

1 **Shifts in diversification rates and host jump frequencies shaped the diversity of host range among**  
2 ***Sclerotiniaceae* fungal plant pathogens**

3 Olivier Navaud<sup>1</sup>, Adelin Barbacci<sup>1</sup>, Andrew Taylor<sup>2</sup>, John P. Clarkson<sup>2</sup>, Sylvain Raffaele<sup>1\*</sup>

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5 <sup>1</sup> LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France

6 <sup>2</sup> Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Warwick CV35

7 9EF, UK

8

9 \* Correspondence: [sylvain.raffaele@inra.fr](mailto:sylvain.raffaele@inra.fr)

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15 **Abstract**

16 The range of hosts that a parasite can infect in nature is a trait determined by its own evolutionary  
17 history and that of its potential hosts. However, knowledge on host range diversity and evolution at  
18 the family level is often lacking. Here, we investigate host range variation and diversification trends  
19 within the *Sclerotiniaceae*, a family of Ascomycete fungi. Using a phylogenetic framework, we  
20 associate diversification rates, the frequency of host jump events, and host range variation during the  
21 evolution of this family. Variations in diversification rate during the evolution of the *Sclerotiniaceae*  
22 define three major macro-evolutionary regimes with contrasted proportions of species infecting a  
23 broad range of hosts. Host-parasite co-phylogenetic analyses pointed towards parasite radiation on  
24 distant hosts long after host speciation (host jump or duplication events) as the dominant mode of  
25 association with plants in the *Sclerotiniaceae*. The intermediate macro-evolutionary regime showed a  
26 low diversification rate, high frequency of duplication events, and the highest proportion of broad  
27 host range species. Consistent with previous reports on oomycete parasites, our findings suggest that  
28 host jump and radiation, possibly combined with low speciation rates, could associate with the  
29 emergence of generalist pathogens. These results have important implications for our understanding  
30 of fungal parasites evolution and are of particular relevance for the durable management of disease  
31 epidemics.

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## 33 INTRODUCTION

34           The host range of a parasite has a central influence on the emergence and spread of disease  
35 (Woolhouse & Gowtage-Sequeria, 2005). There is a clear demarcation between specialist parasites  
36 that can only infect one or a few closely related host species, and generalists that can infect more  
37 than a hundred unrelated host species (Woolhouse et al., 2001, Barrett et al., 2009). Host  
38 specialization, when lineages evolve to infect a narrower range of hosts than related lineages, is a  
39 frequent occurrence in living systems and can be driven by a parasite sharing habitat with only a  
40 limited number of potential hosts. There are also clear examples of parasite adaptations that restrict  
41 the use of co-occurring potential hosts. For instance, the red rust fungal pathogen *Coleosporium*  
42 *ipomoeae* infects fewer *Ipomoea* species from its native community than when inoculated to  
43 nonnative communities, implying that evolution within local communities has narrowed pathogen  
44 host range (Chappell & Rausher, 2016). Some isolates of the rice blast fungus *Magnaporthe oryzae*  
45 are able to infect *Oryza sativa* japonica varieties but not indica varieties co-occurring in the Yuanyang  
46 area of China, because their genome harbors numerous avirulence effector genes (Liao et al., 2016).  
47 It has been proposed that specialization results from trade-offs between traits needed to infect a  
48 wide range of hosts (Futuyma & Moreno, 1988, Joshi & Thompson, 1995). It is frequently associated  
49 with the loss of traits that are not required to infect a particular host, such as the loss of lipid  
50 synthesis in parasitoid wasps (Visser et al., 2010), and the loss of secondary metabolism and  
51 carbohydrate active enzymes in powdery mildew fungal pathogens (Spanu et al., 2010). Specialization  
52 is sometimes considered as an evolutionary dead end (Moran, 1988), since gene losses are often  
53 irreversible and may lead specialist lineages "down a blind alley" that limits transitions back to  
54 generalism (Haldane, 1951, Day et al., 2016). There is nevertheless evidence for transitions from  
55 specialist to generalist parasitism (Johnson et al., 2009, Hu et al., 2014). For instance in plant  
56 pathogens, *Pseudoperonospora cubensis* differs from other downy mildew oomycete pathogens in  
57 that it is able to infect a wide range of Cucurbits (Thines & Choi, 2015). For many parasite lineages  
58 however, knowledge on host range diversity and evolution at the macro-evolutionary level is lacking.

59           *Sclerotiniaceae* is a family of Ascomycete fungi of the class Leotiomycetes which includes  
60 numerous plant parasites. Among the most studied are the grey mould pathogen *Botrytis cinerea*,  
61 considered to be one of the 10 most devastating plant pathogens (Dean et al., 2012), and the white  
62 and stem mold pathogen *Sclerotinia sclerotiorum*. Both are economically important pathogens in  
63 agriculture that infect hundreds of host plant species (Bolton et al., 2006, Mbengue et al., 2016). The  
64 *Sclerotiniaceae* family also includes host specialist parasites such as *Ciborinia camelliae* that causes

65 flower blight on *Camellia* (Denton-Giles et al., 2013), *Sclerotinia glacialis* that specifically infects  
66 *Ranunculus glacialis* (Graf & Schumacher, 1995), and *Monilinia oxycocci* causing the cottonball  
67 disease on cranberry (McManus et al., 1999). Other species from the *Sclerotiniaceae* have  
68 intermediate host range (tens of plant species) such as *Sclerotinia trifoliorum*, *S. subarctica* and *S.*  
69 *borealis* (Farr & Rossman, 2016, Clarkson et al., 2010). While *S. sclerotiorum* and *B. cinerea* are  
70 considered as typical necrotrophic pathogens, rapidly killing host cells to cause disease, the  
71 *Sclerotiniaceae* include species with diverse lifestyles. For instance, the poplar pathogen *Ciborinia*  
72 *whetzellii* and several *Myriosclerotinia* species are biotrophs that can live as endophytes (Schumacher  
73 & Kohn, 1985, Andrew et al., 2012), while *Coprotinia minutula* is coprophilous (Elliott, 1967). How  
74 this remarkable diversity evolved remains elusive. To gain insights into this question, knowledge of  
75 phylogenetic relationships and host range diversity at the macro-evolutionary level is needed.  
76 Specifically, ancestral state reconstruction can provide insights into how fungal host range has  
77 changed over time. The relationship between host range and diversification rates is an active area of  
78 research in insect ecology (Hamm & Fordyce, 2015, Hardy & Otto, 2014). For instance, a positive  
79 correlation was reported between species richness in the butterfly family *Nymphalidae* and the  
80 diversity of plants they feed upon (Janz et al., 2006). However in contrast, some studies found a  
81 negative relationship between host-plant breadth and diversification rate (Hardy & Otto, 2014).  
82 Transitions between feeding strategies generally appeared to be associated with shifts in insect  
83 diversification rates (Janz & Nylin, 2008, Hardy & Otto, 2014). Estimating fungal species divergence  
84 times in the *Sclerotiniaceae* will allow testing of whether biological diversification is related to host  
85 range variation.

86 Host range is a trait determined not only by the evolutionary history of a parasite, but also by  
87 that of its potential hosts (Poulin & Keeney, 2008). Accounting for host association patterns should  
88 therefore prove useful to understand host range evolution in the *Sclerotiniaceae*. The Leotiomycete  
89 class diverged less than 200 million years ago (Prieto & Wedin, 2013, Beimforde et al., 2014) and  
90 likely radiated with the diversification of flowering plants (Smith et al., 2010). Molecular phylogenetic  
91 studies distinguished *Myriosclerotinia*, *Sclerotinia sensu stricto*, *Botrytis* and *Botryotinia*, and  
92 *Monilinia sensu stricto* as monophyletic clades within the *Sclerotiniaceae* family (Holst-Jensen et al.,  
93 1998, Holst-Jensen et al., 2004). A phylogenetic analysis of *Monilinia* species suggested that co-  
94 speciation with host plants was the dominant pattern in this clade (Holst-Jensen et al., 1997). By  
95 contrast, there was no evidence for co-speciation with host plants in a phylogenetic analysis of  
96 *Botrytis* species (Staats et al., 2005). *Botrytis* species were thus proposed to have evolved through  
97 host jumps to unrelated host plants followed by adaptation to their new hosts (Staats et al., 2005,

98 Dong et al., 2015). Host-parasite co-phylogenetic analyses are required to test whether variations in  
99 the frequency of host jumps may have impacted on host range variation in the *Sclerotiniaceae*.

100 In this study, we performed a phylogenetic analysis on 105 *Sclerotiniaceae* species to reveal  
101 multiple independent shifts and expansions of host range. We show that three macro-evolutionary  
102 regimes with distinct diversification rates and dominant host-association patterns have shaped the  
103 diversity of the *Sclerotiniaceae*, and lead to contrasted proportions of broad host range species.  
104 Specifically, we highlight an increased emergence of broad host range parasites during the transition  
105 between macro-evolutionary regimes dominated by distinct patterns of host-pathogen association.  
106 These results suggest that reduced diversification rates and high host-jump frequency could associate  
107 with the emergence of generalist pathogens.

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## 109 MATERIALS AND METHODS

### 110 Taxon and host range data selection

111 We used all 105 *Sclerotiniaceae* species for which at least *ITS* marker sequence data were available in  
112 the Genbank database. As outgroups we selected 56 *Rutstroemiaceae* species and 39 representative  
113 species of Leotiomycetes and Sordariomycetes for a total of 200 species. Host range data were  
114 obtained from (Boland & Hall, 1994, Melzer et al., 1997), Index Fungorum  
115 (<http://www.indexfungorum.org>), the SMML fungus-host distribution database (Farr & Rossman,  
116 2016) and references therein. In total, we retrieved 7101 fungus-host association records that did not  
117 show strong geographic or crop/wild species bias (**Supplementary Figure 1**). Calibrated trees  
118 deciphering the relationships between the host families based on 7 gene regions (18S rDNA, 26S  
119 rDNA, *ITS*, *matK*, *rbcl*, *atpB*, and *trnL-F*) were extracted from (Qian & Zhang, 2014) and updated with  
120 (Hedges et al., 2015). The tree of host families used for cophylogenetic analyses is provided as  
121 **Supplementary File 1**.

### 122 Initial phylogenetic analysis

123 *ITS* sequences were aligned using MAFFT version 7 (Kato & Standley, 2013). The alignment was  
124 manually adjusted to minimize possible homoplastic positions using Seaview 4 (Gouy et al., 2010). We  
125 retained gaps shorter than 41 positions present in a maximum of 39 sequences, with ungapped  
126 blocks being at least five characters long with a maximum of 5% ambiguous nucleotides per position.  
127 Unalignable and autapomorphic regions were excluded from the analysis, yielding an alignment with  
128 797 informative sites (**Supplementary File 2**). Maximum-likelihood Phylogeny was inferred with  
129 PhyML 3 (Guindon et al., 2010) with Smart Model Selection which permits an automatic substitution  
130 model selection supporting the general time-reversible model with gamma distribution using 6  
131 substitution rate categories (GTR+G6) model as the best fit. Statistical branch support was inferred  
132 with the SH-like approximate likelihood ratio test (SH-aLRT) (Anisimova et al., 2011) and bootstrap  
133 analysis with 100 replicates (**Supplementary File 3 and 4**). The topology of the tree was confirmed by  
134 three additional methods. First, we used a neighbor-joining approach in FastME 2.0 (Lefort et al.,  
135 2015) with the LogDet substitution model and tree refinement by subtree pruning and regrafting  
136 (**Supplementary File 5**). Second, we used the parsimony ratchet approach implemented in the  
137 phangorn package in R (Schliep, 2010) (**Supplementary File 6**). Third, we used Bayesian analysis in  
138 MrBayes 3.2.6 (Ronquist et al., 2012) using the GTR substitution model with gamma-distributed rate  
139 variation across sites and a proportion of invariable sites in 100 million MCMC generations, sampling

140 parameters every 1,000, removing 10% of the tree files as burn-in. The resulting trees were edited  
141 with Figtree (available at <http://tree.bio.ed.ac.uk/software/figtree/>).

