EVOLUTION AND DIVERSITY OF THE BASIDIOLICHEN CLADE *DICTYONEMA* (AGARICALES: HYGROPHORACEAE)

by

Manuela Dal-Forno

A Dissertation
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Doctor of Philosophy
Environmental Science and Public Policy

Committee:

_____ Dr. James D. Lawrey, Dissertation Director

_____ Dr. Robert Lücking, Committee Member

_____ Dr. Andrea Weeks, Committee Member

_____ Dr. Donald Seto, Committee Member

_____ Dr. Albert Torzilli,
Graduate Program Director

_____ Dr. Robert Jonas, Department Chairperson

_____ Dr. Donna Fox, Associate Dean,
Student Affairs & Special Programs,
College of Science

_____ Dr. Peggy Agouris, Dean,
College of Science

Date: _____ Spring Semester 2015
George Mason University
Fairfax, VA

Evolution and Diversity of the basidiolichen clade *Dictyonema* (Agaricales: Hygrophoraceae)

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

By

Manuela Dal-Forno
Master of Science
Universidade Federal do Paraná, 2009
Bachelor of Science
Universidade de Santa Cruz do Sul, 2006

Director: James D. Lawrey, Professor
Department of Biology

Spring Semester 2015
George Mason University
Fairfax, VA

Acknowledgments

I am thankful to many people, institutions and organizations for their support during this research project. There are four main people that I am very thankful to in many ways—James D. Lawrey, Robert Lücking, Masoumeh Sikaroodi and Patrick Gillevet—whose help, knowledge shared, encouragement and trust made this project happen.Thank you all so much!

I would also like to thank two additional committee members, Andrea Weeks and Donald Seto, for all their comments, suggestions and to keep pushing me forward.

I am indebted to the following people for sending or personally giving material of *Dictyonema*: Adriano A. Spielmann, Alba Yanez-Ayabaca, Alice Gerlach, Andre Aptroot, Andreas Beck, Bibiana Moncada, Dan Blanchon, Edier Soto Medina, Emerson Gumboski, Fabiane Lucheta, Flavio Beilke, Frank Bungartz, Frauke Ziemmeck, Fredy Nugra, Gothamie Weerakoon Cloonan, Harry Jonitz, Joel Mercado-Diaz, Jose Luis Chaves, Luciana Canêz, Luis Fernando Coca, Marcela Cáceres, Marcelo Sulzbacher, Martin Kukwa, Michel Benatti, Michaela Schmull, Natália Mossmann Koch, Peter Nelson, Pieter Van den Boom, Richard Harris, Robert Lücking, Roger Rosentreter, Sionara Eliasaro, Suzana de Azevedo Martins, Troy McMullin, and William Buck.

I would also like to thank these colleagues for helping or assisting me with collections in the field: Adriano A. Spielmann, Alba Yanez-Ayabaca, Bianca Regina da Hora, Agnes Elisete Luchi, Marcelo P. Marcelli, Emerson Gumboski, Frank Bungartz, Harry Jonitz, Jose Luis Chaves, Bibiana Moncada, and Robert Lücking.

I would also like to thank Masoumeh Sikaroodi, Patrick Gillevet, Smriti Bhattarai, Vrinda Schula, Kimiya Dadkhah, and Swati Almeida Dalmet for all their help in the molecular lab. I also thank Fernanda Craig for helping me with curating collections.

Many thanks to the Environmental Sciences and Policy (ESP) and Biology Departments faculty and staff: Al Torzilli, Larry Rockwood, David Luther, Sharon Bloomquist, Roslyn Cress, Joanne Anderson, and Rebekah A. Flis, for all their help in the process, including granting me a Graduate Teaching Assistantship. Also thanks to the many professors I took courses with for their knowledge shared and to make me think above and beyond.

I also acknowledge help from the Organization for Tropical Studies (OTS) and the participants of a lichen OTS course held in the spring of 2012, during which we were permitted to collect in several areas of Costa Rica, which resulted in many *Dictyonema* collections.

I thank the American Bryological and Lichenological Society (ABLS) for travel support in two meetings (Botany 2013 and Botany 2014).

The members of the Brazilian Group of Lichenogists (Grupo Brasileiro de Liquenólogos) are thanked for their support and fruitful discussions throughout the years. In additional, I am grateful for their help in collections in the meetings: 5ª REBEL (2010), 6º EGBL (2012), 7ª REBEL (2013), and 7º EGBL (2014). I would also like to thank Patrícia Jungbluth and Suzana de Azevedo Martins for inviting me to give talks in the EGBLs from 2012 and 2014, respectively, and getting funding for the corresponding trips.

Financial support for this study was provided by grant DEB 0841405 from the National Science Foundation to George Mason University: 'Phylogenetic Diversity of Mycobionts and Photobionts in the Cyanolichen Genus *Dictyonema*, with Emphasis on the Neotropics and the Galapagos Islands' (PI: J. Lawrey; CoPIs: R. Lücking, P. Gillevet), which awarded me Graduate Research Assistantships for several years.

I also thank the The Charles Darwin Foundation for their support to the Galapagos Lichen Inventory, which permitted access to *Dictyonema* specimens freshly collected in the Archipelago. For this effort I am especially indebted to Frank Bungartz and the Galapagos National Park, particularly Washington Tapia and Galo Quedaza, for granting specimen export permits. The lichen inventory is part of the Census of Galapagos Biodiversity by Charles Darwin Foundation.

There are many family members and friends that I would like to thank for their support and encouragement, most especially my husband, Morgan Robert Gostel, my parents Silvani and Luiz Dal Forno, my sister Ana Julia Dal Forno, my niece Lara Dal Forno Pereira, and members of the entire Gostel family. Thanks also to my many friends in the EVPP program (especially Lilly Parker and Mirian Tsuchiya), and outside the program as well, for their help, unconditional friendship and rich discussions.

Finally I thank George Mason University for providing me all resources, personnel and positive environment I needed while performing research during this program.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

EVOLUTION AND DIVERSITY OF THE BASIDIOLICHEN CLADE *DICTYONEMA* (AGARICALES: HYGROPHORACEAE)

Manuela Dal-Forno, PhD.

George Mason University, 2015

Dissertation Director: Dr. James D. Lawrey

Lichenized basidiomycetes represent a small group within Basidiomycota, with *Dictyonema* s.l. the clade with the highest number of species. This clade occurs world-wide, but has its highest diversity in the tropics and especially in tropical montane regions, such as the endangered paramos. The group has different morphologies, with crustose, filamentous, squamulose and foliose thalli, and the basidiocarps may be cyphelloid, corticioid or steroid. The photobionts are cyanobacteria of the genus *Rhizonema*, which at present are thought to be entirely lichenized. Recent field observations and laboratory investigations indicate that more than 300 species belong to this clade of lichenized fungi alone, but genus and species concepts are still to be established. Prior to these investigations, taxonomic concepts for the clade were based largely on anatomy, with morphological differences being treated as intraspecific variation, giving an accepted number of only five species. In an attempt to elucidate the

evolution of the group and the diversity of species in *Dictyonema* s.l., morphological, anatomical, ecological and molecular analyses were performed using more than 800 samples from 19 countries. The sequences were generated by standard Sanger and/or next-generation sequencing (NGS) procedures, the later using pyrosequencing with the 454 GS Junior, and then phylogenetic reconstructions were done using the markers ITS, nuLSU and *RPB2* for the fungal partner and 16S for the photosynthetic partner. Morphological characters were intensively reanalyzed using features of the thallus, the basidiocarps and numerous additional characteristics (i.e., hairs, sutures, and soredia). Anatomical characters were also collected, including thickness of the thallus and its layers, shape and size of cyanobacteria and hyphae, among others. Results of these molecular, morphological, anatomical and ecological analyses indicated that the group is much more diverse than previously thought, and that anatomical and morphological differences, long thought to indicate forms within the same species, are instead indicative of a far larger number of distinct species. The multilocus and individual marker analyses indicated that the group is monophyletic with five genus-level groups: *Cyphellostereum*, sister genus to the rest of species, *Dictyonema* s.str. forming a paraphyletic transitional group, and three additional monophyletic groups representing three genera, *Acantholichen*, *Cora* and *Corella*. So far, Brazil, Colombia and Costa Rica are the only countries in which all genera have been found, although many other countries have been poorly sampled. In the Galapagos Islands, focused sampling during this study augmented collections made previously by local lichenologists and visiting collectors since the 1970's. Based on previous collections in the Islands, at least eight species of

basidiolichens had been reported to occur there, but most were identified using existing names since no molecular data were available. The present study yielded 90 sequenced specimens representing ten species of the *Dictyonema* clade, most of these from Santa Cruz, Isabela, and Floreana. Molecular, morphological and anatomical investigations indicate that these species are new and mostly endemic to the Islands. Results also demonstrated that the genus *Acantholichen*, originally thought to be monotypic, is like other genera in the *Dictyonema* clade, highly endemic and made up of more than a single species. Here I present five new species to this genus, based on differences in squamules and acanthohyphidia (spiny apical cells of hyphae present in lower and upper surfaces), both unique characters to separate this genus from others within the *Dictyonema* clade. The project also investigated the diversity of the cyanobacterial *Rhizonema* photobionts of *Dictyonema* s.l., generating 16S sequences from 560 *Dictyonema* specimens and 21 additional unrelated cyanolichens in the Ascomycota. Analyses of these sequences indicated that, unlike the diversity of the fungal partner in these lichens, very few *Rhizonema* lineages are present, suggesting that these photobionts are largely shared among mycobionts (from the same or different species, including some ascolichens). These ecological interactions are similar to those of human domestication of crop species and may explain the unusual distributions of fungal and cyanobacterial symbionts throughout the Neotropics. In conclusion, the results of this study support the idea that *Dictyonema* s.l, previously considered to represent only few species in a single genus, actually includes a remarkably high diversity of species in several, distinct genera, differing in morphology, anatomy, substrate ecology and geographical distribution.

Chapter 1 – Introduction and Objectives

Introduction

Dictyonema C. Agardh ex Kunth s.l. is a large group of lichenized basidiomycetes that have a variety of morphologies, from crustose to foliose thalli, cyphelloid to stereoid basidiocarps, and all with *Rhizonema* Lücking & Barrie as its cyanobacterial photobiont (Dal-Forno et al., 2013; Lawrey et al., 2009; Lücking et al., 2009a, 2014a; Oberwinkler, 1970, 1984; Parmasto, 1978). The species in this clade have a unique combination of rare features in lichens, since they are basidiolichens (most lichens are Ascomycota) and cyanolichens (lichens mostly have a green algae as photobiont). The distribution of the species is mainly pantropical, with a few species in temperate zones (Lücking et al., 2014a; Parmasto, 1978).

Until recently, the most widely used concepts to classify this group were those of Parmasto (1978), who adopted a relatively wide species concept, emphasizing basidiocarp anatomy and treating morphological differences largely as phenotypic variation. Consequently, his monograph synonymized several previously named taxa under five species in a single genus, *Dictyonema*.

Before Parmasto, however, there were many names used to classify species in *Dictyonema* s.l. They represented several different genera and families, based on growth

form, presence of clamp connections, and photobiont (Hariot, 1891, 1892; Metzner, 1934). Foliose forms were usually treated as *Cora* Fr. (additionally *Corella* Vain., *Wainiocora* Tomas. or *Gyrolophium* Kunze), whereas filamentous forms were assigned to either *Laudatea* Johow, *Dictyonema*, or *Rhipidonema* Mattir., depending on appressed or shelf-like growth and the absence or presence of clamp connections (Lücking et al., 2013a; Parmasto, 1978). On the other hand, some authors included many different growth forms (i.e., filamentous and foliose) into a single species and regarded differences as ontogenetic and ecological variation (Larcher and Vareschi, 1988; Möller, 1893; Oberwinkler, 1970).

Although Parmasto (1978) accepted five species only, he acknowledged variation within these species. The problem with Parmasto's approach, despite its large and representative sampling, is that he exclusively worked with herbarium specimens, which is disadvantageous in this group, especially in foliose forms, due to the quick loss of key characters such as color, texture, lobe arrangement, etc., as the species are taken from their natural habitat. The other incongruence with his monograph is that he accepted "five lichen species", but at the same time stated that only two fungal species were involved in the symbiosis. According to the International Code of Nomenclature, this is incorrect, since names of lichens always apply to the fungal component [ICN Art. 13.1(d)] and hence there cannot be two 'fungal' and five 'lichen' species at the same time (Lücking et al., 2013a).

Chaves et al. (2004) were the first after Parmasto (1978) to address specifically the *Dictyonema* group, describing two new species and providing a key to a total of eleven

different morphotypes. Until the work of Chaves et al. (2004), most collections of *Dictyonema* in herbaria have been identified to one of the two main species, *D. sericeum* (Sw.) Berk. for filamentous forms (without clamp-connections) and *D. glabratum* (Spreng.) D. Hawksw. [= *Cora pavonia* (Sw.) Fr.] for foliose forms, both thought to be common and widespread in tropical montane to Andean habitats (Hawksworth, 1988; Parmasto, 1978).

Lücking (2008) also mentions four different morphologies of foliicolous *Dictyonema* under two species, *D. sericeum* fo. *membranaceum* Metzer and *D. phyllogenum* (Müll. Arg.) Zahlbr., the later with three differ forms. Both studies (Chaves et al., 2004; Lücking, 2008) challenged Parmasto's concepts, but stated that molecular analyses were necessary to further investigate if morphotypes recognized by Parmasto (1978) were not better recognized as distinct species instead of forms.

The first detailed molecular study of the *Dictyonema* group was by Lawrey et al. (2009), who found that *Dictyonema* s.l. could possibly be divided into three genera, *Cyphellostereum* D. A. Reid, *Dictyonema* s.str., and *Cora*, and in addition included the genus *Acantholichen* P. M. Jørg. Before this study, the only molecular phylogenies to include *Dictyonema* species were those of Gargas et al. (1995), who included one species, and Ertz et al. (2008), who included two species. The study by Yánez et al. (2012) was the first to employ the molecular findings of Lawrey et al. (2009) in the development of an inventory of Galapagos basidiolichens. However, none of these included sufficiently thorough sampling to resolve the structure within the *Dictyonema* clade.

The four genera shown in the phylogeny of Lawrey et al. (2009) are well-distinguished morphologically and anatomically: *Cyphellostereum* and *Dictyonema* have homomerous filamentous thalli formed by fibrils, that is, with the mycobiont forming a hyphal sheath around the cyanobacterial trichomes, while *Acantholichen* and *Cora* have heteromerous thallus with chroococcoid packets of cyanobacteria. *Dictyonema* s.str. and *Cyphellostereum* have very similar morphologies; however, the first has jigsaw-puzzle-shaped hyphae surrounding wide cyanobacterial filaments, whereas the second presents the sheath consisting of irregular hyphae and much thinner cyanobacterial filaments. In addition, *Cyphellostereum* has cyphelloid basidiocarps emerging from an undifferentiated lichenized thallus, and *Dictyonema* s.str. has steroid-corticioid basidiocarps that develop mostly from the underside of a lichenized thallus. *Acantholichen* forms a microsquamulose thallus with acanthohyphidia, spiny hyphae located on the upper and lower surface giving a powdery appearance to the thallus, while species of *Cora* have foliose-macrosquamulose thalli and produce corticioid basidiocarps on the lower surface.

Besides these four genera, a fifth genus was recently segregated, *Corella* (Dal-Forno et al., 2013). *Corella brasiliensis* Vain., a basidiolichen that resembles species in *Cora* (Metzner, 1934; Vainio, 1890; Xavier Filho and Vicente, 1979), is morphologically and anatomically quite distinct from *Dictyonema glabratum* [= *Cora glabrata* (Spreng.) Fr.]; however Parmasto (1978) included *Corella brasiliensis* as a synonym of the latter and regarded it a juvenile form. Recent analyses of Dal-Forno et al. (2013) showed that *Corella* forms a monophyletic genus sister to *Acantholichen*, despite its similarity to *Cora* in thallus morphology. *Corella* has a paraplectenchymatous cortex under the

microscope, which makes visible the cyanobacteria underneath, giving a dark bluish appearance to these species, generally making possible to distinguish the two foliose genera in the field.

The taxonomic significance of the cyanobacterial photobionts in this clade also changed substantially with recent phylogenetic studies. Historically, photobionts in *Dictyonema* were assigned to *Scytonema* C.Agardh ex Bornet & Flahault for filamentous forms and *Chroococcus* Nägeli for foliose and microsquamulose forms (Henssen, 1963; Oberwinkler, 1970, 1984; Parmasto, 1978; Roskin, 1970; Ryan, 2001). It was not until Lücking et al. (2009a) that the *Dictyonema* clade, along with two genera of ascolichens, *Coccocarpia* Pers. and *Stereocaulon* Hoffm., was found to have a previously undescribed lineage of cyanobacteria, *Rhizonema,* which has a uniquely lichenized lifestyle (Lücking et al., 2014a). The same study discovered that this clade of cyanobacteria belongs to the order Nostocales, which is known to form close symbiotic relationships with eukaryotes (e.g., the interaction between the cyanobacteria *Anabaena* and the fern *Azolla*).

Table 1 - General characteristics of accepted genera in *Dictyonema* s.l.

Genus	Thallus morphotype	Basidiocarp	Shape of *Rhizonema*
Acantholichen	Microsquamulose	Unknown	Chroococcoid
Cora	Foliose	Steroid-corticioid	Chroococcoid
Corella	Foliose	Unknown	Chroococcoid
Cyphellostereum	Filamentous	Cyphelloid	Filamentous
Dictyonema	Filamentous	Steroid-corticioid	Filamentous

Objectives

The overall goal of my dissertation research was to clarify the evolution and species diversity of the globally-distributed but mainly tropical clade *Dictyonema* s.l. using molecular, morphological, anatomical and ecological data. Specifically my objectives were: (1) To produce comprehensive uni- and multigene phylogenies of *Dictyonema* s.l. using representatives of all the major clades, with emphasis on the internal transcribed spacer (ITS) barcoding locus, employing next-generation sequencing techniques where appropriate; (2) to provide new species and genus concepts for the group; (3) to generate a protocol to standardize the study and identification of the species belonging to the different genera; (4) to compile a list of currently accepted names within the *Dictyonema* clade; (5) provide a key to identify the five genera; (6) to investigate evolutionary transitions in thallus morphology and reproductive structures in the *Dictyonema* clade by comparing basal groups such as *Cyphellostereum* to the more derived groups such as *Cora* (Chapter 2); (7) to elucidate the diversity of the genus *Acantholichen* using morphological, anatomical, ecological and phylogenetic data (Chapter 3); (8) to reassess the diversity of the *Dictyonema* clade in the Galapagos Islands including phylogenetic data (Chapter 4); and (9) to compare and discuss the diversity between mycobiont versus photobiont using multiple datasets (Chapter 5).

