Do closely related species interact with similar partners? Testing for phylogenetic signal in bipartite interaction networks

Running title: Measuring phylogenetic signal in interactions

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Supplementary data: https://github.com/BPerezLamarque/Phylosignal_network/blob/master/Supplementary_figures_phylo_signal_network.pdf

Abstract

Whether interactions between species are conserved on evolutionary time-scales has spurred the development of both correlative and process-based approaches for testing phylogenetic signal in interspecific interactions: do closely related species interact with similar partners? Here we use simulations to test the statistical performances of the two approaches that are the most widely used in the field: Mantel tests and the Phylogenetic Bipartite Linear Model (PBLM). Mantel tests investigate the correlation between phylogenetic distances and dissimilarities in sets of interacting partners, while PBLM is a process-based approach that relies on strong assumptions on how interactions evolve. We find that PBLM often detects phylogenetic signal when it should not. Simple Mantel tests instead have infrequent false positives and moderate statistical power; however, they often artifactually detect that closely related species interact with dissimilar partners. Partial Mantel tests, which are used to partial out the phylogenetic signal in the number of partners, actually fail at correcting for this confounding effect, and we instead recommend to evaluate the significance of Mantel tests with network permutations constraining the number of partners. We also explore the ability of simple Mantel tests to analyze clade-specific phylogenetic signal. We provide general guidelines and an application on an interaction network between orchids and mycorrhizal fungi.

Keywords: ecological network, phylogenetic signal, Mantel tests, clade-specific signal, species interactions, mycorrhizal symbiosis.

Introduction

Species in ecological communities engage in numerous types of interspecific interactions, such as pollination, mycorrhizal symbioses, herbivory, and parasitism (Bascompte et al. 2003; Fontaine et al. 2011; Martos et al. 2012; Bascompte and Jordano 2013), which are often summarized using bipartite interaction networks (Bascompte & Jordano 2013; Fig. 1). Understanding the processes that shape these interaction networks, including the role of evolutionary history, is a major focus of ecology and evolution (Rezende et al. 2007; Futuyma and Agrawal 2009; Vázquez et al. 2009; Gómez et al. 2010; Krasnov et al. 2012; Elias et al. 2013; Rohr and Bascompte 2014; Braga et al. 2021). One way to assess the role of evolutionary history in shaping contemporary interactions is to test for phylogenetic signal in species interactions, *i.e.* whether closely related species interact with similar sets of partners (Peralta 2016).

Testing for phylogenetic signal in a trait for a given clade, *i.e.* whether a trait is phylogenetically conserved, is mainstream (Felsenstein 1985; Blomberg et al. 2003; Münkemüller et al. 2012). One approach (the 'correlative' approach) is to perform a Mantel test between phylogenetic and trait distances (Mantel 1967); another approach (the 'process-based' approach) relies on trait evolution models such as Pagel's λ (Pagel 1999) or Blomberg's K (Blomberg et al. 2003). The process-based approach has a higher ability to detect an existing phylogenetic signal (power) and a lower propensity to infer a phylogenetic signal when it should not (false positive; Harmon & Glor 2010): The correlative approach should therefore only be used when the process-based approach is not applicable, *e.g.* if the 'trait' data is expressed in terms of pairwise distances.

Testing for phylogenetic signal in species interactions falls in the category of cases where the 'trait' data are pairwise distances, here the between-species dissimilarity in sets of interacting species. Simple Mantel tests have therefore been widely used in this context (*e.g.* Cattin *et al.* 2004; Rezende *et al.* 2007; Elias *et al.* 2013;

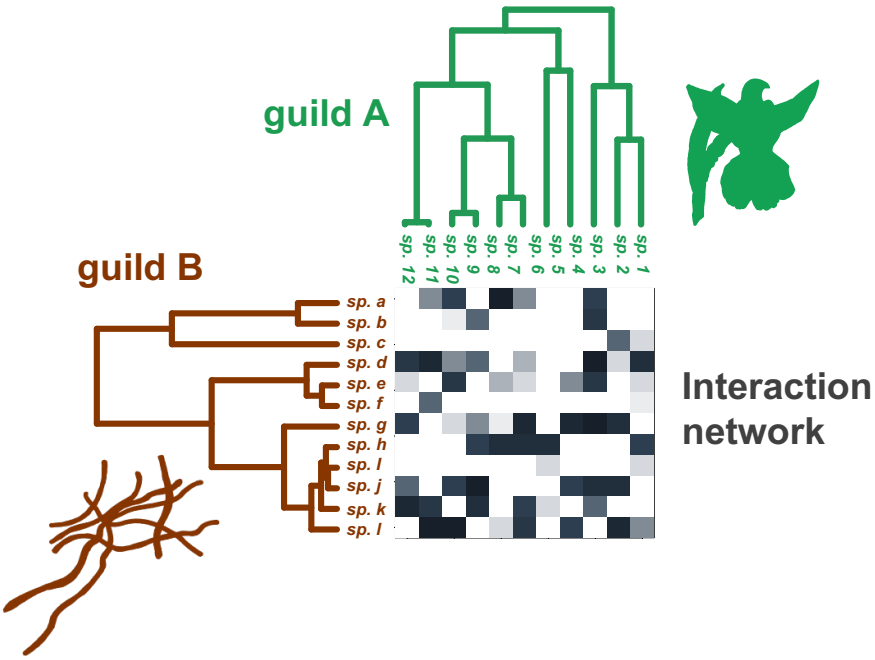
Fontaine & Thébault 2015). Partial Mantel tests have also been used to test whether the phylogenetic signal reflects more the identity of the interacting partners than their number, as similarity in the number of partners can increase the value of similarity metrics ("phylogenetic signal in the number of partners"; Rezende *et al.* 2007; Jacquemyn *et al.* 2011; Aizen *et al.* 2016). Mantel tests, that are easy and fast to run and that do not rely on strong hypotheses, have therefore been vastly used to test for phylogenetic signal in species interactions in empirical networks (Cattin et al. 2004; Rezende et al. 2007; Jacquemyn et al. 2011; Elias et al. 2013; Fontaine and Thébault 2015). Besides these correlative approaches, several process-based approaches have been developed (Ives and Godfray 2006; Rafferty and Ives 2013; Hadfield et al. 2014; Li et al. 2020). The first of these approaches, the Phylogenetic Bipartite Linear Model (PBLM, Ives & Godfray 2006) has been widely used to test for phylogenetic signal in species interactions in a variety of networks, *e.g.* in host-parasite, plant-fungus, and pollination networks (Ives and Godfray 2006; Martos et al. 2012; Martín González et al. 2015; Xing et al. 2020). In short, PBLM assumes that interaction strengths between species from the two guilds are determined by (unobserved) traits that evolve on the two phylogenies each following a simplified Ornstein-Uhlenbeck process (Blomberg et al. 2003). PBLM performs a phylogenetic regression to infer the Ornstein-Uhlenbeck parameters, which are then interpreted in terms of phylogenetic signal (Ives & Godfray 2006). Other models have been developed more recently (Rafferty and Ives 2013; Hadfield et al. 2014; Li et al. 2020), including the phylogenetic generalized linear mixed model (PGLMM; Rafferty and Ives 2013) that uses linear mixed models to infer phylogenetic signals in both the number of partners and species interactions. Yet, the higher computational requirements of these methods have prevented their widespread use on empirical networks. PBLM thus remains the method frequently used in empirical studies (*e.g.* Xing et al. 2020; Corro et al. 2021).

Mantel tests and PBLM sometimes provide contradictory conclusions on empirical data and this is difficult to interpret because the statistical performances of

the two approaches have never been compared (Peralta 2016). Importantly, the statistical performances of PBLM have not been tested. Here, we use simulations to perform a comparative analysis of the statistical performances of these approaches. We consider both weighted and unweighted bipartite interaction networks between species from two guilds A and B (Fig. 1). Our results lead us to propose alternative approaches for measuring phylogenetic signal in interaction networks. We also investigate the ability of Mantel tests to detect the presence of phylogenetic signal in the different clades of a phylogenetic tree, as phylogenetic signal may be localized. Finally, we provide general guidelines and illustrate them on an orchid-fungus mycorrhizal network identified across the oceanic island of Réunion (Martos et al. 2012).

Figure 1: Illustration of the data used to test for phylogenetic signal in species interactions

Toy example of an interaction network between orchids (in green) and mycorrhizal fungi (in brown) with associated phylogenetic trees. The bipartite interaction network between two guilds A (here the orchids) and B (the fungi) is represented by a matrix which elements indicate either whether or not species interact (*i.e.* 1 if they do and 0 otherwise, 'unweighted' or 'binary' network) or the frequency of the interaction ('weighted' network; for example here we indicated the number of times a given pairwise interaction has been observed using shades of gray from white (no interaction) to dark gray (many interactions)). Each guild is also characterized by a rooted phylogenetic tree, used to compute phylogenetic distances between pairs of species.



