Distinguishing cophylogenetic signal from phylogenetic congruence clarifies the interplay between evolutionary history and species interactions

Running title: Cophylogenetic signal does not imply phylogenetic congruence

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Abstract:

Interspecific interactions, including host-symbiont associations, can profoundly affect the evolution of the interacting species. Given the phylogenies of host and symbiont clades and knowledge of which host species interact with which symbiont, two questions are often asked: "Do closely related hosts interact with closely related symbionts?" and "Do host and symbiont phylogenies mirror one another?". These questions are intertwined and can even collapse under specific situations, such that they are often confused one with the other. However, in most situations, a positive answer to the first question, hereafter referred to as "cophylogenetic signal", does not imply a close match between the host and symbiont phylogenies. It suggests only that past evolutionary history has contributed to shaping present-day interactions, which can arise, for example, through present-day trait matching, or from a single ancient vicariance event that increases the probability that closely related species overlap geographically. A positive answer to the second, referred to as "phylogenetic

congruence", is more restrictive as it suggests a close match between the two phylogenies, which may happen, for example, if symbiont diversification tracks host diversification or if the diversifications of the two clades were subject to the same succession of vicariance events.

Here we apply a set of methods (ParaFit, PACo, and eMPRess), which significance is often interpreted as evidence for phylogenetic congruence, to simulations under three biologically realistic scenarios of trait matching, a single ancient vicariance event, and phylogenetic tracking with frequent cospeciation events. The latter is the only scenario that generates phylogenetic congruence, whereas the first two generate a cophylogenetic signal in the absence of phylogenetic congruence. We find that tests of global-fit methods (ParaFit and PACo) are significant under the three scenarios, whereas tests of event-based methods (eMPRess) are only significant under the scenario of phylogenetic tracking. Therefore, significant results from global-fit methods should be interpreted in terms of cophylogenetic signal and not phylogenetic congruence; such significant results can arise under scenarios when hosts and symbionts had independent evolutionary histories. Conversely, significant results from event-based methods suggest a strong form of dependency between hosts and symbionts evolutionary histories. Clarifying the patterns detected by different cophylogenetic methods is key to understanding how interspecific interactions shape and are shaped by evolution.

Keywords: cophylogeny, codiversification, phylogenetic methods, symbiosis, parasitism, coevolution.

53 **Introduction:**

54

55 Antagonistic or mutualistic interactions, such as parasitism, herbivory, seed
56 dispersal, or pollination, are key components of ecological communities (Bascompte
57 and Jordano 2013; Mittelbach and McGill 2019). Patterns of interactions (*i.e.* who
58 interacts with whom) are shaped by evolutionary history through a variety of
59 processes. For example, interspecific interactions may be constrained to species with
60 matching traits, as frequently observed in pollination or host-parasite interaction
61 networks (Muchhala and Thomson 2009; Morand et al. 2015), in which case species
62 evolutionary history matters as soon as the traits involved in the interactions are
63 evolutionarily conserved. Interspecific interactions may also be constrained by
64 historical contingencies such as past dispersal and/or geographic events: for instance,
65 if lineages have been geographically isolated following a vicariance event, these
66 lineages do not interact simply because they do not co-occur (Althoff et al. 2014; Perez-
67 Lamarque et al. 2022a). Interspecific interactions can also be transmitted from
68 generation to generation on evolutionary time scales, as in the case of symbionts that
69 are vertically transmitted from parental to descendant hosts (Bright and Bulgheresi
70 2010). Reciprocally, interactions affect the evolution of the interacting species, for
71 instance through disruptive selection, stabilizing selection, or coevolution. Over
72 macroevolutionary scales, such effects can leave an imprint on the phylogenetic trees
73 of the interacting clades (Harmon et al. 2019; Hembry and Weber 2020; Hayward et al.
74 2021). An extreme case corresponds to phylogenetic tracking, which can happen, for
75 example, when host speciation events lead to the subsequent speciation of symbionts
76 that are closely associated with them (Fahrenholz 1912). Analyzing interacting species
77 through the lens of their past evolutionary history is therefore fundamental for
78 understanding how interspecific interactions shape and are shaped by evolution.

79

80 Given host-symbiont cophylogenetic data, *i.e.* a phylogenetic tree for both the
81 host and the symbiont clades and knowledge of which host species interact with which

symbiont, two patterns are often investigated (Supplementary Box 1): (i) whether closely-related hosts interact with closely-related symbionts, hereafter referred to as "cophylogenetic signal", and (ii) whether host and symbiont phylogenies mirror one another (with pairs of interacting hosts and symbionts that tend to occupy similar positions in the two trees), hereafter referred to as "phylogenetic congruence" (Blasco-Costa et al. 2021). By definition, phylogenetic congruence can occur only when the number of host and symbiont species is similar, with mostly 'one-to-one' interactions between one host and one symbiont species. In this case, patterns of cophylogenetic signal and phylogenetic congruence tend to collapse, such that they can be studied interchangeably. In this context, some cophylogenetic methods, referred to as global-fit methods, have been developed to test for phylogenetic congruence using linear algebra techniques, such as the fourth-corner statistics (ParaFit, Legendre et al. 2002) and procrustean superposition (PACo, Balbuena et al. 2013). Numerous cophylogenetic systems observed in nature, however, are characterized by 'many-to-many' interactions, as illustrated by densely-connected networks of interspecific interactions (Ronquist and Nylin 1990; Bascompte and Jordano 2013; Pichon et al. 2023). The same global fit methods have been regularly applied to such systems, and interpreted as tests of phylogenetic congruence (see *e.g.* Fuzessy et al. 2022; Suzuki et al. 2022 for recent examples). If, under these situations, global-fit methods actually do not measure phylogenetic congruence, this can lead to a misinterpretation of the results.