Chapter 2 – From mushrooms to lichens: The *Dictyonema* clade (Basidiomycota: Hygrophoraceae) as a model of evolutionary transitions to the lichenized lifestyle in fungi

Dal-Forno, M., Lawrey, J.D., Sikaroodi, M., Bhattarai, S., Gillevet, P.M., Sulzbacher, M., and Lücking, R. (2013). Starting from scratch: Evolution of the lichen thallus in the basidiolichen *Dictyonema* (Agaricales: Hygrophoraceae). Fungal Biol. *117*, 584–598.

Abstract

In recent decades, species and genus concepts in *Dictyonema* s.l. were broad and based mainly on thallus anatomy. These concepts resulted from synonymizing many good, valid older names, with all of the previously recognized diversity being represented under a single genus with few species. To elucidate the evolution and diversity in *Dictyonema* s.l., we generated a dataset of 68 new sequences of the nuclear large subunit rDNA (nuLSU), the internal transcribed spacer (ITS), and the RNA polymerase II subunit (*RPB2*). We performed a phylogenetic analysis with a concatenated dataset of 29 species-level lineages of *Dictyonema* s.l. in which nearly all markers were available. The multilocus phylogeny obtained via maximum likelihood and Bayesian approaches indicates the presence of five genus-level groups: *Cyphellostereum*, *Dictyonema* s.str.,

Acantholichen, *Cora*, and *Corella*. Lichens in all genera form associations with a unique lineage of obligately lichenized cyanobacteria, known as *Rhizonema* (Nostocales). The clade is of recent origin, many species of which are thought to have evolved with the uplift of the Andes, and is nested within a larger clade of extant non-lichenized agaric mushrooms in the genus *Arrhenia*, which makes *Dictyonema* attractive as a model for understanding the origin of lichen symbioses. Our analyses indicate a progressive development of the lichenized thallus from loosely organized filamentous crusts with separate, cyphelloid basidiocarps in *Cyphellostereum*, to filamentous crusts with derived hyphal sheath and steroid-corticioid basidiocarps partially incorporated into the lichen thallus in *Dictyonema*, to squamulose-foliose thalli with steroid-corticioid basidiocarps entirely supported by the lichen thallus in *Cora*. Such a trend represents a remarkable evolutionary integration of lichenized and reproductive tissues in *Dictyonema* s.l., supporting the hypothesis that, at least in this case, lichenized thalli may have evolved from reproductive structures in non-lichenized ancestors.

Introduction

Lichens are successful and remarkable living symbiotic organisms in terms of numbers of species, diversity and complexity of lichenized morpho-anatomical features, and in the number of lineages that have independently achieved this type of symbiosis (Ahmadjian, 1993; Kirk et al., 2008; Nelsen et al., 2009; Schoch et al., 2009). This symbiotic habit has evolved numerous times in separate fungal lineages, with wide

distribution within the major clades of ascomycetes, and several (but fewer) origins in the basidiomycetes (Gargas et al., 1995). Yet, the origin of lichenization is a matter of debate, as are the evolutionary steps that have led to the development of the integrated lichen thallus (Eriksson, 2005; Gargas et al., 1995; Hawksworth, 2005; Lücking et al., 2009a; Lutzoni et al., 2001; Schoch et al., 2009).

One theory suggests that, because the lichen thallus is structurally complex, whereas the mycelium of non-lichenized fungi is usually undifferentiated, the lichen thallus evolved from fungal stromata or reproductive fungal tissue (Jahns, 1988; Moser-Rohrhofer, 1969; Poelt and Wunder, 1967). Indeed, in lichens such as *Cladonia*, the vertical thallus is derived from reproductive tissue (Jahns, 1988, 1970). Several studies have shown that the photobiont can also affect lichen thallus morphogenesis, to the point that the same lichen fungus is able to form morphologically distinct thalli with different photosynthetic partners (Armaleo and Clerc, 1991; Brodo and Richardson, 1978; Jahns, 1988; James and Henssen, 1976; Sanders, 2001a; Stocker-Wörgötter, 2002; Takahashi et al., 2006; Tønsberg and Goward, 2001; Tschermak-Woess, 1995). These theories were discussed and studied in connection with lichenized Ascomycota, but except for supported sister-group relationships between rock-inhabiting fungi and lichenized forms in the Arthoniales and Verrucariales (Gueidan et al., 2008; Ruibal et al., 2009), no lineages are known in this phylum that show direct transitions from non-lichenized to lichenized forms.

Approximately 20% of all fungi are lichenized, and these represent 40% of all Ascomycota (Kirk et al., 2008). Current lichenized fungi species are estimated in

approximately 18,000. Nearly all of these species belong in the phylum Ascomycota. Few members of the Basidiomycota are lichenized and these are mainly found in the orders Agaricales, Cantharellales, Corticiales, and Lepidostromatales (Ertz et al., 2008; Fischer et al., 2007; Hibbett et al., 2007; Hodkinson et al., 2014; Lawrey et al., 2009; Nelsen et al., 2007; Sulzbacher et al., 2012, **Table 1**).

Table 2 - Classification of currently accepted genera of basidiolichens
Shaded area represents genera treated in this dissertation

Agaricales
Hygrophoraceae
***Dictyonema* s.l.**
Acantholichen
Cora
Corella
Cyphellostereum
Dictyonema s.str.
***Lichenomphalia* s.l.**
Lichenomphalia
Semiomphalina
Protolichenomphalia

Cantharellales
Clavulinaceae
Ichthyoclavula
Multiclavula
Corticiales
Corticiaceae
Marchandiomphalina
Lepidostromatales
Lepidostromataceae
Ertzia
Lepidostroma
Sulzbacheromyces

Most lichenized basidiomycetes can be found in the family Hygrophoraceae (Agaricales) forming two clades: *Lichenomphalia* s.l. and *Dictyonema* s.l. (Lawrey et al., 2009). Despite their placement within the same fungal family, both present many different characteristics. *Lichenomphalia* forms agaricoid-omphalinoid mushrooms that arise from lichenized granules or squamules containing a green algal (*Coccomyxa*) photobiont, while *Dictyonema* species form a variety of basidiocarps (cyphelloid to steroid-corticioid), but not agaricoid, and a lichenized thallus containing a *Rhizonema*

photobiont, a lineage of cyanobacteria so far known to be only lichenized (Chaves et al., 2004; Lawrey et al., 2009; Lücking et al., 2009a, 2014a; Redhead et al., 2002).

Until recently, it was considered to represent five species in a single genus, *Dictyonema* (Parmasto, 1978), but subsequent revisionary taxonomic and molecular phylogenetic studies suggested that this number is a gross underestimate of the real diversity (Chaves et al., 2004; Lawrey et al., 2009).

Initial studies (Lawrey et al., 2009) indicated that what was previously considered a single genus (Parmasto, 1978), *Dictyonema*, actually forms a clade containing three genera, the filamentous *Cyphellostereum* and *Dictyonema*, the foliose *Cora*, and additionally included the microsquamulose *Acantholichen.*

Dictyonema is a large clade of basidiolichens that exhibit a variety of different patterns of thallus development, basidiocarps, thallus structure, relationship with the photobiont, as well as substrates they grow on. They can develop crustose-filamentous, shelf-like filamentous, squamulose and foliose thalli, and the basidiocarps can be cyphelloid, corticioid and steroid. The numerous morphological and anatomical characters of the group indicate there may be many undescribed species in the group, representing a remarkable diversity of growth forms; however, the current circumscription of the group does not reflect this diversity.

The earlier phylogeny (Lawrey et al., 2009) indicated a transition in the *Dictyonema* clade from unstructured to complex vegetative thalli and the simultaneous gradual incorporation of the basidiocarps into thallus, which lends support to the hypothesis that the lichen thallus evolved from fruiting body structures of non-lichenized

ancestors in these basidiolichens. Since *Dictyonema* s.l. is hitherto the only lichenized lineage in which direct, extant, non-lichenized ancestors have been established in the genera *Arrhenia* and *Eonema* (Lawrey et al., 2009), this clade is an excellent model to investigate the evolution of the lichen thallus. However, testing this hypothesis required a larger molecular dataset representing more specimens and sequences.

We therefore proposed to increase the sampling of this group (both taxonomically and geographically) and to use a combination of the large subunit (nuLSU) and the internal transcribed spacer (ITS) partition of the nuclear ribosomal DNA, and sequences between the conserved domains 6 and 7 of the protein-coding second largest subunit of the RNA polymerase II gene (*RPB2*), to expand the previous phylogenetic analyses (Lawrey et al. 2009) and test hypotheses regarding thallus evolution in the group.

Materials and methods

Taxon sampling

For this study, a small dataset was produced consisting of 29 ingroup species-level terminals, and the outgroup *Eonema pyriforme* used by Lawrey et al. (2009), including 68 new sequences obtained from specimens of *Acantholichen*, *Cyphellostereum*, *Cora*, *Corella* and *Dictyonema* species collected from 13 countries in North, Central, and South America, Europe, and southeast Asia (**Table 2**).

Microscopy

Morphology and anatomy analyses. All specimens were examined with a LEICA MS5 (Wetzlar, Germany) and an OLYMPUS SZX12 (Shinjuku, Japan) dissecting microscope and a ZEISS Axioscop 2 (Jena, Germany) and an OLYMPUS BH-2 (Shinjuku, Japan) compound microscope. Sections of the thallus are studied in water without staining. Microphotographs are taken with DAGE MTI DC-330 3CCD (Michigan City, Indiana, USA) and JENOPTIC ProgRes C3 and C5 (Jena, Germany) digital microscope cameras attached to the aforementioned microscopes. Macrophotos are taken *in situ* with CANON Powershot SX20IS (Ota, Japan) and NIKON F301 (Tokyo, Japan) digital cameras. Additional photos in the laboratory were taken with a DSLR Sony α33 digital camera.

A list of characters was developed to represent the known morphological diversity of the group. As described in the introduction, the group had been poorly circumscribed and there was a critical need for an objective and extensive list of characters to be studied in every sample. The detailed protocols for studying each morphotype can be found in **Appendix 1** (*Cyphellostereum*/*Dictyonema, Cora*/*Corella*. For detailed characteristics on *Acantholichen*, see Chapter 2 in this dissertation).

Chemical data

In addition to morphology and anatomy, we analyzed 64 samples of *Dictyonema* (8), *Acantholichen* (1), *Cora* (50), and *Corella* (5) by means of thin-layer

chromatography (Orange et al., 2001) to detect the possible presence of secondary substances, as suggested by an earlier study (Piovano et al., 1995).

Molecular data

Genomic DNA was extracted from lichenized thalli using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogene, Illkirch, France) according to the manufacturer's protocol with slight modifications. About 10 ng of extracted DNA were subjected to a standard PCR in a 25 mL reaction volume using either Taq Gold polymerase (Applied Biosystems, Foster City, CA, USA) or Bio-X-Act Long Mix (Bioline USA, Taunton, MA, USA) according to manufacturer's protocols. Sequence data were obtained from the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA cistron (ITS1, 5.8S, and ITS2, approximately 700bp), large subunit (nuLSU) nuclear ribosomal DNA (approximately 1470 bp), and partial DNA-directed RNA polymerase II second largest subunit (*RPB2*), between the 6 and 7 conserved domains (approximately 1000bp). The PCR products were visualized on a 1% agarose gel with ethidium bromide and then were purified with magnetic beads (Agencourt Bioscience, Beverly, MA, USA). The purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The primers used were: ITS5 and ITS4 for ITS (White et al., 1990); LR0R, LR3R, LR5, LR7 and LR16 for nuLSU (http://www.biology.duke.edu/ fungi/mycolab/ primers.htm) and b*RPB2*-6F or bRBP2-5F and bRBP2-7R, bRBP2-7R2 or b*RPB2*-7.1R for *RPB2* (Denton et al., 1998; Liu et al., 1999; Matheny, 2005). The sequencing reactions were then purified using Sephadex G-

50 (Sigma-Aldrich, St. Louis, MO, USA), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems).

The data collected were analyzed using ABI software, and 500–700 bases were collected for each primer used. These sequences were then assembled together with the software Sequencher version 5.0 (Gene Codes, Ann Arbor, MI, USA) for manual corrections in base calling and to make contiguous alignments of overlapping fragments.

Sequence alignment and phylogenetic analysis

Newly generated sequences from Sanger and 454 sequencing were aligned using MAFFT (Katoh et al., 2005, --auto option) and the alignment was subjected to maximum likelihood (ML) search using RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2005), with nonparametric bootstrapping using 500 replicates under the GTRGAMMA model. Each gene was first analyzed separately, and then combined (concatenated dataset) for the samples where an additional marker, aside from ITS, was available. This analysis consists of evaluating conflicting clades and takes into consideration nonparametric bootstrap values. It is not a statistical test, but is widely used in phylogenetic studies using multiple markers (Mason-Gamer and Kellogg, 1996). The topologies of individual gene trees are compared and if strongly supported clades (BS higher than 70%) are in disagreements between the different trees that represent conflict and data cannot be combined. Since no conflict was detected, we also subjected our combined dataset to ML.

The individual nuLSU and ITS alignments were subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al., 2010a, 2010b). *RPB2* was not run through GUIDANCE because variation is very low, and alignments were visualized (with and without translation) using Geneious. Altogether, this resulted in an alignment length of 1327 for the nuLSU, 600 for the ITS, and 1021 for the *RPB2* partition, for a total of 2948 sites in the combined dataset. The dataset was also analyzed under a Bayesian framework using MrBAYES 3.1.2 (Huelsenbeck and Ronquist, 2001), with two independent runs, a total chain length of one million generations, and four separate chains each, resampling every 1000 trees and generating a majority rule consensus tree from the tree sample after discarding 25% burnin to obtain posterior probability estimates.

Summary of analysis protocol for each sample

The procedure for analyzing each sample has been the following: (1) In the field, every possible characteristic should be observed *in situ*. As with most other Basidiomycota, specimens in the group quickly lose several characteristics when removed from their natural habitat. Color, texture and arrangement of the thallus, substrate, presence of hairs and basidiocarps are essential features to be observed in the field. If possible, filling out the protocol sheet (**Appendix 1**) in the field makes it easier to remember what characteristics should be observed in fresh material. (2) The material analysis (morphological and anatomical observations and measurements) is finalized in the lab and the protocol of characteristics is followed (**Appendix 1**). (3) The combination

of field and laboratorial observations generates a working name, which is a hypothesis of how each sample may eventually be classified. Generally morpho-groups are used, i.e., "*sericeum*-type" for filamentous forms growing shelf-like, "*applanatum*-type" for forms of *Dictyonema* where fibrils are embedded in a matrix. (4) The sample has its DNA sequenced and placed phylogenetically, which will provide support for rejecting or accepting the working name. (5) The sample is analyzed again and checked to see if placement in the phylogenetic tree makes sense (for example, the presence of a *Cora*-type specimen in *Cyphellostereum* would be highly suspicious and require a new extraction and sequence). (6) The final step is to compilate all specimens under a species name for description and to link them to images available. In cases where good images are missing, samples are photographed in the lab in the dry state and then rehydrated. Specimens for which we have images of fresh specimens are also rehydrated to see if the colors are similar. This is particularly important for the foliose and squamulose forms in the *Cora* clade. In most cases where the recovered color is similar and therefore considered a reliable characteristic, we added this to the protocols. However, since color especially may vary according to the age of the specimen, a date of study is added to the description.

Results

Morphological and anatomical characters

The extensive list of characters to be analyzed proved to provide far more resolution in delimiting genera and species than the mainly mycological features used by

Parmasto (1978). Our working names, developed based on morphological, anatomical and ecological observations, frequently reflected expected positions of the specimens in the phylogenetic trees. In addition, features not taken into consideration before, such as substrate or biogeography, were shown to reflect important patterns. For a detailed list of characters used in the analysis see **Appendix 1**. New species descriptions based on these analyses have since been published (Lücking et al., 2013a, 2014b).

Phylogenetic analysis

The maximum-likelihood analysis of the combined three-gene dataset of selected species sequences resulted in a well-supported topology in which *Cyphellostereum* was monophyletic and sister to the remaining species (**Figure 1**). *Dictyonema* s.str. forms a paraphyletic grade, and *Acantholichen*, *Cora*, and *Corella* emerges as a supported, monophyletic clade. *Acantholichen* plus *Corella* formed a supported, monophyletic clade sister to *Cora*. No support was found in the backbone of the *Dictyonema* s.str. grade.

Bayesian analysis of the same dataset resulted in a congruent topology with strong support for the aforementioned clades (tree not shown but posterior probabilities plotted on the maximum likelihood tree in **Figure 1**). Thus, the phylogenetic analysis supports the recognition of up to five genera: *Cyphellostereum*, *Dictyonema* s.str., *Acantholichen*, *Corella* and *Cora*.

Combined analysis (morphology and phylogeny)

The combined molecular-morphological analysis indicates that the lichenized thallus progresses from appressed, crustose filamentous forms in *Cyphellostereum* and

Dictyonema to microsquamulose thalli in *Acantholichen* and macrosquamulose to large foliose forms in *Corella* and *Cora* (**Figure 2**). *Cora* develops a loose, corticiform layer of more or less perpendicular hyphae, while *Corella* has a true, paraplectenchymatous cortex. The photobiont shows a filamentous morphology in the filamentous thallus types and has individual cells in the squamulose and foliose forms. The hyphal sheath in *Cyphellostereum* is composed of irregular, cylindrical hyphae leaving large interspaces, whereas in all other taxa it forms a closed, paraplectenchymatous layer composed of jigsaw-puzzle-shaped cells. This correlates well with the absence of tubular intracellular haustoria in *Cyphellostereum* and their presence in the other genera. Coincidentally, the cyanobacterial hyphae are narrow in *Cyphellostereum* (5–8 μm) and broad in the other genera [8–12(–20) μm].

Cyphellostereum has cyphelloid basidiocarps emerging from the undifferentiated lichenized thallus, in which the basidiocarps do not have any photobiont; instead, the hyphae associated with the cyanobacterial filaments appear to represent vegetative hyphae, as they are thinner and different from the generative hyphae forming the basidiocarps. *Dictyonema* has stereoid-corticioid basidiocarps developed on the underside of the lichenized thallus and usually at least partly overgrown with photobiont filaments; the hyphae associated with the cyanobacterial filaments are thicker (4–6 μm) than in *Cyphellostereum* (2–3 μm) and indistinguishable from the generative hyphae supporting the hymenophore. Species of *Cora* and also *Dictyonema sericeum* have steroid-corticioid hymenophores developing on the lobe underside and completely incorporated into the

thallus. This means the thallus supports the hymenophore, but it is not transformed in shape when fertile.

In addition to the phylogenetic progression in morphological-anatomical features, the three major clades and grades also exhibit distinctive patterns of sequence evolution, particularly in the ITS1 and ITS2 regions (**Figure 3**). Species currently assigned to *Cyphellostereum* have extremely variable, partially unalignable sequence portions in both the ITS1 and ITS2 region (overall alignment confidence score from GUIDANCE = 0.784). Species contained within *Dictyonema* s.str. also have highly length variable ITS1 and ITS2 regions but their alignment is less ambiguous (overall alignment confidence score = 0.862). Species classified as *Cora* have substantially less length variation in the ITS and the level of ambiguity is significantly lower (overall alignment confidence score = 0.954).

