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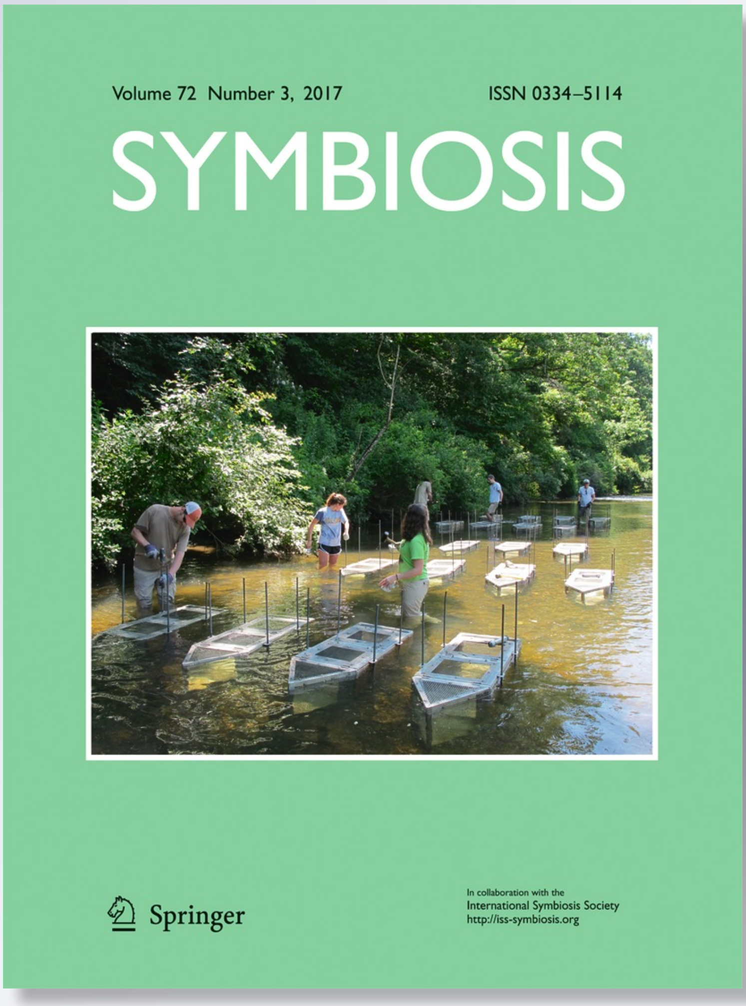

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Phylogenetic signal of photobiont switches in the lichen genus *Pseudocyphellaria* s. l. follows a Brownian motion model

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Abstract Lichen symbioses are defined as a symbiotic relationship between a mycobiont (generally an ascomycete) and one or more photobionts (green algae or/and cyanobacteria). It was proposed that cephalodia emancipation is an evolutionary driver for photobiont switch from chlorophyte to cyanobacteria. In this study we want to test the monophyly of cyanolichens and to measure the phylogenetic signal of the symbiotic relationship between cyanobacteria and a mycobiont partner in the lichen genus *Pseudocyphellaria*. This genus includes some species that have a chlorophyte as primary photobiont (and *Nostoc* in internal cephalodia), while others have only cyanobacteria. In a phylogenetic framework we measure the phylogenetic signal (or phylogenetic dispersion) as well as mapped photobiont switches performing stochastic character mapping. Results show that having cyanobacteria as main photobiont has a strong phylogenetic signal that follows a Brownian motion model. Seven clades in the phylogeny had an ancestor with cyanobacteria. Reversal to a green algae photobiont is rare. Several switches were estimated through evolutionary time suggesting that there was some flexibility in these traits along the phylogeny; however, close relatives retained cyanobacteria as main photobiont throughout the cyanolichen's history. Photobiont switches from green algae to cyanobacteria might enhance ecotypical differentiation. These ecotypes could lead to several speciation events in the new lineage resulting in the phylogenetic signal found in this study. We give insights into the origin of lichen diversity exploring the photobiont switch in a phylogenetic context in *Pseudocyphellaria* s. l. as a model genus.

