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Macroevolution of Specificity in Cyanolichens of the Genus *Peltigera* Section *Polydactylon* (Lecanoromycetes, Ascomycota)

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Abstract.—Patterns of specificity among symbiotic partners are key to a comprehensive understanding of the evolution of symbiotic systems. Specificity of mutualistic partners, within a widespread monophyletic group for which all species are sampled has rarely been explored. Here, we assess the level of specificity between the cosmopolitan lichen-forming fungus (mycobiont) from the genus *Peltigera*, section *Polydactylon*, and its cyanobacterial partner *Nostoc* (cyanobiont). The mycobiont and cyanobiont phylogenies are inferred from five nuclear loci and the *rbc*LX region, respectively. These sequences were obtained from 206 lichen thalli, representing ca. 40 closely related *Peltigera* species sampled worldwide, doubling the number of known species in this group. We found a broad spectrum of specificity for both partners ranging from strict specialists to generalists. Overall, mycobionts are more specialized than cyanobionts by associating mostly with one or a few *Nostoc* phylogroups, whereas most cyanobionts associate frequently with several *Peltigera* species. Specialist mycobionts are older than generalists, supporting the hypothesis that specialization of mycobionts to one or few cyanobionts, is favored through time in geographic areas where species have been established for long periods of time. The relatively recent colonization of a new geographic area (Central and South America) by members of section *Polydactylon* is associated with a switch to a generalist pattern of association and an increased diversification rate by the fungal partner, suggesting that switches to generalism are rare events that are advantageous in new environments. We detected higher genetic diversity in generalist mycobionts. We also found that *Peltigera* species specialized on a single *Nostoc* phylogroup have narrower geographical distributions compared with generalist species. [Cyanobiont; lichen symbiosis; multilocus phylogeny; mutualistic interactions; mycobiont; photobiont; species delimitation.]

Mutualism evolved across all kingdoms and can be linked to major evolutionary and ecological successes such as vertebrates with their microbiome, dinoflagellates in corals, ants with fungi, *Rhizobium* in nodules of legumes, vascular plants with mycorrhizal and endophytic fungi, and lichen-forming fungi with green algae and/or cyanobacteria (Lutzoni et al. 2016; Thompson 1994). Despite the importance of mutualism in shaping the extant natural world, few studies have focused on symbiotic systems with deep phylogenetic histories at a worldwide scale. Such studies could unveil patterns of associations and putative causal evolutionary processses that would otherwise remain undetected in local population studies.

In past decades, several models were developed to account for the evolution of mutualistic interactions. The simplest models, originating from prey–predator or competitor interactions, and based on the Lotka–Volterra equations transposed to mutualism, implied that if both partners are beneficiary, they should reach an infinite population size (May 1982). Modifications of these models to adapt them to the specific case of mutualistic interactions have been proposed (Tainaka et al. 2003; Yoshimura et al. 2003). Other popular models are variants of the prisoners-dilemma model, where both partners benefit from collaborating, but gain more individually from cheating (Trivers 1971; Axelrod and Hamilton 1981). Most of these models, however, have assumed that partners are in direct competition, although their ecological requirements differ, and that payoffs associated with cooperation, cheating, etc. are constant, when in fact they are likely to change as relationships evolve from neutral to beneficial. It was therefore suggested that past investments from one partner will influence future investments by the other partner, which may explain why an initially good investment could be favored, and that the investment varies with time (Doebeli and Knowlton 1998). Law and Lewis (1983; Law 1985) suggested that in mutualistic symbioses the inhabiting symbiont (which is less subjected to the abiotic environment than the exhabitant enclosing it) should show reduced sexual reproduction and genetic diversity, as well as evolve at a slower rate, which could enhance and stabilize the adaptation of the exhabitant to its inhabitant. These would also diminish a potential transition to parasitism (Frank 1996). For example, the alga *Coccomyxa* (inhabitant) is evolving slower than its fungal partner of the genus *Lichenomphalia* (exhabitant; Zoller and Lutzoni 2003). However, fast-evolving inhabitants of slow-evolving exhabitants have been reported for other symbiotic systems (e.g., endosymbiotic bacteria associated with insects, Moran et al. 1995). The Red King hypothesis (opposite to the Red Queen hypothesis for competitors or parasite–host interactions, van Valen 1976) suggests that evolving slower in mutualistic relationships could be advantageous. When a viable equilibrium for the symbiosis has been reached, the slow-evolving partner will have invested less in the co-evolutionary process than the fast-evolving partner, and, therefore, benefited more from the association (Bergstrom and Lachmann 2003, but see also Gokhale and Traulsen 2012).

A broad spectrum of specificity has been reported for mutualistic symbioses, ranging from high reciprocal specificity wherein two species interact only with one another across their geographic ranges, to low reciprocal specificity wherein both species are generalists, each interacting with several partners, with intermediate cases of a specialist species forming a mutualistic association with a generalist species (Ollerton 2006; Otálora et al. 2010; O'Brien et al. 2013). Here, we use the term generalist to refer to a species interacting with a relatively high number of species globally, that is, throughout its geographic range. Generalism should favor the exploitation of different niches and reduce pressure from specific limited resources, whereas specialism potentially optimizes the benefits obtained from a specific partner (de Vienne et al. 2013). According to the theory of the geographic mosaic of coevolution (Thompson 2005), patterns of specificity may vary geographically as interactions between species often evolve in at least slightly different ways across their range. Ideally, the resulting patterns of long-term coevolutionary processes should be studied at a global geographic scale rather than being restricted to a few local populations representing only a small fraction of the geographic range of the interacting species (Thompson and Cunningham 2002; Thompson 2005).

The level of dependence on another species to develop and contribute substantially to the next generation (facultative to obligate) is another intrinsic aspect of symbiotic interactions (Wolin 1985). For example, lichen-forming fungi (mycobionts) are usually obligate mutualists associated with one or several photosynthetic partners (Honegger 1998; Friedl and Büdel 2008). Cases where lichen-forming fungi occur in a free-living saprotrophic stage are extremely rare, for example, *Stictis* (Wedin et al. 2004), and are found in lineages where a loss of lichenization occurred, such as in the case of Stictidaceae within Lecanoromycetes (Lutzoni et al. 2001, see also Chen et al. 2015). Lichen photobionts appear less dependent on the mycobiont, as several are known to grow freely in nature (e.g., *Trentepohlia* and *Nostoc* spp.), and are often more easily isolated *in vitro* (and grow faster) than the fungal partner (Lutzoni and Miadlikowska 2009; McDonald et al. 2013). For example, *Nostoc* strains associated with *Peltigera* have been isolated in axenic cultures (Drew and Smith 1967), whereas this has never been achieved for *Peltigera* mycobionts. Lichen-forming fungi involved in symbioses with cyanobacteria (cyanolichens) have been the most recalcitrant to isolation in pure culture (McDonald et al. 2013).

The mode of transmission of symbionts from one generation to the next (vertically versus horizontally) can also have a major impact on evolutionary processes and specificity. In lichens both modes of transmission occur, either within a single species, or species can use mostly or solely one or the other mode of transmission. In general, horizontal transmission of the photobiont is the most common mode of transmission for lichens (e.g., Otálora et al. 2010; Dal Grande et al. 2012; Werth and Scheidegger 2012). Vertical transmission of the photobiont occurs through thallus fragments and specialized vegetative propagules (e.g., soredia, isidia, phyllidia) containing both the mycobiont and the photobiont. Horizontal transmission occurs when the mycobiont is reproducing sexually and resulting spores have to be sufficiently close to an appropriate photobiont to initiate the next generation of lichen thalli (Lutzoni and Miadlikowska 2009). Photobiont switches (a change of photosynthetic partner by the mycobiont) are common in lichen-forming fungi (Piercey-Normore and DePriest 2001; O'Brien et al. 2013; Magain and Sérusiaux 2014). They can cause changes in thallus morphology, enable colonization of new environments, and drive speciation (Fernández-Mendoza et al. 2011; Magain and Sérusiaux 2014).

Lichen mycobiont–photobiont patterns of associations are challenging to study partly because of unclear species delimitations among the interacting fungi, algae, and cyanobacteria. Lichen-forming fungi, like many other organisms with few morphological features (e.g., Medina et al. 2013), may include many cryptic species (e.g., Leavitt et al. 2011; Lumbsch and Leavitt 2011; Lücking et al. 2014), harbor morphological convergences (e.g., Magain and Sérusiaux 2012; Bendiksby and Timdal 2013; Otálora and Wedin 2013), or exhibit strong morphological plasticity reducing even more the availability of diagnostic traits (Pino-Bodas et al. 2011; Magain et al. 2012). Approximately 16,000 of all known fungal species are lichen-forming (Kirk et al. 2008). This number is likely to be an underrepresentation of the lichen species richness because a) many morphological lichen species occur across extensive geographic areas, and may in fact comprise several genetically isolated and hence phylogenetically unique lineages, which could represent distinct species, b) various taxonomic groups remain understudied, and c) many areas of the world remain poorly explored in terms of their lichen flora (Lücking et al. 2014). For lichen photobionts the situation is worse due to their small unicellular or filamentous growth forms, compounded by phenotypic shifts between the symbiotic (*in vivo*) and cultured (*in vitro*) stages (Vandamme et al. 1996; Beltrami 2008; Flechtner et al. 2013; Fučíková et al. 2014). Moreover, as for prokaryotes in general, the evolutionary histories of cyanobacteria are often obscured by multiple horizontal gene transfers (Doolittle 1999; Oren 2004).

Associations between lichenized fungi and their cyanobacterial *Nostoc* partners have been the subjects of numerous studies. The current paradigm is that a single lichen thallus hosts a single strain (single genotype) of *Nostoc* (Paulsrud and Lindblad 1998), which usually has a wide geographic distribution, and can be found in association with a broad taxonomic range of mycobiont species across continents (Paulsrud et al. 2000). However, cases with more than one photobiont genotype occurring within an individual lichen thallus have also been reported (e.g., Casano et al. 2011). *Nostoc* strains associated with lichen-forming fungi are closely related to strains forming symbiotic associations with bryophytes and angiosperms, as well as to free-living strains (O'Brien et al. 2005). Based on

the close relationships among free-living and symbiotic *Nostoc* strains, it is assumed that the great majority of symbiotic *Nostoc* strains found in lichens can occur in a free-living state, but evidence is still scarce (Oksanen et al. 2002; Wirtz et al. 2003; O'Brien et al. 2005).

Here, we present the results of a macroevolutionary study of the lichen-forming genus *Peltigera* (Peltigerales, Lecanoromycetes) section *Polydactylon* and its *Nostoc* cyanobionts. This section (one of eight for the genus *Peltigera* [Miadlikowska and Lutzoni 2000]) is cosmopolitan and its members are especially abundant in boreal old growth and tropical mountain forests (Martínez et al. 2003). Currently, 19 species are recognized in section *Polydactylon* (e.g., Vitikainen 1994, 1998; Sérusiaux et al. 2009). Most of them reproduce sexually (i.e., apothecia are commonly observed) presumably requiring the reacquisition of a compatible *Nostoc* to establish the next generation of thalli (horizontal transmission of *Nostoc*). Specialized vegetative propagules (isidia, soredia, phyllidia) that enable a codispersal of both partners (vertical transmission of the photobiont) occur only in a few members of this section (e.g., *P. pacifica* Vitik.). However, all species may propagate via simple thallus fragmentation.

The circumscription of morphospecies of section *Polydactylon* has never been evaluated within a comprehensive phylogenetic framework. The extensive morphological variation reported for some species suggests that they may harbor multiple distinct species, which might not share a most recent common ancestor (Miadlikowska et al. 2003). Due to its cosmopolitan distribution at the section level, but more restricted and distinct distributions at the species level, its abundance in many parts of the world, and its association with a single type of photosynthetic symbiont (the cyanobacterium genus *Nostoc*), the monophyletic section *Polydactylon* is a good candidate for a worldwide study of the evolution of specificity.

The aims of this study were to: 1) confirm the delimitation of section *Polydactylon* and its phylogenetic placement within the genus *Peltigera* using multilocus data; 2) evaluate morphospecies within this section using a molecular phylogenetic approach (i.e., as evolving metapopulation lineages, sensu De Queiroz 1998), and species discovery methods based on multilocus data; 3) infer phylogenetic relationships among cyanobionts associated with members of section *Polydactylon* in a broad context of symbiotic and free-living *Nostoc* strains; and 4) explore the biogeographic patterns, specificity, and macroevolution of mycobiont–cyanobiont associations and the factors shaping these patterns.

Materials and Methods

Taxon Sampling

Over 2000 specimens were loaned from herbaria (AMNH, B, BG, CGMS, CONN, DUKE, H, LG, MAF, MEXU, NSPM, NY, O, PTZ, QFA, UBC, UDBC, UGDA, UMEX, UPS) and various private collections, or collected during numerous field trips part of this study (Reunion Island in 2009; Norway, Canada: Québec, USA: North Carolina and Alaska in 2011; Russia, Peru, and Brazil in 2012). Species from the seven remaining sections of the genus *Peltigera* (59 individuals representing sections *Chloropeltigera*, *Peltidea*, *Horizontales*, *Peltigera*, *Retifoveata*, *Phlebia*, and *Hydrothyriae*; Miadlikowska and Lutzoni 2000), as well as outgroup taxa from the order Peltigerales suborder Peltigerinae (five individuals; Miadlikowska and Lutzoni 2004), were also selected for this study (Supplementary Table S1, available on Dryad at http://dx.doi.org/10.5061/dryad.h6v7g).

Molecular Data Acquisition

We extracted DNA from approximately 950 representative, well-preserved, lichen specimens lacking any visible symptoms of fungal infection following two extraction protocols: Cubero et al. (1999) or modified Zolan and Pukkila (1986) using a 2% sodium dodecyl sulphate (SDS) as the extraction buffer. We amplified the internal transcribed spacer (ITS) of the nuclear ribosomal tandem repeat of the mycobiont from about 950 lichen thalli representing a broad geographic and morphological diversity of the group, using the ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) primers. A total of 233 of these specimens from section *Polydactylon* were included in our phylogenetic analyses (shown in Supplementary Table S1, available on Dryad). From these we selected 115 specimens, that each represented a unique ITS haplotype, or in some cases identical haplotypes collected from distant geographic regions (e.g., different continents). We amplified four additional loci, including the nuclear ribosomal large subunit (nrLSU) using primers LR0R and LR7 (Vilgalys and Hester 1990), and three protein-coding genes: RNA polymerase II largest subunit (*RPB1*) using primers RPB1-AF (Stiller and Hall 1997) and RPB1-CR (Matheny et al. 2002), ß-tubulin using the forward primer T1 (O'Donnell and Cigelnik 1997) or bt_34F (O'Brien et al. 2009) and the reverse primer BT2B (Glass and Donaldson 1995), and elongation factor 2 region 1 (*EFT2.1*) using primers EFT2.1_1F (Miadlikowska et al. 2014) and EFT2.1_3R (primer sequence: 5′-ATCCCTGATACCAATGCATGCC-3′). These four loci were also sequenced for 16 specimens from the remaining seven sections of *Peltigera*, as well as four representatives of closely related genera (outgroup) from the suborder Peltigerineae. We included 34 additional specimens from other sections of *Peltigera* and one additional outgroup specimen for which sequences were available in GenBank (Supplementary Table S1, available on Dryad).

We selected a set of 208 specimens from section *Polydactylon* (including 206 for which we sequenced the ITS, and most of the 115 individuals for which multiple loci were sampled), to sequence the *rbc*LX region (i.e., the last 82 amino acids of the RUBISCO large subunit [*rbc*L],

a putative chaperone gene [*rbc*X] and two intergenic spacers; Li and Tabita 1997) of their cyanobiont *Nostoc* using the CX and CW primers (Rudi et al. 1998). We also sequenced the *rbc*LX region for 26 specimens from other sections of the genus *Peltigera*. To this *rbc*LX dataset we added 9 *rbc*LX sequences available in GenBank for section *Polydactylon*, 80 sequences from cyanobionts of other sections of the genus *Peltigera*. We also added 203 GenBank sequences of *rbc*LX representing *Nostoc* associated with other genera of lichenized fungi or other organisms, *Nostoc* that are free-living, and three other closely related cyanobacterial genera (Supplementary Table S1, available on Dryad). This study included a total of 526 *rbc*LX sequences of cyanobacteria.

PCR conditions are provided in Miadlikowska et al. (2014) and literature cited therein. All PCR products were cleaned with ExoSAP (Affymetrix Inc., CA, USA) following the manufacturer's protocol. Sequencing was carried out in 10 µL reactions using: 1 µL of primer (10 µM), 1 µL of purified PCR product, 0.75 µL of Big Dye (Big Dye Terminator Cycle sequencing kit, ABIPRISM version 3.1; Perkin–Elmer, Applied Bio-systems, Foster City, CA), 3.25 µL of Big Dye buffer, and 4 µL of double-distilled water. Automated reaction clean up and visualization was performed at the Duke Genome Sequencing and Analysis Core Facility of the Institute for Genome Sciences and Policies (for details see Gaya et al. 2012).

