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

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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. 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.

Introduction:

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

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

symbiont, two patterns are often investigated (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, 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. In this case, patterns of cophylogenetic signal and phylogenetic congruence tend to collapse, such that they can be studied interchangeably. In this context, cophylogenetic methods have been developed to test for phylogenetic congruence using linear algebra technics, 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 (Bascompte and Jordano 2013; Perez-Lamarque et al. 2022b), and the same methods have been regularly applied to such systems, and interpreted as tests of phylogenetic congruence (see *e.g.* Fuzessy et al. 2022; Suetsugu et al. 2022; Suzuki et al. 2022 for recent examples). If, under these situations, these methods in fact 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 if the diversification of vertically transmitted symbionts tracks host diversification (Fig. 1c – "phylogenetic

tracking"), if symbionts diversify by preferentially switching to closely-related host species ("pseudo-codiversification"), or if the diversifications of the two clades were subject to the same succession of vicariance events (de Vienne et al. 2013; Althoff et al. 2014). Phylogenetic congruence implies cophylogenetic signal, but the reverse is not true.

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 event-based methods, such as TreeMap (Page 1994) 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. We have shown before, in a different context, that global-fit and event-based methods can sometimes output contrasting results (Perez-Lamarque and Morlon 2022), 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, 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.

138 **Simulations of three biologically realistic scenarios:**

139

140 In the first simulated scenario, we assumed that present-day host-symbiont
141 interactions are more likely between species having complementary traits following a
142 trait-matching expression with a unidimensional continuous trait (Fig. 1a – "trait
143 matching"; Supplementary Methods 1). We independently simulated two
144 phylogenetic trees for the host and symbiont clades using a birth-death model and on
145 each tree, we then independently simulated the evolution of traits modulating present-
146 day interactions. By using a Brownian motion for trait evolution, closely-related hosts
147 (resp. symbionts) tend to have similar trait values (*i.e.* phylogenetic signal in species
148 traits). Finally, we assumed that the degree of specialization of the symbionts (*i.e.* the
149 number of hosts that a given symbiont interacts with) follows a Poisson distribution
150 and attributed the present-day host-symbiont interacting pairs following a trait-
151 matching expression. As a result, closely related host species interact with closely
152 related symbiont species (*i.e.* cophylogenetic signal), although the phylogenetic trees
153 were simulated independently and are therefore not congruent. In the second
154 simulated scenario, we assumed that host and symbiont species interact at random as
155 long as they occupy the same biogeographic area (Fig. 1b – "vicariance"). At first, all
156 hosts and symbionts simultaneously occupy a single area and diversify
157 independently, until a vicariance event splits the area into three separate areas. Half
158 of the host and symbiont species are then isolated in separate areas; the other half
159 experiences allopatric speciation as their population is split into two. Following the
160 vicariance event, each host and symbiont lineage diversifies independently in its area,
161 resulting in a phylogenetic signal in biogeographic repartition (*i.e.* closely related
162 species tend to occupy the same area). Although the host and symbiont diversifications
163 are not independent (they both experienced a burst of speciation events at the time of
164 vicariance), the host and symbiont phylogenetic trees are not congruent as they
165 experienced different events of diversification before and after the vicariance (Fig. 1b).
166 Finally, we assumed that the degree of specialization of the symbionts follows a

167 Poisson distribution and randomly attributed host-symbiont present-day interactions
168 within each area. This scenario thus generates cophylogenetic signal but no
169 phylogenetic congruence. In the third simulated scenario, we assumed that the
170 symbiont diversification tracks the host diversification: symbionts species are
171 vertically transmitted over long-time scales among hosts lineages and cospeciate at
172 host speciation events resulting in codiversification (Fig. 1c – "phylogenetic tracking").
173 In addition, we assumed that symbiont lineages experience host transfers and intra-
174 host duplication, and that host lineages can lose their symbionts. The latter processes
175 dampen but maintain the phylogenetic congruence between host and symbiont
176 phylogenies, rendering it more realistic. This third scenario generates both
177 cophylogenetic signal and phylogenetic congruence. For each scenario, we performed
178 1,000 simulations with different host and symbiont species richness values
179 (Supplementary Fig. S1). As expected, our simulations under phylogenetic tracking
180 produce systems with a much higher proportion of one-to-one interactions than
181 simulations under trait matching and vicariance (Supplementary Fig. S2).
182
183 **Outputs of cophylogenetic methods:**
184
185 For each simulated cophylogenetic data, we first applied the global-fit methods
186 ParaFit and PACo (Ref; Supplementary Methods 2). We measured the strength of the
187 cophylogenetic signal using the "ParaFit global statistic" in ParaFit, whereas, in PACo,
188 we used $R^2 = 1 - m^2$, where m^2 is the sum of the squared residuals of the symmetric
189 procrustean superposition (Blasco-Costa et al. 2021). R^2 is comprised between 0 and 1,
190 and R^2 close to 0 (resp. 1) indicates low (resp. high) cophylogenetic signal. Following
191 Legendre et al. (2002) and Hutchinson et al. (2017), the significance of the ParaFit and
192 PACo tests was evaluated by independently shuffling which host species are
193 associated with each symbiont species (hereafter referred to as "null model 1"). For
194 ParaFit, we found significant tests in 39% of the trait-matching simulations, 52% of the
195 vicariance simulations, and 99% of the phylogenetic tracking (Table 1). Significant tests