Keywords Evolutionary driver · Lichenized fungi · Photobiont switch · Speciation · Symbiosis

Lichen symbioses are symbiotic relationships between a mycobiont, most commonly Ascomycota and one or more photobionts, which can be a chlorophyte or cyanobacteria. In the latter case, the cyanobacteria are most commonly from the genus *Nostoc*. Selectivity of each of the two partners has been explored previously, and there are several studies that looked at the diversity of *Nostoc* in symbiosis with fungi (Elvebakk et al. 2008; Fedrowitz et al. 2012; Lohtander et al. 2003; Novis and Smissen 2006; O'Brien et al. 2013) as well as in other organisms (Costa et al. 2001). It has been documented that some mycobionts can establish symbiosis with several photobiont genotypes or several species (Bačkor et al. 2010; Casano et al. 2011; del Campo et al. 2013; Guzow-Krzeminska 2006; Muggia et al. 2013; Piercey-Normore 2006). Most studies focus on one type of photobiont, either chlorophyte or cyanobacteria, but not photobiont switch from a chlorophyte to cyanobacteria (or the other way around).

The process of lichenization is still not clear. It was proposed that lichen surfaces might act as a temporary niche for free-living photobionts, facilitating the establishment of other lichens in the area (Muggia et al. 2013). The lichenization process is linked with the mechanism of dispersal of both partners and how they encounter and form the lichen. The mycobiont reproduces sexually through spores, and free living cyanobacteria, like *Nostoc*, disperse themselves by means of hormogonia (motile filaments) and these serve as infective units for the symbiosis (Dodds et al. 1995; Papaefthimiou

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et al. 2008). However, lichens also reproduce vegetatively through diaspores containing photobiont cells surrounded by fungal cells.

Phylogenies are useful predictors of the outcomes of species interactions, linking ecology and evolution (Agrawal and Fishbein 2006; Weiblen et al. 2006). For example, it has been shown that the interaction of mycorrhizal fungi with different plant species has significant phylogenetic signal (Anacker et al. 2014; Reinhart and Anacker 2014; Reinhart et al. 2012). It was shown that host range of plant pathogens has a phylogenetic signal; the likelihood of a pathogen to infect two plant species decreases with the plant's phylogenetic distance (Gilbert and Webb 2007). In lichens of the family Pannariaceae, photobiont switches and cephalodia emancipation are proposed as evolutionary drivers as they are related with diversification (Magain and Sérusiaux 2014).

We want to assess whether the presence of cyanobacteria as sole photobiont has a phylogenetic signal in *Pseudocyphellaria* s. l. (i.e. related lichen species are more likely to have cyanobacteria as photobiont than expected by chance). Under a Brownian motion model of evolution, character change is proportional to the time of divergence between species. Thus, species that share a more recent common ancestor should have more similar trait values than more distantly related species (Symonds and Blomberg 2014).

Pseudocyphellaria s. l. is a good candidate to study photobiont switch in evolutionary time, as it includes species that have the cyanobacteria *Nostoc* in the photosynthetic layer as the only symbiont (54 %) and species with a green algae (*Chlorella* or *Dictyochloropsis*-like) as main photobiont, and cyanobacteria in internal cephalodia (45 %) (Galloway 1986, 1988). Hence, the species with chlorophyte also have *Nostoc* but not as main photobiont.

In addition, some species in *Pseudocyphellaria* s. l. show different photomorphs (photosymbiodemes), where the same mycobiont ITS genotype is in symbiosis either with cyanobacteria or with chlorophyte. These photomorphs may differ in habitat, morphology, anatomy and chemistry; and both photomorphs derived from the same fungus (Armaleo and Clerc 1991). In the past some authors suggested that each photomorph should have a different species name (Galloway 1988); however under current nomenclature rules they should be regarded as a single species (Armaleo and Clerc 1991; Henskens et al. 2012; Laundon 1995; Rikkinen 2015; Stenroos et al. 2003). Environmental factors (e.g. desiccation and light) can regulate the development of the different photomorphs (Stenroos et al. 2003). It has been suggested that the cyanobacterial photomorphs have an important function in stabilizing and maintaining the symbiosis of the chloromorph thallus by providing nitrogenous compounds from the nitrogen fixation process (Stenroos et al. 2003).

Another important issue is that molecular data for *Pseudocyphellaria* s. l. is available from previous studies (113 taxa) representing 45 species of the 114 species described (Miadlikowska et al. 2002; Moncada et al. 2013, 2014). The molecular phylogenies of *Pseudocyphellaria* s. l. recently published (Moncada et al. 2013, 2014) resolved several new genera, however, for this study we are going to use *Pseudocyphellaria* s. l. since there are several species that make *Pseudocyphellaria s. str.* paraphyletic and have not been assigned to any new genus.