Discussion

Lichens, especially ascolichens, exhibit a remarkable diversity of thallus morphologies, and this variation has traditionally provided the basis for delimiting taxonomic groups. However, it is now apparent that thallus morphology is rarely a good indicator of phylogenetic relationships among lichens (Gaya et al., 2012; Grube and Hawksworth, 2007; Lumbsch and Leavitt, 2011; Rivas-Plata and Lumbsch, 2011). Even among basidiolichens that typically have simpler and less variable vegetative thalli, identical morphologies are known to have arisen independently in unrelated groups, for

example *Multiclavula* and *Sulzbacheromyces* (Ertz et al., 2008; Hodkinson et al., 2014; Sulzbacher et al., 2012).

Our analyses (published in Dal-Forno et al., 2013) indicate a noticeable progression over time in lichenized *Dictyonema* s.l., from a simple, undifferentiated vegetative thallus with separate cyphelloid basidiocarps in the early-diverging *Cyphellostereum* to forms in which basidiocarps themselves appear to dominate the lichen thallus, with individual hymenophores regularly dispersed over the thallus underside, in the late-diverging *Cora*. This suggests that the structure of the basidiocarp and its gradual incorporation into the lichen thallus might be responsible for thallus formation in these lichenized species, and it may also explain why the most derived species of *Cora* and *Corella* closely resemble shelf-like stereoid macrofungi. Oberwinkler (1970) regarded the *Dictyonema* s.l. thallus as lichenized fruiting body, a view shared by other workers (Parmasto, 1978; Ryan, 2001; Slocum, 1980; Trembley et al., 2002). Indeed, Clémençon et al. (2004) remarked that hymenium development in *Cora glabrata* may be interpreted as an aggregation of simple cyphelloid basidiomes on the undersurface of a lichenized 'stroma'.

This interpretation is supported by the observation that in *Dictyonema* s.str., *Acantholichen*, *Corella*, and *Cora*, the hyphae forming the ‘vegetative’ thallus (cortex, medulla, photobiont layer) resemble those producing the hymenophore, and hence can be interpreted as generative hyphae, whereas in *Cyphellostereum*, the hyphae associated with the photobiont filaments strongly differ from the generative hyphae forming the basidiocarp. As a consequence, it is no longer possible to attribute morphological

differentiation in this clade as ontogenetic or ecological variation, as done by previous workers (Larcher and Vareschi, 1988; Möller, 1893; Oberwinkler, 1970, 2001; Parmasto, 1978). Instead, this 'variation' reflects distinct evolutionary patterns resulting in a large number of species- and genus-level clades, a view also accepted by Oberwinkler (2012).

The observed trends in thallus evolution in this clade are restricted to lichenized forms, and are therefore likely caused by lichenization. The immediate, non-lichenized ancestors of *Dictyonema*, *Eonema pyriformis* and the genus *Arrhenia* sensu lato (Barrasa and Rico, 2003; Lawrey et al., 2009; Lodge et al., 2014; Redhead et al., 2002), have mostly dorsiventral, arrhenioid (with gills) or cyphelloid (without gills) basidiocarps, the latter resembling those of *Cyphellostereum*. Most other members of the family Hygrophoraceae have radially symmetrical, agaricoid-omphalinoid basidiocarps, including the lichenized genus *Lichenomphalia*, which forms only crustose to microsquamulose thalli in which the basidiocarps are not integrated into the lichen thallus (Lawrey et al., 2009; Lodge et al., 2014; Lutzoni, 1997; Lutzoni and Vilgalys, 1995; Redhead et al., 2002). We conclude from this that the evolution of dorsiventral basidiocarps in *Arrhenia* and *Eonema* facilitated the subsequent formation of an integrated lichen thallus in *Dictyonema*, since flattened, dorsiventral basidiocarps can incorporate photobionts more easily into their sterile portions than radially symmetrical basidiocarps, as in *Lichenomphalia*, in which the sterile cap is separated from the sterile stipe by the hymenophore. This is also supported by the fact that *Dictyonema* s.l. lichens are substantially more abundant than *Lichenomphalia* and compete successfully with ascolichens with similar morphologies occurring in the same habitats, such as

Coccocarpia, *Coenogonium*, *Normandina*, *Peltigera*, and *Sticta*, among others (Barrasa and Rico, 2003; Bigelow, 1970; Brodo et al., 2001; Chaves et al., 2004; Smith et al., 2009). This view is shared also by Oberwinkler (1984: 748), who stated, regarding the structurally complex thallus in *Cora*, that it was an '*ecologically very well adapted symbiotic structure.*'

In the *Dictyonema* clade, species associate with cyanobacteria in *Rhizonema* that appear to be obligately lichenized and largely tropical, occurring both in *Dictyonema* s.l. and also in other, unrelated but ecologically similar lichens, such as *Coccocarpia* (Lücking et al., 2009a, 2014a). However, in terms of the level of unique associations, the process of lichenization appears to differ in the genera of *Dictyonema* s.l. Species in *Cyphellostereum* commonly harbor diverse mixtures of cyanobacteria and unicellular chlorophytes, as mentioned earlier by Oberwinkler (2001, 2012). This suggests a more loose association of the mycobiont of this genus with diverse photobionts, whereas other genera in the clade generally form associations with a single, dominant photobiont, as apparent from unpublished 454 sequencing data (Chapter 5 has more on this subject). Morphologically, photobionts in *Cyphellostereum* and *Dictyonema* s.str. resemble free-living filiform *Scytonema* trichomes, whereas in the more derived *Cora* and *Corella* clades, they are coiled and significantly reduced in size, forming aggregates of rounded cells similar to *Chroococcus* (Chaves et al., 2004; Clémençon et al., 2004; Lücking et al., 2009a; Parmasto, 1978). This difference was discussed by other authors (Mägdefrau and Winkler, 1967; Möller, 1893; Oberwinkler, 1970; Ryan, 2001; Tomaselli and Caretta, 1969) and even led to the establishment of a separate lichenized genus, *Wainiocora*,

supposedly differing from *Cora* by a *Chroococcus* photobiont (Tomaselli, 1950, 1951). Oberwinkler (1970), Parmasto (1978), and Chaves et al. (2004) suggested that these morphotypes represent the same photobiont, which was later confirmed by molecular data. Phylogenetic analyses indicate that this is not due to a photobiont switch but caused by fungal-specific morphogenetic effects on the photobiont (Lücking et al., 2009a). Thus, in addition to a progressive thallus morphological development, the *Dictyonema* s.l. clade also exhibits a gradual evolution towards more stable symbiotic interactions between its symbionts, with an increased morphological dominance of the mycobiont over the photobiont.

The particular and unique haustoria of *Dictyonema* s.l. have been described and discussed in detail by several authors (Oberwinkler, 1970, 1984, 2001, 2012; Roskin, 1970; Slocum, 1980; Slocum and Floyd, 1977). They are present in *Dictyonema* s.str., *Acantholichen*, *Cora*, and *Corella*, but not in *Cyphellostereum*. We also found a strict correlation between the presence of haustoria, the shape of the hyphal sheath around the photobiont cells, and photobiont morphology. Taxa with haustoria always feature a hyphal sheath formed by jigsaw-puzzle shaped cells, with the sheath being tubular around the photobiont filaments in *Dictyonema* s.str. and orbicular around the irregular photobiont cell groups in *Acantholichen*, *Corella*, and *Cora*. Both haustoria and sheath are absent in *Cyphellostereum*. Remarkably, the cyanobacterial filaments are much narrower in *Cyphellostereum* than in the other genera, which we initially interpreted as a different photobiont species. However, 454 sequencing data indicate the primary photobiont represents the same clade in all observed lichens (Chapter 5 of this

dissertation). The remarkable variation in appearance is explained by the effect of the intracellular haustoria: apparently, the cyanobacterial filaments in *Cyphellostereum*, which lack haustoria, represent the natural cell width, whereas the haustorial hyphae in *Dictyonema*, *Acantholichen*, *Corella*, and *Cora* are caused by lateral 'inflation' of the penetrated photobiont cells. Even in taxa with haustoria present, there is further variation in cell width from species to species (Lücking et al., 2013a).

A differentiated cortex has always been considered a hallmark achievement of lichen thalli, as it provides a certain level of integration and protection of the bionts (Honegger, 2001; Jahns, 1988; Sanders, 2001b). In many lichens, layering of the thallus correlates with the production of particular secondary metabolites in the cortex acting as sun-screens (Bjerke and Dahl, 2002; Fernández et al., 1996; Gauslaa, 2009; Gauslaa and Solhaug, 2001; Lawrey, 1986; Rundel, 1978; Solhaug and Gauslaa, 1996). In the *Dictyonema* clade, only the microsquamulose and foliose forms represented by the genera *Acantholichen*, *Cora* and *Corella* feature a cortex. The cortex in *Cora* is different from the cortex of *Acantholichen* and *Corella*, suggesting an independent evolution, which is supported by their phylogeny, with *Corella* being sister to the squamulose *Acantholichen*, and *Cora* forming a separate clade. The unique, medullary upper cortex in *Cora* and the differences compared to *Corella* were already noted by earlier workers (Metzner, 1934; Ozenda, 1963; Tomaselli, 1950; Tomaselli and Caretta, 1969; Xavier Filho and Vicente, 1979; Zahlbruckner, 1926). Oberwinkler (1970) interpreted the paraplectenchymatous cortex of *Corella* as collapsed hyphae and Parmasto (1978) did not recognize the difference as significant or of taxonomic importance. *Acantholichen* was thought to be

ecorticated and homomerous until Dal-Forno et al. (in prep and Chapter 3) demonstrated that all species now known from the genus produce a thin cortex with acanthohyphidia.

Piovano et al. (1995) reported the presence of atranorin and tenuiorin in material of *Dictyonema glabratum* (now *Cora*). These substances are commonly found in large macrolichens in the Ascomycota, such as Lobariaceae, Parmeliaceae and Peltigeraceae (Huneck and Yoshimura, 1996; Miadlikowska and Lutzoni, 2000; Moncada et al., 2013), and their presence in *Cora* would imply a remarkable level of parallel evolution in completely unrelated lineages with comparable ecology. However, our analysis of 64 samples of *Cora*, *Corella*, *Acantholichen* and *Dictyonema*, indicated the complete absence of acetone-soluble compounds deposited extracellularly in the hyphal walls. This is supported by the color change in wetted *Cora* lichens, which become much darker than in the dry state, very different from Lobariaceae and Parmeliaceae that have atranorin as a cortical compound. We therefore consider the result of Piovano et al. (1995) as artifactual, since it is highly unlikely that only specimens in Chile would produce these substances, whereas samples from Mexico, Costa Rica, Colombia, Ecuador, Bolivia, and Brazil studied by us contained no secondary compounds. Another chemical study (Xavier-Filho et al., 1980) reported phytohaemagglutinin from *Dictyonema sericeum*, *D. glabratum*, and *Corella brasiliensis*, but these are intracellular substances not comparable to extracellular secondary compounds commonly found in lichens. The same applies to the proteins, lipids, and carbohydrates reported by Elifio et al. (2000), Sassaki et al. (2001) and Carbonero et al. (2002) from *D. glabratum*. Phytohaemagglutinin, found for example in legumes, triggers blood agglutination and is also assumed to play a role in

early stages of lichen symbiosis (Lockhart et al., 1978). The occurrence of this substance has not been much studied, but there are reports from *Peltigera* (Lockhart et al., 1978), which makes it unlikely that the shared occurrence in *Dictyonema*, *Cora*, and *Corella* has any phylogenetic significance.

The *Dictyonema* clade is a prime example of how interpretation of morphological differentiation as ontogenetic or ecological variation, even if based on detailed field observations, can lead to misinterpretations about the evolution and classification of a group of organisms. In this case, we refer to the studies by Möller (1893) and Larcher and Vareschi (1988), discussed by others (Oberwinkler, 1970, 2001, 2012; Parmasto, 1978) as potential evidence for ecomorphological and ontogenetic variation in these lichens. Möller (1893) provided a lengthy account on the ontogeny and seasonal variation of *Dictyonema* s.l. lichens depending on ecological conditions, based on field observations over several years. While this study is unique in its approach, it merges different taxa, at the time unknown to the author, to document 'variation'. For example, the nonlichenized, terricolous basidiomata considered by Möller (1893) to represent 'free-living' *Cora* mushrooms are in reality species of *Cyphellostereum*, which often co-occur with *Cora* in the same habitat. He also mentioned that certain *Cora* lichens, with bluish thalli, produced the same cyphelloid basidiocarps, considering this evidence for the conspecificity of all these elements. These are likely to represent *Cora cyphellifera* Dal-Forno, Bungartz & Lücking, a new species described by Lücking et al. (2013a) from northern Ecuador. Möller (1893) also observed foliose *Cora* thalli growing out of filamentous *Dictyonema* and concluded that one and the same fungus was involved and

simply changed its morphology when switching from a *Scytonema*-like to a *Chroococcus*-like photobiont. While such statements were revolutionary for that time and actually hold true in several lichen lineages (Armaleo and Clerc, 1991; Brodo and Richardson, 1978; Jahns, 1988; James and Henssen, 1976; Sanders, 2001a; Stocker-Wörgötter, 2002; Takahashi et al., 2006; Tønsberg and Goward, 2001; Tschermak-Woess, 1995), in this particular case they are incorrect and based on accidental observation of two different lichens growing together. In the hundreds of collections and field individuals of *Dictyonema* observed in this project, there was never evidence of *Cora*-like thalli developing from *Dictyonema*-like forms, and the phylogenetic data clearly do not support this idea. In instances where specimens of *Cora*, *Dictyonema* or *Cyphellostereum* were collected growing closely together, sequence data always showed that they represented different taxa.

The observed variation in ITS sequences among the different clades, with reduced variation in more derived clades (including in size), suggests that *Cyphellostereum* represents a comparatively ancient group that diverged rather early, whereas *Cora* is a relatively young clade. A separate dating study employing a relaxed molecular clock (Lücking, 2012; Lücking et al., 2013b, 2014c) suggests the early divergence of *Dictyonema* s.l. to have taken place about 45 mya during the Eocene, with the *Cyphellostereum* crown node estimated at 35 mya in the late Eocene, whereas *Cora* diversified much later during the early Miocene, about 10–15 mya. These results support the hypothesis that the morphologically primitive *Cyphellostereum* species are relicts from an early radiation, with many species now extinct, whereas *Cora* represents a more

recent radiation, with partially unrecognized species diversity. The relatively young age of the *Dictyonema* clade, together with the evidence of progression in thallus morphology and anatomy, suggests a rare instance when the 'birth' of lichenization can be studied in detail in this clade of Basidiomycota (Lücking et al., 2013b). There are some lineages in Ascomycota, specifically in class Dothideomycetes, which also form filamentous lichens and produce hyphal sheaths comparable to those of *Dictyonema* s.str.; these are the genera *Cystocoleus*, *Racodium*, and *Racoleus* (Gauckler, 1960; Hawksworth et al., 2011; Nelsen et al., 2009). If the above view is correct, these might represent another example of recently emerging lichenized lineages on their way to evolving competitive lichen thalli.

Table 3 - Specimens used for three-marker phylogenetic analysis

Specimens, collection information, and GenBank accession numbers of fungi used in this study.					
			Genbank number		
Species	Collector number	Origin	ITS	LSU	*RPB2*
Acantholichen pannarioides	Bungartz 5593	Galapagos	EU825953	EU825953	KF443265
Corella brasiliensis	Dal-Forno 1271	Brazil	KF443229	KF443255	KF443276
Cora arachnoidea	Hernández 1779	Venezuela	KF443232	KF443256	KF443266
Cora aspera	Lücking 29128	Bolivia	KF443230	KF443257	KF443267
Cora byssoidea	Lücking s.n.	Colombia	KF443234	KF443258	KF443268
Cora hirsuta	Lücking s.n.	Colombia	KF443235	KF443259	KF443270
Cora inversa	Lücking s.n.	Colombia	KF443236	KF443260	KF443271
Cora minor	Navarro s.n.	Costa Rica	EU825968	EU825968	KF443272
Cora pavonia	Lücking s.n.	Ecuador	KF443238	KF443261	KF443275
Cora reticulifera	Lücking 26201	Ecuador	KF443239	KF443262	KF443269
Cora squamiformis	Wilk 7577	Bolivia	KF443240	KF443263	KF443273
Cora strigosa	Paz 3	Peru	KF443241	KF443264	KF443274
Cyphellostereum imperfectum	Lücking 25588	Guatemala	KF443218	KF443243	KF443277
Cyphellostereum nitidum	Rivas Plata 1130	Philippines	–	EU825970	KF443278
Cyphellostereum phyllogenum	Lumbsch s.n.	Fiji	KF443219	KF443244	–
Cyphellostereum pusiolum	Lücking s.n.	Costa Rica	EU825976	EU825976	KF443279
***Cyphellostereum* sp.**	Rivas Plata 2183b	Philippines	KF443220	KF443245	–
Dictyonema aeruginosulum	Nelsen 3754	Costa Rica	EU825955	EU825955	KF443280
Dictyonema hernandezii	Lücking 26258	Ecuador	KF443221	KF443246	KF443281
Dictyonema interruptum	Ertz 10475	Madeira	–	EU825967	KF443282
Dictyonema irpicinum	Lumbsch 19837e	Fiji	–	KF443247	KF443283
Dictyonema metallicum	Lücking 26255	Ecuador	KF443222	KF443248	KF443284
Dictyonema obscuratum	Lücking 23025	Brazil	KF443223	KF443249	–
Dictyonema phyllophilum	Lumbsch 19821	Fiji	KF443224	KF443250	–

Dictyonema schenkianum 1	Lücking 30062	Brazil	KF443225	KF443251	KF443285
Dictyonema schenkianum 2	Lücking 17200	Costa Rica	EU825972	EU825972	KF443286
Dictyonema sericeum 1	Wilk 8868	Bolivia	KF443226	KF443252	–
Dictyonema sericeum 2	Fuentes 4788	Bolivia	KF443227	KF443253	KF443287
Dictyonema sericeum 3	Lücking 25551b	Guatemala	KF443228	KF443254	–
Eonema pyriforme	Hjm 18581	Sweden	EU118605	EU118605	–