## 142 **Ancestral state reconstruction**

143 To infer possible ancestral hosts, we used Reconstruct Ancestral State in Phylogenies 3.1 (RASP) (Yu et  
144 al., 2015). We used the S-DIVA (Statistical-Dispersal Vicariance Analysis), S-DEC (Statistical Dispersal–  
145 Extinction–Cladogenesis model), BBM (Bayesian Binary MCMC) and BayArea methods to verify  
146 congruence between the methods and assess the robustness of their output, as recommended by (Yu  
147 et al., 2015). We report the results of the S-DIVA analysis which is considered the best adapted for  
148 host-parasite association analyses (Yu et al., 2015, Razo-Mendivil & De Leon, 2011). To implement S-  
149 DIVA, we delimited host groups as follows: Vitales (A), Asterids (B), Campanuliids (C), Commelinids  
150 (D), coprophilous (E), core Eudicots (F), Eudicots (G), Fabids (H), Polypodiidae (I), Lamiids (J),  
151 Magnoloidae (K), Malvids (L), Monocotyledones (M), Pinidae (N), allowing a maximum of 4 groups at  
152 each node. Among these groups, the latest divergence is that of Malvids and Fabids, estimated  
153 around 82.8 and 127.2 Mya (Clarke et al., 2011) and predates the emergence of *Sclerotiniaceae*  
154 estimated at ~69.7 Mya in this work, we therefore did not restrict associations in the ancestral  
155 reconstruction analysis. To account for incomplete sampling in this analysis, ancestral state  
156 reconstruction was computed for every plant group by the re-rooting method (Yang et al., 1995)  
157 under an entity-relationship (ER) model available in phytools (Revell, 2012) and derived from the ape  
158 library in R (Paradis et al., 2004). This method based on maximum likelihood, computed for every  
159 nodes of the *Sclerotiniaceae* phylogeny the probability to infect a given group of hosts. We  
160 considered plant groups as hosts when the probability of the ancestral state was >50%. For every  
161 plant group, up to 10% of terminal nodes were pruned randomly 100 times and the ancestral state  
162 reconstructed on pruned trees. For each plant group, we then extracted the variation in the age of  
163 the most ancestral inclusion into the *Sclerotiniaceae* host range (**Supplementary Figure 2**). For all  
164 plant groups, this variation was not significantly different from 0, indicating that ancestral state  
165 reconstruction was robust to tree pruning.

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## 167 **Divergence dating analyses**

168 We used a Bayesian approach to construct a chronogram with absolute times with the program  
169 BEAST 1.8.2. As no fossil is known in this fungal group, we use Sordariomycetes-Leotiomycetes  
170 divergence time to calibrate the tree (~300 Mya, Beimforde et al., 2014). Using our initial phylogeny

171 as a constraint, the partition file was prepared with the BEAUti application of the BEAST package.  
172 Considering that our dataset includes distantly related taxa, subsequent analyses were carried out  
173 using an uncorrelated relaxed clock model with lognormal distribution of rates. We used a uniform  
174 birth-death model with incomplete species sampling as prior on node age, following (Beimforde et  
175 al., 2014) to account for incomplete sampling in our phylogeny. Analyses were run three times for 100  
176 million generations, sampling parameters every 5,000 generations, assessing convergence and  
177 sufficient chain mixing using Tracer 1.6 (Drummond et al., 2012a). We removed 20% of trees as burn-  
178 in, and the remaining trees were combined using LogCombiner (BEAST package), and summarized as  
179 maximum clade credibility (MCC) trees using TreeAnnotator within the BEAST package. The trees  
180 were edited in FigTree. The R packages *ape* (Paradis et al., 2004) and *phytools* (Revell, 2012) were  
181 used for subsequent analyses.

## 182 **Diversification analysis**

183 We used the section containing all 105 *Sclerotiniaceae* species of the chronogram with absolute times  
184 generated by BEAST to perform diversification analyses with three different methods. We first used  
185 Bayesian Analysis of Macroevolutionary Mixtures (BAMM) version 2.5 (Rabosky et al., 2013, Rabosky  
186 et al., 2014), an application designed to account for variation in evolutionary rates over time and  
187 among lineages. The priors were set using the `setBAMMPriors` command in the `BAMMtools` R-  
188 package (Rabosky et al., 2014) . We ran four parallel Markov chains for 5,000,000 generations and  
189 sampled for every 1000 trees. The output and subsequent analyses were conducted with  
190 `BAMMtools`. We discarded the first 10% of the results and checked for convergence and the effective  
191 sample size (ESS) using the *coda* R-package. Lineage-through-time plots with extant and extinct  
192 lineages were computed using the *phytool* package in R (Revell, 2012) with the `drop.extinct=FALSE`  
193 parameter. The exact number of *Sclerotiniaceae* species is currently unknown and no intentional bias  
194 was introduced in data collection (**Supplementary Figure 1**). To detect shifts in diversification rates  
195 taking into account incomplete sampling of the *Sclerotiniaceae* diversity and tree uncertainty, we  
196 used MEDUSA, a stepwise approach based upon the Akaike information criterion (AIC) (Alfaro et al.,  
197 2009, Drummond et al., 2012b). For this analysis, we used the birth and death (bd) model, allowed  
198 rate shifts both at stem and nodes, used the AIC as a statistical criterion with initial  $r=0.05$  and  
199  $\epsilon=0.5$ . Sampling of the fungal diversity is typically estimated around 5% (Hawksworth &  
200 Luecking, 2017). We used random sampling rates between 5 and 100% for each individual species in  
201 100 MEDUSA bootstrap replicates to estimate the impact of sampling richness. Next, we randomly  
202 pruned 1 to 10% of species from the tree in 100 MEDUSA bootstrap replicates to estimate the impact



203 of tree completeness. To control for the impact of divergence time estimates on the diversification  
204 analysis, we altered all branching times in the tree randomly by -15% to +15% in 100 MEDUSA  
205 bootstrap replicates (**Supplementary Figure 3**). Finally, we used RPANDA which includes model-free  
206 comparative methods for evolutionary analyses (Morlon et al., 2016). The model-free approach in  
207 RPANDA compares phylogenetic tree shapes based on spectral graph theory (Lewitus & Morlon,  
208 2015). It constructs the modified Laplacian graph of a phylogenetic tree, a matrix with eigenvalues  
209 reflecting the connectivity of the tree. The density profile of eigenvalues (**Supplementary Figure 4A**)  
210 provides information on the entire tree structure. The algorithm next used k-medoids clustering to  
211 identify eight modalities in the phylogenetic tree of the *Sclerotiniaceae* (**Supplementary Figure 4B-C**).  
212 A post-hoc test comparing Bayesian Information Criterion (BIC) values for randomly bifurcated trees  
213 ( $BIC_{\text{random}}$ ) with that of the tree of the *Sclerotiniaceae* ( $BIC_{\text{Sclerotiniaceae}}$ ) supported the eight modalities  
214 ( $BIC_{\text{random}}/BIC_{\text{Sclerotiniaceae}} = 10.34$ , well above the significance threshold of 4.0). We obtained significant  
215 BIC ratios with two or more modalities, supporting the existence of at least two macro-evolutionary  
216 regimes in the *Sclerotiniaceae*.

217

## 218 **Co-phylogeny analysis**

219 To detect potential codivergence patterns, we tested for co-phylogeny between fungal species and  
220 host family trees with CoRe-PA 0.5.1 (Merkle et al., 2010), PACo (Balbuena et al., 2013) and Jane 4  
221 (Conow et al., 2010) as these programs can accommodate pathogens with multiple hosts. The only 3  
222 species which were not able to interact with an angiosperm host (saprotroph or only described on  
223 Gymnosperms) were excluded from the analysis (namely: *Elliottinia kernerii*, *Coprotinia minutula* and  
224 *Stromatinia cryptomeriae*). We used a full set of 263 host-pathogen associations and a simplified set  
225 of 121 associations minimizing the number of host families involved to control for the impact of  
226 sampling bias (**Table 1**). We also performed the analyses independently for each macro-evolutionary  
227 regime. To estimate cost parameters in CoRe-PA, we ran a first cophylogeny reconstruction with cost  
228 values calculated automatically using the simplex method on 1000 random cycles, with all host  
229 switches permitted, direct host switch, and full host switch permitted (other parameters set to CoRe-  
230 PA default). The best reconstruction had a quality score of 1.348 with total costs 37.076 and  
231 calculated costs of 0.0135 for cospeciation, 0.1446 for sorting, 0.2501 for duplication and 0.5918 for  
232 host switch. With these costs and parameters, we then computed reconstruction with 1000 random  
233 associations to test for the robustness of the reconstructions. Next, we used 100,000 permutations of  
234 the host-pathogen association matrix in PACo to test for the overall congruence between host and

235 pathogen trees. To test for the contribution of each association on the global fit, we performed taxon  
236 jackknifing. We used one-tailed z tests to compare squared residuals distribution for each host-  
237 pathogen link with the median of squared residuals for the whole tree, and infer likely co-  
238 evolutionary and host shift links at  $p < 0.01$ . We used optimal costs calculated by CoRe-PA to run co-  
239 phylogeny reconstruction in Jane 4 Solve Mode with 50 generations and a population size of 1,000.  
240 Statistical tests were performed using 100 random tip mapping with 20 generations and population  
241 size of 100. For the analysis of each macro-evolutionary regime in Jane 4, we used simplified host-  
242 pathogen association set to reduce the amount of Losses and Failure to Diverge predicted and  
243 facilitate comparisons with CoRe-PA and PACo results. We visualized the interactions with TreeMap3  
244 (Charleston & Robertson, 2002), using the untangling function to improve the layout.

245

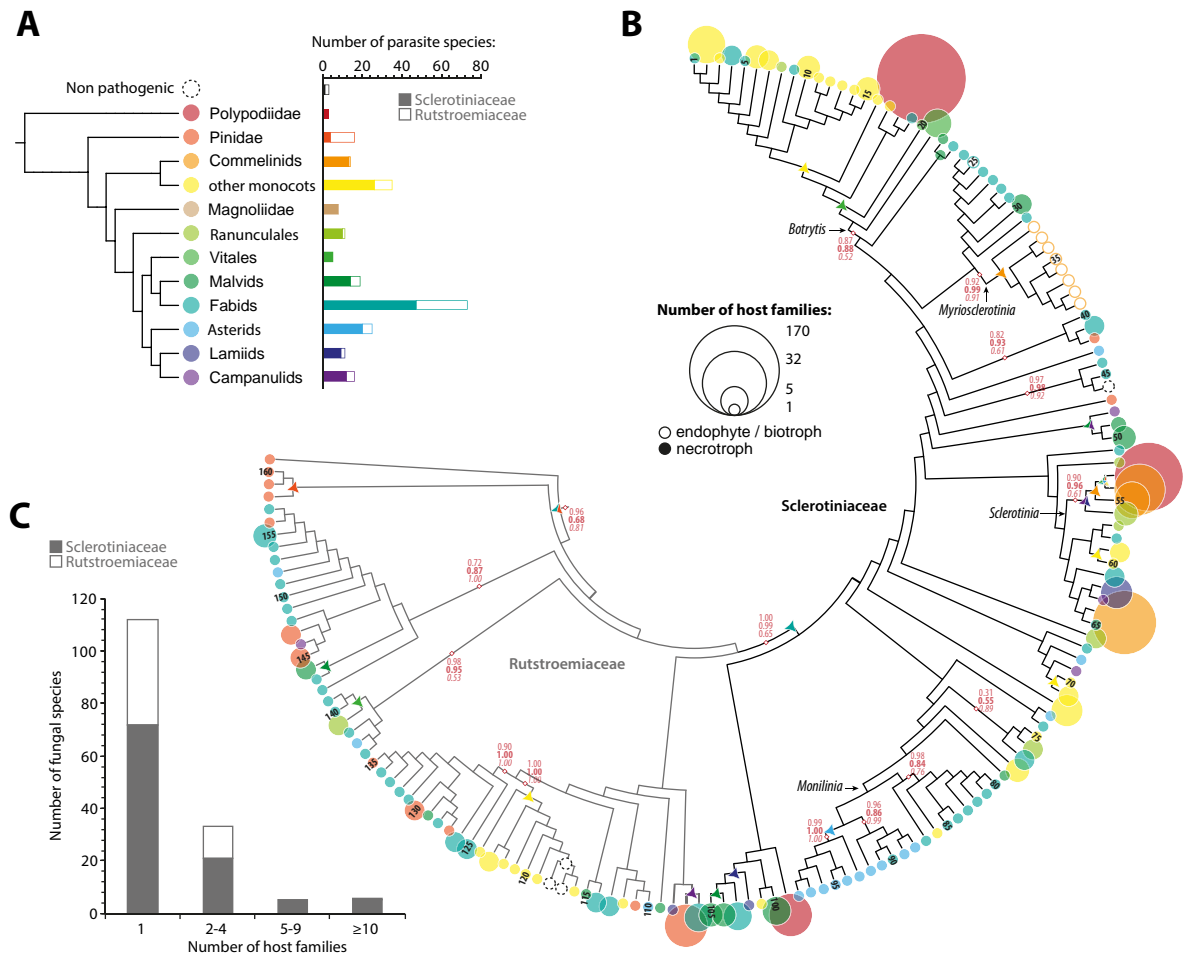
246 **RESULTS**

247 **Multiple independent shifts and expansions of host range in the evolution of the *Sclerotiniaceae***

248 To document the extant diversity in the *Sclerotiniaceae* fungi, we collected information on the host  
249 range of 105 species in this family. For comparison purposes, we also analyzed the host range of 56  
250 species from the *Rutstroemiaceae* family, a sister group of the *Sclerotiniaceae* (**Figure 1A, Table 1**). To  
251 reduce biases that may arise due to missing infection reports, we analyzed host range at the family  
252 level instead of genus or species level. We found one species in the *Sclerotiniaceae* (*Coprotinia*  
253 *minutula*) and two species in the *Rutstroemiaceae* (*Rutstroemia cunicularia* and *Rutstroemia cuniculi*)  
254 reported as non-pathogenic to plants that are coprophilous (Elliott, 1967). Most species in the  
255 *Sclerotiniaceae* are reported necrotrophic pathogens, such as *Botrytis*, *Monilinia* and *Sclerotinia*  
256 species. Exceptions include *Ciborinia whetzellii* which was reported as an obligate biotroph of poplar  
257 (Andrew et al., 2012) and *Myriosclerotinia* species that specifically infect monocot families as  
258 facultative biotrophs or symptomless endophytes (Andrew et al., 2012). The most frequently  
259 parasitized plant group was Fabids (by 47 *Sclerotiniaceae* species and 26 *Rutstroemiaceae* species), a  
260 group of the Rosids (Eudicots) including notably cultivated plants from the Fabales (legumes) and  
261 Rosales (rose, strawberry, apple...) orders. Plants from the order Vitales (e.g. grapevine), from the  
262 Magnoliids, and from the *Polypodiidae* (ferns) were colonized by *Sclerotiniaceae* but not  
263 *Rutstroemiaceae* fungi.