Single Locus and Concatenated Datasets

Sequences were edited using Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, MI) and subjected to BLAST searches (Wheeler et al. 2007) to confirm the fungal or cyanobacterial origin of each sequence fragment. Sequences were aligned manually using MacClade version 4.08 (Maddison and Maddison 2005). Ambiguously aligned regions (*sensu* Lutzoni et al. 2000) were delimited manually and excluded from phylogenetic analyses.

Prior to data concatenation, single-locus phylogenies were generated for all five fungal loci (ITS, LSU, *RPB1*, ß-tubulin, and *EFT*2.1) using RAxML-HPC2 version 7.2.8 (Stamatakis 2006; Stamatakis et al. 2008) as implemented on the CIPRES portal (Miller et al. 2010). Optimal tree and bootstrap searches were conducted with the rapid hill-climbing algorithm for 1000 replicates with the GTRGAMMA substitution model (Rodríguez et al. 1990). Protein-coding genes were partitioned following their codon positions and introns, whereas a partition with two subsets was defined for the ITS (5.8S and ITS1+ITS2). To detect topological incongruence among single-locus datasets, a reciprocal 70% ML bootstrap support criterion was implemented (Reeb et al. 2004; Mason-Gamer and Kellogg 1996). No significant conflict was detected among the single locus datasets, except for the placement of: 1) the *P. scabrosella*/*P.* sp. 7 group (*EFT*2.1 versus all other loci), 2) *P. neopolydactyla* 5 and *P. scabrosa* 3 (*EFT*2.1 versus ITS), and 3) *P. neopolydactyla* 3 (ITS versus LSU and *RPB1*) (see the "Results" section). Because conflicting relationships did not involve major topological rearrangements, we combined the single locus datasets into three concatenated fungal matrices: Matrix 1, consisting of four loci (LSU, *RPB1*, *EFT*2.1, and ß-tubulin) for 106 representatives (42 from section *Polydactylon*, 59 from the remaining sections of *Peltigera*, and 5 outgroup species), which was used to confirm the monophyly and placement of section *Polydactylon* within *Peltigera* (Table 1, Figs. 1 and 2); Matrix 2, consisting of five loci (ITS, LSU, *RPB1*, *EFT*2.1, and ß-tubulin) for 119 representatives of *Peltigera* section *Polydactylon* (loci sequenced as part of this study for 115 specimens, and from Miadlikowska et al. [2014] for the remaining four); and Matrix 3, consisting of Matrix 2 with the addition of recoded characters derived from ambiguous regions of the ITS, LSU, and selected introns of three protein-coding genes, using PICS-ORD (Lücking et al. 2011). The latter computes pairwise distances as sequence identities or cost scores, ordinates the resulting distance matrix by means of Principal Coordinates Analysis, and encodes the principal coordinates as ordered integers for each delimited ambiguous region (Table 1 and Fig. 1).

Four additional single-locus matrices were generated: Matrix 4, consisting of 526 *rbc*LX sequences of cyanobacteria, which were collapsed down to 417 sequences using a 100% similarity criterion in Sequencher; Matrix 5, consisting of ITS sequences from 206 representatives of *Peltigera* section *Polydactylon* for which we sequenced the *rbc*LX region of the co-living *Nostoc*; Matrix 6, containing a subset of Matrix 4 restricted to the symbionts from section *Polydactylon*, consisting of 209 *rbc*LX sequences (206 from *Peltigera* section *Polydactylon* and three outgroup sequences); and Matrix 7, containing one representative of all ITS haplotypes detected in section *Polydactylon* (Table 1, Fig. 1, Supplementary Text B, available on Dryad). All alignments were deposited in TreeBase (19666). In Matrix 2 all five loci were available for 74 of the 119 individuals, whereas sequences from four, three, and two loci were available for 31, 12, and 2 individuals, respectively. For the *rbc*LX datasets (Matrices 4 and 6, Table 1) the two spacers were not alignable, and for this reason they were excluded from phylogenetic analyses.

Phylogenetic Analyses

For maximum likelihood (ML) and Bayesian analyses on Matrices 1 and 2, data subsets were established using PartitionFinder (Lanfear et al. 2012). Thirteen initial subsets within Matrix 1 (LSU, ß-tubulin 1st, 2nd, 3rd codon positions and introns, *RPB1* 1st, 2nd, 3rd codon positions and intron, *EFT*2.1 1st, 2nd, 3rd codon positions and intron), and 16 subsets within Matrix 2 (LSU, ITS1, ITS2, 5.8S, ß-tubulin 1st, 2nd, 3rd codon positions and introns, *RPB1* 1st, 2nd, 3rd codon positions and intron, *EFT*2.1 1st, 2nd, 3rd codon positions and intron) were considered to estimate the optimal partitioning scheme for subsequent phylogenetic analyses. We used the greedy algorithm to explore the nucleotide

TABLE 1. Characterization of matrices used for phylogenetic analyses for both symbiotic partners

Matrix number	Symbiont	Taxonomic breadth	Locus	Number of taxa	Number of char. incl./ Total number of sites	Number of variable char.	Number of parsimony-inf. char.
1	**Mycobiont**	**Genus *Peltigera* (+outgroup)**	**Concatenated: 4**	106	3135/3736 (0.84)	975 (0.31)	776 (0.25)
			LSU	84 (0.80)	1218/1392 (0.88)	244 (0.20)	163 (0.13)
			RPB1	98 (0.93)	638/716 (0.89)	276 (0.43)	235 (0.35)
			EFT2.1	78 (0.74)	784/942 (0.83)	261 (0.33)	216 (0.28)
			ß-tubulin	80 (0.76)	495/686 (0.72)	194 (0.39)	162 (0.33)
2	**Mycobiont**	**Section *Polydactylon***	**Concatenated: 5**	119	3803/4621 (0.82)	566 (0.15)	410 (0.11)
			ITS	119 (1.00)	505/907 (0.56)	150 (0.30)	123 (0.24)
			LSU	118 (0.99)	1252/1374 (0.94)	78 (0.06)	61 (0.05)
			RPB1	109 (0.92)	676/716 (0.94)	87 (0.13)	61 (0.09)
			EFT2.1	84 (0.71)	818/942 (0.87)	120 (0.15)	81 (0.10)
			ß-tubulin	104 (0.87)	552/682 (0.81)	131 (0.24)	84 (0.15)
3	**Mycobiont**	**Section *Polydactylon***	**Concatenated: 6**	119	3907/4769 (0.82)	690 (0.18)	526 (0.13)
			ITS	119 (1.00)	475/907 (0.52)	135 (0.28)	108 (0.23)
			LSU	84 (0.80)	1231/1374 (0.90)	69 (0.06)	53 (0.04)
			RPB1	98 (0.93)	676/716 (0.94)	89 (0.13)	61 (0.09)
			EFT2.1	78 (0.74)	817/942 (0.87)	120 (0.15)	81 (0.10)
			ß-tubulin	80 (0.76)	560/682 (0.82)	129 (0.23)	82 (0.15)
			PICS-ORD char.	119 (1.00)	148	148 (1.00)	141 (0.95)
4	**Cyanobiont**	**Genus *Nostoc* (+outgroup)**	***rbc*LX**	417	636/1162 (0.55)	319 (0.50)	277 (0.44)
5	**Mycobiont**	**Section *Polydactylon***	**ITS**	206	861/907 (0.95)	313 (0.36)	280 (0.33)
6	**Cyanobiont**	**Genus *Nostoc***	***rbc*LX**	209	636/1162 (0.55)	237 (0.37)	176 (0.28)
7	**Mycobiont**	**Section *Polydactylon***	**ITS**	173	563/907 (0.62)	233 (0.41)	195 (0.35)

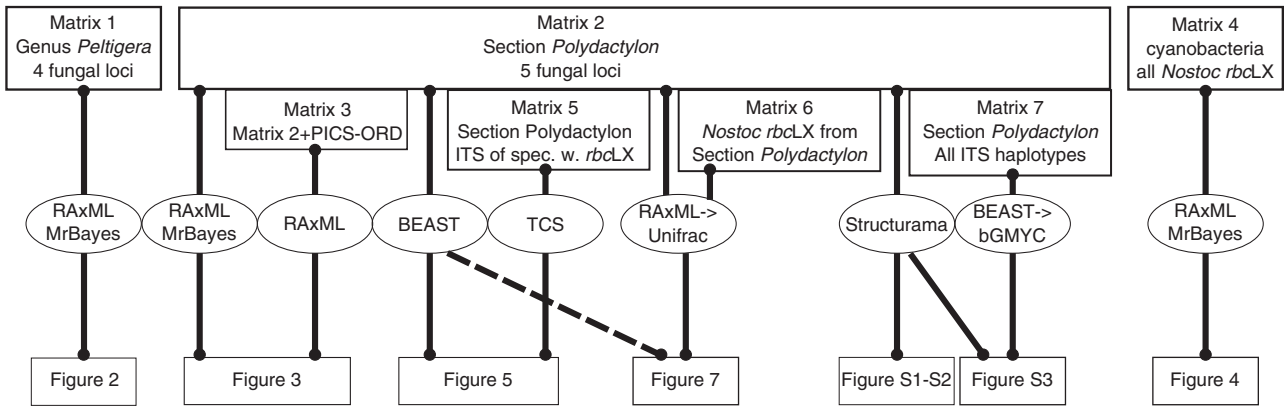

FIGURE 1. Flowchart showing the matrices used for each analysis and the figures reporting the results. Top row: matrices; middle row: software used to perform analyses; bottom row: figures reporting specific analytical results.

substitution models available in RAxML and MrBayes, as well as all the models available, under different selection criteria (AIC, AICc, and BIC) as implemented in PartitionFinder. Matrices 4 and 6 (*Nostoc rbc*LX) were divided into three subsets according to codon positions. Subsets and substitution models selected for all matrices analyzed phylogenetically are included in Supplementary Table S2, available on Dryad.

The final RAxML searches for optimal trees and bootstrap analyses on Matrices 1, 2, 4, and 6, were implemented using the rapid hill-climbing algorithm for 1000 replicates with the GTRGAMMA substitution model. RAxML analyses were performed on Matrix 3 using the same data partition as for Matrix 2 with the addition of one subset to accomodate recoded characters (PICS-ORD) used to capture phylogenetic signal from ambiguously aligned regions (Table 1).

We performed Bayesian analyses on Matrices 1, 2, 4, and 6 using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) as implemented on the CIPRES portal with the partition schemes described in Supplementary Table S2, available on Dryad. For Matrix 1 we used the default priors and completed 50 million generations, sampling every 500th generation. Matrix 2 was partitioned according to the codon-by-locus partition scheme (Table 1 and Supplementary Table S2, available on Dryad) and the best models for each subset of the partition were estimated using MrModelTest (Nylander 2004). We used the default priors and completed 40 million generations, sampling every 1000th generation. For Matrices 4 and 6, the subsets were defined according to codon positions and the GTR+G model was implemented for all subsets. We ran the program for 29 million generations, using

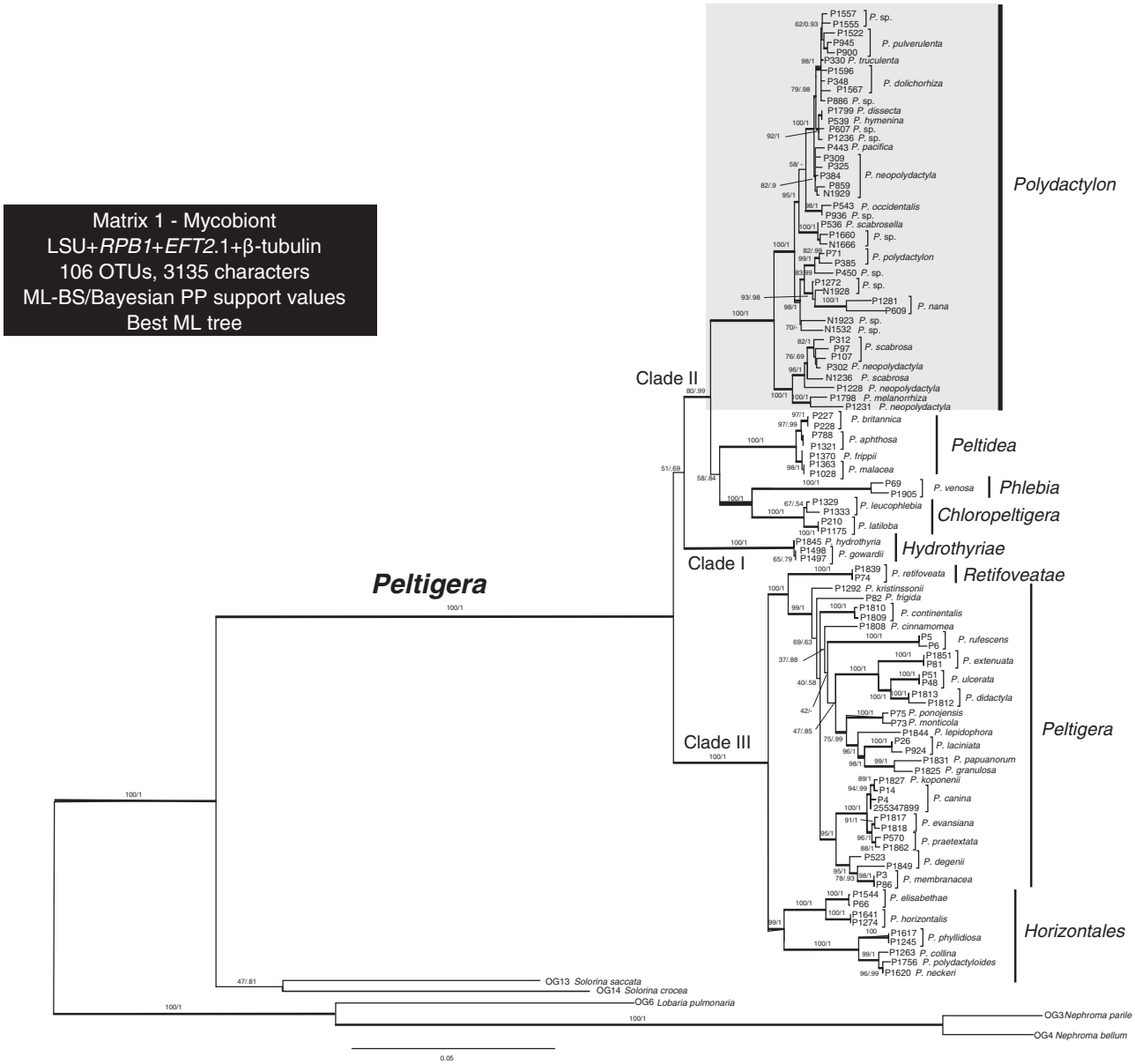

FIGURE 2. Phylogeny of the lichen-forming genus *Peltigera* (mycobiont, i.e., fungal partner). Most likely tree derived from an ML search using Matrix 1 (Table 1, Fig. 1), consisting of 106 OTUs representing 64 species from all (eight) sections of *Peltigera* and five outgroup species selected from the genera *Solorina* (Peltigeraceae), *Lobaria* (Lobariaceae), and *Nephroma* (Nephromataceae). The tree was rooted according to Miadlikowska and Lutzoni (2004). Values associated with each internode represent ML bootstrap support (ML-BS; before slash) and Bayesian posterior probabilities (PP; after slash). Thick internal branches represent internodes with ML-BS $\geqslant$ 70 and PP $\geqslant$ 0.95. Vertical bars delimit sections of the genus *Peltigera* as circumscribed by Miadlikowska and Lutzoni (2000). A gray box highlights the focal section—*Polydactylon*.

default settings, sampling every 1000th generation. Two independent runs, each composed of four chains, were performed for each matrix. We assessed the convergence of chains using Tracer version 1.5 (Rambaut and Drummond 2007) and Are We There Yet (AWTY, Nylander et al. 2008) as implemented on the website http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php, last accessed 3 August 2016.

Matrix 2 was also analyzed with BEAST version 1.7.4 (Drummond and Rambaut 2007) to generate a chronogram (relative time). The optimal nucleotide substitution model was selected using MrModelTest independently for all exons across all loci, and for all introns (see Supplementary Table S2, available on Dryad). We ran BEAST with default priors, unlinking substitution models, but linking clock models (a lognormal relaxed clock) and tree models, for 50 million generations, sampling every 1000th generation. For the protein-coding genes, each codon position was treated as a separate subset and a lognormal distributed prior on the relative rate of the different codon positions was applied. We assessed the convergence of the analysis using Tracer and AWTY.