Methods

Simulating bipartite interaction networks with or without phylogenetic signal in species interactions

We used *BipartiteEvol*, an individual-based eco-evolutionary model (Figure 2; see Maliet *et al.* 2020 for a complete description of the model), to generate interaction networks with or without phylogenetic signal between two guilds interacting in a mutualistic, antagonistic, or neutral way. In short, each individual from guild A (resp. B) is characterized by a multidimensional continuous trait and interacts with one individual from guild B (resp. A). The effect of this interaction on the fitness of each individual from guilds A or B is determined by the distance in trait space of the two interacting individuals, according to a classical trait matching expression parametrized by two parameters α_A and α_B (Supplementary Methods 1, Maliet *et al.* 2020). These parameters determine the nature and specificity of the interaction: positive α_A and α_B correspond to mutualistic interactions, negative α_A and positive α_B to antagonistic interactions (with guild A representing hosts/preys and guild B parasites/predators), high $|\alpha|$ values to scenarios with strong fitness effects (*i.e.* highly specialized interactions), and $|\alpha|$ values close to 0 to more neutral scenarios (Figure 2). *BipartiteEvol* simulates the random death of individuals that are replaced by new ones proportionally to their fitness. At birth, new individuals have a probability μ to mutate, leading to new trait values slightly different from the parental ones (Figure 2). Such mutations can lead to the formation of new species. Networks simulated using *BipartiteEvol* show typical structural properties observed in empirical networks, including significant nestedness and/or modularity according to the sets of simulated parameters (Maliet et al. 2020). For instance, networks simulated with antagonistic interactions ($\alpha_A<0$) tend to be significantly modular, while networks simulated with neutral or mutualistic interactions ($\alpha_A=0$ or $\alpha_A<0$) tend to be nested. Here, instead of

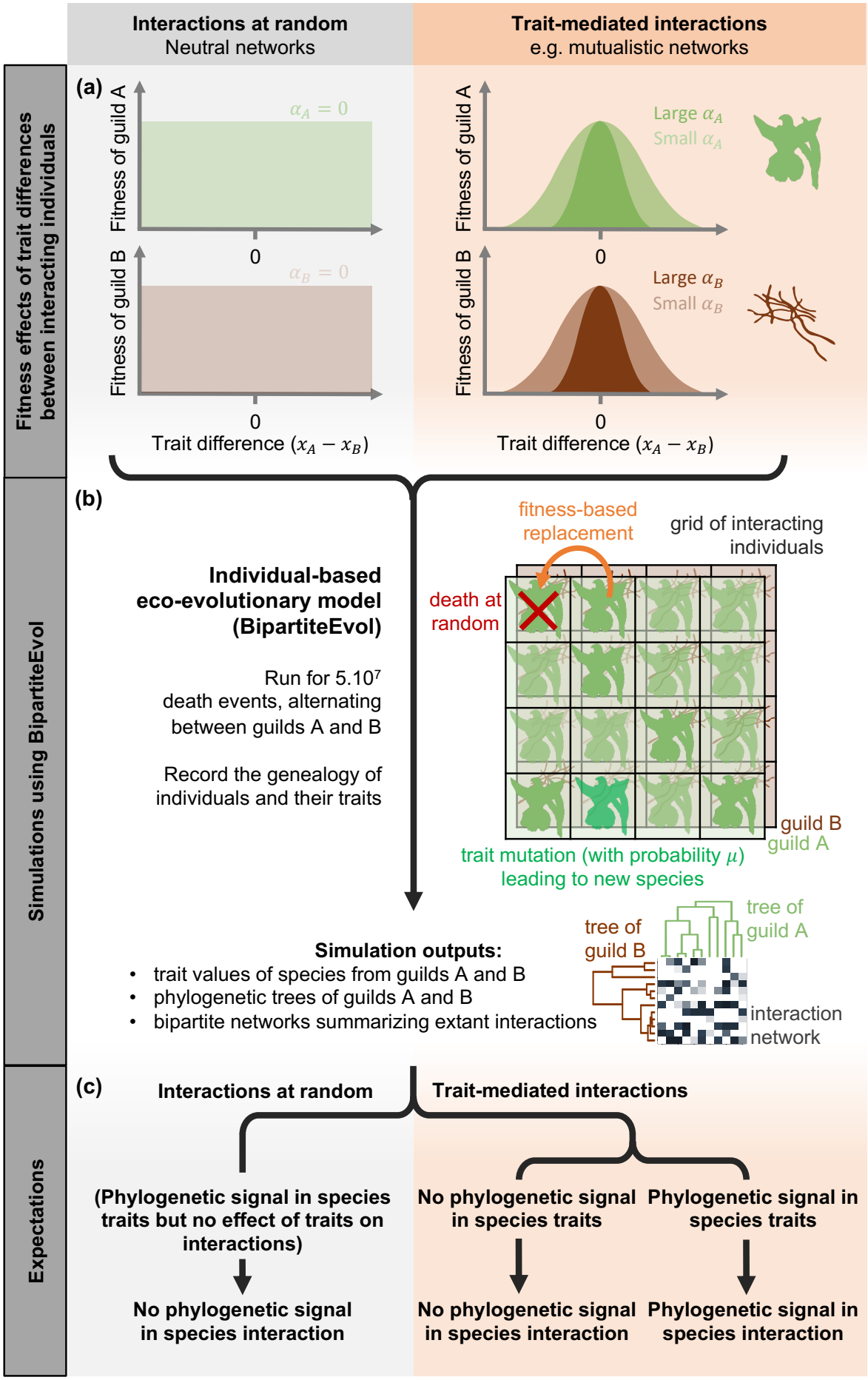
using the species delineation of the original *BipartiteEvol* model (Maliet et al. 2020), we considered that each combination of traits corresponds to a distinct species. This increased our ability to generate phylogenetic signal in the simulated networks, and we show that is does not affect their overall structure (see below).

Figure 2: Simulation scheme used to generate interaction networks with or without phylogenetic signal in species interactions:

(a) The fitness of a given individual is either affected by its trait value and that of the individual it interacts with (right; "mutualistic or antagonistic interactions") or not (left; "neutral interactions"). In the first case, fitness depends on the trait matching between the pair of interacting individuals ($x_A - x_B$), where x_A (resp. x_B) are the trait values of the individual from guild A (resp. B). The strength of the effect of the traits on fitness is modulated by the parameters α_A and α_B ($|\alpha|$ values close to 0 tends toward neutrality, where interactions happen at random).

(b) *BipartiteEvol* assumes that pairs of individuals from guilds A and B interact on a grid of a given size. Each cell of the grid contains one pair of individuals. The process starts with one monomorphic species in each guild. At each time step, one individual of guild A is killed at random and replaced by another individual proportionally to its fitness in the cell. The new individual can mutate with probability μ, in which case the new trait value is drawn from a normal distribution centered on the parental trait. A mutation generates a new species. The same events are applied to an individual from guild B, and the process is repeated a large number of time steps. This simulation model outputs a list of species of guilds A and B with associated trait values, phylogenetic trees, and the bipartite interaction network of extant species.

(c) A phylogenetic signal in species interactions can occur only when trait values modulate interactions (for large α) and there is a phylogenetic signal in trait values. When interactions are completely or quasi random ($\alpha = 0$ or low α), there cannot be a phylogenetic signal in species interactions.


Interactions at random
Neutral networks
Trait-mediated interactions
e.g. mutualistic networks
Fitness effects of trait differences between interacting individuals
(a)
Fitness of guild A
$\alpha_A = 0$
0
Fitness of guild B
$\alpha_B = 0$
0
Trait difference $(x_A - x_B)$
Fitness of guild A
Large α_A
Small α_A
0
Fitness of guild B
Large α_B
Small α_B
0
Trait difference $(x_A - x_B)$
Simulations using BipartiteEvol
(b)
Individual-based eco-evolutionary model (BipartiteEvol)
Run for 5.10^7 death events, alternating between guilds A and B
Record the genealogy of individuals and their traits
fitness-based replacement
grid of interacting individuals
death at random
guild B
guild A
trait mutation (with probability μ) leading to new species
Simulation outputs:
• trait values of species from guilds A and B
• phylogenetic trees of guilds A and B
• bipartite networks summarizing extant interactions
tree of guild B
tree of guild A
interaction network
Expectations
(c)
Interactions at random
Trait-mediated interactions
(Phylogenetic signal in species traits but no effect of traits on interactions)
No phylogenetic signal in species interaction
No phylogenetic signal in species traits
No phylogenetic signal in species interaction
Phylogenetic signal in species traits
Phylogenetic signal in species interaction

185

186 Under the *BipartiteEvol* model, closely related species tend to interact with
187 similar sets of partners, *i.e.* there is a phylogenetic signal in species interactions, if and
188 only if: (1) closely related species have similar traits, *i.e.* there is a phylogenetic signal
189 in species traits, and (2) these traits determine who interacts with whom, *i.e.* $\alpha \neq 0$
190 (Figure 2). Similarly, a negative phylogenetic signal in species interactions, *i.e.* the
191 tendency for closely related species to associate with dissimilar partners, is expected if
192 there is a negative phylogenetic signal in species traits, *i.e.* closely related species have
193 dissimilar traits, and $\alpha \neq 0$.

