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MACROEVOLUTIONARY PERSPECTIVES ON BIOTIC INTERACTIONS

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Species diversification and phylogenetically constrained symbiont switching generated high modularity in the lichen genus *Peltigera*

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Abstract

1. Ecological interactions range from purely specialized to extremely generalized in nature. Recent research has showed very high levels of specialization in the cyanolichens involving *Peltigera* (mycobionts) and their *Nostoc* photosynthetic partners (cyanobionts). Yet, little is known about the mechanisms contributing to the establishment and maintenance of such high specialization levels.
2. Here, we characterized interactions between *Peltigera* and *Nostoc* partners at a global scale, using more than one thousand thalli. We used tools from network theory, community phylogenetics and biogeographical history reconstruction to evaluate how these symbiotic interactions may have evolved.
3. After splitting the interaction matrix into modules of preferentially interacting partners, we evaluated how module membership might have evolved along the mycobionts' phylogeny. We also teased apart the contributions of geographical overlap vs phylogeny in driving interaction establishment between *Peltigera* and *Nostoc* taxa.
4. Module affiliation rarely evolves through the splitting of large ancestral modules. Instead, new modules appear to emerge independently, which is often associated with a fungal speciation event. We also found strong phylogenetic signal in these interactions, which suggests that partner switching is constrained by conserved traits. Therefore, it seems that a high rate of fungal diversification following a switch to a new cyanobiont can lead to the formation of large modules, with cyanobionts associating with multiple closely retated *Peltigera* species.
5. Finally, when restricting our analyses to *Peltigera* sister species, the latter differed more through partner acquisition/loss than replacement (i.e., switching). This pattern vanishes as we look at sister species that have diverged longer ago. This suggests that fungal speciation may be accompanied by a stepwise process of (a) novel partner acquisition and (b) loss of the ancestral partner. This could explain the maintenance of high specialization levels in this symbiotic system where the transmission of the cyanobiont to the next generation is assumed to be predominantly horizontal.

6. *Synthesis*. Overall, our study suggests that oscillation between generalization and ancestral partner loss may maintain high specialization within the lichen genus *Peltigera*, and that partner selection is not only driven by partners' geographical overlap, but also by their phylogenetically conserved traits.

KEYWORDS
biogeography, community phylogenetics, cyanolichens, ecological networks, macroevolution, modularity, specificity, symbiotic history reconstruction

1 | INTRODUCTION

Species neither live nor evolve in isolation. Rather, they enter complex webs, or networks, of interactions (Thompson, 2006). The structure of these networks is expected to drive co-evolution (Guimarães, Pires, Jordano, Bascompte, & Thompson, 2017), populations' stability (May, 1974; Melian & Bascompte, 2002; Pimm, 1979), species coexistence (Bastolla et al., 2009) and community productivity (Poisot, Mouquet, & Gravel, 2013). A major frontier in community ecology is thus to elucidate the drivers of the complex and repeatable patterns, or motifs, that we observe in ecological networks. These drivers can include phenotypic compatibility/complementarity (Eklöf et al., 2013; Junker et al., 2013; Maglianesi, Blüthgen, Böhning-Gaese, & Schleuning, 2014), phenological overlap (Burkle, Marlin, & Knight, 2013; Olesen, Bascompte, Elberling, & Jordano, 2008), or spatial co-occurrence patterns (Bell, Andrew King, Bohan, & Symondson, 2010; Chagnon, Bradley, & Klironomos, 2015). Network structure can also arise from the way new 'immigrant' species connect to the others when they join a network (Maynard, Serván, & Allesina, 2018). For example, it has long been known that preferential attachment of immigrants to already well-connected species (i.e., generalists) will generate both a power-law degree distribution (Barabási & Albert, 1999; Krapivsky & Redner, 2001) and a nested structure in bipartite networks (Medan et al., 2007). Such complexity in network assembly makes it a great challenge to identify the major ecological variables responsible for the establishment, or the avoidance of interactions in ecological networks.

One useful approach to deal with such complexity is to use phylogenies as a way of reducing dimensionality in network studies. In other words, we may not need to capture the myriads of traits (i.e., all the dimensions) responsible for the establishment of interactions between some species pairs but not others: phylogenies might capture sufficient information to make sense of ecological interaction patterns. Indeed, Rossberg, Brannstrom, and Dieckmann (2010) have shown that provided sufficient phylogenetic conservatism of traits, food-web (FW) structure can be synthesized into a single dimension using phylogenetic distance as a proxy for likelihood of establishing, or not, an interaction. In line with this theoretical finding, many empirical studies have found phylogenetic signal in FW structure (Bersier & Kehrli, 2008; Eklöf, Helmus, Moore, & Allesina, 2012; Mouillot, Krasnov, & Poulin, 2008; Rezende, Albert, Fortuna, & Bascompte, 2009), as well as in other types of ecological networks (Chagnon et al., 2015; Donatti et al., 2011; Jacquemyn et al., 2011). Likewise, community phylogenetics has been also widely used in ecology to tease apart deterministic (niche-based) versus stochastic (neutral) mechanisms driving community assembly (e.g., Kembel, 2009, Swenson & Enquist, 2009; Mayfield & Levine, 2010) or to assess potential consequences of species coexistence on macroevolutionary trends (Gerhold, Cahill, Winter, Bartish, & Prinzing, 2015). However, community phylogenetic approaches (either correlating phylogenetic distance with the propensity of coexisting locally, or of sharing a given partner or prey) only map the imprint of phylogeny on current observed patterns, without informing about the evolution of such patterns.

Disentangling how evolutionary mechanisms shape interaction networks through time is a relatively new research avenue. For example, Nuismer, Jordano, and Bascompte (2013) used a co-evolution model to show that when interactions are mediated by phenotype matching, networks should evolve an anti-nested structure, characterized by small subsets of species interacting together if their phenotype is compatible; that is, there could not be a super-generalist taxon, characteristic of a nested architecture. On the other hand, an interaction mediated by any form of threshold (e.g., a predator eating any prey with a smaller body size than its own body size) should evolve a nested architecture. For example, frugivorous birds have been found by Burns (2013) to eat roughly any fruit smaller than their beak in a New Zealand forest. Of course, this simplistic model omits all the other non-evolutionary constraints that may drive network structure (e.g., species encounter rates based on their spatio-temporal distributions), and also omits other potentially relevant evolutionary factors as well, such as cospeciation, heritability of symbionts through vertical transmission, etc. Host-microbiome studies have started to explore such questions tracking the evolution of symbiotic interaction patterns over broad phylogenetic scales (e.g., Sanders et al., 2014). Phylogenetic inference tools have recently been used by Groussin et al. (2017) to evaluate the relative importance of cospeciation and host switch in determining the evolution of mammalian gut microbiome composition. As in other types of networks, strong phylogenetic conservatism has been shown to drive partner acquisition (Ochman et al., 2010), leading to considerable debate regarding the relative importance of vertical versus horizontal transmission of symbionts (Moran & Sloan, 2015; Refrégier et al., 2008).

In this study, we looked at interaction patterns in cyanolichens involving *Peltigera* (Lecanoromycetes, Ascomycota), which are

lichen-forming fungi and *Nostoc*, their photosynthetic and nitrogen-fixing (Darnajoux et al., 2017) cyanobacterial symbionts. This symbiotic association has the particularity to show high levels of partner specificity and modularity, at least for the section *Polydactylon* (Chagnon, Magain, Miadlikowska, & Lutzoni, 2018). These lichen-forming fungi (also referred to as mycobionts) are typically highly specialized on one or very few cyanobacterial phylogroup partners (also referred to as cyanobionts) that commonly associate with multiple *Peltigera* species (asymmetric specificity). The evolution of specialization has fuelled a large body of literature in ecology and evolution (Fisher, 1930; Levins, 1968; MacArthur, 1955; May, 1974) and one question that might be asked when looking at current patterns of specialization is whether selection towards such patterns was directional or stabilizing: in other words, is there a directional evolution towards specialization (Jaenike, 1990) or generalization (Waser, Chittka, Price, Williams, & Ollerton, 1996) or a combination of both, that is, specialization of *Peltigera* and generalization of *Nostoc*, which could be advantageous especially when symbionts are transmitted mostly horizontally (Chagnon et al., 2018; Lu et al., 2018; Lutzoni & Miadlikowska, 2009; Magain, Miadlikowska, Goffinet, Sérusiaux, & Lutzoni, 2017a)? Alternatively, has the specialization level of a guild been stable for a long time due to selection against higher, or lower, levels of specialization (i.e., stabilizing selection)? In this regard, combining a network-based approach with phylogenetic inference tools might provide new insights. Here, we were interested in using the *Peltigera*-*Nostoc* symbiosis as a study system, to test the three following hypotheses:

1. There is an evolutionary trend from generalist ancestral mycobionts towards derived specialists;
2. There is a strong phylogenetic conservatism in *Peltigera* species for *Nostoc* phylogroup selection;
3. Recent speciation events in the genus *Peltigera* are associated more with cyanobiont phylogroup acquisition/loss, rather than replacement/switching to different partners.

To test these hypotheses, we assembled a *Peltigera*-*Nostoc* interaction matrix at a global scale comprising more than one thousand lichen thalli. We combined a network-based approach, community phylogenetics and phylogenetic inference tools to make sense of current patterns of host-symbiont interactions and potential evolutionary mechanisms driving such patterns.

2 | MATERIALS AND METHODS

2.1 | Sample acquisition and interaction network inference

We assembled a dataset of *Peltigera*-*Nostoc* interaction pairs summing up all information from the literature for which we could confidently assess the identity of the *Peltigera* species and *Nostoc* phylogroups or haplotypes (Chagnon et al., 2018; Lu et al., 2018; Magain, Miadlikowska, Goffinet, et al., 2017a; Magain, Miadlikowska, Mueller, et al., 2017b; Magain et al., 2018; Miadlikowska et al., 2014, 2018; O'Brien, Miadlikowska, & Lutzoni, 2005; O'Brien, Miadlikowska, & Lutzoni, 2013; Pardo De la Hoz et al., 2018). The total dataset consisted of 1,026 thalli, or interaction pairs, representing 155 *Peltigera* species and 95 *Nostoc* phylogroups or haplotypes (Tables S1 and S2). For each thallus, DNA sequencing (see below) was used to identify the fungal and cyanobacterial partners. All data on thalli included in this study are available through the dryad data repository (Chagnon, Magain, Miadlikowska, & Lutzoni, 2019).

2.2 | Phylogeny of the genus *Peltigera*

To infer a phylogeny for the entire genus *Peltigera*, we gathered published data from all currently validated species (Chagnon et al., 2018; Magain, Miadlikowska, Goffinet, et al., 2017a; Magain, Miadlikowska, Mueller, et al., 2017b; Magain et al., 2018; Miadlikowska et al., 2014, 2018; Pardo De la Hoz et al., 2018) for seven loci (ITS, nrLSU, β-tubulin, *RPB1*, COR1b, COR3, COR16). We selected one representative per species with the highest number of sequenced loci, or two representatives per species when sets of available loci were mostly non-overlapping between two specimens of a species. We added six species to the outgroup (four thalli representing three *Solorina* species and three thalli representing three *Nephroma* species, Table S1). We generated an additional 159 sequences to fill gaps in the data matrix. These sequences were deposited in GenBank (MK517826-MK517886, MK519281-MK519372, MK520922-MK520926).

