



High diversity, high insular endemism and recent origin in the lichen genus *Sticta* (lichenized Ascomycota, Peltigerales) in Madagascar and the Mascarenes

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ARTICLE INFO

Keywords:

Biogeography
Indian Ocean
Lobariaceae
Photomorph
Radiation

ABSTRACT

Lichen biodiversity and its generative evolutionary processes are practically unknown in the MIOI (Madagascar and Indian Ocean Islands) biodiversity hotspot. We sought to test the hypothesis that lichenized fungi in this region have undergone a rapid radiation, following a single colonization event, giving rise to narrow endemics, as is characteristic of other lineages of plants. We extensively sampled specimens of the lichen genus *Sticta* in the Mascarene archipelago (mainly Réunion) and in Madagascar, mainly in the northern range (Amber Mt and Marojej Mt) and produced the fungal ITS barcode sequence for 148 thalli. We further produced a four-loci data matrix for 68 of them, representing the diversity and geographical distribution of ITS haplotypes. We reconstructed the phylogenetic relationships within this group, established species boundaries with morphological context, and estimated the date of the most recent common ancestor. Our inferences resolve a robust clade comprising 31 endemic species of *Sticta* that arose from the diversification following a single recent (c. 11 Mya) colonization event. All but three species have a very restricted range, endemic to either the Mascarene archipelago or a single massif in Madagascar. The first genus of lichens to be studied with molecular data in this region underwent a recent radiation, exhibits micro-endemism, and thus exemplifies the biodiversity characteristics found in other taxa in Madagascar and the Mascarenes.

1. Introduction

Lichens are formed by the symbiotic associations between a fungus and at least one photosynthetic organism (a green alga and/or a cyanobacterium). About one fifth of all described species of fungi acquire the carbon they need by means of lichenization (i.e., c. 19,000 species; Lücking et al., 2016), which highlights the remarkable evolutionary success of this nutritional strategy (Nash 2008; Lücking et al., 2009; Gueidan et al., 2015). Furthermore, the diversity of lichenized fungi may be vastly underestimated as phylogenetic inferences from molecular data often reveal a robust phylogenetic structure based on putative specific molecular barcodes suggesting that variation in the interspecific morphological traits among lichen thalli may be a poor (Pinos-Bodas et al., 2013, 2015; Mark et al., 2016) or only a conservative predictor of the diversity of the lichenized mycobionts (Moncada et al., 2014; Magain and Sérusiaux, 2015; Magain et al., 2017a, 2017b) and that the morphological space must be explored de novo to enable the diagnosis of the putative cryptic species (e.g., Magain and Sérusiaux,

2015).

The diversity of lichenized fungi is known to be concentrated in scattered regions of the world (Petersen and McCune, 2003; Hodkinson, 2010; Green et al., 2011; Lendemer et al., 2016), and many field lichenologists suspect that additional hotspots may occur, especially in tropical and warm-temperate biomes. Madagascar and the nearby Mascarene archipelago (Mauritius, Réunion and Rodrigues) 640–1,450 km East of it, could be one of them, as this area is a hotspot for any segment of biodiversity and hence integral to the development of world conservation strategies (Myers et al., 2000; Goodman and Benstead, 2003; Cheke and Hume, 2008).

Mauritius, Réunion and Rodrigues are three relatively recent volcanic islands, whereas Madagascar is a geologically diverse assemblage of Gondwanan fragments. Madagascar is composed of plutonic and metamorphic rocks dating back to the Precambrian, volcanic rocks from the Mesozoic to Cenozoic, and sedimentary rocks of the Carboniferous to Quaternary age in the Western parts. This island has been isolated from other landmasses for at least 88 Myrs (Gibbon et al., 2013;

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Ganzhorn et al., 2014). Mauritius emerged between 8 and 10 Myrs ago, followed by Réunion and Rodrigues, which are estimated to be 2–5 and 1.5 Myrs old, respectively (McDougall and Chamalaun, 1969; Bonneville et al., 1988), although recent evidence suggests that Rodrigues is much older (Strijk et al., 2012).

Madagascar and the Mascarene archipelago (the so-called MIOI hotspot for Madagascar and Indian Ocean Islands; Strijk et al., 2012) are world-renowned for their remarkably high rate of endemism (Davis et al., 1994; Myers et al., 2000; Losos and Ricklefs, 2009) notably in angiosperms with 90% endemism in Madagascar (Philippson et al., 2006) and 100% endemism in flagship groups such as amphibians and lemurs (Goodmann and Benstead, 2005). An impressive set of unique taxa is concentrated in this region due to active radiation following colonization events from various sources, including continental Africa, Asia, Australasia and South America (Yoder and Nowak, 2006; Warren et al., 2010; Janssens et al., 2016). Extraordinary migrations “defying biogeography” have also been demonstrated, such as the very recent (< 1.4 Myrs) colonization of Réunion by a tree species of *Acacia*, formerly considered to be endemic to the Hawaiian archipelago and now shown to have a unique distribution composed of two archipelagos approximately 18,000 km apart (Le Roux et al., 2014).

Long distance dispersal occurring after the isolation of the main island (Madagascar) followed by local diversification and radiation is the main evolutionary scenario supported by molecular inferences for explaining the exceptional level of endemism in the MIOI hotspot, but it is not the only conjecture. Vicariance distribution patterns, dating back to the disintegration of Gondwana, are rarer (Strijk et al., 2014), but unambiguously supported by detailed phylogenetical and biogeographical studies (Pirie et al., 2015; Toussaint et al., 2016). The estimated age of *Takhtajania perrieri* (Winteraceae), the most famous plant endemic to NE Madagascar, and its resolution as sister to all other species within this family, distributed across the continents and islands fringing the southern half of the Pacific Ocean, is consistent with the origin of the family in Madagascar and dispersal throughout Gondwana before it split apart (Thomas et al., 2014). A Gondwanan vicariance is thus the scenario retained for explaining the occurrence of this unique genus and species in Madagascar. In another case, the eyeless teleost fishes belonging to the genus *Thyphleotris*, endemic to subterranean karst habitats in southwestern Madagascar, form the sister group to *Milyeringa*, endemic to similar subterranean systems in northwestern Australia. These two taxa likely arose from isolation following the breakup of Gondwana at the end of the Cretaceous period (Chakrabarty et al., 2012).

Diversification patterns and processes for usually neglected taxonomic groups, such as lichenized fungi, are obscure in general, and especially for this part of the world. The lichen flora of these islands is poorly known (van den Boom et al., 2011; Aptroot, 2016), and each detailed study can unveil surprising diversity (e.g. for the genus *Micarea*; Brand et al., 2014), even in families of conspicuous species such as the Parmeliaceae (Masson et al., 2015a, 2015b). As for all biodiversity components in Madagascar, the lichen flora is undoubtedly endangered: a dramatic example is the type locality of the spectacular monospecific genus *Savoronala* (Ertz et al., 2014), discovered in 2008 in the coastal areas of SE Madagascar, which is now considered extinct due to the Rio Tinto QMM ilmenite mining of the dunes where it was found (Google Earth, date of the images: 2/13/2016; see <http://www.ejolt.org/2013/11/rio-tinto-compensation-manipulation-in-southeast-madagascar/>).

Evolutionary radiations have intrigued biologists for more than a century, and our understanding of the patterns and processes associated with these radiations continues to grow and evolve (Simões et al., 2016). Quite surprisingly, species radiations within lichen taxa have seldom been investigated, with only recent studies explicitly dealing with such diversification patterns (Lücking et al., 2005, 2014; Lumbsch et al., 2008; Sérusiaux et al., 2011; de Paz et al., 2012; del Prado et al., 2013; Leavitt et al., 2011, 2012a; 2012b, 2012c, 2013a, 2013b; Gaya

et al., 2015; Divakar et al., 2012; Kraichak et al., 2015a, 2015b; Magain et al., 2017a). The lack of interest of lichenologists for species radiation patterns might be a consequence of the long-standing paradigm that lichen species are mostly widespread – the so-called paradigm “Everything small is everywhere” (Fontaneto, 2011) – and the only biogeographical subdivision demonstrated at world level for these organisms is between a Gondwanan and a Laurasian element (Arcadia, 2013), with few endemic elements, usually assumed to be the result of a slow rate of evolution (Jørgensen, 1983; Karnefelt, 1990). The world-wide analysis of the lichen diversity by Feuerer and Hawksworth (2006) did not point to any putative radiations with or without long-distance dispersal. These authors, however, highlighted species with perplexing distributions and suggested that long-distance dispersal may explain such disjoint distribution patterns.

