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Further photomorphs in the lichen family Lobariaceae from Reunion (Mascarene archipelago) with notes on the phylogeny of *Dendroscocaulon* cyanomorphs

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ABSTRACT. Two new photomorphs in the Lobariaceae have been found on the remote island of Reunion in the Mascarene archipelago: the free-living *Dendroscocaulon*-like cyanomorph of the pantropical *Lobaria discolor*, and the cyanomorph of *Sticta dichotoma*, a species apparently endemic to the western parts of the Indian Ocean, known only from its chloromorph. Inferences from three loci demonstrate that the fungus involved in each morph of either pair belongs to the same species. Phylogenetic analyses resolve all genera of the Lobariaceae as polyphyletic, and all *Dendroscocaulon*-like cyanomorphs within *Lobaria*, except for *D. dendroides*, which belongs to *Sticta*.

KEYWORDS. Peltigerales, *Lobaria discolor*, *Sticta dichotoma*, phylogeny.



A lichen can be described as the intersection of two destinies, that of its mycobiont and photobiont, leading to a composite thallus with distinct, emergent properties (Sanders 2010). Although typically described as the association between two partners, it has long been known that some fungi can recruit both a green alga and a cyanobacterium and form tripartite associations such as in *Nephroma articum* or *Peltigera aphthosa* (Tønsberg & Holtan-Hartwig 1983). Furthermore, genotyping of photobionts reveals that two or more species of green algae may coexist within a single thallus (Bačkor et al. 2010; Casano et al. 2011; Guzew-Krzemiska 2006; Piercey-Normore 2006) and that fungi may be

associated with distinct algal partners along environmental or geographical gradients (Fernández-Mendoza et al. 2011). Such ability extends even to obligatory sterile taxa, and may occur repeatedly over evolutionary timescales (Nelsen & Gargas 2008). Such latitude in specificity for algal partners may allow the fungus to colonize a wider spectrum of ecological niches (Fernández-Mendoza et al. 2011; Yahr et al. 2006), to the extent that the ecological distribution of the fungus may be shaped primarily by that of the photobiont (Peksa & Škaloud 2011; Rikkinen et al. 2002).

The words “photosymbiodemes”, “photopairs” or “photomorphs” apply to the most extreme photobiont switching, from a cyanobacterium to a chlorococcoid alga (Chlorophyta) or vice versa, to form thalli that differ in their physiology and habitat preferences, and in some cases also in their

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morphological and chemical traits. Photomorph pairs are best known from the Peltigerales but have recently also been discovered in the Lecanorales (Lücking 2008) and Peltulales (Aptroot & Schumm 2010). Comparison of discrete genetic loci of the mycobiont involved in the alternative photomorphs in the Peltigerales suggests that they indeed belong to the same species (Armaleo & Clerc 1991; Goffinet & Bayer 1997; Högnabba et al. 2009; Stenroos et al. 2003).

The most spectacular dimorphism is known within the Lobariaceae, where some species of *Lobaria* and *Sticta* form foliose chloromorphs and densely branched, coralloid cyanomorphs. These alternative thalli are either free-living or attached, with typically the cyanomorph emerging from the chloromorph. These cyanomorphs have historically been accommodated in a distinct genus, *Dendriscoaulon* established by Nylander (1885), a concept that should be abandoned considering the polyphyly of the group and more importantly, the conspecificity of the fungal symbionts involved in these thalli and known chloromorphs of *Lobaria* and *Sticta*.

We here report the discovery of two cyanomorphs on the island of Reunion, and test their affinities to sympatric Lobariaceae associated with a green alga. Inferences from ITS data suggest that these cyanomorphs are formed by *Lobaria discolor* and *Sticta dichotoma*, both so far known only as lichenized with a green alga. For the former, the cyanomorph was *Dendriscoaulon*-like and was found free-living, and for the latter, lobes with a green photobiont developed on lobes formed by the cyanomorph. Furthermore, we assess the phylogenetic affinities of *Dendriscoaulon* cyanomorphs throughout the Lobariaceae in continuation of the seminal papers by Stenroos et al. (2003), Takahashi et al. (2006) and Högnabba et al. (2009).

MATERIAL AND METHODS

The material was collected during two field trips to Reunion in 2008 and 2009, the latter being dedicated to a detailed study of Peltigerales. Material from the Albertine Rift in Democratic Republic of Congo (RDC) and accessions from GenBank have

been added to expand our sampling of related taxa. Identification of voucher material follows Galloway (1994, 2001, 2007), Galloway et al. (2001), Swinscow & Krog (1988), and Yoshimura (1971).

