THE UNIVERSITY OF CHICAGO

AN EVOLUTIONARY PERSPECTIVE ON DIVERSITY AND DISTRIBUTION PATTERNS OF LICHENIZED FUNGI IN THE CARIBBEAN

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES

AND THE PRITZKER SCHOOL OF MEDICINE

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

COMMITTEE ON EVOLUTIONARY BIOLOGY

BY

JOEL ALEJANDRO MERCADO-DIAZ

CHICAGO, ILLINOIS

AUGUST 2021

DEDICATION

To each person whom in my absence from Puerto Rico have fought with the ideal of "another future is possible" as a guiding principle. This work is dedicated to you. I will join you soon.

I also want to dedicate this work to the 3,057 lives we lost during Hurricane María. One was too many.

"Porque soy como el árbol talado,
que retoño, ¡aún tengo la vida!"

-J.M. Serrat

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I want to thank my thesis committee, Thorsten Lumbsch, Trevor Price, Andrew Hipp and J. Timothy Wootton for their unconditional support, words of advice and patience. Thanks for having so much faith in me. I am truly humbled. Thorsten, thanks for letting me be part of the amazing "lichen gang" and for always being so supportive of my ideas and endeavors. I will be always indebted to you. In this vein, I want to also thank Robert Lücking who was not formally a committee member, but certainly performed as one from the very beginning. Thanks, Robert, for introducing me to the amazing world of lichens back in 2004, and for providing so much help over all these years. It would have been impossible without your help. To all of you, please know you will always have friend and colleague in Puerto Rico.

Navigating graduate school requires a great team of administrators, and to my luck, I can say I had one. Thanks, in first instance, to Michael Coates and Carolyn Johnson who opened the doors of CEB for me and who supported me from day one. I will be forever grateful for all you did for me. Helping me navigate the last part of the Ph.D. "ride" was Audrey Aronowsky and Marcy Hochberg. Thank you both for all your help and amazing communication. To all of you, be confident that your efforts did not go unnoticed!

The work I present here was accomplished because of the help of my amazing network of friends and collaborators in the insular Caribbean region. Thanks to Yoira Rivera-Queralta, Angel "Motito" Marín (Cuba), Keron Campbell (Jamaica), Lemuel Familia, the late Brígido Peguero (Dominican Republic), Eugenio Santiago-Valentín (Puerto Rico), Cesar Delnatte (Martinique), Malcolm Hodges and Sean Beeching (Georgia, USA) for helping me in all aspects concerning doing fieldwork in your countries and for accompanying me to the field. Melissa Meléndez, Manuela Dal Forno, and Raisa González also participated from fieldwork and are

properly thanked. I am also thankful to the late Richard Harris (NYBG), Eric Schuettpelz (US), Jennifer S. Kluse (LSU), Soili Stenroos (H), Matt Chansler and Alan Fryday (MSC), who provided access to collections, and to Sophie Bedel (Guadeloupe), Stephen Durand (Dominica) and Roger Rosentretter (Idaho, USA) who helped me greatly with collecting permits in their countries.

Each member of the Field Museum "lichen gang" (past and present) helped me in some aspect of my project and I want to distinguish them. This great group is: Matthew P. Nelsen, J.P. Huang, Alejandrina Bárcenas, Todd Widhelm, Felix Grewe, Claudio Ametrano, Ana Otero, Hanna Lindgren, Isabel Distefano and Steve Leavitt. I also want to include in this group the real *Sticta* "guru" and friend Bibiana Moncada, who originally trained me in lab work and have always provided great help with *Sticta*-related work. I also want to recognize four amazing young woman scientists whom I trained in DNA lab work and helped me greatly in these tasks. These are Raisa Munshi, Maddy Janakis, Annalisse Nordgren and Josie Kleve. Ivan Luk also provided help with lab work and is properly thanked. In this regard, I cannot leave out the amazing Pritzker Lab team, Kevin Feldheim, Erica Zahnle and Dylan Maddox who were always there for helping me. You guys were key, thanks!

None of the work presented would have been possible without the support of my funding sources. These include: the CEB-FM Pritzker lab funds, the UChicago Travel Fund, the Mycological Society of America Travel Award, the CEB Henry Hinds Fund, the ABLS Culberson & Hale 2018 Field Lichenology Research Award, the SSB MiniARTS Award, a CEB travel award, the International Association of Lichenology Travel Grant, and a Grainger Bioinformatics Center research award. I also obtained a Ford Foundation Pre-Doctoral fellowship, but I wanted to acknowledge this award and the "Fordies" family separately because

their help went beyond the financial support provided. The annual meetings I participated from helped me regain strength in moments where I was considering dropping out, thus, I owe part of having finished this “ride” to their direct and indirect support.

To my good friends back home, too many to mention by name, but collectively known as “Grupo Sin Nombre”. Thank you, guys, for always being there for me and Johanna, and for always helping us “recharge batteries” in the times most needed. I also owe too much to my Chicago friends, including John S. Park, Mark Bitter, Joe Marcus, Gabriel Vargas, Manny Rocha, Arjun and Haley Biddanda, Cassie Halls, Cony De La Fuente, Helio Tejedor, Rocío López, Daniela Palmer, Shane DuBay, Victoria Flores, Noé De La Sancha, Valentina Gómez, Natalia Ruiz, Natalia Cortéz, Sophia Carryl, Heather Skeen, Charles Washington III, Allison Huangs, Ryan Fuller, and too many others that I am unfairly leaving out. I am looking forward to many more years of friendship with all of you!

Thanks to my parents Elsie Díaz Ortega, George Mercado Montañez, my brother Julián Mercado-Díaz, and my beautiful niece Julianys Mercado Cosme for always supporting me. My extended family Carmen López, Jorge Colón, and Jorge “Georgie” Colón-López were also crucial in this adventure.

Finally, to the real champion, Johanna Colón-López, my beautiful partner. Thank you, honey, for joining me in this adventure in the first place. It would have been impossible without you. Love you!

ABSTRACT

Studying how island biotas evolve and are dynamically shaped by interactions between resident species, their immediate environments and neighboring continental communities have provided basic understanding of ecological and evolutionary processes operating in these ecosystems. However, it remains to be seen how this knowledge applies to biodiverse groups such as lichens since these have been subjected to less scrutiny. To address this, my dissertation implemented integrative taxonomic approaches to shed light on factors that are crucial for the evolution of species and communities of lichenized fungi in tropical islands, specifically those from the Caribbean region. Chapter 1 emphasized using a phylogenetic framework based on multi-locus data from the lichen genus *Sticta* in Puerto Rico to reconstruct evolutionary origins and better quantify species richness within islands. We found that Puerto Rico hosts 16 species, eight of which I described as new. The group also exhibited a 69% degree of inferred endemism and formed polyphyletic assemblages, hinting at multiple colonization events over evolutionary time. Chapter 2 focused on reconstructing the evolutionary history of Caribbean *Sticta* and elucidating patterns of taxonomic and phylogenetic turnover between islands. To this end, I assembled a multi-locus dataset with representative taxa from these islands and other regions, performed macroevolutionary analysis, and estimated taxonomic and phylogenetic beta diversity indices for island-level communities. Ancestral range reconstruction analysis confirmed that most Caribbean species derived from South American ancestors, which first colonized the region nearly 19 Mya. No changes in diversification rates were detected as a result of range expansion to the Caribbean. Phylobetadiversity analysis, on the other hand, showed that taxonomic and phylogenetic turnover was most strongly correlated with variation along environmental gradients. The presumably high diversity of endemic species restricted to high elevation areas in

Hispaniola and Jamaica likely underlies high dissimilarity detected between these communities and those from Puerto Rico and the Lesser Antilles. In Chapter 3, I combined phylogenetic methods with tools for visualization of population structure to revise the phylogeography of *Cladonia sandstedei*, a putative Caribbean endemic that shares parts of its range with the morphologically similar, but chemically different species *C. subtenuis*. This analysis was based on genome-wide data (RADseq) obtained for the southeastern US and Caribbean populations of both species. A major continental clade that showed a poor correlation between chemistry and phylogenetic structure was recovered, suggesting that chemical traits underperform for species discrimination purposes in this group. Strongly divergent island-level clades contrasted with poor separation between an inferred continental population and Jamaican individuals of both species, further hindering clarification of phylogeographic patterns. Cuban and Puerto Rican populations might deserve taxonomic recognition at the species level. Altogether, these projects represent the first attempt to characterize evolutionary events and processes that have shaped species diversity and phylogeographic patterns of lichens in this important biogeographic region. As such, my work ultimately highlights salient features that distinguish lichen evolution in tropical island systems.

INTRODUCTION

Biologists Robert Ricklefs and Eldredge Bermingham stated that the Caribbean islands were an "outstanding natural laboratory for studying processes that establish patterns in the diversity of life" (Ricklefs and Bermingham, 2008). Their statement was grounded on two main facets of these islands' natural history. The first highlights how geographic settings that have resulted from the archipelago's old age, complex geological history, and the different sizes and elevational profiles of its islands (Graham 2003; Woods and Sergile 2001) have provided unique opportunities for exploring complex evolutionary phenomena, including the famous adaptive radiations seen in *Anolis* lizards and other vertebrates (Losos et al. 1998; Hedges 1989; Woods, Borroto Paéz, and Kilpatrick 2001). The second hinges on the distinctive geographic position of the Caribbean islands. Specifically, they argued that by being not too far and not too close from continental regions, these islands were unique in the sense of allowing the evolution of distinctive island forms while simultaneously permitting the occasional inflow of continental elements. It is this combination of factors what separates the Caribbean from other archetypal archipelagos (e.g., Hawaii, Galápagos) in the study of evolution.

One rarely recognized aspect about studies emphasizing the ecology and evolution of insular biotas such as the Caribbean is that they tend to focus primarily on vertebrates as those are usually the best known and understood (Ricklefs and Bermingham 2008). However, vertebrates comprise only 0.7% of the estimated global biodiversity (Chapman 2009). This means that characterization of key ecological and evolutionary processes in these ecosystems have been mostly achieved without considering the most biodiverse groups, specifically invertebrates and fungi, which respectively account for approximately 60% and 13% of the

estimated number of species in the planet (Chapman 2009). There is evidence of bias towards conspicuous organisms in biological and conservation research (Adamo et al. 2021; Mammola et al. 2020; Clark and May 2002), although this is not exclusive to studies on insular biotas. If not addressed, this type of issue might impinge on our ability to correctly infer general ecological and evolutionary principles that we might consider applicable to most organisms evolving in these settings.

Lichens–symbiotic associations between fungi and algae/cyanobacteria– are among these grossly understudied groups of biologically diverse organisms. Although 20,000 species have already been described (Lücking, Hodkinson, and Leavitt 2017), work suggests that nearly 8,000 species (~ 28%) remain to be discovered (Lücking et al. 2009). Many of these species are predicted to occur in tropical forests, such as those in the Caribbean (Lücking et al. 2009; Sipman and Aptroot 2001), but compared to other ecosystems, these areas remain sparsely studied and explored.

In spite of several recent efforts (Mercado-Díaz, Lücking, and Parnmen 2014; Mercado-Díaz et al. 2015), knowledge about species diversity patterns of lichenized fungi in islands of the Caribbean remain highly rudimentary. The current number of known species is considerably below predicted numbers for most islands in the region, particularly in the Greater Antilles (Fig. I.1). In fact, current species diversity knowledge for some islands is below theoretical minimums (Fig. I.1), further attesting to the dearth of basic taxonomic inventories. Recent evidence suggest that species with unique phenotypes and genetic characteristics are present in these islands (Mercado-Díaz, Lücking, and Parnmen 2014; Lücking et al. 2020; Perlmutter, Plata, and Lücking 2018) but work is limited to a handful of lineages. The extent to which these attributes are exclusive to the biota of the region are not well understood. Aspects concerning the

evolutionary history of species assemblages in these islands are also indeterminable due to limited sampling of Caribbean lineages in the few phylogenetic studies that have included samples from this archipelago (e.g. Lumbsch et al. 2014; Nelsen et al. 2014a, 2014b).

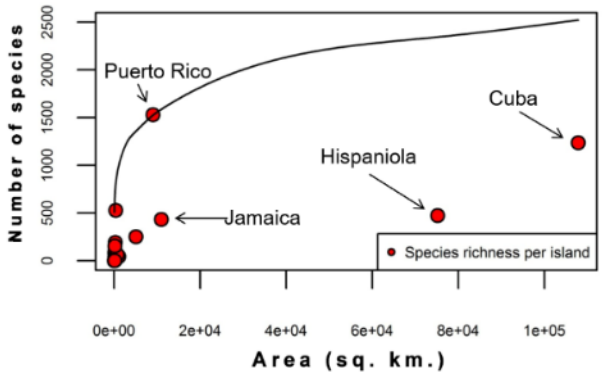

Figure I.1. Predicted (black line) vs. known (red dots) species richness of lichens in islands of the Caribbean. Prediction based on a species-area curve ($S = CA^z$) (From Mercado-Díaz, unpublished).

The work presented here is based on comprehensive sampling throughout the Caribbean region and uses an integrative taxonomic approach to address these knowledge gaps. Specifically, I combine traditional taxonomy with comparative phylogenetic methods to explore diversity patterns and evolutionary origins in the genus *Sticta*. Tools from population genetics are also used to assess phylogeographic patterns in *Cladonia sandstedei*, a presumed Caribbean endemic. This work aims to provide basic understanding about evolutionary processes shaping lichen communities in the Caribbean. As such, it ultimately seeks to highlight key features of lichen evolution in tropical island systems.

Chapter 1: Elucidating species richness in lichenized fungi: the genus *Sticta* (Ascomycota: Peltigeraceae) in Puerto Rico

Comprehensive sampling efforts for the genus *Sticta* were carried out throughout Puerto Rico and DNA sequences, which included important genes for fungal systematics (e.g. ITS, nuLSU, mtSSU), were generated for phylogenetic and species delimitation analyses. Our results show that at least 16 species occur in Puerto Rico, eleven of them potentially endemic to the island. Interestingly, species are scattered across eight separate clades suggesting that Puerto Rican *Sticta* are not derived from a within-island adaptive radiation, but from separate dispersal and in-situ speciation events. This work includes the description of eight new species and is the first exhaustively explore within-island evolutionary relationships and diversity patterns for any lichen group in the region. Results from this project have been published in the journal TAXON (Mercado-Díaz et al. 2020).

Chapter 2: A holistic view of the factors shaping the diversity of the lichen-forming fungal genus *Sticta* in the Caribbean

Chapter 2 emphasizes on elucidating the timing and evolutionary origins of the genus *Sticta* over the broader Caribbean region. Beyond Puerto Rico, sampling was carried out in the islands of Cuba, Jamaica, Hispaniola, Dominica, Guadeloupe, and Martinique and sequences for six genetic markers were generated. Phylogenetic analysis was performed to assess the evolution of geographic ranges and potential diversification associated with the colonization of these islands. A phylobetadiversity analysis was also carried out to characterize patterns of taxonomic and phylogenetic relatedness between insular communities and assess the role of environment and spatial distance in driving these patterns. *Sticta* was found to have arrived at the islands nearly 19 Mya. Although high levels of putative endemism were found (~ 59%), evidence for increased diversification associated with range expansion into the region was equivocal.

Taxonomic and phylogenetic turnover was most strongly correlated with environmental changes rather than with geographic distance. It was concluded that although strong evolutionary links existed between Caribbean and South American biotas, at the scale of the archipelago, species assemblages exhibited complex taxonomic and phylogenetic relationships that were determined by local environments and shared evolutionary histories.

Chapter 3: Genome-wide assessment of putative endemism and phylogeography of *Cladonia sandstedei* (Ascomycota: Cladoniaceae) in the Caribbean

In Chapter 3, I used a reduced genomic data set (RADseq) to help clarify evolutionary relationships and phylogeography of *Cladonia sandstedei* and *C. subtenuis*, two morphologically similar species that have overlapping geographic ranges in the Caribbean and southeastern US. Both species tend to colonize open environments and are distinguished by the presence of major substances, specifically usnic acid in *C. subtenuis* and atranorin in *C. sandstedei*. Phylogenetic reconstructions and population genetics analyses were used to characterize genetic variation between populations. Phylogenetic analysis showed that secondary chemistry was homoplasious. Strong geographic signature in genetic variation was detected in all methods but clarifying species boundaries and geographic ranges remained elusive due to differences found between phylogenetic patterns and population-level genetic clustering. We found evidence suggesting that unrecognized species-level lineages with a *C. sandstedei* phenotype might be present in Puerto Rico and Cuba, but confirmation is constrained by limited sampling. This work demonstrates the utility of RADseq-based phylogenetics and population genetics for investigating genetic variation and phylogeography in lichens with complex taxonomy and evolutionary histories.

CHAPTER 1

ELUCIDATING SPECIES RICHNESS IN LICHEN FUNGI: THE GENUS STICTA (ASCOMYCOTA: PELTIGERACEAE) IN PUERTO RICO

[Published in Taxon as: Mercado-Díaz, Joel A., Robert Lücking, Bibiana Moncada, Todd J. Widhelm, and H. Thorsten Lumbsch. 2020. "Elucidating Species Richness in Lichen Fungi: The Genus Sticta (Ascomycota: Peltigeraceae) in Puerto Rico." Taxon 69 (5): 851–91. https://doi.org/10.1002/tax.12320.]

Abstract Traditional taxonomic studies provide only a limited understanding of species richness within a group. Their usefulness for assessing species diversity could also be limited as many lack sufficient sampling and/or fail to integrate different data types for assessing species boundaries. To explore the challenges and limitations of estimating species richness in lichens, we employed an integrative taxonomic approach to elucidate diversification patterns of the genus *Sticta* (Peltigeraceae) in Puerto Rico. Specimens were collected throughout the island, and a six-locus dataset was generated to infer phylogenetic relationships among Puerto Rican *Sticta* and their continental counterparts. Phylogenetic analysis was combined with species delimitation methods and analysis of morpho-anatomical characters to assess diversity patterns and clarify species-level taxonomy. We found that *Sticta* is represented by 16 species in Puerto Rico and that at least 11 (69%) of them are potentially endemic to the island. We describe eight of these in this work: *S. borinquensis* sp. nov., *S. corymbosa* sp. nov., *S. densiphyllidiata* sp. nov.,

S. guilartensis sp. nov., *S. harrisii* sp. nov., *S. parvilobata* sp. nov., *S. riparia* sp. nov., and *S. tainorum* Merc.-Díaz, sp. nov. These species do not cluster in a monophyletic assemblage but are scattered over the broader *Sticta* phylogeny, indicating at least eight separate dispersal events. Putative endemic species were found to have close allies occurring in South America. Careful re-examination of material revealed phenotypical characters that separate most species, suggesting low levels of cryptic diversity. We highlight that integrating molecular methods and other sources of information in species discovery along with comprehensive sampling efforts can greatly enhance our knowledge about diversity patterns in poorly studied groups and regions. Furthermore, species and ecosystems in the Caribbean are being threatened by substantial human-driven changes (e.g., deforestation, climate change). Consequences of these impacts include reduction in already restricted habitats and potential extinction. We argue that studies analyzing species diversity within a phylogenetic framework could better inform conservation efforts aimed at addressing these challenges.

Keywords biodiversity; Caribbean; evolution; phylogenetics; species delimitation

INTRODUCTION

A crucial task in biodiversity research, ecology and conservation is to quantify species richness—the number of different species in an ecological community. For example, when investigating how intra- and inter-specific interactions influence community assembly, community ecologists need a clear understanding of species occurrence and their abundance (Mittelbach and McGill 2019). Similarly, the establishment of natural reserves depends on having realistic estimates of the number and identity of species present in those areas (Mace

2004).

Intrinsic to obtaining accurate species richness estimates is the capacity of correctly identifying or delimiting species. Species delimitation is the process of identifying how individuals and populations fit into natural groups or species-level clusters (Carstens et al. 2013). Poor knowledge about a species' life history or other key taxonomic attributes can lead to incorrect delimitation of species which can bias diversity estimates and confound inferences of community structure. Efforts that incorporate species delimitation based on molecular approaches, particularly those using DNA barcodes, have been useful in this regard (Hebert et al. 2004; Bickford et al. 2007). Yet, a wider array of datasets are usually needed to meaningfully delimit, discover and identify species (Will, Mishler, and Wheeler 2005). Integrative taxonomic approaches provide means for correctly delimiting species by combining various sources of information including phenotype and molecular data, distribution and ecology, phylogeography and population genetics, and life history traits (Dayrat 2005; Will, Mishler, and Wheeler 2005; Padial and De La Riva 2010). These are therefore ideal for more accurately characterizing species richness.

Integrative taxonomic studies tend to sample the highest possible number of taxa within a clade throughout its entire range, making them valuable for assessing broad diversity and macroevolutionary patterns. These approaches have also helped clarify species boundaries in hard-to-resolve species complexes and have been useful for uncovering cryptic diversity in multiple groups (Meegaskumbura et al. 2002; Damm, Schierwater, and Hadrys 2010; Barrett and Freudenstein 2011; Leavitt et al. 2011). Unfortunately, integrative studies seldom depict detailed species diversity patterns at local scales, hence are of less utility in conservation efforts. Studies focusing on small geographic regions could be help fill these gaps as they permit denser within-

taxon sampling and facilitate consideration of fine-scaled environmental parameters (e.g., habitat type).

Islands are ideal systems to address this issue. They are usually much smaller in size compared to continental areas, which enables a much greater sampling density, likely to fully capture the species richness of a taxon under study. Communities can also be more readily defined because island boundaries are discrete and area is fixed. Evolutionary processes in islands, such as radiations, may also lead to unique traits within a species, helping with species recognition. For example, in Hawaii, species with woody stems within the genus *Geranium* L. are only found in that archipelago (Pax, Price, and Michaels 1997; Kidd and Michaels 2005); whereas endemic species of the mint family have lost their characteristic scent (Morden and Loeffler 1999), a response usually linked to reduced herbivory.

Research on the biotas of the Caribbean islands, among the most biologically rich in the world (Myers et al. 2000), has increased our understanding of diversity patterns in insular regions. They have also provided unique insight into the processes of colonization, diversification and extinction (Ricklefs and Bermingham 2008). This body of work has resulted in better diversity estimates for many groups, including amphibians (Hass and Hedges 1991), rodents (Woods, Borroto Paéz, and Kilpatrick 2001), shrubs (Judd 2001) and lizards from the genus *Anolis*, the latter having undergone spectacular adaptive radiations in this region (Losos et al. 1998; Mahler et al. 2010). Unfortunately, there are comparatively less studies on some of the most diverse groups, such as lichens (Mercado-Díaz and Santiago-Valentín 2010; Mercado-Díaz, Lücking, and Parnmen 2014), organisms formed by a symbiotic relationship between a fungus and at least one photosynthetic partner (i.e., green algae and/or cyanobacteria). Lichens successfully colonize numerous habitats, including tropical forests where half of the estimated

global number of species is predicted to occur (Lücking et al. 2009, 2011).

Although lichens have been collected extensively in the Caribbean region (Imshaug 1957; Mercado-Díaz and Santiago-Valentín 2010), knowledge of island-level species richness is poor. Previous work has suggested that the Greater Antilles are home to nearly 3500 species (Acevedo-Rodríguez 1991), but estimates were not based on systematic inventories. In Cuba, an ongoing taxonomic inventory has identified nearly 1100 valid species names, but predictions suggest that about 2000 species could be present (Lücking et al. 2009). Although current documented richness for Puerto Rico (~1500 spp.) is comparable to estimated values (i.e., 1600 spp.; Lücking et al. [2009]), the taxonomic status of names has not been revised, suggesting that documented diversity is perhaps overestimated and that a considerable proportion of the actual diversity is yet to be discovered. Harris (1989) studied the lichen biota of Puerto Rico, but the taxonomy has not been revised since then. Further work will likely increase the documented species in Puerto Rico and nearby islands.

Sticta (Schreb.) Ach. is a genus of conspicuous foliose macrolichens easily recognized by their large size, formation of photosymbiodemes, and the presence of characteristic pores (i.e., cyphellae) in the lower surface. It is as a monophyletic genus with unresolved affinities with *Pseudocyphellaria* Vain. s.l. (Moncada, Lücking, and Betancourt-Macuase 2013; Widhelm et al. 2019), excluding taxa recently segregated to the genus *Lobaria* (Schreb.) Hoffm.—that is, *L. anomala* (Brodo & Ahti) T.Sprib. & McCune and *L. anthraspis* (Ach.) T.Sprib. & McCune (McCune et al. 2014). It is part of the recently circumscribed subfamily Lobarioideae in Peltigeraceae (Kraichak et al. 2018; Lumbsch and Leavitt 2019). The group has a subcosmopolitan distribution with species largely restricted to humid tropical mountains and few extending to temperate zones (Moncada and Lücking 2012; Moncada, Lücking, and Suárez

2013; Magain and Sérusiaux 2015). It has 200 species currently described (Lücking, Hodkinson, and Leavitt 2017), but more than 500 species are estimated to occur worldwide (Moncada, Lücking, and Suárez 2013). Together with other Peltigeraceae, *Sticta* species are excellent indicators of ecosystem health, and in many areas their diversity is threatened by land use change (Kalwij, Wagner, and Scheidegger 2005; Werth, Wagner, Holderegger, et al. 2006; Ranft et al. 2018).

Harris (1989) recognized nine putative species of *Sticta* in Puerto Rico based on morphology alone (Fig. S.1.1), but proper nomenclature for taxa was lacking. Limited geographic coverage of previous sampling efforts has also been an impediment for elucidating species richness patterns. Here, we used an integrative taxonomic approach to estimate species richness, understand species boundaries, determine levels of endemism and elucidate the evolutionary history of the group in Puerto Rico. We combined information from phylogenetic and species delimitation analyses with a comprehensive taxonomic re-evaluation of the group that included revision of historic material. Specifically, we sought answers to these questions: (1) How many species of *Sticta* occur in Puerto Rico? (2) What proportion of the biota is comprised of endemic species? (3) Does the morphology-based taxonomy proposed by Harris (1989) align with phylogeny? (4) Do *Sticta* species in Puerto Rico represent a monophyletic assemblage, suggesting radiation, or a polyphyletic group, suggesting multiple colonization events?

We include a species accumulation curve showing taxonomic knowledge of the genus as a function of collection efforts through time. This curve highlights the importance of taxonomic revisions and the value in integrating molecular methods with comprehensive sampling efforts to better characterize species richness in lichenized fungi. Formal taxonomic treatment for eight species and a key to identify taxa from Puerto Rico is provided. Conservation-related issues of

Sticta in Puerto Rico and the Caribbean are discussed.

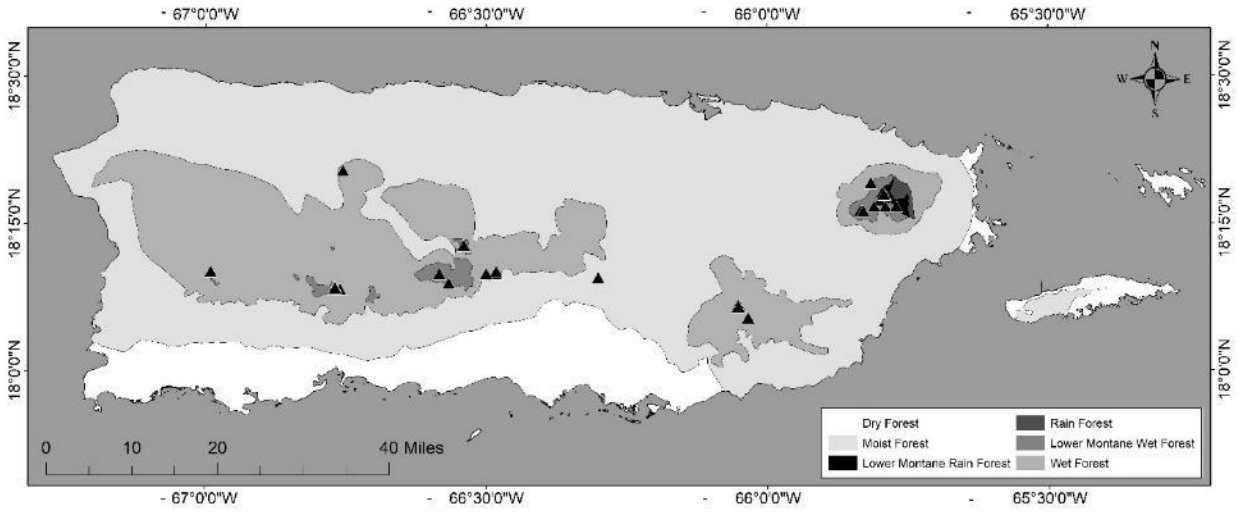

Figure 1.1. Map showing main collecting sites (triangles) of recent sampling of *Sticta* in Puerto Rico. The ecological life zones of Puerto Rico (Ewel and Whitmore 1973) are shown in shades of gray. High humidity and high elevation areas, which are preferred habitat for *Sticta* in the tropics, are represented by darker shades.

MATERIALS AND METHODS

Taxon sampling—Collecting efforts focused on well-conserved, mid- (ca. 200 m) to high-elevation (ca. 1300 m) areas with vegetation classified as humid or rain forests (Fig. 1.1). These are preferred habitat conditions for *Sticta* in the tropics (Moncada 2012). More than 80% of these areas are located inside protected forests that have been extensively surveyed in the past (e.g., El Yunque National Forest, Bosque Estatal de Carite, Bosque Estatal de Toro Negro). Sampling was carried out in these forests and in areas that have not been previously sampled but that contain suitable habitat for *Sticta* (i.e., Bosque Estatal Tres Picachos, Bosque Estatal de Maricao, Area Natural Protegida Cañón San Cristobal, and karstic forests associated with the

Tanamá river). Altogether, these areas contain the majority of *Sticta* habitat present on the island. These sites are also considered centers of high species richness and endemism in plants and contain relict primary forests that survived intense agricultural activities in the island during the early 20th century (Figueroa-Colón 1996). A total of 110 specimens were collected between October 2011 and July 2018 as part of these efforts. Most of this material was used to generate the molecular data presented in this work.

Taxonomic work—In addition to the 110 specimens collected for molecular work, we also inspected 170 historic specimens that are housed in the four largest herbarium collections of *Sticta* from Puerto Rico (i.e., LSU, MSC, NY, US). Altogether, these comprise more than 95% of all existing *Sticta* specimens collected in the island and include material used by R.C. Harris for his key (Harris 1989). Specimens in MICH are duplicates of material housed in NY and US. Recently collected material was brought to NY and side-by-side comparisons were made for determining correspondence with Harris's material. All specimens were inspected under dissecting microscopes, and photographs of each were taken for reference purposes. Thallus morphology of recently collected material was examined using a LEICA MS5 dissecting microscope. To assess microanatomy, sections of thalli and ascomata were cut by hand with a razor blade, mounted in wet slides and examined using ZEISS Axioskop 2 compound microscope. All measurements provided in taxonomic diagnoses below are given in water. High-performance thin-layer chromatography (HPTLC) was done using standard techniques with solvent C following Lumbsch (2002). Locality data for both historic and recently collected specimens was tabulated for assessing geographic distribution of species with respect to suitable *Sticta* habitats in the island. Coordinate data was georeferenced when available using ArcView 10 and overlaid with digital geographic layers (i.e., shapefiles) for ecological life zones (Ewel

and Whitmore 1973) (Fig. 1.1) and natural protected areas (downloaded from www.gis.pr.gov; not shown). Considering both historic collections and recent efforts, localities where *Sticta* has been collected have been sampled more than three times, the only exceptions being the Tanamá river area (sampled once), and the Cañón San Cristobal Natural Protected Area and the Bosque Estatal de Guilarte, which were sampled twice.

Nomenclatural determinations were made based on all data available and analyses performed. Yet, we refrained from recognizing lineages as species when delimitation methods (described below) were in considerable conflict.

DNA extraction, amplification and sequencing—We used the ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, Irvine, California, U.S.A.) and the SIGMA RED Extract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) to extract DNA from a selection of specimens (92). Except for the use of 15 ml of extraction buffer and 15 ml dilution buffer with the SIGMA kit, extractions followed manufacturer's instructions.

Six loci were sequenced, including the internal transcribed spacer (ITS ~ 600 bp), which is the universal barcode for fungi (Schoch et al. 2012), the mitochondrial small subunit (mtSSU ~ 800 bp), the nuclear large subunit (nuLSU ~ 550 bp), the DNA replication licensing factor (*MCM7* ~ 600 bp), the RNA polymerase II largest subunit (*RPB1* ~ 900 bp), and RNA polymerase II second-largest subunit (*RPB2* ~ 700), the latter three being low-copy nuclear protein-coding genes. Except for *RPB2*, these loci were also used in Widhelm et al. (2018).

Primers and PCR conditions used in this study are described in table 2 of Widhelm et al. (2018). We designed new *Sticta*-specific primers for *RPB2* due to problems amplifying this locus with traditional primers. Primer sequences and PCR conditions were as follows: RPB2_*Sticta*_1F: AAGCCGGTGTCTCTCAAGTG, RPB2_*Sticta*_1R:

GGCGCTTTGACTCGTTTGTT, 94°C for 3 min; 34 cycles: 94°C for 45 s, 50°C for 1 min, 72°C for 1.5 min; 72°C for 7 min. PCR amplification was carried out using 6.25 µl MyTaq Red DNA Polymerase (Bioline, Taunton, Massachusetts, U.S.A.), 0.25 µl of each primer (10 µm), 5.25 µl of nuclease-free water and 0.5 µl of diluted genomic DNA (10×) for a total of 12.5 µl per reaction. Amplification products were visualized on 1% agarose gels stained with ethidium bromide and subsequently purified with Exo SAP-IT (USB, Cleveland, Ohio, U.S.A.), following the manufacturer's instructions. Sequencing was performed using Big Dye Terminator v.3.1 (Applied Biosystems, Foster City, California, U.S.A.) and the same primers used for amplification. The cycle sequencing conditions were as follows: 96°C for 1 min; 25 cycles: 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Sequenced products were precipitated with nuclease-free water, EDTA, and 70% EtOH before they were loaded on an ABI 3730 (Applied Biosystems) automatic sequencer. Molecular work was carried out at the Pritzker Laboratory for Molecular Systematics at the Field Museum, Chicago, Illinois, U.S.A.

Phylogenetic analysis—Newly generated sequences are listed in Appendix 1. These sequences were assembled in Geneious v.8.1.7 (https://www.geneious.com) and queried in GenBank's BLASTn suite (Benson et al. 2018) to exclude potential contaminations. The "auto" mode threshold and default settings for MAFFT v.7.017 (Katoh and Standley 2013) plugin in Geneious were used to generate both single-locus and multilocus concatenated alignments. Alignments were visually inspected and manually corrected if needed. Sequences used include those described in table 1 from Widhelm et al. (2018), which include the outgroups *Lobaria pulmonaria* (L.) Hoffm. and *Pseudocyphellaria crocata* (L.) Vain., and sequence data from two additional isolates in GenBank – *Sticta beauvoisii* Delise (Miadlikowska et al. 2006) and *Ricasolia amplissima* (Scop.) De Not. (Appendix 1). The latter was also used as an outgroup. A

total 300 specimens of *Sticta* from Puerto Rico and other parts of the world are included in these alignments. The Gblocks web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to identify and remove ambiguously aligned sites in the ITS alignment, which showed lower levels of sequence conservation. Options for a less stringent selection (allowing for smaller final blocks, gap positions within the final blocks, and less strict flanking positions) were selected (Castresana 2000; Talavera and Castresana 2007; Tan et al. 2015).

We used both Bayesian and maximum likelihood approaches for phylogenetic reconstructions. The program RAxML v.8.1.16 (Stamatakis 2014) was used for maximum likelihood analysis that employed a GTR+Γ substitution model. The bootstrap convergence test using the extended majority-rule consensus tree criterion (auto MRE) was used for *a posteriori* bootstrapping analysis. Topological conflict between individual gene trees was also assessed with RAxML. This analysis entailed searching for the best ML tree under the GTR+Γ model, using at least 100 bootstrap replicates and other default settings. No major conflicts were observed between trees obtained, therefore analysis proceeded with multilocus concatenated datasets. Sequence matrices were partitioned in RAxML using the -q option. For Bayesian analysis, we first evaluated models of DNA evolution for each locus with the program jModelTest v.2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). The models with the lowest Akaike information criterion (AIC) scores were considered best and were selected as follows: ITS: GTR+Γ, *MCM7*: K80+I+Γ, mtSSU: GTR+I+Γ, nuLSU: GTR+I+Γ, *RPB1*: GTR+Γ, *RPB2*: SYM+Γ. We used the Cipres Gateway server (http://www.phylo.org/portal2/login!input.action) to run MrBayes v.3.2.6 (MrBayes on XSEDE) (Miller, Pfeiffer, and Schwartz 2010; Ronquist et al. 2012). Two parallel runs with 30 million

generations, starting with a random tree and employing four simultaneous chains, were used. Heating of chains was set to 0.2. Tree posterior probabilities were estimated by sampling trees using a variant of the Markov Chain Monte Carlo (MCMC) method. Every 1000th tree was sampled to avoid autocorrelation. Parameter values and trees were summarized using a 25% burn-in. The remaining 22,500 trees were pooled to calculate a 50% majority-rule consensus tree. The outputs of MrBayes were inspected in Tracer v.1.5 (Rambaut and Drummond 2009) to assess convergence of different parameters, determine the approximate number of generations at which log likelihood values stabilized and identify the effective sample size (ESS) for each parameter. Additionally, the average standard deviation of split frequencies (Lakner et al. 2008) was monitored to ensure it dropped below 0.1, and the potential scale reduction factor (Gelman and Rubin 1992) for all parameters was examined and found to approach 1.0. Only clades with bootstrap support equal or above 70% under ML and posterior probabilities equal or above 0.95 in Bayesian analysis were considered strongly supported. Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Species delimitation analyses—We assessed species boundaries using three species delimitation methods (i.e., PTP, BPP, GMYC). Details about these methods are provided in the next sections, but in general, these programs perform delimitations by evaluating phylogenetic trees with branches representing either nucleotide substitutions (i.e., PTP) or time (i.e., GMYC). BPP, on the other hand, performs delimitations by simultaneously analyzing multilocus sequence alignments and population data.

Even though PTP and GMYC were designed for single-locus data, both methods are increasingly being applied to multilocus datasets (Luo et al. 2018); therefore, delimitations were done using both ITS and multilocus trees. RAxML trees were used for PTP, whereas two new

ultrametric trees (using both ITS and multilocus alignments) were generated with BEAST v.1.10.4 (Suchard et al. 2018) for GMYC. At least two independent BEAST analyses were run on the CIPRES Science Gateway for the latter. Chain lengths for each of these analyses were of 1×10^8 with a sampling frequency of 10,000. Convergence and mixing of parameters were evaluated in Tracer v.1.6 (Rambaut and Drummond 2009), and effective sample sizes (ESS) were confirmed to be >200. Trees from independent runs were combined in LogCombiner v.1.8.0 (Rambaut and Drummond 2013) after excluding the first 25% of sampled trees as burn-in. A maximum clade credibility tree was generated in TreeAnnotator v.1.8.2 (Rambaut and Drummond 2013b) from the combined posterior distribution of trees using a 0.5 posterior probability cutoff.

Removing identical haplotypes before running PTP and GMYC has been recommended because these methods could be affected by polytomies and zero-length terminal branches (Fujisawa and Barraclough 2013; Talavera, Dincă, and Vila 2013). In our case, analyses with trees including identical sequences did not result in major conflicts. Given that one goal of this work is to compare results between methods, and because removing identical haplotypes might lead to overestimates of parameters in BPP (Yang 2015), species delimitation results highlighted in main figures are based on our complete dataset.

Poisson Tree Processes (PTP)—The Poisson Tree Processes (PTP) is a maximum likelihood point estimate of putative species boundaries on a rooted phylogenetic tree. It uses number of substitutions to model speciation rate or branching events based on the assumption that the number of substitutions between species is significantly higher than number of substitutions within species (Zhang et al. 2013). Because the method requires trees with branches equivalent to the number of substitutions, the delimitation schemes were based on both

multilocus and ITS RAxML trees. Delimitation analysis was run using the bPTP web server (https://species.h-its.org/ptp/).

Our sampling includes many singleton species and generally well-sampled taxa from Puerto Rico. Because it has been suggested that the recent multi-rate implementation of PTP (i.e., mPTP) might underperform compared to PTP-ML when sufficient intraspecific sampling is lacking, (https://groups.google.com/forum/#!topic/ptp-species-delimitation/udcMEZF__P4) results reported here are based on the more general PTP-ML.

Generalized mixed Yule-coalescent (GMYC)—The Generalized mixed Yule-coalescent (GMYC) is a likelihood-based method for delimiting species by fitting within- and between-species branching models to reconstructed gene trees (Fujisawa and Barraclough 2013). It assumes that species are independently evolving entities that accumulate mutations that result in distinctive genetic clusters. These clusters are separated from the rest by longer internal branches (Barraclough, Birky, and Burt 2003; Acinas et al. 2004). Genetic clusters are delimited by optimizing the set of nodes that define shifts between intraspecific and interspecific processes. Optimization consists in finding the maximum likelihood (ML) solution for a model that combines diversification between these processes (Fujisawa and Barraclough 2013).

Ultrametric trees generated in BEAST were used for this analysis. We used the R statistical software package SPLITS v.1.0-19 (Ezard, Fujisawa, and Barraclough 2009) to implement the GMYC species delimitation tool (Pons et al. 2006; Fontaneto et al. 2007). As recommended by Fujisawa and Barraclough (2013), only results from $GMYC_{simple}$ are presented.

Bayesian Phylogenetics Phylogeography (BPP)—BPP uses the multispecies coalescent model to compare different models of species delimitation and species phylogeny in a Bayesian framework. The method accounts for incomplete lineage sorting due to ancestral polymorphism

and gene tree–species tree conflicts (Yang and Rannala 2010, 2014; Rannala and Yang 2013, 2017). BPP requires the use of a fully resolved "guide tree" and *a priori* assignment of samples to individual populations. We used the unguided species delimitation analysis ("A11"), which attempts to merge different populations into one species, while never attempting to split one population into multiple species (Yang 2015). To take full advantage of this feature, populations within each Puerto Rican clade were defined as the least-inclusive subclades that showed high bootstrap support in our multilocus RAxML tree.

Following Leaché and Fujita (2010), we evaluated four different combinations of priors on population size parameters (θ_s) and divergence time at the root of the species tree (τ_0). These priors are assigned an inverse-gamma distribution IG (α, β) with a mean $m = \beta / (\alpha - 1)$ and variance $s^2 = \beta^2 / [(\alpha - 1)^2 \cdot (\alpha - 2)]$. The scenarios evaluated assume relatively small ancestral population sizes and shallow divergences ($\theta_s \sim$ IG [3, 0.002] and $\tau_0 \sim$ [3, 0.002]), small ancestral population sizes and deep divergences ($\theta_s \sim$ IG [3, 0.002] and $\tau_0 \sim$ [3, 0.2)]), large ancestral population sizes and shallow divergences ($\theta_s \sim$ IG [3, 0.2] and $\tau_0 \sim$ [3, 0.002]) and large ancestral population sizes and deep divergences ($\theta_s \sim$ IG [3, 0.2] and $\tau_0 \sim$ [3, 0.2]). The other divergence time parameters were specified by the uniform Dirichlet distribution (Yang and Rannala 2010: equation 2). Each analysis was run at least twice to confirm consistency between runs. Both rjMCMC algorithms (0 and 1) were evaluated. The number of MCMC iterations was 220,000 (burnin = 10000, sample freq. = 2, num. samples = 100000). Sequence divergence in our full taxon sampling is higher than 10%, hence BPP analysis was carried out on clades from Puerto Rico and immediate sister taxa exclusively. Results presented are based on the second combination of priors (i.e., small ancestral population sizes and deep divergences) as they likely fit better the evolutionary history of the group (see Discussion). Results from other combinations

are presented in Table S.1.1. for comparative purposes. Analysis was carried out with BPP v.3.4 (Yang 2015).

Species accumulation over time—A species accumulation curve based on species sampled by different collectors throughout time was generated to illustrate the importance of increasing geographic coverage of sampling and integrating molecular methods for estimating species richness. Species names of historical collections were updated following the taxonomic revision presented here. Collection dates were obtained from specimen labels and used as sampling units. The function SPECACCUM (method = "collector") from the R package "vegan" v.2.5-6 (Oksanen et al. 2019) was used to generate the curve.

RESULTS

Phylogenetic analysis and species delimitation—We obtained new sequence data for 83 specimens from Puerto Rico. These efforts resulted in 298 newly generated sequences for ITS (71), *MCM7* (40), mtSSU (16), nuLSU (59), *RPB1* (46), and *RPB2* (66). Removal of ambiguously aligned regions with Gblocks produced a reduced-sized ITS alignment of 444 bp. Two multilocus concatenated alignments were consequently generated, one using the Gblocks output (4545 bp) and another using the complete ITS alignment (4710 bp). Sequence data for this chapter are available in the supplementary materials of the published paper (https://onlinelibrary.wiley.com/doi/abs/10.1002/tax.12320. Last accessed: 7/15/2021). Even though coverage for mtSSU was low in our final alignments, we decided to keep data from this gene for downstream analyses given that previous work has found no evidence of biased phylogenetic inferences resulting from analyzing datasets with different levels of missing data (Wiens and Morrill 2011). Phylogenetic reconstructions generated with these two concatenated

alignments yielded similar topologies; therefore, trees presented are based on alignments using the complete ITS dataset. Results are highlighted using our MrBayes tree, which also include results from our multilocus species delimitation analyses (Fig. 1.2. [full version in Fig. S.1.2.]). Refer to Fig. S.1.3. for the multilocus RAxML tree. The likelihood value for the two cold chains in our Bayesian trees was −33,953.99 and −33,972.26 whereas the final optimization likelihood for the ML tree was −35,919.15. Instances of conflict between inference methods are highlighted when needed.

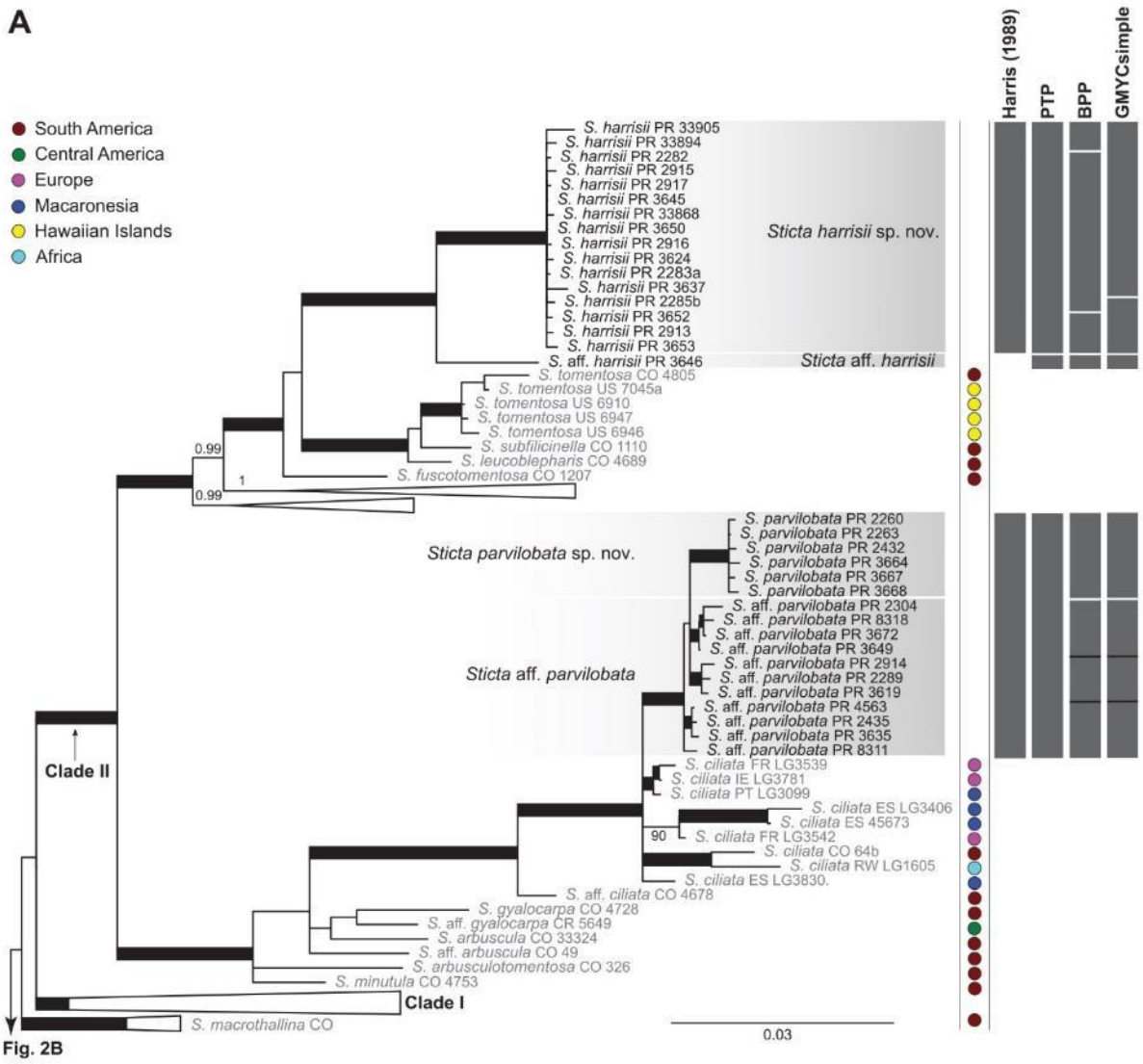

Figure 1.2. Maximum Clade Credibility (MCC) tree obtained from MrBayes based on six nuclear and mitochondrial loci (ITS, MCM7, mtSSU, nuLSU, RPB2, and RPB1). The tree shows species delimitations of Puerto Rican taxa that were used by Harris (1989) or obtained by analyzing the full taxon dataset with PTP, GMYC and BPP (gray boxes to the far right). Black lines separate species that were nested within other delimited species. That is, samples above the upper black line and below the lower black line were delimited as the same species. Missing boxes for Puerto Rican taxa in Harris (1989) indicate that specimens for the taxon in question were not obtained during that effort. Sequence data for 300 specimens of *Sticta* from Puerto Rico (80) and the rest of the world (220) (most in collapsed branches) were used to generate this tree. Clades I-V from Widhelm et al. (2018) are included for reference

purposes (*S. macrothallina* is considered part of Clade II in that work). Geographic origin of samples from other regions is indicated by circles with different colors/patterns in the "Geography" column. Puerto Rican taxa are in black font and are highlighted using a gray gradient. Thickened branches indicate that the clade has both >0.95 posterior probabilities and >70 bootstrap statistical support. Clades supported only by Bayesian analysis show posterior probability values above branches, whereas those exclusively supported by maximum likelihood have bootstrap support below branches.

Multilocus phylogenetic analysis shows that *Sticta* is a well-supported monophyletic clade with a strongly supported sister-group relationship with *Pseudocyphellaria* (Fig. 1.2.). Within the ingroup, material from the island is distributed in 11 monophyletic clades, the exceptions being *S.* aff. *parvilobata* and *S. borinquensis* sp. nov., which are paraphyletic, and *S.* aff. *harrisii*, which is represented by a single specimen (Fig. 1.2., Figs. S.1.2., S.1.3.). Concurrent morpho-anatomical analysis indicated that these clades correspond to distinct species (see Taxonomic treatment), most being closely related to species from South and Central America. *Sticta parvilobata* sp. nov. + *S.* aff. *parvilobata*, on the other hand, have close affinities with *S. ciliata* Tayl., which is widely distributed (Fig. 1.2.).

Our specimens agree with four of the species delimited in Harris (1989) (Fig. 1.2.). Six species (i.e., *S.* aff. *harrisii*, *S. weigelii* (Ach.) Vain. s. str., *S.* aff. *borinquensis*, *S. corymbosa* sp. nov., *S. guilartensis* sp. nov. and *S.* aff. *guilartensis*) were missed in collecting efforts associated with that work, whereas species "*Sticta* sp. 22678" and "*S. trichographis* Fée ined." contained material representative of two species each, *S. parvilobata* + *S.* aff. *parvilobata* and *S. densiphyllidiata* sp. nov. + *S. riparia* sp. nov., respectively.

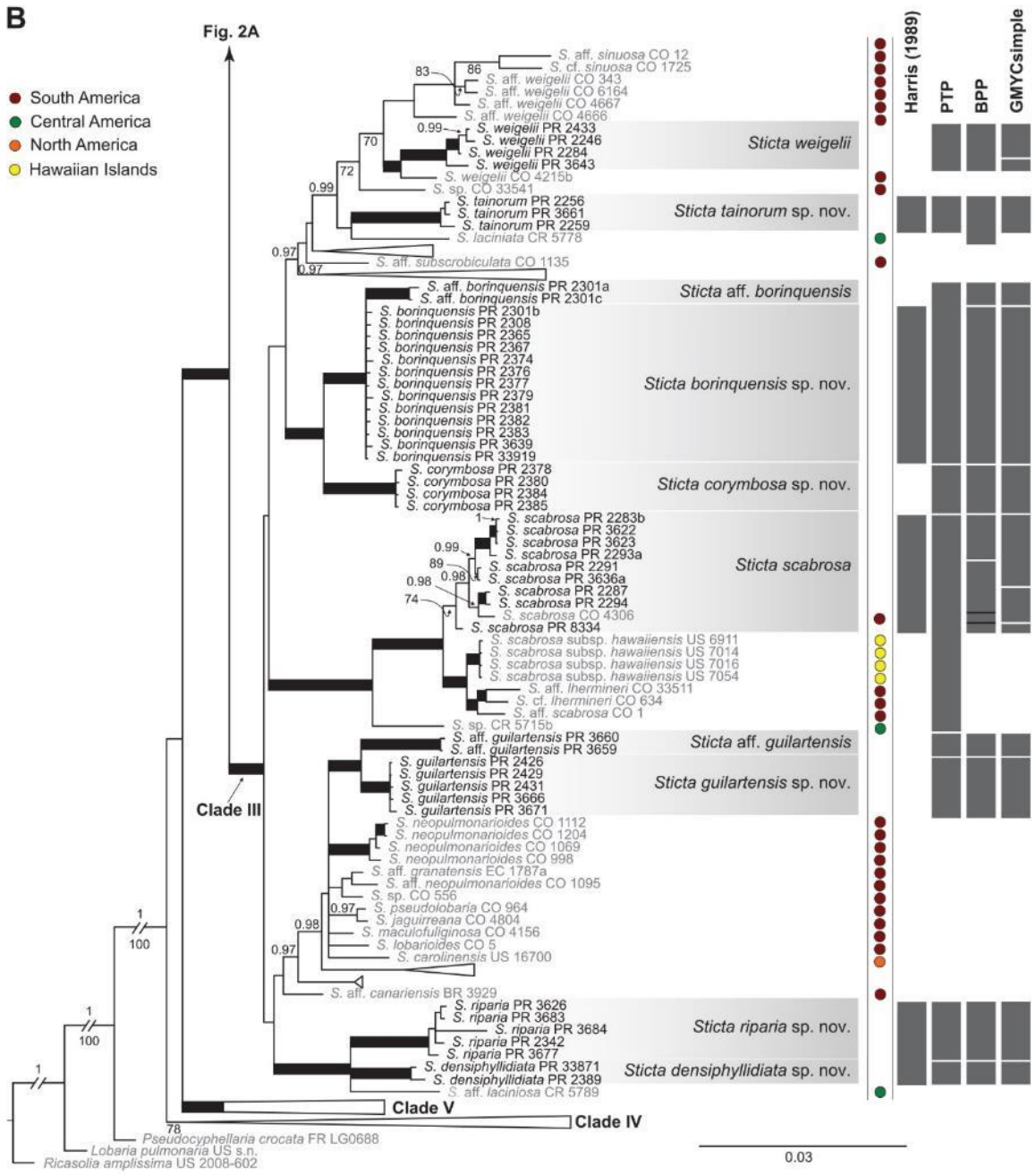

Figure 1.2. Continued.

Species delimitation analyses on our multilocus tree showed that PTP was the most conservative among methods used. It delimited 9 of the 14 species identified in our tree but

failed at separating *Sticta parvilobata* from *S.* aff. *parvilobata*, *S. borinquensis* from *S.* aff. *borinquensis*, and "*S. scabrosa* ined." from several taxa from other regions (Fig.1.2.). BPP and GMYC estimated a slightly higher species diversity, both delimiting 10 of the recognized species. Both methods, however, similarly over split several species, namely *S. harrisii* sp. nov., *S.* aff. *parvilobata* and "*S. scabrosa*". Delimitations on single-locus (ITS) trees, on the other hand, were similar to those from multilocus trees, but were generally more liberal. For instance, multilocus PTP analysis delimited *S. parvilobata* and *S.* aff. *parvilobata* as the same species, but single-locus delimitation regarded them as separate (Fig. S.1.4.). Similarly, single-locus GMYC analysis split *S.* aff. *parvilobata* into three species, whereas GMYC based on our multilocus tree delimited only two species (Fig. S.1.5.).

Results from species delimitation analysis with BPP under all combinations of priors are shown in Table S.1.1. There were several instances of agreement, particularly between delimitations that assumed priors that modelled either shallow or deep divergences. Major differences were linked to priors that assumed large ancestral population sizes. That is, delimitations that assumed large ancestral population sizes tended to delimit less species compared to delimitations assuming small ancestral populations.

Species accumulation over time—Patterns of species accumulation as a function of sampling efforts over time are shown in Fig. 1.3. Drastic increases in the number of species recorded in the island were first observed between 1900 and 1920 when collectors mainly from the United States, which included Elizabeth G. Britton and Nathaniel L. Britton from NY, visited the island (Mercado-Díaz and Santiago-Valentín 2010). Species richness increased during a second period from 1960 to 1970 mostly due to efforts by Ismael Landrón-Concepción during his work on *Ramalina* Ach. (Landrón-Concepción 1972). Species richness remained at 11

species from 1975 to 2011 when efforts for the present work, which included increased sampling efforts and use of molecular data, started (dashed line, Fig. 1.3). As a result, a third increase in number of species recorded for the island was documented between 2011 and 2019.

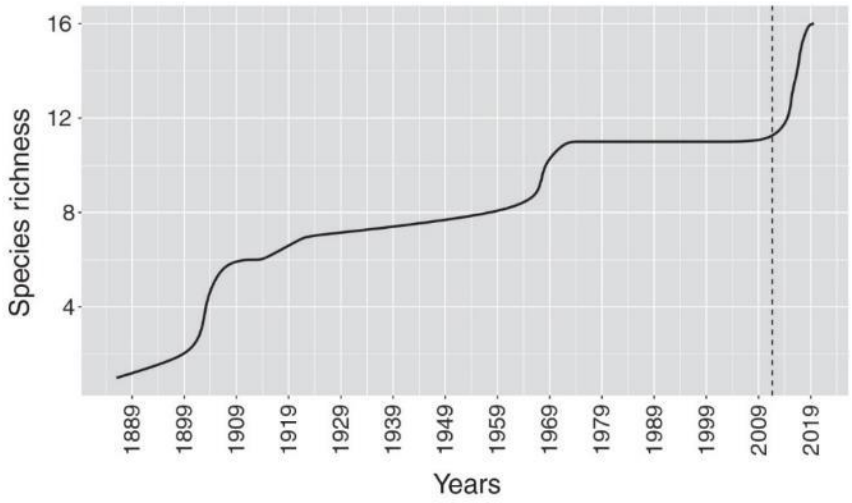

Figure 1.3. Species accumulation curve showing species richness of *Sticta* in Puerto Rico as a function of sampling efforts over time. The black line represents species richness known at a specific sampling year in the horizontal axis. Dashed vertical line in grey represents the year 2011 when we began expanding sampling efforts and incorporating molecular methods for studying *Sticta* species richness in Puerto Rico.

DISCUSSION

Phylogenetic patterns, species richness and potential endemism—Phylogenetic patterns recovered in this work are similar to those observed by others (Moncada, Lücking, and Betancourt-Macuase 2013; Widhelm et al. 2018) and support the placement of *Sticta* as a monophyletic group sister to *Pseudocyphellaria*. (Moncada, Lücking, and Betancourt-Macuase 2013), on the other hand, did not find strong support for this sister relationship. Phylogenetic relationships within this group were recently assessed by Widhelm et al. (2019) using a target

enrichment approach of 400 single-copy nuclear genes. Although conflicting topological patterns among data types and phylogeny reconstruction methods used were observed, *Sticta* was most often recovered as sister to *Yarrumia* D. J. Galloway, a recently segregated genus from *Pseudocyphellaria* (Galloway 2015) not included in (Moncada, Lücking, and Betancourt-Macuase 2013).

We recovered a topology highly similar to the one in Widhelm et al. (2018) and found strong support for the major clades highlighted in that work, except for Clade IV, which was strongly supported only in our maximum likelihood analysis (Fig. 1.2.). Clade V was strongly supported but was recovered as sister to the clade containing Clades I, II, III as opposed of being sister to these clades and Clade IV, as was shown in Widhelm et al. (2018). Lack of support for Clade IV in our Bayesian phylogeny and conflicting placement of Clade V between both studies are likely due to slight differences in parameters used for phylogenetic reconstructions and in attributes of gene datasets analyzed (e.g., an additional marker [*RPB2*] was used in this study). Complex historical processes might also underlie some of these conflicts, as has been recently proposed by Widhelm et al. (2019). These could potentially confound phylogenetic reconstructions and result in clades with short branches and poor support such as the one containing Clades I, II, III and V in Fig. 1.2. Interpretation of phylogenetic relationships among these clades should consequently be approached conservatively.

Most of the species represented in our main phylogeny (11) were recovered as monophyletic clades. Topological relationships between *S. parvilobata* + *S.* aff. *parvilobata* and *S. borinquensis* + *S.* aff. *borinquensis* were not fully resolved and/or had some degree of clade substructure and will require further study with additional data. Moreover, morpho-anatomical and chemical attributes of material representative of most of these species presented unique

combinations of characters that are not known from other species within the genus, the exceptions being *S.* aff. *parvilobata*, which is morphologically cryptic with respect to *S. ciliata* (discussed below), and *S. scabrosa* and *S. weigelii*, which represent well-known, widely distributed species (Moncada 2012; Moncada, Mercado-Díaz, Smith, et al. 2021).

Results from our analysis justify the formal taxonomic recognition of eight new species from Puerto Rico, whereas some of the lineages that are either not well resolved phylogenetically or represent few collections or singletons are labeled with only provisional designations at this moment. To this end, the following species are formally established: *S. borinquensis* sp. nov., *S. corymbosa* sp. nov., *S. densiphyllidiata* sp. nov., *S. guilartensis* sp. nov., *S. harrisii* sp. nov., *S. parvilobata* sp. nov., *S. riparia* sp. nov., and *S. tainorum* sp. nov. (refer to "New Taxa" section). Two phylogenetically recognized lineages (*S.* aff. *borinquensis*, *S.* aff. *guilartensis*) and *S.* aff. *harrisii* are considered distinct species but are not described here due to poor quality or insufficient amount of material to adequately assess morphological characters. We refrained from recognizing new species within *S.* aff. *parvilobata* because genetic variability and lack of distinctive morphological characters in material representative of this group prevented us from confidently separating it from *S. ciliata*. On the other hand, material from *Sticta* sp. 320 Harris (1989), a morphologically distinct species within the island (see below), was not collected during recent efforts. Given the general concordance between morphology and phylogeny that has been recently highlighted for this genus (Moncada, Lücking, and Suárez 2013), there is little doubt that this lineage represents a phylogenetically distinct species-level clade. We refrained from providing a formal description for it because historical material from this species is also scarce. The morphotype referred to as *Sticta* sp. 3725 in Harris (1989), on the other hand, is not considered different from *S. borinquensis* in spite of its K+ purple medullary reaction (refer to

"Remarks" section in the *S. borinquensis* description below). It is therefore presumed to be represented in our phylogeny. Lastly, one specimen in US (*Hale 38304*) is similar to *Sticta* sp. 320 but considered a different species due to several morphological differences (see below). This species will remain undescribed and phylogenetically unresolved until additional material is obtained. Taken together, *Sticta* in Puerto Rico is represented by at least 16 species-level lineages, two solely recognized on morphological grounds (i.e., *Sticta* sp. 320 and *Sticta* sp. 38304). Further work should help determine if *S.* aff. *parvilobata* is comprised of one or several species-level lineages or if its conspecific with the widespread *S. ciliata*.

From a conservative standpoint, if we consider the two confirmed widespread species present on the island (*Sticta scabrosa*, *S. weigelii*) and assume that *S.* aff. *parvilobata* is eventually resolved as a geographic variant of *S. ciliata*, and that *Sticta* sp. 320 and *Sticta* sp. 38304 are found to represent widely distributed species, the minimum number of potentially endemic species level-lineages for Puerto Rico is 11 (i.e., the 8 newly described species + *S.* aff. *harrisii*, *S.* aff. *borinquensis* and *S.* aff. *guilartensis*). Putative endemism for this group in the island might therefore reach 69% (11 out of 16 species), a reasonable figure considering that islands are known to promote speciation and high levels of endemism in many types of organisms (Losos and Ricklefs 2009). In the Caribbean, high endemism has been reported for many groups including reptiles (Scantlebury 2014), amphibians (Hedges 1989; Rodríguez et al. 2010), bats (Dávalos 2007), and plants (Liogier and Martorell 2000; Francisco-Ortega et al. 2007). For lichens, the scenario is less clear because diversity and distribution patterns have been less studied. Nevertheless, studies on *Sticta* and other Lobariaceae from other islands show levels of endemism similar to those reported for other groups and also to those reported here. For instance, previous phylogenetic studies revealed high endemism of 75% in the Hawaiian

archipelago for the genera *Pseudocyphellaria* (Moncada, Reidy, and Lücking 2014) and *Lobariella* Yoshim. (Lücking, Moncada, and Smith 2017), whereas endemism based on morphological species concepts had been estimated at zero percent for these taxa. Recent phylogenetic work suggests that 9 out of the 13 species of *Sticta* found in Hawaii are endemic to this archipelago (i.e. 69% endemism, Moncada, Lücking, and Lumbsch 2020). Simon et al. (2018) showed that most *Sticta* species (31 out of 35 [89%]) found in Madagascar and the Indian Ocean Islands are restricted to either the Mascarene archipelago or a single region in Madagascar. Several other lichen genera from Puerto Rico, such as *Ocellularia* G. Mey. and *Cladestinotrema* Rivas Plata & al., are also believed to contain high number of endemics (Mercado-Díaz, Lücking, and Parnmen 2014). Unfortunately, as stated before, the lichen biota in other islands of the Caribbean is still poorly known, particularly those of the larger islands of Cuba, Jamaica, and Hispaniola. For *Sticta*, only 4, 11 and 2 species have been formally recognized in each of these islands, respectively (Imshaug 1957). This means that potential endemism within Puerto Rico reported here (69%) might be lower because further sampling efforts in these islands could show that some of these lineages are more widely distributed in the Caribbean. For instance, floristic similarities between Puerto Rico and the Lesser Antilles have been previously noted (Dewalt, Ickes, and James 2016) and attributed to exposure to similar climatic regimes and presumed increased migration between these regions during the Pliocene (Beard 1949). This hints that some of the putatively endemic species described here are likely to be found in high-elevation islands in the Lesser Antilles (e.g., Martinique, Guadeloupe, Dominica). Their presence in other islands of the Greater Antilles is also plausible, particularly if we consider the direction of hurricanes and their role as potential agents of long-distance dispersal (Andraca-Gómez et al. 2015). In paleogeographic terms, however, this latter scenario is

less likely because *Sticta* was emerging as an independent evolutionary group (possibly from South America) between the Oligocene and the Miocene (Widhelm et al. 2018), which is the period when Puerto Rico separated from Hispaniola and the rest of the Greater Antilles (Graham 2003). *Sticta* was therefore absent during a period that likely witnessed substantial biotic exchange between these areas. This is supported by our recent delimitation of *S. damicornis* (Sw.) Ach. as a Caribbean endemic occurring only in Cuba, Jamaica and Hispaniola, but not occurring in Puerto Rico (Moncada, Mercado-Díaz, and Lücking 2018).

Evolution of *Sticta* in Puerto Rico—Species from Puerto Rico do not form a single clade as would be expected in the scenario of a single origin and subsequent radiation, e.g., in the case of Madagascar and the Mascarenes (Simon et al. 2018). They form a polyphyletic assemblage of a few, widely distributed species that likely evolved elsewhere and colonized the island, and species that evolved *in situ* after multiple colonization events of ancestral lineages. These colonization events likely happened via long-distance dispersal because the origin of the group (30 million years ago [mya], Widhelm et al. [2018]) postdates the Late Cretaceous (~ 76 mya), when the Proto-Antilles were at their closest distance to North and South America (Hedges, 2006). *Sticta* is also younger than the quasi-continuous land-bridge that allegedly connected South America to the Greater Antilles during the Eo-Oligocene boundary (~ 34 mya) (Iturralde-Vinent and MacPhee 1999; but see Ali 2012), supporting our hypothesis of long-distance dispersal origin for the group.

Although *Sticta* diversity on the island has not originated via major *in situ* radiations, the possibility of active micro-radiations in some clades cannot be ruled out. For example, while results from phylogenetic reconstructions, species delimitation analysis, and evaluation of morpho-anatomical characters did not yield unambiguous evidence for hidden cryptic diversity

within *S.* aff. *parvilobata* and *S. scabrosa*, these analyses made clear that both lineages exhibit considerable haplotype variability at local scales. This hints at the presence of ongoing selective pressures that may lead to the evolution of new species (Levin 2000; Givnish 2010; Madriñán, Cortés, and Richardson 2013). In *S.* aff. *parvilobata*, observed short branching patterns and lack of monophyly suggest that this lineage might be undergoing a rapid radiation and/or contain recently diverged species (Shaw et al. 2003; Leavitt, Grewe, et al. 2016; Widhelm et al. 2019). This agrees with Magain and Sérusiaux (2015) observations on *S. ciliata* as a clade that is in active divergence and dispersion and that may require recognition of additional species. Unique haplotypes of *S. scabrosa* from Puerto Rico were also recently recognized, although they were taxonomically resolved as geographic variants of this species (Moncada, Mercado-Díaz, Smith, et al. 2021). Our finding that a *S. scabrosa* sample from Colombia was, according to several delimitation methods, conspecific with samples from Puerto Rico and Hawaii, supports this hypothesis.

The lack of evidence of major evolutionary radiations for this genus within the island was somewhat surprising. These events are known to be major factors shaping diversity patterns in this region. One example are the multiple radiation events of different *Anolis* ecomorphs in each island of the Greater Antilles (Losos et al. 1998; Mahler et al. 2010; Losos 2009). Likewise, recent work suggests that many genera of endemic seed plants of the Caribbean originated via *in situ* radiations within islands (Nieto-Blázquez, Antonelli, and Roncal 2017). Regional-scale radiations, on the other hand, have been amply documented in multiple groups apart from *Anolis* (e.g., frogs from the genus *Eleutherodactylus* [Hedges 1989; Heinicke, Duellman, and Hedges 2007; Rodríguez et al. 2010], Phylostomid bats [Dávalos 2007], extant and extinct non-volant mammals [MacPhee and Iturralde-Vinent 1995; Van der Geer et al. 2010; Fabre et al. 2014;

Brace et al. 2015], plants [Michelangeli et al. 2008; Perret et al. 2013], etc.), but additional sampling on other islands will be needed to determine if this type of process has contributed to present-day diversity patterns in this group.

Geographic affinities—Phylogenetic analysis revealed that most taxa from Puerto Rico are associated with South American clades, which hints at stronger biogeographic links to that continent. For instance, many of the non-flying terrestrial vertebrate groups (Hedges 2006; Marivaux et al. 2020) and even some invertebrates (e.g. McHugh et al. 2014) have their closest relatives in South America. Less evidence is available for plants, but recent work suggests that most West Indian *Adiantum* L. species originated from immigration events from that continent during the Miocene (Regalado et al. 2018).

These biogeographic affinities are reasonable considering that *Sticta* may have originated in the New World (Widhelm et al. 2018) and that South America is apparently a center of diversity for this group (Moncada, Lücking, and Suárez 2013); however, close links of several clades (e.g., *Sticta harrisii* + *S.* aff. *harrisii* and *S. parvilobata* + *S.* aff. *parvilobata*) to lineages from North and Central America or from extra-Neotropical areas (e.g., Hawaii, Macaronesia, Europe) hint at a more complex scenario. Active dispersers such as birds, bats or freshwater fishes, for example, are believed to have colonized the Caribbean islands from North and Central America (Hedges 2006). In their recent study, (Nieto-Blázquez, Antonelli, and Roncal 2017) found that South America was the main ancestral area for only 5 of the 32 Caribbean endemic seed plant genera they analyzed, which is surprising considering floristic similarities that have been documented for many plant genera between both regions (Acevedo-Rodríguez and Strong 2008). Furthermore, our dataset has a moderate overrepresentation of South American lineages, which might obscure underlying patterns. Molecular approaches of historical biogeography

aimed at reconstructing the ancestral ranges of Caribbean taxa coupled with additional collecting activities in poorly studied regions will be needed to evaluate these patterns in more detail.

Species delimitation analyses—Species delimitation analyses have been amply used for exploring diversity patterns in lichenized fungi, particularly for assessing boundaries within species complexes and/or uncover instances of cryptic speciation (Parnmen et al. 2012; Kraichak, Lücking, et al. 2015; Alors et al. 2016; Widhelm et al. 2016; Zhao et al. 2017). In this work, these methods provided additional means of evaluating species boundaries, particularly in complex clades such as those containing strongly supported subclades (e.g., *Sticta* aff. *parvilobata*) or those where morpho-anatomical differences between lineages were subtle (e.g., *Sticta borinquensis* + *S.* aff. *borinquensis*). For example, congruent with the analysis of morphological data, GMYC and BPP analyses on both multilocus and ITS datasets agreed in separating *S. parvilobata* from *S.* aff. *parvilobata* and *S.* aff. *borinquensis* from *S. borinquensis*. PTP on our multilocus tree, on the other hand, failed to separate these species. This latter analysis also delimited within *S. scabrosa* lineages now considered to represent separate species (Moncada, Mercado-Díaz, Smith, et al. 2021). Differences between GMYC and PTP were somewhat unexpected because when gene flow is presumed absent, both methods tend to produce similar estimates of species limits (e.g. Arrigoni et al. 2016; Del-Prado et al. 2016). These minor incongruences, however, are probably linked to the high number of species analyzed here since PTP has been found to outperform GMYC when fewer species are involved (Luo et al. 2018). Empirical work has also shown that GMYC delimitation could also result in over-splitting (Alors et al. 2016; Eberle, Warnock, and Ahrens 2016; Guillemin et al. 2016), which might explain to some degree the disagreement between boundaries placed by these methods.

In general, species delimitation under the preferred combination of priors in BPP (i.e., θ_s [3, 0.002], τ_0 [3, 0.2]) showed low conflict with delimitations reached by other methods. Although this set of priors not always resulted in the highest posterior probabilities, we opted to favor delimitations based on them as they were thought to better represent the natural history of species in the island. This rationale was based on the relatively old geologic age of Puerto Rico (~ 100 million years; Mitchell 1954), the estimated age of the group (~30 million years) and the idea that effective population sizes in island lineages should be small as they represent only a fraction of the individuals in mainland populations (Nei, Maruyama, and Chakraborty 1975). Other prior combinations were less favored as they seemed to over- or underestimate the number of species. One example is *S. scabrosa.* As mentioned previously, recent work suggests that this species is widespread in the Neotropics and include local haplotypes in the island (Moncada, Mercado-Díaz, Smith, et al. 2021), yet, prior combination θ_s (3, 0.002) and τ_0 (3, 0.002) split our 10 specimens into 7 species. Conversely, large values of θ_s and small τ_0 apparently underestimated true diversity as it was seen with *S. guilartensis* and *S.* aff. *guilartensis*. These two strongly supported monophyletic lineages and morphologically divergent species were delimited as a single species under this prior combination. This agrees with previous work that suggests that large θ_s and small τ_0 tend to favor fewer delimited species (Leaché and Fujita 2010; Yang and Rannala 2010).

Incongruence between delimitation methods was not rampant although it was present in our analyses. Carstens et al. (2013) suggested that incongruence across results from different methods could be indicative of violation of assumptions or could be due to differences in the power to detect cryptic lineages in one or more of the approaches. Uneven sampling has also been linked to poor performance and conflict between delimitation methods (Lim, Balke, and

Meier 2012; Rittmeyer and Austin 2012) and likely explain some of the incongruences detected. In lichenized fungi, conflict between delimitation methods has also been attributed to limitations in placing boundaries in lineages with recent diversification histories (Wei et al. 2016). This might have affected delimitation within *S.* aff. *parvilobata*, which is most likely undergoing an active speciation process. Insufficient data for species delimitation may also explain oversplitting in some methods (e.g., GMYC; Lohse 2009) and might underlie some of the conflict observed between them.

Our work reaffirms the value of species delimitation analyses for integrative studies but also illustrates the risks of using them in isolation. Previous work has suggested that some methods, such as BPP, cannot statistically distinguish genetic structure associated with population isolation vs. species boundaries, and thus might not be effective at diagnosing species (Sukumaran and Knowles 2017; Huang 2018). Other methods are relatively stable under different circumstances (i.e., GMYC) but might have a high incidence of wrongly delimited species and therefore could not be used as sufficient evidence for evaluating some clades (Talavera, Dincă, and Vila 2013). These observations reiterate the importance of analyzing all available data (i.e., genetic, morphological and ecological) when interpreting species delimitations.

It should be noted that although PTP and GMYC have been increasingly applied to multilocus trees (Luo et al. 2018), their use and interpretation should preferably be based on the analysis of single-locus data (Fujisawa and Barraclough 2013; Zhang et al. 2013). We are less concerned about presenting results obtained from analyzing multilocus trees because taxonomic determinations were mostly based on distinctive morphologies and strong branching patterns and clade support. In addition, delimitations produced with them were in general more conservative.

Molecular vs. morphological data—Phylogenetic analysis and species delimitation methods were important for uncovering hidden diversity of this group in the island. These approaches showed that three of the species defined by Harris (1989) were representative of two lineages: *Sticta* sp. 22678 (*S.* aff. *parvilobata* + *S. parvilobata*), *Sticta* sp. 22494 (*S. borinquensis* + *S*. aff. *borinquensis*) and "*Sticta trichographis*" (*S. riparia* + *S. densiphyllidiata*). Interestingly, only *S.* aff. *parvilobata* should be considered cryptic in the sense of lacking unambiguous morphological characters to separate it confidently from *S. ciliata*. *Sticta* aff. *borinquensis* exhibit subtle morphological differences compared to *S. borinquensis* and is better described as a semi-cryptic species, whereas "*S. trichographis*" and *S. riparia* are not cryptic to each other because phenotypic characters useful for separating them were eventually found. Unaccounted diversity resulting from overlooked phenotypic characters is not a rare phenomenon in lichen studies. It is prevalent, particularly in otherwise cryptic lineages hiding within hitherto assumed widespread species (Lücking et al. 2014) including *Sticta* (Moncada, Lücking, and Suárez 2013).

Morphological differences observed were usually sufficient for discriminating between species. Similarly, HPTLC analysis uncovered several unknown secondary compounds that are so far known from several species from the island (Table S.1.2.). The presence of these potentially informative substances reiterates the value of phenotypic characters in this group, which has traditionally been regarded as having a poor secondary chemistry (Moncada 2012). While morphology-based species delimitation in lichenized fungi has been deemed inaccurate in many instances because of limited phenotypic variability between lineages and/or high degrees of homoplasy in morphological characters (Pino-Bodas et al. 2011; Parnmen et al. 2012; Zhao et al. 2017), our results add to the growing body of work challenging the notion of lichens as organisms with few taxonomically useful characters (Printzen 2010). Conversely, they confirm

that molecular-based species delimitations could be supported by previously overlooked phenotypic characters (Lumbsch and Leavitt 2011). They also highlight that at least for island biotas, morphology is still relevant for characterizing species diversity. This is perfectly exemplified by endemic species of *Lobariella* in the Hawaiian archipelago, which bear unique morphologies compared to other species in the genus (Lücking, Moncada, and Smith 2017).

Implications for conservation—Most species of *Sticta* from Puerto Rico discussed in this work (11) are potentially endemic to the island, which means they are likely found nowhere else on the planet. Because their unique genotypes are usually represented by a small number of individuals and because multiple factors threaten their survival, endemic species, especially those from biodiversity hotspots like the Caribbean, are priorities for conservation (Myers et al. 2000). In Puerto Rico, an increasing urban footprint at the expense of forest cover is reducing the habitats available for many species (Lugo, López, and Ramos-González 2004). Climate change may result in habitat shrinkage of high-elevation species, making them more susceptible to extinction (Dirnböck, Essl, and Rabitsch 2011; Jennings et al. 2014). One species likely facing this fate is *S. corymbosa*, known from only a few individuals in peaks of El Yunque National Forest. Being more diverse in humid and shaded high-elevation environments of the island, species of *Sticta* are also threatened by increases in the frequency and magnitude of hurricanes in the Caribbean region (Mann and Emanuel 2006). In fact, photographs of the thallus of the same *S. tainorum* individual that were taken both before and after Hurricane María (September 2017) show considerable browning (Fig. S.1.6.), suggesting damaging effects of increasing solar radiation due to reduced canopy foliage. Measures to further improve the protection of the habitats in which these species thrive will be essential for reducing potential risks of extinction linked to these changes.