The aim of this study is to use a molecular approach to determine if the symbiosis between a nostocacean cyanobacteria as main photobiont and Ascomycota is phylogenetically conserved, specifically:

1) We want to test if species in *Pseudocyphellaria* s. l. with cyanobacteria as sole photobiont form a monophyletic group
2) We want to assess if having cyanobacteria as sole photobiont has phylogenetic signal in *Pseudocyphellaria* s. l. This is to say, if close relative species share cyanobacteria as main photobiont.

1 Materials and methods

1.1 Molecular and data analyses

For the molecular phylogenetic study we downloaded all ITS sequences available in GenBank for *Pseudocyphellaria* (113 representing 45 species, accessed October 2012, Table 1). We added two more species, *P. guilleminii* (Mont.) D. J. Galloway and *P. vaccina* (Mont.) Malme, that were collected in northwestern Patagonia, Río Negro Province, Argentina. Total genomic DNA was isolated using 1X CTAB (Sigma Aldrich) following the protocol described in Cubero and Crespo (2002). Amplifications were carried out using 1X reaction Buffer (Invitrogen provided with the *Taq* polymerase −10 mM Tris HCl, 50 mM KCl, pH 8.3-), 1.5 mM $MgCl_2$, 50 μM dNTPs, 1 unit *Taq* polymerase (Invitrogen), 0.4 μM of each primer, and 1 μl of 1/20 dilution of the genomic DNA. For the mycobiont, the nuclear ribosomal ITS was amplified with universal primers (Gardes and Bruns 1993). The amplification scheme consisted of 5 min at 94 °C followed by 30 cycles of 1 min 94 ° C, 1 min 47 °C and 2 min 72 °C and a final extension of 7 min at 72 °C. Fragments were sequenced with PCR primers by Macrogen Inc. (South Korea).

All sequences were automatically aligned with the software muscle as implemented in Aliview (Larsson 2014) using default settings (only small adjustment were made by eye). The clade now considered as the genus *Anomalobaria* Moncada &

Table 1 Matrix of lichenized fungi species used in the phylogeny reconstruction with the Genbank accession number for the ribosomal internal transcribed spacer and the information of the primary photobiont

ITS Genbank Accession	Lichenized Fungi Species Name	Primary Photobiont
AF351138	Cyanomorph of *P. coriacea* (*Pseudocyphellaria allanii*)	cyanobacteria
AF401970	*Pseudocyphellaria anomala*	cyanobacteria
HQ650697	*Pseudocyphellaria anomala*	cyanobacteria
AF401969	*Pseudocyphellaria anthraspis*	cyanobacteria
AF350300	*Pseudocyphellaria ardesiaca*	cyanobacteria
AB623072	*Pseudocyphellaria argyracea*	cyanobacteria
EU558727	*Pseudocyphellaria argyracea*	cyanobacteria
JQ735974	*Pseudocyphellaria argyracea*	cyanobacteria
AF350299	*Pseudocyphellaria aurata*	chlorophyte
AF401967	*Pseudocyphellaria aurata*	chlorophyte
AF524901	*Pseudocyphellaria aurata*	chlorophyte
EU558728	*Pseudocyphellaria aurata*	chlorophyte
FJ349102	*Pseudocyphellaria aurata*	chlorophyte
JQ735975	*Pseudocyphellaria aurata*	chlorophyte
EU558721	*Pseudocyphellaria berberina*	chlorophyte
EU558722	*Pseudocyphellaria berberina*	chlorophyte
EU558723	*Pseudocyphellaria berberina*	chlorophyte
EU558712	*Pseudocyphellaria* cf. *intricata*	cyanobacteria
EU558713	*Pseudocyphellaria* cf. *intricata*	cyanobacteria
AF351139	*Pseudocyphellaria cinnamomea*	cyanobacteria
EU558729	*Pseudocyphellaria clathrata*	chlorophyte
FJ356150	*Pseudocyphellaria clathrata*	chlorophyte
AF351150	*Pseudocyphellaria corbettii*	chlorophyte
AF351149	*Chloromorph Pseudocyphellaria coriacea*	chlorophyte
EU558706	*Pseudocyphellaria coriifolia*	cyanobacteria
EU558707	*Pseudocyphellaria coriifolia*	cyanobacteria
EU558708	*Pseudocyphellaria coriifolia*	cyanobacteria
EU558709	*Pseudocyphellaria coriifolia*	cyanobacteria
AF350302	*Pseudocyphellaria coronata*	chlorophyte
AF350314	*Pseudocyphellaria crocata*	cyanobacteria
AF401978	*Pseudocyphellaria crocata*	cyanobacteria
AF401979	*Pseudocyphellaria crocata*	cyanobacteria
AF401980	*Pseudocyphellaria crocata*	cyanobacteria
AF401981	*Pseudocyphellaria crocata*	cyanobacteria
AJ437679	*Pseudocyphellaria crocata*	cyanobacteria
AJ437680	*Pseudocyphellaria crocata*	cyanobacteria
AJ437681	*Pseudocyphellaria crocata*	cyanobacteria
AJ437682	*Pseudocyphellaria crocata*	cyanobacteria
AJ437683	*Pseudocyphellaria crocata*	cyanobacteria
AJ437684	*Pseudocyphellaria crocata*	cyanobacteria
AJ888202	*Pseudocyphellaria crocata*	cyanobacteria
AJ888203	*Pseudocyphellaria crocata*	cyanobacteria
AJ888204	*Pseudocyphellaria crocata*	cyanobacteria
AJ888205	*Pseudocyphellaria crocata*	cyanobacteria
AJ888206	*Pseudocyphellaria crocata*	cyanobacteria
AJ888207	*Pseudocyphellaria crocata*	cyanobacteria
AJ888208	*Pseudocyphellaria crocata*	cyanobacteria
AJ888209	*Pseudocyphellaria crocata*	cyanobacteria