Well-preserved lichen specimens lacking any visible symptoms of fungal infection were sampled for DNA isolation. Great care was taken to extract DNA from the target material only, e.g. the *Dendriscoaulon*-like free living cyanomorph (**Fig. 1D**) and the cyano- and chloromorph of *Sticta* growing as an autonomous assemblage (**Figs. 1A & B**). We broadened our sampling to additional species including those originally described based on material collected on Reunion (**Table 1**). Extraction of DNA and PCR amplification were performed following the protocol of Cubero et al. (1999). The following primers were used: (1) for ITS: ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990); (2) for mtSSU: mrSSU1 and mrSSU3R (Zoller et al. 1999); for nLSU: LIC2044, LR0R, LR3R, LR3, and LR6 (Kauff & Lutzoni, 2002; Rehner & Samuels 1994; Vilgalys & Hester 1990). Amplicons were sequenced by Macrogen[®]. Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to BLAST searches to detect potential contamination. The sequences were aligned manually using MacClade 4.05 (Maddison & Maddison 2002). Ambiguous regions were delimited using the online version of GBLOCKS v 0.91b (Castresana 2000) at <http://molevol.cmima.csic.es/castresana/Gblocks.html>, allowing for gap positions within the final blocks then carefully checked manually.

We assembled two matrices. Matrix 1 was assembled to detect the phylogenetic affinities of both photomorphs studied here within the Lobariaceae, a family whose generic delimitations are debated (Högnabba et al. 2009). It includes sequences of three loci, nLSU, ITS and mtSSU for representative species of the three genera of the Lobariaceae (*Lobaria*, *Pseudocyphellaria* and *Sticta*), with a focus on species assumed to be closely related to the photomorphs studied here. *Massalongia carnosa* and *Nephroma antarcticum* were chosen as outgroups as they belong respectively to the Massalongiaceae and Nephromataceae, two strongly supported sister families (Wedin et al. 2007).



Figure 1. Photomorphs of Lobariaceae found on Reunion. **A.** Assemblage of both morphs of the common species *Sticta dichotoma* (Forêt de Bébour, Nov. 2009). **B.** Close-up showing details of chloromorphs lobes overgrowing the cyanomorph. **C.** Most common chloromorph of *Lobaria discolor* (Forêt du Grand Matarum, Nov. 2009). **D.** Free-living *Dendriscoaulon*-like cyanomorph of *Lobaria discolor* (Above St-Philippe, Nov. 2009, N. Magain & E. Sérusiaux s. n., LG). A-C: photographs taken in the field. D. Scale = 2 cm.

Matrix 2 includes ITS sequences only for selected exemplars of Lobariaceae but including all available sequences of *Dendriscoaulon*-like cyanomorph as either free-living or attached photomorphs, to test their affinities within the genera *Lobaria* and *Sticta*. *Massalongia carnosa* was selected as outgroup as it belongs to a family (Massalongiaceae) forming an unresolved clade with the Lobariaceae (Wedin et al. 2007). No representative of *Pseudocyphellaria* was included as no *Dendriscoaulon*-like cyanomorph has ever been detected in that genus.

Congruence between the three loci partitions in matrix 1 was assessed, with datasets considered congruent if relationships characterized by bootstrap proportions for MP and ML or posterior probabilities above 70% or 0.95, respectively, were

identical or at least not in direct conflict among the inferences from individual loci. Since all partitions were shown to be congruent they were concatenated. The two matrices are deposited in TreeBASE under the accession numbers S12431.

For each matrix phylogenetic relationships were reconstructed based on Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian inferences. The MP analysis was performed in PAUP* 4.0b10 (Swofford 2002) with characters equally weighted and gaps treated as missing data. An initial run was performed by using the NNI (Nearest Neighbor Interchange) branch swapping, with 10 trees saved for each of 1000 replicates, and was followed by a second analysis using the Tree Bisection Reconnection (TBR) branch swapping algorithm whereby all saved NNI trees were swapped to

Table 1. Voucher information and associated GenBank accession numbers for nLSU, mtSSU and ITS loci. New accessions are in bold. Collections made in Reunion were gathered by N. Magain and E. Sérusiaux and are deposited at LG. The number following the herbarium acronym LG refers to the lichen DNA database at the University of Liège.