Failing to collect *Sticta* sp. 320 and *Sticta* sp. 38304 suggests that these species are rare or potentially extinct, or perhaps that sampling efforts were inadequate, but this latter scenario is less likely because sampling included all areas surveyed in the past as well as other suitable habitats that were missed in previous efforts. Additionally, the observed increase in recorded species associated with our work (i.e., Fig. 1.3.) demonstrates that our sampling was as exhaustive as those carried out by others in the past. This is supported by the discovery of several morphologically distinct species not documented in Harris (1989) (e.g., *S.* aff. *guilartensis*, *S.* aff. *harrisii*). The rarity or potential extinction of these species is therefore most likely linked to the negative effect of past disturbances and/or other current pressures (e.g., climate change).

Estimates of species richness are essential in conservation assessments and for the implementation of informed conservation policies. As evidenced in our species accumulation curve, accurate quantification of the species is most likely obtained when taxonomic revisions of target groups are coupled with molecular methods and comprehensive sampling within a region. Yet, as a metric to inform conservation efforts, species richness estimates should not be used in isolation (Fleishman, Noss, and Noon 2006). Phylogenetic approaches are valuable in this sense because they also provide insight on natural history aspects (e.g., genetic uniqueness) that are seldom available for species. Phylogeny-based metrics such as phylogenetic diversity and relative phylogenetic endemism are certainly promising in this regard (Rosauer et al. 2009; Thornhill et al. 2016).

TAXONOMY

Previous morphological taxonomy and herbarium revisions—Previous to this work, only two species from Puerto Rico were known from the published literature: *Sticta sinuosa* and

S. weigelii (Imshaug 1957). Several specimens originally identified as *S. sinuosa* by Müller Argoviensis (1888) were examined in NY and US and found to correspond to *S. tainorum*. Excluding this taxon, nearly all species identified in this work would fall under the broad concept of *S. weigelii* (sensu Galloway 1994). We were not able to revise specimens of *S. weigelii* in Müller Argoviensis (1888); therefore, it is unknown to which of the species described here this material corresponds to.

As mentioned before, nine species were recognized by Harris (1989) based on morphological characters. The valid name *Sticta weigelii* was used, but this material was found to correspond to *S. scabrosa*, a species within the *S. weigelii* morphodeme (sensu Moncada, Lücking, and Suárez 2013). None of the specimens evaluated by Harris (1989) correspond to *S. weigelii* sensu stricto. The two other species names in that work (i.e., "*S. trichographis*" and "*S. circumroda*") were never published and are therefore not valid. The taxonomy of *Sticta* sp. 320 in Harris (1989), on the other hand, is more complex. This species resembles *S.* aff. *guilartensis* in the linear-lingulate lobes that do not curl down (see Harris 1989), but differs from it by the presence of scattered dark brown rhizines that project outward along lobe margins (not noted in Harris 1989) and the considerable smaller length/width ratio of the lobes. We refrained from formally describing it here due to lack of molecular data and scarcity of specimens to assess morphology. *Sticta* sp. 3725 was found to be morphologically identical to *S. borinquensis* and is considered conspecific with this species; however, none of our specimens showed the KOH+ purple medullary reaction highlighted in Harris (1989) (refer to "Remarks" under the *S. borinquensis* description below). We re-examined the material in NY studied by Harris (1989) and confirmed the KOH+ reaction of the medulla in those specimens. *Sticta* sp. 22489, on the other hand, was found to be conspecific with *S. borinquensis*. Except for *Sticta* sp. 320, the

taxonomy of the rest of the taxa in Harris (1989), which are also identified with collection numbers, is resolved in this work (see below).

It is worth noting that material from Puerto Rico have also been identified under the names *S. xanthotropa* (Kremp.) D.J.Galloway, *S. mexicana* D. J. Galloway*, S. beauvoisii*, *S. tomentosa* (Sw.) Ach., *S. wrightii* Tuck. and *S. duforii* Delise (http://lichenportal.org/portal/index.php). These determinations apparently followed broad species concepts from other works (e.g. Galloway and Thomas 2004). After revising this material, we determined that none of these species occurred on the island.

Several specimens evaluated during our revision of herbarium material deserve special mention due to their peculiar morphology. Hale's specimen *38304* (US) keys out as *Sticta* sp. 320 in Harris (1989), but several differences in its morphology suggest that its most likely a different species. It shares with *Sticta* sp. 320 the presence of lingulate lobes, branched isidia and a short tomentum, but differs from that species in the absence of dark brown rhizines projecting outwards along lobe margins and presence of a distinctly yellow tinge that is so far absent from any of the Puerto Rican material studied to date. Hale's specimen also resembles *S*. aff. *guilartensis*, in its large thallus with linear-lingulate lobes and a very short tomentum throughout but contrasting to it by its wider lobes and shorter internode distances. On the other hand, B. Fink's specimen *1892* (NY, US), which was identified in Harris (1989) as "*Sticta weigelii* auct.", is regarded here as conspecific with *S. scabrosa*. Its smaller thallus size and lobe widths and relatively smooth upper surface, however, make this material worth of further study.

Lastly, chemical analysis by HPTLC revealed the presence of several unidentified secondary compounds in some species of the island. A general description of chromatographic properties of these substances is provided in Table S.1.2.

Key to the species of *Sticta* in Puerto Rico

1.Photobiont green ..... ***S. tainorum***

1.Photobiont blue-green 2

2.Vegetative propagules present and usually abundant 3

2.Thallus without vegetative propagules or with small marginal lobules. White, reticulated maculae throughout. Known only from Bosque Estatal de Guilarte ..... ***S. guilartensis***

3.Phyllidia present 4

3.Isidia present 11

3.Corymbose, sorediiform isidia along margins present ..... ***S. corymbosa***

4.Tomentum short to pubescent 5

4.Tomentum rather thick 8

5.Lobes rounded to suborbicular. Without marginal rhizines projecting outwards 6

5.Lobes lingulate. With or without marginal rhizines projecting outwards 7

6.Lobes broad (3.5–7 mm), tomentum tan to brown, lower surface cream-colored to light brown, primary tomentum hairs not branching, specimens usually turning reddish with age..... ***S. densiphyllidiata***

6.Lobes narrower (2–4 mm), tomentum brown to dark brown, lower surface greyish-brown to dark brown, primary tomentum hairs sometimes branching, specimens dark brown to gray in herbarium ..... ***S. riparia***

7.Lobes with marginal rhizines projecting outwards ..... ***Sticta* sp. 320**

7.Marginal rhizines absent, lower surface turning yellowish in herbarium..... ***Sticta* sp. 38304**

8.Tomentum towards margins whitish to greyish-brown 9

8.Tomentum towards margins brown to black 10

9.Margins distinctly ciliate and highly dissected due to abundance of branched phyllidia. Lobe internode short (0.4–4 mm). Tomentum becoming sparse towards margins. Upper surface smooth. Apothecia frequent. Cells of basal membrane with numerous papillae. Upper surface never maculate ..... ***S. harrisii***

9.Margins rarely ciliate although tomentum hairs frequently extend outwards resembling cilia. Phyllidia mostly marginal and occasionally laminal, less dense. Lobe internode long (5–7 mm). Tomentum remaining more or less dense towards margins. Upper surface becoming scrobiculate to slightly faveolate, particularly towards center. Apothecia absent to sparse. Cells of basal membrane without papillae. Upper surface occasionally maculate..... ***S. scabrosa***

10.Tomentum brown. Thallus mostly horizontal. Apothecia and pycnidia absent or infrequent. Known only from high-elevation forests to the west of the island..... ***S.* aff. *borinquensis***

10.Tomentum darker brown to blackish, particularly towards the center. Thallus usually ascending. Apothecia and pycnidia frequent. Abundant in high-elevation forests to the east of the island..... ***S. borinquensis***

11.Isidia laminal.....12

11.Isidia marginal 14

12.Lobes lingulate to spathulate, regularly branching. Known only from El Yunque National Forest..... ***S.* aff. *harrisii***

12.Lobes rounded, unbranched to rarely branching.....13

13.Lobes usually broad (10–25 mm), frequently overlapping other lobes, stacked in appearance. Mature lobe apices distinctly revolute. Isidia becoming arbuscular with distinct stipe, forming distinct rounded clusters scattered throughout the thallus. Minutely dotted appearance under high magnification due to small, whitish granules. Distributed throughout the island .....

.....***S.* aff. *parvilobata***

13.Lobes rarely exceeding 12 mm in width, with individual thalli usually scattered throughout the substrate. Mature lobe apices levelled to weakly revolute. Isidia granular to coralloid, with more homogeneous distribution throughout the thallus. Thallus with white microfibrils visible under high magnification. Known from high-elevation forests to the west of the island.....

.....***S. parvilobata***

14.Lobes long, linear, length >3–4 times larger than width ..... ***S.* aff. *guilartensis***

14.Lobes rounded to lingulate, length <3–4 times larger than width 15

15.Tomentum thick, spongy towards center. Lobes rounded. Isidia truly cylindrical, dense .....

.....***S. weigelii***

15.Tomentum thin, pubescent or strigose throughout. Lobes lingulate. Isidia slightly flattened, elongated and branching or simple, scattered and sometimes clustered.....16

16.Marginal rhizines projecting outwards along lobe margins, tomentum strigose, lower surface not turning yellowish in herbarium..... ***Sticta* sp. 320**

16.Marginal rhizines absent, tomentum pubescent, lower surface turning yellowish in herbarium .

..... ***Sticta* sp. 38304**

New taxa

Sticta borinquensis Merc.-Díaz & Lücking, **sp. nov.** [MycoBank # 834856] – Holotype: PUERTO RICO. Mun. Río Grande, Barrio Guzmán Arriba, along El Toro Trail, near Pico El Toro, El Yunque National Forest; 18°16′22″N, 65°50′02″W; 982 m; 28 Dec 2015, *Mercado-Díaz 2381* (F barcode C0243203F; isotype: UPR).

Species is illustrated in Fig. 1.4.

Diagnosis. – Differing from *Sticta scabrosa* in the smooth upper surface, darker brown tomentum and frequent occurrence of apothecia.

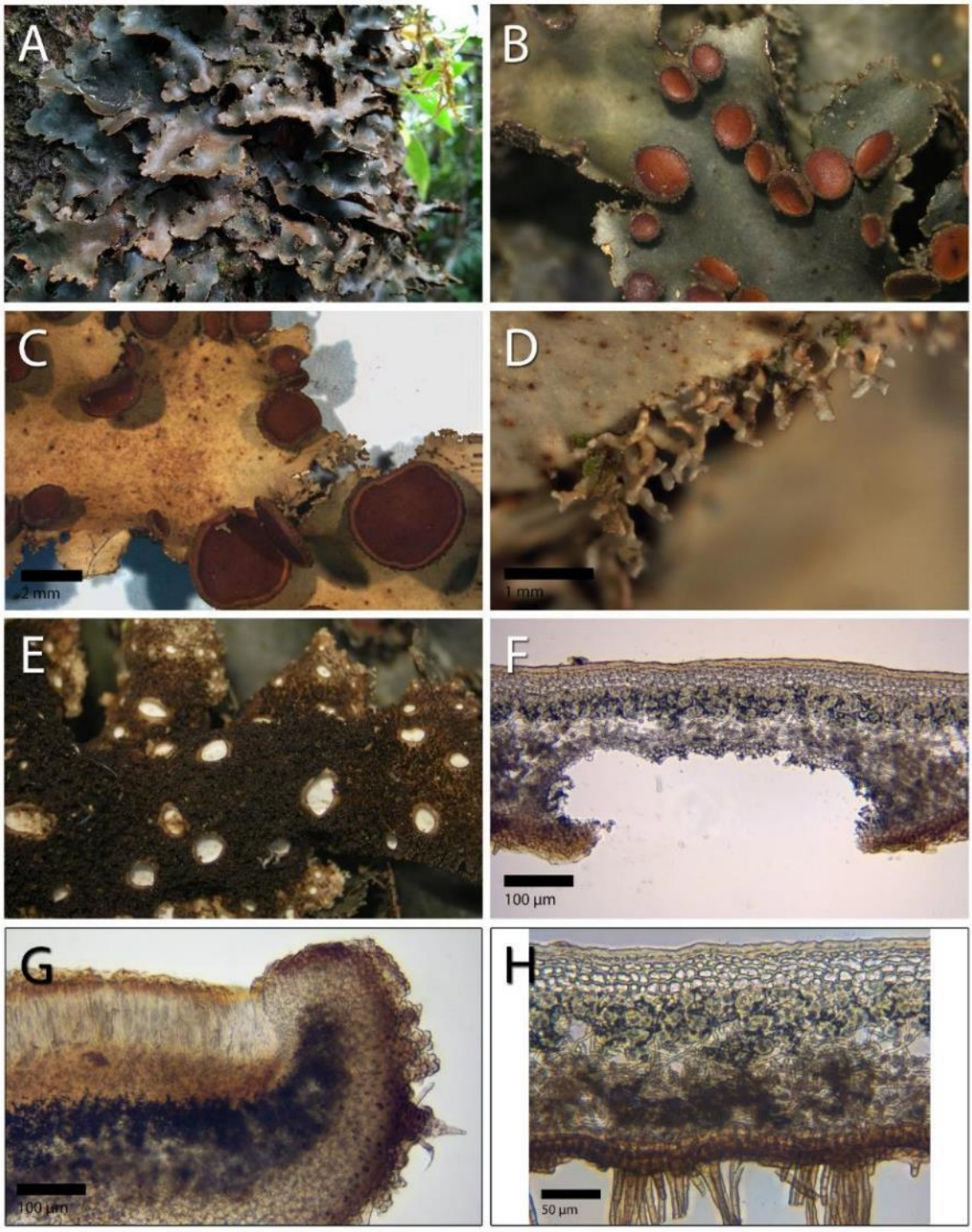

Figure 1.4. *Sticta borinquensis.* **A.** View of thallus in the field. **B.** Lobes with apothecia when fresh. **C.** Lobes with apothecia and phyllidia when dry. **D.** Detail of marginal branched phyllidia. **E.** Lower surface with dark brown tomentum and cyphellae. **F.** Section of cyphellae. **G.** Section of apothecia. **H.** Section through thallus.

Description. – Primary photobiont cyanobacterial (*Nostoc* Vaucher ex Bornet & Flahault). Basal stipe absent. Thallus irregular to orbicular in outline, up to 15 cm diam., densely branched, with 6–10 branches per 5 cm radius, branching polytomous; lobes suborbicular to lingulate, horizontal to ascending, imbricate, involute to undulate, with rounded to irregular, plane to revolute apices; margins entire to crenate, not thickened; lobe internodes 1–5 mm long, (2–)2.5–5(–6) mm broad; thallus resistant, coriaceous. Upper surface smooth, slate grey to olive-grey when fresh and brown-grey to light brown and darkening towards the apices in the herbarium, glossy; surface glabrous, marginal line color brown, without papillae, pruina, or cilia. Maculae sparse to absent, white and irregular. Apothecia sparse, marginal to submarginal, subaggregated, pedicillated, base invagination pronounced, 1–4 mm diam., disc color reddish-brown to brown and slightly glossy to opaque both when fresh and in herbarium, margin dark brown, with lighter inner rim, verrucose and tomentose (particularly when young) to smooth and weakly crenulate when old. Tomentum on apothecial margin denser towards base. Phyllidia abundant, marginal (only laminal when injured), dispersed, mostly linear, simple to branched, becoming coralloid to palmate, with irregular orientation, to 1 mm long and 0.1–0.5 mm broad, same color as thallus, becoming darker with age, glossy, flattened to dorsiventral in section, lingulate to weakly spathulate, basal stalk applanate, with cyphellae initials. Lower surface smooth to uneven, glossy, cream-colored to dark brown becoming blackish towards center; primary tomentum dense, absent towards margin, thick, becoming thinner towards margins, spongy, soft, brown, hairs occasionally whitish toward apices. Rhizines absent. Cyphellae abundant, 21–40 per cm^2 towards the thallus center and 61–100 per cm^2 towards the margin, dispersed, rounded to irregular, urceolate with wide pore, erumpent to prominent, margin

remaining below the level of the primary tomentum, elevated and involute, cream-colored to brown, without tomentum; pore 0.5–2 mm diam.; basal membrane weakly pruinose (more evident in younger cyphellae), white, K+ yellow–ochre, C–, KC–, P–. Medulla compact, white to cream-colored, K+ yellow–ochre, C–, KC–, P–. Borinquensis unknown (major), Harrisii unknown (minor), Unknown 2 (minor). Pycnidia immersed, black. No cephalodia observed.

Upper cortex paraplectenchymatous, 35–45 µm thick, with two differentiated layers, the upper layer sometimes darkened, consisting of 3–4 cell layers with cells 5–20 µm diam., their walls 1–2.5 µm thick and their lumina rounded to isodiametric, 3.5–19 µm diam. Photobiont layer 25–62 µm thick, its cells 8–14 µm diam. Medulla 50–100 µm thick, its hyphae 2.5–3.5 µm broad, without crystals. Lower cortex paraplectenchymatous, 25–35 µm thick, with 2–3 cell layers; cells 5–20 µm diam., their walls 1–2.5 µm thick. Hairs of lower primary tomentum 200–1100 µm long, in fascicles of 12–20 hyphae, unbranched, cylindrical, septate with intertwined apices. Cyphellae internal pore cavity 100–225 µm deep; cells of basal membrane without papillae. Apothecia biatorine, 700–1200 µm high, with stipe; excipulum 75–150 µm broad. Hymenium 80–125 µm high; epihymenium 2.5–5 µm high, dirty orange to light brown, without gelatinous layer above. Ascospores 1–3 septate, 32–43 × 5–9 µm, fusiform, hyaline.

Distribution and ecology. – Although populations of *Sticta borinquensis* have been found in scattered locations in forests to the west of the island, the distribution of this species seems to be centered around high-elevation forests to the east, particularly in El Yunque National Forest. It seems to be mostly epiphytic and individuals have been found on several tree species including *Prestoea acuminata* var. *montana* (Graham) A.J.Hend. & Galeano, *Cecropia schreberiana* Miq. subsp. *schreberiana* and *Clusia* L. spp. It is often found growing in partly shaded conditions in very humid environments.

Etymology. – The epithet refers to "Borinquen", the Taíno name for the island of Puerto Rico.

Remarks. – *Sticta borinquensis* is identified in Harris (1989) as *Sticta* sp. 22494 and is one of the largest species to be found among the cyanobacterial representatives of this genus in the island. Phylogenetic analysis shows that this species is most closely related to *S.* aff. *borinquensis*, which is known only from mountain summits within the Bosque Estatal Tres Picachos to the west of the island and is sympatric with *S. borinquensis* in that forest. Both are morphologically very similar and difficult to separate; however, phylogenetic patterns, nearly consistent species delimitation analyses results and subtle morphological differences convince us of treating *S.* aff. *borinquensis* as a separate species. Unfortunately, more material from *S.* aff. *borinquensis* will be needed before a formal taxonomic description of this species is made. It is worth noting, on the other hand, that maculae are sparsely seen in *S. borinquensis*. Yet, these seem to be more evident in larger, older thalli.

Our revision of historical material revealed that the type specimen for *S. weigelii* f. *schizophylliza* (Nyl.) Hue collected in Guadeloupe (i.e., *Husnot #436*; Nylander 1869) may be conspecific with *S. borinquensis*. This observation was unexpected because (Hue 1901) indicated that this material was similar to a specimen identified by Fée as "*S. circumroda*", an unpublished name covering the taxon now named *S. harrisii* (Harris 1989; see below). Diagnostic characters that place the Guadeloupean specimen closer to *S. borinquensis* are the abundance of linear marginal phyllidia, the brown to black lower cortex and the dense brown tomentum. Yet, given the high endemism that has been recently documented for this group in other tropical island systems (Moncada, Reidy, and Lücking 2014; Dal Forno et al. 2017; Lücking, Moncada, and Smith 2017; Simon et al. 2018) and the lack of additional molecular and morphological data to

assess if this specimen corresponds to *S. borinquensis*, we decided not to take up the infraspecific epithet for the Puerto Rican taxon. Should in the future *S. weigelii* f. *schizophylliza* indeed be shown to be conspecific with *S. borinquensis*, the latter name retains priority at the species level.

Sticta sp. 3725 was separated from *S. borinquensis* (identified in Harris [1989] as *Sticta* sp. 22494) for its medullary K+ purple reaction but is otherwise identical in morphology to *S. borinquensis* and is treated here as the same taxon. This reaction has not been documented in any of the recently collected material from this species, and the reasons for this remain unclear. Considering that some of the old material show signs of poor drying after collecting, it is possible that this reaction is explained by decomposition of otherwise undetectable secondary substances or even by intrathalline fungi that could have grown because of prolonged duration of moisture within medulla.

Additional specimens examined. – PUERTO RICO. Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850–950 m; 9 Jun 1988, *Harris 22497A* (NY). Mun. Jayuya, Bosque Estatal Tres Picachos, trail to Tres Picachos peaks; 18°12′52″N, 66°32′23″W; 1153 m; 18 Aug 2013, *Mercado-Díaz 1958* (UPR). Mun. Luquillo, El Yunque National Forest, near G. González (USFS) "Britton Palm" plot; 18°18′16″N, 65° 47' 43"; 917 m; 27 Sep 2011, *Mercado-Díaz 957* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18°16′18″N, 65°49′52″W; 1006 m; 28 Dec 2015, *Mercado-Díaz 2365* (UPR). Mun. Orocovis, Bosque Estatal de Toro Negro, along Hwy 143, 3.5 mi. E. of Hwy 139; 27 Feb 1981, *Buck 3725* (NY). Refer to Appendix 2. for additional specimens revised.

Sticta corymbosa Merc.-Díaz & Moncada, **sp. nov.** [MycoBank # 834857] – Holotype: PUERTO RICO. Mun. Las Piedras, Barrio El Río, at summit of Pico El Toro, El

Yunque National Forest; 18°16′20″N, 65°49′44″W; 1048 m; 28 Dec 2015, *Mercado-Díaz 2385* (F barcode C0172453F; isotype: UPR).

Species is illustrated in Fig. 1.5.

Diagnosis. – Differing from *Sticta sublimbata* (J. Steiner) Swinscow & Krog in the suborbicular lobes with black marginal cilia and the marginal and laminal corymbose isidia that erode into sorediiform propagules.

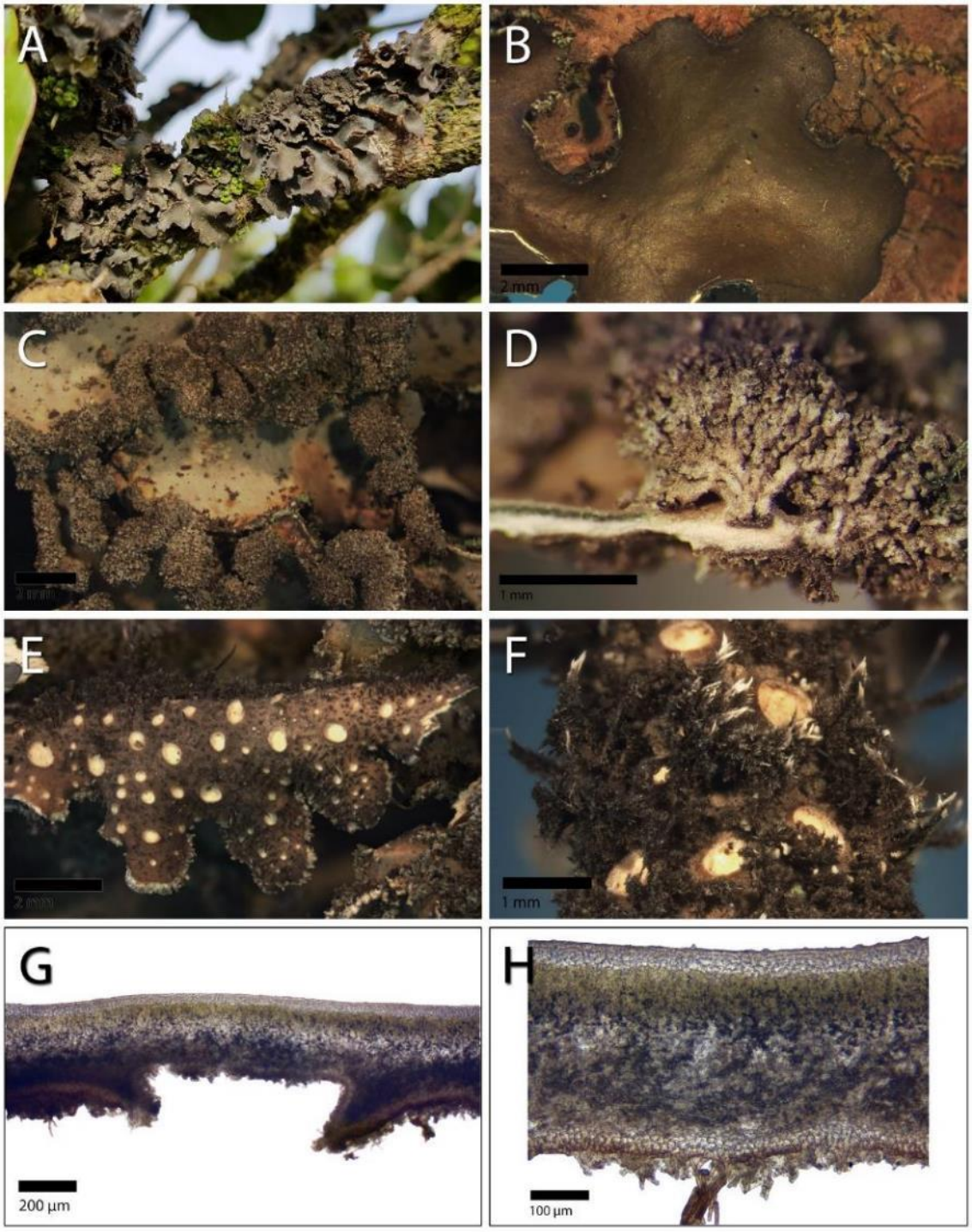

Figure 1.5. *Sticta corymbosa.* **A.** Thallus in the field. **B.** Detail of a young lobe with black marginal cilia. **C.** Tightly packed clusters of marginal corymbose soreediform isidia. **D.** Section of corymbose isidia. **E.** Lower surface with short arachnoid tomentum, rounded cyphellae and rhizines. **F.** Detail of barbulate rhizines. **G.** Section of cyphellae. **H.** Section through thallus.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Basal stipe absent. Thallus irregular in outline, up to 10 cm diam., moderately branched, with 6–10 branches per 5 cm radius, branching pleurotomous to polytomous; lobes suborbicular, horizontal to ascending, adjacent to imbricate, involute, with rounded, plane to undulate apices; margins entire to sinuose and eroding, not thickened; lobe internodes 3–6 mm long, 2–5(–6) mm broad; thallus resistant, coriaceous. Upper surface smooth, grey when fresh and grey to dark brown in herbarium, moderately glossy; surface glabrous, without papillae, pruina or maculae. Cilia sparse, simple, hyaline when young and dark brown to black when old, to 0.5 mm, more evident in younger lobes. Apothecia not observed. Isidia abundant, eroding and becoming sorediiform, both marginal and laminal, aggregated and continuous along margins, branched, corymbose, vertically oriented, to 1.5 mm long and 0.4–1.3 mm broad, darker than thallus (especially near tips), glossy, rounded, granular to cylindrical, basal stalk cylindrical. Lower surface weakly scrobiculate-rugose, grey to brown, becoming darker in older portions of thallus; primary tomentum dense, sparse towards margins, thin throughout, becoming absent in old portions of the thallus, arachnoid, soft, white. Rhizines abundant, thallus centered to dispersed, dark brown to black with white tips, barbulate, to 2 mm. Cyphellae abundant, 40–60 per cm^2 towards the thallus center and 61–100 per cm^2 towards the margin, dispersed, rounded, urceolate with wide pore to cupuliform, prominent, at or above level of primary tomentum; margin elevated and involute to erect, cream-colored to brown, with tomentum near base; pore 0.3–1.5 mm diam.; basal membrane smooth, cream-colored, K+ yellow–orange, C–, KC–, P–. Medulla compact, white to cream-colored, K+ yellow–orange, C–, KC–, P–. Tainorum unknown (major). No pycnidia or cephalodia observed.

Upper cortex paraplectenchymatous, 35–50 μm thick, with two differentiated layers,

consisting of 4–5 cell layers with cells 7–20 µm diam., their walls 2–3.5 µm thick and their lumina rounded to isodiametric, 4–18 µm diam. Photobiont layer 85–125 µm thick, its cells 5–12 µm diam. Medulla 100–200 µm thick, its hyphae 2.5–5 µm broad, without crystals. Lower cortex paraplectenchymatous, 25–45 µm thick, with 2–3 cell layers; cells 4–20 µm diam., their walls 2.5–5 µm thick. Hairs of lower primary tomentum 25–75 µm long, dispersed, branched, apically moniliform, septate with free apices. Cyphellae internal pore cavity 100–300 µm deep; basal membrane cell papillae absent.

Distribution and ecology. – *Sticta corymbosa* is an epiphytic species known only from the summit of Pico El Toro in El Yunque National Forest. Highly humid and open to partly shaded environments are therefore the assumed preferred habitat for this species. Because collecting activities in other mountain summits in this forest have failed to detect it, we suspect this is the only locality where this species is to be found. If true, *S. corymbosa* would be the species with the smallest geographical range in the island.

Etymology. – This name alludes to the shape (i.e., corymbose) of the isidia that are abundant along the lobe margins of this species.

Remarks. – *Sticta corymbosa* is a morphologically distinct species within the *S. limbata* (Sm.) Ach. morphodeme (sensu Moncada, Lücking, and Suárez 2013). Although it resembles *S. sublimbata* in many aspects, the presence of tightly packed sorediiform and corymbose isidia both along the margins and in the surface, as well as the presence of black marginal cilia makes it easy to distinguish from that species. It also has very distinctive barbulate rhizines and a thin, arachnoid primary tomentum. Within Puerto Rico, it is most closely related to *S. borinquensis*.

Because it thrives at a mountain summit, *S. corymbosa* was thought to have been most affected by Hurricane María in September 2017. Fortunately, several healthy individuals were

observed during a recent survey in July 2018. Given the locality it was found, we believe that *S. corymbosa* is one of the most susceptible species to climate change in the island. This is because it has been proposed that climate change may reduce the habitat size of high elevation species (Dirnböck, Essl, and Rabitsch 2011; Jennings et al. 2014). Because of its unique habitat preferences and apparently small population size, we suspect that *S. corymbosa* is perhaps the most threatened species of *Sticta* in Puerto Rico. Additional population-level studies will be required to further determine the degree of threat that this species might currently be facing.

Additional specimens examined. – PUERTO RICO. Mun. Las Piedras, Barrio El Río, El Yunque National Forest, at summit of Pico El Toro; 18°16′20″N, 65°49′44″W; 1048 m; 28 Dec 2015, *Mercado-Díaz 2378* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta densiphyllidiata Merc.-Díaz & Lücking, **sp. nov.** [MycoBank # 834859] – Holotype: PUERTO RICO: Mun. Río Grande, Barrio Mameyes II, along Mt. Britton Trail near Mt Britton Tower, El Yunque National Forest; 18°18′05″N, 65°47′34″W; 909 m; 4 Oct 2011, *Lücking & Mercado-Díaz 33871* (F barcode C0172458F; isotype: UPR).

Species is illustrated in Fig. 1.6.

Diagnosis. – Differing from *Sticta beauvoisii* in the suborbicular lobes, shorter tomentum, presence of phyllidia and frequent reddish tinge of thalli in herbarium.

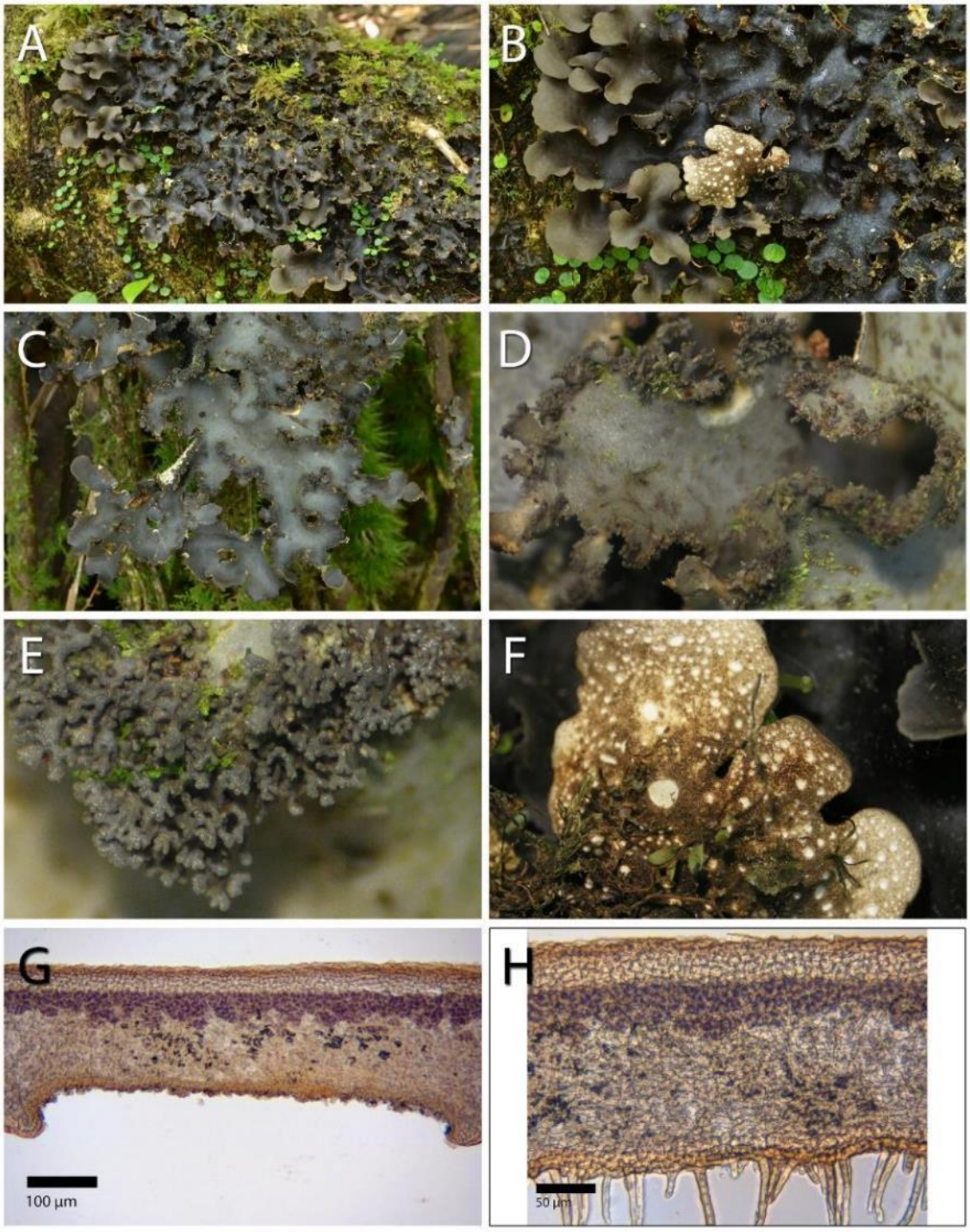

Figure 1.6. *Sticta densiphyllidiata.* **A.** Thallus in the field. **B.** Close-up of thallus in the field showing upper and lower surface. **C.** Upper surface of branched lobes. **D.** Detail of lobes with marginal phyllidia. **E.** Detail branched coralloid to palmate phyllidia. **F.** Detail of lower surface with short brown tomentum and cyphellae. **G.** Section of cyphellae. **H.** Section through thallus.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Basal stipe absent. Thallus orbicular to irregular in outline, up to 20 cm diam., moderately branched, with 4–6 branches per 5 cm radius, branching polytomous; lobes suborbicular, horizontal, imbricate, involute to canaliculate, with rounded, plane to weakly revolute apices; margins entire, not thickened; lobe internodes 3–8 mm long, (2–)3.5–7(–11) mm broad; thallus resistant, coriaceous. Upper surface smooth, grey to dark olive-grey when fresh and grey to light brown, occasionally turning reddish in herbarium, opaque; surface glabrous, without papillae, pruina, maculae or cilia. Apothecia not observed. Phyllidia abundant, marginal (only laminal when injured), dispersed but occasionally clustered and imbricated, branched, coralloid to palmate, with oblique to vertical orientation, to 0.8 mm long and 0.1–0.8 mm broad, darker than thallus, slightly glossy, flattened, cylindrical to spathulate, basal stalk subcylindrical. Lobules sparse, marginal, dispersed, unbranched, horizontal, to 3 mm long, 1–3 mm broad, usually associated with injured areas. Gall-like structures protruding from margins frequent, dispersed, unbranched, irregularly oriented, and without photobiont cells (type material only). Lower surface smooth to weakly rugose, cream-colored (fresh) becoming light brown to reddish in herbarium; primary tomentum thin and dense throughout, but becoming naked in old portions of the thallus, pubescent to hirsute, soft, tan to brown, hairs occasionally whitish toward apices. Rhizines sparse, toward thallus center, penicillated. Cyphellae abundant, 41–60 per cm^2 towards the thallus center and 100–200 per cm^2 towards the margin, dispersed, rounded, urceolate with wide pore, prominent, margin at or above level of primary tomentum, elevated and involute, cream-colored, without tomentum; pore 0.4–1.8 mm diam.; basal membrane pruinose, cream-colored, K– or K+ weak pink, C–, KC–, P–. Medulla compact, white to cream-colored, K– or K+ weak pink, C–, KC–, P–. Riparia unknown

(major). No pycnidia or cephalodia observed.

Upper cortex paraplectenchymatous, 32–50 µm thick, with two differentiated layers, the upper layer darkened, consisting of 4–5 cell layers with cells 5–15 µm diam., their walls 1.2–2.5 µm thick and their lumina rounded to isodiametric, 3.75–12.5 µm diam. Photobiont layer 35–75 µm thick, its cells 10–15 µm diam., upper margin in line with cortex. Medulla 75–130 µm thick, its hyphae 2–3 µm broad, without crystals. Lower cortex paraplectenchymatous, 20–30 µm thick, with 2–3 cell layers; cells 5–15 µm diam., their walls 2–3.5 µm thick. Hairs of lower primary tomentum 75–250 µm long, in fascicles of 6–12 hyphae, unbranched, cylindrical, septate with free or intertwined apices. Cyphellae internal pore cavity 50–150 µm deep; basal membrane cell papillae absent.

Distribution and ecology. – *Sticta densiphyllidiata* is one of the most commonly encountered species in well-preserved rainforests to the east of the island, particularly in El Yunque National Forest. Highly humid and shaded environments seem to be the preferred habitat for this species. It is most commonly found growing on rocks but could also grow epiphytically.

Etymology. – This name refers to the high density of branched phyllidia that are usually found along the lobe margins of this species.

Remarks. – Within Puerto Rico, *Sticta densiphyllidiata* is most closely related to the morphologically similar *S. riparia*. It can be distinguished from that species by the generally larger lobes and lighter lower cortex color. Specimens of *S. densiphyllidiata* also tend to turn reddish when in herbarium. Material belonging to this species was identified by Harris (1989) as "*S. trichographis*", a name attributed to Fée and not validly published in (Hue 1901), referring to Husnot's specimen n. 437 collected in Guadeloupe (Nylander 1869). Hue regarded this specimen conspecific with *S. weigelii* but noted that that material from Guadaloupe was saxicolous and had

a reddish tinge in herbarium, which agrees with *S. densiphylllidiata*. Although this hints that *S. densiphyllidiata* might be more widely distributed in the Lesser Antilles, additional collecting efforts and molecular work will be needed to confirm its presence in those islands. On the other hand, several individuals of *S. densiphyllidiata* were encountered during collecting efforts in El Yunque after Hurricane María hit Puerto Rico in September, 2107. Thalli from these individuals had white spots and necrotic areas, which suggests that this species is susceptible to increased solar radiation due to reduced canopy foliage.

Additional specimens examined. – PUERTO RICO. Mun. Humacao, El Yunque National Forest, Luquillo Experimental Forest, on moist rocky slopes near the base of a waterfall; Jul 1969, *Rundel s.n.* (US). Mun. Luquillo, El Yunque National Forest, near G. González (USFS) "Britton Palm" plot; 18°18′16″N, 65°47′43″W; 917 m; 27 Sep 2011, *Mercado-Díaz 958* (UPR). Mun. Naguabo, El Yunque National Forest, Sierra de Naguabo, Barrio de Maizales, on rock in ravine; 8 Mar 1914; 600 m; *Britton & Cowell 2195* (NY). Mun. Río Grande, El Yunque National Forest, trail to LFDP, El Verde Field Station; 18°19′13″N, 65°48′56″W; 415 m; 12 Dec 2015, *Mercado-Díaz 2389* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta guilartensis Merc.-Díaz, **sp. nov.** [MycoBank # 834860] – Holotype: PUERTO RICO. Mun. Adjuntas, Barrio Guilarte, Bosque Estatal Guilarte, along trail to Pico Guilarte; 18°08′37″N, 66°46′08″W; 1100 m; 30 Jul 2018, *Mercado-Díaz 3666* (F barcode C0172455F; isotype: UPR).

Species is illustrated in Fig. 1.7.

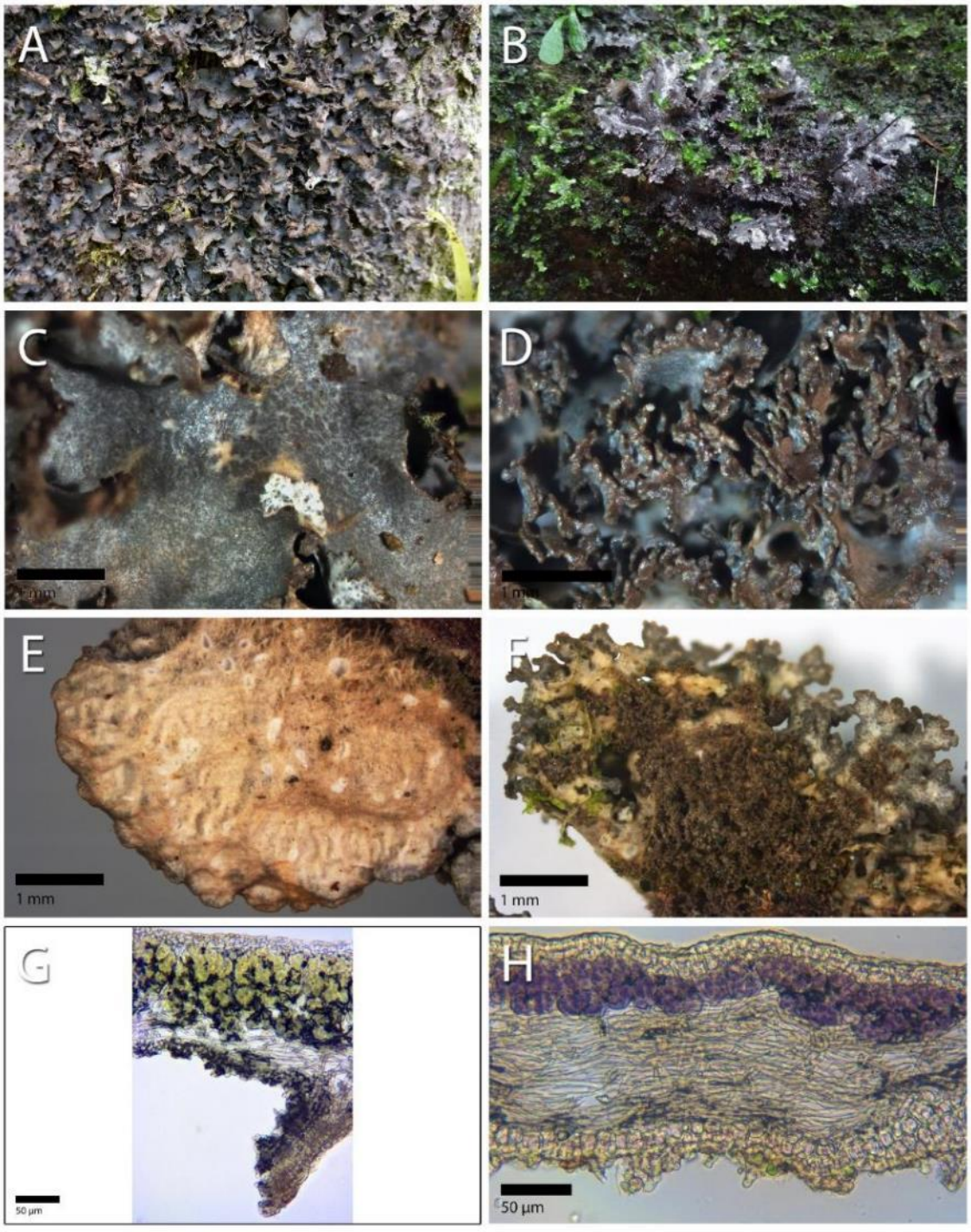

Figure 1.7. *Sticta guilartensis.* **A.** View of dry thallus in the field. **B.** View of moistened thallus in the field. **C.** Reticulated maculae on thallus upper surface. **D.** Detail of marginal lobules. **E.** Rugose lower surface near lobe margins and cyphellae. **F.** Lower primary tomentum and underside of marginal lobules. **G.** Section through cyphellae. **H.** Section through thallus.

Diagnosis. – Differing from the cyanomorph of *Sticta lobarioides* Moncada & Coca in the glossy, gray to brown upper surface, absence of fasciculate cilia and the presence of marginal lobules.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Basal stipe absent. Thalli irregular in outline, up to 10 cm diam. but frequently aggregating and forming patches >30 cm diam., densely branched, with more than 10 branches per 5 cm radius, branching pleurotomous to polytomous; lobes flabellate to suborbicular, adnate to horizontal, imbricate, involute to undulate, with rounded to undulate apices; margins irregular to crenate, not thickened; internodes 0.5–3 mm long, 1–3 mm broad; thallus fragile, papyraceous. Upper surface smooth but becoming somewhat faveolate with age, grey to brown, moderately glossy; surface glabrous, without papillae, pruina or cilia, although minute marginal hyaline projections of up to 0.05mm are sometimes observed. Maculae abundant, white, reticulated. Apothecia not observed. Lobules abundant, marginal, sometimes appearing to emerge from the lower surface, dispersed, simple or branched, coralloid to palmate, imbricated, horizontal to oblique, to 1 mm long and 0.1–0.8 mm broad, darker than thallus, glossy, applanate to dorsiventral in section, spathulate to lobuliform, basal stalk applanate. Lower surface irregular, becoming scrobiculate to costillate near margins, white to cream-colored. Primary tomentum sparse, absent towards margin, thick, becoming thinner towards margin, hirsute to weakly spongy, soft, tan to brown. Secondary tomentum pubescent, white to cream-colored. Rhizines not observed. Cyphellae abundant, 21–40 per cm^2 towards the thallus center and 41–60 per cm^2 towards the margin, dispersed, irregular to angular, cupuliform to pseudocyphelloid, erumpent to prominent, at or below level of the primary tomentum; margin erect to elevated and weakly involute, white to cream-colored, without

tomentum; pore 0.1–0.6 mm diam.; basal membrane smooth with pruinose appearance, white, K+ light yellow, C–, KC–, P–. Medulla lax, white, K+ light yellow, C–, KC–, P–. No substances detected by HPTLC. No pycnidia or cephalodia observed.

Upper cortex paraplectenchymatous, 12.5–22 µm thick, homogeneous, sometimes with darkened outer cortex, consisting of (1–)2–3(–4) cell layers with cells 5–17 µm diam., their walls 1–2.5 µm thick and their lumina rounded to isodiametric, 4–15.5 µm diam. Photobiont layer 25–90 µm thick, its cells 12–20 µm diam. Medulla 15–112 µm thick, its hyphae 2.5–3.5 µm broad, without crystals. Lower cortex paraplectenchymatous, 20–30 µm thick, with 2–3 cell layers; cells 6–20 µm diam., their walls 1.5–3 µm thick. Hairs of lower primary tomentum 100–300 µm long, dispersed, unbranched, cylindrical, septate with intertwined apices. Hairs of lower secondary tomentum 12–50 µm long, dispersed, unbranched, cylindrical, septate with free apices. Cyphellae internal pore cavity 80–150 µm deep; basal membrane cell papillae absent.

Distribution and ecology. – Sticta guilartensis is known from a single locality in Bosque Estatal de Guilarte in the central-west region of the island. It has been found growing on rocks as well as in roots and trunks of several trees including *Prestoea acuminata* var. *montana*. It seems to prefer shaded, very humid conditions and is commonly found growing among bryophytes.

Etymology. – This species is named after the forest where this species was found: Bosque Estatal de Guilarte.

Remarks. – Sticta guilartensis is morphologically similar to the cyanomorph of species that form photosymbiodemes, particularly to *S. lobarioides*, a recently described species from Colombia that is found in well-preserved forests (Moncada, Coca, and Lücking 2013). For *S. lobarioides*, it is the chloromorph counterpart that is most commonly found in those forests, suggesting that pristine ecological conditions are most likely a pre-requisite for the chloromorphs

to occur. Because Puerto Rico underwent substantial land degradation at the beginning of the 20th century (Grau et al. 2003), the likelihood for a chloromorph of *S. guilartensis* to be found is low, but it may have been present before that period. Within Puerto Rico, it is most closely related to the morphologically dissimilar *S.* aff. *guilartensis*, an undescribed species from the western mountains of the island.

Even though lichen collecting efforts have occurred in the past, to our knowledge, no species of *Sticta* have been collected in Bosque Estatal de Guilarte previous to our efforts. This species may have escaped detection in the past because of its broad resemblance to other genera of cyanolichens like *Leptogium* (Ach.) Gray, with whom it shares the foliose growth habit, dark color when wet, and occurrence in humid microhabitats. On the other hand, during our examination of the *S. guilartensis* material, we noted the frequent presence of clusters of free living *Nostoc* on the upper thallus surface. An interesting avenue of research would be to determine if the presence of these clusters is due to random growth of foreign *Nostoc* on the surface or if it resulted from the growth of previously lichenized *Nostoc* that escaped lichenization.

Additional specimens examined. – PUERTO RICO: Mun. Adjuntas, Bosque Estatal Guilarte, Along trail to Pico Guilarte; 18°08′24″N, 66°46′12″W; 1100 m; 27 Dec 2016, *Mercado-Díaz 2426* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta harrisii Merc.-Díaz, Moncada & Lücking, **sp. nov.** [MycoBank # 834861] – Holotype: PUERTO RICO. Mun. Naguabo, Barrio Río Blanco, at the beginning of Trailwinds trail, El Yunque National Forest; 18°16′48″N, 65°47′24″W; 667 m; 26 Jul 2016, *Mercado-Díaz 2913* (F barcode C0172454F; isotype: UPR).

Species is illustrated in Fig. 1.8.

Diagnosis. – Differing from *Sticta tomentosa* in the absence of a basal stipe and the presence of a thick tomentum and branched phyllidia.

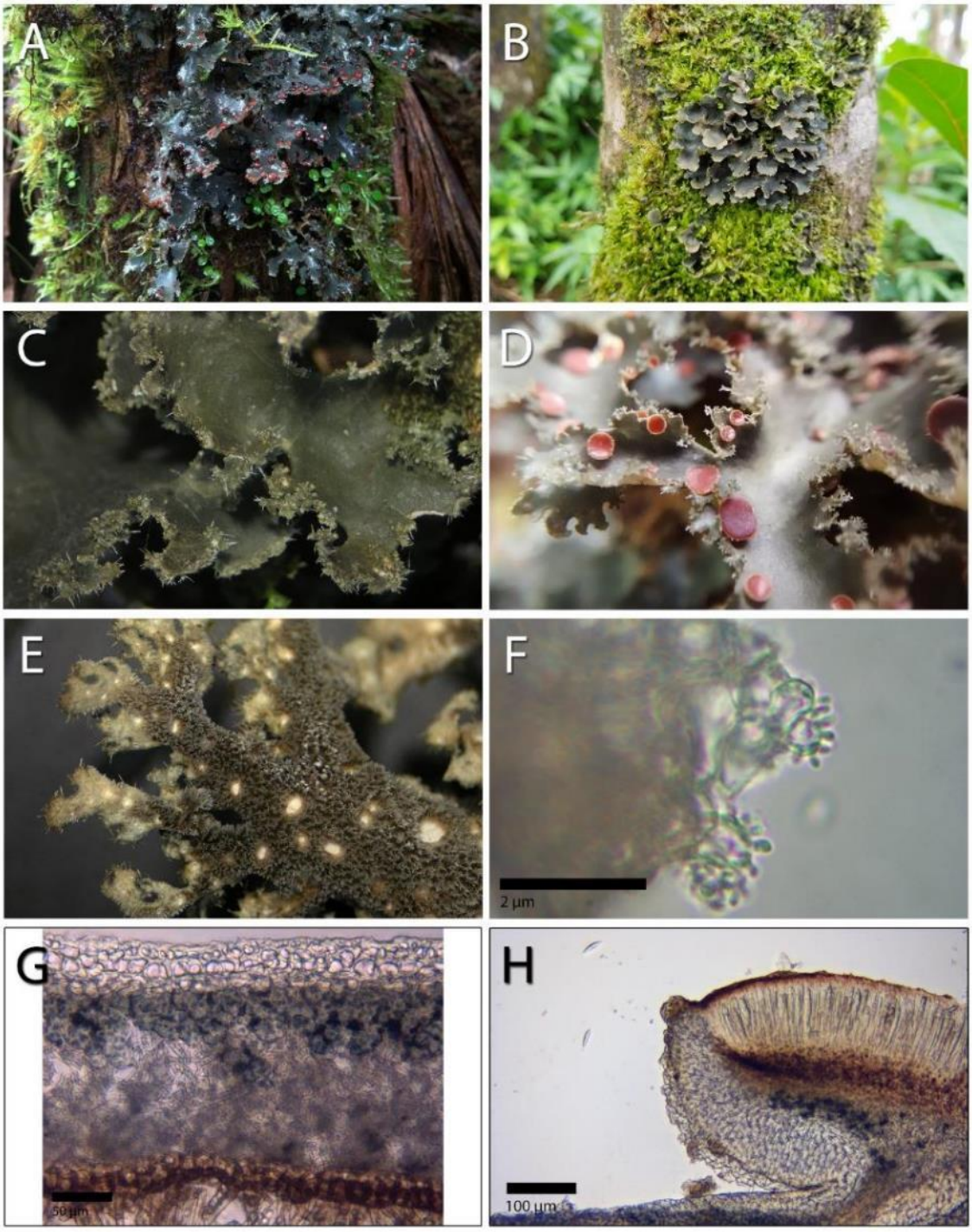

Figure 1.8. *Sticta harrisii* **A.** View of mature thallus in the field. **B.** View of rosette-shaped thallus in the field. **C.** Lobes with white marginal cilia**. D.** Lobes with submarginal and marginal apothecia and scattered marginal phyllidia. **E.** Lower surface with brown-gray primary tomentum and cyphellae. **F.** Detail of cyphellae basal membrane cells with numerous papillae. **G.** Section through thallus. **H.** Section of apothecia.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Basal stipe absent. Thallus irregular in outline, sometimes rosette-shaped, up to 15 cm diam., densely branched, with 6–10 branches per 5 cm radius, branching pleurotomous to polytomous; lobes flabellate to lingulate, horizontal to ascending, adjacent to imbricate, involute becoming undulate, with irregular, plane or weakly revolute apices, margins crenate to dissected, not thickened; lobe internodes 0.2–4 mm long, (1–)1.5–4(–5) mm broad; thallus resistant, subcoriaceous. Upper surface smooth, brownish grey to olive-grey both when fresh and in the herbarium, marginal line color same as lobe surface, moderately glossy; surface glabrous, without papillae, pruina, and maculae; Cilia marginal, abundant, simple and tapering, white, to 0.5 mm. Apothecia sparse to abundant, submarginal to marginal, dispersed to subaggregated, subpedicillated, base invagination pronounced, 0.4–1.5 mm diam., disc color dirty orange to brown and glossy when fresh to dark brown and opaque in herbarium, margin entire to minutely verrucose, rarely ciliate (mostly when young), cream-colored. Phyllidia abundant, marginal, dispersed, branched, simple to coralloid and becoming isidioid toward apices, irregularly oriented, up to 2 mm long and 0.1–2 mm broad, same color as thallus but becoming darker with age, slightly glossy, weakly flattened to dorsiventral in section, subcylindrical to squamiform, basal stalk flattened with cyphellae initials. Lower surface smooth to sinuose, pale white to cream-colored; primary tomentum dense, sparse or absent towards margin, thick, becoming thinner toward margins, spongy, soft, tan to dark brown, becoming yellowish when old, hairs occasionally whitish toward apices; secondary tomentum pubescent, pale white to cream-colored. Rhizines absent. Cyphellae abundant, 21–40 per cm^2 towards the thallus center and 41–60 per cm^2 towards the margin, dispersed, rounded to irregular (especially near margins), urceolate with wide pore to cupuliform, prominent, margin

remaining below the level of the primary tomentum, elevated and involute to erect, pale white to cream-colored, without tomentum; pore 0.2–1.5 mm diam.; basal membrane smooth to weakly pruinose, white, K+ yellow to dirty orange, C–, KC–, P–. Lower cortex K+ yellow, C-, KC+ yellow-orange, P–. Medulla compact, white, K+ yellow to dirty orange, C–, KC–, P–. Harrisii unknown (major). Pycnidia and cephalodia not seen.

Upper cortex paraplectenchymatous, 20–50 µm thick, homogeneous, consisting of 2–3(–4) cell layers with cells 5–23 µm diam., their walls 2–6 µm thick and their lumina rounded to isodiametric, 3.5–22 µm diam. Photobiont layer 50–75 µm thick, its cells 12.5–17.5 µm diam. Medulla 50–125 µm thick, its hyphae 2.5–3.5 µm broad, without crystals, becoming moniliform in apices. Lower cortex paraplectenchymatous, 20–35 µm thick, with 1–2 cell layers; cells 10–22 µm diam., their walls 2–5 µm thick. Hairs of lower primary tomentum 150–1750 µm long, in fascicles of more than 12 hyphae, unbranched, cylindrical, septate with intertwined apices. Hairs of lower secondary tomentum 20–50 µm long, dispersed, unbranched, cylindrical, with free apices. Cyphellae internal pore cavity 75–350 µm deep; cells of basal membrane with numerous papillae. Apothecia biatorine, 300–800 µm high, with stipe; excipulum 70–150 µm broad, minutely papillose. Hymenium 80–120 µm high; epihymenium 2.5–5 µm high, dirty orange to dark brown, without gelatinous layer above. Ascospores 1–septate, 25–42 × 5–7.5 µm, fusiform, hyaline.

Distribution and ecology. – Sticta harrisii is restricted to high-elevation rain forests to the east of the island, specifically in El Yunque National Forest and Bosque Estatal de Carite. It is mostly epiphytic and has been found growing on individuals of *Heterotrichum cymosum* (Wendl.) Urban and *Cecropia schreberiana* var. *schreberiana*, as well as on vines, ferns and less frequently on rocks. It seems to prefer shaded environments with high humidity.

Etymology. – This species is named after lichenologist Richard C. Harris, who prepared the first formal taxonomic treatment of lichens for the island and the first key to species of *Sticta* in Puerto Rico.

Remarks. – *Sticta harrisii* has a somewhat variable morphology that is apparently determined by age and/or microhabitat. Under shaded and humid conditions, mature individuals of this species usually feature moderately sized thalli that spread more horizontally on the substrate (Fig. 1.8.A). Other individuals, however, exhibit smaller, rosette-shaped thalli that have a higher density of imbricately arranged lobes (Fig. 1.8.B). It is most similar to *S. tomentosa* with respect to its greyish color, smooth surface, marginal cilia, rather light-colored tomentum and presence of apothecia, but its easily separated from that species by the presence of phyllidia. It is also remotely reminiscent of species within the *S. weigelii* morphodeme, most similar perhaps to "*S. pseudobeauvoisii* ined." (see Moncada 2012), with which it shares the presence of applanate, dorsiventral phyllidia, but from which differs in the distinctly lighter tomentum towards the margins and the highly dissected lobes. Within Puerto Rico, it is most closely related to *S.* aff. *harrisii*, a new, but still undescribed species from El Yunque National Forest.

Material in NY corresponding to *Sticta harrisii* was identified by Harris (1989) as "*S. circumroda*", a name attributed to Fée that was not validly published in (Hue 1901). According to Hue (1901), material originally identified by Fée as "*S. circumroda*" was similar to a specimen collected by Husnot in Guadeloupe (i.e., *Husnot #436*; Nylander 1869) that corresponded to *S. weigelii* f. *schizophylliza* (Nyl.) Hue. Curiously, high-resolution images showed that this material is more similar to *S. borinquensis*, a new species from Puerto Rico described here (see above). The characteristic marginal cilia and paler lower cortex and tomentum toward margins that characterize *S. harrisii* are in fact absent in this material.

Specimens referred to as "*Sticta schizophylliza* ined." in Moncada, Lücking, and Suárez (2013); i.e., *Lücking 33894*, *Lücking 33905*, *Lücking 33868*), on the other hand, correspond to this species.

Several individuals of *Sticta harrisii* were encountered by the first author during recent collecting efforts in El Yunque National Forest after Hurricane María hit Puerto Rico in September 2017. Different to other species discussed here (i.e., *S. densiphyllidiata* and *S. tainorum*), thalli from these individuals did not have necrotic areas and showed no signs of browning, which would be indicative of damage due to increase solar radiation after defoliation caused by the hurricane. On the contrary, *S. harrisii* individuals were more conspicuous and appeared to be abundant than before the hurricane. This suggests that *S. harrisii* might be better at taking advantage of newly available resources (i.e., opened substrate space) after this type of disturbances when compared to other sympatric species.

Additional specimens examined. – PUERTO RICO. Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850–950 m; 9 Jun 1988, *Harris 22497* (NY). Mun. Luquillo, El Yunque National Forest, near G. González (USFS) "Britton Palm" plot; 18°18′16″N, 65°47′43″W; 917 m; 27 Sep 2011, *Mercado-Díaz 960* (UPR). Mun. Río Grande, El Yunque National Forest, along trail to El Toro Peak from El Verde; 18°16′18″N, 65°49′53″W; 1006 m; 28 Dec 2015, *Mercado-Díaz 2375* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network's antennas; 18°06′42″N, 66°03′00″W; 865 m; 29 Jan 2015, *Mercado-Díaz 2282* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta parvilobata Merc.-Díaz, **sp. nov.** [MycoBank # 834862] – Holotype: PUERTO RICO. Mun. Adjuntas, Barrio Guilarte, Bosque Estatal de Guilarte, along trail to Pico Guilarte; 18°08′37″N, 66°46′08″W; 1100 m; 30 Jul 2018, *Mercado-Díaz 3668* (F barcode

C0172456F; isotype: UPR).

Species is illustrated in Fig. 1.9.

Diagnosis. – Differing from *Sticta ciliata* in the smaller lobes, presence of microfibrils in upper surface, branched isidia that are rarely strongly arbuscular, and frequent occurrence of maculae in central portion of young lobes.

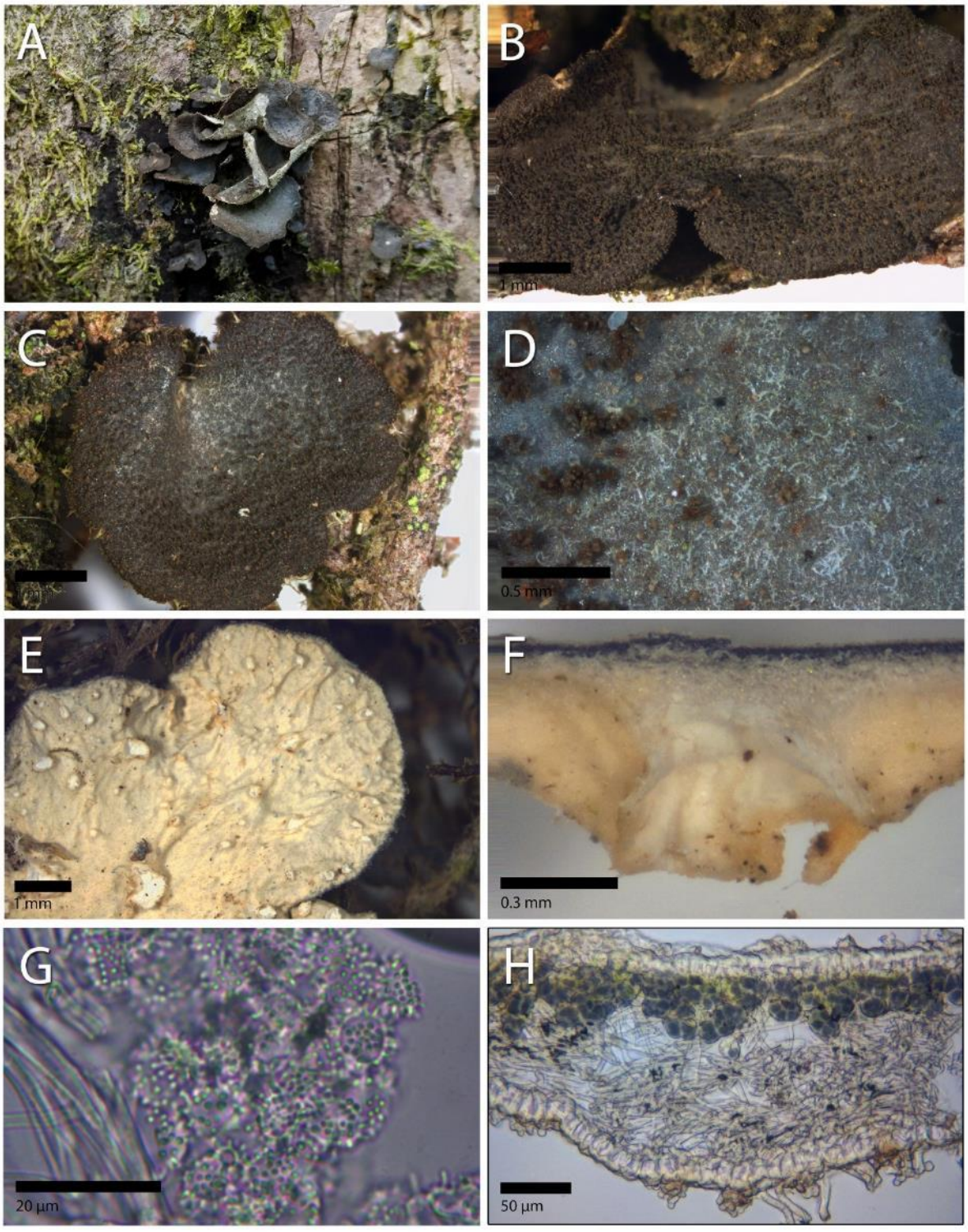

Figure 1.9. *Sticta parvilobata.* **A.** Thallus in the field. **B.** Lobes upper surface with laminal isidia. **C.** Young lobe with maculae in the center. **D.** Microfibrils in the upper surface. **E.** Lower surface with cyphellae. **F.** Section of cyphellae. **G.** Detail of cyphellae basal membrane cell papillae. **H.** Section through thallus.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Thallus flabellate when young, eventually orbicular in outline, up to 2 cm diam., sparsely branched, with 0–2 branches per 5 cm radius, branching pleurotomous to polytomous, occasionally in clusters of overlapping lobes; lobes suborbicular, ascending, interspaced to imbricate, plane to involute, with plane to weakly revolute apices, margins entire to weakly crenate, not thickened; lobe internodes 0.05–1 mm long, 4–12 mm broad; thallus fragile, papyraceous. Upper surface smooth to scrobiculate-faveolated when old and pitted with scars of broken isidia, grey to brownish grey, both when fresh and in herbarium, marginal line color same as lobe surface, opaque; surface glabrous but with pubescent appearance under high magnification due to small, whitish microfibrils; without papillae or pruina. Cilia scarce, but sometimes abundant in young lobes, nearly absent with age, simple, white or pale, to 0.5 mm. Minute maculae frequent in young lobes, reticulated, white, more evident toward central portions of thallus. Apothecia not observed. Isidia abundant, laminal extending towards margins, dispersed to subaggregated, branched, simple to coralloid, vertically oriented, up to 0.5 mm long and 0.02–0.06 mm broad, same color as thallus, opaque to weakly glossy, rounded in section, granular to globular, basal stalk cylindrical. Lobules rare, marginal, dispersed, unbranched, horizontal, to 1 mm and 0.6–1.4 mm broad, same color as thallus, opaque, dorsiventral in section, lobuliform, basal stalk cylindrical. Lower surface costillate to scrobiculate, cream-colored; primary tomentum sparse but sometimes dense in points of attachment to substrate, becoming sparser towards margins, thin throughout, hirsute to fasciculated, soft, white to cream-colored; secondary tomentum pubescent, pale white to cream-colored. Rhizines absent. Cyphellae sparse, 1–20 per cm^2 towards the thallus center and 21–40 per cm^2 towards the margin, dispersed, irregular to angular, cupuliform to pseudocyphelloid,

prominent to suprasessile, levelled or above the level of the primary tomentum, margin erect to weakly revolute, white, tomentum occasionally present; pore 0.1–1.8 mm diam.; basal membrane smooth, white, K+ yellow to weak orange, C–, KC–, P–. Medulla lax, white, K+ yellow to weak orange, C–, KC–, P–. No substances detected by HPTLC. Pycnidia and cephalodia not seen.

Upper cortex paraplectenchymatous, 10–15 µm thick, homogeneous, consisting of 1(2) cell layers with cells 7–15 µm diam., their walls 1.5–2.5 µm thick and their lumina rounded to isodiametric, 5–14 µm diam. Photobiont layer 25–70 µm thick, its cells 10–20 µm diam. Medulla 25–65 µm thick, its hyphae 2–3 µm broad, without crystals. Lower cortex paraplectenchymatous, 10–14 µm thick, with 1 (2) cell layers; cells 8–17 µm diam., their walls 1.5–2.5 µm thick. Hairs of lower primary tomentum 100–400 µm long, in fascicles of more than 6–12 hyphae, mostly unbranched, septate but septa less evident in older hairs, with free to intertwined, cylindrical apices. Hairs of lower secondary tomentum 12–25 µm long, dispersed, occasionally branching, septate, moniliform, with free apices. Cyphellae internal pore cavity 50–400 µm deep; cells of basal membrane with numerous papillae.

Distribution and ecology. – *Sticta parvilobata* has only been found in high-elevation forests of the Bosque Estatal de Toro Negro and Bosque Estatal de Guilarte, two natural protected areas in the central-west region of the Cordillera Central. It is therefore considered to have a western distribution within the island. It is epiphytic and is commonly found growing among bryophytes.

Etymology. – This name refers to the generally smaller lobes (compared to *S. ciliata*) of mature individuals of this species.

Remarks. – *Sticta parvilobata* is closely related to *S. ciliata*, a lineage characterized by thalli formed by single, suborbicular to palmate lobes that frequently overlap each other, simple

laminal isidia, papillose cyphellae basal membrane cells and marginal white cilia (Magain and Sérusiaux 2015). It is very similar to *S.* aff. *parvilobata* from Puerto Rico, but distinguishable from it by its generally smaller-sized mature lobes, shorter branched isidia that are usually more evenly distributed in the surface, occasional presence of microfibrils in the upper surface and minutely maculate young lobes. Also, mature lobe margins in *S.* aff. *parvilobata* are usually more sharply revolute than in *S. parvilobata*. Harris (1989) referred to both *S. parvilobata* and *S.* aff. *parvilobata* as *Sticta* sp. 22678. Both lineages form a monophyletic Puerto Rican clade with unresolved affinities to *S. ciliata*. While more work will be needed to clarify phylogenetic relationships, results from phylogenetic and morphoanatomical analyses, in combination with molecular species delimitation approaches and geographical patterns convinced us of treating *S. parvilobata* as a separate species. Conversely, while *S.* aff. *parvilobata* exhibited some degree of genetic structure within the island, genetic signal was not sufficiently robust to resolve relationships between subclades. Additionally, this lineage is nearly morphologically indistinguishable from *S. ciliata*, which made resolving boundaries within this clade more challenging.

Sticta parvilobata is distributed in the western region of the island; therefore, material that resembles this species but is collected closer to the east most likely belong to *S.* aff. *parvilobata*. Specimens collected to the west, however, would need to be carefully inspected for proper identification given that both species are sympatric in this region. Lastly, even though apothecia are known to occur in *S. ciliata* (Magain and Sérusiaux 2015), these reproductive structures have not been observed in *S. parvilobata* and *S.* aff. *parvilobata*. More specimens will be needed to corroborate their presence in material from the island.

Additional specimens examined. – PUERTO RICO. Mun. Adjuntas, Barrio Guilarte,

Bosque Estatal de Guilarte, along trail to Pico Guilarte; 18°08′24″N, 66°45′36″W; 927 m; 27 Dec 2016, *Mercado-Díaz 2432* (UPR). Mun. Orocovis, Barrío Bauta Abajo, Along El Bolo trail, Bosque Estatal de Toro Negro; 18°10′19″N, 66°29′07″W; 927 m; 22 Jan 2015, *Mercado-Díaz 2260* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta riparia Merc.-Díaz, **sp. nov.** [MycoBank # 834863] – Holotype: PUERTO RICO. Mun. Aibonito, Barrio Asomante, San Cristobal Canyon, on rock face by the river; 18°09′34″N, 65°18′05″W; 465 m; 31 Jul 2018, *Mercado-Díaz 3677* (F barcode C0172457F; isotype: UPR).

Species is illustrated in Fig. 1.10.

Diagnosis. – Differing from *Sticta densiphyllidiata* in the darker lower surface, smaller lobes, presence of pycnidia and occasionally branched primary tomentum hairs.

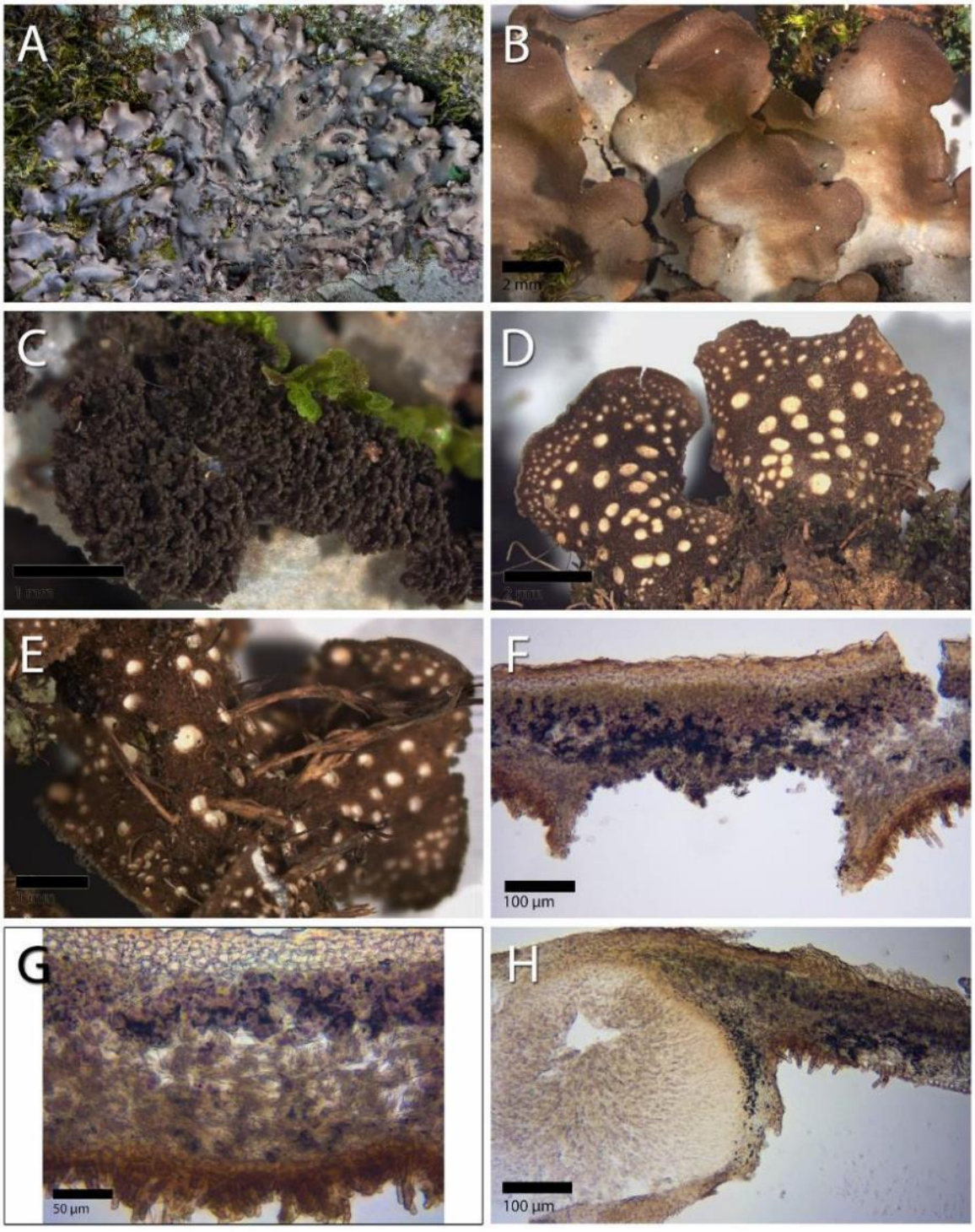

Figure 1.10. *Sticta riparia.* **A.** Thallus in the field. **B.** Close-up of upper surface and lobes configuration. **C.** Detail of marginal, agglutinated phyllidia. **D.** Lower surface with short brown tomentum and cyphellae. **E.** Detail of long, whitish to cream-colored penicillated rhizines. **F.** Section of cyphellae. **G.** Section through thallus. **H.** Section through thallus with internal pycnidia in initial stages of development.

Description. – Primary photobiont cyanobacterial (*Nostoc*). Basal stipe absent. Thalli mostly orbicular in outline, up to 10 cm diam. but frequently aggregating and forming patches >50 cm diam., densely branched, with 6–10 branches per 5 cm radius, branching pleurotomous to polytomous; lobes suborbicular, adnate to horizontal, imbricate, undulate, with rounded, undulate to weakly revolute apices, margins entire, not thickened; lobe internodes 1–3 mm long, (1.5–)2–4(–5.5) mm broad; thallus resistant, subcoriaceous. Upper surface smooth, grey to brown both when fresh and in herbarium, becoming darker brown towards lobes, especially in exposed conditions, marginal line color slightly darker to same as lobe surface, opaque to weakly glossy; surface glabrous, without papillae, pruina, maculae or cilia. Apothecia not observed. Phyllidia abundant, marginal, aggregated, branched, palmate and sometimes isidiate in appearance due to strong imbrication of phyllidia, oblique, up to 0.5 mm long and 0.1–0.5 mm broad, dark brown, glossy, flattened in section, spathulate, basal stalk applanate. Lower surface smooth to weakly scrobiculate toward margins, light greyish-brown to dark brown, becoming darker towards center; primary tomentum dense, absent towards margin, thin, becoming thinner towards margin, pubescent to hirsute, soft, brown. Rhizines sparse, dispersed or towards thallus center, simple becoming penicillate, whitish to cream-colored, up to 3 mm. Cyphellae abundant, 41–60 per cm^2 towards the thallus center and 101–200 per cm^2 towards the margin, dispersed, rounded to irregular, urceolate with wide pore, erumpent to prominent, at or above level of primary tomentum; margin levelled to elevated and involute, cream-colored to light brown, without tomentum; pore 0.1–0.8 mm diam.; basal membrane smooth, white, K+ weak yellow, C–, KC–, P–. Medulla compact, white, K+ weak yellow, C–, KC–, P–. Riparia unknown (major). Pycnidia erumpent, brown, protruding to the lower surface when mature (resembling a tubercle).

Cephalodia not seen.

Upper cortex paraplectenchymatous, 25–40 µm thick, homogeneous, darkening towards outer cortex, consisting of (2–)3–4 cell layers with cells 5–15 µm diam., their walls 1.25–3 µm thick and their lumina rounded to isodiametric, 3.5–14 µm diam. Photobiont layer 40–75 µm thick, its cells 12–18 µm diam. Medulla 50–90 µm thick, its hyphae 2.5 µm broad, without crystals. Lower cortex paraplectenchymatous, 12.5–25 µm thick, with 1–2(–3) cell layers; cells 5–15 µm diam., their walls 1–2.5 µm thick. Hairs of lower primary tomentum 50–150 µm long, dispersed, occasionally branching, cylindrical, highly septate with free moniliform apices. Cyphellae internal pore cavity 80–150 µm deep; cells of basal membrane without papillae.

Distribution and ecology. – *Sticta riparia* has been collected mostly along riverbanks or areas not far from rivers along the Cordillera Central, at elevations not higher than 800 m. It seems to prefer well-conserved areas, but it has also been collected in secondary forests in both semi-open and shaded conditions. Although this species has been found growing on tree branches, rocks seem to be its preferred substrate. *Sticta riparia* has been collected at 270 m (Tanamá river, near entrance to Radio Telescopio cave), which is lowest recorded elevation for any of the species that occur on the island.

Etymology. – The name alludes to the common occurrence of this species near rivers or riverbanks.