Species Discovery Methods

Species delimitation of *Peltigera* (mycobiont) was assessed with Structurama (Huelsenbeck et al. 2011) and bGMYC (Pons et al. 2006; Reid and Carstens 2012) (Fig. 1 and Supplementary Fig. S3, available on Dryad). For Structurama, we analyzed three sets of taxa derived from Matrix 2, representing each of the three major clades separately: the polydactyloid clade with 25 individuals, the dolichorhizoid clade with 70 individuals, and the scabrosoid clade with 24 individuals. We coded alleles for six loci: ITS1, ITS2, ß-tubulin, *EFT2*.1, LSU, and *RPB1*. For the polydactyloid clade dataset, we also coded alleles of the 5.8S. We applied different priors (see Supplementary Figs. S1 and S2, available on Dryad) to detect their effect on species delimitation. Analyses were run for 1 million generations, sampling every 1000th generation. The gamma hyperprior shape parameter was set to vary from 1 to 50 for the dolichorhizoid clade, and from 1 to 20 for the polydactyloid and scabrosoid clades. For the dolichorhizoid clade (the most speciose group within section *Polydactylon*) we tested the effects of various fixed hyperpriors (ranging from 5 to 35) on the expected numbers of populations, and completed analyses of 20 million generations for three different priors (gamma shape = 5, 20, and 40) to detect if the results would converge, regardless of the priors, by performing more MCMC generations. For each clade, we selected the gamma shape that gave the most coherent results (favoring monophyletic, as well as morphologically and geographically homogeneous species; see the "Results" section) and performed the final analyses with these priors for 20 million generations.

For the bGMYC analyses we determined the best substitution model using MrModelTest and analyzed Matrix 7 (containing all unique ITS haplotypes recovered in section *Polydactylon*; Table 1; Fig. 1) using BEAST. Because of the low level of resolution obtained for the dolichorhizoid clade resulting from section-wide analyses (due to a high proportion of characters that were excluded because they were ambiguously aligned), we performed another BEAST analysis restricted to the haplotypes from the dolichorhizoid clade (which enabled us to align unequivocally more sites). For the analysis across section *Polydactylon*, we ran BEAST with a strict molecular clock (other clocks tested provided similar results and therefore we chose the simplest model), for 50 million generations, sampling one tree every 500,000th generation for a final set of 100 trees. For the analysis restricted to the dolichorhizoid clade, we ran BEAST for 30 million generations, sampling one tree every 2000th generation; excluded the first 10% of trees as burn-in, and then randomly selected 100 trees from the posterior distribution (13,500 trees). bGMYC analyses were executed on these two sets of 100 trees for 50,000 generations with a burn-in of 40,000 generations, with threshold values of 2 and 100 and a starting point of 1,1,25. A species was circumscribed if the posterior probability for grouping a set of haplotypes together was higher than the posterior probability of any grouping containing one of these haplotypes. Discrepancies between Structurama and bGMYC were resolved following the most inclusive (broadly defined) species circumscriptions using monophyly as a grouping criterion.

Haplotype Network Reconstruction

We generated haplotype networks using TCS version 1.21 (Clement et al. 2000) based on those 206 mycobiont ITS haplotypes for which we sequenced the *rbc*LX of their co-living *Nostoc* symbiont (Matrix 5, Table 1 and Fig. 1). We divided the dataset into six subgroups based on sequence similarity (scabrosoid, polydactyloid without *nana* 1, *nana* 1, *scabrosella*-sp. 7, *occidentalis*-sp. 6, and the remaining individuals from the dolichorhizoid group) so that none of the sites were ambiguously aligned, and therefore, all sites could be included in these six separate TCS analyses. Haplotypes were connected using a parsimony criterion with the 0.95 threshold value, and gaps considered as a 5th state. When different paths of equal number of changes occurred in the ITS haplotype networks, the path favoring indels rather than substitutions was selected because of the high frequency of short indels in the ITS spacers (Gaya et al. 2011).

Estimating Similarity among Nostoc *and* Peltigera *Groups Based on their Spatio-temporal Association Networks*

We performed three analyses with UniFrac (Lozupone and Knight 2005; Lozupone et al. 2006) as implemented on the website http://bmf2.colorado.edu/UniFrac: We compared 1) the phylogenetic breadth of *Nostoc* phylogroups associated with each mycobiont species using the *Nostoc* tree derived from Matrix 6 (Fig. 1); 2) *Nostoc* phylogroup composition in different biogeographic regions (as illustrated by the map in Fig. 3) using the *Nostoc* tree derived from Matrix 6; and 3) *Peltigera* species composition in different biogeographic regions (map in Fig. 3) using the ML tree of *Peltigera* section *Polydactylon* based on Matrix 2 (Fig. 3).

Analyses 2 and 3 were performed twice, once with the biogeographic regions defined as depicted in Fig. 3, and a second time with finer geographic divisions: 1) the three northern regions (NA, WP, and EP) were further split into arctic-boreal and temperate subregions; 2) the neotropic (NT) region was divided into South and Central America; and 3) Australasia was subdivided by considering New Zealand and Papua New Guinea as distinct subregions. Mycobiont species represented by a single thallus or a single photobiont sequence (*P. melanorrhiza*, *P.* "*hawaiensis*", *P.* sp. 5, *P. pulverulenta* 3, *P. nana* 2, *P. macra*) were excluded from the analyses and the Oriental region was omitted from the coding scheme due to the limited number of specimens available from this region.

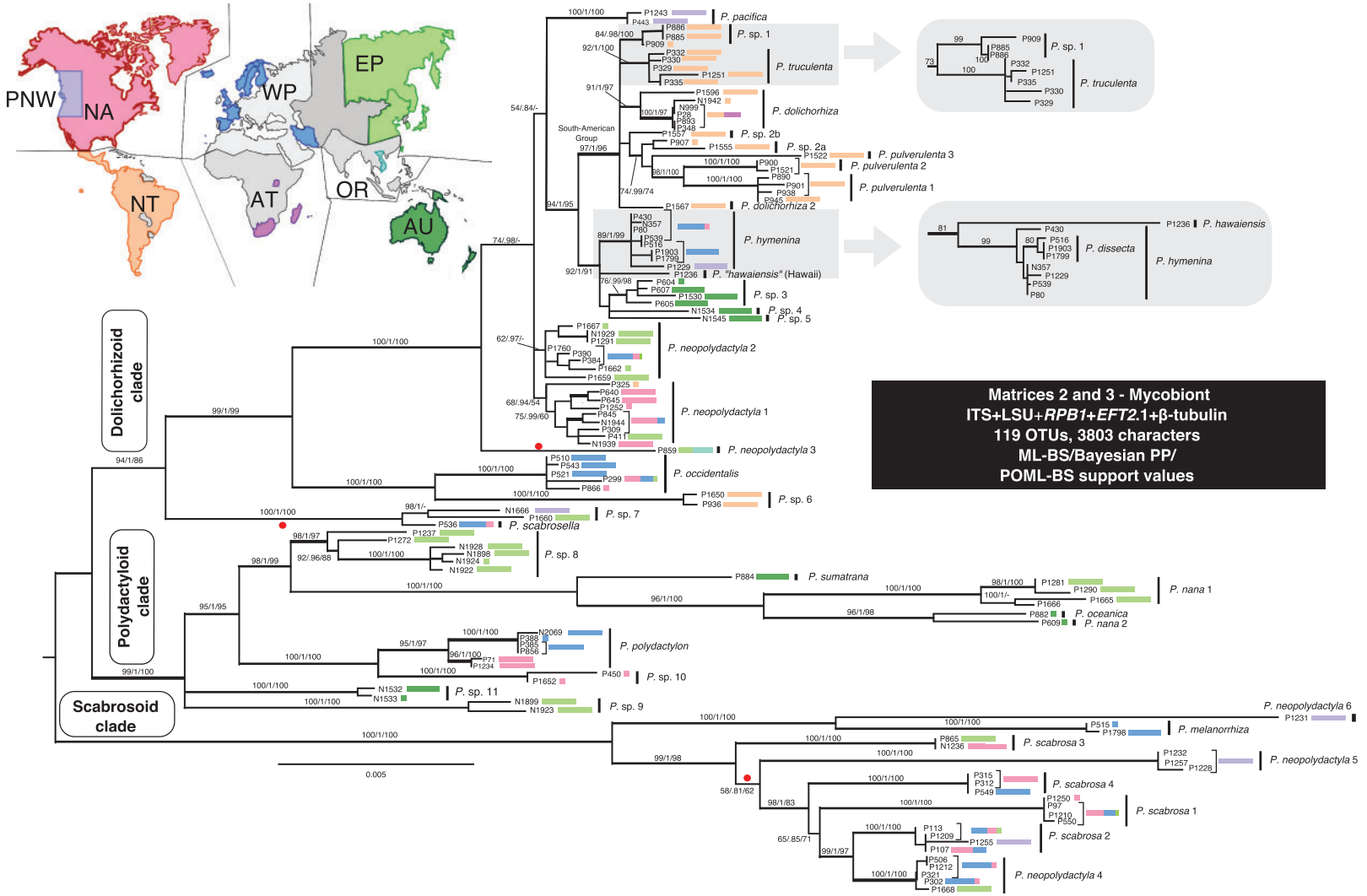

FIGURE 3. Phylogeny of the lichen-forming genus *Peltigera* section *Polydactylon* (mycobiont, i.e., fungal partner). Most likely (ML) tree using Matrix 2 (Table 1, Fig. 1), consisting of 119 OTUs representing 39 putative monophyletic species. The tree was rooted according to the topology presented in Fig. 2. Values associated with each internode represent ML bootstrap support (ML-BS; first value), Bayesian posterior probability (PP; middle value) and ML bootstrap support derived from the analyses on Matrix 3 (Fig. 1), which incorporated recoded ambiguously aligned characters (using PICS-ORD) excluded from the alignments (POML-BS; last value). Thick internal branches represent internodes with ML-BS $\geqslant$ 70%, POML-BS $\geqslant$ 70%, and PP $\geqslant$ 0.95. Colored horizontal bars (color scheme according to the map on the top left corner) following each OTU or group of OTUs (delimited with square brackets) indicate the geographic origin of specimens (see Supplementary Text Part A, available on Dryad, for the delimitation of geographic regions) included in the phylogenetic analysis, as well as other available specimens (not included in Matrix 2) that have identical ITS sequences with OTUs included in the phylogenetic analysis. The proportion of each color inside these bars corresponds to the relative number of specimens from each region. Unique ITS haplotypes have a square instead of a horizontal bar. Vertical black bars delimit recognized or putative species. Two gray boxes in the dolichorhizoid clade indicate polytomies that were resolved when recoded characters (PICS-ORD) were added to Matrix 2 to form Matrix 3. The corresponding parts of the phylogeny resulting from the ML search based on Matrix 3 are shown in gray boxes with round corners. Abbreviations used in the map in the top left corner refer to the following regions: Afrotropics (AT), Australasia (AU), East Palearctic (EP), Nearctic (NA), Neotropics (NT), Oriental (OR), Pacific North West (PNW), and West Palearctic (WP). Red dots refer to the placement of the minor conflicts detected in the single-locus topologies (see Materials and Methods "Single Locus and Concatenated Datasets"). Scale = nucleotide substitution/site.

Ancestral State Inferences of Peltigera–Nostoc *Mutualistic Interactions*

We inferred ancestral states for cyanobiont pools (subgroups) that include *Nostoc* phylogroups shared by *Peltigera* species. These pools correspond to networks formed by *Nostoc* phylogroups associated with the same *Peltigera* species. These networks unveiled three cyanobiont pools: *occidentalis*, *dolichorhiza*, and *scabrosella*. *Peltigera* species associated with *Nostoc* phylogroups of the *occidentalis* pool, for example, were never found associated with *Nostoc* phylogroups of the *dolichorhiza* or *scabrosella* pools. We hypothesized that each *Peltigera* species can only associate with *Nostoc* phylogroups from one of the three *Nostoc* pools. Therefore, we considered these three pools as potential character states for our ancestral state reconstruction analyses.

To infer ancestral states, we used SIMMAP version 1.5.2 (Bollback 2006), with default settings, on 2000 chronograms obtained from the BEAST analysis of Matrix 2. The number of chronograms was determined by a sensitivity analysis where we progressively increased the number of trees until the addition of more trees did not change the results in a way that would impact our conclusions. We also used BEAST directly to infer ancestral states by completing 10 million generations and sampling every 1000th generation when analyzing Matrix 2. Finally, we used BayesTraits version 1.0 (Pagel et al. 2004) on the same subset of 2000 trees derived from the BEAST analysis of Matrix 2. We constrained selected branches (ancestors) on certain states, and compared the harmonic mean of the iterations by calculating Bayes factors to determine which ancestral state leads to the highest likelihood (Pagel and Meade 2004). We performed sensitivity analyses, increasing the number of trees and the number of iterations until the results were not influenced by the addition of more trees and/or iterations. We used the reversible jump function and a gamma hyperprior of mean and variance varying from 0 to 10 and completed 20 million iterations for each constrained state.

Diversification Analyses of Peltigera *Section* Polydactylon

We first conducted a phylogenetic analysis on a modified Matrix 2, that is, with a single representative per species, using *BEAST (Heled and Drummond 2010). For each species, we selected one of the representatives with the highest number of loci available. We used a strict molecular clock and ran the analysis for 50 million generations, sampling every 1000th generation. We discarded 10% of the trees (burn-in) and generated a majority rule consensus species tree based on the remaining 45,000 trees.

We performed BiSSE analyses (Binary State Speciation and Extinction; Maddison et al. 2007) as implemented in the R package diversitree (FitzJohn 2012) on the mycobiont species tree obtained with *BEAST, testing whether being a specialist versus non-specialist (i.e., a *Peltigera* species found in association with a single versus several *Nostoc* phylogroups, respectively) plays a key role in the diversification process within the section *Polydactylon*. Character states for mycobiont species for which the level of specificity to *Nostoc* phylogroups was unknown were coded as missing data.

To determine whether major shifts in diversification rates occurred in the section *Polydactylon*, we used MEDUSA (Alfaro et al. 2009) with default settings and an AIC criterion as implemented in the R package geiger (Harmon et al. 2008) based on the species tree generated with *BEAST derived from the modified Matrix 2, where each species was represented by a single specimen. We also used BAMM (Rabosky 2014) as implemented in the R package BAMMtools (Rabosky 2014) on the same tree. We let the program determine the best priors, then ran it for 2,000,000 generations, sampling every 1000th generation, using default settings.

Results

Phylogeny of the Mycobiont Peltigera *at the Genus and Section* Levels

The genus *Peltigera* and its eight sections as defined in Miadlikowska and Lutzoni (2000) are monophyletic and highly supported (Fig. 2). All well-supported relationships (Fig. 2) are congruent with the results shown in Miadlikowska et al. (2014) including three major clades within the genus *Peltigera* (Clades I–III).

Within section *Polydactylon*, we recognize three main and highly supported lineages, named scabrosoid, polydactyloid, and dolichorhizoid clades (Fig. 3). The last two clades share a most recent common ancestor. Overall, relationships across section *Polydactylon* are highly supported. However, the dolichorhizoid clade includes a few species complexes that are not fully resolved. As predicted based on phenotypic diversity and broad geographic distributions, some "well-established" species, such as *P. neopolydactyla* and *P. scabrosa*, are polyphyletic. In contrast, some putative narrow endemics are conspecific with more widespread species (e.g., *P. dissecta* nested within *P. hymenina*). Many individuals are part of monophyletic entities outside all currently recognized species (*P.* spp. 1–11; Fig. 3). Overall, our phylogeny of section *Polydactylon* suggests that its species richness might be at least twice as high as currently recognized. This is remarkable given that *Peltigera* species form large conspicuous foliose thalli that are regularly collected, and that multiple studies aimed at revising morphological species diversity (e.g., Holtan-Hartwig [1993] and Vitikainen [1994] for Europe).

With a few minor exceptions (i.e., within species), no significant conflict was detected between the topologies generated by the phylogenetic analyses of Matrices 2 and 3 (i.e., without and with PICS-ORD characters, respectively; Table 1). Most relationships were highly supported by both analyses and a few poorly supported

internodes received complementary high support from one or the other analysis. The average bootstrap support for the topology without versus with PICS-ORD characters was 74.55% (73 internodes supported above 70%) and 82.15% support (81 internodes supported above 70%), respectively (Fig. 3). However, we also noticed a decrease in ML-BS values caused by the addition of PICS-ORD characters, especially at some deeper internodes. This pattern of higher values toward the tip of the tree and lower values for deeper internodes was also observed in previous studies when ambiguously aligned regions were recoded with INAASE or other methods (see Lutzoni et al. 2000 and Miadlikowska et al. 2003).

Comparison of Species Discovery Methods

When implemented on the entire section *Polydactylon*, bGMYC analyses performed well on the polydactyloid and scabrosoid clades (corroborated by morphological and geographical data) but did not on the dolichorhizoid clade. A bGMYC analysis restricted to the dolichorhizoid clade, which enabled the inclusion of more sites (which were ambiguously aligned and excluded from the analysis of the entire section), improved the results even if phylogenetic uncertainty associated with a rapid radiation detected in this clade (see the results of the diversification analyses below) led to low support values. The results of Structurama are highly dependent on the shape parameter used (see Supplementary Text Part C, available on Dryad, for a discussion on the sensitivity of the priors).