196 for PACo were even more frequent, reaching 50%, 68%, and 100% of the simulations
197 in the three scenarios, respectively (Table 1). Qualitatively similar results were
198 obtained when evaluating the significance by shuffling the host species labels
199 (hereafter referred to as "null model 2"; Supplementary Table S1). Global-fit methods
200 thus tend to measure cophylogenetic signal in general rather than specifically
201 phylogenetic congruence. Corroborating this result, we found that, under scenarios of
202 trait matching or vicariance, global-fit tests are more likely to be significant when there
203 are frequent many-to-many host-symbiont interactions (Supplementary Table S2;
204 Supplementary Figs. S3 & S4; generalized linear models (GLM): p-values<0.05), while
205 we would expect the contrary if these tests measured phylogenetic congruence. As
206 expected, global-fit tests were more often significant in simulations with more
207 interacting species (Supplementary Table S3, Supplementary Figs. S3 & S4).
208
209 Second, we applied the event-based method eMPRess to each simulation.
210 eMPRess reconciles the host and symbiont tree topologies by fitting, using maximum
211 parsimony, events of host transfers, duplications, and losses, each event being
212 associated with a given cost. We chose eMPRess over all the other existing event-based
213 methods, as it can be automatically and rapidly run using the command line. As with
214 most event-based methods, eMPRess does not handle symbiont species that interact
215 with multiple hosts (Dismukes et al. 2022). However, symbionts often interact with
216 several hosts in nature, and also in our simulated scenarios of trait matching and
217 vicariance. We thus tested two strategies for running eMPRess: (i) subsampling one
218 host at random per symbiont species (Su et al. 2022) or (ii) randomly generating
219 bifurcating sub-trees for symbionts with multiple hosts, such that each tip in the
220 symbiont tree is associated with a single host (Satler et al. 2019). A reconciliation is
221 considered significant if its total cost is lower than 95% of the costs of reconciliations
222 obtained after shuffling the host species labels ("null model 2"). Phylogenetic trees are
223 considered congruent if the reconciliation estimates less host transfer than
224 cospeciation events (Groussin et al. 2017; Perez-Lamarque and Morlon 2022). When

225 subsampling one host per symbiont species, the eMPRess reconciliation was
226 significant in only 5% of the trait-matching simulations and 16% of the vicariance
227 simulations, and congruent in none (Table 1). When generating random bifurcations
228 in the symbiont tree, it was significant in 34% and 80% of the trait-matching and
229 vicariance simulations, respectively, but congruent in none (Table 1). Indeed, eMPRess
230 estimated on average 5 times more host transfers than cospeciations for both scenarios
231 of trait matching and vicariance (Table 1; Supplementary Fig. S5). In contrast, when
232 simulating phylogenetic tracking, eMPRess gave significant reconciliations in 100% of
233 the simulations, 92% of which were congruent (Table 1). Results were qualitatively
234 similar when choosing alternative event costs (Supplementary Tables S4, S5, & S6).
235 Among all existing event-based methods, we only tried eMPRess for computational
236 reasons. Yet, many of these other methods, e.g. TreeMap or Jane (Page 1994; Conow et
237 al. 2010), are also cost-based and use maximum parsimony, so we suspect that they
238 would behave as eMPRess. More sophisticated methods, such as the version of the
239 amalgamated likelihood estimation (ALE) that considers branching orders in addition
240 to tree topology (Szöllősi et al. 2013), may perform even better than eMPRess as it
241 would provide only time-consistent reconciliations (Maestri et al. 2023). Overall, our
242 findings suggest that event-based methods can specifically detect patterns of
243 phylogenetic congruence and distinguish them from a "simple" cophylogenetic signal.
244