194 We used the *sim.BipartiteEvol* function from the R-package RPANDA (Morlon
195 et al. 2016; R Core Team 2022) to simulate a total of 2,400 interaction networks. To
196 obtain a wide range of network sizes, we considered a total number of 500, 1,000, 2,000,
197 3,000, 4,000, or 5,000 pairs of interacting individuals per simulation. For each size, we
198 simulated the evolution of 100 neutral networks (α_A=0 ; α_B=0), 120 mutualistic
199 networks (**i**: α_A=1; α_B=1; **ii**: α_A=0.1; α_B=0.1; **iii**: α_A=0.01; α_B=0.01; **iv**: α_A=1; α_B=0.1; **v**: α_A=1;
200 α_B=0.01; and **vi**: α_A=0.1; α_B=0.01) and 180 antagonistic networks (**i**: α_A=-1; α_B=1; **ii**: α_A=-
201 0.1; α_B=0.1; **iii**: α_A=-0.01; α_B=0.01; **iv**: α_A=-1; α_B=0.1; **v**: α_A=-1; α_B=0.01; **vi**: α_A=-0.1; α_B=1;
202 **vii**: α_A=-0.1; α_B=0.01; **viii**: α_A=-0.01; α_B=1; **ix**: α_A=-0.01; α_B=0.1). Each individual was
203 characterized by a six-dimensional trait, and trait mutation occurred at birth with
204 probability μ=0.01. Upon mutation, the new trait values were drawn independently in
205 a normal distribution centered on the parental traits and with a variance of 1. We
206 followed the interacting individuals during 5.10^7 death events. At the end, we
207 extracted for each guild a species tree from its genealogy by randomly selecting one
208 individual per species (Fig. S1), we also recorded the number of individuals belonging
209 to each species, and counted the number of occurrences of each interspecific
210 interaction; we then reconstructed the corresponding weighted interaction network.

211 First, we evaluated whether these simulations generated realistic networks by
212 comparing their structure with that of empirical networks. Empirical networks were
213 gathered from the Web of Life database (web-of-life.es (Fortuna et al. 2014)) and the
214 database of Michalska-Smith & Allesina (2019). We compared the structures of

simulated *versus* empirical networks in terms of connectance, nestedness, and modularity (Supplementary Methods 2).

Second, we separated the 2,400 simulated networks between those for which we should expect a phylogenetic signal in species interactions and those for which we should not (Figure 2). We did not expect phylogenetic signal in species interactions in neutral networks and in non-neutral networks with no phylogenetic signal in species traits. Conversely, we expected phylogenetic signal in non-neutral networks with phylogenetic signal in species traits. We evaluated phylogenetic signal in species traits using two approaches. First, for simplicity and consistency with the rest of the paper, we used Mantel tests (Pearson correlation) between phylogenetic distances and trait distances computed as the Euclidian distances between trait values for each species pair. Second, given that process-based approaches usually perform better (Harmon and Glor 2010), we used a multivariate extension of Pagel's λ (Pagel 1999) implemented in R (Goolsby 2015); we assessed the significance of the phylogenetic signal in species traits with likelihood ratio tests comparing the inferred Pagel's λ model to a null model where λ=0 (*i.e.* no phylogenetic signal).

Computing phylogenetic signal in species interactions

We computed phylogenetic signal in species interactions in the simulated networks using Mantel tests and PBLM, as well as the computationally-intensive PGLMM for the smallest networks. Complete descriptions of these methods are available in Supplementary Methods 3. Mantel tests, PBLM, and PGLMM rely on different strategies to evaluate the significance of the phylogenetic signal, and it could be argued that results of these tests are not directly comparable. Our approach is to follow the methodologies traditionally used in empirical studies and compare their conclusions (detection or not of a phylogenetic signal).

243 Mantel tests: We evaluated the phylogenetic signal in species interactions in guilds A
244 and B separately using simple Mantel tests between phylogenetic and ecological (set
245 of interacting partners) distances. Ecological distances were measured both without
246 accounting for evolutionary relatedness of the interacting partners, using (weighted or
247 unweighted) Jaccard, and accounting for relatedness using (weighted or unweighted)
248 UniFrac distances (Supplementary Methods 3 (Lozupone et al. 2011)). Accounting for
249 evolutionary relatedness of the interacting partners can be particularly relevant for
250 organisms with uncertain species delineations (e.g. microorganisms delineated using
251 only molecular data (Martos et al. 2012; Sanders et al. 2014)). We used Pearson,
252 Spearman, and Kendall correlations (R) by extending the *mantel* function in the R-
253 package ecodist (Goslee and Urban 2007); the significance of each correlation was
254 evaluated using 10,000 permutations, except for the computationally intensive Kendall
255 correlation (100 permutations only). For each network, we considered that there was
256 a significant positive phylogenetic signal (resp. negative phylogenetic signal) if the
257 correlation coefficient (R) was higher (resp. lower) than >95% of the randomized
258 correlations; we computed the p-value of each one-tailed Mantel test as the fraction of
259 the randomized correlations above (resp. below) the original value.
260
261 PBLM: To estimate phylogenetic signal based on PBLM, we modified the function *pblm*
262 from the R-package picante (Kembel et al. 2010) to more efficiently perform matrix
263 inversions and handle large interaction networks. In short, the parameters d_A and d_B
264 of the Ornstein-Uhlenbeck processes of PBLM were estimated using generalized least
265 squares (Ives & Godfray 2006). d_A and d_B are interpreted as a measure of phylogenetic
266 signal in species interactions: if d=0, there is no effect of the phylogeny (similar as
267 evolution on a star phylogeny, *i.e.* no phylogenetic signal); 0<d<1 generates stabilizing
268 selection (*i.e.* phylogenetic signal) and d>1 disruptive selection (*i.e.* negative
269 phylogenetic signal). We followed Ives & Godfray (2006; Supplementary Methods 3)
270 by considering that the phylogenetic signal is significant when the mean square error
271 (MSE) of the model is smaller than that obtained using star phylogenies (MSE_{star}); we

also used a more stringent criterion by considering that the signal is significant when the MSE is at least 5% lower than MSE_{star}. Finally, we applied the bootstrapping method of Ives & Godfray (2006; Supplementary Methods 3) to the smallest networks. A single PBLM inference can take several days to run (time measured on an Intel 2.8 GHz MacOSX laptop) on networks of intermediate sizes (*e.g.* between 50 and 100 species per guild), which prevented us from applying the bootstrap approach to large networks; we therefore only tested this approach on networks simulated with 500 individuals (*i.e.* a total of 400 networks).

PGLMM: We performed analyses of the statistical performances of PGLMM (Rafferty and Ives 2013) using the function *pglmm* in the R-package phyr (Li et al. 2020). Following the procedure used in Lajoie and Kembel (2021), we fitted for each network different models accounting or not for phylogenetic signals in both the number of partners and in the species interactions in both clades, using restricted maximum likelihood and evaluating significance with likelihood ratio tests. Because fitting these models can require large amount of memory (*e.g.* >80 Gb for some networks with >50 species per guild) and long computation time (Fig. S2), we only tested this approach on networks simulated with 500 individuals. We fitted the PGLMM using either a Gaussian or a Poisson distribution of abundances for weighted networks, and a binomial distribution (presence/absence data) for unweighted networks (Li et al. 2020).

Confounding effect of the phylogenetic signal in the number of partners

To test the performances of the partial Mantel test at measuring phylogenetic signal in species interactions while controlling for the number of partners (Supplementary Methods 3), we first performed partial Mantel tests between phylogenetic and ecological distances, while controlling for pairwise differences in the number of partners, on the networks simulated with *BipartiteEvol*. There is no reason that *BipartiteEvol* simulations generate a phylogenetic signal in the number of partners,

301 and we verified this by performing Mantel tests between phylogenetic distances and
302 pairwise differences in the number of partners. These analyses thus assess whether
303 partial Mantel tests lose power compared to simple Mantel tests in the absence of
304 phylogenetic signal in the number of partners. If they do not suffer power loss, partial
305 Mantel tests applied to *BipartiteEvol* simulations should be significant when simple
306 Mantel tests are significant.
307
308 Second, we assessed whether partial Mantel tests successfully correct for
309 phylogenetic signal in the number of partners using networks simulated under a
310 process that generate phylogenetic conservatism in the number, but not the identity,
311 of interacting partners (*i.e.* partial Mantel tests should not be significant when applied
312 to such networks). To simulate network with only phylogenetic conservatism in the
313 number of partners in guild A, we first simulated phylogenetic trees for guilds A and
314 B using *pbtree* (R-package phytools; Revell 2012) with a number of species uniformly
315 sampled between 40 and 150 by guild. Next, we simulated the number of partners of
316 the species from guild A using an Ornstein-Uhlenbeck process with an attraction
317 toward 0, a variance of 0.1 (noise of the Brownian motion), and a selection strength (a_A)
318 ranging from 5 (strong stabilizing effect, weak phylogenetic signal) to 0 (Brownian
319 motion, strong phylogenetic signal). We computed the number of partners per species
320 by calibrating the simulated values between 1 and the number of species in guild B
321 and taking the integer part. For each a_A value (5, 1, 0.5, 0.05, or 0), we performed 100
322 simulations using *mvSIM* (R-package mvMORPH; Clavel *et al.* 2015). Finally, for each
323 species in A, we attributed the corresponding number of partners in B at random to
324 obtain binary networks. We checked that our simulations indeed generated a signal in
325 the number of partners by performing simple Mantel tests between phylogenetic
326 distances and pairwise differences in the number of partners. Finally, we performed
327 on each simulated network a partial Mantel test between phylogenetic and ecological
328 distances, while controlling for pairwise differences in number of partners.
329