For section *Polydactylon* we expected a high number of undiscovered species. Although both methods agreed in the total number of fungal species in section *Polydactylon* (37 or 38 species assigned by Structurama, and 37 species by bGMYC) and the overall assignment of individuals to delimited species, several discrepancies (involving splitting versus lumping of species) distinguished the two approaches, especially in the dolichorhizoid clade (Supplementary Fig. S3, available on Dryad). The final estimations of Structurama for the dolichorhizoid clade were 18 species (gamma shape = 19); 11 species (gamma shape = 3) for the polydactyloid clade; and 8 species (gamma shape = 5) for the scabrosoid clade (Supplementary Figs. S1, S2, and S3, available on Dryad). bGMYC delimited 21 species within the dolichorhizoid clade, and 8 species in each of the remaining two clades (Supplementary Fig. S3, available on Dryad).

Our proposed species assignments within the dolichorhizoid clade (22 putative species), the polydactyloid (eight species) and scabrosoid clades (eight species; outermost dotted line delimitations in Supplementary Fig. S3, available on Dryad) were based on the consensus of both methods (when possible), monophyly, morphological traits (including from type specimens), and geographical distributions of the studied taxa. Only 14 species names are currently available for these 38 monophyletic putative species. The remaining 24 newly delimited species (awaiting formal description) represent predominantly allopatric or sympatric cryptic entities collected mostly from poorly explored regions of the world. The actual geographic ranges of species in section *Polydactylon* are more restricted than previously reported based on phenotypic traits alone (Supplementary Table S3, available on Dryad; Martínez et al. 2003).

Phylogeny of the Nostoc *Cyanobiont*

Our *rbc*LX phylogeny (Fig. 4) revealed *Nostoc* as a non-monophyletic group similar to previous studies (O'Brien et al. 2005; Svenning et al. 2005; Otálora et al. 2010). We recovered *Nostoc* clades I and II, as well as the three subclades (1–3) of clade II, in agreement with Otálora et al. (2010). However, significant support was obtained only for *Nostoc* clade I and subclade 2 of clade II (Fig. 4). Subclade 3 is composed of a large polytomy of numerous small, often well-supported and internally resolved subgroups. Moreover, the Bayesian analyses on the cyanobacterial *rbc*LX did not fully converge according to AWTY. Nearly all cyanobionts sampled from *Peltigera* belong to the broadly defined genus *Nostoc*, clade II, subclade 3. Only a few *Nostoc* strains found in *Peltigera* thalli belong to subclade 2 (Fig. 4).

In our phylogeny, cluster I from O'Brien et al. 2013 was not retrieved as monophyletic (not annotated in Fig. 4), but the remaining five clusters (II–VI) recognized by O'Brien et al. (2013) are represented. Here, we defined 14 new *Nostoc* phylogroups (VII–XX; Fig. 4) corresponding to well-supported clades of *Nostoc* associated with mycobionts from section *Polydactylon*. To the set of 30 unique *Nostoc* haplotypes (HT 1-30) defined by O'Brien et al. (2013) we added 17 (HT 31-47) new unique haplotypes associating with section *Polydactylon* and 15 haplotypes (HT 48-62) associating with other sections of *Peltigera* (Fig. 4).

Patterns of Association between Peltigera *Species and* Nostoc *Phylogroups*

Three main association patterns occurred within section *Polydactylon*: specialist mycobionts with non-specialist cyanobionts, non-specialists associating with non-specialists, and specialists associating with specialists (Figs. 5 and 6a). One-third of *Peltigera* species in section *Polydactylon* were associated with only one *Nostoc* phylogroup, being thus potentially strict specialists (Fig. 6b). Less than a quarter of *Nostoc* phylogroups are known to associate with only one *Peltigera* species (Fig. 6d), and we anticipate this proportion will shrink with additional sampling of *Peltigera* and members of the order Peltigerales in general. Overall, nearly three quarters of *Peltigera* species of section *Polydactylon* associate with one or two different *Nostoc* phylogroups, whereas more than half of their *Nostoc* phylogroups associate with more than two *Peltigera* species (Fig. 6b,d). Therefore, the main trend involves *Peltigera* species specializing on very few *Nostoc* phylogroups, whereas these cyanobionts tend to

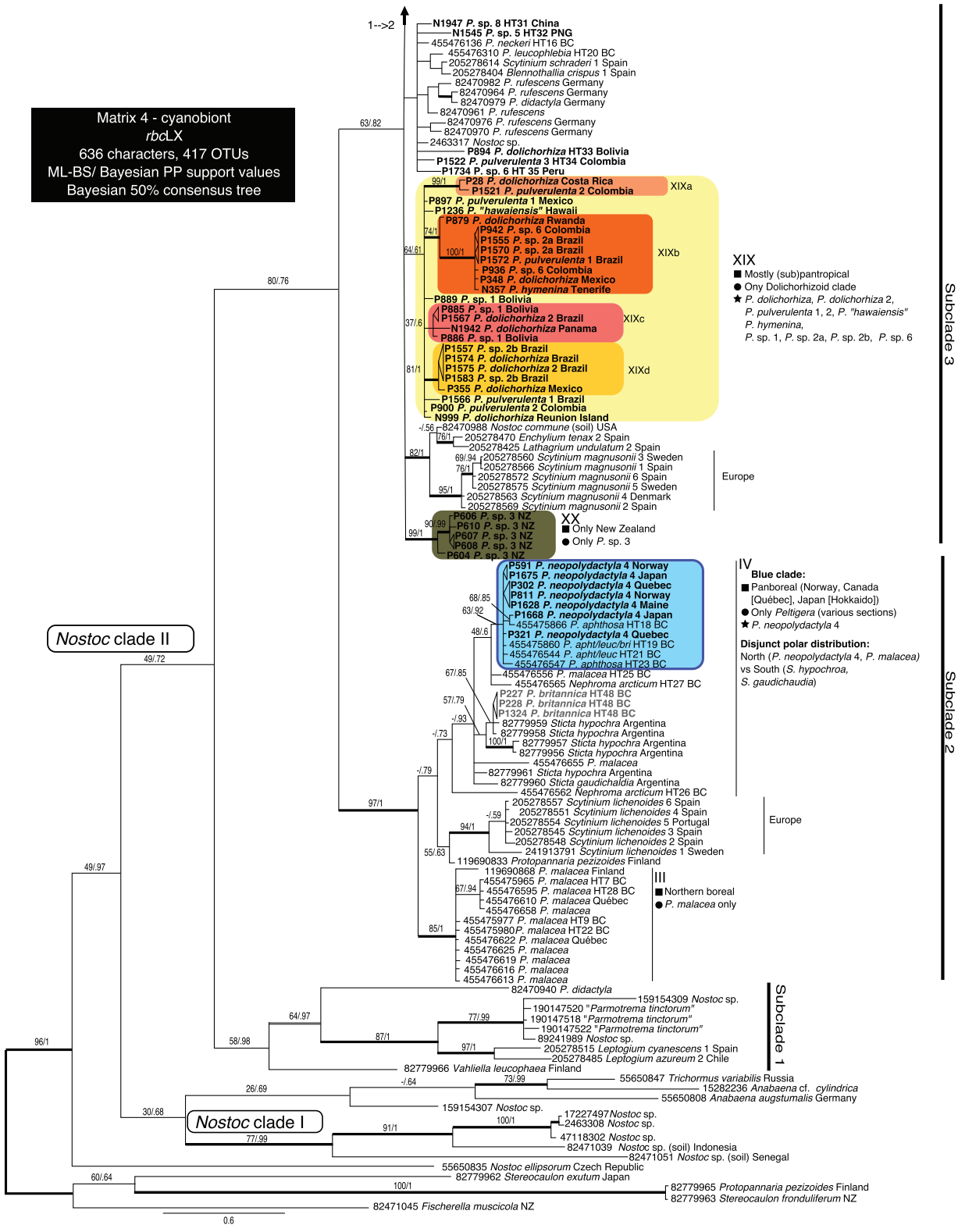

FIGURE 4. Phylogeny of *Nostoc* (cyanobiont). This is a 50% majority rule consensus tree of 26,100 trees that resulted from a Bayesian analysis of Matrix 4 (*rbc*LX dataset; Fig. 1) representing 417 unique *rbc*LX haplotypes. The tree was rooted according to Otálora et al. (2010). Values associated with each internode represent ML bootstrap support (ML-BS; before slash) and Bayesian posterior probability support (PP; after slash). Thick internodes received ML-BS $\geqslant$ 70 and PP $\geqslant$ 0.95. Clades and subclades of *Nostoc* were defined according to Otálora et al. (2010). Newly sequenced *Nostoc* cyanobionts associated with mycobionts from section *Polydactylon* are shown in bold, whereas those from other sections of *Peltigera* are in gray bold. Geographic origin is provided after the name of each terminal OTU and for published sequences downloaded from GenBank

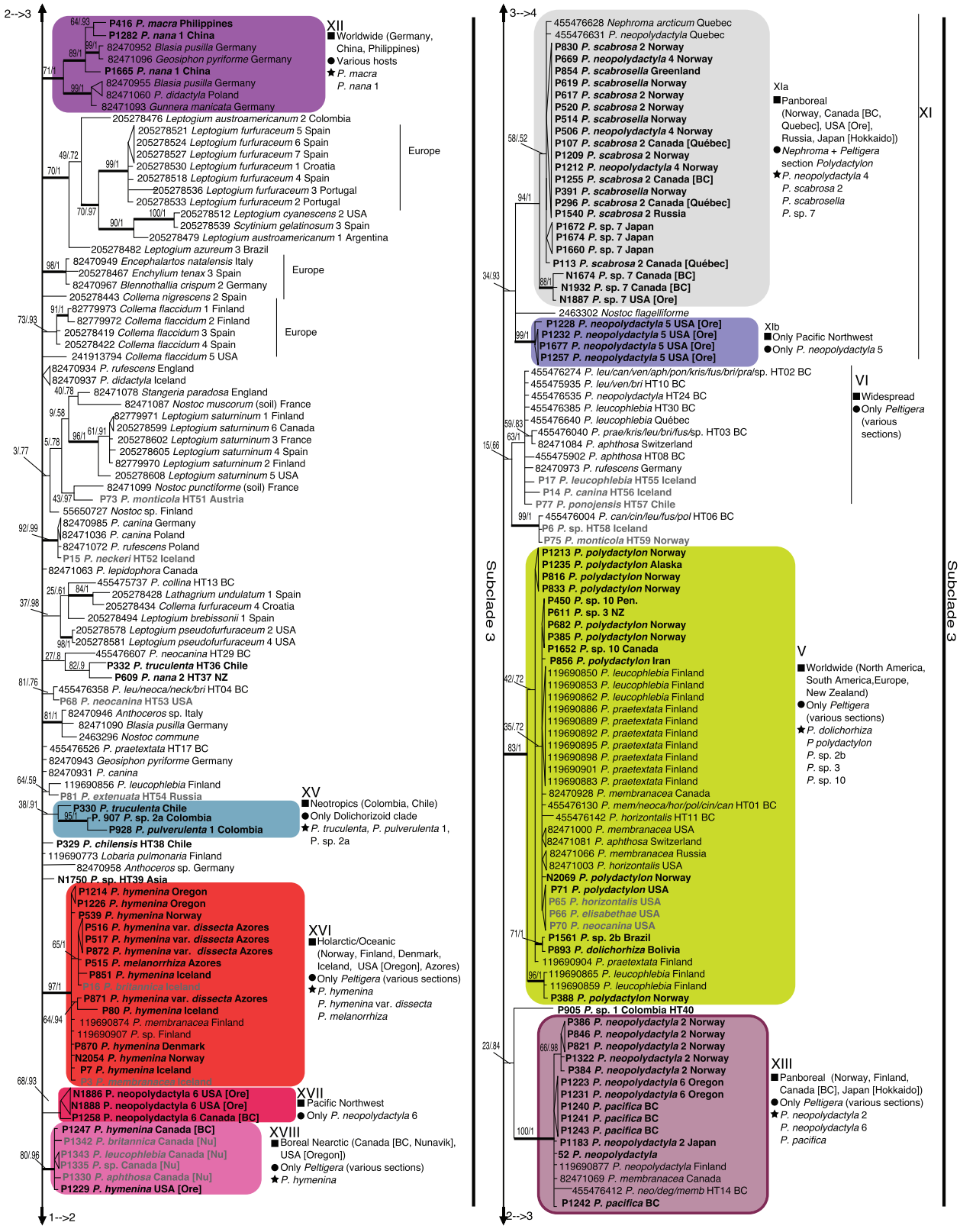

FIGURE 4. Continued

(indicated by GB number), whenever possible. Recognized phylogroups of *Nostoc* are represented by Roman numbers; phylogroups II–VI refer to O'Brien et al. (2013), whereas phylogroups VII–XX (defined here) represent significantly or moderately supported monophyletic groups encompassing *Nostoc* associated with representatives of section *Polydactylon*. Colors were attributed to each phylogroup (and four subclades within phylogroup XIX) except the ones defined by O'Brien et al. (2013), which do not contain any of the newly added *Nostoc* sequences from section *Polydactylon*. Geographic range (full squares), mycobiont affinity within the genus *Peltigera* (full circles) and associated mycobiont

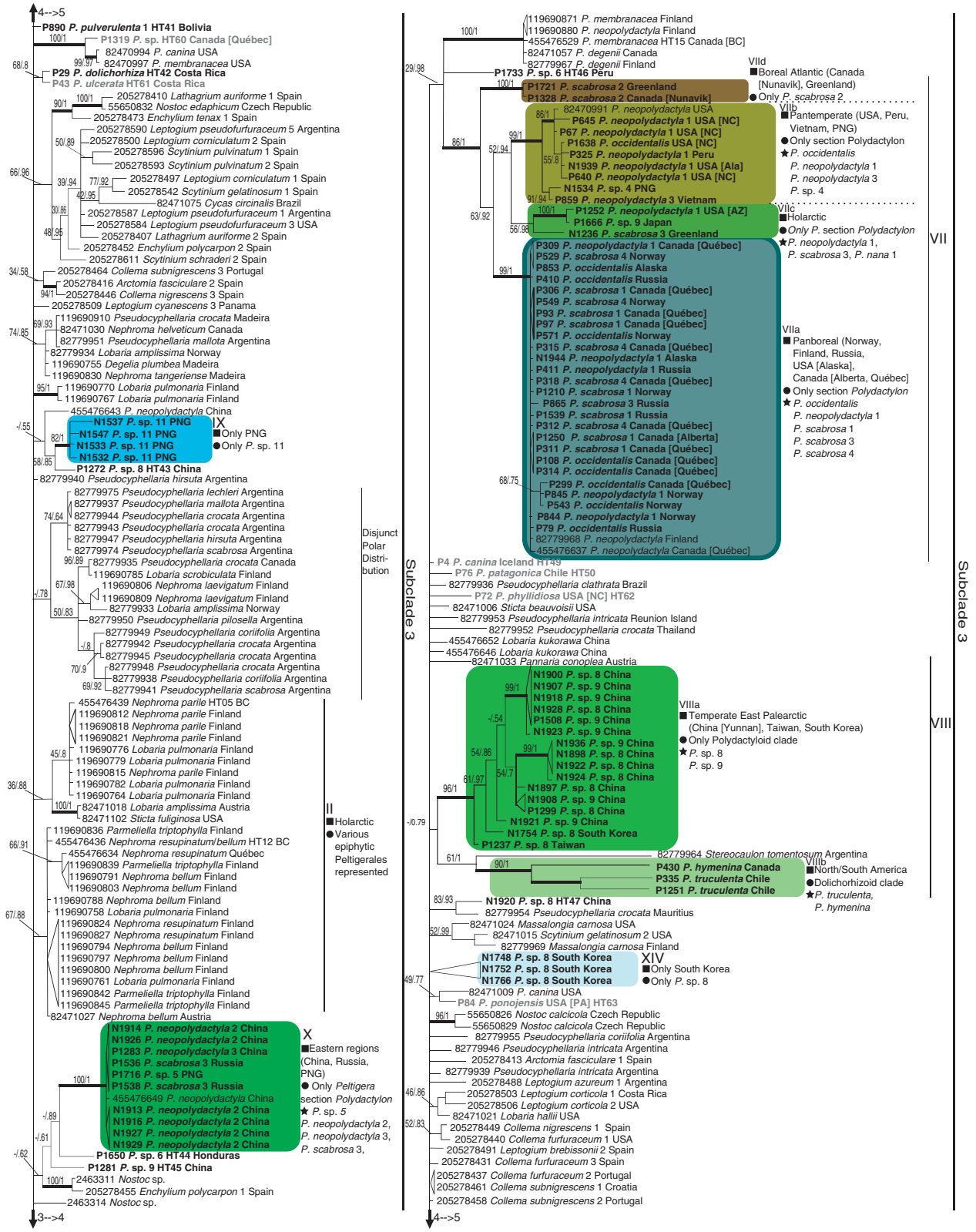

FIGURE 4. Continued

species within section *Polydactylon* (full stars) are provided for each *Nostoc* phylogroup and selected clades. When associated with identical *rbc*LX haplotype, a terminal branch representing different mycobionts was replaced by a cone (horizontally oriented) comprising all collapsed individuals. Abbreviations for *Peltigera* species are: apht = *P. aphthosa*, bri = *P. britannica*, leuc = *P. leucophlebia*, neoca = *P. neocanina*, neck = *P. neckeri*, can = *P. canina*, ven = *P. venosa*, pon = *P. ponojensis*, kri = *P. kristinsonii*, fus = *P. fuscopraetextata*, pra = *P. praetextata*, cin = *P. cinnamomea*, pol = *P. polydactylon*, mem = *P. membranacea*, hor = *P. horizontalis*, neo = *P. neopolydactyla*. Abbreviations for geographic regions are: BC = British Columbia, Ala = Alabama, Ore = Oregon, NZ = New Zealand, Pen = Pennsylvania, NC = North Carolina, AZ = Arizona, PNG = Papua New Guinea, Nu = Nunavik. Scale = nucleotide substitution/site.

diversify their association across many *Peltigera* species. Only two species (*P. hymenina* and *P. dolichorhiza*) were found to associate with more than three *Nostoc* phylogroups, some of these phylogroups associating with more than three species of *Peltigera* (Fig. 6a). We found two cases of one-to-one specificity involving a specialist *Peltigera* (*P. neopolydactyla* 5 and *P.* sp. 11) with a specialist *Nostoc* (phylogroups XIb and IX, respectively).