In the framework of a project on the global phylogeny of the diverse and conspicuous lichen genus *Sticta* (Schreber) Ach. (Moncada et al., 2014), populations of this genus were extensively sampled on the islands of Réunion and Mauritius, as well as in several parts of Madagascar (mainly in two National Parks in the NW part of the island: Amber Mountain and Marojejy). *Sticta* is an important component of humid forest ecosystems world-wide, contributing to nutrient and water cycles (Zotz et al., 1998), and is a sensitive indicator of human-induced disturbance and global change (Ramírez-Morán et al., 2016). *Sticta* species generally display a leafy appearance and are characterized by the presence of small circular pits on the lower thallus surface (i.e., “cyphellae”), a characteristic fishy scent when wet and associated with cyanobacteria (caused by the production of trimethylamine), and production of metabolites rarely soluble in acetone (Galloway, 1997). Three types of symbiotic associations can be distinguished within the genus: (1) the fungus is only associated with a cyanobacterium; (2) the fungus is only associated with a coccoid green alga; (3) the fungus is associated with a green alga or a cyanobacterium to produce two distinct forms, i.e., the so-called photomorphs, that can grow independently or form composite thalli (Armaleo and Clerc, 1991; Goffinet and Bayer, 1997; Heidmarsson et al., 1997; Magain et al., 2012). The genus has a subcosmopolitan distribution with centers of distribution in the tropical montane forests, especially in the Páramo ecosystem biome in the Andes (Moncada et al., 2014). *Sticta* species within the region under consideration here (MIOI) mostly occur in montane forests, which are roughly delimited by the 11 and 15 °C mean annual isotherms (Fig. 1A). In the MIOI region, five endemic species are currently recognized within the genus: *S. macrophylla* Delise, and *S. plumbea* Delise, which are associated with a cyanobacterium; *S. variabilis* Ach., which is associated with a green alga; *S. dichotoma* (Bory) Delise, and *Sticta caperata* (Nyl.) Nyl., which can associate with both kinds of photosynthetic partners.

Here we assess the diversity of the genus *Sticta* in the MIOI based on an extensive sample of populations from all islands, using a coalescent-based species delimitation method based on inferences from molecular data complemented by information from morphological and biological traits. Using multiple molecular markers and appropriate statistical methods in a phylogenetic context, we infer the spatial and temporal patterns of colonization and speciation at the scale of the MIOI hotspot. The specific aims of this study were to address: (1) the taxonomic diversity and distribution patterns of MIOI *Sticta*, (2) the phylogenetic relationships between these species, and (3) the age and possible origin of this group.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

We extensively sampled collections made within the Mascarene Islands since 2008, except for the island of Rodrigues. On Madagascar, *Sticta* populations were sampled in the region of Ambalamanakana, in October 2008 by D. Ertz, E. Fischer, D. Killmann, and E. Sérusiaux, and

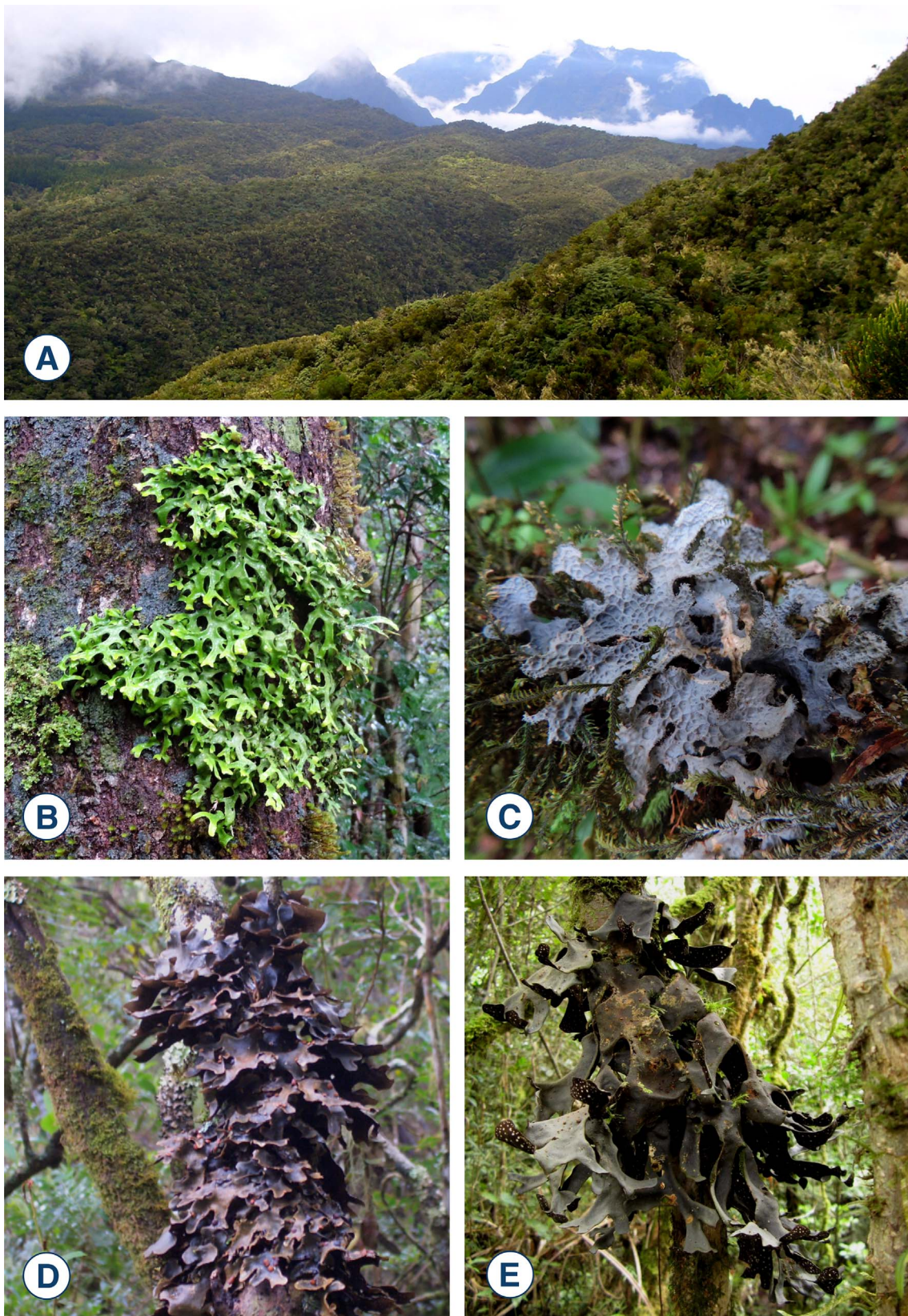


Fig. 1. Representatives of MIOI *Sticta* species and their habitat. (A) Mountain rainforest in Réunion. (B) *Sticta* sp. 20. (C) *Sticta* sp. 23. (D) *S. macrophylla*. (E) *Sticta* sp. 22. Photographs taken in the field by E.Sérusiaux (A–D) and B. Goffinet (E).

in October 2014 in two National Parks, i.e., Marojejy and Amber Mountain, by D. Ertz, E. Fischer, B. Goffinet, and E. Sérusiaux. Samples from Réunion were collected in June 2008 by M. Brand, E. Sérusiaux, and P. van den Boom, in November 2009 by N. Magain and E. Sérusiaux, and in July 2013 by E. Sérusiaux. The diversity on Mauritius was sampled by E. Sérusiaux in 2013. In all localities, the sampling was extensive but parsimonious. Identification of these collections was based on van den Boom et al. (2011) and Swinscow & Krog (1988). Two specimens of *Sticta ainoae* collected in January 2015 by B. Goffinet in Southern Chile were included given their morphological similarities to specimens of *Sticta* from the Mascarene Islands. For the same reason, we also included one specimen of *S. stipitata* collected in 2010 by G. Kantvilas in Tasmania (Australia). Only recent collections of *Sticta* could be included in the phylogenetic study, as DNA extraction from specimens older than two years typically failed to yield DNA suitable for amplification.

Well-preserved specimens without any visible symptoms of parasitism were selected for DNA extraction. DNA was isolated either using Nucleospin Plant II Midi kits (Macherey-Nagel, Bethlehem, Pennsylvania, USA) following the manufacturer's guidelines, or following the protocol by Cubero et al. (1999). The internal transcribed spacer (ITS), which has been adopted as a universal barcode for fungi (Schoch et al., 2012), was amplified and sequenced using primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). The PCR conditions were as follows: 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min, 50 °C for 1 min, and 70 °C for 1 min, with a final extension of 70 °C for 10 min. We sampled all morphotypes seen for the genus *Sticta* assumed to belong to a regional lineage in the MIOI and produced ITS sequences for 151 collections (Supplemental Table S1).

