497 spore size and level of endemism (Supplementary Fig. 39).
498
499 Finally, Tajima's estimator of Glomeromycotina genetic diversity was significantly
500 and positively correlated with niche width (PC1) for all Glomeromycotina delineations and
501 minimal number of sequences per Glomeromycotina unit considered, and in particular
502 with abiotic aspects of the niche (PC2) in many cases (Fig. 4e-h; Supplementary Fig. 33).
503 Genetic diversity was not correlated with speciation rate (Supplementary Fig. 33), latitude,
504 habitat, climatic zone (MCMCglmm: P>0.05), or spore size (PGLS: P>0.05).
505
506 **Simulation results:**
507
508 When we simulated the evolution of a short DNA gene mimicking the SSU rRNA
509 marker and used it to delineate species, we found that the number of EU99 delineated units
510 was generally lower than the number of simulated species (~10% to 20% lower;

511 Supplementary Figure 2b). Hence, even the EU99 delineation tends to lump together some
512 closely related species. As expected, this lumping resulted in an artefactual inference of a
513 decline of speciation rates toward the present, but this artifactual decline was significantly
514 smaller in magnitude than that observed in Glomeromycotina (Figure 5). Hence, these
515 analyses suggest that the lumping of species resulting from the use of a small, slowly
516 evolving marker is unlikely to fully explain the strong temporal decline in speciation rate
517 we found in Glomeromycotina.

518 ## *Discussion*

519

520 **Glomeromycotina species delineations, diversity, and phylogeny:**

521

522 It is difficult to delineate species in Glomeromycotina, which are poorly
523 differentiated morphologically and mainly characterized by environmental sequences
524 (Bruns et al., 2018). Our GMYC analyses suggest that Glomeromycotina species-like units
525 correspond to SSU rRNA haplotypes with a sequence similarity between 98.5 and 99%.
526 With this criterion of species delineation, we estimate that there are between 1,300 and
527 2,900 Glomeromycotina 'species'. These estimates are largely above the number of
528 currently described morphospecies or VT (Supplementary Note 1) but remain low in
529 comparison with other fungal groups, like the Agaricomycetes that include taxa forming
530 ectomycorrhiza (Varga et al., 2019).

531

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

547 including longer reads (Krehenwinkel et al., 2019; Tedersoo, Albertsen, Anslan, &
548 Callahan, 2021) and additional genomic information, with the aim of reconstructing better
549 supported, comprehensive phylogenies and efforts to conduct diversification analyses
550 despite uncertainty in the data for groups where better data is not yet available.
551
552 **Glomeromycotina diversify slowly:**
553
554 We found speciation rates for Glomeromycotina an order of magnitude lower than
555 rates typically found for macro-eukaryotes (Maliet et al., 2019; Upham et al., 2019), like
556 plants (Zanne et al., 2014), or Agaricomycetes (Varga et al., 2019). Low speciation rates in
557 Glomeromycotina may be linked to their multinucleate hyphal state (Yildirir, Malar,
558 Kokkoris, & Corradi, 2020), to their occasional long-distance dispersal that homogenizes
559 populations globally over evolutionary timescales (Savary et al., 2018), and/or to the fact
560 that they are generalist obligate symbionts (Morlon, Kemps, Plotkin, & Brisson, 2012).
561 Regardless of the proximal cause, and contrary to Agaricomycetes for example, which
562 present a large diversity of species, morphologies, and ecologies, Glomeromycotina have
563 poorly diversified in the last 500 Myr despite their ubiquity; their niche space is restricted
564 to plant roots and the surrounding soil because of their obligate dependence on plants for
565 more than 400 Myr (Rich, Nouri, Courty, & Reinhardt, 2017; Tisserant et al., 2013).
566
567 Our estimates of speciation rates were highly variable across lineages. We reported
568 the highest speciation rates in Glomeraceae and Diversisporaceae. Speciation rates in
569 Paraglomeraceae and Archaeosporaceae, which are thought to be less beneficial for the
570 plants than the fast diversifying Glomeraceae and Diversisporaceae (Säle et al., 2021), were
571 an order of magnitude lower. We can therefore speculate that good symbiotic abilities may
572 favor diversification, although this remains to be tested in further investigations.
573
574 We found little evidence for species extinction in Glomeromycotina, including at
575 mass extinction events. Because Glomeromycotina are relatively widespread and have a