We further built networks of *Nostoc* phylogroups (*Nostoc* pools) that are shared by *Peltigera* species (Fig. 7b). UniFrac analyses clustered *Peltigera* species into five groups based on the *Nostoc* phylogroups with which they associate (Fig. 7a). The fusion of these *Nostoc* networks and UniFrac clusters formed the *Nostoc* pools and their internal cores shown in Fig. 7b. One subset of *Peltigera* species associates with *Nostoc* from the *scabrosella* pool (except *P. neopolydactyla* 5, which associates with a unique *Nostoc* phylogroup), two subsets (cores 1 and 2) associate with the *occidentalis* pool, and two subsets (*dolichorhiza* and *polydactylon* cores) associate with the *dolichorhiza* pool (Fig. 7a–c). *Nostoc* phylogroups found with *P.* spp. 8 and 9 (two Asian species, Fig. 5) could not be assigned to any pool, most likely due to our low sampling from Asia.

Macroevolution of Peltigera–Nostoc *Association Networks*

Both ancestral state reconstruction methods converged on the same ancestral states for the nodes of interest for this study (Fig. 7d). However, the degree of confidence varied considerably. In most cases, the greatest level of confidence resulted from SIMMAP analyses. The Bayes factors obtained with BayesTraits cannot be directly compared with the other results, but when testing the character state reconstructed as ancestral by other methods against the other states, BayesTraits always generated a significant positive value favoring the reconstructed state. Based on SIMMAP analyses, the most recent common ancestor of section *Polydactylon* was associated with *Nostoc* from the *occidentalis* pool (Fig. 7d), which are currently widespread and commonly found in association with *Peltigera* from boreal regions. The *occidentalis* pool was also reconstructed by all methods as the ancestral *Nostoc* pool for the most recent common ancestors of the scabrosoid and dolichorhizoid clades.

Diversification Analyses

BiSSE analyses revealed a similar extinction rate for specialist and non-specialist *Peltigera* species (2.96×10^2, 1.44×10^2, respectively), but the speciation rates were much higher for non-specialists, compared with specialists (4.2×10^2, 6.36×10^{-10}, respectively). Similarly, we detected a higher rate of transition from non-specialists to specialists than vice versa (1.72×10^2, 9.91×10^{-9}, respectively). A model with constrained equal rates was significantly rejected by AIC.

The best model selected by MEDUSA and by BAMM (PP = 0.53 with BAMM) detected one major acceleration in diversification rate (within the dolichorhizoid clade, see the asterisk on Fig. 7d), which is associated with a southward colonization from the boreal biome to temperate and tropical biomes, and to the Southern Hemisphere in general, as well as a major switch from the *occidentalis* to the *dolichorhiza* pool of cyanobacteria (see Figs. 5 and 7).

Specificity, Range, Genetic Diversity, and Age of Peltigera *Species*

The average distance between the farthest apart localities where two conspecific fungal specimens were sampled is 7854 km for non-specialists, and 3473 km for specialists (the difference is significant according to a Welch's unpaired *t*-test, *P*-value = 0.019). The average latitudinal range for non-specialists is 25.14° and 8.7° for specialists (Welch's *P*-value = 0.005).

All *Peltigera* species distributed across latitudinal ranges spanning at least 20° associate with at least two different *Nostoc* phylogroups, except *P. polydactylon*, which is specialized on a cosmopolitan *Nostoc* (phylogroup V, Supplementary Fig. S4, available on Dryad). Similarly, all *Peltigera* species spread over 10,000 km or more associate with at least two *Nostoc* phylogroups. This includes species that are generalists across their range and species that specialize on a different phylogroup in a different bioclimatic zone.

The average number of ITS haplotypes within non-specialist *Peltigera* species (7.4) is twice that of specialists (3.2), a difference that is significant (Welch's test *P*-value = 0.0135; Fig. 8c). Specialist *Peltigera* species are on average twice as old as non-specialists, that is, 6.9 versus

FIGURE 5. Associations of *Nostoc* phylogroups with *Peltigera* species within a geographic and evolutionary context. The main tree represents a simplified chronogram (BEAST analysis of Matrix 2, Fig. 1), where each putative *Peltigera* species from section *Polydactylon* (36 of 38 species) as shown in Supplementary Fig. S3, available on dryad, is represented by one terminal branch. OTUs for which *Nostoc* data were not available were collapsed together with their closest relatives for which *rbc*LX data were available. Bayesian posterior probabilities from BEAST analysis are provided above branches. Thicker internodes indicate PP $\geqslant$ 0.95. Background colors extending from the terminal branches represent geographic regions where thalli were sampled (as shown on the map on the top left corner, see Supplementary Text Part A, available on Dryad, for the delimitation of geographic regions). Abbreviations refer to the following regions: Arcto-Boreal (BO), Afrotropics (AT), Australasia (AU), Temperate East Palearctic (EP), Temperate Nearctic (NA), Neotropics (NT), Oriental (OR), Pacific North West (PNW), and Temperate West Palearctic (WP). Haplotype networks resulted from TCS analyses based on ITS sequences from all sampled individuals of the 36 putative *Peltigera* species (Fig. 1). Haplotypes were not connected at *P*-values below 0.95. Circles in the haplotype networks represent sampled mycobiont haplotypes, while small black dots represent putative (unsampled) haplotypes with a difference of one mutation (including indels)

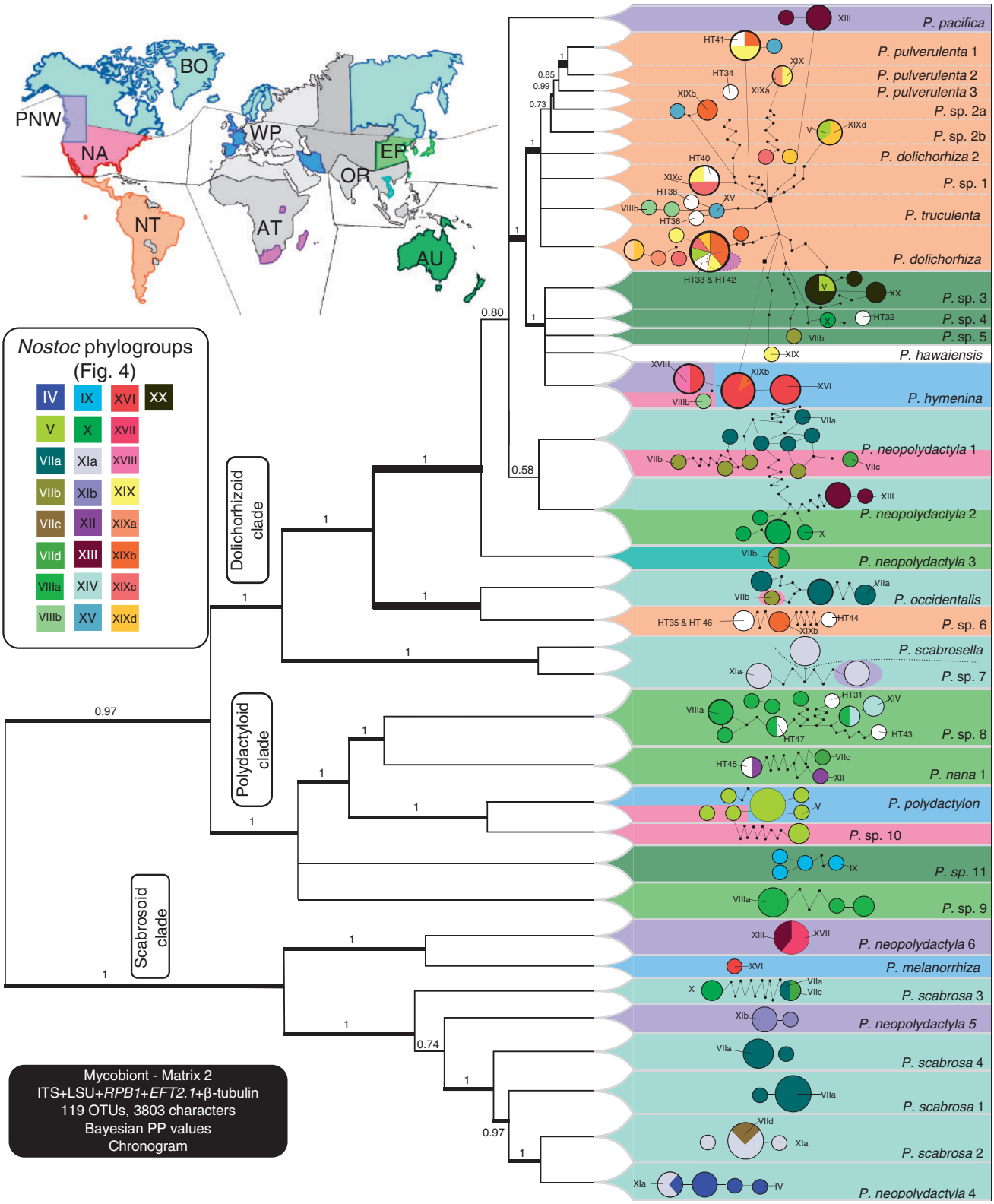

FIGURE 5. Continued

compared with the next adjacent haplotype(s). The sizes of the circles are proportional to the number of *Peltigera* mycobionts with identical ITS sequences. The colors within circles of the fungal haplotype networks correspond to phylogroups of *Nostoc* (as defined in Fig. 4, and represented in the legend provided here) in association with each individual mycobiont (i.e., sampled from the same thallus). White circles, or fractions of circles, indicate *Nostoc* haplotypes that were placed outside of all defined phylogroups (Fig. 4). Their haplotype numbers are provided, preceded by HT.

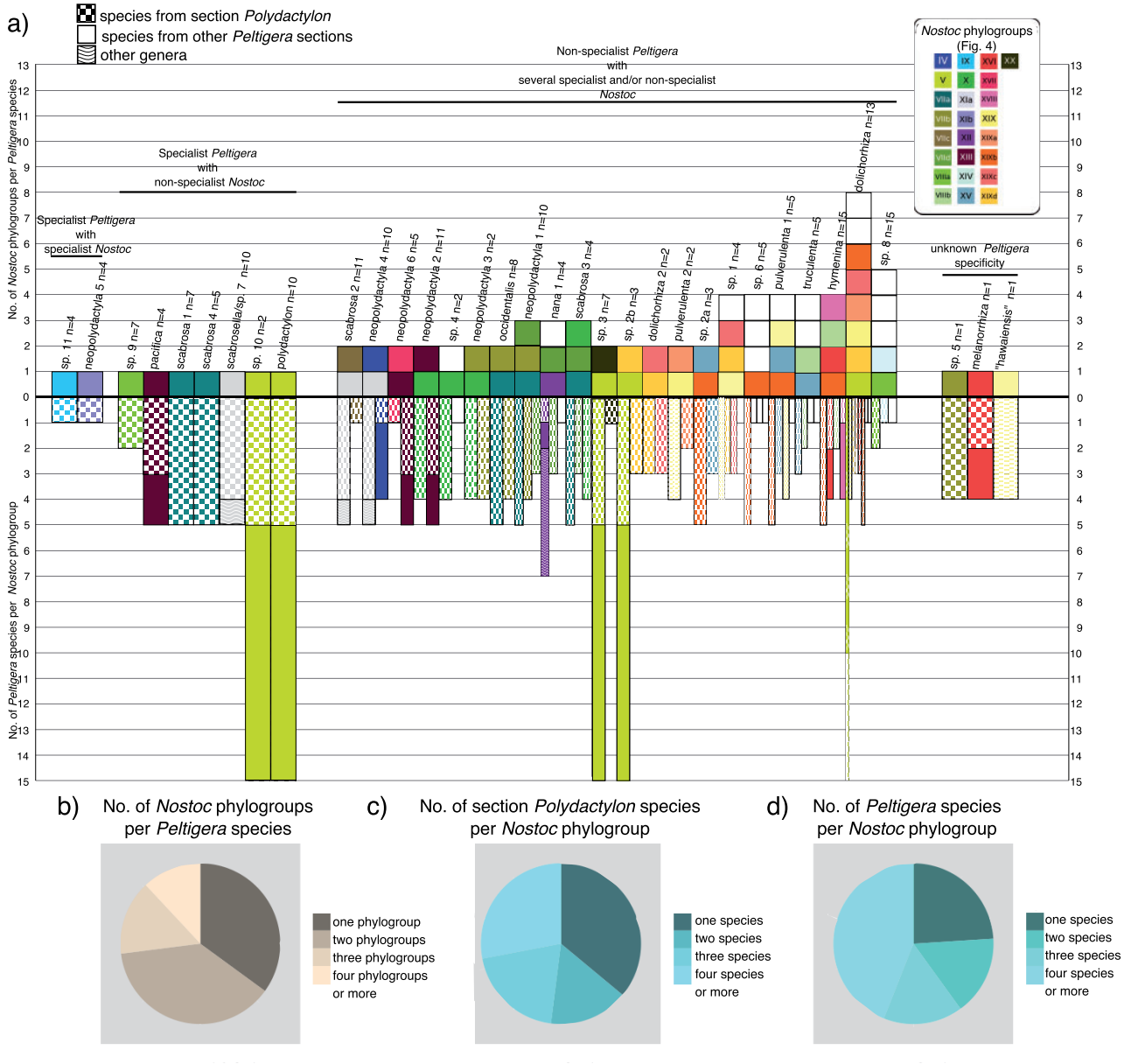

FIGURE 6. Comparison of specificity for *Peltigera* species and their *Nostoc* phylogroups. (a) Upper scale: number of *Nostoc* phylogroups associated with each *Peltigera* species (section *Polydactylon*). *N* indicates the number of lichen thalli sampled for each *Peltigera* species. Lower scale: number of *Peltigera* species (section *Polydactylon*) associated with each *Nostoc* phylogroup. Colors correspond to *Nostoc* phylogroups defined in Fig. 4 and also shown in the legend of Fig. 5. Pie charts summarize the level of specificity for the mycobiont and cyanobiont: (b) proportion of *Peltigera* species in section *Polydactylon* associated with one to four or more *Nostoc* phylogroups, (c) proportion of *Nostoc* phylogroups associated with one to four or more *Peltigera* species from section *Polydactylon*, and (d) proportion of *Nostoc* phylogroups associated with one to four or more *Peltigera* species globally.

3.3 relative units of time, respectively (Welch's *P*-value = 0.034; Fig. 8d).