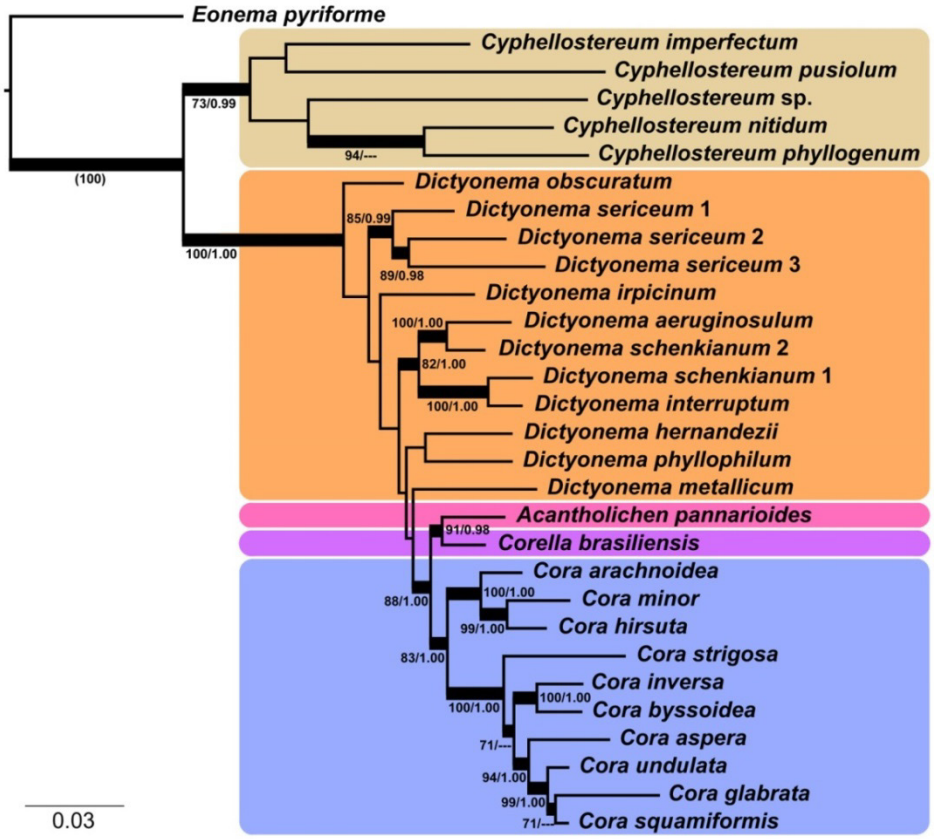


Figure 1 - Best-scoring maximum likelihood tree obtained from a three-gene dataset via RAxML
Supported branches are indicated by thick lines and bootstrap support values as well as posterior probabilities from a separate Bayesian analysis are given. The five genus-level clades and grades (*Cyphellostereum*, *Dictyonema*, *Acantholichen*, *Corella*, *Cora*) are colored

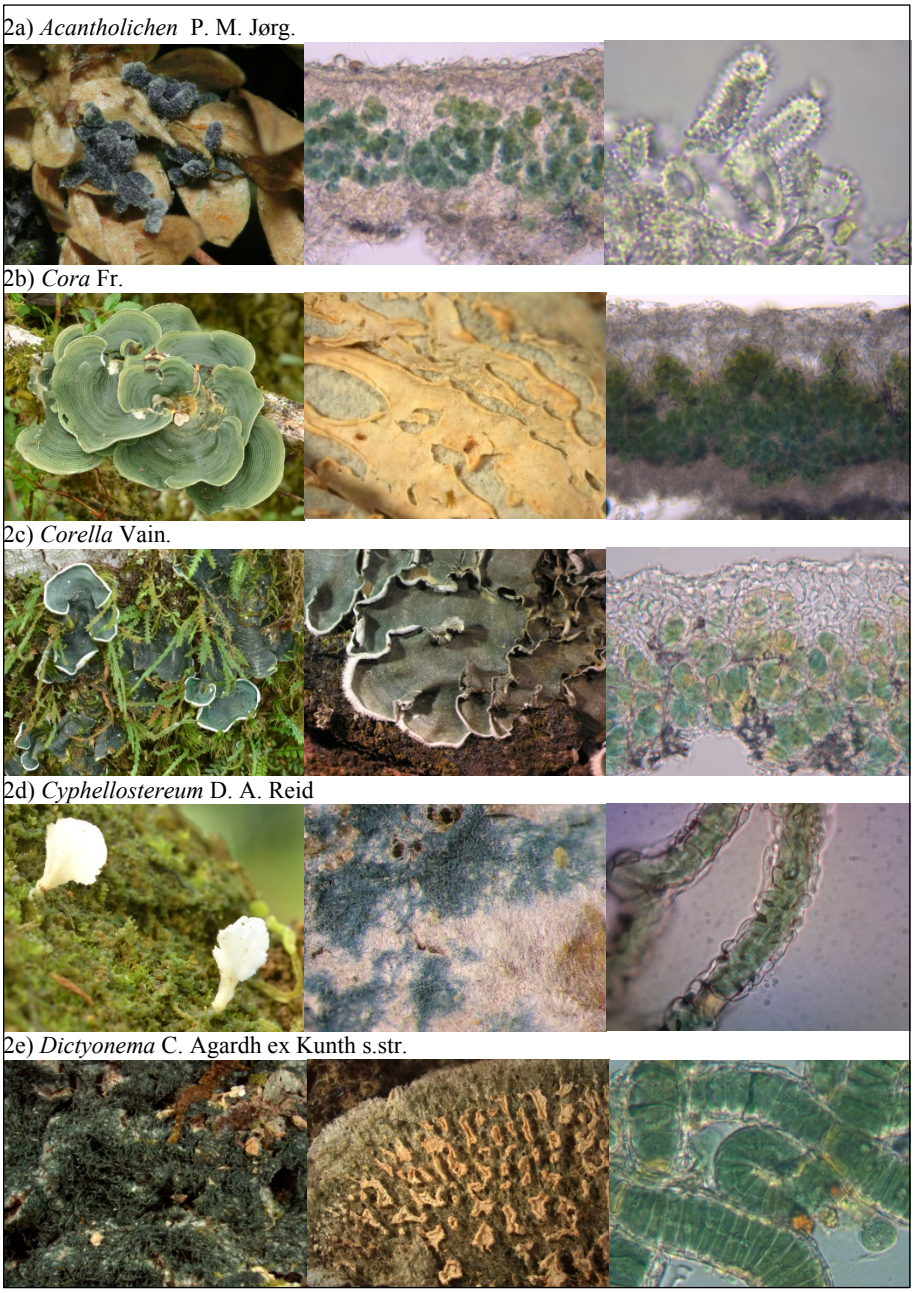


Figure 2 - Photographs of the five morphological groups in *Dictyonema* s.l.
(Photo credits: morphology: Robert Lücking, anatomy: Manuela Dal-Forno)

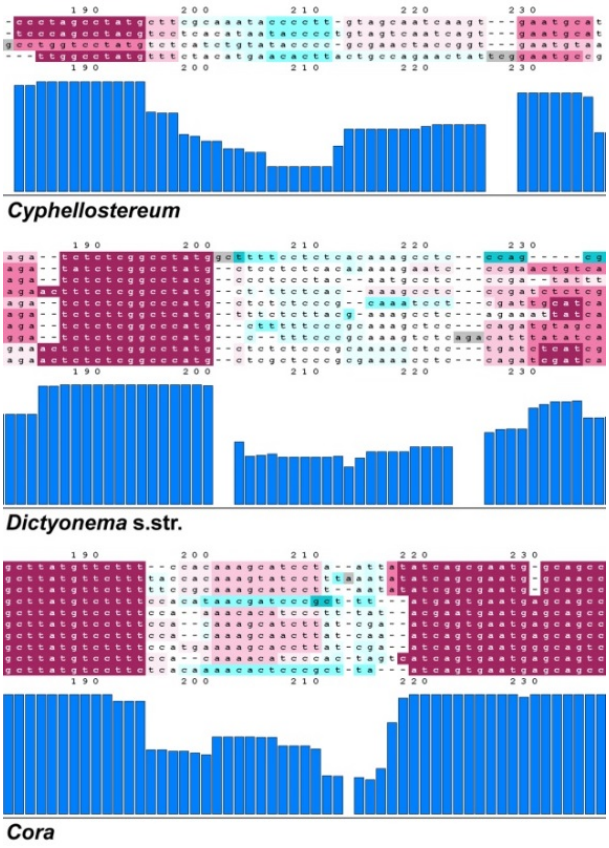


Figure 3 - Selected ITS alignments obtained via the GUIDANCE web server for three genera
Purple colors denote high and blue colors denote low confidence scores, also expressed by the columns height

Chapter 3 - From one to six: Unrecognized species diversity in the genus *Acantholichen* (lichenized Basidiomycota: Hygrophoraceae)

In review

Dal-Forno, M.; Lücking, R.; Bungartz, F.; Yánez-Ayabaca, A.; Marcelli, M. P.; Spielmann, A. A.; Coca, L.F.; Chaves, J. L.; Aptroot, A.; Sikaroodi, M.; Gillevet, P. M.; Sipman, H. J. M.; Lawrey, J. D. 2015. From one to six: unrecognized species diversity in the genus *Acantholichen* P. M. Jørg. (lichenized Basidiomycota: Hygrophoraceae).

Abstract

This chapter presents a taxonomic revision of the lichenized basidiomycete genus *Acantholichen*, species of which produce a characteristic blue-grey, microsquamulose thallus with spiny apical hyphal cells known as acanthohyphidia. Since its discovery, the genus was thought to be monospecific, only including the generic type, *Acantholichen pannarioides*. However, a detailed morphological and anatomical study of recently collected specimens from the Galapagos, Costa Rica, Brazil and Colombia, combined with a molecular phylogenetic analysis with three genetic markers (ITS, nuLSU and *RPB2*), revealed a much more diverse and widespread species assemblage. Based on the results of these analyses, five new species are described in the genus: *A. albomarginatus*

Dal-Forno, Marcelli & Lücking, *A. campestris* Dal-Forno, Spielmann & Lücking, *A. galapagoensis* Dal-Forno, Bungartz & Lücking, *A. sorediatus* Dal-Forno, Sipman & Lücking, and *A. variabilis* Dal-Forno, Coca & Lücking. We also provide an identification key to all species, as well as anatomical and morphological descriptions, photographs and a table comparing main characteristics of each species.

Introduction

The genus *Acantholichen* was established by Jørgensen (1998) for *A. pannarioides*, based on material originally set aside as a possible sterile ascolichen in the genus *Parmeliella*. The species is characterized by a gelatinous, microsquamulose thallus, dark blue to slightly grey, with spiny apical cells on both thallus surfaces, giving it a white-pruinose appearance. Jørgensen (1998) recognized these spiny cells as acanthohyphidia, structures known to be produced by mushroom-forming basidiomycetes. Since no sexual structures were observed in the specimen and no other lichen species are known to produce acanthohyphidia, the species was described as a new basidiolichen. At the time of its description, the genus and species appeared to be tropical, with specimens collected in the moist montane forests of South and Central America (Jørgensen, 1998).

In a later molecular phylogenetic study, Lawrey et al. (2009) confirmed the identity of *A. pannarioides* as a lichenized basidiomycete and placed it in the *Dictyonema* clade in the family Hygrophoraceae of the Agaricales. *Dictyonema* had never before been

considered an agaric genus, having previously been assigned to several families in different orders, including Atheliaceae, Aphyllophoraceae, Corticiaceae, Thelephoraceae, and more recently Phanerochaetaceae (Parmasto, 1978; Zmitrovich et al., 2006). Members of the *Dictyonema* clade are entirely lichenized and have a cyanobacterial *Rhizonema* photobiont that is only known from lichens (Lücking et al., 2009a, 2014a). *Acantholichen* shares some morphological characteristics, such as the paraplectenchymatous cortex, with other lineages in the clade, most notably those in the foliose genus *Corella*, to which *Acantholichen* appears to be most closely related (Dal-Forno et al., 2013).

Recent investigations indicate that the *Dictyonema* clade may be a source of a remarkable number of undescribed species (Lücking et al., 2013a, 2014c). Studies have revealed a previously unrecognized diversity of basidiolichens in a variety of phylogenetic groups, not only in the *Dictyonema* clade (Chaves et al., 2004; Dal-Forno et al., 2013; Lücking et al., 2013a, 2014c; Yánez et al., 2012), but also in the chlorolichen genera *Lichenomphalia* (Redhead et al., 2002), *Lepidostromatales* (Ertz et al., 2008; Hodkinson et al., 2012, 2014; Sulzbacher et al., 2012) and *Multiclavula* (Nelsen et al., 2007). Our hypothesis is that *Acantholichen* may also represent a potentially important source of new species that warrants further study.

For this study we obtained several distinctive new specimens of *Acantholichen* from the Galapagos Islands, Costa Rica, Brazil, and Colombia. These specimens are at first glance similar in color and outward appearance to *A. pannarioides*, but exhibit phenotypic differences indicating they may represent separate species. We combined a

detailed morphological and anatomical study of the specimens with a molecular phylogeny obtained with three commonly used markers (ITS, nuLSU and *RPB2*) to: (1) test the hypothesis that the specimens represent new species of *Acantholichen* in the Hygrophoraceae; (2) provide a preliminary assessment of the ecology, distribution and degree of endemism of the known species; and (3) provide descriptions, diagnoses and a key to all species.

Materials and methods

Taxon sampling

Our entire *Acantholichen* dataset included 17 samples (**Table 4**), most of them collected during field trips between the years of 2010–2014 throughout South and Central America. For our phylogenetic analyses, we also used *Corella brasiliensis* as the outgroup, since *Corella* has been shown to be the sister genus of *Acantholichen* by Dal-Forno et al. (2013).

Light microscopy

All specimens were examined with a LEICA MS5 (Wetzlar, Germany), an OLYMPUS SZX12 (Shinjuku, Japan) and Stereostar Zoom Stereoscopic (Reichert, Austria) dissecting microscopes and a ZEISS Axioskop 2 (Jena, Germany), an OLYMPUS BH-2 (Shinjuku, Japan) and a NIKON Optiphot (Japan) compound microscopes. Sections of the thallus were studied in water only and microphotographs of them were taken with DAGE MTI DC-330 3CCD (Michigan City, IN, USA) and

JENOPTIK ProgRes C3 and C5 (Jena, Germany) digital microscope cameras attached to the aforementioned microscopes. Macrophotos were taken in situ with CANON Powershot SX20IS (Ota, Japan), NIKON F301 (Tokyo, Japan) and Sony Alpha 33 DSLR digital cameras.

Table 4 - GenBank numbers and voucher information of specimens used in the phylogenetic analysis

Species	Collector and lab control #	Herbaria	Locality	GenBank # *RPB2*	GenBank # ITS	GenBank # nuLSU
Acantholichen albomarginatus	Dal-Forno & Marcelli 2043 (MDF543)	GMUF	Brazil, Minas Gerais	-		
Acantholichen campestris	Spielmann et al. 10243b (DIC595b)	GMUF	Brazil, Santa Catarina			
Acantholichen galapagoensis	Dal-Forno 1204 (MDF057)	GMUF	Galapagos, Santa Cruz			
Acantholichen galapagoensis	Dal-Forno 1205 (MDF058)	GMUF	Galapagos, Santa Cruz			
Acantholichen galapagoensis	Aptroot 64679 (MDF089)	GMUF	Galapagos, Santa Cruz	-		
Acantholichen galapagoensis	Bungartz 4125 (MDF090)	GMUF	Galapagos, Isabela			
Acantholichen galapagoensis	Aptroot 65187 (MDF091)	GMUF	Galapagos, Isabela	-		-
Acantholichen galapagoensis	Aptroot 65554 (MDF092)	GMUF	Galapagos, Santiago	-		-
Acantholichen galapagoensis	Nugra 400 (MDF093)	GMUF	Galapagos, Santa Cruz			
Acantholichen galapagoensis	Nugra 379 (MDF094)	GMUF	Galapagos, Santa Cruz			
Acantholichen galapagoensis	Bungartz 8152 (MDF100)	GMUF	Galapagos, Santa Cruz			
Acantholichen galapagoensis	Bungartz 8577 (MDF101)	GMUF	Galapagos, San Cristobal	-		-
Acantholichen galapagoensis	Bungartz 5593 (DIC064)	GMUF	Galapagos, Santa Cruz	AGV75855	EU825953.2	EU825953.2
Acantholichen pannarioides	Dal-Forno 1752 (MDF352)	GMUF	Costa Rica, Las Alturas			
Acantholichen sorediatus	Sipman 48329 (DIC060)	GMUF	Costa Rica, Cartago	-	-	EU825952.2
Acantholichen sorediatus	Lücking s.n. (DIC335)	F	Costa Rica, Puntarenas	-		
Acantholichen variabilis	Coca 5209 (MDF679)	GMUF	Colombia, Valle del Cauca	-		
Cora arachnoidea	Hernández 1779 (DIC279)	VEN	Venezuela	KF443232	KF443256	KF443266
Cora aspera	Lücking 29128 (DIC110)	F	Bolivia	KF443230	KF443257	KF443267
Cora byssoidea	Lücking 25901 (DIC151)	F	Colombia	KF443234	KF443258	KF443268
Cora hirsuta	Lücking 25900 (DIC152)	F	Colombia	KF443235	KF443259	KF443270
Cora inversa	Lücking 25903 (DIC149)	F	Colombia	KF443236	KF443260	KF443271
Cora minor	Navarro s.n. (DIC 063)	F	Costa Rica	EU825968	EU825968	KF443272
Cora ciferrii	Lücking s.n. (DIC215)	F	Ecuador	KF443238	KF443261	KF443275
Cora reticulifera	Lücking 26201 (DIC119)	F	Ecuador	KF443239	KF443262	KF443269
Cora squamiformis	Wilk 7577 (DIC146)	KRAM	Bolivia	KF443240	KF443263	KF443273
Cora strigosa	Paz 3 (DIC107)	F	Peru	KF443241	KF443264	KF443274
Corella brasiliensis	Dal-Forno 1271 (MDF017)	GMUF	Brazil	KF443276	KF443229	KF443255
Cyphellostereum imperfectum	Lücking 25588 (DIC115a)	F	Guatemala	KF443218	KF443243	KF443277
Cyphellostereum pusiolum	Lücking s.n. (R04)	F	Costa Rica	EU825976	EU825976	KF443279
Dictyonema aeruginosulum	Nelsen 3754 (DIC053)	F	Costa Rica	EU825955	EU825955	KF443280
Dictyonema discocarpum	Fuentes 4788 (DIC136)	KRAM, F	Bolivia	KF443227	KF443253	KF443287
Dictyonema hernandezii	Lücking 26258 (DIC122)	F	Ecuador	KF443221	KF443246	KF443281
Dictyonema metallicum	Lücking 26255 (DIC123)	F	Ecuador	KF443222	KF443248	KF443284
Dictyonema obscuratum	Lücking 23025 (DIC113)	F	Brazil	KF443223	KF443249	–
Dictyonema* aff. *schenkianum	Lücking 17200 (DIC059)	F	Costa Rica	EU825972	EU825972	KF443286
Eonema pyriforme	Hjm 18581 (OUTGROUP)	GB	Sweden	EU118605	EU118605	–

Molecular data

Genomic DNA was extracted from lichenized thalli (around 2 mm^2 of squamules) using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogene, Illkirch, France) according to the manufacturer's protocol with slight modifications. About 10 ng of extracted DNA were subjected to a standard PCR in a 20 µL reaction volume using either Taq Gold polymerase (Applied Biosystems, Foster City, CA, USA) or Bio-X-Act Long Mix (Bioline USA, Taunton, MA, USA) according to manufacturer's protocols. Sequence data were obtained from the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA cistron (ITS1, 5.8S, and ITS2, approximately 700bp), large subunit (nuLSU) nuclear ribosomal DNA (approximately 1470 bp), and partial DNA-directed RNA polymerase II second largest subunit (*RPB2*), between the 6 and 7 conserved domains (approximately 1000bp). The PCR products were visualized on a 1% agarose gel with ethidium bromide and then were purified with magnetic beads (Agencourt Bioscience, Beverly, MA, USA). The purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The primers used were: ITS5 and ITS4 for ITS (White et al. 1990); LR0R, LR3R, LR5, LR7 and LR16 for nuLSU (http://www.biology.duke.edu/ fungi/mycolab/ primers.htm) and b*RPB2*-6F or bRBP2-5F and bRBP2-7R, bRBP2-7R2 or b*RPB2*-7.1R for *RPB2* (Denton et al., 1998; Liu et al,. 1999; Matheny, 2005). The sequencing reactions were then purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, MO, USA), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed

using ABI software, and 500-700 bases were collected for each primer used. For one sample (*Acantholichen sorediatus* R. Lücking s.n.) we were not able to get a sequence through Sanger sequencing and therefore applied pyrosequencing techniques according to Lücking et al. (2014d).