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

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

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

662 **Correlates of Glomeromycotina recent speciation rates:**

663

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

676

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

691 Glomeromycotina with large spores experience long-distance dispersal (Davison et al.,
692 2018; Kivlin, 2020). Thus, if large spores might limit dispersal at smaller (*e.g.* intra-
693 continental) scales in Glomeromycotina (Bueno & Moora, 2019; Chaudhary, Nolimal, Sosa-
694 Hernández, Egan, & Kastens, 2020), this does not seem to affect speciation rates.
695
696 In Glomeromycotina, intraspecific variability is an important source of functional
697 diversity (Munkvold, Kjøller, Vestberg, Rosendahl, & Jakobsen, 2004; Savary et al., 2018)
698 and their genetic diversity may indicate the intraspecific variability on which selection can
699 act, potentially leading to speciation. Here, geographically widespread Glomeromycotina
700 species appear to be more genetically diverse, as previously suggested by population
701 genomics (Savary et al., 2018), but do not necessarily speciate more frequently. Along with
702 a decoupling between genetic diversity and lineage-specific speciation rate, this suggests
703 that the accumulation of genetic diversity in the SSU region among distant subpopulations
704 is not enough to spur Glomeromycotina speciation.
705
706 **Analyzing diversification dynamics using a short marker gene:**
707
708 Short DNA regions, like those used in metabarcoding surveys, typically do not
709 allow to robustly delineate species, estimate global-scale diversity, and reconstruct
710 phylogenetic trees. As these three aspects can all affect results of diversification analyses
711 (Moen & Morlon, 2014), such analyses are rarely performed with these types of data. Yet,
712 for many species-rich groups of organisms, in particular microorganisms, no other data
713 currently provide a thorough representation of diversity at the "species" level. Hence,
714 these data, although far from ideal, are the only one that can be used to study the past
715 diversification of such groups (see Lewitus et al., 2018; Louca et al., 2018 for antecedents).
716 The approach we took here is to recognize all these potential sources of uncertainty and
717 biases and to test the robustness of our results. We demonstrated the usefulness of this
718 approach: while some results inevitably depend on the choices made for species
719 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.

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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 GiHub 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.

1097 **Tables:**

1098

1099 **Table 1: Estimation of the total diversity of Glomeromycotina:**

1100 Estimated sampling fraction using the Bayesian Diversity Estimation Software (BDES;
1101 Quince et al., 2008) for the different species delineations (VT or EU) assuming a Sichel
1102 species abundance distribution. The estimated number of units corresponds to the median
1103 value and we indicated the 95% confidence interval. We indicated the sampling fractions
1104 for each delineation, computed as the number of observed VT or EUs divided by the
1105 corresponding BDES estimates of global Glomeromycotina diversity.

1106 The Sichel distribution was selected compared to other distributions (log-normal, log-
1107 Student, and inverse gaussian) based on lowest deviance information criterion (DIC).

1108

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%)