Remarks. – Together with *Sticta densiphyllidiata*, *S. riparia* is referred to in Harris (1989) as "*S. trichographis* Fée ined.". It is similar to that species in many respects, such as the presence of small, branched to palmate phyllidia along the margins and the very short tomentum which becomes absent towards lobe margins. However, *S. riparia* differs from *S. densiphyllidiata* by its lobes of smaller average size, presence of pycnidia and generally darker lower cortex. Based on

our molecular data, *S. riparia* and *S. densiphylllidiata* are closely related to each other and also to *S. laciniosa* D.J.Galloway, a species with a green algal photobiont that apparently lacks a cyanomorph counterpart. It is worth noting that although specimen *Mercado-Díaz 3684* is well nested within the *Sticta riparia* clade and is considered representative of that species, this material exhibits a pale-beige lower cortex and lighter grey upper surface which is not typical for this species. Its longer branch length also suggests some degree of incipient divergence within the clade. More material and additional sequences from this variant will be needed to further clarify these issues.

Additional specimens examined. – PUERTO RICO: Mun. Aibonito, San Cristobal Canyon, on rock face by the river; 18°09′34″N, 65°18′05″W; 465 m; 31 Jul 2018, *Mercado-Díaz 3678* (UPR). Mun. Arecibo, Barrio Esperanza, Surroundings of Río Tanamá, near to entrance of the Radiotelescopio cave; 18°20′30″N, 66°45′19″W; 185 m; 13 Apr 2015, *Mercado-Díaz 2342* (UPR). Mun. Orocovis, Distr. Ponce: Cordillera Central, above Villalba, Doña Juana recreation area; 800–1000 m; 2 Jun 1988, *Harris 22034* (NY). Mun. Patillas, Carite State Forest, Charco Azul Recreation Area, in camping area by the river; 18°05′28″N, 66°02′04″W; 599 m; 15 Jul 2018, *Mercado-Díaz 3626* (UPR). Refer to Appendix 2. for additional specimens revised.

Sticta tainorum Merc.-Díaz, **sp. nov.** [MycoBank # 834864] – Holotype: PUERTO RICO. Mun. Orocovis, Barrio Bauta Abajo, along trail to observation tower, Toro Negro State Forest; 18°10′15″N, 66°28′52″W; 1036 m; 22 Jan 2015, *Mercado-Díaz 2259* (F barcode C0172450F; isotype: UPR).

Species is illustrated in Fig. 1.11.

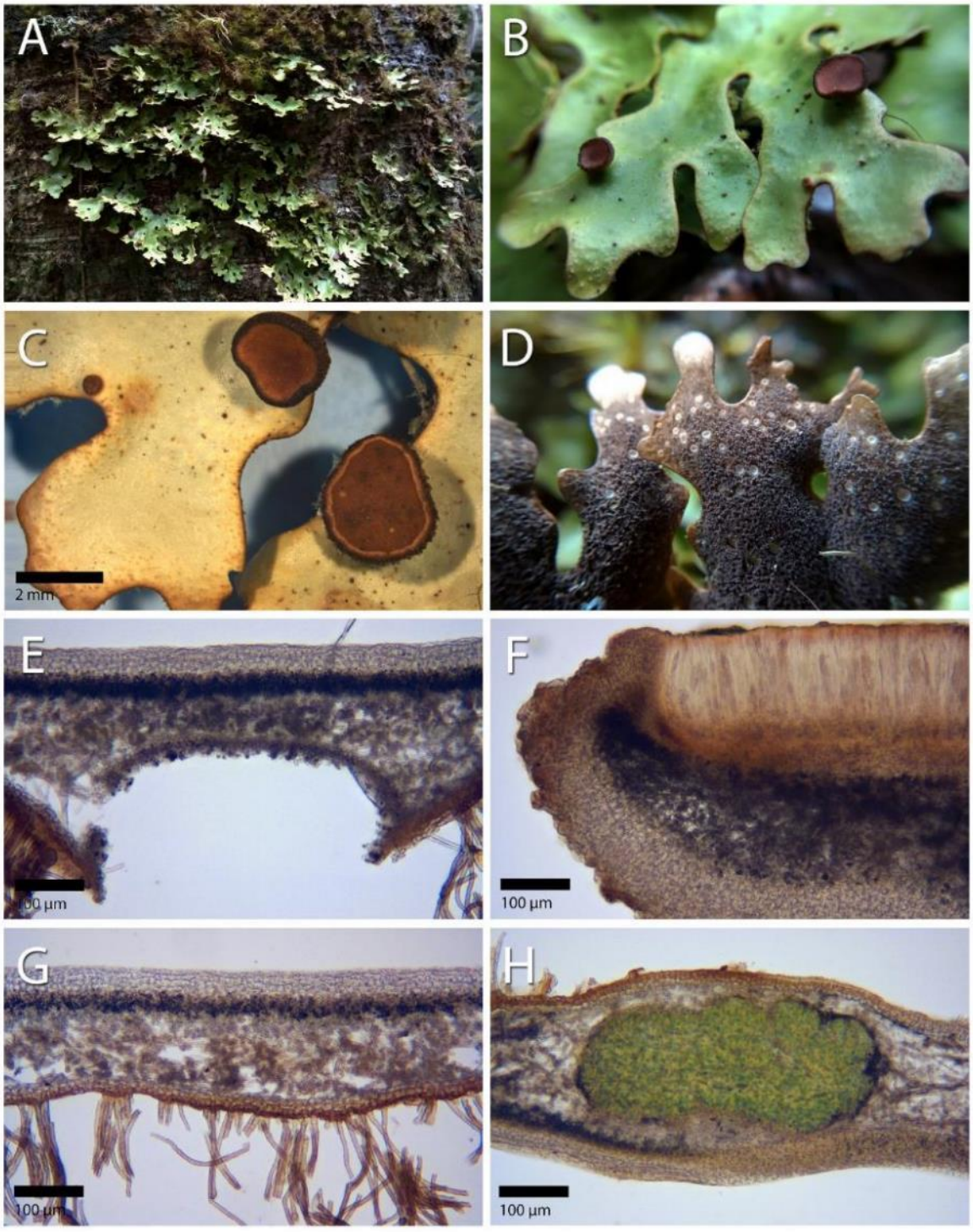

Figure 1.11. *Sticta tainorum* **A.** Thallus in the field. **B.** Lobes upper surface with apothecia when fresh **C.** Detail of lobes and apothecia when dry. **D.** Lower surface with short brown tomentum and small cyphellae. **E.** Section of cyphellae. **F.** Section of apothecia. **G.** Section through thallus. **H.** Section of internal cephalodia.

Diagnosis. – Differing from *Sticta damicornis* f. *rudiuscula* Vain. in the lighter thallus color, narrower lobes and presence of sparse, simple papillae.

Description. – Primary photobiont a green alga. Basal stipe absent. Thallus irregular to orbicular in outline, up to 30 cm diam., densely branched, with 6–10 branches per 5 cm radius, branching anisotomic to pleurotomous; lobes lingulate to flabellate, horizontal to ascending, interspaced to adjacent, plane to involute, with rounded to truncate, plane to revolute apices; margins entire, slightly thickened; lobe internodes 2–7 mm long, 2–5(–9) mm broad; thallus resistant, coriaceous. Upper surface smooth, green when fresh and greenish grey to tan and darkening toward margins in the herbarium, glossy; surface glabrous, sometimes with submarginal, simple papillae. No pruina, cilia or maculae observed. Apothecia sparse, marginal, dispersed to subaggregated, pedicillated, base invagination pronounced, 1–3 mm diam., disc color reddish-brown both when fresh and in herbarium, darkening with age, opaque, margin cream-colored to brown, verrucose, becoming weakly crenulate and occasionally tomentose. Lobules absent to sparse, marginal, dispersed, unbranched, simple, with horizontal orientation, to 3 mm long and 1–2 mm broad, same color as thallus, moderately glossy, dorsiventral in section, lobuliform, basal stalk applanate. Lower surface smooth, cream-colored to light brown; primary tomentum dense, sparse towards margin, thin throughout, hirsute to strigose towards margins becoming spongy towards center, soft, brown. Rhizines sparse, submarginally aggregated, fasciculated, dark brown, to 2 mm. Cyphellae abundant, 40–60 per cm^2 towards the thallus center and 61–100 per cm^2 towards the margin, dispersed, rounded, urceolate with wide pore, erumpent to prominent, margin remaining below the level of the primary tomentum, elevated and involute, cream-colored, without tomentum; pore 0.2–1 mm diam.; basal membrane pruinose, white, K+

yellow–orange, C–, KC–, P–. Medulla compact, cream colored, K+ yellow–orange, C–, KC–, P–. Tainorum unknown (major) Harrisii unknown (minor), Unknown 1 (minor). Pycnidia immersed, brown. Cephalodia internal.

Upper cortex paraplectenchymatous, 25–35 µm thick, homogeneous, consisting of 3–4 cell layers with cells 5–17 µm diam., their walls 1.5–2.5 µm thick and their lumina rounded to isodiametric, 4–14 µm diam. Photobiont layer 15–30 µm thick, its cells 2.5–5 µm diam. Medulla 75–150 µm thick, its hyphae 2.5–3.5 µm broad, without crystals. Lower cortex paraplectenchymatous, 20–30 µm thick, with 2–3 cell layers; cells 5–18 µm diam., their walls 2–3 µm thick. Hairs of lower primary tomentum 200–700 µm long, in fascicles of 6–12 hyphae, unbranched, cylindrical, septate with free apices. Cyphellae internal pore cavity 100–200 µm deep; basal membrane cell papillae absent. Apothecia biatorine, 800–1100 µm high, with stipe; excipulum 100–130 µm broad, rarely with tomentum. Hymenium 100–120 µm high; epihymenium 2.5–5 µm high, dirty orange without gelatinous layer above. Ascospores 3-septate, 25–35 × 5–8.5 µm, fusiform, hyaline.

Distribution and ecology. – *Sticta tainorum* is the only green *Sticta* found in Puerto Rico. It is restricted to high-elevation, well-conserved forests to the west of the island, specifically near Pico Doña Juana in the Bosque Estatal de Toro Negro. *S. tainorum* is a rather rare species known only from a few trees in that mountain. Individuals of this species may cover large areas of the trunks in this forest.

Etymology. – This species is dedicated to the Taíno people, the indigenous people that inhabited Puerto Rico before the Spanish invasion.

Remarks. – *Sticta tainorum* is identified in Harris (1989) as *Sticta* sp. 1155. Delimitation analysis with BPP suggested that this species and a specimen of *S. laciniata* Ach. from Costa

Rica were the same species. Considering the poor support for their sister relationship in our multilocus tree and the fact that other species delimitation methods considered them separate, we argue they should be treated as different species. In terms of morphology, *S. tainorum* it is most similar to the type material of *S. sinuosa* Pers. collected in Brazil and *S. sinuosa* var. *flavicans* Müll.Arg. collected in Jamaica. Because of their geographic proximity it is possible that the material from Puerto Rico and Jamaica belong to the same species. Yet, we have generally observed differences between the lichen biota of Cuba and Jamaica on one hand and Puerto Rico on the other, the latter showing stronger affinities with the Lesser Antilles. Therefore, we are not taking up the infraspecific epithet "flavicans" for the present taxon. It easily distinguished from the recently established *S. aongstroemii* Dal-Forno et al. (Dal Forno, Moncada, and Lücking 2018) and the Caribbean endemic *S. damicornis* (Moncada, Mercado-Díaz, and Lücking 2018) by the shape of the lobes, being lingulate-flabellate in *S. tainorum* vs. linear in *S. aongstroemii* and *S. damicornis*. This species also resembles *Sticta damicornis* f. *rudiuscula*, a putative endemic species from Colombia that is known only from the type specimen (Moncada 2012).

As highlighted above, living individuals of *S. tainorum* were examined in the field after Hurricane María (Sept. 2017). These thalli showed considerable browning, possibly resulting from prolonged exposure to sunlight resulting from canopy defoliation (Fig. S.1.6.). High resistance of desiccated thalli due to extreme climatic conditions (such as increased irradiation) is a general feature in lichens (Kranner et al. 2008); however, experimental increases in temperature and light simulating the effects of logging on other members within Peltigerales (i.e., *Lobaria pulmonaria*) showed that increased solar irradiation can have lethal consequences on natural populations (Gauslaa and Solhaug 1999). We, therefore, suggest that increased irradiation currently threaten the long-term persistence of *S. tainorum* populations within the

island. Because this species is also known from just few tree individuals in the Toro Negro State Forest, studies to assess the conservation status of this species are urgently needed.

Additional specimens examined. – PUERTO RICO. Mun. Adjuntas, unk. elev., 6 Jan 1886, *Sintenis L.123* (NY, US). Mun. Orocovis, Toro Negro State Forest, along trail to observation tower; 18°10′15″N, 66°28′52″W; 1036 m; 22 Jan 2015, *Mercado-Díaz 2256* (UPR). Mun. Ponce, San Narciso; 900 m; 6–8 Feb 1923, *N.L. & E.G. Britton 7313* (NY, US). Mun. Utuado, upper slopes of Mount Morales; approx. 900 m; 19 Mar 1906, *Howe 1155* (NY). Refer to Appendix 2. for additional specimens revised.

CONCLUSIONS

The Caribbean islands have long been recognized as an important region for global biodiversity. Although past studies using a variety of molecular and taxonomic approaches advanced our knowledge on biotic richness for many groups in this region, none until this study focused on lichens. We showed that species richness of *Sticta* in Puerto Rico is higher than previously assumed and that most species (~ 69%) are potentially endemic to the island. Furthermore, phylogenetic analyses showed that species from Puerto Rico do not form a monophyletic clade. This suggests that the current species assemblages resulted from multiple colonization events and that evolutionary radiations did not play a major role in the diversification of *Sticta* within the island. Evolutionary relationships inferred from our phylogenies also suggested stronger biogeographic links to South America, but ancestral area reconstruction studies will be needed to properly assess geographic affinities.

One interesting finding was observing that similar to Harris (1989), most species delimited in this work could still be separated using morpho-anatomical characters, reiterating

the validity of morphology-based species delimitations in some groups of lichens. Although this observation has been previously made for *Sticta* and other members within Lobarioideae, its significance for biodiversity research and conservation in the Caribbean cannot be overstated. The capability of separating species based on morphology provides a straightforward way of documenting species richness, an important metric used by community ecologists to describe the unique biodiversity in these islands. It also opens the door to more frequent integration of lichens in biodiversity inventories and conservation initiatives and more participation of non-experts in this type of efforts. This is particularly important in areas where local biotas are under threat, such as the Caribbean islands which have been severely affected by past agricultural activities and are affected by anthropogenically driven factors such as urban sprawl and climate change.

Our study adds to numerous studies highlighting the importance of integrating molecular methods for obtaining more accurate estimates of lichen species richness in a region. It also shows that inferences of species diversity should be made preferentially after repeated sampling of areas with suitable habitat for the target taxa. To conclude, it is worth noting that patterns uncovered for *Sticta* in this study could also exist for other groups and other islands in the region. Unfortunately, most aspects about the diversity, distribution and conservation status of the lichen biota of the Caribbean remain poorly known. More studies applying methods similar to those used here will be critical for filling these knowledge gaps. They should also demonstrate that Caribbean biodiversity hotspot still have much to contribute towards the study of ecological and evolutionary processes, as well as conservation of biodiversity in island ecosystems.

CHAPTER 2

A HOLISTIC VIEW OF THE FACTORS SHAPING THE DIVERSITY OF THE LICHEN-FORMING FUNGAL GENUS *STICTA* (LICHENIZED ASCOMYCOTA: PELTIGERALES) IN THE CARIBBEAN

Abstract Phylogenetic approaches to macroevolution have provided unique insight into evolutionary relationships, ancestral ranges, and diversification patterns in lichenized fungi. Phylogenetic frameworks have also been developed to assess how environmental and/or spatial variables shape species diversity and distribution patterns at different spatial/temporal scales, but lichen studies implementing these are still scarce. Here, we combine phylogeny-based ancestral range reconstruction and diversification analysis with a community phylogenetics approach to reconstruct evolutionary origins and assess patterns of taxonomic and phylogenetic relatedness between island communities of the lichen genus *Sticta* in the Caribbean. Sampling was carried out in the Greater Antilles (Cuba, Jamaica, Hispaniola, and Puerto Rico) and Lesser Antilles (Dominica, Guadeloupe, and Martinique). Data for six molecular loci were obtained for 64 candidate Caribbean species and used to perform both macroevolutionary phylogenetics, which also included worldwide taxa, and phylobetadiversity/clustering analyses, which emphasized island-level communities. Our work uncovered high levels of island endemism (~ 59%) in Caribbean *Sticta*. We estimate initial colonization of the region occurred about 19 Mya from a South American ancestor. Reverse migration events by Caribbean lineages to South America were also inferred. We found no evidence for increased diversification rates associated with range expansion into the Caribbean. Taxonomic and phylogenetic turnover was most strongly correlated with environmental variation rather than with geographic distance. We observed less

dissimilarity among communities from the Dominican Republic and Jamaica than between these islands and the Lesser Antilles/Puerto Rico. High levels of hidden diversity and endemism in Caribbean *Sticta* reaffirm that islands are crucial for the maintenance of global lichen biodiversity. Strong evolutionary links exist between Caribbean and South American biotas but at regional scales, species assemblages exhibit complex taxonomic and phylogenetic relationships that are determined by local environments and shared evolutionary histories.

Keywords Lobarioid Peltigeraceae, Caribbean, Diversification, Biogeography, Phylobetadiversity, Biodiversity

INTRODUCTION

Studies on Caribbean Island biotas have had a profound impact on our understanding of the evolution and diversification of a variety of lineages. This archipelago has been pivotal for exploring many biogeographical processes such as colonization and extinction dynamics in island systems (Ricklefs 2009), and demonstrated the importance of repeated evolution of similar phenotypes in insular adaptive radiations (Losos et al. 1998). As such, the Caribbean represents a vital biogeographic region for evaluating how ecological and evolutionary processes interact to shape biodiversity patterns in natural systems (Ricklefs and Bermingham 2008).

Like other insular regions, our understanding of the ecology and evolution of Caribbean biotas has been mostly shaped by observations on vascular plants and vertebrate assemblages. For the Caribbean, the most notable example is that of *Anolis* lizards which underwent an impressive adaptive radiation in these islands (Losos et al. 1998). Work in many other groups,

including plants (Hidalgo et al. 2020; Cervantes et al. 2016) and birds (Ricklefs and Lovette 1999; Bellemain, Bermingham, and Ricklefs 2008), have also been instrumental. However, plants and vertebrates comprise less 5% of the species in the planet (based on Chapman [2009]), thus they represent a small proportion of the biodiversity in most regions, including the Caribbean. Further efforts to evaluate how diversification, adaptive radiation, and community assembly processes operate in groups like invertebrates and fungi, which collectively account for nearly 70% of the global biodiversity, will ultimately be key to understand the extent to which ecological and evolutionary principles derived from studying plants and vertebrate assemblages are generalizable to other systems.

Advances in molecular phylogenetic approaches have been critical for elucidating evolutionary and biogeographic patterns and processes in fungi and other highly diverse taxa. A notable case within this group are lichens, symbiotic organisms formed by an association between a main exhabitant fungus, a photosynthetic partner (green algae and/or cyanobacterium), and specific components of the microbiome contained in the lichen thallus, including bacteria and cortical fungi (Hawksworth and Grube 2020). It was generally believed that, at the species level, lichens were more widespread than vascular plants and animals (Lücking 2003; Feuerer and Hawksworth 2007; Galloway 1979; Smith 1993); however, molecular data have shown that diverse lineages, often with a distinct geographic structure and sometimes without discernable phenotypic differentiation, are frequently found within nominal species previously considered to be widespread (Crespo and Lumbsch 2010; Leavitt, Kraichak, et al. 2016; Singh et al. 2015; Onuţ-Brännström, Tibell, and Johannesson 2017; Widhelm et al. 2021; Dal Forno et al. 2017). These discoveries had a major impact on studies focusing on island lichens as they soon revealed that insular biotas were much more than subsets of continental

lineages, but diverse species assemblages with truly unique evolutionary histories (Dal Forno et al. 2017; Sérusiaux et al. 2011; Moncada, Reidy, and Lücking 2014; Mercado-Díaz, Lücking, and Parnmen 2014; Simon et al. 2018).

The currently available information about species richness and the underlying evolutionary mechanisms and biogeographical histories that led to extant diversity patterns is relatively sparse for lichens in the Caribbean. Only a few phylogenetic studies, focused on taxonomic revisions of genera, have included material from this region. Notably, many have uncovered previously unrecognized, endemic species- and even genus-level lineages (Lücking et al. 2017; Mercado-Díaz, Lücking, and Parnmen 2014; Mercado-Díaz et al. 2020; Lücking et al. 2020), suggesting that species richness and the phylogenetic diversity represented in these island communities are likely underestimated. Recent work by Mercado-Díaz et al. (2020) on the genus *Sticta* in Puerto Rico was the first to integrate phylogenetic approaches, quantitative species delimitation methods, and a thorough analysis of phenotype characters, to disentangle the evolutionary history of a lichenized fungal lineage in this region. The study revealed that present-day assemblages were constituted by several widespread species, but also by presumed endemics that evolved from lineages apparently derived from South American ancestors. The authors noted, however, that suggested geographic affinities and potential evolutionary micro-radiations proposed for some clades represented tentative hypotheses in need of further studies, preferably utilizing ancestral range reconstruction and diversification analysis. In addition, molecular sampling for taxa in Mexico and Central America was limited.

Sticta (Schreb.) Ach. is a genus of lichenized fungi recognizable by their large foliose thalli, the presence of conspicuous, well-defined lower surface pores (i.e. cyphellae), and the ability to form associations with both cyanobacteria and green algae, sometimes by the same

fungus and even the same individual. Although only Puerto Rican *Sticta* have been thoroughly studied in the Caribbean (Mercado-Díaz et al. 2020), the recent circumscription of *S. damicornis* as a presumably restricted Greater Antillean endemic (Moncada, Mercado-Díaz, and Lücking 2018) suggests complex distribution patterns for the genus in this region. To explore these issues in more detail, we carried out comprehensive sampling and generated molecular data to infer evolutionary relationships of taxa and the degree of regional endemism. Ancestral range reconstruction was performed to assess geographic affinities and infer the timing of colonization events. We also evaluated if colonizing the Caribbean increased diversification rates. For many organisms, species distributions exhibit strong geographic structure within this region as is seen in many plant genera (Acevedo-Rodríguez and Strong 2008; Maunder et al. 2011; Nieto-Blázquez, Antonelli, and Roncal 2017; Roncal et al. 2020). We combined a taxonomic and phylogenetic beta diversity approach to ask if similar patterns of geographic structure are detected in Caribbean *Sticta*. When analyzed in tandem with environmental (e.g. elevation, climate) and spatial (geographic distance) variables, analysis of phylogenetic turnover (i.e. phylogenetic beta diversity, or "phylobetadiversity") allow us to better assess how local processes (e.g. environmental filtering) interact with regional processes (e.g. speciation, dispersal) to produce current species diversity and distribution patterns (Graham and Fine 2008; Leprieur et al. 2012).

Here we test whether presumed South American affinities hypothesized in a previous study on Puerto Rican species (Mercado-Díaz et al. 2020), are true for the Caribbean in general or whether more complex historical patterns can be found. We anticipate that range expansion over the islands of the Caribbean triggered in-situ evolution and high levels of endemism. In this connection, we study whether speciation and/or extinction rate shifts leading to evolutionary

radiations in some lichen clades, as found in Madagascar (Simon et al. 2018), are apparent in the Caribbean as well. Lastly, high dispersal ability inferred for *Sticta* (Widhelm et al. 2018) coupled with the relatively short distances between islands should result in patterns of taxonomic and phylogenetic turnover that are more strongly correlated with environmental gradients than with geographic distance. *Sticta* is most diverse in wet montane forests and alpine grasslands (páramos in the Neotropics) (Moncada 2012; Moncada, Lücking, and Lumbsch 2020), therefore high elevation communities (e.g. Hispaniola and Jamaica) are expected to be more diverse and less dissimilar among each other than they are to communities in lower elevations (e.g. Lesser Antilles).

MATERIALS AND METHODS

Sampling and DNA sequencing—Sampling for this study encompasses more than 100 collecting sites scattered throughout the Greater- (Cuba, Hispaniola, Jamaica, Puerto Rico) and the Lesser Antilles (Dominica, Martinique, Guadeloupe). These sites were clustered around 19 sampling areas (Fig. 2.1, described in Table S.2.1). Details of sampling in Puerto Rico (treated as a single sampling area) is described elsewhere (Mercado-Díaz et al. 2020). Collecting efforts were carried out in well preserved, low- (ca. 90 m) to high-elevation (ca. 2,136 m) forests. Sampling localities occur in vegetation types broadly classified as Tropical and Subtropical Moist Broadleaf Forests and Coniferous Forests. These areas contain preferred habitat types for *Sticta*, including Upper Montane and Cloud Forests. Sampling efforts in the region started in 2015 and have yielded a total of 595 specimens to date (deposited at: Field Museum [F], Herbario del Jardín Botánico de la Universidad de Puerto Rico, Río Piedras [UPR], Universidad

Distrital “Francisco José de Caldas” [UDBC], and Botanischer Garten und Botanisches Museum Berlin [B]). Part of this material was used to generate our molecular data.

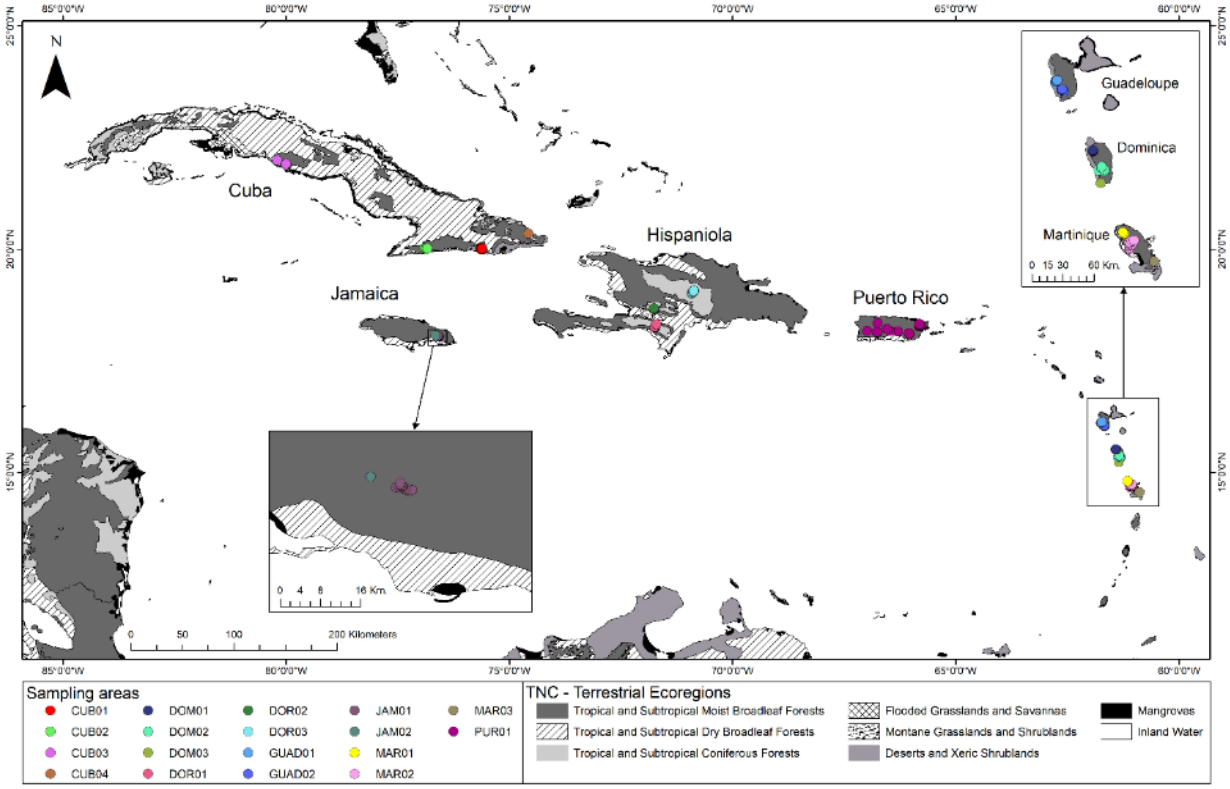

Figure 2.1. Map of sampling areas (colored dots) of *Sticta* in the Caribbean. Terrestrial ecoregions from The Nature Conservancy (TNC) are used as a base layer. Refer to Table S.2.1 for additional information on sampling areas.

Sequences from six gene regions were generated and used for phylogenetic analysis of the fungal symbionts. The loci include the internal transcribed spacer (ITS ~ 600 bp), which is the universal barcode for fungi (Schoch et al. 2012), the mitochondrial small subunit (mtSSU ~ 800 bp), the nuclear large subunit (nuLSU ~ 550 bp), the DNA replication licensing factor (MCM7 ~ 600 bp), the RNA polymerase II largest subunit (RPB1 ~ 900 bp), and the RNA polymerase II second largest subunit (RPB2 ~ 700), the latter three being low-copy nuclear

protein-coding genes. DNA extraction and amplification procedures are further described in Appendix 3.

Filtering and candidate species delimitation—Newly generated Caribbean sequences were assembled in Geneious 8.1.7 (https://www.geneious.com) and queried in the BLASTn suite in GenBank (Benson et al. 2018) for initial assessment. After confirming correspondence to the genus *Sticta*, single-locus alignments of these new sequences were assembled using the "auto" mode threshold and default settings for MAFFT 7.017 (Katoh and Standley 2013) plugin in Geneious. We generated a first set of Maximum Likelihood (ML) trees based on RAxML (see below for procedures) to assess congruence between these datasets. The program *compat*, which detects topological conflict between supported clades in phylogenetic trees (Kauff and Lutzoni 2003, 2002), was further used to assess conflicting placement of individuals in single-gene topologies. This analysis was based on a 70% bootstrap threshold and allowed us to identify potentially problematic sequences.

Taxonomical knowledge on Caribbean *Sticta* is rudimentary with most known names corresponding to widespread taxa and/or species with ranges that so far exclude the Caribbean region (e.g. *Sticta filix* [Ranft et al. 2018], *Sticta fuliginosa* and *Sticta sylvatica* [Magain and Sérusiaux, 2015]). To circumvent poor knowledge about taxa represented in our material, candidate species were obtained using an integrative taxonomic approach. Results from these analyses and methodological details will be reported elsewhere (Mercado-Díaz et al. in prep), but in short, they entailed using the filtered Caribbean ITS dataset to generate a phylogenetic tree that included 448 Caribbean specimens and 2,130 worldwide samples with tentatively assigned species names (Moncada unpubl.). This 2,578-tips tree was also subjected to single-locus species delimitation analysis with the Poisson Tree Processes (PTP) model (Zhang et al. 2013) and the

General Mixed Yule Coalescent (GMYC) (Fujisawa and Barraclough 2013). Candidate species-level lineages were further validated by assessing morphological characters in representative specimens. For each candidate species, we identified the samples for which we obtained the highest amount of sequence data (ITS + additional loci) and used these in subsequent analyses.

Alignment assembly, partitioning schemes, and substitution models—Single-gene alignments for a broader taxon sampling were generated once candidate Caribbean species were obtained. These alignments included representative sequences for each candidate Caribbean species, sequences representing Widhelm et al. (2018) global taxonomic sampling, and sequences for three outgroup species. We generated a second set of ML trees (RAxML) based on these alignments to corroborate congruence between single gene topologies. Since no major conflicts were detected between single-locus trees, concatenated alignments were assembled. Species with at least two of the six targeted loci were considered for concatenation and only one individual per taxon was included in alignments. Despite having limited data for *Sticta* aff. *laciniosa*-2 (ITS only), this species was included in concatenated datasets as it represented one of the less common "green" *Sticta* species.

The best partitioning scheme and optimal molecular substitution models for concatenated alignments were determined using partition models in IQ-TREE 2.0.5 (Minh et al. 2020; Chernomor, Von Haeseler, and Minh 2016) and IQ-TREE implementation of PartitionFinder v1.1.1 (Lanfear et al. 2012) and ModelFinder (Kalyaanamoorthy et al. 2017) (Table S.2.2.). This analysis was run using the -spp option which allows each partition to have its own evolutionary rate, and the -m TESTMERGEONLY option which implements the greedy algorithm of PartitionFinder. Seventeen pre-delimited character sets were analyzed in IQ-TREE. These included the intragenic regions within ITS (i.e. 18S, ITS1, 5.8S, ITS2 and 28S), codon positions

in protein coding genes (i.e. MCM7, RPB1, RPB2), an intron within RPB1, and the genes mtSSU and nuLSU. Separate model selection for MrBayes (see below) was performed using the *-mset* mrbayes option due to lower number of substitution models available for this program.

Phylogenetic analysis—Phylogenetic analysis proceeded with RAxML and the program MrBayes v.3.2.6 (Ronquist et al. 2012). For RAxML, we performed *a posteriori* bootstrapping analysis with the bootstrap convergence test using the extended majority-rule consensus tree criterion (auto MRE). Our concatenated dataset was subjected to partitioned analysis (-q option) which allowed for estimation and optimization of individual alpha-shape parameters, GTR rates, and empirical base frequencies. MrBayes analysis was performed using two parallel Markov Chain Monte Carlo (MCMC) runs with four chains each. The number of generations was set at 30 million using a sampling frequency of 1,000. A 25% burn-in was used to summarize sampled trees and parameter values. Post-burnin trees were pooled to calculate the 50% majority-rule consensus tree. Convergence of chains was assessed in Tracer v.1.5 (Rambaut and Drummond 2009). Clades were considered supported if bootstrap values were equal or above 70% or if posterior probabilities were equal or above 0.95. Trees were visualized using FigTree v.1.4.2 (Rambaut 2012). Major clades identified by Widhelm et al. (2018) (Clades I-V) were also added to these to facilitate interpretation. Congruence between RAxML and MrBayes trees was assessed with *ivy* (https://github.com/rhr/ivy).

The Gblocks web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), which is used to identify and remove gaps and non-conserved sites in alignments (Castresana 2000) was used in preliminary analysis to evaluate if a low degree of sequence conservation in ITS affected phylogenetic reconstructions. Since no major differences were found between

filtered and unfiltered datasets, and to reduce chances of eliminating potentially informative sites (Tan et al. 2015), phylogenetic analyses were based on unfiltered datasets.

Divergence dating—Divergence times were estimated in BEAST v2.6.1 (Bouckaert et al. 2014) using input files prepared in BEAUTi (Bouckaert et al. 2014). We carried out concatenation analyses allowing clock and tree models to be linked and sites models to be unlinked. Transition rates and base frequencies from the model selection results were fixed and branch rates were estimated using relaxed lognormal molecular clocks.

A Calibrated Yule Model was used for divergence time estimation. The birth rate was assigned an exponential distribution with mean= 0.1. Default gamma distribution was used for ucldStdev whereas an exponential distribution (mean=1) was applied to ucldMean. In line with recent estimates for the origin of *Sticta* (Widhelm et al. 2018, 2019), two-node calibrations were used to date our tree. The calibration constrained the age of the MRCA for the crown of this genus to 25 ± 8 MY and was assigned a log-normal distribution with M = 3.2 and S = 0.2. The second node calibration was also assigned a log-normal distribution with M = 4.17 and S = 0.1 and constrained the age for the MRCA between *Sticta* and *Pseudocyphellaria* to 65 ± 10 MY. Following previous studies (Widhelm et al. 2018; Mercado-Díaz et al. 2020; Widhelm et al. 2019), both node calibrations were forced to be monophyletic.

Two independent BEAST analyses using the RAxML topology as the starting tree and a chain length of 5.0×10^7 generations were run. Tree and log files had a sampling frequency of 5,000. Convergence and mixing of parameters were evaluated in Tracer v.1.6 (Rambaut and Drummond 2009) and effective sample sizes (ESS) were confirmed to be > 200. Trees from independent runs were combined in LogCombiner v.1.8.0 (Rambaut and Drummond 2013a) after excluding the first 10% of sampled trees in each run as burn-in. A maximum clade credibility

(MCC) tree was generated in TreeAnnotator v.1.8.2 (Rambaut and Drummond 2013b) from the combined posterior distribution of trees (18,000) using a 0.5 posterior probability cutoff and node heights set at "common ancestors".

It is worth noting that we consistently confronted convergence issues with several parameters when analysis with BEAST was carried out using a partitioning-by-gene scheme. This was seen under different parameter configurations and when using both a full dataset (6-loci) and a reduced 4-loci dataset that excluded both RPB1 and RPB2, which had data for only 25% and 39% of the species, respectively. Many factors might underlie this type of issue, such as overparameterization (Zheng and Wiens 2015) and problematic accessions. In our case, proper mixing and convergence were achieved when a 4-loci (i.e. ITS, MCM7, mtSSU, nuLSU) dataset with a partitioning scheme based on the greedy algorithm of PartitionFinder was used for analyses. Divergence dating results are therefore based on this latter alignment.

Geographic range evolution—To reconstruct the geographic origins of *Sticta* in the Caribbean, species were coded with respect to their occurrence in nine broad biogeographic regions: Afrotropical (AF), Australasia (AU), Caribbean (CA), Central America (CAM), Hawaii (HA), North America (NA), Oriental (OR), Palearctic (PA), and South America (SA). This geographic coding broadly follow biogeographic realms from Wallace (1876), except that we separated Central America and the Caribbean as distinct biogeographic areas, and included the Hawaiian region. Species distributions for extra-Caribbean species mostly followed (Widhelm et al. 2018) with updates in the distribution of several species obtained from Moncada et al. (2020).

We used the RASP platform (Y. Yu et al. 2015) to reconstruct ancestral ranges based on the Dispersal–Extinction–Cladogenesis (DEC) model from Ree and Smith (2008). Analysis was carried out using our MCC tree and considered tree uncertainty for the interpretation of results.

Considering that the maximum number of biogeographic regions that a species within our sampling occurs is six, the analysis was set to reconstruct a maximum of six ranges at ancestral nodes. Range reconstruction was modelled without disallowing ranges which is in line with presumed high dispersal capabilities of *Sticta* and lichens in general (Werth 2011; Widhelm et al. 2018). Analyses were carried both with and without dispersal constraints. Additional details about these analyses and the dispersal constraints matrix can be found in Appendix 4 and Table S.2.3., respectively.

Some recent biogeographic analyses employed the DEC+j model from Matzke, 2014 in cases where the likelihood of founder event speciation was thought to be high (e.g. island clades). DEC+J is a Maximum Likelihood model similar to the original DEC model by (Ree and Smith 2008), but it incorporates a free parameter "j" which accounts for "jump dispersal" or "founder event" speciation (Matzke 2014). We did not use this approach since in our case, the focus was inferring ancestral ranges and not testing hypotheses related to processes of range evolution (e.g. jump dispersal). There are also concerns raised in terms of the propensity of DEC+j to exacerbate issues related to inflating the contributions of cladogenetic relative to anagenetic events (Ree and Sanmartín 2018); therefore, we consider our adherence to the DEC model warranted.

Diversification analyses—Trait-dependent diversification analysis was performed using the Geographic State-Dependent Speciation and Extinction (GeoSSE) model (Goldberg, Lancaster, and Ree 2011) as implemented in the R package *diversitree* (Fitzjohn 2012). GeoSSE is unique among other models within the SSE framework as it allows testing hypotheses related to the link between macroevolutionary rates and the geographic distribution of lineages (Goldberg, Lancaster, and Ree 2011). Species assigned to three geographic character states (i.e.

endemic to the Continental Neotropics "A" or the Caribbean "B", and present in both regions "AB") were analyzed. This process entailed evaluating ten macroevolutionary scenarios which allowed us to evaluate if colonization of the Caribbean triggered changes speciation and/or extinction rates and assess potential dispersal asymmetries. To do this, we constructed a full, unconstrained model in which speciation (sA, sB, sAB), extinction (xA, xB), and dispersal (dA, dB) could vary between areas, and then fitted nine different constraints. Additional details are provided in Appendix 5.

Model inadequacy (i.e. potential for inflated Type I error rates) has been raised as an issue likely affecting SSE models (Rabosky and Goldberg, 2015; but see Caetano et al. 2018). In GeoSSE models, inadequacies seem to be most closely linked to assigning species membership to particular geographic regions and/or to uncertainties related to tree topology (e.g. polytomies) (Alves, Diniz-Filho, and Villalobos 2017). To assess the potential influence of these factors, we adjusted simulation analyses from Alves et al. (2017) to test for model inadequacy in our GeoSSE inference. Additional methodological details from these simulations and are provided in Appendix 6.

As an update to previous analyses (Widhelm et al. 2018), we also investigated heterogeneity in rates of speciation and extinction in our multilocus time-calibrated tree using the Bayesian analysis of macroevolutionary mixtures program BAMM (Rabosky 2014). Methodological details for this analysis are provided in Appendix 7.

Taxonomic and phylogenetic turnover of Caribbean *Sticta* communities

Taxonomic and phylogenetic beta diversity matrices—Taxonomic beta diversity provides useful means to estimate the amount of overlap in species composition between islands (Baselga 2010; Koleff, Gaston, and Lennon 2003). Phylogenetic beta diversity, on the other hand, adds a temporal dimension to beta diversity and is better defined as the phylogenetic distance (branch lengths) between samples of individual organisms between any two sites (Graham and Fine 2008).

To assess beta diversity within the Caribbean, we first generated a community data matrix stratified by island for the species present in the region. Thus, communities analyzed are defined by island membership (island-level communities) and the totality of species in the dataset represents our regional species pool. Since we lacked abundance estimates, this community matrix was based on presence/absence data. We quantified Taxonomic Beta Diversity (TBD), which was treated as species composition dissimilarity between island communities, using the *Jaccard* index. Calculation of this index was accomplished using the "vegdist" function in *vegan* R package (Oksanen et al. 2019).

To estimate Phylogenetic Beta Diversity (PBD), the phylogenetic analog of TBD, we followed methods documented above for phylogenetic reconstructions with BEAST and generated a time-calibrated tree that only included Caribbean species. A RAxML tree was also produced and used as the starting topology for this analysis. Since convergence/mixing issues were not confronted during preliminary analysis, this tree was based on a 6-gene dataset. We used our BEAST tree and our community data matrix to calculate two PBD dissimilarity metrics. These are categorized as "terminal" (tPBD), which are sensitive to turnover near the tip of trees (i.e. *Unifrac*) and "basal" (bPBD), which are sensitive to turnover deeper in the phylogeny (i.e. $D_{Rao's}$) (Swenson 2011). The R package *picante* (Kembel et al. 2010) was used for these

calculations, specifically the functions "unifrac" (*Unifrac*) and "raoD" ($D_{Rao's}$). Additionally, to better understand how communities differ in terms of their composition and evolutionary history, we decomposed taxonomic and phylogenetic beta diversity indices into components accounting for "true" turnover and "nestedness" (or "phylogenetic diversity gradients" if using a PBD metric) (Baselga 2010; Leprieur et al. 2012). Decomposition was accomplished with the functions "phylo.beta.pair" and "beta.pair" from the R package *betapart* (Baselga, Orme, and Villéger 2013). Since tools for decomposing beta diversity have only been developed for *Jaccard* and *Unifrac*, decomposition was limited to these two indices.

We carried out preliminary analysis to assess correspondence of *Unifrac* and $D_{Rao's}$ to other tPBD (e.g. 1 - *Phylosor*, D_{nn}) and bPBD (e.g. D_{pw}, $H_{Rao's}$) metrics that have been developed and found that these yielded analogous patterns (not shown). Similarly, previous work has demonstrated that 1 - *PhyloSor* and D_{pw} are largely redundant with *Unifrac* and $D_{Rao's}$, respectively (Jin, Cadotte, and Fortin 2015; Swenson 2011). In line with these results and observations, taxonomic and phylogenetic beta diversity analyses presented here focused exclusively on *Jaccard*, *Unifrac* and $D_{Rao's}$.

Taxonomic richness and phylogenetic diversity are often correlated, therefore to make better inferences about observed patterns, we calculated phylogenetic diversity ("PD") for each island using the "pd.calc" function of the R package *caper* (Orme et al. 2014). Obtained values were plotted against "species richness" and "maximum elevation" per island to facilitate interpretation of results.

Environmental and geographic distances—We used data on elevation, precipitation, maximum and minimum temperatures, evapotranspiration, the Normalized Difference Vegetation Index (NDVI, Huete, Jackson, and Post, 1985), the Enhanced Vegetation Index (EVI,

Liu and Huete, 1995), and the Terrain Ruggedness Index (TRI, Riley et al. 1999) to generate an environmental distance matrix for island-level communities. These parameters influence or have the potential of influencing species diversity patterns of lichens at both local and regional scales (Armstrong 2015; Nupoor, Manoj, and Partha Sarathi 2015). We used specimen locality data to estimate mean, median, and maximum elevation values for each island. Strong covariation between these parameters was found during preliminary analysis; thus, only maximum elevation was kept for downstream analysis. Likewise, positive correlations between other environmental variables could potentially inflate differences between islands in terms of their environmental distances. To account for this, a principal component analysis (PCA) was carried out on our environmental matrix and the first two PCA axes were extracted to create an Euclidean distance matrix which was used for subsequent analysis.

Cloud computing for visualization of remotely sensed data was used to obtain data for environmental parameters. Inter-island geographic distances were obtained by combining geographic information systems and R. Procedures to obtain these estimates are further described in Appendix 8.

Influence of environmental and geographic distances on TBD and PBD—We plotted geographical and environmental distances against TBD and PBD metrics to visualize associations between them. Mantel tests were used to assess the significance of the correlation between these measures. Because low statistical power and/or spatial autocorrelation biases might affect assessments with Mantel tests, we used the Procrustes superimposition method (Peres-Neto and Jackson 2001) to corroborate correlations that yielded statistically significant associations. TBD and PBD metrics were subjected to ordination analysis with Principal Coordinate Analysis (PCoA) and resulting axes were used for Procrustean analysis. Functions

"mantel" and "protest" from the R package *vegan* (Oksanen et al. 2019) were used to perform these tests.

Null modeling of *Sticta* communities—We used a null modelling approach to ask if taxonomic and phylogenetic relatedness between *Sticta* communities in the Caribbean differed from random expectation. To do this, we first used the "randomizeMatrix" function in *picante* to execute 100 randomizations of our community data matrix. As we were only interested in evaluating turnover deviations from null expectation, the argument null.model() from "randomizeMatrix" was set to "richness". Using the functions described above, we calculated TBD and PBD metrics for each of these 100 null communities. Following from previous work (Graham et al. 2009; Leprieur et al. 2012), we used a standardized effect sizes (SES) approach to evaluate if observed values for our TBD and PBD metrics differed significantly from values estimated for null communities. SES values greater than 1.96 were considered indicative of higher-than-expected turnover (more dissimilarity between communities) whereas SES values below -1.96 were indicative of lower-than-expected turnover (less dissimilarity between communities).

Within-region clustering of Caribbean communities—Cluster analysis was performed to further evaluate how *Sticta* communities partitioned into island-level clusters. This was based on the "fuzzy" C-means clustering algorithm which allow objects to have variable degrees of membership to different clusters or groups (Bezdek 1981). The R base function "hclust" (method = ward.D2) was first used to obtain cluster dendrograms for all TBD and PBD distance matrices. "Fuzzy" clustering for TBD and PBD metrics was then carried out with the "hier.vegclustdist" function from the R package *vegclust* (de Cáceres, Font, and Oliva 2010). This function allows simultaneous runs of the "vegclustdist" function (a distance-based equivalent of the function

"vegclust") using different numbers of clusters. Starting cluster configurations in "hier.vegclustdist" derived from "hclust" objects which were obtained from cluster dendrograms generated above. Minimum (cmin) and maximum (cmax) number of clusters evaluated were 2 and 3, respectively.

Evaluation statistics were calculated with the "vegclustIndex" function. Maximum values of the normalized partition coefficient (PCN) were used as criteria to assign cluster membership. Geographic mapping of cluster dendrograms for each index evaluated was performed with the function "plot.to.map" from the R package *phytools* (Revell 2012).

RESULTS

Molecular data and phylogenetic analysis—A total of 637 sequences, including 189 newly generated sequences (ITS: 41, MCM7: 28, mtSSU: 24, nuLSU: 38, RPB1: 6, RPB2: 52) were used for analyses presented in this work (Table S.2.4). Analysis with RAxML and MrBayes using 4- and 6-loci yielded similar results, therefore results are based on the six-locus dataset and are presented using the MrBayes tree (Fig. 2.2). The likelihood value for the two cold chains in our Bayesian trees was -37,725.67 and -37,736.21 whereas the final optimization likelihood for the ML tree was -38,090.91. Final alignments will be available on an online repository (to be decided).

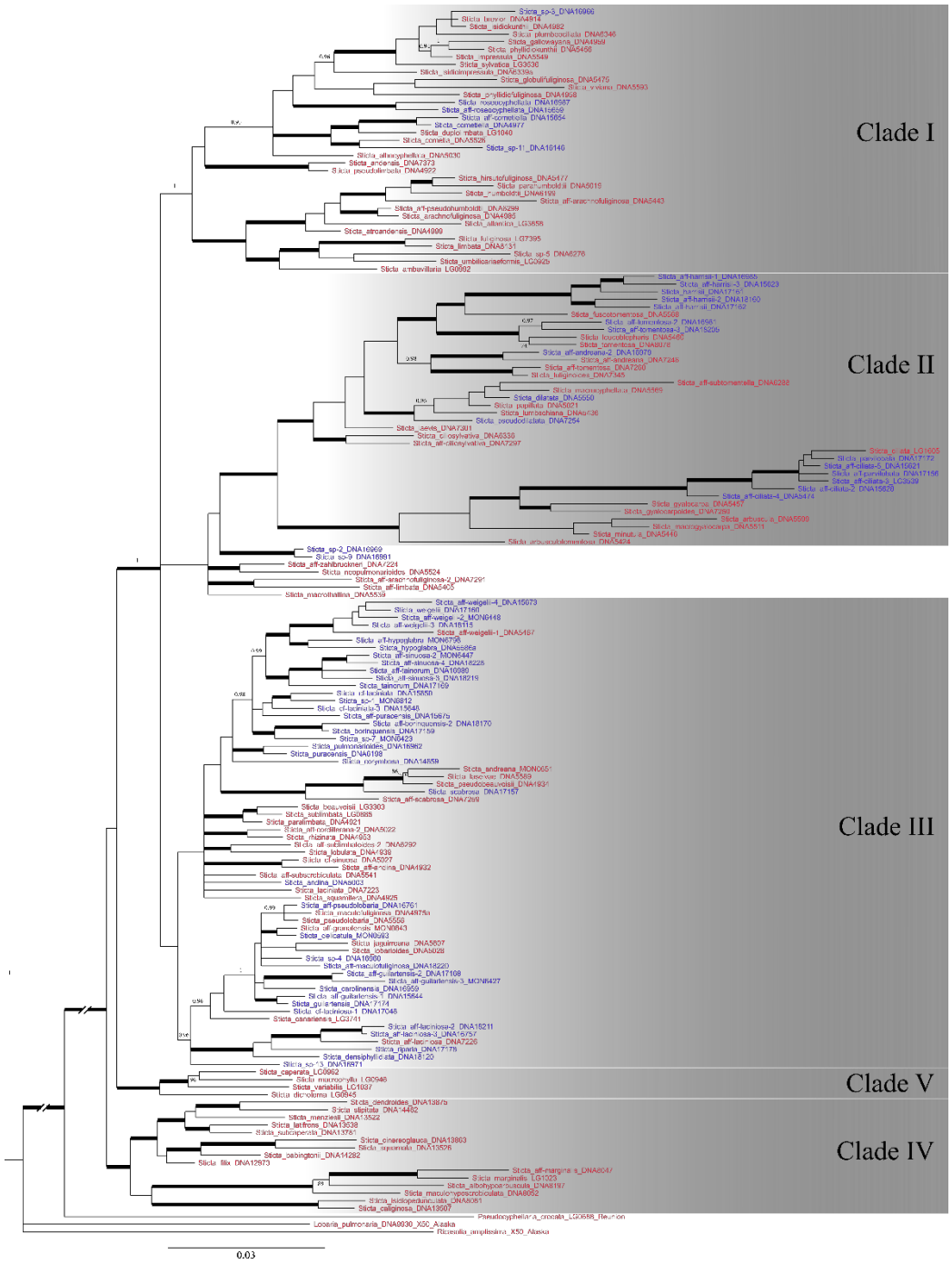

Figure 2.2. 50% majority rule consensus tree obtained from MrBayes based on six nuclear and mitochondrial loci (ITS, MCM7, nuLSU, RPB1, RPB2, mtSSU). Species of *Sticta* occurring in the Caribbean (endemic or not) are colored in purple. Species so far not known from this region are shown in red.

We found sixty-four candidate species of *Sticta* in the Caribbean. Two of these species had insufficient sequence data, therefore only 62 are included in our multilocus phylogeny (Fig. 2.2). Of these species, 38 (59%) are only known from this region, whereas 26 (41%) are also recorded from elsewhere. Phylogenetically, *Sticta* was recovered as a monophyletic group sister to the genus *Pseudocyphellaria*. We recovered the five major ingroup clades reported by Widhelm et al. (2018) with different degrees of statistical support. Three of these clades, Clades II, IV, and V were strongly supported by both RAxML and MrBayes. Seven species (i.e. *Sticta* sp-9, *Sticta* sp-2, *S. neopulmonarioides*, *S.* aff. *zahlbruckneri*, *S. latior*, *S. inversa,* and *S. macrothallina*) were excluded from Clade II as several of these were nested in other clades in Widhelm et al. (2018). Eighteen Caribbean species were found within Clade II, with two additional ones present within the excluded set. While Clade IV's status as the earliest diverging group was strongly supported, the relationship of Clade V to the rest of the clades in the tree remained unresolved. Clade I, which contains the smallest number of species occurring in the Caribbean (6), was only strongly supported in MrBayes. Clade III, on the other hand, includes the highest number of species with Caribbean affinities (38) but methods did not yield strong support for its monophyly. Altogether, Clades I, II, and III and the seven species excluded from Clade II formed a strongly supported clade according to RAxML and MrBayes, but relationships among them remain unresolved.

Divergence dating and biogeographic analysis—According to our BEAST analysis (Fig. S.2.1), the divergence of *Sticta* from *Pseudocyphellaria* occurred about 63.3 Mya (95% Highest Posterior Density (HPD): 51.3–75 Mya). The origins of *Sticta*, on the other hand, date back to the late Oligocene, about 26.2 Mya (HPD: 17.9–34.5 Mya).

The DEC model without dispersal constraints yielded a higher likelihood (lnL = -455.8) compared to the model with dispersal constraints (lnL = -459.8) and is therefore used to highlight results. About 16.5 dispersal events from the Continental Neotropics (i.e. SA + CAM) to the Caribbean were estimated by DEC whereas 18 were inferred to have happened in the reverse direction.

Ancestral ranges are plotted only for strongly supported clades (Fig. 2.3.). Multiple areas (i.e. SA, AF, HA, AU) yielded the highest probability for the ancestral range of the crown node for *Sticta* (~ 67%, not shown). The earliest diverging clades had the most probable geographic origins in the Afrotropics (Clade V) and the Australasian region (Clade IV). Clades I, II, and III, which include all Neotropical species in our tree, share a common ancestor with an ancestral range traced back to South America (Fig. 2.3., node "A"). The earliest putative arrival of *Sticta* to the Caribbean is linked to the common ancestor of all species within Clade III (node "C"). This species likely colonized the Caribbean from South America during the early Miocene about 19 Mya (HPD: 12.6–26.3 Mya). Within this Clade, the Caribbean was inferred to be the most probable ancestral range for two nodes ("E" and "F") that diversified into species with both Caribbean and extra-Caribbean distributions. Taxa that originated from node "E" (~11 Mya, HPD: 5.6–16.4 Mya) are mostly Caribbean endemics, except for *Sticta riparia* which dispersed to South America from the Caribbean and *Sticta* aff. *laciniosa* which only occurs in that continent. Most species that originated from node "F" (~14 Mya, HPD: 9.2–19.7 Mya) have at present a strictly Caribbean distribution. Some species including *Sticta laselvae*, *S. andreana,* and *S. pseudobeauvoisii*, however, seem to have ancestors in this region but now are found only in South America. Besides their presence in South and/or Central America, the geographic span of other species that originated from node "F" extends, in some cases, to the Afrotropics (i.e. *S.*

weigelii, S. aff. *weigelii-3)* and Hawaii (*S. scabrosa*). Species within both Clades I and II, on the other hand, were inferred to have originated from South American ancestors (nodes "B" and "D", respectively). Except for *Sticta* sp. 3 which derived from a South/Central American ancestor, the rest of the species with Caribbean distribution within Clade I have an inferred South American origin. All Caribbean taxa in Clade II originated from South American ancestors that spread to the Caribbean and remained widely distributed (e.g. *S.* aff. *ciliata-4*, *S.* aff. *ciliata-2*, *S. dilatata*, *S. pseudodilatata*) or diverged to become Caribbean endemics (e.g. *S. parvilobata*, *S.* aff. *ciliata-5*, all species within the "*harrisii*/aff. *harrisii*" group).

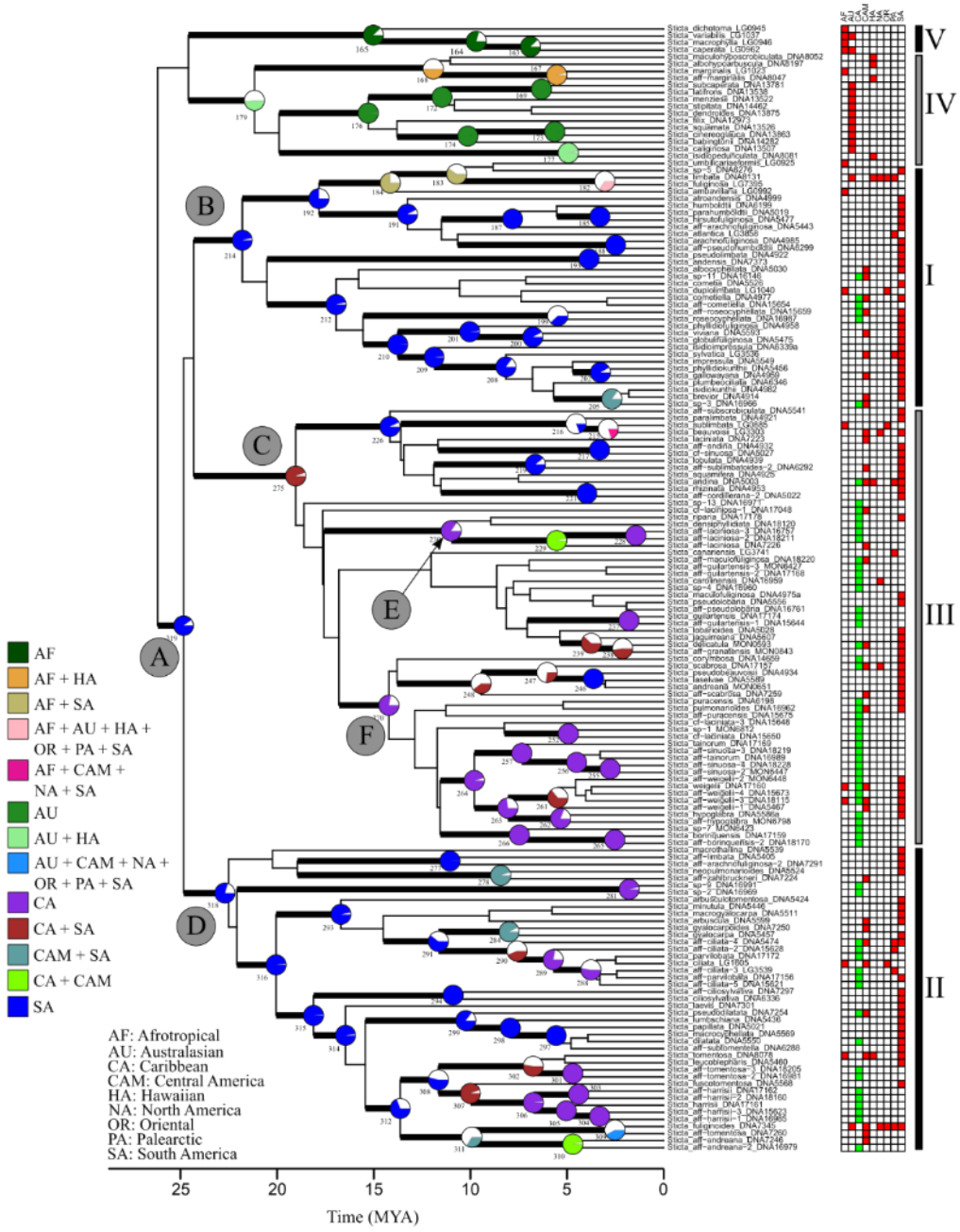

Figure 2.3. Ancestral range reconstruction analysis without dispersal constraints based on our 4-loci MCC tree (outgroups removed). Matrix to the right indicates the presence of species in the Caribbean (green) and the other biogeographic regions analyzed (red). Major clades are identified with black and gray bars to the far right. Labeled nodes indicate the common ancestors for Clades I, II, and III ("A"), Clade I ("B"), Clade II ("D"), Clade III ("C"), and the earliest ancestors of potential Caribbean origin (Nodes "E" and "F"). Ancestral ranges reconstructed are only shown for strongly supported clades.

Ancestral ranges reconstructed with dispersal constraints were in broad agreement with those without restrictions (Fig. S.2.2). Differences concerning strongly supported nodes containing Caribbean taxa are described in detail in Table S.2.5. In general, probabilities for the most likely ancestral ranges were lower using dispersal constraints. South America was more frequently recovered as the single ancestral range of many nodes that were otherwise inferred to be Caribbean or both South American and Caribbean. This was particularly prevalent in Clade III. Additionally, Central America was more frequently included in ancestral areas reconstructed for several nodes in Clade II. Ancestral ranges inferred for all major nodes highlighted in Fig. 2.3. were identical between these analyses, except for node "D" which was recovered as both South American and Caribbean, and node "E" which had Central American and Caribbean origins.

Diversification analyses—The distribution of geographic character states in the 119-tip MCC tree used for GeoSSE analysis is shown in Fig. S.2.3. Model selection results as well as parameter estimates for full and constrained GeoSSE models analyzed are provided in Table S.2.6. Models without between-region speciation (sAB ~ 0) and without dependence of dispersal rates (dA ~ dB), as well as those assuming no dispersal from the Caribbean (dB ~ 0) and no dispersal from the Continental Neotropics (dA ~ 0) were statistically supported. This result was the same both when the root was unfixed and when the root was fixed for the Continental Neotropics. Only the model with no dispersal from the Caribbean (dB ~ 0) was supported when the root was fixed to the Caribbean.

For the most part, speciation, and extinction rates for the Continental Neotropics and the Caribbean converged to similar values (Fig. 2.4.A, B). In contrast, between-region speciation

rates were slightly lower than rates in individual areas (Fig. 2.4.A) and dispersal rates from the Caribbean were slightly higher than rates from the Continental Neotropics (Fig. 2.4.C). The magnitude of differences was more pronounced for dispersal rates, whereas speciation and extinction rates showed similar rate differences (Fig. 2.4.D).

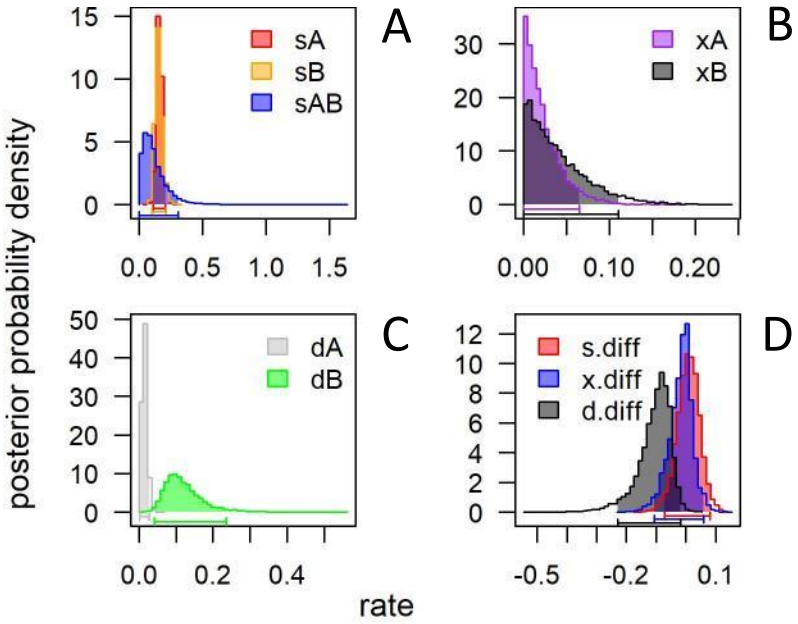

Figure 2.4. Posterior probability distributions for **A.** speciation rates, **B.** extinction rates, **C.** dispersal rates, and **D.** rate differences for our full, unfixed root GeoSSE model. Region "A" represents the Continental Neotropics and Region "B" corresponds to the Caribbean region. Both geographical regions are denoted as "AB".

Model inadequacy tests of our GeoSSE analysis show that for all transition rates evaluated and for both neutral and random traits simulated on our MCC tree, there is a high chance of incorrectly rejecting the null hypotheses of no between-region speciation and no dependence on dispersal rates (Fig. S.2.4., Fig S.2.5.). The only slight deviation from this generalization was observed when testing for no between-region speciation, specifically the

simulation of neutral traits with a 0.05 transition rate. In this simulation, the proportion in which the null would be correctly rejected was 55% (Fig S.2.4.).

The frequency for zero number of regime shifts in the posterior distribution of samples in our BAMM analysis was 0.92 which suggests that distinct rate shifts are likely absent in our multilocus MCC tree. As hinted in the net diversification through time plot, decreases in diversification rate in the mean phylorate plot are at first slightly pronounced but decelerate over time (Figure S.2.6.). Accordingly, a steady increase in the number of lineages is also observed.

Taxonomic and phylogenetic turnover of Caribbean *Sticta* communities

Effects of environmental and geographic distance on TBD and PBD—Data for environmental parameters generated for each island-level community is shown in Table S.2.7. PCA axis 1 (Variance explained = 71%) was most strongly correlated with maximum elevation and max/min temperature whereas PCA axis 2 (Variance explained = 17%) was most strongly correlated with precipitation and TRI (ruggedness) (Table S.2.8).

Correlation analysis with Mantel tests shows a significant association between all TBD and PBD metrics and environmental distances (Fig. 2.5.). These relationships were further supported in our Procrustes analysis (Fig. S.2.7.). In contrast, association of these indices with geographic distance was not statistically significant. (Fig. S.2.8.).

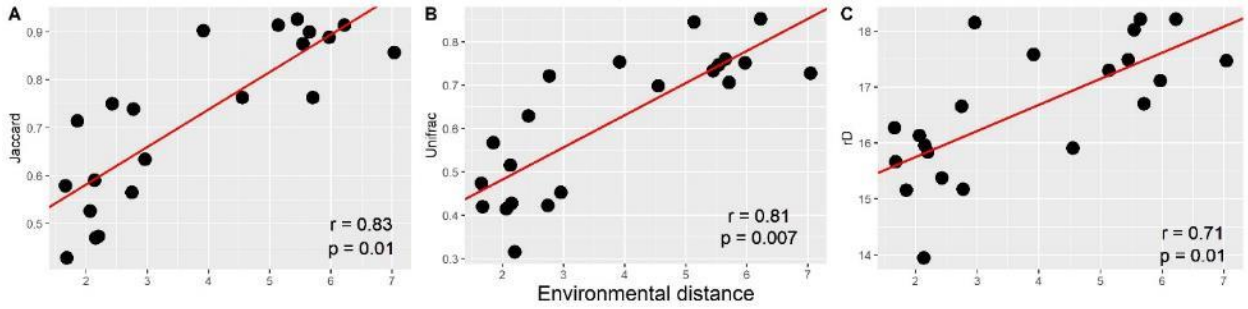

Figure 2.5. Relationship between TBD, tPBD and bPBD metrics with environmental distance. **A.** Jaccard, **B.** UniFrac, **C.** $D_{Rao's}$. All associations were statistically significant according to Mantel tests.

A strong positive correlation between PD and species richness per island was also observed. These variables were positively correlated with maximum elevation (Fig. 2.6). Partitioning of *Jaccard* and *Unifrac* indices into "true" turnover and "nestedness" (*Jaccard*) or "phylogenetic diversity gradients" (*Unifrac*) components showed that taxonomic and phylogenetic dissimilarities are mostly driven by "true" turnover (Fig. S.2.9.).

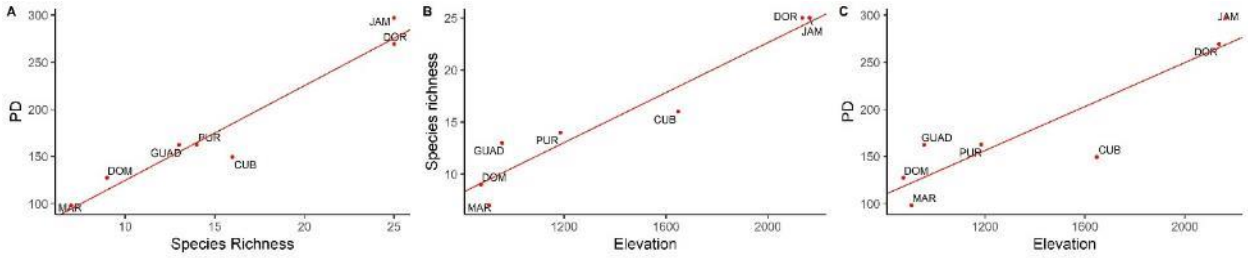

Figure 2.6. Relationships between **A.** species richness vs. phylogenetic diversity, **B.** species richness vs. elevation, **C.** phylogenetic diversity vs. elevation, in *Sticta* communities from islands in the Caribbean.

Between-island taxonomic and phylogenetic dissimilarities—Analysis of dissimilarities between islands estimated with TBD and PBD metrics show that the Lesser Antilles (Dominica, Guadeloupe, Martinique) and Puerto Rico are taxonomically similar (white to light blue [*Jaccard*]), and as a group, they are more dissimilar to the islands of Jamaica and Hispaniola (dark blue) (Fig. 2.7.A). Cuba, on the other hand, does not reflect strong patterns of dissimilarity with any of the other islands, although tends to be slightly less dissimilar to Dominica. Phylogenetic dissimilarity as evaluated with *Unifrac* mirror patterns observed with the *Jaccard* index, whereas analysis with the $D_{Rao's}$ metric reflects less dissimilarity between Cuba and Dominica and high dissimilarity between Hispaniola vs. Martinique, Jamaica, and Guadeloupe.

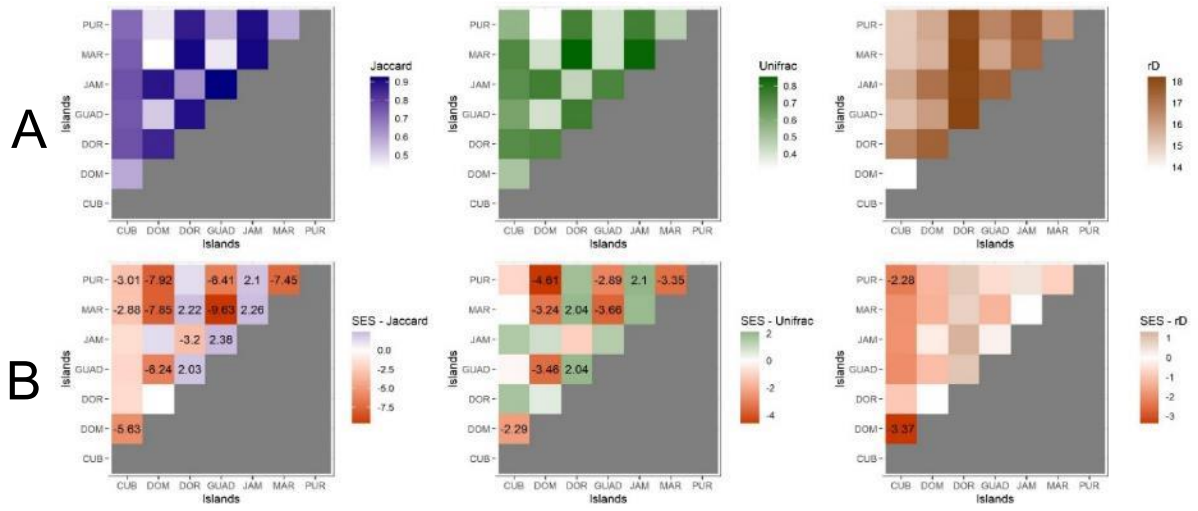

Figure 2.7. Heatmaps illustrating taxonomic and phylogenetic dissimilarities between Caribbean islands. Dissimilarities were estimated from TBD and PBD indices calculated for **A.** empirical, and **B.**100 null (simulated) communities. Standardized Effect Sizes (SES) values are shown in grid cells in B. whenever these were lower or higher than |1.96|. Darker colors in A) indicate higher dissimilarities and darker brick red colors in B indicate high similarities.

With few exceptions, results from our null modeling/SES analysis broadly validate the aforementioned patterns (Fig. 2.7.B). Taxonomic dissimilarity seems to be greatest between the Lesser Antilles (excluding Dominica) vs. Hispaniola + Jamaica. The Lesser Antilles as a whole, and Puerto Rico, are strongly similar and some islands show strong affinities with Cuba (i.e. Puerto Rico, Martinique, Dominica). Puerto Rico is strongly dissimilar to Jamaica. Patterns observed with SES - *Unifrac* resemble those with SES – *Jaccard* but are less pronounced. Strong phylogenetic dissimilarity was only observed between Hispaniola vs. Guadeloupe + Martinique, and Puerto Rico vs. Jamaica. Cuba remained similar only to Dominica. Significantly less dissimilarity between Cuba and Dominica + Puerto Rico was the only major pattern that emerged from SES – $D_{Rao's}$.

Within-region clustering—Analysis using fuzzy C-means clustering broadly align with patterns observed from our null modeling/SES approach. When the number of clusters is set at two, both *Jaccard* and *Unifrac* indices converge at splitting Jamaica and Hispaniola from the rest of the islands (Fig. 2.8.). Using three clusters still split these two islands from the rest, but also recovers Cuba as a separate community. Differences between these two indices are only observed in the Lesser Antilles + Puerto Rico cluster. Here Puerto Rico is either separated from the rest of the Lesser Antilles (i.e. *Jaccard*) or merged with Dominica (*Unifrac*). No clusters were assigned when evaluating the $D_{Rao's}$ index.

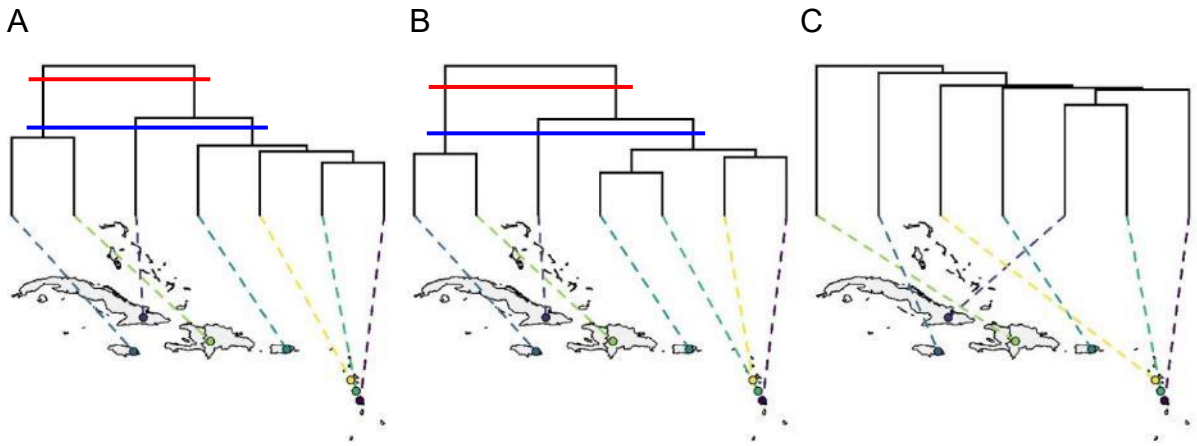

Figure 2.8. Results from fuzzy C-means cluster analysis using **A.** taxonomic (*Jaccard*) and both **B.** terminal (*UniFrac*) and **C.** basal ($D_{Rao's}$) phylogenetic beta diversity indices to assess partitioning of Caribbean islands' *Sticta* communities. Cluster membership based on the maximum value of the normalized partition coefficient (PCN) is shown using red lines (two clusters) and blue lines (three clusters). No clusters were assigned when evaluating $D_{Rao's}$. Dendrograms are based on the "hclust" function in R.

DISCUSSION

Diversity, endemism, and phylogenetic patterns—Thirty-eight out of the 64 species of *Sticta* we have recorded in the Caribbean islands have not been found elsewhere, and hence are potential endemics. Not long ago, this degree of diversity and endemism in insular lichens would have been considered striking. However, an increasing number of phylogenetic studies are showing that high endemism in lichen fungi is a common phenomenon in islands. Puerto Rican *Sticta* endemics may be as high as 69% (Mercado-Díaz et al. 2020). Additional work on *Sticta* in other island systems, such as the Hawaiian Archipelago (Moncada, Lücking, and Lumbsch 2020) and the Madagascar and Indian Ocean Islands (Simon et al. 2018), along with findings from studies in related (e.g. *Lobariella*, *Pseudocyphellaria* [Moncada et al. 2014; Lücking et al. 2017])

and other genera (e.g. *Dictyonema*, *Nephroma*, [Dal Forno et al. 2017; Sérusiaux et al. 2011]) support this view, with inferred rates of insular endemism between 69% and 100%.

We were able to recover the five major clades identified by Widhelm et al. (2018), albeit with variable degrees of statistical support. Like that study, evidence supporting the monophyly of Clades II, IV and V was found, but Clades I and III remained ambiguous. As suggested in Mercado-Díaz et al. (2020), variation in parameter settings might partly explain these differences. In spite of this, our BEAST analysis showed strong support for Clades I and III, resembling Widhelm et al. (2018) findings. Differences in sampling might also underlie these patterns because our BEAST tree was generated with a dataset that excluded RPB2. However, using RPB2 yielded a result analogous to Mercado-Díaz et al. (2020), specifically placing Clade IV as the earliest diverging clade as opposed to Clade V as inferred in Widhelm et al. (2018). This suggests that using multiple phylogenetic inference methods to analyze datasets with different degrees of sampling might be critical for properly evaluating evolutionary relationships in this group. Further attesting to these issues was the placement of seven species that were nested in multiple clades in Widhelm et al. (2018) but found to form a paraphyletic grade associated with Clade II in our study. Phylogenetic studies have demonstrated that deep-time evolutionary relationships within the tribe to which *Sticta* belongs (i.e. Lobarioideae, Lumbsch and Leavitt [2019]) are difficult to resolve (Moncada, Lücking, and Betancourt-Macuase 2013), even when using genome-scale datasets (Widhelm et al. 2019). Broader taxonomic and genetic sampling will hopefully shed light on these unresolved aspects of *Sticta* evolution.

Timing and geographic origins of Caribbean *Sticta*—The estimated crown age for *Sticta* (~ 26.2 Mya) is highly similar to the one obtained in Widhelm et al. (2019) (~ 25.2 Mya) and reaffirm that the group likely emerged during the Late Oligocene.

Ancestral range reconstruction based on nine biogeographic regions provided further resolution compared to a previous study (Widhelm et al. 2018). For instance, the early diverging Clade IV was found to be more narrowly restricted to the Afrotropics. The common ancestor to Clades I, II, and III, which gave rise to all Caribbean species around the early Miocene, was also inferred to have originated in South America. South American affinities have been suggested for many Caribbean lineages, including *Sticta* in Puerto Rico (Mercado-Díaz et al. 2020). Other notable examples include non-volant terrestrial vertebrates (Hedges 2006; Marivaux et al. 2020), spiders (McHugh et al. 2014), fishes (Weaver et al. 2016), and several groups of plants (Santiago-Valentín and Olmstead 2003; Regalado et al. 2018; Filipowicz and Renner 2012). Intriguingly, our analysis also showed that some lineages might have derived from Central American ancestors. Indeed, several Central American species for which we had limited sequence data emerged as sister to Caribbean lineages during preliminary analysis. Sampling bias issues could have therefore influenced our reconstructed ranges. If patterns with *Sticta* mirror in any way what has been previously observed in some plant groups like *Coccoloba* or members within the subfamily Acalyphoideae (Koenemann and Burke 2020; Cervantes et al. 2016), further investigation into these affinities might potentially reveal that directions and frequency of colonization events between both regions are, in fact, underestimated.

As indicated, the earliest colonization event of *Sticta* in the Caribbean presumably occurred about 19 Mya (node "C", Fig 2.3.) which imply that the islands must have had suitable habitat for colonization during this period. Fragmentary mega fossils from Puerto Rico together with pollen and spore assemblages from this island and Haiti (Graham and Jarzen 1969; Graham 1990) demonstrate that upland vegetation was in fact present, providing support for this colonization scenario.

