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Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus *Peltigera*

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Abstract
Identifying the drivers and evolutionary consequences of species interactions is a major goal of community ecology. Network-based analyses can provide mathematical tools to detect non-random patterns of interactions, and potentially help predicting the consequences of such patterns on evolutionary dynamics of symbiotic systems. Here, we characterize the structure of a lichen network at a very fine phylogenetic scale, by identifying the photosynthetic partners (i.e., cyanobacteria of the genus *Nostoc*) of lichenized fungi belonging to a monophyletic section of a single genus (i.e., section *Polydactylon* of the genus *Peltigera*), worldwide. Even at such a fine phylogenetic scale, we found that interactions were highly modular and anti-nested, indicating strong preferences in interactions. When considering local *Peltigera* communities, i.e., datasets at small spatial scales with only a slightly broader phylogenetic range, interactions remained modular but were asymmetric, with generalist *Nostoc* partners interacting with specialized *Peltigera* species. This asymmetry was not detected with our global spatial scale dataset. We discuss these results in the light of lichen community assembly, and explore how such interaction patterns may influence coevolution in lichens and the evolutionary stability of the mutualism in general.

Keywords Cyanolichens · Partner selection · Mutualism · *Nostoc* · Symbiosis

Introduction

One fundamental goal of community ecology has typically been to determine why species occur in some places but not in others (Vellend 2016), and why some species interact preferentially. This latter line of inquiry was first applied to trophic interactions (e.g., Ulanowicz and Kemp 1979; Pimm and Lawton 1980; Ulanowicz 1986, 1997), but quickly reached mutualistic associations (e.g., Herrera 1982; Jordano 1987). During the last decade, there has been

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a renewed interest in multispecies interactions in community ecology with the application of network-based tools to uncover interaction patterns (Dupont et al. 2003, 2009; Olesen et al. 2007). These tools were identified as potentially useful to (1) elucidate the assembly mechanisms responsible for such structure, and (2) determine the consequences of such patterns on community dynamics (e.g., species persistence, secondary extinctions) and on the coevolution of species (Thompson 2009).

The bulk of the studies on ecological networks has focused on plant–animal interactions (e.g., pollination, frugivory, myrmecophytic plants) (Bascompte et al. 2003; Guimarães et al. 2006; Burns 2013). In those systems, network-based approaches have contributed to highlight how interaction patterns resulted from the joint influence of species abundances (Vázquez et al. 2005), phenotypic traits (Stang et al. 2007, 2009; Donatti et al. 2011; Maglianesi et al. 2015) and evolutionary history (Rezende et al. 2007; Gómez et al. 2010). A number of network studies have also been conducted on plant–fungal symbioses, e.g., mycorrhizae (Montesinos-Navarro et al. 2012; Martos et al. 2012; Chagnon et al. 2015) and fungal endophytes (Chagnon et al. 2016). Likewise, in those systems, interaction patterns were

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shown to be structured by host traits (Kembel et al. 2014; Chagnon et al. 2015, 2016), habitat preferences (Martos et al. 2012; Torrecillas et al. 2014) or species abundances (Chagnon et al. 2015). These studies have provided insights on the establishment of mutualistic interactions between fungi and photosynthetic partners, and allowed the exploration of the similarities and differences with plant–animal networks (Toju et al. 2014). The ecologically and evolutionarily successful lichen symbiosis, involving primarily fungi (mycobionts) and photosynthetic algae and/or cyanobacteria (photobionts), still has not been characterized from a network perspective. Yet, there is a clear interest in using this symbiosis as a model system, including the possibility to pose novel hypotheses regarding interaction establishment, and maintenance of mutualism.

About one-fifth of all known species of fungi are lichenized (Feuerer and Hawksworth 2007; Kirk et al. 2008). Unlike mycorrhizae and fungal endophytes, the symbiotic unit, the lichen thallus, is visible and often distinct, which enables efficient sampling design. Moreover, in lichens, the mycobionts are often more dependent on photobionts than vice versa (Nash 2008; Lutzoni and Miadlikowska 2009). The fungal partner forming the thallus (the mycobiont) cannot live independently from its photobiont unless a loss of lichenization is at play in a specific fungal clade (Lutzoni et al. 2001; Wedin et al. 2004). This could lead us to expect that specialization is rarely reciprocal in this symbiosis: if mycobionts are so dependent upon compatible photobionts, then there must be a pressure to remain generalist and broadly compatible with many photobionts, as any compatible partner is better than none. However, if these associations with various photobionts result in a range of fitness levels for the mycobiont, natural selection could lead to the specialization of a fungal species on a few optimal photobionts. If some of these photobionts are advantageous to many closely related lichen-forming species, this would result in a few generalist photobionts associating with many specialist fungal species, as observed by Magain et al. (2017a) and Lu et al. (2018). This asymmetric specialization could be key to the maintenance of the lichen symbiosis, where the photobiont is transmitted mostly horizontally (i.e., the symbiosis needs to be re-established) from one generation to the next. Such asymmetric specialization is characteristic of nested interaction patterns in ecological networks (Vázquez and Aizen 2004; Joppa et al. 2010). Finding strong nestedness in lichen networks would be in line with previous studies showing that nestedness is generally prevalent in natural mutualistic networks (e.g., Bascompte et al. 2003; Fontaine et al. 2011). However, the ability for some lichens to co-disperse their mycobiont and photobiont together in specialized structures (such as soredia or isidia; i.e., vertical transmission of photobionts), or through thallus fragmentation, suggests that there may be some highly intimate interactions among

subsets of mycobionts and photobionts (Otálora et al. 2010; Dal Grande et al. 2012). This should translate, at the network level, in high modularity and rather symmetrical specialization. Thus, network-based analyses have the potential to discriminate between competing hypotheses explaining the establishment and maintenance of lichenic interactions.

Studies on specificity of symbiotic associations in cyanolichens from various genera: *Leptogium*, *Collema* and *Degelia* (Otálora et al. 2010, 2013) indicated a link between high reciprocal specificity and vertical transmission of the photobiont, and restricted ecological niches. It was also suggested that the substrate determines the sets of cyanobionts available for sharing among mycobionts of terrestrial and epiphytic lichen guilds (Rikkinen et al. 2002) and that associations between lichen-forming fungi and their photobionts can be best explained by the interplay of vertical and horizontal transmissions of photobionts (Rikkinen 2003). However, the study by Magain et al. (2017a) supports the hypothesis that mycobiont specificity towards a specific pool of *Nostoc* phylogroups is inherited through time at a macroevolutionary scale with occasional switches from one cyanobiont pool to another.