Because each locus was analysed separately in previous studies to detect potential topological conflicts, with appropriate corrections made when needed (Chagnon et al., 2018; Magain, Miadlikowska, Goffinet, et al., 2017a; Magain, Miadlikowska, Mueller, et al., 2017b; Magain et al., 2018; Miadlikowska et al., 2014, 2018; Pardo De la Hoz et al., 2018), we concatenated these seven loci using in-house PERL scripts (Magain, 2018). Our concatenated dataset consisted of 205 specimens and 10,064 characters. We delimited and excluded ambiguously aligned sites manually using Mesquite v. 3.11 (Maddison & Maddison, 2016), resulting in a dataset of 5,736 characters. Our dataset was divided into 13 subsets (according to codon positions and non-coding regions for β-tubulin and *RPB1*, and locus delimitations for the remaining five loci). The optimal partitions were estimated using PartitionFinder v. 1.0.1 (Lanfear, Calcott, Ho, & Guindon, 2012) by searching all models using the greedy algorithm and the BIC criterion.

The best tree (phylogram) was inferred using maximum likelihood (ML) as the optimization criterion (RAxML-HPC2 v.7.2.8; Stamatakis, 2006; Stamatakis, Hoover, & Rougemont, 2008) as implemented on the CIPRES portal (Miller, Pfeiffer, & Schwartz, 2010). Searches for optimal trees and bootstrap analyses were conducted with the rapid hill-climbing algorithm for 1,000 replicates with the GTRGAMMA substitution model (Rodriguez, Oliver, Marin, & Medina, 1990).

We estimated relative divergence times (chronogram) using BEAST v1.8.4 (Drummond, Suchard, Xie, & Rambaut, 2012) as implemented on the CIPRES portal, with linked clocks (with a lognormal relaxed clock model) and linked trees, and substitution models

following the best scheme retrieved with the PartitionFinder analysis. We ran the program for 100 million generations, sampling every 10,000th generation, and discarded 25% of generations as burn-in.

2.3 | Phylogenetic distances among *Peltigera* species

For both the phylogram and the chronogram, we pruned our 199-OTU phylogenetic tree, to include only one representative per species, resulting in a 175-OTU tree (Figure S1), using the *drop.tip* function of the R (R core team, 2018) package ape (Paradis, Claude, & Strimmer, 2004). We then computed pairwise phylogenetic distances between all species using the function *cophenetic.phylo*.

2.4 | Similarity-based distances among cyanobionts

Because the phylogeny of *Nostoc* is incompletely resolved and poorly supported (Figure S2; see also Magain, Miadlikowska, Goffinet, et al., 2017a), we computed pairwise similarity distances as a proxy for phylogenetic distances among *Nostoc* phylogroups and distinct haplotypes. We assembled a dataset containing each of the 95 *Nostoc* groups (phylogroups and distinct haplotypes) represented in our interaction matrix, and computed pairwise distances corrected with the General Time Reversible (GTR) model, using PAUP v. 4.0a (Swofford, 2001).

2.5 | Statistical analyses

2.5.1 | Hypothesis 1: Evolutionary trend from generalism to specialism

We tested this hypothesis only for *Peltigera*, because this analysis requires a well-resolved and well-supported phylogeny. For computational feasibility, we split our global *Peltigera* 1-tip-per-species chronogram (Figure S1) into two monophyletic groups, one clade comprising sections *Chloropeltigera*, *Peltidea*, *Phlebia* and *Polydactylon* (POLY clade), and the other encompassing sections *Horizontales*, *Peltigera* and *Retifoveatae* (PELT clade; sections follow Miadlikowska & Lutzoni, 2000). Prior to the analyses, we excluded *Peltigera* species for which we had no information about their interactions with *Nostoc* cyanobionts.

For each of these two clades, we ran ancestral area reconstructions using BioGeoBears v. 0.2.1 (Matzke, 2013a, 2013b) with models DEC and DEC + J. We considered the modules in which each *Peltigera* species belonged as the ancestral areas to infer. Unlike ancestral state reconstructions of traits, biogeographical models allow nodes to be reconstructed as several character states, corresponding to broader areas (or in our case, larger [or broader] ancestral modules than currently delimited modules). In the DEC model, dispersion (parameter D) corresponds to the addition of a new area (in our case a new module or phylogroup) along a phylogenetic branch, and extinction (parameter E) the loss of a module or phylogroup. At cladogenesis (C), areas of children species split into the ancestral area, or a subset of it. The difference between the two models is the J parameter for founder effects. In our scenario, this founder effect would represent a speciation event linked to a contemporaneous change of module (i.e., a switch to an ecologically drastically different set of *Nostoc* phylogroups), whereas in the DEC model, acquisition of new modules (equivalent to dispersal) only occurs along branches of the tree. At speciation events, distributions (modules or phylogroups) of species are the same, or a subset, of the ancestral one. For further computational feasibility, we allowed a maximum of three ancestral modules per node. For the POLY clade, we reconstructed 17 modules for 61 species. For the PELT clade, we reconstructed 17 modules for 87 species.

We ran the same analyses on the same two clades using *Nostoc* phylogroups, instead of modules. For computational feasibility, we allowed a maximum of five reconstructed phylogroups per node. We therefore only tested the 12 most widespread phylogroups for the POLY clade, and the 10 most widespread phylogroups for the PELT clade, and coded all other rare phylogroups as a 13th or 11th state, respectively.

It should be noted that criticisms against the DEC + J model were raised (e.g., Ree & Sanmartin, 2018), but we considered that in our case, the J parameter was ideal to capture the effects of cladogenesis events linked with contemporaneous changes of modules or phylogroups. The two models cannot be directly compared by a statistical test, because they are not nested version of each other (Ree & Sanmartin, 2018). Because the DEC + J model resulted in much higher likelihood values, we only discuss the DEC + J reconstructions below. However, reconstructed nodes with the DEC model were globally similar.

2.5.2 | Hypothesis 2: Phylogenetic conservatism of mycobionts in the selection of cyanobionts

We evaluated whether genetic distances between cyanobionts among thalli pairs could be explained by phylogenetic distances among *Peltigera* species in the same thalli. Under phylogenetically conserved interactions, we would expect that closely related *Peltigera* species would host phylogenetically related cyanobionts. However, at a global scale, we must take into account the fact that some mycobiont-cyanobiont pairs have much higher probabilities of encountering each other. If the spatial distribution across the globe is also phylogenetically constrained, this might be falsely interpreted as phylogenetic constraints on partner selection. To tease apart the two mechanisms, we generated null interaction matrices that randomized interaction patterns with the two following constraints: (a) forbidding interactions among pairs of mycobionts and cyanobionts that do not overlap in their spatial distribution in our dataset and (b) forcing the total number of interactions recorded (i.e., network connectance) to be the same as in the original dataset (see R code in file S1). Imposing such biological constraints on null models, as opposed to architectural constraints (such as fixing row and column marginal totals through swap-based approaches), is considered to be more ecologically relevant (Lessard, Belmaker, Myers, Chase, & Rahbek, 2012; Perez-Neto, Olden, & Jackson, 2001). Using these null matrices, we then compared the amount of variance in cyanobiont genetic

distances among thalli pairs that could be explained by mycobiont inter-species phylogenetic distances, to what was measured in the original dataset.

We also looked at phylogenetic conservatism in interactions by splitting our interaction matrix into modules, and looking at phylogenetic dispersion within versus among modules. These modules correspond to subgroups of species that preferentially interact together. They were generated using a simulated annealing optimization algorithm (see the detailed code in File S1). In a nutshell, we started by allocating species to a random set of modules, to calculate an initial modularity state. Following other major algorithms recently developed, we used Barber's modularity *Q* (Barber, 2007; Beckett, 2016; Dormann & Strauss, 2014; Marquitti, Guimarães, Pires, & Bittencourt, 2014). Then, for each iteration, the algorithm swapped module affiliation for one row (here, mycobionts, i.e., *Peltigera* species) and one column (here, cyanobionts, i.e., *Nostoc* phylogroups and distinct haplotypes). If these swaps yielded a module comprising at least one mycobiont but no cyanobiont (or vice versa), module affiliation for the mycobionts (or cyanobionts) belonging to this module was swapped. The maximal number of modules was set to the minimum between the number of rows and the number of columns in the matrix. Here, we had 155 mycobionts and 95 cyanobionts, so the maximal number of modules was set to 95. After each iteration, modularity *Q* was recalculated and the swaps made in the iteration were accepted with a probability *p*. This probability depended on (a) their impact on modularity and (b) the time that had passed since the beginning of the algorithm. In the earlier phases of the algorithm, even swaps that decreased modularity *Q* by 0.1 had roughly a probability of 0.5 of being accepted, but as the algorithm progressed, it became increasingly stringent and accepted modularity-decreasing swaps with a probability approaching 0 (Figure S3). Swaps that increased modularity *Q* were always accepted ($p = 1$). While the main goal of modularity analyses in studies on ecological networks is typically to test the significance of the overall modularity *Q* metric, or to test the ecological drivers of such modules (e.g., species traits, Olesen, Bascompte, Dupont, & Jordano, 2007; phylogeny, Chagnon et al., 2015; environmental filtering, Torrecillas et al., 2014), here we were interested in assigning a support value to each of our modules. In other words, we were interested in differentiating which modules constantly took part in the optimal solution during the algorithmic optimization, versus other modules that were only infrequently part of the modules configuration. To do so, we ran our chain for 2×10^5 generations and saved the set of modules for every generation. We plotted the modularity *Q* against the number of generations to assess the convergence of our chain (Figure S4). We converted our sets of modules to newick format using R (code in file S1). We sampled every 10th set of modules to generate a set of 2×10^4 sets of modules, from which we estimated the stability of each module throughout the algorithmic process (i.e., proportional frequency of each module) by building a Majority-Rule Consensus set of modules with PAUP v. 4.0a. We tested the effect of different values of burn-in by discarding sets of modules with $Q < 0$, $Q < 0.7$, $Q < 0.71$, $Q < 0.72$ and $Q < 0.7225$, and no burn-in, respectively. We did not use the term posterior probability because it is probably not adequate here, because our modularity algorithm is a simulated annealing-based optimization, not a Bayesian inference. Indeed, our function aims at maximizing modularity *Q*, but does not calculate the probability that a given set of modules generates the observed interaction matrix. Nevertheless, the frequency of a given module during the cooling of our simulated annealing chain may hold significant biological information. If a module contributes very strongly to the whole network modularity, it is unlikely that a random swap disassembling it will be accepted during the chain. Thus, this module will be included during most of the time steps in the chain. On the other hand, a 'weak' module contributing little to network modularity might frequently be disassembled and reassembled during the chain cooling. Hence, the frequency of a given module during the algorithm may be used as a proxy for the strength, or support of this module.

To test for phylogenetic conservatism in ecological interactions, we calculated phylogenetic dispersion within versus outside modules, and compared it to expectations based on a random scenario. We used distance matrices described above, and implemented the analysis using the R package *picante* (Kembel et al., 2010). We used a mean nearest neighbour taxon distance (MNTD) approach, thus evaluating whether species within modules had a higher probability of having a closely related neighbour than expected by chance. For this calculation, we omitted modules comprising only one mycobiont and/or one cyanobiont.

2.5.3 | Hypothesis 3: Symbiont switching versus acquisition/loss during diversification

As for hypothesis 2, and for the same reason, we only conducted this analysis for *Peltigera*. We were interested to see if what typically accompanies a diversification event is either the acquisition of an additional symbiont or the loss of some of the multiple symbionts found with the ancestral *Peltigera* species throughout its distribution. This is in contrast to a complete switch, that is, complete replacement of ancestral symbionts by new symbionts. Based on our previous observations of interaction patterns in section *Polydactylon* of the genus *Peltigera*, where we found high specialization levels by *Peltigera* species on *Nostoc* phylogroups that were more generalists than their *Peltigera* partners (Chagnon et al., 2018; Lu et al., 2018), we hypothesized that during fungal speciation, we would find either no change in *Nostoc* partner or *Nostoc* symbiont switching, rather than acquisitions or losses. We further hypothesized that shifts in *Nostoc* partners may be a stepwise process, whereby a fungus first acquires a new cyanobiont, to then lose their ancestral *Nostoc* partner. This mechanism could be favoured by natural selection if there is a cost to maintain compatibility with many partners. For example, if it involves the maintenance of different signalling pathways involving genes for specific lectins (Singh & Walia, 2014) or small secreted proteins (Plett et al., 2014). This implies that more recent speciation events should have a distinct signature from more ancient speciations. Recent divergences should show a stronger contribution of partner acquisition/loss, while older splits should rather reflect

turnover, or switch in *Nostoc* partners, because no traces of the ancestral partner can be found in current populations.