In order to obtain an optimal phylogenetic resolution at all levels of this putative clade and to evaluate its relationships within the genus, we amplified three additional markers for 68 selected specimens representing the haplotype diversity in ITS sequences. For each ITS haplotype, we included accessions from all islands where it was detected. Therefore, the geographical range of each haplotype is also represented in the dataset (Supplemental Table S1). We amplified the 5' portion of the ribosomal nuclear locus LSU with primers LR0R (Vilgalys and Hester, 1990) and either LR7 (Vilgalys and Hester, 1990) or LIC2044 (Kauff and Lutzoni, 2002), the mitochondrial ribosomal locus mtSSU, using primers SSU1 and SSU3R (Zoller et al., 1999), and a portion of the protein-coding gene *RPB1* with primers RPB1STF (5'-GCADCAGCCTDAGCTGACA-3') and RPB1STR (5'-GGCGTAAGTC-WATCCTCTC-3'). The PCR conditions for the locus LSU were identical to those used for the marker ITS. Amplification of mtSSU was run with the following conditions: 94 °C for 5 min, followed by 37 cycles of 95 °C for 1 min, 54 °C for 50 s, and 72 °C for 1 min, with a final extension of 70 °C for 8 min. Amplification of *RPB1* was run with the following conditions: and 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 70 °C for 1 min.

PCR reactions were performed using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA) and following the manufacturer's guidelines. The quality and size of the amplicons were checked by visual inspection on a 1% w/v agarose gel stained by SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Amplicons were sequenced by Macrogen, Inc. (Seoul, South Korea). The sequences obtained were edited using Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

2.2. Phylogenetic analyses

In order to test the monophyly of the presumed clade, we expanded the four-loci dataset by adding a worldwide set of representatives of 22 species of *Sticta*, including those which are morphologically very similar to some MIOI species, such as *S. canariensis* (a species producing photomorphs), and conspicuous green Australasian and Patagonian species. These species are representative of all major lineages of *Sticta*

and the four aforementioned loci were available for most of them (Högnabba et al., 2009; Magain and Sérusiaux, 2015). Four species belonging to the presumably local endemic lineage and derived from previous phylogenetic studies (Magain et al., 2012; Magain and Sérusiaux, 2015) were also added to the dataset. *Pseudocyphellaria citrina* (Gyeln.) Lücking, Moncada & S.Stenroos (= *crocata* auct. europ.; see Lücking et al., 2017a) isolate LG 688, collected in La Gomera (Canary Islands) was used as an outgroup, based on the topology of the Lobariaceae recently published by Moncada et al. (2013a, 2013b). For each single-locus dataset, DNA sequences were first aligned using MAFFT run in "auto" mode (Katoh et al., 2002, 2009) and eventually manually adjusted using Geneious 7.1.3 (Biomatters Ltd., Auckland, New Zealand). Ambiguous regions were excluded from the analyses manually. Congruence of the four fungal loci was assessed by comparing single-locus phylogenetic trees produced with RAXML v.8.2.3 (Stamatakis, 2006; Stamatakis et al., 2008) as implemented on the CIPRES portal (Miller et al., 2010; www.phylo.org), searching for the best ML tree and bootstrapping with 1000 pseudoreplicates in the same run, using GTR+G model and the default settings. No significant conflict with bootstrap values $\geq 70\%$ was detected. A maximum likelihood analysis was also performed using RAXML v.8.2.3 on the CIPRES Science Gateway using the rapid hill-climbing algorithm and bootstrapping with 1000 pseudoreplicates under a GTR+G model of evolution for each partitioned subset (ITS, LSU, mtSSU, *RPB1*). A Bayesian analysis was performed using MrBayes v.3.2.6 (Ronquist et al., 2003) with model partitioning on the CIPRES Science Gateway. The corrected Akaike Information Criterion (AIC; Akaike 1974; Posada and Buckley, 2004) in jModelTest 2.1.6 (Darriba et al., 2012) was used to select the model of sequence evolution for the Bayesian analysis of each single-locus dataset, and run on the same gateway (ITS: GTR+I+G; LSU: GTR+I+G; mtSSU: GTR+I+G; *RPB1*: GTR+G). Two separate runs were performed with four chains running for 10^6 generations, sampling every 100th generation and discarding the first 25% of the trees as burn-in period. Convergence, mixing, and effective sample sizes (ESS) of parameters were checked in Tracer 1.6 (Rambaut and Drummond, 2007). A majority rule consensus species tree was built for the 75,000 remaining trees, and Posterior Probabilities (PP) were inferred from this tree. The results of the Bayesian and maximum likelihood analyses were visualized with FigTree version 1.4.2 (Rambaut, 2012). The combined matrix and the resulting trees have been deposited in TreeBASE (Accession No. S20682).

We further inferred the maximum likelihood tree for the ITS complete dataset (154 accessions, plus *S. hypochra* and *S. gaudichaldia* as outgroups) under the GTR+I+G model (based on the AIC) with Garli v. 2.01 (Zwickl, 2006); branch support was estimated from 250 bootstrap pseudoreplicates.

A significant conflict was detected within the single clade including *Sticta* spp. 6, 7, 8 and 9, endemic to Madagascar as the topologies inferred from ITS only versus four loci are not congruent. Further, an insertion of seven bp in the internal transcribed spacer 2 (ITS2) region is restricted to all accessions from Amber Mt belonging to that clade; because of ambiguous alignments, this indel was excluded from all analyses. To further analyze the genealogical relationships among the haplotypes resolved within the two clades including all but two species new to science found in Madagascar (clades B and C in Fig. 2), we constructed an ITS haplotype network using the program *rcs* version 1.21 (Clement et al., 2000). This software implements the statistical parsimony estimation as described by Templeton et al. (1992). Connection limit of the networks was not fixed and gaps were treated as missing character state.

2.3. Species delimitation analysis

We used the General Mixed Yule Coalescent model (GMYC; Pons et al., 2006) to generate species hypotheses. We applied this method to the four-loci ML tree from which we excised the clade of interest (the