Network structure may not only reflect community assembly rules, but could also suggest potential evolutionary consequences of given interaction patterns. For example, asymmetric specialization may lead to an imbalance in the reciprocal adaptation of the partners to each other. While a specialist experience strong selection pressures to adapt to its only partner, if the latter is also interacting with many other species (i.e., is a generalist), then it may not reciprocally adapt to the specialist (Vázquez et al. 2007; Guimarães et al. 2007). Guimarães et al. (2011) have shown, using a theoretical model of evolution, that generalists should have more impact on trait evolution in networks, and favor trait convergence among species at a given trophic level, to adapt to their core of generalist partners. Moreover, the presence of well-defined modules (i.e., groups of species that preferentially interact) in a network could suggest that most coevolutionary processes will take place within modules, in such a way that species belonging to the same modules will progressively co-adapt to each other. The presence of well-defined modules may thus suggest a tight coevolutionary history between sets of mycobionts and photobionts (e.g., Olesen et al. 2007; Danieli-Silva et al. 2012).

Here, we characterize the network of interactions involving lichenized fungi belonging to the section *Polydactylon* of the genus *Peltigera* (Peltigerales, Lecanoromycetes) at two spatial scales (global and local). These fungi form macrolichens with cyanobacterial partners of the genus *Nostoc* (Magain et al. 2017a). Section *Polydactylon* was shown to include ca. 40 species with different profiles of specificity towards their *Nostoc* partner, from strict specialists to generalists (Magain et al. 2017a, b). Previous studies demonstrated

that *Peltigera* have high specificity toward their cyanobacterial partners, both locally and globally, but the degree of specificity is variable and likely influenced by factors such as the identity and phylogenetic relationships of the species, as well as geographical and ecological factors (O'Brien et al. 2013; Magain et al. 2017a; Lu et al. 2018), whereas the specificity of *Nostoc* appears lower but still ranging from strict specialists to generalists (O'Brien et al. 2005; Magain et al. 2017a). Our study was conducted primarily at a global spatial scale, allowing a better understanding of the ecological drivers as well as potential evolutionary consequences of these interactions at a fine phylogenetic scale, i.e., involving closely related species of the genus *Peltigera* that are sharing a most recent common ancestor, as part of the monophyletic section *Polydactylon*. To evaluate the effect of spatial scales on the driving mechanisms for network structure, we also characterized network structure at local spatial scales in British Columbia (Canada) for the entire communities of *Peltigera* species growing side by side, using datasets from O'Brien et al. (2009, 2013).

Materials and methods

Data acquisition

Our global dataset consists of 250 lichen specimens representing section *Polydactylon* of the genus *Peltigera* [200 from Magain et al. (2017a), 18 from Magain et al. (2017b), and 32 newly sequenced individuals (dataset 1 in Supplementary Table S1)]. These specimens were loaned from herbaria or private collections, or collected throughout the world by members of the Lutzoni lab and collaborators (Supplementary Figure S1) from a broad diversity of habitats (Magain et al. 2017a). For each thallus, both the mycobiont (fungal partner, *Peltigera*) and photobiont (photosynthetic partner, the nitrogen-fixing cyanobacterium *Nostoc*, also referred to as the cyanobiont) were identified using a molecular approach (Magain et al. 2017a, b). Briefly, for the mycobiont, the nuclear ribosomal RNA [Internal Transcribed Spacer (ITS) region and the 5′ end of the large subunit (nrLSU)], three protein-coding genes [partial RNA polymerase II largest subunit (*RPB1*), β-tubulin, and the elongation factor 2 region 1 (*EFT2*.1)] and three Collinear Orthologous Regions (COR), each containing one intergenic spacer, were amplified and sequenced (Magain et al. 2017a, b). We used species delimitations from Magain et al. (2017b), which were based on a consensus of five different methods [BPP, Yang and Rannala (2010); spedeSTEM, Ence and Carstens (2011); Structurama, Huelsenbeck et al. (2011); bGMYC, Reid and Carstens (2012); and bPTP, Zhang et al. (2013)]. For the cyanobiont, the *rbc*LX locus, coding for the RuBisCO large subunit (*rbc*L), and a chaperone gene (*rbc*X) were amplified and sequenced to identify *Nostoc* haplotypes and delimit well-supported monophyletic phylogroups as proxies for species. Detailed information about the molecular data acquisition and phylogenetic methods can be found in Magain et al. (2017a, b).

For the 32 new specimens added for this study, we sequenced 28 ITS of *Peltigera* and 32 *rbc*LX of their *Nostoc* partner (Supplementary Table S1) using the same methods described in Magain et al. (2017a). These sequences were included in the alignments of Magain et al. (2017b) using MacClade v. 4.08 (Maddison and Maddison 2005). The identification of the fungal and cyanobacterial partners was first attempted by sequence similarity (BLASTn; Altschul et al. 1990). If sequences were not identical to reference sequences, their identity was confirmed by performing a phylogenetic analysis with RAxML (Stamatakis 2006; Stamatakis et al. 2008) and bootstrapping, using the same parameters as in Magain et al. (2017b).

Based on this sampling of 250 specimens (dataset 1), we built an interaction matrix, with mycobiont species as columns, photobiont phylogroups as rows, and each cell representing the number of thalli in our dataset where the corresponding mycobiont and photobiont putative species were found within the same thallus (i.e., representing the frequency for each interacting partner pair). Thus, our dataset provided a quantitative (i.e., weighted) estimate of interactions, which allowed us to conduct the network-level analyses described below.

We used two additional datasets (datasets 2 and 3, Supplementary Table S1), which include all *Peltigera* species (i.e., not restricted to section *Polydactylon*) sampled from two lichen communities in British Columbia (BC), Canada (O'Brien et al. 2009, 2013). One of the local scale datasets (dataset 2) encompasses samples from 159 *Peltigera* thalli, which were separated by a few centimeters to ~20 m. These thalli were collected from the Spahats Creek site, located in southern British Columbia (O'Brien et al. 2013). The second local scale dataset (dataset 3) includes 235 thalli collected from five different sites in southern BC (Ghost Lake, Barkersville, Cameron Ridge, Battle Creek and Spahats Creek), with a maximal distance among sites of about 150 km (O'Brien et al. 2005, 2009, 2013). Using these two datasets of 159 and 235 specimens, respectively, we built interaction matrices the same way as described for the global scale sampling.

Network analyses

Various network-level analyses were conducted on our global and our two local datasets of lichen interactions, to answer the following three questions: (1) Are there specific groups of mycobionts and photobionts that preferentially interact together? (2) Is there evidence for reciprocal

specialization [or for the absence of it, as commonly found in ecological networks, e.g., Joppa et al. (2009)]? (3) How might the interaction patterns observed in our datasets impact the evolution of the mycobionts and photobionts?