Patterns of cophylogenetic signal and phylogenetic congruence can reflect different processes (Fig. 1). The occurrence of cophylogenetic signal simply suggests that past evolutionary history has contributed to shaping present-day interactions. For instance, a cophylogenetic signal can arise if interactions are shaped by evolutionarily conserved traits (Fig. 1a "trait matching") or by past biogeographic events (Fig. 1b "vicariance"), even if the diversification of the host and symbiont clades was not influenced by the interactions between host and symbiont species. The occurrence of

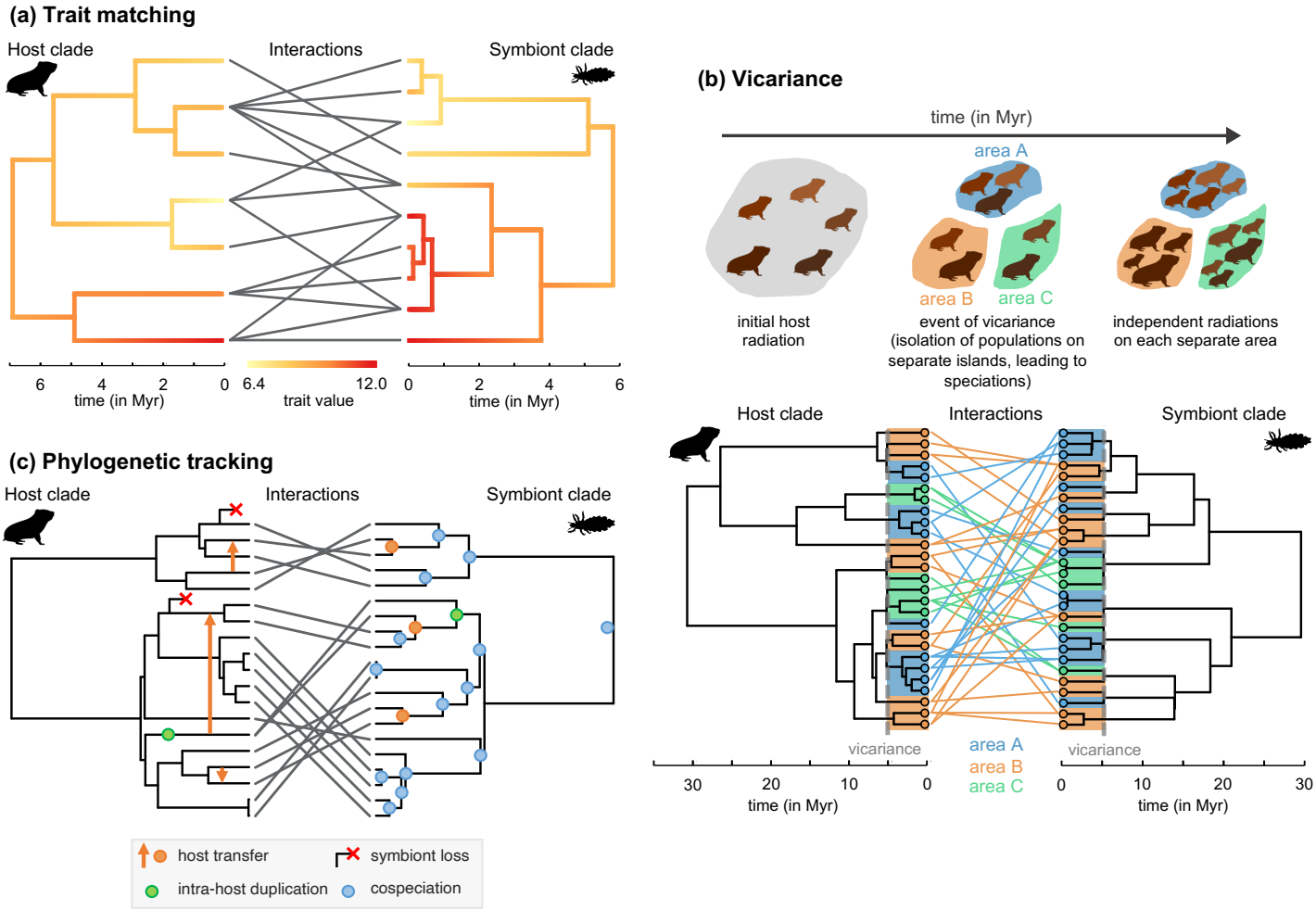
phylogenetic congruence, on the other hand, suggests a potential (co-)dependency between the host and symbiont diversifications, causing both phylogenetic trees to look alike. For instance, phylogenetic congruence can emerge (i) if the diversification of vertically transmitted symbionts tracks host diversification, generating a pattern of concomitant diversification events happening in both host and symbiont clades, referred to as "codiversification" (Fig. 1c "phylogenetic tracking"), (ii) if the diversifications of the two clades were subject to the same succession of vicariance events, also resulting in a pattern of concomitant codiversification, or (iii) under preferential host switching, *i.e.* if symbionts diversify by preferentially transferring to closely-related host species, generating a pattern of phylogenetic congruence without concomitant divergence times, referred to as "pseudo-codiversification" (de Vienne et al. 2013; Althoff et al. 2014). Coevolution, *i.e.* the reciprocal evolutionary changes in interacting lineages induced by selective pressures exerted by one another, may also generate phylogenetic congruence in some cases (*e.g.* aphid-associated bacterial endosymbionts (Jousselin et al. 2009)), but is not a necessary nor sufficient condition for observing phylogenetic congruence (Poisot 2015). Phylogenetic congruence implies cophylogenetic signal, but the reverse is not true.

Figure 1: Three mock examples of host-symbiont systems (here represented as gophers and lice) that generate a cophylogenetic signal. Only the third example also generates phylogenetic congruence.

(a) Trait matching. Here host and symbiont phylogenetic trees evolve independently. Traits also evolve independently on each phylogeny following Brownian motion processes, which results in a phylogenetic signal in species traits (*i.e.* closely related species tend to have similar trait values; see the color gradient). Host-symbiont interactions at present are more likely between species having complementary traits, following a trait-matching expression. Because of trait matching, closely related host species interact with closely related symbiont species (*i.e.* cophylogenetic signal), although the phylogenetic trees are independent (*i.e.* no phylogenetic congruence).