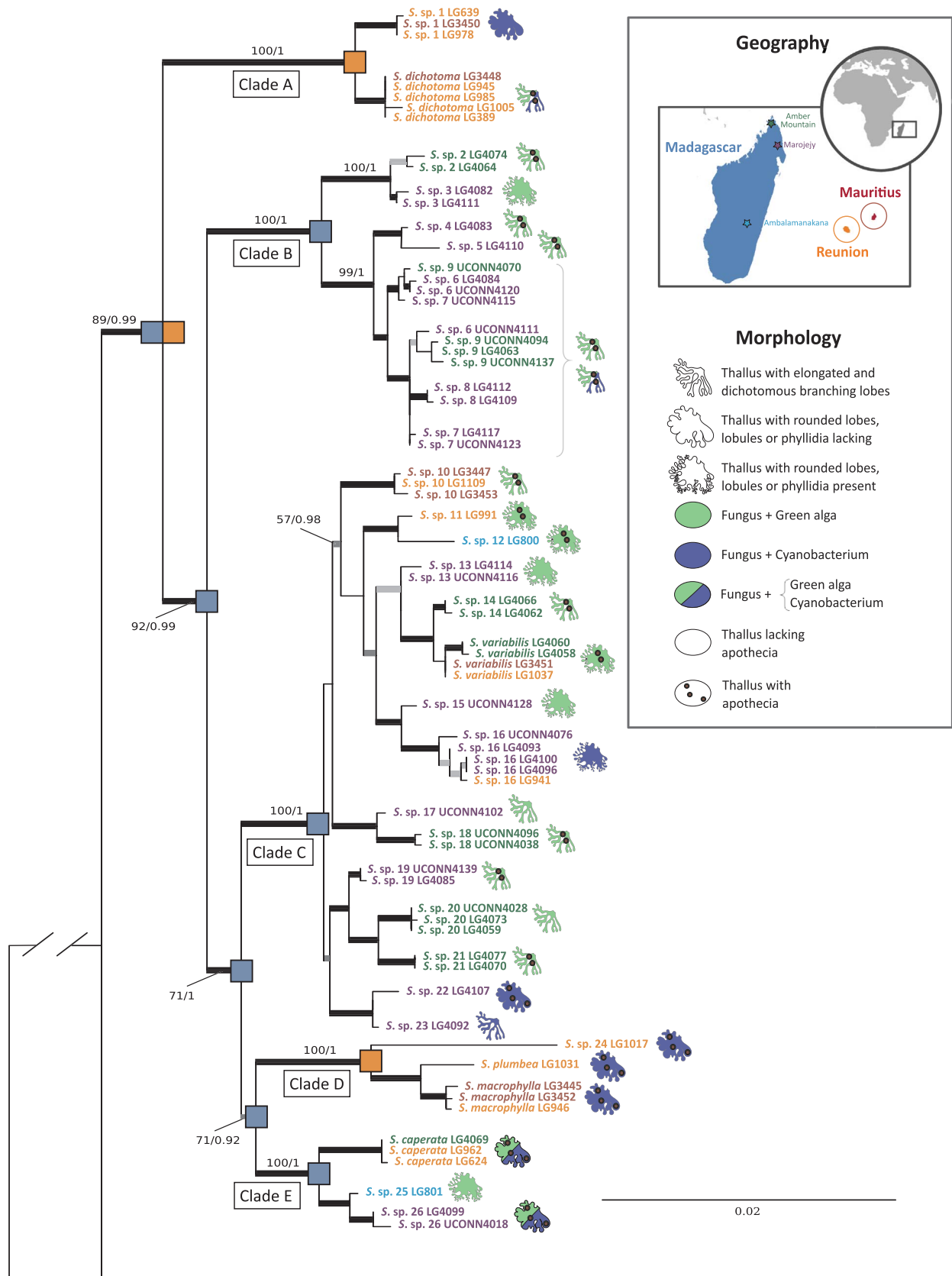


Fig. 2. Single most likely phylogenetic tree of the genus *Sticta* obtained from inferences of four-loci molecular data (ITS, nuLSU, mtSSU, and *RPB1*) and ancestral area reconstruction for species in the MIOI *Sticta* lineage. DNA accession numbers are indicated for the MIOI species. Values above branches represent ML bootstrap and Bayesian PP values, respectively. Thick black branches have BS ≥ 70 and Bayesian PP ≥ 0.95 , dark grey branches have BS < 70 but PP ≥ 0.95 and light grey branches have PP < 0.95 but BS ≥ 70 . Colors in the taxa names represent the origin of the samples. Colored squares at nodes indicate the most likely ancestral area reconstructed using the DIVALIKE + J model. Schematic thallus drawings (right to taxa labels) show the main morphological features of the MIOI species.

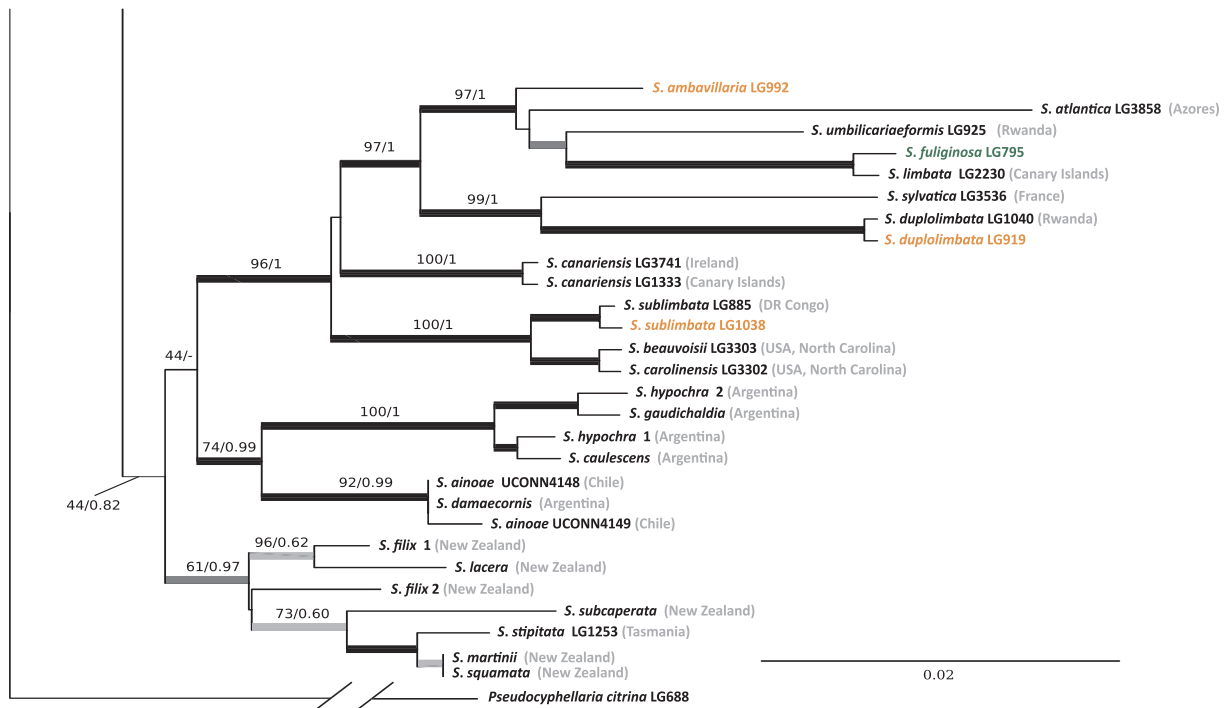


Fig. 2. (continued)

monophyletic group formed only by species from MIOI) and thus included 68 accessions. The tree was made ultrametric by the *chronos* function implemented in the R package Ape (Paradis et al., 2004), assuming a clock-like model. The GMYC analysis was performed on the Exelixis Lab web server (<http://species.h-its.org/gmyc/>), employing the single threshold method.

For each specimen included in this study, several morphological and biological traits were examined and compiled in order to highlight diagnostic characters for each putative species, and to evaluate their distribution throughout the clade (Supplemental Table S1). Three traits were projected onto the phylogenetic tree (Fig. 2): (1) type of photosynthetic partner (coccoid green alga, or cyanobacterium, or both); (2) thallus fertility (thallus lacking apothecia, thallus with apothecia); and (3) general lobe configuration (thallus with elongated and dichotomous branching lobes, thallus with rounded lobes and without lobules or phyllidia, thallus with rounded lobes and lobules or phyllidia). In the case of intermediate morphology for the latter trait, the character “thallus with elongated and dichotomous branching lobes” has been given priority.

2.4. Dating analysis

We inferred the age of the most recent common ancestor (MRCA) of the MIOI species by using a secondary calibration strategy. We constructed a three-loci dataset (LSU, mtSSU, *RPB1*) including 169 specimens from across most major clades within the Peltigerales, 22 specimens of the Lecideales as the outgroup, following the most recent phylogenetic tree of the Lecanoromycetes (Miadlikowska et al., 2014), as well as 30 specimens belonging to the *Stictis* clade of interest (Supplemental Table S2). The number of specimens within the MIOI clade was reduced in order to use an inter-species sequence dataset appropriate for analyses involving a birth-death speciation process model (Gernhard, 2008). For each single-locus dataset DNA sequences were first aligned using MAFFT run in “auto” mode (Katoh et al., 2002; Katoh et al., 2009) and eventually manually adjusted using Geneious 7.1.3 (Biomatters Ltd., Auckland, New Zealand). Ambiguous alignment sites were excluded using the GBLOCKS server 0.91b, with settings allowed to produce the least stringent selection (Castresana, 2000). The single-

gene alignments were then combined, as the congruence of the loci was evaluated by Miadlikowska et al. (2014) and demonstrated as reliable. The combined matrix is available in TreeBASE (Accession No. S20682). We used BEAST 1.8.2 (Drummond and Rambaut, 2007; Drummond et al., 2012) implementing a secondary calibration point by using the age estimate for the Peltigerales by Prieto and Wedin (2013). Consistent with the results of this detailed study based on a thorough and careful selection of fossils used as time markers, the common ancestor of Peltigerales was constrained to 142, 146, 149, or 150 Myrs (normal distribution; mean = 142, 146, 149, or 150; standard deviation = 10). The dataset was partitioned by gene, with unlinked subset. Models of sequence evolution for each subset were determined by the corrected Akaike Information Criterion (Akaike, 1974; Posada and Buckley, 2004) in jModelTest 2.1.6 (Darriba et al., 2012) on the CIPRES Science Gateway (LSU: GTR + I + G; mtSSU: HKY + I + G; *RPB1*: GTR + I + G). We employed an uncorrelated lognormal relaxed clock model (Drummond et al., 2006) and a birth-death speciation process model (Gernhard, 2008). BEAST analyses were run for 90 million generations, sampling parameters every 1000th generation, and performed on the CIPRES Science Gateway. Convergence, mixing, and effective sample sizes (ESS) of parameters were examined in Tracer 1.6 (Rambaut and Drummond, 2007) to ensure all the effective sample size (ESS) values were larger than 200. A burn-in of 12,000 trees was removed from each run. A maximum credibility tree with a cut-off of 0.5 of posterior probabilities was generated with the remaining 78,000 trees in TreeAnnotator version 1.8.2 (BEAST package) and visualized with FigTree version 1.4.2 (Rambaut, 2012).