Table 1 (continued)

ITS Genbank Accession	Lichenized Fungi Species Name	Primary Photobiont
EU558699	*Pseudocyphellaria crocata*	cyanobacteria
EU558700	*Pseudocyphellaria crocata*	cyanobacteria
EU558701	*Pseudocyphellaria crocata*	cyanobacteria
EU558702	*Pseudocyphellaria crocata*	cyanobacteria
EU558703	*Pseudocyphellaria crocata*	cyanobacteria
EU558704	*Pseudocyphellaria crocata*	cyanobacteria
EU558705	*Pseudocyphellaria crocata*	cyanobacteria
JQ735976	*Pseudocyphellaria crocata*	cyanobacteria
AF351151	*Pseudocyphellaria dissimilis*	cyanobacteria
EU558725	*Pseudocyphellaria endochrysea*	chlorophyte
AF351152	*Pseudocyphellaria episticta*	chlorophyte
AF350311	*Pseudocyphellaria faveolata*	chlorophyte
AF351143	*Pseudocyphellaria fimbriatoides*	cyanobacteria
EU558717	*Pseudocyphellaria freycinetii*	chlorophyte
EU558724	*Pseudocyphellaria freycinetii*	chlorophyte
AF351144	*Pseudocyphellaria glabra*	chlorophyte
AF350313	*Pseudocyphellaria granulata*	chlorophyte
KX372567[a]	*Pseudocyphellaria guilleminii*	cyanobacteria
KX372568[a]	*Pseudocyphellaria guilleminii*	cyanobacteria
KX372569[a]	*Pseudocyphellaria guilleminii*	cyanobacteria
EU558710	*Pseudocyphellaria hirsuta*	cyanobacteria
EU558711	*Pseudocyphellaria hirsuta*	cyanobacteria
AF351145	*Pseudocyphellaria homoeophylla*	chlorophyte
AF350315	*Pseudocyphellaria hookeri*	cyanobacteria
AF351148	*Pseudocyphellaria intricata*	cyanobacteria
EU558726	*Pseudocyphellaria intricata*	cyanobacteria
JQ735977	*Pseudocyphellaria intricata*	cyanobacteria
AF351140	Cyanomorph of *P. lividofusca* (*Pseudocyphellaria knightii*)	cyanobacteria
EU558716	*Pseudocyphellaria lechleri*	cyanobacteria
AF350312	*Pseudocyphellaria lindsayi*	chlorophyte
AF351153	*Chloromorph Pseudocyphellaria lividofusca*	chlorophyte
AF351147	*Pseudocyphellaria maculata*	cyanobacteria
AJ437689	*Pseudocyphellaria maculata*	cyanobacteria
EU558715	*Pseudocyphellaria mallota*	cyanobacteria
EU558739	*Pseudocyphellaria mallota*	cyanobacteria
AF351146	*Pseudocyphellaria multifida*	chlorophyte
AF350316	Cyanomorph of *P. rufovirescens* (*Pseudocyphellaria murrayi*)	cyanobacteria
AF351141	*Pseudocyphellaria neglecta*	cyanobacteria
AJ437685	*Pseudocyphellaria neglecta*	cyanobacteria
AJ437686	*Pseudocyphellaria neglecta*	cyanobacteria
AJ437687	*Pseudocyphellaria neglecta*	cyanobacteria
AJ437688	*Pseudocyphellaria neglecta*	cyanobacteria
AJ888210	*Pseudocyphellaria neglecta*	cyanobacteria
AJ888211	*Pseudocyphellaria neglecta*	cyanobacteria
EU558730	*Pseudocyphellaria obvoluta*	chlorophyte
AF401971	*Pseudocyphellaria perpetua*	cyanobacteria
AF401972	*Pseudocyphellaria perpetua*	cyanobacteria