(b) Vicariance: Here hosts and symbionts interact at random as long as they occupy the same biogeographic area. They occupy a single area until a vicariance event (*i.e.* the formation of a biogeographic barrier) splits this area into three separate areas, isolating populations and leading to speciation (a species occupying two areas at the time of vicariance immediately experiences allopatric speciation). Following the vicariance event, each host and symbiont lineage radiates independently on its area, without dispersal between areas, which results in a phylogenetic signal in biogeographic repartition (*i.e.* closely related species tend live on the same area). Because interactions happen at random within each area, this scenario generates cophylogenetic signal without phylogenetic congruence.

(c) Phylogenetic tracking: Here symbiont species are vertically transmitted over long-time scales along host lineages and hosts speciations concomitantly lead to symbiont speciations ("cospeciation") resulting in a pattern of codiversification. In addition, symbiont lineages can experience horizontal host transfers from a donor host to a receiver host (with replacement of the previous symbiont lineage), intra-host duplication, and host lineages can lose their symbionts. This scenario generates both cophylogenetic signal and phylogenetic congruence. The greater the number of host transfers, intra-host duplications, and symbiont loss, the lower the phylogenetic congruence, as these events disrupt the symbiont phylogeny with regard to the host phylogeny (Ronquist 2003a,b).



Here, in an effort to clarify the conclusions that can be drawn from various cophylogenetic methods, we analyze their outputs under various evolutionary scenarios, using simulations. Cophylogenetic methods can be divided into two main types of approaches (see de Vienne et al. (2013) and Dismukes et al. (2022) for reviews of these methods): the global-fit methods mentioned above, such as ParaFit and PACo, and the event-based methods, such as TreeMap (Page 1994a, 1995), TreeFitter (Ronquist 2003a), Jane (Conow et al. 2010), or eMPRess (Santichaivekin et al. 2021). Event-based methods try to reconciliate the host and symbiont phylogenies by fitting a set of reconciliation events (e.g. cospeciation, host transfer, intra-host duplication, or symbiont loss) to the symbiont phylogeny (Page 1994b; Ronquist 2003b). We have

shown before, in a different context, that global-fit and event-based methods can sometimes output contrasting results (Perez-Lamarque and Morlon 2023), suggesting that they measure different patterns. We evaluate here their outputs under three biologically realistic scenarios of host-symbiont evolution by trait matching, vicariance, and phylogenetic tracking. The latter is the only scenario that generates phylogenetic congruence when simulating frequent cospeciations, whereas the first two generate a cophylogenetic signal in the absence of phylogenetic congruence. We find that global-fit methods provide a test of cophylogenetic signal rather than phylogenetic congruence, whereas event-based methods provide a test of phylogenetic congruence, and discuss the implications of these results for the study of cophylogenetic systems.

Simulations of three biologically realistic scenarios:

In the first simulated scenario, we assumed that present-day host-symbiont interactions are more likely between species having complementary traits following a trait-matching expression with a unidimensional continuous trait (Fig. 1a – "trait matching"; Supplementary Methods 1). We independently simulated two phylogenetic trees for the host and symbiont clades using a birth-death model and on each tree, we then independently simulated the evolution of traits modulating present-day interactions. By using a Brownian motion for trait evolution, closely-related hosts and closely-related symbionts tend to have similar trait values (*i.e.* phylogenetic signal in species traits). Finally, we assumed that the degree of specialization of the symbionts (*i.e.* the number of hosts that a given symbiont interacts with) follows a Poisson distribution with parameter λ=1.5 and attributed the present-day host-symbiont interacting pairs following a trait-matching expression. As a result, closely related host species interact with closely related symbiont species (*i.e.* cophylogenetic signal), although the phylogenetic trees were simulated independently and are therefore not congruent. We generated 1,000 simulations with varying clade sizes (from 10 to 200

202 species per clade; Supplementary Methods 1) and replicated the simulations with
203 fewer host species associated with each symbiont species by using a Poisson
204 distribution with parameter λ=1.
205
206 In the second simulated scenario, we assumed that host and symbiont species
207 interact at random as long as they occupy the same biogeographic area. At first, all
208 hosts and symbionts simultaneously occupy a single area and diversify
209 independently, until a vicariance event splits the area into three separate areas (Fig. 1b
210 – "vicariance"; Supplementary Methods 1). Half of the host and symbiont species are
211 then isolated in separate areas; the other half experiences allopatric speciation as their
212 population is split into two. Following the vicariance event, each host and symbiont
213 lineage diversifies independently in its area, resulting in a phylogenetic signal in
214 biogeographic repartition (*i.e.* closely related species tend to occupy the same area).
215 Although the host and symbiont diversifications are not independent (they both
216 undergo a burst of speciation events at the time of vicariance), the host and symbiont
217 phylogenetic trees are not congruent as they experienced different events of
218 diversification before and after the vicariance (Fig. 1b). Finally, we assumed that the
219 degree of specialization of the symbionts follows a Poisson distribution with
220 parameter λ=1.5 and randomly attributed host-symbiont present-day interactions
221 within each area. This scenario thus produces cophylogenetic signal but no
222 phylogenetic congruence. We generated 1,000 simulations with varying clade sizes
223 (Supplementary Methods 1) and replicated the simulations with fewer host species
224 associated with each symbiont species (λ=1).
225
226 In the third simulated scenario, we assumed that the symbiont diversification
227 tracks the host diversification. Symbionts species are vertically transmitted over long-
228 time scales from host generation to host generation (at the level of host individual or
229 at the level of the whole host lineage) and cospeciate at host speciation events resulting
230 in codiversification (Fig. 1c – "phylogenetic tracking"; Supplementary Methods 1). In