Question 1 Preferential interactions were identified using modularity analyses, which have become fairly popular in community ecology to detect higher level specificity in interactions. Modularity analyses were designed to not only determine if different species interact with different partners, but to also discover if there are groups of species (called modules) that share common preferred partners (e.g., Donatti et al. 2011; Mello et al. 2011). In other words, in a modular network we can partition species into modules in such a way that there will be much more interactions within rather than outside modules. Although modularity remains a simple clustering technique similar to other commonly used methods in community ecology (e.g., *k*-means, hierarchical; Chagnon 2016), one appealing property of modularity analysis is that the algorithms developed to optimize it often use a powerful optimization technique called simulated annealing. Such optimization strategy has been developed in computational sciences to solve very complex problems (sometimes referred to as NP-hard problems) within a reasonable time frame (Nourani and Andresen 1999). Although computationally intensive, this technique is particularly efficient at uncovering global maxima in optimization problems (here, the very most modular state of an interaction network). We investigated the level of modularity in our dataset following Dormann and Strauss (2014), using the *bipartite* R package (Dormann et al. 2009) and compared it to 1000 random scenarios to determine whether our lichen network was more modular than expected by chance alone. Those random scenarios (i.e., random interaction matrices) were generated under a conservative null model (*vaznull* implemented in *bipartite*) that aims at best preserving the total number of interactions in a network, as well as the total number of interactions for each species individually. In a nutshell, this null model uses an iterative procedure to progressively fill a random matrix with interactions, following the marginal totals of the matrix. Thus, at each iteration, a matrix cell that corresponds to a generalist photobiont and a generalist mycobiont has a much higher probability of receiving an interaction as opposed to a cell corresponding to specialist mycobionts and photobionts. After a high number of iterations, interactions are added or removed to the random matrix to ensure that the final total number of interactions mirrors the number of interactions in the original matrix. We preferred a null model that uses a probabilistic approach over a swap-based approach to fill the random matrices' cells, because in low connectance matrices such as ours, swap-based approach can be overly conservative or even fail to converge to a solution in a

reasonable time (see Supplementary Methods for detailed information and discussion on the null model).

Because the sampling for dataset 1 was conducted at a global geographical scale, modularity could arise in this dataset simply because some mycobionts and photobionts are endemic to a restricted part of the world and, therefore, would be found interacting with each other more frequently than with the rest of the taxa in the dataset. This would not necessarily reflect a preferential and selective process of association between mycobionts and photobionts. To control for this biogeographical issue in structuring our modules, we performed a variance partitioning of the Bray–Curtis distance matrix among the different thalli to see whether the identity of a photobiont in a lichen thallus was best predicted by: (1) the geographic region where a lichen was sampled (2) the biome in which it was sampled, or (3) the identity of the mycobiont in the sampled thallus. This analysis is implemented in the *adonis* function of the R package *vegan* (Oksanen et al. 2017). We evaluated, also using *adonis*, how photobiont partner identity could be predicted by mycobiont identity within each region or biome.

Furthermore, we characterized network structure of datasets 2 and 3 collected at local scales (O'Brien et al. 2009, 2013). Collectively, these data provided interaction networks at much finer spatial scales (especially dataset 2, with thalli often within a few centimeters from each other and nearly overlapping), which should prevent modularity arising from environmental preferences: at finer spatial scales, the abiotic environment is expected to be much more homogeneous.

Question 2 Reciprocal specialization was characterized using nestedness analyses, commonly used in network-based and β-diversity studies. In interaction networks, nestedness refers to a pattern opposed to reciprocal specialization; i.e., where specialists are interacting mostly with generalists, but not vice versa. To measure nestedness in our datasets, we used the weighted version of the *NODF* index (Almeida-Neto et al. 2008) developed by Almeida-Neto and Ulrich (2011), hereafter referred to as *wNODF*. To evaluate the statistical significance of *wNODF*, we compared the observed *wNODF* value to 1000 random values generated using the same null model as described for our modularity analysis (see Supplementary Methods). We also complemented *wNODF* analysis with SDR analysis, developed by Podani and collaborators (Podani and Schmera 2011; Podani et al. 2013). This analysis, originally intended to characterize structural patterns in meta-communities (i.e., species × sites matrices, instead of species × species matrices for interaction networks), aims at explaining variation in partner selection in a network. Briefly, when comparing the symbiotic partners of two species, the SDR framework partitions variation into three additive components: component S is their similarity in partner choice, component D is the variation in the number

of interactions, and component R is the variation in the identity of the partners for these two species. Since these three components sum to 1, the SDR framework allows to visualize the trends present at the whole network level in a two-dimensional, ternary triangle. The D component of SDR is closely related to the concept of nestedness in ecological networks (i.e., the idea that the set of interactions by a more specialized species can always be included, or nested, within a set of interactions exhibited by more generalist species). However, it remains debated whether one method or the other is superior to characterize nestedness in a matrix (e.g., Ulrich and Almeida-Neto 2012; Podani and Schmera 2012). There is thus value in corroborating *wNODF* analysis with SDR analysis. Here, as for *wNODF* analyses, we took advantage of our quantitative data on species interactions to perform quantitative (not binary) SDR analyses, thus taking into account not only whether a mycobiont and a photobiont interacted together, but also at which frequency they did so.

Question 3 To explore how interaction patterns might give feedback on the evolution of the mycobionts and photobionts, we looked at interaction strength symmetry. Herein we use the term symmetric interaction to denote an interaction where two partners share the same proportion of time or effort with one another. For example, a case scenario where species *A* spends all its interaction events with species *B*, while species *B* is involved in many more interactions with other partners would be highly asymmetric. For lichens, the thallus offers a convenient definition of an "interaction event". Interaction symmetry is expected to impact the evolution of symbiotic systems (Vázquez et al. 2007) and, therefore, be potentially highly relevant to our understanding of symbiotic systems such as lichens. Here, we characterized interaction symmetry following Vázquez et al. (2007). Briefly, for a pair of species *i* and *j*, the effect of species *i* on species *j* (s_{ij}) is the proportion of interactions of species *j* that involve species *i*. This implies the logical assumption that if species *i* interacts almost always with species *j*, then it will experience a higher selection pressure to adapt to its partner and derive a higher fitness benefit from this interaction than if it would interact frequently with many different species. Then, for this species pair, we calculated the difference between the reciprocal effects of species *i* and *j* on each other (i.e., $d_{ij} = s_{ij} - s_{ji}$). For a given species, its asymmetry (*A*) value is defined as the mean of its "*d*-values" for all its partners. A_i is close to 1 if species *i* exerts strong effects on its partners while experiencing little reciprocal effects from them, and close to -1 in the reverse situation. Because the effect of a species *i* on *j* (i.e. s_{ij}) should be linked to the selection pressure that *j* experiences to become better adapted to *i*, looking at the symmetry in these effects should inform about coevolution in a network.