264 We next used the internal transcribed spacer (*ITS*) region of rDNA sequences to construct a  
265 phylogenetic tree of the 105 *Sclerotiniaceae* and 56 *Rutstroemiaceae* species (**Figure 1B, Sup. File 1 -**  
266 **7**). This marker is the only one available for all species and is currently considered as the universal  
267 marker for taxonomic use (Schoch et al., 2012). Maximum likelihood, neighbor joining, parsimony and  
268 Bayesian analyses yielded convergent tree topologies. The tree topologies confirmed the familial  
269 classification recognized in previous phylogenies (Holst-Jensen et al., 1997, Holst-Jensen et al., 1998,  
270 Andrew et al., 2012), identifying a clearly supported *Botrytis* genus including 23 species; a  
271 *Myriosclerotinia* genus including 8 species; a *Sclerotinia* genus *sensu stricto* including 4 *Sclerotinia*;  
272 and a *Monilinia* genus including 16 *Monilinia* species and 3 other species. *Botrytis calthae* and  
273 *Amphobotrys ricini* were the only two species with imprecise positions in maximum likelihood  
274 reconstructions, due to the *ITS* marker being insufficient to infer complete phylogenetic placement  
275 (Staats et al., 2005, Lorenzini & Zapparoli, 2016).

276



**Figure 1. Multiple independent shifts and expansions of host range in the evolution of the *Sclerotiniaceae*.** (A) Distribution of plant hosts of parasites from the *Sclerotiniaceae* and *Rutstroemiaceae* fungi. (B) Maximum likelihood *ITS* phylogeny of 105 *Sclerotiniaceae* and 56 *Rutstroemiaceae* species obtained by showing host range information and ancestral host reconstruction. Host range is shown as circles at the tip of branches, sized according to the number of host families and colored as in (A) according to the earliest diverging plant group in host range. Numbers at the tip of branches refer to species listed in Supplementary Table 1. Branch support indicated in light red for major clades corresponds to SH-aLRT (regular), bootstrap (bold) and Bayesian posterior probabilities (italics). Reconstructed ancestral host is shown as triangles at intermediate nodes when a change compared to previous node is predicted. Endophytes and biotrophic parasites are shown with empty circles. (C) Distribution of *Sclerotiniaceae* and *Rutstroemiaceae* species according to their number of host families.

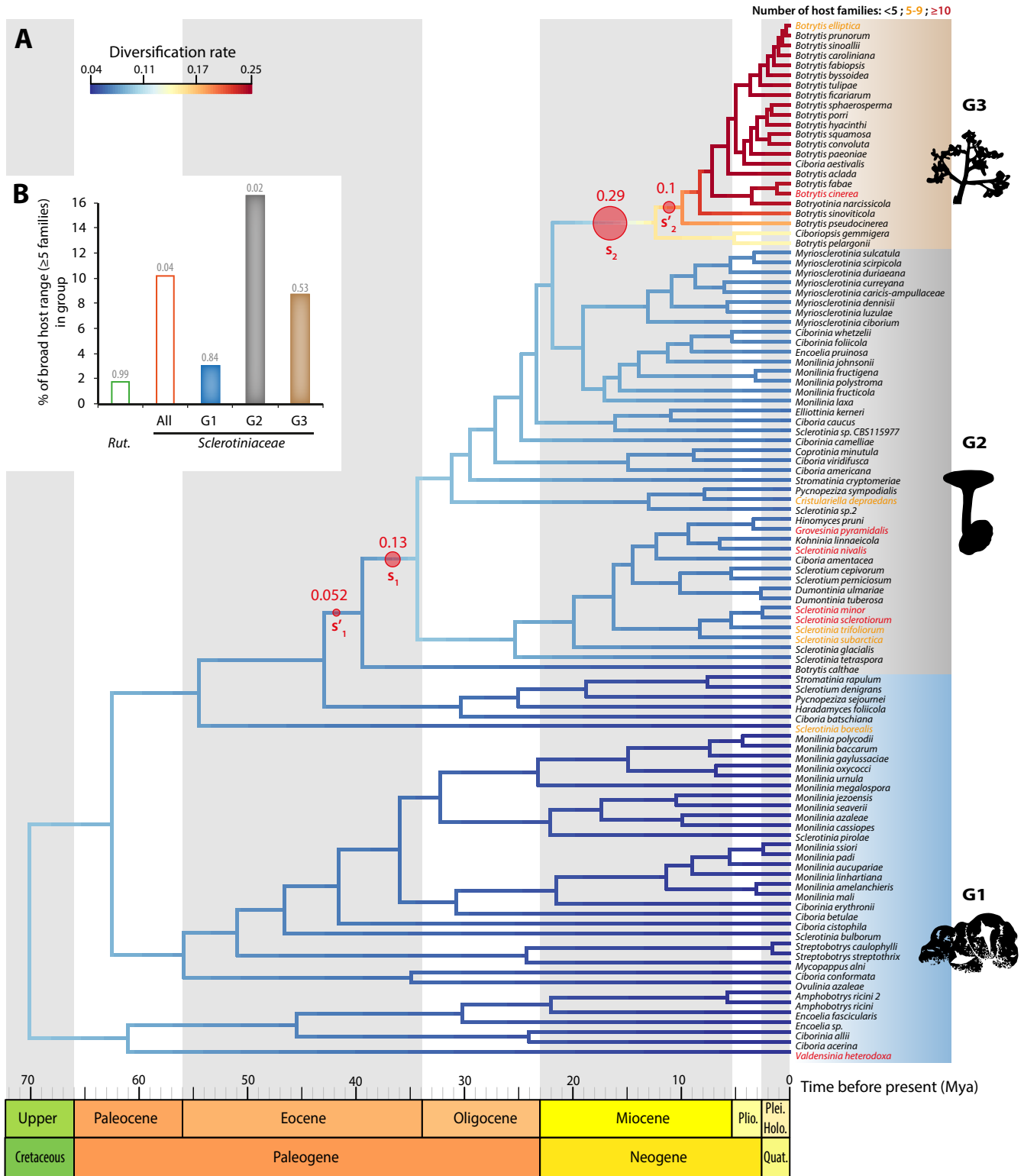
277 We used RASP (Yu et al., 2015) and phytools (Revell, 2012) to reconstruct the ancestral host range  
278 across the phylogeny (**Figure 1B, Supplementary Figure 1, Supplementary Figure 5**). This identified  
279 Fabids as the most likely ancestral hosts of the *Sclerotiniaceae* family (relative probability of 100% in  
280 S-DIVA and S-DEC analyses). Random tree pruning in phytools indicated that this result is robust to  
281 sampling biases. Over 48% of the *Sclerotiniaceae* species are pathogens of host plants that evolved  
282 prior to the divergence of the Fabids, suggesting numerous host jumps in this family of parasites.  
283 Notably, a jump to Malvids and then to Monocots was identify at the base of the *Botrytis* genus (87%  
284 and 85% probability in S-DIVA respectively), a jump to Commelinids occurred in the *Myriosclerotinia*  
285 genus (91% probability in S-DIVA), a jump to the Ranunculales occurred at the base of the *Sclerotinia*  
286 genus (76% probability in S-DIVA); a jump to Asterids was found at the base of a major group of  
287 *Monilinia* (89% probability in S-DIVA).

288 A total of 73 *Sclerotiniaceae* species (69.5%) and 41 (73.2%) *Rutstroemiaceae* infected a single host  
289 family (**Figure 1C, Supplementary Table 1**). *Moellerodiscus lentus* was the only *Rutstroemiaceae*  
290 species infecting hosts from more than 5 plant families whereas eleven *Sclerotiniaceae* species  
291 (10.4%) exhibited this trait, including *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotinia minor* and  
292 *Grovesinia pyramidalis*, each of which colonizes plants from more than 30 families. Each of these  
293 species belong to a clearly distinct phylogenetic group with a majority of species infecting a single  
294 host family. This may result from radiation following host jumps (Choi & Thines, 2015) and suggests  
295 that the ability to colonize a broad range of plant was acquired multiple times independently through  
296 the evolution of the *Sclerotiniaceae*.

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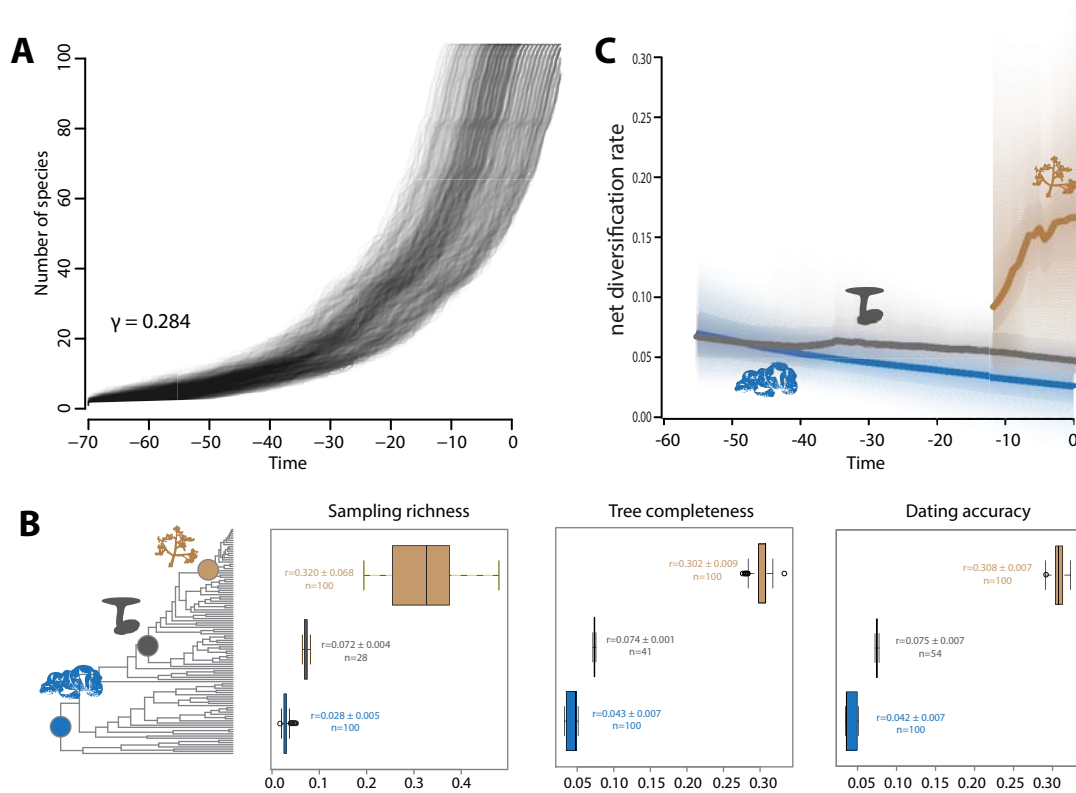
## 298 **Two major diversification rate shifts in the evolution of the *Sclerotiniaceae***

299 To test for a relationship between host range variation and biological diversification in the  
300 *Sclerotiniaceae*, we estimated divergence times for the fungal family *Sclerotiniaceae* using the *ITS*  
301 marker in a Bayesian framework. A calibrated maximum clade credibility chronogram from these  
302 analyses is shown in **Figure 2A** (and **Supplementary Figure 6**). This showed that *Sclerotiniaceae* fungi  
303 shared a most recent common ancestor between 33.9 and 103.5 million years ago (Mya), with a  
304 mean age of 69.7 Mya. The divergence of *Botrytis pseudocinerea*, estimated from the *ITS* dataset,  
305 occurred ca. 3.35-17.8 Mya (mean age 9.8 Mya) and is similar to a previous estimate of 7-18 Mya  
306 (Walker et al., 2011).