2.5. Ancestral range reconstruction analysis

We attempted to reconstruct ancestral areas along the inferred four-loci phylogeny in order to assess the geographical point of origin for the radiation detected in this study. To avoid circularity, the dated phylogeny obtained with BEAST was not used here. Instead, we used the excised ingroup clade of interest from the ML tree previously obtained. The tree was made ultrametric by the *chronos* function implemented in the R package Ape (Paradis et al., 2004), assuming a clock-like model. The R package BioGeoBEARS (Matzke, 2013) was used to assess the

biogeographic history of the MIOI species. This program allows the use of various models, including Dispersal–Extinction–Cladogenesis (DEC; [Ree and Smith, 2008](#)), a likelihood interpretation of Dispersal–Vicariance Analysis (DIVALIKE; [Ronquist, 1997](#); [Matzke, 2013](#)), and a likelihood interpretation of BayArea (BAYAREALIKE; [Landis et al., 2013](#)). Furthermore, BioGeoBEARS also implements these biogeographic models supplemented with a founder-event dispersal parameter (“+J”; [Matzke, 2014](#)). Using this R package, we performed ancestral area reconstructions and compared six models in a statistical framework: DEC, DEC+J, DIVALIKE, DIVALIKE+J, BAYAREALIKE, and BAYAREALIKE+J. Two geographical coding strategies were followed, coding each specimen with its geographical origin: (1) the three islands (Madagascar, Mauritius and Réunion) were regarded as three distinct geographical regions, or (2) the two Mascarene Islands (Mauritius and Réunion) were coded as a single geographical region (Mascarene Archipelago). For both analyses, neither range nor dispersal constraint was applied, and the maximum number of areas that any species may occupy was set equal to the number of geographical regions considered.

3. Results

3.1. Phylogenetic relationships

The ITS, LSU, mtSSU, and *RPB1* loci were amplified and sequenced for 151, 65, 62, and 56 specimens, respectively. Together with publicly available sequences, a combined matrix of 97 specimens was assembled. The combined dataset comprised 4,520 characters, of which 4,123 were retained after removal of ambiguously aligned regions. The ML tree inferred from the ITS complete dataset (154 accessions, plus *S. hypochra* and *S. gaudichaldia* as outgroups) is shown as a supplementary file (Supplemental Fig. S1). The single most likely phylogenetic tree inferred from the four-loci dataset is presented in Fig. 2.

The ML analysis yielded a tree with a likelihood value of $\ln L = -13310.91$; the likelihood value of the consensus Bayesian trees were $\ln L = -12628.22$ and -12631.46 for the cold chains. ML and Bayesian analyses yielded congruent, well-supported topologies. Regardless of the analytical method used, MIOI species formed a strongly supported, monophyletic group (Bootstrap Support [BS] = 89, Posterior Probabilities [PP] = 0.99) within the genus *Sticta*. Most relationships within this clade of interest are fully resolved and supported. Five main lineages can be distinguished, two of them comprising only species endemic to the Mascarene archipelago (clades A and D in Fig. 2). A single lineage (clade B in Fig. 2) contained only species endemic to Madagascar and the other two (clades C and E in Fig. 2) contained species endemic to Madagascar, to Réunion, to Mauritius and Réunion, or to Madagascar and Réunion.

All other *Sticta* species formed a poorly supported lineage, sister to the MIOI clade (BS = 45, PP = 0.82), but with reasonably well-resolved internal branches. Indeed, this sister group comprised three well-supported clades: (1) one composed of large foliose species restricted to Australasia (BS = 61, PP = 0.97), whose primary photobiont is a coccoid green alga, and several species, which also develop fruticose cyanobacterial thalli (*S. filix*, *S. latifrons* and *S. stipitata*; [Magain et al., 2012](#); [Moncada et al., 2013b](#)); (2) one with large foliose species restricted to southern South America (BS = 75, PP = 0.99), whose primary photobiont is either a coccoid green alga [*S. aimoae* and *S. ‘damacornis’* (the type of the latter species was collected in the Caribbean region and thus this epithet most likely represents a different species from the one occurring in Patagonia)] or a cyanobacterium (*S. caulescens*, *S. gaudichaldia* and *S. hypochra*) (3) one comprising the remaining species (BS = 96, PP = 1), which display a wide variety of thallus morphology and geographical distribution and are associated with either a cyanobacterium (predominantly) or a green alga, or with both (*S. canariensis*). It should be underlined that the latter clade also contains widespread species which can also be found in Madagascar

and the Mascarenes (e.g., *S. ambavillaria* and *S. fuliginosa*).

3.2. Species delimitation and identification, and distribution range

Species numbers were allocated to the specimens belonging to undescribed species. This assignment took into account the results provided by the GMYC and TCS analyses examined hereafter.

Five validly published epithets are available for the species studied in this paper. Each of them is briefly reviewed here:

- *Sticta caperata* (Nyl.) Nyl. was described from Réunion and lectotyped by [Galloway \(1998\)](#); it is known from Australia (New South Wales), Madagascar, Comoro Islands, and several archipelagos in the western Pacific ([Galloway, 2001](#)). We confirm the occurrence of this species in Madagascar where it was found on Amber Mt. Other records are not confirmed by the world-wide ITS sequences gathered by B. Moncada and R. Lücking (pers. comm.). Previously, this species was only known to primarily associate with a green alga photobiont. However, evidence for its photomorphic capabilities is noted for the first time, with the presence of an individual that primarily associates with cyanobacteria.
- *S. dichotoma* (Bory) Delise was described from Mauritius in the Mascarene archipelago ([Galloway, 1995](#)). It is locally common in montane humid forests of Réunion, and is also present in the nearby island of Mauritius. It was reported from the Marojejy Mt in Madagascar by [des Abbayes \(1956\)](#), but all Madagascan morphotypes similar to *S. dichotoma* in this study were resolved as distinct species (*Sticta* sp. 4 and *Sticta* sp. 5). Reports from Tanzania ([Krog, 2000](#)) and Panama ([Büdel et al., 2000](#)) are doubtful as no similar or closely related sequences were found in the dataset of world-wide ITS sequences gathered by B. Moncada and R. Lücking (pers. comm.), particularly from the Neotropics.
- *S. macrophylla* Delise (Fig. 1D) was described from Mauritius by [Delise \(1825\)](#). It is a most remarkable species in the montane forests of Réunion and Mauritius because of its large size (specimens reaching c. 15–20 cm in diameter) and because of its ecological preference for small branches or trunks. It was reported from Madagascar, from the Marojejy Mt by [des Abbayes \(1956\)](#) but Madagascan specimens matching this morphotype are here resolved as *Sticta* sp. 22. It has also been reported from Panama ([Büdel et al., 2000](#)) but no similar or closely related sequences were found in the dataset of world-wide ITS sequences gathered by B. Moncada and R. Lücking (pers. comm.).
- *S. plumbea* Delise was described from Réunion and was lectotyped by [Galloway \(1995\)](#). It is similar to *S. macrophylla* but differs by its smaller size. Based on our sampling it appears indeed to be restricted to Réunion where it is not common. Both *S. macrophylla* and *S. plumbea* form, together with *Sticta* sp. 24, a strongly supported clade, endemic to the Mascarene archipelago (clade D in Fig. 2).
- *S. variabilis* Ach. was described from Réunion and lectotyped by [Galloway \(1995\)](#) and further reported from East Africa, Mauritius, the Comoros Islands, Australia and Papua New Guinea ([Galloway, 2001](#)). Our ITS sequences confirm its presence in Mauritius and Madagascar (Amber Mt and Marojejy Mt). It is one of three species within the clade studied here that occurs in Madagascar and the Mascarenes, the other species being *S. caperata* and *Sticta* sp. 16. *S. variabilis* produces phyllidia usually in large quantities and is thus likely prone to effective dispersal. No closely related sequences could be obtained from other collections across the world.