1109

1110

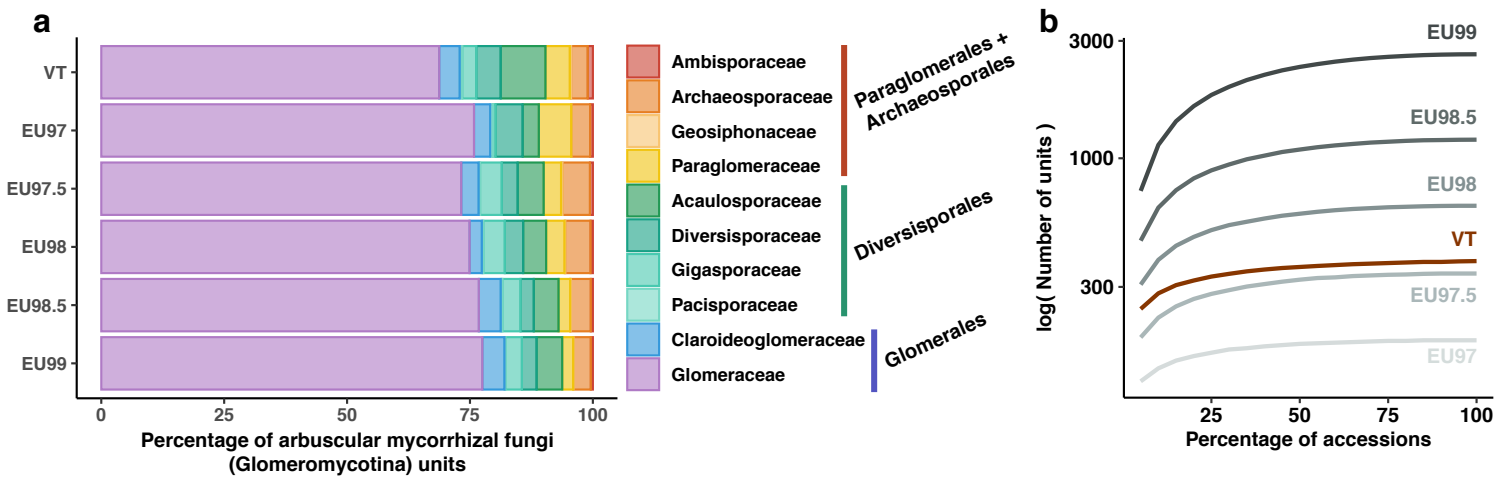
1111 # Figures

1112

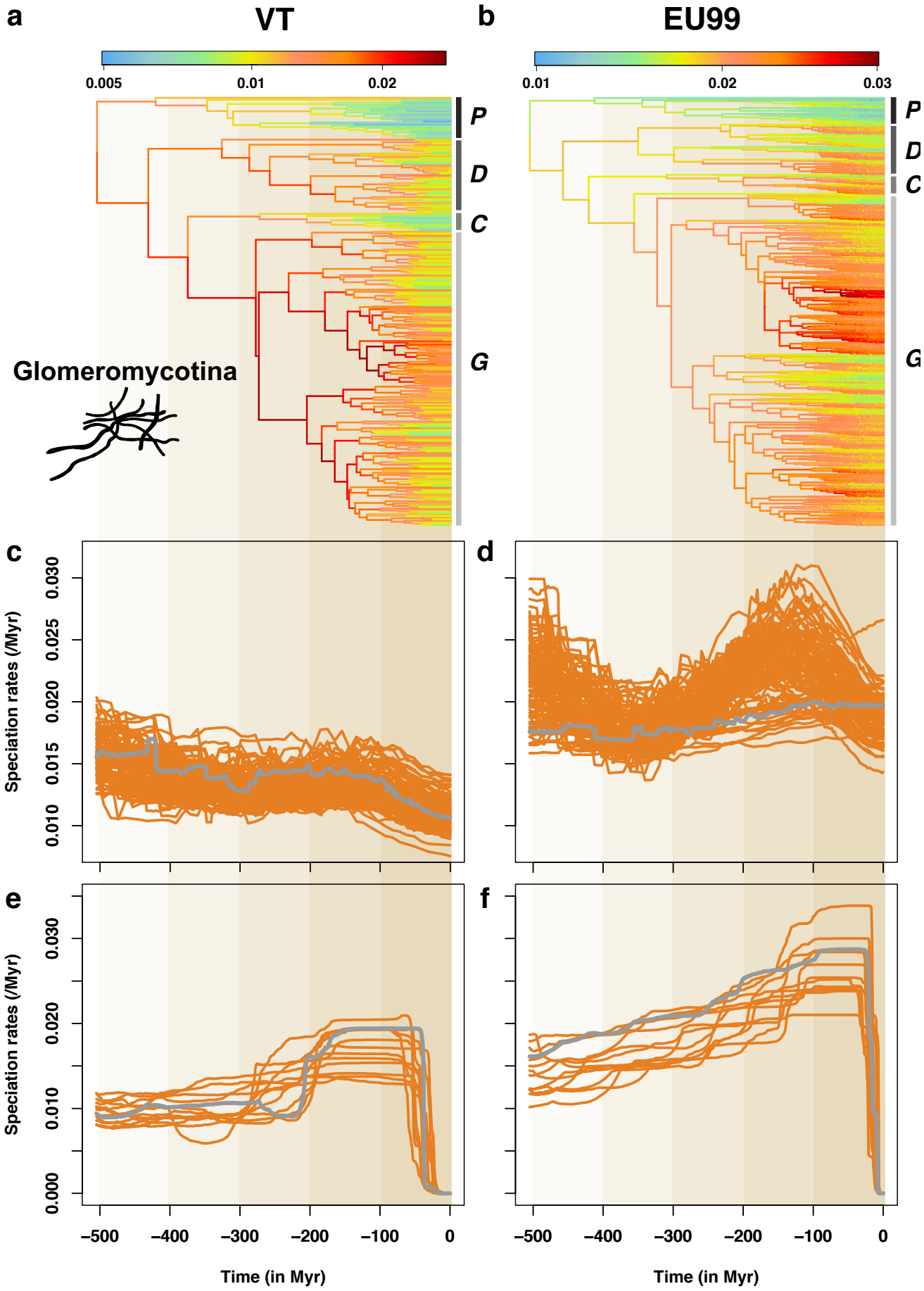
1113

1114 **Figure 1: Molecular-based species delineations of Glomeromycotina (arbuscular**
1115 **mycorrhizal fungi) give consistent results and indicate a nearly complete sampling.**
1116 We compared the *virtual taxa* (VT) delineation from (Öpik et al., 2010) with newly-
1117 developed automatic delineations into *evolutionary units* (EUs) based on an average
1118 threshold of similarity and a criterion of monophyly.
1119 **(a)** The proportion of Glomeromycotina units (VT or EUs) in each Glomeromycotina family
1120 reveals constant proportions across delineations, although Glomeraceae tend to be
1121 relatively less abundant compared with the other Glomeromycotina family in the VT
1122 delineation. The main Glomeromycotina orders are indicated on the right of the charts:
1123 Paraglomerales + Archaeosporales, Diversisporales, and Glomerales (Glomeraceae +
1124 Claroideoglomeraceae).
1125 **(b)** Rarefaction curves indicating the number of Glomeromycotina units as a function of
1126 the percentage of sampled Glomeromycotina accession revealed that the
1127 Glomeromycotina sampling in MaarjAM is close to saturation for all delineations (VT or
1128 EUs). Rarefactions were performed 100 times every 5 percent and the median of the 100
1129 replicates is represented here.