Sampling completeness

Our global sampling (dataset 1) identifies 250 interactions between lichenized fungi from the section *Polydactylon* of the genus *Peltigera*, and cyanobacterial partners from the genus *Nostoc*. Given the worldwide scale at which this survey was performed, 250 thalli may seem a small number. It is well known that sampling effort will impact patterns that are uncovered through network-based analyses. While it is difficult in itself to fully describe species composition in communities, it is even harder to detect all realized interactions among them (e.g., Nielsen and Bascompte 2007; Dorado et al. 2011; Chacoff et al. 2012; Rivera-Hutinel et al. 2012; Fort et al. 2016). Thus, before going further with network analyses, we verified that our sampling completeness was adequate to appropriately characterize network structure. We generated rarefaction curves for network connectance (i.e., the proportion of potential interactions between sets of species that are realized in the field), modularity and nestedness. We also followed methodology outlined by Chao et al. (2009) to evaluate our sampling completeness and the number of additional thalli that would have been necessary to fully sample our network of interactions (see Supplementary Methods). We also conducted these analyses for our two additional, local samplings (datasets 2 and 3) in British Columbia.

Results

Sampling effort

In our global sampling (dataset 1), we observed 91 different interaction pairs among our 42 *Peltigera* species and 43 photobiont phylogroups and unique haplotypes. Using the Chao2 estimator (Chao et al. 2009), we calculated that a complete sampling of our network could have uncovered 130 interactions. This means that our sample size (i.e., 250 thalli) allowed us to detect 70% of the interactions potentially realized between our taxa pool. Likewise, for our two local datasets, sampling completeness was estimated to be 81% for Spahats Creek (dataset 2) and 90% for all five BC sites combined (dataset 3). We were also able to estimate that about 1030 additional thalli would be necessary to detect all interactions in our global dataset, highlighting the difficulty to detect rare interaction pairs. Rarefaction curves (see Supplementary Methods) show that there is a link between sampling effort and network modularity and nestedness. However, these plots also suggest that additional sampling would only strengthen the results that we already found (see sections below), and thus our conclusions appear to be robust to sampling effort.

Modularity and preferential interactions

Figure 1 shows the modules revealed by our global survey of *Peltigera* section *Polydactylon* (dataset 1). Rows and columns are ordered to pack the modules of preferentially interacting taxa along the matrix diagonal (i.e., not following phylogenetic relationships among mycobionts and photobionts, but rather to reflect preferences in interactions). The network modularity was statistically higher than in randomized matrices ($P = 0.035$). The identity of the photobiont in a thallus was best predicted by the mycobiont identity (39%), while the effects of the region or the biome where the thallus was sampled remained marginal (region = 3%, biome = 6%). This finding was corroborated by additional analyses showing that the effect of mycobiont identity in predicting photobiont identity remained significant even within regions and biomes (except for Brazil, where the mycobiont and the photobiont identities were unrelated) (Supplementary Table S2). In our local datasets (2 and 3), the trend for modularity was in fact even stronger (dataset 2: $P < 0.001$; dataset 3: $P < 0.001$),
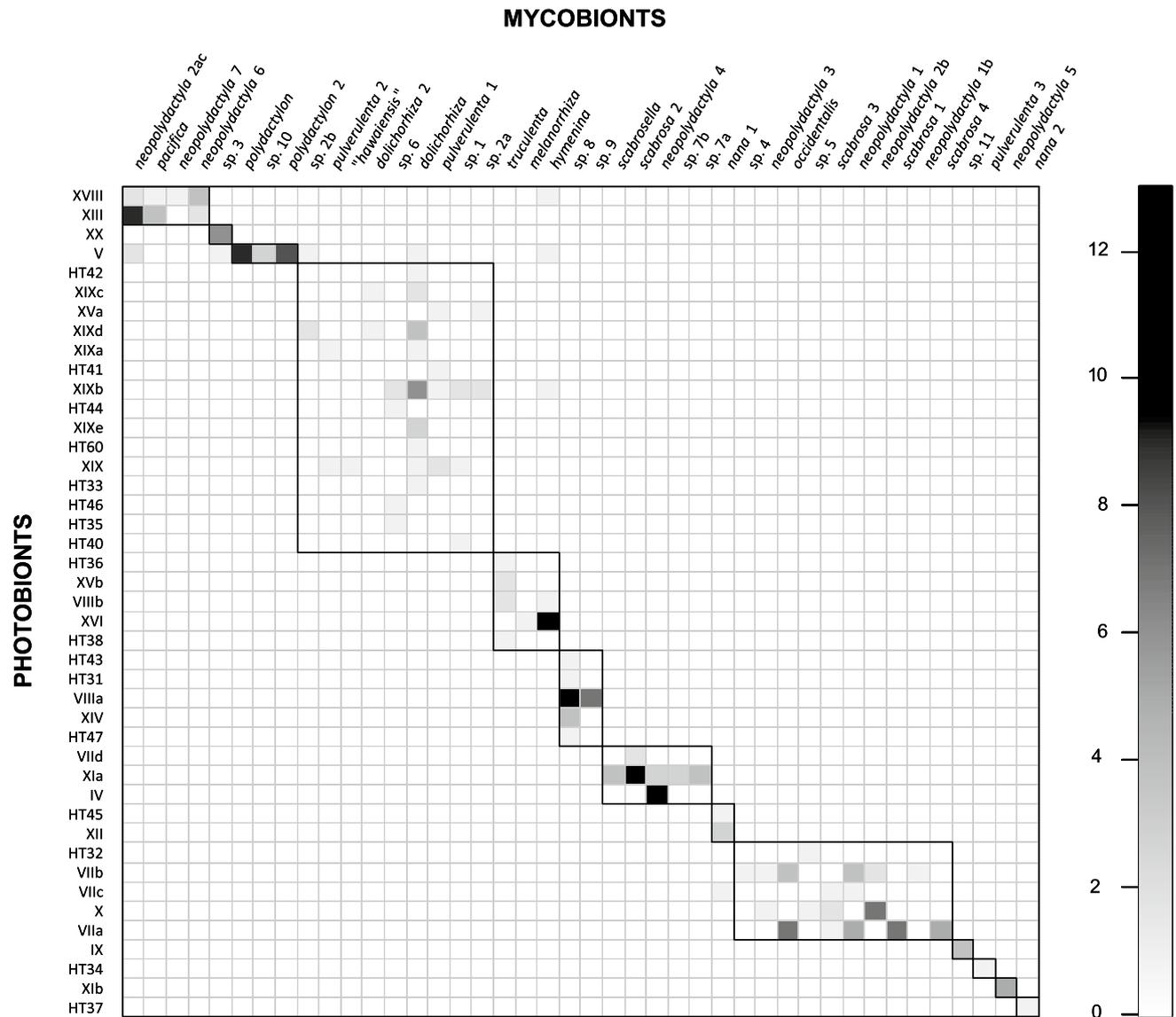

Fig. 1 Graphical depiction of the modularity analysis through the simulated annealing algorithm, showing significant modules in dataset 1. Each column represents a mycobiont species (*Peltigera*, section *Polydactylon*) as defined by Magain et al. (2017b). Species are only designated by their epithet, i.e., omitting the genus name *Peltigera*. Each row represents a photobiont phylogroup (roman numerals) or haplotype (HT) sensu Magain et al. (2017a). In each cell the shade of gray is proportional to the frequency of a specific mycobiont–photobiont pair (i.e., number of thalli found with the same mycobiont and photobiont pair). The modules defined by the algorithm are delimited by dark lines and aligned along the matrix diagonal. See Supplementary Figure S2 for results from the binary modularity analysis

indicating that modularity is not scale sensitive in this symbiotic system (Supplementary Figure S3).