245 We investigated whether the effect size of the global-fit methods, *i.e.* values of
246 the ParaFit global statistic and PACo's R^2, can be used as indicators of phylogenetic
247 congruence (Blasco-Costa et al. 2021). The ParaFit global statistic varies with the total
248 number of species, and is thus difficult to interpret in itself; it cannot be used to
249 distinguish phylogenetic congruence from cophylogenetic signal (Fig. 2;
250 Supplementary Fig. S3). In contrast, for PACo, we found that R^2 is generally higher
251 than 0.25 when there is a pattern of phylogenetic congruence (in 99% of the scenario
252 of phylogenetic tracking), whereas it is lower than 0.5 when there is a pattern of
253 cophylogenetic signal alone (in 99% of the scenarios of trait-matching or vicariance;

254 Fig. 2). Hence, a significant test with $R^2 > 0.50$ is strong support for phylogenetic
255 congruence (although not definite evidence, it may be possible to obtain higher R^2
256 values under scenarios without congruence with different simulation choices),
257 whereas a significant test with $R^2 < 0.25$ suggests that the system presents a
258 cophylogenetic signal without phylogenetic congruence. A significant test with R^2
259 values between 0.25 and 0.50 are harder to interpret, as R^2 tends to correlate positively
260 with the ratio of one-to-one interactions and R^2 values above 0.25 are sometimes
261 reported in simulations without phylogenetic congruence (Supplementary Fig. S4).
262 PACo alone is therefore often not sufficient to identify a pattern of phylogenetic
263 congruence.
264
265 **Concluding remarks:**
266
267 Using simulations, we have assessed the ability of different cophylogenetic
268 methods to distinguish phylogenetic congruence from cophylogenetic signal. The
269 distinction is important, as these patterns are indicative of different processes,
270 phylogenetic congruence revealing more intricate evolutionary histories between
271 hosts and symbionts than cophylogenetic signal (Fig. 3). We have shown that global-
272 fit methods typically return significant results as soon as there is cophylogenetic signal
273 in species interactions, including in the absence of phylogenetic congruence, meaning
274 that they cannot distinguish the two patterns. In contrast, event-based methods can be
275 used to specifically detect phylogenetic congruence.
276
277 Several recent studies have interpreted significant results of global-fit
278 approaches as evidence for phylogenetic congruence and interpreted them as signs of
279 codiversification (*e.g.* Fuzessy et al. 2022; Suetsugu et al. 2022; Suzuki et al. 2022). Our
280 findings suggest that there is evidence for cophylogenetic signal in the studied
281 systems, which is already insightful in itself, but that little can be said about
282 phylogenetic congruence and codiversification before event-based methods are

283 applied. Applying event-based methods could drastically change the biological
284 conclusions that have been drawn.
285
286 Our results suggest that the complementary use of PACo (fast and easy to run)
287 and eMPRess (more computationally intensive but more informative) can be most
288 useful for analyzing cophylogenetic data, with a careful interpretation of the results
289 (Fig. 3). One could begin by using PACo; if the test is not significant, there is neither
290 cophylogenetic signal nor phylogenetic congruence in the data, suggesting
291 independent evolution. If the PACo test is significant but with a low R^2 ($R^2<0.25$), there
292 is a cophylogenetic signal but no congruence, suggesting that evolutionary history
293 played a role in shaping present-day interactions, but that there was not a strong co-
294 dependency during the evolutionary history of the two clades. If the PACo test is
295 significant with a higher R^2 ($R^2>0.25$), there is a cophylogenetic signal, and potentially
296 also phylogenetic congruence, but the latter needs to be validated using eMPRess, as
297 $R^2>0.25$ also frequently occur in systems without phylogenetic congruence. Before
298 running eMPRess, if some symbiont species interact with several host species, we
299 recommend randomly sampling one host species per symbiont (rather than, for
300 example, generating random bifurcations in the symbiont tree, which seems to foster
301 false positives). If the eMPRess test supports phylogenetic congruence (significant
302 reconciliation with a larger number of cospeciation events compared with the number
303 of host transfers), this suggests a strong co-dependency during the evolutionary
304 history of the two clades, such as phylogenetic tracking, successive vicariance events,
305 or pseudo-codiversification.
306
307 An advantage of global-fit methods is that their hypothesis testing is rather
308 flexible. One can easily imagine designing more constrained randomization strategies,
309 for example, to specifically test the influence of biogeography or trait matching on the
310 observed cophylogenetic signal (Perez-Lamarque and Morlon 2022). In parallel, data
311 augmentation and other machine-learning technics 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.