Sequence alignment and phylogenetic analysis

New sequences were assembled together with the software Sequencher version 5.0 (Gene Codes, Ann Arbor, MI, USA) for manual corrections in base calling and to make contigs of overlapping fragments. That resulted in 35 new sequences which were then assembled with three sequences from GenBank using BIOEDIT 7.09 (Hall, 1999) and automatically aligned with the program MAFFT using the -auto option (Katoh et al., 2005). The concatenated dataset alignment was subjected to analysis of ambiguously aligned regions using the GUIDANCE web server (Penn et al., 2010a, 2010b) and since the alignments were highly scored (overall 0.97) no regions were removed. The alignments were subjected to maximum likelihood (ML) search using RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2005), with non-parametric bootstrapping using 500 replicates under the GTRGAMMA model. Each gene was first analyzed separately and all data were eventually combined since no conflict was detected after visual inspection of tree topologies (Mason-Gamer and Kellogg, 1996). For the concatenated dataset, when sequences were lacking for determined sample, we used the missing data approach, that is, inclusion of gaps for those regions. The concatenated dataset was also analyzed under a Bayesian framework using MrBAYES 3.1.2 (Huelsenbeck and Ronquist, 2001), with

two independent runs, a total chain length of five million generations, and four separate chains each, resampling every 200 trees and generating a 50% majority rule consensus tree from the sampled trees after discarding 25% burnin to obtain posterior probability estimates. We also ran an additional Bayesian analysis under the same parameters, but partitioning the dataset into the three different loci. All trees were visualized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Photoshop CS5.1.

To test if the genus would remain monophyletic with the addition of multiple specimens, we downloaded from GenBank sequences from the *Dictyonema* clade corresponding to species previously published by Dal-Forno et al. (2013) and Lücking et al. (2013a, 2014b). For *Acantholichen*, only species in which all three loci are available were used for this analysis (and a single specimen representing the species when many were available). We performed a MAFFT alignment (Katoh and Toh, 2010; Katoh et al., 2005) which was then subjected to ML search in RAxML under the same settings described above.

Results

Sequences aligned resulted in an alignment length of 713bp for the ITS region, 1472bp for the nuLSU, and 1017bp for the *RPB2*, for a total of 3202 sites in the combined dataset, with 250 variable sites. No regions in the alignments were deleted. The final ML optimization likelihood was -5648.546362. For the analysis in MrBayes for the partitioned data, the average standard deviation of split frequencies reached a number inferior of 0.01 after 110000 generations. The average arithmetic mean and harmonic

means between chains were -5697.91 and -5722.99, respectively. The 50% majority rule consensus tree was generated from a total of 1102 trees in 2 files (sampling 828 of them).

The phylogenetic tree inferred by maximum likelihood only, including representative taxa from other genera in the *Dictyonema* clade, recovered *Acantholichen* as monophyletic (**Figure 4**) and shows *Corella* to be a genus sister to *Acantholichen.*

Phylogenetic trees inferred by maximum likelihood and Bayesian inference (**Figure 4**) were congruent in topology. Analysis of the partitioned and non-partitioned datasets recovered the same tree topology with similar support values, and we show only the results from the analysis of partitioned data in **Figure 4b**. Analysis of the combined three-marker dataset suggests that there are six distinct species, a result that agrees with our detailed morphological and anatomical observations. With the moderate to high support for species relationships within the genus, it is now possible to define distinct species based on phylogenetic, morphological and geographic structure.

Our phylogeny indicates that *Acantholichen sorediatus* is sister to the rest of the species in the genus with high support in BI (**Figure 4b**). The relationship of *A. albomarginatus* to *A. pannarioides*, *A. variabilis*, *A. campestris* and *A. galapagoensis* does not appear to be strongly supported in ML (70%), but shows high support in BI (pp = 1). Two species newly described in this paper (*A. variabilis* and *A. campestris*) appear to be closely related and together form a clade sister to *A. galapagoensis*.

Discussion

Our multi-locus phylogeny (ITS, nuLSU, *RPB2*), combined with anatomical and morphological characters, indicates that *Acantholichen* as currently defined is monophyletic and that at least six distinct species in the genus can be distinguished, one from the Galapagos Islands, two from Brazil, one from Colombia and two from Costa Rica, including the type species, *A. pannarioides* (Jørgensen, 1998). The *Acantholichen* clade is monophyletic and sister to the genus *Corella* in a large, diverse and entirely lichenized clade, *Dictyonema* s.l., an important element within the agaric family Hygrophoraceae (Dal-Forno et al., 2013; Lodge et al., 2014).

Prior to studies beginning with Chaves et al. (2004), the taxonomy of basidiolichens assigned to *Dictyonema* had received little attention, with the exception of Oberwinkler (1970, 2001, 2012) and Parmasto (1978). The monotypic lichen *Acantholichen pannarioides*, when first described (Jørgensen, 1998), was not known to be a member of this group until the molecular phylogenetic study of Lawrey et al. (2009) established its position in the *Dictyonema* clade. Apart from *Acantholichen*, the genera now recognized in this clade (Dal-Forno et al., 2013) are *Cora*, *Dictyonema* s.str., *Corella* and *Cyphellostereum*, all containing lichenized basidiomycetes with a cyanobacterial *Rhizonema* photobiont.

All presently recognized *Acantholichen* species share the same overall aspect, with microsquamulose thalli and acanthohyphidia that are not found elsewhere in the *Dictyonema* clade. However, they share the paraplectenchymatous cortex as a synapomorphy with the genus *Corella*. *Acantholichen* species differ mainly in color, size,

arrangement and overall appearance of the squamules, and shape, size and location of their acanthohyphidia. Thallus colors include various hues of blue, green and grey, all typical colors of *Dictyonema* s.l. caused by the *Rhizonema* photobiont (Lücking et al. 2009a, 2014a). The cyanobacterial *Rhizonema* forms short threads of angular-rounded to applanate cells which are surrounded by a jigsaw puzzle-shaped hyphal sheath a unique characteristic within the *Cora*-clade.

The characteristic spiny hyphae, acanthohyphidia, found in *Acantholichen* species are not exclusive to this genus. Although not found in other lichenized fungi, acanthohyphidia are reported for many non-lichenized basidiomycetes, such as *Xylobolus frustulatus* (Pers.) Boidin (Stereaceae), the ceramic fungus. It was the presence of these characteristic hyphae that led Jørgensen (1998) to propose *Acantholichen* as a new genus of basidiolichens. Morphologically, they are similar to the spiny periphysoids and/or periphyses in *Fissurina* and *Acanthothecis* (Graphidaceae, Ascomycota) (Staiger, 2002), but the latter are much smaller in size and often difficult to discern. To date, the function of acanthohyphidia is unknown. However, at least in *Acantholichen*, we can hypothesize that they may serve as water repellants or regulators. When rehydrating specimens with acanthohyphidia on the entire surface, they seemed to not absorb water immediately, only when extra pressure is added with forceps. However, specimens with acanthohyphidia mostly on the margins of squamules absorb water quickly, as usually happens in other genera within the *Dictyonema* s.l. clade. Obviously, this hypothesis requires further experimental testing.

The exact phylogenetic position of *Acantholichen* within the *Dictyonema* clade was not well established until Dal-Forno et al. (2013) showed for the first time that *Corella* is a sister to *Acantholichen* and not to *Cora*, as had been hypothesized earlier based on similar morphology and anatomy. This result led to a reassessment of the relationships within the *Dictyonema* clade, especially the *Cora*-clade (*Acantholichen*, *Cora* and *Corella*), and possible evolutionary scenarios to explain these relationships. For example, the close relationship of *Acantholichen* and *Corella* could be supported potentially by similar cortical structures in these genera. *Acantholichen* and *Corella* species have the upper cortex formed by multi-layers of rounded to angular and densely packed hyphae, while *Cora* species have the cortex formed by loosely interwoven elongated hyphae. It is also open to question whether in the *Acantholichen*-*Corella* clade, the foliose or microsquamulose growth form is ancestral. Since the sister clade *Cora* is entirely foliose, the most parsimonious hypothesis would be for *Acantholichen* to be derived from a foliose or macrosquamulose form similar to *Corella* and some *Cora* species.

Another observation is that, at present, fertile specimens of *Corella* and *Acantholichen* have never been found. This does not necessarily mean that they have permanently lost the ability to reproduce sexually. With our current data (including several yet undescribed species in the genus *Corella*), we are not discarding the possibility that fertile structures could be found in known or new species in both genera.

Our discovery of several new species of *Acantholichen*, combined with reports of newly described basidiolichens in other clades, supports the notion that lichenized species

represent an important source of undescribed biodiversity in the Basidiomycota. This appears to be especially true in the *Dictyonema* s.l. clade to which *Acantholichen* belongs. An example of this is the discovery by Lücking et al. (2014c) of a remarkable number of unrecognized species in what Parmasto (1978) considered to be a single species, *Cora pavonia* (= *Dictyonema glabratum*). Over 120 morphologically and genetically distinct new species were discovered, with a predicted number well over 400 species (Lücking et al., 2014c). This study emphasizes the need to establish biologically meaningful species recognition guidelines for this and other groups in *Dictyonema* s.l., and also for increased and targeted collecting in biodiversity hotspots predicted to support and maintain undescribed species. The remarkable species richness in *Cora*, a group familiar to collectors, has only recently been established, so there is currently no way of predicting actual species richness of *Acantholichen* based on limited collections, especially given the minute size of *Acantholichen* squamules, which can easily be mistaken for something else. At least some part of the perceived lower diversity for this genus must be ascribed to a far lower sampling of specimens that are simply unnoticed in the field.

Similarly, as shown in the genus *Cora* in Lücking et al. (2014c), preliminary data suggests high levels of local endemism in *Acantholichen* species, such as *A. galapagoensis*, which so far is only known from the Galapagos Islands. However, more collections of all species are essential to clarify the actual distributions and ecologies. Prior to this study, the prevailing view was that any small microsquamulose blue-green-grey lichen with acanthohyphidia corresponds to *A. pannariodes*, a species suggested by

Jørgensen (1998) to have a wide distribution throughout the moist montane regions of the Neotropics. Our results now may indicate that *Acantholichen* species seem much more restricted in their distribution; some species (like *A. galapagoensis*) possibly local endemics. From the relatively few specimens so far examined, it seems likely that all newly described species, and even *A. pannorioides* itself, may occupy relatively small distribution areas. It is entirely possible that many additional species will still be discovered, and that these species will be adapted to occupy much narrower habitats than previously anticipated in basidiolichens in general.

Overall, our studies are beginning to establish a similar pattern for species in all of the described clades in *Dictyonema* s.l. An unexpectedly high, hidden biodiversity is present within many of the clades, and the distribution of their members appears highly influenced by local geographical conditions. Yet, much of the preferred habitat of these species in tropical montane forests of South and Central America has not been surveyed, suggesting that a significant portion of this biodiversity remains to be discovered.

Our study also emphasizes the need to not only examine species using molecular tools, but to study their morphology and anatomy using fresh collections, documenting these details with photographs, ideally in the field. Many important characteristics still present in fresh material are lost over time, especially important features like color and texture. This is certainly the case for all members of *Dictyonema* s.l., including *Acantholichen* (**Figures 9c** and **9d**, for example), in which one of the most important distinguishing characteristics, the natural color, changes drastically in the herbarium. This may explain why previous investigators encountered much difficulty recognizing species-

level variability when examining the dried herbarium specimens of these basidiolichens. Our studies demonstrate the importance of observing, photographing and measuring these lichens when fresh, in much the same way that other mushroom-forming agarics are treated.

Taxonomic treatment

Acantholichen P.M. Jørg., Bryologist 101(3): 444 (1998)

Thallus morphology. Microsquamulose, squamules with a coarsely pruinose appearance, individual squamules scattered or growing closely together and sometimes even merging; their shape either round and broad or elongated and thin, each squamule basally attached to the substrate, rarely centrally (one species), squamules, if distinctly elongated, typically proliferating from their tips; typically distinctly swollen, rarely ± flattened (one species); ca. 0.1–2 (–3) mm wide and 0.1–2(–3) mm long; not, sparsely, moderately or highly branching; their color ranging from mostly blue and green hues when fresh, to dark grey and blue when dry, rarely distinctly whitened along their margin, with or without soredia, which can give the thallus an additional granulose appearance.

Thallus anatomy. Heteromerous, squamules 100–240 µm thick in section, with a distinct, paraplectenchymatous (5–)10–30 µm thick upper cortex, formed by 2–3 layers of cells, a 70–150 µm thick photobiont layer, and a 10–100 µm thick medulla of more or less dense, irregular to rounded or elongated hyphae; the lower side lacking a cortex.

Upper cortex and lower surface with acanthohyphidia, i.e., modified hyphal terminal cells with pin-like outgrowths (Kirk et al., 2008), that can be small to large, 4–50 µm long, 4–10(–12) µm wide, and are always conspicuously spiny, these cells that can be irregular, subglobose, pyriform, elongate, subclavate or clavate, they spread across the entire lamina, or can be more abundant along the margin of the squamules; no clamp-connections observed among hyphae. Typically, acanthohyphidia can be more abundant on the lower surface. Photobiont *Rhizonema*, forming clusters of 8–17 µm diam. of coiled cyanobacterial filaments ('chroococcoid'), wrapped within a dense hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Sexual reproduction structures unknown.

Chemistry. All spot tests negative (P–, K–, KC–, C–), UV–, no secondary metabolites detected by thin-layer chromatography.

Distribution and ecology. At present, the genus is known only from South and Central America, inhabiting mostly habitats at high altitudes (only in Galapagos as low as 500 m.a.s.l.), in moist and cloudy forests, in areas with high light intensity. Species of *Acantholichen* have been found on a wide range of different substrates, but typically accompanied by and/or overgrowing bryophytes and other lichens. *Acantholichen campestris* is the only known species that grows on rock while the other species grow on bark and wood, often overgrowing mosses and liverworts. One specimen in Galapagos (*A. Aptroot 63214*) was collected on liverworts growing on soil. All species show high levels of endemism. Jørgensen (1998) commented that the genus is most likely to occur in moist montane regions of South and Central America.

Remarks. Due to its unique microsquamulose thallus with coarsely pruinose appearance, *Acantholichen* is morphologically very different from other members of the *Dictyonema* clade. As originally pointed out by Jørgensen (1998), members of the genus resemble species of the Pannariaceae, but all species of the lichenized basidiomycete *Acantholichen* are easily distinguished from these lichenized ascomycetes by the presence of acanthohyphidia. Phylogenetically, *Acantholichen* is sister to *Corella*¸ both in turn forming a sister clade to *Cora* (Dal-Forno et al., 2013). The three genera form a clade characterized by a heteromerous thallus, with chroococcoid shaped cyanobacterial cells of the genus *Rhizonema. Corella* is a genus that is morphologically very similar to *Cora*, but it shares with *Acantholichen* the same paraplectenchymatous cortex. The unshaved chin appearance described by Jorgenson (1998) is very typical of this genus.

A summary of the morpho-anatomical characters found to be most useful in distinguishing the different species is provided in **Table 5**. For most species, no single character can be used to differentiate them from all others. However, each species exhibits a unique combination of characters. Five new species are recognized and described here.

Key to currently recognized species of *Acantholichen*

1.1 Squamules granular to flattened, with conspicuous white margins; acanthohyphidia may be sparse to absent in some parts of the lamina, but are always abundant along the margins .....***A. albomarginatus***

1.2 Squamules not granular to flattened, but distinctly swollen (inflated), margins not conspicuously white, but in some species more abundantly pruinose than the squamule surface..... 2

2.1 Acanthohyphidia up to 50 µm long, elongate to clavate..... 3

2.2 Acanthohyphidia up to 20 µm long, pyriform, subglobose, subclavate and/or irregularly shaped..... 4

3.1 Squamules dark blue when fresh, turning dark bluish grey when dried, acanthohyphidia variable in size (10–50 µm) ..... ***A. variabilis***

3.2 Squamules greyish blue green when fresh, turning dark blue when dried, acanthohyphidia typically quite large (30–50 µm) ..... ***A. pannarioides***

4.1. Squamules very sparsely branched, forming soredia along the margins .... ***A. sorediatus***

4.2. Squamules moderately to abundantly branched, lacking soredia 5

5.1 Squamules intricately branched, but not appearing fruticose, when fresh with a distinct olivaceous hue; endemic to the Galapagos Islands ..... ***A. galapagoensis***

5.2 Squamules abundantly branched, of a microfruticose appearance, not olivaceous .... ***A. campestris***

Table 5 - Characters distinguishing species in *Acantholichen*
Measurements, observations and photographs made under a stereoscope – *Appearance of squamules:* flattened vs. swollen (inflated); granulose, broad (wider than longer) or elongated (long and thin); *attachment of the squamules:* squamules attached at the base or in the center, distinctly elongated squamules proliferating from their tips; *growth:* scattered squamules vs. many agglomerating and growing together; *branching pattern:* squamules without any branching, or with very sparsely, sparsely, moderately, abundantly branched squamules to microfruticulose; *soredia:* present vs. absent; *color fresh:* shades of green, blue and grey based on *in situ* observations and photographs; *color dry:* shades of green, blue and grey, based on color observed months after collecting and/or herbarium material; *acanthohyphidia location:* predominantly marginal, laminal (evenly across the surface), observed under the stereoscope. Measurements and photographs made in water with a 400X and/or 1000X magnification under a light microscope – *Acanthohyphidia size:* small, medium and large, and *shape*: elongate to clavate, irregular, subglobose, pyriform elongate or clavate

	A. albomarginatus	*A. campestris*	*A. galapagoensis*	*A. pannarioides*	*A. sorediatus*	*A. variabilis*
Appearance of squamules	Mostly flat; partly broad, partly granulose	Swollen; slightly broad to mostly elongated	Swollen; elongated	Swollen; broad to elongated	Swollen; broad	Swollen; broad to elongated
Attachment of the squamules	squamules attached in the center	squamules attached at the base and proliferating from their tips	squamules attached at the base and proliferating from their tips	squamules attached at the base	squamules attached at the base	squamules attached at the base
Growth of the squamules	Scattered to many growing together	Growing together	Many growing together	Scattered to slightly growing together	Scattered	Scattered to slightly growing together
Branching pattern	Sparsely to moderately branched	Abundantly branched to microfruticulose	Abundantly branched	Moderately branched	Not or very sparsely branched	Moderately to abundantly branched
Soredia	Present, across the entire thallus	Absent	Absent	Absent	Present, marginal	Absent
Color fresh	Blue green with white margins	Not available (bluish grey when rehydrated)	Light blue-grey to olivaceous	Greyish blue green	Green	Dark blue
Color dried	Dark bluish green and grey	Grey	Dark grey	Dark blue	Dark bluish grey	Dark bluish grey
Acanthohyphidia location	Predominantly marginal, but also across the lamina	Laminal (evenly across the surface)	Laminal (evenly across the surface)	Laminal (evenly across the surface)	Predominantly marginal, but also across the lamina	Laminal (evenly across the surface)
Acanthohyphidia size and shape	Small (≤ 15 µm), mostly irregular, rarely subglobose	Small (≤ 15 µm), pyriform	Small (≤ 15 µm, rarely up to 20 µm), subglobose to pyriform	Large (up to 50 µm), elongate to clavate	Medium (15–20 µm), irregular, pyriform to subclavate	Small, medium and large (10–50 µm), subglobose, pyriform to subclavate when short, elongate to clavate when large
Geographical location	Brazil – Southeast (this study)	Brazil – South (this study)	Galapagos Islands (this study)	Costa Rica (this study, Jørgensen 1998); Venezuela and Ecuador (Jørgensen 1998)	Costa Rica (this study)	Colombia (this study)

The Species

Acantholichen albomarginatus Dal-Forno, Marcelli & Lücking sp. nov.