Our work suggests that after diverging from the ancestor to Clade III, two lineages inferred to be of Caribbean origin (i.e. nodes "E" and "F", Clade III) gave rise to species that eventually re-colonized South America and other regions. Re-colonization of continents by island lineages is highly debated as theory assumes unidirectional movements of species from continents to islands (MacArthur and Wilson 2001). Underlying this prediction are "island syndromes" which involve the loss of dispersal power and defensive traits in island clades (Whittaker and Fernández-Palacios 2001). However, reverse colonization of continents by insular lineages has been documented in animals (Table 1 in Bellemain and Ricklefs [2008]) and plants (Carine et al. 2004; Herrando-Moraira et al. 2019). Recent work on plant lineages from the Caribbean islands, which are regarded as favorable for reverse colonization (Bellemain and Ricklefs 2008), support this view (Nieto-Blázquez, Peña-Castillo, and Roncal 2020; Cano et al. 2018). Organisms assumed to have relative high dispersal abilities, such as lichens and other spore-dispersed organisms, should display increased capacity for reverse colonization. This has been evidenced in several bryophyte studies (Hutsemékers et al. 2011; Patiño and Vanderpoorten 2015; Laenen et al. 2011) and one study on lichens (Sérusiaux et al. 2011). However, until uncertainty in reconstructions and sampling limitations are properly addressed, reverse colonization from the Caribbean to the continent should remain a working hypothesis.

***Sticta* diversification in the Caribbean—**In agreement with the lack of significant rate shifts inferred in BAMM, our GeoSSE analysis suggested that the colonization of the Caribbean islands did not trigger changes in diversification rates. This is consistent with results from studies on several plant groups, including the family Podocarpaceae, and the tribes Chrysophileae and Sabalae (Nieto-Blázquez, Peña-Castillo, and Roncal 2020; Cano et al. 2018). It is likely that our relatively small dataset might have introduced sampling artifacts (Davis et al. 2013).

Uncertainties in reconstructions and the influence of extinction imply rate changes may remain undetected. Issues such as inaccurate rate estimates due to lack of fossil data (Didier et al. 2017) and the possibility of life-history traits (e.g. high dispersal capacity) influencing the detection of bursts of lineage-splitting events (Claramunt et al. 2012) might have also played a role, but deserve further investigation.

Higher "out of the Caribbean" dispersal rates (dB) might appear counterintuitive because ancestral colonization of the region was inferred to be mostly South American. Yet, dispersal events from the Caribbean to SA and CAM that were inferred by DEC were also slightly higher, thus aligning with this finding. This strengthens the view of reverse colonization instances from the Caribbean as potentially important events in the biogeographic history of *Sticta* in this region. On the other hand, support for a model favoring the influence of between-region speciation (sAB) suggests that allopatric speciation of widespread species may have had a heightened role in Caribbean *Sticta* diversification. However, caution should be exercised when interpreting these results since similar to what has been predicted and tested in the past (Rabosky and Goldberg 2015; Alves, Diniz-Filho, and Villalobos 2017), our simulations suggested the presence of model inadequacy issues. This is worsened by topological uncertainty associated with poorly supported clades. It, thus, remains to be elucidated how dispersal asymmetries and speciation of widespread species along regional boundaries influence *Sticta* diversification in this important biogeographic region.

Community assembly over evolutionary time—Multiple colonization events over evolutionary time have shaped the current diversity of *Sticta* in the Caribbean. This has led to communities represented by species at different stages of range evolution, including 1) lineages that expanded their ranges from elsewhere such *S. andina*, *S. delicatula*, *S. scabrosa,* and all

Caribbean species in Clade I, 2) lineages that evolved in-situ and are endemic, and 3) lineages that were endemic but expanded their range to other areas more recently (e.g. *S. riparia*, *S. weigelii*, *S.* aff. *parvilobata*, etc.). In tandem with findings from other studies, the presence of species at these different evolutionary stages brings to focus the process of diversification and community assembly in island lichens. Islands at the early stages of this process will have biotas conformed by lineages that colonized from elsewhere and/or recently evolved endemics. This is best exemplified by the *Sticta* biota of the relatively young Hawaiian archipelago (1–5 My). These communities were apparently derived from Australasian and South American species that expanded their range to these islands and either retained their widespread distribution or evolved into new Hawaiian endemics (Moncada, Lücking, and Lumbsch 2020). Nine of the 12 species of Hawaiian *Pseudocyphellaria* are considered putative endemics (Moncada, Reidy, and Lücking 2014), whereas all basidiolichens from the geologically young (~ 4 My) Galápagos islands are endemic (Dal Forno et al. 2017), suggesting analogous processes of community assembly. Caribbean assemblages in many other groups are also constituted by endemics and extra-Caribbean species as has been seen in the plant genus *Philodendron* (Canal et al. 2019).

With sufficient time and contingent on the geographical and ecological context, lichen assemblages in islands can go through evolutionary radiation phases which could lead to drastic increases in the number of island species. Insular radiations (particularly adaptive radiations) are a rather well-documented phenomenon in many groups (Losos and Mahler, 2010, but see Rundell and Price, 2009 and Simões et al. 2016) but examples in lichens are still scarce, although a growing body of evidence is emerging. This includes the *Sticta* biota of Madagascar (Simon et al. 2018) with a radiation following a single colonization event (~11 Mya) and smaller radiations in *Lobariella* and *Sticta* in Hawaii (Lücking, Moncada, and Smith 2017; Moncada, Lücking, and

Lumbsch 2020), and *Rocella* in the Galápagos (Tehler et al. 2009). Evidence for species radiations are particularly notable in Caribbean plant genera (Filipowicz and Renner 2012; Hidalgo et al. 2020; Cervantes et al. 2016; Appelhans et al. 2012).

The expansion of island endemics to the mainland likely hinges on multiple factors and is not expected to be a derived feature of all insular assemblages. Although work on lichens is still wanting, the availability of vacant niches in continental areas might be important. This was recently demonstrated by Hutsemékers et al. (2011) who showed that a severe bottleneck in continental populations of the moss *Rynchostegium riparioides* during the last glacial maximum likely facilitated back colonization of this species from Macaronesia to Europe. Wind currents with a predominant island-to-mainland direction might also be determinant since establishment is ultimately a function of invasion frequency. Lastly, tradeoffs between the proximity of archipelagos to continents and the age of the lineages are also potentially influential. As has been indicated, *Sticta* has putatively high dispersal abilities (Widhelm et al. 2018); thus, the degree of remoteness should not be the only factor precluding instances of reverse colonization. For Hawaiian endemics, short time scales for geographic range expansion might be more limiting since these species are relatively young (< 6 My, Moncada et al. [2020]). Endemics from the Caribbean islands that gave rise to reverse colonizing species were slightly older than those from Hawaii, but in this case, closeness to South and Central America likely facilitated range expansion to the continent. Madagascar, on the other hand, have attributes keen to reverse colonization (e.g. the island is geologically old [~ 88 My] and moderately close to the African continent [< 430 Km.]), but *Sticta* endemics from this island are also of young age (< 11 Mya, Simon et al. [2018]) which has likely limited these type of events. Furthermore, morphoanatomical characterizations will be needed to understand the role of life history

attributes since traits facilitating rather than limiting dispersal might have evolved in many of these insular endemics. Direct evidence documenting dispersal of lichen propagules by migratory birds is still limited, thus it is also difficult to determine if reverse colonization could be linked to this type of dispersal. It has certainly been invoked to explain discontinuous distribution patterns in several lichens (Garrido-Benavent and Pérez-Ortega 2017), hence it is possible it might have enhanced the connectivity between the Caribbean and the mainland.

Taxonomic and phylogenetic turnover of Caribbean *Sticta* communities

Environmental structuring of taxonomic and phylogenetic beta diversity—A significant positive correlation between taxonomic beta diversity and environmental gradients suggests that at ecological scales, environmental filtering plays a pivotal role for Caribbean *Sticta* community assembly. Most of the variance in our environmental PCA (71%) was explained by axis 1, thus we attribute observed variation to changes in elevation and concomitant fluctuations in temperature. Although alpha-diversity along elevational gradients have been well characterized for lichens in continental ecosystems (Wolf, 1993; Baniya et al. 2010; Bässler et al. 2016; Soto-Medina et al. 2019), taxonomic turnover as a function of variation along environmental axes (particularly elevation), especially in the tropics, is less understood. Our work documented increases in species richness with elevation, however, partitioning of beta diversity components showed that patterns were being driven by 'true' turnover. This agrees with studies evidencing strong variation in macro- and micro-lichen species composition along altitudinal gradients in mountainous neotropical areas (Wolf 1993; Soto-Medina et al. 2019) and attests to the compositional "uniqueness" that characterize these communities.

Correlations of environmental PCA axes with PBD metrics mirror those with TBD and further indicate that irrespective of geographic distance between islands, *Sticta* species within the same elevational/temperature community tend to be more closely related to each other than they are to species in other elevational zones in the same island. Communities at opposite extremes along elevational gradients are therefore phylogenetically most distant within this region. These findings reiterate the importance of environmental filtering as a major factor regulating species composition in these island level communities, but most importantly, they suggest that these communities are apparently tracking key environmental attributes of their habitats over evolutionary time. Phylogenetic niche conservatism might therefore represent a salient feature of assembly processes in these communities. Niche conservatism associated with environmental conditions is a predominant pattern in most empirical studies on phylobetadiversity, but studies on groups other than plants and animals are scarce. One example is the study by Wang et al. (2013) which showed that phylogenetic dissimilarity was strongest among habitat types in bacterial communities of subsurface lake environments distributed throughout China. Peixoto et al. (2014) work on bats uncovered strong spatial distance effects on global-scale patterns of phylobetadiversity, however, they noted that strong environmental gradients may influence assemblages occurring in adjacent biogeographic regions. This reaffirms the influence of environmental gradients on phylogenetic turnover, although it also suggests that spatial scale ultimately determines the detectability of their effects.

Determining the importance of phylogenetic niche conservatism vs. evolutionary lability in island-level communities of Caribbean *Sticta* will certainly require additional efforts, particularly testing for phylogenetic signal at the metacommunity level (Pillar and Duarte 2010). Results from other efforts evaluating phylogenetic structure of lichen communities would also be

ideal for comparative purposes, but studies are still scarce. Lücking et al. (2016) showed that *Parmeliaceae* communities in major biomes of Mexico exhibit different degrees of phylogenetic clustering. More recently, Nascimento et al. (2021) suggested that underlying differences in climatic and edaphic conditions seen between major vegetation types from Brazil were indirectly linked to contrasting patterns of phylogenetic overdispersion and clustering of lichen metacommunities in those areas. Additional work using phylogenetic frameworks to disentangle the links between environmental variation and species distributions can also be informative. Moncada et al. (2021) is notable in this respect as they showed that divergent genetic structuring in geographically overlapping populations of *S. scabrosa* (tropical lowland species with a "weedy" character) and *S. andina* (upper montane, cloud forest/paramo specialist) were likely driven by autecological preferences. Yet, population-level studies in lichens often uncover cases of cryptic speciation in putatively widespread lineages (Alors et al. 2016; Otálora et al. 2010; Fernández-Mendoza and Printzen 2013). This stresses the need for additional assessment of geographic distance as a potential driver of phylogenetic structuring in many of these communities.

Relatedness of *Sticta* communities among Caribbean islands—Between-island dissimilarity matrices and fuzzy C-means clustering revealed concordant patterns. Significantly low taxonomic and phylogenetic dissimilarities between *Sticta* from the Lesser Antilles and Puerto Rico suggest that both areas have similar species composition and high degree of shared evolutionary history. Stronger affinities between Puerto Rico and the Lesser Antilles have been suggested for some biological groups, most notably plants (Dewalt, Ickes, and James 2016; Nieto-Blázquez, Peña-Castillo, and Roncal 2020). Land bridges that presumably connected these areas in the past (Iturralde-Vinent and MacPhee 1999; Philippon et al. 2020) cannot be invoked

to explain these patterns since these predate the origin of the genus (this study, Widhelm et al. [2018]). Hurricanes, on the other hand, might partly explain these similarities since prevailing tracks seem to connect more often the Lesser Antilles to Puerto Rico (https://www.nhc.noaa.gov/climo/ [last accessed: 2/1/2021]). Nonetheless, links of Puerto Rican species to Greater Antillean communities have also been proposed (Acevedo-Rodríguez and Strong 2008), constraining further generalizations in this respect. The degree of shared phylogenetic history between communities from Jamaica and Hispaniola was not different from null expectations, despite species composition between them being significantly similar. Additional sampling of communities in these two islands, particularly at low elevations, will be critical to disentangle these patterns of association.

To interpret significant taxonomic and phylogenetic dissimilarities observed between Hispaniola and some of the Lesser Antilles, and between Jamaica and Puerto Rico, it is important to emphasize that island-level communities from Hispaniola and Jamaica yielded the highest taxonomic and phylogenetic diversities in the region (Fig. 2.6.). As mentioned previously, 'true' turnover explained most variation in beta diversity (taxonomic and phylogenetic) (Fig. S.2.9.) which attest to the compositional and evolutionary distinctiveness of these communities. These observations, together with findings from our null modelling approach, suggest that small ranged species potentially restricted to high elevation environments in Hispaniola and Jamaica are likely driving these dissimilarities. These are likely paleoendemics that have a low degree of shared evolutionary history with species from communities at lower elevations (see Table 1 in Graham and Fine, 2008). Overall, this agrees with putative high endemism that we have observed in the region but also aligns with the notion of higher genetic variability conducive to speciation that is seen in groups that have evolved affinities to cold, high

elevation environments, like some lineages in *Sticta* (Moncada, Mercado-Díaz, Magain, et al. 2021) and other groups of plants and birds (Madriñán, Cortés, and Richardson 2013; Ryan et al. 2007; Hughes and Atchison 2015). Significantly low phylogenetic dissimilarity observed between Cuba and the islands of Puerto Rico and Dominica in terms of the basal metric $D_{Rao's}$ deserves further investigation. These findings suggest that shared environmental tolerances between these communities evolved during the early diversification of *Sticta* in this region.

CONCLUSIONS

Our study revealed that *Sticta* is represented by at least 64 species in the Caribbean, 38 of these potentially endemic to this region. This fraction of endemics is comparable to what has been found for lichens in other archipelagos emphasizing the importance of island systems for the maintenance of biodiversity in this group. Although further work will be needed to better characterize geographic affinities with Central America, we showed that Caribbean *Sticta* diversity has a predominant South American ancestry. In addition, after diverging from broadly distributed species, several putative Caribbean lineages expanded their range back to South America, thus exemplifying potential cases of reverse colonization. We have not found any evidence that range expansion to the Caribbean triggered increased diversification.

To our knowledge, this is the first study to implement a phylobetadiversity approach to explore patterns of taxonomic and phylogenetic relatedness in lichen communities. In line with known habitat preferences for *Sticta*, we confirmed that niche differences linked to environmental variation along elevational gradients are major drivers of taxonomic and phylogenetic turnover in island-level communities from the Caribbean. Less dissimilarity was seen between high elevation communities of Hispaniola and Jamaica and between low elevation

assemblages in the Lesser Antilles and Puerto Rico. Taxonomic and phylogenetic diversity was positively correlated with elevation. This suggests that small ranged endemic species abundant in high elevation environments and species with wider distribution in the Lesser Antilles and Puerto Rico drive most of the taxonomic and phylogenetic turnover observed. These findings provide a broad picture of community assembly in Caribbean *Sticta* over ecological and evolutionary time but also highlight the notable contribution of Hispaniola and Jamaican communities to *Sticta* diversity in this region. Additional work at smaller spatial scales would still be needed to further disentangle patterns of relatedness, particularly between communities within each island.

Our study demonstrates the important contribution that Caribbean lichens make to global biodiversity. It also adds to the growing body of work demonstrating that unique evolutionary patterns that characterize island lineages are not exclusive to vascular plants or vertebrate assemblages, but are also evident in speciose, understudied groups such as lichens.

CHAPTER 3

GENOME-WIDE ASSESSMENT OF PUTATIVE ENDEMISM AND PHYLOGEOGRAPHY OF *CLADONIA SANDSTEDEI* (ASCOMYCOTA: CLADONIACEAE) IN THE CARIBBEAN

Abstract *Cladonia sandstedei* is a cushion-forming lichen that colonizes open environments and is distributed throughout the Caribbean and southeastern United States. It co-occurs in parts of its range with *C. subtenuis*, a morphologically similar species that is distinguished from the former by the presence of usnic acid. Preliminary phylogenetic analysis with several barcoding loci revealed that these species were closely related, but relationships were inconsistent among markers. Here, we combined phylogenetic, and population genomic analysis based on RADseq data to clarify evolutionary relationships and phylogeography of these species. Both approaches indicate strong geographic structure in genetic variation. Continental *C. sandstedei* was more closely related to continental C. *subtenuis* suggesting homoplasy of secondary chemistry as a trait for separating species. While phylogenetic analysis showed that continental samples formed a clade that was separate from island-specific clades, population genetic de-novo clustering merged populations from Cuba and Puerto Rico, and populations from Jamaica and the continent. These results yield contrasting phylogeographic and species delimitation scenarios which prevented us from confidently clarifying species boundaries and geographic ranges. However, analyses consistently separated Cuban and Puerto Rican samples as distinct genetic groups hinting that unrecognized cryptic species with a *C. sandstedei* phenotype might inhabit these islands. Better characterization of populations in Cuba, Jamaica and Hispaniola, and the

southern tip of Florida is needed to assess the generality of our observations and determine potential taxonomic changes. Our work reaffirms the power of combining RADseq-based phylogenetics and population genetics to disentangle taxonomic and evolutionary histories in poorly understood, closely related and phenotypically similar lichen-forming fungal species.

Keywords Lichenized fungi, Phylogenetics, Population Genetics, Phylogeography, Endemism, Caribbean

INTRODUCTION

A defining character of our planet's biosphere is the uneven distribution of species diversity (Boenigk, Wodniok, and Glücksman 2015). Contrasting spatial patterns in global biodiversity are multifaceted, but are often attributed to limited taxonomic knowledge of biodiverse regions (e.g. tropics), heterogeneous climates and to areas with high accumulation of endemic species (Cowling 2001; Freeman and Pennell 2021). Multiple areas around the globe fit these characteristics, including the insular Caribbean region. Well known for its high degree of endemism in animals and vascular plants (Nieto-Blázquez, Antonelli, and Roncal 2017; Losos 2009), the Caribbean is also emerging as a region with unaccounted diversity in less-studied groups, including lichenized fungi (Mercado-Díaz et al. 2020; Moncada, Mercado-Díaz, and Lücking 2018; Mercado-Díaz, Lücking, and Parnmen 2014).

Cladonia sandstedei (Abbayes) Ahti is a cushion-forming "reindeer" lichen that is considered primarily a Caribbean endemic, but has documented occurrences in southeastern United States (Ahti 2000, 1984) (Fig. 3.1.A, Fig. 3.2.). It is a soil-dwelling species that mostly

colonizes open environments but can also be found in partly shaded situations. A peculiar aspect about *C. sandstedei* is its disjunct distribution within Puerto Rico. Within this island, populations are known from high elevation forests in the Maricao State Forest and from sea-level scrub-type ecosystems of the Tortugero Lagoon in Vega Baja (Ahti 2000). This pattern contrasts with its tendency to occur in high elevation environments in other islands of the region (i.e. Cuba, Jamaica, and Hispaniola). Apart from elevational differences and concomitant climate variation, the two areas in Puerto Rico also exhibit contrasting soil types. Specifically, Maricao features serpentinite-derived soils (Ricart-Pujals and Padrón-Vélez 2010) whereas the Vega Baja site is characterized by white siliceous sands (Lugo et al. 2001). In the southeastern US and the Bahamas, the species is mostly distributed in low elevation habitats with variable soil types.

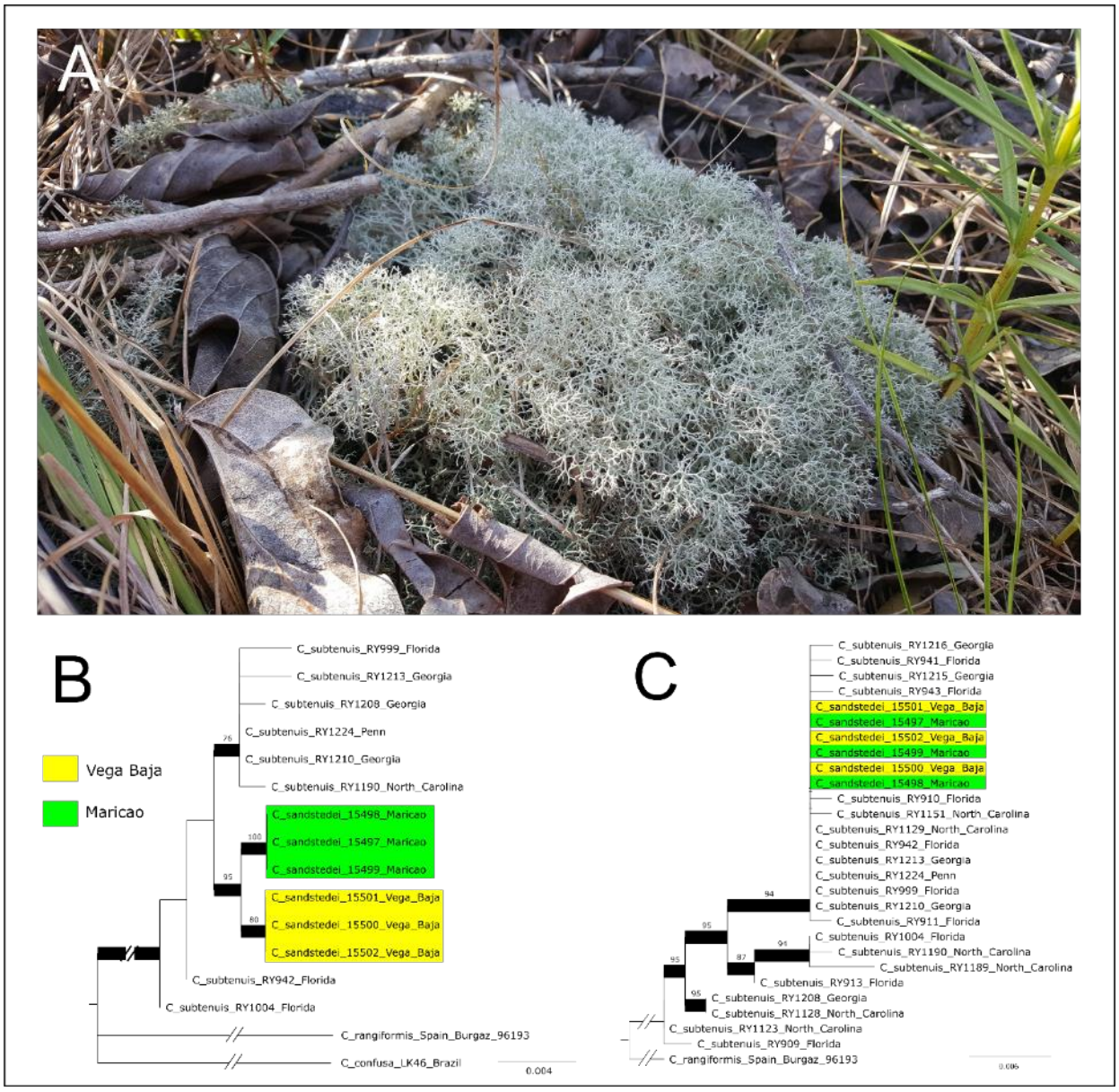

Figure 3.1. A. Habit of *Cladonia sandstedei* from the population in Vega Baja, Puerto Rico and phylogenetic trees (ML) based on gene markers **B.** RPB2 and **C.** EF1 for a selection of *C. sandstedei* individuals from main populations from Puerto Rico (Vega Baja and Maricao) and continental samples of *C. subtenuis*.

Cognizant of the importance of environmental differences as drivers of lichenized fungi diversification (Kraichak et al. 2015; Huang et al. 2019), it was initially hypothesized that

morphologically and chemically indistinguishable populations of *Cladonia sandstedei* within Puerto Rico represented separate, cryptic lineages. To test this, a preliminary phylogenetic analysis using several barcoding loci was carried out (Appendix 9, Table S.3.1., S.3.2.), but results were inconclusive. Gene histories showed that both populations were either genetically indistinguishable or separate genetic entities (Fig. 3.1.B, C). Most importantly, it was found that *C. sandstedei* was consistently nested within *C. subtenuis*, a species similarly distributed in the Caribbean and southeastern US, but further extending its range to Central and Eastern North America. (Fig. 3.2). Several markers have resolved species-level relationships in some groups of *Cladonia* (Kanz et al. 2015), but others suffer from insufficient resolution to confidently place boundaries between species (Pino-Bodas et al. 2011, 2013; Stenroos et al. 2002). In the case of these two species, their secondary chemistry is assumed to be taxonomically informative. *C. subtenuis* has usnic acid as major product whereas *C. sandstedei* typically lacks this substance and instead produces atranorin. Yet, these chemical signatures are not definitive since individuals lacking atranorin have been identified as *C. sandstedei* and samples with both usnic acid and atranorin have been assigned to *C. subtenuis* (Ahti 2000). A further complication is that these supposedly rare chemotypes are also typically associated with Caribbean material (Ahti 2000). Moreover, potassium hydroxide spot tests (K), which are typically used for taxonomic purposes, often fail to produce definitive results (Mercado-Díaz, pers. obs.). These species are also morphologically very similar, with *C. sandstedei* branching being slightly more dense and isotomic (Ahti, 2000, Rosentreter et al., 2015) As expected, these issues can lead to erroneous taxonomic identifications and biased inferences about species' evolutionary relationships.

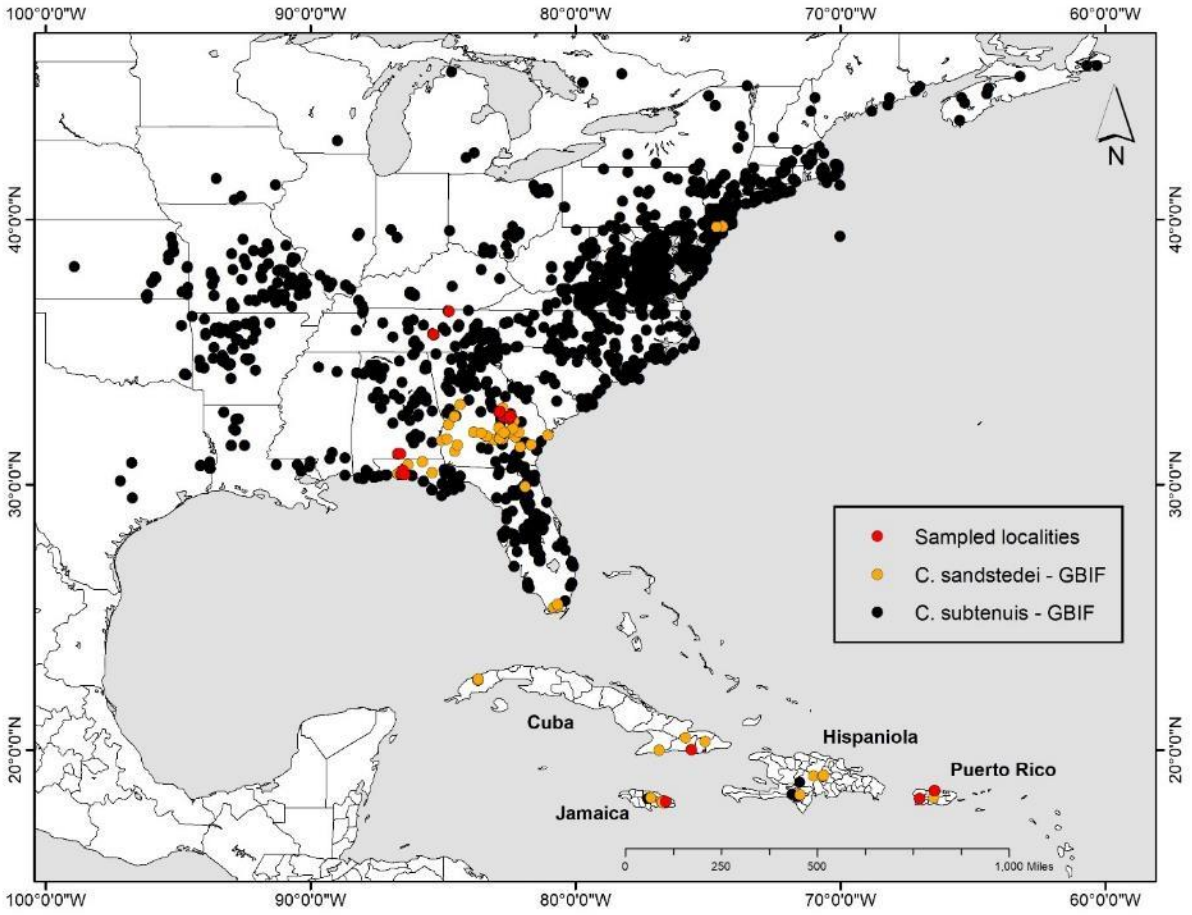

Figure 3.2. Sampling localities (red dots) used in this study for populations of *Cladonia sandstedei* and *C. subtenuis* in the Caribbean and continental United States. Black dots show distribution of *C. subtenuis* whereas orange dots the distribution of *C. sandstedei* based on Global Biodiversity Inventory Facility (GBIF) occurrence records.

Considering limitations related to the use of barcoding loci and issues inherent to phenotypic characters that might lack sufficient discriminatory power, resolving species boundaries between *C. sandstedei* and *C. subtenuis* and understanding the phylogeographic structure of populations throughout the Caribbean remains a challenge. One alternative is to reassess phylogenetic relationships using a wider sampling of genomic regions. This approach was recently implemented in a multilocus study by Stenroos et al., (2019) which found support

for eleven major clades within *Cladonia*. Nonetheless, species-level relationships were poorly resolved in several clades which suggest that more extensive sampling of the genome might be needed to further clarify relationships at shallower taxonomic levels.

Next generation sequencing (NGS) approaches, particularly those categorized as reduced-representation sequencing or "genotype-by-sequencing" techniques, are increasingly becoming the option of choice for generating comprehensive genome-wide datasets, particularly in non-model organisms. Among these, Restriction site-Associated DNA sequencing, or RADseq, is prominent as it allows the discovery and genotyping of high-throughput single nucleotide polymorphisms (SNP) at reasonable cost and without requiring prior genomic information of the taxa under study (Andrews et al. 2016; Narum et al. 2013). RADseq approaches have proved useful for disentangling the genetic basis of poorly understood ecological and evolutionary phenomena in diverse taxa (see Table 1 in Narum et al., 2013). Notable examples include the identification of markers linked to key ecological traits in three-spine sticklebacks (Baird et al. 2008), studies assessing patterns of introgression and hybridization between native and invasive trout species in western US (Hohenlohe et al. 2013, 2011) and groundbreaking work resolving species-level relationships in recently diverged Lake Victoria cichlid species (Wagner et al., 2013). By relaxing the prerequisite of prior identification of loci, RADseq-based methods have allowed researchers to perform multiple analyses that were previously unattainable for non-model organisms. This have led to an explosion of studies in many groups, including investigations in lichenized fungi (Grewe et al. 2018; Widhelm et al. 2021; Alonso-García et al. 2021).

Phylogenetic and population genetic studies in lichenized fungi have greatly benefitted from RADseq-based methods. From a taxonomic standpoint, population genetic analysis based

on RADseq data has clarified species-level relationships, such as recently diverged lineages within *Rhizoplaca melanophthalma* species complex (i.e. the *porteri* group) (Grewe et al. 2017). Similarly, a phylogenomic study using RADseq clearly delimited members of the *Usnea antarctica*/*Usnea aurantiacoatra* "species pair" into separate, potentially species-rank level lineages (Grewe et al. 2018). A recent RADseq study argued that the lack of genetic divergence between populations of *Pseudocyphellaria glabra* in areas throughout the southern hemisphere resulted from frequent long-distance dispersal (Widhelm et al. 2021), further demonstrating the power of RADseq approaches to disentangle the evolutionary history and phylogeography of poorly understood groups.

Here, we implement a RADseq approach to evaluate phylogenetic relationships and explore genetic structure of *Cladonia sandstedei* and *C. subtenuis* populations from the Caribbean and southeastern United States. We assess the degree of divergence between *C. sandstedei* populations within the Caribbean and describe evolutionary relationships between these lineages and those from the continent. Ordination techniques will be used to explore population structure and evaluate genetic admixture. We anticipate considerable genetic differentiation associated with geography. As such, *C. sandstedei* populations within Puerto Rico will likely emerge as distinct genetic clusters, separated from other Caribbean and continental populations. Due to lower barriers to dispersal, degree of admixture and genetic variability is expected to be higher among continental lineages. Phylogenetic patterns will be used to evaluate the usefulness of secondary chemistry for species discrimination, however, no specific patterns are anticipated since secondary chemistry in *Cladonia* is highly variable (Stenroos et al. 2002), sometimes concordant with phylogeny-based species delimitations (Pino-Bodas et al. 2012) but occasionally overestimating diversity (Pino-Bodas, Martín, and Burgaz 2012).

MATERIALS AND METHODS

Sampling and taxonomic work—We sampled at localities where either one or both species have been historically collected according to GBIF (Fig. 3.2). Both *Cladonia sandstedei* and *C. subtenuis* produce cushion-shaped, subglobose "heads" which we regard as separate individuals (Fig 3.1A). Most specimens were collected in the United States, specifically in the states of Alabama (37), Florida (34), Georgia (65) and Tennessee (4). In Puerto Rico, we collected a total of 31 samples identified as *C. sandstedei*: 15 from populations in Maricao and 16 from Vega Baja. Seven samples from Cuba were identified as *C. sandstedei* but four, which were collected as part of a separate effort, where in the same herbarium sheet suggesting these are possibly clonal. These were still processed separately since each represented individual subglobose "heads". Among four samples from Jamaica, three were identified as *C. subtenuis* and one as *C. sandstedei*. A minimum distance of 5 m between individuals was kept, except for populations in Maricao which occur in relatively restricted patches (~100 m^2). Herbarium vouchers were deposited in F.

Preliminary taxonomic identification of samples relied on assessment of thallus color and gross morphology. Spot-tests (K) were used to determine the presence of atranorin, but these can occasionally be misleading, therefore High-Performance Thin Layer Chromatography (HPTLC) was also carried out. Besides detecting atranorin, this method can also be used to detect usnic acid. Solvent C was used for all HPTLC analysis which followed Lumbsch (2002).

DNA extraction and single-locus sequencing—Small fragments for each sample were soaked in acetone to remove secondary substances. These fragments were manually grinded with mortar and pestle. Next, DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep

(Zymo Research, Irvine, California, U.S.A.) following the manufacturer's instructions. A Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to corroborate DNA concentration of samples whereas the quality of isolated DNA was assessed via gel electrophoresis. DNA isolation was carried out at the Field Museum Pritzker DNA Lab. These extractions were used for both single-locus and RAD sequencing. RADseq libraries were created and sequenced the University of Wisconsin-Madison Biotechnology Center. Of the 182 specimens collected for this work, only three samples from Cuba were not processed for phylogenomic analysis due to poor DNA quality.

RADseq library preparation and sequencing—DNA was submitted to the University of Wisconsin-Madison Biotechnology Center. DNA concentration was verified using the Quant-iT™ PicoGreen® dsDNA kit (Life Technologies, Grand Island, NY). Libraries were prepared as in (Elshire et al. 2011) with minimal modification; in short, 50 ng of DNA was digested using the 5-bp cutter ApeKI (New England Biolabs, Ipswich, MA) after which barcoded adapters amenable to Illumina sequencing were added by ligation with T4 ligase (New England Biolabs, Ipswich, MA). Adapter ligation proceeded in batches of 96 samples which were then pooled and amplified to provide library quantities amenable for sequencing. Adapter dimers were removed by SPRI bead purification. Quality and quantity of the finished libraries were assessed using the Agilent Bioanalyzer High Sensitivity Chip (Agilent Technologies, Inc., Santa Clara, CA) and Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY), respectively. Single-end sequencing for an initial set of 15 samples were sequenced on a HiSeq2500 (Illumina Inc.). Paired-end sequencing for an additional set of 164 samples were subsequently sequenced a NovaSeq6000 (Illumina Inc.). For both efforts, sequencing targeted 1.3M reads/sample. Images were analyzed using the standard Illumina Pipeline, version 1.8.2.

RADseq dataset assembly—The *process_radtags* pipeline from Stacks v.2.3 (Rochette, Rivera-Colón, and Catchen 2019) was used to demultiplex raw paired-end reads obtained from the sequencers. Quality control was also carried out with *process_radtags*. This entailed cleaning (remove uncalled bases) and filtering out reads with low quality scores (Phred score = 33) as well as removing adapters by trimming reads to a 55bp length. Quality control with *process_radtags* was also done for the set of 15 single-end libraries which were previously demultiplexed. Parameters and command-line settings used are provided in Appendix 10. FastQC (Banraham Bioinformatics, Cambridge, UK) reports were used to guide quality control.

To increase the number of individuals used for downstream analysis, we combined the 15 single-end reads with forward reads of the paired-end sequence dataset. A RADseq data assembly was carried out with ipyRAD (Eaton and Overcast 2020) using a reference-based approach which allowed to filter for mycobiont loci. Mapping and subsequent filtering of reads was performed using the genome generated by Alonso-García et al. (2021) obtained by merging the genomes of *C. grayi*, *C. macilenta*, *C. metacorallifera*, *C. uncialis*, and the transcriptome of *C. rangiferina*. To facilitate interpretation, raw Illumina RAD sequences are referred to as "reads" and "loci" refers to clustered reads per individual sample. The final matrices are alignments of homologous loci from multiple samples with nucleotide substitutions referred to as "SNP". Single-end sequences were processed in ipyRAD by setting the datatype to "gbs", ploidy to haploid ("1"), clustering of reads within and between individuals to a similarity threshold of "0.90" and a minimum coverage of 4. Seven samples were removed from datasets as they either had less than 1,000 recovered loci or belonged to non-target taxa. Subsequent phylogenetic and population genomic analysis was carried out using filtered ipyRAD output files, such as ".unlinked_snps", ".alleles" and ".vcf".

Phylogenetic analysis—Unlinked SNP files (i.e. matrices limited to one SNP per RAD locus) from the filtered RADseq dataset were used for phylogenetic reconstructions with RAxML v8.2.11 (Stamatakis 2014). This analysis entailed searching for the best scoring ML tree under the ASC_GTRGAMMA model with ascertainment bias correction (--asc-corr=lewis). The bootstrap convergence test using the extended majority-rule consensus tree criterion (autoMRE) was used for a posteriori bootstrapping analysis. Phylogenetic trees were first inspected in FigTree v1.4.3 (Rambaut 2012) then plotted with R package "ggtree" (G. Yu 2020).

Population genomics—Population genetic analysis first required to generate a reduced dataset that included all sites with a minor allele frequency (MAF) greater than or equal to 0.05 and a minimum coverage of 80%. This was accomplished using the program vcftools v.0.1.15 (Danecek et al. 2011). This dataset was converted to a genlight object using the R package "vcfR" (Knaus and Grünwald 2017). The package "adegenet 2.0.2" (Jombart et al., 2010; Jombart and Ahmed, 2011) was then used to convert this file to a genind object. Additional information settings for haploid genomes and sample population membership were subsequently appended to the dataset. Population genetics analyses were executed in R using both genlight and genind objects.

Population structure was initially explored using Principal Component Analysis (PCA). This analysis was based on the genlight object and was run with the *glPca* function from "adegenet". The first three principal components were retained. Visualization of PCA scores was carried out with the R package "ggplot2". Colors and ellipses (level = 95%) denote major clades inferred in our RAxML tree which correspond to the island-specific clades of Jamaica and Cuba, main clades corresponding to populations within Puerto Rico (i.e. Maricao and Vega Baja) and the single clade recovered for continental samples (see below). To further characterize genetic

variation within these clades, basic population-level statistics were computed in using the "Gene Flow and Genetic differentiation" option in DnaSP (Librado and Rozas 2009).

We also used Discriminant Analysis of Principal Components (DAPC) as implemented in "adegenet" to explore grouping of samples that might be linked to population structure. This is a non-parametric method that attempts to summarize genetic variation between groups while overlooking within-group variation (Jombart, Balloux, and Dray 2010). In essence, the method performs a Principal Components Analysis (PCA) transformation of the data which produce a set of uncorrelated variables (principal components) amenable for Discriminant Analysis (DA). Resembling Bayesian clustering methods, individuals are probabilistically assigned to groups (clusters).

Both a-priori grouping and de-novo clustering of samples was performed with DAPC. As with PCA, clades for each Caribbean island and the major clade for continental US were used as a-priori groups. Since we were interested in evaluating potential influence of contrasting habitat preferences on genetic structuring within Puerto Rico, population from Maricao and Vega Baja were kept separate. For de-novo clustering, the function *find.clusters* was used to assign samples to groups. Group assignment was based on the "diffNgroup" criterion using the lowest-score Bayesian information criterion (BIC) value for selecting the "best" number of populations (K). For both a-priori and de-novo clustering analyses, an "a-score" optimization approach was used to select an ideal number of principal components (PC) to retain for DAPC analysis. This entailed running an initial DAPC using 30 PC's and then using the function *optim.a.score* to determine the final number of PC's to retain. Differentiation between populations was carried out by visualizing discriminant functions and principal components of the DAPC using the function *scatter*. Group membership barplots from DAPC were generated in *ggplot2*.

Lastly, we used fineRADstructure (Malinsky et al. 2018) to better understand recent shared ancestry between individuals. This program uses haplotype linkage to summarize nearest-neighbor haplotype relationships between individuals and infer populations. A co-ancestry matrix is used to visualize relationships. As a first step, the ".alleles" file from ipyrad was converted into a fineRADstructure format using the *finerad_input.py* script from fineRADstructure-tools (https://github.com/edgardomortiz/fineRADstructure-tools). During this process, the dataset was reduced to contain only unlinked loci (default parameter) and a minimum sample number of four (--minsample 4). Following authors recommendations, the *sampleLD.R* script from fineRADstructure was then used to re-order loci. A co-ancestry matrix for a haploid dataset (-p 1) was generated with RADpainter and individuals were assigned to populations using fineSTRUCTURE Markov chain Monte Carlo (MCMC) clustering algorithm. The following arguments were provided for the latter: -x 100,000, -z 100,000, and -y 1,000. This clustering algorithm was also used to generate a simple coalescent tree that allowed exploring the relationships between inferred populations. For this step, the following arguments were used: -m T and -x 10,000. Visualization and generation of co-ancestry figure was carried out in the program "Finestructure GUI" (Lawson et al. 2012) after uploading the co-ancestry matrix, the inferred MCMC clusters (populations) and the coalescent tree.

RESULTS

RADseq data processing—Of the 182 samples collected, 172 samples were included in final genomic datasets. An average of 2,053,710 (SD = 369,260; range 747,109–2,963,310) raw reads per sample were recovered (Table S.3.3.). An average of 16% of reads (SD = 5%) successfully mapped to the set of reference genomes. A statistically significant association

between within-sample clusters and the number of mapped reads was found ($R^2 = 0.76$, $p < 0.001$, Fig. S.3.1.), whereas the number of within-sample clusters was strongly correlated with the final number of loci used for analysis ($R^2 = 0.88$, $p < 0.01$, Fig. S.3.2.). Retained samples yielded an average of 14,133 loci (SD = 4,075) which were used for downstream analysis. Samples excluded from genomic datasets were still used for chemical characterization.

Phylogenetic analysis—Results from the phylogenetic analysis of the 172 samples of *C. sandstedei* and *C. subtenuis* are shown in Fig. 3.3. Samples from the Caribbean and southeastern US are separated into two main clades each having strong statistical support. Within the Caribbean clade, we found strong stratification by island with samples from Cuba and Jamaica forming separate, strongly supported clades. All samples from Puerto Rico clustered together with strong support, with Vega Baja individuals forming a strongly supported subclade and Maricao individuals forming a paraphyletic grade. All Caribbean samples contained atranorin including the three *C. subtenuis* individuals from Jamaica.

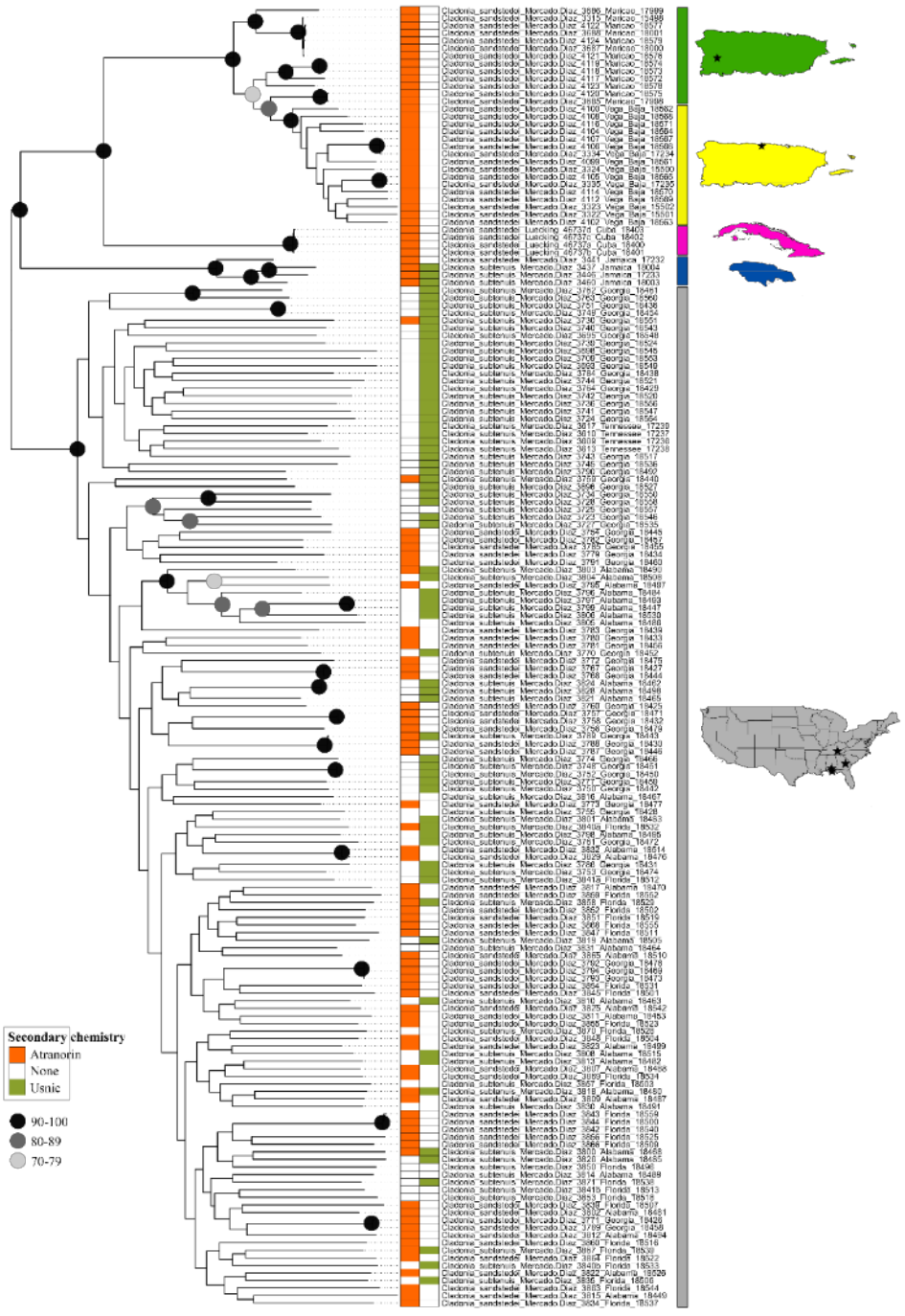

Figure 3.3. RAD-seq based phylogenetic analysis (RAxML) of *C. sandstedei* and *C. subtenuis* samples from the Caribbean and southeastern United States. Presence/absence of main substances are shown to the left of tip labels. Colored maps of sample areas are used to indicate geographic origin of specimens: Maricao, PR (green), Vega Baja, PR (yellow), Cuba (magenta), Jamaica (navy blue) and United States (gray). Black stars on maps for geographic regions indicate approximate sampling localities. Shaded circles on nodes indicate different levels of bootstrap support (%).

The continental clade included samples identified both as *Cladonia sandstedei* and *C. subtenuis* (Fig. 3.3.). Two small subclades, one with five *C. subtenuis* samples from Georgia and another comprised of one *C. sandstedei* and seven *C. subtenuis* individuals from Alabama, were strongly supported. Outside these and several pairs or triplets of genetically similar individuals, relationships within this clade were unresolved. This finding reflected the secondary chemistry patterns with no clear separation between atranorin- vs. usnic acid-producing samples. Other chemotypes were also evident, with some samples producing both substances and others lacking them altogether (Fig. 3.3.).

Population genetic structure—A total of 2,862 SNPs were included in the reduced dataset used for population genomic analyses. Most variation in our PCA is associated with PC axis 1 (17.3%) (Fig. 3.4.) which reflect a geographical gradient in genetic variation. Continental individuals exhibit high genetic variability, with samples spanning a broad range of values along PC2. Jamaican and Cuban samples show low within-island variation and cluster between individuals from the continent and Puerto Rico. Strong overlap on PCA space was also detected between populations from Puerto Rico which exhibit comparatively moderate genetic variation. In terms of population-level statistics estimated with DnaSP, the continental clade yielded the highest values followed by populations from Vega Baja, Maricao, Jamaica and Cuba (Table 3.1). Samples from Cuba are potentially clonal as they were found to represent a single haplotype.

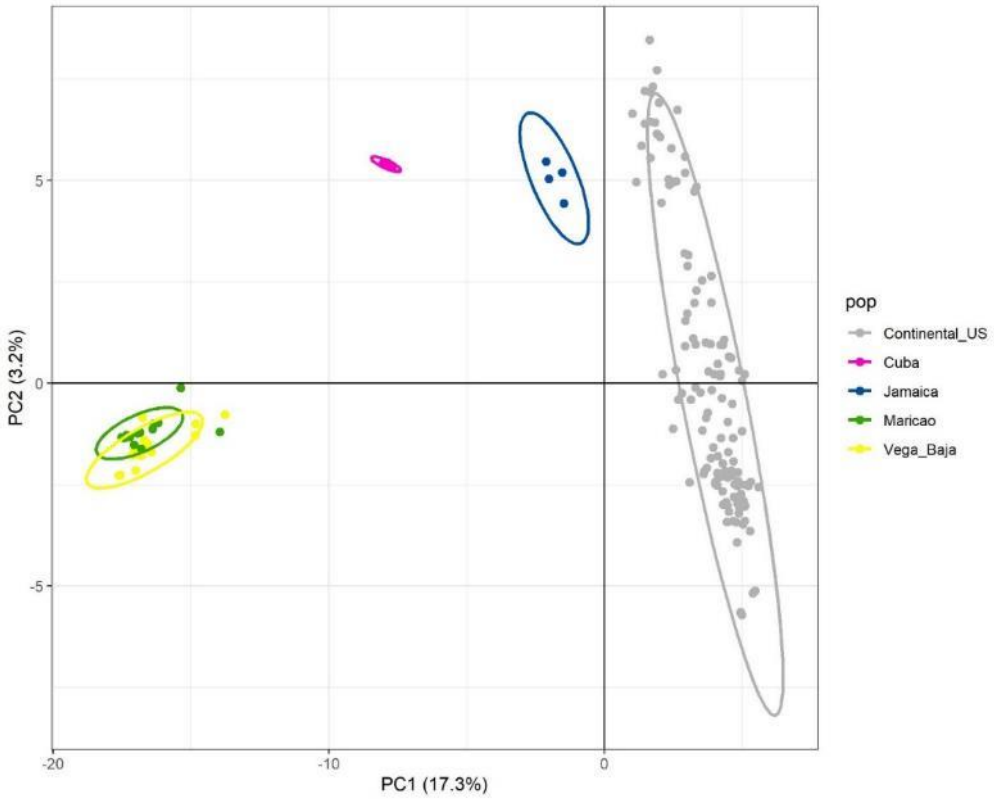

Figure 3.4. Principal component analysis based on a reduced 2,862 SNPs dataset for Caribbean and southeastern US samples of *C. sandstedei* and. *C. subtenuis*.

Table 3.1. Population-level statistics of genetic differentiation for a-priori groups. Statistics include the number of representative sequences for each group (Num. seq.), the number of segregating sites (S), the number of haplotypes (h), haplotype diversity (Hd), the average number of nucleotide differences (K), nucleotide diversity (π), and nucleotide diversity with Jukes-Cantor correction (πJC).

	Num. seq.	S	h	Hd	K	π	π JC
Continental US	135	121	122	0.99834	32.42753	0.2367	0.28623
Cuba	4	0	1	0	0	0	0
Jamaica	4	13	4	1	7	0.05109	0.05307
Puerto Rico, Maricao	13	34	6	0.78205	12.33333	0.09002	0.09938
Puerto Rico, Vega Baja	16	41	13	0.96667	15.70833	0.11466	0.12515

De-novo clustering at K = 4 yielded the lowest BIC score for selecting the "best" number of populations (Fig. S.3.3.). Seven PCs and three (all) discriminant functions were retained for this analysis. At this level, genomic variation associated with the continent was split into two weakly separated clusters: cluster 1, which merged several continental samples and individuals from Jamaica, and cluster 3, which contained most continental samples (Fig. 3.5.). Several continental samples represented admixed individuals. Cluster 2 is composed of all samples from Vega Baja and a few samples from Maricao, whereas Cuban samples are grouped together with the rest of Maricao samples (cluster 4). These two clusters show considerable scatter along discriminant functions.

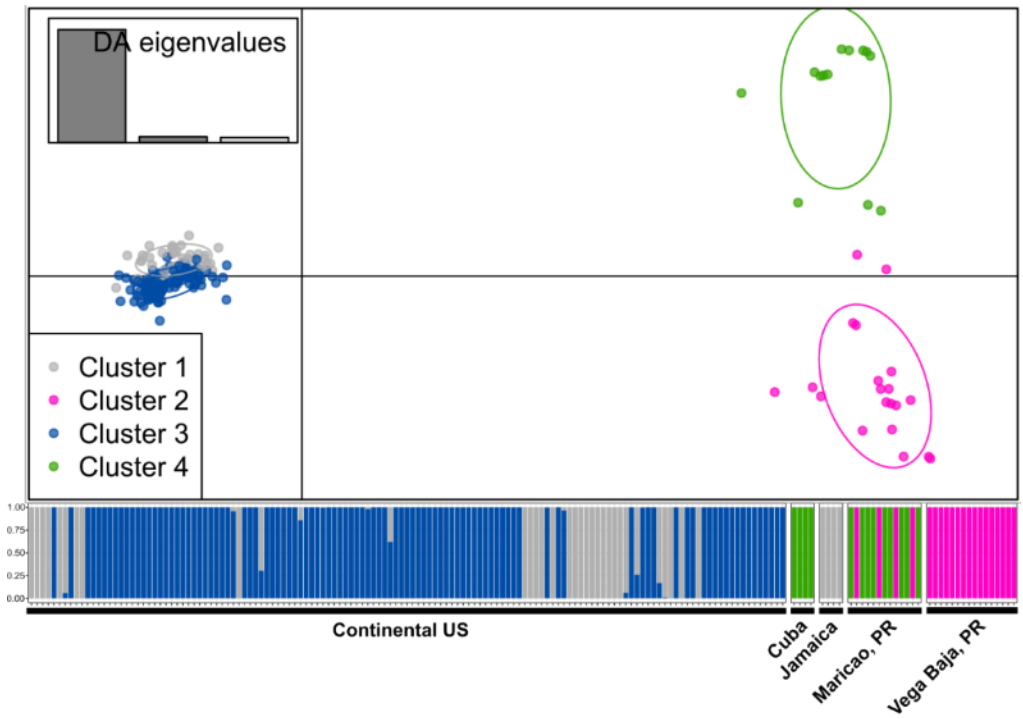

Figure 3.5. Results from de-novo clustering with DAPC at K = 4. Upper part shows the scatterplot for discriminant functions whereas the lower part shows a barplot with assigned membership probabilities. Each dot and bar represent an individual.

We explored de-novo clustering at K = 3 and K = 5 since these also yielded comparatively low BIC scores. Nine PCs and two discriminant functions were retained for K = 3 analysis which merged Jamaican and continental samples into two clusters (1 and 2) (Fig. S.3.4.). Continental samples had several admixed individuals whereas Jamaican samples were unambiguously assigned to cluster 1. Samples from Cuba, Maricao and Vega Baja, on the other hand, were grouped into cluster 3. Lastly, at K = 5, seven PCs and four discriminant functions were retained. Clustering of continental samples was similar to K = 3 and K = 4, with samples either assigned to one of two clusters (2 and 4) or representing admixed individuals (Fig. S.3.5.). A small set of continental samples were grouped into an additional cluster (cluster 5) whereas Jamaican individuals were unambiguously assigned to cluster 4. Samples from Cuba and Puerto Rico form two weakly separated clusters, one containing two samples from Maricao and all individuals from Cuba and Vega Baja (cluster 3), and the other containing the rest of Maricao samples (cluster 1), some showing some degree of admixture with cluster 3.

Five PCs together with four (all) discriminant functions were retained for DAPC analysis based on a-priori groupings. This analysis shows clear separation between continental samples and Caribbean samples (Fig. S.3.6.). Within the Caribbean, samples from Jamaica and Cuba are unambiguously assigned to separate groups. Although several Maricao samples were assigned with high probability to Vega Baja, group membership for most samples was concordant with geographic location. Yet, individuals from these two populations failed to separate along discriminant function space. Continental samples form a single group with no observed admixture.

Finally, fineRADstructure analysis inferred a total of 23 populations (Fig. 3.6.). Levels of shared co-ancestry mirrored patterns of clade support in our RAxML tree. Thus, shared ancestry was higher in samples within each island and in continental samples that formed strongly supported clades. It is worth noting that co-ancestry between Cuban samples ("Pop 14") and Puerto Rican samples ("Pop 3", "Pop 11", "Pop 8", "Pop16") was higher than co-ancestry between Jamaican samples ("Pop 12") and Puerto Rican samples. Moreover, levels of shared ancestry between Jamaican and Cuban samples resembled the degree of co-ancestry observed between Cuban and Puerto Rican samples.

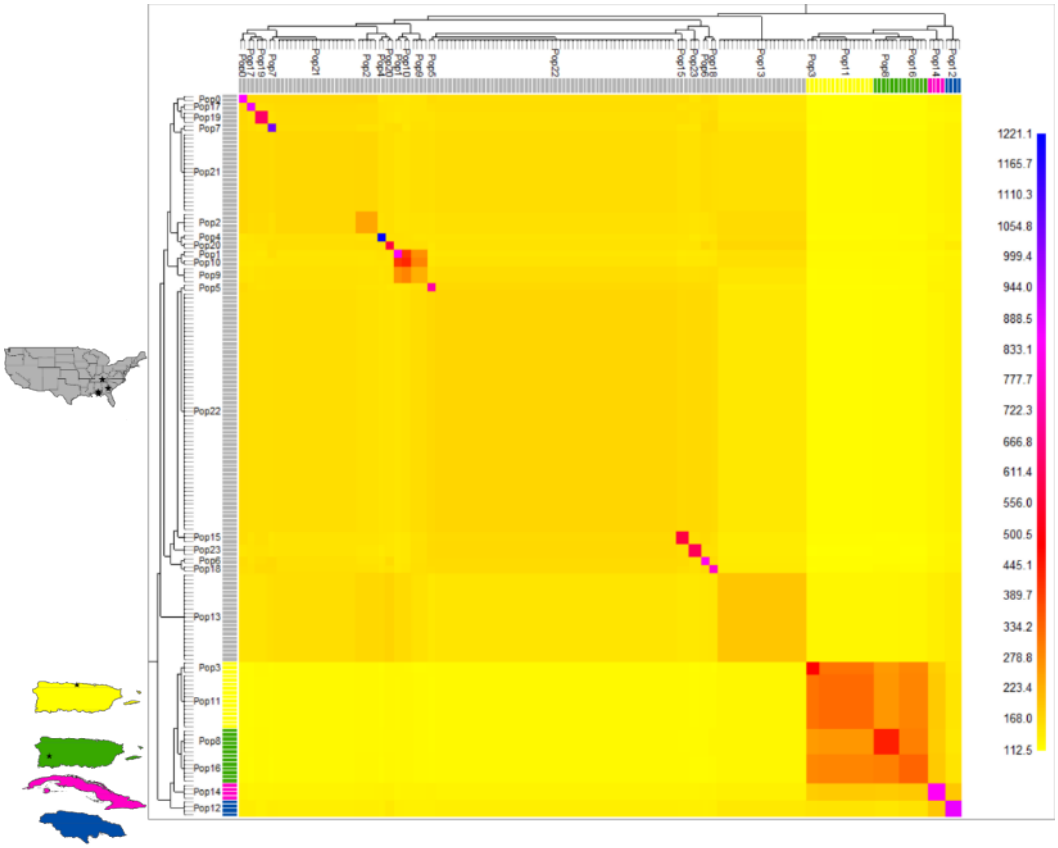

Figure 3.6. fineRADstructure co-ancestry matrix showing recent shared ancestry between *C. sandstedei* and *C. subtenuis* individuals from the Caribbean and southeastern US. Populations inferred are numbered from 1-23; these broadly correspond to strongly supported clades in phylogenetic analysis. Indication of the geographic origin of samples follow the same scheme used in Fig. 3.3.

DISCUSSION

Phylogenetic patterns—Our phylogenetic analyses show strong geographic signal. This is especially evident in the Caribbean where strongly supported island-specific clades were inferred for *C. sandstedei* from Cuba and Puerto Rico, and *C. sandstedei*/*C. subtenuis* from Jamaica. Strong divergence associated with these clades resemble patterns observed in other metazoan lineages and plant groups from this region, many of which are considered putative

endemics (Alonso, Crawford, and Bermingham 2012; Rodríguez et al. 2010; Matos-Maraví et al. 2014; Reynolds et al. 2013; Judd 2001; Michelangeli et al. 2008; Nieto-Blázquez, Peña-Castillo, and Roncal 2020). Phylogenetic studies of lichen-forming fungal species with predominantly Caribbean distributions are scant, but evidence to date suggest that lineages likely restricted to the Caribbean exhibit similar degrees of phylogenetic uniqueness (Mercado-Díaz et al. 2020; Lücking et al. 2020). Increased genetic diversity resulting from regional-specific evolutionary processes likely underlie observed patterns, however, degree of divergence as interpreted from branch lengths should be interpreted with caution. A simulation study demonstrated that without proper correction, phylogenetic analysis based on SNP data might introduce systematic errors in phylogenetic inferences, including biases in branch lengths (Bertels et al. 2014). Overestimated branch lengths might result even when ascertainment bias corrections, like the one performed in this work (i.e. asc_corr=lewis), are carried out (Leaché et al., 2015). This type of issue might also affect topological patterns which can certainly have negative impacts on downstream analyses and/or the interpretation of results (Lewis 2001; Leaché et al. 2015).

Contrasting patterns of phylogenetic support in continental vs. island clades and the lack of clear phylogenetic signal in secondary chemistry deserve further consideration. Despite representing different chemotypes, *C. sandstedei* and *C. subtenuis* individuals from the continent were not recovered as separate clades. Similarly, Jamaican samples from both species were more closely related to each other than they were to any other sample, suggesting that separation between these species based on secondary chemistry does not reflect their phylogenetic relationships. The homoplasy in chemical signature might lead to patterns of overestimated diversity as evidenced in Pino-Bodas et al. (2012b). In contrast, *C. sandstedei* from Cuba and Puerto Rico showed homogeneous secondary chemistry but samples were placed in distinct,

island-specific clades which could result in underestimated diversity if chemistry is used to group taxa. Secondary chemistry is not useful for delimitation purposes between these species, and applies also to subordinate taxonomic ranks that have been previously proposed (cf. Ahti, 2000). There is also insufficient evidence to determine abundance patterns of particular chemical profiles. Furthermore, we note that strongly supported clade substructure observed by Yahr, Vilgalys, and DePriest (2006) in their ITS-based study of *C. subtenuis* in the US contrasts with the lack of phylogenetic structure observed within the continent. Additional work is required to further characterize the underlying causes for these contrasting signals.

C. sandstedei and *C. subtenuis* are also known for Hispaniola (Ahti 2000) which was not included in our collecting efforts. Sampling for both species in Cuba and Jamaica was also limited, particularly populations of *C. subtenuis* from Cuba. Extended sampling in these islands along with concurrent genetic characterization will help evaluate if our phylogenetic generalizations extend to populations in these areas.

Population genomics—Analysis of population genomic structure provide additional details about genetic variation. As suggested by the PCA, and further validated in the phylogenetic analysis, genetic differences along an island-to-continent gradient represent the strongest axis of variation in our data. Continental samples showed the largest scatter of PC scores and yielded the highest haplotype and nucleotide diversity values which suggests that genetic diversity and variation is highest in continental vs. insular lineages. Such observations broadly follow expectations from population genetics theory which predict higher base pair differences in gene copies from larger (e.g. continent) vs. smaller (e.g. island) populations (Kimura 1983). Yet, conclusions are hard to draw due to the limited number of island samples included. Despite this shortcoming, genetic variation at local and regional scales have been

previously documented for continental populations of *C. subtenuis*. For instance, Beard and DePriest (1996) detected restriction site polymorphisms associated with the small subunit (SSU) ribosomal DNA marker in individuals from the same locality and between individuals from different populations in the US. Between-population differences in the accumulation of nucleotide changes in one group I intron were also observed (Beard and DePriest 1996). Yahr, Vilgalys, and DePriest al. (2006) found high diversity in fungal ITS genotypes (32) in a set of 79 samples collected throughout Eastern US. Further characterization of genetic diversity in island lineages is still required, which stresses the need of increasing sampling efforts particularly of individuals with *C. sandstedei* phenotype for which genetic data was unavailable before the present work.

Clustering analysis with DAPC further helped visualizing potential boundaries of population subdivision in our data. Both a-priori and de-novo clustering agreed in separating most insular populations from continental individuals. A-priori clustering mirrored patterns observed in our phylogeny and PCA analysis. Except for several samples from Maricao, this method assigned 100% membership probabilities to individuals into their respective a-priori groups.

Groupings recovered when exploring optimal K values for de-novo clustering provided key insight into potentially hidden patterns of genetic structuring. Splitting of genetic variation at the continental level into two or more clusters represented a consistent feature. Several evolutionary processes might be invoked to explain such patterns including sympatric speciation processes at their early stages or genetic structuring associated with isolation by distance between populations. To address the latter, we performed a separate analysis evaluating if genetic dissimilarity correlated with geographic distance between individuals (Appendix 11).

Although the correlation was weak, we found the association between these variables to be statistically significant suggesting that distance between populations might partly explain the observed splitting (Fig. S.3.7.). Genetic partitioning at the continental level is somewhat surprising because landscape connectivity should promote substantial gene flow in groups like *Cladonia* which have putatively high dispersal capabilities (Alonso-García et al., 2021; Myllys et al., 2003). For instance, ITS-based population genetic analysis in *C. subtenuis* has detected weak genetic structure (F_{st}) between widely dispersed populations in the continental US (Yahr, Vilgalys, and DePriest 2006). Moreover, recent work by Alonso-García et al., (2021) found that populations of *C. stellaris* distributed along a continental-level latitudinal gradient in Canada lacked significant genetic differentiation.

De-novo clustering with DAPC consistently grouped Jamaican samples with continental clusters, thus, partitioning of genetic variation into island vs. continental populations might not be as clear cut as the phylogenetic results suggested. Specifically, grouping by DAPC suggest these belong to the same population, whereas strong phylogenetic divergence hint at potentially different species-level lineages. However, our study is limited by relatively small samples from the Greater Antilles and no samples from the southern tip of Florida, where the species have also been reported (see Fig. 3.2.). Potential biases deriving from using SNP data to reconstruct phylogenies also limit the extent to which these could be used to infer phylogeographic patterns. On the other hand, potential methodological artifacts might underlie grouping of samples with DAPC. Previous work has shown that clustering methods and similar tools for visualization of population structure could be sensitive to uneven sampling (Puechmaille 2016; Wang 2017; Shringarpure and Xing 2014). Studies on sampling bias sensitivity in DAPC are still wanting, but a recent study found that de-novo clustering was inaccurate in situations with high migration

rates (Miller, Cullingham, and Peery 2020). We did not explicitly quantify migration, but effects of this type of biases on our analyses cannot be discarded. These issues highlight that attempting to circumscribe the taxonomy and phylogeography of these species at this stage might be premature.

DAPC merging of Cuban samples with clusters from Puerto Rico raise competing scenarios analogous to those described above. Specifically, these populations might either represent a single lineage (de-novo clustering) or correspond to at least two lineages (phylogeny) As before, these analyses should be interpreted with caution as they could similarly be affected by limited sampling efforts (particularly in Cuba), methodological artifacts and/or SNP-related phylogenetic biases. Yet, divergence of Cuban and Puerto Rican samples is consistent in both phylogenetic and population-level analyses suggesting that formal taxonomic recognition at the species-level might still be required to better characterize the lineages present in these islands.

Candidate populations inferred by fineRADstructure corresponded to strongly supported clades in our phylogenetic analysis which reaffirms that genetic variation in our data is geographically structured. Since the method is optimized for detecting recent coalescence, the analysis hints at relatively recent shared ancestry as a driver of observed phylogenetic patterns. Levels of shared co-ancestry, particularly among samples within Jamaica, Cuba and several strongly supported subpopulations within the continent, were similar to co-ancestry observed for *Usnea aurantiacoatra* and *U. antarctica* in a recent RADseq-based study that concluded these were different species (Grewe et al. 2018). On the other hand, lower co-ancestry associated with the rest of the samples resembled patterns observed by Alonso-García et al. (2021) for clade-defined populations of *Cladonia stellaris* in northeastern North America.

The finding that populations from Cuba and Puerto Rico were genetically isolated from the rest was somewhat surprising considering life history attributes in this group, particularly its preference for relatively open habitats. These areas are often associated with higher levels of gene flow and thus wider distributions (e.g. *Sticta scabrosa*, Moncada et al., 2021). Underlying this pattern are presumed high dispersal capacities in these species which result from small propagules (e.g. spores, minute thallus fragments) assumed to be carried away easily by wind currents and/or animal vectors. In fact, although clonal propagation was not rejected, Yahr, Vilgalys, and DePriest (2006) suggested that patterns of association between mycobionts and photobionts in *C. subtenuis* detected in their study aligned better with apparent landscape-scale level spore dispersal. Notably, one study focusing on populations of *C. subcervicornis* occurring in open environments along several islands off the west coast of Norway found that populations in one island were strongly genetically differentiated from the rest (Printzen and Ekman 2003). Considering the proximity between these islands (< 50 km), their data suggest that *Cladonia* species from open environments might also experience effective barriers to dispersal even over short distances. In Puerto Rico, preferences for relatively rare soil types might also help explain moderate genetic divergence associated with populations within this island. Dissecting how these processes operate, not only in this system, but in insular populations of other species are certainly a next "frontier" in population genetic studies in lichens.

CONCLUSIONS

We found that genetic variation in *C. sandstedei* and *C. subtenuis* show considerable geographic structuring in the Caribbean and southeastern US. No correlation was found between our results and current phenotype-based delimitation of these species. While phylogenetic

reconstruction yielded distinct continental and island-specific clades, population genomic analysis suggested more diffuse boundaries. Cuba and Puerto Rico might harbor potentially unrecognized species-level lineages with *C. sandstedei* phenotype since separation of these populations from the rest was consistent in both phylogenetic and population-level analyses. Our work illustrates the importance of jointly using phylogenetic and population genetic frameworks to assess the phylogeography of poorly understood, sympatrically distributed species of phenotypically similar lichens. Adherence to these frameworks translate into more efficient ways to delimit species. This ultimately allows for better characterization of the lineages that populate our ecosystems.

REFERENCES

Acevedo-Rodríguez, Pedro. 1991. "Angiosperms in the Greater Antilles." *Flora of the Greater Antilles Newsletter*. Vol. 1. New York. https://www.nybg.org/bsci/fga/Newsletter/FGANno1.htm.

Acevedo-Rodríguez, Pedro, and Mark T. Strong. 2008. "Floristic Richness and Affinities in the West Indies." *Botanical Review* 74 (1): 5–36. https://doi.org/10.1007/s12229-008-9000-1.

Acinas, Silvia G., Vanja Klepac-Ceraj, Dana E. Hunt, Chanathip Pharino, Ivica Ceraj, Daniel L. Distel, and Martin F. Polz. 2004. "Fine-Scale Phylogenetic Architecture of a Complex Bacterial Community." *Nature* 430 (6999): 551. https://doi.org/10.1038/nature02649.

Adamo, Martino, Matteo Chialva, Jacopo Calevo, Filippo Bertoni, Kingsley Dixon, and Stefano Mammola. 2021. "Plant Scientists' Research Attention Is Skewed towards Colourful, Conspicuous and Broadly Distributed Flowers." *Nature Plants* 7 (5): 574–78. https://doi.org/10.1038/s41477-021-00912-2.

Ahti, Teuvo. 1984. "The Status of *Cladina* as a Genus Segregated from *Cladonia*" *Beihefte Zur Nova Hedwigia* 79: 25–61. https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=9079262.

———. 2000. *Cladoniaceae – Flora Neotropica Monograph 78.* Bronx, NY: The New York Botanical Garden Press.

Ali, Jason R. 2012. "Colonizing the Caribbean: Is the GAARlandia Land-Bridge Hypothesis Gaining a Foothold?" *Journal of Biogeography* 39 (3): 431–33. https://doi.org/10.1111/j.1365-2699.2011.02674.x.

Alonso-García, Marta, Felix Grewe, Serge Payette, and Juan Carlos Villarreal A. 2021. "Population Genomics of a Reindeer Lichen Species from North American Lichen Woodlands." *American Journal of Botany* 108 (1): 159–71. https://doi.org/10.1002/ajb2.1601.

Alonso, Roberto, Andrew J. Crawford, and Eldredge Bermingham. 2012. "Molecular Phylogeny of an Endemic Radiation of Cuban Toads (Bufonidae: *Peltophryne*) Based on Mitochondrial and Nuclear Genes." *Journal of Biogeography* 39 (3): 434–51. https://doi.org/10.1111/j.1365-2699.2011.02594.x.

Alors, David, H. Thorsten Lumbsch, Pradeep K. Divakar, Steven D. Leavitt, and Ana Crespo. 2016. "An Integrative Approach for Understanding Diversity in the *Punctelia rudecta* Species Complex (Parmeliaceae, Ascomycota)." *PLoS ONE* 11 (2): e0146537. https://doi.org/10.1371/journal.pone.0146537.

Alves, Davi Mello Cunha Crescente, José Alexandre Felizola Diniz-Filho, and Fabricio Villalobos. 2017. "Geographical Diversification and the Effect of Model and Data Inadequacies: The Bat Diversity Gradient as a Case Study." *Biological Journal of the Linnean Society* 121 (4): 894–906. https://doi.org/10.1093/biolinnean/blx030.

Andraca-Gómez, Guadalupe, Mariano Ordano, Karina Boege, César A. Domínguez, Daniel Piñero, Rubén Pérez-Ishiwara, Jacqueline Pérez-Camacho, Maikel Cañizares, and Juan Fornoni. 2015. "A Potential Invasion Route of *Cactoblastis cactorum* within the Caribbean Region Matches Historical Hurricane Trajectories." *Biological Invasions* 17 (5): 1397–

1406. https://doi.org/10.1007/s10530-014-0802-2.

Andrews, Kimberly R., Jeffrey M. Good, Michael R. Miller, Gordon Luikart, and Paul A. Hohenlohe. 2016. "Harnessing the Power of RADseq for Ecological and Evolutionary Genomics." *Nature Reviews Genetics* 17 (2): 81–92. https://doi.org/10.1038/nrg.2015.28.

Appelhans, Marc S., Paul J.A. Keßler, Erik Smets, Sylvain G. Razafimandimbison, and Steven B. Janssens. 2012. "Age and Historical Biogeography of the Pantropically Distributed Spathelioideae (Rutaceae, Sapindales)." *Journal of Biogeography* 39 (7): 1235–50. https://doi.org/10.1111/j.1365-2699.2012.02686.x.

Armstrong, Richard A. 2015. "The Influence of Environmental Factors on the Growth of Lichens in the Field." In *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection, Volume 1*, edited by Dalip Kumar Uptreti, Pradeep K. Divakar, Vertika Shukla, and Rajesh Bajpai, 1–18. New Delhi: Springer. https://doi.org/10.1007/978-81-322-2181-4_1.

Arrigoni, Roberto, Michael L. Berumen, Chaolun Allen Chen, Tullia I. Terraneo, Andrew H. Baird, Claude Payri, and Francesca Benzoni. 2016. "Species Delimitation in the Reef Coral Genera *Echinophyllia* and *Oxypora* (Scleractinia, Lobophylliidae) with a Description of Two New Species." *Molecular Phylogenetics and Evolution* 105: 146–59. https://doi.org/10.1016/j.ympev.2016.08.023.

Baird, Nathan A., Paul D. Etter, Tressa S. Atwood, Mark C. Currey, Anthony L. Shiver, Zachary A. Lewis, Eric U. Selker, William A. Cresko, and Eric A. Johnson. 2008. "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers." *PLoS ONE* 3 (10): e3376. https://doi.org/10.1371/journal.pone.0003376.

Baniya, Chitra Bahadur, Torstein Solhøy, Yngvar Gauslaa, and Michael W. Palmer. 2010. "The Elevation Gradient of Lichen Species Richness in Nepal." *Lichenologist* 42 (1): 83–96. https://doi.org/10.1017/S0024282909008627.

Barraclough, Timothy G., C. William Birky, and Austin Burt. 2003. "Diversification in Sexual and Asexual Organisms." *Evolution* 57 (9): 2166–72. https://doi.org/10.1111/j.0014-3820.2003.tb00394.x.

Barrett, Craig F., and John V. Freudenstein. 2011. "An Integrative Approach to Delimiting Species in a Rare but Widespread Mycoheterotrophic Orchid." *Molecular Ecology* 20 (13): 2771–86. https://doi.org/10.1111/j.1365-294X.2011.05124.x.

Baselga, Andrés. 2010. "Partitioning the Turnover and Nestedness Components of Beta Diversity." *Global Ecology and Biogeography* 19 (1): 134–43. https://doi.org/10.1111/j.1466-8238.2009.00490.x.

Baselga, Andrés, C. David L. Orme, and Sébastien Villéger. 2013. "Betapart: Partitioning Beta Diversity into Turnover and Nestedness Components." *Search.r-Project.org*. https://search.r-project.org/CRAN/refmans/betapart/html/betapart-package.html.

Bässler, Claus, Marc W. Cadotte, Burkhard Beudert, Christoph Heibl, Markus Blaschke, Johannes Heribert Bradtka, Thomas Langbehn, Silke Werth, and Jorg Müller. 2016. "Contrasting Patterns of Lichen Functional Diversity and Species Richness across an Elevation Gradient." *Ecography* 39 (7): 689–98. https://doi.org/10.1111/ecog.01789.

Beard, J. S. 1949. *The Natural Vegetation of the Windward and Leeward Islands*. 1st. Amen House, London: Oxford University Press.

Beard, Karen H., and Paula T. DePriest. 1996. "Genetic Variation within and among Mats of the Reindeer Lichen, *Cladina subtenuis*." *Lichenologist* 28 (2): 171–81. https://doi.org/10.1006/lich.1996.0015.

Bellemain, Eva, Eldredge Bermingham, and Robert E. Ricklefs. 2008. "The Dynamic Evolutionary History of the Bananaquit (*Coereba flaveola*) in the Caribbean Revealed by a Multigene Analysis." *BMC Evolutionary Biology* 8 (1). https://doi.org/10.1186/1471-2148-8-240.

Bellemain, Eva, and Robert E. Ricklefs. 2008. "Are Islands the End of the Colonization Road?" *Trends in Ecology and Evolution* 23 (8): 461–68. https://doi.org/10.1016/j.tree.2008.05.001.

Benson, Dennis A., Mark Cavanaugh, Karen Clark, Ilene Karsch-Mizrachi, James Ostell, Kim D. Pruitt, and Eric W. Sayers. 2018. "GenBank." *Nucleic Acids Research* 46 (D 1): D41–47. https://doi.org/10.1093/nar/gkx1094.

Bertels, Frederic, Olin K. Silander, Mikhail Pachkov, Paul B. Rainey, and Erik Van Nimwegen. 2014. "Automated Reconstruction of Whole-Genome Phylogenies from Short-Sequence Reads." *Molecular Biology and Evolution* 31 (5): 1077–88. https://doi.org/10.1093/molbev/msu088.

Bezdek, James C. 1981. *Pattern Recognition with Fuzzy Objective Function Algorithms*. *Pattern Recognition with Fuzzy Objective Function Algorithms*. New York: Springer. https://doi.org/10.1007/978-1-4757-0450-1.

Bickford, David, David J. Lohman, Navjot S. Sodhi, Peter K. L. Ng, Rudolf Meier, Kevin Winker, Krista K. Ingram, and Indraneil Das. 2007. "Cryptic Species as a Window on Diversity and Conservation." *Trends in Ecology and Evolution* 22 (3): 148–55. https://doi.org/10.1016/j.tree.2006.11.004.

Boenigk, Jens, Sabina Wodniok, and Edvard Glücksman. 2015. "Distribution of Present-Day Biodiversity." In *Biodiversity and Earth History*, edited by Jens Boenigk, Sabina Wodniok, and Edvard Glücksman, 157–225. Berlin: Springer. https://doi.org/10.1007/978-3-662-46394-9_3.

Bouckaert, Remco, Joseph Heled, Denise Kühnert, Tim Vaughan, Chieh Hsi Wu, Dong Xie, Marc A. Suchard, Andrew Rambaut, and Alexei J. Drummond. 2014. "BEAST 2: A Software Platform for Bayesian Evolutionary Analysis." *PLoS Computational Biology* 10 (4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537.