Name	Voucher	ITS	nLSU	mtSSU
<i>Dendriscoaulon dendroides</i> (Nyl.) R. Sant.	NEW ZEALAND: Thomas et al. (2002)	AF350303	—	—
<i>Dendriscoaulon</i> sp. 1	REUNION (LG 1020)	JQ735971	JQ735988	JQ736005
<i>Dendriscoaulon</i> sp. 2	CHINA: Takahashi et al. (2006)	AB239338	—	—
<i>Dendriscoaulon</i> sp. 3	CHINA: Hur et al. (2005)	DQ001281	—	—
<i>Dendriscoaulon</i> sp. 4	CHINA: Takahashi et al. (2006)	AB239337	—	—
<i>Dendriscoaulon</i> sp. 5	CHINA: Takahashi et al. (2006)	AB239340	—	—
<i>Dendriscoaulon umhausense</i> (Auersw.) Degel.	UNITED KINGDOM: Kelly et al. (2011)	FR799178	—	—
<i>Lobaria subexornata</i> Yoshim.	MEXICO: Stenroos et al. (2003), Högnabba et al. (2009)	AF524902	EU558770	EU558804
<i>Lobaria amplissima</i> (Scop.) Forss.	NORWAY: Stenroos et al. (2003), Högnabba et al. (2009)	AF524924	EU558749	EU558805
<i>Lobaria discolor</i> (Bory) Hue	REUNION (LG 1035)	JQ735972	JQ735989	JQ736006
<i>Lobaria linita</i> (Ach.) Rabenh.	JAPAN: Stenroos et al. (2003), Högnabba et al. (2009)	AF524914	EU558800	EU558809
<i>Lobaria orientalis</i> (Asahina) Yoshim.	JAPAN: Stenroos et al. (2003), Högnabba et al. (2009)	AF524907	EU558796	EU558810
<i>Lobaria patinifera</i> (Taylor) Hue	REUNION (LG 947)	JQ735973	JQ735990	—
<i>Lobaria pulmonaria</i> (L.) Hoffm.	RUSSIA: Stenroos et al. (2003), Högnabba et al. (2009)	AF524910	EU558797	EU558811
<i>Lobaria quercizans</i> Michaux	CANADA: Stenroos et al. (2003), Högnabba et al. (2009)	AF524921	EU558747	EU558814
<i>Lobaria retigera</i> (Bory) Trevis.	CHINA: Lohtander et al. (2002) (ITS & mtSSU), and CANADA: Wiklund et Wedin (2003) (LSU)	AY124094	AY340550	AY124159
<i>Lobaria sachalinensis</i> Asahina	JAPAN: Stenroos et al. (2003), Högnabba et al. (2009)	AF524906	EU558795	EU558815
<i>Lobaria scrobiculata</i> (Scop.) DC.	FINLAND: Stenroos et al. (2003), Högnabba et al. (2009)	AF524913	EU558787	EU558816
<i>Massalongia carnosa</i> (Dickson) Körber	FINLAND: Högnabba et al. (2009)	EU558742	EU558697	EU558817
<i>Nephroma antarcticum</i> (Wulfen) Nyl.	ARGENTINA: Stenroos et al. (2003), Högnabba et al. 2009	AF524897	EU558743	EU558818
<i>Pseudocyphellaria argyracea</i> (Delise) Vain.	REUNION (LG 1024)	JQ735974	JQ735991	JQ736007
<i>Pseudocyphellaria aurata</i> (Ach.) Vain.	REUNION (LG 1246)	JQ735975	JQ735992	JQ736008
<i>Pseudocyphellaria clathrata</i> (De Not.) Malme	BRAZIL: Högnabba et al. (2009)	EU558729	EU558784	EU558828
<i>Pseudocyphellaria crocata</i> (L.) Vain.	REUNION (LG 688)	JQ735976	JQ735993	JQ736009
<i>Pseudocyphellaria freycinetii</i> (Delise) Malme	ARGENTINA : Högnabba et al. (2009)	EU558717	EU558771	EU558843
<i>Pseudocyphellaria intricata</i> (Delise) Vain.	D.R. CONGO (LG 883)	JQ735977	JQ735994	JQ736010
<i>Sticta ambavillaria</i> (Bory) Ach.	REUNION (LG 992)	JQ735978	JQ735995	JQ736011
<i>Sticta caperata</i> (Nyl.) Bory	REUNION (LG 962)	JQ735979	JQ735996	JQ736012
<i>Sticta caulescens</i> De Not.	ARGENTINA: Högnabba et al. (2009)	EU558737	EU558793	EU558858

Table 1. Continued.