Table 1 (continued)

ITS Genbank Accession	Lichenized Fungi Species Name	Primary Photobiont
AF401973	*Pseudocyphellaria perpetua*	cyanobacteria
AF401974	*Pseudocyphellaria perpetua*	cyanobacteria
AF401975	*Pseudocyphellaria perpetua*	cyanobacteria
AF401976	*Pseudocyphellaria perpetua*	cyanobacteria
AF401977	*Pseudocyphellaria perpetua*	cyanobacteria
AJ888212	*Pseudocyphellaria perpetua*	cyanobacteria
EU558741	*Pseudocyphellaria perpetua*	cyanobacteria
AF350301	*Pseudocyphellaria pickeringii*	chlorophyte
EU558740	*Pseudocyphellaria pilosella*	cyanobacteria
AF350298	*Pseudocyphellaria poculifera*	chlorophyte
AF401968	*Pseudocyphellaria rainierensis*	chlorophyte
AF351142	*Chloromorph Pseudocyphellaria rufovirescens*	chlorophyte
EU558720	*Pseudocyphellaria scabrosa*	cyanobacteria
KX372570[*]	*Pseudocyphellaria vaccina*	chlorophyte
KX372571[*]	*Pseudocyphellaria vaccina*	chlorophyte
KX372572[*]	*Pseudocyphellaria vaccina*	chlorophyte
AF351154	*Pseudocyphellaria wilkinsii*	chlorophyte

[a] sequences generated in this study

Lücking (*P. anomala* Brodo & Ahti and *P. anthraspis* (Ach.) H. Magn.) was used to root the tree.

1.2 Mycobiont phylogeny

Phylogenetic analyses were performed using the likelihood criterion in PhyML (Guindon et al. 2010). The model of sequence evolution (GTR+G) was selected with MrModeltest (Nylander 2004). PhyML was carried out using the South of France Bioinformatics Platform web server (www.atgc-montpellier.fr/phyml/ accessed 10/07/13). The tree search was started with a neighbour joining tree with the NNI branch swapping option. Branch support was assessed with 1000 bootstrap replicates.

1.3 Ancestral state reconstruction and character mapping

A matrix with photobiont type (cyanobacteria or green algae) for each *Pseudocyphellaria* species was assembled with information taken from the literature (Galloway 1986, 1988, 1992). The three reported photosymbiodemes were considered as different individuals in the matrix and were indicated as cyanomorph or chloromorph of the corresponding species. This matrix (Table 1) and the topology resulted from the Likelihood analysis were used to study the evolution of the symbiosis with cyanobacteria in *Pseudocyphellaria*. We inferred ancestral photobionts and mapped photobiont switches performing Stochastic character mapping (Bollback 2006) on the maximum likelihood tree using 1000 simulations (i.e. 1000 stochastic maps) with the make.simmap function in the phytools package (Revell 2012) in R (R Development Core Team 2008). This method implies a continuous-time Markov process to model character change (Huelsenbeck et al. 2003). We used an equal rate (ER) and an all-rates-different (ARD) model, where each rate is a unique parameter model to estimate ancestral characters and their rate of change across the tree. We used the fit.discrete function in GEIGER (Harmon et al. 2008) to calculate the likelihood of each model. We performed a likelihood ratio test to find the model (ER or ARD) that best fits the data.

The transition matrix between states, Q, for the stochastic mapping was estimated from the data as a single maximum-likelihood point estimate. Hence, the most likely value was used for all simulations. Results were summarized on the tree using the density.map function in phytools (Revell 2012).