The GMYC analysis of the concatenated phylogeny supported the existence of 28 putative species in the MIOI clade (Supplemental Fig. S2). These clusters are consistent with the morphological trait combinations, except for the two following cases, where the GMYC analysis does not discriminate the additional species:

- *Sticta* sp. 14 differs from *S. variabilis* by the lack of phyllidia (abundant in *S. variabilis*) and typically more robust and elongate lobes;
- *Sticta* sp. 22 differs from *Sticta* sp. 23 by the large thalli with a smooth upper surface (Fig. 1E) versus the smaller thalli with a conspicuously scrobiculate upper surface, rather similar to a representative of the *Lobaria pulmonaria* group (Fig. 1C).

Due to the discordance between the four-loci and the ITS-only phylogeny and to include the insertion of seven bp detected in ITS2 of several collections from the Amber Mt, a TCS analysis was conducted for the clades B and C (see Fig. 2), and yielded the ITS haplotype network shown in Supplemental Fig. S3. The TCS undeniably supports one additional taxonomic unit (considered here as a further species) in the clade formed by the species 6 to 9 (clade B in Fig. 2).

The incongruence with the concatenated phylogeny is potentially due to incomplete lineage sorting or hybridization since the ITS region and *RPB1* convey different phylogenetic signals for this group of species; the exclusion of many ambiguous sites in ITS disguises this discrepancy. Additionally, the TCS haplotype network reinforces the notes about morphology discussed above by differentiating *Sticta* sp. 14 from *S. variabilis*, and *Sticta* sp. 22 from *Sticta* sp. 23.

Altogether, based on molecular data enhanced by morphological traits, the MIOI clade comprises 31 species. Twenty species associate with a green alga; seven associate with a cyanobacterium; four species exhibit photomorphism (i.e., they associate with both types of photosynthetic partners). Several species are morphologically very similar, and each morphological “main” trait, such as dichotomous lobes with a green photobiont (Fig. 1B), large thalli with rounded to elongated lobes with a cyanobacterium as photobiont, or production of phyllidia, occurs in several species and several lineages throughout the group. However, as soon as the locality where the material was collected is known, identification becomes much easier and in most cases, straightforward. Formal descriptions of all species recognized here will be published in a forthcoming paper.

All species delimited here have a restricted distribution within the MIOI, except *S. variabilis*, which occurs on Mauritius, Réunion, and in the two main montane ranges sampled in Madagascar (Supplemental Table S3). This geographic distribution of the species is confirmed by inferences from the ITS complete dataset (154 samples; Supplemental Table S1, Fig. S1), and therefore is unlikely to be an effect of under-sampling. Five geographical regions were sampled: Amber Mt, Marojejy Mt and Ambalamanakana forest in Madagascar, as well as Mauritius and Réunion in the Mascarene archipelago. If we consider these as five localities, one species occurs in four of them (*S. variabilis*), another in three (*Sticta* sp. 16), six in two (*S. caperata*, *S. dichotoma*, *S. macrophylla*, *Sticta* sp. 1 and *Sticta* sp. 10, *Sticta* sp. 15), and all others (23 species) occur in a single one (Supplemental Table S3). If we aggregate the three localities in Madagascar in a single one, and we consider the Mascarene archipelago as a single entity, 22 species occur in Madagascar and 10 in the Mascarenes; only three occur in both (*S. caperata*, *S. variabilis*, and *Sticta* sp. 16). Within Madagascar, all endemic species (21) are endemic to a single locality; within the Mascarenes, the seven endemic species are either restricted to Réunion (four species) or occur on both Mauritius and Réunion (three species); no species is endemic to Mauritius.

3.3. Dating and biogeographical analyses

The combined dataset used in the divergence time analysis comprised 7,686 characters, and 2,906 after trimming and removal of ambiguously aligned regions. The mean divergence time of the MIOI clade is estimated in the Late Miocene at 10.57 Myrs (95% highest posterior density [HPD] interval: 6.62–15.28) when the Peltigerales clade is calibrated to 150 Myrs with a standard deviation of 10 Myrs (Fig. 3). In the resulting maximum clade credibility tree from BEAST, this clade is also strongly supported (PP = 0.99). The MRCA of the genus

Sticta has an estimated mean age of 17.19 Myrs, also in the Miocene period (HPD interval: 11.98–23.42). Inferences based on slightly different calibration ages for the Peltigerales (mean = 142, 146, 149; standard deviation = 10) did not yield any significant modification in age estimates for the target nodes (Supplemental Table S4).

The ancestral area reconstruction method using a DIVALIKE+J model (J = the founder-event jump dispersal parameter) was selected as the best-fitting model, having the lowest AICc score, whether the three considered islands were regarded as three or two distinct geographical regions (Table 1). The DIVALIKE+J model inferred Madagascar+Réunion as the most likely ancestral range, when the three islands were regarded as three different geographical entities. When Réunion and Mauritius were coded as a single geographical region (Mascarene archipelago), the most likely ancestral range was Madagascar+Mascarenes. Reconstructions inferred with the first strategy (three distinct geographical regions), and the DIVALIKE+J model, were projected onto the non-ultrametric ML tree (Fig. 2) for major nodes. Likelihood-ratio tests (LRT) indicated that the data likelihood conferred by models supplemented with a founder-event dispersal parameter “J” was significantly better than the data likelihood conferred by the models without this additional parameter: the very small p-values obtained (Table 1) indicated that the difference was statistically highly significant. In other words, the models involving founder-event dispersal always fitted the data much better.

4. Discussion

4.1. High species richness

Phylogenetic inferences based on our extensive population sample highlight an unsuspectedly high diversity, with 31 putative species compared to the five morphospecies currently recognized. This hypothesis is congruent with studies of *Sticta* in the Neotropics, which led Moncada et al. (2014) to predict that the global diversity of *Sticta* may be four to five times higher than currently estimated (rising from 120 to about 500 species). Several papers published in the last fifteen years described no less than 43 species new to science, even from continental Western Europe and Eastern North America (Aptroot, 2008; McDonald et al., 2003; Øvstedal and Gremmen, 2010; Lumbsch et al., 2011; Moncada and Lücking, 2012; Suárez and Lücking, 2013; Moncada et al., 2013b, 2013c, 2015; Ariyawansa et al., 2015; Lendemer and Goffinet, 2015; Magain and Sérusiaux, 2015). Of the 31 statistically supported species within the MIOI lineage only five could be assigned a validly published epithet [*S. caperata* (Nyl.) Nyl., *S. dichotoma* Delise, *S. macrophylla* Delise, *S. plumbea* Delise, and *S. variabilis* Ach.], a 1:6 ratio resulting from extensive sampling of the localities visited (Amber Mt and Marojejy Mt in Madagascar, Mauritius and Réunion in the Mascarene archipelago). This ratio could be even higher considering that the Ambalamanakana area, a small remnant of the montane forests that covered parts of the central plateau in Madagascar (McConnell and Kull, 2014) was only sparsely sampled, but was shown here to harbor two distinct species. Furthermore, only a modest section of the montane forests in Madagascar was sampled, since other suitable localities in the central and southern parts of the island were not explored.

4.2. High insular and small-range endemism

Strong patterns of endemism are shown for *Sticta* within the MIOI: most species (28) are restricted to either Madagascar, or to one of both of the Mascarene Islands. Only one species (*S. variabilis*) is confirmed from all three islands (Madagascar, Mauritius and Réunion), and two (*S. caperata* and *Sticta* sp. 16) occur only in Madagascar and Réunion. Moreover, two of the five MIOI clades are strictly endemic to the Mascarene archipelago: clade A with two species and clade D with three (Fig. 2A). This pattern further strengthens the degree of mostly narrow endemism in this archipelago, for which new and extraordinary