330 Given the poor performances of partial Mantel tests (see Results), we tested
331 three alternative approaches to partial out the confounding effect of the number of
332 partners in measures of phylogenetic signals. First, we tested whether using sequential
333 Mantel tests would provide a good alternative: based on simple Mantel tests, we
334 consider that there is a phylogenetic signal in the identity of the partners if there is a
335 phylogenetic signal in species interactions and no phylogenetic signal in the number
336 of partners. Second, we tested the use of methods that directly partition ecological
337 distance metrics into a part due to the dissimilarity in the number of partners and a
338 part due to the dissimilarity in the identity of the partners, *i.e.* "species turnover"
339 (Baselga 2010). We used the betapart R-package (Baselga and Orme 2012) to extract the
340 part of the unweighted Jaccard distances due to species turnover and tested its
341 correlation with phylogenetic distances using a simple Mantel test. Third, we designed
342 specific network permutations to test for the significance of the Mantel correlation
343 between phylogenetic distances and ecological distances while accounting for the
344 number of partners. To measure whether the phylogenetic signal observed in guild A
345 is not due to a phylogenetic signal in the number of partners, instead of shuffling the
346 distance matrix as in a regular Mantel test (Supplementary Methods 3), we
347 randomized the interaction network by keeping constant the number of partners per
348 species from guild A while permuting the partner identities. Because this third
349 approach requires recomputing the ecological distances for each permutation, it is
350 much slower than regular Mantel tests (Fig. S2) and we thus used only 1,000
351 permutations. We applied these three methods to all our simulated networks.
352
353 **Effect of phylogenetic uncertainty, sampling asymmetry, and network**
354 **heterogeneity on measures of phylogenetic signal in species interactions**
355
356 Unlike simulations, such as those provided by *BipartiteEvol*, empirical bipartite
357 networks suffer from (i) uncertainty in the phylogenetic reconstructions, *e.g.* in the
358 microbial partners' tree when studying host-associated microbiota, which often

359 prevents accounting for evolutionary relatedness (*i.e.* using UniFrac distances), (ii)
360 sampling asymmetry, *i.e.* one side of the network is more thoroughly sampled than
361 the other, and (iii) network heterogeneity, *i.e.* different sub-clades in the network have
362 different levels of phylogenetic signal. We performed additional analyses to
363 investigate the effect of these aspects on phylogenetic signals in species interactions
364 measured using simple Mantel tests.
365
366 First, we tested the effect of phylogenetic uncertainty in the partners' tree on the
367 measure of phylogenetic signal when evolutionary relatedness is accounted for (*i.e.*
368 using UniFrac distances). We performed these analyses to assess whether accounting
369 for the partners' evolutionary relatedness remains advantageous (see Results) when
370 phylogenetic uncertainty is high. To add some variability in the phylogenetic tree of
371 guild B (resp. A) used to compute the UniFrac distances between species pairs from
372 guild A (resp. B), we first simulated, on the original partners tree, the evolution of a
373 short DNA sequence and then reconstructed the tree from the simulated DNA
374 alignment using neighbor-joining (*nj* function, R-package APE (Paradis et al. 2004)).
375 We used *simulate_alignment* (R-package HOME; Perez-Lamarque & Morlon 2019) to
376 simulate sequences of length 75, 150, 300, 600, or 1,200 base-pairs, with 30% of variable
377 sites, and a substitution rate of 1.5 (shorter fragments should result in noisier
378 phylogenies). To assess the uncertainty of these reconstructed phylogenetic trees
379 compared with the original trees, we computed the correlations between the pairwise
380 phylogenetic distances in both trees.
381
382 Second, we tested the influence of sampling asymmetry on measures of
383 phylogenetic signal. Empirical networks are often an incomplete representation of the
384 actual interactions between two guilds because they are under-sampled, and
385 frequently, in an asymmetrical way. For instance, by sampling targeted species from
386 guild A, observed networks are constituted by few species from guild A which have
387 the complete set of their partners and by often more species from guild B which have

388 an incomplete set of their partners (as they likely interact with unsampled species from
389 guild A). We tested the influence of such sampling asymmetry by selecting only 10%
390 of the most abundant species from guild A in each simulated network (while retaining
391 at least 10 species) and computed phylogenetic signal in these asymmetrically-
392 subsampled networks.
393
394 Third, both Mantel tests and PBLM neglect the heterogeneity within networks.
395 Indeed, a non-significant phylogenetic signal at the level of the entire network can
396 potentially hide a sub-clade of species presenting significant phylogenetic signal.
397 Alternatively, a phylogenetic signal in the entire network may be driven by only two
398 sub-clades of guilds A and B, while the other sub-clades present no significant
399 phylogenetic signal. To explore the potential heterogeneity of the phylogenetic signal
400 within one guild, one possibility is to apply Mantel tests to the sub-networks formed
401 by a given sub-clade (*e.g.* Song *et al.* 2020). For each node of the tree of guild A having
402 at least 10 descendants, we estimated the clade-specific phylogenetic signal using a
403 Mantel test investigating whether closely related species from this sub-clade of A tend
404 to interact with similar partners (and *vice-versa* for guild B). Using UniFrac distances,
405 we performed the Mantel tests with 100,000 permutations, and introduced a
406 Bonferroni correction for multiple testing to keep a global risk of false positives of 5%.
407 To test this approach, we simulated networks with known sub-clade signal by
408 artificially combining networks simulated under neutrality with networks simulated
409 with the mutualistic parameters **v** (see Results). We grafted each "mutualistic"
410 phylogenetic tree from guilds A and B within a "neutral" phylogenetic tree by
411 randomly selecting a branch, such that it creates a separate module with strong
412 phylogenetic signal. Such simulations could correspond to the evolution of a different
413 niche, *e.g.* terrestrial *versus* epiphytic plants associating with different mycorrhizal
414 fungi (Martos et al. 2012). We then performed our clade-specific analysis of
415 phylogenetic signal and investigated in which nodes we recovered a significant
416 phylogenetic signal.

417

418 **General guidelines and illustration with application on the orchid-fungus**
419 **mycorrhizal network from La Réunion**

420

421 We used our results and other empirical considerations to provide general
422 guidelines for testing for phylogenetic signal in interaction networks. We illustrated
423 these guidelines by applying them in a network between orchids and mycorrhizal
424 fungi from La Réunion island (Martos et al. 2012). This network encompasses 70 orchid
425 species (either terrestrial or epiphytic species) and 93 molecularly-identified fungal
426 partners (defined according to 97% sequence similarity; Martos *et al.* 2012). We
427 gathered the maximum-likelihood plant and fungal phylogenies on TreeBASE (Study
428 Accession S12721), calibrated the orchid phylogeny using a relaxed clock with *chronos*
429 (Paradis 2013), and set the divergence between Orchidoideae and Epidendroideae at
430 65 million years (Givnish et al. 2015). To obtain a species-level orchid phylogeny,
431 missing species were grafted in the phylogeny by arbitrarily adding 10 million-year-
432 old polytomies in the corresponding unresolved genera, namely *Habenaria*, *Benthamia*,
433 *Cynorkis*, *Phaius*, *Liparis*, *Bulbophyllum*, and *Polystachya*.

434 # Results

435

436 **Expected phylogenetic signal in species interactions in *BipartiteEvol* networks**

437

438 *BipartiteEvol* simulations resulted in interaction networks with a large range of
439 sizes for guilds A and B, from less than 50 to more than 250 species (Fig. S3). These
440 simulated networks had similar structural properties as empirical networks, including
441 in terms of connectance, nestedness, and modularity (Fig. S4). This means that
442 networks simulated using *BipartiteEvol* are realistic and cover a large range of
443 structures encountered in natural interaction networks.