**Figure 2. Two major diversification rate shifts in the evolution of the Sclerotiniaceae.** (A) Dated ITS-based species tree for the Sclerotiniaceae with diversification rate estimates. The divergence times correspond to the mean posterior estimate of their age in millions of years calculated with BEAST. Branches of the tree are color-coded according to diversification rates determined with BAMM. Major rate shifts identified in BAMM are shown as red circles, noted  $s_1$ ,  $s_1'$ ,  $s_2$  and  $s_2'$  and labeled with the posterior distribution in the 95% credible set of macro-evolutionary shift configurations. Species names are shown in black if host range includes less than 5 plant families, in yellow for 5 to 9 plant families and in red for 10 or more plant families. Diversification rate shifts define three macro-evolutionary regimes noted G1, G2 and G3 and boxed in blue, grey and brown respectively. (B) Distribution of broad host range (5 or more host families) parasites in Rutstroemiaceae, Sclerotiniaceae and under each macro-evolutionary regime of the Sclerotiniaceae. P-values calculated by random permutations of host ranges along the tree are indicated above bars. Holo., Holocene; Mya, Million years ago; Plei., Pleistocene; Plio., Pliocene; Quat., Quaternary; Rut., Rutstroemiaceae.

307 Using this time-calibrated phylogeny, we calculated speciation and diversification rates using three  
308 different methods, to control for the limits of individual methods (Rabosky et al., 2017). First, we  
309 used the BAMM framework, which implements a Metropolis Coupled Markov chain Monte Carlo  
310 method to calculate diversification rates along lineages (Rabosky, 2014) (**Figure 2A, Supplementary**  
311 **Figure 7A-C**). The analysis identified two significant rate shifts ( $s_1$  and  $s_2$ , accounting for > 30% of the  
312 posterior distribution in the 95% credible set of macro-evolutionary shift configurations) each  
313 immediately adjacent to a minor rate shift ( $s_1'$  and  $s_2'$  respectively;  $\leq 10\%$  of the posterior distribution)  
314 within two different clades (**Figure 2A**). Each shift affects a specific clade and is not detected  
315 coincidentally across the whole phylogeny. The earliest shift occurred between 34.2 and 42.7 Mya. It  
316 resulted in an increase of instantaneous diversification rate from  $\sim 0.05$  lineage/million year in  
317 *Monilinia* and related clades to  $\sim 0.08$  lineage/million year in *Sclerotinia* and derived clades. The  
318 second shift occurred between 9.8 and 21.7 Mya. It resulted in a further increase of instantaneous  
319 diversification to  $> 0.15$  lineage/million year in *Botrytis* lineage. These two shifts defined three distinct  
320 macro-evolutionary regimes in the *Sclerotiniaceae*. Second, to determine how speciation rates  
321 changed over time, we computed lineage-through-time plots for the *Sclerotiniaceae* tree and 1000  
322 trees in which we altered all branching times randomly by -15% to +15% to control for the sensitivity  
323 to divergence time estimates (**Figure 3A**). We observed an exponential trend of species accumulation  
324 consistent with a late burst of cladogenesis or early extinction (Pybus  $\gamma=0.284$ ). Third, we used  
325 MEDUSA (Alfaro et al., 2009, Drummond et al., 2012b) to detect shifts in diversification rate in the  
326 *Sclerotiniaceae* phylogeny. MEDUSA reported two shifts, corresponding to shifts  $s_1$  and  $s_2'$  identified  
327 in BAMM (**Sup. Fig. 3D**). We used a bootstrap approach to test for the sensitivity of these shifts to  
328 sampling richness, tree completeness and dating accuracy (**Figure 3B, Supplementary Figure 3**). Shift  
329  $s_2'$  was detected in 100% of the bootstraps. Shift  $s_1$  was the second most frequent shift detected in  
330 our bootstrap analysis although it appeared sensitive to sampling richness in particular. Estimates of  
331 speciation rates in MEDUSA were robust to sampling richness, tree completeness and dating accuracy  
332 ( $r \sim 0.038$  in G1,  $r \sim 0.074$  in G2 and  $r \sim 0.31$  in G3). These values were consistent with diversification rate  
333 estimated in BAMM for each regime (**Figure 3C**). Fourth, we used model-free analysis of branching  
334 patterns in phylogenetic trees with R-PANDA (Morlon et al., 2016). The spectrum of eigenvalues  
335 suggested eight modes of division in the phylogeny of the *Sclerotiniaceae* (**Supplementary Figure 4A-**  
336 **B**). Comparison to randomly bifurcating trees suggested that these modalities are significant (BIC  
337 ratio 0.09). The modalities identified by RPANDA k-means clustering algorithm overlapped with the  
338 three macro-evolutionary regimes identified previously (**Supplementary Figure 4C**). Overall, these  
339 analyses converged towards the identification of three macro-evolutionary regimes in the



**Figure 3. Robustness of diversification rate shifts identification in the *Sclerotiniaceae* phylogeny.** (A) Lineage-through-time plots for the *Sclerotiniaceae* tree and 1000 trees in which branching times were altered randomly by -15% to +15% to control for the sensitivity to divergence time estimates. Pybus  $\gamma$  for the *Sclerotiniaceae* tree is provided. (B) Frequency (n) of diversification rate shift detection and diversification rate estimates (r) in a 100 MEDUSA bootstrap replicates in which sampling richness, tree completeness and divergence times were randomly altered. Labels indicate average diversification rate estimates (r) for each macro-evolutionary regime (blue for G1, grey for G2, brown for G3), with standard deviation of the mean for a 100 replicates. (C) Net diversification rates over time estimated by BAMM for each macro-evolutionary regime (blue for G1, grey for G2, brown for G3).



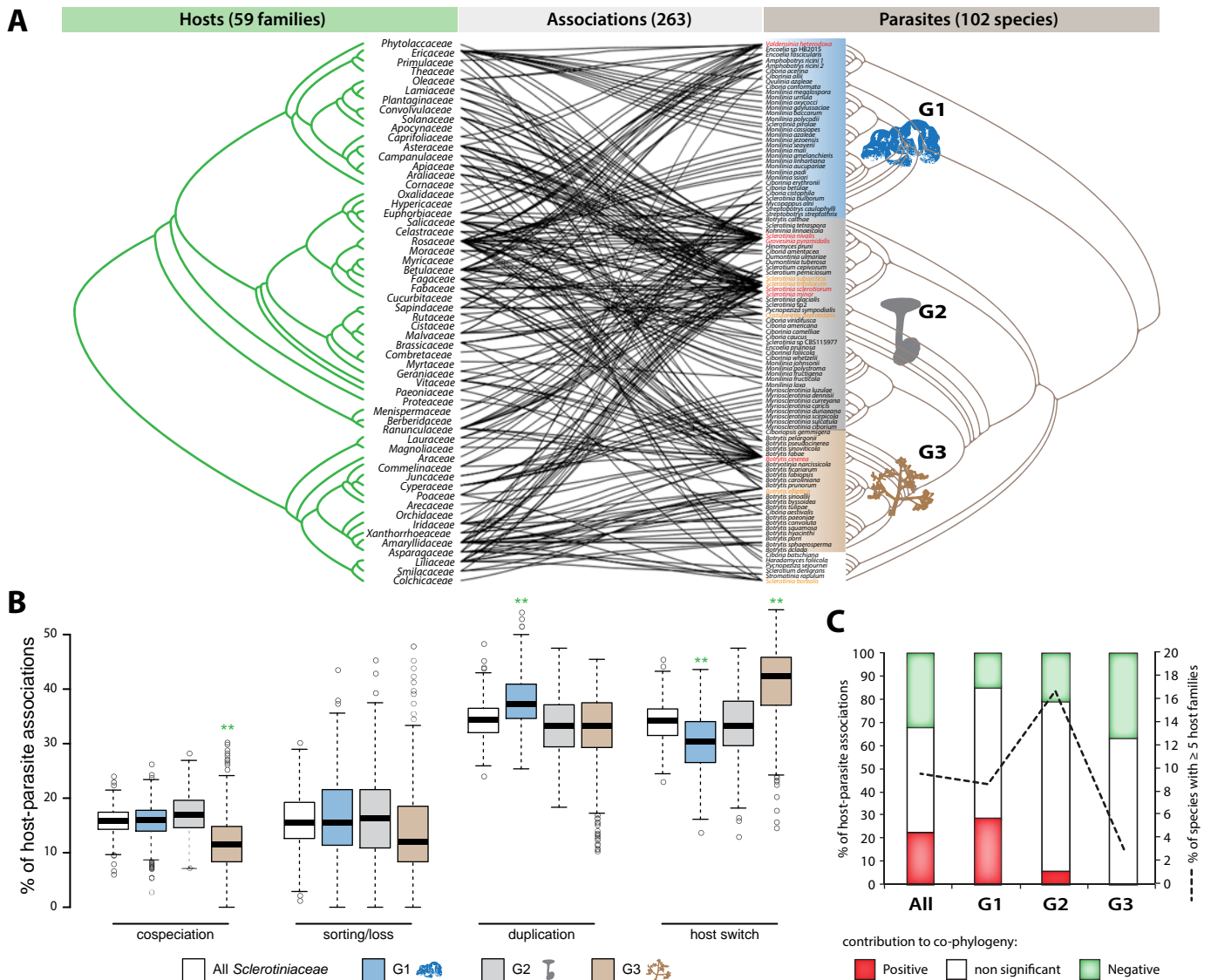
340 *Sclerotiniaceae*: species in regime G1 are early diverging and showed low diversification rates, they  
341 encompassed notably *Monilinia*, *Encoelia* and some *Ciboria* species. Regime G2 had intermediate  
342 diversification rates and encompassed notably *Myriosclerotinia* species and most *Sclerotinia* species.  
343 Regime G3 corresponding to *Botrytis* genus was the most recently diverged and presented the  
344 highest diversification rates.

345 We compared the proportion of broad host range ( $\geq 5$  plant families) fungal species that emerged in  
346 the *Rutstroemiaceae*, in the *Sclerotiniaceae*, and under each the three macro-evolutionary regimes in  
347 the *Sclerotiniaceae* (**Figure 2B**). We randomly permuted host range values across the tree 10,000  
348 times to estimate the p-values of these proportions occurring by chance. We counted one (1.8%,  $p=$   
349 0.9959) broad host range species in the *Rutstroemiaceae* (*Moellerodiscus lentus*) and eleven (10.4%,  
350  $p= 0.0359$ ) in the *Sclerotiniaceae*. In the *Sclerotiniaceae*, regime G2 with intermediate diversification  
351 rates showed the highest proportion of broad host range species (16.2%,  $p=0.0163$ ), followed by  
352 regime G3 (8.7% of broad host range species,  $p=0.537$ ) and regime G1 (5.1% of broad host range  
353 species,  $p=0.8407$ ). These analyses suggest that the evolutionary history of regime G2 could have  
354 favored the emergence of broad host range parasites in the *Sclerotiniaceae*.

355

### 356 **Diversification rate shifts associate with variations in rates of co-speciation, duplication and host** 357 **jump in the *Sclerotiniaceae***

358 Within the last 50 Ma the world has experienced an overall decrease in mean temperatures but with  
359 important fluctuations that dramatically modified the global distribution of land plants (Zachos et al.,  
360 2001, Donoghue & Edwards, 2014, Nürk et al., 2015). We hypothesized that modifications in the  
361 distribution of plants could have an impact on host association patterns in *Sclerotiniaceae* parasites.  
362 To test this hypothesis, we performed host-parasite co-phylogeny reconstructions using CoRe-PA  
363 (Merkle et al., 2010), PACo (Balbuena et al., 2013) and Jane 4 (Conow et al., 2010) (**Table 1**). We  
364 found that hosts of *Sclerotiniaceae* fungi include  $\sim 1800$  plant species. To consider this overall host  
365 diversity in a co-phylogenetic analysis, we used a tree including 56 plant families, a tree of 102  
366 *Sclerotiniaceae* parasitic species, and 263 host-parasite interactions (**Sup. File 3**). The resulting  
367 tanglegram (**Figure 4A**) highlighted clear parasite duplications notably on *Ericaceae* and *Rosaceae* in  
368 the G1 group of the *Sclerotiniaceae* (*Monilinia* species). There was no obvious topological congruence  
369 between the plant tree and groups G2 and G3 of the *Sclerotiniaceae*. To take into account sampling  
370 bias, we performed co-phylogeny reconstructions using the full set of 263 host-parasite associations



**Figure 4. Diversification rate shifts associate with variations in rates of co-speciation, duplication and host switch in *Sclerotiniaceae* fungi.** (A) Tanglegram depicting the associations between 102 *Sclerotiniaceae* species and 59 plant families. The three macro-evolutionary regimes are indicated by colored boxes on the *Sclerotiniaceae* tree. Fungal species labels are color-coded as in figure 2. (B) Proportion of co-speciation, sorting/loss, duplication and host switches in host-*Sclerotiniaceae* associations as predicted by CoRe-PA in 1,000 co-phylogeny reconstructions. \*\* indicate large effect size in a macro-evolutionary compared to the complete set of associations as assessed by Cohen's d test. (C) Proportion of host-*Sclerotiniaceae* associations contributing significantly and positively (likely co-speciation), non significantly and significantly and negatively (likely host switch) to co-phylogeny in PACo analysis. The black dotted line indicates the percentage of broad host range species (5 or more host families) in each group.