231 addition, we assumed that symbiont lineages experience a given number of host
232 transfers from a donor host to a receiver host (with replacement of the previous
233 symbiont lineage); this number was uniformly sampled between 0 and half the
234 number of extant host species. Finally, intra-host duplication occurs at rate 0.001 event
235 per million year per lineage and host lineages can lose their symbionts with a
236 probability of 0.1 at present. The latter processes (transfer, duplication and loss)
237 dampen but maintain the phylogenetic congruence between host and symbiont
238 phylogenies, rendering it more realistic. This third scenario of phylogenetic tracking
239 with frequent cospeciations produces both cophylogenetic signal and phylogenetic
240 congruence. We generated 1,000 simulations with varying clade sizes (from 10 to 200
241 host species; Supplementary Methods 1). We also replicated the simulations with less
242 cospeciations and more host transfers (number uniformly sampled between 50% and
243 75% of the number of extant host species) and intra-host duplications (rate of 0.0015
244 event per million year per lineage). Under these simulations of phylogenetic tracking
245 with infrequent cospeciations, the pattern of phylogenetic congruence is greatly erased
246 as cospeciation events represent a minority of simulated events.
247
248 We therefore obtained a total of 6,000 simulations with different host and
249 symbiont species richness values and ratios of one-to-one interactions (Supplementary
250 Figs. S1 & S2). As expected, our simulations under phylogenetic tracking produced
251 systems with a much higher proportion of one-to-one interactions than simulations
252 under trait matching and vicariance, which were mainly constituted of many-to-many
253 interactions, especially under low symbiont specialization (λ=1.5, Supplementary Fig.
254 S2).
255
256 **Fitting cophylogenetic methods:**
257
258 For each simulated cophylogenetic data, we first applied the global-fit methods
259 ParaFit and PACo using the functions *parafit* and *PACo* from the R-packages ape

260 (Paradis et al. 2004) and paco (Hutchinson et al. 2017) respectively, amended to avoid
261 technical issues when the number of host or symbiont species is low (Perez-Lamarque
262 and Morlon 2023). We measured the strength of the cophylogenetic signal using the
263 "ParaFit global statistic" in ParaFit, whereas, in PACo, we ran the symmetric option
264 for the procrustean superposition to obtain R^2, defined as $R^2 = 1 - m^2$, where m^2 is the
265 sum of the squared residuals of the symmetric procrustean superposition (Blasco-
266 Costa et al. 2021). R^2 is comprised between 0 and 1, and R^2 close to 0 indicates low
267 cophylogenetic signal, whereas R^2 close to 1 indicates high cophylogenetic signal.
268 Following Legendre et al. (2002) and Hutchinson et al. (2017), the significance of the
269 ParaFit and PACo tests was first evaluated using 10,000 randomizations obtained by
270 independently shuffling which host species are associated with each symbiont species,
271 hereafter referred to as "null model 1". Second, following Ronquist (1998) and
272 Sanmartín and Ronquist (2004), we assessed their significance by randomly shuffling
273 the host species labels, hereafter referred to as "null model 2". We also investigated
274 whether the effect size of the global-fit methods, *i.e.* values of the ParaFit global statistic
275 and PACo's R^2, can be used as indicators of phylogenetic congruence (Blasco-Costa et
276 al. 2021). Finally, we tested whether global-fit approaches were more likely to be
277 significant when the ratio of one-to-one interactions was low using generalized linear
278 models.
279
280 Second, we applied the event-based method eMPRess to each simulation
281 (Santichaivekin et al. 2021). eMPRess reconciles the host and symbiont tree topologies
282 by using maximum parsimony to fit events of host transfers, duplications, and losses;
283 each event being associated with a given cost. We chose eMPRess over all the other
284 existing event-based methods, as it can be automatically and rapidly run using the
285 command line. As with most event-based methods, eMPRess does not handle
286 symbiont species that interact with multiple hosts (Dismukes et al. 2022). However,
287 symbionts often interact with several hosts in nature, and also in our simulated
288 scenarios of trait matching and vicariance. We thus tested two strategies for running

289 eMPRess following Sanmartín and Ronquist (2002): (i) subsampling one host at
290 random per symbiont species (Su et al. 2022) or (ii) randomly generating bifurcating
291 sub-trees for symbionts with multiple hosts, such that each tip in the symbiont tree is
292 associated with a single host (Satler et al. 2019). We used the command-line version of
293 eMPRess with the commands "python empress_cli.py reconcile" to reconcile the trees
294 and "python empress_cli.py p-value" to assess the significance. We tested 5 different
295 combinations of cost values for duplication (d), host transfer (t) or loss (l) events: (i)
296 d=1, t=1, l=1; (ii) d=4, t=1, l=1; (iii) d=2, t=1, l=2; (iv) d=4, t=2, l=1; or (v) d=2, t=3, l=1. A
297 reconciliation is considered significant if its total cost is lower than 95% of the costs of
298 1,000 reconciliations obtained after randomly shuffling the host species labels ("null
299 model 2"; Ronquist 1998; Sanmartín and Ronquist 2004). Phylogenetic trees are
300 considered congruent if the reconciliation estimates less host transfer than
301 cospeciation events (Groussin et al. 2017; Perez-Lamarque and Morlon 2023).
302
303 **Outputs of cophylogenetic methods:**
304
305 For ParaFit, we found significant tests in 34% of the trait-matching simulations,
306 45% of the vicariance simulations, and 98% of the phylogenetic tracking (obtained with
307 "null model 1" and λ=1 or 1.5; Table 1). Significant tests for PACo were even more
308 frequent, reaching 48%, 63%, and 99% of the simulations in the three scenarios,
309 respectively (Table 1). Qualitatively similar results were obtained when evaluating the
310 significance by shuffling the host species labels ("null model 2"; Supplementary Table
311 S1). As expected, global-fit tests were more often significant in simulations with higher
312 numbers of host and symbiont species (Supplementary Table S2, Supplementary Figs.
313 S3 & S4). In trait matching and vicariance simulations, when increasing the ratio of
314 one-to-one host-symbiont interactions (*i.e.* reducing the mean number of associated
315 hosts per symbiont by using λ=1), global-fit tests were less often significant (Table 1;
316 Supplementary Table S3; Supplementary Figs. S3 & S4; generalized linear models: p-
317 values<0.05). In other words, global-fit tests are actually more likely to be significant