444 Using Mantel tests, we found a significant phylogenetic signal in species traits
445 for most antagonistic and neutral simulations (Fig. S5A). In contrast, for many
446 mutualistic simulations, closely related species often did not tend to have similar traits,
447 except when α_B=0.01 (*i.e.* mutualistic parameters **iii**, **v**, and **vi**; Fig. S5A). When α_B were
448 higher (*i.e.* mutualistic parameters **i**, **ii**, and **iv)**, we suspect stabilizing selection to
449 occur and erase the phylogenetic signal in the traits (Maliet et al. 2020): we therefore
450 do not expect phylogenetic signal in species interactions for these simulations, which
451 represent ~40% of the mutualistic simulations. In addition, we found a negative
452 phylogenetic signal in species traits (suggesting that closely related species have
453 dissimilar traits) in less than 1% of the simulations (Fig. S5A). Given that we do not
454 expect *BipartiteEvol* to generate a negative phylogenetic signal in species traits and
455 given that the risk of false positives of a Mantel test is 5%, these 1% of networks with
456 a negative phylogenetic signal in species traits are likely false-positives. We removed
457 them when evaluating the performance of the different approaches and we therefore
458 do not expect negative phylogenetic signal in species interactions for the networks we
459 tested, *i.e.* closely related species should not tend to associate with dissimilar partners.
460 Results were similar with Pagel's λ, with a significant phylogenetic signal in species
461 traits for almost all antagonistic and neutral simulations, and in ~65% of the

462 mutualistic simulations (Fig. S5B). Mantel tests and Pagel's λ lead to identical
463 conclusions for >95% of the simulated networks.
464
465 **Computing phylogenetic signal in species interactions in *BipartiteEvol* networks**
466
467 Using Mantel tests, as expected, we did not find a significant phylogenetic
468 signal in species interactions for most neutral networks or for networks with no signal
469 in species traits (Figs. 3 & S6): the false positive rate was below 5%, corresponding to
470 the risk of false positives of the test (Table S1), with one notable exception for small
471 networks when using weighted Jaccard distances and Pearson correlations (~8% of
472 false positives). Conversely, we detected a significant unexpected negative
473 phylogenetic signal in more than 10% of the simulated networks, in particular in the
474 small ones (Figs. 3 & S6).
475
476 Many mutualistic or antagonistic networks where we expected a phylogenetic
477 signal in species interactions (*i.e.* non-neutral networks with signal in species traits)
478 presented no significant signal with Mantel tests (Figs. 3 & S6), in particular those
479 simulated with low α_A and α_B values (*e.g.* antagonism **vii**), where non-neutral effects
480 were weak. Mantel tests measuring phylogenetic signal in species interactions were
481 most often not significant unless the phylogenetic signal in species traits was strong
482 (R>0.6; Fig. S7). Even when the phylogenetic signal in species traits was very strong
483 (R>0.9), the phylogenetic signal in species interactions was not significant in many
484 networks. In mutualistic networks, phylogenetic signals in species interactions were
485 present only when there was a large asymmetry in the effects of trait matching on the
486 fitnesses of the species from guilds A or B (case **v**: α_A=1; α_B=0.01), *i.e.* when only one
487 guild was specialized. Conversely, in antagonistic networks, phylogenetic signals
488 were found mainly when trait matching had a strong impact on the fitness of guild B
489 (the obligate parasites/predators; $\alpha_B \geq 0.1$). Additionally, when phylogenetic signal was

significant in one guild, it was generally also significant in the other; in antagonistic networks, the signal was usually higher in guild A compared to guild B (Fig. S6).

Figure 3: Statistical performances of the simple Mantel tests and the Phylogenetic bipartite linear model (PBLM; Ives & Godfray, 2006)

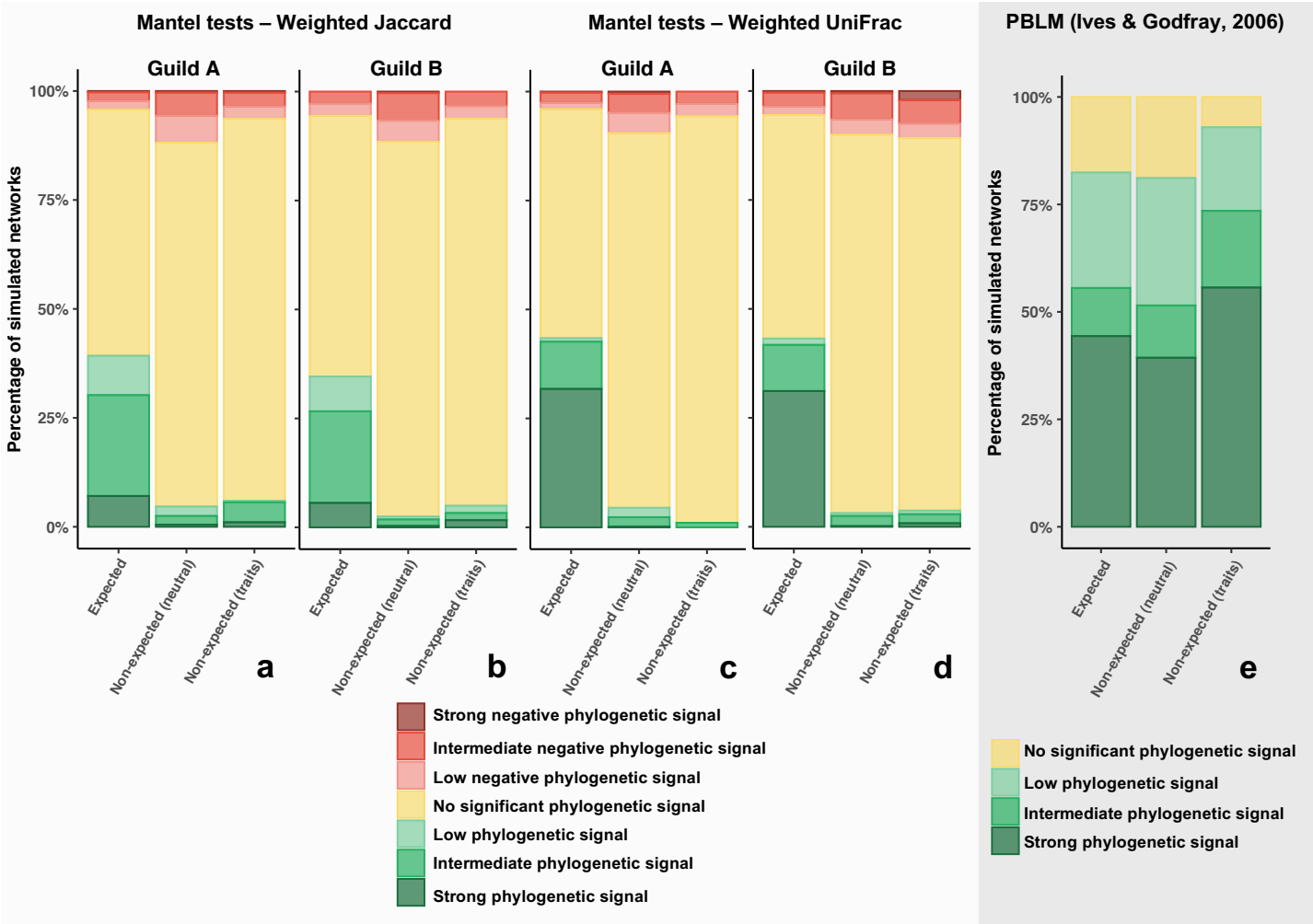
For each panel, the simulations are divided between networks where phylogenetic signal in species interactions is expected (*i.e.* networks (i) simulated with an effect of the traits on individual fitness - antagonistic and mutualistic simulations - and (ii) presenting traits that are phylogenetically conserved according to a Mantel test – see Fig. S5A) and networks where phylogenetic signal in species interactions is not expected (*i.e.* neutral simulations ($\alpha = 0$) or simulated networks where we observed no phylogenetic signal in the traits). Results are similar when the expectations are based on Pagel's λ to measure the phylogenetic signals in species traits (Fig. S11).

a-d: Phylogenetic signals in species interactions estimated using simple Mantel tests with Pearson correlation (R) in the guilds A (a, c) and B (b, d). The different panels in rows correspond to the 2 tested ecological distances: weighted Jaccard (a, b) or weighted UniFrac (c, d) distances. One-tailed Mantel tests between phylogenetic distances and ecological distances were performed using 10,000 permutations. In each panel, the bars indicate the percentage of simulated networks that present a significant positive correlation (in green; p-value>0.05 for the test of phylogenetic signal), a significant negative correlation (in red; p-value>0.05 for the test of negative phylogenetic signal), or no significant correlation (in yellow; both p-values>0.05). Significant phylogenetic signals (resp. significant negative phylogenetic signals) are shaded from light green (resp. red) to dark green (resp. red) according to the strength of the signal: we arbitrarily considered a "low signal" when R<0.05 (resp. R>-0.05), an "intermediate signal" when 0.05<R<0.15 (resp. -0.05>R>-0.15), and a "strong signal" when R>0.15 (resp. R<-0.15).

e: Phylogenetic signals estimated using PBLM. For a given combination of parameters, the bar indicates the percentage of simulated networks that present no significant (in yellow; $MSE \geq MSE_{star}$) or a significant (green; $MSE < MSE_{star}$) phylogenetic signal. Phylogenetic signals are shaded from light green to dark green according to the strength of the signal: we arbitrarily considered a "low signal" when $d_A < 0.05$ and $d_B < 0.05$, an "intermediate signal" when $d_A > 0.05$ or $d_B > 0.05$, and a "strong signal" when $d_A > 0.15$ or $d_B > 0.15$. PBLM were run on the weighted networks.