To test this hypothesis, we used additive partitioning of β-diversity, frequently used in community ecology (Legendre, 2014). This partitioning stems from the idea that differences in species composition between two sites (here, differences in *Nostoc* partners between two mycobiont species) can originate from (a) abundance difference or (b) species turnover (Williams, 1996). Abundance difference is directly linked to the concept of nestedness in biogeography and ecological networks (Bascompte, Jordano, Melian, & Olesen, 2003; Patterson & Atmar, 1986), where species with fewer partners have their interactions nested within the interactions of species with more interactions. Methods have been developed to additively partition the dissimilarity between pairs of sites (or here, pairs of *Peltigera* species) into its abundance difference component (hereafter labelled *D*) and its species replacement component (hereafter *R*) (Baselga, 2013; Podani, Ricotta, & Schmera, 2013). Both were tested with our data (following Legendre, 2014), but yielded qualitatively similar results. Here, we will only report results using Podani's method and Ružička dissimilarity index (Ružička, 1958) for pairwise mycobiont comparisons. We also note that our dataset is not suited to provide strict estimations of the relative importance of *D* versus *R* in Ružička mycobionts' pairwise dissimilarity, because our sampling design did not strictly control for sampling effort per mycobiont species, which results in some mycobionts being better sampled than others. This naturally induces some dissimilarity allocated to the *D* component, that is, some mycobionts having more thalli (the equivalent of some sites having more individuals). For example, if two mycobionts shared the same unique *Nostoc* partner, but with one mycobiont being sampled from five thalli and the other from only two thalli, this would result in a non-null dissimilarity between them, which would be fully explained by the *D* component in this additive partitioning framework. Therefore, results must be interpreted with caution, taking into account that the *D* component of mycobiont dissimilarity is probably an inflated estimate for most pairwise comparisons. Nevertheless, the goal of the analysis is to compare the relative importance of *D* versus *R* between sister *Peltigera* species (i.e., species sharing a most recent common ancestor), as opposed to all other pairwise mycobiont comparisons. We have no logical reason to expect the bias explained above to be over- or under-represented among sister species as opposed to any other pairwise mycobiont comparisons.

Finally, because we inferred a chronogram for the genus *Peltigera*, where branch lengths represent relative time, we were able to evaluate, for sister species, if the relative contribution of *D* versus *R* varies as a function of evolutionary time using Pearson's correlation. Our expectation was that fungal speciation events would not necessarily imply a partner switch right away, but over time this divergence between sister species would eventually lead to a partner switch/turnover (i.e., the *R* component). In other words, we expected the *R* component to be positively associated with time since divergence when comparing sister species, and vice versa for the *D* component. Figure S5 explains graphically how such a stepwise partner switch may drive an initially high *D* component right after speciation and a larger *R* component later after the loss of one ancestral partner.

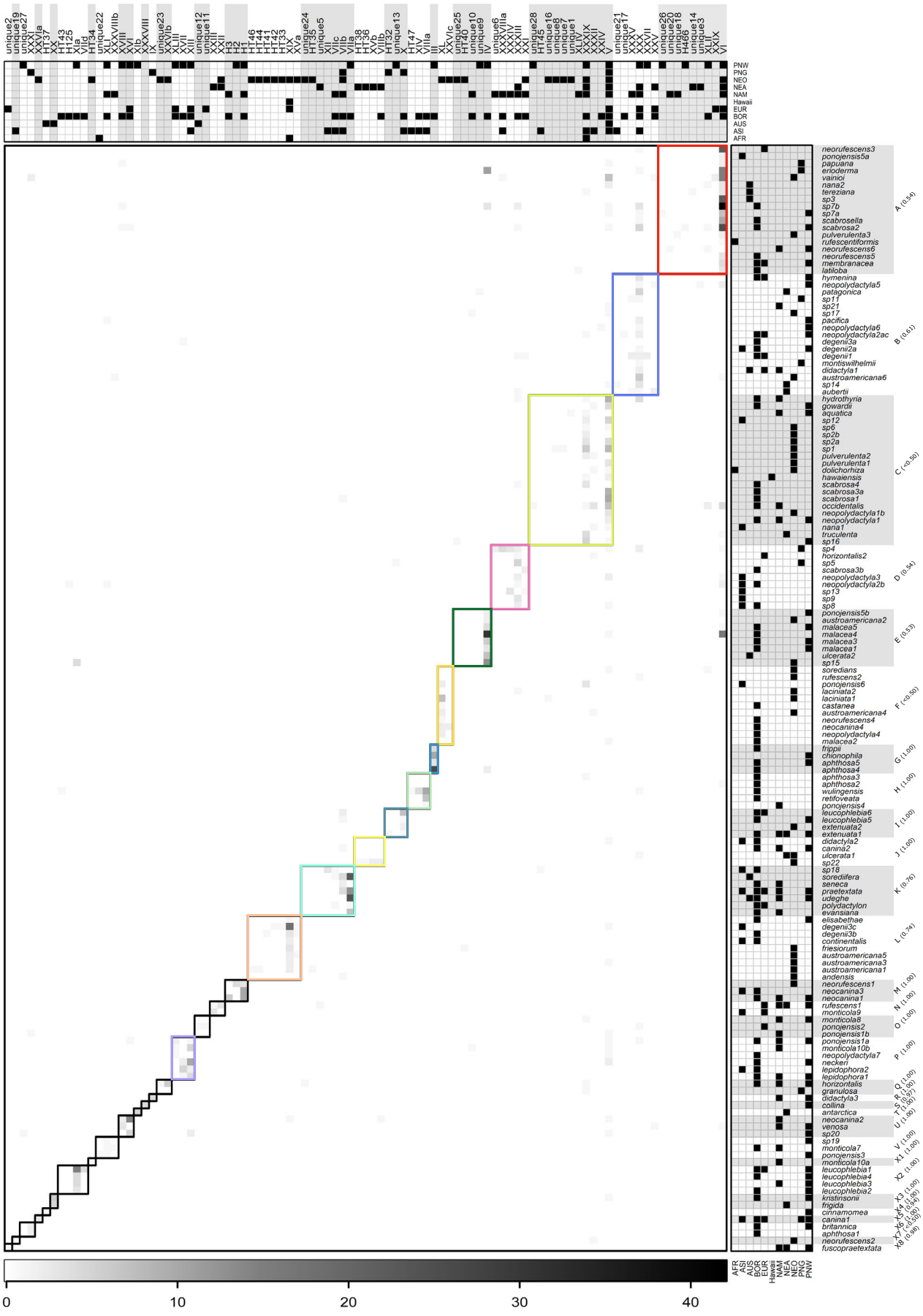
3 | RESULTS

Our interaction network between *Peltigera* mycobionts and *Nostoc* cyanobionts comprised 1,026 thalli, yielding 324 binary interactions (Figure 1). Although this dataset is a major effort in uncovering interactions at a global scale, our rarefaction analyses suggest that up to 594 interactions could have been uncovered in this dataset with a 'complete' sampling. This is based on rarefaction analyses with Hill numbers (order q = 0) following Chao et al. (2014) (Figure S6). Given the lack of saturation in interactions we wanted to make sure that the network structure uncovered through our analyses was robust to sampling effort. We thus calculated two widely used network metrics (i.e., modularity and nestedness), over a gradient of sampling effort ranging between ~ 50 and our full 1,026 thalli dataset. Confirming what we had found in prior analyses on the section *Polydactylon* of the genus *Peltigera* (Chagnon et al., 2018), we found that modularity and nestedness did vary with sampling effort, but with trends that increasingly diverged from random expectations as sampling effort increased (Figure S7). In other words, further sampling would have only strengthened our conclusions about network structure. Thus, overall, we are confident that our global dataset is well suited to provide a robust test to our three main hypotheses.

3.1 | Hypothesis 1: Evolutionary trend from generalism to specialism and eco-evolutionary drivers of modules

We found no evidence for a directional evolution from generalism to specialism. Instead, when inferring module affiliations through time, the DEC + J model greatly improved the likelihood score compared to the DEC model (POLY clade, DEC model, LnL = −175.05 vs. DEC + J model,

FIGURE 1 The interaction matrix and modules uncovered through our global sampling. In the main matrix, shades of grey are proportional to the number of thalli (as shown on the scale) into which a given cyanobiont (columns) was found in association with a corresponding mycobiont (rows). The boxes delineate the modules found through simulated annealing. We used alternating white and grey backgrounds to facilitate visual allocation of given cyanobionts and mycobionts to a given module. On the top and right panels, we show the geographical distribution of the cyanobionts and mycobionts, respectively, in each of the 11 biogeographical regions sampled in our study (AFR = Africa, ASI = Asia, AUS = Australasia, BOR = Boreal biome, EUR = Europe, NAM = North America, NEA = Argentina and Chile, NEO = Neotropics, PNW = Pacific Northwest, PNG = Papua New Guinea): a filled cell means that at least one thallus of this cyanobiont/mycobiont has been sampled from the corresponding region in our study. Module ID is shown on the right part of the figure, with the module frequency/support value shown in parenthesis. The module ID and colours correspond to those shown in Figure 2. [Colour figure can be viewed at wileyonlinelibrary.com]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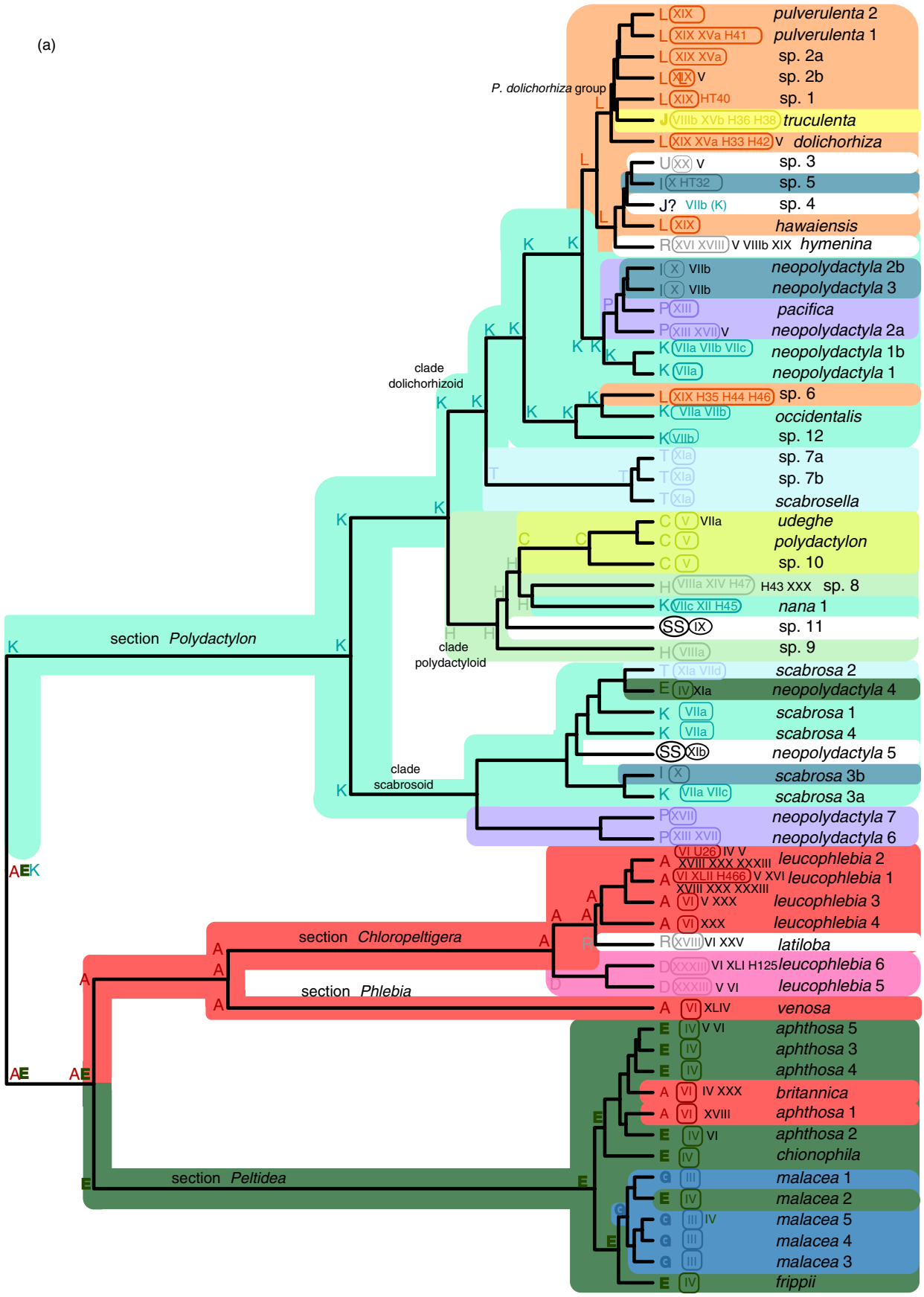
(a)
P. dolichorhiza group
pulverulenta 2
pulverulenta 1
sp. 2a
sp. 2b
sp. 1
truculenta
dolichorhiza
sp. 3
sp. 5
sp. 4
hawaiensis
hymenina
neopolydactyla 2b
neopolydactyla 3
pacifica
neopolydactyla 2a
neopolydactyla 1b
neopolydactyla 1
sp. 6
occidentalis
sp. 12
sp. 7a
sp. 7b
scabrosella
clade dolichorhizoid
udeghe
polydactylon
sp. 10
sp. 8
nana 1
sp. 11
sp. 9
clade polydactyloid
scabrosa 2
neopolydactyla 4
scabrosa 1
scabrosa 4
neopolydactyla 5
scabrosa 3b
scabrosa 3a
clade scabrosoid
neopolydactyla 7
neopolydactyla 6
section Polydactylon
leucophlebia 2
leucophlebia 1
leucophlebia 3
leucophlebia 4
latiloba
leucophlebia 6
leucophlebia 5
section Chloropeltigera
section Phlebia
venosa
aphthosa 5
aphthosa 3
aphthosa 4
britannica
aphthosa 1
aphthosa 2
chionophila
malacea 1
malacea 2
malacea 5
malacea 4
malacea 3
frippii
section Peltidea