1.4 Photobiont phylogenetic signal

The matrix with photobiont type (cyanobacteria or green algae) and the topology resulted from the likelihood analysis with its branch lengths were used for the comparative analysis. The existence of a phylogenetic signal (or phylogenetic dispersion) was tested by calculating the statistic D for binary traits (Fritz and Purvis 2010). This measure of phylogenetic signal is based on the sum of sister clade differences in a given phylogenetic tree. This statistic and its significance were tested using the caper package for R (Orme 2013). We performed 10,000 permutations based on random or Brownian motion model of evolution to test the significance of D. These two distributions were compared to the observed phylogenetic pattern for the presence of cyanobacteria as main photobiont. If the statistic D <0 it suggests a highly clustered trait, if D is around 0 it indicates a Brownian motion model, D = 1 indicates a random mode of evolution, and D > 1 suggests phylogenetic overdispersion (Fritz and Purvis 2010). Hence, D approaching or below 0 means phylogenetically informative traits otherwise, D approaching or exceeding 1 indicate phylogenetically labile traits. Density plots of the distributions of the two simulations relative to the observed D value were drawn.

2 Results

2.1 Mycobiont phylogeny and ancestral photobiont reconstruction

The resulted topology of the relationships between species of *Pseudocyphellaria* s. l. is in agreement with previous studies (Moncada et al. 2013). The relationships of the five major clades at the spine of the tree are not resolved (Fig. 1). The model of trait evolution that best fitted the data was the ARD model (likelihood -133.37). The ARD model was significantly better ($p = 0.002$) than the ER model (likelihood −137.98). Hence, we chose the ARD model to best represent the rate of trait evolution in the tree.

The ancestral photobiont reconstruction in the species of *Pseudocyphellaria* s. l. is shown in Fig. 1. The marginal