318 when there are frequent many-to-many host-symbiont interactions, while we would
319 expect the contrary if these tests measured phylogenetic congruence. Overall, these
320 results indicate that global-fit methods tend to measure cophylogenetic signal in
321 general rather than specifically phylogenetic congruence.
322
323 In terms of interpretation of the effect size of the global-fit methods, the ParaFit
324 global statistic varies with the total number of species, and is thus difficult to interpret
325 in itself: it cannot be used to distinguish phylogenetic congruence from cophylogenetic
326 signal (Fig. 2; Supplementary Fig. S3). In contrast, for PACo, we found that R^2 is
327 generally higher than 0.25 when there is a pattern of phylogenetic congruence (in 99%
328 of the scenario of phylogenetic tracking with frequent cospeciations), whereas it is
329 lower than 0.5 when there is a pattern of cophylogenetic signal alone (in 99% of the
330 scenarios of trait-matching or vicariance; Figs. 2 & 3). Hence, a significant test with R^2
331 > 0.50 is strong support for phylogenetic congruence (although not definite evidence,
332 it may be possible to obtain higher R^2 values under scenarios without congruence with
333 different simulation choices), whereas a significant test with $R^2 < 0.25$ suggests that the
334 system presents a cophylogenetic signal without phylogenetic congruence (Fig. 3). A
335 significant test with R^2 values between 0.25 and 0.50 is harder to interpret, as R^2 tends
336 to correlate positively with the ratio of one-to-one interactions and R^2 values above
337 0.25 are sometimes reported in simulations without phylogenetic congruence
338 (Supplementary Fig. S4). PACo alone is therefore often not sufficient to identify a
339 pattern of phylogenetic congruence.
340
341 For eMPRess, when subsampling one host per symbiont species, the
342 reconciliation was significant in only 5% of the trait-matching simulations and 16% of
343 the vicariance simulations (the two scenarios generating cophylogenetic signal
344 without phylogenetic congruence), and congruent in none (Table 1). When generating
345 random bifurcations in the symbiont tree, it was significant in 34% and 80% of the trait-
346 matching and vicariance simulations, respectively, but congruent in none (Table 1).

347 Indeed, eMPRess estimated on average 5 times more host transfers than cospeciations
348 for both scenarios of trait matching and vicariance (Table 1; Fig. 2; Supplementary Fig.
349 S5). In contrast, when simulating phylogenetic tracking with a majority of cospeciation
350 events, eMPRess gave significant reconciliations in 100% of the simulations, 92% of
351 which were congruent (Table 1). Results were qualitatively similar when choosing
352 alternative event costs (Supplementary Tables S4, S5, & S6). In all simulated scenarios,
353 significant reconciliations were more frequent when the number of host and symbiont
354 species were larger (Supplementary Tables S4, S5, & S6). When simulating
355 phylogenetic tracking with infrequent cospeciations, eMPRess reconciliations were
356 still significant in 99% of the simulations, but congruent in only 34% of them; this is
357 expected, given that the number of cospeciations is lower than the number of host
358 transfers in these simulations (Fig. 2; Table 1; Supplementary Tables S7). Overall, our
359 findings suggest that event-based methods can specifically detect patterns of
360 phylogenetic congruence and distinguish them from a "simple" cophylogenetic signal.
361
362 **Table 1: ParaFit and PACo (global-fit methods) are often significant in all simulated**
363 **scenarios, including scenarios of trait matching and vicariance that do not generate**
364 **phylogenetic congruence, whereas eMPRess (an event-based method) supports**
365 **phylogenetic congruence only under scenarios of phylogenetic tracking with**
366 **frequent cospeciations, which generate phylogenetic congruence:** This table
367 indicates the percentages of simulations for which ParaFit, PACo, or eMPRess output
368 a significant test, under three scenarios (Fig. 1): (a) present-day interactions dictated
369 by trait matching (with more (λ=1.5) or less (λ=1) host species associated with each
370 symbiont species), (b) present-day interactions at random following a single vicariance
371 event (with more (λ=1.5) or less (λ=1) host species associated with each symbiont
372 species), or (c) present-day interactions resulting from phylogenetic tracking (with
373 frequent or infrequent cospeciation events). For eMPRess, we report the percentage of
374 significant reconciliations based on permutations alone (P) or based on selecting
375 among these the ones that have more cospeciation than host transfer events (P+C). We

376 consider eMPRess to support phylogenetic congruence when conditions P and C are
377 met (in bold). Results by clade size are given in Supplementary Tables S1-S7. eMPRess
378 results correspond to host-symbiont reconciliations ran with relative costs d=4, t=1,
379 and l=1 for duplications, host transfers, and losses, respectively (other costs
380 combinations provided qualitatively similar results – see Supplementary Tables S4-
381 S6).

382

Methods		(a) Trait matching		(b) Vicariance		(c) Phylogenetic tracking	
		λ=1.5	λ=1	λ=1.5	λ=1	Frequent co-speciations	Infrequent co-speciations
Global-fit methods	**Percentage of significant tests using ParaFit**	39%	29%	52%	38%	99%	96%
	Percentage of significant tests using PACo	50%	45%	68%	59%	100%	99%
Event-based methods	**Percentage of significant tests using eMPRess with one host per symbiont**	P: 5% **P+C: 0%**	P: 8% **P+C: 0%**	P: 16% **P+C: 0%**	P: 18% **P+C: 0%**	P: 100% **P+C: 92%**	P: 99% **P+C: 34%**
	Percentage of significant tests using eMPRess with random bifurcations	P: 34% **P+C: 0%**	P: 23% **P+C: 0%**	P: 80% **P+C: 0%**	P: 58% **P+C: 0%**		