FIGURE 2

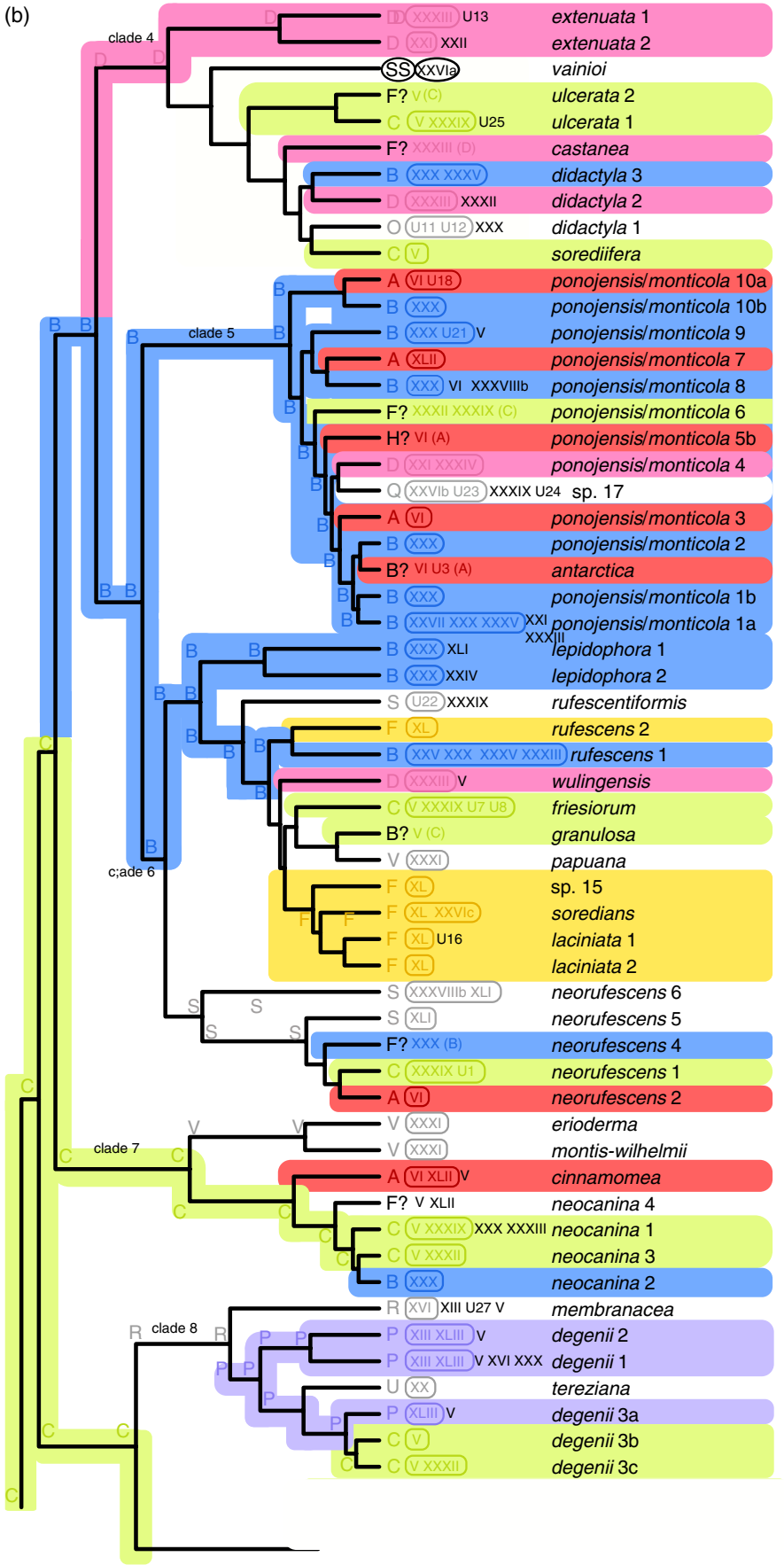
(b)
clade 4
clade 5
clade 6
clade 7
clade 8
extenuata 1
extenuata 2
vainioi
ulcerata 2
ulcerata 1
castanea
didactyla 3
didactyla 2
didactyla 1
sorediifera
ponojensis/monticola 10a
ponojensis/monticola 10b
ponojensis/monticola 9
ponojensis/monticola 7
ponojensis/monticola 8
ponojensis/monticola 6
ponojensis/monticola 5b
ponojensis/monticola 4
sp. 17
ponojensis/monticola 3
ponojensis/monticola 2
antarctica
ponojensis/monticola 1b
ponojensis/monticola 1a
lepidophora 1
lepidophora 2
rufescentiformis
rufescens 2
rufescens 1
wulingensis
friesiorum
granulosa
papuana
sp. 15
sorediens
laciniata 1
laciniata 2
neorufescens 6
neorufescens 5
neorufescens 4
neorufescens 1
neorufescens 2
erioderma
montis-wilhelmii
cinnamomea
neocanina 4
neocanina 1
neocanina 3
neocanina 2
membranacea
degenii 2
degenii 1
tereziana
degenii 3a
degenii 3b
degenii 3c

FIGURE 2

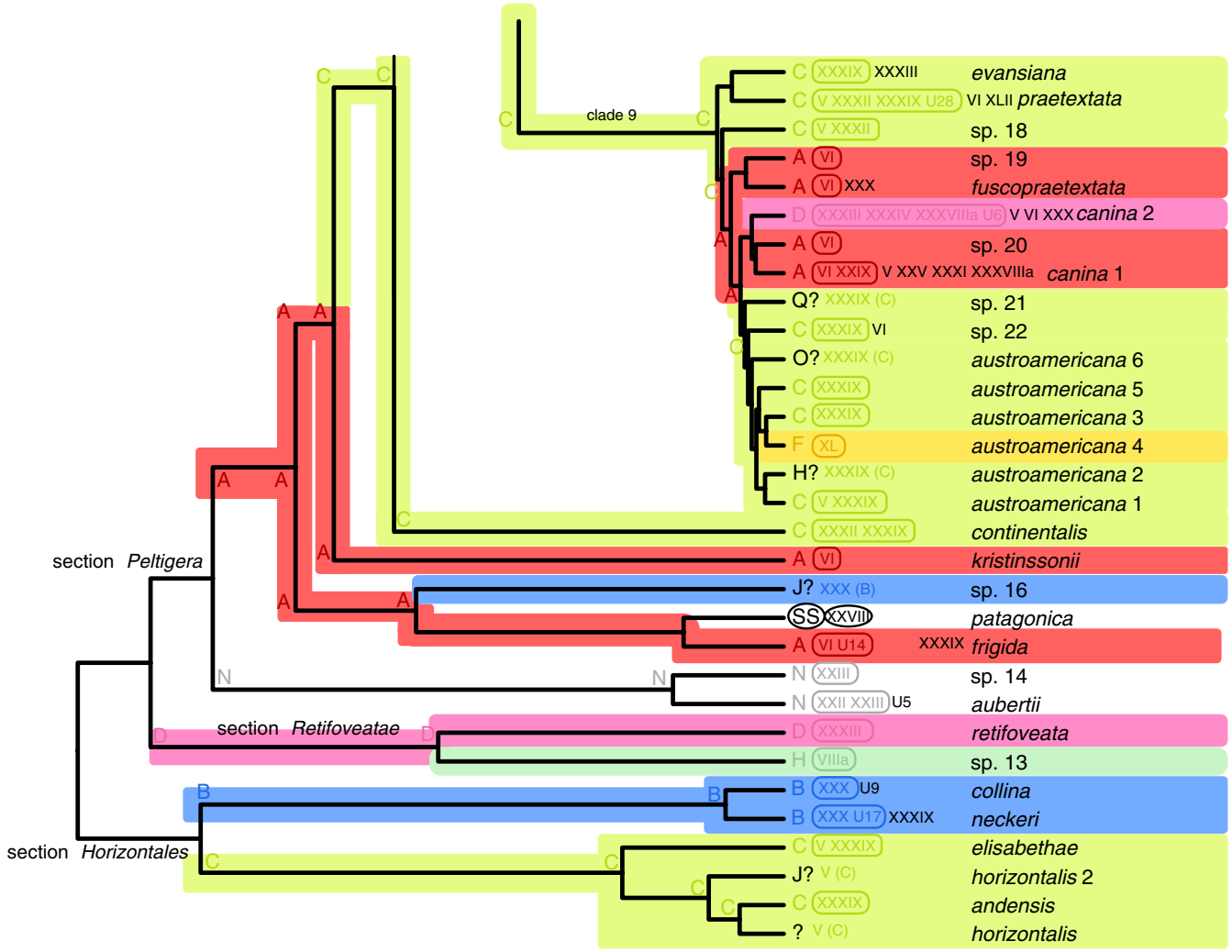

FIGURE 2 Close-ups of the seven-locus chronogram of the genus *Peltigera*: (a) POLY clade, that is, sections *Chloropeltigera*, *Peltidea*, *Phlebia* and *Polydactylon*, and (b) PELT clade, that is, sections *Horizontales*, *Peltigera* and *Retifoveatae*. Branch lengths are proportional to relative time, and each terminal tip represents a species indicated on the far right, or putative species resulting from recent studies (Magain, Miadlikowska, Goffinet, et al., 2017a; Magain et al., 2018; Pardo De La Hoz et al., 2018; Miadlikowska et al., 2018). The coloured capital letters immediately to the right of the terminal branch tips represent the network modules of each species, based on results of the modularity analyses (Figure 1). Further on the right, the *Nostoc* phylogroup partners of each *Peltigera* species are shown. Each *Nostoc* phylogroup that belongs to the same module as its *Peltigera* species partner share the same colour inside a rounded box. *Nostoc* phylogroups belonging to different modules are shown in black outside of the rounded boxes. Inferences of ancestral modules on internal nodes were generated using BioGeoBears with the DEC + J model. Background colours represent the current and inferred ancestral modules each *Peltigera* species belongs to. Rare modules have no background colours. Strict 1:1 specialist associations forming distinct modules are represented in a circle with SS. Modules represented with a question mark are presumably incorrectly reconstructed by the modularity analyses (species in modules with none of their partners) and the module of their cyanobiont was used for the colour-coding. Branches and nodes with very low probabilities for all states, or with several states reconstructed, have no background colour. [Colour figure can be viewed at wileyonlinelibrary.com]