Figure 5

Type: Brazil: Minas Gerais: Itamonte, Parque Nacional do Itatiaia, Estrada das Prateleiras, 22°21′39.7″S, 44°43′57.1″W, alt. 2190 m, cloud forest, on the edge of forest, by the road, dense vegetation on road side banks, growing on bryophytes and liverworts, 06-Jan-2013, *Dal-Forno*, *M.* & *Marcelli*, *M. P.* 2043 (GMUF, holotype; SP, F, isotypes).

Description. Thallus granular to microsquamulose; when not completely granulose (soredia), squamules are broad, flattened, ca. 0.1–1 mm across, little to moderately branched, often merging and closely adhering, and thus forming an almost continuous crust up to 5 mm across with a center attached with detached margins, blue green when fresh, dark bluish green and grey when dry, the closely adhered parts typically rimmed with a white margin (hypha only, no photobiont). Pruinose appearance present frequently, but not always on all lamina (certain squamules have scattered acanthohyphidia). Thallus in cross section 100–120 µm thick, dominated by a thick photobiont layer, and with a thin cortex and medulla. Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia small, 4–14 µm long and 4–7 µm wide, mostly irregular, but sometimes subglobose; present on the upper and lower surfaces, but most abundant along the thallus margins, where they sometimes become

much pronounced and then resemble a prothallus; generally profuse across the upper surface of squamules that have not merged into a crust. Soredia present and abundant.

Distribution and ecology. This species is known from a single but large collection growing on mosses and hepatics in a high-elevation cloud forest of the renowned Itatiaia National Park, Brazil, a part of the Atlantic Forest biome. With the high number of lichens and bryophytes present, the location is locally known as "the lichenologist and bryologist heaven". Due to the unique morphology of *Acantholichen albomarginatus*, it is easily overlooked, even by the most careful lichenologists, since it looks like a mass of bluish grey hyphae (from a distance) resembling a non-lichenized cyanobacterium or just developing hyphae.

Etymology. The epithet refers to the characteristic white margins of well-developed squamules.

Remarks. The difference in color from the lamina and margins reflects location and abundance of the acanthohyphidia across the thallus surface. The larger squamules have overall very few acanthohyphidia on the thallus lamina; mostly they are found along the margins, which is particularly apparent if viewed in cross section under the microscope. *Acantholichen albomarginatus* therefore also morphologically distinctly differs from all other species by this white margin contrasting with the blue green lamina. Overall it has a far less "pruinose" appearance, which can easily be observed under the stereoscope or with a good hand lens. This character may, however, be well developed only in large thalli. The single collection presently known is a large, well developed specimen and it is difficult to assess if smaller developmental stages may resemble other

Acantholichen species. Nevertheless, the holotype of this species is clearly distinct from all other *Acantholichen* species. Only *A. sorediatus* also has acanthohyphidia similarly concentrated along the squamule margin, but it distinctly differs by the way its soredia are formed. In *A. albomarginatus* the whole thallus is microsquamulose to granular and thus in parts irregularly dissolves into sorediate granules. That is not the case for *A. sorediatus*, where soredia are restricted to the margin of its squamules. *Acantholichen sorediatus*, further differs by its vivid green color and the way each squamule is attached at the base, not the center of the squamule. The color of *A. albomarginatus* is blue green and it is the only species that has centrally attached squamules.

Acantholichen campestris Dal-Forno, Spielmann & Lücking, sp. nov.

Figure 6

Type: Brazil: Santa Catarina: Campo Alegre, Campos do Quiriri, 26°01′35″S, 48°58′57.4″W, alt. 1380m, on exposed rocky outcrops, on top of the mountain, growing on liverwort and lichens on rock, 03-Feb-2012, *Spielmann, A. A.; Canêz, L. S.; Gumboski E. L.* 10243b (GMUF, holotype; CGMS, isotype).

Description. Thallus microsquamulose; squamules slightly broad to mostly elongated, attached basally to the substrate and proliferating from the tips, swollen, 0.1–1 mm wide and 0.1–1 mm long, richly branching, thus becoming almost microfruticose, grey when dry (fresh material not seen, but bluish grey when rehydrated). Overall uniform white pruinose appearance. Soredia absent. Thallus in cross section 120–140 µm thick, dominated by a thick photobiont layer, and with a thin cortex and medulla.

Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia small, 10–15 µm long and 6–8 µm wide, pyriform.

Distribution and ecology. This is the only species of *Acantholichen* presently known to overgrow lichens and liverworts that inhabit rock, not bark. The Campos do Quiriri is part of the Brazilian Atlantic Forest biome, under a subcategory classified as high-altitude fields ("Campos de Altitude"), featuring a dominance of low vegetation, shrubs and small trees. This species was found on the top of the mountain, in an area with many rocky outcrops.

Etymology. The epithet denotes to "field", a reference to the ecosystem where the type species is found.

Remarks. This species of *Acantholichen* shares many characteristics with the other species described here, such as a small acanthohyphidia and an overall swollen appearance of squamules. However, the species differs from all others in forming a continuous thallus, where individual squamules repeatedly branch and proliferate, giving the thallus an overall microfruticose aspect, the squamules often growing into almost erect, vertical structures. All other species grow more or less prostrate on the substrate. *Acantholichen campestris* is also the only species so far known from rocks growing outside densely forested humid habitat. Unfortunately it was not possible to document this species with photographs in the field as the material was discovered only by accident among a herbarium specimen of *Cora*. Nevertheless, this collection was relatively recent

and sufficiently fresh for the molecular analysis and it is therefore possible that the color of rehydrated specimens does not differ significantly from that of fresh specimens.

Acantholichen galapagoensis Dal-Forno, Bungartz & Lücking sp. nov.

Figure 7 and 19

Type: Ecuador: Galapagos Islands: Isla Santa Cruz, along trail from Bellavista to El Puntudo, upper *Cinchona* forest, 0°39′002″S, 90°20′42″W, alt. 684 m, dense forest of *Cinchona pubescens*, some life trees but mostly dead trees due to management control of the invasive trees, on bryophyte, growing over *Frullania* sp., 23-Jun-2010, *Dal-Forno, M.* 1205 (CDS, holotype; GMUF, F, isotypes).

Description. Thallus microsquamulose; squamules elongated, attached basally to the substrate and proliferating from the tips, and moderately swollen, 0.1–0.2(–0.3) mm broad, up to 2(–3) mm long, abundantly branched and typically intricately tiled, many growing together, occasionally thus shading one another, light blue grey to olivaceous when fresh, darker olivaceous grey when dry, the shaded parts becoming necrotic and pale beige to orange. Overall uniform pruinose appearance. Soredia absent. Thallus in cross section 130–160 µm thick, dominated by a thick photobiont layer, and with a thin cortex and medulla. Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia typically small, rarely of moderate size, 12–16(–20) × 6–10 µm, subglobose to pyriform.

Distribution and ecology. All material of *Acantholichen* collected in Galapagos belongs to the same species and it is considered here to be endemic to this archipelago. In general, specimens do not grow directly on trees or shrubs, but typically establish on epiphytic liverworts and mosses, which in turn are common on a variety of substrates: introduced trees (*Cinchona pubescens*, *Psidium guava*), native shrub (*Zanthoxylon fagara*) and endemic trees (*Scalesia pedunculata, Psychotria* spp.); one specimen was even collected on a soil inhabiting bryophyte (*Campylopus* sp.). The populations overgrowing *Frullania* (Jubulaceae, Marchantiophyta) on the introduced tree *Cinchona* in Santa Cruz represent the best developed material. The type specimen was collected in this particular habitat, where the endemic *Acantholichen galapagoensis* is surprisingly abundant. At the type locality, individual thalli are among the largest and best developed specimens known, covering dead tree trunks and forming thalli of up to 1 m length, indicating that the species thrives particularly well in these humid highlands around El Puntudo and Cerro Crocker. In contrast, collections from all other islands are mostly minute and not well developed, with the notable exception of collections from Cerro Azúl (one of the highest and the southernmost volcano of Isabela), where specimens grow exuberant and abundantly on the dead basal sheaths of fronds of the endemic Galapagos tree fern (*Cyathea weatherbyana*). Because *Cinchona* is a tree introduced to the archipelago, one can assume that the endemic, now threatened tree ferns represent the original, natural habitat of this endemic *Acantholichen*.

Etymology. The epithet refers to the type locality.

Remarks. Our studies demonstrate that this species could be endemic to the Galapagos Islands. A single material from continental Ecuador examined [L. Arvidsson & D. Nilson 1145 (GB)] seems more similar morphologically to *A. sorediatus*, due to the presence of soredia and appearance of squamules.This afore mentioned specimen remains to be the only Ecuadorian specimen known, since our attempts in collecting fresh ones during a 10-day trip across the country were not successful.

The phylogeny places *A. galapagoensis* as a sister of the clade containing *A. campestris* and *A. variabilis*. The most diagnostic characteristics are the olivaceous thalli of elongated and intricate, often overlapping or "tiled" squamules; this structural arrangement of the squamules immediately differentiates *A. galapagoenis* from other *Acantholichen* species. Another characteristic of this taxon is that when well developed, the thallus can get unusually large, occasionally covering bryophytes on tree trunks up to 1 m length. Additionally, in large specimens, the thallus center squamules often become necrotic. Because of the tiled, overlapping growth of the squamules, squamules in the thallus center regularly become shaded and are then unable to photosynthesize; these areas then lose the characteristic pigmentation of the photobiont and become beige (**Figures 7A and 7B**). Squamules of other *Acantholichen* species are mostly broader and rarely grow overlapping each other, thus typically do not become necrotic. The species was first reported from the archipelago as *Acantholichen pannarioides* (Jørgensen 1998; Yánez et al. 2012), but the molecular data presented here and a thorough morphological and anatomical analysis of all material clearly indicates that all reports are based on one

and the same species, which is endemic to the Galapagos. Throughout the archipelago no other lichen closely resembles *A. galapagoensis* (Bungartz et al., 2013a).

Additional material examined (paratypes): *Galapagos*: Isla Isabela, Volcán Alcedo, alt. 1100 m, on bryophytes, growing over hepatics on *Zanthoxylum fagara*, 07-Mar-2006, *Aptroot, A.* 65187 (CDS 31771, GMUF); alt. 1066 m, on bark of *Zanthoxylum fagara*, 06-Mar-2006, *Bungartz, F.* 4125 (CDS 28152, GMUF); –Isla San Cristóbal, Cerro San Joaquín, alt. 771 m, on bark, branches and twigs of *Miconia robinsoniana*, 24-Aug-2008, *Bungartz, F.* 8577 (CDS 41223, GMUF, F). –Isla Santa Cruz, along trail from Bellavista to El Puntudo, alt. 684 m, on bryophyte, growing over *Frullania* sp., 23-Jun-2010, *Dal-Forno, M.* 1202 (CDS 44753, GMUF), *Dal-Forno, M.* 1204 (CDS 44755, GMUF, F); alt. 733 m, on bark, trunk of *Cinchona pubescens*, 08-Feb-2007, *Bungartz, F.* 5593 (CDS 33035, GMUF, F, B); close to El Puntudo, alt. 735 m, on bark of *Scalesia pedunculata*, 23-Feb-2007, *Nugra, F.* 400 (CDS35155, GMUF, F); eastern slope below the summit of El Puntudo, alt. 780 m, on bark of *Cinchona*, 28-Feb-2006, *Aptroot, A.* 64679 (CDS 31253, GMUF, F); NE-slope of El Puntudo, alt. 813 m, on bark of *Cinchona pubescens*, 10-Aug-2008, *Bungartz, F.* 8152 (CDS 40798, GMUF, F); behind El Puntudo, previously farm of Don Benito, alt. 732 m, on bark of *Cinchona pubescens*, 03-Feb-2007, *Nugra, F.* 379 (CDS 35134, GMUF, F);–Isla Santiago, permanent plot # 11 Pampa dentro, alt. 870 m, on bark of *Zanthoxylum fagara*, 24-Mar-2006, *Aptroot, A.* 65554 (CDS 32142, GMUF, F).

Acantholichen pannarioides P.M. Jørg., Bryologist 101(3): 444 (1998)

Figure 8

Type: Costa Rica: Heredia, NE of Heredia, S slope of Barva volcano, "Calle Cienaga', Concepcion, alt. 1580-1700 m, 17-Feb-1990, *Döbbeler*, *P.* & *Poelt*, *J.* s.n. (GZU).

Description. Thallus microsquamulose; squamules broad to slightly elongated, basally attached to the substrate, swollen, up to 2 mm long, 1.5–1.8 mm wide, moderately branched, greyish blue green with slightly paler margins when fresh, dark blue when dry. Overall uniform white pruinose appearance. Soredia absent.Thallus in cross section 200–240 µm thick, with a thick photobiont layer, a thin cortex and an unusually well-developed thick medulla with abundant acanthohyphidia. Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia large, 28–50 µm long and 6.5–9.5 µm wide, elongate to clavate.

Distribution and ecology. Our single recent collection of this taxon, identified by comparison to description and images (light and SEM microscope) of the type material, corroborates the distribution pattern, as our specimen was found in a wet montane forest in the Cordillera Talamanca of Costa Rica, where the material grows on an old fence post along with species of *Heterodermia* and *Frullania*. Jørgensen (1998) also considered material from Venezuela (BG), continental Ecuador (GB) and the Galapagos (COLO) as part of *A. pannarioides.* The Galapagos specimens are here described as new (*A. galapagoensis*), as well as the one from Ecuador (*A. sorediatus*), but we have had no

access to the Venezuelan material as it was not found in the cited herbaria. Therefore it is presently not possible to confirm that this species occurs outside Costa Rica; most likely, the specimen from Venezuela represents one of the other species distinguished here or perhaps an entirely novel taxon.

Remarks. The acanthohyphidia in *Acantholichen pannarioides* are considerably longer than in the other taxa, except for *A. variabilis*, in which they can be as large, but are overall much more variable in size. Morphologically, the two species show very different color when fresh, *A. pannarioides* is grey blue green with a lighter greenish hue towards the tips, while *A. variabilis* is dark blue. *A. campestris* also resembles *A. pannarioides*, but the squamules of the latter species are always sparse, growing separate and are considerably broader. *Acantholichen campestris* by comparison develops a much more continuous thallus with richly branching squamules that look almost microfruticose.

Material examined: Costa Rica: Puntarenas Province, Las Alturas Biological Station, near the Panama border on the western slopes of the Talamancan range, montane rain forest, 8°56′43″N, 82°50′00″W, alt. 1500 m, growing mostly on wood from fence, but also on mosses, 25-May-2012, *Dal-Forno*, *M.* 1752 (GMUF, INBio).

Acantholichen sorediatus Dal-Forno, Sipman & Lücking sp. nov.

Figure 9

Type: Costa Rica: Puntarenas, San Vito de Coto Brus, Las Cruces Biological Station; 82°58′ W, 8°47′ N, alt. 1200 m; on ridge beyond Río Java, undergrowth of

disturbed primary forest; growing on trunk, with other lichens (*Hypotrachyna*, *Normandina*, *Leptogium*), Oct-2004, *Lücking, R.* s.n. (F).

Description. Thallus granular to microsquamulose; granules initially 0.1 mm in diam,, eventually forming larger, broad squamules, basally attached to the substrate, swollen, 0.25–1.5 mm wide and 0.3–0.9 mm long, not to moderately branched, green when fresh, but becoming dark bluish grey when dry. White pruinose appearance due to acanthohyphidia on both upper and lower surface, though these spiny hyphae are more abundant along the margin of the squamules, where granular, ecorticate soredia are formed. Soredia present, marginal and frequent. Thallus in cross section 130–190 µm thick, dominated by a thick photobiont layer, and with a thin cortex and medulla. Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia of moderate size, 15–20 µm long and 5–8 µm wide, irregular, pyriform to subclavate.

Distribution and ecology. The type locality is part of the Cordillera Central in Costa Rica, an area with active volcanos. This ecology appears similar to areas where specimens of *A. galapagoensis* grow on Santa Cruz Island in the Galapagos. Nevertheless, specimens of *A. sorediatus* are not only well separated using molecular data, they are also morphologically very distinct. Both specimens examined grow directly on the bark, while other species tend to grow on bryophytes.

Etymology. The epithet refers to the marginal soredia found in this species.

Remarks. The most remarkable feature of this species is the formation of true soredia along the margin of its squamules. The species has acanthohyphidia that are mostly irregular and of relatively uniform, moderate size, in that regard somewhat similar to *Acantholichen albomarginatus*, though typically slightly longer. These two species also share a granulose-sorediate thallus morphology, but the color (green vs. blue green) and squamules appearance (swollen vs. flattened) of both species are very distinguishable. *Acantholichen campestris* also resembles *A. sorediatus*, but this species does not produce soredia, although its highly pruinose squamules can be very densely branched along their rim, a characteristic that could be mistaken for the formation of soredia.