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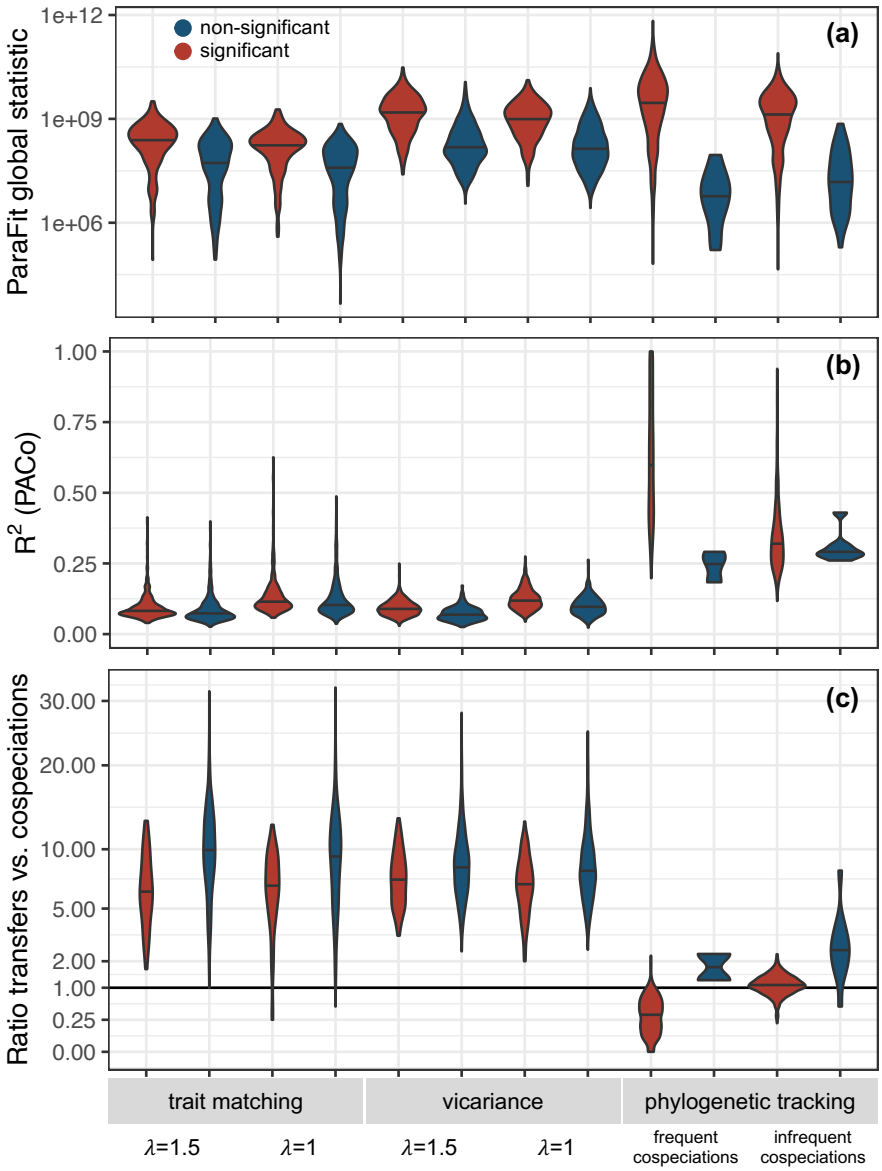
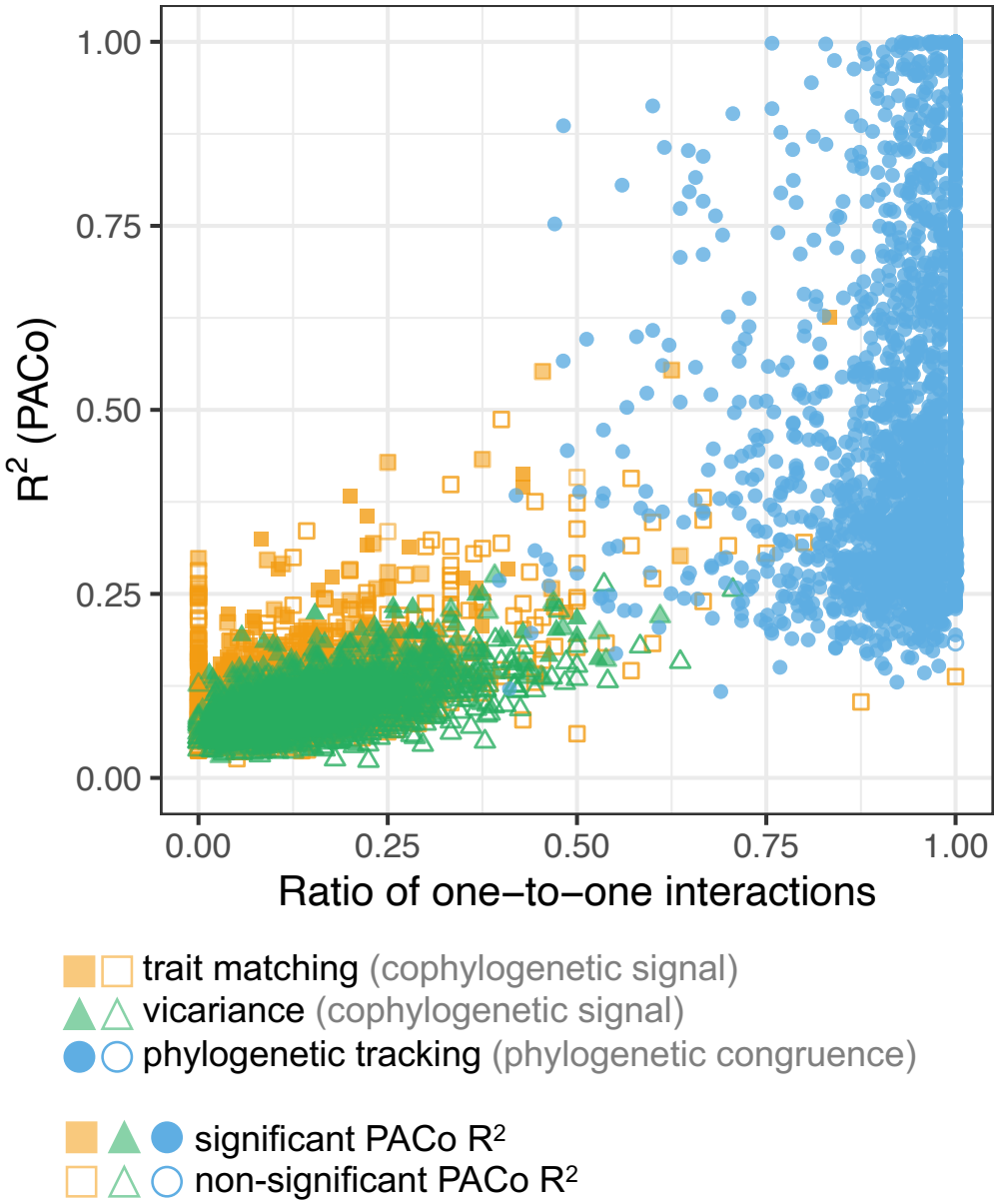
Figure 2: eMPRess correctly distinguishes patterns of phylogenetic congruence (scenario of phylogenetic tracking with frequent cospeciations) from cophylogenetic signal alone (scenarios of trait matching and vicariance), whereas ParaFit cannot and PACo can only in some cases: Distribution of ParaFit global statistics (a), PACo's R^2 (b), and the ratio between the number of host transfers and the number of cospeciations in eMPRess reconciliations (c) as a function of the test significance and the simulation scenario of trait matching, vicariance, or phylogenetic tracking. Here, we only reported eMPRess reconciliations obtained when subsampling one host per symbiont species (with relative costs d=4, t=1, and l=1), but similar results were observed when using random bifurcations (Supplementary Fig. S5).