LnL = −127.74; PELT clade, DEC model lnL = −317.58 vs. DEC + J model LnL = −216.10; Table S3). Interestingly, the J parameter explains all changes (POLY clade, $d = 0$, $e = 0$, $J = 0.0277$; PELT clade $d = 0$, $e = 0$, $J = 0.0541$), which means that under this model all changes to a *Nostoc* in a different module are associated with founder effects linked to speciation events.

Likewise, when inferring ancestral phylogroup affiliations using the same *Peltigera* clades (Table S3), the likelihoods generated with the DEC + J model are still better than with DEC, but the differences are not as large as for modules (lnL of −256.84 vs. −247.08 for the POLY clade, −383.25 vs. −361.39 for the PELT clade; Table S3). Moreover, for the DEC + J model, the *d* parameter (dispersion; in this case, the acquisition of a new phylogroup) is larger than the *j* parameter for clades POLY and PELT (Table S3). Collectively, these reconstruction results suggest that the evolution of *Peltigera* did not proceed from large ancestral modules to smaller modules. Instead, modules were replaced by other modules (corresponding to a drastic change of *Nostoc* symbionts), and these switches were linked to speciation events. However, changes in phylogroup associations, especially acquisitions, without immediate replacement, are frequent within modules.

Eight modules consist of only one *Peltigera* species and one *Nostoc* phylogroup (Figure 1). These modules include 1-to-1 strict

reciprocal specialists, or rare *Peltigera* species sampled once. Strict specialists display no obvious phylogenetic or geographic trend except that they are endemic to a specific region: for example, *P. neopolydactyla* 5 is endemic to Oregon and British Columbia, *P.* sp. 11, is endemic to Papua New Guinea, *P. patagonica* is endemic to Southern Chile/Argentina, and *P. vainioi*, is endemic to the Andes (Figure 1).

Biogeographical factors are also shaping the detected modules. For example, the ancestor of section *Polydactylon* was part of module K, which is mostly boreal (Magain, Miadlikowska, Goffinet, et al., 2017a) (Figures 1 and 2a). Sympatric species of temperate and boreal zones of Asia and Pacific Northwest seem to be associated with an amphi-berengian module P (i.e., *P. neopolydactyla* 2a, *P. pacifica*, *P. neopolydactyla* 6, *P. neopolydactyla* 7 from section *Polydactylon*, and *P. degenii* 1, *P. degenii* 2, *P. degenii* 3a from section *Peltigera*. In the *P. dolichorhiza* group, the colonization of the Neotropics is linked to a switch to module L. The only species of that group to escape the Neotropics and disperse to boreo-temperate regions of Southern Chile and Argentina, *P. truculenta*, further switched to module J. The independent colonization of the Neotropics by *Peltigera* sp. 6 also resulted in a switch from module K to L (Figure 2a). In the polydactyloid clade, the colonization of tropical Asia is linked to a switch to module H, followed by a switch to module C for the *P. polydactylon/udeghe/*sp. 10 clade when returning to boreo-temperate regions of the northern hemisphere. Interestingly, dispersion events to the Neotropics were not associated to switches to the same modules in sections *Polydactylon* versus *Peltigera*, the two most species-rich sections of the genus *Peltigera*. In section *Polydactylon*, two independent switches to module L were observed, whereas in section *Peltigera*, two switches to module F occurred (*P. laciniata* group, and *P. rufescens* 2) (Figure 2).

In the tri-membered (one mycobiont with both a green algal and a cyanobacterial photobiont, the latter restricted to small localized structures called cephalodia) and generalist sections *Chloropeltigera* and *Phlebia*, all species associate with phylogroup VI (part of module A) but some species are part of other modules (e.g., D) because they associate more frequently with other *Nostoc* phylogroups (Figure 2a). Interestingly, in section *Peltidea* (the only section to include both bi-membered and tri-membered *Peltigera* species), the ancestor associated with module E, but some tri-membered species (*P. britannica* and *P. aphthosa* 1) seem to have transitioned back to module A, including phylogroup VI (Figure 2a).

3.2 | Hypothesis 2: Phylogenetic conservatism of mycobionts in the selection of cyanobionts

We found various lines of evidence for phylogenetic conservatism in partner selection between *Peltigera* and *Nostoc*. The network is strongly modular (Figure 1), which indicates a strong pattern of preferential interactions among mycobionts and cyanobionts. More closely related *Peltigera* species were much more likely to host (a) the same cyanobionts (pseudo-$F_{155,1026}$ = 9.07, R^2 = 0.62, p < 0.001) or at least (b) cyanobionts with low genetic distances (pseudo-$F_{155,1026}$ = 18.01, R^2 = 0.76, p < 0.001). Without controlling for geographic overlap in mycobionts' and cyanobionts' distributions, the mycobionts phylogenetic distances explained ~76% of the variation in cyanobionts' variation across thalli. This amount of explained variation was never met in our null matrices taking into account geographic distribution (hence the p < 0.001 value). In these null matrices, mycobionts phylogenetic distances explained roughly 50%–60% of the variation in cyanobionts variation among thalli (Figure S8). This 50%–60% value suggests that mycobionts' geographic distributions are phylogenetically conserved, that is, closely related mycobionts tend to be present in similar geographic areas, and thus share closely related cyanobionts. Nevertheless, a 16%–26% of variation (i.e., 76% minus 50%–60%) in observed data cannot be explained by a null model taking geographic distributions into account. We also note from Figure 1 that no apparent trend can be seen with regard to modules' distribution across the globe. In other words, the great majority of modules do not appear to be constrained to a single biogeographical region.

We also found that mycobionts sharing the same module often tended to be more closely related than expected by chance alone (Figure 3. Indeed, out of 22 modules (which comprised more than one mycobiont), 12 were shown to host more closely related mycobionts than expected by chance. The non-significant results of this phylogenetic clustering analysis within modules mostly came from very small modules comprising only two species. This might be regarded as a statistical artefact, because such small module size inflates the variance in the null scenarios for phylogenetic clustering (see wide error bars on Figure 3 for small modules). Figure 2 shows in more details the phylogenetic conservatism in module affiliation, with some modules being found in only specific sections of the genus *Peltigera* (e.g., module B is only found in section *Peltigera*, or modules L and K are only in section *Polydactylon*). For the cyanobionts, the phylogenetic clustering trend was much weaker, with only three modules showing a significant signal of phylogenetic clustering (Figure S9), again restricted to larger modules.

3.3 | Hypothesis 3: Symbiont switching versus acquisition/loss during species diversification

Our β-diversity partitioning analyses revealed that recent speciation events are more associated with partner abundance/richness differences (i.e., the *D* component in our decomposition) than with partner replacement/switching (*R*). Indeed, for sister species, the cyanobiont partners of one mycobiont were often a nested subset of the cyanobiont partners of its sister species. In other words, cases of partner replacement (i.e., cyanobiont *A* being more frequent with mycobiont *X* and cyanobiont *B* more frequent with mycobiont *Y*, and vice versa) were less frequent among sister species (Figure 4a). Interestingly, sister species pairs that diverged more recently were less likely to show evidence of partner switching/replacement (*R* component) than the ones that had diverged longer ago (and of course, vice versa for the *D* component, as *R* and *D* come from an additive partitioning of total dissimilarity) (Figure 4b,c). As a cautionary note, we highlight, that the higher *D* component of sister species, and particularly those that diverged recently, can be explained either by variation in the number of thalli sampled for the two mycobionts compared, or a 'real' biological signal of partner acquisition/loss (and not just a sampling artefact).

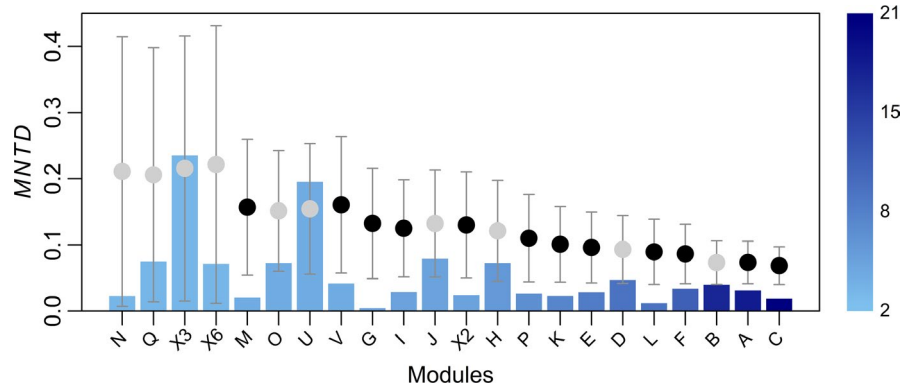

FIGURE 3 Phylogenetic clustering (for mycobionts) in our delineated modules. On the *x*-axis, the 22 modules comprising more than 1 mycobiont are ordered from the smallest to the largest. Module size is shown by the shade of gray (see colour scale on the right), that is, the number of *Peltigera* species per module. The bars represent observed value of mean nearest taxon distance (MNTD) for each module, while the circles show the null expectations from a null model shuffling the tree tips. These results are shown with their standard deviations (1,000 null scenarios per module). Black circles indicate significant clustering, while grey circles indicate non-significant trends [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Globally, interaction patterns between *Peltigera* and *Nostoc* are highly specialized. However, many studies on ecological networks have showed how specialization can be overestimated because of incomplete sampling effort (e.g., Chacoff et al., 2012), leading to the discovery of what Brooks and McLennan (2002) coined 'faux specialists'. Here, our recorded connectance (i.e., proportion of all possible pairwise interactions that are actually realized/observed) was 0.022, or 2.2%. A rarefaction analysis following Chao et al. (2014) showed that even with a 'complete' sampling of the system (3,500 thalli would be needed for detecting 90% of all interactions and 4,500 thalli would be needed to reach 95%), connectance would only have reached ~5%. This remains considerably lower than other study systems. Fortuna et al. (2010) did a meta-analysis on datasets with connectance of 0.09–0.19 for pollination networks, 0.15–0.28 for host-parasite networks and 0.29–0.49 for seed dispersion networks (values representing 25th and 75th quantiles, after reanalyzing their published data). Thus, our study seems to reveal true patterns of specialization, and not sampling artefacts.