1130



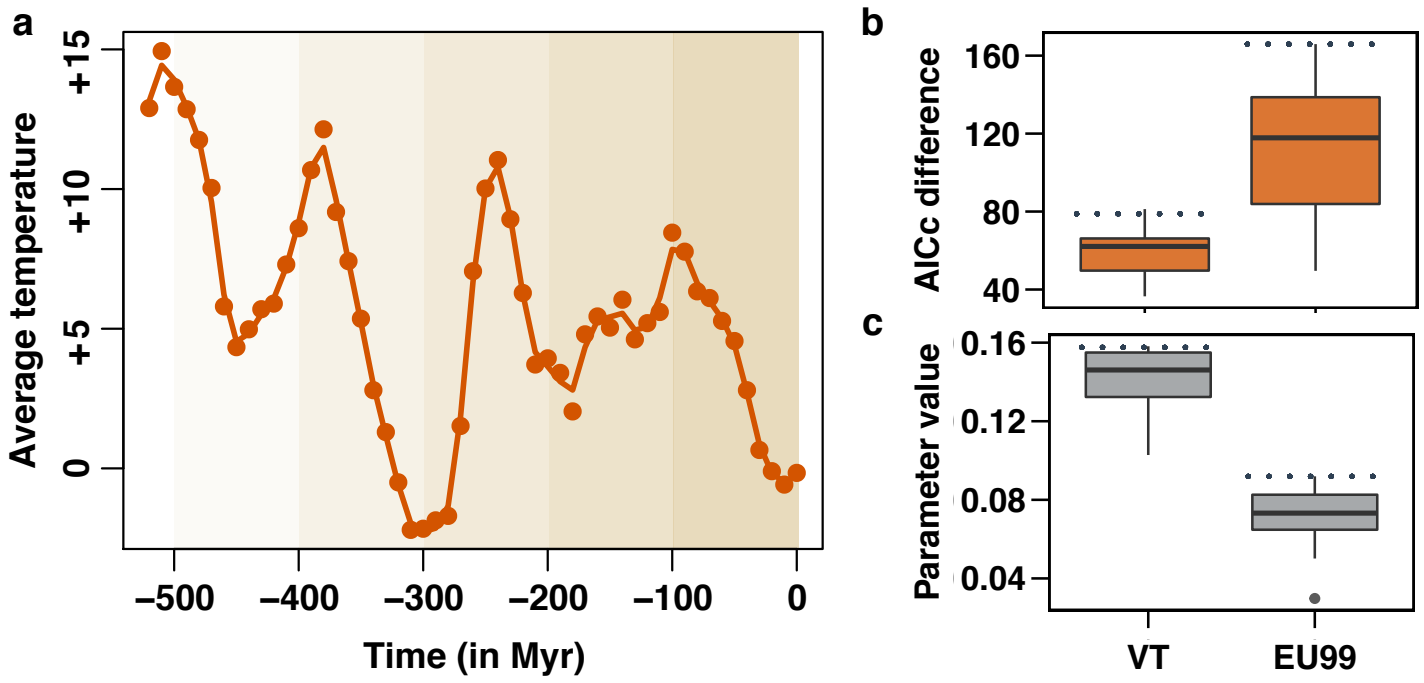
1131 **Figure 2: The speciation dynamic of Glomeromycotina (arbuscular mycorrhizal fungi)**
1132 **varies significantly through time and between lineages.**
1133
1134 **(a-b):** Glomeromycotina consensus phylogenetic trees corresponding to the VT (a) and
1135 EU99 (b) species delineations. Branches are colored according to the lineage-specific
1136 speciation rates estimated by ClaDS using the BDES estimated sampling fraction: lineages
1137 with low and high speciation rates are represented in blue and red, respectively.
1138 The main Glomeromycotina clades are indicated with the following letters: *P* =
1139 Paraglomerales + Archaeosporales, *D* = Diversisporales, *C* = Claroideoglomeraceae, and *G*
1140 = Glomeraceae.
1141
1142 **(c-d):** Mean speciation rates through time estimated by ClaDS, for the VT (c) and EU99 (d)
1143 delineations and using the BDES estimated sampling fraction. The mean speciation rate
1144 corresponds to the maximum *a posteriori* (MAP) of the mean speciation rate across all
1145 fungal lineages back in time (including extinct and unsampled lineages). Orange and grey
1146 lines represent the independent replicate trees and the consensus tree, respectively:
1147 because some of the 12 replicate trees showed different trends, we replicated ClaDS
1148 inferences using 100 replicate trees. Unlike most replicate trees, the EU99 consensus tree
1149 tends to present a limited decline in speciation rates, which reinforces the idea that
1150 consensus trees can be misleading (Janzen & Etienne, 2017).
1151
1152 **(e-f):** Mean speciation rates through time estimated by CoMET, for the VT (c) and EU99 (d)
1153 delineations and using the BDES estimated sampling fraction. Orange and grey lines
1154 represent the 12 independent replicate trees and the consensus tree, respectively.
1155


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)
0.000
0.010
0.015
0.020
0.025
0.030
−500
−400
−300
−200
−100
0
Time (in Myr)