DISCUSSION

Discovery of Previously Unknown Biodiversity

Our study has unveiled previously unknown biodiversity for both the fungal and cyanobacterial partners of *Peltigera* section *Polydactylon*. With this study, we have circumscribed 38 *Peltigera* species, whereas only 14 species names could be attributed to these lineages (Vitikainen 1994, 1998; Sérusiaux et al. 2009). Unsuspected biodiversity composed mainly of cryptic species is frequently encountered in phylogenetic studies of lichen-forming fungi (see e.g., Lumbsch et al. 2011), but rarely at this scale, where we nearly tripled the known number of species (but see Lücking et al. 2014). Similarly, our study unveiled 25 *Nostoc* phylogroups associating with these 38 *Peltigera*

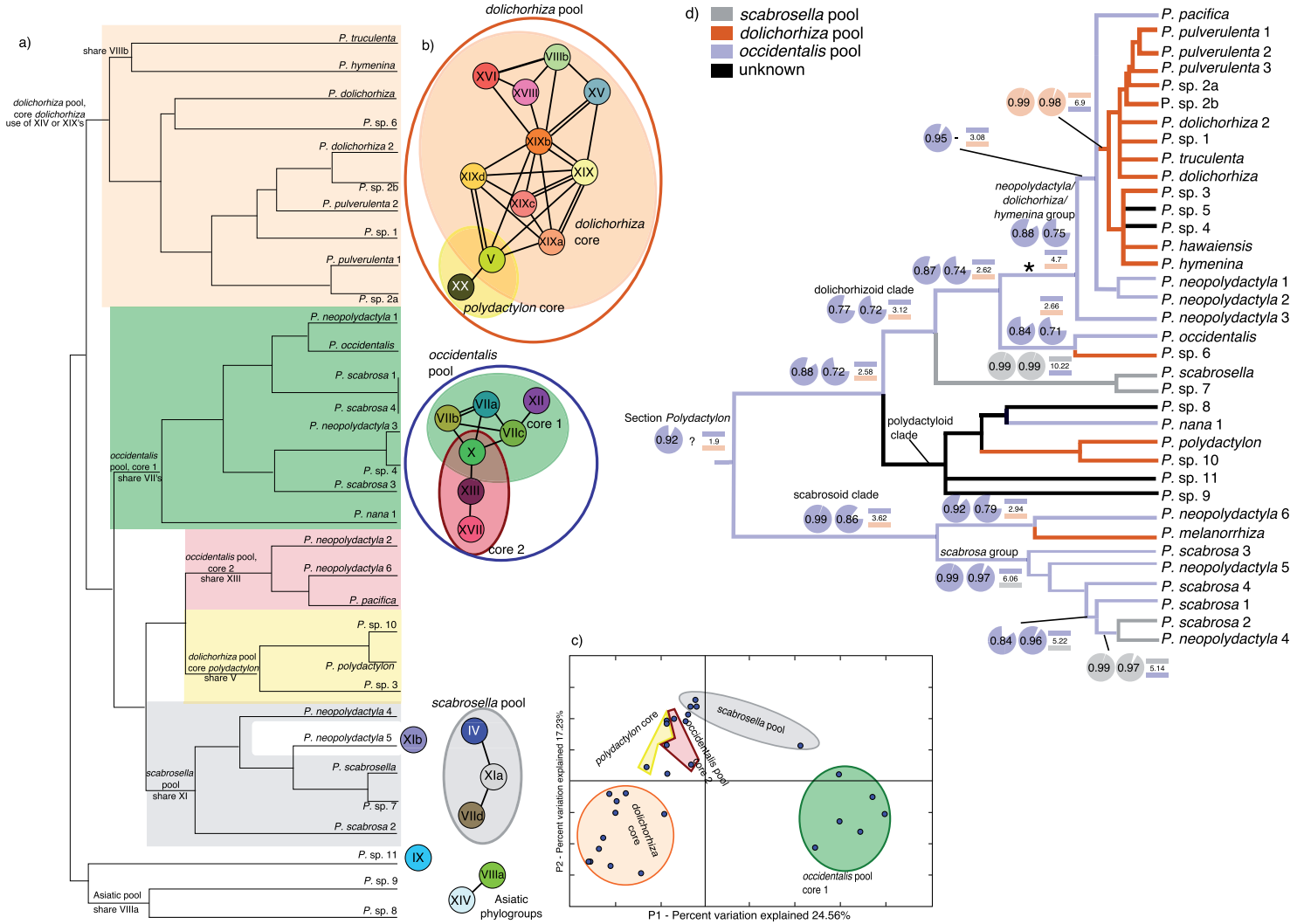

FIGURE 7. *Peltigera–Nostoc* association networks and evolution for *Peltigera* section *Polydactylon*. a) UniFrac clustering of *Peltigera* species based on the respective set of *Nostoc* phylogroups with which they associate. Color shades on the tree define clusters of *Peltigera* species sharing at least one *Nostoc* phylogroups as shown in panel b. b) Delimitation of *Nostoc* pools within clusters of *Peltigera* species. Colored circles with Roman numbers represent *Nostoc* phylogroups (as defined in Fig. 4 and shown in legends of Figs. 5 and 6) where each line represents a *Peltigera* species that was found associated with the two connected *Nostoc* phylogroups. Colored background inside the *Nostoc* pools indicate the sets of *Nostoc* phylogroups associated clusters (cores) as defined and colored in panel a. Unique *Nostoc* phylogroups are shown outside of the three delimited *Nostoc* pools. c) PCA from the UniFrac analysis. Each dot represents a *Peltigera* species and their proximity reflects similarity in the respective set of *Nostoc* phylogroups with which they associate. Colored circles correspond to *Nostoc* pools and their cores as defined in panels a and b. d) Reconstruction of the ancestral pool of *Nostoc* associated with *Peltigera* species depicted in the chronogram presented in Fig. 5. Three main pools of *Nostoc*, as delimited in panel b, were coded. Internal branches are colored according to the pool of *Nostoc* reconstructed as the ancestral state. Pie charts associated with nodes summarize the results from three different analyses. The first pie charts represent posterior probabilities generated with SIMMAP, the second pie charts represent posterior probabilities generated with BEAST, the third values represent the Bayes factor for the ancestral state color above that number compared with the state color below that number, from the BayesTraits analysis. The colors correspond to the state (*Nostoc* pool) that was reconstructed as ancestral with significant value. The asterisk shows the branch where a drastic increase in diversification rate was detected using MEDUSA and BAMM.

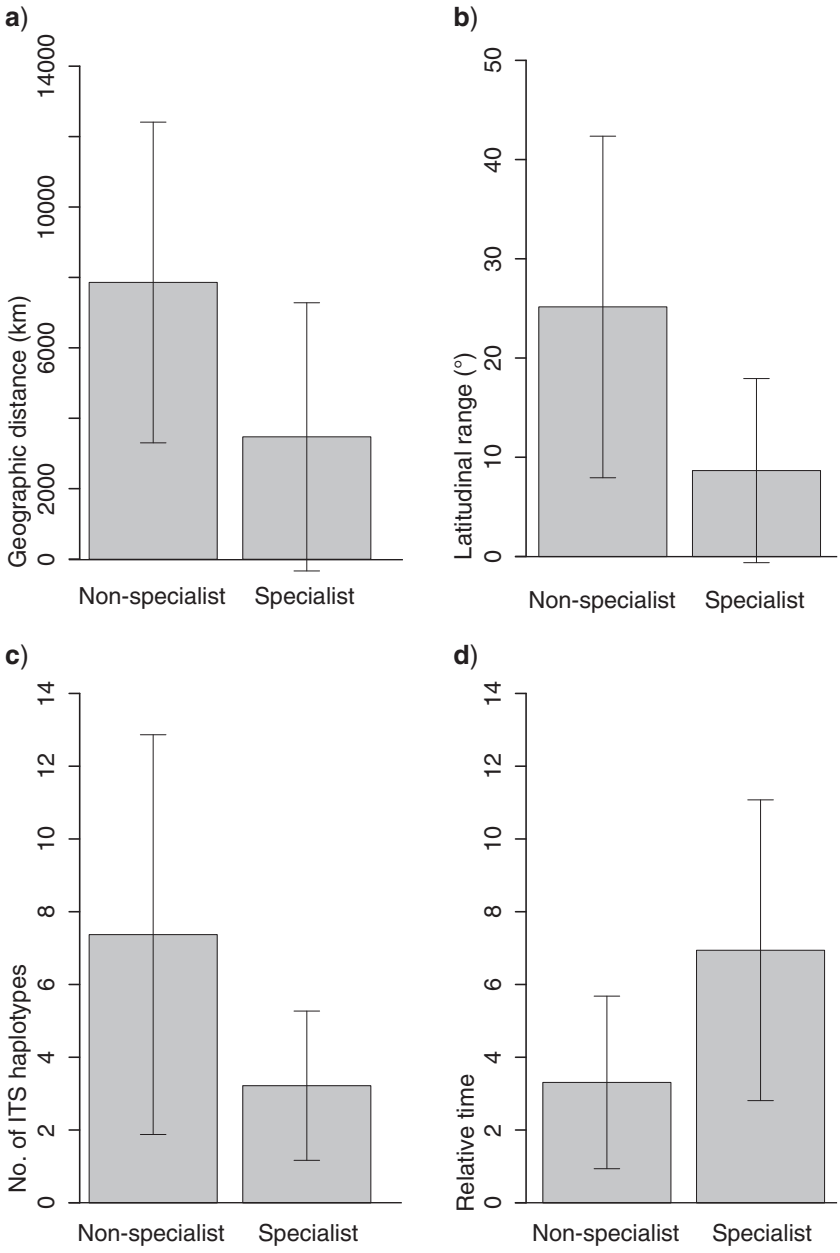

FIGURE 8. Comparisons between specialist and non-specialist *Peltigera* species. a) Distance in kilometers. b) Latitudinal range. c) Diversity defined by the number of ITS haplotypes. d) Relative age of species, based on the terminal branch lengths as shown in the chronogram of Figure 5. Specialists were defined as *Peltigera* species (from section *Polydactylon*) known to associate with only one phylogroup of *Nostoc*. The remaining species were considered to be non-specialists. Bars represent the standard deviation.

species, and an additional 17 unique *Nostoc* haplotypes (outside of phylogroups). Although only a few hundred lichen photobionts are recognized so far (Friedl and Büdel 2008) compared with the high species richness of described lichen mycobionts (estimated at 16,000 species; Kirk et al. 2008) our study reveals that their diversity may not be much lower than that of the mycobionts, and hence that lichenized fungi have a much broader range of partners to choose from.

Of the 25 *Nostoc* phylogroups found in association with species of section *Polydactylon*, 22 are exclusively associated with the genus *Peltigera*, and 18 are restricted to section *Polydactylon*. However, for many parts of the world (Papua New Guinea, New Zealand, or South America for instance) most of the available sequences were generated as part of this study for which only *Peltigera* populations were targeted. In a few cases, a *Nostoc* phylogroup associated with *Peltigera* species can also be associated with lichen-forming species representing different lichen genera (e.g., phylogroup XIa) or even be associated with plants (e.g., phylogroup XII, Figs. 4 and 5).

Without this thorough phylogenetic reconstruction and species delimitation, the potential for specificity between symbionts would have been underestimated and widespread generalism might have been inferred across the section. This more balanced biodiversity between a monophyletic group of lichen-forming fungi and their cyanobionts at a worldwide scale provided an unprecedented eco-phylogenetic framework to revisit hypotheses of symbiotic associations, and to gain a more comprehensive understanding of the patterns and evolution of specificity in symbiotic systems.

Patterns of Association and Specificity between Peltigera *Species of Section* Polydactylon *and their* Nostoc *Cyanobionts*

Associations of myco- and photobionts within section *Polydactylon* span the whole spectrum of specificity, wherein either partner can be a strict specialist or a generalist. These associations and levels of specialization are not random. They are structured geographically, ecologically, and phylogenetically. The specialization of *Peltigera* species to one *Nostoc* phylogroup can be seen as an adaptive process toward optimized fitness to a set of local biotic and abiotic environmental factors. The association of some *Peltigera* species with more than one *Nostoc* phylogroup can be driven by natural selection resulting in adaptations to different bioclimatic zones (geographical range expansion). For example, *P. neopolydactyla* 1 and *P. occidentalis* are local specialists (they associate with distinct *Nostoc* phylogroups in different regions) by partnering with phylogroup VIIa in the boreal region across all continents but with phylogroup VIIb in temperate USA or the very rare phylogroup VIIc in Arizona (Fig. 5). *Peltigera neopolydactyla* 2 associates with phylogroup XIII throughout the boreal zone but with phylogroup X in temperate Asia (Fig. 5). Fernández-Mendoza et al. (2011) found a similar pattern of association for the lichen-forming species *Cetraria aculeata* and its green algal photobiont *Trebouxia jamesii*, as well as Elvebakk et al. (2008) for three species of the lichen-forming genus *Pannaria* and their *Nostoc* photobionts. Generalists interacting with generalists should promote large geographic ranges and broad niche spectra. This type of interaction would be advantageous for lichen-forming species and *Nostoc* phylogroups colonizing new geographic areas or habitats. Most South American *Peltigera* species from section *Polydactylon* are generalists, associating with several *Nostoc* phylogroups (Fig. 5). Moreover, they associate with most (9 of 14) of the unique *Nostoc rbc*LX haplotypes (i.e., single haplotypes outside of delimited phylogroups; Fig. 4) that we found as part of this study (Fig. 6).

Except for the two cases of reciprocal one-to-one specificity involving phylogroups IX and XIb paired with *P.* sp. 11 and *P. neopolydactyla* 5, respectively (Figs. 5 and 6), *Nostoc* specialists are very rare within section *Polydactylon*. By default, cases of high specificity are favored by low sampling, which means that overall we are overestimating high specificity for the photobiont because we only sampled *Nostoc* associated with *Peltigera*. We do not have convincing cases of specialist cyanobionts associated with generalist *Peltigera* species because they involve *Nostoc* haplotypes that have been sampled only once. Specialization by *Nostoc* phylogroups toward one *Peltigera* species may exist, but these *Peltigera* species are also specialists. A specialist fungus associated with one or two generalist cyanobionts may represent an optimal equilibrium between high specificity resulting in the adaptation of the symbiotic thalli to a local set of environmental factors and low specificity to maximize the probability of finding an appropriate photobiont through horizontal transmission.

This trend of higher specialization by the mycobiont compared with its cyanobiont, already detected in *Peltigera*, to some extent, by O'Brien et al. (2005, 2013) and Myllys et al. (2007), might relate to the unequal level of dependency, where in general, the mycobiont is more dependent on the cyanobiont than vice-versa (Lutzoni and Miadlikowska 2009), and to the mode of transmission of the cyanobiont from one generation of lichen thalli to the next, which is mostly horizontal in section *Polydactylon* (the mycobiont needs to re-establish an association with a photobiont at the onset of every generation). Under such circumstances, it is likely that natural selection would lean toward *Peltigera* species associating with generalist *Nostoc* phylogroups, which should be more abundant in nature.

It is assumed that vertical transmission of the photobiont (through vegetative propagules that contain both partners of the lichen thallus) would facilitate a one-to-one reciprocal specialization, and promote coevolution and/or cospeciation of the partners. Otálora et al. (2010) reported five species of *Collema* and *Leptogium* (Collemataceae, Peltigerales) exhibiting a one-to-one specificity with their cyanobacterial partners (whereas most mycobiont species in this family are generalists). These fungal specialist species reproduce mainly by vegetative propagules, and grow exclusively on old trees in very humid conditions, which is concordant with the expectation of vertical transmission and narrow ecological amplitudes for strict specialists (Otálora et al. 2010). Similarly, when comparing the asexually reproducing species *Degelia atlantica* with the sexually reproducing *D. plumbea*, Otálora et al. (2013) reported that the genetic diversity was lower in both partners in the asexual species (this species exhibiting a narrower ecological niche) compared with the sexually reproducing species. For the two cases of such extreme specialization of both partners found in this study (*P. neopolydactyla* 5 and *P.* sp. 11 with phylogroup XIb and IX, respectively) none of these species have specialized vegetative propagules and there is no reason to believe that these species would reproduce mostly by thallus fragmentation. Therefore, the high reciprocal specificity detected here is likely to be determined genetically, for example, by highly specific interspecies signaling

as reported for *P. malacea* and its *Nostoc* phylogroup (O'Brien et al. 2013), rather than the result of vertical transmission.

Joneson et al. (2010) reported that extracellular communication between lichen symbionts can occur without cellular contact, and the authors identified a variety of fungal genes that are involved in self and non-self recognition. Lectins secreted by the fungal partner can play an important role in recognizing compatible photobiont cells (Galun and Kardish 1995), and in their communications with both green algae (Legaz et al. 2004) and cyanobacteria (Vivas et al. 2010). Therefore, the pairing of *Peltigera* species with cyanobionts is complex, involving genetic processes, and the observed patterns of specificity are not simply the result of the presence/absence or dominance of certain *Nostoc* phylogroups in a given locality, as illustrated by sympatric populations of *Peltigera* species that are specialized on different phylogroups in the boreal zone (*P. neopolydactyla* 1, *P. occidentalis*, *P. scabrosa* 1, and *P. scabrosa* 4 with phylogroup VIIa, *P. neopolydactyla* 2 with phylogroup XIII, *P. polydactylon* with phylogroup V, and *P. scabrosella* with phylogroup XIa).

Biogeographic and Climatic Factors Shaping Peltigera–Nostoc *Associations*

Because *Peltigera* species appear more dependent on their cyanobiont than vice versa, we wondered if the geographical range on *Nostoc* determines the distribution range of *Peltigera* species. As for most organisms, climate is an important factor shaping *Nostoc* distributions, as *Nostoc* phylogroups tend to be restricted to a single bioclimatic zone (Figs. 4 and 5 and Supplementary Fig. S4, available on Dryad). Most *Nostoc* phylogroups have extensive longitudinal ranges, but rather narrow latitudinal spectra (Supplementary Fig. S4a, available on Dryad). Thus, like for green algal photobionts (Fernández-Mendoza et al. 2011; Peksa Škaloud 2011) climate plays a major role in shaping the distribution of cyanobacterial photobionts. Phylogroup V is unusual in being present across a wide range of latitudes and longitudes (Fig. 4 and Supplementary Fig. S4, available on Dryad). In general, the geographic ranges of *Nostoc* phylogroups reflect their level of specialization, with generalist *Nostoc* phylogroups spanning more extensive geographical distributions than specialist *Nostoc*.