The evolution and maintenance of specialization has fuelled a large number of publications outside the study of mutualism. Indeed, other systems consistently leaning towards high specialization are host-parasites systems (e.g., Agosta, Janz, & Brooks, 2010). In parasitology, the 'Stockholm paradigm' has been coined to explain the evolutionary maintenance of specialization, despite the occurrence of host shifts in the system. Two major components of this paradigm are (a) ecological fitting and (b) the oscillation hypothesis. In the context of interaction networks, ecological fitting (Agosta & Klemens, 2008; Janzen, 1985) refers to the notion of partner switching without the prerequisite for de novo adaptation to this new partner. This is closely related to the concept of exaptation, whereby a specialist could have a realized fitness on a different partner outside of its current partner range. The oscillation hypothesis refers to alternative cycles of novel partner acquisition and loss of ancestral partner as an explanation for partner switching in interaction networks (Janz & Nylin, 2008). Interestingly, this hypothesis developed by parasitologists is in striking agreement with our *SDR* analyses on lichen symbionts (Figure 4). Indeed, the very fact that the *D* component and the *R* component are respectively larger and smaller for recently diverged species suggests that partner switching in the *Peltigera-Nostoc* system seems to follow such an oscillatory dynamics of partner acquisition-loss (Figure S5).

In line with this Stockholm paradigm, our results suggest that specialization is not an evolutionary dead-end in *Peltigera-Nostoc* cyanolichens. Specialists do seem to maintain the ability to expand partner range and perform partner switching. This is also corroborated by the fact that our reconstruction analysis of modules along the mycobionts' phylogeny was best represented by a model incorporating founder effects. In other words, our biogeographical reconstruction analysis did not support a model with ancestral lineages bearing all modules and progressively losing some during diversification (i.e., no directional trend from generalism to specialism). This model also evidenced coincidences between new module emergences and fungal speciation events. This could be explained by what has been termed the 'taxon pulse hypothesis' (Erwin, Ball, Whitehead, & Halpern, 1979). This hypothesis states that some conditions (e.g., rapid environmental change, sudden range expansion) can promote speciation through cycles of expansion and isolation. In the context of the evolution of interaction networks, it could be envisaged that such conditions could promote both speciation and partner switch. Indeed, rapid environmental change can prime partner switching if a new partner becomes more favourable in this new environment. On the other hand, range expansion can promote novel contacts between pairs of partners not used to encounter each other in their ancestral distribution. Several cases of partner switching have been associated with periods of climate change and/or range expansion (reviewed in Agosta et al., 2010). This is totally plausible in our study system where some specific conditions/events seem to have primed the emergence of new modules and the diversification of *Peltigera*. For example, a *Peltigera* lineage within section *Polydactylon* colonized South America, which led to a switch to new cyanobiont

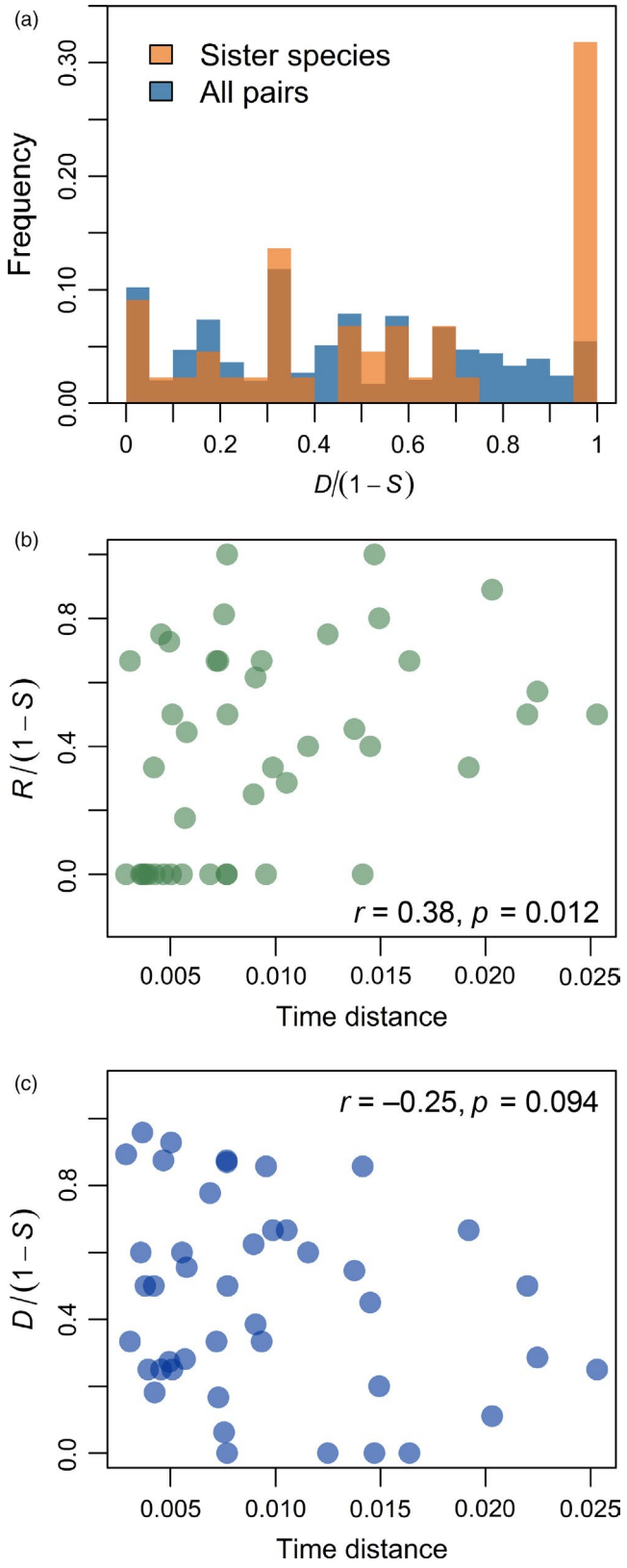

FIGURE 4 β-diversity decomposition analyses for sister species. In (a), we show that variation in cyanobionts between sister species can be better explained by richness/abundance differences (i.e., *D* as a fraction of total pairwise dissimilarity, (1-*S*)), as compared with all other pairwise mycobiont comparisons. In (b and c), we show that sister species that have diverged longer ago tend towards higher partner replacement (*R*) component and lower richness/abundance differences (*D*) component. In these latter plots, each point represents a pairwise comparison between two sister species [Colour figure can be viewed at wileyonlinelibrary.com]

phylogroups, selection towards generalism, a burst of speciation in this lineage, and the formation of a new module (module L, Figures 1 and 2a; see also Magain, Miadlikowska, Goffinet, et al., 2017a). In fact, fungal speciation and partner switching may not be independent from each other. For example, Braga, Araujo, et al. (2018a) recently found that new partners can create heterogeneity in geographic mosaics: a partner switch may thus further prime fungal diversification in a geographic mosaic of co-evolution (Thompson, Thompson2005).

We observed a strong phylogenetic conservatism in interactions between *Peltigera* species and *Nostoc* symbionts. This may not seem surprising given the very intimate nature of the lichen interaction (sensu Guimarães et al., 2007) resulting from more than 400 million years of evolution (Honegger, Edwards, & Axe, 2012; Lutzoni et al., 2018). In this context, it could be expected that compatible interactions are mediated by a large number of conserved traits, and thus reducing evolutionary lability. Such conservatism was also found in other 'intimate' systems such as orchid mycorrhizae (Shefferson et al., 2010). However, this needs not to be the rule: arbuscular mycorrhizal fungi, also 'intimate' symbionts in their host plants, and a symbiosis that probably originated before lichens (Lutzoni et al., 2018), have been found to be less similar among closely related hosts (meta-analysis by Reinhart & Anacker, 2014; Veresoglou & Rillig, 2014). Here, interestingly, our phylogenetic partner conservatism remained significant even after controlling for the geographical overlap of cyanobionts and their *Peltigera* partners (Figure S8). This is in line with Braga, Razzolini, and Boeger (2015) showing that phylogeny was a stronger driver of host-parasites networks than geography. However, it is likely that our coarse geographic resolution hides finer scale partitioning of the environment. If *Peltigera* species are not distributed randomly within our geographic regions, in a way that is linked to phylogeny, habitat partitioning may still be the underlying cause for part of the phylogenetically conserved partner selection observed in our system (Jüriado, Kaasalainen, Jylhä, & Rikkinen, 2019). For example, Lu et al. (2018) observed that along a latitudinal gradient crossing the entire boreal belt, some *Peltigera* species were restricted to specific portions of the gradient, well correlated to climatic variables such as total precipitation and mean temperature during the warmest quarter of the year. This study demonstrated that appropriate *Nostoc* phylogroup availability was not limiting for the *Peltigera* species sampled along this intra-biome latitudinal gradient. Future work should test for phylogenetic bioclimatic niche conservatism in *Peltigera* to verify whether environmental filtering could be a driver of phylogenetically constrained *Nostoc* partner selection observed in our study.

Overall, our study shows how the combination of phylogenetics, ancestral biogeographical inferences, network-based and β-diversity analytical tools can yield novel insights into the evolution of symbiotic interactions. However, major unknowns remain to be solved to better understand why there seems to be such a strong pressure

in these cyanolichens to remain highly specialized. Indeed, in many other mutualistic symbioses, some species evolve a more generalist strategy, and this gradient in generalism is at the core of the typically nested interaction patterns in these communities (e.g., Almeida-Neto, Guimarães, Guimarães, Loyola, & Ulrich, 2008; Podani & Schmera, 2011). It is possible that the very slow growth rate and life history of lichens, as opposed to other organisms, may constrain opportunism in that it would be very costly to engage in an intimate interaction with a sub-optimal partner. Conversely, fast growing plant roots can establish interactions with various compatible rhizobial or mycorrhizal partners to then screen for preferred partners through either sanctions towards uncooperative symbionts (Kiers, Rousseau, West, & Denison, 2003) or preferential reward towards beneficial partners (Bever, Richardson, Lawrence, Holmes, & Watson, 2009). A major frontier for this field of research remains the estimation of the reliability in partner availability across spatial scales for mycobionts: how can widely spread species across large biomes (e.g., *Peltigera occidentalis*) remain so selective in their partnership with *Nostoc* cyanobacteria? One necessary condition is to not be limited by partner availability across its home range (Douglas, 1998). We still have to determine how *Nostoc* partner availability varies across space in the environment, and how other potential hosts (e.g., bryophytes, vascular plants) contribute to this regional partner pool.

It is important to note that the largest modules in this study, modules A-C, include the most broadly distributed *Nostoc* phylogroups (VI, XXX, V and XXXIX), all of which are the most generalist *Nostoc* phylogroups in this symbiotic system. Therefore, it is possible that *Nostoc* phylogroups that are the most broadly distributed geographically, enable the interaction with the largest number of *Peltigera* species. A large fraction of these fungal species resulted from multiple speciation events subsequent to the establishment of a mutualistic interaction with a broadly distributed *Nostoc*, and continuous association with the same *Nostoc* (phylogenetic conservatism) through time. This not only provides a potential explanation for the maintenance of specialization by the mycobiont, but also the asymmetry of specificity in lichens, which is resulting from a gradual increase in generalism by broadly distributed *Nostoc* partners that are hosting an increasingly large number of *Peltigera* species sharing a most recent common ancestor. This is in agreement with the results from Lu et al. (2018), where *Peltigera* species have narrower latitudinal ranges than their broadly distributed generalist *Nostoc* partners.

Finally, another emergent finding from our study is how evolutionary trends in our mutualistic interaction networks were found to be closely aligned with theories put forth in parasitology, that is, antagonistic networks. This suggests that some overarching laws may govern the evolution of specialized interaction networks in general, notwithstanding the nature of the interaction itself. This paves the way for more exciting work to develop broader hypotheses on the evolution of symbioses in general. The combination of network-based tools with macroevolutionary models appears to be a particularly promising avenue of research (e.g., Braga, Guimarães, Wheat, Nylin, & Janz, 2018b).