371 and a simplified set of 121 associations. Reconstructions were also performed independently on each  
372 of the three macro-evolutionary regimes. CoRe-PA classifies host-pathogen associations into (i)  
373 cospeciation, when speciation of host and pathogen occur simultaneously, (ii) duplication, when  
374 pathogen speciation occurs independently of host speciation, (iii) sorting or loss, when a pathogen  
375 remains associated with a single descendant host species after host speciation, and (iv) host switch  
376 (also designated as host jump) when a pathogen changes host independently of speciation events  
377 (Merkle et al., 2010). The CoRe-PA reconstructions indicated that duplications and host switch each  
378 represented ~34% of host associations from the full set of host-*Sclerotiniaceae* associations.  
379 Cospeciation and sorting each represented ~16% of host associations. The analysis of each macro-  
380 evolutionary regime indicated that G1 is characterized by high duplication and low host jump  
381 frequencies and G3 by low cospeciation and sorting frequencies and high host jump frequencies  
382 (**Figure 4B**). We next used Procrustean superimposition in PACo (Balbuena et al., 2013) to identify  
383 host-pathogen associations that contribute significantly to co-phylogeny between *Sclerotiniaceae* and  
384 host plant families. The PACo analysis suggested that overall, the *Sclerotiniaceae* lineages are not  
385 randomly associated with their host families ( $P < 0.01$ ). The PACo analysis also includes taxon  
386 jackknifing to test for the relative contribution of each host-pathogen association in the co-phylogeny  
387 pattern. In the full set of host-*Sclerotiniaceae* associations, ~21% contributed positively and  
388 significantly to cophylogeny, likely representing cospeciation events, while ~31% contributed  
389 negatively and significantly to cophylogeny, therefore likely representing host jump events (**Table 1**).  
390 Taxon jackknifing on individual macro-evolutionary regime revealed a high frequency of associations  
391 with positive contribution to cophylogeny (likely cospeciation) in G1, and a high frequency of  
392 associations with negative contribution to cophylogeny (likely host switch) in G3, in good agreement  
393 with the CoRe-PA analysis. In addition to the types of host-parasite association mentioned before,  
394 Jane 4 identifies 'failure to diverge' associations, corresponding to events when a host speciates and  
395 the parasite remains on both new host species (Conow et al., 2010). Jane 4 analysis for the whole  
396 *Sclerotiniaceae* family (simplified set of associations) identifies losses as the dominant form of host  
397 association (~65%), followed by duplications (~15%) and host jumps (~12%) while failure to diverge  
398 (~5.5%) and cospeciation (~3%) were rare events (**Table 1**). Consistent with CoRe-PA and PACo  
399 analyses, Jane 4 found the highest rate of host jumps in regime G3 (~42%). Unlike previous analyses,  
400 Jane 4 found the highest rate of cospeciation in G3 (~9.7%). G1 was characterized by a high  
401 duplication rate while G2 was characterized by a high rate of losses and failure to diverge events but  
402 the lowest host jump rate. Overall, our co-phylogeny analyses converged towards the conclusion that  
403 cospeciations represent a minor proportion of host associations in the *Sclerotiniaceae* and that the

404 proportion of host jumps varied markedly between the three macro-evolutionary regimes, with G3  
405 showing the highest host jump rate (between 33% and 42%).

## 406 DISCUSSION

407 Our analyses lead to a model in which the extant diversity of *Sclerotiniaceae* fungi is the  
408 result of three macro-evolutionary regimes characterized by distinct diversification rates and host  
409 association patterns. Patterns of co-phylogeny decreased from regime G1 to G3, while the frequency  
410 of inferred host jump events increased from G1 to G3. Regime G2, which includes the highest  
411 proportion of broad host range parasites, showed a frequency of host jumps intermediate between  
412 G1 and G3. Our co-phylogeny analyses are consistent with the view that long-term plant-pathogen  
413 co-speciation is rare (deVienne et al., 2013). The decrease in host-pathogen co-phylogeny signal from  
414 G1 to G3 regime (**Figure 4**) could indicate more frequent true co-speciation events in early diverging  
415 *Sclerotiniaceae* species, or could result from host jumps being restricted to closely related hosts for  
416 G1 species whereas G2 and G3 species progressively gained the ability to jump to more divergent  
417 hosts (deVienne et al., 2007, deVienne et al., 2013). Low diversification rates, notably in regime G2,  
418 may have resulted from increased extinction rates. It is conceivable that a high extinction rate of  
419 specialist parasites during regime G2 is responsible for the reduced clade diversification rates, while  
420 increasing the apparent frequency of emergence of generalist species. This phenomenon, consistent  
421 with the view of specialization as “an evolutionary dead-end” (leading to a reduced capacity to  
422 diversify), is notably supported in *Tachinidae* parasitic flies and hawkmoths pollinating *Ruellia* plants  
423 (Day et al., 2016). An analysis of *Papilionoidea* and *Heliconii* revealed lower rates of diversification for  
424 butterfly species feeding on a broad range of plants compared to specialist butterflies (Hardy & Otto,  
425 2014, Day et al., 2016). Consistently, theoretical models of sympatric speciation predict that  
426 competition for a narrow range of resources (specialization) to be a strong driver of speciation  
427 (Dieckmann & Doebeli, 1999). Similar to plant-feeding insects, the diversity of fungal and oomycete  
428 pathogens is considered largely driven by host jumps rather than host specialization that may follow  
429 (Hardy & Otto, 2014, Choi & Thines, 2015). Low diversification rates in G2 may also result from a  
430 strong increase in diversification rate during the transition from regime G2 to G3. Our ancestral state  
431 reconstruction analysis inferred a jump to monocots at the base of regime G3 (**Figure 1**). A recent  
432 study on *Hesperiidae* butterflies reported a strong increase in diversification coincident with a switch  
433 from dicot-feeders to monocot-feeders (Sahoo et al., 2017). As postulated for *Hesperiidae*, the  
434 emergence of open grasslands and global temperature decrease may have affected diversification in  
435 the *Sclerotiniaceae*.

436 Competition for resources is likely to lead to speciation if host range expansion is costly  
437 (Ackermann et al., 2004). Notably, if different host families are scattered in space and  
438 phylogenetically related hosts are clustered, the cost of host range expansion is expected to increase,  
439 due to higher costs for dispersal or tradeoffs with other traits (Ackermann et al., 2004). In agreement  
440 with this theory, genomic signatures associated with metabolic cost optimization were stronger in  
441 generalist than specialist fungal parasites (Badet et al., 2017). Strong climate oscillations during the  
442 Cenozoic Era, leading to population isolation through range fragmentations and dispersal events,  
443 likely contributed to the radiation of plant lineages (Nyman et al., 2012). For instance, major events in  
444 the diversification of *Brassicaceae* plant family coincide with glaciations and arid conditions of the  
445 Eocene-Oligocene and the Oligocene-Miocene transitions (Hohmann et al., 2015). In addition, the  
446 mid-Miocene (20 to 10 Mya) corresponds to the emergence of the first open grassland habitats in  
447 northern Eurasia (Strömberg, 2011). The fragmentation and diversification of plant host populations  
448 may have increased the cost for host range expansion in fungal pathogens. A jump to monocots at  
449 the base of *Sclerotiniaceae* regime G3 ~10 Mya may have favored the conquest of grassland habitats  
450 and the rapid diversification of specialist species over the emergence of generalists in this group.

451 Similar to herbivore diet breadth (Forister et al., 2015), host range in the *Sclerotiniaceae*  
452 shows a continuous distribution from specialists to broad host range generalists, with the majority of  
453 species being specialists. Mathematical analyses and studies of the host range of insect herbivores  
454 suggest that host range expansion could involve the emergence of a “pre-adaptation” followed by the  
455 colonization of new hosts (Janz & Nylin, 2008). The existence of such pre-existing enabler traits has  
456 been proposed as a facilitator for several shifts in plant species distribution (Donoghue & Edwards,  
457 2014). Notably, the pre-existence of a symbiotic signaling pathway in algae is thought to have  
458 facilitated the association of land plants with symbiotic fungi (Delaux et al., 2015). In the plant genus  
459 *Hypericum*, increased diversification rates in the Miocene Epoch were likely facilitated by adaptation  
460 to colder climates (Nürk et al., 2015). In the bacterial pathogen *Serratia marcescens* and in yeast,  
461 adaptation to temperature change was associated with improved tolerance to other stresses (Ketola  
462 et al., 2013, Caspeta & Nielsen, 2015). Analysis of the complete predicted proteomes of *S. borealis*, *S.*  
463 *sclerotiorum* and *Botrytis cinerea* have revealed protein signatures often associated with cold  
464 adaptation in the secreted protein of all three species (Badet et al., 2015). Cold tolerance might have  
465 been a pre-adaptation that facilitated the emergence of generalist parasites under low diversification  
466 rates (regime G2) and a rapid diversification following host jumps on fragmented host populations  
467 (regime G3). Indeed, *Sclerotinia borealis*, *S. glacialis*, *S. subarctica* and *S. nivalis*, that diverged during

468 regime G2, are largely restricted to hemiboreal climates (circumboreal region) and can have a lower  
469 optimal growth temperature than their sister species (Saito, 1997, Hoshino et al., 2010).

470 These findings suggest that global climate instability and host diversification in the Cenozoic  
471 might have impacted on the diversity of fungal parasites within the *Sclerotiniaceae*. This effect could  
472 have been direct, through the emergence of cold adaptation as an enabling trait, or indirect through  
473 changes in host population structures and host-parasite association patterns. Knowledge on the  
474 dynamics of pathogen evolution increases the understanding of the complex interplay between host,  
475 pathogen and environment governing the dynamics of disease epidemics. These evolutionary  
476 principles are useful for the design of disease management strategies (Vander Wal et al., 2014) and  
477 provide new insights into the factors that influenced the diversity of extant fungal parasite species.

478

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484

#### 485 **Authors contributions:**

486 ON: collected data, performed analyses, writing original draft and revised manuscript

487 AB: performed analyses, writing revised manuscript

488 AT: collected data, revised manuscript

489 JPC: funding acquisition, collected data, revised manuscript

490 SR: Supervision, funding acquisition, project administration, writing original draft and revised  
491 manuscript

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672 **DATA ACCESSIBILITY**

- 673 - Species identifiers: Genbank accessions provided in Supplementary Table 1  
674 - DNA sequences and alignments : provided as supplementary file 2  
675 - Phylogenetic trees: provided as supplementary file 1, 3, 4 and 5

676

677 **FIGURE LEGENDS**

678 **Figure 1. Multiple independent shifts and expansions of host range in the evolution of the**  
679 *Sclerotiniaceae*. **(A)** Distribution of plant hosts of parasites from the *Sclerotiniaceae* and  
680 *Rutstroemiaceae* fungi. **(B)** Maximum likelihood *ITS* phylogeny of 105 *Sclerotiniaceae* and 56  
681 *Rutstroemiaceae* species showing host range information and ancestral host reconstruction. Host  
682 range is shown as circles at the tips of branches, sized according to the number of host families and  
683 colored as in (A) according to the earliest diverging plant group in host range. Numbers at the tips of  
684 branches refer to species listed in Supplementary Table 1. Branch support indicated in light red for  
685 major clades corresponds to SH-aLRT (regular), bootstrap (bold) and Bayesian posterior probabilities  
686 (italics). Reconstructed ancestral host is shown as triangles at intermediate nodes when a change  
687 compared to the previous node is predicted. Endophytes and biotrophic parasites are shown with  
688 empty circles. **(C)** Distribution of *Sclerotiniaceae* and *Rutstroemiaceae* species according to their  
689 number of host families.

690 **Figure 2. Two major diversification rate shifts in the evolution of the *Sclerotiniaceae*.** **(A)** Dated *ITS*-  
691 based species tree for the *Sclerotiniaceae* with diversification rate estimates. The divergence times  
692 correspond to the mean posterior estimate of their age in millions of years calculated with BEAST.  
693 Mean age is shown for selected nodes with bars showing 95% confidence interval of the highest  
694 posterior density (HPD). Branches of the tree are color-coded according to diversification rates  
695 determined with BAMM. Major rate shifts identified in BAMM are shown as red circles, noted  $s_1$ ,  $s'_1$ ,  
696  $s_2$  and  $s'_2$  and labeled with the posterior distribution in the 95% credible set of macro-evolutionary  
697 shift configurations. Species names are shown in black if host range includes less than 5 plant  
698 families, in yellow for 5 to 9 plant families and in red for 10 or more plant families. Diversification rate  
699 shifts define three macro-evolutionary regimes noted G1, G2 and G3 and boxed in blue, grey and  
700 brown respectively. **(B)** Distribution of broad host range (5 or more host families) parasites in  
701 *Rutstroemiaceae*, *Sclerotiniaceae* and under each macro-evolutionary regime of the *Sclerotiniaceae*.  
702 P-values calculated by random permutations of host ranges along the tree are indicated above bars.

703 Holo., Holocene; Mya, Million years ago; Plei., Pleistocene; Plio., Pliocene; Quat. Quaternary; Rut.,  
704 *Rutstroemiaceae*.