Name	Voucher	ITS	nLSU	mtSSU
<i>Sticta cyphellulata</i> (Müll.Arg.) Hue	REUNION (LG 1023)	JQ735980	JQ735997	JQ736013
<i>Sticta dichotoma</i> Bory 1 (free living chloromorph)	REUNION (LG 945)	JQ735981	JQ735998	JQ736014
<i>Sticta dichotoma</i> Bory 2 (chloromorph attached to cyanomorph)	REUNION (LG 984)	JQ735982	JQ735999	JQ736015
<i>Sticta dichotoma</i> Bory 3 (cyanomorph attached to chloromorph)	REUNION (LG 985)	JQ735983	JQ736000	JQ736016
<i>Sticta duplolibata</i> (Hue) Vain.	REUNION (LG 1040)	JQ735984	JQ736001	JQ736017
<i>Sticta filix</i> (Sw.) Nyl.	NEW ZEALAND: Thomas et al. (2002)	AF350304	—	—
<i>Sticta gaudichaudia</i> Delise	ARGENTINA: Högnabba et al. (2009)	EU558736	EU558792	EU558860
<i>Sticta hypochroa</i> Vain.	ARGENTINA: Högnabba et al. (2009)	EU558714	EU558767	EU558861
<i>Sticta lacera</i> (Hook. f. & Taylor) Müll. Arg.	NEW ZEALAND: Thomas et al. (2002)	AF350305	—	—
<i>Sticta latifrons</i> A.Rich.	NEW ZEALAND: Thomas et al. (2002)	AF350307	—	—
<i>Sticta macrophylla</i> Bory	REUNION (LG 946)	JQ735985	JQ736002	JQ736018
<i>Sticta oroborealis</i> Goward & Tønsberg	CANADA: Tønsberg & Goward (2001)	AF208015	—	—
<i>Sticta sublimbata</i> (Steiner) Swinscow & Krog	D. R. CONGO (LG 885)	JQ735986	JQ736003	JQ736019
<i>Sticta variabilis</i> Ach.	REUNION (LG 1037)	JQ735987	JQ736004	JQ736020
<i>Sticta weigelii</i> (Ach.) Vain.	GUYANA : Stenroos et al. (2003), Högnabba et al. (2009)	AF524905	EU558794	EU558865
<i>Sticta wrightii</i> Tuck.	CHINA: Takahashi et al. (2006)	AB239343	—	—

completion with no limit to the number of trees saved. Throughout the analysis, MulTrees option was activated. Support for the branches was estimated using the bootstrap approach (Felsenstein 1985) with a heuristic search algorithm on 500 pseudoreplicates, all other parameter settings being identical. Bootstrap frequencies (MPBS) were obtained by constructing a majority-rule consensus tree of all trees saved during the analysis.

Models of evolution for the ML and Bayesian analyses were selected based on the Akaike Information Criterion (Akaike 1974; Posada & Buckley 2004) as implemented in MrModeltest v2.3 (Nylander 2004). We used RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) for the ML analysis on the CIPRES gateway (Miller et al. 2010). Support for each branch was evaluated using the “fast bootstrap” with 1000 pseudoreplicates. Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MC³) in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). No prior values were assumed. Four parallel runs were performed each

using four independent chains (three heated and one cold chain), with a single tree saved every 100th generation for a total of 4,000,000 generations. The incremental heating scheme was set by default. We used TRACER v1.4.1 (Rambaut & Drummond 2007) to plot the log-likelihood values of the sample points against generation time, and determine when stationarity was achieved. Consequently the first 400,000 generations were deleted as the burn-in of the chain. A majority-rule consensus tree with average branch lengths was constructed for the remaining trees using the sumt option of MrBayes. Phylogenetic trees were visualized using FigTree v1.2.3 (Rambaut 2009).

Branches support values were considered significant when MP bootstrap (MPBS) > 75%, ML bootstrap (MLBS) > 75% and Bayesian posterior probabilities (PP) > 95%.

RESULTS

For both matrices, the General Time Reversible substitution model accommodating a proportion of