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AUTHORS' CONTRIBUTIONS

P.-L.C., N.M., J.M. and F.L. conceived the study; P.-L.C. and N.M. conducted statistical analyses; P.-L.C. wrote a first draft of the manuscript. All authors participated in revising the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.b537cs5 (Chagnon et al., 2019).

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REFERENCES

Agosta, S. J., Janz, N., & Brooks, D. R. (2010). How specialists can be generalists: Resolving the "parasite paradox" and implications for emerging infectious disease. *Zoologia*, *27*(2), 151–162. https://doi.org/10.1590/S1984-46702010000200001

Agosta, S. J., & Klemens, J. A. (2008). Ecological fitting by phenotypically flexible genotypes: Implications for species associations, community assembly and evolution. *Ecology Letters*, *11*(11), 1123–1134. https://doi.org/10.1111/j.1461-0248.2008.01237.x

Almeida-Neto, M., Guimarães, P., Guimarães, P. R., Loyola, R. D., & Ulrich, W. (2008). A consistent metric for nestedness analysis in ecological systems: Reconciling concept and measurement. *Oikos*, *117*(8), 1227–1239. https://doi.org/10.1111/j.0030-1299.2008.16644.x

Barabási, A. L., & Albert, R. (1999). Emergence of scaling in random networks. *Science*, *286*(5439), 509–512. https://doi.org/10.1126/science.286.5439.509

Barber, M. J. (2007). Modularity and community detection in bipartite networks. *Physical Review E*, *76*(6), 066102. https://doi.org/10.1103/PhysRevE.76.066102.

Bascompte, J., Jordano, P., Melian, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Baselga, A. (2013). Separating the two components of abundance-based dissimilarity: Balanced changes in abundance vs. abundance gradients. *Methods in Ecology and. Evolution*, *4*(6), 552–557. https://doi.org/10.1111/2041-210X.12029

Bastolla, U., Fortuna, M. A., Pascual-García, A., Ferrera, A., Luque, B., & Bascompte, J. (2009). The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, *458*(7241), 1018–1020. https://doi.org/10.1038/nature07950

Beckett, S. J. (2016). Improved community detection in weighted bipartite networks. *Royal Society Open Science*, *3*(1), 140536. https://doi.org/10.1098/rsos.140536.

Bell, J. R., Andrew King, R., Bohan, D. A., & Symondson, W. O. C. (2010). Spatial co-occurrence networks predict the feeding histories of polyphagous arthropod predators at field scales. *Ecography*, *33*(1), 64–72. https://doi.org/10.1111/j.1600-0587.2009.06046.x

Bersier, L.-F., & Kehrli, P. (2008). The signature of phylogenetic constraints on food-web structure. *Ecological Complexity*, *5*(2), 132–139. https://doi.org/10.1016/j.ecocom.2007.06.013

Bever, J. D., Richardson, S. C., Lawrence, B. M., Holmes, J., & Watson, M. (2009). Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters*, *12*(1), 13–21. https://doi.org/10.1111/j.1461-0248.2008.01254.x

Braga, M. P., Araujo, S. B. L., Agosta, S., Brooks, D., Hoberg, E., Nylin, S., … Boeger, W. A. (2018a). Host use dynamics in a heterogeneous fitness landscape generates oscillations in host range and diversification. *Evolution*, *72*(9), 1773–1783. https://doi.org/10.1111/evo.13557

Braga, M. P., Guimarães, P. R., Wheat, C. W., Nylin, S., & Janz, N. (2018b). Unifying host-associated diversification processes using butterfly–plant networks. *Nature Communications*, *9*(1), 5155. https://doi.org/10.1038/s41467-018-07677-x

Braga, M. P., Razzolini, E., & Boeger, W. A. (2015). Drivers of parasite sharing among neotropical freshwater fishes. *Journal of Animal Ecology*, *84*(2), 487–497. https://doi.org/10.1111/1365-2656.12298

Brooks, D. R., & McLennan, D. A. (2002). *The nature of diversity: An evolutionary voyage of discovery*. Chicago: Chicago University Press.

Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. *Science*, *339*(6127), 1611–1615. https://doi.org/10.1126/science.1232728

Burns, K. C. (2013). What causes size coupling in fruit–frugivore interaction webs? *Ecology*, *94*(2), 295–300. https://doi.org/10.1890/12-1161.1

Chacoff, N. P., Vázquez, D. P., Lomáscolo, S. B., Stevani, E. L., Dorado, J., & Padrón, B. (2012). Evaluating sampling completeness in a desert plant-pollinator network. *Journal of Animal Ecology*, *81*(1), 190–200. https://doi.org/10.1111/j.1365-2656.2011.01883.x

Chagnon, P.-L., Bradley, R. L., & Klironomos, J. N. (2015). Trait-based partner selection drives mycorrhizal network assembly. *Oikos*, *124*(12), 1609–1616. https://doi.org/10.1111/oik.01987

Chagnon, P. L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2018). Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus *Peltigera*. *Oecologia*, *187*(3), 767–782. https://doi.org/10.1007/s00442-018-4159-6

Chagnon, P. L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2019). Data from: Species diversification and phylogenetically constrained symbiont switching generated high modularity in the lichen genus *Peltigera*. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.b537cs5

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*, *84*(1), 45–67. https://doi.org/10.1890/13-0133.1

Darnajoux, R., Xhang, X., McRose, D. L., Miadlikowska, J., Lutzoni, F., Kraepiel, A. M. L., & Bellenger, J.-P. (2017). Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: Importance of molybdenum availability and implications for current biological nitrogen fixation estimates. *New Phytologist*, *213*, 680–689. https://doi.org/10.1111/nph.14166

Donatti, C. I., Guimarães, P. R., Galetti, M., Pizo, M. A., Marquitti, F. M. D., & Dirzo, R. (2011). Analysis of a hyper-diverse seed dispersal network: Modularity and underlying mechanisms. *Ecology Letters*, *14*(8), 773–781. https://doi.org/10.1111/j.1461-0248.2011.01639.x

Dormann, C. F., & Strauss, R. (2014). A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution*, *5*(1), 90–98. https://doi.org/10.1111/2041-210X.12139.

Douglas, A. E. (1998). Host benefit and the evolution of specialization in symbiosis. *Heredity*, *81*(6), 599–603. https://doi.org/10.1038/sj.hdy.6884550

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*(8), 1969–1973. https://doi.org/10.1093/molbev/mss075

Eklöf, A., Helmus, M. R., Moore, M., & Allesina, S. (2012). Relevance of evolutionary history for food web structure. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1733), 1588–1596. https://doi.org/10.1098/rspb.2011.2149

Eklöf, A., Jacob, U., Kopp, J., Bosch, J., Castro-Urgal, R., Chacoff, N. P., … Allesina, S. (2013). The dimensionality of ecological networks. *Ecology Letters*, *16*(5), 577–583. https://doi.org/10.1111/ele.12081

Erwin, T. L., Ball, G. E., Whitehead, D. R., & Halpern, A. L. (1979). *Carabid Beetles – Their evolution, natural history and classification*. The Hague, Netherlands: Springer

Fisher, R. A. (1930). *The genetical theory of natural selection*. Oxford: Oxford University Press.

Fortuna, M. A., Stouffer, D. B., Olesen, J. M., Jordano, P., Mouillot, D., Krasnov, B. R., … Bascompte, J. (2010). Nestedness versus modularity in ecological networks: Two sides of the same coin? *The Journal of Animal Ecology*, *79*(4), 811–817. https://doi.org/10.1111/j.1365-2656.2010.01688.x

Gerhold, P., Cahill, J. F., Winter, M., Bartish, I. V., & Prinzing, A. (2015). Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). *Functional Ecology*, *29*(5), 600–614. https://doi.org/10.1111/1365-2435.12425

Groussin, M., Mazel, F., Sanders, J. G., Smillie, C. S., Lavergne, S., Thuiller, W., & Alm, E. J. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature Communications*, *8*, 14319. https://doi.org/10.1038/ncomms14319

Guimarães, P. R., Pires, M. M., Jordano, P., Bascompte, J., & Thompson, J. N. (2017). Indirect effects drive coevolution in mutualistic networks. *Nature*, *550*(7677), 511–514. https://doi.org/10.1038/nature24273

Guimarães, P. R., Rico-Gray, V., Oliveira, P. S., Izzo, T. J., dos Reis, S. F., & Thompson, J. N. (2007). Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Current Biology*, *17*(20), 1797–1803. https://doi.org/10.1016/j.cub.2007.09.059

Honegger, R., Edwards, D., & Axe, L. (2012). The earliest records of internally stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh Borderland. *New Phytologist*, *197*, 264–275. https://doi.org/10.1111/nph.12009

Jacquemyn, H., Merckx, V., Brys, R., Tyteca, D., Cammue, B. P. A., Honnay, O., & Lievens, B. (2011). Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus *Orchis* (Orchidaceae). *New Phytologist*, *192*(2), 518–528. https://doi.org/10.1111/j.1469-8137.2011.03796.x

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, *21*(1), 243–273. https://doi.org/10.1146/annurev.es.21.110190.001331

Janz, N., & Nylin, S. (2008). The oscillation hypothesis of host-plant range and speciation. In K. Tilmon (Ed.), *Specialization, speciation, and radiation: The evolutionary biology of herbivorous insects* (pp. 203–215). Berkely, CA: University of California Press.

Janzen, D. H. (1985). On ecological fitting. *Oikos*, *45*(3), 308–310. https://doi.org/10.2307/3565565

Junker, R. R., Blüthgen, N., Brehm, T., Binkenstein, J., Paulus, J., Martin Schaefer, H., & Stang, M. (2013). Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Functional Ecology*, *27*(2), 329–341. https://doi.org/10.1111/1365-2435.12005

Jüriado, I., Kaasalainen, U., Jylhä, M., & Rikkinen, J. (2019). Relationships between mycobiont identity, photobiont specificity and ecological preferences in the lichen genus *Peltigera* (Ascomycota) in Estonia

(northeastern Europe). *Fungal Ecology*, *39*, 45–54. https://doi.org/10.1016/j.funeco.2018.11.005

Kembel, S. W. (2009). Disentangling niche and neutral influences on community assembly: Assessing the performance of community phylogenetic structure tests. *Ecology Letters*, *12*(9), 949–960. https://doi.org/10.1111/j.1461-0248.2009.01354.x

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., … Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kiers, E. T., Rousseau, R. A., West, S. A., & Denison, R. F. (2003). Host sanctions and the legume–*Rhizobium* mutualism. *Nature*, *425*(6953), 78–81. https://doi.org/10.1038/nature01931

Krapivsky, P. L., & Redner, S. (2001). Organization of growing random networks. *Physical Review E*, *63*(6), 066123. https://doi.org/10.1103/PhysRevE.63.066123

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*(6), 1695–1701. https://doi.org/10.1093/molbev/mss020

Legendre, P. (2014). Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography*, *23*(11), 1324–1334. https://doi.org/10.1111/geb.12207

Lessard, J.-P., Belmaker, J., Myers, J. A., Chase, J. M., & Rahbek, C. (2012). Inferring local ecological processes amid species pool influences. *Trends in Ecology & Evolution*, *27*(11), 600–607. https://doi.org/10.1016/j.tree.2012.07.006

Levins, R. (1968). *Evolution in changing environments*. Princeton, NJ: Princeton University Press.