Additional material examined (paratypes): Costa Rica: Cartago, Irazú Volcano National Park, part of the Cordillera Volcanica Central Conservation Area, summit of the Irazu Volcano, 25 km ENE of San Jose, access road to crater and surroundings, 9°59′N, 83°51′W, alt. 3300 m, alpine paramo zone, disturbed paramo vegetation, on bark (stem), 6-Jul-2002, *Sipman, H. J. M.* 48329 (B, GMUF, F).

Acantholichen variabilis Dal-Forno, Coca & Lücking sp. nov.

Figure 10

Type: Colombia: Valle del Cauca: Cerro San Antonio (= Cerro de la Horqueta), Dagua, 10 km by via El Mar, 03°29′40″N, 76°37′25″W**,** alt. 1946 m, lower montane wet forest, in forest edge, high light intensity, on moss, 08-April-2014, *Coca, L. F.* 5209 (FAUC, holotype; CUVC, GMUF, F, isotypes).

Description. Thallus microsquamulose; squamules broad to elongated, basally attached to the substrate, swollen, 0.1–1 mm wide, 0.1–0.5 mm long, moderately to abundantly branched, dark blue when fresh but becoming dark bluish grey when dry. Overall uniform pruinose appearance. Soredia absent. Thallus in cross section 110–130 µm thick, dominated by a thick photobiont layer, and with a thin cortex and medulla. Photobiont *Rhizonema*, clusters of densely coiled cyanobacterial filaments wrapped within a hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia highly variable in size, small, medium or large, 10–50 µm long and 8–10(–12) µm wide, subglobose, pyriform to subclavate when short and elongate to clavate when large.

Distribution and ecology. This species is so far only known from a single collection from lower montane wet forest in Colombia. It shares a similar ecology with *A. albomarginatus*, in which both species are found in forests of high altitude, in habitats that are wet, but nevertheless exposed to high light intensity, such as the edge of the forest.

Etymology. The epithet refers to the variable size of the acanthohyphidia in this species.

Remarks. This species much resembles the type species *Acantholichen pannarioides* overall in morphology and anatomy. However, when fresh, the material is more swollen, rounder and much darker in color than that of *A. pannarioides*, which remains distinctly greenish even when fully hydrated. The dried squamules get a little flattened, thus to some extent resembling *A. albomarginatus*, but differing by the location

of acanthohyphidia on the squamules (in *A. albomarginatus* these are not as evenly distributed across the surface, especially where individual squamules fuse into a crust and show a white margin). Another very distinct characteristic of *A. variabilis* is the highly variable size of its acanthohyphidia. Even across one single squamule these cells vary considerably from very small (10 μm) to large (50 μm) long.

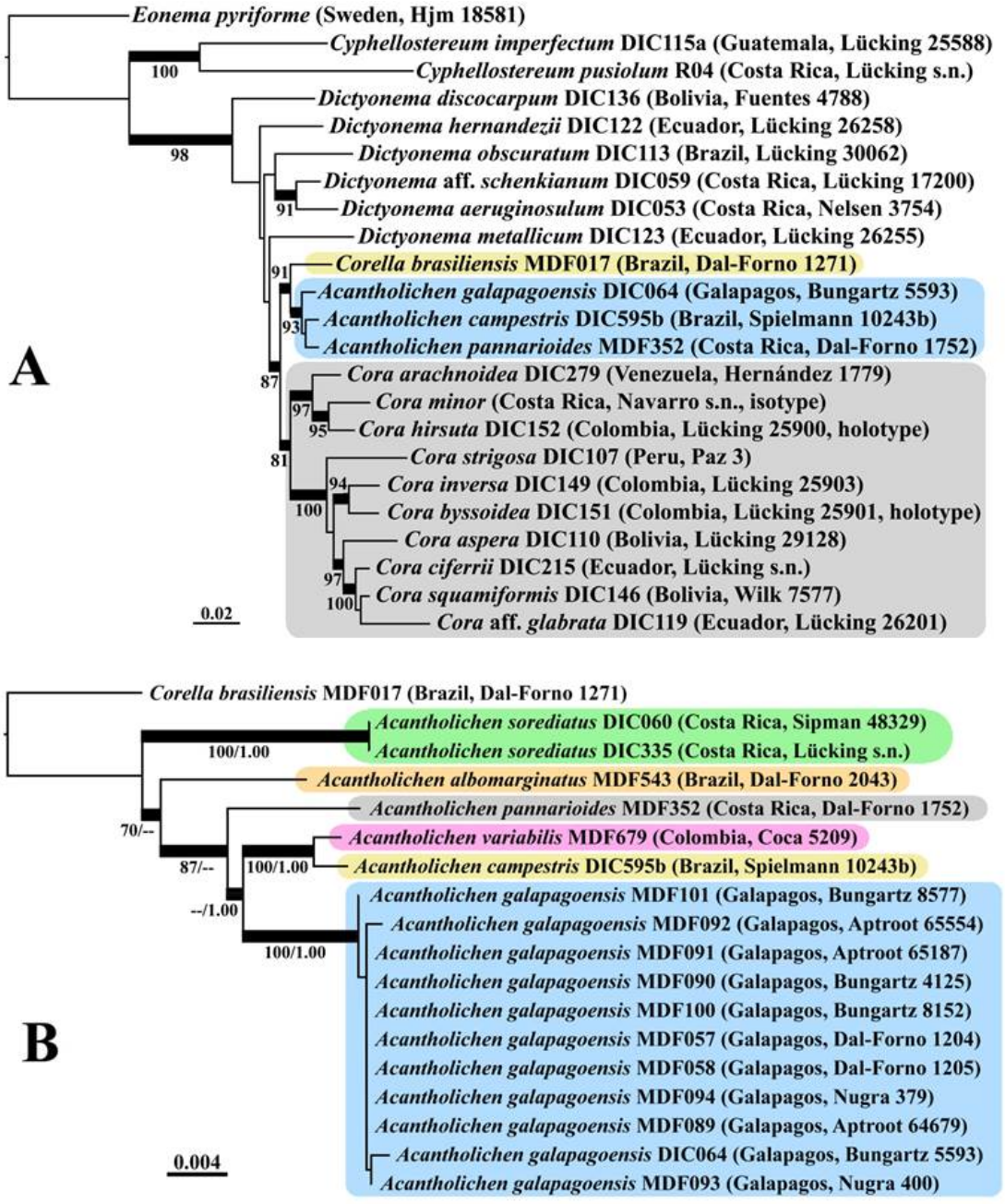


Figure 4 - Phylogenetic trees inferred by maximum likelihood (RAxML) using three loci (ITS, nuLSU, RPB2) A- *Dictyonema* clade: 22 specimens plus Eonema pyriformis as outgroup; B- *Acantholichen*: 17 specimens with Corella brasiliensis as the outgroup. Support values are given below the branches for ML for both trees and Bayesian Inference (BI) with partitioned data for b only. Branches were thickened for moderate to strong values (ML bootstrap values above 70 and BI posterior probabilities above 0.95)

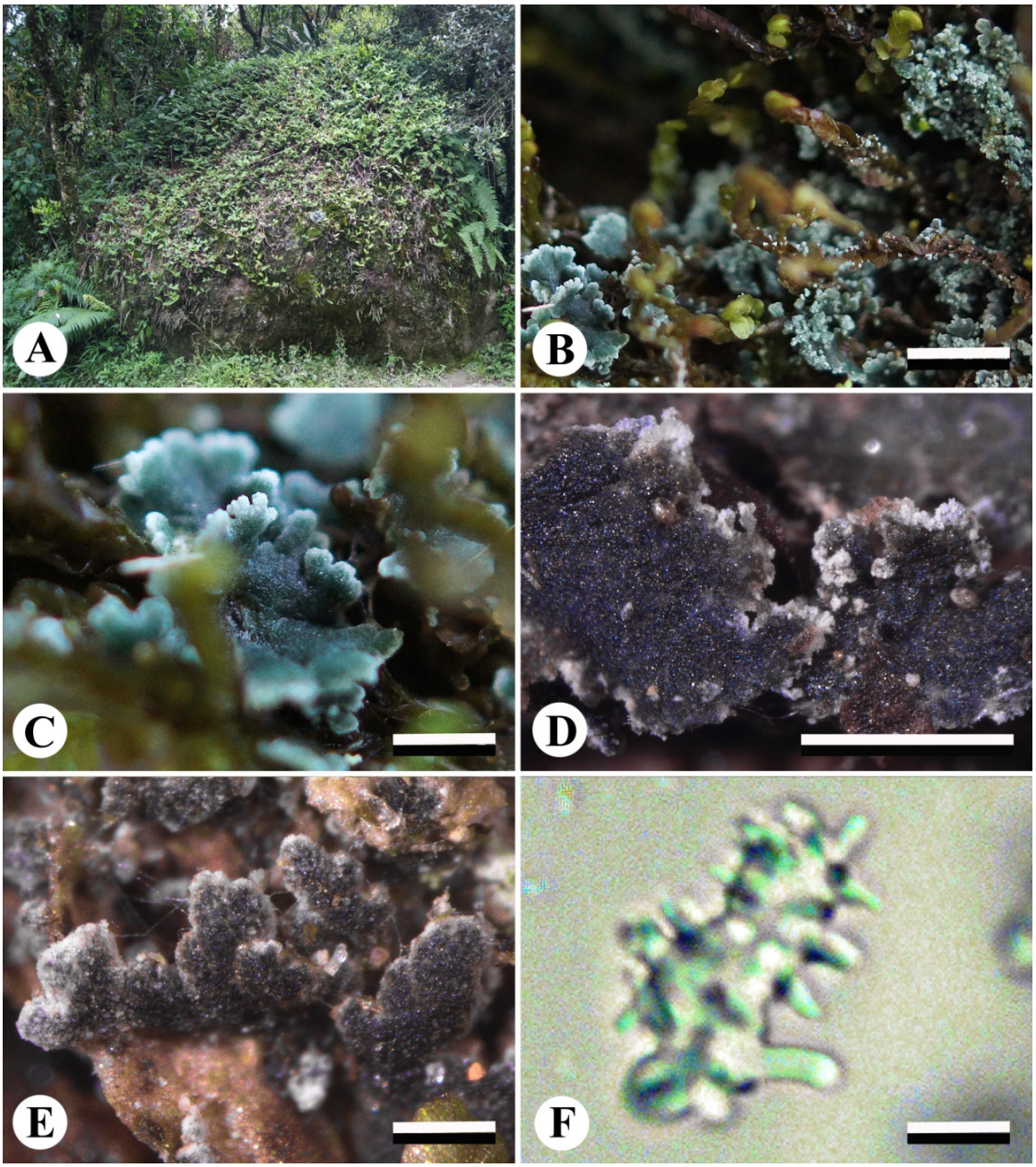


Figure 5 - *Acantholichen albomarginatus* (holotype, M. Dal-Forno & M. P. Marcelli 2043)
A- Habitat where the species grows. B, C - Growth aspect and color in fresh material (scale bar = 3 mm and 1 mm, respectively). D, E - Growth aspect and color in dry material (scale bar = 1 mm and 0,1 mm, respectively). F- acanthohyphidia (scale bar = 5 µm)

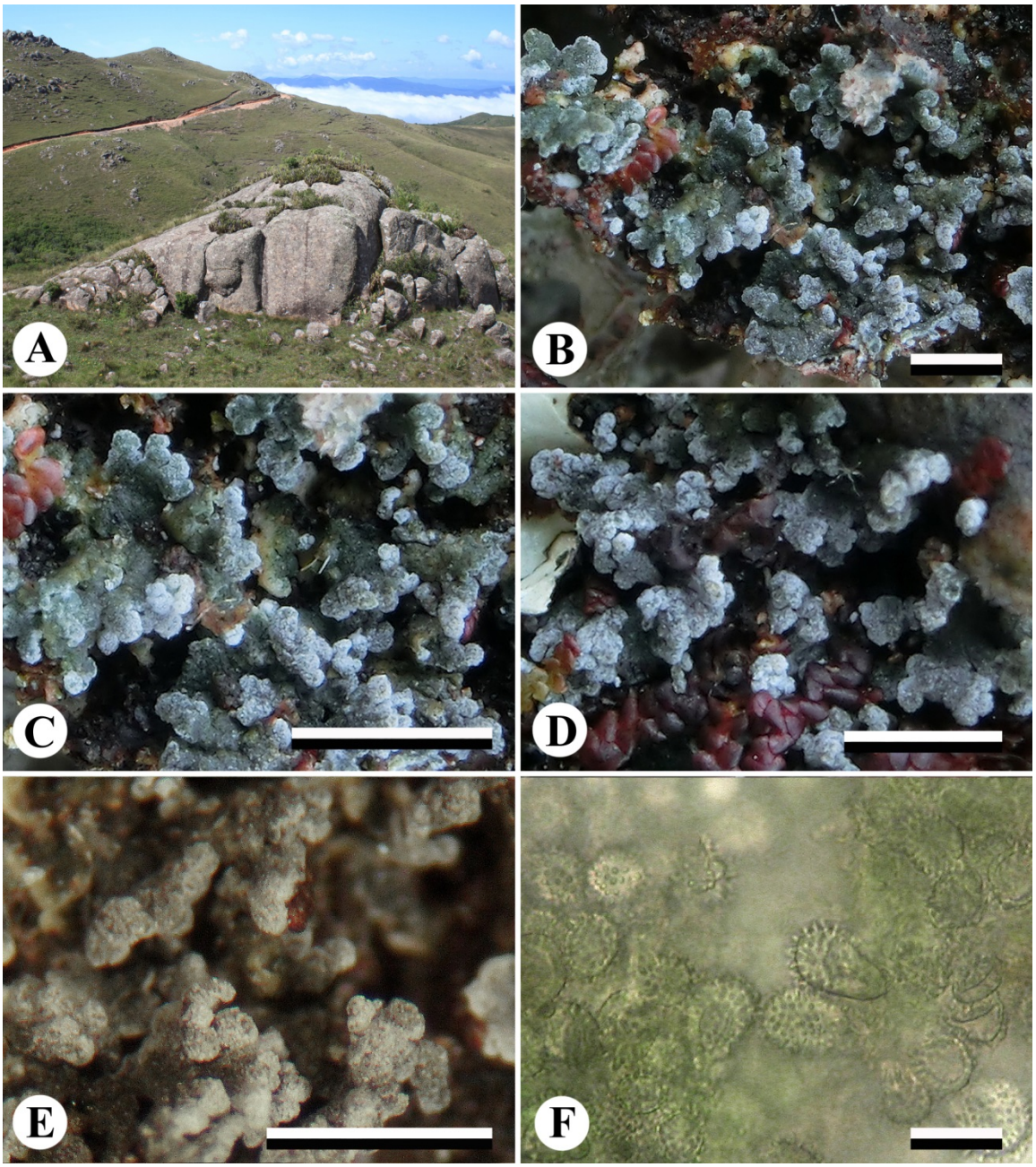


Figure 6 - *Acantholichen campestris* (holotype, A.A. Spielmann, L.S. Canêz & E.L. Gumboski 10243b)
A- Habitat where the species grow. B, C, D- Growth aspect and color in rehydrated material (scale bar = 1 mm). E- Growth aspect and color in dry material (scale bar = 1 mm). F- Acanthohyphidia (scale bar = 15 µm)

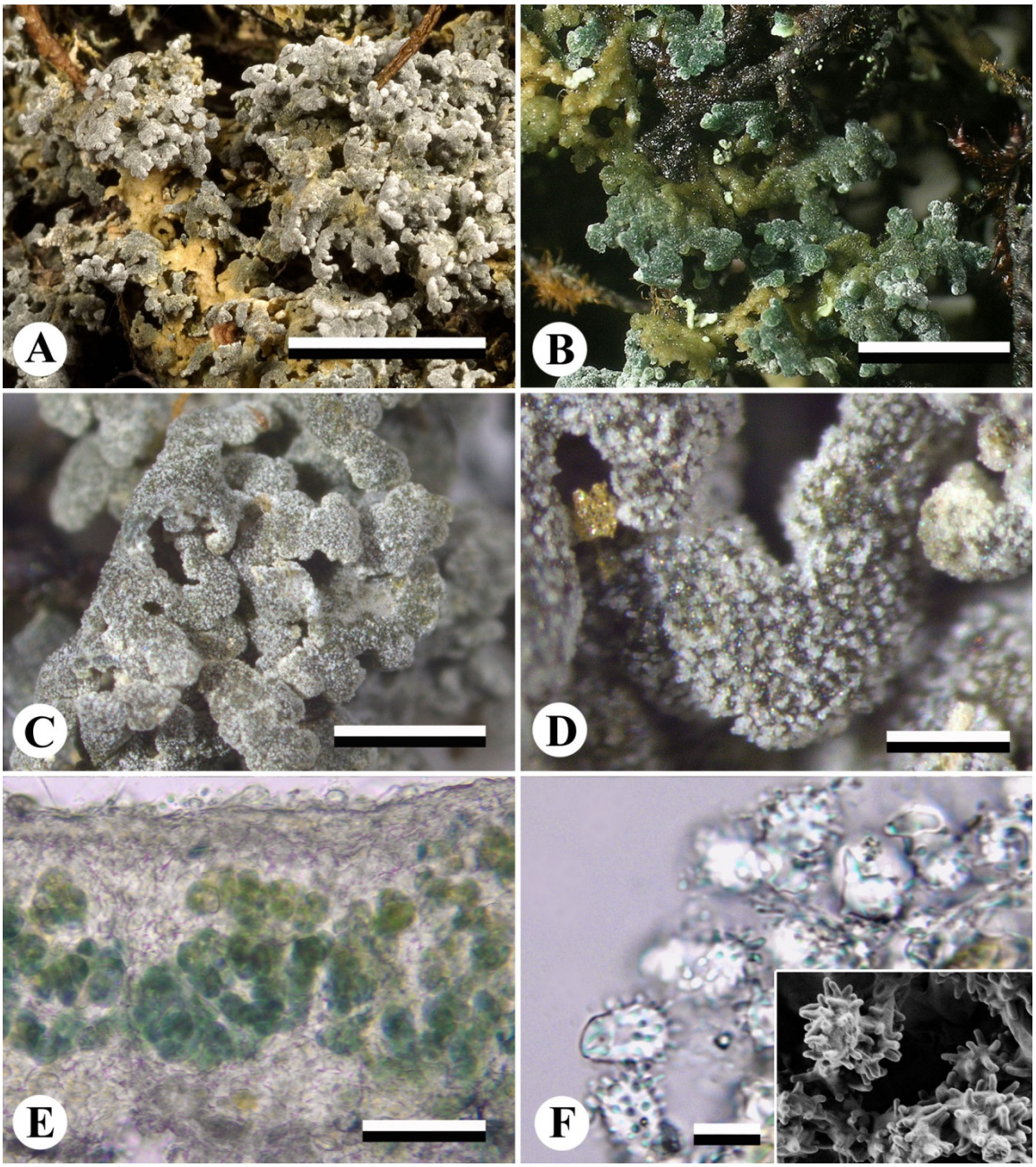


Figure 7 - *Acantholichen galapagoensis* (multiple specimens)
A- Growth aspect and color in fresh material, showing yellowish brown bases in old parts of the thallus (paratype, F. Bungartz 5593, scale bar = 1 mm). B- Growth aspect and color in fresh wetted material (holotype, M. Dal-Forno 1205, scale bar = 1 mm). C, D- Growth aspect and color in dry material (paratype, F. Bungartz 5593, scale bar = 1 mm, and 0,1 mm, respectively). E- Heteromerous thallus cross section, showing cortex, photobiont layer and medulla (paratype, F. Bungartz 5593, scale bar = 50 µm). F- Acanthohyphidia (scale bar = 10 µm), inlay SEM image of the acanthohyphidia (isotype, M. Dal-Forno 1205)