Nestedness and SDR analyses

The weighted nestedness (*wNODF*) analyses for dataset 1 indicated that the interaction network was significantly less nested than expected under random scenarios (mycobionts, $P = 0.003$; photobionts, $P = 0.015$), a pattern that is sometime referred to as anti-nestedness (Poulin and Guégan 2000). This finding was corroborated by the SDR analyses, where the pairwise comparisons aligned on the left side of the ternary triangle, consistent with what is expected under anti-nestedness (Podani et al. 2013, Fig. 2). Similar trends were found in local datasets: the trend was significant for dataset 3 (mycobionts, $P = 0.004$; photobionts, $P = 0.016$), but not

significant for dataset 2 (mycobionts, $P = 0.264$; photobionts, $P = 0.140$) (Supplementary Figure S4).

Symmetry in interaction strength

We found evidence for high prevalence of symmetric interactions at the global scale (dataset 1; Fig. 3). However, for both the mycobionts and the photobionts, some species had consistently highly negative asymmetry scores, which could represent a passive sampling effect (Vázquez et al. 2007). Rare species, which by definition have low interaction frequencies, always tend to have a smaller effect on their partners than vice versa, unless their only partners are also very rare. Therefore, species with highly negative asymmetry values might be simply rare species in the dataset. We did observe such correlation between species frequency and asymmetry scores. However, for
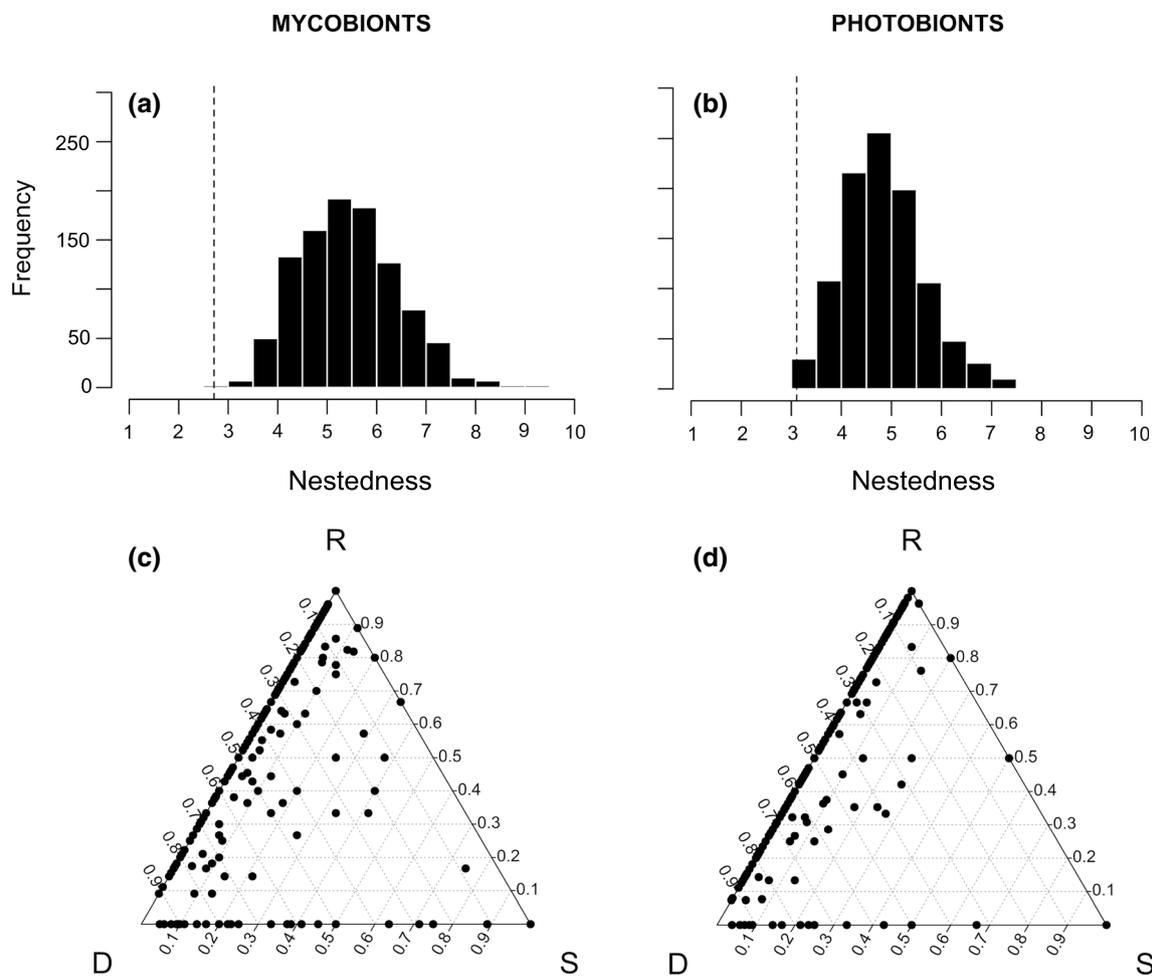