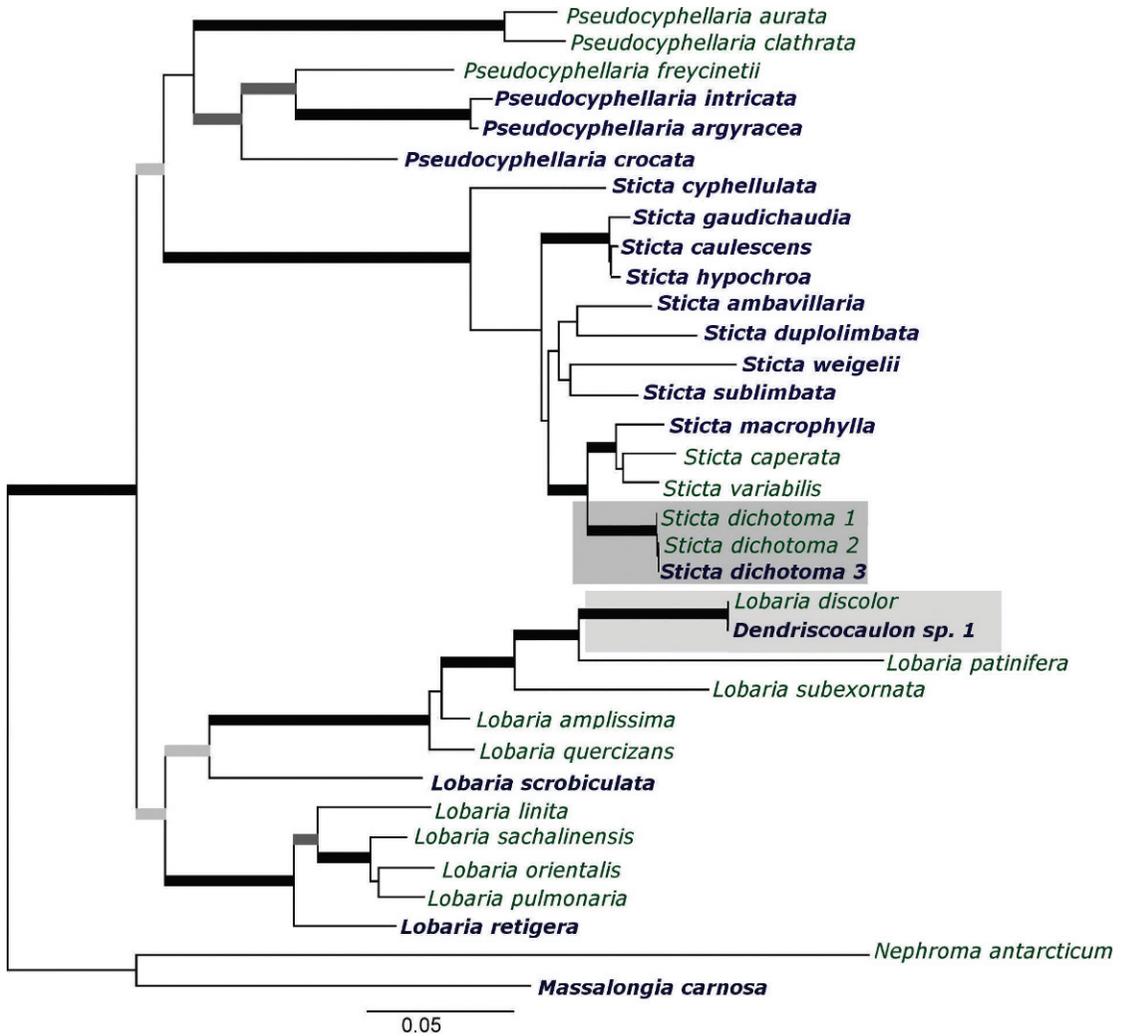


Figure 2. Most likely phylogenetic tree of the Lobariaceae, including the two photomorphs found in Reunion (*Lobaria discolor* and its free-living *Dendriscoaulon*-like cyanomorph; *Sticta dichotoma*, 1 free-living chloromorph, 2 chloromorph attached to cyanomorph, 3 cyanomorph attached to chloromorph). Concatenated matrix with nLSU, ITS and mtSSU sequences. Black branches are characterized by bootstrap percentages (MPBS and MLBS) above 75% and posterior probabilities (PP) above 0.95; dark grey branches have MLBS > 75% and PP > 0.95 but MLBS < 75%; and light grey branches have PP > 0.95 but MPBS MLBS < 75%. Taxa in bold blue are associated with *Nostoc* as the main photobiont while taxa not bold but in green are associated with a green alga.

invariant sites and a rate heterogeneity distributed with a parameter gamma among six categories “GTR + I + Γ” (Rodríguez et al. 1990) was selected.

Matrix 1 was composed of nuLSU, ITS and mtSSU sequences for 34 exemplars of the Lobariaceae. Of the 2825 characters, 781 characters were excluded, 1415 constant and 402 potentially parsimony-informative. Under MP analysis, 35 equally parsimonious trees were retained, with a tree length of 1774 steps, CI = 0.526 and RI = 0.661. The

single most likely tree had a likelihood score of -11510.547757.

Matrix 2 was composed of ITS sequences for 32 exemplars of Lobariaceae comprising all available *Dendriscoaulon*-like cyanomorphs sequences, and included 664 characters, of which 289 were excluded, 229 were constant and 99 were potentially parsimony-informative. Under MP analysis, 34 equally parsimonious trees were retained, with a tree length of 337 steps, CI = 0.562 and RI = 0.766. The