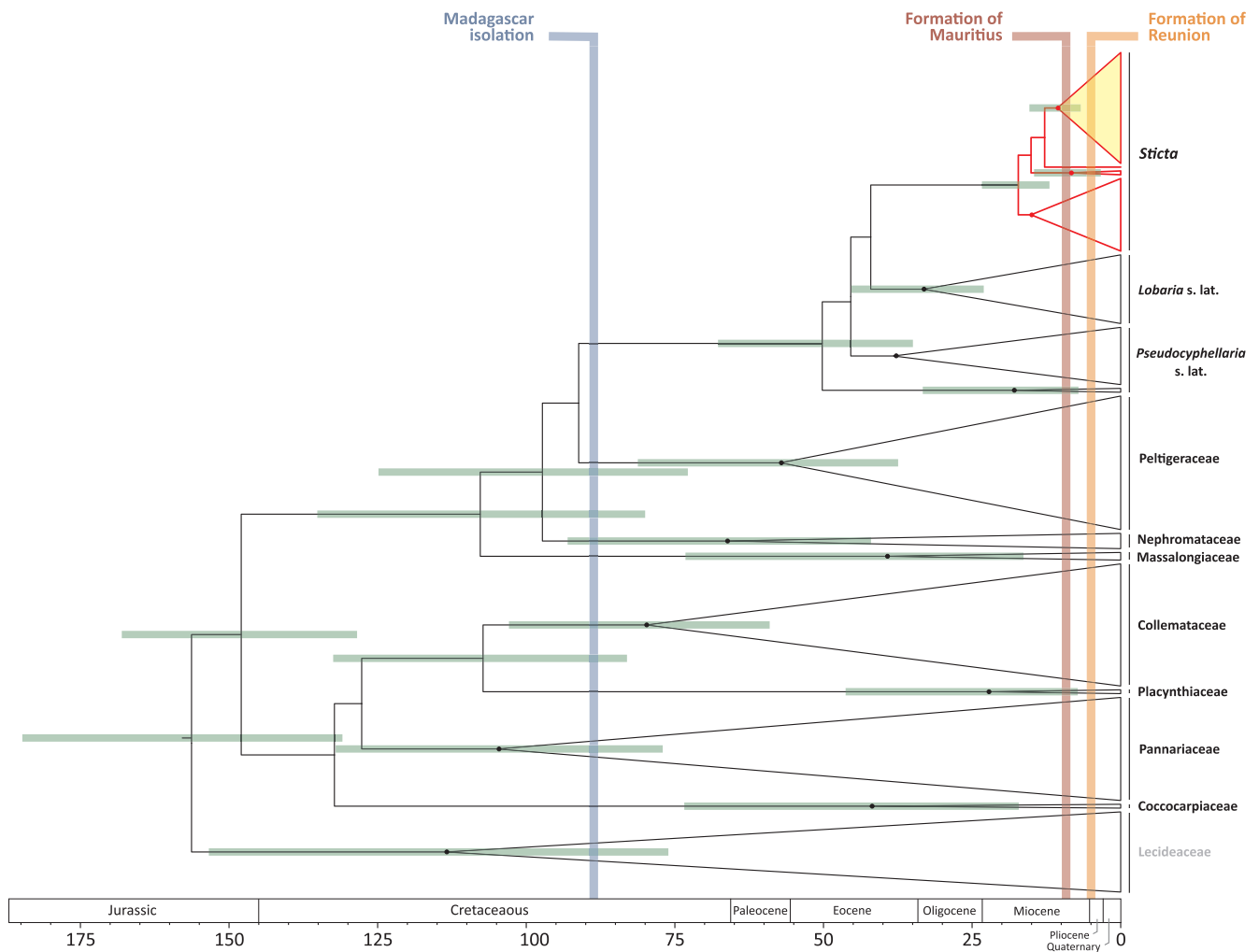


Fig. 3. Maximum clade credibility chronogram obtained in the BEAST analysis for the Peltigerales order, showing mean age estimates and 95% high posterior density intervals (for nodes with posterior probability > 0.9), obtained from the LSU-mtSSU-RPB1 dataset. The MIOI *Sticta* clade is highlighted in yellow. The whole genus *Sticta* is highlighted by red outlines. Taxa from the Peltigerales appear in black, and those of the Lecideaceae (outgroup) in gray. Three major geological events are highlighted: the isolation of Madagascar from the Indian subcontinent about 88 Mya (in blue), the emergence of Mauritius 8–10 Mya (in red) and of Réunion about 5 Mya (in orange). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Reconstruction of the ancestral area for the clade of endemic MIOI *Sticta* species under different models and geographical coding strategies implemented in BioGeoBEARS. Models favored by AICc, for each pair of competing conditional models, are indicated in bold.

	Model	Ln L	AICc	P-value (LRT)	Most likely ancestral range
Three geographic regions (Madagascar, Mauritius, Réunion)	DEC	-66.46	137.1	2.5 10 ⁻⁹	Madagascar + Réunion
	DEC + J	-48.69	103.7		Madagascar + Réunion
	DIVA	-66.04	136.3	2.6 10 ⁻⁹	Madagascar + Réunion
	DIVA + J	-48.31	103		Madagascar + Réunion
	BAYAREALIKE	-114.2	232.7	7.8 10 ⁻³⁰	Madagascar + Réunion
	BAYAREALIKE + J	-49.88	106.1		Réunion
Two geographic regions (Madagascar, Mascarenes)	DEC	-34.44	73.07	8.4 10 ⁻⁵	Madagascar + Mascarenes
	DEC + J	-26.71	59.8		Madagascar + Mascarenes
	DIVA	-33.27	70.73	2.0 10 ⁻⁴	Madagascar + Mascarenes
	DIVA + J	-26.24	58.85		Madagascar + Mascarenes
	BAYAREALIKE	-58.01	120.2	7.2 10 ⁻¹⁵	Madagascar + Mascarenes
	BAYAREALIKE + J	-27.74	61.85		Réunion

discoveries continue to be published across taxonomic groups (Klopper et al., 2013; Le Péchon et al., 2013, 2015; Hennequin et al., 2014; Hume, 2014; Stone, 2014; Strijk et al., 2014; Braldler et al., 2015; Hugel, 2015). Such a high level of endemism reported here is not an artifact of poor uneven sampling in view of the large number of populations included in this study (Appendix A).

Underrepresentation of species in Mauritius within this radiation, and especially the absence of species endemic to this island, may be explained by historical factors. Indeed, unlike the younger island of Réunion, Mauritius has lost most of its original volcanic mass as a result of erosion (including giant landslides) and subsidence. Hence, the island, with a maximum altitude of only 828 m, does not harbor a

significant area of montane forests where representatives of the Peltigerales, including *Sticta* species, could grow and thrive. Moreover, most of the original vegetation has been lost to agriculture, plantation of exotic trees and invasive species. With the loss of the emblematic dodo (*Raphus cucullatus*), hundreds of other species, and most of the original vegetation cover, Mauritius represents a dramatic example of the tremendous impact of human contact on islands biomes (Cheke and Hume, 2008; Florens, 2013). Thus, we may consider that a significant part of the lichen flora in Mauritius is now extinct and that our study only incorporates the few surviving taxa.

The two main montane forest massifs (Amber Mt and Marojejy Mt) harbor separate phylogeographic entities as often demonstrated for many emblematic endemic taxa on the island (Wilmé et al., 2006; Guillaumet et al., 2008; Vences et al., 2009), including among others, frogs (Wollenberg et al., 2008), amphibians (Vietes et al., 2009), leaf-tailed geckos *Uroplatus* (Ratsoavina et al., 2013) and tenrecs (Everson et al., 2016). Throughout Madagascar, including the montane forests where the genus *Sticta* thrives, many species have small distributions that cannot be explained by adaptation to a broad vegetation biome (Ganzhorn et al., 2014). It is beyond the scope of this paper to discuss the mechanisms and processes that produce such a fascinating diversity. However, it is interesting to note that the very first group of lichens (*Sticta*) studied in detail in Madagascar shows a pattern of micro-endemicity: Amber Mt, a rather recent volcanic zone (active from the Miocene to the Pleistocene: Bardintzeff et al., 2010) has six endemic species; the Marojejy, a montane massif made of Precambrian rocks hosts another set of 14 endemic species; the tiny forest remnant sampled at Ambalamanakana has two endemic species. This suggests that, in addition to regional patterns of endemism, MIOI *Sticta* also display microendemicity; each forest massif in Madagascar acts as an island harboring its own unique species.

4.3. Radiation triggered by a single colonization event

The majority of species of *Sticta* in the MIOI biodiversity hotspot arose from a unique ancestor, as previously suggested by molecular studies, which included a subset of these species (Magain et al., 2012; Magain and Sérusiaux, 2015). This result, together with ancestral geographical reconstruction and divergence event-dating, strongly supports the occurrence of a local species-rich radiation initiated by a single long-distance dispersal event. In addition, models in our biogeographic analysis supplemented with a founder-event dispersal parameter produced the best statistical fit to the data, thus strongly supporting the hypothesis of a single radiation followed by multiple dispersal events between islands and in situ diversification processes.