Brace, Selina, Samuel T. Turvey, Marcelo Weksler, Menno L.P. Hoogland, and Ian Barnes. 2015. "Unexpected Evolutionary Diversity in a Recently Extinct Caribbean Mammal Radiation." *Proceedings of the Royal Society B: Biological Sciences* 282 (1807): 20142371. https://doi.org/10.1098/rspb.2014.2371.

Brikiatis, Leonidas. 2014. "The de Geer, Thulean and Beringia Routes: Key Concepts for Understanding Early Cenozoic Biogeography." *Journal of Biogeography* 41 (6): 1036–54. https://doi.org/10.1111/jbi.12310.

Cáceres, Miquel de, Xavier Font, and Francesc Oliva. 2010. "The Management of Vegetation

Classifications with Fuzzy Clustering." *Journal of Vegetation Science* 21 (6): 1138–51. https://doi.org/10.1111/j.1654-1103.2010.01211.x.

Caetano, Daniel S., Brian C. O'Meara, and Jeremy M. Beaulieu. 2018. "Hidden State Models Improve State-Dependent Diversification Approaches, Including Biogeographical Models." *Evolution* 72 (11): 2308–24. https://doi.org/10.1111/evo.13602.

Canal, Dubán, Nils Köster, Marcela Celis, Thomas B. Croat, Thomas Borsch, and Katy E. Jones. 2019. "Out of Amazonia and Back Again: Historical Biogeography of the Species-Rich Neotropical Genus *Philodendron* (Araceae)." *Annals of the Missouri Botanical Garden* 104 (1): 49–68. https://doi.org/10.3417/2018266.

Cano, Ángela, Christine D. Bacon, Fred W. Stauffer, Alexandre Antonelli, Martha L. Serrano-Serrano, and Mathieu Perret. 2018. "The Roles of Dispersal and Mass Extinction in Shaping Palm Diversity across the Caribbean." *Journal of Biogeography* 45 (6): 1432–43. https://doi.org/10.1111/jbi.13225.

Carine, Mark A., Stephen J. Russell, Arnoldo Santos-Guerra, and Javier Francisco-Ortega. 2004. "Relationships of the Macaronesian and Mediterranean Floras: Molecular Evidence for Multiple Colonizations into Macaronesia and Back-Colonization of the Continent in *Convolvulus* (Convolvulaceae)." *American Journal of Botany* 91 (7): 1070–85. https://doi.org/10.3732/ajb.91.7.1070.

Carstens, Bryan C., Tara A. Pelletier, Noah M. Reid, and Jordan D. Satler. 2013. "How to Fail at Species Delimitation." *Molecular Ecology* 22 (17): 4369–83. https://doi.org/10.1111/mec.12413.

Castresana, Jose. 2000. "Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis." *Molecular Biology and Evolution* 17 (4): 540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Cervantes, Angélica, Susy Fuentes, Jorge Gutiérrez, Susana Magallón, and Thomas Borsch. 2016. "Successive Arrivals since the Miocene Shaped the Diversity of the Caribbean Acalyphoideae (Euphorbiaceae)." *Journal of Biogeography* 43 (9): 1773–85. https://doi.org/10.1111/jbi.12790.

Chapman, Arthur. 2009. "Numbers of Living Species in Australia and the World." *Report for the Australian Biological Resources Study*. 2nd ed. Canberra. https://ci.nii.ac.jp/naid/10030098112/.

Chernomor, Olga, Arndt Von Haeseler, and Bui Quang Minh. 2016. "Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices." *Systematic Biology* 65 (6): 997–1008. https://doi.org/10.1093/sysbio/syw037.

Claramunt, Santiago, Elizabeth P. Derryberry, J. V. Jr. Remsen, and Robb T. Brumfield. 2012. "High Dispersal Ability Inhibits Speciation in a Continental Radiation of Passerine Birds." *Proceedings of the Royal Society B: Biological Sciences* 279 (1733): 1567–74. https://doi.org/10.1098/rspb.2011.1922.

Clark, J. Allan, and Robert M. May. 2002. "Taxonomic Bias in Conservation Research." *Science* 297 (5579): 191b – 192. https://doi.org/10.1126/science.297.5579.191b.

Cowling, Richard M. 2001. "Endemism." In *Encycolopedia of Biodiversity*, edited by Simon

Asher Levin, 2nd ed., 497–507. San Diego: Academic Press.

Crespo, Ana M., and H. Thorsten Lumbsch. 2010. "Cryptic Species in Lichen-Forming Fungi." *IMA Fungus* 1 (2): 167–70. https://doi.org/10.5598/imafungus.2010.01.02.09.

Dal Forno, Manuela, Frank Bungartz, Alba Yánez-Ayabaca, Robert Lücking, and James D. Lawrey. 2017. "High Levels of Endemism among Galapagos Basidiolichens." *Fungal Diversity* 85 (1): 45–73. https://doi.org/10.1007/s13225-017-0380-6.

Dal Forno, Manuela, Bibiana Moncada, and Robert Lücking. 2018. "*Sticta aongstroemii*, a Newly Recognized Species in the *S. damicornis* Morphodeme (Lobariaceae) Potentially Endemic to the Atlantic Forest in Brazil." *Lichenologist* 50 (6): 691–96. https://doi.org/10.1017/S0024282918000403.

Damm, Sandra, Bernd Schierwater, and Heike Hadrys. 2010. "An Integrative Approach to Species Discovery in Odonates: From Character-Based DNA Barcoding to Ecology." *Molecular Ecology* 19 (18): 3881–93. https://doi.org/10.1111/j.1365-294X.2010.04720.x.

Danecek, Petr, Adam Auton, Goncalo Abecasis, Cornelis A. Albers, Eric Banks, Mark A. DePristo, Robert E. Handsaker, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27 (15): 2156–58. https://doi.org/10.1093/bioinformatics/btr330.

Darriba, Diego, Guillermo L. Taboada, Rama Doallo, and David Posada. 2012. "JModelTest 2: More Models, New Heuristics and Parallel Computing." *Nature Methods* 9 (8): 772 p. https://doi.org/10.1038/nmeth.2109.

Dávalos, Liliana M. 2007. "Short-Faced Bats (Phyllostomidae: Stenodermatina): A Caribbean Radiation of Strict Frugivores." *Journal of Biogeography* 34 (2): 364–75. https://doi.org/10.1111/j.1365-2699.2006.01610.x.

Davis, Matthew P., Peter E. Midford, and Wayne Maddison. 2013. "Exploring Power and Parameter Estimation of the BiSSE Method for Analyzing Species Diversification." *BMC Evolutionary Biology* 13 (38): 1–12. https://doi.org/10.1186/1471-2148-13-38.

Dayrat, Benoit. 2005. "Towards Integrative Taxonomy." *Biological Journal of the Linnean Society* 85 (3): 407–15. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1095-8312.2005.00503.x.

Del-Prado, Ruth, Pradeep Kumar Divakar, H. Thorsten Lumbsch, and Ana M. Crespo. 2016. "Hidden Genetic Diversity in an Asexually Reproducing Lichen Forming Fungal Group." *PLoS ONE* 11 (8): e0161031. https://doi.org/10.1371/journal.pone.0161031.

Dewalt, Saara J., Kalan Ickes, and Arlington James. 2016. "Forest and Community Structure of Tropical Sub-Montane Rain Forests on the Island of Dominica, Lesser Antilles." *Caribbean Naturalist*, no. 1: 116–37. https://data.fs.usda.gov/research/pubs/iitf/ja_iitf_2016_DeWalt001.pdf.

Didier, Gilles, Marine Fau, and Michel Laurin. 2017. "Likelihood of Tree Topologies with Fossils and Diversification Rate Estimation." *Systematic Biology* 66 (6): 964–87. https://doi.org/10.1093/sysbio/syx045.

Dirnböck, Thomas, Franz Essl, and Wolfgang Rabitsch. 2011. "Disproportional Risk for Habitat Loss of High-Altitude Endemic Species under Climate Change." *Global Change Biology* 17 (2): 990–96. https://doi.org/10.1111/j.1365-2486.2010.02266.x.

Eaton, Deren A.R., and Isaac Overcast. 2020. “Ipyrad: Interactive Assembly and Analysis of RADseq Datasets.” *Bioinformatics* 36 (8): 2592–94. https://doi.org/10.1093/bioinformatics/btz966.

Eberle, Jonas, Rachel C.M. Warnock, and Dirk Ahrens. 2016. “Bayesian Species Delimitation in *Pleophylla* chafers (Coleoptera) - The Importance of Prior Choice and Morphology.” *BMC Evolutionary Biology* 16 (94): 1–16. https://doi.org/10.1186/s12862-016-0659-3.

Elshire, Robert J., Jeffrey C. Glaubitz, Qi Sun, Jesse A. Poland, Ken Kawamoto, Edward S. Buckler, and Sharon E. Mitchell. 2011. “A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species.” *PLoS ONE* 6 (5): e19379. https://doi.org/10.1371/journal.pone.0019379.

Ewel, John J., and Jacob L. Whitmore. 1973. “The Ecological Life Zones of Puerto Rico and the U.S.Virgin Islands.” *Forest Service Research Paper ITF-18*. Río Piedras. https://doi.org/10.1017/CBO9781107415324.004.

Ezard, Thomas, Tomochika Fujisawa, and Timothy G. Barraclough. 2009. “Splits: SPecies’ LImits by Threshold Statistics.” *R Package Version*.

Fabre, Pierre Henri, Julia T. Vilstrup, Maanasa Raghavan, Clio Der Sarkissian, Eske Willerslev, Emmanuel J.P. Douzery, and Ludovic Orlando. 2014. “Rodents of the Caribbean: Origin and Diversification of Hutias Unravelled by next-Generation Museomics.” *Biology Letters* 10 (7): 1–5. https://doi.org/10.1098/rsbl.2014.0266.

Fernández-Mendoza, Fernando, and Christian Printzen. 2013. “Pleistocene Expansion of the Bipolar Lichen *Cetraria aculeata* into the Southern Hemisphere.” *Molecular Ecology* 22 (7): 1961–83. https://doi.org/10.1111/mec.12210.

Feuerer, Tassilo, and David L. Hawksworth. 2007. “Biodiversity of Lichens, Including a World-Wide Analysis of Checklist Data Based on Takhtajan’s Floristic Regions.” *Biodiversity and Conservation* 16 (1): 85–98. https://doi.org/10.1007/s10531-006-9142-6.

Figueroa-Colón, Julio C. 1996. “Phytogeographical Trends, Centers of High Species Richness and Endemism, and the Question of Extinctions in the Native Flora of Puerto Rico.” *Annals of the New York Academy of Sciences* 776 (1): 89–102. https://doi.org/10.1111/j.1749-6632.1996.tb17414.x.

Filipowicz, Natalia, and Susanne S. Renner. 2012. “*Brunfelsia* (Solanaceae): A Genus Evenly Divided between South America and Radiations on Cuba and Other Antillean Islands.” *Molecular Phylogenetics and Evolution* 64 (1): 1–11. https://doi.org/10.1016/j.ympev.2012.02.026.

Fitzjohn, Richard G. 2012. “Diversitree: Comparative Phylogenetic Analyses of Diversification in R.” *Methods in Ecology and Evolution* 3 (6): 1084–92. https://doi.org/10.1111/j.2041-210X.2012.00234.x.

Fleishman, Erica, Reed F. Noss, and Barry R. Noon. 2006. “Utility and Limitations of Species Richness Metrics for Conservation Planning.” *Ecological Indicators* 6 (3): 543–53. https://doi.org/10.1016/j.ecolind.2005.07.005.

Fontaneto, Diego, Elisabeth A. Herniou, Chiara Boschetti, Manuela Caprioli, Giulio Melone, Claudia Ricci, and Timothy G. Barraclough. 2007. “Independently Evolving Species in

Asexual Bdelloid Rotifers." *PLoS Biology* 5 (4): e87. https://doi.org/10.1371/journal.pbio.0050087.

Francisco-Ortega, Javier, Eugenio Santiago-Valentín, Pedro Acevedo-Rodríguez, Carl Lewis, John Plpoly, Alan W. Meerow, and Mike Maunder. 2007. "Seed Plant Genera Endemic to the Caribbean Island Biodiversity Hotspot: A Review and a Molecular Phylogenetic Perspective." *Botanical Review* 73 (3): 183–234. https://doi.org/10.1663/0006-8101(2007)73[183:SPGETT]2.0.CO;2.

Freeman, Benjamin G., and Matthew W. Pennell. 2021. "The Latitudinal Taxonomy Gradient." *Trends in Ecology and Evolution* 0 (0). https://doi.org/10.1016/j.tree.2021.05.003.

Fujisawa, Tomochika, and Timothy G. Barraclough. 2013. "Delimiting Species Using Single-Locus Data and the Generalized Mixed Yule-Coalescent Approach: A Revised Method and Evaluation on Simulated Data Sets." *Systematic Biology* 62 (5): 707–24. https://doi.org/10.1093/sysbio/syt033.

Galloway, David J. 1979. "Biogeographical Elements in the New Zealand Lichen Flora." In *Plants and Islands*, edited by David Bramwell, 201–24. London: Academic Press. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C14&q=Biogeographical+elements+in+the+New+Zealand+lichen+flora&btnG=.

———. 2015. "Contributions to a History of New Zealand Lichenology 5*. James Murray (1923–1961)." *Phytotaxa* 198 (1): 1–67. https://doi.org/10.11646/phytotaxa.198.1.1.

Galloway, D. J., and M .A. Thomas. 2004. "*Sticta*." In *Lichen Flora of the Greater Sonoran Desert Region Vol. II*, edited by Thomas H Nash III, Bruce D Ryan, Paul Diederich, Corinna Gries, and Frank Bungartz, 513–24. Tempe: Tempe: Lichen Unlimited. Arizona State University.

Galloway, David J. 1994. "Studies on the Lichen Genus *Sticta* (Schreber) Ach .: II . I. Southern South American Species." *Lichenologist* 26 (3): 223–82.

Garrido-Benavent, Isaac, and Sergio Pérez-Ortega. 2017. "Past, Present, and Future Research in Bipolar Lichen-Forming Fungi and Their Photobionts." *American Journal of Botany* 104 (11): 1660–74. https://doi.org/10.3732/ajb.1700182.

Gauslaa, Y., and K. A. Solhaug. 1999. "High-Light Damage in Air-Dry Thalli of the Old Forest Lichen *Lobaria pulmonaria*—Interactions of Irradiance, Exposure Duration and High Temperature." *Journal of Experimental Botany* 50 (334): 697–705. https://academic.oup.com/jxb/article-abstract/50/334/697/525813.

Geer, Alexandra Van der, George Lyras, John de Vos, and Michael Dermitzakis. 2010. *Evolution of Island Mammals: Adaptation and Extinction of Placental Mammals on Islands*. 1st ed. Chichester: Wiley-Blackwell. https://doi.org/10.1002/9781444323986.

Gelman, Andrew, and Donald B. Rubin. 1992. "Inference from Iterative Simulation Using Multiple Sequences." *Statistical Science* 7 (4): 457–72. https://doi.org/10.1214/ss/1177011136.

Givnish, Thomas J. 2010. "Ecology of Plant Speciation." *Taxon* 59 (5): 1329–66. https://doi.org/10.2307/20774033.

Goldberg, Emma E., Lesley T. Lancaster, and Richard H. Ree. 2011. "Phylogenetic Inference of

Reciprocal Effects between Geographic Range Evolution and Diversification." *Systematic Biology* 60 (4): 451–65. https://doi.org/10.1093/sysbio/syr046.

Gorelick, Noel, Matt Hancher, Mike Dixon, Simon Ilyushchenko, David Thau, and Rebecca Moore. 2017. "Google Earth Engine: Planetary-Scale Geospatial Analysis for Everyone." *Remote Sensing of Environment* 202: 18–27. https://doi.org/10.1016/j.rse.2017.06.031.

Graham, Alan. 1990. "Late Tertiary Microfossil Flora from the Republic of Haiti." *American Journal of Botany* 77 (7): 911–26. https://doi.org/10.2307/2444507.

———. 2003. "Geohistory Models and Cenozoic Paleoenvironments of the Caribbean Region." *Systematic Botany* 28 (2): 378–86. https://doi.org/10.1043/0363-6445-28.2.378.

Graham, Alan, and David M. Jarzen. 1969. "Studies in Neotropical Paleobotany. I. The Oligocene Communities of Puerto Rico." *Annals of the Missouri Botanical Garden* 56 (3): 308. https://doi.org/10.2307/2394849.

Graham, Catherine H., and Paul V A Fine. 2008. "Phylogenetic Beta Diversity: Linking Ecological and Evolutionary Processes across Space in Time." *Ecology Letters* 11 (12): 1265–77. https://doi.org/10.1111/j.1461-0248.2008.01256.x.

Graham, Catherine H., Juan L. Parra, Carsten Rahbek, and Jimmy A. McGuire. 2009. "Phylogenetic Structure in Tropical Hummingbird Communities." *Proceedings of the National Academy of Sciences of the United States of America* 106 (SUPPL. 2): 19673–78. https://doi.org/10.1073/pnas.0901649106.

Grau, H. Ricardo, T. Mitchell Aide, Jess K. Zimmerman, Eileen H. Helmer, and Xioming Zou. 2003. "The Ecological Consequences of Socioeconomic and Land-Use Changes in Postagriculture Puerto Rico." *BioScience* 53 (12): 1159–68. https://doi.org/10.1641/0006-3568(2003)053[1159:TECOSA]2.0.CO;2.

Grewe, Felix, Jen Pen Huang, Steven D. Leavitt, and H. Thorsten Lumbsch. 2017. "Reference-Based RADseq Resolves Robust Relationships among Closely Related Species of Lichen-Forming Fungi Using Metagenomic DNA." *Scientific Reports* 7 (9884): 1–11. https://doi.org/10.1038/s41598-017-09906-7.

Grewe, Felix, Elisa Lagostina, Huini Wu, Christian Printzen, and H. Thorsten Lumbsch. 2018. "Population Genomic Analyses of RAD Sequences Resolves the Phylogenetic Relationship of the Lichen-Forming Fungal Species *Usnea antarctica* and *Usnea aurantiacoatra*." *MycoKeys* 43: 91–113. https://doi.org/10.3897/mycokeys.43.29093.

Guillemin, Marie Laure, Loretto Contreras-Porcia, María Eliana Ramírez, Erasmo C. Macaya, Cristian Bulboa Contador, Helen Woods, Christopher Wyatt, and Juliet Brodie. 2016. "The Bladed Bangiales (Rhodophyta) of the South Eastern Pacific: Molecular Species Delimitation Reveals Extensive Diversity." *Molecular Phylogenetics and Evolution* 94: 814–26. https://doi.org/10.1016/j.ympev.2015.09.027.

Guindon, Stéphane, and Olivier Gascuel. 2003. "A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood." *Systematic Biology* 52 (5): 696–704. https://doi.org/10.1080/10635150390235520.

Harris, Richard. 1989. *Working Keys to the Lichen-Forming Fungi of Puerto Rico*. Bronx: Published by the author.

Hass, Carla A., and S. Blair Hedges. 1991. “Albumin Evolution in West Indian Frogs of the Genus *Eleutherodactylus* (Leptodactylidae): Caribbean Biogeography and a Calibration of the Albumin Immunological Clock.” *Journal of Zoology* 225 (3): 413–26. https://doi.org/10.1111/j.1469-7998.1991.tb03825.x.

Hawksworth, David L., and Martin Grube. 2020. “Lichens Redefined as Complex Ecosystems.” *New Phytologist* 227 (5): 1281–83. https://doi.org/10.1111/nph.16630.

Hebert, Paul D.N., Erin H. Penton, John M. Burns, Daniel H. Janzen, and Winnie Hallwachs. 2004. “Ten Species in One: DNA Barcoding Reveals Cryptic Species in the Neotropical Skipper Butterfly *Astraptes fulgerator*.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (41): 14812–17. https://doi.org/10.1073/pnas.0406166101.

Hedges, S. Blair. 1989. “An Island Radiation: Allozyme Evolution in Jamaican Frogs of the Genus *Eleutherodactylus* (Leptodactylidae).” *Caribbean Journal of Science* 25 (3–4): 123–47.

———. 2006. “Paleogeography of the Antilles and Origin of West Indian Terrestrial Vertebrates.” *Annals of the Missouri Botanical Garden* 93 (2): 231–44. https://doi.org/10.1007/s00586-014-3395-7.

Heinicke, Matthew P., William E. Duellman, and S. Blair Hedges. 2007. “Major Caribbean and Central American Frog Faunas Originated by Ancient Oceanic Dispersal.” *Proceedings of the National Academy of Sciences* 104 (24): 10092–97. https://doi.org/10.1073/pnas.0611051104.

Herrando-Moraira, Sonia, Sergi Massó, Deborah Zani, Jordi López-Pujol, and Roser Vilatersana. 2019. “The Impact of Pleistocene Sea-Level Oscillations on Plant Genetic Diversity: The Case of the Western Mediterranean Endemic *Carduncellus dianius* (Asteraceae).” *Botanical Journal of the Linnean Society* 191 (3): 399–420. https://doi.org/10.1093/botlinnean/boz058.

Hidalgo, Banessa Falcón, Susy Fuentes Bazan, Rosalina Berazaín Iturralde, and Thomas Borsch. 2020. “Phylogenetic Relationships and Character Evolution in Neotropical *Phyllanthus* (Phyllanthaceae), with a Focus on the Cuban and Caribbean Taxa.” *International Journal of Plant Sciences* 181 (3): 284–305. https://doi.org/10.1086/706454.

Hijmans, Robert J. 2019. “Geosphere: Spherical Trigonometry. R Package Version 1.5-10.” https://cran.r-project.org/package=geosphere.

Hohenlohe, Paul A., Stephen J. Amish, Julian M. Catchen, Fred W. Allendorf, and Gordon Luikart. 2011. “Next-Generation RAD Sequencing Identifies Thousands of SNPs for Assessing Hybridization between Rainbow and Westslope Cutthroat Trout.” *Molecular Ecology Resources* 11 (SUPPL. 1): 117–22. https://doi.org/10.1111/j.1755-0998.2010.02967.x.

Hohenlohe, Paul A., Mitch D. Day, Stephen J. Amish, Michael R. Miller, Nick Kamps-Hughes, Matthew C. Boyer, Clint C. Muhlfeld, Fred W. Allendorf, Eric A. Johnson, and Gordon Luikart. 2013. “Genomic Patterns of Introgression in Rainbow and Westslope Cutthroat Trout Illuminated by Overlapping Paired-End RAD Sequencing.” *Molecular Ecology* 22 (11): 3002–13. https://doi.org/10.1111/mec.12239.

Huang, Jen-Pan. 2018. “What Have Been and What Can Be Delimited as Species Using Molecular Data under the Multi-Species Coalescent Model? A Case Study Using Hercules Beetles (Dynastes; Dynastidae).” *Insect Systematics and Diversity* 2 (2): 1–10. https://doi.org/10.1093/isd/ixy003.

Huang, Jen-Pan, Ekaphan Kraichak, Steven D. Leavitt, Matthew P. Nelsen, and H. Thorsten Lumbsch. 2019. “Accelerated Diversifications in Three Diverse Families of Morphologically Complex Lichen-Forming Fungi Link to Major Historical Events.” *Scientific Reports* 9 (1). https://doi.org/10.1038/s41598-019-44881-1.

Hue, A. M. 1901. “Lichenes Extra-Europaei.” *Nouvelles Archives Du Musém d’Histoire Naturelle* sér. 4 (3): 21–146.

Huete, Alfredo R., Chris Justice, and Wim van Leeuwen. 1999. “MODIS Vegetation Index (MOD 13), EOS MODIS Algorithm—Theoretical Basis Document.” Greenbelt, MD.

Huete, Alfredo R., Ray D. Jackson, and Don F. Post. 1985. “Spectral Response of a Plant Canopy with Different Soil Backgrounds.” *Remote Sensing of Environment* 17 (1): 37–53. https://doi.org/10.1016/0034-4257(85)90111-7.

Hughes, Colin E., and Guy W. Atchison. 2015. “The Ubiquity of Alpine Plant Radiations: From the Andes to the Hengduan Mountains.” *New Phytologist* 207 (2): 275–82. https://doi.org/10.1111/nph.13230.

Hutsemékers, Virginie, Peťer Szoẅeńyi, A. Jonathan Shaw, Juana Mariá Gonzaĺez-Mancebo, Jesuś Munõz, and Alain Vanderpoorten. 2011. “Oceanic Islands Are Not Sinks of Biodiversity in Spore-Producing Plants.” *Proceedings of the National Academy of Sciences of the United States of America* 108 (47): 18989–94. https://doi.org/10.1073/pnas.1109119108.

Imshaug, Henry A. 1957. *Catalogue of West Indian Lichens*. 1st ed. Kingston: Bulletin of the Institute of Jamaica.

Iturralde-Vinent, Manuel A., and RDE MacPhee. 1999. “Paleogeography of the Caribbean Region: Implications for Cenozoic Biogeography.” *Bulletin of the American Museum of Natural History* 238: 1–95. http://hdl.handle.net/2246/1642.

Jennings, L.N., J. Douglas, E. Treasure, and G. González. 2014. “Climate Change Effects in El Yunque National Forest, Puerto Rico, and the Caribbean Region.” *General Technical Report SRS-GTR-193*. Asheville, NC.

Jin, Lanna S., Marc W. Cadotte, and Marie Josée Fortin. 2015. “Phylogenetic Turnover Patterns Consistent with Niche Conservatism in Montane Plant Species.” *Journal of Ecology* 103 (3): 742–49. https://doi.org/10.1111/1365-2745.12385.

Jombart, Thibaut, and Ismaïl Ahmed. 2011. “Adegenet 1.3-1: New Tools for the Analysis of Genome-Wide SNP Data.” *Bioinformatics* 27 (21): 3070–71. https://doi.org/10.1093/bioinformatics/btr521.

Jombart, Thibaut, François Balloux, and Stéphane Dray. 2010. “Adephylo: New Tools for Investigating the Phylogenetic Signal in Biological Traits.” *Bioinformatics* 26 (15): 1907–9. https://doi.org/10.1093/bioinformatics/btq292.

Judd, Walter S. 2001. “Phylogeny and Biogeography of *Lyonia* Sect. Lyonia (Ericaceae).” In

Biogeography of the West Indies: Patterns and Perspectives, edited by C. A. Woods and F. E. Sergile, 2nd ed., 63–75. Boca Ratón: CRC Press.

Kalwij, Jesse M., Helene H. Wagner, and Christoph Scheidegger. 2005. "Effects of Stand-Level Disturbances on the Spatial Distribution of a Lichen Indicator." *Ecological Applications* 15 (6): 2015–24.

Kalyaanamoorthy, Subha, Bui Quang Minh, Thomas K.F. Wong, Arndt Von Haeseler, and Lars S. Jermiin. 2017. "ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates." *Nature Methods* 14 (6): 587–89. https://doi.org/10.1038/nmeth.4285.

Kamvar, Zhian N., Javier F. Tabima, and Niklaus J. Grünwald. 2014. "Poppr: An R Package for Genetic Analysis of Populations with Clonal, Partially Clonal, and/or Sexual Reproduction." *PeerJ* 2: e281. https://doi.org/10.7717/peerj.281.

Kanz, Birgit, Wolfgang von Brackel, Rainer Cezanne, Marion Eichler, Marie- Luise Hohmann, Dietmar Teuber, and Christian Printzen. 2015. " DNA Barcodes for the Distinction of Reindeer Lichens: A Case Study Using *Cladonia rangiferina* and *C. stygia*." *Herzogia* 28 (2): 445–64. https://doi.org/10.13158/heia.28.2.2015.445.

Katoh, Kazutaka, and Daron M. Standley. 2013. "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability." *Molecular Biology and Evolution* 30 (4): 772–80. https://doi.org/10.1093/molbev/mst010.

Kauff, Frank, and François Lutzoni. 2002. "Phylogeny of the Gyalectales and Ostropales (Ascomycota, Fungi): Among and within Order Relationships Based on Nuclear Ribosomal RNA Small and Large Subunits." *Molecular Phylogenetics and Evolution* 25 (1): 138–56. https://doi.org/10.1016/S1055-7903(02)00214-2.

———. 2003. "Compat.Py - a Program to Detect Topological Conflict Betweeen Supported Clades in Phylogenetic Trees." http://lutzonilab.org/downloadable-programs/.

Kembel, Steven W., Peter D. Cowan, Matthew R. Helmus, William K. Cornwell, Helene Morlon, David D. Ackerly, Simon P. Blomberg, and Campbell O. Webb. 2010. "Picante: R Tools for Integrating Phylogenies and Ecology." *Bioinformatics* 26 (11): 1463–64. https://doi.org/10.1093/bioinformatics/btq166.

Kidd, S. A., and H. J. Michaels. 2005. "Molecular Phylogenetics of the Hawaiian Geraniums." *MS. Thesis*. Bowling Green State University.

Kimura, Motoo. 1983. *The Neutral Theory of Molecular Evolution*. New York: Cambridge University Press.

Knaus, Brian J., and Niklaus J. Grünwald. 2017. "Vcfr: A Package to Manipulate and Visualize Variant Call Format Data in R." *Molecular Ecology Resources* 17 (1): 44–53. https://doi.org/10.1111/1755-0998.12549.

Koenemann, Daniel Mark, and Janelle M. Burke. 2020. "A Molecular Phylogeny for the Genus *Coccoloba* (Polygonaceae) with an Assessment of Biogeographic Patterns." *Systematic Botany* 45 (3): 567–75. https://doi.org/10.1600/036364420x15935294613536.

Koleff, Patricia, Kevin J. Gaston, and Jack J. Lennon. 2003. "Measuring Beta Diversity for Presence-Absence Data." *Journal of Animal Ecology* 72 (3): 367–82. https://doi.org/10.1046/j.1365-2656.2003.00710.x.

Kraichak, Ekaphan, Pradeep K. Divakar, Ana Crespo, Steven D. Leavitt, Matthew P. Nelsen, Robert Lücking, and H. Thorsten Lumbsch. 2015. "A Tale of Two Hyper-Diversities: Diversification Dynamics of the Two Largest Families of Lichenized Fungi." *Scientific Reports* 5 (1): 1–9. https://doi.org/10.1038/srep10028.

Kraichak, Ekaphan, Jen-Pan Huang, Matthew P. Nelsen, Steven D. Leavitt, and H. Thorsten Lumbsch. 2018. "A Revised Classification of Orders and Families in the Two Major Subclasses of Lecanoromycetes (Ascomycota) Based on a Temporal Approach." *Botanical Journal of the Linnean Society* 188 (3): 233–49. https://doi.org/10.1093/botlinnean/boy060.

Kraichak, Ekaphan, Robert Lücking, Andre Aptroot, Andreas Beck, Patrick Dornes, Volker John, James C. Lendemer, et al. 2015. "Hidden Diversity in the Morphologically Variable Script Lichen (*Graphis scripta*) Complex (Ascomycota, Ostropales, Graphidaceae)." *Organisms Diversity and Evolution* 15 (3): 447–58. https://doi.org/10.1007/s13127-015-0219-5.

Kranner, Ilse, Richard Beckett, Ayala Hochman, and Thomas H. Nash. 2008. "Desiccation-Tolerance in Lichens: A Review." *Bryologist* 111 (4): 576–93. https://doi.org/10.1639/0007-2745-111.4.576.

Laenen, Benjamin, Aurélie Désamoré, Nicolas Devos, A. Jonathan Shaw, Juana Maria González-Mancebo, Mark A. Carine, and Alain Vanderpoorten. 2011. "Macaronesia: A Source of Hidden Genetic Diversity for Post-Glacial Recolonization of Western Europe in the Leafy Liverwort *Radula lindenbergiana*." *Journal of Biogeography* 38 (4): 631–39. https://doi.org/10.1111/j.1365-2699.2010.02440.x.

Lakner, Clemens, Paul Van Der Mark, John P. Huelsenbeck, Bret Larget, and Fredrik Ronquist. 2008. "Efficiency of Markov Chain Monte Carlo Tree Proposals in Bayesian Phylogenetics." *Systematic Biology* 57 (1): 86–103. https://doi.org/10.1080/10635150801886156.

Landrón-Concepción, Ismael. 1972. "The Lichen Genus Ramalina Ach. in the West Indies with Notes on Its Role in the Vegetation of Puerto Rico." Michigan State University, East Lansing, MI, USA. http://agris.fao.org/agris-search/search.do?recordID=US201300325718.

Lanfear, Robert, Brett Calcott, Simon Y.W. Ho, and Stephane Guindon. 2012. "PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses." *Molecular Biology and Evolution* 29 (6): 1695–1701. https://doi.org/10.1093/molbev/mss020.

Lawson, Daniel John, Garrett Hellenthal, Simon Myers, and Daniel Falush. 2012. "Inference of Population Structure Using Dense Haplotype Data." *PLoS Genetics* 8 (1): 1002453. https://doi.org/10.1371/journal.pgen.1002453.

Leaché, Adam D., Barbara L. Banbury, Joseph Felsenstein, Adrián Nieto Montes De Oca, and Alexandros Stamatakis. 2015. "Short Tree, Long Tree, Right Tree, Wrong Tree: New Acquisition Bias Corrections for Inferring SNP Phylogenies." *Systematic Biology* 64 (6): 1032–47. https://doi.org/10.1093/sysbio/syv053.

Leaché, Adam D., and Matthew K. Fujita. 2010. "Bayesian Species Delimitation in West African Forest Geckos (*Hemidactylus fasciatus*)." *Proceedings of the Royal Society B* 277 (1697): 3071–77. https://doi.org/10.1098/rspb.2010.0662.

Leavitt, Steven D., Johnathon D. Fankhauser, Dean H. Leavitt, Lyndon D. Porter, Leigh A. Johnson, and Larry L. St. Clair. 2011. "Complex Patterns of Speciation in Cosmopolitan 'Rock Posy' Lichens - Discovering and Delimiting Cryptic Fungal Species in the Lichen-Forming *Rhizoplaca melanophthalma* Species-Complex (Lecanoraceae, Ascomycota)." *Molecular Phylogenetics and Evolution* 59 (3): 587–602. https://doi.org/10.1016/j.ympev.2011.03.020.

Leavitt, Steven D., Felix Grewe, Todd Widhelm, Lucia Muggia, Brian Wray, and H. Thorsten Lumbsch. 2016. "Resolving Evolutionary Relationships in Lichen-Forming Fungi Using Diverse Phylogenomic Datasets and Analytical Approaches." *Scientific Reports* 6: 1–11. https://doi.org/10.1038/srep22262.

Leavitt, Steven D., Ekaphan Kraichak, Jan Vondrak, Matthew P. Nelsen, Mohammad Sohrabi, Sergio Perez-Ortega, Larry L. St Clair, and H. Thorsten Lumbsch. 2016. "Cryptic Diversity and Symbiont Interactions in Rock-Posy Lichens." *Molecular Phylogenetics and Evolution* 99: 261–74. https://doi.org/10.1016/j.ympev.2016.03.030.

Leprieur, Fabien, Camille Albouy, Julien de Bortoli, Peter F. Cowman, David R. Bellwood, and David Mouillot. 2012. "Quantifying Phylogenetic Beta Diversity: Distinguishing between 'true' Turnover of Lineages and Phylogenetic Diversity Gradients." *PLoS ONE* 7 (8): e42760. https://doi.org/10.1371/journal.pone.0042760.

Levin, Donald A. 2000. *The Origin, Expansion, and Demise of Plant Species*. 1st ed. New York: Oxford University Press.

Lewis, Paul O. 2001. "A Likelihood Approach to Estimating Phylogeny from Discrete Morphological Character Data." *Systematic Biology* 50 (6): 913–25. https://doi.org/10.1080/106351501753462876.

Librado, P., and J. Rozas. 2009. "DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data." *Bioinformatics* 25 (11): 1451–52. https://doi.org/10.1093/bioinformatics/btp187.

Lim, Gwynne S., Michael Balke, and Rudolf Meier. 2012. "Determining Species Boundaries in a World Full of Rarity: Singletons, Species Delimitation Methods." *Systematic Biology* 61 (1): 165–69. https://doi.org/10.1093/sysbio/syr030.

Liogier, Henri Alain, and Luis Felipe Martorell. 2000. *Flora of Puerto Rico and Adjacent Islands: A Systematic Synopsis*. 2nd ed. San Juan: Editorial de la Universidad de Puerto Rico.

Liu, Hui Qing, and Alfredo Huete. 1995. "Feedback Based Modification of the NDVI to Minimize Canopy Background and Atmospheric Noise." *IEEE Transactions on Geoscience and Remote Sensing* 33 (2): 457–65. https://doi.org/10.1109/36.377946.

Liu, Yajuan J., Sally Whelen, and Benjamin D. Hall. 1999. "Phylogenetic Relationships among Ascomycetes: Evidence from an RNA Polymerse II Subunit." *Molecular Biology and Evolution* 16 (12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092.

Lohse, Konrad. 2009. "Can MtDNA Barcodes Be Used to Delimit Species? A Response to Pons et al. (2006)." *Systematic Biology* 58 (4): 439–42. https://doi.org/10.1093/sysbio/syp039.

Losos, Jonathan B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of*

Anoles. 1st ed. Berkeley: University of California Press..

Losos, Jonathan B., Todd R. Jackman, Allan Larson, Kevin De Queiroz, and Lourdes Rodríguez-Schettino. 1998. "Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards." *Science* 279 (5359): 2115–18. https://doi.org/10.1126/science.279.5359.2115.

Losos, Jonathan B., and D. Luke Mahler. 2010. "Adaptive Radiation: The Interaction of Ecological Opportunity, Adaptation, and Speciation." In *Evolution since Darwin: The First 150 Years*, edited by Walter Eanes, Jeffrey Levinton, and Douglas J. Futuyma, 1st ed., 381–420. Sunderland: Sinauer.

Losos, Jonathan B., and Robert E. Ricklefs. 2009. "Adaptation and Diversification on Islands." *Nature* 457 (7231): 830–36. https://doi.org/10.1038/nature07893.

Lücking, Robert. 2003. "Takhtajan's Floristic Regions and Foliicolous Lichen Biogeography: A Compatibility Analysis." *Lichenologist* 35 (1): 33–54. https://doi.org/10.1006/lich.2002.0430.

Lücking, Robert, Manuela Dal-Forno, Masoumeh Sikaroodi, Patrick M. Gillevet, Frank Bungartz, Bibiana Moncada, A. Yanez-Ayabaca, José Luis Chaves, Luis Fernando Coca, and James D. Lawrey. 2014. "A Single Macrolichen Constitutes Hundreds of Unrecognized Species." *Proceedings of the National Academy of Sciences* 111 (30): 11091–96. https://doi.org/10.1073/pnas.1403517111.

Lücking, Robert, Manuela Dal Forno, Bibiana Moncada, Luis Fernando Coca, Leidy Yasmín Vargas-Mendoza, André Aptroot, Laura Juliana Arias, et al. 2017. "Turbo-Taxonomy to Assemble a Megadiverse Lichen Genus: Seventy New Species of *Cora* (Basidiomycota: Agaricales: Hygrophoraceae), Honouring David Leslie Hawksworth's Seventieth Birthday." *Fungal Diversity* 84 (1): 139–207. https://doi.org/10.1007/s13225-016-0374-9.

Lücking, Robert, Brendan P. Hodkinson, and Steven D. Leavitt. 2017. "The 2016 Classification of Lichenized Fungi in the Ascomycota and Basidiomycota-Approaching One Thousand Genera." *Bryologist* 119 (4): 361–416. https://doi.org/10.1639/0007-2745-119.4.361.

Lücking, Robert, Bibiana Moncada, Harrie Sipman, Priscylla Bezerra Sobreira, Carlos Viñas, Jorge Gutíerrez, and Timothy Flynn. 2020. "*Saxiloba*: A New Genus of Placodioid Lichens from the Caribbean and Hawaii Shakes up the Porinaceae Tree (Lichenized Ascomycota: Gyalectales)." *Plant and Fungal Systematics* 65 (2): 577–85. https://doi.org/10.35535/pfsyst-2020-0031.

Lücking, Robert, Bibiana Moncada, and Clifford W. Smith. 2017. "The Genus *Lobariella* (Ascomycota: Lobariaceae) in Hawaii: Late Colonization, High Inferred Endemism and Three New Species Resulting from Micro-Radiation." *Lichenologist* 49 (6): 673–91. https://doi.org/10.1017/S0024282917000470.

Lücking, Robert, Eimy Rivas-Plata, Jose Luis Chaves, Loengrin Umaña, and Harrie J M Sipman. 2009. "How Many Tropical Lichens Are There... Really?" *Bibliotheca Lichenologica* 100: 399–418. Berlin - Stuttgart: J. Cramer. https://doi.org/10.3917/ctf.042.0285.

Lücking, Robert, Frederick Seavey, Ralph S Common, Sean Q Beeching, Othmar Breuss, William R Buck, Lee Crane, et al. 2011. "The Lichens of Fakahatchee Strand Preserve State Park, Florida: Proceedings from the 18th Tuckerman Workshop." *Bulletin of the Florida*

Museum of Natural History. Vol. 49. Gainesville: University of Florida.

Lücking, Robert, Jose Luis Villaseñor, María De Los Ángeles Herrera-Campos, Rosa Emilia Pérez-Pérez, Robert S. Egan, Theodore L. Esslinger, and Thomas H. Nash III. 2016. "Phylogenetic Structure of Metacommunities in Mexican Parmeliaceae (Lichenized Asomycota: Lecanorales)." *Bibliotheca Lichenologica* 110: 27–54.

Luebert, Federico, and Maximilian Weigend. 2014. "Phylogenetic Insights into Andean Plant Diversification." *Frontiers in Ecology and Evolution* 2 (Article 27): 1–17. https://doi.org/10.3389/fevo.2014.00027.

Lugo, Ariel E., Leopoldo Miranda-Castro, Abel Vale, Tania del Mar López, Enrique Hernández-Prieto, Andres García-Martinó, Alberto R. Puente-Rolón, et al. 2001. "Puerto Rican Karst-a Vital Resource." *General Technical Report WO-65*. Washington, DC. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C14&q=puerto+rican+karst+a+vital+resource&btnG=.

Lugo, Ariel E, Tania del Mar López, and Olga Ramos-González. 2004. "Urbanización de Los Terrenos En La Periferia de El Yunque." *General Technical Report WO-66*. Washington, DC. https://www.researchgate.net/profile/Tania_Lopez-Marrero/publication/316635565_Urbanizacion_de_los_terrenos_en_la_Periferia_de_El_Yunque/links/5908e0aa458515ebb49547e4/Urbanizacion-de-los-terrenos-en-la-Periferia-de-El-Yunque.pdf.

Lumbsch, H. Thorsten. 2002. "Analysis of Phenolic Products in Lichens for Identification and Taxonomy." In *Protocols in Lichenology*, edited by I. Kraner, R. P. Beckett, and A. K. Varma, 1st ed., 281–95. Berlin: Springer. https://doi.org/10.1007/978-3-642-56359-1.

Lumbsch, H. Thorsten, Ekaphan Kraichak, Sittiporn Parnmen, Eimy Rivas Plata, André Aptroot, Marcela E.S. Cáceres, Damien Ertz, et al. 2014. "New Higher Taxa in the Lichen Family Graphidaceae (Lichenized Ascomycota: Ostropales) Based on a Three-Gene Skeleton Phylogeny." *Phytotaxa* 189 (1): 039–051. https://doi.org/10.11646/phytotaxa.189.1.5.

Lumbsch, H. Thorsten, and Steven D. Leavitt. 2011. "Goodbye Morphology? A Paradigm Shift in the Delimitation of Species in Lichenized Fungi." *Fungal Diversity* 50 (1): 59–72. https://doi.org/10.1007/s13225-011-0123-z.

———. 2019. "Introduction of Subfamily Names for Four Clades in Cladoniaceae and Peltigeraceae (Lecanoromycetes)." *Mycotaxon* 134 (2): 271–73. https://doi.org/10.5248/134.271.

Luo, Arong, Cheng Ling, Simon Y.W. Ho, and Chao Dong Zhu. 2018. "Comparison of Methods for Molecular Species Delimitation across a Range of Speciation Scenarios." *Systematic Biology* 67 (5): 830–46. https://doi.org/10.1093/sysbio/syy011.

MacArthur, Robert H., and Edward O. Wilson. 2001. *The Theory of Island Biogeography*. *The Theory of Island Biogeography (MPB-1)*. Princeton, NJ.: Princeton University Press.

Mace, Georgina M. 2004. "The Role of Taxonomy in Species Conservation." *Philosophical Transactions of the Royal Society B: Biological Sciences* 359 (1444): 711–19. https://doi.org/10.1098/rstb.2003.1454.

MacPhee, RDE., and Manuel A. Iturralde-Vinent. 1995. "Origin of the Greater Antillean Land

Mammal Fauna, 1: New Tertiary Fossils from Cuba and Puerto Rico." *American Museum Novitates* 3141: 1–31.

Madriñán, Santiago, Andrés J. Cortés, and James E. Richardson. 2013. "Páramo Is the World's Fastest Evolving and Coolest Biodiversity Hotspot." *Frontiers in Genetics* 4: 1–7. https://doi.org/10.3389/fgene.2013.00192.

Magain, Nicolas, and Emmanuël Sérusiaux. 2015. "Dismantling the Treasured Flagship Lichen *Sticta fuliginosa* (Peltigerales) into Four Species in Western Europe." *Mycological Progress* 14: 97. https://doi.org/10.1007/s11557-015-1109-0.

Mahler, D. Luke, Liam J. Revell, Richard E. Glor, and Jonathan B. Losos. 2010. "Ecological Opportunity and the Rate of Morphological Evolution in the Diversification of Greater Antillean Anoles." *Evolution* 64 (9): 2731–45. https://doi.org/10.1111/j.1558-5646.2010.01026.x.

Malinsky, Milan, Emiliano Trucchi, Daniel John Lawson, and Daniel Falush. 2018. "RADpainter and FineRADstructure: Population Inference from RADseq Data." *Molecular Biology and Evolution* 35 (5): 1284–90. https://doi.org/10.1093/molbev/msy023.

Mammola, Stefano, Nicoletta Riccardi, Vincent Prié, Ricardo Correia, Pedro Cardoso, Manuel Lopes-Lima, and Ronaldo Sousa. 2020. "Towards a Taxonomically Unbiased European Union Biodiversity Strategy for 2030." *Proceedings of the Royal Society B: Biological Sciences* 287 (1940): 20202166. https://doi.org/10.1098/rspb.2020.2166.

Mann, Michael E., and Kerry A. Emanuel. 2006. "Atlantic Hurricane Trends Linked to Climate Change." *Eos, Transactions, American Geophysical Union* 87 (24): 233–41. https://doi.org/10.1029/2006EO240001.

Marivaux, Laurent, Jorge Vélez-Juarbe, Gilles Merzeraud, François Pujos, Lázaro W. Viñola López, Myriam Boivin, Hernán Santos-Mercado, et al. 2020. "Early Oligocene Chinchilloid Caviomorphs from Puerto Rico and the Initial Rodent Colonization of the West Indies." *Proceedings of the National Academy of Sciences of the United States of America Series B* 287 (1920): 20192806. https://doi.org/10.1098/rspb.2019.2806.

Matos-Maraví, Pável, Rayner Núñez Águila, Carlos Peña, Jacqueline Y. Miller, Andrei Sourakov, and Niklas Wahlberg. 2014. "Causes of Endemic Radiation in the Caribbean: Evidence from the Historical Biogeography and Diversification of the Butterfly Genus *Calisto* (Nymphalidae: Satyrinae: Satyrini)." *BMC Evolutionary Biology* 14 (199): 1–18. https://doi.org/10.1186/s12862-014-0199-7.

Matzke, Nicholas J. 2014. "Model Selection in Historical Biogeography Reveals That Founder-Event Speciation Is a Crucial Process in Island Clades." *Systematic Biology* 63 (6): 951–70. https://doi.org/10.1093/sysbio/syu056.

Maunder, Michael, Melissa Abdo, Rosalina Berazain, Colin Clubbe, Francisco Jiménez, Ángela Leiva, Eugenio Santiago-Valentín, Brett Jestrow, and Javier Francisco-Ortega. 2011. "The Plants of the Caribbean Islands: A Review of the Biogeography, Diversity and Conservation of a Storm-Battered Biodiversity Hotspot." In *The Biology of Island Floras*, edited by David Bramwell and Juli Caujapé-Castells, 154–78. New York: Cambridge University Press. https://doi.org/10.1017/CBO9780511844270.007.

McCune, Bruce, Roger Rosentreter, Toby Spribille, Othmar Breuss, and Tim Wheeler. 2014.

"Montana Lichens: An Annotated List." *Monographs in North American Lichenology* 2: 1–183.

McHugh, Anne, Carol Yablonsky, Greta Binford, and Ingi Agnarsson. 2014. "Molecular Phylogenetics of Caribbean Micrathena (Araneae:Araneidae) Suggests Multiple Colonisation Events and Single Island Endemism." *Invertebrate Systematics* 28 (4): 337–49. https://doi.org/10.1071/IS13051.

Meegaskumbura, H., F. Bossuyt, R. Pethiyagoda, K. Manamendra-Arachchi, M. Bahir, M. C. Milinkovitch, and C. J. Schneider. 2002. "Sri Lanka: An Amphibian Hot Spot." *Science* 298 (5592): 379. https://doi.org/10.1126/science.298.5592.379.

Mercado-Díaz, Joel A., William A. Gould, Grizelle González, and Robert Lücking. 2015. "Lichens in Puerto Rico: An Ecosystem Approach." *Gen. Tech. Rep. IITF-GTR-46.* https://data.fs.usda.gov/research/pubs/iitf/IITF_GTR_46.pdf.

Mercado-Díaz, Joel A., Robert Lücking, Bibiana Moncada, Todd J. Widhelm, and H. Thorsten Lumbsch. 2020. "Elucidating Species Richness in Lichen Fungi: The Genus *Sticta* (Ascomycota: Peltigeraceae) in Puerto Rico." *Taxon* 69 (5): 851–91. https://doi.org/10.1002/tax.12320.

Mercado-Díaz, Joel A., Robert Lücking, and Sittiporn Parnmen. 2014. "Two New Genera and Twelve New Species of Graphidaceae from Puerto Rico: A Case for Higher Endemism of Lichenized Fungi in Islands of the Caribbean?" *Phytotaxa* 189 (1): 186–203. https://doi.org/10.11646/phytotaxa.189.1.14.

Mercado-Díaz, Joel A., and Eugenio Santiago-Valentín. 2010. "Lichenological Studies in Puerto Rico: History and Current Status." *Harvard Papers in Botany* 15 (1): 93–101. https://doi.org/10.3100/025.015.0124.

Miadlikowska, Jolanta, Frank Kauff, Valérie Hofstetter, Emily Fraker, Martin Grube, Josef Hafellner, Valérie Reeb, et al. 2006. "New Insights into Classification and Evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from Phylogenetic Analyses of Three Ribosomal RNA- and Two Protein-Coding Genes." *Mycologia* 98 (6): 1088–1103. https://doi.org/10.1080/15572536.2006.11832636.

Michelangeli, F. A., W. S. Judd, D. S. Penneys, J. D. Skean, E. R. Bécquer-Granados, R. Goldenberg, and C. V. Martin. 2008. "Multiple Events of Dispersal and Radiation of the Tribe Miconieae (Melastomataceae) in the Caribbean." *The Botanical Review* 74 (1): 53–77. https://doi.org/10.1007/s12229-008-9004-x.

Miller, Joshua M., Catherine I. Cullingham, and Rhiannon M. Peery. 2020. "The Influence of a Priori Grouping on Inference of Genetic Clusters: Simulation Study and Literature Review of the DAPC Method." *Heredity* 125 (5): 269–80. https://doi.org/10.1038/s41437-020-0348-2.

Miller, Mark A., Wayne Pfeiffer, and Terri Schwartz. 2010. "Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees." In *2010 Gateway Computing Environments Workshop (GCE 2010)*, 1–8. New Orleans. https://doi.org/10.1109/GCE.2010.5676129.

Minh, Bui Quang, Heiko A. Schmidt, Olga Chernomor, Dominik Schrempf, Michael D. Woodhams, Arndt Von Haeseler, Robert Lanfear, and Emma Teeling. 2020. "IQ-TREE 2:

New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era." *Molecular Biology and Evolution* 37 (5): 1530–34. https://doi.org/10.1093/molbev/msaa015.

Mitchell, R. C. 1954. "A Survey of the Geology of Puerto Rico." Agricultural Experimental Station, Río Piedras.

Mittelbach, Gary G., and Brian J. McGill. 2019. *Community Ecology*. 2nd ed. New York: Oxford University Press. https://doi.org/10.1093/oso/9780198835851.001.0001.

Moncada, Bibiana. 2012. "El Género *Sticta* (Schreb .) Ach . En Colombia : Taxonomía , Ecogeografía e Importancia." Universidad Nacional de Colombia. http://www.bdigital.unal.edu.co/11296/1/190844.2012.pdf.

Moncada, Bibiana, Luis Fernando Coca, and Robert Lücking. 2013. "Neotropical Members of *Sticta* (Lichenized Ascomycota: Lobariaceae) Forming Photosymbiodemes, with the Description of Seven New Species." *Bryologist* 116 (2): 169–200. https://doi.org/10.1639/0007-2745-116.2.169.

Moncada, Bibiana, and Robert Lücking. 2012. "Ten New Species of *Sticta* and Counting: Colombia as a Hot Spot for Unrecognized Diversification in a Conspicuous Macrolichen Genus." *Phytotaxa* 74: 1–29. https://doi.org/http://dx.doi.org/10.11646/phytotaxa.74.1.1.

Moncada, Bibiana, Robert Lücking, and Luisa Betancourt-Macuase. 2013. "Phylogeny of the Lobariaceae (Lichenized Ascomycota: Peltigerales), with a Reappraisal of the Genus *Lobariella*." *Lichenologist* 45 (2): 203–63. https://doi.org/10.1017/S0024282912000825.

Moncada, Bibiana, Robert Lücking, and H. Thorsten Lumbsch. 2020. "Rewriting the Evolutionary History of the Lichen Genus *Sticta* (Ascomycota: Peltigeraceae Subfam. Lobarioideae) in the Hawaiian Islands." *Plant and Fungal Systematics* 65 (1): 95–119. https://doi.org/10.35535/pfsyst-2020-0005.

Moncada, Bibiana, Robert Lücking, and Alejandra Suárez. 2013. "Molecular Phylogeny of the Genus *Sticta* (Lichenized Ascomycota: Lobariaceae) in Colombia." *Fungal Diversity* 64 (1): 205–31. https://doi.org/10.1007/s13225-013-0230-0.

Moncada, Bibiana, Joel A. Mercado-Díaz, and Robert Lücking. 2018. "The Identity of *Sticta damicornis* (Ascomycota: Lobariaceae): A Presumably Widespread Taxon Is a Caribbean Endemic." *Lichenologist* 50 (5): 591–97. https://doi.org/10.1017/S0024282918000373.

Moncada, Bibiana, Joel A. Mercado-Díaz, Nicolas Magain, Brendan P. Hodkinson, Clifford W. Smith, Frank Bungartz, Rosa Emilia Pérez-Pérez, et al. 2021. "Phylogenetic Diversity of Two Geographically Overlapping Lichens: Isolation by Distance, Environment, or Fragmentation?" *Journal of Biogeography* 48 (3): 676–89. https://doi.org/10.1111/jbi.14033.

Moncada, Bibiana, Joel A. Mercado-Díaz, Clifford W. Smith, Frank Bungartz, Emmanuël Sérusiaux, H. Thorsten Lumbsch, and Robert Lücking. 2021. "Two New Common, Previously Unrecognized Species in the *Sticta weigelii* Morphodeme (Ascomycota: Peltigeraceae)." *Willdenowia* 51 (1): 35–45. https://doi.org/10.3372/wi.51.51103.

Moncada, Bibiana, Brendon Reidy, and Robert Lücking. 2014. "A Phylogenetic Revision of Hawaiian *Pseudocyphellaria* Sensu Lato (Lichenized Ascomycota: Lobariaceae) Reveals

Eight New Species and a High Degree of Inferred Endemism." *Bryologist* 117 (2): 119–60. https://doi.org/10.1639/0007-2745-117.2.119.

Montes, C., A. Cardona, C. Jaramillo, A. Pardo, J. C. Silva, V. Valencia, C. Ayala, et al. 2015. "Middle Miocene Closure of the Central American Seaway." *Science* 348 (6231): 226–29. https://doi.org/10.1126/science.aaa2815.

Morden, C. W., and W. Loeffler. 1999. "Fragmentation and Genetic Differentiation among Subpopulations of the Endangered Hawaiian Mint *Haplostachys haplostachya* (Lamiaceae)." *Molecular Ecology* 8 (4): 617–25. https://doi.org/10.1046/j.1365-294X.1999.00601.x.

Müller Argoviensis, J. 1888. "Lichenes Portoricenses Ab Egregio Sintenis Lectis, in Hujus Collect. Exs. Sub Citatis Numeris Editi, et a Cl. Dr. Urban Communicati, Adjunctis Nonnullis a Barone Egger in St. Domingo Lectis, Quos Determinavit Dr. J. Müller." *Flora* 71: 490–496.

Myers, Norman, Russell A. Mittermeler, Cristina G. Mittermeler, Gustavo A.B. Da Fonseca, and Jennifer Kent. 2000. "Biodiversity Hotspots for Conservation Priorities." *Nature* 403: 853–58. https://doi.org/10.1038/35002501.

Myllys, Leena, Soili Stenroos, Arne Thell, and Teuvo Ahti. 2003. "Phylogeny of Bipolar *Cladonia arbuscula* and *Cladonia mitis* (Lecanorales, Euascomycetes)." *Molecular Phylogenetics and Evolution* 27 (1): 58–69. https://doi.org/10.1016/S1055-7903(02)00398-6.

Narum, Shawn R., C. Alex Buerkle, John W. Davey, Michael R. Miller, and Paul A. Hohenlohe. 2013. "Genotyping-by-Sequencing in Ecological and Conservation Genomics." *Molecular Ecology* 22 (11): 2841–47. https://doi.org/10.1111/mec.12350.

Nascimento, Edvaneide Leandro de Lima, Leonor Costa Maia, Marcela E. S. Cáceres, and Robert Lücking. 2021. "Phylogenetic Structure of Lichen Metacommunities in Amazonian and Northeast Brazil." *Ecological Research* 36 (3): 440–63. https://doi.org/10.1111/1440-1703.12206.

Nei, Masatoshi, Takeo Maruyama, and Ranajit Chakraborty. 1975. "The Bottleneck Effect and Genetic Variability in Populations." *Evolution* 29 (1): 1. https://doi.org/10.2307/2407137.

Nelsen, Matthew P., Robert Lücking, Carrie J. Andrew, André Aptroot, Marcela E.S. Cáceres, Joel A. Mercado-Díaz, Eimy Rivas Plata, and H. Thorsten Lumbsch. 2014. "Molecular Phylogeny Reveals the True Colours of Myeloconidaceae (Ascomycota: Ostropales)." *Australian Systematic Botany* 27 (1): 38–47. https://doi.org/10.1071/SB13040.

Nelsen, Matthew P., Robert Lücking, André Aptroot, Carrie J. Andrew, Marcela E. S. Cáceres, Eimy Rivas Plata, Cécile Gueidan, et al. 2014. "Elucidating Phylogenetic Relationships and Genus-Level Classification within the Fungal Family Trypetheliaceae (Ascomycota: Dothideomycetes)." *Taxon* 63 (5): 974–92. https://doi.org/10.3978/j.issn.2078-6891.2015.008.

Nieto-Blázquez, María Esther, Alexandre Antonelli, and Julissa Roncal. 2017. "Historical Biogeography of Endemic Seed Plant Genera in the Caribbean: Did GAARlandia Play a Role?" *Ecology and Evolution* 7 (23): 10158–74. https://doi.org/10.1002/ece3.3521.

Nieto-Blázquez, María Esther, Lourdes Peña-Castillo, and Julissa Roncal. 2020. "Historical Biogeography of Caribbean *Podocarpus* Does Not Support the Progression Rule." *Journal of Biogeography* 48 (3): 690–702. https://doi.org/10.1111/jbi.14034.

Nupoor, Prasad, Semwal Manoj, and Roy Partha Sarathi. 2015. "Remote Sensing and GIS for Biodiversity Conservation." In *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection, Volume 1*, edited by Dalip Kumar Uptreti, Pradeep K. Divakar, Vertika Shukla, and Rajesh Bajpai, 151–80. New Delhi: Springer. https://doi.org/10.1007/978-81-322-2181-4_7.

Nylander, W. 1869. "Énumération Des Lichens Récoltés Par H. Husnot Aux Antilles Françaises." *Bull. Soc. Linn. Normandie* sér. 2 (3): 259–89.

Oksanen, Author Jari, F. Guillaume Blanchet, Micheal Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, R. B. Minchin, et al. 2019. "Vegan: Community Ecology Package." *R package version 2.5-6.* https://cran.r-project.org/package=vegan.

Olson, D. M., E. Dinerstein, E. D. Wikramanayake, N. D. Burgess, G. V. N. Powell, E. C. Underwood, J. A. D'Amico, et al. 2001. "Terrestrial Ecoregions of the World: A New Map of Life on Earth." *BioScience* 51 (11): 933–38. https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2.

Onuţ-Brännström, Ioana, Leif Tibell, and Hanna Johannesson. 2017. "A Worldwide Phylogeography of the Whiteworm Lichens *Thamnolia* Reveals Three Lineages with Distinct Habitats and Evolutionary Histories." *Ecology and Evolution* 7 (10): 3602–15. https://doi.org/10.1002/ece3.2917.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2014. "Caper: Comparative Analyses of Phylogenetics and Evolution in R." *R package version 0.5.2/ R121*. http://r-forge.r-project.org/projects/caper/.

Otálora, Mónica A.G., Isabel Martínez, Gregorio Aragón, and M. Carmen Molina. 2010. "Phylogeography and Divergence Date Estimates of a Lichen Species Complex with a Disjunct Distribution Pattern." *American Journal of Botany* 97 (2): 216–23. https://doi.org/10.3732/ajb.0900064.

Padial, José M., and Ignacio De La Riva. 2010. "A Response to Recent Proposals for Integrative Taxonomy." *Biological Journal of the Linnean Society* 101 (3): 747–56. https://doi.org/10.1111/j.1095-8312.2010.01528.x.

Parnmen, Sittiporn, Achariya Rangsiruji, Pachara Mongkolsuk, Kansri Boonpragob, Aparna Nutakki, and H. Thorsten Lumbsch. 2012. "Using Phylogenetic and Coalescent Methods to Understand the Species Diversity in the *Cladia aggregata* Complex (Ascomycota, Lecanorales)." *PLoS ONE* 7 (12): e52245. https://doi.org/10.1371/journal.pone.0052245.

Patiño, Jairo, and Alain Vanderpoorten. 2015. "Macaronesia Is a Departure Gate of Anagenetic Speciation in the Moss Genus *Rhynchostegiella*." *Journal of Biogeography* 42 (11): 2122–30. https://doi.org/10.1111/jbi.12583.

Pax, Denise L., Robert A. Price, and Helen J. Michaels. 1997. "Phylogenetic Position of the Hawaiian Geraniums Based on RbcL Sequences." *American Journal of Botany* 84 (1): 72–78. https://doi.org/10.2307/2445884.

Peixoto, Franciele P., Pedro Henrique P. Braga, Marcus Vinicius Cianciaruso, José Alexandre F. Diniz-Filho, and Daniel Brito. 2014. "Global Patterns of Phylogenetic Beta Diversity Components in Bats." *Journal of Biogeography* 41 (4): 762–72. https://doi.org/10.1111/jbi.12241.

Peres-Neto, Pedro R., and Donald A. Jackson. 2001. "How Well Do Multivariate Data Sets Match? The Advantages of a Procrustean Superimposition Approach over the Mantel Test." *Oecologia* 129 (2): 169–78. https://doi.org/10.1007/s004420100720.

Perlmutter, Gary B., Eimy Rivas Plata, and Robert Lücking. 2018. "Is *Stirtonia alba* in North America? Resolving a Nomenclatural Impasse and Assessing the Taxonomic Status of the Arthonia Alba Complex." *Bryologist* 121 (1): 80–86. https://doi.org/10.1639/0007-2745-121.1.080.

Perret, Mathieu, Alain Chautems, Andréa Onofre De Araujo, and Nicolas Salamin. 2013. "Temporal and Spatial Origin of Gesneriaceae in the New World Inferred from Plastid DNA Sequences." *Botanical Journal of the Linnean Society* 171 (1): 61–79. https://doi.org/10.1111/j.1095-8339.2012.01303.x.

Philippon, Mélody, Jean Jacques Cornée, Philippe Münch, Douwe J. J. Van Hinsbergen, Marcelle BouDagher-Fadel, Lydie Gailler, Lydian M. Boschman, et al. 2020. "Eocene Intra-Plate Shortening Responsible for the Rise of a Faunal Pathway in the Northeastern Caribbean Realm." *PLoS ONE* 15 (10): e0241000. https://doi.org/10.1371/journal.pone.0241000.

Pillar, Valério D., and Leandro D. S. Duarte. 2010. "A Framework for Metacommunity Analysis of Phylogenetic Structure." *Ecology Letters* 13 (5): 587–96. https://doi.org/10.1111/j.1461-0248.2010.01456.x.

Pino-Bodas, Raquel, Teuvo Ahti, Soili Stenroos, María P. Martín, and Ana Rosa Burgaz. 2012. "*Cladonia conista* and *C. humilis* (Cladoniaceae) Are Different Species." *Bibliotheca Lichenologica* 108: 161–76. https://www.researchgate.net/profile/Soili_Stenroos/publication/259219025_Cladonia_conista_and_C_humilis_Cladoniaceae_are_different_species/links/00b7d52cd4fd579919000000.pdf.

Pino-Bodas, Raquel, Ana Rosa Burgaz, María P. Martín, and H. Thorsten Lumbsch. 2011. "Phenotypical Plasticity and Homoplasy Complicate Species Delimitation in the *Cladonia gracilis* Group (Cladoniaceae, Ascomycota)." *Organisms Diversity and Evolution* 11 (5): 343–55. https://doi.org/10.1007/s13127-011-0062-2.

Pino-Bodas, Raquel, María P. Martín, and Ana R. Burgaz. 2012. "*Cladonia subturgida* and *C. iberica* (Cladoniaceae) Form a Single, Morphologically and Chemically Polymorphic Species." *Mycological Progress* 11 (1): 269–78. https://doi.org/10.1007/s11557-011-0746-1.

Pino-Bodas, Raquel, María P. Martín, Ana R. Burgaz, and H. Thorsten Lumbsch. 2013. "Species Delimitation in *Cladonia* (Ascomycota): A Challenge to the DNA Barcoding Philosophy." *Molecular Ecology Resources* 13 (6): 1058–68. https://doi.org/10.1111/1755-0998.12086.

Pons, Joan, Timothy G. Barraclough, Jesus Gomez-Zurita, Anabela Cardoso, Daniel P. Duran, Steaphan Hazell, Sophien Kamoun, William D. Sumlin, and Alfried P. Vogler. 2006.

"Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects." *Systematic Biology* 55 (4): 595–609. https://doi.org/10.1080/10635150600852011.

Printzen, Christian. 2010. "Lichen Systematics: The Role of Morphological and Molecular Data to Reconstruct Phylogenetic Relationships." In *Progress in Botany 71*, edited by Ulrich Lüttge, Wolfram Beyschlag, Burkard Büdel, and Dennis Francis, 233–75. Berlin: Springer. https://doi.org/10.1007/978-3-642-02167-1_10.

Printzen, Christian, and Stefan Ekman. 2003. "Local Population Subdivision in the Lichen *Cladonia subcervicornis* as Revealed by Mitochondrial Cytochrome Oxidase Subunit 1 Intron Sequences." *Mycologia* 95 (3): 399–406. https://doi.org/10.1080/15572536.2004.11833084.

Puechmaille, Sebastien J. 2016. "The Program Structure Does Not Reliably Recover the Correct Population Structure When Sampling Is Uneven: Subsampling and New Estimators Alleviate the Problem." *Molecular Ecology Resources* 16 (3): 608–27. https://doi.org/10.1111/1755-0998.12512.

Rabosky, Daniel L. 2014. "Automatic Detection of Key Innovations, Rate Shifts, and Diversity-Dependence on Phylogenetic Trees." *PLoS ONE* 9 (2): e89543. https://doi.org/10.1371/journal.pone.0089543.

Rabosky, Daniel L., and Emma E. Goldberg. 2015. "Model Inadequacy and Mistaken Inferences of Trait-Dependent Speciation." *Systematic Biology* 64 (2): 340–55. https://doi.org/10.1093/sysbio/syu131.

Rabosky, Daniel L., Michael Grundler, Carlos Anderson, Pascal Title, Jeff J. Shi, Joseph W. Brown, Huateng Huang, and Joanna G. Larson. 2014. "BAMMtools: An R Package for the Analysis of Evolutionary Dynamics on Phylogenetic Trees." *Methods in Ecology and Evolution* 5 (7): 701–7. https://doi.org/10.1111/2041-210X.12199.

Rambaut, Andrew. 2012. "FigTree v.1.4.0." Edingburgh: University of Edinburgh, Institute of Evolutionary Biology. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, Andrew, and Alexei J Drummond. 2009. "Tracer v.1.5." http://beast.bio.ed.ac.uk/Tracer.

———. 2013a. "LogCombiner v.1.8.2." https://beast.community/logcombiner.

———. 2013b. "TreeAnnotator, v.1.8.0." https://beast.community/treeannotator.

Ranft, Hannah, Bibiana Moncada, Peter J. De Lange, H. Thorsten Lumbsch, and Robert Lücking. 2018. "The *Sticta filix* Morphodeme (Ascomycota: Lobariaceae) in New Zealand with the Newly Recognized Species *S. dendroides* and *S. menziesii*: Indicators of Forest Health in a Threatened Island Biota?" *Lichenologist* 50 (2): 185–210. https://doi.org/10.1017/S0024282917000706.

Rannala, Bruce, and Ziheng Yang. 2013. "Improved Reversible Jump Algorithms for Bayesian Species Delimitation." *Genetics* 194 (1): 245–53. https://doi.org/10.1534/genetics.112.149039.

———. 2017. "Efficient Bayesian Species Tree Inference under the Multispecies Coalescent." *Systematic Biology* 66 (5): 823–42. https://doi.org/10.1093/sysbio/syw119.

Ree, Richard H., and Isabel Sanmartín. 2018. "Conceptual and Statistical Problems with the DEC+J Model of Founder-Event Speciation and Its Comparison with DEC via Model Selection." *Journal of Biogeography* 45 (4): 741–49. https://doi.org/10.1111/jbi.13173.

Ree, Richard H., and Stephen A. Smith. 2008. "Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis." *Systematic Biology* 57 (1): 4–14. https://doi.org/10.1080/10635150701883881.

Regalado, Ledis, Josmaily Lóriga, Julia Bechteler, Andreas Beck, Harald Schneider, and Jochen Heinrichs. 2018. "Phylogenetic Biogeography Reveals the Timing and Source Areas of the *Adiantum* Species (Pteridaceae) in the West Indies, with a Special Focus on Cuba." *Journal of Biogeography* 45 (3): 541–51. https://doi.org/10.1111/jbi.13119.

Rehner, Stephen A. 2001. "Primers for Elongation Factor 1-Alpha (EF1-Alpha)." http://www.aftol.org/pdfs/EF1primer.pdf.

Revell, Liam J. 2012. "Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things)." *Methods in Ecology and Evolution* 3 (2): 217–23. https://doi.org/10.1111/j.2041-210X.2011.00169.x.

Reynolds, R. Graham, Matthew L. Niemiller, S. Blair Hedges, Alex Dornburg, Alberto R. Puente-Rolón, and Liam J. Revell. 2013. "Molecular Phylogeny and Historical Biogeography of West Indian Boid Snakes (*Chilabothrus*)." *Molecular Phylogenetics and Evolution* 68 (3): 461–70. https://doi.org/10.1016/j.ympev.2013.04.029.

Ricart-Pujals, Juan L. R., and Rubén Padrón-Vélez. 2010. *Sinópsis Anotada y Comentada de La Flora Del Bosque Estatal de Maricao*. San Juan, PR: United States Forest Service, USDA.

Ricklefs, Robert E. 2009. "Dynamics of Colonization and Extinctions on Islands." In *The Theory of Island Biogeography Revisited*, edited by Jonathan B. Losos and Robert E. Ricklefs, 1st ed., 388–414. New Jersey: Princeton University Press. https://doi.org/https://doi.org/10.1515/9781400831920.388.

Ricklefs, Robert E., and Eldredge Bermingham. 2008. "The West Indies as a Laboratory of Biogeography and Evolution." *Philosophical Transactions of the Royal Society B: Biological Sciences* 363 (1502): 2393–2413. https://doi.org/10.1098/rstb.2007.2068.

Ricklefs, Robert E., and Irby J. Lovette. 1999. "The Roles of Island Area per se and Habitat Diversity in the Species-Area Relationships of Four Lesser Antillean Faunal Groups." *Journal of Animal Ecology* 68 (6): 1142–60. https://doi.org/10.1046/j.1365-2656.1999.00358.x.

Riley, Shawn J, Stephen D DeGloria, and Robert Elliot. 1999. "A Terrain Ruggedness Index That Quantifies Topographic Heterogeneity." *Intermountain Journal of Sciences* 5 (1–4): 23–27.

Rittmeyer, Eric N., and Christopher C. Austin. 2012. "The Effects of Sampling on Delimiting Species from Multi-Locus Sequence Data." *Molecular Phylogenetics and Evolution* 65 (2): 451–63. https://doi.org/10.1016/j.ympev.2012.06.031.

Rochette, Nicolas C., Angel G. Rivera-Colón, and Julian M. Catchen. 2019. "Stacks 2: Analytical Methods for Paired-End Sequencing Improve RADseq-Based Population Genomics." *Molecular Ecology* 28 (21): 4737–54. https://doi.org/10.1111/mec.15253.

Rodríguez, Ariel, Miguel Vences, Bruno Nevado, Annie Machordom, and Erik Verheyen. 2010. "Biogeographic Origin and Radiation of Cuban *Eleutherodactylus* Frogs of the Auriculatus Species Group, Inferred from Mitochondrial and Nuclear Gene Sequences." *Molecular Phylogenetics and Evolution* 54 (1): 179–86. https://doi.org/10.1016/j.ympev.2009.08.023.

Roncal, Julissa, María Esther Nieto-Blázquez, Agustín Cardona, and Christine D. Bacon. 2020. "Historical Biogeography of Caribbean Plants Revises Regional Paleogeography." In *Neotropical Diversification: Patterns and Processes*, edited by Rull Valentí and Ana Carolina Carnaval, 521–46. Cham: Springer. https://doi.org/10.1007/978-3-030-31167-4_20.

Ronquist, Fredrik, Maxim Teslenko, Paul Van Der Mark, Daniel L. Ayres, Aaron Darling, Sebastian Höhna, Bret Larget, Liang Liu, Marc A. Suchard, and John P. Huelsenbeck. 2012. "MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space." *Systematic Biology* 61 (3): 539–42. https://doi.org/10.1093/sysbio/sys029.

Rosauer, Dan, Shawn W. Laffan, Michael D. Crisp, Stephen C. Donnellan, and Lyn G. Cook. 2009. "Phylogenetic Endemism: A New Approach for Identifying Geographical Concentrations of Evolutionary History." *Molecular Ecology* 18 (19): 4061–72. https://doi.org/10.1111/j.1365-294X.2009.04311.x.

Rosentreter, Roger, Ann M. DeBolt, and Laurel Kaminsky. 2015. *Field Oriented Keys to the Florida Lichens*. Boise: Florida Museum. https://www.floridamuseum.ufl.edu/herbarium/lib/pdf/Rosentreter_Field_oriented_keys_to_the_Florida_lichens_Dec_2015.pdf (last accessed: 4/7/2021).

Rundell, Rebecca J., and Trevor D. Price. 2009. "Adaptive Radiation, Nonadaptive Radiation, Ecological Speciation and Nonecological Speciation." *Trends in Ecology and Evolution* 24 (7): 394–99. https://doi.org/10.1016/j.tree.2009.02.007.

Ryan, Peter G., Paulette Bloomer, Coleen L. Moloney, Tyron J. Grant, and Wayne Delport. 2007. "Ecological Speciation in South Atlantic Island Finches." *Science* 315 (5817): 1420–23. https://doi.org/10.1126/science.1138829.

Santiago-Valentín, Eugenio, and Richard G. Olmstead. 2003. "Phylogenetics of the Antillean Goetzeoideae (Solanaceae) and Their Relationships within the Solanaceae Based on Chloroplast and ITS DNA Sequence Data." *Systematic Botany* 28 (2): 452–60. https://doi.org/10.1043/0363-6445-28.2.452.

Scantlebury. 2014. "Patterns of Adaptive Radiation in Insular Reptiles and Amphibians." Rochester: University of Rochester.

Schoch, Conrad L, Keith A. Seifert, Sabine Huhndorf, Vincent Robert, John L. Spouge, C. André Levesque, Wen Chen, and Fungal Barcoding Consortium. 2012. "Nuclear Ribosomal Internal Transcribed Spacer (ITS) Region as a Universal DNA Barcode Marker for Fungi." *Proceedings of the National Academy of Sciences* 109 (16): 6241–46. https://doi.org/10.1073/pnas.1117018109.

Sérusiaux, Emmanuël, Juan Carlos Villarreal A., Tim Wheeler, and Bernard Goffinet. 2011. "Recent Origin, Active Speciation and Dispersal for the Lichen Genus *Nephroma* (Peltigerales) in Macaronesia." *Journal of Biogeography* 38 (6): 1138–51. https://doi.org/10.1111/j.1365-2699.2010.02469.x.

Shaw, A. J., C. J. Cox, B. Goffinet, W. R. Buck, and S. B. Boles. 2003. “Phylogenetic Evidence of a Rapid Radiation of Pleurocarpous Mosses (Bryophyta).” *Evolution* 57 (10): 2226–41. https://doi.org/10.1111/j.0014-3820.2003.tb00235.x.

Shringarpure, Suyash, and Eric P. Xing. 2014. “Effects of Sample Selection Bias on the Accuracy of Population Structure and Ancestry Inference.” *G3: Genes, Genomes, Genetics* 4 (5): 901–11. https://doi.org/10.1534/g3.113.007633.

Simões, M., L. Breitkreuz, M. Alvarado, S. Baca, J. C. Cooper, L. Heins, K. Herzog, and B. S. Lieberman. 2016. “The Evolving Theory of Evolutionary Radiations.” *Trends in Ecology and Evolution* 31 (1): 27–34. https://doi.org/10.1016/j.tree.2015.10.007.

Simon, Antoine, Bernard Goffinet, Nicolas Magain, and Emmanuël Sérusiaux. 2018. “High Diversity, High Insular Endemism and Recent Origin in the Lichen Genus *Sticta* (Lichenized Ascomycota, Peltigerales) in Madagascar and the Mascarenes.” *Molecular Phylogenetics and Evolution* 122: 15–28. https://doi.org/10.1016/j.ympev.2018.01.012.

Singh, Garima, Francesco Dal Grande, Pradeep K. Divakar, Jürgen Otte, Steven D. Leavitt, Katarzyna Szczepanska, Ana Crespo, et al. 2015. “Coalescent-Based Species Delimitation Approach Uncovers High Cryptic Diversity in the Cosmopolitan Lichen-Forming Fungal Genus *Protoparmelia* (Lecanorales, Ascomycota).” *PLoS ONE* 10 (5): e0124625. https://doi.org/10.1371/journal.pone.0124625.

Sipman, Harrie J. M., and André Aptroot. 2001. “Where Are the Missing Lichens?” *Mycological Research* 105 (12): 1433–39. https://doi.org/10.1017/S0953756201004932.

Smith, Clifford W. 1993. “Notes on Hawaiian Parmelioid Lichens.” *Bryologist* 96 (3): 326. https://doi.org/10.2307/3243860.

Soto-Medina, Edier, Robert Lücking, Philip A. Silverstone-Sopkin, and Alba Marina Torres. 2019. “Changes in Functional and Taxonomic Diversity and Composition of Corticolous Lichens in an Altitudinal Gradient in Colombia.” *Cryptogamie, Mycologie* 40 (6): 97–115. https://doi.org/10.5252/cryptogamie-mycologie2019v40a6.

Stamatakis, Alexandros. 2014. “RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies.” *Bioinformatics* 30 (9): 1312–13. https://doi.org/10.1093/bioinformatics/btu033.

Stenroos, Soili, Jaakko Hyvönen, Leena Myllys, Arne Thell, and Teuvo Ahti. 2002. “Phylogeny of the Genus *Cladonia* s.Lat. (Cladoniaceae, Ascomycetes) Inferred from Molecular, Morphological, and Chemical Data.” *Cladistics* 18 (3): 237–78. https://doi.org/10.1006/clad.2002.0202.

Stenroos, Soili, Raquel Pino-Bodas, Jaakko Hyvönen, H. Thorsten Lumbsch, and Teuvo Ahti. 2019. “Phylogeny of the Family Cladoniaceae (Lecanoromycetes, Ascomycota) Based on Sequences of Multiple Loci.” *Cladistics* 35 (4): 351–84. https://doi.org/10.1111/cla.12363.

Suchard, Marc A, Philippe Lemey, Guy Baele, Daniel L Ayres, Alexei J Drummond, and Andrew Rambaut. 2018. “Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10.” *Virus Evolution* 4 (1): vey016. https://doi.org/10.1093/ve/vey016.