Fig. 2 Reciprocity of specialization assessed using nestedness and beta-diversity analyses (SDR; *S* partner similarity, *D* partner richness difference, *R* partner replacement) in dataset 1. Histograms of the null distributions of nestedness values (wNODF) are presented for **a** mycobionts and **b** photobionts. Dashed vertical lines represent the observed values in the dataset. **c**, **d** The ternary triangles representing SDR simplex decompositions of beta-diversity following Podani et al. (2013). Each point in such triangles represents a pairwise comparison of **c** mycobiont species or **d** photobiont phylogroups
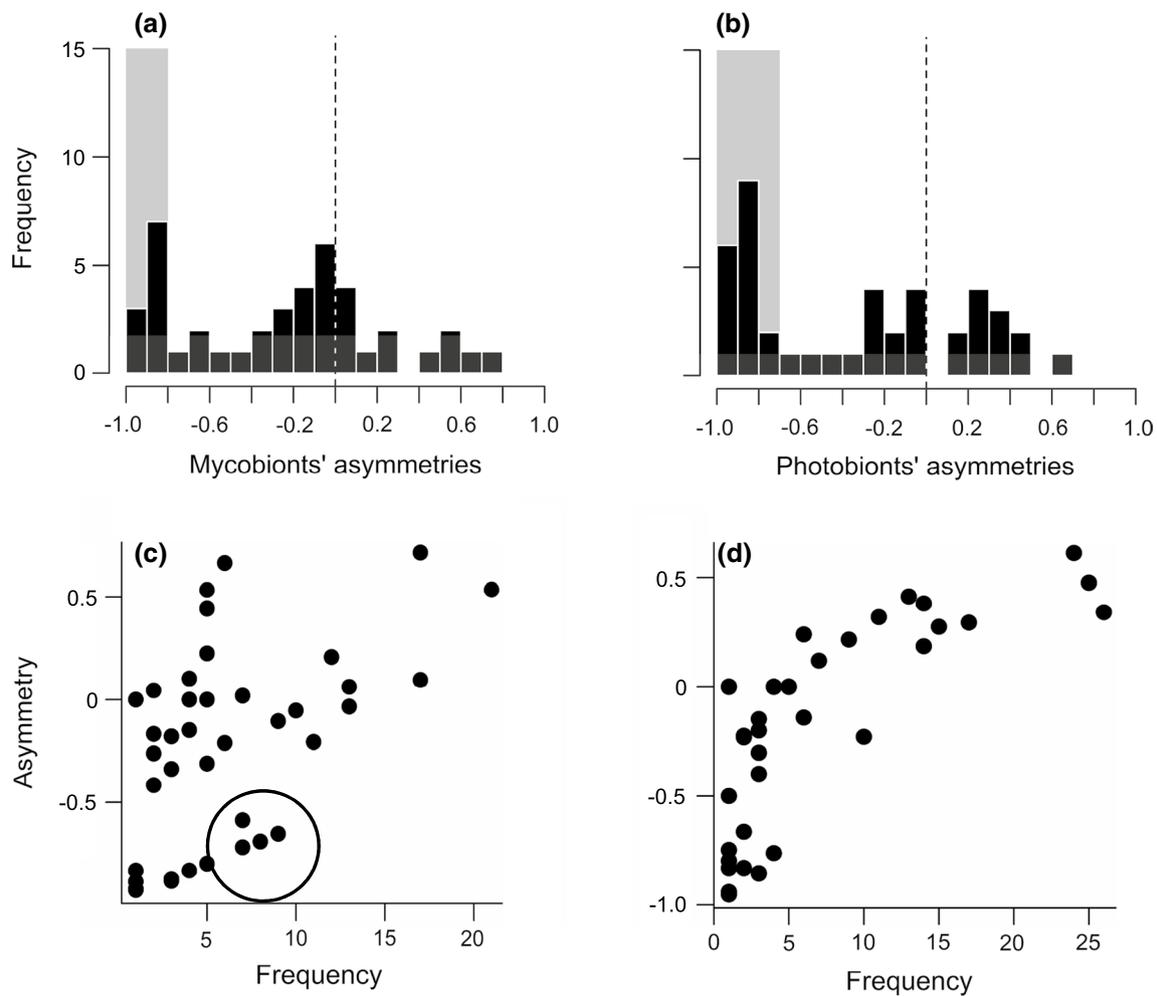
Fig. 3 Interaction symmetry among mycobionts and photobionts in dataset 1. We show the frequency distributions of asymmetry scores for **a** mycobionts and **b** photobionts. The areas shaded in gray represent the portion of species with highly negative asymmetry scores that seem to diverge from the main, modal scores centered around 0.

We also plot the asymmetry scores against the number of sampled thalli where a given **c** mycobiont or **d** photobiont was found. The circle in **c** represents the mycobionts for which asymmetry scores remain low even with a reasonable sampling effort (i.e., true cases of highly asymmetric specialization and dependence)

the mycobionts in the global dataset, there were also a number of species that had been sampled in several thalli, but continue to have highly negative asymmetry values. These are mycobionts truly specialized asymmetrically on generalist photobionts (e.g., phylogroup VIIa, see Fig. 1), a trend we did not detect for photobionts, which tend to be less dependent on the mycobiont (i.e., more generalists, with more phylogroups having positive asymmetry scores above 0.2 than the mycobiont; Fig. 3).

Conversely, in our local datasets 2 and 3, we found a trend for mycobionts to have mostly negative asymmetry scores (Supplementary Figure S3), which typically corresponded to interactions with a highly generalist photobiont partner having a positive asymmetry score. In dataset 3, there were also a few specialized photobionts found only

once, and these were found to have negative asymmetry scores, for reasons explained in the previous paragraph.

Discussion

In this study, we found preferential interactions among mycobionts and photobionts, as indicated by the significant modularity of the network at the global scale. However, modularity can arise in a network when species are segregated in space and/or time (e.g., Martos et al. 2012; Martín González et al. 2012; Torrecillas et al. 2014) such that all partners in the network are not available to each other. It would thus be incautious to directly link this pattern to a preferential partner selection, which implies a behavioral

choice among multiple options. However, two additional lines of evidence allow us here to suggest that in our system, modularity truly arises from preferential, or selective, interactions: (1) the mycobiont identity in a thallus was a better predictor of the photobiont identity than the region of the globe, or the biome where the thallus was sampled; (2) modularity remained highly significant even at small spatial scales, where partner availability is not expected to prevent any interaction.

This result is in line with trends found in other lichen symbioses where mycobionts were highly selective in their photobiont choice, even at regional to global spatial scales (e.g., Yahr et al. 2004; Lindgren et al. 2014; Leavitt et al. 2015). For example, the extensive survey of the lichen-forming fungi in the family Parmeliaceae (Leavitt et al. 2015) revealed that the *Oropogon* fungal genus was highly selective towards a few *Trebouxia* algal partners, even though mycobiont species were sampled over a broad geographic range. Likewise, Lindgren et al. (2014) have found that different *Bryoria* mycobiont species interacted with clearly distinct subsets of the *Trebouxia* algal phylogeny. These studies, and others as well (e.g., Yahr et al. 2006, but see Leavitt et al. 2013) show that high selectivity in mycobiont–photobiont interactions is not unique to cyanolichens (i.e., involving a cyanobacterial photobiont; Stenroos et al. 2006, and this study).

However, our results stand in contrast with many studies showing that some mycobionts and/or photobionts are endemic to restricted regions or parts of the world, either because of historical effects or because of their response to environmental filters (Fernández-Mendoza et al. 2011; Peksa and Škaloud 2011; Nadyeina et al. 2014; Dal Grande et al. 2014), which can drive their interaction patterns. Indeed, interaction establishment between mycobionts and photobionts is a hierarchical process (Yahr et al. 2006) that intuitively relies on these sequential steps when the lichen-forming fungus is reproducing sexually (e.g., ascospores): the partners must (1) co-occur and encounter each other, and (2) establish a compatible interaction. Then an additional filter can determine the fitness outcome of the interaction for the partners, and perhaps drive selectivity patterns observed in the field.