705 **Figure 3. Robustness of diversification rate shifts identification in the *Sclerotiniaceae* phylogeny. (A)**

706 Lineage-through-time plots for the *Sclerotiniaceae* tree and 1000 trees in which branching times were  
707 altered randomly by -15% to +15% to control for the sensitivity to divergence time estimates. Pybus  $\gamma$   
708 for the *Sclerotiniaceae* tree is provided. **(B)** Frequency (n) of diversification rate shift detection and  
709 diversification rate estimates (r) in a 100 MEDUSA bootstrap replicates in which sampling richness,  
710 tree completeness and divergence times were randomly altered. Labels indicate average  
711 diversification rate estimates (r) for each macro-evolutionary regime (blue for G1, grey for G2, brown  
712 for G3), with standard deviation of the mean for a 100 replicates. **(C)** Net diversification rates over  
713 time estimated by BAMM for each macro-evolutionary regime (blue for G1, grey for G2, brown for  
714 G3).

715

716 **Figure 4. Diversification rate shifts associate with variations in rates of co-speciation, duplication**

717 **and host switch in *Sclerotiniaceae* fungi. (A)** Tanglegram depicting the associations between 102  
718 *Sclerotiniaceae* species and 59 plant families. The three macro-evolutionary regimes are indicated by  
719 colored boxes on the *Sclerotiniaceae* tree. Fungal species labels are color-coded as in figure 2. **(B)**  
720 Proportion of co-speciation, sorting/loss, duplication and host switches in host-*Sclerotiniaceae*  
721 associations as predicted by CoRe-PA in 1,000 co-phylogeny reconstructions. \*\* indicate large effect  
722 size in a macro-evolutionary compared to the complete set of associations as assessed by Cohen's d  
723 test. **(C)** Proportion of host-*Sclerotiniaceae* associations contributing significantly and positively (likely  
724 co-speciation), non significantly and significantly and negatively (likely host switch) to co-phylogeny in  
725 PACo analysis. The black dotted line indicates the percentage of broad host range species (5 or more  
726 host families) in each group.

727 **SUPPLEMENTARY FILES**

728 **Supplementary Figure 1.** We analyzed the 7101 records from the fungus-host distribution database  
729 that served as a basis for our study on the *Sclerotiniaceae*. Host species were classified as crops  
730 according to the USDA Natural Resources Conservation Service database  
731 (<https://plants.usda.gov/npk/main>), other cultivated plants, ornamental plants and "others",  
732 including largely wild plant species. We found that 13% of the Fungus-Host database entries related  
733 to crops, 20% to cultivated plants and 9.5% to ornamentals (A). A total of 37% of the Fungus-Host

734 database entries were from the USA, 17% from Europe, leaving a total of 46% of the entries that were  
735 neither from the USA or Europe (B). These distributions do not reveal a strong bias towards crops or  
736 USA/Europe records in the records used for this analysis. (C) Relationship between fungal pathogen  
737 host range and the number of records in the database for each plant family. Dotted line shows linear  
738 regression of the data.

739 **Supplementary Figure 2.** Reconstruction of ancestral host range by the re-rooting method under an  
740 entity-relationship model and robustness of the results to tree pruning. (A) Posterior probability for  
741 the presence of Fabids in *Sclerotiniaceae* at each internal node (red), Fabids being the most likely  
742 ancestral host group of the *Sclerotiniaceae* family in S-DIVA and S-DEC analyses. (B) Sensitivity to tree  
743 pruning for the date of inclusion of plant groups into *Sclerotiniaceae* pathogen host range. The graph  
744 shows the % of variation for the earliest date of inclusion into *Sclerotiniaceae* pathogen host range  
745 upon pruning of 10% of the phylogenetic tree 100 times randomly. Error bars show 95% confidence  
746 intervals. (C) Posterior probability for the presence of *Rosaceae* (a family in the Fabids) in  
747 *Sclerotiniaceae* host groups at each internal node (red).

748 **Supplementary Figure 3.** Assessment of the robustness of diversification rate shifts predicted by  
749 MEDUSA to sampling richness (A-B), tree completeness (C-D) and dating accuracy (E-F). The figure  
750 shows the frequency of detection of a diversification rate shift at every internal node of the tree in  
751 100 bootstrap MEDUSA analyses (A, C and E), as well as the total number of shifts predicted in each  
752 of the 100 bootstrap analyses (B, D and F).

753 **Supplementary Figure 4.** Diversification rates analysis with R-PANDA. (A) Spectral density plot of the  
754 *Sclerotiniaceae*. (B) Eigenvalues ranked in descending order for the *Sclerotiniaceae*, the eigengap  
755 between values 8 and 9 is shown by an arrow and indicates eight modalities in this phylogeny. (C)  
756 Location of the eight modalities on the *Sclerotiniaceae* phylogeny, indicated by different colors. The  
757 major rate shifts identified by BAMM are shown as circles on the phylogeny and delimit the shift  
758 between modes 1/7/8 to modes 5/6/3 and finally modes 2/4.

759 **Supplementary figure 5.** RASP phylogram showing extant and reconstructed ancestral host range for  
760 161 *Sclerotiniaceae* and *Rutstroemiaceae* species. The ancestral reconstruction shows the  
761 probabilities associated with the optimal distribution at major nodes. It was generated using the S-  
762 DIVA method and the phylogenetic tree provided in supplementary file 2 as a template. The code for  
763 host groups is as follows: Vitales (A), Asterids (B), Campanuliids (C), Commelinids (D), coprophilous

764 (E), core Eudicots (F), Eudicots (G), Fabids (H), Polypodiidae (I), Lamiids (J), Magnoloidae (K), Malvids  
765 (L), Monocotyledones (M), Pinidae (N).

766 **Supplementary Figure 6.** Chronotree of the 105 *Sclerotiniaceae* species generated with BEAST.  
767 Estimated mean age of all intermediate nodes is indicated (in million years), with bars showing the  
768 95% confidence interval of the highest posterior density (HPD).

769 **Supplementary Figure 7.** Diversification rates analysis with BAMM and Medusa. (A) The eight most  
770 probable shift configurations in the 95% credible set of shift configurations predicted by BAMM with  
771 the posterior probability of each configuration indicated on top. (B) Density distribution of net  
772 diversification rates calculated by BAMM. The three peaks of density reflect three macro-evolutionary  
773 regimes in the *Sclerotiniaceae*. (C) Net diversification rates as a function of time calculated by BAMM  
774 through the complete *Sclerotiniaceae* phylogeny (105 species), showing a first a relatively sharp  
775 decrease (70 to 55 Mya), followed by a slow decrease (55 to 17 Mya) and a recent increase (17 Mya  
776 to present) in diversification rates. (D) Diversification rate shifts identified by MEDUSA.

777 **Supplementary File 1.** Phylogenetic tree of host plant families used for co-phylogenetic analyses in  
778 this work (newick format).

779 **Supplementary File 2.** Curated multiple ITS sequence alignment for 200 Leotiomycece species,  
780 including 105 *Sclerotiniaceae* and 56 *Rutstroemiaceae* species. This alignment includes 797  
781 informative sites and was used to generate the phylogenetic tree shown in Figure 1 and downstream  
782 analyses (fasta format).

783 **Supplementary File 3.** Phylogenetic tree of 200 Leotiomycece species including 161 *Sclerotiniaceae*  
784 and *Rutstroemiaceae* species used in Figure 1, obtained by maximum likelihood approach and  
785 featuring SH-aLRT branch support (newick format).

786 **Supplementary File 4.** Same phylogenetic tree as in supplementary file 3 including bootstrap from  
787 100 replicates as branch support (newick format).

788 **Supplementary File 5.** Time calibrated phylogenetic tree of the 105 *Sclerotiniaceae* species used in  
789 Figure 2.

790 **Supplementary File 6.** List of host-parasite associations tested for co-phylogenetic analyses.

791 **Supplementary Table 1.** List of *Sclerotiniaceae* and *Rutstroemiaceae* species used for phylogenetic  
792 analysis and their corresponding host range. 1 refers to position in the tree shown in Figure 1; 2 refers

793 to the code used in RASP analysis (Sup. Figure 5). NA, not applicable; Rutst., *Rutstroemiaceae*; Sclero.  
794 *Sclerotiniaceae*.

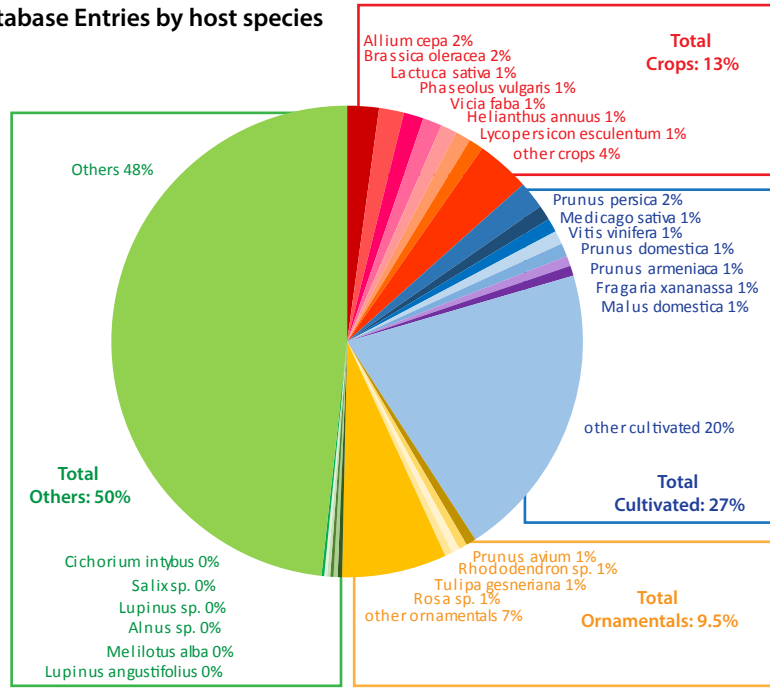
795 **TABLE 1. Results of the cophylogeny analyses under CoRe-PA optimized cost settings using various**  
 796 **methods and host association sets.** <sup>a</sup> refers to the set of host association tested for cophylogeny: ‘Full  
 797 set’ corresponds to the complete list of all plant-*Sclerotiniaceae* associations; ‘Simplified’ corresponds  
 798 to a reduced set covering the whole *Sclerotiniaceae* family; ‘G1’, ‘G2’,and ‘G3’ corresponds to  
 799 associations involving *Sclerotiniaceae* species from macro-evolutionary regime G1, G2 or G3 only. <sup>b</sup>  
 800 standard deviation correspond to frequencies calculated for 1000 reconstruction with randomized  
 801 host-parasite associations. <sup>c</sup> In PACo taxon jackknifing, associations that contributed significantly and  
 802 positively to cophylogeny were classified as ‘cospeciation’, significantly and negatively as ‘host switch’  
 803 and associations with no significant contribution to cophylogeny are classified as either Sorting/loss  
 804 or Duplication. <sup>d</sup> values correspond to CoRe-Pa total reconstruction costs, p-value of the observed  
 805 host-parasite association matrix  $m^2$  in 100,000 permutations with PACo, or the p-value of the  
 806 observed reconstruction cost in 100 random tip mappings in Jane 4. FD, failure to diverge.

Method	Host associations <sup>a</sup>	Event frequency (% of host associations)					Cost / p-val <sup>d</sup>
		Cospeciation	Sorting/Loss	Duplication	Host switch	FD	
<b>CoRe-PA<sup>b</sup></b>	<b>Full set (263)</b>	<b>15.82 ± 2.34</b>	<b>15.90 ± 4.80</b>	<b>34.35 ± 3.35</b>	<b>33.94 ± 3.49</b>		<b>38.21 ± 0.79</b>
	<b>Simplified (121)</b>	<b>9.63 ± 2.63</b>	<b>11.09 ± 4.97</b>	<b>40.20 ± 3.25</b>	<b>39.07 ± 4.00</b>		<b>40.44 ± 0.81</b>
	G1 only (68)	15.75 ± 3.19	16.32 ± 7.35	37.78 ± 4.67	30.14 ± 5.23		13.95 ± 0.44
	G2 only (130)	17.03 ± 3.57	16.21 ± 7.58	33.16 ± 5.27	33.60 ± 5.63		14.57 ± 0.47
	G3 only (65)	11.78 ± 5.17	14.38 ± 8.69	32.86 ± 6.75	40.98 ± 6.87		8.95 ± 0.34
<b>PACo<sup>c</sup></b>	<b>Full set (263)</b>	<b>20.91</b>	<b>48.29</b>	<b>30.79</b>			<b>p&lt;1.0<sup>-05</sup></b>
	<b>Simplified (121)</b>	<b>15.70</b>	<b>57.02</b>	<b>27.27</b>			<b>p&lt;1.0<sup>-05</sup></b>
	G1 only (68)	27.94	55.88	16.18			p=2.0 <sup>-05</sup>
	G2 only (130)	6.15	71.54	22.31			p= 0.00045
	G3 only (65)	0.00	66.15	33.85			p= 0.00607
<b>Jane 4</b>	<b>Full set (263)</b>	<b>0.86</b>	<b>67.73</b>	<b>8.00</b>	<b>3.57</b>	<b>19.83</b>	<b>1291 (p&lt;0.01)</b>
	<b>Simplified (121)</b>	<b>2.90</b>	<b>65.22</b>	<b>14.78</b>	<b>11.59</b>	<b>5.51</b>	<b>719.47 (p&lt;0.01)</b>
	G1 simple (43)	1.39	41.67	25.00	26.39	5.56	206.98 (p<0.01)
	G2 simple (54)	2.28	75.80	11.87	3.65	6.39	374.08 (p<0.01)
	G3 simple (24)	9.68	25.81	19.35	41.94	3.23	105.41 (p=0.13)