Sukumaran, Jeet, and L. Lacey Knowles. 2017. “Multispecies Coalescent Delimits Structure, Not Species.” *Proceedings of the National Academy of Sciences* 114 (7): 1607–12.

https://doi.org/10.1073/pnas.1607921114.

Swenson, Nathan G. 2011. “Phylogenetic Beta Diversity Metrics, Trait Evolution and Inferring the Functional Beta Diversity of Communities.” *PLoS ONE* 6 (6): e21264. https://doi.org/10.1371/journal.pone.0021264.

Talavera, Gerard, and Jose Castresana. 2007. “Improvement of Phylogenies after Removing Divergent and Ambiguously Aligned Blocks from Protein Sequence Alignments.” *Systematic Biology* 56: 564–77. https://doi.org/10.1080/10635150701472164.

Talavera, Gerard, Vlad Dincă, and Roger Vila. 2013. “Factors Affecting Species Delimitations with the GMYC Model: Insights from a Butterfly Survey.” *Methods in Ecology and Evolution* 4 (12): 1101–10. https://doi.org/10.1111/2041-210X.12107.

Tan, Ge, Matthieu Muffato, Christian Ledergerber, Javier Herrero, Nick Goldman, Manuel Gil, and Christophe Dessimoz. 2015. “Current Methods for Automated Filtering of Multiple Sequence Alignments Frequently Worsen Single-Gene Phylogenetic Inference.” *Systematic Biology* 64 (5): 778–91. https://doi.org/10.1093/sysbio/syv033.

Tehler, Anders, Martin Irestedt, Frank Bungartz, and Mats Wedin. 2009. “Evolution and Reproduction Modes in the *Roccella galapagoensis* Aggregate (Roccellaceae, Arthoniales).” *Taxon* 58 (2): 438–56. https://doi.org/10.1002/tax.582010.

Thornhill, Andrew H., Brent D. Mishler, Nunzio J. Knerr, Carlos E. González-Orozco, Craig M. Costion, Darren M. Crayn, Shawn W. Laffan, and Joseph T. Miller. 2016. “Continental-Scale Spatial Phylogenetics of Australian Angiosperms Provides Insights into Ecology, Evolution and Conservation.” *Journal of Biogeography* 43 (11): 2085–98. https://doi.org/10.1111/jbi.12797.

Wagner, Catherine E., Irene Keller, Samuel Wittwer, Oliver M. Selz, Salome Mwaiko, Lucie Greuter, Arjun Sivasundar, and Ole Seehausen. 2013. “Genome-Wide RAD Sequence Data Provide Unprecedented Resolution of Species Boundaries and Relationships in the Lake Victoria Cichlid Adaptive Radiation.” *Molecular Ecology* 22 (3): 787–98. https://doi.org/10.1111/mec.12023.

Wallace, Alfred Russel. 1876. *Geographical Distribution of Animals : With a Study of the Relations of Living and Extinct Faunas as Elucidating the Past Changes of the Earth’s Surface. Geographical Distribution of Animals : With a Study of the Relations of Living and Extinct Faunas as Elucidating the Past Changes of the Earth’s Surface.* London: McMillan. https://doi.org/10.5962/bhl.title.46581.

Wang, Jianjun, Ji Shen, Yucheng Wu, Chen Tu, Janne Soininen, James C. Stegen, Jizheng He, Xingqi Liu, Lu Zhang, and Enlou Zhang. 2013. “Phylogenetic Beta Diversity in Bacterial Assemblages across Ecosystems: Deterministic versus Stochastic Processes.” *ISME Journal* 7 (7): 1310–21. https://doi.org/10.1038/ismej.2013.30.

Wang, Jinliang. 2017. “The Computer Program Structure for Assigning Individuals to Populations: Easy to Use but Easier to Misuse.” *Molecular Ecology Resources* 17 (5): 981–90. https://doi.org/10.1111/1755-0998.12650.

Weaver, Pablo F., Alexander Cruz, Steven Johnson, Julia Dupin, and Kathleen F. Weaver. 2016. “Colonizing the Caribbean: Biogeography and Evolution of Livebearing Fishes of the Genus *Limia* (Poeciliidae).” *Journal of Biogeography* 43 (9): 1808–19.

https://doi.org/10.1111/jbi.12798.

Wei, Xinli, Bruce McCune, H. Thorsten Lumbsch, Hui Li, Steven D. Leavitt, Yoshikazu Yamamoto, Svetlana Tchabanenko, and Jiangchun Wei. 2016. "Limitations of Species Delimitation Based on Phylogenetic Analyses: A Case Study in the *Hypogymnia hypotrypa* Group (Parmeliaceae, Ascomycota)." *PLoS ONE* 11 (11): e0163664. https://doi.org/10.1371/journal.pone.0163664.

Werth, Silke. 2011. "Biogeography and Phylogeography of Lichen Fungi and Their Photobionts." In *Biogeography of Microscopic Organisms Is Everything Small Everywhere?*, edited by Diego Fontaneto, 1st ed., 191–208. New York: Cambridge University Press. https://doi.org/10.1017/CBO9780511974878.011.

Werth, Silke, Helene H. Wagner, Felix Gugerli, Rolf Holderegger, Daniela Csencsics, Jesse M. Kalwij, and Christoph Scheidegger. 2006. "Quantifying Dispersal and Establishment Limitation in a Population of an Epiphytic Lichen." *Ecology* 87 (8): 2037–46. https://doi.org/10.1890/0012-9658(2006)87[2037:QDAELI]2.0.CO;2.

Werth, Silke, Helene H. Wagner, Rolf Holderegger, Jesse M. Kalwij, and Christoph Scheidegger. 2006. "Effect of Disturbances on the Genetic Diversity of an Old-Forest Associated Lichen." *Molecular Ecology* 15 (4): 911–21. https://doi.org/10.1111/j.1365-294X.2006.02838.x.

Whittaker, Robert J., and José María Fernández-Palacios. 2001. *Island Biogeography: Ecology, Evolution, and Conservation*. 2nd ed. Cambridge: Cambridge University Press.

Widhelm, Todd J., Francesca R. Bertoletti, Matt J. Asztalos, Joel A. Mercado-Díaz, Jen-Pan Huang, Bibiana Moncada, Robert Lücking, et al. 2018. "Oligocene Origin and Drivers of Diversification in the Genus *Sticta* (Lobariaceae, Ascomycota)." *Molecular Phylogenetics and Evolution* 126: 58–73. https://doi.org/10.1016/j.ympev.2018.04.006.

Widhelm, Todd J., Robert S. Egan, Francesca R. Bertoletti, Matt J. Asztalos, Ekaphan Kraichak, Steven D. Leavitt, and H. Thorsten Lumbsch. 2016. "Picking Holes in Traditional Species Delimitations: An Integrative Taxonomic Reassessment of the *Parmotrema perforatum* Group (Parmeliaceae, Ascomycota)." *Botanical Journal of the Linnean Society* 182 (4): 868–84. https://doi.org/10.1111/boj.12483.

Widhelm, Todd J., Felix Grewe, Jen-Pan Huang, Joel A. Mercado-Díaz, Bernard Goffinet, Robert Lücking, Bibiana Moncada, Roberta Mason-Gamer, and H. Thorsten Lumbsch. 2019. "Multiple Historical Processes Obscure Phylogenetic Relationships in a Taxonomically Difficult Group (Lobariaceae, Ascomycota)." *Scientific Reports* 9: 8968. https://doi.org/10.1038/s41598-019-45455-x.

Widhelm, Todd J., Felix Grewe, Jen-Pan Huang, Karolis Ramanauskas, Roberta Mason-Gamer, and H. Thorsten Lumbsch. 2021. "Using RADseq to Understand the Circum-Antarctic Distribution of a Lichenized Fungus, *Pseudocyphellaria glabra*." *Journal of Biogeography* 48 (1): 78–90. https://doi.org/10.1111/jbi.13983.

Wiens, John J., and Matthew C. Morrill. 2011. "Missing Data in Phylogenetic Analysis: Reconciling Results from Simulations and Empirical Data." *Systematic Biology* 60 (5): 719–31. https://doi.org/10.1093/sysbio/syr025.

Will, Kipling W., Brent D. Mishler, and Quentin D. Wheeler. 2005. "The Perils of DNA

Barcoding and the Need for Integrative Taxonomy." *Systematic Biology* 54 (5): 844–51. https://doi.org/10.1080/10635150500354878.

Wolf, Jan H. D. 1993. "Diversity Patterns and Biomass of Epiphytic Bryophytes and Lichens Along an Altitudinal Gradient in the Northern Andes." *Annals of the Missouri Botanical Garden* 80 (4): 928. https://doi.org/10.2307/2399938.

Woods, Charles A., Rafael Borroto Paéz, and C. William Kilpatrick. 2001. "Insular Patterns and Radiations of West Indian Rodents." In *Biogeography of the West Indies: Patterns and Perspectives*, edited by C. A. Woods and F. E. Sergile, 2nd ed., 335–53. Boca Ratón: CRC Press. https://doi.org/10.1201/9781420039481.

Woods, Charles A., and Florence E. Sergile. 2001. *Biogeography of the West Indies: Patterns and Perspectives*. 2nd. ed. Boca Ratón: CRC Press. https://doi.org/10.1201/9781420039481.

Yahr, Rebecca, Rytas Vilgalys, and Paula T. DePriest. 2006. "Geographic Variation in Algal Partners of *Cladonia subtenuis* (Cladoniaceae) Highlights the Dynamic Nature of a Lichen Symbiosis." *New Phytologist* 171 (4): 847–60. https://doi.org/10.1111/j.1469-8137.2006.01792.x.

Yang, Ziheng. 2015. "The BPP Program for Species Tree Estimation and Species Delimitation." *Current Zoology* 61 (5): 854–65. https://doi.org/10.1093/czoolo/61.5.854.

Yang, Ziheng, and Bruce Rannala. 2010. "Bayesian Species Delimitation Using Multilocus Sequence Data." *Proceedings of the National Academy of Sciences of the United States of America* 107 (20): 9264–69. https://doi.org/10.1073/pnas.0913022107.

———. 2014. "Unguided Species Delimitation Using DNA Sequence Data from Multiple Loci." *Molecular Biology and Evolution* 31 (12): 3125–35. https://doi.org/10.1093/molbev/msu279.

Yu, Guangchuang. 2020. "Using ggtree to Visualize Data on Tree-Like Structures." *Current Protocols in Bioinformatics* 69 (1): e96. https://doi.org/10.1002/cpbi.96.

Yu, Yan, Alan J. Harris, Christopher Blair, and Xingjin He. 2015. "RASP (Reconstruct Ancestral State in Phylogenies): A Tool for Historical Biogeography." *Molecular Phylogenetics and Evolution* 87: 46–49. https://doi.org/10.1016/j.ympev.2015.03.008.

Zhang, Jiajie, Paschalia Kapli, Pavlos Pavlidis, and Alexandros Stamatakis. 2013. "A General Species Delimitation Method with Applications to Phylogenetic Placements." *Bioinformatics* 29 (22): 2869–76. https://doi.org/10.1093/bioinformatics/btt499.

Zhao, Xin, Samantha Fernández-Brime, Mats Wedin, Marissa Locke, Steven D. Leavitt, and H. Thorsten Lumbsch. 2017. "Using Multi-Locus Sequence Data for Addressing Species Boundaries in Commonly Accepted Lichen-Forming Fungal Species." *Organisms Diversity and Evolution* 17 (2): 351–63. https://doi.org/10.1007/s13127-016-0320-4.

Zheng, Yuchi, and John J. Wiens. 2015. "Do Missing Data Influence the Accuracy of Divergence-Time Estimation with BEAST?" *Molecular Phylogenetics and Evolution* 85: 41–49. https://doi.org/10.1016/j.ympev.2015.02.002.

APPENDIX 1. Voucher information and GenBank accession numbers for taxa used in phylogenetic analyses in Chapter 1. Information presented in the following order: Species, authority, country, large political subunit, collector, collector number, herbarium and GenBank accession numbers for ITS, MCM7, mtSSU, nuLSU, RPB1, RPB2, respectively. Species names and newly generated sequences are highlighted in bold. Missing sequences are indicated by a dash (–).

Lobaria pulmonaria (L.) Hoffm.: U.S.A., Michigan, *Widhelm s.n.* (F), MG367435, MF984336, MG754091, MG063078, MG754080, –. ***Pseudocyphellaria crocata*** (L.) Vain.: France, Réunion, *Magain & Sérusiaux LG0688* (LG), JQ735976, –, JQ736009, JQ735993, KT281770, –. ***Ricasolia amplissima*** (Scop.) De Not.: U.S.A., Alaska, *Dillman 2008-602* (WSL), KX385118, –, KC494188, –, –, KX385158. ***Sticta ambavillaria*** (Bory) Ach.: France, Réunion, *Magain & Sérusiaux LG0992* (LG), JQ735978, –, JQ736011, JQ735995, –, –. ***Sticta andensis*** (Nyl.) Trevis.: Colombia, Cundinamarca, *Lücking & Moncada 35422* (B, F, UDBC), KC732548, MF984317, MG754134, MG062956, –, –. ***Sticta*** aff. ***andensis*** (Nyl.) Trevis.: Colombia, Cundinamarca, *Moncada 4009* (B, F, UDBC), KC732467, MF984316, MG754142, MG062955, –, –. ***Sticta andina*** Moncada, Lücking & Sérusiaux: Colombia, Boyacá , *Suárez 212* (B, F, UDBC), MG367388, MF984249, MG754100, MG062970, MG754086, –; Colombia, Cundinamarca, *Alfonso 4* (B, F, UDBC), KC732537, MF984248, MG754099, MG062967, MG754084, –; Colombia, Cundinamarca, *Moncada 4592* (B, F, UDBC), KC732548, MF984247, MG754101, MG062966, –, –; Colombia, Cundinamarca, *Moncada 4802* (B, F, UDBC), KC732688, MF984321, MG754161, MG062969, –, –; Colombia, Cundinamarca, *Moncada 4814* (B, F, UDBC), KC732753, MF984250, MG754159, MG062972, MG754085, –; Colombia, Cundinamarca, *Moncada 4944* (B, F, UDBC), MG367394, MF984245, MG754163, MG062968, –, –; Colombia, Cundinamarca, *Moncada & Lücking 4594* (B, F, UDBC), KC732712, –, –, MG062975, –, –; Ecuador, –, *Dal Forno 1773* (B, F), MG367415, MF984251, MG754160, MG062971, –, –. ***Sticta*** aff. ***andina*** Moncada, Lücking & Sérusiaux: Colombia, Boyacá , *Simijaca 1698* (B, F, UDBC), KC732546, MF984246, MG754105, MG062974, MG754083, –; Colombia, Cundinamarca, *Moncada 3119* (B, F, UDBC), KC732486, –, –, MG062973, –, –. ***Sticta*** aff. ***andreana*** (Müll. Arg.) Zahlbr.: Costa Rica, San José, *Moncada 5620* (B, F, CR), MG367402, MF984284, –, MG063062, –, –. ***Sticta arachnofuliginosa*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4007* (B, F, UDBC), KC732524, MF984306, –, MG062946, –, –. ***Sticta arbuscula*** Moncada & Lücking: Colombia, Cundinamarca, *Lücking & Moncada 33324* (B, F, UDBC), KC732682, –, –, MG063046, –, –. ***Sticta*** aff. ***arbuscula*** Moncada & Lücking: Colombia, Boyacá , *Fonseca 49* (B, F, UDBC), KC732619, –, MG754092, MG063045, MG754090, –. ***Sticta arbusculotomentosa*** Moncada & Betanc.: Colombia, Cundinamarca, *Betancourt 326* (B, F, UDBC), KC732572, MF984220, –, MG063041, –, –. ***Sticta atlantica*** Magain & Sérus.: Ireland, Munster, *Sérusiaux LG3747* (LG), KT281734, –, KT281690, KT281645, –, –; Portugal, Azores, *Sérusiaux LG3858* (LG), KT281737, –, KT281693, KT281648, KT281784, –. ***Sticta atroandensis*** Moncada & Lücking: Colombia, Boyacá , *Fonseca 23* (B, F, UDBC), KC732533, MF984310, –, MG062952, MG754082, –. ***Sticta babingtonii*** D.J. Galloway: New Zealand, Northland, *de Lange 12640* (AK, B, F), MF373808, MF984256, MG754167, MG063012, –, –. ***Sticta beauvoisii*** Delise: Colombia, Boyacá , *Suárez 318* (B, F, UDBC), KC732707, MF984328, –, MG062958, –, –; U.S.A, North Carolina, *Quedensley 16699* (F), MG754194, MF984244, –, MG062957, –, –; U.S.A., Georgia, *Fraker et al. 872* (DUKE), –, –, DQ986867, DQ986769, –, DQ992456; U.S.A., North Carolina, *Goffinet 11137* (LG), KT281725, –, KT281681, KT281636, KT281787, –; U.S.A., North Carolina, *Goffinet 11141* (LG), KT281724, –, KT281680, KT281635, KT281786, –. ***Sticta borinquensis*** Merc.-Díaz & Lücking: Puerto Rico, Jayuya, *Mercado-Díaz 2308* (F, UPR), **MN065850**, –, –, –, –, –; Puerto Rico, Jayuya, *Mercado-Díaz 2301b* (F, UPR), **MN065849**, –, –, **MN065965**, **MN066014**, **MN066067**; Puerto Rico, Rio Grande, *Lücking 33919* (F, UPR), MG367397, MF984263, –, MG062976, **MN066016**, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2365* (F, UPR), **MN065851**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2367* (F, UPR), **MN065852**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2374* (F, UPR), **MN065853**, –, –, –, –, **MN066068**; Puerto Rico, Rio Grande, *Mercado-Díaz 2376* (F, UPR), **MN065854**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2377* (F, UPR), **MN065855**, –, –, –, –, **MN066069**; Puerto Rico, Rio Grande, *Mercado-Díaz 2379* (F, UPR), **MN065856**, –, –, –, –, **MN066070**; Puerto Rico, Rio Grande, *Mercado-Díaz 2381* (F, UPR), **MN065857**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2382* (F, UPR), **MN065858**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2383* (F, UPR), **MN065859**, –, –, –, –, **MN066071**; Puerto Rico, Rio Grande,

Mercado-Díaz 3639 (F, UPR), **MN065860**, –, –, **MN065966**, **MN066017**, **MN066122**. ***Sticta*** aff. ***borinquensis*** Merc.-Díaz & Lücking: Puerto Rico, Jayuya, *Mercado-Díaz 2301a* (F, UPR), **MN065847**, –, –, **MN065963**, **MN066015**, **MN066065**; Puerto Rico, Jayuya, *Mercado-Díaz 2301c* (F, UPR), **MN065848**, –, –, **MN065964**, –, **MN066066**. ***Sticta brevior*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4590b* (B, F, UDBC), MG367386, –, MG754108, MG062929, –, –. ***Sticta caliginosa*** D.J. Galloway: New Zealand, Manawatu-Manganui, *Lücking et. al. 39038* (AK, B, F), MF373767, –, MG754136, MG063036, –, –; New Zealand, Waikato, *Lücking et. al. 39060a* (AK, B, F), MF373760, MF984229, MG754135, MG063035, –, –. ***Sticta*** aff. ***caliginosa*** D.J. Galloway: U.S.A., Hawaii, *Moncada 6949* (F), MG367425, MF984211, MG754137, MG063037, –, –. ***Sticta canariensis*** (Bory) Bory ex Delise: Ireland, Munster, *Sérusiaux LG3741* (LG), KT281733, –, KT281689, KT281644, KT281779, –; Spain, Canary Islands, *Sérusiaux LG1333* (LG), KT281700, –, KT281658, KT281612, KT281752, –. ***Sticta*** aff. ***canariensis*** (Bory) Bory ex Delise: Brazil, Santa Catarina, *Gumboski 3929* (B, F, JOI), MG367417, –, –, MG063000, –, –. ***Sticta caperata*** (Nyl.) Nyl.: France, Réunion, *Magain & Sérusiaux LG0962* (LG), JQ735979, –, JQ736012, JQ735996, KT281745, –. ***Sticta carolinensis*** T. McDonald: U.S.A, North Carolina, *Quedensley 16700* (F), –, MF984234, MG754116, MG063074, –, –. ***Sticta*** cf. ***lhermineri*** (Nyl. ex Stizenb.) Vain.: Colombia, Casanare, *Vargas & Herrera 634* (B, F, UDBC), MG367393, MF984331, –, MG063009, –, –. ***Sticta*** cf. ***sinuosa*** Pers.: Colombia, Boyacá , *Simijaca 1725* (B, F, UDBC), KC732554, MF984296, –, MG062977, –, –. ***Sticta ciliata*** Taylor: Colombia, Casanare, *Vargas & Herrera 64b* (B, F, UDBC), KC732699, MF984325, –, MG063040, –, –; France, Brittany, *Gérault LG3539* (LG), KT281718, –, KT281674, KT281630, KT281774, –; France, Brittany, *Gérault LG3542* (LG), KT281714, –, KT281670, KT281626, KT281772, –; Ireland, Munster, *Sérusiaux LG3781* (LG), KT281716, –, KT281672, KT281628, KT281773, –; Portugal, Azores, *Divakar LG 3099)* (LG), KT281715, –, KT281671, KT281627, KT281762, –; Rwanda, –, *Sérusiaux LG1605* (LG), KT281717, –, KT281673, KT281629, KT281763, –; Spain, Canary Islands, *Sérusiaux LG3406* (LG), KT281713, –, KT281669, KT281625, KT281780, –; Spain, Canary Islands, *Sérusiaux LG3830* (LG), KT281719, –, KT281675, KT281631, KT281775, –; Spain, Canary Islands, *van den Boom 45673* (LG), KT281712, –, KT281668, KT281624, –, –. ***Sticta*** aff. ***ciliata*** Taylor: Colombia, Valle del Cauca, *Moncada 4678* (B, F, UDBC), KC732607, MF984324, MG754144, MG063039, –, –. ***Sticta cinereoglauca*** Hook. f. & Taylor: New Zealand, Chatham Islands, *de Lange CH2449* (AK, B, F), MG367380, MF984224, –, MG063027, –, –; New Zealand, Hawke's Bay, *Lücking et. al. 38646* (AK, B, F), MF373798, MF984241, MG754140, MG063029, –, –; New Zealand, Hawke's Bay, *Lücking et. al. 38776* (AK, B, F), MF373794, MF984242, MG754139, MG063028, –, –. ***Sticta cometia*** Ach.: Colombia, Riseralda, *Coca 1067* (B, F, UDBC), KC732626, MF984222, MG754178, MG062927, –, –. ***Sticta cometiella*** Vain.: Colombia, Cesar, *Moncada 4209* (B, F, UDBC), KC732517, MF984221, MG754177, MG062926, –, –. ***Sticta*** aff. ***cordillerana*** Gyeln.: Colombia, Boyacá , *Simijaca 1731* (B, F, UDBC), KC732553, MF984252, MG754120, MG062963, –, –. ***Sticta corymbosa*** Merc.-Díaz & Moncada: Puerto Rico, Rio Grande, *Mercado-Díaz 2378* (F, UPR), **MN065843**, –, –, **MN066002**, –, **MN066097**; Puerto Rico, Rio Grande, *Mercado-Díaz 2380* (F, UPR), **MN065844**, –, –, **MN066003**, **MN066054**, **MN066098**; Puerto Rico, Rio Grande, *Mercado-Díaz 2384* (F, UPR), **MN065845**, –, –, **MN066004**, –, **MN066099**; Puerto Rico, Rio Grande, *Mercado-Díaz 2385* (F, UPR), **MN065846**, –, –, **MN066005**, –, **MN066100**. ***Sticta dendroides*** (Nyl.) Moncada, Lücking & de Lange,: New Zealand, Hawke's Bay, *Lücking et. al. 38734* (AK, B, F), MF373799, MF984272, MG754188, MG063025, –, –; New Zealand, Manawatu-Manganui, *Lücking et. al. 39007* (AK, B, F), MF373805, MF984253, –, MG063026, –, –; New Zealand, Waikato, *Lücking et. al. 39060b* (AK, B, F), –, MF984233, –, MG063073, –, –. ***Sticta densiphyllidiata*** Merc.-Díaz & Lücking: Puerto Rico, Rio Grande, *Lücking 33871* (F, UPR), MG367398, MF984239, –, MG062987, –, **MN066081**; Puerto Rico, Rio Grande, *Mercado-Díaz 2389* (F, UPR), **MN065890**, **MN065905**, –, **MN065980**, –, **MN066080**. ***Sticta dichotoma*** Bory ex Delise: France, Réunion, *Magain & Sérusiaux LG0945* (LG), JQ735981, –, JQ736014, JQ735998, KT281743, –; France, Réunion, *Magain & Sérusiaux LG0984* (LG), JQ735982, –, JQ736015, JQ735999, KT281746, –. ***Sticta dilatata*** (Nyl.) Vain.: Colombia, Risaralda, *Coca 1077a* (B, F, UDBC), KC732647, –, MG754125, MG063057, –, –. ***Sticta*** aff. ***dilatata*** (Nyl.) Vain.: Costa Rica, San José, *Moncada 5675* (B, F, CR), MG367405, MF984214, MG754127, MG063058, –, –. ***Sticta duplolimbata*** (Hue) Vain.: France, Réunion, *Magain & Sérusiaux LG1040* (LG), JQ735984, –, JQ736001, JQ736017, KT281751, –; Rwanda, –, *Sérusiaux LG0919* (LG), KT281696, –, KT281654, KT281651, KT281741, –. ***Sticta filix*** (Sw.) Nyl.: New Zealand, Manawatu-Manganui, *Lücking et. al. 39034* (AK, B, F), MF373766, –, –, MG063011, –, –; New Zealand, North Island, *de Lange 12284* (AK, B, F), MG367379, MF984228, –, MG063010, –, –. ***Sticta fuliginoides*** Magain & Sérus.: France, Brittany, *Séité LG3551* (LG), KT281729, –, KT281685, KT281640, KT281777, –; France, Grand Est, *Sérusiaux LG1421* (LG), KT281701,

–, KT281659, KT281613, KT281753, –; Ireland, Munster, *Sérusiaux LG3733* (LG), KT281732, –, KT281688, KT281643, KT281781, –; Spain, Canary Islands, *Sérusiaux LG3012* (LG), KT281722, –, KT281678, KT281634, KT281765, –; United Kingdom, England, *Magain LGS4* (LG), KT281738, –, KT281694, KT281649, KT281785, –; United Kingdom, Wales, – *SN739972* (B, F), KC732454, MF984215, –, MG063047, –, –. ***Sticta*** aff. ***fuliginoides*** Magain & Sérus.: Colombia, Cundinamarca, *Moncada & Lücking 4786* (B, F, UDBC), KC732709, MF984304, –, MG063048, –, –. ***Sticta fuliginosa*** (Dicks.) Ach.: Australia, Tasmania, *Lumbsch et al. 2376* (F, UPR), MG754192, MF984305, MG754180, MG062943, –, –; Brazil, Rio Grande do Sul, *Gumboski 3536* (B, F, JOI), MG367419, MF984303, MG754184, MG062939, –, –; France, Brittany, *Bouffinier LG3537* (LG), KT281727, –, KT281683, KT281638, KT281766, –; France, Réunion, *Magain & Sérusiaux LG0989* (LG), KT281698, –, KT281656, KT281610, KT281747, –; Ireland, Munster, *Sérusiaux LG3729* (LG), KT281731, –, KT281687, KT281642, KT281768, –; Madagasgar, Fianarantosa, *Sérusiaux LG0795* (LG), KT281695, –, KT281653, KT281609, KT281740, –; Portugal, Azores, *Divakar LG3100* (LG), KT281704, –, KT281662, KT281616, KT281756, –; Rwanda, –, *Sérusiaux LG1611* (LG), KT281702, –, KT281660, KT281614, KT281754, –; South Africa, –, *Goffinet 10242* (LG), KT281703, –, KT281661, KT281615, KT281755, –; Spain, Canary Islands, *Sérusiaux LG3010* (LG), KT281721, –, KT281677, KT281633, KT281776, –; U.S.A., Hawaii, *Moncada 6978* (F), MG367426, –, MG754185, MG062941, –, –; U.S.A., Hawaii, *Moncada 6979* (F), MG367427, MF984300, MG754186, MG062944, –, –; U.S.A., Hawaii, *Moncada 7026* (F), MG367432, MF984301, MG754182, MG062942, –, –; United Kingdom, England, *Magain LGS9* (LG), KT281739, –, –, KT281650, KT281769, –. ***Sticta*** aff. ***fuliginosa*** (Dicks.) Ach.: Canada, British Columbia, *Goward 09_246b* (LG), KT281723, –, KT281679, –, –, –; Spain, Canary Islands, *Sérusiaux LG2229* (LG), KT281705, –, KT281663, KT281617, KT281757, –; Spain, Canary Islands, *van den Boom 46379* (LG), KT281720, –, KT281676, KT281632, KT281764, –; U.S.A., Oregon, *McCune s.n.* (F, hb. McCune), MG367377, MF984203, –, –, MG754081, –. ***Sticta fuscotomentosa*** Moncada, Coca & Lücking: Colombia, Risaralda, *Coca 1207* (B, F, UDBC), KC732661, MF984280, MG754126, MG063070, –, –. ***Sticta gallowayana*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4637* (B, F, UDBC), KC732496, MF984285, –, MG062934, MG754087, –. ***Sticta globulifuliginosa*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4757* (B, F, UDBC), KC732608, –, –, MG062924, –, –. ***Sticta*** aff. ***granatensis*** Nyl.: Ecuador, –, *Dal Forno 1787a* (B, F), MG367416, –, MG754117, MG062990, –, –. ***Sticta guilartensis*** Merc.-Díaz: Puerto Rico, Adjuntas, *Mercado-Díaz 2426* (F, UPR), **MN065863**, **MN065908**, –, **MN065955**, –, **MN066060**; Puerto Rico, Adjuntas, *Mercado-Díaz 2429* (F, UPR), **MN065861**, **MN065909**, –, **MN065956**, **MN066032**, **MN066061**; Puerto Rico, Adjuntas, *Mercado-Díaz 2431* (F, UPR), –, **MN065910**, –, **MN065957**, –, **MN066062**; Puerto Rico, Adjuntas, *Mercado-Díaz 3666* (F, UPR), **MN065862**, **MN065907**, –, **MN065958**, **MN066030**, **MN066114**; Puerto Rico, Adjuntas, *Mercado-Díaz 3671* (F, UPR), **MN065864**, **MN065906**, **MN065954**, **MN065959**, **MN066031**, **MN066120**. ***Sticta*** aff. ***guilartensis*** Merc.-Díaz: Puerto Rico, Orocovis, *Mercado-Díaz 3659* (F, UPR), **MN065866**, –, –, **MN066013**, **MN066033**, **MN066104**; Puerto Rico, Orocovis, *Mercado-Díaz 3660* (F, UPR), **MN065865**, –, **MN065941**, **MN066012**, **MN066034**, **MN066103**. ***Sticta gyalocarpa*** (Nyl.) Trevis.: Colombia, Cundinamarca, *Moncada 4728* (B, F, UDBC), KC732594, MF984327, MG754111, MG063043, MG754089, –. ***Sticta*** aff. ***gyalocarpa*** (Nyl.) Trevis.: Costa Rica, San José, *Moncada 5649* (B, F, CR), MG367403, MF984326, –, MG063044, –, –. ***Sticta harrisii*** Merc.-Díaz, Moncada & Lücking: Puerto Rico, Patillas, *Mercado-Díaz 3624* (F, UPR), **MN065836**, **MN065916**, –, **MN065996**, **MN066041**, **MN066112**; Puerto Rico, Rio Grande, *Lücking 33868* (F, UPR), **MN065835**, –, –, **MN065995**, **MN066048**, **MN066095**; Puerto Rico, Rio Grande, *Lücking 33894* (F, UPR), **MN065834**, –, –, **MN065994**, **MN066046**, **MN066093**; Puerto Rico, Rio Grande, *Lücking 33905* (F, UPR), KC732774, MF984282, MG754190, MG063072, **MN066047**, **MN066094**; Puerto Rico, Rio Grande, *Mercado-Díaz 2913* (F, UPR), **MN065830**, **MN065918**, –, **MN065991**, –, **MN066091**; Puerto Rico, Rio Grande, *Mercado-Díaz 2915* (F, UPR), **MN065831**, **MN065919**, –, **MN065992**, –, **MN066092**; Puerto Rico, Rio Grande, *Mercado-Díaz 2916* (F, UPR), **MN065832**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2917* (F, UPR), **MN065833**, –, –, **MN065993**, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 3637* (F, UPR), –, **MN065915**, –, –, **MN066040**, **MN066123**; Puerto Rico, Rio Grande, *Mercado-Díaz 3645* (F, UPR), **MN065838**, **MN065914**, **MN065947**, **MN066000**, **MN066039**, **MN066111**; Puerto Rico, Rio Grande, *Mercado-Díaz 3650* (F, UPR), **MN065839**, **MN065913**, **MN065948**, **MN065999**, **MN066044**, **MN066113**; Puerto Rico, Rio Grande, *Mercado-Díaz 3652* (F, UPR), **MN065840**, **MN065912**, **MN065946**, **MN065998**, **MN066043**, **MN066110**; Puerto Rico, Rio Grande, *Mercado-Díaz 3653* (F, UPR), **MN065841**, **MN065911**, **MN065945**, **MN065997**, **MN066042**, **MN066109**; Puerto Rico, San Lorenzo, *Mercado-Díaz 2282* (UPR), MG367376, MF984281, –, MG063071, **MN066045**,

MN066089; Puerto Rico, San Lorenzo, *Mercado-Díaz 2283a* (F, UPR), –, **MN065917**, –, **MN065989**, –, **MN066090**; Puerto Rico, San Lorenzo, *Mercado-Díaz 2285b* (F, UPR), **MN065837**, –, –, **MN065990**, –, **MN066096**. ***Sticta*** aff. ***harrisii*** Merc.-Díaz, Moncada & Lücking: Puerto Rico, Rio Grande, *Mercado-Díaz 3646* (F, UPR), **MN065842**, **MN065898**, **MN065949**, **MN066001**, **MN066038**, **MN066108**. ***Sticta hirsutofuliginosa*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4731* (B, F, UDBC), KC732610, MF984311, MG754152, MG062950, –, –. ***Sticta humboldtii*** Hook.: Colombia, Valle del Cauca, *Diaz-Escandón L2* (B, F, UDBC), KC732702, MF984312, MG754118, MG062951, –, –. ***Sticta*** aff. ***humboldtii*** Hook.: Colombia, Cundinamarca, *Moncada 4733* (B, F, UDBC), KC732580, MF984309, MG754154, MG062948, –, –. ***Sticta impressula*** (Nyl.) Zahlbr.: Colombia, Risaralda, *Coca 1014* (B, F, UDBC), KC732646, MF984287, MG754110, MG062931, –, –. ***Sticta isidiokunthii*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4630* (B, F, UDBC), KC732522, MF984288, MG754189, MG062930, MG754088, –. ***Sticta jaguirreana*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4804* (B, F, UDBC), MG754195, –, MG754162, MG062999, –, –. ***Sticta laciniata*** Ach.: Costa Rica, San José, *Moncada 5778* (B, F, CR), MG367399, –, MG754179, MG062984, –, –. ***Sticta*** aff. ***laciniosa*** D.J. Galloway: Costa Rica, San José, *Moncada 5789* (B, F, CR), MG367401, MF984240, –, MG062988, –, –. ***Sticta laevis*** (Nyl.) Vain.: Colombia, Boyacá, *Fonseca 259* (B, F, UDBC), MG367409, MF984206, –, MG063052, –, –. ***Sticta latifrons*** A. Rich.: New Zealand, Chatham Islands, *de Lange CH2517* (AK, B, F), MF373763, MF984230, MG754173, MG063015, –, –; New Zealand, Waikato, *Lücking et. al. 38815* (AK, B, F), MF373800, –, –, MG063016, –, –. ***Sticta leucoblepharis*** Mont.: Colombia, Valle del Cauca, *Moncada 4689* (B, F, UDBC), KC732597, MF984276, –, MG063063, –, –. ***Sticta*** aff. ***lherminieri*** (Nyl. ex Stizenb.) Vain.: Colombia, Valle del Cauca, *Lücking & Moncada 33511* (B, F, UDBC), KC732673, MF984269, MG754145, MG063008, –, –. ***Sticta limbata*** (Sm.) Ach.: Canada, British Columbia, *Goward 09–246a* (LG), KT281710, –, –, KT281622, –, –; France, Brittany, *Gérault LG3544* (LG), KT281728, –, KT281684, KT281639, KT281767, –; Portugal, Azores, *Divakar LG3105* (LG), KT281709, –, KT281667, KT281621, –, –; Portugal, Azores, *Sérusiaux LG3868* (LG), KT281711, –, –, KT281623, KT281761, –; Spain, Canary Islands, *Sérusiaux LG2230* (LG), KT281706, –, KT281664, KT281618, KT281758, –; Spain, Canary Islands, *van den Boom 46085* (LG), KT281708, –, KT281666, KT281620, KT281760, –; United Kingdom, Scotland, *Coppins LG2690* (LG), KT281707, –, KT281665, KT281619, KT281759, –. ***Sticta*** aff. ***limbata*** (Sm.) Ach.: Brazil, Rio Grande do Sul, *Gumboski 3560* (B, F, JOI), MG367418, MF984294, MG754183, MG062945, –, –; U.S.A., Hawaii, *Moncada 6995* (F), MG367428, MF984298, MG754181, MG062940, –, –; U.S.A., Oregon, *McCune s.n.* (F, hb. McCune), MG367378, MF984292, –, –, –, –. ***Sticta lobarioides*** Moncada & Coca: Colombia, Cundinamarca, *Alfonso 5* (B, F, UDBC), KC732555, MF984238, MG754113, MG062992, –, –. ***Sticta lumbschiana*** Moncada & Lücking: Colombia, Cundinamarca, *Lücking & Moncada 33370* (B, F, UDBC), KC732575, MF984212, MG754124, MG063055, –, –. ***Sticta macrocyphellata*** Moncada & Coca: Colombia, Risaralda, *Coca 1267* (B, F, UDBC), KC732662, MF984313, –, MG063056, –, –. ***Sticta macrophylla*** Bory ex Delise: France, Réunion, *Magain & Sérusiaux LG0946* (LG), JQ735985, –, JQ736018, JQ736002, KT281744, –. ***Sticta macrothallina*** Moncada & Coca: Colombia, Risaralda, *Coca 1115* (B, F, UDBC), KC732629, MF984208, MG754122, MG063034, –, –; Colombia, Risaralda, *Coca 1210* (B, F, UDBC), KC732655, MF984314, MG754106, MG063032, –, –; Colombia, Risaralda, *Coca 1376* (B, F, UDBC), KC732637, –, MG754107, MG063033, –, –. ***Sticta maculofuliginosa*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4156* (B, F, UDBC), KC732514, MF984235, –, –, –, –. ***Sticta marginalis*** Bory: France, Réunion, *Magain & Sérusiaux LG1023* (LG), JQ735980, –, JQ736013, JQ735997, KT281748, –. ***Sticta*** aff. ***marginalis*** Bory: U.S.A., Hawaii, *Moncada 6916* (F), MG754196, –, MG754095, MG062921, –, –. ***Sticta menziesii*** Hook. f. & Taylor: New Zealand, Manawatu-Manganui, *Lücking et. al. 39001* (AK, B, F), MF373788, MF984254, –, MG063014, –, –; New Zealand, Manawatu-Manganui, *Lücking et. al. 39011* (AK, B, F), –, MF984257, –, MG063076, –, –; New Zealand, Waikato, *Lücking et. al. 39050* (AK, B, F), MF373761, MF984225, MG754191, MG063013, –, –; New Zealand, Waikato, *Lücking et. al. 39062a* (AK, B, F), –, MF984255, –, MG063075, –, –. ***Sticta minutula*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4753* (B, F, UDBC), KC732583, MF984297, –, MG063042, –, –. ***Sticta neopulmonarioides*** Moncada & Coca: Colombia, Risaralda, *Coca 949* (B, F, UDBC), KC732625, MF984204, MG754115, –, –, –; Colombia, Risaralda, *Coca 998* (B, F, UDBC), KC732652, –, –, MG062995, –, –; Colombia, Risaralda, *Coca 1069* (B, F, UDBC), KC732651, –, –, MG062994, –, –; Colombia, Risaralda, *Coca 1112* (B, F, UDBC), KC732628, –, –, MG062989, –, –; Colombia, Risaralda, *Coca 1204* (B, F, UDBC), KC732636, MF984236, –, MG062993, –, –. ***Sticta*** aff. ***neopulmonarioides*** Moncada & Coca: Colombia, Risaralda, *Coca 1095* (B, F, UDBC), KC732654, –, –, MG062997, –, –. ***Sticta papillata*** Moncada & Lücking: Colombia, Cundinamarca,

Alfonso 3 (B, F, UDBC), KC732552, MF984232, MG754123, MG063053, –, –; Colombia, Cundinamarca, *Lücking & Moncada 35400* (B, F, UDBC), MG367414, MF984283, MG754133, MG063054, –, –. ***Sticta parahumboldtii*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4016* (B, F, UDBC), KC732550, MF984308, MG754151, MG062949, –, –. ***Sticta parvilobata*** Merc.-Díaz: Puerto Rico, Adjuntas, *Mercado-Díaz 2432* (F, UPR), **MN065878**, –, –, **MN065968**, –, **MN066074**; Puerto Rico, Adjuntas, *Mercado-Díaz 3664* (F, UPR), **MN065877**, **MN065923**, –, **MN065978**, **MN066020**, **MN066119**; Puerto Rico, Adjuntas, *Mercado-Díaz 3667* (F, UPR), **MN065879**, **MN065922**, –, **MN065975**, **MN066019**, **MN066117**; Puerto Rico, Adjuntas, *Mercado-Díaz 3668* (F, UPR), **MN065880**, **MN065921**, –, **MN065974**, **MN066018**, **MN066118**; Puerto Rico, Orocovis, *Mercado-Díaz 2260* (UPR), MG367375, MF984323, **MN065939**, MG063038, **MN066024**, **MN066072**; Puerto Rico, Orocovis, *Mercado-Díaz 2263* (F, UPR), **MN065876**, **MN065924**, –, **MN065967**, **MN066025**, **MN066073**. ***Sticta*** aff. ***parvilobata*** Merc.-Díaz: Puerto Rico, Adjuntas, *Mercado-Díaz 2435* (F, UPR), **MN065884**, –, –, –, –, –; Puerto Rico, Adjuntas, *Mercado-Díaz 3672* (F, UPR), **MN065889**, **MN065902**, –, **MN065973**, –, **MN066078**; Puerto Rico, Cayey, *Mercado-Díaz 2289* (F, UPR), **MN065885**, –, –, **MN065972**, **MN066023**, **MN066079**; Puerto Rico, Jayuya, *Coca 4563* (F, UPR), **MN065882**, –, –, –, **MN066029**, **MN066076**; Puerto Rico, Jayuya, *Mercado-Díaz 2304* (F, UPR), **MN065829**, **MN065904**, –, **MN065971**, **MN066028**, **MN066077**; Puerto Rico, Jayuya, *Moncada 8311* (F, UPR), **MN065828**, **MN065903**, –, **MN065969**, **MN066026**, –; Puerto Rico, Jayuya, *Moncada 8318* (F, UPR), **MN065881**, –, –, **MN065970**, **MN066027**, **MN066075**; Puerto Rico, Patillas, *Mercado-Díaz 3619* (F, UPR), **MN065886**, **MN065901**, –, **MN065976**, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 2914* (F, UPR), **MN065883**, –, –, –, –, –; Puerto Rico, Rio Grande, *Mercado-Díaz 3635* (F, UPR), **MN065887**, **MN065900**, –, **MN065979**, **MN066022**, **MN066124**; Puerto Rico, Rio Grande, *Mercado-Díaz 3649* (F, UPR), **MN065888**, **MN065899**, **MN065950**, **MN065977**, **MN066021**, **MN066115**. ***Sticta*** aff. ***peltigerella*** (Nyl.) Trevis.: Colombia, Cundinamarca, *Buitrago 24* (B, F, UDBC), MG367410, MF984216, MG754158, MG063049, –, –. ***Sticta phyllidiofuliginosa*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4051* (B, F, UDBC), KC732495, MF984329, –, –, –, –. ***Sticta phyllidiokunthii*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4758* (B, F, UDBC), KC732593, MF984291, MG754112, MG062932, –, –; Colombia, Risaralda, *Coca 1206* (B, F, UDBC), KC732638, MF984286, MG754109, MG062933, –, –. ***Sticta plumbeociliata*** Moncada, A. Suárez & Lücking: Colombia, Cundinamarca, *Moncada 4820* (B, F, UDBC), KC732767, MF984290, –, MG062935, –, –. ***Sticta pseudohumboldtii*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4928* (B, F, UDBC), KC732736, MF984307, –, MG062947, –, –. ***Sticta pseudolobaria*** Moncada & Coca: Colombia, Risaralda, *Coca 964* (B, F, UDBC), KC732650, –, –, MG062996, –, –. ***Sticta rhizinata*** Moncada & Lücking: Colombia, Cundinamarca, *Moncada 4638* (B, F, UDBC), KC732491, –, MG754097, MG062962, –, –. ***Sticta riparia*** Merc.-Díaz: Puerto Rico, Aibonito, *Mercado-Díaz 3677* (F, UPR), **MN065892**, **MN065926**, –, **MN066007**, **MN066036**, **MN066107**; Puerto Rico, Aibonito, *Mercado-Díaz 3683* (F, UPR), **MN065894**, **MN065925**, –, **MN066006**, –, **MN066106**; Puerto Rico, Aibonito, *Mercado-Díaz 3684* (F, UPR), **MN065893**, **MN065928**, **MN065942**, **MN066008**, **MN066035**, **MN066105**; Puerto Rico, Arecibo, *Mercado-Díaz 2342* (UPR), MG367373, MF984275, –, MG062986, **MN066037**, **MN066082**; Puerto Rico, Patillas, *Mercado-Díaz 3626* (F, UPR), **MN065891**, **MN065927**, **MN065951**, –, –, **MN066125**. ***Sticta scabrosa*** Moncada, Mercado-Díaz & Bungartz: Colombia, Cesar, *Moncada 4306* (B, F, UDBC), MG367387, MF984258, –, –, –, –; Puerto Rico, Cayey, *Mercado-Díaz 2287* (F, UPR), **MN065871**, **MN065933**, –, **MN065983**, **MN066051**, **MN066085**; Puerto Rico, Cayey, *Mercado-Díaz 2291* (UPR), MG367374, MF984334, –, MG063002, **MN066052**, **MN066086**; Puerto Rico, Cayey, *Mercado-Díaz 2294* (F, UPR), **MN065872**, **MN065935**, –, **MN065985**, –, **MN066088**; Puerto Rico, Cayey, *Mercado-Díaz 2293a* (F, UPR), **MN065827**, **MN065934**, –, **MN065984**, **MN066053**, **MN066087**; Puerto Rico, Patillas, *Mercado-Díaz 3622* (F, UPR), **MN065873**, **MN065931**, **MN065953**, **MN065988**, –, –; Puerto Rico, Patillas, *Mercado-Díaz 3623* (F, UPR), **MN065874**, **MN065930**, **MN065952**, **MN065987**, **MN066049**, –; Puerto Rico, Rio Grande, *Mercado-Díaz 3636a* (F, UPR), **MN065875**, **MN065929**, –, **MN065986**, –, –; Puerto Rico, San Lorenzo, *Mercado-Díaz 2283b* (F, UPR), **MN065870**, **MN065932**, –, **MN065982**, **MN066050**, **MN066084**; Puerto Rico, Villalba, *Moncada 8334* (F, UPR), **MN065869**, –, –, **MN065981**, –, **MN066083**. ***Sticta*** aff. ***scabrosa*** Moncada, Mercado-Díaz & Bungartz: Colombia, Boyacá , *Ardila 1* (B, F, UDBC), KC732478, MF984265, MG754143, MG063007, –, –. ***Sticta scabrosa* subsp. *hawaiiensis*** Moncada, Lücking & C.W. Sm.: U.S.A., Hawaii, *Moncada 6911* (F), MG367422, –, MG754148, MG063003, –, –; U.S.A., Hawaii, *Moncada 7014* (F), MG367429, MF984266, MG754147, MG063004, –, –; U.S.A., Hawaii, *Moncada 7016* (F), MG367431, MF984267, MG754149, MG063005, –, –; U.S.A., Hawaii, *Moncada 7054* (F), MG367430, MF984268, MG754146, MG063006, –, –. ***Sticta*** aff. ***sinuosa*** Pers.: Colombia,

Boyacá , *Barragán 12* (B, F, UDBC), KC732476, MF984295, –, –, –, –. ***Sticta*** sp. –: Brazil, São Paulo, *Lücking 30122* (B, F, SP), KC732568, MF984319, –, MG062954, –, –; Colombia, Boyacá, *Fonseca 65* (B, F, UDBC), MG367407, MF984213, –, –, –, –; Colombia, Boyacá, *Fonseca 255* (B, F, UDBC), MG367408, MF984209, MG754174, MG063061, –, –; Colombia, Boyacá , *Álvaro 41218a* (B, F, UDBC), KC732482, MF984271, MG754098, MG062960, –, –; Colombia, Boyacá , *Álvaro 41218b* (B, F, UDBC), KC732727, –, –, MG062961, –, –; Colombia, Casanare, *Vargas & Herrera 556* (B, F, UDBC), MG367391, MF984237, MG754119, MG062998, –, –; Colombia, Cauca, *Diaz-Escandón L1* (B, F, UDBC), KC732701, MF984243, MG754175, –, –, –; Colombia, Cundinamarca, *Moncada 4026* (B, F, UDBC), KC732470, MF984217, –, MG062965, –, –; Colombia, Cundinamarca, *Moncada 4588* (B, F, UDBC), KC732557, –, MG754114, MG062937, –, –; Colombia, Cundinamarca, *Moncada 4746* (B, F, UDBC), KC732581, MF984289, MG754150, MG062928, –, –; Colombia, Cundinamarca, *Moncada 4870* (B, F, UDBC), MG367395, MF984205, –, MG063060, –, –; Colombia, Cundinamarca, *Moncada 4987* (B, F, UDBC), KC732732, MF984333, –, MG062964, –, –; Colombia, Cundinamarca, *Moncada 4992* (B, F, UDBC), KC732761, MF984219, –, MG062936, –, –; Colombia, Cundinamarca, *Moncada 6131* (B, F, UDBC), MG367412, MF984207, MG754176, MG062938, –, –; Colombia, Cundinamarca, *Pérez Perez 1* (B, F, UDBC), MG367411, –, MG754153, –, –, –; Colombia, Valle del Cauca, *Lücking & Moncada 33541* (B, F, UDBC), KC732667, MF984322, MG754104, MG063001, –, –; Costa Rica, San José, *Moncada 5715b* (B, F, CR), –, MF984264, MG754121, MG063077, –, –; U.S.A., Hawaii, *Moncada 6920* (F), MG367423, MF984302, MG754093, MG062922, –, –; U.S.A., Hawaii, *Moncada 7056* (F), MG367434, MF984210, MG754094, MG062923, –, –. ***Sticta squamata*** D.J. Galloway: New Zealand, Auckland, *Lücking et. al. 39200* (AK, B, F), MG367382, MF984226, MG754168, MG063031, –, –; New Zealand, Gisborne, *Lücking et. al. 38562* (AK, B, F), MG367381, MF984260, MG754138, MG063030, –, –. ***Sticta stipitata*** C. Knight: Australia, Tasmania, *Lumbsch et al. 2210* (F, UPR), MG754197, MF984274, MG754141, MG063024, –, –. ***Sticta subcaperata*** (Nyl.) Nyl.: New Zealand, Auckland, *Knight s.n.* (B, F), MG754193, MF984223, MG754187, MG063021, –, –; New Zealand, Bay of Plenty, *Lücking et. al. 38436* (AK, B, F), MG367383, MF984270, MG754172, MG063018, –, –; New Zealand, Hawke's Bay, *Lücking et. al. 38656* (AK, B, F), MG367384, MF984227, MG754171, MG063019, –, –; New Zealand, Manawatu-Manganui, *Lücking et. al. 38949* (AK, B, F), MG754200, MF984273, –, MG063022, –, –; New Zealand, Manawatu-Manganui, *Lücking et. al. s.n.* (AK, B, F), MG367385, MF984261, –, MG063020, –, –; New Zealand, Waikato, *Lücking et. al. 38819* (AK, B, F), MG754199, –, MG754170, MG063023, –, –; New Zealand, Waikato, *Lücking et. al. 39061* (AK, B, F), MG754198, MF984231, MG754169, MG063017, –, –. ***Sticta subfilicinella*** Moncada, Coca & Lücking: Colombia, Risaralda, *Coca 1110* (B, F, UDBC), KT354937, –, –, MG063064, –, –. ***Sticta sublimbata*** (J. Steiner) Swinscow & Krog: Democratic Republic of the Congo, –, *Sérusiaux LG0885* (LG), JQ735986, –, JQ736019, JQ736003, KT281771, –; France, Réunion, *Magain & Sérusiaux LG1038* (LG), KT281699, –, KT281657, KT281611, KT281750, –. ***Sticta*** aff. ***sublimbata*** (J. Steiner) Swinscow & Krog: Colombia, Cundinamarca, *Valbuena 126* (B, F, UDBC), KC732466, –, –, MG062959, –, –. ***Sticta*** aff. ***subscrobiculata*** (Nyl.) Gyeln.: Colombia, Risaralda, *Coca 1135* (B, F, UDBC), KC732639, –, MG754096, MG062985, –, –. ***Sticta*** aff. ***subtomentella*** (C. Knight ex Shirley) Zahlbr.: Colombia, Risaralda, *Coca 1363* (B, F, UDBC), KC732730, MF984259, –, MG063059, –, –. ***Sticta sylvatica*** (Huds.) Ach.: France, Brittany, *Gérault LG3536* (LG), KT281726, –, KT281682, KT281637, KT281788, –; France, Grand Est, *Sérusiaux LG3837* (LG), KT281736, –, KT281692, KT281647, KT281783, –; Ireland, Munster, *Sérusiaux LG3780* (LG), KT281735, –, KT281691, KT281646, KT281782, –; United Kingdom, England, *Wolseley LG3723* (LG), KT281730, –, KT281686, KT281641, KT281778, –. ***Sticta*** aff. ***sylvatica*** (Huds.) Ach.: Colombia, Boyacá , *Suárez 306* (B, F, UDBC), KC732724, MF984335, –, MG062953, –, –. ***Sticta tainorum*** Merc.-Díaz: Puerto Rico, Orocovis, *Mercado-Díaz 2256* (UPR), MG367371, MF984330, **MN065944**, **MN065960**, –, **MN066063**; Puerto Rico, Orocovis, *Mercado-Díaz 2259* (F, UPR), **MN065867**, **MN065936**, –, **MN065961**, –, **MN066064**; Puerto Rico, Orocovis, *Mercado-Díaz 3661* (F, UPR), **MN065868**, **MN065937**, **MN065940**, **MN065962**, **MN066055**, **MN066121**. ***Sticta tomentosa*** (Sw.) Ach.: Colombia, Cundinamarca, *Moncada 4805* (B, F, UDBC), KC732690, MF984279, MG754128, MG063065, –, –; U.S.A., Hawaii, *Moncada 6910* (F), MG367420, –, MG754130, MG063066, –, –; U.S.A., Hawaii, *Moncada 6946* (F), MG367424, MF984278, MG754131, MG063069, –, –; U.S.A., Hawaii, *Moncada 6947* (F), MG367421, MF984277, MG754132, MG063067, –, –; U.S.A., Hawaii, *Moncada 7045a* (F), MG367433, –, MG754129, MG063068, –, –. ***Sticta*** aff. ***tomentosa*** (Sw.) Ach.: Costa Rica, San José, *Moncada 5653* (B, F, CR), MG367404, MF984218, MG754156, MG063050, –, –; Costa Rica, San José, *Moncada 5694* (B, F, CR), MG367406, MF984315, MG754157, MG063051, –, –. ***Sticta umbilicariiformis*** Hochst. ex Flot.: Rwanda, –,

Sérusiaux LG0925 (LG), KT281697, –, KT281655, KT281652, KT281742, –. ***Sticta variabilis*** Ach.: France, Réunion, *Magain & Sérusiaux LG1037* (LG), JQ735987, –, JQ736020, JQ736004, KT281749, –. ***Sticta viviana*** Alej. Suárez & Lücking: Colombia, Cundinamarca, *Lücking & Moncada 33311* (B, F, UDBC), KC732680, –, MG754155, MG062925, –, –. ***Sticta weigelii*** (Isert.) Ach.: Colombia, Cesar, *Moncada 4215b* (B, F, UDBC), KC732483, MF984262, MG754102, MG062982, –, –; Puerto Rico, Adjuntas, *Mercado-Díaz 2433* (F, UPR), **MN065896**, –, –, **MN066009**, **MN066059**, –; Puerto Rico, Maricao, *Mercado-Díaz 2246* (UPR), MG367370, MF984332, **MN065943**, MG062978, **MN066056**, **MN066101**; Puerto Rico, Rio Grande, *Mercado-Díaz 3643* (F, UPR), **MN065895**, **MN065938**, –, **MN066011**, **MN066057**, **MN066116**; Puerto Rico, San Lorenzo, *Mercado-Díaz 2284* (F, UPR), **MN065897**, –, –, **MN066010**, **MN066058**, **MN066102**. ***Sticta*** aff. ***weigelii*** (Isert.) Ach.: Colombia, Casanare, *Vargas & Herrera 343* (B, F, UDBC), MG367392, MF984293, MG754166, MG062979, –, –; Colombia, Cundinamarca, *Moncada 6164* (B, F, UDBC), MG367413, –, MG754164, MG062981, –, –; Colombia, Valle del Cauca, *Moncada & Lücking 4666* (B, F, UDBC), KC732710, MF984320, MG754165, MG062980, –, –; Colombia, Valle del Cauca, *Moncada & Lücking 4667* (B, F, UDBC), MG367390, MF984299, MG754103, MG062983, –, –. ***Sticta*** aff. ***zahlbruckneri*** B. de Lesd.: Costa Rica, San José, *Moncada 5785* (B, F, CR), MG367400, MF984318, –, MG062991, –, –.

APPENDIX 2. Additional specimens examined for eight new species of *Sticta* from Puerto Rico described in Chapter 1. The specimens are sorted alphabetically based on municipality ("Mun.").

Sticta borinquensis Merc.-Díaz & Lücking, spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Harris 22503* (NY). Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Harris 22494* (NY). Mun. Jayuya, Bosque Estatal Tres Picachos, trail to Tres Picachos peaks; 18° 12' 52" N, 66° 32' 23" W; 1153 m; Mar 29, 2015, *Mercado-Díaz 2308* (UPR). Mun. Luquillo, El Yunque National Forest, Dwarf ridgetop forest, Mt. Britton; 1000 m; Mar, 1985, *McCune 14787* (NY). Mun. Luquillo, El Yunque National Forest, trail to El Yunque; 850 m; Mar, 1985, *McCune 14773* (NY). Mun. Luquillo, El Yunque National Forest, Dwarf ridgetop forest, Mt. Britton; 1000 m; Mar, 1985, *McCune 14785* (NY). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 10* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 12* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 17* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 27* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 33* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 39* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 41* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 61* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 28, 1967, *Landrón-Concepción 72* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 96* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 108* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 122* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 124* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 175* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 179* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 183* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km. 13.7; Jun 29, 1967, *Landrón-Concepción 232* (MSC). Mun. Luquillo, El Yunque National Forest, Espíritu Santo River, PR-186 El Verde; Jun 23 & 30, 1967, *Landrón-Concepción 288* (MSC). Mun. Luquillo, El Yunque National Forest, East Peak, PR-186 Km. 7.7; Jul 10–11, 1967, *Landrón-Concepción 963* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1008* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1016* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1020* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1024* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1032* (MSC). Mun. Luquillo, El Yunque National Forest, Palo Colorado Association; Jul 11, 1967, *Landrón-Concepción 1044* (MSC). Mun. Luquillo, El Yunque National Forest, Palm Brake, Route to El Toro, PR-191 Km 14; Jul 14, 1967, *Landrón-Concepción 1108* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jun 27, 1963, *Imshaug 29514* (MSC). Mun. Luquillo, El Yunque National Forest, The Pinnacles; Jun 28, 1963, *Imshaug 29551* (MSC). Mun. Luquillo, El Yunque National Forest, South of Mt. Britton on route PR-191; Jun 29, 1963, *Imshaug 29573* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jun 27, 1963, *Imshaug 29503A* (MSC). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16' 18" N, 65° 49' 52" W; 1006 m; Dec 28, 2015, *Mercado-Díaz 2374* (UPR). Mun. Río Grande, El Yunque National Forest, along

El Toro trail; 18° 16’ 22” N, 65° 50’ 2” W; 982 m; Dec 28, 2015, *Mercado-Díaz 2376* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 18” N, 65° 49’ 22” W; 1006 m; Dec 28, 2015, *Mercado-Díaz 2377* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 22” N, 65° 50’ 2” W; 982 m; Dec 28, 2015, *Mercado-Díaz 2381* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 22” N, 65° 50’ 2” W; 982 m; Dec 28, 2015, *Mercado-Díaz 2382* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 18” N, 65° 49’ 52” W; 1006 m; Dec 28, 2015, *Mercado-Díaz 2383* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 22” N, 65° 50’ 2” W; 982 m; Dec 28, 2015, *Mercado-Díaz 2367* (UPR). Mun. Río Grande, El Yunque National Forest, along El Toro trail; 18° 16’ 20” N, 65° 49’ 44” W; 1049 m; Dec 28, 2015, *Mercado-Díaz 2379* (UPR). Mun. Río Grande, El Yunque National Forest, Elfin forest; 18° 18’ 4” N, 65° 47’ 35” W; 909 m; Apr 8, 2011, 2015, *Lücking & Mercado-Díaz 33919* (F). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16’ 24” N, 65° 50’ 2” W; 976 m; Jul 19, 2018, *Mercado-Díaz 3638* (UPR). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16’ 24” N, 65° 50’ 4” W; 980 m; Jul 26, 2018, *Mercado-Díaz 3639* (UPR).

Sticta corymbosa Merc.-Díaz & Moncada spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Las Piedras, Barrio El Río, El Yunque National Forest, at summit of Pico El Toro; 18º 16' 20” N, 65º 49' 44” W; 1048 m; Dec 28, 2015, *Mercado-Díaz 2380* (UPR). Mun. Las Piedras, Barrio El Río, El Yunque National Forest, at summit of Pico El Toro; 18º 16' 20” N, 65º 49' 44” W; 1048 m; Dec 28, 2015, *Mercado-Díaz 2384* (UPR). Mun. Las Piedras, Barrio El Río, El Yunque National Forest, at summit of Pico El Toro; 18º 16' 19” N, 65º 49' 45” W; 1048 m; Jul 26, 2018, *Mercado-Díaz 3654* (UPR).

Sticta densiphyllidiata Merc.-Díaz & Lücking, spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Humacao, El Yunque National Forest, about 9 mi. south of Mameyes; Jun 16, 1970, *Tucker 8645* (LSU). Mun. Luquillo, El Yunque National Forest; Jul 7, 1968, *Griffin III s.n.* (LSU). Mun. Luquillo, El Yunque National Forest, foothills of El Yunque, Luquillo Mountains; Jul 17, 1902, *Wilson 316* (NY). Mun. Luquillo, El Yunque National Forest, El Verde, vicinity of El Verde Biological Station; Feb 23-24, 1981, *Buck 3419* (NY). Mun. Luquillo, El Yunque National Forest, Catalina-Yunque trail, Luquillo Mountains, on rock; Feb 23-26, 1923, *EG Britton 7765* (NY, US). Mun. Luquillo, El Yunque National Forest, El Verde, PR-186 Km 7.7; Jun 2 & Jun 14, 1967, *Landrón-Concepción 385* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 992* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 993* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 995* (MSC). Mun. Luquillo, El Yunque National Forest, Valley of La Mina river; Jun 30, 1963, *Imshaug 29606* (MSC). Mun. Luquillo, El Yunque National Forest, Valley of La Mina river; Jun 30, 1963, *Imshaug 29616* (MSC). Mun. Naguabo, El Yunque National Forest, Río Prieto and adjacent hills, on rock; 690-1035 m; Aug 10-15, 1914, *Schafer 3696* (NY, US). Mun. Naguabo, El Yunque National Forest, Sierra de Naguabo, Barrio de Maizales, on rock in ravine; 600 m; Mar 8, 1914, *NL Britton & Cowell 3091* (NY). Mun. Naguabo, El Yunque National Forest, Sierra de Naguabo, Barrio de Maizales, on rock in ravine; 600 m; Mar 8, 1914, *NL Britton & Cowell 3094* (NY). Mun. Río Grande, El Yunque National Forest, trail to LFDP, El Verde Field Station; 18° 19’ 13” N, 65° 48’ 56” W; 415 m; Jun 19, 2013, *Mercado-Díaz 2004* (UPR). Mun. Río Grande, El Yunque National Forest, northwest slopes of low mountains, ca. one km northwest of El Yunque; 500 m; Dec 9, 1963, *Merrill-King s.n.* (US). Mun. Río Grande, El Yunque National Forest, El

Verde Experimental Station, off Rt. 186 at Km. 19.1, on rock in rain forest; 2000 ft.; Jan 22, 1974, *Schmitt 1693* (US).

Sticta guilartensis Merc.-Díaz spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Adjuntas, Bosque Estatal Guilarte, Along trail to Pico Guilarte; 18º 8' 24" N, 66º 46' 12" W; 1100 m; Dec 27, 2016, *Mercado-Díaz 2429* (UPR). Mun. Adjuntas, Bosque Estatal Guilarte, Along trail to Pico Guilarte; 18º 8' 37" N, 66º 46' 8" W; 1100 m; Jul 30, 2018, *Mercado-Díaz 3669* (UPR). Mun. Adjuntas, Bosque Estatal Guilarte, Along trail to Pico Guilarte; 18º 8' 34" N, 66º 46' 9" W; 1133 m; Jul 30, 2018, *Mercado-Díaz 3670* (UPR). Mun. Adjuntas, Bosque Estatal Guilarte, Along trail to Pico Guilarte; 18º 8' 34" N, 66º 46' 9" W; 1133 m; Jul 30, 2018, *Mercado-Díaz 3671* (UPR). Mun. Adjuntas, Barrio Guilarte, along trail to Pico Guilarte, Bosque Estatal de Guilarte; 18º 8' 35" N, 66º 46' 11" W; 1095 m; Dec 12, 2016, *Mercado-Díaz 2431* (UPR).

Sticta harrisii Merc.-Díaz, Moncada & Lücking spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Humacao, El Yunque National Forest, Palm Brake, route to El Toro; 710 m; Jul 14, 1967, *Landrón-Concepción 1108* (NY). Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Harris 22490* (NY). Mun. Humacao, El Yunque National Forest, Recreation Area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Harris 22479* (NY). Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Harris 22477* (NY). Mun. Humacao, El Yunque National Forest, recreation area, trail up to Mt. Britton; 850-950 m; Jun 9, 1988, *Buck 16166* (NY). Mun. Humacao, El Yunque National Forest, Mt. El Toro, trail from El Verde, side on Hwy 186; 1000-1074 m; Jun 4, 1988, *Buck 16041* (NY). Mun. Luquillo, El Yunque National Forest, Dwarf ridgetop forest, Mt. Britton; 1000 m; Mar, 1985, *McCune 14786* (NY). Mun. Luquillo, El Yunque National Forest, Catalina-Yunque trail; Feb 23-26, 1923, *Britton E.G. 7762* (NY, US). Mun. Luquillo, El Yunque National Forest, Catalina-Yunque trail; Feb 23-26, 1923, *Britton E.G. 7757* (NY). Mun. Luquillo, El Yunque National Forest, Summit and upper slopes of Pico del Este, roadside and cloud forest; 1051 m; Mar 5, 1981, *Buck 4149* (NY). Mun. Luquillo, El Yunque National Forest, along trail up to Mt. Britton; 941 m; Feb 24, 1981, *Buck 3527* (NY). Mun. Luquillo, El Yunque National Forest; 3700 ft.; Jul 12, 1902, *Wilson 165* (NY). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 43* (MSC). Mun. Luquillo, El Yunque National Forest, PR-191 Km 11.0; Jun 27, 1967, *Landrón-Concepción 95* (MSC). Mun. Luquillo, El Yunque National Forest, PR-930 Km 1.5; Jun 28, 1967, *Landrón-Concepción 104* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29, 1967, *Landrón-Concepción 269* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 270* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 271* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 277* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 278* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 279* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 280* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jun 29 & Jul 11, 1967, *Landrón-Concepción 281* (MSC). Mun. Luquillo, El Yunque National Forest, El Verde, PR-186 Km 7.7; Jun 2 & Jun 4, 1967, *Landrón-Concepción 339* (MSC). Mun. Luquillo, El Yunque National Forest, East Peak; Jul 10, 1967, *Landrón-Concepción 925* (MSC). Mun. Luquillo, El Yunque National Forest, East Peak; Jul 10, 1967, *Landrón-Concepción 926* (MSC). Mun. Luquillo, El Yunque National Forest, East Peak, PR-186 Km 7.7; Jul 10–11, 1967, *Landrón-Concepción 982* (MSC). Mun. Luquillo, El Yunque National Forest, La Mina; Jul 29 & Jul 11, 1967, *Landrón-Concepción 987* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11,

1967, *Landrón-Concepción 1003* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1010* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1014* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1019* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1045* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1046* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jul 11, 1967, *Landrón-Concepción 1048* (MSC). Mun. Luquillo, El Yunque National Forest, Route to El Toro, PR-191 Km 14; Jul 14, 1967, *Landrón-Concepción 1101* (MSC). Mun. Luquillo, El Yunque National Forest, Ridge from Mt. Britton to the Pinnacles; Jul 14, 1967, *Landrón-Concepción 1115* (MSC). Mun. Luquillo, El Yunque National Forest, Ridge from Mt. Britton to the Pinnacles; Jul 14, 1967, *Landrón-Concepción 1115* (MSC). Mun. Luquillo, El Yunque National Forest, Ridge from Mt. Britton to the Pinnacles; Jul 14, 1967, *Landrón-Concepción 1123* (MSC). Mun. Luquillo, El Yunque National Forest, Ridge from Mt. Britton to the Pinnacles; Jul 14, 1967, *Landrón-Concepción 1124* (MSC). Mun. Luquillo, El Yunque National Forest, Ridge from Mt. Britton to the Pinnacles; Jul 14, 1967, *Landrón-Concepción 1125* (MSC). Mun. Luquillo, El Yunque National Forest, Mt. Britton; Jun 27, 1963, *Imshaug 29509* (MSC). Mun. Luquillo, El Yunque National Forest; Jun 27, 1968, *Lowy s.n.* (LSU). Mun. Luquillo, El Yunque National Forest; Jun 27, 1968, *Lowy s.n.* (LSU). Mun. Luquillo, El Yunque National Forest; Jun 21, 1968, *Lowy 5316* (LSU). Mun. Luquillo, El Yunque National Forest; Jul 7, 1968, *Griffin III s.n.* (LSU). Mun. Luquillo, El Yunque National Forest, near G. González (USFS) "Britton Palm" plot; 18° 18' 16" N, 65° 47' 43" W; 917 m; Sep 27, 2011, *Mercado-Díaz 956* (UPR). Mun. Río Grande, El Yunque National Forest, along road PR-9338, in front of entrance to Mt. Britton trail; 18° 17' 55" N, 65° 47' 28" W; 755 m; Jul 26, 2016, *Mercado-Díaz 2915* (UPR). Mun. Río Grande, El Yunque National Forest, along Tradewinds trail; 18° 16' 48" N, 65° 47' 24" W; 667 m; Jul 26, 2016, *Mercado-Díaz 2916* (UPR). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16' 18" N, 65° 49' 53" W; 1006 m; Jul 19, 2018, *Mercado-Díaz 3637* (UPR). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16' 19" N, 65° 49' 50" W; 900 m; Jul 26, 2018, *Mercado-Díaz 3645* (UPR). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16' 19" N, 65° 49' 50" W; 900 m; Jul 26, 2018, *Mercado-Díaz 3647* (UPR). Mun. Río Grande, El Yunque National Forest, trail to Pico El Toro from Cubuy; 18° 16' 19" N, 65° 49' 50" W; 900 m; Jul 26, 2018, *Mercado-Díaz 3648* (UPR). Mun. Río Grande, El Yunque National Forest, near Pico El Toro summit; 18° 16' 20" N, 65° 49' 45" W; 1040 m; Jul 26, 2018, *Mercado-Díaz 3650* (UPR). Mun. Río Grande, El Yunque National Forest, near Pico El Toro summit; 18° 16' 20" N, 65° 49' 45" W; 1040 m; Jul 26, 2018, *Mercado-Díaz 3651* (UPR). Mun. Río Grande, El Yunque National Forest, along trail to Pico El Toro; 18° 16' 20" N, 65° 49' 50" W; 980 m; Jul 26, 2018, *Mercado-Díaz 3652* (UPR). Mun. Río Grande, El Yunque National Forest, along trail to Pico El Toro; 18° 16' 19" N, 65° 49' 50" W; 980 m; Jul 26, 2018, *Mercado-Díaz 3653* (UPR). Mun. Río Grande, El Yunque National Forest, along Tradewinds trail; 18° 16' 48" N, 65° 48' 36" W; 823 m; Jul 26, 2016, *Mercado-Díaz 2917* (UPR). Mun. Río Grande, El Yunque National Forest, along Mt. Britton trail; 18° 18' 05" N, 65° 47' 34" W; 760-940 m; Oct 4, 2011, *Lücking & Mercado-Díaz 33866* (UPR). Mun. Río Grande, El Yunque National Forest, along Mt. Britton trail; 18° 18' 00" N, 65° 47' 31" W; 812 m; Apr 10, 2011, *Lücking & Mercado-Díaz 33868* (UPR). Mun. Río Grande, El Yunque National Forest, at Mt. Britton; 18° 18' 3.6" N, 65° 47' 35" W; 909 m; Oct 4, 2011, *Lücking & Mercado-Díaz 33894* (UPR). Mun. Río Grande, El Yunque National Forest, along Mt. Britton trail; 18° 18' 3.6" N, 65° 47' 35" W; 909 m; Apr 8, 2011, *Lücking & Mercado-Díaz 33864* (UPR). Mun. Río Grande, El Yunque National Forest, at Mt. Britton; 18° 18' 3.6" N, 65° 47' 35" W; 909 m; Apr 8, 2011, *Lücking & Mercado-Díaz 33905* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network's antennas; 18° 6' 36" N, 66° 3' 5" W; 885 m; Jan 29, 2015, *Mercado-Díaz 2285* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network's

antennas; 18° 6’ 36” N, 66° 3’ 5” W; 885 m; Jan 29, 2015, *Mercado-Díaz 2283a* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network’s antennas; 18° 6’ 36” N, 66° 3’ 5” W; 885 m; Jan 29, 2015, *Mercado-Díaz 2285b* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network’s antennas; 18° 6’ 36” N, 66° 3’ 00” W; 885 m; Jan 29, 2015, *Mercado-Díaz 2288* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network’s antennas; 18° 6’ 36” N, 66° 3’ 00” W; 885 m; Jan 29, 2015, *Mercado-Díaz 2290* (UPR). Mun. San Lorenzo, Bosque Estatal de Carite, along road that access TV network’s antennas; 18° 6’ 36” N, 66° 3’ 5” W; 885 m; Jul 16, 2018, *Mercado-Díaz 3624* (UPR).

Sticta parvilobata Merc.-Díaz

Additional specimens examined: PUERTO RICO. Mun. Adjuntas, Barrio Guilarte, Bosque Estatal de Guilarte, along trail to Pico Guilarte; 18º 8' 41” N, 66º 46' 8” W; 1058 m; Jul 30, 2018, *Mercado-Díaz 3664* (UPR). Mun. Adjuntas, Barrio Guilarte, Bosque Estatal de Guilarte, along trail to Pico Guilarte; 18º 8' 37” N, 66º 46' 8” W; 1100 m; Jul 30, 2018, *Mercado-Díaz 3667* (UPR). Mun. Adjuntas, Barrio Guilarte, Bosque Estatal de Guilarte, along trail to Pico Guilarte; 18º 8' 34” N, 66º 46' 8” W; 1138 m; Jul 30, 2018, *Mercado-Díaz 3672* (UPR). Mun. Orocovis, Barrio Bauta Abajo, Toro Negro State Forest, along El Bolo trail; 18º 10' 19” N, 66º 29' 7” W; 927 m; Jan 22, 2015, *Mercado-Díaz 2263* (UPR).

Sticta riparia **Merc.-Díaz**

Additional specimens examined: PUERTO RICO. Mun. Aibonito, San Cristobal Canyon, rock inside riparian forest; 18º 9' 35” N, 65º 18' 4” W; 465 m; Jul 31, 2018, *Mercado-Díaz 3683* (UPR). Mun. Orocovis, Valleys near Dona Juana Waterfall, Toro Negro. Among rocks; 700 m; Mar 3, 1922, *NL Britton, EG Britton, MS Brown 6395* (NY, US).

Sticta tainorum Merc.-Díaz spec. nov.

Additional specimens examined: PUERTO RICO. Mun. Orocovis, Toro Negro State Forest, along trail to observation tower; 18º 10' 14” N, 66º 28' 52” W; 1037 m; Jul 27, 2018, *Mercado-Díaz 3661* (UPR).

Table S.1.1. Number of inferred species within Puerto Rican clades and their posterior probabilities under different prior settings obtained using rjMCMC algorithm 1 from BPP. Posterior probability values and number of species under algorithm 0 are included within parentheses whenever they differ from algorithm 1. Preferred prior combination in this work highlighted in bold. Refer to Yang (2015) for more information on prior settings and rjMCMC algorithms.

Taxa	Prior settings	Posterior probability	Number of inferred species
Sticta scabrosa[a]	θ_s (3, 0.002), τ_0 (3, 0.002)	0.42	7
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.38**	**3**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.89	1
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.64	2
Sticta harrisii Merc.-Díaz, Moncada & Lücking + ***Sticta* aff. *harrisii***	θ_s (3, 0.002), τ_0 (3, 0.002)	0.042 (0.036)	5 (4)
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.4 (0.70)**	**4 (3)**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.48 (0.98)	1 (2)
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.99	2
Sticta guilartensis Merc.-Díaz + ***Sticta* aff. *guilartensis***	θ_s (3, 0.002), τ_0 (3, 0.002)	0.88	2
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.97**	**2**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.98	1
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.98 (1)	2 (1)
Sticta riparia Merc.-Díaz + ***Sticta densiphyllidiata***[b] *Merc.-Díaz & Lücking*[2]	θ_s (3, 0.002), τ_0 (3, 0.002)	0.26	6
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.97**	**3**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.83 (0.97)	1 (2)
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.96 (0.80)	2 (1)
Sticta tainorum[c] Merc.-Díaz	θ_s (3, 0.002), τ_0 (3, 0.002)	0.75	2
	θ_s (3, 0.002), τ_0 (3, 0.2)	**1 (0.97)**	**1 (2)**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.81	1
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.98	2
Sticta parvilobata Merc.-Díaz + ***Sticta* aff. *parvilobata***[d]	θ_s (3, 0.002), τ_0 (3, 0.002)	0.015	7
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.059 (0.050)**	**3 (4)**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.48	1
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.97	1
Sticta borinquensis Merc.-Díaz & Lücking + ***Sticta* aff. *borinquensis*** + ***Sticta corymbosa*** Merc.-Díaz & Moncada	θ_s (3, 0.002), τ_0 (3, 0.002)	0.018	3
	θ_s (3, 0.002), τ_0 (3, 0.2)	**0.49**	**3**
	θ_s (3, 0.2), τ_0 (3, 0.002)	0.68	1
	θ_s (3, 0.2), τ_0 (3, 0.2)	0.95	2
Sticta weigelii[e]	θ_s (3, 0.002), τ_0 (3, 0.002)	0.49	3

θ_s (3, 0.002), τ_0 (3, 0.2)	**0.72**	**1**
θ_s (3, 0.2), τ_0 (3, 0.002)	0.97	1
θ_s (3, 0.2), τ_0 (3, 0.2)	0.74	1

[a.] Analyzed clade includes nested *Sticta scabrosa* specimen from Colombia (i.e. *S_scabrosa*_CO_4306).

[b.] Analyzed clade includes sister lineage from Costa Rica (i.e. *S_aff_laciniosa*_CR_5789), but numbers reported here exclude this taxon.

[c.] Analyzed clade includes sister taxon from Costa Rica (i.e. *S_laciniata*_CR_5778) but numbers reported here exclude this taxon.

[d.] Analyzed clade include specimens identified as *S. ciliata* and *S.* aff. *ciliata*, but these were excluded from the numbers reported here. The single species reported for the last two sets of priors merged specimens from *S. parvilobata* and *S.* aff. *parvilobata* with *S. ciliata* and *S.* aff. *ciliata*.

[e.] Analyzed clade includes a sister taxon from Colombia (*S_weigelii*_CO_4215b) but numbers reported here exclude this taxon. The delimitation obtained using the last set of priors merged samples from Puerto Rico with this sample.

Table S.1.2. Descriptive summary of unknown secondary metabolites found in several *Sticta* species from Puerto Rico that were analyzed in Chapter 1.