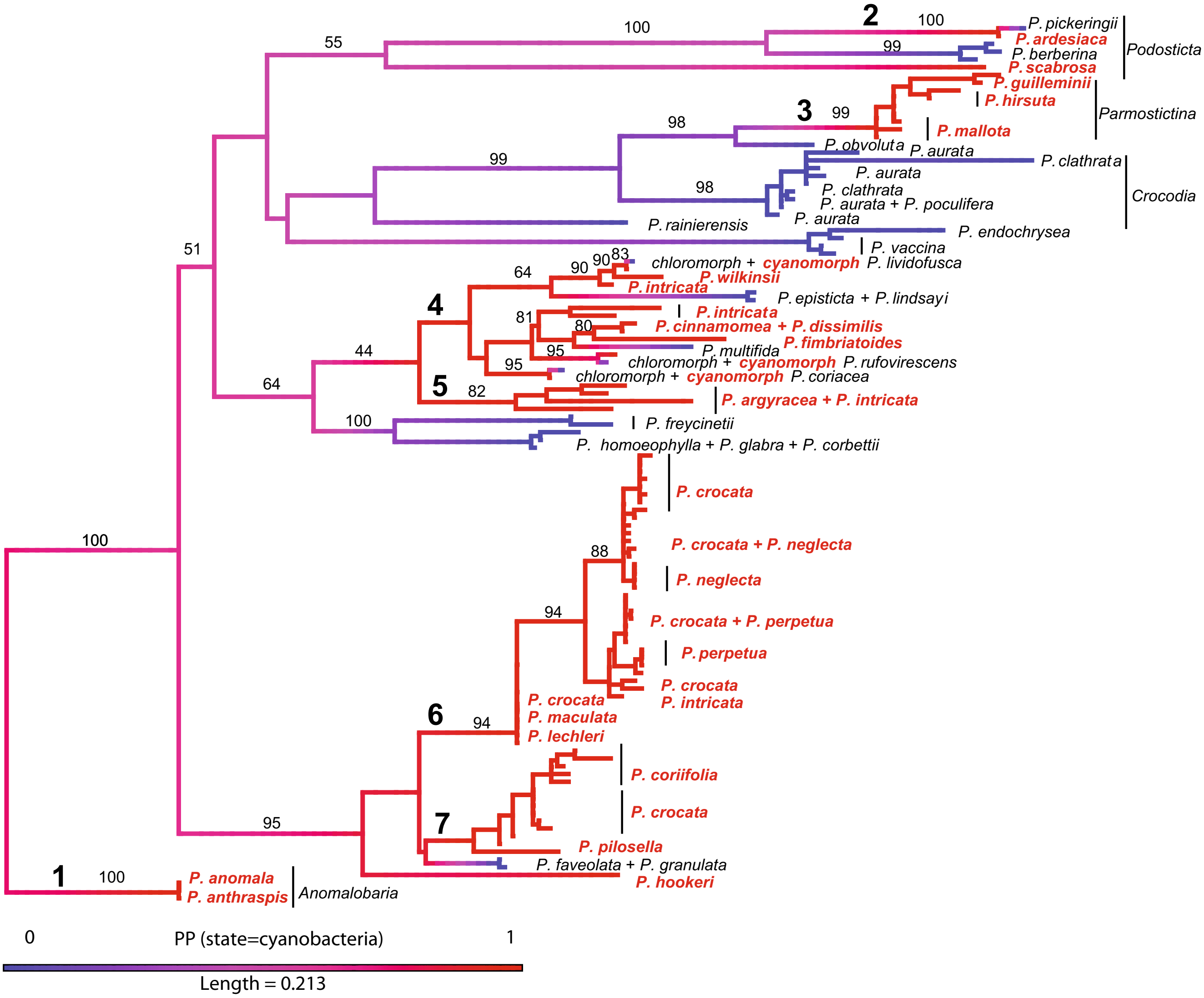

Fig. 1 Density map from stochastic mapping in the most likely tree of *Pseudocyphellaria* s. l. species using ITS marker, bootstrap support values are above branches, branch color indicates the probability of having cyanobacteria, color scale is indicated (PP = 0 *blue*, PP = 1 *red*). Numbers indicate clades that have marginal probabilities >0.98 of having *Nostoc*. Taxa with cyanobacteria as main photobiont are in *bold red*

probability that the most recent common ancestor of all *Pseudocyphellaria* species had cyanobacteria is 0.75. The stochastic mapping analysis estimated a mean of 22 events of photobiont switches in the 1000 stochastic maps. There are 7 switches from green algae to cyanobacteria. The likelihood rate estimation for the transition matrix Q was 7.49 for the switches from green algae to cyanobacteria and 5.25 from cyanobacteria to green algae. The stochastic mapping estimated 43 % of mean time spent in the state green algae and 57 % in cyanobacteria.

There are seven clades for which the ancestor has posterior probability of 1 (or very close to one, >0.98) of being a cyanobacterium (Fig. 1, clades numbered 1 to 7). Clade 1 (BS = 100) is formed by *P. anomala* and *P. anthraspis*, now in the newly circumscribed genus *Anomalobaria* (Moncada et al. 2013). Clade 2 (BS = 99) with *P. ardesiaca* D. J. Galloway and the species *P. scabrosa* R. Sant., are in the newly proposed genus *Podosticta*. Clade 3 (BS = 99) involves the species of *P. hirsuta* (Mont.) Malme*, P. mallota* (Tuck.) H. Magn., and *P. guillemini* (Mont.) D. J. Galloway, which were circumscribed within *Parmostictina* Nyl. The fourth clade (BS = 64) includes many New Zealand species and is the clade where the three cases of photosymbiodemes (same mycobiont with a chlorophyte or a cyanobacteria as photobiont) were recorded. Several species in this clade have the same ITS sequence revealing a taxonomic problem. Clade 5 (BS = 82) includes the species *P. argyracea* (Delise) Vain. and *P. intricata* (Delise) Vain.. Clade 6 (BS = 94) includes individuals of *P. crocata* (L.) Vain.*, P. neglecta* (Müll. Arg.) H. Magn.*, P. perpetua* McCune & Miadl., *P. maculata* D. J. Galloway and *P. lechleri* (Müll. Arg.) Du Rietz. Finally Clade 7 (BS = 70) includes individuals of *P. crocata, P. coriifolia (*Müll. Arg.) Malme and *P. pilosella* Malme. Within some of these clades there are reversals to green algae photobiont.

2.2 Photobiont phylogenetic signal

The distribution of cyanobacteria as main photobiont across the genus *Pseudocyphellaria* s. l. shows high levels of phylogenetic signal ($D = 0.109$). Simulation tests showed that the phylogenetic pattern follows a Brownian motion model ($p = 0.208$, Fig. 2). It also showed that D was significantly different from 1 ($p < 0\ .0001$).