In each panel, the first bar indicates the statistical power of the test, whereas the second and third bars indicate the false positive rate of the test. Note that the strength the phylogenetic signals (based on the R and d values) are not directly comparable.

Results discriminating the simulated networks of different sizes and with different sets of parameters are available in Figures S6 & S8.



535 The statistical power of Mantel tests measuring phylogenetic signal in species
536 interactions seems to be modulated by network size, as phylogenetic signals were less
537 often significant but generally stronger in smaller networks (Fig. S6). Moreover,
538 Mantel tests based on Pearson correlations had higher power than Spearman and
539 Kendall correlations (Fig. S6) and weighted UniFrac distances performed better than
540 other ecological distances in terms of power in the context of these simulations (Fig.
541 S6; Table S2).
542
543 When using mean square errors to evaluate the significance of PBLM, we found
544 a significant phylogenetic signal in species interactions in most of the simulated
545 networks including when we did not expect any (Fig. 3e). The strength and the
546 significance of the inferred phylogenetic signals were independent of the strength of
547 the phylogenetic signal in species traits (Fig. S7). The propensity of PBLM to detect
548 phylogenetic signal decreased in large unweighted networks, but the false positives
549 remained >30%, including when using a more stringent significance cutoff (Figs. S8).
550 Similar results were obtained when bootstrapping to evaluate the significance (Fig.
551 S9). PGLMM on weighted networks with a Gaussian or Poisson distribution had
552 slightly lower but still frequent false positives (>25% or 20%, respectively) and
553 intermediate statistical power (<50%) when measuring phylogenetic signals in species
554 interactions (Fig. S10). PGLMM also often artifactually detected phylogenetic signals
555 in the number of partners (Fig. S10). Conversely, PGLMM on unweighted networks
556 never detected any significant signal (Fig. S10).
557
558 We inferred similar statistical performances of both Mantel tests and PBLM
559 when we used Pagel's λ to evaluate phylogenetic signal in species traits (Figs. S7 and
560 S11).
561

562 **Confounding effect of the phylogenetic signal in the number of partners**

563

564 As expected, tests of phylogenetic signal in the number of partners were non-
565 significant in the large majority of the *BipartiteEvol* networks, especially the larger ones
566 (Fig. S12). We did however observe significant correlations between ecological
567 distances and differences in the number of partners (Fig. S13). Partial Mantel tests
568 testing for phylogenetic signal in species interactions while accounting for
569 phylogenetic signal in the number of partners had similar false positive rates and
570 power as simple Mantel tests (Figs. S6 & S14; Table S2). Sequential Mantel tests
571 decreased the statistical power by less than 2% (Table S2). Partitioning the ecological
572 distances before running the Mantel test reduced the power by only 5% and resulted
573 in less than 1.5% of false positives, but also resulted in an artefactual detection of
574 negative phylogenetic signal in 9% of the simulations (Table S2; Fig. S15). Finally,
575 Mantel tests with network permutations keeping the number of partners constant
576 increased the statistical power by ~5% (Table S2), but resulted in an artefactual
577 detection of (positive or negative) phylogenetic signal in ~10% of the simulations when
578 using Jaccard distances (Fig. S16).

579

580 Networks simulated with phylogenetic conservatism in the number, but not the
581 identity of partners covered a realistic range of sizes (Fig. S17). As expected, Mantel
582 tests revealed significant phylogenetic signals in the number of partners in >60% of
583 these networks, with an increasing percentage of significant tests with decreasing a_A
584 (*i.e.* increasing conservatism in the number of partners; Fig. S18). We found significant
585 correlations between differences in the number of partners and ecological distances in
586 most of these simulated networks (Fig. S19), generating a confounding effect. As a
587 result, simple Mantel tests testing for phylogenetic signal in species interactions
588 without accounting for phylogenetic signal in the number of partners were frequently
589 significant (>30%; Fig. S20; Table S3). Partial Mantel tests controlling for differences in
590 the number of partners slightly decreased the proportion of false-positives, but it

591 remained very high (>25% of false positives; Fig. S21). In addition, partial Mantel tests
592 detected a spurious significant negative phylogenetic signal in species interactions in
593 >15% of the networks (Fig. S21). Conversely, only few networks with a significant
594 simple Mantel test in species interactions did not produce a significant simple Mantel
595 test in the number of partners, such that sequential Mantel tests had only ~7% of false
596 positives (Table S3). Partitioning the ecological distances before running the Mantel
597 test (Fig. S22) or using Mantel tests with network permutations keeping the number of
598 partners constant (Fig. S23) had even lower false positive rates (~4% and ~5%
599 respectively; Table S3). However, partitioning the ecological distances led to an
600 artefactual detection of negative phylogenetic signals in more than 30% of the
601 simulated networks (Fig. S23).
602
603 **Effect of phylogenetic uncertainty, sampling asymmetry, and network**
604 **heterogeneity on measures of phylogenetic signal in species interactions**
605
606 As expected, phylogenetic uncertainty in the partner's tree increased when the
607 length of the simulated DNA sequence used for phylogenetic reconstruction decreased
608 (Fig. S24), resulting in a decreased statistical power of Mantel tests using UniFrac
609 distances (Fig. S25). However, even when the simulated DNA sequences were the
610 shortest (75 base pairs), resulting in very noisy reconstructed partners' trees (Fig. S26),
611 the statistical power of the Mantel tests using UniFrac distances decreased by less than
612 5% (Fig. S25).
613
614 Our results on the statistical performance of tests of phylogenetic signal were
615 similar when considering sampling asymmetry (Figs. S27-30): PBLM spuriously
616 detected phylogenetic signal when it should not, and Mantel tests had decent
617 statistical performances, especially when using weighted UniFrac distances. In
618 addition, the correlations of the Mantel tests in guild A were generally higher when
619 significant (Fig. S29).

620

621 Our clade-specific tests of phylogenetic signal using Mantel tests while
622 correcting for multiple testing recovered a significant phylogenetic signal in 82% of the
623 nodes where mutualism originated (Fig. S31), as well as in most of the ascending
624 nodes. Conversely, we did not find spurious phylogenetic signals in nodes with only
625 neutrally-evolving lineages (Fig. S31).

626

627 **General guidelines and illustration with application on the orchid-fungus**
628 **mycorrhizal network from La Réunion**

629

630 Figure 4 provides general guidelines based on our results and empirical
631 considerations for accurate tests of phylogenetic signal in interaction networks. We
632 applied these guidelines on the orchid-fungus mycorrhizal network from La Réunion
633 (available in Martos et al. (2012)). First (step 1), simple Mantel tests of phylogenetic
634 signal in species interactions for fungi and orchids revealed a significant but low
635 phylogenetic signal (R<0.10) on the orchid side using Jaccard distances; however, the
636 significance disappeared with UniFrac distances (Table S4). Similarly, marginally not-
637 significant and low phylogenetic signals were detected in the mycorrhizal fungi side
638 (R<0.04; Table S4). Next (step 2), results were qualitatively similar when using Mantel
639 tests with permutations keeping the number of partners constant, suggesting that the
640 phylogenetic signal in species interaction did not result from a phylogenetic signal in
641 the number of partners. Our investigation of clade-specific phylogenetic signals in
642 species interactions in orchids (option 1) revealed a significant phylogenetic signal in
643 Angraecinae, a sub-tribe composed of 34 epiphytic species (Mantel test: R=0.37;
644 Bonferroni-corrected p-value=0.016; Fig. 5) interacting with 53 fungi, suggesting that
645 closely related Angraecinae tend to interact with more similar mycorrhizal fungi.
646 When we checked the robustness of the significant phylogenetic signal detected in
647 Angraecinae (option 2) by subsampling the Angraecinae clade down to 10 species, we
648 still recovered a significant signal in species interactions in both cases (Fig. S32).

Figure 4: Recommended guidelines to measure phylogenetic signal in species interactions within bipartite ecological networks.

This guideline is composed of two fixed steps followed by two optional ones and can be applied as soon as a bipartite interaction network (with or without abundances) and at least the phylogenetic tree of guild A are available. The phylogenetic tree does not need to be binary, rooted, or ultrametric. For each step, an example of the corresponding function available in the R-package RPANDA is indicated in grey.

Step 1: The first step consists in testing for phylogenetic signal in species interactions for guild A (*i.e.* whether closely related species from guild A tend to interact with similar partners from guild B) using a one-tailed simple Mantel test. This step requires to pick an ecological distance (*e.g.* UniFrac or Jaccard distances) and a type of correlation (Pearson correlation by default).

Step 2: Next, to assess whether a phylogenetic signal in species interactions really comes from the identity of species interactions (and not from a phylogenetic signal in the number of partners), the second step consists in testing whether the phylogenetic signal in guild A remains significant when the significance of the Mantel correlation is evaluated using network permutations keeping the number of partners constant.

Option 1: Clade-specific phylogenetic signal in guild A can be tested using simple Mantel tests while correcting for multiple testing (*e.g.* Bonferroni correction). It can be used to test whether some clades present different intensities of phylogenetic signal (*e.g.* because of higher specificity).