Attribute	**Harrisii unknown**	**Tainorum unknown**	**Borinquensis unknown**	**Riparia unknown**	**Unknown 1**	**Unknown 2**
Rf class	2-3	3	5-6	1-2	4	6
Absolute Rf (x100)	20	33	63	10	40	74-75
Color daylight, before heating		light yellowish peach	faint pink-light orange			
Color daylight, after heating	tan	tan	tan/light brown		faint gray/brown	+bluish/purple
Color UV SW, before heating	charred	charred	charred	charred		
Color UV LW, before heating	+faint white/bluish	+++blue	+light blue/white		+dark orange/red	
Color UV SW, after heating	faint white/bluish	+purplish blue	+blueish-purple			
Color UV LW, after heating	+white/bluish	+purplish blue			opaque	
Species in which it is found	*S. harrisii* Merc.-Díaz, Moncada & Lücking (major), *S. tainorum* Merc.-Díaz (traces, occasional), *S. borinquensis Merc.-Díaz & Lücking* (traces, occasional)	*S. corymbosa* Merc.-Díaz & Moncada (major), *S. weigelii* (major), *S. tainorum* (major)	*S. borinquensis* (major)	*S. riparia* Merc.-Díaz (major), *S. densiphyllidiata* Merc.-Díaz & Lücking (major)	*S. tainorum* (minor)	*S. borinquensis* (minor)

STICTA (Schreber) Ach.

1. Photobiont green; lobes short linear; branching irregular; medulla KOH+ yellow..... Sticta sp. 1155
1. Photobiont blue-green..... 2

2. Lobes with large, usually branched lobules; isidia forming only where injured..... 3
2. Lobes with small granular to coralloid isidia; lobules lacking..... 5

3. Medulla KOH+ purplish; lobes short, linear to rounded, papery, flat..... Sticta sp. 3725
3. Medulla KOH-..... 4

4. Thalli small and delicate; pale and shiny below, rhizines sparse..... S. circumroda Fée ined.
4. Thalli larger; lobes linear, canaliculate, coriaceous; dark below, rhizines abundant..... Sticta sp. 22494

5. Isidia laminal, small and dark; underside white, wrinkled; thalli round, small, ca. 1-2 cm diameter..... Sticta. sp. 22678
5. Isidia marginal (laminal only along cracks or injuries)..... 6

6. Hairs of tomentum producing spherical "buds".. S. weigelii auct.
6. Hairs of tomentum smooth..... 7

7. Underside with well developed, long tomentum; lobes linear, irregularly branched; margins with projecting colorless hairs..... Sticta sp. 22489
7. Underside naked or with short, sparse tomentum..... 8

8. Lobes rounded; margins curled down; old herbarium specimens reddish; on rock..... S. trichographis Fée ined.
8. Lobes linear-lingulate; margins not curled down; on trees..... Sticta sp. 320

Figure S.1.1. Original taxonomic key for *Sticta* morphospecies from Puerto Rico that were recognized in Harris (1989).

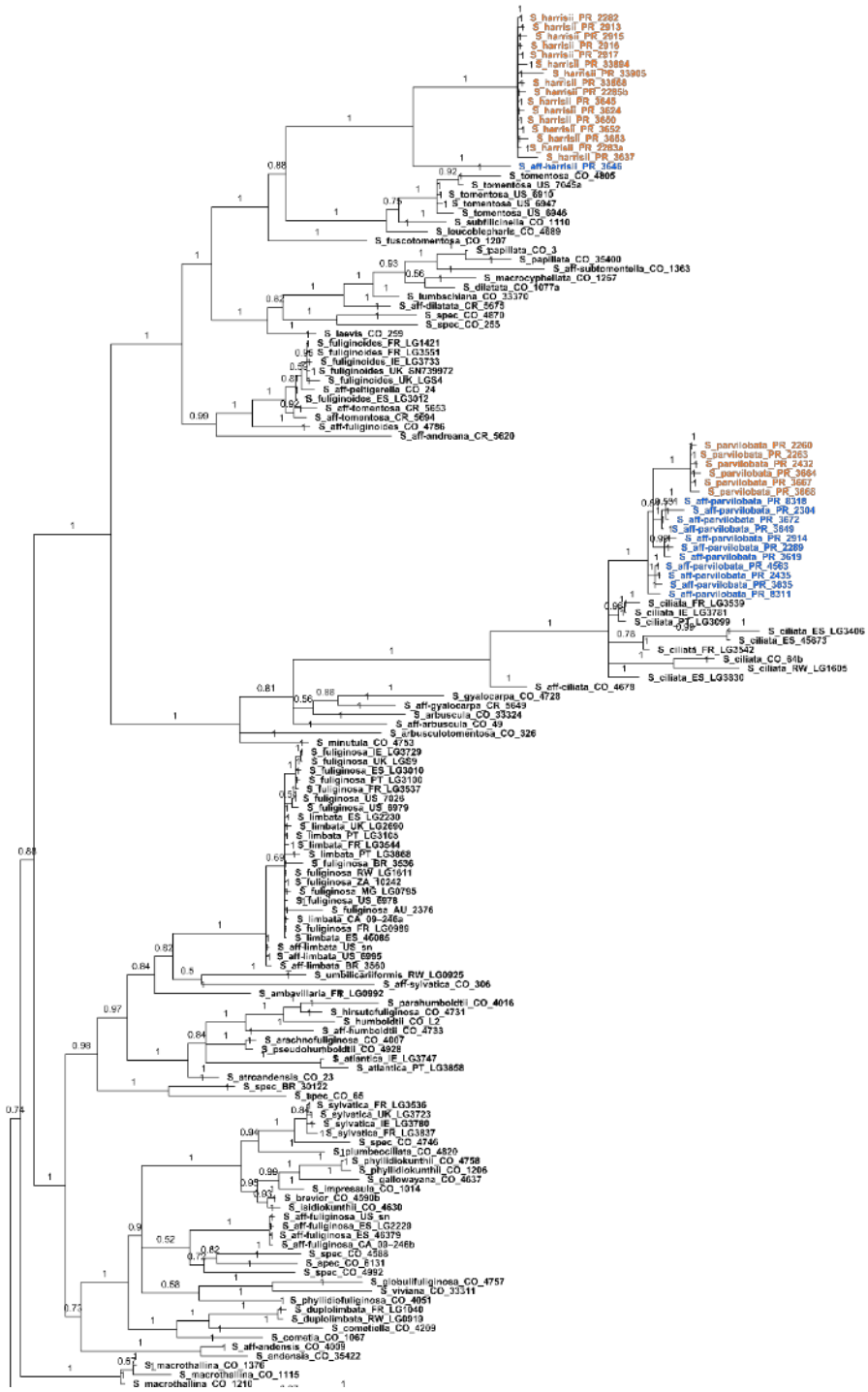

Figure S.1.2. Maximum clade credibility (MCC) tree obtained from MrBayes based on six nuclear and mitochondrial loci (ITS, MCM7, nuLSU, RPB1, RPB2, mtSSU) for 300 specimens of Sticta from Puerto Rico (83) and the rest of the world (217). Sequences from Puerto Rico are highlighted in orange and blue. Bayesian posterior probabilities are indicated above branches. Scale represents number of substitutions per site.

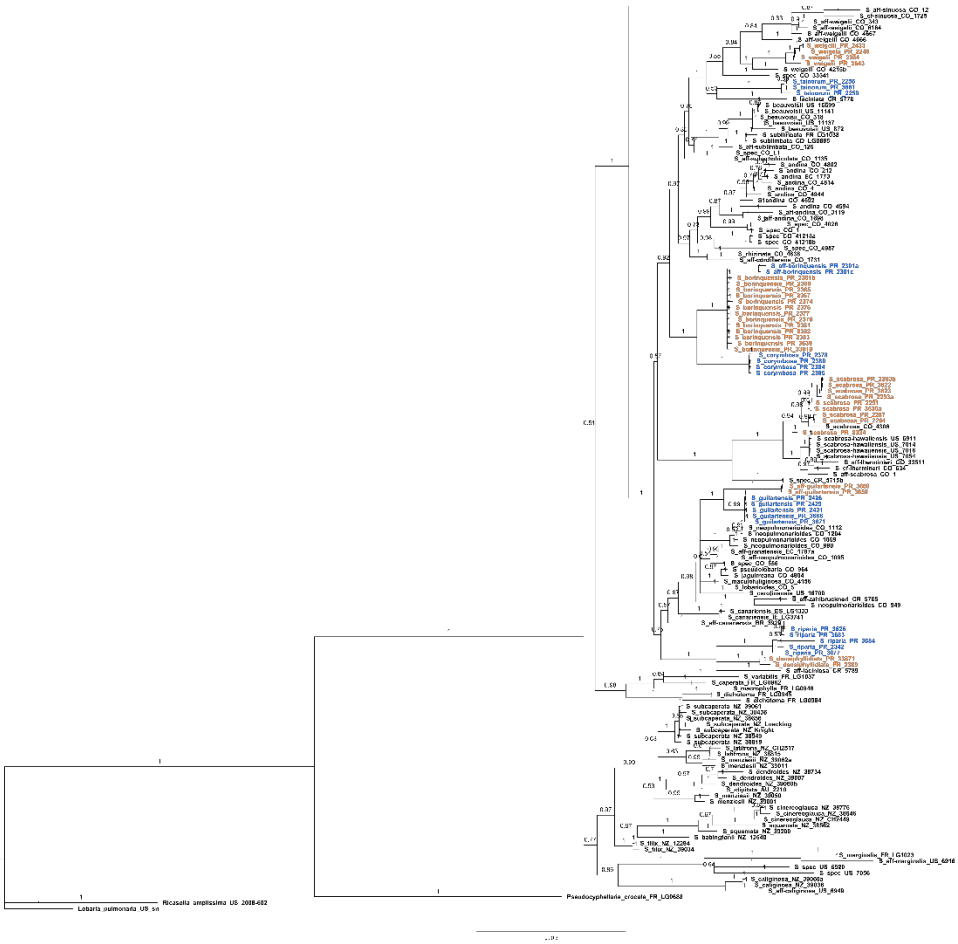

Figure S.1.2. Continued.

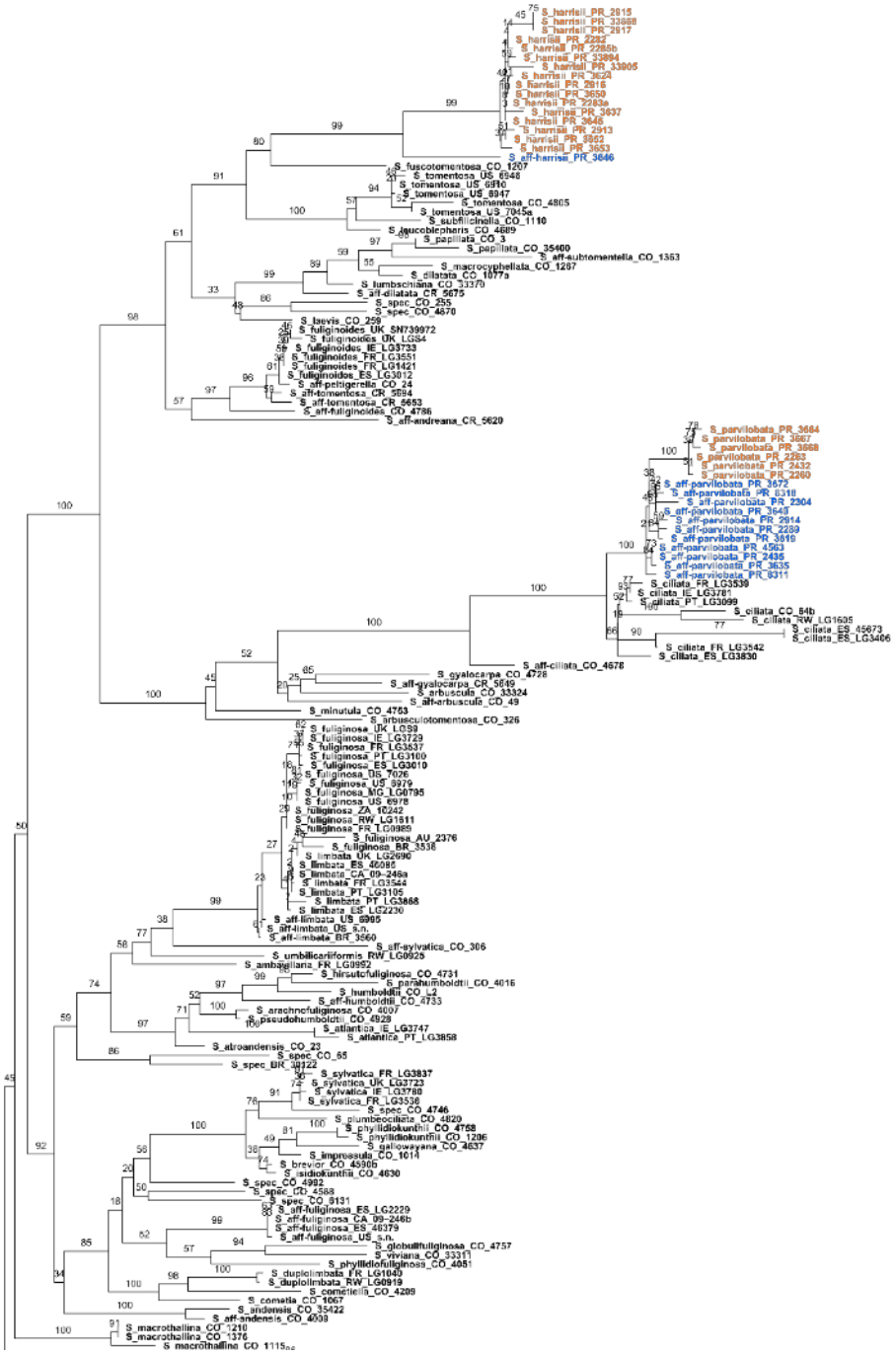

Figure S.1.3. Best-scoring maximum likelihood tree obtained from RAxML based on six nuclear and mitochondrial loci (ITS, MCM7, mtSSU, nuLSU, RPB2, and RPB1) for 300 specimens of *Sticta* from Puerto Rico (80) and the rest of the world (220). Sequences from Puerto Rico are highlighted in orange and blue. Bootstrap values are indicated above branches.

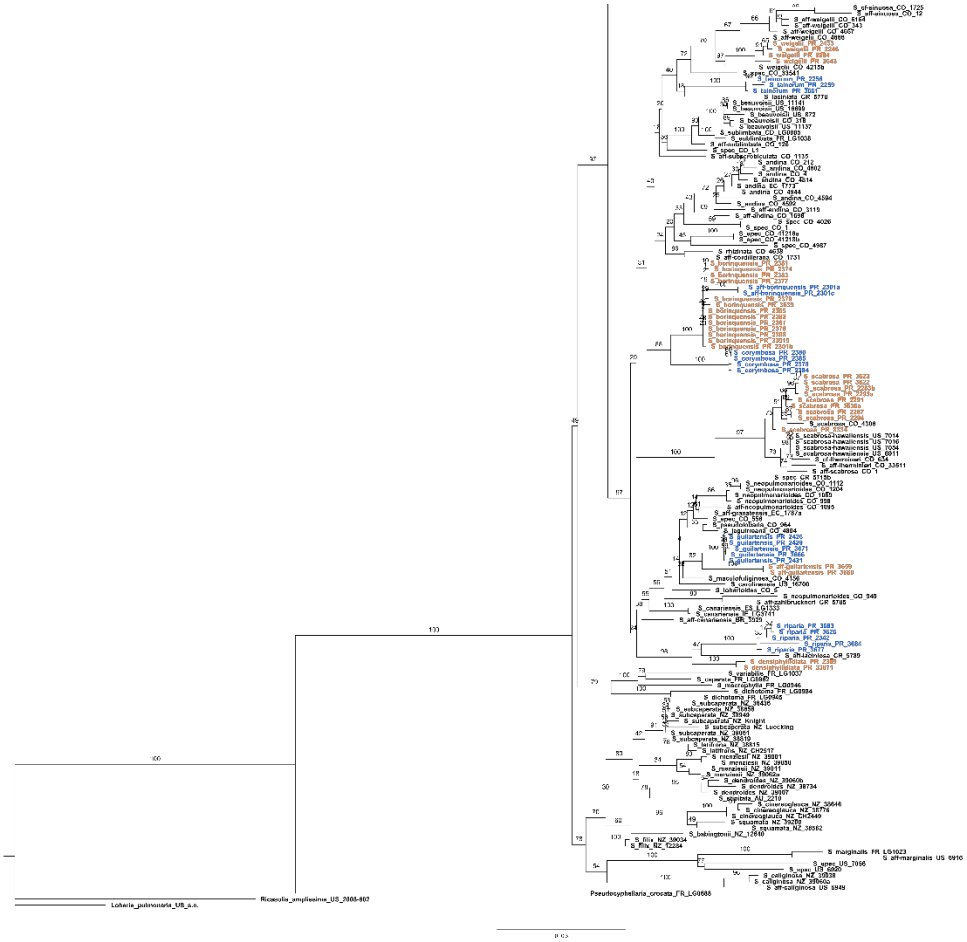

Figure S.1.3. Continued.

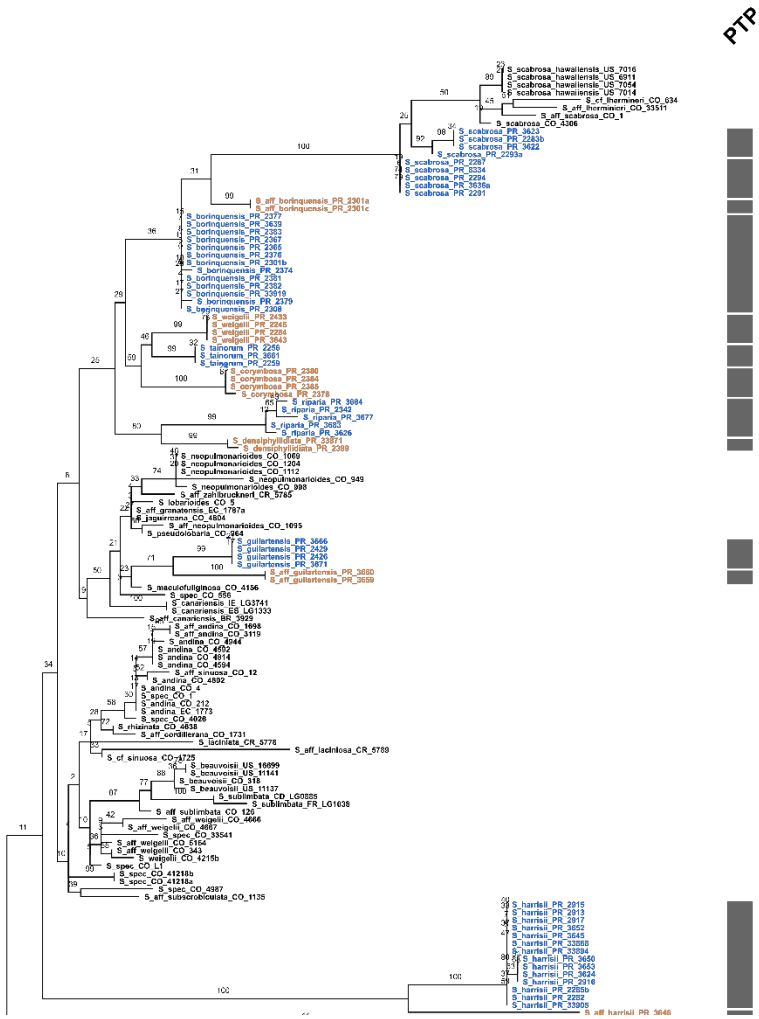

Figure S.1.4. Best-scoring maximum likelihood tree obtained from RAxML based on ITS for 300 specimens of *Sticta* from Puerto Rico (80) and the rest of the world (220). Species boundaries delimited by PTP are indicated in gray boxes. Sequences from Puerto Rico are highlighted in orange and blue. Bootstrap values are indicated above branches.

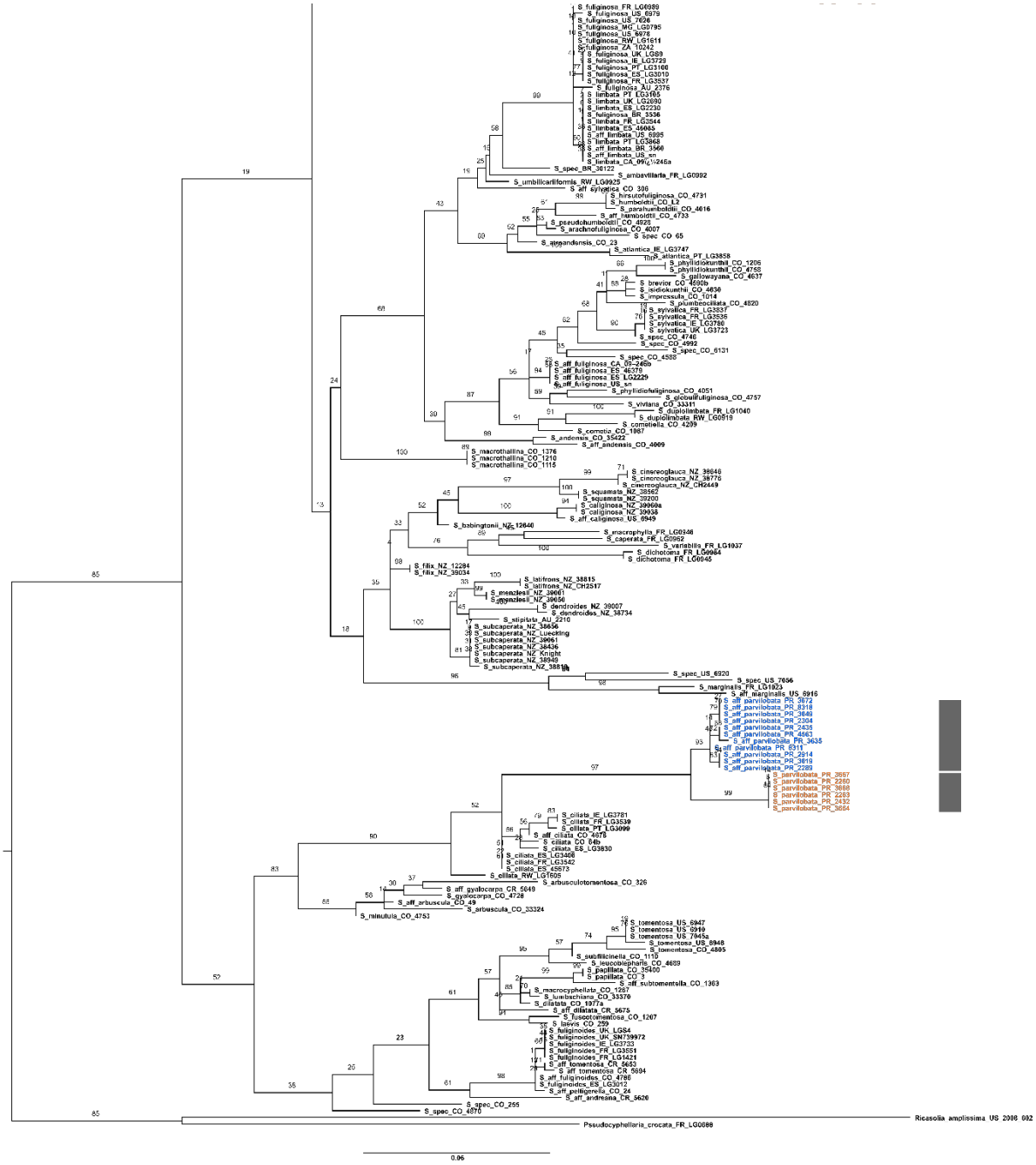

Figure S.1.4. Continued.

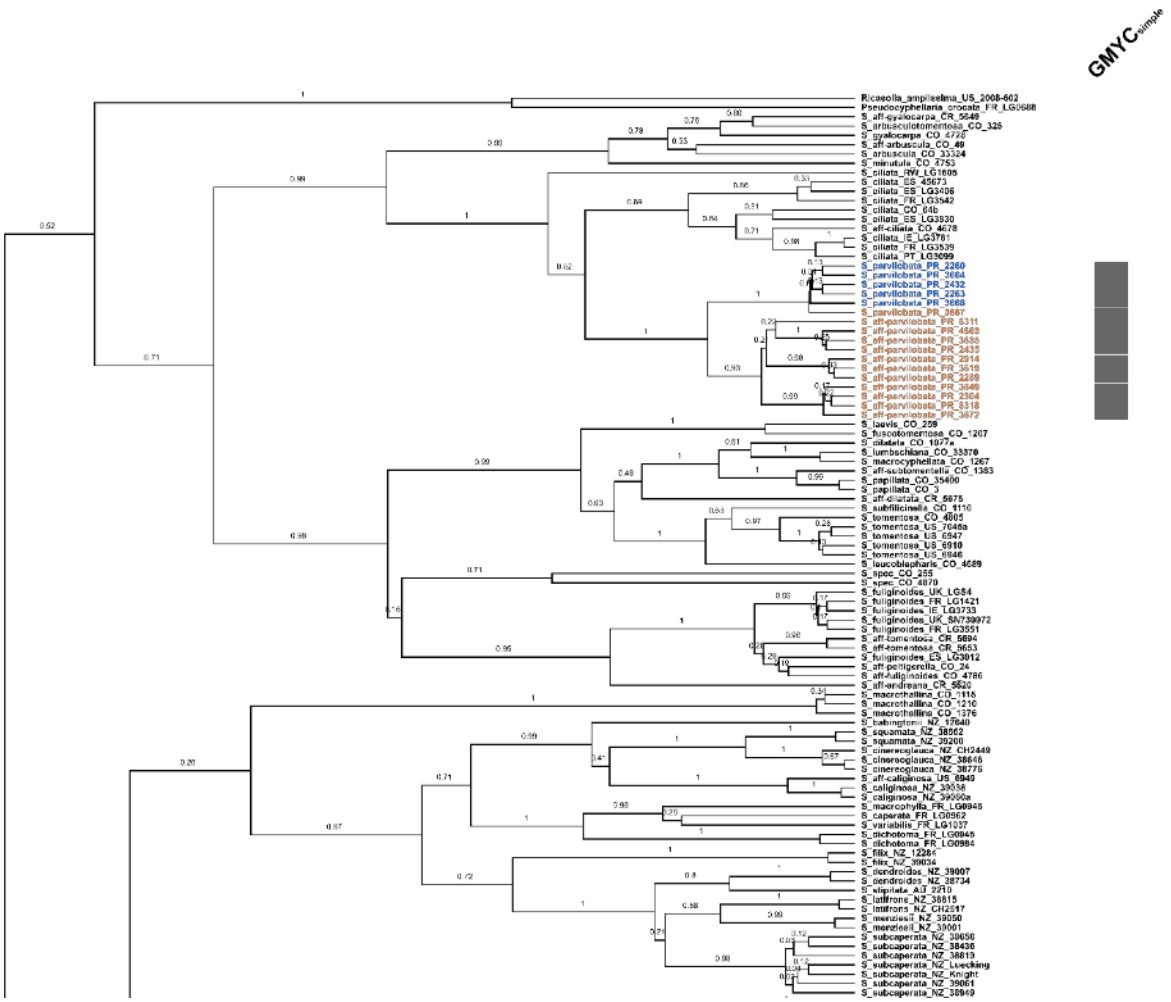

Figure S.1.5. Ultrametric maximum clade credibility (MCC) tree obtained from analysis of ITS sequences for 300 specimens of *Sticta* from Puerto Rico (80) and the rest of the world (220) in BEAST. Species boundaries delimited by GMYC are indicated in gray boxes. Sequences from Puerto Rico are highlighted in orange and blue. Bayesian posterior probabilities are indicated above branches.

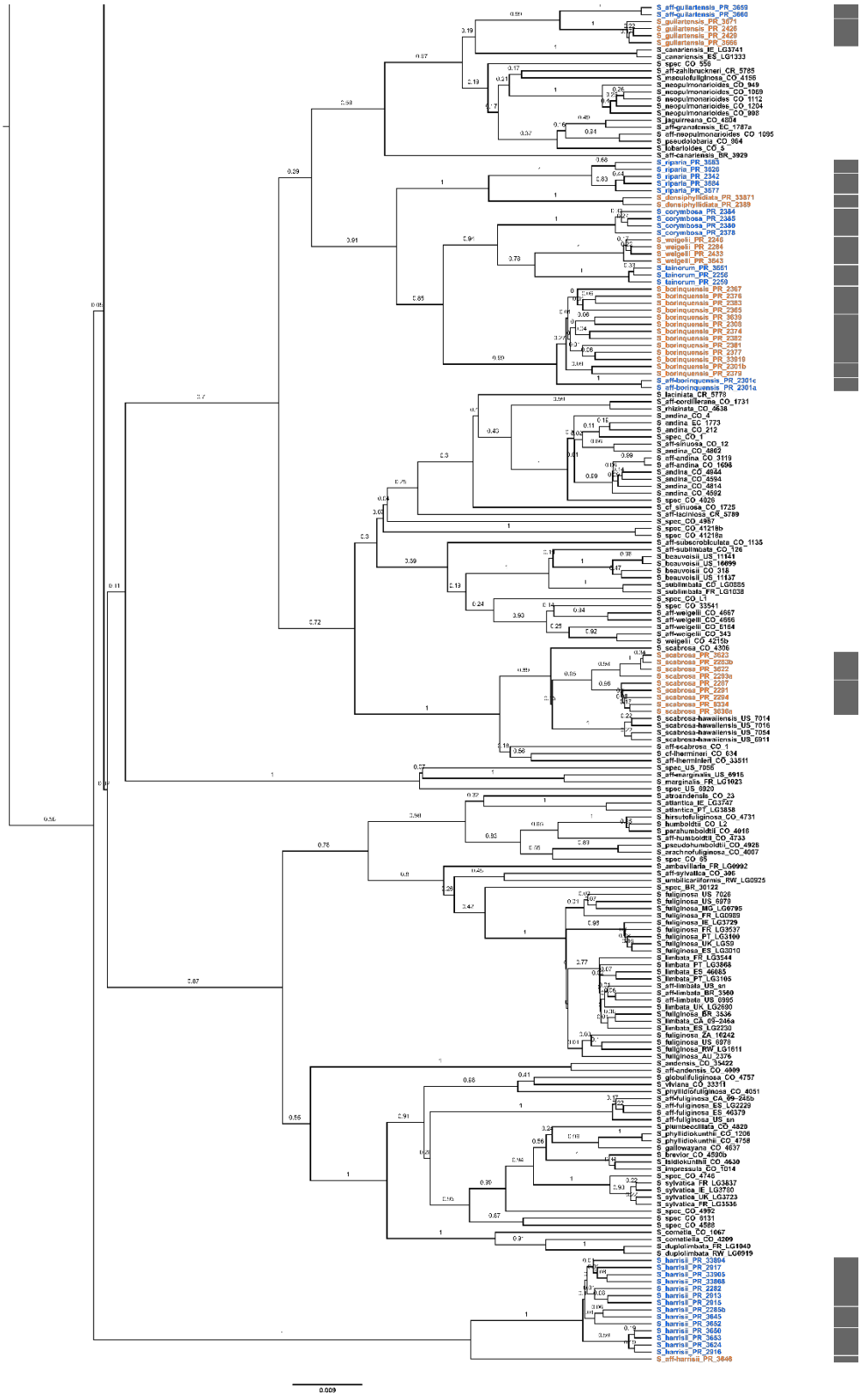

Figure S.1.5. Continued.

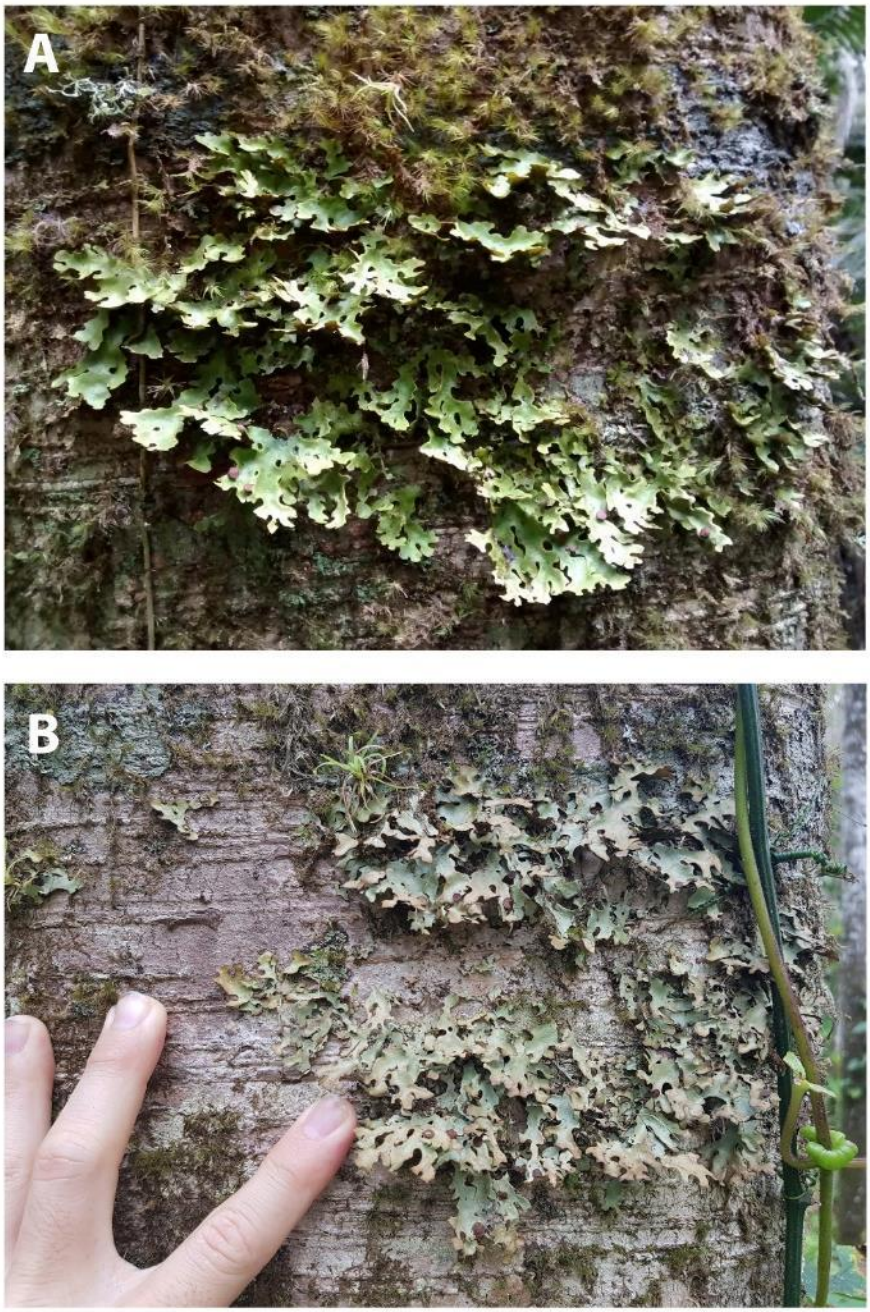

Figure S.1.6. Photographs taken before and after Hurricane María of the living individual of specimen Mercado-Diaz 2256 corresponding to *Sticta tainorum*. **A.** Bright green thallus of this individual surrounded by a healthy bryophyte flora. Photograph date: January 2014. **B.** Thallus showing considerable browning, especially near lobe margins. Surrounding bryophyte flora with signs of high mortality and reduced cover. Photograph date: July 2018.

APPENDIX 3. Additional details about DNA extraction, amplification and sequencing used in Chapter 2

DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research, Irvine, CA, USA). Small portions of thalli were removed and manually grinded with mortar and pestle. Liquid nitrogen was used to facilitate tissue breakdown. Apart from these steps, extractions followed manufacturer's instructions.

Primers and PCR conditions used in this study are described in detail in Mercado-Díaz et al. (2020) and Widhelm et al. (2018). Briefly, PCR amplification was carried out using MyTaq™ Red DNA Polymerase (Bioline, Taunton, MA, USA) using previously reported aliquots of primers, water and template DNA. Amplification products were visualized on 1% agarose gels and subsequently purified with Exo SAP-IT (USB, Cleveland, OH, USA). Cycle sequencing was performed using Big Dye Terminator v.3.1 (Applied Biosystems, Foster City, CA, USA) and the same primers used for amplification. An ABI 3730 (Applied Biosystems) automatic sequencer was used to obtain sequences. Molecular work was carried out at the Pritzker Laboratory for Molecular Systematics at the Field Museum, Chicago, IL, USA.

APPENDIX 4. Details about DEC analysis with dispersal limitations used in Chapter 2

Dispersal limitations were set by analyzing area–dispersal matrices which allocated different dispersal probabilities to different periods of time (see table below). We applied a dispersal constraint scheme similar to (Cano et al. 2018) which assigns a dispersal probability of $p = 1$ for dispersal between adjacent areas, $p = 0.5$ for dispersal over the Caribbean Sea or over non-adjacent areas and 0.01 for dispersal over oceans. Sensitivity tests were not carried out since no significant differences in terms of loglikelihoods for biogeographic reconstruction have been found under different dispersal probabilities (e.g. $p = 0.1$ vs. $p = 0.001$) (Cano et al. 2018).

We evaluated the four time periods defined by Cano et al. 2018 using adjustments that accounted for our biogeographic areas: (1) 90–33 Mya: increase probability of dispersal between NA and PA, (2) 33–15 Mya: land bridges connecting NA and PA were no longer available (Brikiatis 2014); (3) 15–7 Mya: Panama Isthmus closure (Montes et al. 2015); and (4) 7 Mya-present: final uplift of the Northern Andes acting as a barrier for dispersal between Amazonia and the Chocó region (Luebert and Weigend 2014). Results are presented in Fig. S.2.2.

APPENDIX 5. Details about GeoSSE analysis used in Chapter 2.

Analysis with GeoSSE requires lineages to be assigned one of three geographic character states: either present in one of two regions (i.e. endemic to region "A" or region "B") or present in both regions (i.e. "AB" distribution). Accordingly, and following indications above, we used BEAST to generate a time-calibrated tree (119 tips) that included species restricted to either the continental Neotropics (65) or the Caribbean islands (36), and species that occur in both regions (18). Sampling fractions were based on the global ITS dataset used for candidate species delimitation and were set as follows: 70% (both regions), 35% (endemic to the continental Neotropics), and 90% (endemic to the Caribbean).

ML model construction and constraining were carried out with diversitree functions "make.geosse" and "constrain", respectively. Parameter estimates for the different models were obtained with the function "find.mle". Models were compared using likelihood ratio tests. A posterior probability distribution of parameter estimates for the full model was also generated with the "mcmc" function in diversitree (nsteps = 10,000). The chain started with parameter estimates obtained from Maximum Likelihood and used a broad exponential prior probability distribution of 1/2. A burnin of 1,000 was applied. Lastly, root states of two additional unconstrained models were fixed to either the continental Neotropics or the Caribbean. We compare model selection and parameter estimates from fixed and unfixed root models to better understand dispersal asymmetries.

APPENDIX 6. Details about GeoSSE simulation analysis performed in Chapter 2.

We used four different transition rates (q) (0.05, 0.1, 1, and 10) to simulate the evolution of neutral and random traits on our MCC tree. Only simulated trees (100) with three states and more than 10% of species in each state were allowed to avoid biases related to sampling (Davis, Midford, and Maddison 2013). Simulation was similar for both trait types, except that tip states were reshuffled for random traits trees. A full GeoSSE model was fit to simulated trees using the same sampling fraction of our empirical analysis. Two additional (null) models, one without between-region speciation (sAB ~ 0) and another without regional dependence of dispersal rates (dA ~ dB) were generated by constraining the full (alternative) model. Models were compared using likelihood ratio tests and p-values were extracted to estimate error rates (i.e. visualize how often the null models where rejected when they were true).

APPENDIX 7. Methodological details about BAMM analysis performed in Chapter 2.

BAMM is a statistical framework that uses a reversible jump Markov chain Monte Carlo (rjMCMC) sampler to ultimately identify the number and location of so called "rate shifts", transitions in evolutionary parameters along branches of a phylogenetic tree. Except for Clades IV and V, sampling fractions used by Widhelm et al. (2018) were updated according to the global ITS dataset used for candidate species delimitation and set using the "SamplesProbsFilename" argument in the control file (Clade I: 65%, Clade II: 60%, Clade III: 50%). Outgroups were removed from analysis as Widhelm et al. (2018) showed they had no noticeable effect on BAMM inferences. The function "setBAMMpriors" from the R package BAMMtools (Rabosky et al. 2014) was used to find appropriate prior parameters. We ran four parallel chains of 10,000,000 generations with sampling frequency set at 5000. Output files "mcmcout" and "eventdata" were analyzed with BAMMtools and used to assess convergence, calculate effective sample sizes (ESS) of parameters and visualize rates. The R package coda v. 0.19-4 (Plummer et al. 2006) was used to estimate ESS values. Twenty percent of trees were discarded as burnin. Our phylorate plot was generated with the "plot.bammdata" function, the net diversification rate through time plot with the function "plotRateThroughTime" whereas the lineage through time plot was obtained with the "ltt.plot" function.

APPENDIX 8. Environmental and geographic parameters for phylobetadiversity analysis used in Chapter 2.

- **Environmental distances**

Environmental parameters used for phylobetadiversity were obtained using GIS analysis and cloud computing for visualization of remotely sensed data. To do this, we first loaded specimen locality data in ArcGIS (ESRI, 2016) and defined 10 km2 quadrats that captured most of the locality points within each of the sampling areas identified in Table S.2.1. We characterized climate patterns for these areas by uploading and analyzing quadrats in the ClimateEngine web browser (http://climateengine.org/app), a cloud computing tool that uses Google's Earth Engine (Gorelick et al. 2017) for on-demand processing of satellite and climate data. The TerraClimate dataset, which is based on WorldClim and CRU Ts4.0 and JRA55 data, was used for obtaining data on precipitation (which was characterized using the Standardized Precipitation Index (SPI) (McKee, 1993) and maximum and minimum temperatures. Data from the USGS MODIS Eta was used to estimate reference evapotranspiration (Eto). Estimates for the NDVI and the EVI indices were obtained using data from the USGS Modis Terra/Aqua sensor. Both NDVI and EVI are vegetation vigor, or "greenness" metrics but EVI minimizes adverse effects that derive from the soil background and atmospheric nuances (A. Huete, Justice, and van Leeuwen 1999). Single-point estimates for all parameters were obtained by averaging monthly or bi-monthly values recorded for each island in 2019. On the other hand, values for the TRI index were obtained by implementing the original algorithm (Riley, DeGloria, and Elliot 1999). This analysis was carried out in Google Earth Engine and used the Shuttle Radar Topography Mission (SRTM, ver. 4) digital elevation dataset. Due to computing limitations, it was necessary to add a 1.2 scale factor to obtain TRI estimates. Calculations were therefore based on a 36 m resolution value (30 m [native SRTM resolution] x 1.2 [scaling parameter]). This resulted in ruggedness ranging from 0.39 (less rugged) to 0.99 (more rugged). TRI estimates were first obtained for our sampling area quadrats and then averaged by island.

- **Geographic distances**

To generate a matrix of inter-island geographic distances, we first used ArcGIS to draw for each island a polygon with vertices representing a single georeferenced sampling locality within each of our sampling areas. We then used the "Calculate Geometry" function in ArcGIS to obtain coordinates for polygon centroids. These coordinates were uploaded in R and the function "distm" (fun = distGeo) from the R package geosphere (Hijmans 2019) was used to calculate linear distances (in kilometers) between island centroids.

Table S.2.1. Details about collecting areas for *Sticta* in the Caribbean. Vegetation types follow the "Terrestrial Ecoregions, Major Habitat Types, Biogeographic Realms" map from The Nature Conservancy (Olson et al. 2001). Due to similarities observed, two localities were attributed to ID "CUB03". The geographic distribution of localities is shown in Fig. 2.1.

ID	Geographic area	Island	Province	Vegetation type	Elevational range (m)	Collection dates
CUB01	Paisaje Natural Protegido Gran Piedra	Cuba	Santiago de Cuba	Tropical and Subtropical Moist Broadleaf Forests	920-1131	June 19 – July 17, 2016
CUB02	Parque Nacional Turquino	Cuba	Granma / Santiago de Cuba	Tropical and Subtropical Moist Broadleaf Forests	878-1955	July 7– July 9, 2016
CUB03	Parque Natural Protegido Topes de Collantes, Trinidad	Cuba	Sancti Spiritus	Tropical and Subtropical Moist Broadleaf Forests	650-860	March 19, 2018
CUB03	Las Vegas de Matagua, Cumanayagua	Cuba	Cienfuegos	Tropical and Subtropical Moist Broadleaf Forests	665	March 21, 2018
CUB04	El Yunque	Cuba	Guantánamo	Tropical and Subtropical Moist Broadleaf Forests	40-560	Februrary 27, 2019
JAM01	Blue Mountains National Park	Jamaica	St. Thomas / Portland	Tropical and Subtropical Moist Broadleaf Forests	1684-2165	April 8 – April 23, 2018
JAM02	Cinchona Botanical Garden	Jamaica	St. Andrew	Tropical and Subtropical Moist Broadleaf Forests	1670-1680	April 8 – April 23, 2018
DOR01	Parque Nacional Sierra de Bahoruco	Dominican Republic	Pedernales	Tropical and Subtropical Coniferous Forests	1410-1739	July 12 – July 14, 2017
DOR02	Parque Nacional Sierra de Neiba	Dominican Republic	Independencia	Tropical and Subtropical Coniferous Forests	1684-1911	July 25 – July 26, 2017
DOR03	Parque Nacional Jose del Carmen Ramirez	Dominican Republic	San Juan / Manabao	Tropical and Subtropical Coniferous Forests	1115-2136	July 18 – July 21, 2017
PUR01	Puerto Rico	Puerto Rico	Several municipalities	Tropical and Subtropical Moist Broadleaf Forests	200-1,200	Continuously since 2015

Table S.2.1. Continued.

GUAD01	Pitons de Bouillante – Parc National de la Guadeloupe	Guadeloupe	Vieux-Habitants	Tropical and Subtropical Moist Broadleaf Forests	621-957	May 2, 2019
GUAD02	Sentier d' interpretation du Matouba – Parc National de la Guadeloupe	Guadeloupe	Saint-Claude	Tropical and Subtropical Moist Broadleaf Forests	678-914	April 30 – May 4, 2019
DOM01	Syndicate Visitor Center – Morne Diablotin National Park	Dominica	St. Peter	Tropical and Subtropical Moist Broadleaf Forests	587-873	July 31 – Aug 11, 2017 and April 14 – 21, 2019
DOM02	Mourne Trois Pitons National Park	Dominica	St. Paul / St. George	Tropical and Subtropical Moist Broadleaf Forests	360-828	July 31 – Aug 11, 2017 and April 14 – 21, 2019
DOM03	Soufriere Sulfur Springs National Park	Dominica	St. Mark	Tropical and Subtropical Moist Broadleaf Forests	90-100	July 31 – Aug 11, 2017 and April 14 – 21, 2019
MAR01	Sentier PR Montagne Pelée par l' Aileron	Martinique	St. Pierre	Tropical and Subtropical Moist Broadleaf Forests	625-906	April 21 -28, 2019
MAR02	Piton Boucher / Piton Alma / Morne Bellevue / Mourne de Lorrain	Martinique	Fort-de-France / Gros Morne / La Trinite / St. Joseph	Tropical and Subtropical Moist Broadleaf Forests	408-745	April 21 -28, 2019
MAR03	Montagne du Vauclin	Martinique	Saint Esprit	Tropical and Subtropical Moist Broadleaf Forests	430-450	April 21 -28, 2019

Table S.2.2. Partitioning schemes and best fit substitution models for multilocus datasets used in analyses presented in this work. Information on number of samples included in each analysis, number of loci and percent missing data is summarized first. Other columns show the following data: number of sequences in partition (Seq), total number of sites in partition (Site), number of unique site patterns (Unique), number of parsimony-informative sites (Infor), best substitution model for partition (Model), Bayesian information criterion scores (BIC). RAxML analysis used the same partitioning scheme as MrBayes but was based on the GTRGAMMA model..

Analysis	Number of species	# loci included	Missing data	Partitions	Seq	Site	Unique	Infor	Model	BIC
MrBayes	162	6	22.70%	18s_rRNA+28S_rRNA+MCM7_pos2+RPB1_pos2+RPB2	162	930	385	20	HKY+F+G4	4252.31
				ITS1+ITS2	161	412	376	236	GTR+F+G4	20171.5
				5-8_rRNA+MCM7_pos1	162	373	169	38	K2P+I+G4	3389.3
				MCM7_pos3	114	216	205	136	K2P+G4	8645.25
				mtSSU	107	1189	653	221	GTR+F+I+G4	15803.7
				nuLSU	152	594	295	103	SYM+I+G4	8401.77
				RPB1_pos1+RPB2_pos1	85	645	252	22	GTR+F+I	3269.07
				RPB1_pos3+RPB1_intron+RPB2_pos3	85	709	556	306	SYM+G4	11839.7
				Alignment length:		**5068**				
Divergence dating + biogeographic reconstruction + BAMM (BEAST)	162	4	16.57%	18s_rRNA+28S_rRNA+MCM7_pos2	161	285	163	8	K2P+G4	1614.75
				ITS1+ITS2	161	412	376	236	TIM2+F+G4	19921
				5-8_rRNA+MCM7_pos1	162	373	169	38	K2P+I+G4	3427.69
				MCM7_pos3	114	216	205	136	K2P+G4	8727.05
				mtSSU	107	1189	653	221	TVM+F+I+G4	15753.2
				nuLSU	152	594	295	103	TIM2e+I+G4	8412.02
				Alignment length:		**3069**				

Table S.2.2. Continued.

GeoSSE (BEAST)	119	4	20.59%	18s_rRNA+5-8_rRNA+28S_rRNA+MCM7_pos1+nuLSU	119	1036	466	116	TNe+I+G4	9535.6
				ITS1+ITS2	118	412	324	184	TIM2+F+G4	14021.9
				MCM7_pos2	89	216	96	4	K2P	933.664
				MCM7_pos3	89	216	198	122	K2P+G4	6887.52
				mtSSU	67	1189	497	144	TPM3u+F+I+G4	10294.7
				Alignment length:		**3069**				
Phylo-betadiversity (BEAST)	62	6	10.01%	18s_rRNA+MCM7_pos1+nuLSU+RPB1_pos1+RPB2_pos1	63	1483	531	98	TIM+F+I+G4	8285.17
				ITS1+ITS2	63	412	282	158	TIM+F+G4	8255.41
				5-8_rRNA+28S_rRNA+MCM7_pos2+RPB1_pos2+RPB2_p	63	1059	300	15	TIM2+F+I	3984.91
				MCM7_pos3+RPB1_pos3+RPB1_intron+RPB2_pos3	58	925	548	258	TIM3e+G4	10456.8
				mtSSU	40	1189	397	107	TPM3u+F+I+G4	7478.22
				Alignment length:		**5068**				

Table S.2.3. Matrix of dispersal constraint multipliers indicating the probability of dispersal between each set of areas in different time periods. SA: South America, CA: Caribbean, CAM: Central America, NA: North America, AF: Afrotropical, PA: Palearctic, OR: Oriental, HA: Hawaiian, AU: Australasian.

Time: 0-7 MY

	SA	CA	CAM	NA	AF	PA	OR	HA	AU
SA	1	0.5	1	0.01	0.01	0.01	0.01	0.01	0.01
CA	0.5	1	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CAM	1	0.5	1	1	0.01	0.01	0.01	0.01	0.01
NA	0.01	0.5	1	1	0.01	0.01	0.01	0.01	0.01
AF	0.01	0.01	0.01	0.01	1	0.5	0.5	0.01	0.01
PA	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
OR	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
HA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1	0.01
AU	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

Time: 7-15 MY

	SA	CA	CAM	NA	AF	PA	OR	HA	AU
SA	1	0.5	1	0.5	0.01	0.01	0.01	0.01	0.01
CA	0.5	1	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CAM	1	0.5	1	1	0.01	0.01	0.01	0.01	0.01
NA	0.5	0.5	1	1	0.01	0.01	0.01	0.01	0.01
AF	0.01	0.01	0.01	0.01	1	0.5	0.5	0.01	0.01
PA	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
OR	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
HA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1	0.01
AU	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

Time: 15-33 MY

	SA	CA	CAM	NA	AF	PA	OR	HA	AU
SA	1	0.5	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CA	0.5	1	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CAM	0.5	0.5	1	1	0.01	0.01	0.01	0.01	0.01
NA	0.5	0.5	1	1	0.01	0.01	0.01	0.01	0.01
AF	0.01	0.01	0.01	0.01	1	0.5	0.5	0.01	0.01
PA	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
OR	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
HA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1	0.01
AU	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

Time: 33-90 MY

	SA	CA	CAM	NA	AF	PA	OR	HA	AU
SA	1	0.5	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CA	0.5	1	0.5	0.5	0.01	0.01	0.01	0.01	0.01
CAM	0.5	0.5	1	1	0.01	0.01	0.01	0.01	0.01
NA	0.5	0.5	1	1	0.01	1	0.01	0.01	0.01
AF	0.01	0.01	0.01	0.01	1	0.5	0.5	0.01	0.01
PA	0.01	0.01	0.01	1	0.5	1	1	0.01	0.01
OR	0.01	0.01	0.01	0.01	0.5	1	1	0.01	0.01
HA	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1	0.01
AU	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1

Table S.2.4. Voucher information and GenBank accession numbers for taxa used in phylogenetic analyses in Chapter 2. Newly generated sequences tentatively identified with an "x".

ID	Taxon Corrected	Country	Collector	Voucher #	ITS	MCM7	mtSSU	nuLSU	RPB1	RPB2
DNA9930	*Lobaria pulmonaria*	U.S.A.	Widhelm	*s.n.*	MG367435	MF984336	MG754091	MG063078	MG754080	–
LG0688	*Pseudocyphellaria crocata*	France	Magain & Sérusiaux	*LG0688*	JQ735976	–	JQ736009	JQ735993	KT281770	–
RX50	*Ricasolia amplissima*	U.S.A.	Dillman	*2008-602*	KX385118	–	KC494188	–	–	KX385158
DNA4932	*Sticta aff. andina*	Colombia	Barragán	*12*	KC732476	MF984295	–	–	–	–
DNA7246	*Sticta aff. andreana*	Costa Rica	Moncada	*5620*	MG367402	MF984284	–	MG063062	–	–
DNA16979	*Sticta aff. andreana-2*	Jamaica	Mercado-Díaz	*3401*	x	–	x	x	–	x
DNA5443	*Sticta aff. arachnofuliginosa*	Colombia	Moncada	*4733*	KC732580	MF984309	MG754154	MG062948	–	–
DNA7291	*Sticta aff. arachnofuliginosa-2*	Colombia	Fonseca	*65*	MG367407	MF984213	–	–	–	–
DNA18170	*Sticta aff. borinquensis-2*	Martinique	Mercado-Díaz	*3981*	x	x	–	x	–	x
DNA15628	*Sticta aff. ciliata-2*	Dominican Republic	Mercado-Díaz	*3062e*	x	x	–	x	x	x
LG3539	*Sticta aff. ciliata-3*	France	Gérault	*LG3539*	KT281718	–	KT281674	KT281630	KT281774	–
DNA5474	*Sticta aff. ciliata-4*	Colombia	Moncada	*4678*	KC732607	MF984324	MG754144	MG063039	–	–
DNA15621	*Sticta aff. ciliata-5*	Dominican Republic	Mercado-Díaz	*3122b*	x	–	–	x	–	x
DNA7297	*Sticta aff. ciliosylvativa*	Colombia	Fonseca	*255*	MG367408	MF984209	MG754174	MG063061	–	–
DNA15654	*Sticta aff. cometiella*	Dominican Republic	Mercado-Díaz	*3034a*	x	x	x	x	–	x
DNA5022	*Sticta aff. cordillerana-2*	Colombia	Simijaca	*1731*	KC732553	MF984252	MG754120	MG062963	–	–
DNA7382	*Sticta aff. granatensis*	Ecuador	Dal Forno	*1787a*	MG367416	–	MG754117	MG062990	–	–
DNA15644	*Sticta aff. guilartensis-1*	Dominican Republic	Mercado-Díaz	*3072b*	x	x	x	x	x	
DNA17168	*Sticta aff. guilartensis-2*	Puerto Rico	Mercado-Díaz	*3660*	MN065865	x	MN065941	MN066012	MN066034	MN066103
MON6427	*Sticta aff. guilartensis-3*	Cuba	Mercado-Díaz	*44749*	x	x	–	x	–	x
DNA17162	*Sticta aff. harrisii*	Puerto Rico	Mercado-Díaz	*3646*	MN065842	MN065898	MN065949	MN066001	MN066038	MN066108
DNA16985	*Sticta aff. harrisii-1*	Jamaica	Mercado-Díaz	*3372*	x	–	–	x	–	x
DNA18160	*Sticta aff. harrisii-2*	Martinique	Mercado-Díaz	*3973a*	x	x	x	x	–	x
DNA15623	*Sticta aff. harrisii-3*	Dominican Republic	Mercado-Díaz	*3131e*	x	–	x	x	–	x

Table S.2.4. Continued.

MON6798	*Sticta aff. hypoglabra*	Cuba	Mercado-Díaz	*46012*	x	–	–	x	–	x
DNA7226	*Sticta aff. laciniosa*	Costa Rica	Moncada	*5789*	MG367401	MF984240	–	MG062988	–	–
DNA18211	*Sticta aff. laciniosa-2*	Guadeloupe	Mercado-Díaz	*4043*	x	–	–	–	–	–
DNA16757	*Sticta aff. laciniosa-3*	Jamaica	Mercado-Díaz	*3542a*	x	x	x	x	–	x
DNA5405	*Sticta aff. limbata*	Brazil	Lücking	*30122*	KC732568	MF984319	–	MG062954	–	–
DNA18220	*Sticta aff. maculofuliginosa*	Guadeloupe	Mercado-Díaz	*4057*	x	x	x	x	–	x
DNA8047	*Sticta aff. marginalis*	U.S.A.	Moncada	*6916*	MG754196	–	MG754095	MG062921	–	x
DNA17156	*Sticta aff. parvilobata*	Puerto Rico	Mercado-Díaz	*3635*	MN065887	MN065900	x	MN065979	MN066022	MN066124
DNA6299	*Sticta aff. pseudohumboldtii*	Colombia	Moncada	*4928*	KC732736	MF984307	–	MG062947	–	–
DNA16761	*Sticta aff. pseudolobaria*	Jamaica	Mercado-Díaz	*3350*	x	x	–	x	–	x
DNA15675	*Sticta aff. puracensis*	Dominican Republic	Mercado-Díaz	*3155*	x	–	x	–	x	x
DNA15659	*Sticta aff. roseocyphellata*	Dominican Republic	Mercado-Díaz	*3062b*	x	x	–	–	x	x
DNA7259	*Sticta aff. scabrosa*	Costa Rica	Moncada	*5715b*	–	MF984264	MG754121	MG063077	–	–
MON6447	*Sticta aff. sinuosa-2*	Cuba	Mercado-Díaz	*45173*	x	x	–	x	–	x
DNA18219	*Sticta aff. sinuosa-3*	Guadeloupe	Mercado-Díaz	*4056*	x	–	–	x	–	x
DNA18228	*Sticta aff. sinuosa-4*	Guadeloupe	Mercado-Díaz	*4078*	x	x	–	x	–	x
DNA6292	*Sticta aff. sublimbatoides-2*	Colombia	Moncada	*4987*	KC732732	MF984333	–	MG062964	–	–
DNA5541	*Sticta aff. subscrobiculata*	Colombia	Coca	*1135*	KC732639	–	MG754096	MG062985	–	–
DNA6288	*Sticta aff. subtomentella*	Colombia	Coca	*1363*	KC732730	MF984259	–	MG063059	–	–
DNA16989	*Sticta aff. tainorum*	Jamaica	Mercado-Díaz	*3369a*	x	x	–	x	–	x
DNA7260	*Sticta aff. tomentosa*	Costa Rica	Moncada	*5694*	MG367406	MF984315	MG754157	MG063051	–	–
DNA16981	*Sticta aff. tomentosa-2*	Jamaica	Mercado-Díaz	*3384a*	x	x	x	x	–	x
DNA18205	*Sticta aff. tomentosa-3*	Guadeloupe	Mercado-Díaz	*4036*	x	x	–	x	–	–
DNA5467	*Sticta aff. weigelii-1*	Colombia	Moncada & Lücking	*4667*	MG367390	MF984299	MG754103	MG062983	–	–
MON6448	*Sticta aff. weigelii-2*	Cuba	Mercado-Díaz	*45218*	x	x	x	x	–	x
DNA18115	*Sticta aff. weigelii-3*	Dominica	Mercado-Díaz	*3884*	x	x	x	x	x	x
DNA15673	*Sticta aff. weigelii-4*	Dominican Republic	Mercado-Díaz	*3066*	x	–	x	x	x	x
DNA7224	*Sticta aff. zahlbruckneri*	Costa Rica	Moncada	*5785*	MG367400	MF984318	–	MG062991	–	–

Table S.2.4. Continued.

DNA5030	*Sticta albocyphellata*	Colombia	Moncada	*4588*	KC732557	–	MG754114	MG062937	–	–
DNA8197	*Sticta albohypoarbuscula*	U.S.A.	Moncada	*7056*	MG367434	MF984210	MG754094	MG062923	–	x
LG0992	*Sticta ambavillaria*	France	Magain & Sérusiaux	*LG0992*	JQ735978	–	JQ736011	JQ735995	–	–
DNA7373	*Sticta andensis*	Colombia	Lücking & Moncada	*35422*	KC732548	MF984317	MG754134	MG062956	–	–
DNA5003	*Sticta andina*	Colombia	Alfonso	*4*	KC732537	MF984248	MG754099	MG062967	MG754084	x
DNA6237	*Sticta andreana*	Colombia	Vargas & Herrera	*634*	MG367393	MF984331	–	MG063009	–	–
DNA4985	*Sticta arachnofuliginosa*	Colombia	Moncada	*4007*	KC732524	MF984306	–	MG062946	–	–
DNA5599	*Sticta arbuscula*	Colombia	Lücking & Moncada	*33324*	KC732682	–	–	MG063046	–	–
DNA5424	*Sticta arbusculotomentosa*	Colombia	Betancourt	*326*	KC732572	MF984220	–	MG063041	–	–
LG3858	*Sticta atlantica*	Azores		*3858*	KT281737	–	KT281693	KT281648	KT281784	–
DNA4999	*Sticta atroandensis*	Colombia	Fonseca	*23*	KC732533	MF984310	–	MG062952	MG754082	–
DNA14282	*Sticta babingtonii*	New Zealand	de Lange	*12640*	MF373808	MF984256	MG754167	MG063012	–	–
LG3303	*Sticta beauvoisii*	U.S.A.	Goffinet	*11137*	KT281725	–	KT281681	KT281636	KT281787	–
DNA17159	*Sticta borinquensis*	Puerto Rico	Mercado-Díaz	*3639*	MN065860	x	x	MN065966	MN066017	MN066122
DNA4914	*Sticta brevior*	Colombia	Moncada	*4590b*	MG367386	–	MG754108	MG062929	–	–
DNA13507	*Sticta caliginosa*	New Zealand	Lücking et. al.	*39060a*	MF373760	MF984229	MG754135	MG063035	–	–
LG3741	*Sticta canariensis*	Ireland	Sérusiaux	*LG3741*	KT281733	–	KT281689	KT281644	KT281779	–
LG0962	*Sticta caperata*	France	Magain & Sérusiaux	*LG0962*	JQ735979	–	JQ736012	JQ735996	KT281745	–
DNA16959	*Sticta carolinensis*	Jamaica	Mercado-Díaz	*3356b*	x	x	–	x	–	x
DNA15650	*Sticta cf. laciniata*	Dominican Republic	Mercado-Díaz	*3149*	x	–	–	x	–	–
DNA15648	*Sticta cf. laciniata-3*	Dominican Republic	Mercado-Díaz	*3119a*	x	–	–	x	–	–
DNA17048	*Sticta cf. laciniosa-1*	Dominican Republic	Mercado-Díaz	*2962*	x	x	x	x	–	x
DNA5027	*Sticta cf. sinuosa*	Colombia	Simijaca	*1725*	KC732554	MF984296	–	MG062977	–	–
LG1605	*Sticta ciliata*	Rwanda	Sérusiaux	*LG1605*	KT281717	–	KT281673	KT281629	KT281763	–
DNA6336	*Sticta ciliosylvativa*	Colombia	Moncada	*4870*	MG367395	MF984205	–	MG063060	–	–
DNA13863	*Sticta cinereoglauca*	New Zealand	Lücking et. al.	*38646*	MF373798	MF984241	MG754140	MG063029	–	–
DNA5526	*Sticta cometia*	Colombia	Coca	*1067*	KC732626	MF984222	MG754178	MG062927	–	–
DNA4977	*Sticta cometiella*	Colombia	Moncada	*4209*	KC732517	MF984221	MG754177	MG062926	–	x

Table S.2.4. Continued.

DNA1465	*Sticta corymbosa*	Puerto Rico	Mercado-Díaz	*2380*	MN065844	–	–	MN066003	MN066054	MN066098
DNA6179	*Sticta delicatula*	Colombia	Vargas & Herrera	*556*	MG367391	MF984237	MG754119	MG062998	–	–
DNA1387	*Sticta dendroides*	New Zealand	Lücking et. al.	*38734*	MF373799	MF984272	MG754188	MG063025	–	–
DNA1812	*Sticta densiphyllidiata*	Dominica	Mercado-Díaz	*3892*	x	x	x	x	–	x
LG0945	*Sticta dichotoma*	France	Magain & Sérusiaux	*LG0945*	JQ735981	–	JQ736014	JQ735998	KT281743	–
DNA5550	*Sticta dilatata*	Colombia	Coca	*1077a*	KC732647	–	MG754125	MG063057	–	–
LG1040	*Sticta duplolimbata*	France	Magain & Sérusiaux	*LG1040*	JQ735984	–	JQ736001	JQ736017	KT281751	–
DNA1297	*Sticta filix*	New Zealand	de Lange	*12284*	MG367379	MF984228	–	MG063010	–	–
DNA7345	*Sticta fuliginoides*	Colombia	Buitrago	*24*	MG367410	MF984216	MG754158	MG063049	–	–
DNA7395	*Sticta fuliginosa*	Brazil	Gumboski	*3536*	MG367419	MF984303	MG754184	MG062939	–	x
DNA5568	*Sticta fuscotomentosa*	Colombia	Coca	*1207*	KC732661	MF984280	MG754126	MG063070	–	–
DNA4959	*Sticta gallowayana*	Colombia	Moncada	*4637*	KC732496	MF984285	–	MG062934	MG754087	x
DNA5475	*Sticta globulifuliginosa*	Colombia	Moncada	*4757*	KC732608	–	–	MG062924	–	–
DNA1717	*Sticta guilartensis*	Puerto Rico	Mercado-Díaz	*3671*	MN065864	MN065906	MN065954	MN065959	MN066031	MN066120
DNA5457	*Sticta gyalocarpa*	Colombia	Moncada	*4728*	KC732594	MF984327	MG754111	MG063043	MG754089	–
DNA7250	*Sticta gyalocarpoides*	Costa Rica	Moncada	*5649*	MG367403	MF984326	–	MG063044	–	–
DNA1716	*Sticta harrisii*	Puerto Rico	Mercado-Díaz	*3645*	MN065838	MN065914	MN065947	MN066000	MN066039	MN066111
DNA5477	*Sticta hirsutofuliginosa*	Colombia	Moncada	*4731*	KC732610	MF984311	MG754152	MG062950	–	–
DNA6199	*Sticta humboldtii*	Colombia	Díaz-Escandón	*L2*	KC732702	MF984312	MG754118	MG062951	–	–
DNA5586	*Sticta hypoglabra*	Colombia	Lücking &	*33541*	KC732667	MF984322	MG754104	MG063001	–	–
DNA5549	*Sticta impressula*	Colombia	Coca	*1014*	KC732646	MF984287	MG754110	MG062931	–	–
DNA6339	*Sticta isidioimpressula*	Colombia	Moncada	*4992*	KC732761	MF984219	–	MG062936	–	–
DNA4982	*Sticta isidiokunthii*	Colombia	Moncada	*4630*	KC732522	MF984288	MG754189	MG062930	MG754088	–
DNA8081	*Sticta isidiopedunculata*	U.S.A.	Moncada	*6949*	MG367425	MF984211	MG754137	MG063037	–	x
DNA5607	*Sticta jaguirreana*	Colombia	Moncada	*4804*	MG754195	–	MG754162	MG062999	–	–
DNA7223	*Sticta laciniata*	Costa Rica	Moncada	*5778*	MG367399	–	MG754179	MG062984	–	–
DNA7301	*Sticta laevis*	Colombia	Fonseca	*259*	MG367409	MF984206	–	MG063052	–	–
DNA5589	*Sticta laselvae*	Colombia	Lücking &	*33511*	KC732673	MF984269	MG754145	MG063008	–	–

Table S.2.4. Continued.

DNA13538	*Sticta latifrons*	New Zealand	de Lange	*CH2517*	MF373763	MF984230	MG754173	MG063015	–	–
DNA5460	*Sticta leucoblepharis*	Colombia	Moncada	*4689*	KC732597	MF984276	–	MG063063	–	–
DNA8131	*Sticta limbata*	U.S.A.	Moncada	*6995*	MG367428	MF984298	MG754181	MG062940	–	x
DNA5028	*Sticta lobarioides*	Colombia	Alfonso	*5*	KC732555	MF984238	MG754113	MG062992	–	x
DNA4939	*Sticta lobulata*	Colombia	Álvaro	*41218a*	KC732482	MF984271	MG754098	MG062960	–	x
DNA5436	*Sticta lumbschiana*	Colombia	Lücking & Moncada	*33370*	KC732575	MF984212	MG754124	MG063055	–	x
DNA5569	*Sticta macrocyphellata*	Colombia	Coca	*1267*	KC732662	MF984313	–	MG063056	–	–
DNA5511	*Sticta macrogyalocarpa*	Colombia	Fonseca	*49*	KC732619	–	MG754092	MG063045	MG754090	–
LG0946	*Sticta macrophylla*	France	Magain & Sérusiaux	*LG0946*	JQ735985	–	JQ736018	JQ736002	KT281744	–
DNA5539	*Sticta macrothallina*	Colombia	Coca	*1115*	KC732629	MF984208	MG754122	MG063034	–	–
DNA4975a	*Sticta maculofuliginosa*	Colombia	Moncada	*4156*	KC732514	MF984235	–	–	–	x
DNA8052	*Sticta maculohyposcrobiculata*	U.S.A.	Moncada	*6920*	MG367423	MF984302	MG754093	MG062922	–	x
LG1023	*Sticta marginalis*	France	Magain & Sérusiaux	*LG1023*	JQ735980	–	JQ736013	JQ735997	KT281748	–
DNA13522	*Sticta menziesii*	New Zealand	Lücking et. al.	*39050*	MF373761	MF984225	MG754191	MG063013	–	–
DNA5446	*Sticta minutula*	Colombia	Moncada	*4753*	KC732583	MF984297	–	MG063042	–	–
DNA5524	*Sticta neopulmonarioides*	Colombia	Coca	*949*	KC732625	MF984204	MG754115	–	–	–
DNA5021	*Sticta papillata*	Colombia	Alfonso	*3*	KC732552	MF984232	MG754123	MG063053	–	x
DNA5019	*Sticta parahumboldtii*	Colombia	Moncada	*4016*	KC732550	MF984308	MG754151	MG062949	–	–
DNA4921	*Sticta paralimbata*	Colombia	Valbuena	*126*	KC732466	–	–	MG062959	–	–
DNA17172	*Sticta parvilobata*	Puerto Rico	Mercado-Díaz	*3667*	MN065879	MN065922	–	MN065975	MN066019	MN066117
DNA4958	*Sticta phyllidiofuliginosa*	Colombia	Moncada	*4051*	KC732495	MF984329	–	–	–	–
DNA5456	*Sticta phyllidiokunthii*	Colombia	Moncada	*4758*	KC732593	MF984291	MG754112	MG062932	–	–
DNA6346	*Sticta plumbeociliata*	Colombia	Moncada	*4820*	KC732767	MF984290	–	MG062935	–	–
DNA4934	*Sticta pseudobeauvoisii*	Colombia	Ardila	*1*	KC732478	MF984265	MG754143	MG063007	–	–
DNA7254	*Sticta pseudodilatata*	Costa Rica	Moncada	*5675*	MG367405	MF984214	MG754127	MG063058	–	–
DNA4922	*Sticta pseudolimbata*	Colombia	Moncada	*4009*	KC732467	MF984316	MG754142	MG062955	–	–
DNA5556	*Sticta pseudolobaria*	Colombia	Coca	*964*	KC732650	–	–	MG062996	–	–
DNA16962	*Sticta pulmonarioides*	Jamaica	Mercado-Díaz	*3436a*	x	x	–	x	–	x

Table S.2.4. Continued.

DNA6198	*Sticta puracensis*	Colombia	Díaz-Escandón	*L1*	KC732701	MF984243	MG754175	–	–	–
DNA4953	*Sticta rhizinata*	Colombia	Moncada	*4638*	KC732491	–	MG754097	MG062962	–	x
DNA17178	*Sticta riparia*	Puerto Rico	Mercado-Díaz	*3684*	MN065893	MN065928	MN065942	MN066008	MN066035	MN066105
DNA16987	*Sticta roseocyphellata*	Jamaica	Mercado-Díaz	*3374b*	x	x	x	x	–	x
DNA17157	*Sticta scabrosa*	Puerto Rico	Mercado-Díaz	*3636a*	MN065875	MN065929	x	MN065986	–	x
MON6812	*Sticta sp-1*	Cuba	Mercado-Díaz	*46701*	x	x	–	x	–	x
DNA16146	*Sticta sp-11*	Dominican Republic	Mercado-Díaz	*3129b*	x	x	x	x	–	x
DNA16971	*Sticta sp-13*	Jamaica	Mercado-Díaz	*3510a*	x	x	x	x	–	x
DNA16969	*Sticta sp-2*	Jamaica	Mercado-Díaz	*3571*	x	–	x	x	–	x
DNA16966	*Sticta sp-3*	Jamaica	Mercado-Díaz	*3463a*	x	x	–	x	–	x
DNA16960	*Sticta sp-4*	Jamaica	Mercado-Díaz	*3462a*	x	–	x	x	–	x
DNA6276	*Sticta sp-5*	Colombia	Suárez	*306*	KC732724	MF984335	–	MG062953	–	–
MON6423	*Sticta sp-7*	Cuba	Mercado-Díaz	*44655*	x	–	x	x	–	x
DNA16991	*Sticta sp-9*	Jamaica	Mercado-Díaz	*3425b*	x	–	–	x	–	–
DNA13526	*Sticta squamata*	New Zealand	Lücking et. al.	*38562*	MG367381	MF984260	MG754138	MG063030	–	–
DNA4925	*Sticta squamifera*	Colombia	Moncada	*4026*	KC732470	MF984217	–	MG062965	–	–
DNA14462	*Sticta stipitata*	Australia	Lumbsch et al.	*2210*	MG754197	MF984274	MG754141	MG063024	–	–
DNA13781	*Sticta subcaperata*	New Zealand	Lücking et. al.	*38436*	MG367383	MF984270	MG754172	MG063018	–	–
LG0885	*Sticta sublimbata*	D.R. Congo	Sérusiaux	*LG0885*	JQ735986	–	JQ736019	JQ736003	KT281771	–
LG3536	*Sticta sylvatica*	France	Gérault	*LG3536*	KT281726	–	KT281682	KT281637	KT281788	–
DNA17169	*Sticta tainorum*	Puerto Rico	Mercado-Díaz	*3661*	MN065868	MN065937	MN065940	MN065962	MN066055	MN066121
DNA8078	*Sticta tomentosa*	U.S.A.	Moncada	*6946*	MG367424	MF984278	MG754131	MG063069	–	x
LG0925	*Sticta umbilicariiformis*	Rwanda	Sérusiaux	*LG0925*	KT281697	–	KT281655	KT281652	KT281742	–
LG1037	*Sticta variabilis*	France	Magain & Sérusiaux	*LG1037*	JQ735987	–	JQ736020	JQ736004	KT281749	–
DNA5593	*Sticta viviana*	Colombia	Lücking & Moncada	*33311*	KC732680	–	MG754155	MG062925	–	–
DNA17160	*Sticta weigelii*	Puerto Rico	Mercado-Díaz	*3643*	MN065895	MN065938	x	MN066011	MN066057	MN066116

Table S.2.5. Differences in ancestral ranges inferred with and without dispersal constraints for strongly supported nodes containing Caribbean taxa. Refer to page 98 for acronym meanings.

Clade	Node #	Taxa	+ dispersal constraints	- dispersal constraints
I	199	*S. roseocyphellata, S. aff. roseocyphellata*	CA	SA
I	205	*S. brevior, Sticta sp. 3, S. isidiokunthii*	CAM	SA, CAM
I	208	*S. sylvatica, S. impressula, S. phyllidiokunthii, S. gallowayana, S. plumbeociliata, S. isidiokunthii, S brevior, Sticta sp. 3*	SA, PA	SA
II	288	*S. parvilobata, S. ciliata, S. aff. ciliata-3, S. aff. parvilobata, S. aff. ciliata-5*	CA, CAM, AF, OR	CA
II	290	*S. aff. ciliata-4, S. aff. ciliata-2, S. parvilobata, S. ciliata, S. aff. ciliata-3, S. aff. parvilobata, S. aff. ciliata-5*	CA	SA, CA
II	291	*S. gyalocarpoides, S. gyalocarpa, S. aff. ciliata-4, S. aff. ciliata-2, S. parvilobata, S. ciliata, S. aff. ciliata-3, S. aff. parvilobata, S. aff. ciliata-5*	CAM	SA
II	311	*S. aff. andreana, S. aff. tomentosa, S. fuliginoides*	CA, CAM, NA, PA, OR, AU	SA, CAM
II	312	*S. tomentosa, S. leucoblepharis, S. aff. tomentosa-3, S. aff. tomentosa-2, S. fuscotomentosa, S. aff. harrisii, S. aff. harrisii-2, S. harrisii, S. aff. harrisii-3, S. aff. harrisii-1, S. fuliginoides, S. aff. tomentosa, S. aff. andreana, S. aff. andreana-2*	CA, CAM, NA, PA, OR, AU	SA
II	318	All species within Clade II *(*Node "D" in Fig. 2.3*)*	SA, CA	SA
III	230	*S. riparia, S. densiphyllidiata, S. aff. laciniosa, S. aff. laciniosa2, S. aff. laciniosa-3 (*Node "E" in Fig. 2.3*)*	CA, CAM	CA
III	238	*S. delicatula, S. granatensis*	SA, CAM, CA	SA, CA
III	239	*S. jaguirreana, S. delicatula, S. aff. granatensis*	SA	SA, CA
III	247	*S. andreana, S. laselvae, S. pseudobeauvoisii, S. scabrosa*	SA	SA,CA
III	248	*S. aff. scabrosa, S. andreana, S. laselvae, S. pseudobeauvoisii, S. scabrosa*	SA	SA, CA
III	261	*S. aff. weigelii-2, S. weigelii, S. aff. weigelii-4, S. aff. weigelii-3, S. aff. weigelii-1*	SA	SA, CA
III	263	*S. aff. weigelii-2, S. weigelii, S. aff. weigelii-4, S. aff. weigelii-3, S. aff. weigelii-1, S. hypoglabra, S. aff. hypoglabra*	SA	CA
III	264	*S. tainorum, S. aff. sinuosa-3, S. aff. tainorum, S. aff. sinuosa-4, S. aff. sinuosa-2, S. aff. weigelii-2, S. weigelii, S. aff. weigelii-4, S. aff. weigelii-3, S. aff. weigelii-1, S. hypoglabra, S. aff. hypoglabra*	SA, CA	CA

Table S.2.6. Model selection results and parameter estimates for three full GeoSSE models (unfixed root, fixed root: Cont. Neo., fixed root: Caribbean) and nine models where constraints on speciation (sA, sB, sAB), extinction (xA, xB), and dispersal (dA, dB) were performed to evaluate different macroevolutionary scenarios.

				full		full (fixed root: Cont. Neo.)		full (fixed root: Caribbean)		Rates						
Models	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)	ChiSq	Pr(>|Chi|)	ChiSq	Pr(>|Chi|)	sA	sB	sAB	xA	xB	dA	dB
full	7	-451.6	917.3	–	–	–	–	–	–	0.15	0.1	0.08	0	0	0	0.1
full (fixed root: Cont. Neo.)	7	-451	916	–	–	–	–	–	–	0.15	0.1	0.09	0	0	0	0.1
full (fixed root: Caribbean)	7	-454.8	923.6	–	–	–	–	–	–	0.15	0.2	0.07	0	0.1	0	0.1
no.sAB (sAB ~ 0)	6	-453.8	919.5	4.24	0.04	5.52	0.02	-2.14	1	0.18	0.1	0	0	0	0	0.1
eq.div (sA ~ sB, xA ~ xB)	5	-451.8	913.7	0.43	0.81	1.71	0.43	-5.95	1	0.15	0.2	0.08	0	0	0	0.1
eq.disp (dA ~ dB)	6	-455.8	923.5	8.24	0	9.52	0	1.85	0.17	0.16	0.1	0.06	0	0	0	0
eq.sp (sA ~ sB)	6	-451.8	915.7	0.43	0.51	1.71	0.19	-5.95	1	0.15	0.2	0.08	0	0	0	0.1
eq.sp_no.sAB (sA ~ sB, sAB ~ 0)	5	-453.8	917.7	4.4	0.11	5.68	0.06	-1.98	1	0.16	0.2	0	0	0	0	0.1
eq.sp_eq.ex_no.sAB (sA ~ sB, sAB ~ 0, xA ~ xB)	4	-454.1	916.2	4.93	0.18	6.21	0.1	-1.46	1	0.16	0.2	0	0	0	0	0.1
eq.ex_no.sAB (xA ~ xB, sAB ~ 0)	5	-453.8	917.6	4.38	0.11	5.65	0.06	-2.01	1	0.17	0.2	0	0	0	0	0.1
no.dB (dB ~ 0)	6	-458.2	928.4	13.1	0	14.42	0	6.76	0.01	0.17	0.1	0.06	0	0	0	0
no.dA (dA ~ 0)	6	-454.9	921.9	6.62	0.01	7.89	0	0.23	0.63	0.12	0.1	0.28	0	0	0	0.2

Table S.2.7. Values for environmental variables obtained for island-level communities.