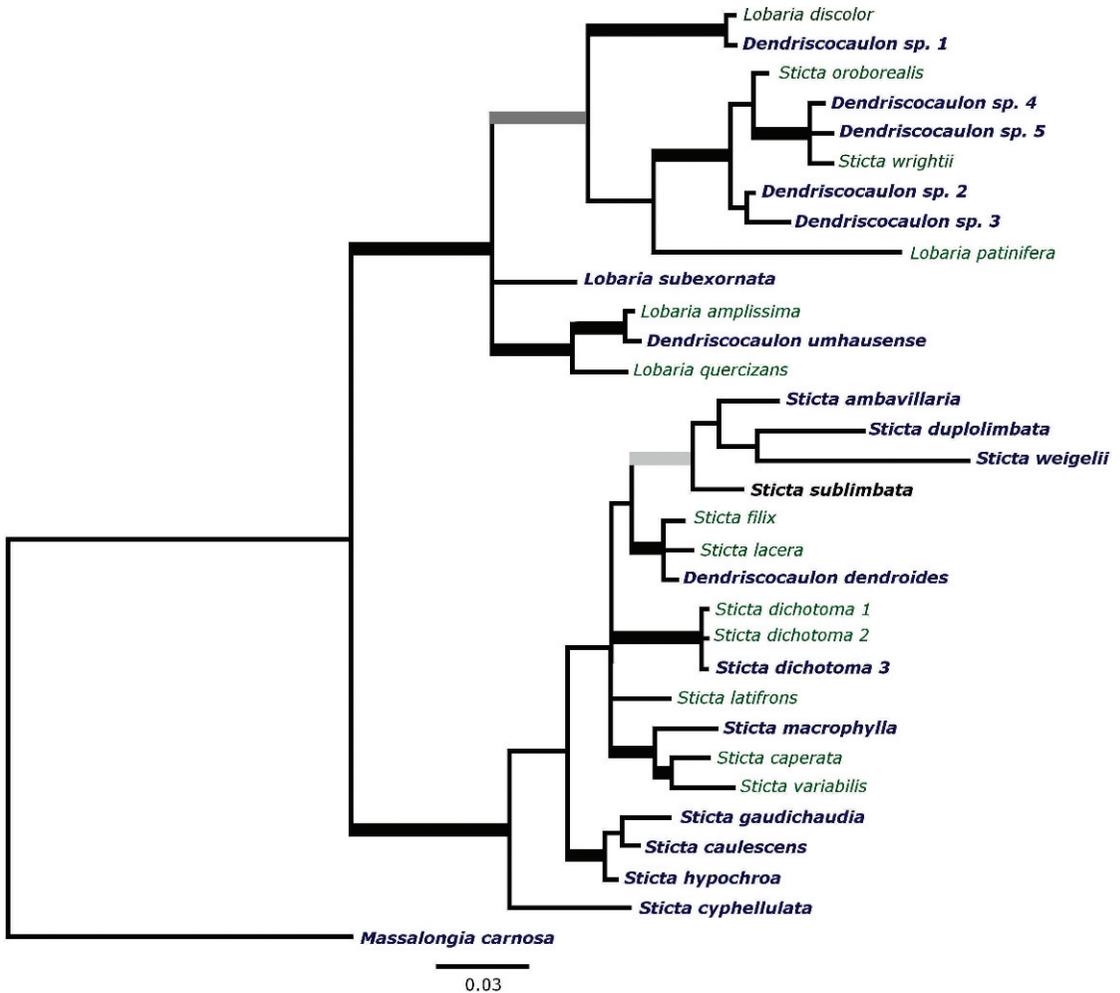


Figure 3. 50% consensus-tree of the Bayesian analysis of selected species in the genera *Lobaria* and *Sticta* including all available accessions of *Dendriscoaulon*-like cyanomorphs. Matrix with ITS sequences only. Black branches are characterized by bootstrap percentages (MPBS and MLBS) above 75% and posterior probabilities (PP) above 0.95; dark grey branches have MLBS > 75% and PP > 0.95 but MLBS < 75%; and light grey branches have PP > 0.95 but MPBS and MLBS < 75%. Taxa in bold blue are associated with *Nostoc* as the main photobiont while taxa not bold but in green are associated with a green alga.

single most likely tree had a likelihood score of -2329.674464 .

The phylogenetic inference within the Lobariaceae (Fig. 2) suggests a) that *Lobaria* is composed of two clades (i.e., the strongly supported *pulmonaria* group and a clade comprising *L. scrobiculata* sister to the strongly supported *L. amplissima* group), b) that the monophyly of *Pseudocyphellaria* comprising the robust *aurata* and *argyrea* groups and *P. crocata*, is unsupported, and c) that *Sticta* is a strongly supported but poorly resolved genus. Within this phylogenetic scenario,

the sample of *Dendriscoaulon* from Reunion is resolved as a member of *Lobaria*. Furthermore, the sequences of three loci obtained for this sample are identical to their homologous sequences in chlorolichen *L. discolor*. Similarly the genotypes of the mycobiont in the attached foliose cyano- and chloromorphs are identical. They differ only by a single substitution in ITS2 from the mycobiont of an independent chloromorph of *S. dichotoma*, collected in a different locality on the island.