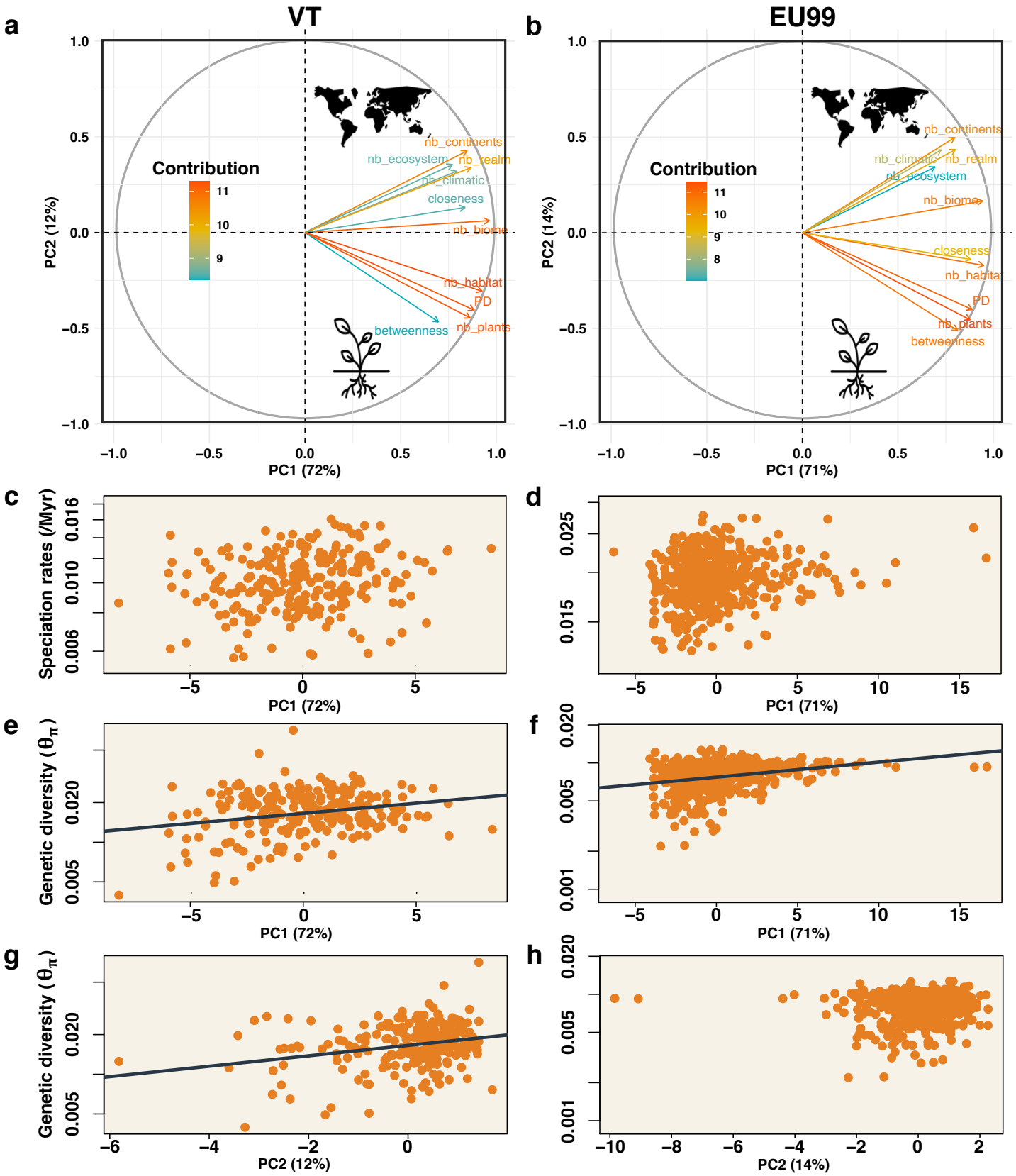
1156

1157 **Figure 3: Temperature-dependent diversification models reveal that global temperature**
1158 **positively associates with the speciation rates of Glomeromycotina (arbuscular**
1159 **mycorrhizal fungi) in the last 500 million years.**
1160
1161 **(a):** Average global temperature in the last 500 million years (Myr) relative to the average
1162 temperature of the period 1960-1990. The smoothed orange line represents cubic splines
1163 with 33 degrees of freedom used to fit temperature-dependent models of
1164 Glomeromycotina diversification with RPANDA. This default smoothing was estimated
1165 using the R function *smooth.spline*.
1166
1167 **(b):** AICc difference between the best-supported time-dependent model and the
1168 temperature-dependent model in RPANDA, for the VT (left) and EU99 (right) delineations,
1169 using the BDES estimated sampling fraction. An AICc difference greater than 2 indicates
1170 that there is significant support for the temperature-dependent model.
1171
1172 **(c):** Parameter estimations of the temperature-dependent models (speciation rate ~
1173 exp(parameter * temperature)). A positive parameter value indicates a positive effect of
1174 temperature on speciation rates.
1175
1176 For both delineations, the boxplots represent the results obtained for the consensus tree
1177 and the 12 independent replicate trees. Boxplots indicate the median surrounded by the
1178 first and third quartiles, and whiskers extend to the extreme values but no further than 1.5
1179 of the inter-quartile range. The horizontal dotted lines highlighted the values estimated for
1180 the consensus trees. Compared to the replicate trees, the consensus trees tends to present
1181 extreme values (stronger support for temperature-dependent model), which reinforces the
1182 idea that consensus trees can be a misleading representation (Janzen & Etienne, 2017).