Option 2: The robustness of the findings can be tested by looking at how the conclusions might be affected by phylogenetic uncertainty (*e.g.* using a Bayesian posterior of tree) or sampling bias. The potential effect of sampling bias can be investigated by subsampling all clades to the same number of species.

If a phylogenetic tree for guild B is available, all these steps can be replicated to test for phylogenetic signal in species interaction in guild B.

Phylogenetic signal in guild A:

Step 1: Is there a phylogenetic signal in species interactions ?
(simple Mantel test)
(i) choice of ecological distances (Jaccard, UniFrac…)
(ii) with or without interaction abundances

```
RPANDA::phylosignal_network(network, tree_A, tree_B,
   method = "GUniFrac", correlation = "Pearson")
```

Yes

Step 2: Is there still a phylogenetic signal in species interactions when accounting for the signal in the number of partners ?
(simple Mantel test with permutations keeping the number of partners per species constant)

```
RPANDA::phylosignal_network(network,
tree_A, tree_B, method = "GUniFrac",
      correlation = "Pearson",
     permutations="nbpartners")
```

No

Option 1: Investigate clade-specific phylogenetic signals (simple Mantel tests with Bonferroni correction)

```
RPANDA::phylosignal_sub_network(network, tree_A, tree_B,
      method = "GUniFrac", correlation = "Pearson")
```

Option 2: Test the robustness of the findings to phylogenetic uncertainty and/or sampling bias

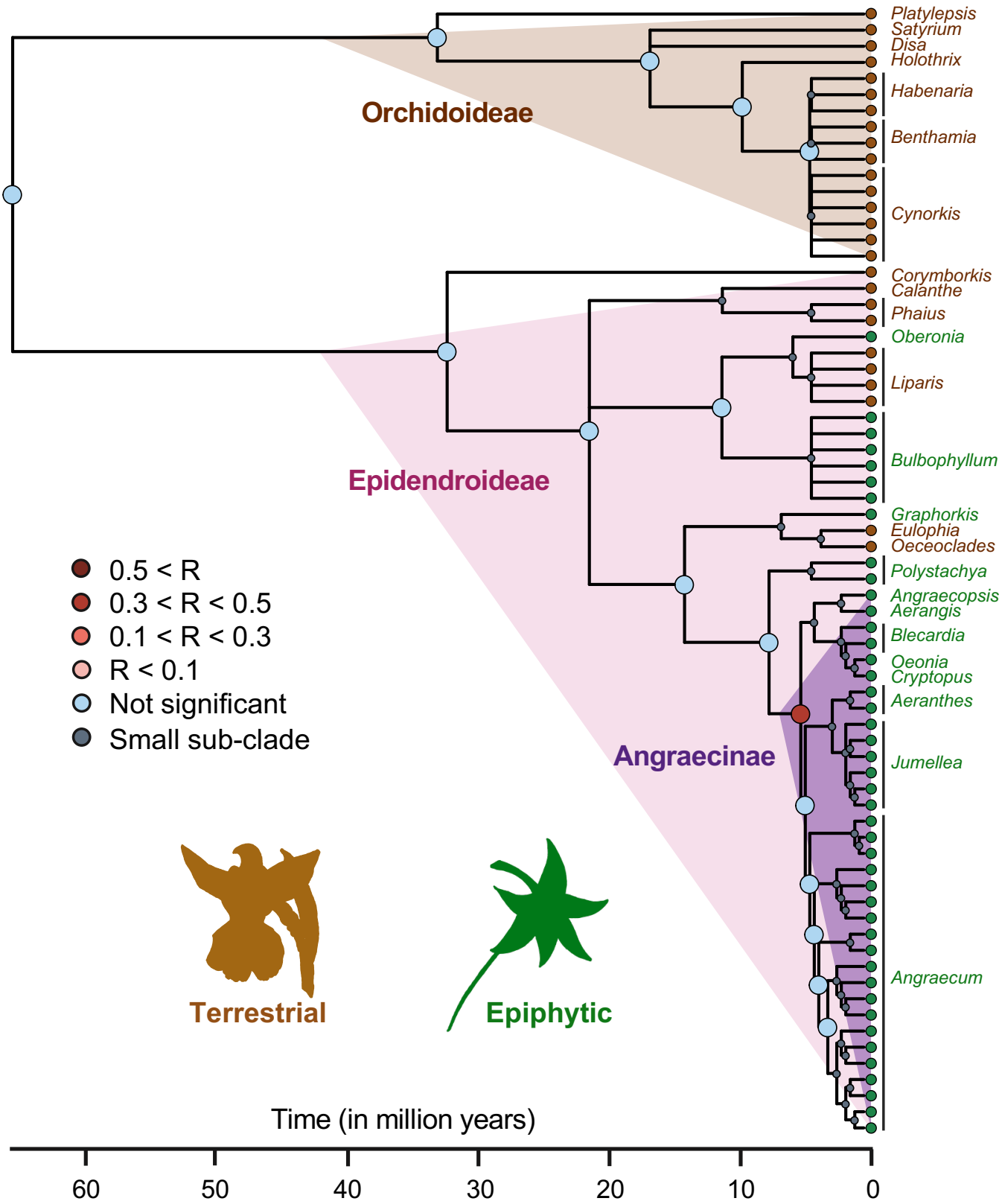
(repeat for guild B)

Figure 5: Empirical application on an orchid-fungus interaction network from La Réunion island (Martos *et al.,* 2012): the clade-specific analyses of phylogenetic signal in species interactions revealed a significant phylogenetic signal in the epiphytic subtribe Angraecinae.

The orchid phylogeny (Martos *et al.,* 2012) is represented with its nodes colored according to the results of the Mantel test performed on the corresponding sub-network: in blue if non-significant, in grey when the node has less than 10 descendent species (the Mantel test was not performed), and in red when the phylogenetic signal is significant. Each one-tailed simple Mantel test was performed using the Pearson correlation and 100,000 permutations and its significance was evaluated while correcting for multiple testing (Bonferroni correction).

For each species, its habitat (terrestrial or epiphytic) is indicated at the tips of the tree and the main orchid clades are highlighted in colors. Only the genera are indicated at the tips of the tree (see Supplementary Figure S32 for the species list).

692


Orchidoideae
Epidendroideae
Angraecinae
Platylepsis
Satyrium
Disa
Holothrix
Habenaria
Benthamia
Cynorkis
Corymborkis
Calanthe
Phaius
Oberonia
Liparis
Bulbophyllum
Graphorkis
Eulophia
Oeceoclades
Polystachya
Angraecopsis
Aerangis
Blecardia
Oeonia
Cryptopus
Aeranthes
Jumellea
Angraecum
0.5 < R
0.3 < R < 0.5
0.1 < R < 0.3
R < 0.1
Not significant
Small sub-clade
Terrestrial
Epiphytic
Time (in million years)
60
50
40
30
20
10
0

Discussion:

We used simulations to perform a comparative analysis of the statistical performances of Mantel tests and the Phylogenetic bipartite linear model (PBLM; Ives & Godfray 2006) for testing for phylogenetic signal in species interactions. Our results highlight the weaknesses of PBLM and partial Mantel tests, and advocate for the use of regular Mantel tests and Mantel tests with network permutations keeping the number of partners per species constant.

The Phylogenetic bipartite linear model (PBLM) is widely used to test for phylogenetic signal in species interactions, however we found that it has very frequent false positives (>30%). PBLM assumes that the interaction strength between two species is determined by the product of two unobserved traits evolving on the phylogenies of guilds A and B respectively, according to two independent Ornstein-Uhlenbeck processes with the selection strengths d_A and d_B (Supplementary Methods 3). PBLM tests the significance of d_A and d_B, which measure the phylogenetic signal of the unobserved traits. A species with a high trait value will have high interaction strengths with many partner species (*i.e.* it is a generalist species), while a species with a low trait value will have low interaction strengths with most partner species, except with the few species with high trait values (*i.e* it is a specialist species). Therefore, we suspect d_A and d_B to measure phylogenetic signals in the number of partners rather than in species interactions. However, we also found significant d_A and d_B in the absence of phylogenetic signal in the number of partners, suggesting that PBLM is sensitive to model misspecification (it relies on strong hypotheses on how the number of partners evolves). In any case, our results suggest that PBLM should not be used as a routine for measuring phylogenetic signal in species interactions.