3 Discussion

Photobiont switch in *Pseudocyphellaria* s. l. has phylogenetic signal that follows Brownian motion model of evolution (i.e. evolution towards randomly fluctuating selective optima). This suggests a conservatism of the photobiont through evolutionary time. Hence, loosing green algae results in

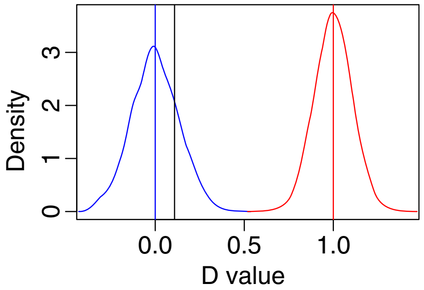

Fig. 2 Distribution of simulations under a phylogenetic random model (*blue line*) and a Brownian threshold model (*red line*), the estimated D in *black*

maintenance of cyanobacteria as main photobiont among close relatives. Once the symbiosis of cyanobacteria with the mycobiont is established reversal to a green algae photobiont is rare. Although the stochastic mapping estimated several switches from cyanobacteria to green algae this is evidenced only in four cases in the tips (Fig. 1). Interestingly, the most recent common ancestor of all photosymbiodemes has 0.80 or higher probability of having cyanobacteria. But unfortunately this part of the tree is unresolved so no conclusion can be made. The taxa involved in these clades (mostly found in New Zealand) need taxonomic revision as many have the same ITS sequence.

Species of *Pseudocyphellaria* s. l. with cyanobacteria as sole photobiont are not monophyletic. They were found in many clades across the phylogeny. However, most clades that had cyanobacteria as main photobiont were composed of several species and rarely included species with green algae. The ancestor of all *Pseudocyphellaria* s. l. species most likely had cyanobacteria as photobiont ($p = 0.75$). The stochastic mapping suggests several switches along the branches of the phylogeny, and seven switches from green algae to cyanobacteria. These estimated switches suggest that there was some flexibility in these traits along the phylogeny. However, the unilineal descent systems were retained throughout the cyanolichen's history, in contrast to a scenario where the type of photobiont is gained randomly. We think that once the green algae are displaced from the thallus by cyanobacteria, regaining green algae as main photobiont is difficult. This might be the reason why photobiont type is not randomly distributed in the phylogeny. This is supported by the model of trait evolution selected, which contemplates two different rates for gaining and loosing cyanobacteria.

In addition, we believe that the phylogenetic signal is high because seven highly supported clades, which contain mostly cyanobacterial species, were reconstructed with a cyanobacterial ancestor. We expect that the missing taxa of *Pseudocyphellaria* cyanolichens will also form monophyletic clades.

Several lichen species that have green algae as photobiont can also have cyanobacteria in cephalodia. According to

Galloway (1988) all *Pseudocyphellaria* species have internal cephalodia. The speciation process can be hypothesized to be linked to cephalodia emancipation. Thus, these structures can provide the new photobiont with subsequent loss of the green algae photobiont leading to a new evolutionary lineage. Magain and Sérusiaux (2014) proposed this mechanism of photobiont switch and cephalodia emancipation as evolutionary drivers for Pannariaceae.

Previous studies that focus on one lichen species and one type of photobiont suggest there exists ecological specialization for both lichen partners (in terms of the photobiont lineage), and that this selectivity is environment dependent (Blaha et al. 2006; Ortiz-Álvarez et al. 2015; Piercey-Normore 2006). Thus, it greatly facilitates lichen ecotypical differentiation explaining the wide ecological niches and distributional area of many lichens (Fernández-Mendoza et al. 2011; Yahr et al. 2006). It would be interesting to deepen if the habitat-specific phenomenon proposed by Rodríguez et al. (2008) can be adapted to the mycobiont associations. This would imply that with an specific lineage photobiont or the preference for one type of them, the mycobiont will adapt to specific habitat conditions. In this perspective the mycobiont, could respond physiologically to stress caused by ecological changes overtime. This could be achieved by mitigating the stress effect through the association with a specific lineage photobiont. Recently, it was suggested lichenized fungi that have both a broad ecological niche and photobiont selectivity can overcome certain ecological factors by the associations with different photobionts (Muggia et al. 2014). Probably, photobiont switch from green algae to cyanobacteria enhances ecotypical differentiation and competition leading to several speciation events in the new lineage resulting in the phylogenetic signal found in this study. This study gives insights into the origin of lichen diversity exploring the photobiont switch in a phylogenetic context in *Pseudocyphellaria* s. l. as a model genus.

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