Currently, three local radiations have been demonstrated within the Peltigerales, in addition to the one discussed here:

- (1) *Lobariella* in Hawaii, with a recent 'micro-radiation', which gave rise to three species on the archipelago (Lücking et al., 2017b).
- (2) *Nephroma* in Macaronesia (Sérusiaux et al., 2011), with 5 endemic species belonging to two sister clades and originating from a most recent common ancestor shared with one widely distributed taxon, either *N. parile* or *N. laevigatum*.
- (3) *Peltigera* Sect. *Polydactylon*, *dolichorhiza* group (Magain et al., 2017a), with a large radiation in Central and South America including c. 28 species, most of them still undescribed.

Our dating analysis suggests a recent radiation of the MIOI lineage, which most likely occurred in the Late Miocene, with a divergence time estimated at 10.57 Myrs for this node (Fig. 3), similarly to Madagascan generic endemic angiosperms, which predominantly originated during the Miocene or after (Buerki et al., 2013). Based on stratigraphic and potassium-argon dating, Mauritius is estimated to be the first island generated by the Mascarene volcanic hotspot between 8 and 10 Myrs ago, whereas Réunion and Rodrigues are likely younger with age

estimates of about 5 and 1.5 Myrs, respectively (McDougall and Chamalaun, 1969; Bonneville et al., 1988). On the other hand, Madagascar became isolated much earlier: the island detached from Gondwana about 165 Myrs ago (Coffin and Rabinowitz, 1987) and split from the Indian subcontinent about 88 Myrs ago (Storey et al., 1995), isolating Madagascar in the Indian Ocean. Therefore, a recent diversification of *Sticta* and its subsequent and even younger radiation in the MIOI (c. 17 Myrs and c. 11 Myrs, respectively) are inconsistent with the general hypothesis that southern hemisphere lichen floras are mainly formed by the "paleoaustral element", and hence species of Gondwanan origin (Jørgensen, 1983; Galloway, 1987). Instead the MIOI lineage of *Sticta* seemingly arose via long-distance dispersal during the Miocene. Even if the secondary calibration approach used here is less accurate compared to a direct calibration strategy, ancient vicariance following the separation of Madagascar from other parts of the Gondwana can be ruled out. Further, the estimated divergence time of the genus *Sticta* resulted in a mean age of 17.19 Myrs, which is roughly similar to the node age of *Sticta* estimated by Lücking (2012; mentioned in Moncada et al., 2014) at 26 Myrs. Therefore, we are confident that our divergence time analysis yielded a reliable temporal framework for this species-rich radiation triggered by a single colonization event.

Lichens disperse by aposymbiotic or symbiotic propagules. The latter may facilitate establishment since both symbionts are dispersed at once. Various species of *Sticta* produce specialized symbiotic diaspores, such as isidia, soredia, phyllidia, or the simplest of which is a thallus fragment. However, many species lack such propagules and rely on meiotic spores for dispersal. Establishment may then be hampered by the availability of a suitable photobiont. Furthermore, effective establishment, that is the establishment of sexually reproducing populations, may require, in the case of strictly outcrossing (i.e., heterothallic) species, the presence of suitable mates. It is not known if species of *Sticta* are homo- (i.e., self-fertile) or heterothallic. However, 22 species in the MIOI clade are fertile, suggesting that either the species or their ancestor are homothallic. Alternatively, in the event of a single long-distance dispersal of a heterothallic species (i.e., where distinct individuals bearing different mating types merge to produce sexual spores), the following radiation would have led to a set of strictly sterile species, unless a second dispersal event imported the complementary mating type in the region. Of course, dispersal of a symbiotic propagule, which might have included both mating strains, would overcome barriers to sexual reproduction in a heterothallic species. A survey of studies of mating types in lichenized fungi suggests that heterothallism is more common than homothallism (i.e., 26 versus four; Ludwig et al., 2017). This, in conjunction with the fact that the most closely related of these species (*Lobaria pulmonaria*, Lobariaceae) is not self-fertile, pleads in favor of a MIOI clade composed of heterothallic species. Establishment of a heterothallic species following dispersal of a aposymbiotic diaspore may appear unlikely, except if the founder event was followed by a conversion to homothallism as hypothesized at the local scale in *L. pulmonaria* (Singh et al., 2012). Be that as it may, empirical research on lichen mating systems is still scarce, and determining which kind of sexual system was harbored by the MRCA cannot be settled for now.

The estimated age of diversification of *Sticta* in MIOI is consistent with the timing of emergence of Mauritius. Furthermore, our ancestral area reconstruction suggests a shared common ancestor on Madagascar and the Mascarenes, and does not exclude the possibility of the origin of the common ancestor on the Mascarene Islands, which at that time consisted only of Mauritius. Examples of similar radiation patterns can be found in other groups, such as in *Badula* (Primulaceae; Strijk et al., 2014). In such cases, it is possible that neighboring islands in the Indian Ocean may have functioned as stepping stones for long distance dispersals, while simultaneously acting as sources of increased biodiversity. Since the MIOI lineage has no supported sister group in our study, the origin of the dispersal and founding event for the radiation in the MIOI is uncertain; nevertheless, it conceivably dispersed from other areas in the Southern Hemisphere, either Australia/New Zealand or

southern South America. In fact, the taxa from these two geographic regions form two distinct well-supported lineages, likely candidates for sister group (Fig. 2). However, the ambiguity in the relationships among major clades of *Sticta* leaves open the hypothesis that the MIOI lineage is sister to the remainder of *Sticta* species, and thus that the genus actually originated in this area.

5. Conclusions

The present study demonstrates the existence of a well-supported monophyletic lineage within the genus *Sticta*, which is endemic to three islands of the Western Indian Ocean: Madagascar, Mauritius and Réunion (MIOI hotspot). This clade comprises an assemblage of mostly narrow endemic species six times more diverse than previously recognized, highlighting the extent of the undiscovered diversity within lichen-forming fungi in this region, especially in the genus *Sticta*. Our results strongly support a local species-rich radiation starting in the Late Miocene, that is concomitant with the emergence of the Mascarene archipelago. Thus, the following biogeographic scenario can be tentatively suggested for the MIOI *Sticta* species: their most recent common ancestor reached either Madagascar or the Mascarenes, via a single long dispersal event, most likely from an area in the Southern Hemisphere; thereafter migrations between the three islands shaped the observed evolutionary patterns and local speciation, including within each Madagascar montane area, giving rise to a rich complex of small-range endemic species.

Data accessibility

Alignments and trees have been deposited in TreeBASE (Accession No. S20682):

<http://purl.org/phylo/treebase/phyloids/study/TB2:S20682>

Acknowledgments

Field studies in several parts of Africa were made possible with the help and advice of local authorities and colleagues. We would like to mention in particular Rogerlala Andriamiandrisoa, Eberhard Fischer, Damien Ertz, Dorothee Killmann and Tahina Razafindrahaja for our field trips in Madagascar. We also benefited from the help of the Missouri Botanical Garden offices at Antananarivo (in particular Faranirina Lantoarisoa) and Claudine Ah-Peng for the preparation of field trips to Amber Mountain and Marojeje National Parks. Further the support of the *Parc Botanique et Zoologique de Tzimbazaza* at Antananarivo and of the *Ministère de l'Environnement, de l'Ecologie et des Forêts* in Madagascar (i.e., the Madagascar National Parks Services) has been crucial for the success of our field trips. We thank Gintaras Kantvilas for the gift of a recently collected *Sticta stipitata* from Tasmania. This project is partially funded by the National Science Foundation through grant DEB-1354631 to B.G. Field trip in the Mascarenes were made possible with the help and advice from the *Parc National de La Réunion*, especially through the courtesy of Benoît Lequette, Claudine Ah-Peng and Dominique Strasberg of the University of La Réunion in Saint-Denis. We thank them all most sincerely. Maarten Brand and Pieter van den Boom were great companions during the first field trip to La Réunion in 2008. We further would like to thank very warmly Bruno Dennetière, the curator of the Lichen Herbarium in Paris, and his colleagues for their warm welcome and help during the visit of two of us (N.M. and E.S.) for the study of the type collections of *Sticta* from Mauritius and Réunion. Collection of Chilean species was made possible by the Chilean National Forest Corporation (CONAF) under permit 06/2014 to B.G., and facilitated by Dr. Reinaldo Vargas (Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile). We would like to acknowledge Laurent Gohy for technical assistance at the University of Liège. We are grateful to Dinah Parker for the careful reading of the manuscript. A.S. is a PhD student at the

University of Liège and acknowledges the financial support by FRIA, a grant of the Belgian Research Foundation (F.R.S.-FNRS). Finally, we thank the referees for their critical and helpful notes and suggestions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ympev.2018.01.012>.

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