398 **Figure 3: PACo's statistic (R^2) tends to increase with the ratio of one-to-one**

399 **interactions; however, PACo tests tend to be more often significant when the ratio**

400 **of one-to-one interaction is low under scenarios of trait matching and vicariance.**



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403 **Discussion:**

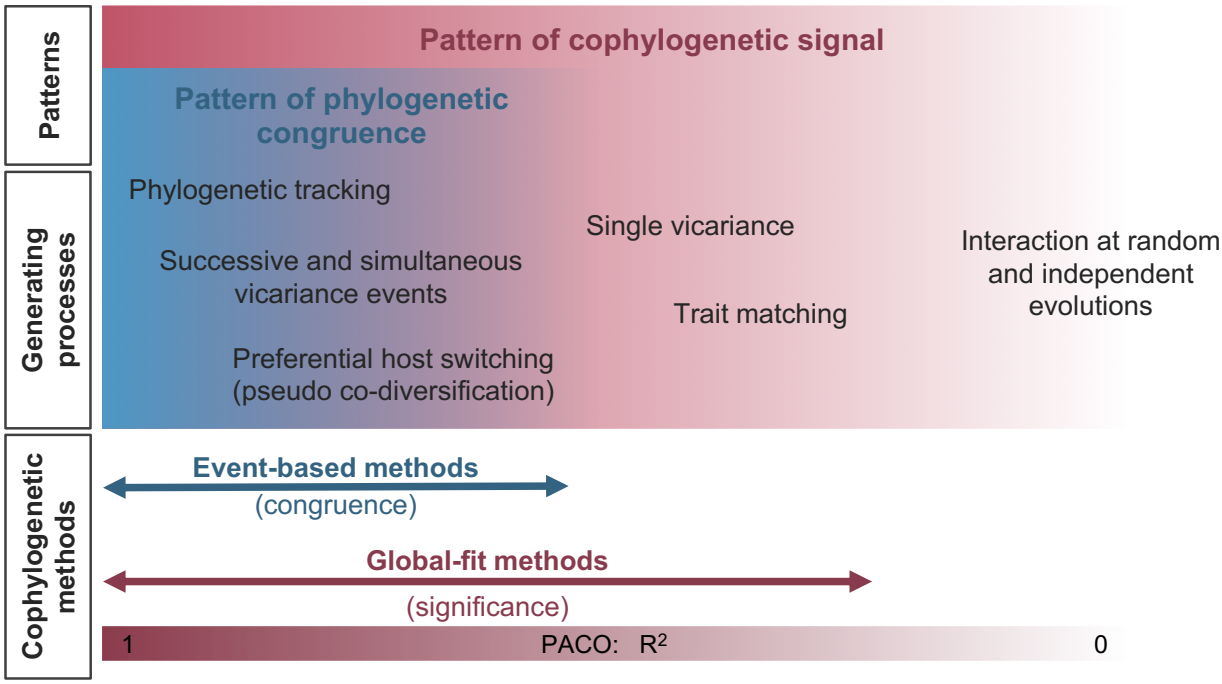
404

405 Using simulations, we have assessed the ability of different cophylogenetic
406 methods to distinguish phylogenetic congruence from cophylogenetic signal. The
407 distinction is important, as these patterns are indicative of different processes,
408 phylogenetic congruence revealing more intricate evolutionary histories between
409 hosts and symbionts than cophylogenetic signal (Fig. 4). We have shown that global-
410 fit methods typically return significant results as soon as there is cophylogenetic signal
411 in species interactions, including in the absence of phylogenetic congruence, meaning
412 that they cannot distinguish the two patterns. In contrast, event-based methods can be
413 used to specifically detect phylogenetic congruence.

414

415 **Figure 3: Patterns of cophylogenetic signal and phylogenetic congruence can be**
416 **generated by various processes:**
417 Event-based and global-fit methods differently measure these patterns: Event-based
418 methods can robustly identify phylogenetic congruence, whereas global-fit methods
419 measure cophylogenetic signal. Some of the statistics of global-fit methods (e.g. the R^2
420 of PACo) can inform whether the cophylogenetic signal may be due to phylogenetic
421 congruence: a low R^2 ($R^2<0.25$) indicates that there is a cophylogenetic signal but no
422 phylogenetic congruence, whereas a higher R^2 ($R^2>0.25$) suggests there is a
423 cophylogenetic signal and potentially also phylogenetic congruence, but the latter
424 needs to be validated using eMPRess, as $R^2>0.25$ also frequently occur in systems
425 without phylogenetic congruence (Fig. 3).