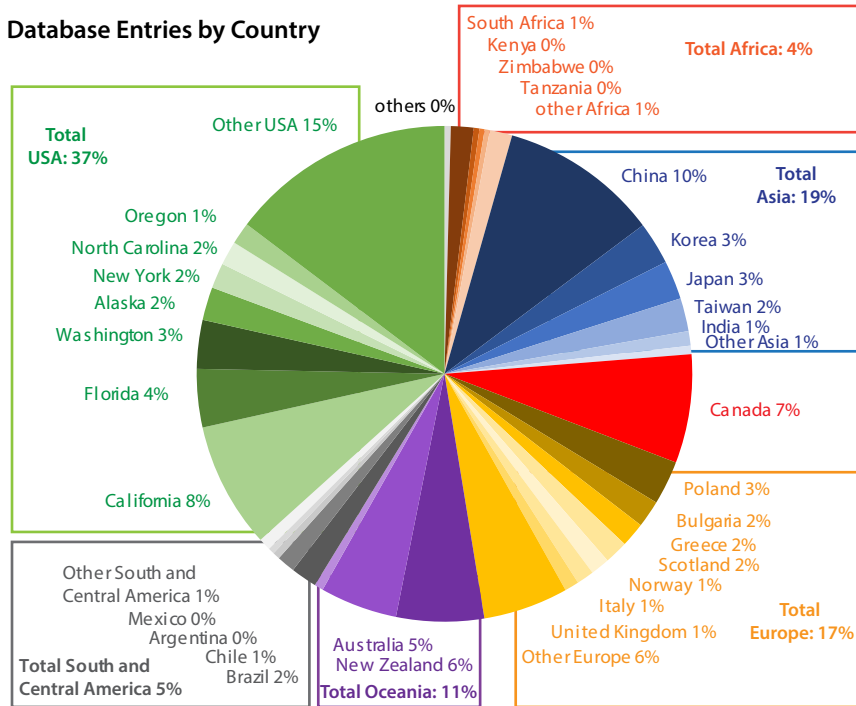
807



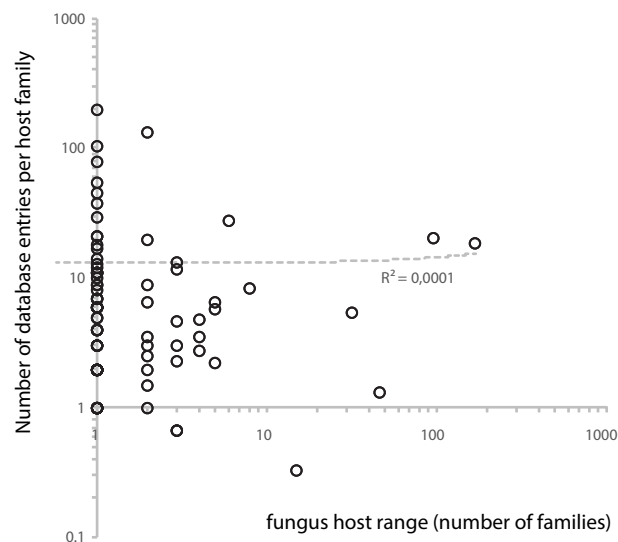
### A Database Entries by host species

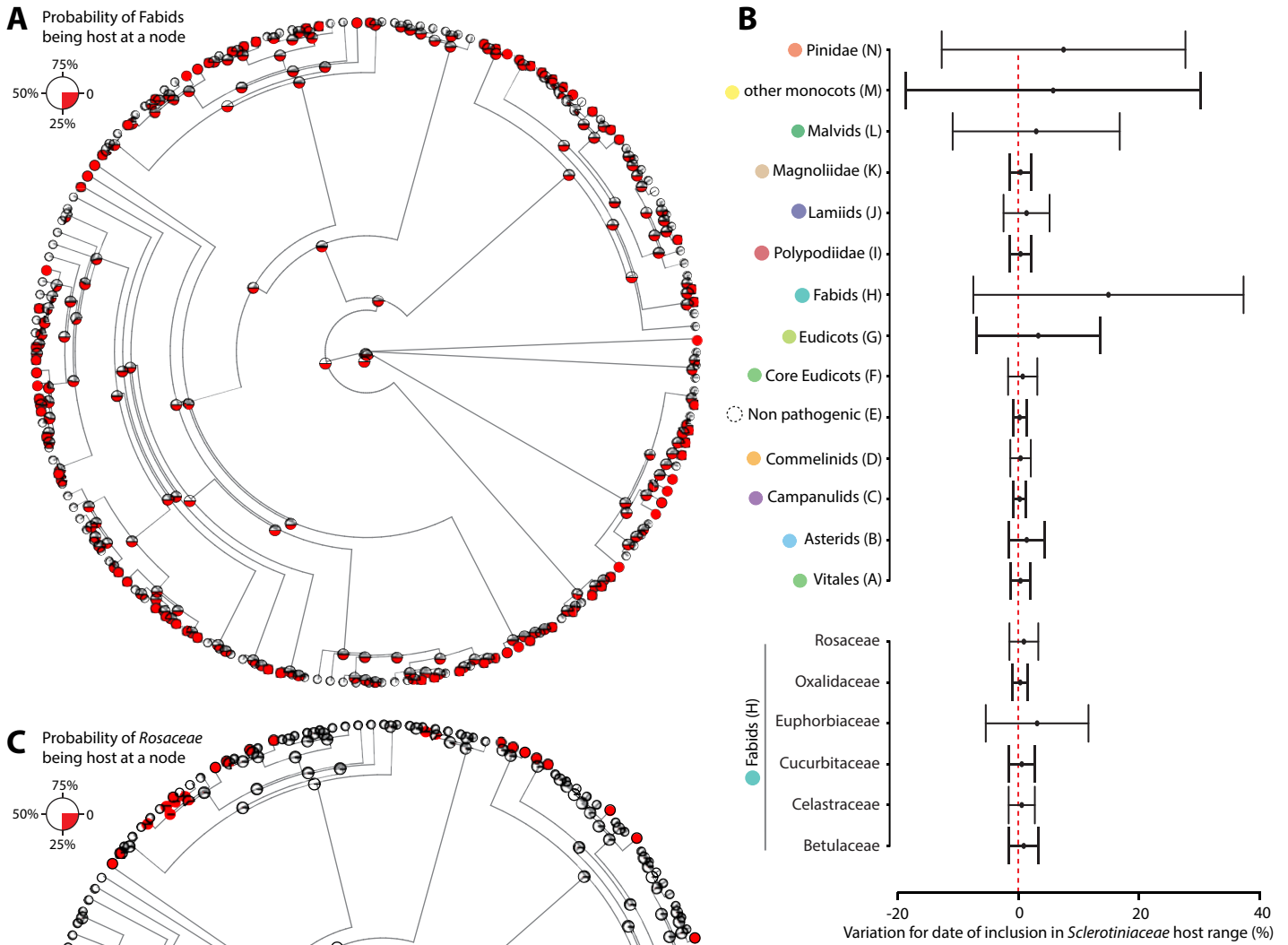


### B Database Entries by Country

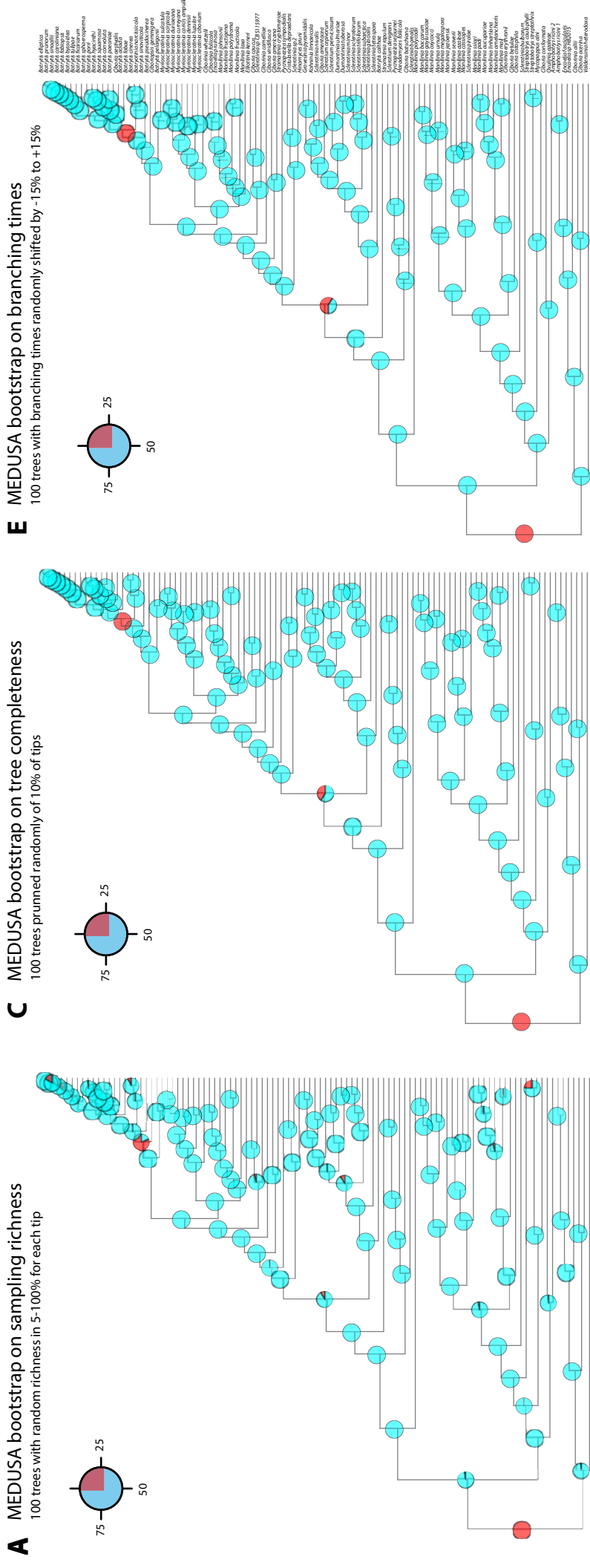


### C

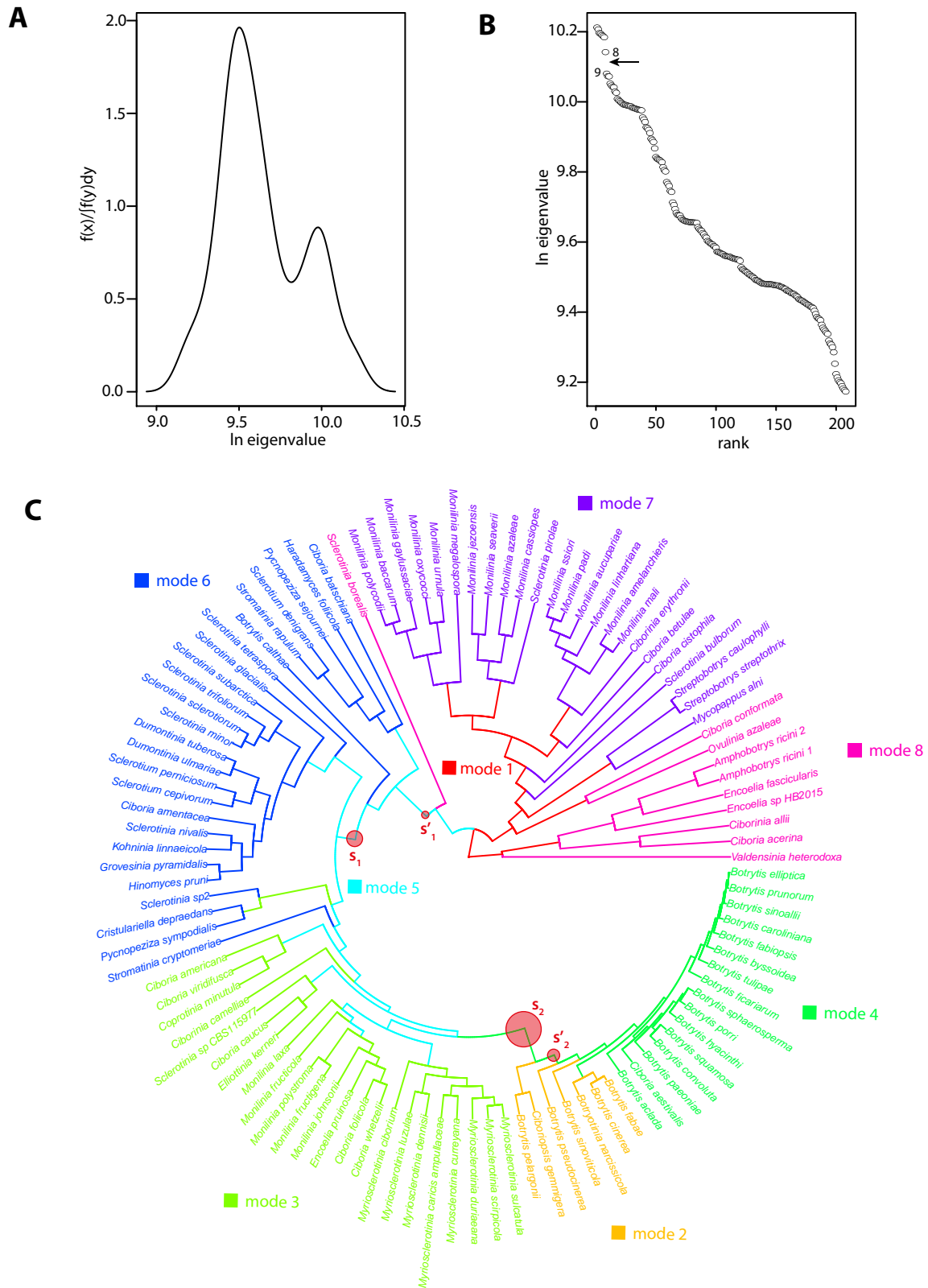