This pattern is mirrored by the *Peltigera* species of section *Polydactylon*: the range of fungal species associated with more than one *Nostoc* phylogroup is broader than that of specialists that are associated with only one phylogroup (Fig. 8a,b). As expected, most *Peltigera* species restricted to the boreal areas were restricted to boreal-only *Nostoc* phylogroups (*P. scabrosa* 1, 2, 4, *P. neopolydactyla* 4, *P. scabrosella-P*. sp. 7 associated with phylogroups VIIa, XIa, IV, VIId), whereas boreal species with distributions extending to the temperate zone (*P. neopolydactyla* 1, 2, *P. occidentalis*) switch to a temperate *Nostoc* phylogroup (VIIb, VIIc, X; Figs. 4 and 5 and Supplementary Fig. S4, available on Dryad). Other broadly distributed species such as *P. hymenina* and *P. polydactylon* are associated with *Nostoc* phylogroups that have ranges covering more than one bioclimatic zone (phylogroup XVI for *P. hymenina*, and phylogroup V for *P. polydactylon*). Therefore, being a generalist, a local specialist in multiple biogeographical regions, or a strict specialist on a widespread phylogroup, are three viable strategies that can result in a broad geographical range for *Peltigera* species. Studies on other taxa in Peltigerales (Collemataceae and the genus *Degelia*, Otálora et al. 2010, 2013), also suggested that specialist species have more restricted niches and distributions compared with generalists. However, asexual reproduction was also an important factor in these studies, whereas in section *Polydactylon* similar patterns were detected even with specialist species reproducing sexually.

However, the bioclimatic range of the *Nostoc* phylogroup, and consequently the availability of an appropriate *Nostoc* partner, is not the factor restricting the distribution of *Peltigera* species. For example, *Peltigera* species associated with the nearly cosmopolitan *Nostoc* phylogroup V (*P. polydactylon*, *P*. sp. 10, *P. dolichorhiza*, *P*. sp. 2b and 3) have more limited distributions than their *Nostoc* partner. This is true for several *Peltigera* species, even within a bioclimatic zone, like *P. pacifica* (restricted to Pacific Northwest), which associates with a cyanobiont (phylogroup XIII) that is present throughout the circumboreal belt (Fig. 5, Supplementary Fig. S4b, available on Dryad). Overall, the distribution of *Nostoc* phylogroups exceeded the distribution of *Peltigera* species in the section *Polydactylon*, both in terms of geographic distance and latitudinal range. This pattern emerged even though we underestimated the distribution of *Nostoc* phylogroups by restricting our study to species from section *Polydactylon*.

Symbiotic Switches across Space and Time

Most *Peltigera* species associated with *Nostoc* from the *scabrosella* and *occidentalis* pools (Fig. 7) are sympatric (for instance in boreal forests), and phylogroups from these two pools were frequently sampled in the same localities. Yet, no *Nostoc* phylogroups from distinct pools were found associating with the same species of *Peltigera*, despite the boreal regions being our most intensively sampled biome. This suggests that we can rule out the hypothesis that *Peltigera* mycobionts can associate with all *Nostoc* phylogroups present in a specific locality.

In the *dolichorhiza* pool, two common species, *P. hymenina* and *P. polydactylon*, were never found with the same *Nostoc* phylogroup, despite being partially sympatric. Therefore, specificity occurs even inside these *Nostoc* pools (Figs. 4, 5, and 7b). Associations are also sometimes geographically and phylogenetically structured, that is, in some cases *Peltigera* species associate with several closely related *Nostoc* phylogroups as exemplified by *P. neopolydactyla* 1 and *P. occidentalis*

associating with *Nostoc* phylogroups VIIa–c (*occidentalis* pool) and species from the South American group associating with phylogroups XIXa–d (*dolichorhiza* core) (Fig. 7b).

The scabrosoid clade is the result of the earliest split within section *Polydactylon*, and the dominance of boreal species in this clade strongly suggests that they originated in boreal forests in association with *Nostoc* from the *occidentalis* pool (Figs. 5 and 7). So far, the majority of *Nostoc* phylogroups from the *occidentalis* pool was found only with species from section *Polydactylon*. Contrary to the *occidentalis* pool, several phylogroups from the *dolichorhiza* and *scabrosella* pools are associated with mycobionts from other sections of *Peltigera*.

The origin and radiation of the South American group (within the dolichorizoid clade) was correlated with a switch from the *occidentalis* to the *dolichorhiza Nostoc* pool (Fig. 7d). *Peltigera* sp. 6 also shifted from the *occidentalis* to the *dolichorhiza* pool, as part of an independent colonization of South America by this lineage. A switch from the *occidentalis* to the *dolichorhiza* pool also occurred along the branch leading to *P. melanorrhiza*, a species restricted to the Azores. Switches of *Nostoc* pools are therefore frequently linked with a change of geographic distribution, but not necessarily, as exemplified by a switch from the *occidentalis* to the *scabrosella* pool in the scabrosoid clade (Figs. 5 and 7).

Contribution of Specificity to the Diversification of Peltigera

The much lower transition rate from specialists to generalists, versus generalists to specialists, suggests that specialization is frequent and could occur through time in many lineages, whereas transitions to generalism would be rare events. Otálora et al. (2010) concluded that in Collemataceae, extreme cases of one-to-one reciprocal specialization between *Nostoc* and these lichen-forming fungi were derived states, evolving several times independently from generalist ancestors. We found higher speciation rates for generalists. We also found a rapid radiation in the clade including the group of generalist species almost exclusively present in the Neotropics (Figs. 5 and 7). The only member in this clade that specializes on a single phylogroup, *P. pacifica*, has a very narrow distribution (Pacific Northwest) perhaps due to the fact that the species rarely produces apothecia and disperses mainly asexually by vegetative propagules (involving a vertical transmission of *Nostoc*). The other two species resulting from an early split within this clade, *P. neopolydactyla* 1 and 2, are local specialists associating with one *Nostoc* phylogroup locally, while species in the South American group are generalists (Fig. 5).

Whether this radiation is driven by the generalist profile of the species in this group, by the dispersion to a new geographical area, by the colonization of a tropical environment, or by a combination of these is not known. The recency of this radiation could mean that as these species spread to South America, they were exposed to new habitats with novel *Nostoc* phylogroups. Under this evolutionary context, the ability to associate with many different *Nostoc* phylogroups would be advantageous, favoring generalism. We hypothesize that transitions to generalism are rare events, linked with a spread to new biogeographical areas, which can result in higher speciation rates. The ages of *Peltigera* species (i.e., the amount of time since they diverged from their most recent common ancestor) could also be linked to their levels of specificity, as we found that specialist species were older than generalists. Indeed, the fact that generalists are only found on short branches, representing young species, suggests that species do not stay generalist for long periods of time, and specialization might be required for the species to persist, explaining the high rates of transition from generalist to specialist and the fact that specialist species are older in general.

In newly invaded geographical areas (such as in the Neotropics for the dolichorhizoid clade), the dominant association pattern involves generalist *Peltigera* associating with a mixture of generalists and rare specialist *Nostoc* strains (Fig. 6a). This might represent the first stage of a process of specialization of the mycobiont subjected to new selective pressures. The next stage could be the differential specialization across the geographical range as observed in *P. neopolydactyla* 1 and *P. neopolydactyla* 2, eventually leading to the specialization to a single cyanobiont in a given bioclimatic zone, as exemplified in this group by *P. pacifica*.

Peltigera–Nostoc *Associations in Light of Theories on Mutualism*

Law and Lewis (1983) suggested that the "inhabitant" (corresponding to the photobiont for most lichens), should be under selective pressure to reproduce asexually, and have a lower rate of evolution compared with the exhabitant (corresponding to the mycobiont in lichen symbiosis) or closely related free-living taxa. It is generally assumed that only the fungal partner reproduces sexually in lichens (Büdel and Scheiddeger 2008). We found common instances of a low level of genetic variation or no variation at all within a single *Nostoc* phylogroup associated with a single species of *Peltigera*, or occasionally with closely related species (for instance *P. scabrosa* 4 and *P. scabrosa* 1), which can share the same *Nostoc* haplotypes (Figs. 4 and 5). Identical *Nostoc* haplotypes were frequently identified across a large geographic scale (Fig. 4 and Supplementary Fig. S4, available on Dryad). These *Nostoc* haplotypes were identical even in the extremely variable spacer region (unalignable across the *Nostoc* strains included in this study). This broad distribution of highly similar *Nostoc* strains (based on *rbc*LX sequences) can be a signature of low evolutionary rates in *Nostoc* involved in lichen symbioses, as well as low rates of nucleotide substitutions due to purifying selection for an

optimal association with specific lichen-forming species. However, this pattern of highly similar *Nostoc* strains covering large areas could also be explained by efficient long-distance dispersal mechanisms for certain *Nostoc* phylogroups.

Correspondingly, because cyanobionts are predominantly transmitted horizontally in *Peltigera*, the presence of the same *Nostoc* haplotype within and among different species of *Peltigera* can be explained by a parallel acquisition of the same cyanobiont, rather than coevolution of a fast-evolving exhabitant with a slow-evolving *Nostoc* partner. Recent studies (see Sachs et al. 2011) on a large variety of microbial symbionts demonstrated that the Law and Lewis paradigm was too simplistic. Indeed, the mutualistic framework set by Law and Lewis (1983) involving a positive frequency-dependent selection, evolutionary stasis, and high asexuality of one symbiont is consistent in some symbiotic associations, but highly incoherent in others (Sachs et al. 2011), and therefore, there is probably a continuum of different stages between an arms race in parasite–host systems and the Law and Lewis paradigm.

The Red King hypothesis (Bergstrom and Lachmann 2003) states that in mutualistic interactions, while both partners need to find a viable equilibrium to maintain the symbiosis, the slower partner wins the race because, by reaching the equilibrium more slowly, it can invest less and benefit more from the symbiosis. Specialists may be under strong selection to meet the requirements of the high specificity for their symbiotic partner and hence are genetically less diverse. In early-diverged species from section *Polydactylon* (*P*. sp. 11, *P*. sp. 9, *P*. *scabrosa* 1, *P. scabrosa* 4, *P. neopolydactyla* 5, *P. neopolydactyla* 6) both partners are highly stable (a single or very few similar ITS haplotypes per *Peltigera* species associated with one or a few *Nostoc* haplotypes). However, this would require a predominant role of clonal reproduction by the specialist fungi to explain the observed pattern because the ITS spacers are not under strong purifying selection.

Therefore, our results support the hypotheses of both Law and Lewis (1983) and Bergstrom and Lachmann (2003) by demonstrating that both symbionts can benefit when involved in a mutualistic relationship for a long time. This long-term specialization results in a low rate of evolution leading to the reduced genetic diversity and slow diversification of both partners, because the most frequent or best-adapted haplotype is positively selected (Law and Lewis 1983) to maintain the optimal benefits in the symbiosis.

Because we do not see a high level of specialization for the photobiont, it is very likely that in the process of lichenization, the mycobiont is capturing the photobiont (shared by other species), rather than the photobiont infecting the mycobiont. The fact that the lichen-forming fungus (*Peltigera*) is highly dependent on *Nostoc* (never found free-living) but not vice-versa also supports the fungal capture of *Nostoc*. As a consequence, the mycobiont might be adapting to the photobiont more than *Nostoc* adapting to *Peltigera*. Slow evolution of the cyanobiont (embedded in long-lived thalli) can be explained by a reduced selective pressure from the environment and a high selection from the mycobiont to maintain the relationship with the optimal cyanobacterial partner. A strong specialization of the mycobiont toward a single cyanobiont may limit its ablility to switch to a different *Nostoc* partner and might explain why strict *Peltigera* specialists cannot expand to new regions (and have narrower geographic ranges compared with generalists).

We hypothesize two categories of fungal species regarding their symbiotic status: 1) a "suboptimal" status where partners that associated recently, and/or experienced new environmental conditions, would be evolving faster, driven by positive selection leading to an optimal association, and where associations with many different cyanobionts would be advantageous during this selective process, and 2) an "optimal" status, where the mycobiont is specialized to interact with one or two *Nostoc* partners, for example, and the association has reached an optimal equilibrium under specific environmental conditions. This is a case where a *Peltigera* species–*Nostoc* phylogroup pair is drastically more successful in a given area, compared with the same mycobiont species associated with other *Nostoc* phylogroups.

With time, local specialists might become strict specialists. For example, *P. neopolydactyla* 1 (Fig. 5) could speciate to form two species, one in a temperate zone in North America specializing on phylogroup VIIb, and a second species in boreal region, specializing on phylogroup VIIa. Similarly, populations of *P. neopolydactyla* 2 might split into two taxa, one in its temperate range in Asia, in association with phylogroup X, and another in its boreal range in association with phylogroup XIII (Fig. 5). In the case of *P. neopolydactyla* 2, it seems that specimens from Yunnan associating with *Nostoc* phylogroup X are already genetically distinct from the boreal populations associating with phylogroup XIII (they do not share ITS haplotypes, and form two monophyletic lineages; Fig. 5) whereas in *P. neopolydactyla* 1, there is no such clear distinction among populations as the same haplotype was found in association with *Nostoc* phylogroup VIIa and VIIb. The ability to switch cyanobionts can facilitate the expansion of mycobionts to new environments where the former cyanobiont is not available, to avoid competition for cyanobionts from the co-existing mycobionts, or to choose a better-adapted cyanobiont in a changing environment. These observations are in agreement with the geographic mosaic of coevolution theory where a species may adapt and become specialized on another species differentially within different geographic regions (Thompson 2005). However, switches from specialization to generalism are also possible, and might represent rare events and transition states, when dispersion to new habitats and the presence of new *Nostoc* phylogroups make it advantageous to associate with several partners.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h6v7g.

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References

Alfaro M.E., Santini F., Brock C., Alamillo H., Dornburg A., Rabosky D.L., Carnevale G., Harmon L.J. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. USA. 106:13410–13414.

Axelrod R., Hamilton W.D. 1981. The evolution of cooperation. Science 211:1390–1396.

Beltrami O. 2008. Assessing taxonomic issues with the genera *Anabaena*, *Aphanizomenon* and *Nostoc* using morphology, 16S rRNA and efp genes [Doctoral dissertation]. [Ontario (Canada)]: University of Waterloo.

Bendiksby M., Timdal E. 2013. Molecular phylogenetics and taxonomy of Hypocenomyce *sensu lato* (Ascomycota: Lecanoromycetes): Extreme polyphyly and morphological/ecological convergence. Taxon 62:940–956.

Bergstrom C.T., Lachmann M. 2003. The Red King effect: When the slowest runner wins the coevolutionary race. Proc. Natl. Acad. Sci. USA. 100:593–598.

Bollback J.P. 2006. SIMMAP: Stochastic character mapping of discrete traits on phylogenies. BMC Bioinformatics 7:88.

Büdel B., Scheidegger C. 2008. Thallus morphology and anatomy. In: Nash T.H., editor. Lichen biology. Cambridge: Cambridge University Press. p. 37–64.

Casano L.M., del Campo E.M., Garcia-Breijo F.J., Reig-Armiñana J., Gasulla F., Del Hoyo A., Guera A., Barreno E. 2011. Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? Environ. Microbiol. 13:806–818.

Chen K.-H., Miadlikowska J., Molnár K., Arnold A.E., U'Ren J.M., Gaya E., Gueidan C., Lutzoni F. 2015. Phylogenetic analyses of eurotiomycetous endophytes reveal their close affinities to Chaetothyriales, Eurotiales and a new order—Phaeomoniellales. Mol. Phylogenet. Evol. 85:117–130.

Clement M., Posada D., Crandall K.A. 2000. TCS: A computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1659.

Cubero O.F., Crespo A., Fatehi J., Bridge P.D. 1999. DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. Plant Syst. Evol. 216:243–249.

Dal Grande F., Widmer I., Wagner H.H., Scheidegger C. 2012. Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. Mol. Ecol. 21:3159–3172.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: Howard D.J., Berlocher S.H., editors. Endless forms: Species and speciation. Oxford: Oxford University Press. p. 57–75.

Doebeli M., Knowlton N. 1998. The evolution of interspecific mutualisms. Proc. Natl. Acad. Sci. USA. 95:8676–8680.

Doolittle W. F. 1999. Phylogenetic classification and the universal tree. Science 284:2124–2128.

Drew E.A., Smith, D.C. 1967. Studies in the physiology of lichens. VII. The physiology of the *Nostoc* symbiont of *Peltigera polydactyla* compared with cultured and free-living forms. New Phytol. 66:379–388.

Drummond A.J., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Elvebakk A., Papaefthimiou D., Robertsen E.H., Liaimer A. 2008. Phylogenetic patterns among *Nostoc* cyanobionts within bi- and tripartite lichens of the genus *Pannaria*. J. Phycol. 44:1049–1059.

Fernández-Mendoza F., Domaschke S., García M.A., Jordan P., Martín M.P., Printzen C. 2011. Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. Mol. Ecol. 20:1208–1232.