426



Given that global-fit methods detect cophylogenetic signal rather than phylogenetic congruence, Mantel tests measuring phylogenetic signal in species interactions could be used in place of these methods (Perez-Lamarque et al. 2022b). It would be useful, in the future, to compare the behavior of Mantel tests to global-fit approaches. Regarding event-based methods other than eMPRess, such as TreeMap (Page 1994a, 1995), TreeFitter (Ronquist 2003a), or Jane (Conow et al. 2010), we expect them to have similar behaviors given that they are also cost-based and use maximum parsimony. There are however notable differences between them; for example, eMPRess (like TreeMap or Jane) sets a null cost to cospeciation events, therefore implicitly favoring cospeciation over other reconciliation events (host transfers, intra-host duplication, and symbiont loss), while TreeFitter (Ronquist 2003a) allows setting a positive cost to cospeciation events. Given that cospeciation events may not be that frequent in nature (Ronquist 1995), TreeFitter is probably less likely to overestimate cospeciation events in the reconciliation. More sophisticated probabilistic methods, such as the version of the amalgamated likelihood estimation (ALE) that considers branching orders in addition to tree topology (Szöllősi et al. 2013), may also perform better as it would provide only time-consistent reconciliations (Maestri et al. 2023).

446

447 Several recent studies have interpreted significant results of global-fit
448 approaches as evidence for phylogenetic congruence and signs of codiversification
449 (*e.g.* Fuzessy et al. 2022; Suzuki et al. 2022). Our findings suggest that there is evidence
450 for cophylogenetic signal in the studied systems, which is already insightful in itself,
451 but that little can be said about phylogenetic congruence before event-based methods
452 are applied. Applying event-based methods could drastically change the biological
453 conclusions that have been drawn.

454

455 Our results suggest that the complementary use of PACo (fast and easy to run)
456 and eMPRess (more computationally intensive but more informative) can be most
457 useful for analyzing cophylogenetic data, with a careful interpretation of the results
458 (Fig. 4). We recommend to begin by using PACo; if the test is not significant, there is
459 neither cophylogenetic signal nor phylogenetic congruence in the data, suggesting
460 independent evolution. If the PACo test is significant but with a low R^2 ($R^2<0.25$), there
461 is a cophylogenetic signal but no congruence, suggesting that evolutionary history
462 played a role in shaping present-day interactions, but that there was not a strong co-
463 dependency during the evolutionary history of the two clades. If the PACo test is
464 significant with a higher R^2 ($R^2>0.25$), there is a cophylogenetic signal, and potentially
465 also phylogenetic congruence, but the latter needs to be validated using eMPRess, as
466 $R^2>0.25$ also frequently occur in systems without phylogenetic congruence. Before
467 running eMPRess, if some symbiont species interact with several host species, we
468 recommend randomly sampling one host species per symbiont (rather than, for
469 example, generating random bifurcations in the symbiont tree, which seems to foster
470 false positives). If the eMPRess test supports phylogenetic congruence (significant
471 reconciliation with a larger number of cospeciation events compared with the number
472 of host transfers), this suggests a strong co-dependency during the evolutionary
473 history of the two clades, such as phylogenetic tracking, successive vicariance events,
474 or preferential host switching. As eMPRess uses only the tree topologies and not the

branch lengths, no conclusion can be drawn about the concomitance of divergence times in the host and symbionts trees. Additional analyses are thus needed to be able to distinguish phylogenetic congruence with concomitant divergence times (pattern of codiversification arising, *e.g.* from phylogenetic tracking or successive vicariance events) from phylogenetic congruence with non-concomitant divergence times (pattern of pseudo-codiversification arising, *e.g.* from preferential host switching; Ronquist 2003b; de Vienne et al. 2013). One possibility is to check whether eMPRess reconciliations include time-inconsistent host transfers (*i.e.* "back-in-time" transfers between non-contemporary host lineages; Maestri et al. 2023), which would suggest pseudo-codiversification.

An advantage of global-fit methods is that their hypothesis testing is rather flexible. One can easily imagine designing more constrained randomization strategies, for example, to specifically test the influence of biogeography or trait matching on the observed cophylogenetic signal (Perez-Lamarque and Morlon 2023). In parallel, data augmentation and other machine-learning techniques may allow overriding the computational bottleneck that limits the implementation of more complex process-based models. Altogether, such advancements will facilitate linking patterns to processes in cophylogenetic systems.

Concluding remarks:

To conclude, our results imply that using global-fit methods alone is not sufficient to robustly assess a pattern of phylogenetic congruence. In a given cophylogenetic system, finding both significant global-fit tests and significant reconciliations using event-based approaches suggests that there is a pattern of phylogenetic congruence that can be linked to various processes such as phylogenetic tracking, successive vicariance events, or pseudo-codiversification. In contrast, finding significant global-fit tests but no significant reconciliations with event-based

approaches suggests that phylogenetic congruence is unlikely; the cophylogenetic signal in this system may rather emerge from processes such as trait matching or biogeographical contingency, but not from phylogenetic tracking or pseudo-codiversification. Clearly distinguishing patterns of cophylogenetic signal and phylogenetic congruence and carefully interpreting outputs of cophylogenetic methods are key if we are to understand the processes that shape present-day communities.

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Supplementary Information:

Supplementary information can be found on DRYAD: 10.5061/dryad.7wm37pvzn (temporary link: https://datadryad.org/stash/share/u1-stLek8-xsOgCKnCdGzPXl6KaQopUuY3h1NNOfCeI)

Code availability:

All the codes for generating the simulations and performing the analyses are available through the following link:
https://github.com/BPerezLamarque/Scripts/tree/master/Cophylogenetic_signal.
Upon publication, all the codes for generating the simulations and performing the analyses will be available on DRYAD: https://doi.org/10.5061/dryad.7wm37pvzn (temporary link: https://datadryad.org/stash/share/u1-stLek8-xsOgCKnCdGzPXl6KaQopUuY3h1NNOfCeI)

Conflicts of interests:

The authors declare no conflict of interests.

Author contributions:

BPL and HM designed the study, BPL performed the analyses, and both authors wrote the manuscript.

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