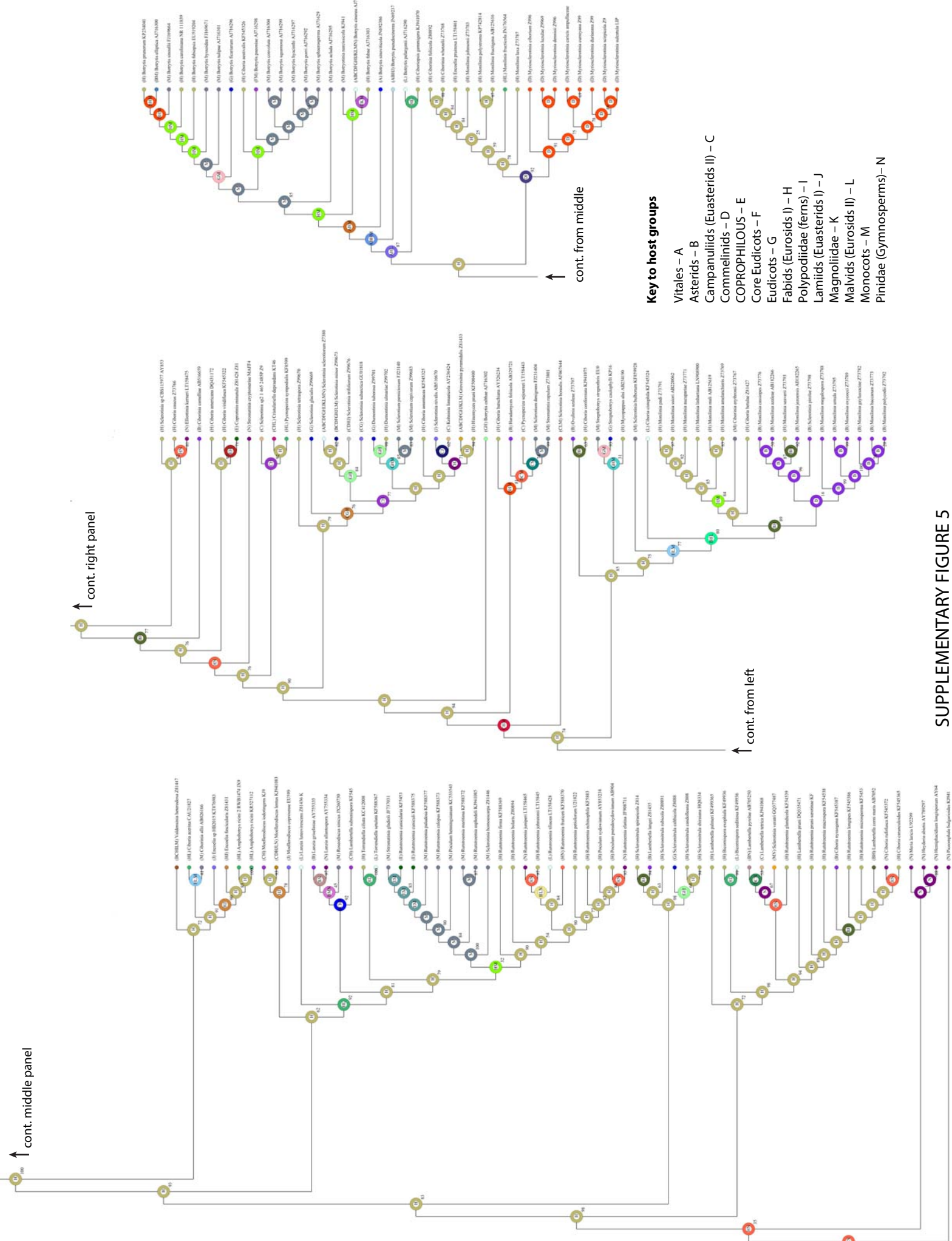
SUPPLEMENTARY FIGURE 2



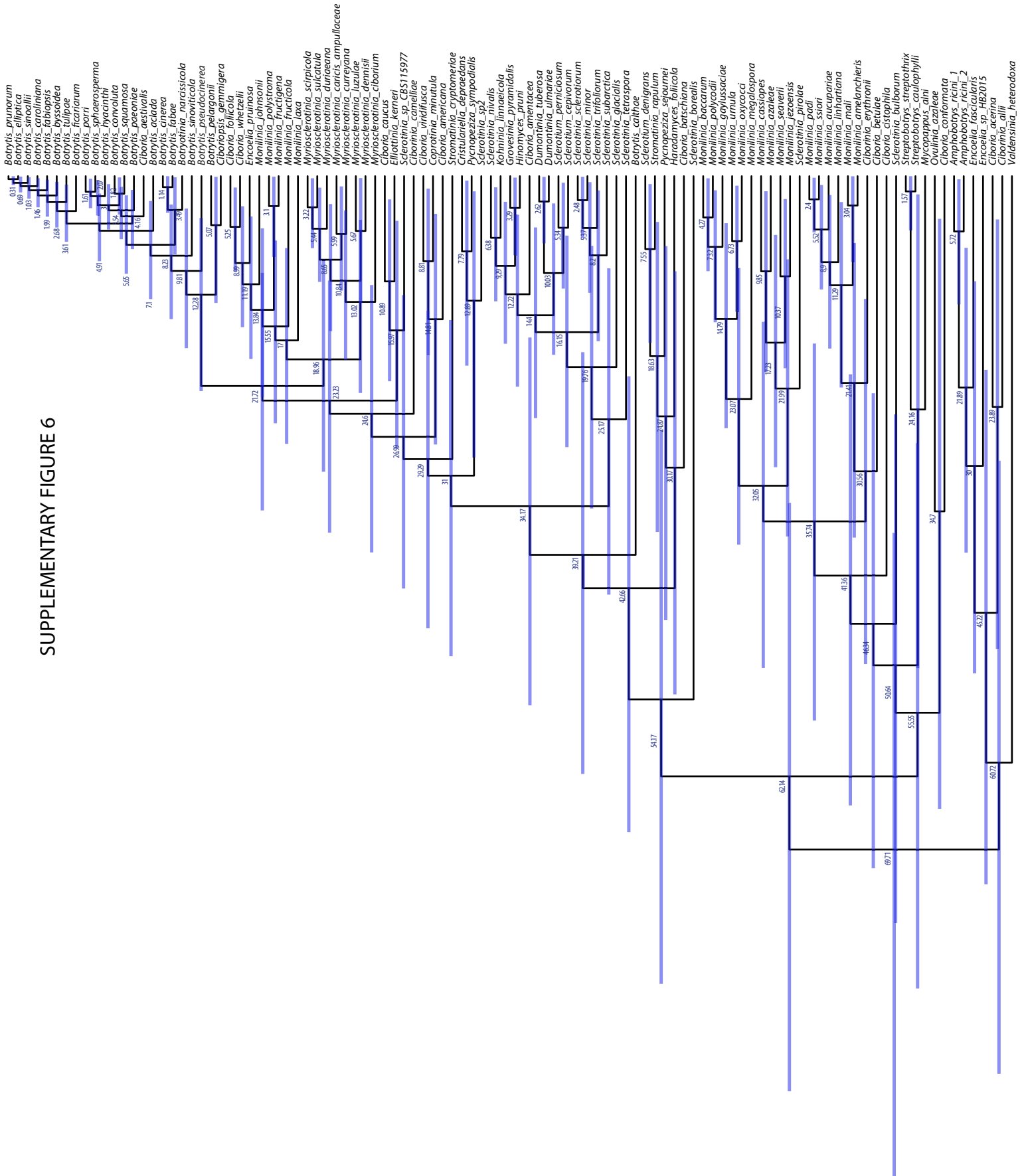
SUPPLEMENTARY FIGURE 3

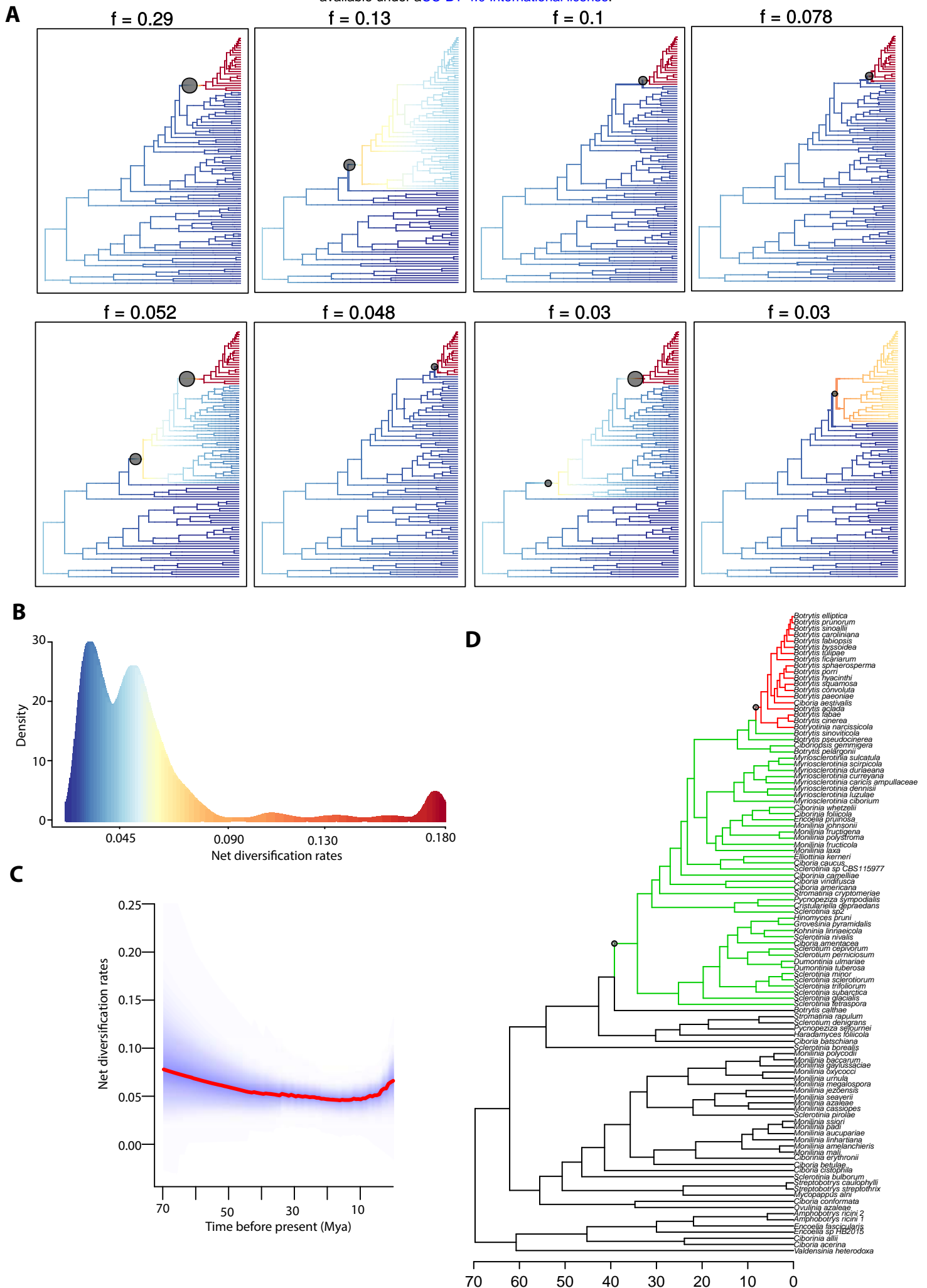


SUPPLEMENTARY FIGURE 4



SUPPLEMENTARY FIGURE 6





SUPPLEMENTARY FIGURE 7