Here, we found high specificity even at the smallest spatial scale under investigation, where environmental heterogeneity was unlikely to drive mycobionts' and photobionts' spatial distribution. This is congruent, though, with another study on the epiphytic mycobiont *Evernia mesomorpha* showing that this fungus was not sharing its algal partner with other epiphytic mycobionts co-occurring locally (Piercey-Normore 2009). The next frontier will be to evidence what physiological/molecular traits drive these preferential associations. Such data would allow to better predict future interaction establishment and potentially

coextinction cascades (e.g., Gravel et al. 2013). It has been argued that mycobionts forming fruticose lichens may select more strongly for drought-adapted photobionts (Leavitt et al. 2015), and there is empirical evidence that the substrate of lichenized fungi can predict to some extent their photobiont identity (e.g., Rikkinen et al. 2002; Elvebakk et al. 2008). However, studies have yet to uncover the biochemical traits that mediate compatibility between mycobionts and photobionts (Lindgren et al. 2014).

Our network was also found to be anti-nested, that is, to show a lower level of nestedness than expected under a random scenario. As for modularity, this trend was found to be robust to spatial scales of sampling (although the trend was not significant for dataset 2, which was caused by the presence of one highly generalist photobiont). Given the very low connectance (5%) of our network, low nestedness values were not surprising as it is well known that nestedness covaries positively with connectance (e.g., Almeida-Neto et al. 2008). However, since our null model controlled for connectance (such that every randomized matrix had the same connectance as the original dataset), the anti-nestedness pattern found here cannot simply be ascribed to a low network connectance.

Many theoretical studies have suggested that nestedness should stabilize mutualistic networks, potentially because it prevents coextinction cascades (e.g., Memmott et al. 2004; Burgos et al. 2007; Thébault and Fontaine 2010). Fontaine et al. (2011) have interpreted the high prevalence of nestedness in natural mutualistic communities as a validation of such theoretical result. However, here, it must be stressed that our global sampling scheme (dataset 1) does not reflect a community per se and, therefore, it would be irrelevant to interpret our results in the light of the above-mentioned studies. With such a global sampling scheme, our results on nestedness may evidence potential trade-offs in partner acquisition by lichen mycobionts and photobionts. For example, our anti-nestedness trend means that when two species share two partners, it is highly likely that species #1 will interact more frequently with partner #1, and species #2 will interact more frequently with partner #2. Anti-nestedness may thus indicate a potential trade-off that photobionts and mycobionts face when selecting partners, i.e., being a better partner for a given species implies being a worse partner for another species and, therefore, generalists here would be doomed to be "jack-of-all-trades but masters of none" (Wilson and Yoshimura 1994).

Such anti-nestedness has been reported in antagonistic networks involving marine fishes and their ectoparasites (Poulin and Guégan 2000), which could be explained by an analogous trade-off between being able to exploit many hosts versus efficiently exploiting each host (Poulin 1998). This goes against the niche-breadth hypothesis suggesting that generalist mycobionts and photobionts interacting

with many potential partners would increase in abundance or frequency because of this particular ability to interact with many different partners (Krasnov et al. 2004; Drovetski et al. 2014). Such generalism could increase, for example, the reliability to find a compatible partner in a novel environment, which is critical for lichens lacking vertical transmission systems and reproducing sexually (Belinchón et al. 2015). Here, our results rather suggest that the range of potential partners is not the main factor limiting niche breadth and range expansion for *Peltigera* (section *Polydactylon*) fungi and *Nostoc* photobionts, which is in agreement with the results from Lu et al. (2018) at an intrabiome scale. This contradicts other results showing that generalist mycobionts achieve a wider geographical niche (Wirtz et al. 2003; Muggia et al. 2014). High generalism has been hypothesized to be a favorable attribute allowing the colonization of novels habitats and thus favoring range expansion (Magain et al. 2017a). Conversely, it remains unknown whether highly specialized lichenized fungi experience a cost for such specialization, where a slight decrease in their preferred photobionts can drive their rarity. This would parallel the trend observed for the terrestrial orchid *Caladenia huegelii* specializing on a narrow subset of mycorrhizal fungi essential for its germination that seems to explain its rarity in Western Australia (Swarts et al. 2010).

By showing anti-nestedness for a mutualistic lichen network, our results also stand in contrast with other studies frequently reporting that mutualistic systems tend to be more nested, and antagonistic systems more compartmented (i.e., modular; e.g., Thébault and Fontaine 2010; Wardhaugh et al. 2015). This is in line, though, with results demonstrating that within mutualisms, interactions of high intimacy (that is, closed and sustained interactions, such as when a symbiont spends a significant portion of its life cycle with a host) should be more modular and less nested (Guimarães et al. 2007; Fontaine et al. 2011). This prediction stems from the idea that intimate interactions require more physiological integration of the partners, and thus place more constraints on the establishment of interactions with many different partners bearing different traits (Price 1980). However, this prediction is not coherent with the high levels of nestedness found in some mycorrhizal networks (e.g., Montesinos-Navarro et al. 2012), which are obviously intimate and involve the fungi placing a significant portion of their biomass within the plants' roots, and initiating a complex biochemical and molecular dialog with the host (e.g., Plett et al. 2014; Bonfante and Genre 2015). Similar nested patterns were found for endolichenic fungi [i.e., cryptic fungal communities inhabiting lichen thalli (lichen mycobiota)], which should also be considered an intimate interaction (Chagnon et al. 2016), while intimate interactions between plant leaves and their fungal endophytes showed

higher host-specificity and modularity based on a culture-dependent approach (Chagnon et al. 2016).

Collectively, these findings and our current study highlight that our coarse delineation of interaction types as "mutualistic" or "antagonistic", or as having high or low "intimacy" fails at predicting the architecture of interaction networks. Instead, it seems much more probable that the network structure rather reflects more basic properties related to the system, or how it has been sampled. For example, as the spatial scale of a study increases, for a given sampling effort, it should be expected that the proportion of interactions that remain undetected among the large pool of species found within the area of the study will increase, thus decreasing connectance. This could reduce nestedness (Almeida-Neto et al. 2008). Moreover, as environmental heterogeneity increases in a given study area, the odds increase that some species pairs never encounter each other because they remain in their own respective preferred or suitable habitat (e.g., Torrecillas et al. 2014; Fernández-Mendoza et al. 2011; Nadyeina et al. 2014). Thus, network modularity should increase. This issue has been recognized when dealing with species co-occurrence patterns, and trying to infer potential positive or negative interactions from these patterns (e.g. Ovaskainen et al. 2010; Bar-Massada and Belmaker 2017). Network structure may also not necessarily be caused by sampling artifacts, but rather reflect more subtle characteristics of the interaction investigated. For example, Chagnon et al. (2016) compared the network structure for two types of "intimate" interactions (endolichenic and leaf endophytic fungi) that were sampled following the same culture-based protocol, in the same sites, and found them to show very contrasted structures. As a result, network structure does tell us some information about the properties of an interaction: it may simply be subtler than the interaction being mutualistic or antagonistic, or intimate or not.