Inferences from ITS loci (Fig. 3) retrieved two strongly supported groups: a) one comprising all

accessions of *Sticta* (except *S. oroborealis* and *S. wrightii*) and *Dendriscoaulon dendroides* which is resolved within a strongly supported group with *S. filix* and *S. lacera*; and b) one composed of all accessions of *Lobaria* and all other accessions of *Dendriscoaulon*. Within the latter *D. umhausense* shares a common ancestor with *L. amplissima*, the exemplar from Reunion is sister to *L. discolor*, and all samples compose a strongly supported group with *Sticta oroborealis* and *S. wrightii*. *Dendriscoaulon* 4 & 5 share a common ancestor with *S. wrightii* but their relationships remain ambiguous, whereas samples 2 & 3 are sister and potentially closely related to *S. oroborealis*.

DISCUSSION

The species producing photomorphs in Reunion.

Lobaria discolor (Fig. 1C) is a pantropical species (Joshi & Awasthi 1982; Yoshimura 1991, 1998), described from Reunion (Delise 1825) where it is common in montane forests ("Bois de couleurs des Hauts"; Doumenge & Renard 1989). Throughout its range, it is quite variable as reflected by the numerous varieties and forms recognized by Yoshimura (1971); the taxonomic status of these taxa has never been assessed within a phylogenetic framework. The species was only known to associate with a green alga as primary photobiont. Its cyanomorph, reported for the first time, was found free-living in a heavily disturbed forest at c. 650 m elev. on the SE part of the island, where the green photomorph has not been seen. It is a typical *Dendriscoaulon* morph with a bushy habit, numerous arbusculoid and coralloid bluish-grey secondary branches, and attached to the substrate by a single conspicuous pale orange, terete or more typically slightly flattened rooting stalk (Fig. 1D).

Sticta dichotoma Delise (Figs. 1A & B) has also been described from Reunion (Delise 1825) and its distribution range seems to be restricted to the western parts of the Indian Ocean: Reunion (where it is locally very abundant), Madagascar (no data published are available but a picture of a collection from Madagascar is available at <http://www.tropicallichens.net/3127.html>) and a single locality in NE Tanzania (Krog 2000). The chloromorph is almost exclusively present on Reunion and the

assemblage of its chloromorph developing over its cyanomorph has been detected only once. The thallus developed by either photobiont is similar, although the cyanomorph has more irregularly dichotomous lobes with a maculate upper surface versus regularly dichotomous lobes and a smooth shiny upper surface in the chloromorph.

Phylogenetic distribution of Dendriscoaulon.

The Lobariaceae are one of nine families composing the Peltigerales (Wedin et al. 2007, 2011), and include three genera of large foliose lichen forming fungi: *Lobaria*, *Pseudocyphellaria* and *Sticta*. These genera are defined by anatomical features of their lower surface, namely the presence of cyphellae (*Sticta*), pseudocyphellae (*Pseudocyphellaria*) or neither (*Lobaria*). Such straightforward morphological diagnosis has been widely adopted but recent phylogenetic inferences from sequence data reveal that neither cyphellae nor pseudocyphellae are derived characters that arose once and were retained by all descendant species (synapomorphies). Indeed, all three genera as currently defined, are polyphyletic: *Pseudocyphellaria anomala* Brodo & Ahti, *P. anthraspis* (Ach.) H. Magn. and *P. rainierensis* Imsh. are included in *Lobaria* (Högnabba et al. 2009), and *Sticta oroborealis* Goward & Tønsberg and *S. wrightii* Tuck., two *Sticta* species known to develop *Dendriscoaulon* cyanomorphs (Takahashi et al. 2006; Tønsberg & Goward 2001), are also resolved within *Lobaria*. Furthermore, the monophyly of *Lobaria* and *Pseudocyphellaria* as currently circumscribed is supported only by Bayesian analysis (Fig. 2).

The genus *Lobaria* has been split into several genera (Yoshimura 1998, 2002): *Lobarina* (Vain.) Cromb. for *L. scrobiculata*, *Lobariella* Yoshim. (= *Lobaria* subgenus *Durietzia* C. W. Dodge) for the *L. crenulata* group, all remaining species belonging to *Lobaria* (Schreb.) Hoffm. (type species: *L. pulmonaria*). *Lobarina* was segregated on the basis of denuded portions of the thallus lower surface made of periclinal hyphae (versus sclero- or paraplectenchymatous lower cortex for all other species of *Lobaria* s. l.), whereas *Lobariella* was diagnosed by the production of pseudocyphellae on the upper surface of the thallus (versus absent in all other species). Phylogenetic inference (Högnabba et al. 2009) suggests, however, that species of *Lobaria* compose two well-supported

clades: the *L. amplissima* group with the two species of *Lobariella* (*L. crenulata* and *L. subexornata*) forming a robust group nested in it, and the *L. pulmonaria* group including *L. scrobiculata*. Our results reveal a similar pattern except for *L. scrobiculata*, resolved as sister to the *amplissima* group. All phylogenetic reconstructions converge on all species producing *Dendriscoaulon*-like cyanomorphs, including *Sticta oroborealis* Goward & Tønsberg and *S. wrightii* Tuck., being resolved in a single clade (the *Lobaria amplissima* group), except for *D. dendroides*, which is unambiguously resolved in *Sticta* (Fig. 2).