1183


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

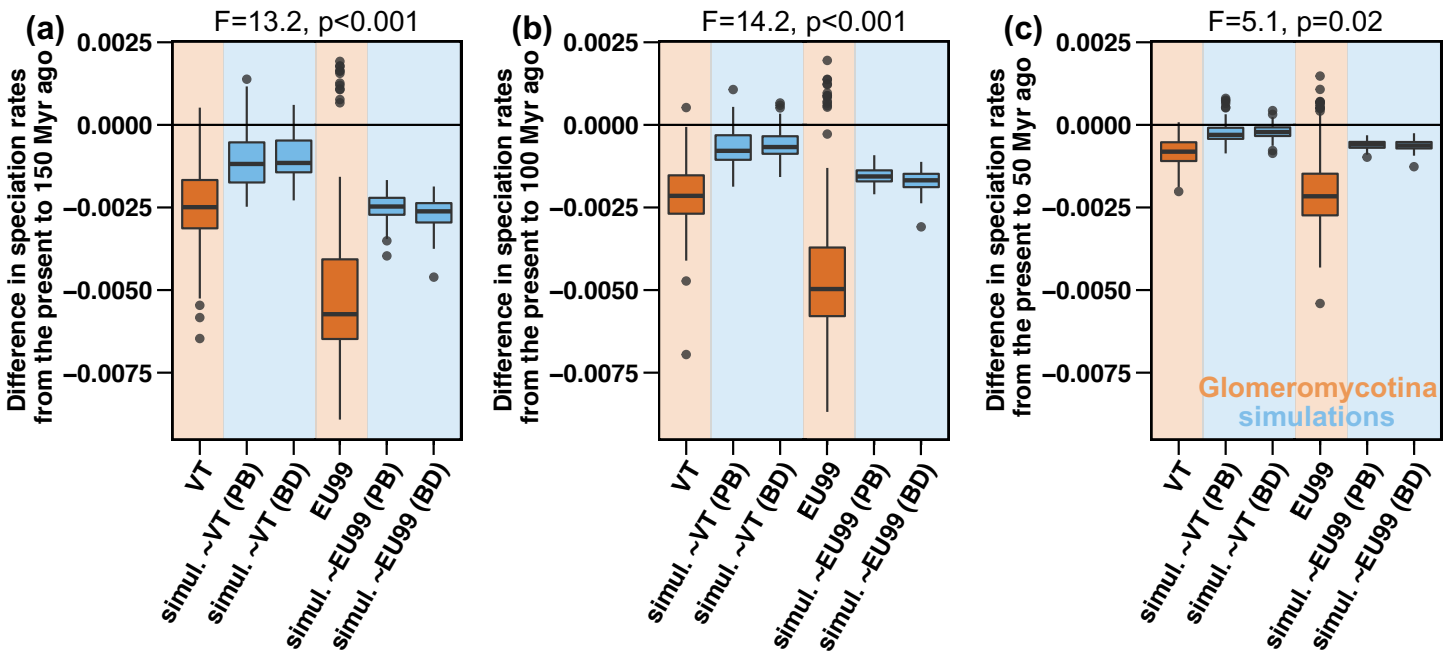
1184 **Figure 4: Abiotic and biotic correlates of speciation rates and genetic differentiation in**
1185 **Glomeromycotina (arbuscular mycorrhizal fungi)**
1186 **(a-b):** Projection of 10 abiotic and biotic variables on the two principal coordinates
1187 according to the VT (a) or EU99 (b) delineations. Principal coordinate analysis (PCA) was
1188 performed for the Glomeromycotina units represented by at least 10 sequences. Colors
1189 represent the contribution of the variable to the principal coordinates. The percentage for
1190 each principal coordinate (PC) indicates its amount of explained variance.
1191 Tested variables were: the numbers of continents on which the Glomeromycotina unit
1192 occurs (nb_continent), of realms (nb_realm), of ecosystems (nb_ecosystems), of habitats
1193 (nb_habitats), of biomes (nb_biomes), and climatic zones (nb_climatic) (Öpik et al., 2010),
1194 as well as information about the associated plant species of each unit, such as the number
1195 of plant partners (nb_plants), the phylogenetic diversity of these plants (PD), and the
1196 betweenness and closeness measurement of each fungal unit in the plant-fungus
1197 interaction network (see Methods).
1198
1199 **(c-d):** Speciation rates as a function of the PC1 coordinates for each VT (c) or EU99 (d) unit.
1200 Only the Glomeromycotina consensus tree is represented here (other replicate trees are
1201 presented in Supplementary Fig. 33).
1202
1203 **(e-h):** Genetic diversity (Tajima's $\theta\pi$ estimator) as a function of the PC1 (e-f) or PC2 (g-h)
1204 coordinates for each VT (e-g) or EU99 (f-h) unit. Only the Glomeromycotina consensus tree
1205 is represented here (other replicate trees are presented in Supplementary Fig. 33). The grey
1206 lines indicate the statistically significant linear regression between the two variables
1207 inferred using MCMCglmm.