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 vicariance, 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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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.

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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435 **Box 1: Definitions**

436

Host-symbiont bipartite interaction network: A network representing which symbiont species interact with which host, here encoded by a matrix with host species in rows and symbiont species in columns, and 1/0 representing the presence/absence of interaction, respectively.

Codiversification/Co-cladogenesis: Pattern of concomitant diversification events happening in both host and symbiont clades. Codiversification can occur due to processes of phylogenetic tracking or successive vicariance events affecting both clades.

Coevolution: Process of reciprocal evolutionary changes induced by reciprocal selective pressures in two (or more) interacting lineages.

Cophylogenetic signal: Pattern depicting the tendency of closely related species to interact with closely related partners.

Cophylogenetics: Study of the link between the host and symbiont evolutionary histories and extant interactions.

Cospeciation: Concomitant event of host and symbiont speciations.

Degree of specialization: Number of species interacting with a given focal species.

Event-based methods: Cophylogenetic methods reconciling the host and symbiont phylogenies by fitting reconciliation events (e.g. cospeciation, host transfer, duplication, or loss) on the symbiont phylogeny.

Global-fit methods: Cophylogenetic methods evaluating the presence of cophylogenetic signal in a bipartite network.

Intra-host duplications: Process of symbiont speciation within a host lineage.

Host transfer (syn. host switch, host shift): Process of transmission of a symbiont from a donor host to a receiver host.

Phylogenetic congruence: Pattern of high similarity of the phylogenetic trees of interacting host and symbiont clades in terms of topology and relative branch

lengths. If host and symbiont divergence times are matching, phylogenetic congruence can correspond to codiversification.

Phylogenetic signal: Pattern depicting the tendency of closely related species to have similar traits.

Phylogenetic tracking: Process of host (resp. symbiont) speciations generating subsequent symbiont (resp. host) speciations (e.g. because of vertical transmission that isolates symbiont populations between the two daughter host lineages). Alternatively, symbionts can experience events of host transfers, intra-host duplication, or loss. Phylogenetic tracking usually generates a pattern of codiversification.

Preferential host switching: Tendency of symbionts to experience host transfers toward closely related host species; when the transfer results in a speciation event in the symbiont lineage, this tends to generate phylogenetic congruence. This does not imply co-diversification though, as the divergence times of the symbionts may be much more recent than those of the hosts. We specifically refer to this resulting pattern as **pseudo-codiversification.**

Symbiont loss: Extinction of a symbiont in a given host lineage.

Tree topology: Branching pattern of the different nodes of a phylogenetic tree that does not consider branch lengths (*i.e.* evolutionary time).

Vertical transmission: Process of symbiont inheritance from host generation to host generation (at the level of individual or at the level of the whole lineage). Over long timescales, it can generate phylogenetic tracking.

Vicariance: Process of biogeographic changes leading to the isolation of populations and subsequent allopatric speciation. By simultaneously affecting hosts and symbionts, vicariance can lead to cophylogenetic signal, and potentially phylogenetic congruence if events of vicariance occur repetitively.

437

438 **Table 1: ParaFit and PACo (global-fit methods) are often significant in all simulated**
439 **scenarios, including scenarios of trait matching and vicariance that do not generate**
440 **phylogenetic congruence, whereas eMPRess (an event-based method) supports**
441 **phylogenetic congruence only under phylogenetic tracking which generates**
442 **phylogenetic congruence:**
443 The table indicates the percentages of simulations for which ParaFit, PACo, or
444 eMPRess output a significant test, under three scenarios (Fig. 1): (a) present-day
445 interactions dictated by trait matching, (b) present-day interactions at random
446 following a single vicariance event, or (c) present-day interactions resulting from
447 phylogenetic tracking. For eMPRess, we report the percentage of significant
448 reconciliations based on permutations alone (P) or based on selecting among these the
449 reconciliations that have more cospeciation than host transfer events (P+C). We
450 consider eMPRess to support phylogenetic congruence when conditions P and C are
451 met (in bold). Results by clade size are given in Supplementary Tables S1-S6. eMPRess
452 results correspond to host-symbiont reconciliations ran with relative costs d=4, t=1,
453 and l=1 for duplications, host transfers, and losses, respectively; other costs
454 combinations provided qualitatively similar results – see Supplementary Tables S4-
455 S6).