Our study provides a framework for exploring the potential evolutionary consequences of interaction patterns in the lichen network. Coevolution in symbiotic systems has long been a matter of debate (Thompson 2009). The recognition that coevolution takes place in species-rich communities (e.g., microbiota), with many potential indirect effects among species, has led some researchers to argue that coevolution among species is diffuse (Inouye and Stincombe 2001; Strauss and Irwin 2004) and, consequently, difficult to predict in any reductionist way (Levin et al. 1990). However, recent advances in ecological network theory have suggested that looking at symmetry of interactions could be a useful tool to explore the coevolutionary process (Vázquez et al. 2007).

This might be especially true in our study system, section *Polydactylon*, where the number of generalists and interacting taxa is low, and thus, so are the potential indirect coevolutionary effects. Contrary to many mutualistic networks

investigated to date (e.g., Bascompte et al. 2006; Vázquez et al. 2007), we found that interaction strengths were fairly symmetrical in this lichen group at the global scale. However, at the local scale (in BC), a different pattern emerged, whereby a few generalist photobionts disproportionately influence their specialized mycobionts. The same pattern was observed for *Peltigera* in the province of Québec at an intraboreal scale (Lu et al. 2018). Some examples of asymmetrical specialization were also evidenced at the global scale (e.g., mycobionts specialized on *Nostoc* phylogroup VIIa). This is in line with our current overall understanding of the lichen symbiosis involving an asymmetry in dependence, whereby mycobionts are more dependent on their photoautotrophic partners than vice versa (Lutzoni and Miadlikowska 2009). This is reflected by the inability (so far) to grow the mycobiont (*Peltigera*), and the relative ease to isolate the cyanobiont in pure culture. Moreover, *Peltigera*, as most lichen-forming fungi, has never been observed in a free-living state, whereas there is evidence suggesting that *Nostoc* phylogroups partnering with *Peltigera* can be free-living in nature (O'Brien et al. 2005; Zúñiga et al. 2017).

The issue of dependence on the partners also has important ramifications to the evolutionary stability of mutualisms: heavy reliance on a partner could promote the emergence of a cheating strategy in this partner (e.g., Steidinger and Bever 2014). Some have suggested that a host keeping symbionts captive within a given compartment (e.g., rhizobia in nodules) could be prone to cheat on these symbionts (Johnstone and Bshary 2002, but see Porter and Simms 2014). Here, the lichen mutualism represents a conflicting example whereby the mycobionts keep the photobionts within the thallus, but are also more dependent on them than vice versa. From the asymmetry analyses presented here, we could expect photobionts to be in the driver seat, even if they are sometime perceived as "captive" within fungal biomass. Indeed, at the local scales, specialized mycobionts appear to experience a stronger pressure to adapt to their generalist photobiont than vice versa. This could in fact promote cheating on the mycobionts by the photobionts. On the other hand, it is well known that mutualistic interactions can be stabilized against cheating by mechanisms that allow to favor beneficial interactions and/or terminate detrimental interactions (e.g., Ferriere et al. 2002; Kiers et al. 2003). For example, the ability of plants to preferentially reward most beneficial mycorrhizal fungi has been suggested to stabilize the mutualism (Bever et al. 2009; Kiers et al. 2011). The existence of similar mechanisms for mycobionts to favor interactions with cooperating photobionts may stabilize the lichen mutualism in a similar way. Horizontal transmission of the photobiont, which enhances the possibility for photobiont switches, may represent such mechanism. Interestingly, the pool of free-living photobionts, even sometimes observed at the surface of existing thalli (Muggia et al. 2013), may

provide a propagule pool for mycobionts to screen for cooperating partners over time.

Finally, the network study presented here represents an interesting addition to other studies on cyanolichens trying to identify higher level structure in mycobiont–photobiont partnerships. Indeed, Rikkinen et al. (2002) have shown that lichen guilds (i.e., the *Peltigera* guild and the *Nephroma* guild) hosted distinct *Nostoc* photobionts, which is analogous to a modular network structure. Here, our study uncovers guilds within guilds, in that the *Peltigera* guild of Rikkinen et al. (2002) is probably itself subdivided into smaller modules. This has interesting implications for potential interactions among mycobionts following the perspective of Rikkinen et al. (2002) that mycobionts being part of the same guild may in a way facilitate each other in their joined effort to maintain a pool of shared cyanobionts. This should be explored in future studies using co-occurrence network analyses at local to regional scales. Recently developed statistical approaches could identify pairs of mycobionts that co-occurred more frequently than what is predicted based on their habitat preferences or geographical ranges (Ovaskainen et al. 2017). The sharing of photobionts within guilds could be one cause explaining such co-occurrences. However, the study of Magain et al. (2017a) revealed that these modules are phylogenetically structured to various degrees, i.e., *Peltigera* species sharing the same set of *Nostoc* phylogroups are closely related (part of distinct clades). This is suggesting that specificity to a specific shared pool of *Nostoc* phylogroups is inherited and contribute to the pattern of associations found in lichen communities. This is observed also across the class Lecanoromycetes where specific monophyletic groups of lichens are specialized on specific groups of photobionts (Miadlikowska et al. 2006, 2014).

Conclusion and future directions

Our datasets revealed preferential interactions among *Peltigera* mycobionts and *Nostoc* photobionts at both global and local geographical scales, as reflected by our modularity analyses. Those preferential interactions are also visible in an anti-nestedness pattern. Apparently, there may be a trade-off, especially for the mycobiont, whereby interacting more with a given partner implies interacting less with others and interacting with many taxa implies interacting less with any given partner. It should be noted that specialized interaction patterns may seem surprising at such a fine phylogenetic scale (i.e., a single section within a single genus, *Peltigera*). More specialized interactions may be the norm for interactions being sampled at broader phylogenetic scales [e.g., leaf-fungal endophytic networks where a broad array of Pezizomycotina fungi are interacting with hosts ranging from bryophytes to ligneous angiosperms and

gymnosperms, see Chagnon et al. (2016)], because species that have diverged for extensive periods of time are expected to bear contrasted traits, which should make them select different interacting partners (Rezende et al. 2007; Gómez et al. 2010). Here such high specialization among closely related *Peltigera* species may be the intriguing exception rather than the rule in fungal–photobiont systems, but a common trend in cyanolichens and perhaps lichens in general. This will require further research at various phylogenetic scales in lichens (perhaps starting by integrating various sections of this species rich, *Peltigera* genus).

Future research will be necessary to better understand the relative importance of ecological versus evolutionary mechanisms in driving the assembly of this lichen network. Do preferential interactions arise because species select partners that maximize their fitness (evolutionary and ecological mechanism), or because of past coevolution in various geographic areas (Thompson 2009)? Also, what allows a photobiont to become highly generalist? Is it due to specific traits, or simply the result of a high local abundance? If so, then different photobionts may become generalists in different regions, which could create a geographic mosaic of *Peltigera* adaptation to their most common local photobiont. Sampling along gradients using transects at intermediate geographical scales such as within biomes (e.g., Lu et al. 2018) and across inter-biome ecotones might unveil the trends and patterns needed to better understand the eco-evolutionary mechanisms shaping photoautotrophs–heterotrophs symbiotic interactions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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