a
VT
Contribution
11
10
9
nb_continents
nb_ecosystem
nb_realm
nb_climatic
closeness
nb_biome
nb_habitat
PD
nb_plants
betweenness
PC1 (72%)
PC2 (12%)
b
EU99
Contribution
11
10
9
8
nb_continents
nb_climatic
nb_realm
nb_ecosystem
nb_biome
closeness
nb_habitat
PD
nb_plants
betweenness
PC1 (71%)
PC2 (14%)
c
Speciation rates (/Myr)
PC1 (72%)
d
PC1 (71%)
e
Genetic diversity (θ_π)
PC1 (72%)
f
PC1 (71%)
g
Genetic diversity (θ_π)
PC2 (12%)
h
PC2 (14%)

1208

1209 **Figure 5: Artefactual species lumping and lack of phylogenetic resolution in the SSU**
1210 **rRNA region are not enough to explain the temporal decline in speciation rates detected**
1211 **in Glomeromycotina.**
1212 Comparison of the magnitude of the decline in speciation rates observed in
1213 Glomeromycotina (in orange) and on simulated data (in blue). The intensity of the
1214 slowdown is measured as the difference between the mean speciation rate estimated at
1215 present and the mean speciation rate estimated 150 Myr ago (a), 100 Myr ago (b), or 50 Myr
1216 ago (c). Negative differences indicate a speciation rate decline. Sequence alignments were
1217 simulated on phylogenetic trees obtained under a scenario of constant speciation rate and
1218 no extinction (*i.e.* pure birth "PB") and constant speciation and extinction rates (*i.e.* birth
1219 death "BD"), with characteristics mimicking the slow evolution of the SSU rRNA marker.
1220 We simulated phylogenies with two different net diversification rates, such that we
1221 obtained simulations with total numbers of species similar to the total numbers of VT or
1222 EU99 units (Supplementary Fig. 2). Boxplots indicate the median surrounded by the first
1223 and third quartiles, and whiskers extend to the extreme values but no further than 1.5 of
1224 the inter-quartile range. Each boxplot represents results for the consensus trees and the 12
1225 independent replicate trees for each of the 10 simulations, and for the consensus trees and
1226 the 100 independent replicate trees for the Glomeromycotina. Differences between the
1227 magnitude of the decline measured in Glomeromycotina (VT or EU99) and in the
1228 corresponding simulations were tested using linear models (reported at the top of the
1229 plots).
1230

1231


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