456

Methods		(a) Trait matching	(b) Vicariance	(c) Phylogenetic tracking
Global-fit methods	**Percentage of significant tests using ParaFit**	39%	52%	99%
	Percentage of significant tests using PACo	50%	68%	100%
Event-based methods	**Percentage of significant tests using eMPRess with one host per symbiont**	P: 5% **P+C: 0%**	P: 16% **P+C: 0%**	P: 100% **P+C: 92%**
	Percentage of significant tests using eMPRess with random bifurcations	P: 34% **P+C: 0%**	P: 80% **P+C: 0%**	

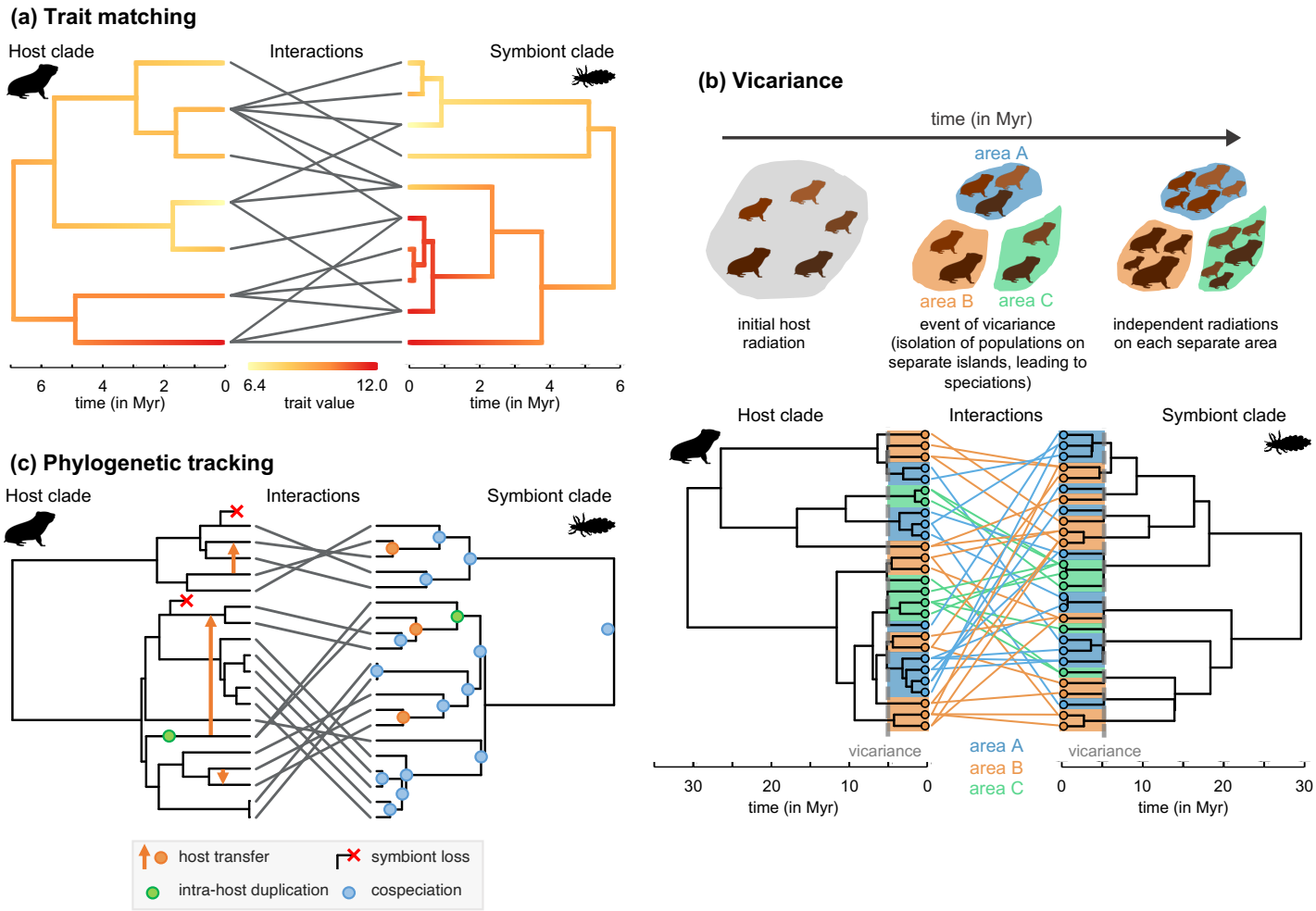
457

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.