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming genus *Peltigera*. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

Lutzoni, F., & Miadlikowska, J. (2009). Lichens. Quick Guide. *Current Biology*, *19*, R502–R503. https://doi.org/10.1016/j.cub.2009.04.034

Lutzoni, F., Nowak, M. D., Alfaro, M. E., Reeb, V., Miadlikowska, J., Krug, M., … Magallón, S. (2018). Contemporaneous radiations of fungi and plants linked to symbiosis. *Nature Communications*, *9*, 5451. https://doi.org/10.1038/s41467-018-07849-9

MacArthur, R. (1955). Fluctuations of animal populations and a measure of community stability. *Ecology*, *36*(3), 533–536. https://doi.org/10.2307/1929601

Maddison, W. P., & Maddison, D. R. (2016). Mesquite: A modular system for evolutionary analysis. *Version*, *3*, 11.

Magain, N. (2018). PLexus, a PERL package to handle DNA matrices. Retrieved from https://github.com/NicolasMagain/PLexus

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017a). Macroevolution of specificity in cyanolichens of the genus *Peltigera* section *Polydactylon* (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99.

Magain, N., Miadlikowska, J., Mueller, O., Gajdeczka, M., Truong, C., Salamov, A. A., … Lutzoni, F. (2017b). Conserved genomic collinearity as a source of broadly applicable, fast evolving, markers to resolve species complexes: A case study using the lichen-forming genus *Peltigera* section *Polydactylon*. *Molecular Phylogenetics and Evolution*, *117*, 10–29. https://doi.org/10.1016/j.ympev.2017.08.013

Magain, N., Truong, C., Goward, T., Niu, D., Goffinet, B., Sérusiaux, E., … Miadlikowska, J. (2018). Species delimitation at a global scale reveals high species richness with complex biogeography and patterns of symbiont association in *Peltigera* section *Peltigera* (lichenized Ascomycota: Lecanoromycetes). *Taxon*, *67*(5), 836–870.

Maglianesi, M. A., Blüthgen, N., Böhning-Gaese, K., & Schleuning, M. (2014). Morphological traits determine specialization and resource use in plant–hummingbird networks in the neotropics. *Ecology*, *95*(12), 3325–3334. https://doi.org/10.1890/13-2261.1

Marquitti, F. M. D., Guimarães, P. R., Pires, M. M., & Bittencourt, L. F. (2014). MODULAR: software for the autonomous computation of modularity in large network sets. *Ecography*, *37*(3), 221–224. https://doi.org/10.1111/j.1600-0587.2013.00506.x.

Matzke, N. J. (2013a). BioGeoBEARS: Biogeography with Bayesian (and likelihood) evolutionary analysis in R scripts. R package, version 0.21. Retrieved from https://phylo.wikidot.com/biogeobears

Matzke, N. J. (2013b). Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers Biogeogr.*, *5*, 242–248. Retrieved from https://escholarship.org/uc/item/44j7n141. https://doi.org/10.21425/F5FBG19694

May, R. M. (1974). *Stability and complexity in model ecosystems*. Princeton, NJ: Princeton University Press.

Mayfield, M. M., & Levine, J. M. (2010). Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters*, *13*(9), 1085–1093. https://doi.org/10.1111/j.1461-0248.2010.01509.x

Maynard, D. S., Serván, C. A., & Allesina, S. (2018). Network spandrels reflect ecological assembly. *Ecology Letters*, *21*(3), 324–334. https://doi.org/10.1111/ele.12912

Medan, D., Perazzo, R. P. J., Devoto, M., Burgos, E., Zimmermann, M. G., Ceva, H., & Delbue, A. M. (2007). Analysis and assembling of network structure in mutualistic systems. *Journal of Theoretical Biology*, *246*(3), 510–521. https://doi.org/10.1016/j.jtbi.2006.12.033

Melian, C. J., & Bascompte, J. (2002). Food web structure and habitat loss. *Ecology Letters*, *5*(1), 37–46. https://doi.org/10.1046/j.1461-0248.2002.00280.x

Miadlikowska, J., & Lutzoni, F. (2000). Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. *International Journal of Plant Sciences*, *161*(6), 925–958.

Miadlikowska, J., Magain, N., Pardo de la Hoz, C., Niu, D., Goward, T., Sérusiaux, E., & Lutzoni, F. (2018). Species in section Peltidea (aphthosa group) of the genus Peltigera remain cryptic after molecular phylogenetic revision. *Plant and Fungal Systematics*, *63*(2), 45–64. https://doi.org/10.2478/pfs-2018-0007.

Miadlikowska, J., Richardson, D., Magain, N., Ball, B., Anderson, F., Cameron, R., … Lutzoni, F. (2014). Phylogenetic placement, species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming Ascomycota, Lecanoromycetes). *American Journal of Botany*, *101*(7), 1141–1156. https://doi.org/10.3732/ajb.1400267

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*, *IEEE*, 1–8.

Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: Helpful or hollow? *PLOS Biology*, *13*(12), e1002311. https://doi.org/10.1371/journal.pbio.1002311

Mouillot, D., Krasnov, B. R., & Poulin, R. (2008). High intervality explained by phylogenetic constraints in host–parasite webs. *Ecology*, *89*(7), 2043–2051. https://doi.org/10.1890/07-1241.1

Nuismer, S. L., Jordano, P., & Bascompte, J. (2013). Coevolution and the architecture of mutualistic networks. *Evolution*, *67*(2), 338–354. https://doi.org/10.1111/j.1558-5646.2012.01801.x

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2005). Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus *Peltigera*. *European Journal of Phycology*, *40*(4), 363–378.

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2013). Assessing population structure and host specialization in lichenized cyanobacteria. *New Phytologist*, *198*(2), 557–566. https://doi.org/10.1111/nph.12165

Ochman, H., Worobey, M., Kuo, C.-H., Ndjango, J.-B.-N., Peeters, M., Hahn, B. H., & Hugenholtz, P. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology*, *8*(11), e1000546. https://doi.org/10.1371/journal.pbio.1000546

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104.

Olesen, J. M., Bascompte, J., Elberling, H., & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, *89*(6), 1573–1582. https://doi.org/10.1890/07-0451.1

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Pardo-De la Hoz, C. J., Magain, N., Lutzoni, F., Goward, T., Restrepo, S., & Miadlikowska, J. (2018). contrasting symbiotic patterns in two closely related lineages of trimembered lichens of the genus Peltigera. *Frontiers in Microbiology*, *9*, https://doi.org/10.3389/fmicb.2018.02770

Patterson, B. D., & Atmar, W. (1986). Nested subsets and the structure of insular mammalian faunas and archipelagos. *Biological Journal of the Linnean Society*, *28*(1–2), 65–82. https://doi.org/10.1111/j.1095-8312.1986.tb01749.x

Peres-Neto, P. R., Olden, J. D., & Jackson, D. A. (2001). Environmentally constrained null models: Site suitability as occupancy criterion. *Oikos*, *93*(1), 110–120. https://doi.org/10.1034/j.1600-0706.2001.930112.x

Pimm, S. L. (1979). Complexity and stability: Another look at Macarthur's original hypothesis. *Oikos*, https://doi.org/10.2307/3544322

Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssieres, A., Deveau, A., Melton, S. J., … Martin, F. (2014). Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences*, *111*(22), 8299–8304. https://doi.org/10.1073/pnas.1322671111

Podani, J., Ricotta, C., & Schmera, D. (2013). A general framework for analyzing beta diversity, nestedness and related community-level phenomena based on abundance data. *Ecological Complexity*, *15*, 52–61. https://doi.org/10.1016/j.ecocom.2013.03.002

Podani, J., & Schmera, D. (2011). A new conceptual and methodological framework for exploring and explaining pattern in presence - absence data. *Oikos*, *120*(11), 1625–1638. https://doi.org/10.1111/j.1600-0706.2011.19451.x

Poisot, T., Mouquet, N., & Gravel, D. (2013). Trophic complementarity drives the biodiversity-ecosystem functioning relationship in food webs. *Ecology Letters*, https://doi.org/10.1111/ele.12118

R Core Team. (2018). *R: A language and environment for statistical computing*.

Ree, R. H., & Sanmartín, I. (2018). Conceptual and statistical problems with the DEC+ J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography*, *45*(4), 741–749. https://doi.org/10.1111/jbi.13173

Refrégier, G., Le Gac, M., Jabbour, F., Widmer, A., Shykoff, J. A., Yockteng, R., … Giraud, T. (2008). Cophylogeny of the anther smut fungi and their caryophyllaceous hosts: Prevalence of host shifts and importance of delimiting parasite species for inferring cospeciation. *BMC Evolutionary Biology*, *8*(1), 100. https://doi.org/10.1186/1471-2148-8-100

Reinhart, K. O., & Anacker, B. L. (2014). More closely related plants have more distinct mycorrhizal communities. *AoB PLANTS*, *6*, plu051-plu051. https://doi.org/10.1093/aobpla/plu051

Rezende, E. L., Albert, E. M., Fortuna, M. A., & Bascompte, J. (2009). Compartments in a marine food web associated with phylogeny, body mass, and habitat structure. *Ecology Letters*, *12*(8), 779–788. https://doi.org/10.1111/j.1461-0248.2009.01327.x

Rodriguez, F., Oliver, J., Marin, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, *142*, 485–501. https://doi.org/10.1016/S0022-5193(05)80104-3

Rossberg, A. G., Brannstrom, A., & Dieckmann, U. (2010). Food-web structure in low- and high-dimensional trophic niche spaces. *Journal of The Royal Society Interface*, *7*(53), 1735–1743. https://doi.org/10.1098/rsif.2010.0111

Ružička, M. (1958). Anwendung mathematisch-statistischer Methoden in der Geobotanik (synthetische Bearbeitung von Aufnahmen). *Biologia, Bratislava*, *13*, 647–661.

Sanders, J. G., Powell, S., Kronauer, D. J. C., Vasconcelos, H. L., Frederickson, M. E., & Pierce, N. E. (2014). Stability and phylogenetic correlation in gut microbiota: Lessons from ants and apes. *Molecular Ecology*, *23*(6), 1268–1283. https://doi.org/10.1111/mec.12611

Shefferson, R. P., Cowden, C. C., McCormick, M. K., Yukawa, T., Ogura-Tsujita, Y., & Hashimoto, T. (2010). Evolution of host breadth in broad interactions: Mycorrhizal specificity in east Asian and North American rattlesnake plantains (*Goodyera spp.*) and their fungal hosts. *Molecular Ecology*, *19*(14), 3008–3017. https://doi.org/10.1111/j.1365-294X.2010.04693.x

Singh, R. S., & Walia, A. K. (2014). Characteristics of lichen lectins and their role in symbiosis. *Symbiosis*, *62*(3), 123–134. https://doi.org/10.1007/s13199-014-0278-y

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, *57*, 758–771. https://doi.org/10.1080/10635150802429642

Swenson, N. G., & Enquist, B. J. (2009). Opposing assembly mechanisms in a Neotropical dry forest: Implications for phylogenetic and functional community ecology. *Ecology*, *90*(8), 2161–2170. https://doi.org/10.1890/08-1025.1

Swofford, D. L. (2001). Paup*: Phylogenetic analysis using parsimony (and other methods) 4.0. B5.

Thompson, J. N. (2005). *The geographic mosaic of coevolution*. Chicago, IL: Chicago University Press.

Thompson, J. N. (2006). Mutualistic webs of species. *Science*, *312*(5772), 372–373. https://doi.org/10.1126/science.1126904

Torrecillas, E., del Mar Alguacil, M., Roldán, A., Díaz, G., Montesinos-Navarro, A., & Torres, M. P. (2014). Modularity reveals the tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gypsum soils. *Applied and Environmental Microbiology*, *80*(17), 5457–5466. https://doi.org/10.1128/AEM.01358-14.

Veresoglou, S. D., & Rillig, M. C. (2014). Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant and Soil*, *377*(1–2), 395–406. https://doi.org/10.1007/s11104-013-2008-2

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Williams, P. H. (1996). Mapping variations in the strength and breadth of biogeographic transition zones using species turnover. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1370), 579–588. https://doi.org/10.1098/rspb.1996.0087

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