FitzJohn R.G. 2012. Diversitree: Comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Flechtner V.R., Pietrasiak N., Lewis L.A. 2013. Newly revealed diversity of eukaryotic algae from wilderness areas of Joshua Tree National Park (JTNP). Monogr. West N. Am. Nat. 6:43–63.
Frank S.A. 1996. Host-symbiont conflict over the mixing of symbiotic lineages. Proc. R. Soc. Lond. B Biol. Sci. 263:339–344.
Friedl T., Büdel B. 2008. Photobionts. In: Nash T.H., editor. Lichen biology. Cambridge: Cambridge University Press. p. 9–26.
Fučíková K., Lewis P.O., Lewis L.A. 2014. Widespread desert affiliation of trebouxiophycean algae (Trebouxiophyceae, Chlorophyta) including discovery of three new desert lineages. Phycol. Res. 62:294–305.
Galun M., Kardish N. 1995. Lectins as determinants of symbiotic specificity in lichens. Cryptogamic Botany 5:144.
Gardes M., Bruns T.D. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113–118.
Gaya E., Högnabba F., Holguin A., Molnar K., Fernández-Brime S., Stenroos S., Arup U., Søchting U., Van den Boom P., Lücking R., Sipman H.J.M., Lutzoni F. 2012. Implementing a cumulative supermatrix approach for a comprehensive phylogenetic study of the Teloschistales (Pezizomycotina, Ascomycota). Mol. Phylogenet. Evol. 63:374–387.
Gaya E., Redelings B.D., Navarro-Rosinés P., Llimona X., De Cáceres M., Lutzoni F. 2011. Align, or not to align? Resolving species complexes within the *Caloplaca saxicola* group as a case study. Mycologia 103:361–378.
Glass N.L., Donaldson G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microb. 61:1323–1330.
Gokhale C.S., Traulsen A. 2012. Mutualism and evolutionary multiplayer games: Revisiting the Red King. Proc. R. Soc. Lond. **B** Biol. Sci. 279:4611–4616.
Harmon L.J., Weir J.T., Brock C.D., Glor R.E., Challenger W. 2008. GEIGER: Investigating evolutionary radiations. Bioinformatics 24:129–131.
Holtan-Hartwig J. 1993. The Lichen Genus 'Peltigera', Exclusive of the 'P. Canina' Group, in Norway. Botanical Garden and Museum-University of Oslo.
Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27:570–580.
Honegger R. 1998. The lichen symbiosis—what is so spectacular about it? Lichenologist 30:193–212.
Huelsenbeck J.P., Andolfatto P., Huelsenbeck E.T. 2011. Structurama: Bayesian inference of population structure. Evol. Bioinform. Online 7:55.
Huelsenbeck J.P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.
Joneson S., Armaleo D., Lutzoni F. 2010. Fungal and algal gene expression in early developmental stages of lichen-symbiosis. Mycologia 103:291–306.
Kirk P., Cannon P., Minter D., Stalpers J. editors. 2008. Ainsworth & Bisby's dictionary of the fungi. 10th ed. Wallingford, UK: CABI Publishing.
Lanfear R., Calcott B., Ho S.Y., Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29: 1695–1701.
Law R. 1985. Evolution in a mutualistic environment. In: Boucher D.H., editor. The biology of mutualism: Ecology and evolution. Oxford: Oxford University Press. p. 145–170.
Law R., Lewis D. 1983. Biotic environments and the maintenance of sex—some evidence from mutualistic symbioses. Biol. J. Linn. Soc. 20:249–276.
Leavitt S.D., Fankhauser J.D., Leavitt D.H., Porter L.D., Leigh A.J., St Clair L.L. 2011. Complex patterns of speciation in cosmopolitan "rock posy" lichens—discovering and delimiting cryptic fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota). Mol. Phylogenet. Evol. 59:587–602.
Legaz M.-E., Fontaniella B., Millanes A.-M., Vicente C. 2004. Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. Eur. J. Cell Biol. 83:435–446.
Li L.A., Tabita L.R. 1997. Maximum activity of recombinant ribulose 1,5 bisphosphate carboxylase/oxygenase of *Anabaena* sp. strain CA requires the product of the *rbcX* gene. J. Bacteriol. 179:3793–3796.
Lozupone C., Hamady M., Knight R. 2006. UniFrac—an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7:371.
Lozupone C., Knight R. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol.71:8228–8235.
Lücking R., Hodkinson B.P., Stamatakis A., Cartwright R.A. 2011. PICS-Ord: Unlimited coding of ambiguous regions by pairwise identity and cost scores ordination. BMC Bioinformatics 12:10.
Lücking R., Dal-Forno M., Sikaroodi M., Gillevet P.M., Bungartz F., Moncada B., Yánez-Ayabaca A., Chaves J.L., Coca L.F., Lawrey J.D. 2014. A single macrolichen constitutes hundreds of unrecognized species. Proc. Natl. Acad. Sci. USA. 111:11091–11096.
Lumbsch H.T., Chaves-Chaves J.L., Umaña-Tenorio L., Lücking, R. 2011. One hundred new species of lichenized fungi: A signature of undiscovered global diversity. Un centenar de nuevas especies de hongos liquenizados: Una firma de la diversidad mundial por descubrir. Phytotaxa (18):1–127.
Lumbsch H.T., Leavitt S.D. 2011. Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. Fungal Divers. 50:59–72.
Lutzoni F., Miadlikowska J. 2009. Lichens. Quick guide. Curr. Biol. 19:R502–R503.
Lutzoni F., Nowak M.D., Alfaro M.E., Reeb V., Miadlikowska J., Swofford D., Arnold A.E., Hibbett D., Hilu K., James T.Y., Quandt D., Magallón S. 2016. Synchronized radiations of fungi and plants linked to symbiosis (in review).
Lutzoni F., Pagel M., Reeb V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940.
Lutzoni F., Wagner P., Reeb V., Zoller S. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. 49:628–651.
Maddison D., Maddison W. 2005. MacClade v. 4.08. Sinauer Associates.
Maddison W.P., Midford P.E., Otto S.P. 2007. Estimating a binary character's effect on speciation and extinction. Syst. Biol. 56:701–710.
Magain N., Goffinet B., Sérusiaux E. 2012. Further photomorphs in the lichen family Lobariaceae from Reunion (Mascarene archipelago) with notes on the phylogeny of *Dendriscocaulon* cyanomorphs. Bryologist 115:243–254.
Magain N., Sérusiaux E. 2012. A further new species in the lichen genus Arctomia: A. borbonica from Reunion (Mascarene archipelago). MycoKeys 4:9–21.
Magain N., Sérusiaux E. 2014. Do photobiont switch and cephalodia emancipation act as evolutionary drivers in the lichen symbiosis? A case study in the Pannariaceae (Peltigerales). PLoS One 9:e89876.
Martínez I., Burgaz A.R., Vitikainen O., Escudero A. 2003. Distribution patterns in the genus *Peltigera* Willd. Lichenologist 35:301–323.
Mason-Gamer R., Kellogg E. 1996. Testing for phylogenetic conflict among molecular datasets in the tribe Triticeae (Gramineae). Syst. Biol. 45:524–545.
Matheny P.B., Liu Y.J., Ammirati J.F., Hall B.D. 2002. Using *RPB1* sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). Am. J. Bot. 89:688–698.
May R.M. 1982. Mutualistic interactions among species. Nature 296:803–804.
McDonald T.R., Gaya E., Lutzoni F. 2013. Twenty-five cultures of lichenizing fungi available for experimental studies on symbiotic systems. Symbiosis 59:165–171.
Medina R., Lara F., Goffinet B., Garilleti R., Mazimpaka V. 2013. Unnoticed diversity within the disjunct moss *Orthotrichum tenellum* sl validated by morphological and molecular approaches. Taxon 62:1133–1152.
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. Int. J. Plant Sci. 161:925–958.
Miadlikowska J., Lutzoni F. 2004. Phylogenetic classification of peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. Am. J. Bot. 91:449–464.

Miadlikowska J., Lutzoni F., Goward T., Zoller S., Posada D. 2003. New approach to an old problem: Incorporating signal from gap-rich regions of ITS and rDNA large subunit into phylogenetic analyses to resolve the *Peltigera canina* species complex. Mycologia 95:1181–1203.
Miadlikowska J., Richardson D., Magain N., Ball B., Anderson F., Cameron R., Lendemer J., Truong C., Lutzoni F. 2014. Phylogenetic placement, species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming Ascomycota, Lecanoromycetes). Am. J. Bot. 101:1141–1156.
Miller M.A., Pfeiffer W., Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 2010, IEEE. p. 1–8.
Moran N.A., Dohlen C.D., Baumann P. 1995. Faster evolutionary rates in endosymbiotic bacteria than in cospeciating insect hosts. J. Mol. Evol. 41:727–731.
Myllys L., Stenroos S., Thell A., Kuusinen M. 2007. High cyanobiont selectivity of epiphytic lichens in old growth boreal forest of Finland. New Phytol. 173:621–629.
Nylander J. 2004. MrModeltest v2. Uppsala University: Evolutionary Biology Centre. (Program distributed by the author.)
Nylander J.A., Wilgenbusch J.C., Warren D.L., Swofford D.L. 2008. AWTY (are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–583.
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*. Eur. J. Phycol. 40:363–378.
O'Brien H.E., Miadlikowska J., Lutzoni F. 2009. Assessing reproductive isolation in highly diverse communities of the lichen-forming fungal genus *Peltigera*. Evolution 63:2076–2086.
O'Brien H.E., Miadlikowska J., Lutzoni F. 2013. Assessing population structure and host specialization in lichenized cyanobacteria. New Phytol. 198:557–566.
O'Donnell K., Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogenet. Evol. 7:103–116.
Oksanen I., Lohtander K., Paulsrud P., Rikkinen J. 2002. A molecular approach to cyanobacterial diversity in a rock-pool community involving gelatinous lichens and free-living *Nostoc* colonies. Ann. Bot. Fenn. 39:93–100.
Ollerton J. 2006. "Biological barter": Patterns of specialization compared across different mutualisms. In: Waser N.M., Ollerton J., editors. Plant pollinator interactions: from specialization to generalization. Chicago: University of Chicago Press. p. 411–435.
Oren A. 2004. Prokaryote diversity and taxonomy: Current status and future. Phil. Trans. R. Soc. Lond. B 359:623–638.
Otálora M.A., Martínez I., O'Brien H.E., Molina M.C., Aragón G., Lutzoni F. 2010. Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). Mol. Phylogenet. Evol. 56:1089–1095.
Otaìlora M.A., Salvador C., Martiìnez I., Aragoìn G. 2013. Does the reproductive strategy affect the transmission and genetic diversity of bionts in cyanolichens? A case study using two closely related species. Microb. Ecol. 65:517–530.
Otálora M.A., Wedin M. 2013. *Collema fasciculare* belongs in Arctomiaceae. Lichenologist 45:295–304.
Pagel M., Meade A. 2004. BayesTraits manual. Available online at http://www.evolution.rdg.ac.uk/BayesTraits.html.
Pagel M., Meade A., Barker D. 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53:673–684.
Paulsrud P., Lindblad P. 1998. Sequence variation of the tRNALeu intron as a marker for genetic diversity and specificity of symbiotic cyanobacteria in some lichens. Appl Environ Microbiol 64: 310–315.
Paulsrud P., Rikkinen J., Lindblad P. 2000. Spatial patterns of photobiont diversity in some *Nostoc*-containing lichens. New Phytol. 146:291–299.
Peksa O.E., Škaloud P. 2011. Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). Mol. Ecol. 20:3936–3948.
Piercey-Normore M.D., DePriest P.T. 2001. Algal switching among lichen symbioses. Am. J. Bot. 88:1490–1498.
Pino-Bodas R., Burgaz A.R., Martin M.P., Lumbsch H.T. 2011. Phenotypical plasticity and homoplasy complicate species delimitation in the *Cladonia gracilis* group (Cladoniaceae, Ascomycota). Org. Divers. Evol. 11:343–355.
Pons J., Barraclough T.G., Gomez-Zurita J., Cardoso A., Duran D.P., Hazell S., Kamoun S., Sumlin W.D., Vogler A.P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55:595–609.
Rabosky D.L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS One 9:e89543.
Rabosky D.L., Grundler M., Anderson C., Shi, J.J., Brown J.W., Huang H., Larson J.G. 2014. BAMMtools: An R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol. Evol. 5:701–707.
Rambaut A., Drummond A. 2007. Tracer v1. 4. Available online at http://tree.bio.ed.ac.uk/software/tracer/.
Reeb V., Lutzoni F., Roux C. 2004. Multilocus phylogenetic circumscription of the lichen-forming fungi family Acarosporaceae and its position within the Ascomycota. Mol. Phylogenet Evol 32:1036–1060.
Reid N., Carstens B. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: A Bayesian implementation of the general mixed Yule-coalescent model. BMC Evol. Biol. 12:196.
Rodríguez F.J., Oliver J.L., Marín A., Medina J.R. 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol 142:485–501.
Rudi K., Skulberg O.M., Jakobsen K.S. 1998. Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. J. Bacteriol. 180:3453–3461.
Sachs J.L., Essenberg C.J., Turcotte M.M. 2011. New paradigms for the evolution of beneficial infections. Trends Ecol. Evol. 26:202–209.
Sérusiaux E., Goffinet B., Miadlikowska J., Vitikainen O. 2009. Taxonomy, phylogeny and biogeography of the lichen genus *Peltigera* in Papua New Guinea. Fungal Divers. 38:185–224.
Stamatakis A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.
Stamatakis A., Hoover P., Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Syst. Biol. 57:758–771.
Stiller J.W., Hall B.D. 1997. The origin of red algae: Implications for plastid evolution. Proc. Natl. Acad. Sci. USA. 94:4520–4525.
Svenning M.M., Eriksson T., Rasmussen U. 2005. Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses. Arch. Microbiol. 183:19–26.
Tainaka K., Yoshida N., Terazawa N., Nakagiri N., Hashimoto T., Takeuchi Y., Yoshimura J. 2003. The effect of mutualism on community stability. J. Phys. Soc. Jpn. 72:956–961.
Thompson J.N. 1994. The coevolutionary process. Chicago: University of Chicago Press.
Thompson J.N. 2005. The geographic mosaic of coevolution. Chicago: University of Chicago Press.
Thompson J.N., Cunningham B.M. 2002. Geographic structure and dynamics of coevolutionary selection. Nature 417:735–738.
Trivers R.L. 1971. The evolution of reciprocal altruism. Q. Rev. Biol. 46:35–57.
van Valen L. 1976. The red queen lives. Nature 260:575.
Vandamme P., Pot B., Gillis M., de Vos P., Kersters K., Swings J. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60:407–438.
de Vienne D., Refrégier G., López-Villavicencio M., Tellier A., Hood M., Giraud T. 2013. Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. New Phytol. 198:347–385.
Vilgalys R., Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172:4238–4246.
Vitikainen O. 1994. Taxonomic revision of *Peltigera* (lichenized Ascomycotina) in Europe. Acta Bot. Fenn. 152:1–96.
Vitikainen O. 1998. Taxonomic notes on neotropical species of *Peltigera*. In: Marcelli M.P., Seaward M.R., editors. Lichenology in Latin

America: History, current knowledge and applications. Sao Paulo: Companhia de Tecnologia de Saneamento Ambiental. p. 135–139.
Vivas M., Sacristan M., Legaz M., Vicente C. 2010. The cell recognition model in chlorolichens involving a fungal lectin binding to an algal ligand can be extended to cyanolichens. Plant Biol. 12:615–621.
Wedin M., Döring H., Gilenstam G. 2004. Saprotrophy and lichenization as options for the same fungal species on different substrata: Environmental plasticity and fungal lifestyles in the Stictis–Conotrema complex. New Phytol. 164:459–465.
Werth S., Scheidegger C. 2012. Congruent genetic structure in the lichen-forming fungus *Lobaria pulmonaria* and its green-algal photobiont. Mol. Plant Microbe Interact. 25:220–230.
Wheeler D.L., Barrett T., Benson D.A., Bryant S.H., Canese K., Chetvernin V., Church D.M., DiCuccio M., Edgar R., Federhen S., Geer L.Y., Kapustin Y., Khovayko O., Landsman D., Lipman D.J., Madden T.L., Maglott D.R., Ostell J., Miller V., Pruitt K.D., Schuler G.D., Sequeira E., Sherry S.T., Sirotkin K., Souvorov A., Starchenko G., Tatusov R.L., Tatusova T.A., Wagner L., Yaschenko E. 2007. Database resources of the national center for biotechnology information. Nucleic Acids Res. 35:D5–D12.
White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications. 18: 315–322.
Wirtz N., Lumbsch H.T., Green T.G.A., Türk R., Pintado A., Sancho L., Schroeter B. 2003. Lichen fungi have low cyanobiont selectivity in maritime Antarctica. New Phytol. 160:177–183.
Wolin C.L. 1985. The population dynamics of mutualistic systems. In: Boucher D.H., editor. The biology of mutualism: Ecology and evolution. Oxford: Oxford University Press. p. 248–269.
Yoshimura J., Amagai H., Suzuki T., Togashi T., Miyazaki T. 2003. Population dynamics of some mutualistic relationships. Proceedings of the International Congress on Modelling and Simulation. Townsville. p. 730–735.
Zolan M., Pukkila P. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. Mol. Cell. Biol. 6:195–200.
Zoller S., Lutzoni F. 2003. Slow algae, fast fungi: Exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*. Mol. Phylogenet. Evol. 29:629–640.