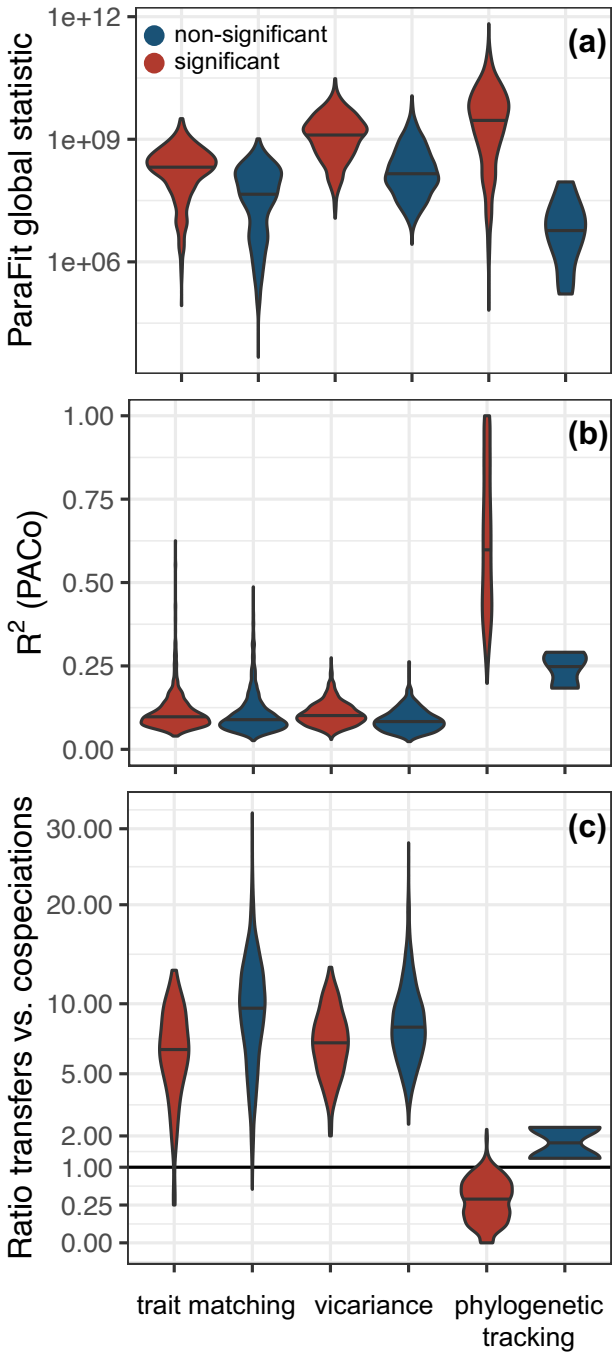
(a) Trait matching. Here host and symbiont phylogenetic trees evolve independently. Traits also evolve independently on each phylogeny following Brownian motion processes, which results in a phylogenetic signal in species traits (i.e. closely related species tend to have similar trait values, see the 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 an event of vicariance 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 migration, 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 cospeciate at host speciation events resulting in codiversification. In addition, symbiont lineages can experience horizontal host transfers (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.