The phylogenetic affinities of *Sticta oroborealis* and *S. wrightii* within the *Lobaria* clade is inconsistent with the current morphological definition of these genera, since *Lobaria* lacks cyphellae, a diagnostic feature of *Sticta*, on its lower surface. The phylogenetic significance of cyphellae is further questioned by the resolution of three species of *Pseudocyphellaria* (i.e., *P. anomala*, *P. anthraspis* and *P. rainierensis*), a genus defined by pseudocyphellae on its lower surface, within *Lobaria*, a genus lacking pseudocyphellae on its lower surface. Furthermore, pseudocyphellae on the upper surface are used as a diagnostic character for the segregation of *Lobariella* whereas they occur in several species of *Pseudocyphellaria*, e.g. the *P. argyrea* group (Swinscow & Krog 1988). Whether cyphellae and pseudocyphellae on either thallus surface have been acquired independently in multiple lineages or repeatedly lost during the diversification of the Lobariaceae remains to be critically tested.

Morphological classification of the photomorphs. James and Henssen (1976) recognize four photomorphs morphotypes in their seminal paper on “cephalodia”.

- A. *Dendriscoaulon*-like cyanomorph attached by a conspicuous rooting stalk, with chloromorph developing dorsiventral lobes;
- B. dorsiventral thallus as the chloromorph and attached or free-living *Dendriscoaulon*-like cyanomorph;
- C. as the former but further with chloromorph developing dorsiventral lobes on the *Dendriscoaulon*;
- D. dorsiventral thallus as cyanomorph and chloromorph developing over it and also forming a dorsiventral thallus.

All four types occur in the Lobariaceae. Type (A) occurs in *Sticta* s. str. with *Dendriscoaulon dendroides*, an Australasian, free-living cyanomorph,

sometimes found attached to species of *Sticta* belonging to the *latifrons-filix* group (Galloway 2007). It is also found in the *Lobaria amplissima* group, with species such as *Sticta oroborealis* and *S. wrightii*. Type (B) belongs to the *L. amplissima* group, and the identity of fungi in both morphs has been demonstrated on molecular basis for *L. amplissima* (Stenroos et al. 2003) and here for *L. discolor*. Other cases mentioned by James and Henssen (1976), such as *L. ornata* or *S. glomulifera* have not been further documented. Type (C) could be considered as a more morphologically complex variant of the former, with the chloromorph growing over the cyanomorph, which is growing on the chloromorph. James and Henssen (1976) mentioned two examples, one with *L. amplissima* and the other with *L. cf. erosa*. Foliose cyanomorphs (Type D) are best known within *Nephroma* and *Peltigera* (Goffinet & Bayer 1997; Tønsberg & Holtan-Hartwig 1983; White & James 1988) [see illustration of *P. britannica* (Gyeln.) Holtan-Hartwig & Tønsberg in Brodo et al. (2001)]. In such cases, both morphs develop rather similar thalli, except that the upper surface is most usually rather dull and typically maculate (white-marbled) in the cyanomorph and mostly shiny and smooth in the chloromorph.

Type (D) is indeed rare in the Lobariaceae, but is hereby shown to characterize *S. dichotoma* on Reunion, and is otherwise well documented for *Sticta canariensis* (Flörke) Delise for which the cyanomorph was originally distinguished and named *S. dufourii* Delise. The latter develops a margin minutely dissected in small, terete to coralloid isidia compared to the entire margin of the chloromorph. In another case within *Sticta* (unidentified species in Brazil: Sanders, 2001), both photomorphs develop similar thalli, except for a dull and maculate surface in the cyanomorph and a smooth and shiny one for the chloromorph. However, the composite thalli could not be identified to species level and no molecular analysis could be conducted to support that a single fungus was involved in the assemblage. A further case is hereby reported with *S. dichotoma*, for which the fungus is identical in both photomorphs as demonstrated by their strictly identical sequences for the three loci studied here. Phylogenetic inferences demonstrate that none of these morphotypes represent an autapomorphic character within the

Lobariaceae. However, as long as the relationships within the whole family are not fully resolved, the evolution of such characters as those offered by the photomorphs cannot be assessed.

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