Island	Elev (mean)	Elev (median)	Elev (max)	Precip	MaxTemp	MinTemp	Eta	NDVI	EVI	TRI
Cuba	941.75	941.75	1648	127.6	27.44	18.65	113.8	0.86	0.6	0.7
Dominica	619.18	612.25	873	123.9	28.25	20.65	132	0.86	0.6	0.6
Dominican Republic	1635.66	1605	2136	115.6	22.03	10.07	88.43	0.78	0.5	0.5
Guadeloupe	808.67	824.25	957	97.2	27.1	19.7	120.5	0.85	0.5	0.7
Jamaica	1728.33	1696.25	2165	158.2	22.35	15.55	106.6	0.79	0.5	0.9
Martinique	676.57	710	906	130.6	28	20.9	129.4	0.8	0.6	0.7
Puerto Rico	901.32	919	1185	150.6	26.6	17.17	125.6	0.83	0.6	0.5

Table S.2.8. PCA loadings for environmental variables used for taxonomic and phylobetadiversity analysis.

	Elev_max	**Precip**	**MaxTemp**	**MinTemp**	**Eta**	**NDVI**	**EVI**	**TRI**
PC1	0.896205	0.17576	-0.97938	-0.94513	-0.93532	-0.83248	-0.86843	0.0428
PC2	-0.2058	-0.82049	0.123948	-0.22217	-0.19563	0.17084	-0.26831	-0.78763
PC3	-0.14505	-0.5123	-0.01639	0.23162	-0.00599	-0.13405	-0.32312	0.58615
PC4	-0.35574	0.17733	0.036813	0.024802	0.2782	-0.48476	-0.24374	-0.1842
PC5	0.081449	-0.03977	0.150745	0.054781	-0.09383	-0.15689	0.09707	-0.01537
PC6	0.015511	-0.02051	-0.03331	0.010065	0.026	-0.01489	0.02431	-0.00873
PC7	0.589854	-0.12828	-0.65193	-0.41624	-0.63308	-0.62892	-0.67955	0.56683

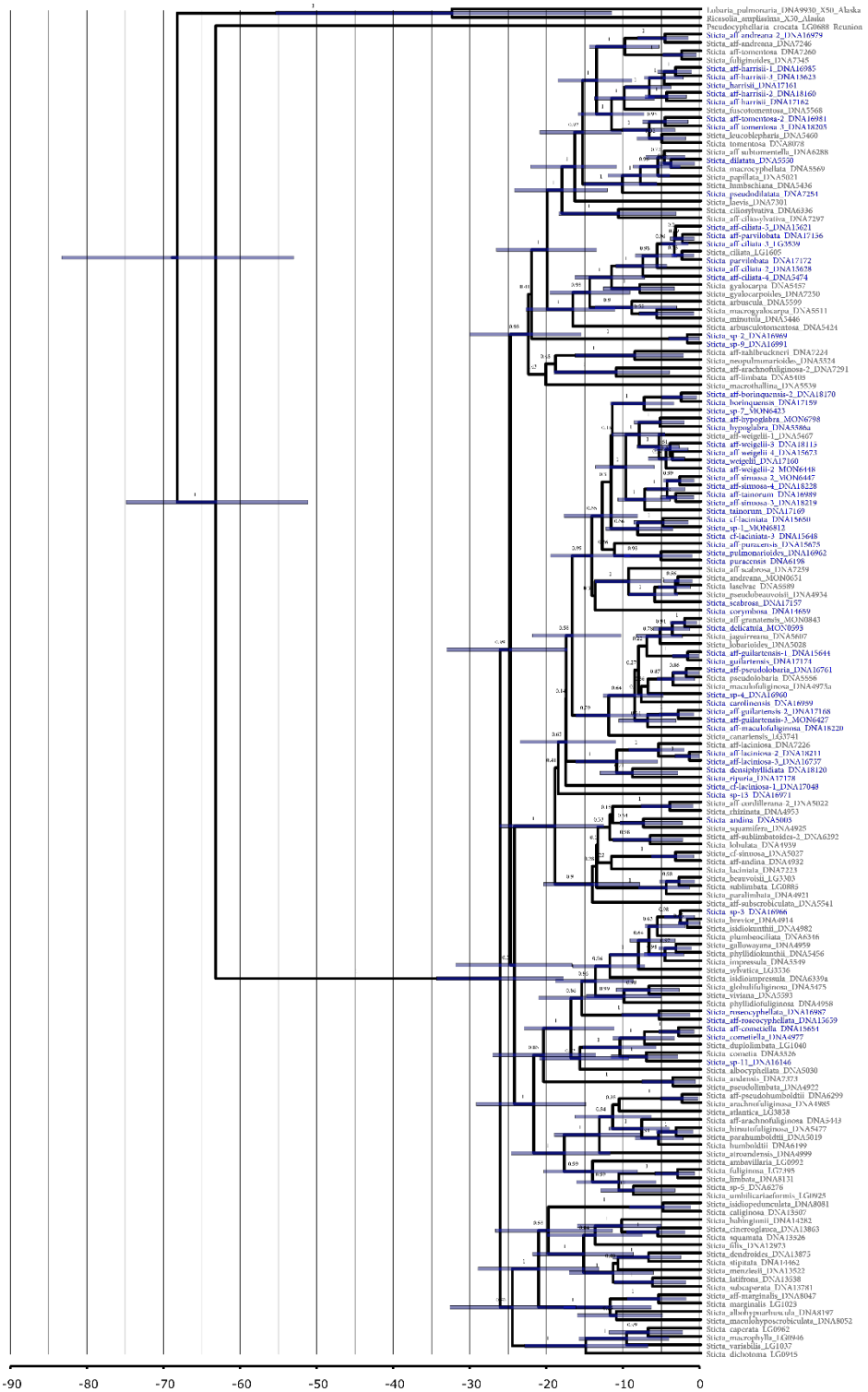

Figure S.2.1. Maximum Clade Credibility tree from BEAST. Caribbean taxa are highlighted in blue. Node bars show 95% HPD values whereas values above branches indicate posterior probabilities. Horizontal scale in millions of years.

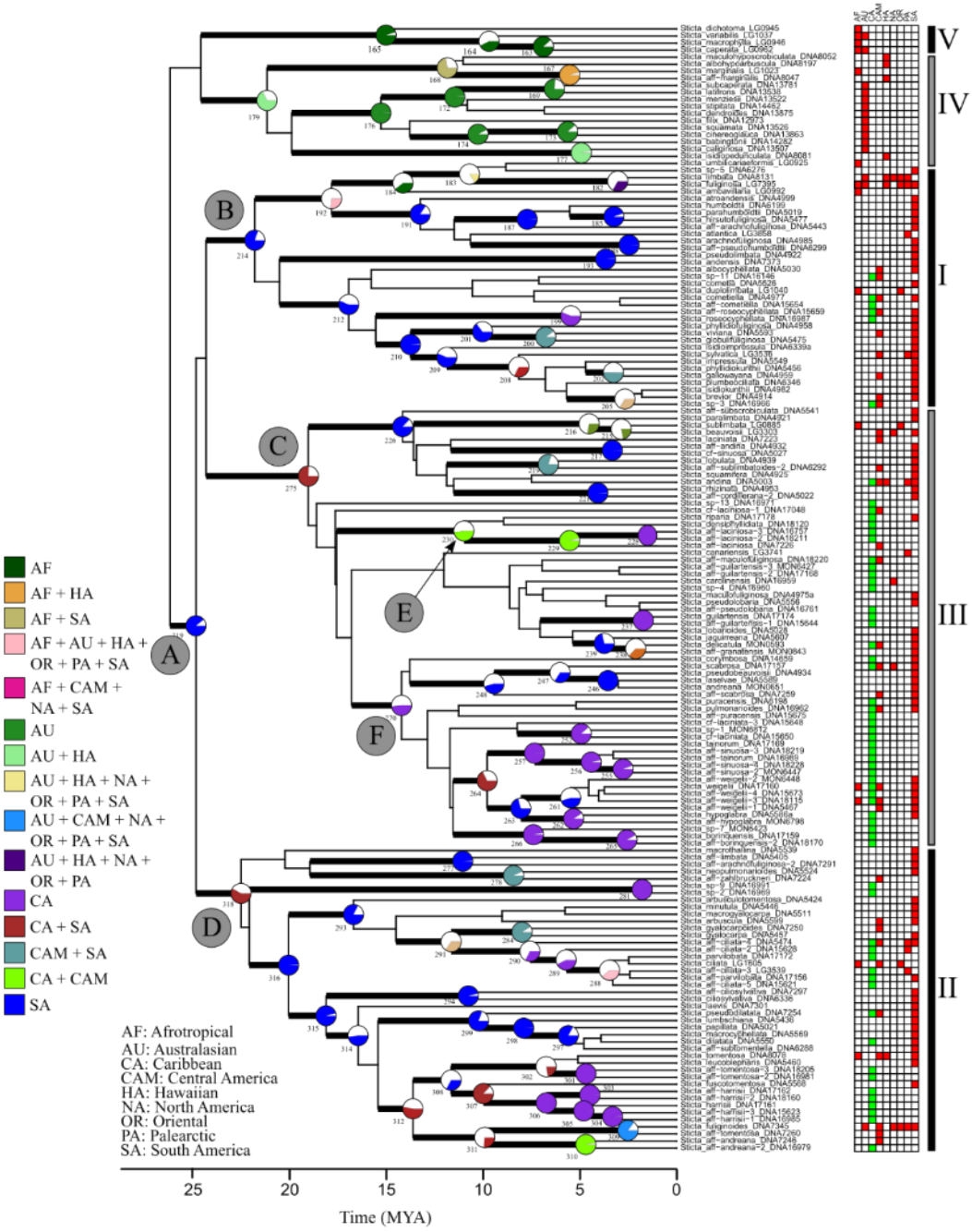

Figure S.2.2. Ancestral range reconstruction analysis based on DEC models using our 4-loci MCC tree (outgroups removed) and a matrix of dispersal constraints multipliers. Matrix to the right indicates presence of species in the Caribbean (green) and the other biogeographic regions analyzed (red). Labeled nodes indicate common ancestor for Clades I, II and III ("A"), common ancestor for Clade I ("B"), common ancestor to Clade III ("C"), common ancestor for Clade II ("D") and earliest ancestors of potential Caribbean origin (Nodes "E" and "F"). Ancestral ranges reconstructed are only shown for strongly supported clades.

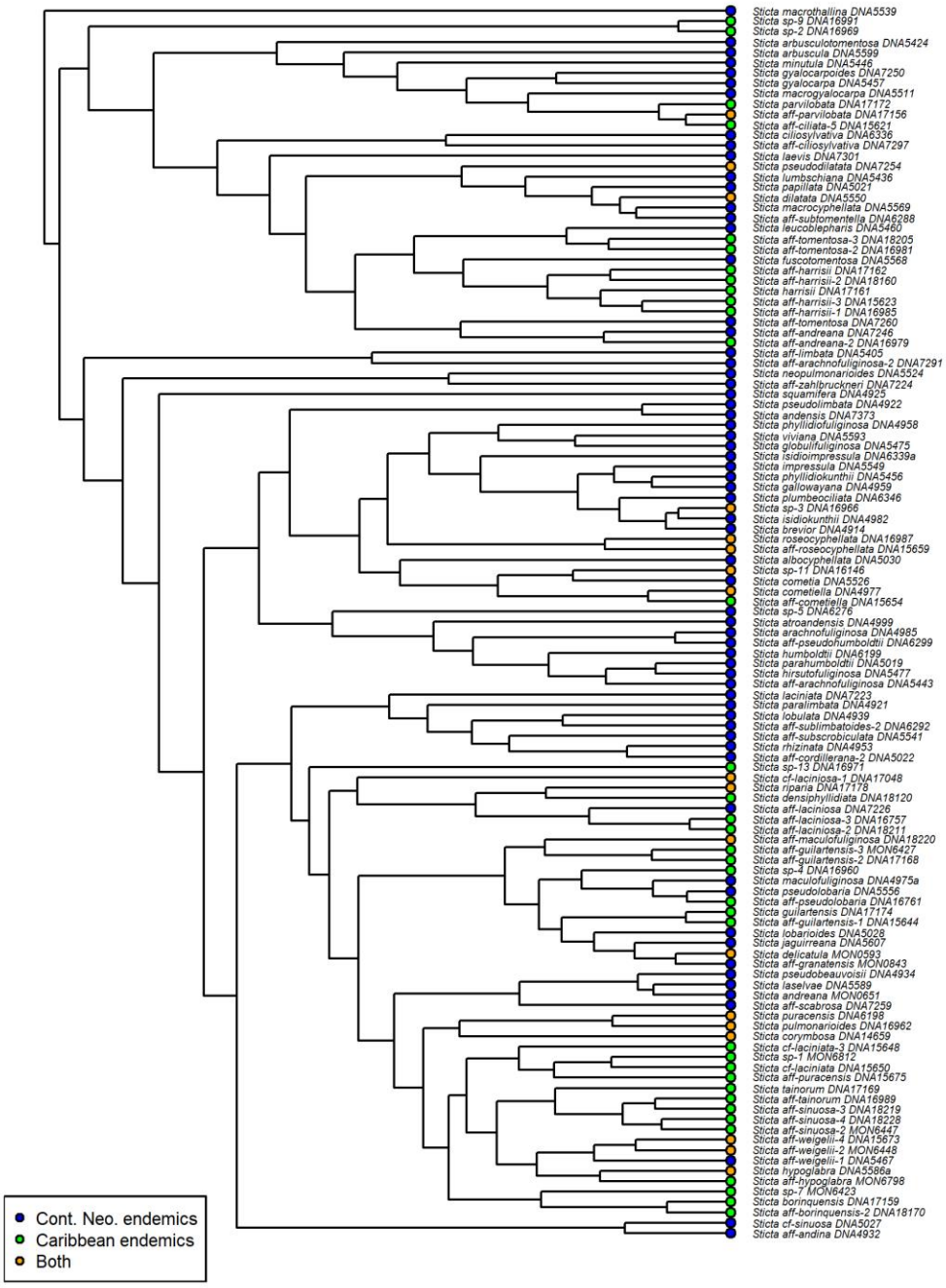

Figure S.2.3. Distribution of geographic character states in 119-tip MCC tree used for GeoSSE analysis.

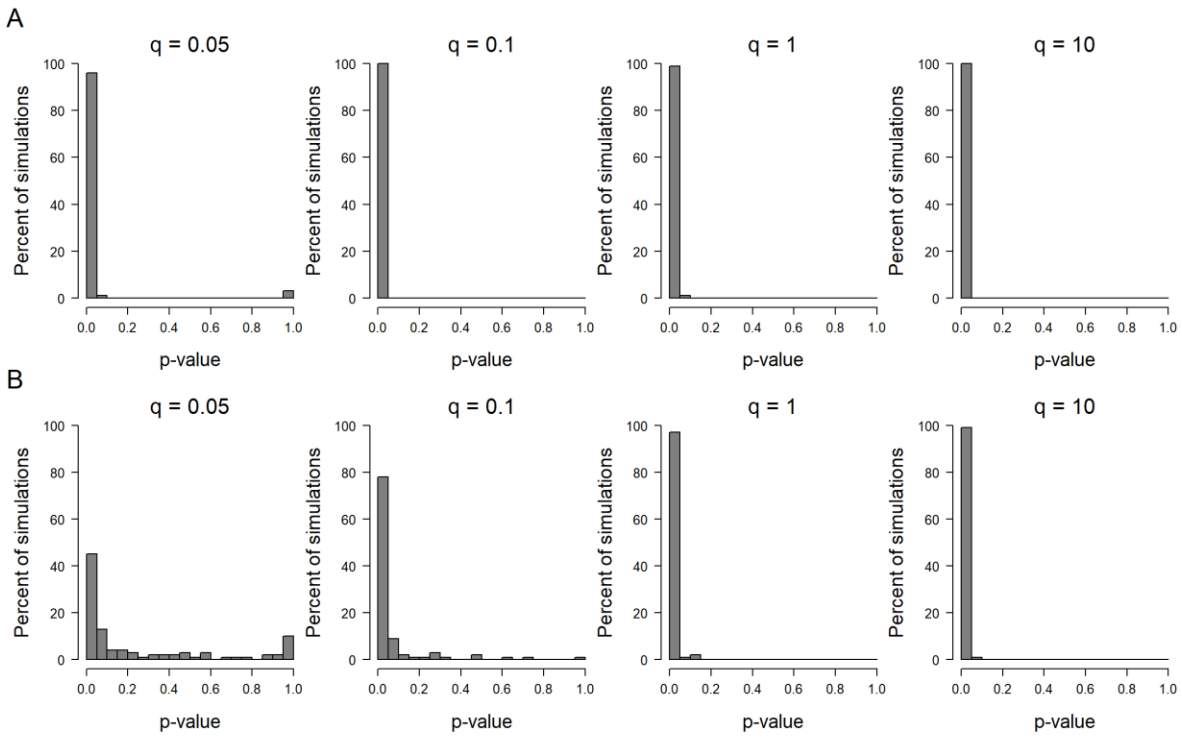

Figure S.2.4. Results from simulation analysis to estimate error rates of between-region speciation in GeoSSE. The evolution of three-state **A.** random and **B.** neutral traits were simulated on our MCC tree using four transition rates: 0.05, 0.1, 1, 10. Bars indicate the distribution of p-values obtained for all simulations. In each graph, the first bar to the left (p-values < 0.05) indicate the proportion of simulations in which the null hypothesis (sAB ~ 0) was incorrectly rejected in favor of the alternative hypothesis of differences in between-region speciation.

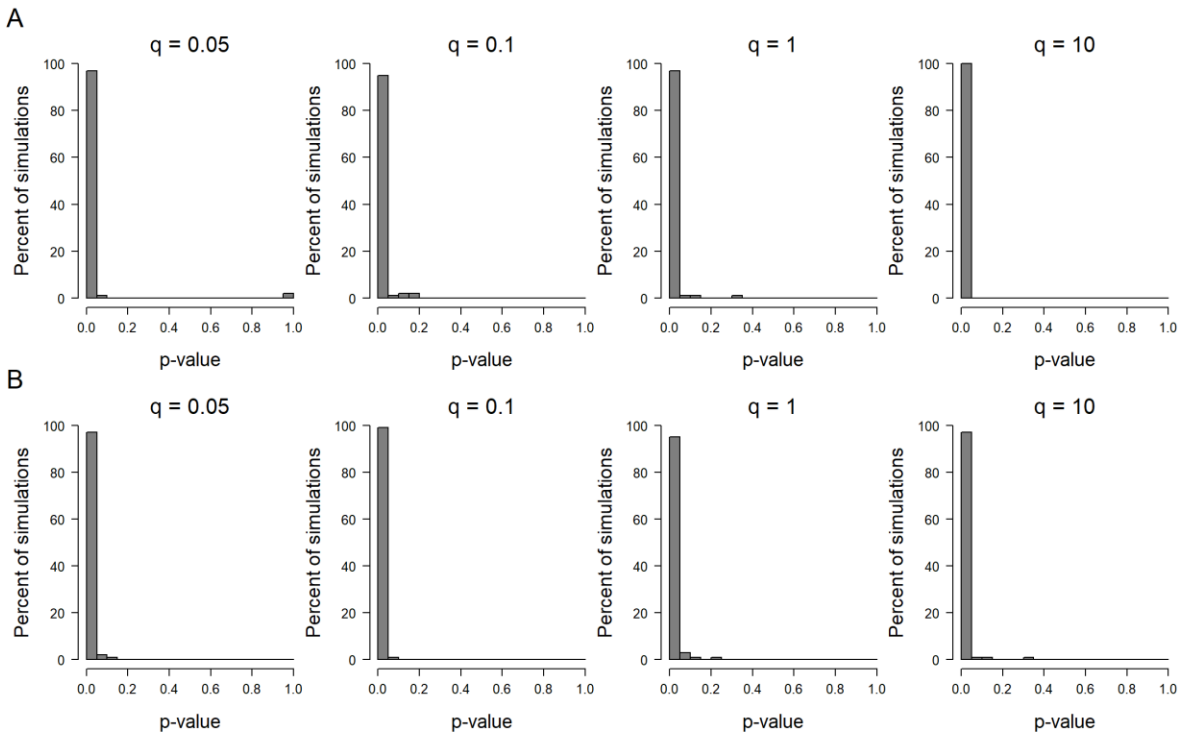

Figure S.2.5. Results from simulation analysis to estimate error rates in dispersal asymmetries inferred in GeoSSE. The evolution of three-state **A.** random and **B.** neutral traits were simulated on our MCC tree using four transition rates: 0.05, 0.1, 1, 10. Bars indicate the distribution of p-values obtained for all simulations. In each graph, the first bar to the left (p-values < 0.05) indicate the proportion of simulations in which the null hypothesis (dA ~ dB) was incorrectly rejected in favor of the alternative hypothesis of dispersal asymmetries between regions.

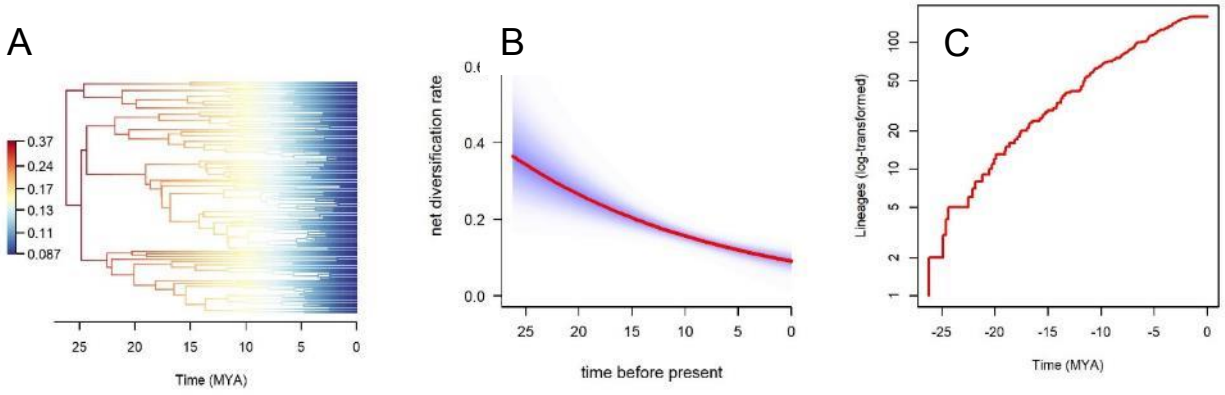

Figure S.2.6. Results from state-independent diversification analysis using Bayesian Analysis of Macroevolutionary Mixtures (BAMM) based on our 162 tips multilocus tree (outgroups removed). **A.** Mean phylorate plot on diversification rate (breaksmethod = 'jenks'), **B.** Net diversification through time plot, C. Lineage through time plot

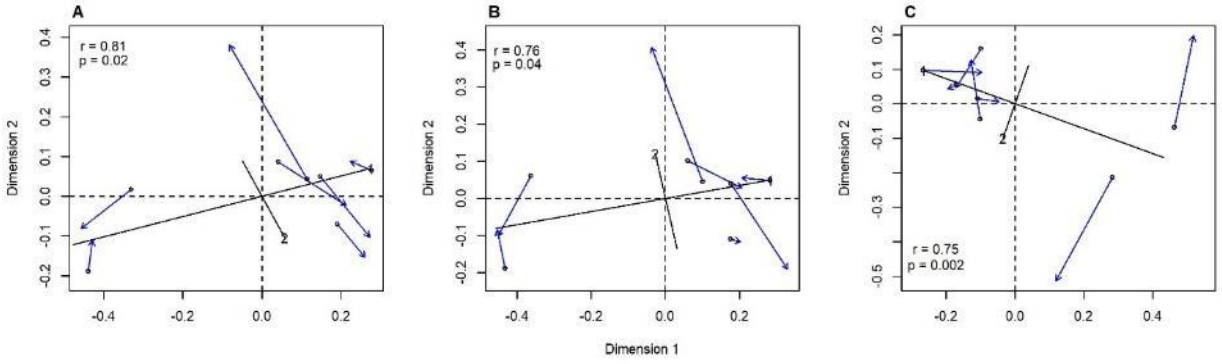

Figure S.2.7. Procrustes superimposition to assess the relationship between TBD, tPBD and bPBD metrics with environmental distances. **A.** *Jaccard*, **B.** *Unifrac*, **C.** $D_{Rao's}$. All associations were statistically significant.

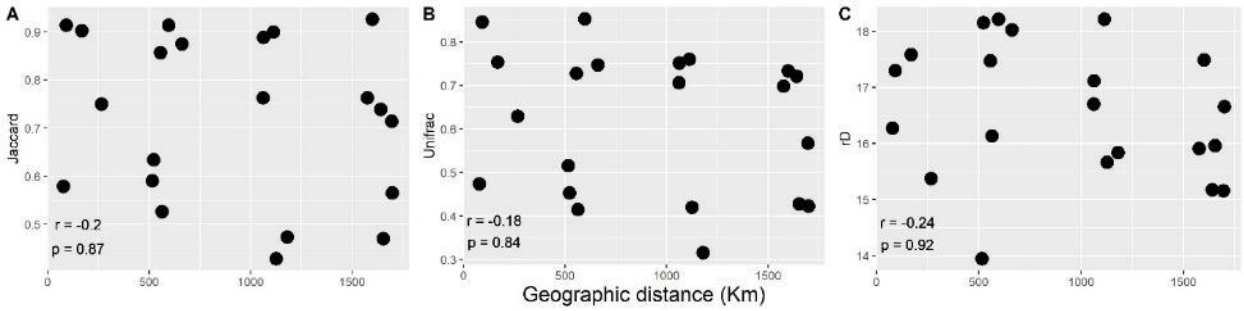

Figure S.2.8. Relationship between TBD, tPBD and bPBD metrics with geographic distance. **A.** *Jaccard*, **B.** *Unifrac*, **C.** $D_{Rao's}$. None of the associations were statistically significant according to Mantel tests.

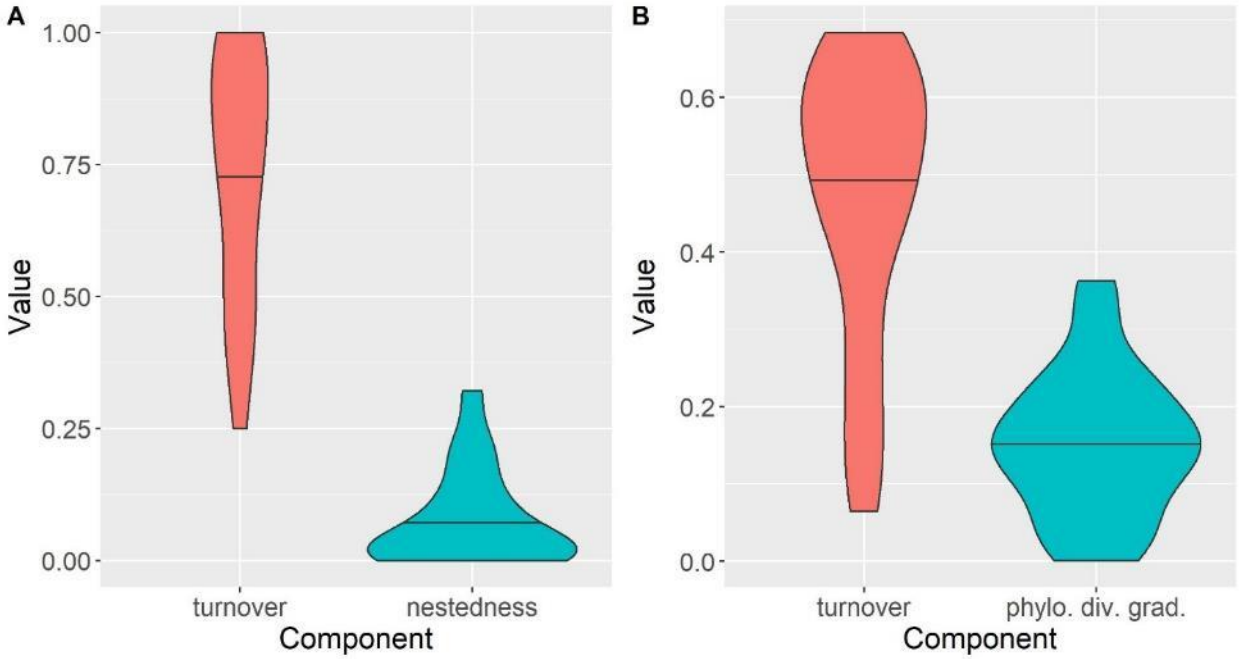

Figure S.2.9. Distribution of values for "true" turnover and "nestedness" or "phylogenetic diversity gradients" components of **A.** *Jaccard* and **B.** *Unifrac*.

APPENDIX 9. Methodological details about barcoding sequencing of *Cladonia sandstedei* individuals from Puerto Rico performed for Chapter 3.

Exploratory analysis of genetic divergence between Puerto Rican populations of C. sandstedei was carried out by generating single-locus data for three samples from Maricao and three samples from Vega Baja. This work entailed obtaining sequences for the Translation Elongation Factor 1-Alfa (EF1) and the RNA polymerase I subunit II (RPB2). Primers and PCR conditions used in this study are described in Table S.3.1. PCR amplification and sequencing followed protocols described in Mercado-Díaz et al. (2020). Reference sequences were downloaded from GenBank or obtained from Rebecca Yahr (Table S.3.2.).

APPENDIX 10. *process_radtags* command-line usage implemented for processing RADseq data used in Chapter 3.

1- Single-end sequences were already demultiplexed (ipyrad), thus *process_radtags* was only used for quality control. No barcode file required:

process_radtags -p /home/FM/jmercado/CladRad/C_sandstedei_demultiplexed_files/ -o /home/FM/jmercado/CladRad/stacks/samples_original –inline_null -e apeKI -t 55 -r -c -q

2- Paired-end sequencing quality control and demultiplexing reads (two plates):

process_radtags -P -p /home/FM/jmercado/CladRad/201113_AHLTJKDSXY/Plate1 -o /home/FM/jmercado/CladRad/stacks/Plate1 -b /home/FM/jmercado/CladRad/201113_AHLTJKDSXY/Plate1/GBS-ApeKI-1-96_barcodes2_stacks.txt –inline_null -e apeKI -t 55 -r -c -q

process_radtags -P -p /home/FM/jmercado/CladRad/201113_AHLTJKDSXY/Plate2 -o /home/FM/jmercado/CladRad/stacks/Plate2 -b /home/FM/jmercado/CladRad/201113_AHLTJKDSXY/Plate2/GBS-ApeKI-2-68_barcodes2_stacks.txt –inline_null -e apeKI -t 55 -r -c -q

APPENDIX 11. Methodological details and summary of results for genetic dissimilarity vs. geographic distance correlation analysis performed in Chapter 3.

Genetic dissimilarity and geographic distances between continental individuals were computed to assess if isolation by distance could partly explain population partitioning found using de-novo clustering with DAPC. We used the function *diss.dist* from the R package "poppr" (Kamvar, Tabima, and Gŕunwald 2014) to calculate pairwise allelic distances between individuals. For geographic distances, sample coordinates were tabulated and the function *distm* (fun = distGeo) from the R package "geosphere" (Hijmans 2019) was used to calculate physical distance between these samples. Matrices were converted to distance objects with the R function *as.dist*. Statistical significance of correlation was assessed using a mantel test (function *mantel* in R package "vegan"(Oksanen et al. 2019)).

We found weak correlation between genetic dissimilarity and geographic distance between continental individuals ($r=0.11$). However, the association between these variables was found to be significant ($p=0.001$) (Fig S6).

Table S.3.1. Primers and PCR conditions used for single-locus sequencing.

Locus	Primer	Primer sequence 5'-3'	PCR protocol	Reference
Translation elongation factor 1-alpha (~ 1,000 bp) Program: EF1TD	EF1-526f	GTC GTY GTY ATY GGH CAY GT	94°C for 4 mins;10 cycles: 94 °C for 30 s, 66 °C for 30 s (decreasing 1 °C per cycle), 72 °C for 90 s; 30 cycles: 94 °C for 30 s, 56 °C for 30 s, 72 °C for 90 s; 72 °C for 7 mins	(Rehner 2001)
	EF1-1567R	ACH GTR CCR ATA CCA CCR ATC TT		
RNA polymerase II subunit 2 (RPB2) (~ 800 bp) Program: IGS52_2	RPB2-5f	GAY GAY MGW GAT CAY TTY GG	94˚C for 3 min; 34 cycles: 94˚C for 45 s, 50˚C for 60 s, 72˚C for 90 s; 72˚C for 7 min	(Y. J. Liu, Whelen, and Hall 1999)
	RPB2-7cR	CCC ATR GCT TGY TTR CCC AT		

Table S.3.2. Samples and GenBank accession numbers used for barcoding sequencing. Asterisks denote pending GenBank accession numbers. Exclamation marks show sequences obtained from R. Yahr that are not available in GenBank.

ID	Species	Area	EF1	RPB2
LK46	*Cladonia confusa*	Brazil		KP941559
Burgaz 96193	*Cladonia rangiformis*	Spain	JN811444	JF288838
DNA15497	*Cladonia sandstedei*	Maricao, PR	*	*
DNA15498	*Cladonia sandstedei*	Maricao, PR	*	*
DNA15499	*Cladonia sandstedei*	Maricao, PR	*	*
DNA15500	*Cladonia sandstedei*	Vega Baja, PR	*	*
DNA15501	*Cladonia sandstedei*	Vega Baja, PR	*	*
DNA15502	*Cladonia sandstedei*	Vega Baja, PR	*	*
RY1004	*Cladonia subtenuis*	Florida, USA	DQ490098	DQ522287
RY1123	*Cladonia subtenuis*	North Carolina, USA	DQ490096	
RY1128	*Cladonia subtenuis*	North Carolina, USA	DQ490101	
RY1129	*Cladonia subtenuis*	North Carolina, USA	DQ490093	
RY1151	*Cladonia subtenuis*	North Carolina, USA	DQ490095	
RY1189	*Cladonia subtenuis*	North Carolina, USA	DQ490105	
RY1190	*Cladonia subtenuis*	North Carolina, USA	DQ490104	DQ522286
RY1208	*Cladonia subtenuis*	Georgia, USA	!	DQ522282
RY1210	*Cladonia subtenuis*	Georgia, USA	!	DQ522283
RY1213	*Cladonia subtenuis*	Georgia, USA	!	DQ522284
RY1215	*Cladonia subtenuis*	Georgia, USA	DQ490102	
RY1216	*Cladonia subtenuis*	Georgia, USA	DQ490100	
RY1224	*Cladonia subtenuis*	Pennsylvania, USA	!	DQ522289
RY909	*Cladonia subtenuis*	Florida, USA	DQ490103	
RY910	*Cladonia subtenuis*	Florida, USA	DQ490091	
RY911	*Cladonia subtenuis*	Florida, USA	DQ490097	
RY913	*Cladonia subtenuis*	Florida, USA	DQ490092	
RY941	*Cladonia subtenuis*	Florida, USA	DQ490094	
RY942	*Cladonia subtenuis*	Florida, USA	!	DQ522285
RY943	*Cladonia subtenuis*	Florida, USA	DQ490099	
RY999	*Cladonia subtenuis*	Florida, USA	!	DQ522288

Table S.3.3. ipyRAD assembly statistics. Summary statistics are at the bottom of the table.

Sample	Raw reads	Reads mapped to reference	Percent reads mapped	Within-sample clusters	Number of loci
C_cf_sandstedei_18003_Mercado-Diaz_3460_Jamaica.	1601624	378646	24%	54862	18719
C_sandstedei_15498_Mercado-Diaz_3315_Maricao.	1432995	146834	10%	31667	6958
C_sandstedei_15500_Mercado-Diaz_3324_Vega_Baja.	864958	152257	18%	32286	7437
C_sandstedei_15501_Mercado-Diaz_3322_Vega_Baja.	1232113	213463	17%	38690	10375
C_sandstedei_15502_Mercado-Diaz_3323_Vega_Baja.	1116468	186670	17%	35759	8994
C_sandstedei_17232_Mercado-Diaz_3441_JM.	1976240	559296	28%	59133	23092
C_sandstedei_17233_Mercado-Diaz_3446_JM.	1356432	451418	33%	53834	20097
C_sandstedei_17234_Mercado-Diaz_3334_Vega_Baja.	2265323	419982	19%	52178	18771
C_sandstedei_17235_Mercado-Diaz_3335_Vega_Baja.	1776408	331574	19%	44742	14878
C_sandstedei_17998_Mercado-Diaz_3685_Maricao.	1816283	417997	23%	56504	20872
C_sandstedei_17999_Mercado-Diaz_3686_Maricao.	1701586	365376	21%	49986	17201
C_sandstedei_18000_Mercado-Diaz_3687_Maricao.	1941878	449827	23%	45869	17606
C_sandstedei_18001_Mercado-Diaz_3688_Maricao.	1994937	453126	23%	51651	18925
C_sandstedei_18004_Mercado-Diaz_3437_JM.	1468070	385644	26%	56911	19431
C_sandstedei_18400_Luecking_46737a_CU.	1864539	501810	27%	53197	19271
C_sandstedei_18401_Luecking_46737b_CU.	2190512	515539	24%	53111	19673
C_subtenuis_17236_Mercado-Diaz_3609_Tennessee.	1669340	219339	13%	38832	10147
C_subtenuis_17237_Mercado-Diaz_3610_Tennessee.	747109	126218	17%	28801	5882
C_subtenuis_17238_Mercado-Diaz_3613_Tennessee.	899198	177568	20%	34544	8248
C_subtenuis_17239_Mercado-Diaz_3617_Tennessee.	1664363	248756	15%	40817	11571
Cladonia_arbuscula_Mercado-Diaz_3769_Georgia_18458.	1933127	254595	13%	39522	12097
Cladonia_arbuscula_Mercado-Diaz_3784_Georgia_18438.	2155473	381779	18%	46440	16334
Cladonia_cf-rangiferina_Mercado-Diaz_3749_Georgia_18454.	1617252	168773	10%	34435	8412
Cladonia_cf-rangiferina_Mercado-Diaz_3761_Georgia_18472.	1802794	216124	12%	42400	10135
Cladonia_cf-rangiferina_Mercado-Diaz_3762_Georgia_18461.	1959741	206570	11%	41444	9810
Cladonia_cf-rangiferina_Mercado-Diaz_3764_Georgia_18429.	1991437	177683	9%	37860	8182
Cladonia_cf-rangiferina_Mercado-Diaz_3812_Alabama_18494.	1838304	306333	17%	42435	14169
Cladonia_cf-rangiferina_Mercado-Diaz_3845_Florida_18501.	1982751	277500	14%	41583	12747
Cladonia_cf-rangiferina_Mercado-Diaz_3863_Florida_18544.	2709971	431185	16%	50698	17871
Cladonia_cf-rangiferina_Mercado-Diaz_3864_Florida_18522.	2181408	283682	13%	42499	13084
Cladonia_cf-rangiferina_Mercado-Diaz_3865_Florida_18510.	1823188	268948	15%	40066	13083
Cladonia_cf-rangiferina_Mercado-Diaz_3867_Florida_18539.	2438797	258823	11%	39070	11313

Cladonia_cf-rangiferina_Mercado-Diaz_3869_Florida_18534.	2335644	478080	20%	52631	18126
Cladonia_cf-rangiferina_Mercado-Diaz_3870_Florida_18528.	2019650	375394	19%	45354	15783
Cladonia_cf-rangiferina_Mercado-Diaz_3871_Florida_18538.	2502130	349348	14%	45438	15261
Cladonia_cf-sandstedei_Mercado-Diaz_3757_Georgia_18471.	2005072	278382	14%	39705	12912
Cladonia_cf-sandstedei_Mercado-Diaz_3758_Georgia_18432.	2161897	286895	13%	41686	13429
Cladonia_cf-sandstedei_Mercado-Diaz_3785_Georgia_18455.	1752734	345383	20%	44564	15099
Cladonia_cf-sandstedei_Mercado-Diaz_3807_Alabama_18488.	1788122	334870	19%	44938	15226
Cladonia_cf-sandstedei_Mercado-Diaz_3809_Alabama_18487.	1804853	129299	7%	29563	6273
Cladonia_cf-sandstedei_Mercado-Diaz_3840a_Florida_18532.	2499291	525398	21%	62348	19940
Cladonia_cf-sandstedei_Mercado-Diaz_3841b_Florida_18513.	1981072	337583	17%	48703	14667
Cladonia_cf-sandstedei_Mercado-Diaz_3850_Florida_18496.	1714035	344748	20%	42020	15476
Cladonia_cf-sandstedei_Mercado-Diaz_3851_Florida_18519.	2614868	490392	19%	49095	17076
Cladonia_cf-sandstedei_Mercado-Diaz_3853_Florida_18518.	2113408	514389	24%	51042	20128
Cladonia_cf-subtenuis_Mercado-Diaz_3748_Georgia_18451.	1438077	158568	11%	13990	4677
Cladonia_cf-subtenuis_Mercado-Diaz_3752_Georgia_18450.	1904075	319818	17%	44192	15065
Cladonia_cf-subtenuis_Mercado-Diaz_3840b_Florida_18533.	2245033	394336	18%	49846	16216
Cladonia_cf-subtenuis_Mercado-Diaz_3841a_Florida_18512.	1992596	452083	23%	59396	20486
Cladonia_cf_rangiferina_Mercado-Diaz_3750_Georgia_18442.	1882353	182321	10%	34393	8733
Cladonia_cf_rangiferina_Mercado-Diaz_3753_Georgia_18474.	2030779	169334	8%	32197	8820
Cladonia_cf_sandstedei_Mercado-Diaz_3848_Florida_18504.	1785319	318215	18%	36672	13623
Cladonia_cf_subtenuis_Mercado-Diaz_3770_Georgia_18452.	2013373	289371	14%	40011	12219
Cladonia_sandstedei_Luecking_46737c_Cuba_18402.	1924842	420039	22%	46357	16851
Cladonia_sandstedei_Luecking_46737d_Cuba_18403.	1547485	411871	27%	54565	20035
Cladonia_sandstedei_Mercado-Diaz_3695_Georgia_18548.	2055178	327449	16%	42344	12619
Cladonia_sandstedei_Mercado-Diaz_3727_Georgia_18535.	2483136	402357	16%	44084	14792
Cladonia_sandstedei_Mercado-Diaz_3754_Georgia_18445.	2117850	245557	12%	38654	11787
Cladonia_sandstedei_Mercado-Diaz_3756_Georgia_18479.	2188759	217418	10%	42560	9985
Cladonia_sandstedei_Mercado-Diaz_3759_Georgia_18440.	1937337	274018	14%	40289	12371
Cladonia_sandstedei_Mercado-Diaz_3760_Georgia_18425.	1855693	200213	11%	42742	9049
Cladonia_sandstedei_Mercado-Diaz_3767_Georgia_18427.	1717412	204464	12%	37971	10688
Cladonia_sandstedei_Mercado-Diaz_3768_Georgia_18444.	2144659	241029	11%	42935	11010
Cladonia_sandstedei_Mercado-Diaz_3771_Georgia_18426.	1923934	131037	7%	34351	6333
Cladonia_sandstedei_Mercado-Diaz_3772_Georgia_18475.	2134855	258783	12%	40464	12103
Cladonia_sandstedei_Mercado-Diaz_3773_Georgia_18477.	2075016	182692	9%	32302	8800

Cladonia_sandstedei_Mercado-Diaz_3779_Georgia_18434.	1996331	349634	18%	44811	15727
Cladonia_sandstedei_Mercado-Diaz_3780_Georgia_18433.	2164801	314326	15%	42178	14078
Cladonia_sandstedei_Mercado-Diaz_3781_Georgia_18456.	1964150	280404	14%	45162	13628
Cladonia_sandstedei_Mercado-Diaz_3782_Georgia_18457.	2133334	225456	11%	41073	10311
Cladonia_sandstedei_Mercado-Diaz_3783_Georgia_18439.	2102700	279127	13%	43353	13137
Cladonia_sandstedei_Mercado-Diaz_3787_Georgia_18446.	2197841	279517	13%	40377	13337
Cladonia_sandstedei_Mercado-Diaz_3788_Georgia_18430.	2140048	498007	23%	53564	20578
Cladonia_sandstedei_Mercado-Diaz_3789_Georgia_18443.	1814380	242888	13%	39627	11760
Cladonia_sandstedei_Mercado-Diaz_3791_Georgia_18460.	1827727	175708	10%	39298	8123
Cladonia_sandstedei_Mercado-Diaz_3792_Georgia_18478.	2031109	309619	15%	40828	14233
Cladonia_sandstedei_Mercado-Diaz_3793_Georgia_18473.	1980781	289119	15%	43895	14270
Cladonia_sandstedei_Mercado-Diaz_3794_Georgia_18469.	2014844	313684	16%	42067	13845
Cladonia_sandstedei_Mercado-Diaz_3795_Alabama_18497.	1947173	172467	9%	29980	8002
Cladonia_sandstedei_Mercado-Diaz_3800_Alabama_18468.	1784409	271075	15%	40840	13117
Cladonia_sandstedei_Mercado-Diaz_3801_Alabama_18483.	1890917	185997	10%	33347	9316
Cladonia_sandstedei_Mercado-Diaz_3803_Alabama_18490.	1747489	198432	11%	36238	10493
Cladonia_sandstedei_Mercado-Diaz_3804_Alabama_18508.	1929751	272818	14%	42928	13562
Cladonia_sandstedei_Mercado-Diaz_3811_Alabama_18453.	2029449	405816	20%	49772	16002
Cladonia_sandstedei_Mercado-Diaz_3817_Alabama_18470.	1861809	321822	17%	51125	16017
Cladonia_sandstedei_Mercado-Diaz_3818_Alabama_18480.	1976588	228879	12%	43927	11467
Cladonia_sandstedei_Mercado-Diaz_3819_Alabama_18505.	1725142	214559	12%	38492	10253
Cladonia_sandstedei_Mercado-Diaz_3822_Alabama_18526.	2535369	366999	14%	46666	15681
Cladonia_sandstedei_Mercado-Diaz_3823_Alabama_18499.	1782121	166964	9%	34845	8056
Cladonia_sandstedei_Mercado-Diaz_3826_Alabama_18485.	1730782	158737	9%	32271	8066
Cladonia_sandstedei_Mercado-Diaz_3832_Alabama_18514.	1770832	240392	14%	36917	10981
Cladonia_sandstedei_Mercado-Diaz_3834_Florida_18537.	2600089	462063	18%	48708	16870
Cladonia_sandstedei_Mercado-Diaz_3839_Florida_18507.	1531486	331095	22%	47133	16294
Cladonia_sandstedei_Mercado-Diaz_3842_Florida_18540.	2223743	449738	20%	46117	16281
Cladonia_sandstedei_Mercado-Diaz_3843_Florida_18559.	2252526	651936	29%	52810	20588
Cladonia_sandstedei_Mercado-Diaz_3844_Florida_18500.	1892282	373098	20%	42238	15425
Cladonia_sandstedei_Mercado-Diaz_3847_Florida_18511.	1573446	513963	33%	49158	19562
Cladonia_sandstedei_Mercado-Diaz_3852_Florida_18502.	2035926	297246	15%	48061	14414
Cladonia_sandstedei_Mercado-Diaz_3854_Florida_18531.	2176005	437571	20%	47886	16308
Cladonia_sandstedei_Mercado-Diaz_3855_Florida_18523.	2282613	405909	18%	45778	15135

Cladonia_sandstedei_Mercado-Diaz_3856_Florida_18525.	2369310	471205	20%	49579	16575
Cladonia_sandstedei_Mercado-Diaz_3858_Florida_18529.	2352866	359714	15%	43190	14089
Cladonia_sandstedei_Mercado-Diaz_3859_Florida_18552.	2963310	429643	14%	50815	17149
Cladonia_sandstedei_Mercado-Diaz_3860_Florida_18516.	2112414	407742	19%	48191	17414
Cladonia_sandstedei_Mercado-Diaz_3866_Florida_18509.	1433676	282111	20%	41031	12970
Cladonia_sandstedei_Mercado-Diaz_3868_Florida_18555.	2279445	309888	14%	43436	12733
Cladonia_sandstedei_Mercado-Diaz_4099_Vega_Baja_18561.	2718763	740721	27%	57959	23046
Cladonia_sandstedei_Mercado-Diaz_4100_Vega_Baja_18562.	2414564	633153	26%	53575	20281
Cladonia_sandstedei_Mercado-Diaz_4102_Vega_Baja_18563.	2265758	539667	24%	52857	19877
Cladonia_sandstedei_Mercado-Diaz_4104_Vega_Baja_18564.	2154703	334928	16%	43229	13712
Cladonia_sandstedei_Mercado-Diaz_4105_Vega_Baja_18565.	2516490	527616	21%	53053	18407
Cladonia_sandstedei_Mercado-Diaz_4106_Vega_Baja_18566.	2423248	547625	23%	57844	21365
Cladonia_sandstedei_Mercado-Diaz_4107_Vega_Baja_18567.	2085736	440471	21%	48054	16783
Cladonia_sandstedei_Mercado-Diaz_4108_Vega_Baja_18568.	2599622	483589	19%	56985	19247
Cladonia_sandstedei_Mercado-Diaz_4112_Vega_Baja_18569.	2056087	414643	20%	51076	17937
Cladonia_sandstedei_Mercado-Diaz_4114_Vega_Baja_18570.	2388531	540339	23%	43454	15941
Cladonia_sandstedei_Mercado-Diaz_4116_Vega_Baja_18571.	2373700	554269	23%	54024	19565
Cladonia_sandstedei_Mercado-Diaz_4117_Maricao_18572.	2035521	365865	18%	45906	14631
Cladonia_sandstedei_Mercado-Diaz_4118_Maricao_18573.	2255784	402960	18%	48655	15818
Cladonia_sandstedei_Mercado-Diaz_4119_Maricao_18574.	2222395	550134	25%	53129	19367
Cladonia_sandstedei_Mercado-Diaz_4120_Maricao_18575.	1814111	413522	23%	52612	17885
Cladonia_sandstedei_Mercado-Diaz_4121_Maricao_18576.	2415782	538548	22%	56904	21352
Cladonia_sandstedei_Mercado-Diaz_4122_Maricao_18577.	2546331	479663	19%	50702	17649
Cladonia_sandstedei_Mercado-Diaz_4123_Maricao_18578.	2156875	361599	17%	45625	14552
Cladonia_sandstedei_Mercado-Diaz_4124_Maricao_18579.	2634580	397732	15%	43606	15075
Cladonia_subtenuis_Mercado-Diaz_3693_Georgia_18549.	2500569	465908	19%	50594	16229
Cladonia_subtenuis_Mercado-Diaz_3696_Georgia_18527.	2762574	513635	19%	51500	18293
Cladonia_subtenuis_Mercado-Diaz_3698_Georgia_18545.	2714562	475199	18%	56583	18596
Cladonia_subtenuis_Mercado-Diaz_3709_Georgia_18553.	2671856	384448	14%	48608	15729
Cladonia_subtenuis_Mercado-Diaz_3723_Georgia_18546.	2486181	519237	21%	55739	20044
Cladonia_subtenuis_Mercado-Diaz_3724_Georgia_18554.	2663291	392455	15%	46250	15205
Cladonia_subtenuis_Mercado-Diaz_3725_Georgia_18557.	2493433	439410	18%	51749	17816
Cladonia_subtenuis_Mercado-Diaz_3728_Georgia_18558.	2563454	431253	17%	47431	16253
Cladonia_subtenuis_Mercado-Diaz_3730_Georgia_18551.	2673877	535078	20%	51941	18451

Cladonia_subtenuis_Mercado-Diaz_3734_Georgia_18550.	2604297	456948	18%	50187	16356
Cladonia_subtenuis_Mercado-Diaz_3736_Georgia_18556.	2313125	285286	12%	45960	13127
Cladonia_subtenuis_Mercado-Diaz_3739_Georgia_18524.	2306297	388843	17%	45504	14581
Cladonia_subtenuis_Mercado-Diaz_3740_Georgia_18543.	2607416	475415	18%	46928	16133
Cladonia_subtenuis_Mercado-Diaz_3741_Georgia_18547.	2412558	473263	20%	49710	16705
Cladonia_subtenuis_Mercado-Diaz_3742_Georgia_18520.	2600134	462345	18%	45905	15653
Cladonia_subtenuis_Mercado-Diaz_3743_Georgia_18517.	2217104	422650	19%	49598	17706
Cladonia_subtenuis_Mercado-Diaz_3744_Georgia_18521.	1969738	348264	18%	48910	15950
Cladonia_subtenuis_Mercado-Diaz_3745_Georgia_18536.	2489893	530199	21%	53611	19244
Cladonia_subtenuis_Mercado-Diaz_3751_Georgia_18436.	2087308	197368	9%	39161	9172
Cladonia_subtenuis_Mercado-Diaz_3755_Georgia_18428.	1988187	224792	11%	42776	10856
Cladonia_subtenuis_Mercado-Diaz_3763_Georgia_18560.	2582124	431159	17%	47790	15928
Cladonia_subtenuis_Mercado-Diaz_3774_Georgia_18466.	1877706	141010	8%	32603	6988
Cladonia_subtenuis_Mercado-Diaz_3777_Georgia_18459.	2124609	528448	25%	49049	18728
Cladonia_subtenuis_Mercado-Diaz_3786_Georgia_18431.	1926757	364587	19%	51011	16837
Cladonia_subtenuis_Mercado-Diaz_3790_Georgia_18492.	1863472	214675	12%	34953	9771
Cladonia_subtenuis_Mercado-Diaz_3796_Alabama_18484.	1911782	202422	11%	36078	10077
Cladonia_subtenuis_Mercado-Diaz_3797_Alabama_18493.	1944450	230923	12%	35889	11063
Cladonia_subtenuis_Mercado-Diaz_3798_Alabama_18495.	1794718	238248	13%	37448	11447
Cladonia_subtenuis_Mercado-Diaz_3799_Alabama_18447.	2088744	275384	13%	45889	13563
Cladonia_subtenuis_Mercado-Diaz_3802_Alabama_18481.	1960967	257048	13%	41598	12000
Cladonia_subtenuis_Mercado-Diaz_3805_Alabama_18486.	1926304	199913	10%	38069	10023
Cladonia_subtenuis_Mercado-Diaz_3806_Alabama_18530.	2602415	372835	14%	45253	14463
Cladonia_subtenuis_Mercado-Diaz_3808_Alabama_18515.	2022996	276651	14%	42521	12958
Cladonia_subtenuis_Mercado-Diaz_3810_Alabama_18463.	2004775	286411	14%	38752	12445
Cladonia_subtenuis_Mercado-Diaz_3813_Alabama_18482.	2152494	187085	9%	34390	9082
Cladonia_subtenuis_Mercado-Diaz_3814_Alabama_18489.	1937351	279286	14%	40234	12415
Cladonia_subtenuis_Mercado-Diaz_3815_Alabama_18449.	2140612	206786	10%	39154	9526
Cladonia_subtenuis_Mercado-Diaz_3816_Alabama_18467.	2157277	336679	16%	43113	14851
Cladonia_subtenuis_Mercado-Diaz_3821_Alabama_18465.	2129513	97868	5%	26004	4306
Cladonia_subtenuis_Mercado-Diaz_3824_Alabama_18462.	1758747	253367	14%	42904	12459
Cladonia_subtenuis_Mercado-Diaz_3825_Alabama_18542.	2571518	278407	11%	39827	11839
Cladonia_subtenuis_Mercado-Diaz_3828_Alabama_18498.	1666839	161845	10%	30981	8045
Cladonia_subtenuis_Mercado-Diaz_3829_Alabama_18476.	1941332	208832	11%	34355	9977

Cladonia_subtenuis_Mercado-Diaz_3830_Alabama_18491.	1651487	190140	12%	35590	9420
Cladonia_subtenuis_Mercado-Diaz_3831_Alabama_18464.	1946153	166017	9%	37375	7966
Cladonia_subtenuis_Mercado-Diaz_3835_Florida_18506.	1721152	143012	8%	30408	6828
Cladonia_subtenuis_Mercado-Diaz_3857_Florida_18503.	1763226	404083	23%	45456	16369
Mean	**2053710**	**337744**	**16%**	**44195**	**14133**
SD	**369260**	**126891**	**5%**	**7560**	**4075**
Min	**747109**	**97868**	**5%**	**13990**	**4306**
Max	**2963310**	**740721**	**33%**	**62348**	**23092**

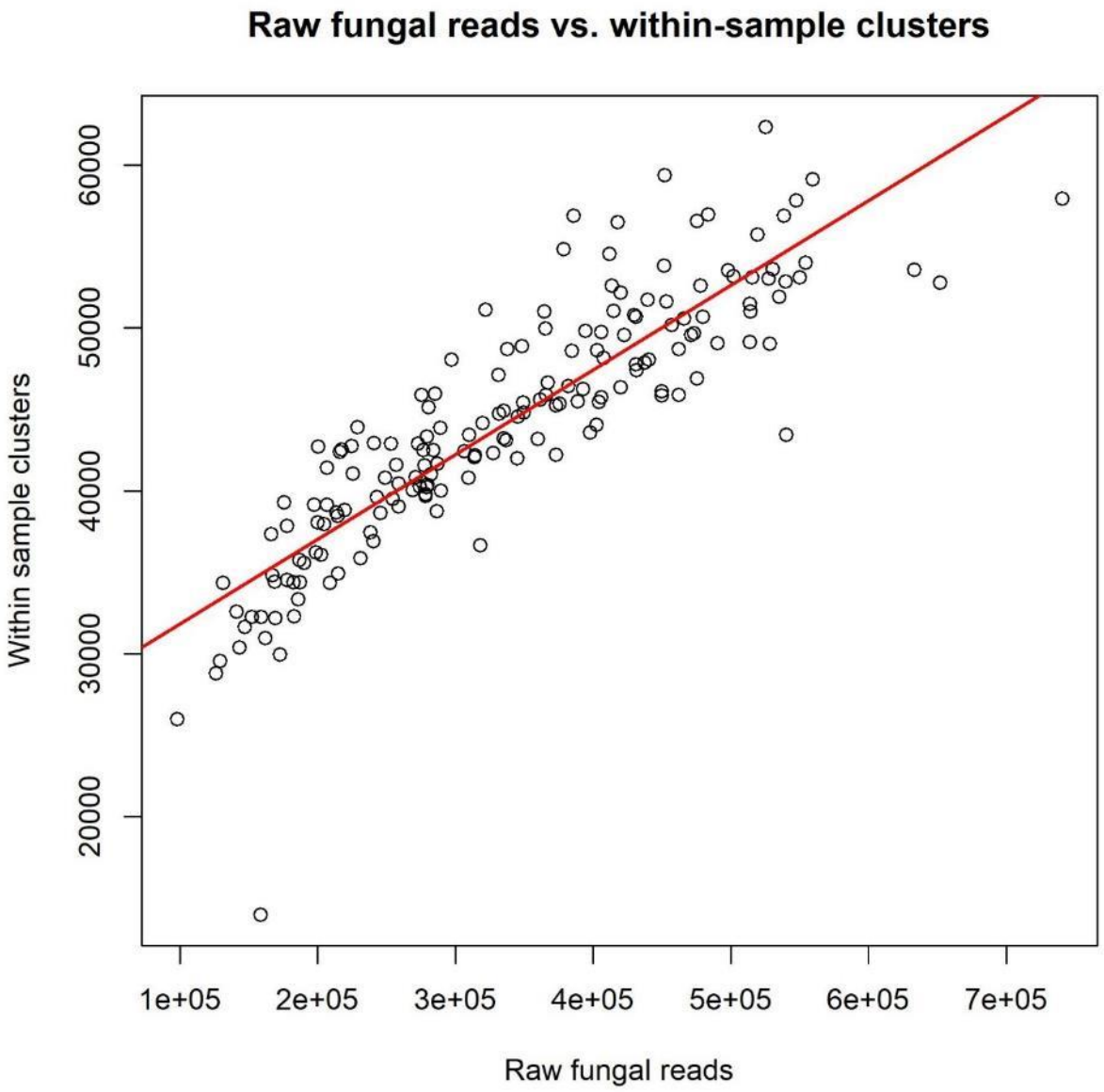

Figure S.3.1. Association between the raw number of mapped reads and within-sample clusters (loci) in analyzed RADseq data.

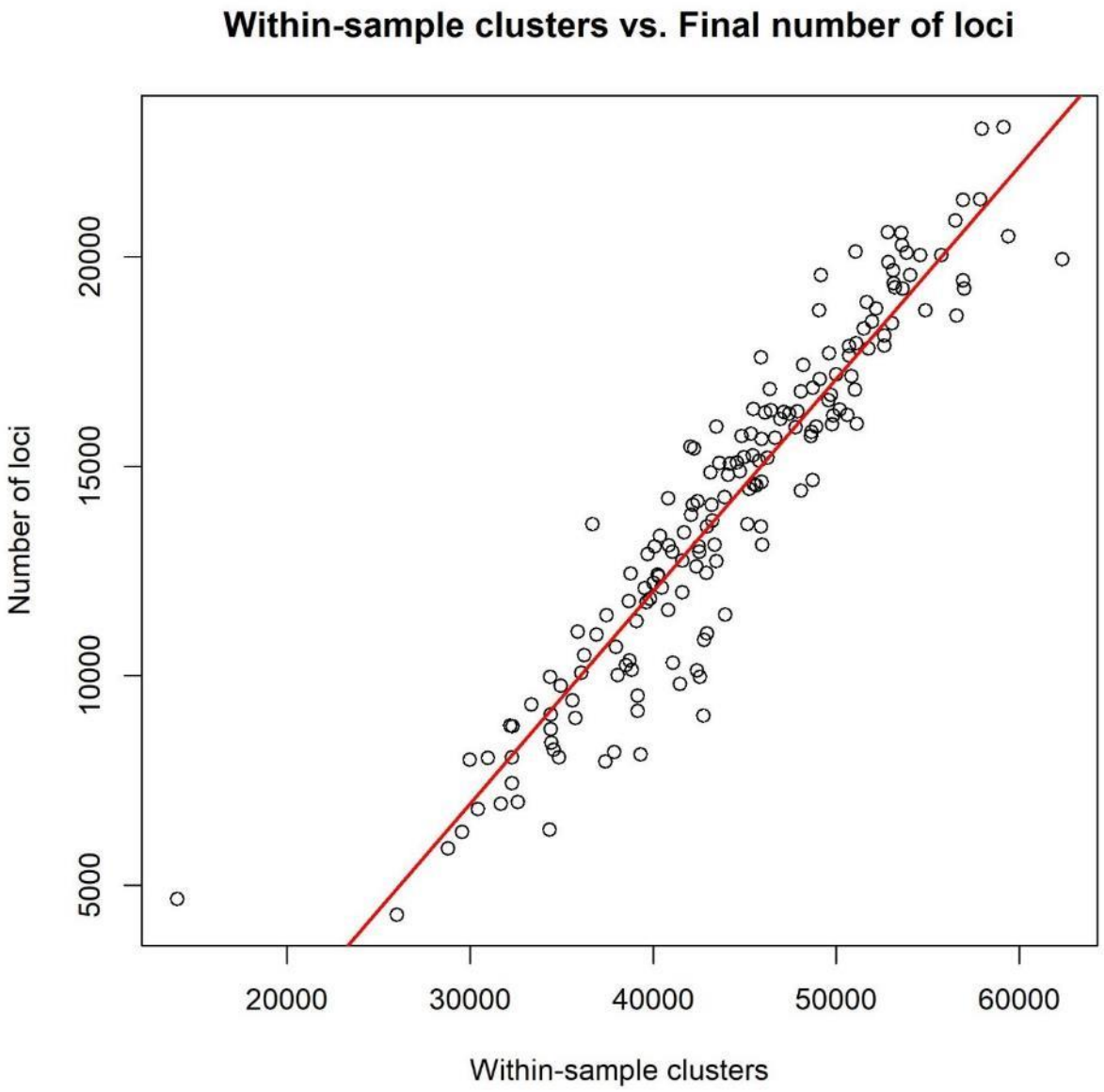

Figure S.3.2. Association between within-sample clusters (loci) and the final number of loci in analyzed RADseq data.

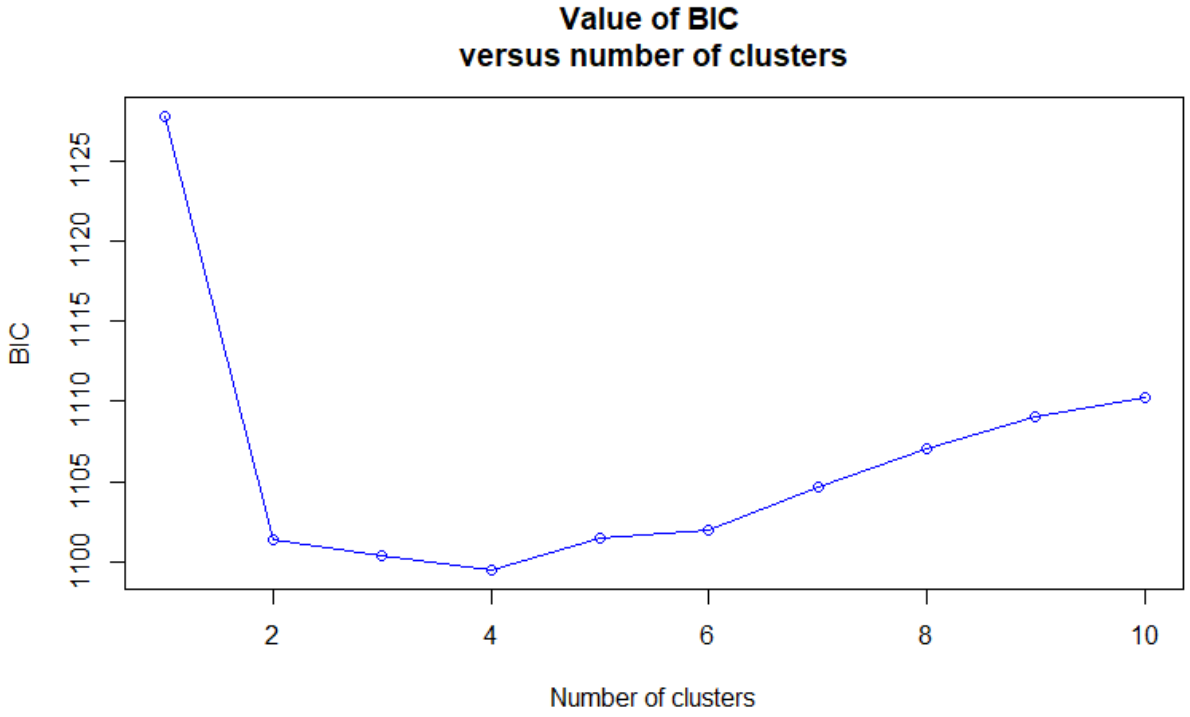

Figure S.3.3. Plot for selecting the "best" number of populations (K) based on a Bayesian information criterion. Part of the function *find.clusters* (R package "adegenet").

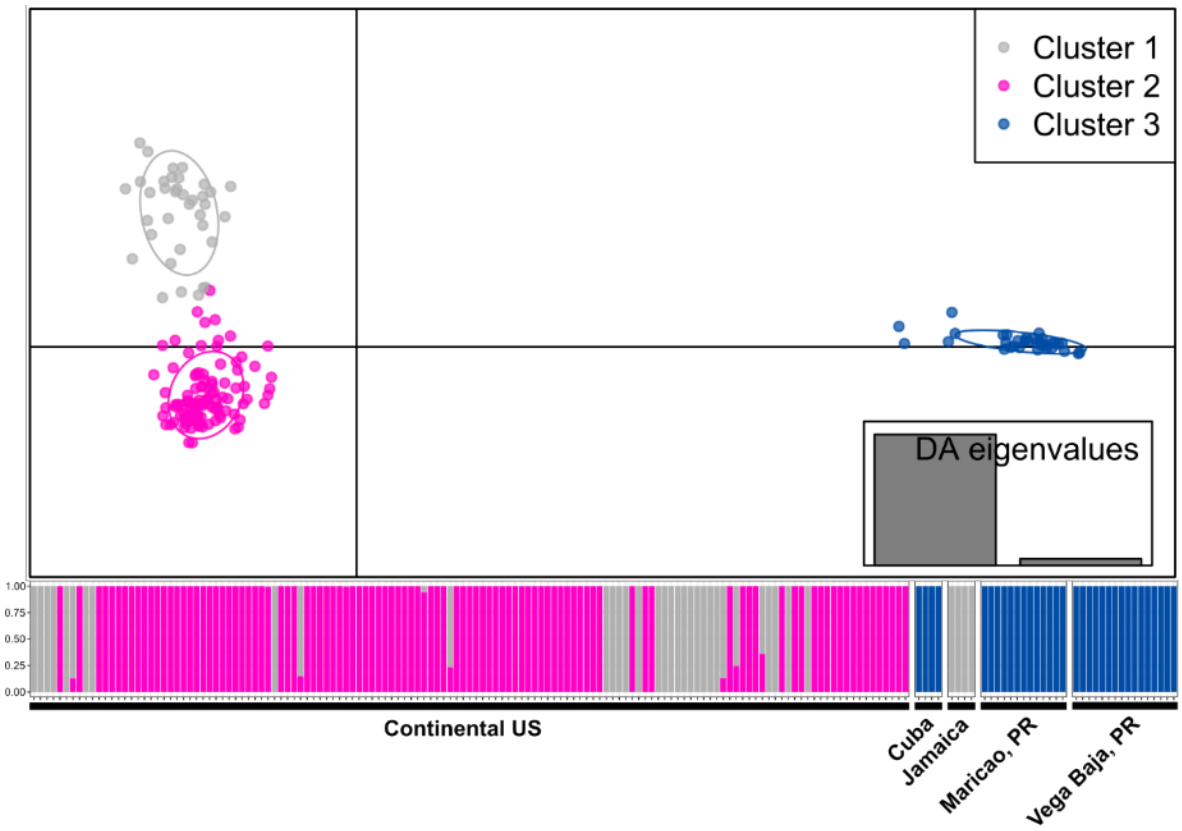

Figure S.3.4. Results from de-novo clustering with DAPC at K = 3. Upper part shows scatterplot for discriminant functions whereas the lower part show barplot with assigned membership probabilities. Each dot and bar represent an individual.

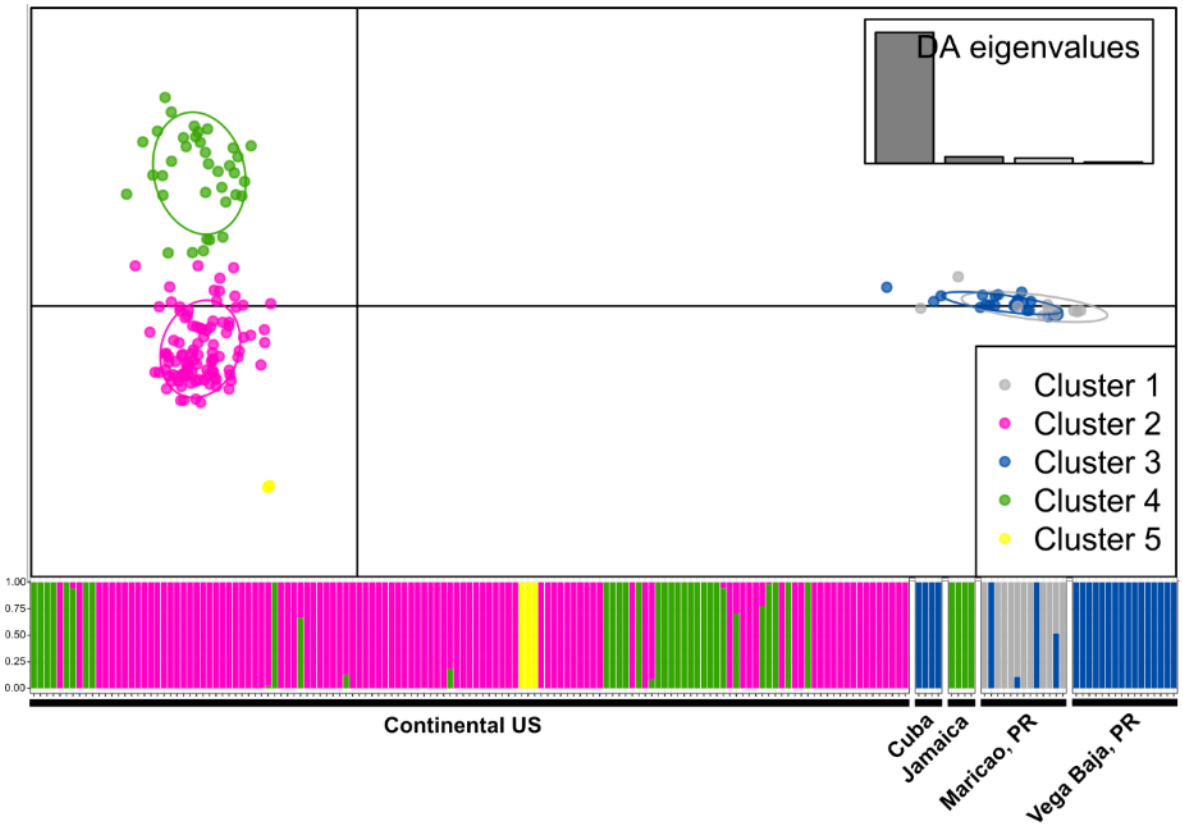

Figure S.3.5. Results from de-novo clustering with DAPC at K = 5. Upper part shows scatterplot for discriminant functions whereas the lower part show barplot with assigned membership probabilities. Each dot and bar represent an individual.

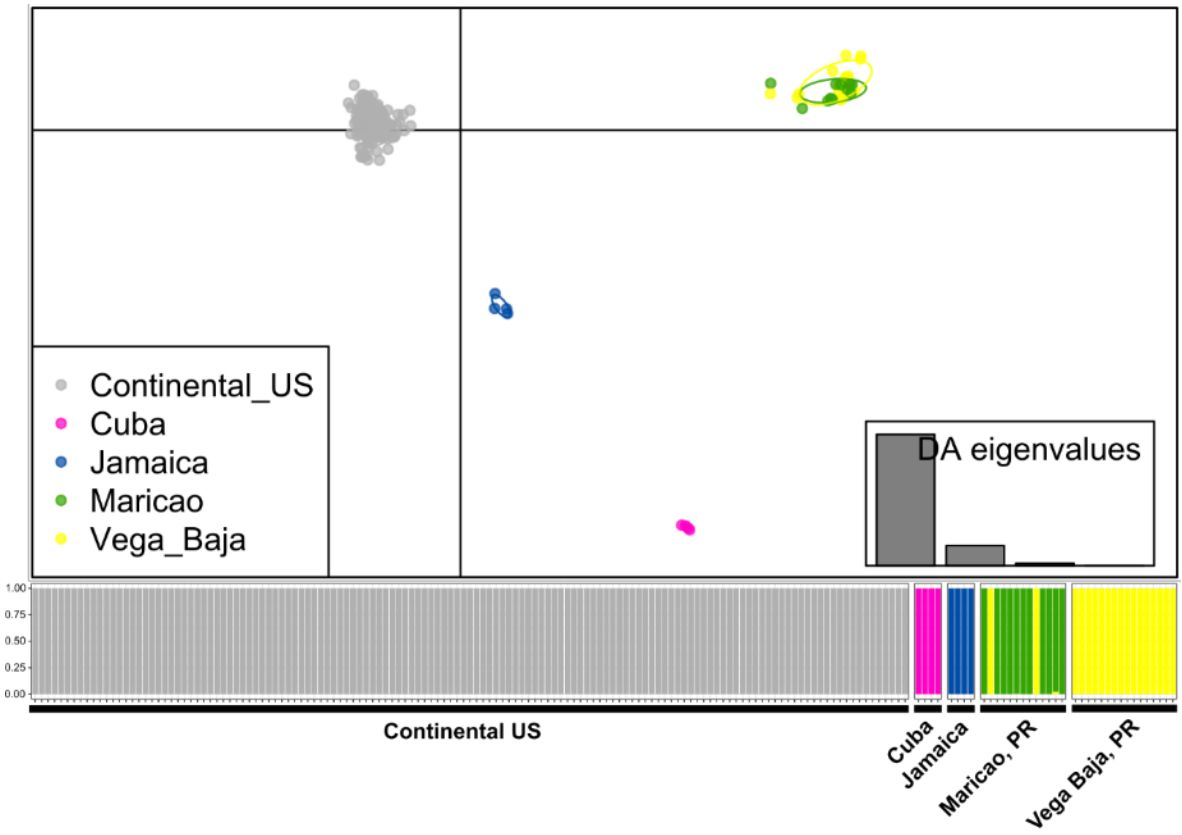

Figure S.3.6. Results from a-priori clustering with DAPC. Upper part shows scatterplot for discriminant functions whereas the lower part show barplot with assigned membership probabilities. Each dot and bar represent an individual.

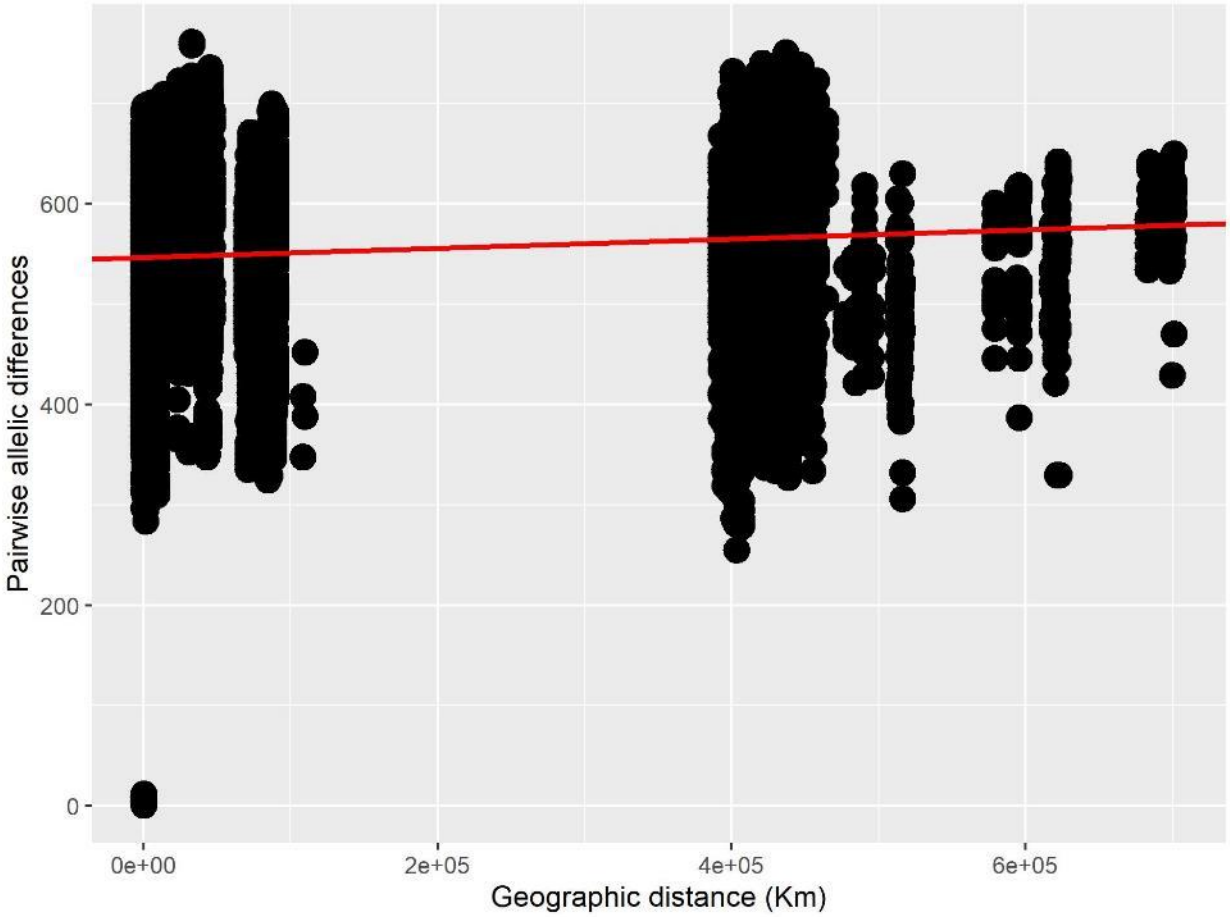

Figure S.3.7. Correlation between geographic distance and genetic dissimilarity (as pairwise allelic differences) between continental individuals. The red line denotes the linear regression function.