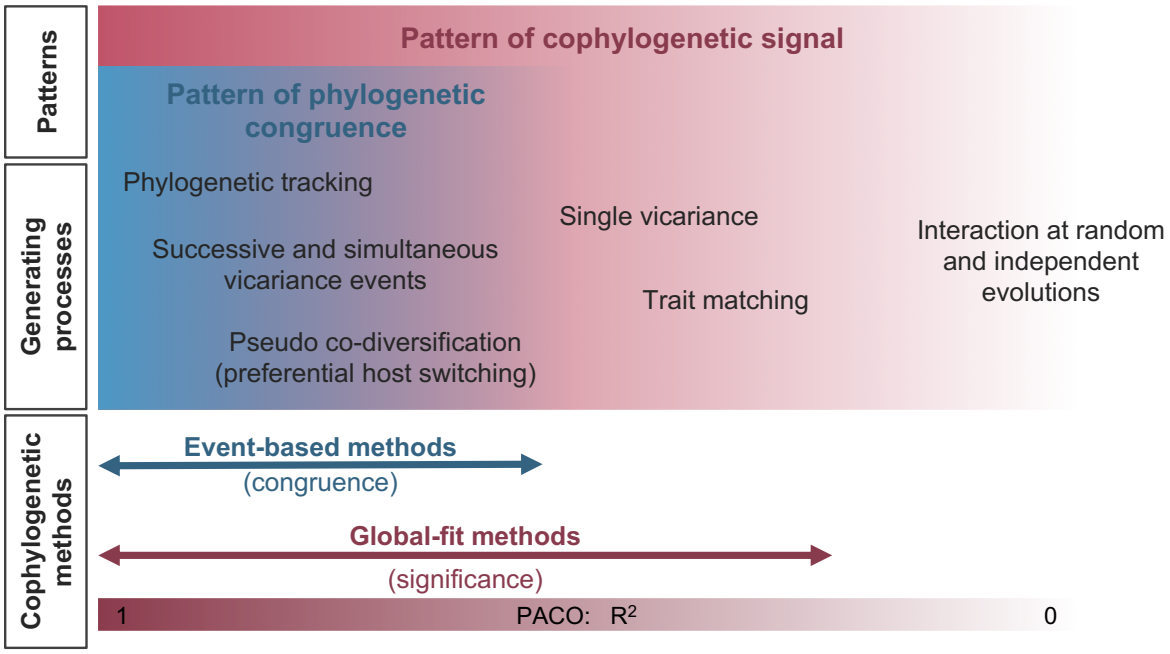

(a) Trait matching
Host clade
Interactions
Symbiont clade
6
4
2
0
time (in Myr)
6.4
12.0
trait value
0
2
4
6
time (in Myr)
(b) Vicariance
time (in Myr)
area A
area B
area C
initial host radiation
event of vicariance (isolation of populations on separate islands, leading to speciations)
independent radiations on each separate area
Host clade
Interactions
Symbiont clade
vicariance
vicariance
area A
area B
area C
30
20
10
0
time (in Myr)
0
10
20
30
time (in Myr)
(c) Phylogenetic tracking
Host clade
Interactions
Symbiont clade
host transfer
symbiont loss
intra-host duplication
cospeciation

485 **Figure 2: eMPRess correctly distinguishes patterns of phylogenetic congruence**
486 **(scenario of phylogenetic tracking) from cophylogenetic signal alone (scenarios of**
487 **trait matching and vicariance), whereas ParaFit cannot and PACo can only in some**
488 **cases:**
489 Distribution of ParaFit global statistics (a), PACo's R^2 (b), and the ratio between the
490 number of host transfers and the number of cospeciations in eMPRess reconciliations
491 (c) as a function of the simulation scenario and the test significance. Here, we only
492 reported eMPRess reconciliations obtained when subsampling one host per symbiont
493 species, but similar results were observed when using random bifurcations
494 (Supplementary Fig. S5).


non-significant
significant
(a)
ParaFit global statistic
1e+12
1e+09
1e+06
(b)
R2 (PACo)
1.00
0.75
0.50
0.25
0.00
(c)
Ratio transfers vs. cospeciations
30.00
20.00
10.00
5.00
2.00
1.00
0.25
0.00
trait matching
vicariance
phylogenetic tracking

495

496 **Figure 3: Patterns of cophylogenetic signal and phylogenetic congruence can be**
497 **generated by various processes:**
498 Event-based and global-fit methods differently measure these patterns: Event-based
499 methods can robustly identify phylogenetic congruence, whereas global-fit methods
500 measure cophylogenetic signal. Some of the statistics of global-fit methods (e.g. the R^2
501 of PACo) can inform whether the cophylogenetic signal may be due to phylogenetic
502 congruence: a low R^2 (R^2<0.25) indicates that there is a cophylogenetic signal but no
503 phylogenetic congruence, whereas a higher R^2 (R^2>0.25) suggests there is a
504 cophylogenetic signal and potentially also phylogenetic congruence, but the latter
505 needs to be validated using eMPRess, as R^2>0.25 also frequently occur in systems
506 without phylogenetic congruence (Figure 2).
507



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