Other process-based approaches that extend PBLM (Rafferty and Ives 2013; Hadfield et al. 2014; Li et al. 2020) allow to infer parameters thought to reflect the

722 phylogenetic structure of interactions networks, while controlling for phylogenetic
723 signal in the number of parterns as well as heterogeneity in sampling effort (Hadfield
724 *et al.*, 2014). Our analyses using the PGLMM approach (Rafferty and Ives 2013) on the
725 smallest simulated networks suggested that it also has frequent false positives and
726 intermediate statistical power when using weighted interactions. It would have been
727 ideal to also test this approach on larger networks, but this was prohibited by their
728 computational cost (Fig. S2). Indeed, fitting PGLMM can require >80 Gb of memory
729 for some networks and our application of the Bayesian approach of Hadfield *et al.*
730 (2014) ran several days (on an Intel 2.8 GHz, MacOSX laptop) without reaching
731 convergence. Because of these high computational demands, these methods are
732 typically not used to measure phylogenetic signal in species interactions in empirical
733 studies, which is either done using Mantel tests or PBLM (see Fontaine and Thébault
734 2015; Xing et al. 2020; Corro et al. 2021 for recent examples). Future model
735 developments of such approaches would thus benefit from faster inferences and our
736 results highlight the need to thoroughly test these approaches with simulations before
737 they are applied to empirical systems and biological conclusions are drawn.
738
739 We found that simple Mantel tests have a moderate statistical power and a
740 reasonable false positive rate (<5%) when testing for phylogenetic signal in species
741 interactions. Not surprisingly, these tests have a higher power for larger simulated
742 networks. The fact that Mantel tests have a moderate power for measuring
743 phylogenetic signals in species interactions corroborates the findings about Mantel
744 tests in other contexts (Harmon and Glor 2010; Guillot and Rousset 2013). Hence,
745 although simple Mantel tests might fail at detecting low phylogenetic signal, we can
746 trust their results when they are significant. On the contrary, we found a high
747 proportion of simulated networks (5-10%) presenting a significant negative
748 phylogenetic signal in species interactions, suggesting that closely related species
749 would tend to associate with dissimilar partners. Yet, we did not expect such an
750 outcome in our simulations because we did not observe any negative phylogenetic

751 signal in species traits. False-positives are therefore frequent when testing for negative
752 phylogenetic signal using simple Mantel tests and detection of such signal in empirical
753 networks should be interpreted with caution.
754
755 In addition, Pearson correlations performed better than Spearman and Kendall
756 correlations, which is somewhat surprising, as correlations between phylogenetic and
757 ecological distances are not particularly expected to be linear: Spearman and Kendall
758 correlations have less stringent hypotheses, as they only assume monotonicity
759 (Supplementary Methods 3), but they probably lose information. We also reported that
760 using ecological distances that consider interaction abundances, such as weighted
761 Jaccard or UniFrac distances, significantly improves the detection of phylogenetic
762 signal. Using UniFrac distances, which rely on the phylogenetic relatedness of the
763 partners, can be particularly relevant when species delineation is somewhat arbitrary,
764 *e.g.* in microbial systems, as it is less sensitive to species delineation than Jaccard
765 distances. In addition, results obtained with UniFrac distances were only moderately
766 influenced by the phylogenetic uncertainty in the partner's tree, which should thus not
767 prevent the use of UniFrac distances. In the context of our *BipartiteEvol* simulations,
768 which assume that species interactions are mediated by some phylogenetically-
769 conserved traits on both sides of the network, we found that UniFrac distances
770 outperform Jaccard distances. We note however that a significant phylogenetic signal
771 in UniFrac or Jaccard distances can reflect different evolutionary processes, such as
772 one where the traits involved in the interaction are evolutionary conserved on both
773 sides of the networks in the case of UniFrac, and on only one side of the network in
774 the case of Jaccard (Calatayud et al. 2016). Therefore, choosing between one or the
775 other metric (or using both) can also be dictated by the question at stake. Also, if
776 communities of interactors differ mainly in terms of recently diverged species, Jaccard
777 distances may perform better, as UniFrac distances emphasize differences in long
778 branches rather than recent splits (Sanders et al. 2014).
779

780 We also found that multiple simple Mantel tests combined with a Bonferroni
781 correction perform rather well to investigate clade-specific phylogenetic signals. Such
782 an approach can therefore be valuable for measuring local phylogenetic signal in large
783 "meta-networks", such as those describing host-microbiota phylosymbiosis (Song et
784 al. 2020), which likely have heterogeneous phylogenetic signals across the network.
785
786 While simple Mantel tests have satisfactory statistical performances, these tests do
787 not control for the potential confounding effect of phylogenetic signal in the number
788 of partners. Partial Mantel tests are frequently used for investigating phylogenetic
789 signal in species interactions while controlling for signal in the number of partners;
790 however, we found that they often detected significant signals in species interactions
791 when we simulated signals in only the number of partners. Thus, partial Mantel tests
792 fail at discerning whether evolutionary relatedness strictly affects the identity of
793 partners, independently of the total number of partners associated with each species
794 (Rezende et al. 2007). This corroborates the poor statistical performances of partial
795 Mantel tests frequently observed in other contexts (Harmon and Glor 2010; Guillot and
796 Rousset 2013). Among the alternative possibilities we tested, using sequential Mantel
797 tests, *i.e.* testing first for phylogenetic signal in species interactions, and if significant
798 testing for phylogenetic signal in the number of partners, has both high statistical
799 power and a low false positive rate. Yet, if both Mantel tests are significant, it does not
800 say whether the signal is entirely due to the signal in the number of partners and
801 therefore, sequential Mantel tests likely have very low power in this case.
802 Alternatively, using methods that can explicitly partition ecological distances into a
803 part due to dissimilarities in the number of partners *versus* the identity of the partners
804 appears promising, although we detected a slight power decrease in our simulations
805 and >30% of artefactual negative phylogenetic signals when partitioning unweighted
806 Jaccard distances. Other partitioning approaches may give better results and should
807 require further attention, as they offer a direct quantification of the contribution of the
808 species identity *versus* the number of partners in the phylogenetic signal (Baselga 2010;

809 Leprieur et al. 2012; Calatayud et al. 2016). Finally, performing a Mantel test with
810 network permutations designed to keep the number of partners associating with each
811 species constant while shuffling their identity has infrequent false positives and does
812 not decrease the statistical power. Therefore, if there is still signal while constraining
813 the number of partners, then we can safely conclude that evolutionary relatedness
814 affects the identity of partners. We thus recommend using such network permutations
815 to correct for the confounding effect of the phylogenetic signal in the number of
816 partners (Figure 4).
817
818 By definition, phylogenetic signals in species interactions measure general patterns
819 that are not informative of the processes at play (Losos 2008). A better understanding
820 of the ecological and evolutionary processes playing a role in the assembly of
821 interaction networks (Harmon et al. 2019) will require developing integrative process-
822 based approaches, for instance inference machineries for eco-evolutionary models
823 such as *BipartiteEvol*. Classical inferences (generalized least-squares or likelihood-
824 based approaches) might be challenging for such complex models (Hadfield et al.
825 2014), but strategies such as machine learning provide promising alternatives.
826
827 In the mycorrhizal network from La Réunion, we found non-significant or weak
828 phylogenetic signals in species interactions at the level of the entire orchid-fungus
829 network, suggesting these interactions are generally poorly conserved over long
830 evolutionary timescales (Jacquemyn et al. 2011; Martos et al. 2012; Perez-Lamarque et
831 al. 2022). Conversely, clade-specific Mantel tests detected a significant phylogenetic
832 signal in the Angraecinae epiphytic clade that is experiencing a radiation in La
833 Réunion island. This signal is likely produced by the different orchids genera in
834 Angraecinae associating with specific fungal clades (Martos et al. 2012). Thus, our
835 results corroborate a trend toward mycorrhizal specialization in epiphytic orchids
836 compared with terrestrial species (Xing et al. 2019), as the epiphytic habitats might

837 require particular adaptations and stronger dependences toward specific mycorrhizal
838 fungi.
839
840 Interaction networks are increasingly being analyzed to unravel the
841 evolutionary processes shaping their structure and to predict their stability. Currently-
842 used tools for measuring phylogenetic signals are clearly misleading. The approach
843 we propose based on Mantel tests may have a limited statistical power, but it avoids
844 false positive, and it is flexible as it allows using different ecological distances and/or
845 permutation strategies. By emphasizing the limits of current tests of phylogenetic
846 signal, we hope to stimulate new developments in the statistical adjustment to
847 empirical data of process-based models for the evolution of interaction networks.

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Author contributions:

All authors designed the study. BPL performed the analyses, BPi performed the analyses on the network structures, and FM gathered the empirical data. BPL and HM wrote the first draft of the manuscript and all authors contributed to revisions.

Data accessibility:

The R functions used to measure phylogenetic signals in bipartite interaction networks, including (simple, partial, and clade-specific) Mantel tests and PBLM, are available in the R-package RPANDA (Morlon et al. 2016) (functions *phylosignal_network* and *phylosignal_sub_network*). A tutorial and the simulated networks can be found at https://github.com/BPerezLamarque/Phylosignal_network. Amended functions of *BipartiteEvol* are also included in RPANDA.

The scripts for simulating the networks and for measuring the phylogenetic signals in species interactions are available at:

https://github.com/BPerezLamarque/Phylosignal_network/tree/master/simulations

Supplementary data (including Supplementary Methods, Tables, and Figures) are available at:
https://github.com/BPerezLamarque/Phylosignal_network/blob/master/Supplementary_figures_phylo_signal_network.pdf

Conflict of Interest statement:

The authors declare that there is no conflict of interest with the content of this article.

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