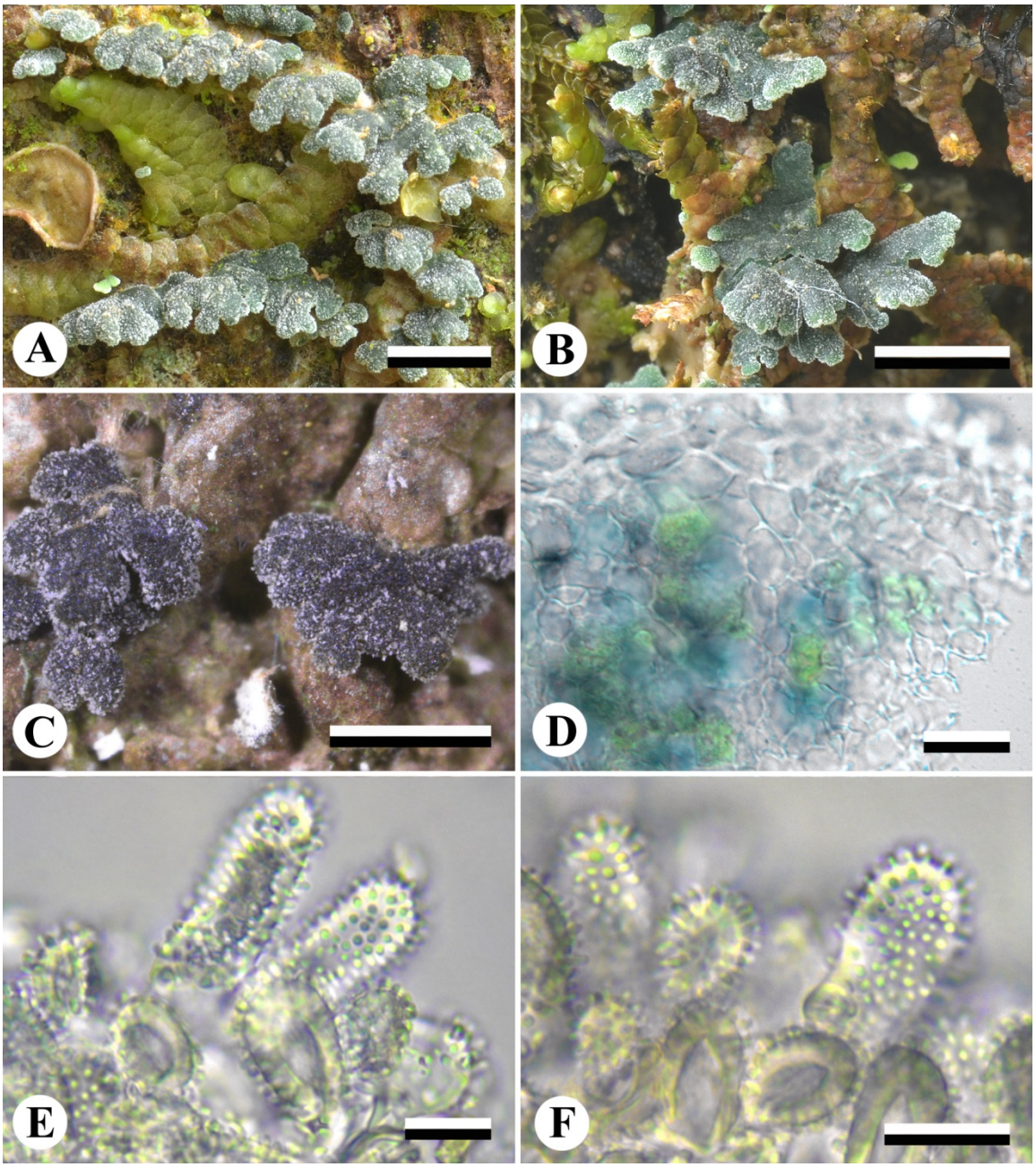


Figure 8 - *Acantholichen pannarioides* (M. Dal-Forno 1752)
A, B- Growth aspect and color in fresh material (scale bar = 2 mm). C- Growth aspect and color in dry material (scale bar = 1 mm). D- Paraplectenchymatous cortex (scale bar = 10 µm). E, F- Acanthohyphidia (scale bar = 10 µm)

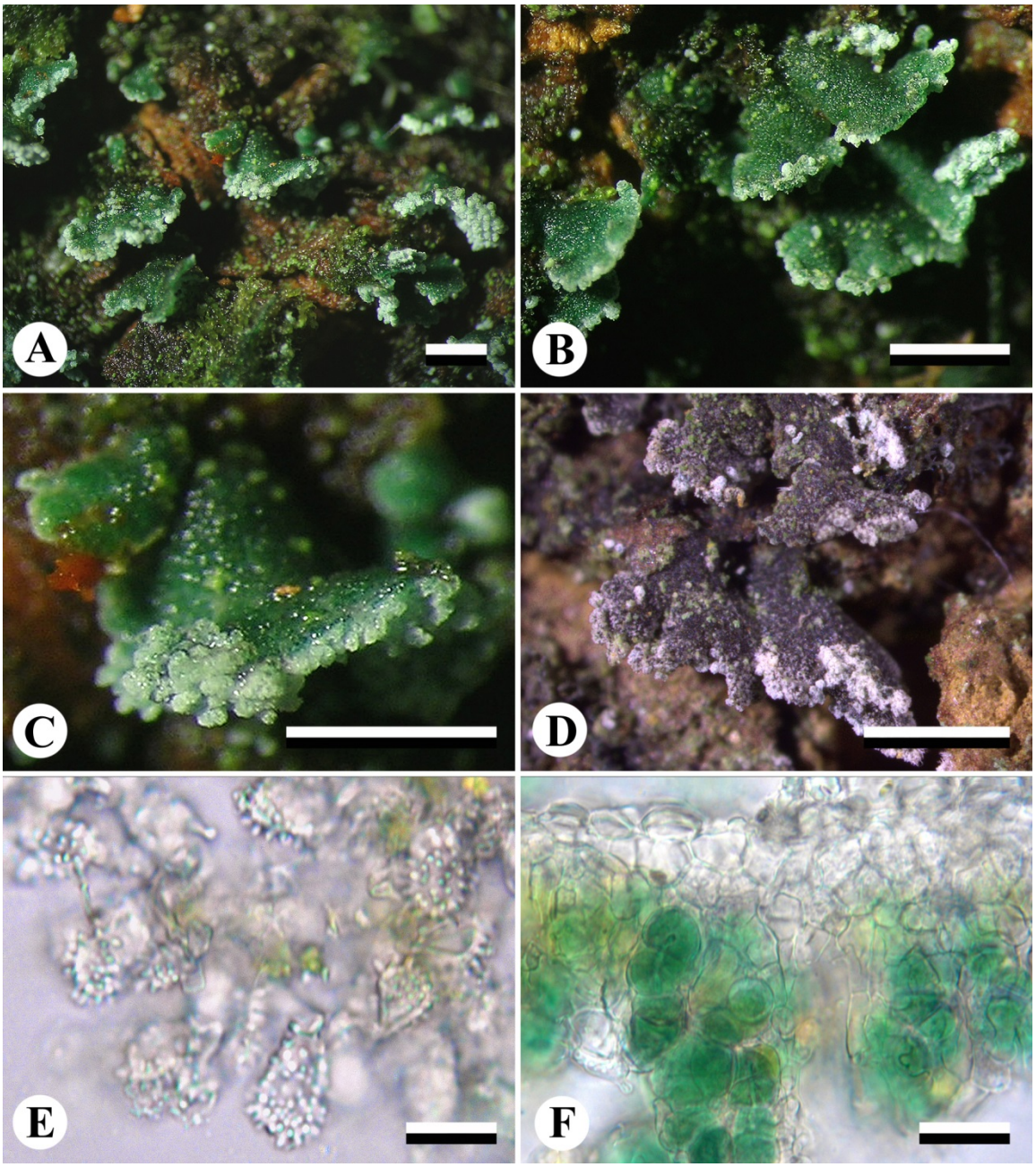


Figure 9 - *Acantholichen sorediatus* (holotype, R. Lücking s.n.)
A, B, C - Growth aspect and color in fresh material (scale bar = 1 mm). D- Growth aspect and color in dry material (scale bar = 1 mm). E- Acanthohyphidia (scale bar = 10 µm). F- Paraplectenchymatous cortex (scale bar = 20 µm)

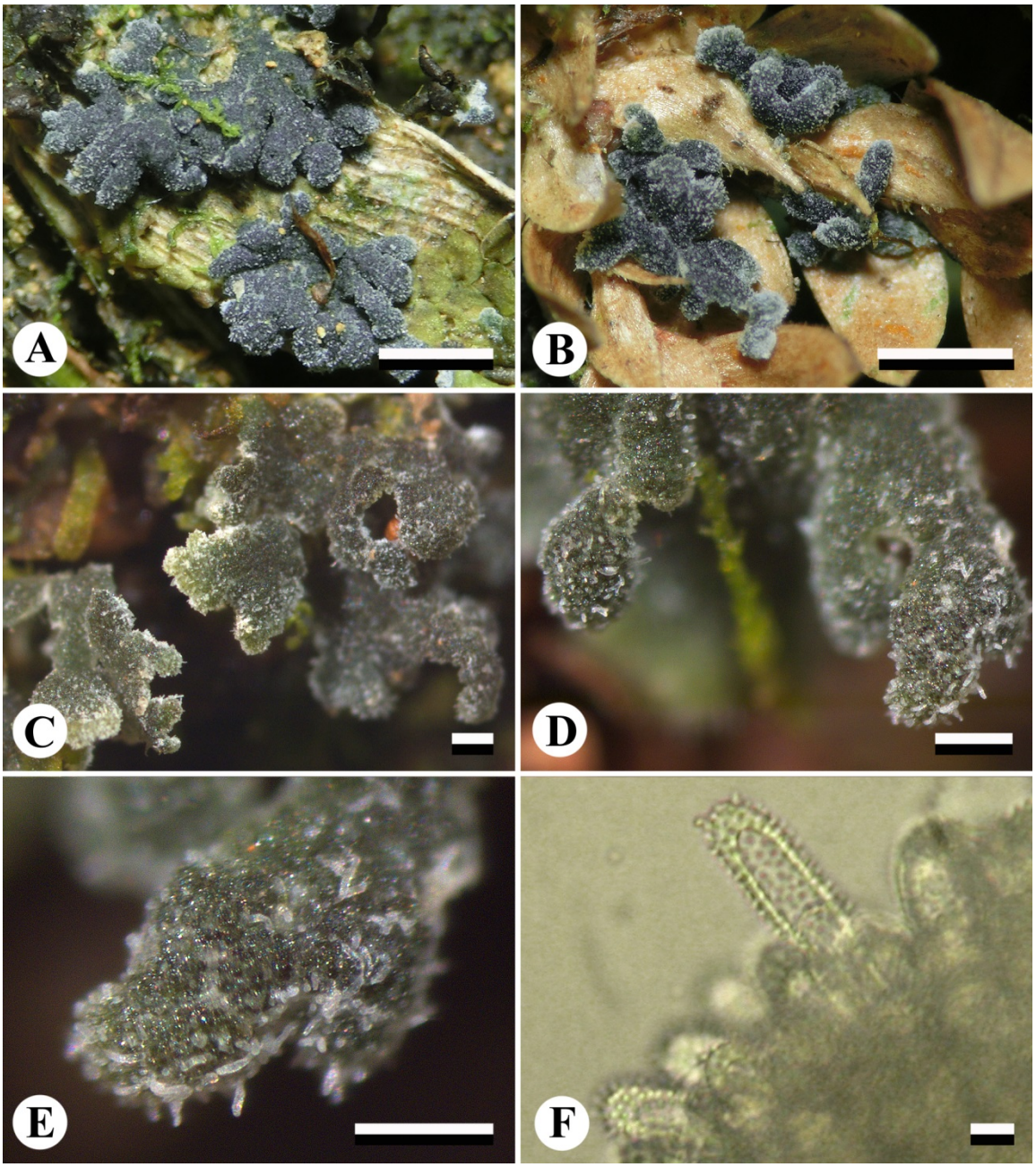


Figure 10 - *Acantholichen variabilis* (holotype, L. F. Coca 5209)
A, B- Growth aspect and color in fresh material (scale bar = 1 mm). C, D- Growth aspect and color in dry material (scale bar = 0,1 mm). E- Close up showing acanthohyphidia (scale bar = 0,1 mm). F- Acanthohyphidia (scale bar = 10 µm)

Chapter 4 – High levels of endemism in Galapagos Islands basidiolichens of the *Dictyonema* clade: An updated assessment including molecular data and taxonomic novelties

Abstract

This study is an assessment of the present state of knowledge concerning the diversity of basidiolichens in the Galapagos Islands. Based on collections from the Islands made in the past and by us recently, all basidiolichens in the Galapagos belong to the *Dictyonema* clade. After a previously published taxonomic account, here we include for the first time a molecular phylogenetic study of 90 specimens in the genera *Acantholichen*, *Cora*, *Cyphellostereum*, and *Dictyonema*, making use of two nuclear ribosomal DNA markers (ITS and nuLSU). A detailed morphological and anatomical revision is also incorporated. Ten basidiolichen species are now known from the Islands, among them three that have been published elsewhere: *Acantholichen galapagoensis* Dal-Forno, Bungartz & Lücking, *Dictyonema pectinatum* Dal Forno, Yánez & Lücking, and *Dictyonema galapagoense* Yánez, Dal Forno & Bungartz, here recombined as *Cyphellostereum galapagoense* (Yánez, Dal Forno & Bungartz) Dal-Forno, Bungartz & Lücking. An additional seven species are here proposed as new to science: *Cora galapagoensis* Dal-

Forno, Bungartz & Lücking, *Cora santacruzensis* Dal-Forno, Bungartz & Lücking, *Cyphellostereum floreanum* Dal-Forno, Bungartz & Lücking, *Dictyonema barbatum* Dal-Forno, Bungartz & Lücking, *Dictyonema bungartziana* Dal-Forno, Yánez & Lücking, *Dictyonema ramificans* Dal-Forno, Yánez & Lücking and *Dictyonema subobscuratum* Dal-Forno, Bungartz & Lücking. Based on our phylogenetic analysis, including a large number of samples from the South American continent, it appears that of these ten species, only two (*D. pectinatum* and *D. subobscuratum*) are found outside of the Galapagos Islands, indicating a potentially high level of endemism of 80% in these lichens for the archipelago.

Introduction

The Galapagos Islands represent an isolated, self-contained ecosystem long considered a living laboratory of evolution (Bensted-Smith, 2002; Carlquist, 1974; Darwin, 1859; Williamson, 1981). Assuming that species only rarely managed to colonize the islands from South and Central America, evolutionary opportunities permitted them to fill unoccupied ecological niches after they arrived. Many groups of organisms therefore exhibit high levels of endemism in the archipelago. Estimates of endemism from biodiversity reports are 32–43% for vascular plants, ~50% for invertebrates (excluding insects), 47% for insects, and 59% for land vertebrates (Tye et al., 2002). Endemism in lichens is considered to be much lower, around 5-10% (Weber, 1986; Yánez et al., 2013).

The first publication mentioning lichens in the Galapagos is that of Hooker (1847), who included three collections originally made by Charles Darwin. Weber (1986) briefly summarizes lichen collections subsequent to this first paper, mentioning that most of these were gathered by collectors without specific lichen knowledge and identified and reported in the literature by experts only much later (Dodge, 1935; Farlow, 1902; Howell, 1942; Linder, 1934; Stewart, 1912). William Weber himself was responsible for obtaining most of the modern lichen collections from the Galapagos, beginning with a 6-week expedition in 1964 and including additional visits after that. His extensive work led to multiple publications (Weber, 1981, 1966, 1986; Weber and Beck, 1985; Weber et al., 1977), with a comprehensive list including 196 species (Weber, 1986). Since then, there have been three updated checklists, two by Elix and McCarthy (1998, 2008), who included 229 and 253 species, respectively, and the most recent one by (Bungartz et al., 2013a), in which 579 species are accepted, along with an additional group of specimens representing names under revision (unidentified taxa, preliminary identifications, etc.). The substantial increase by over 300 additional species is due to the work of Frank Bungartz, who spent several years (from 2005 to 2015) on the islands focusing on a complete inventory of the lichen biota, partially supported by other visiting lichenologists and students, such as André Aptroot, María Herrera-Campos, Alba Yánez-Ayabaca Fredy Nugra, and Adriano Spielmann.

In addition to general floristic treatments of lichens in the Galapagos, specific revisionary treatments dedicated to certain groups have been published recently, among them studies of *Ramalina* Ach. (Aptroot and Bungartz, 2007), *Collema* F.H. Wigg. and

Leptogium (Ach.) Gray (Bungartz, 2008), *Rocella* DC. (Tehler et al., 2009), Graphidaceae (Bungartz et al., 2010), *Bulbothrix* Hale (Bungartz et al., 2013b), *Lepraria* Ach. and *Septotrapelia* Aptroot & Chaves (Bungartz et al., 2013c), and Cladoniaceae (Yánez et al., 2013). These also include a first treatment of Galapagos basidiolichens (Yánez et al., 2012), based entirely on morphological and anatomical studies.

Prior to the 2012 treatment, four basidiolichen taxa had been reported from the Galapagos Islands, partially under different names, all members of the *Dictyonema* clade:

1. *Cora pavonia* (Sw.) Fr. in Dodge (1935), also cited in Weber (1966), later moved to *Dictyonema montanum* (Sw.) Parm. by Weber (1986) and followed by Elix and McCarthy (1998).
2. *Dictyonema sericeum* (Fr.) Mont. by Linder (1934), updated to *Dictyonema guadalupense* (Rabenh.) Zahlbr. by Weber (1986) and followed by Elix and McCarthy (1998).
3. *Dictyonema* sp. (Weber, 1993) (an unidentified, appressed filamentous, crustose species).
4. *Acantholichen pannarioides* P. M. Jørg. (Jørgensen, 1998; Lawrey et al., 2009; Lücking et al., 2009a).

These four species represent one foliose form (*Cora pavonia*), one filamentous, shelf-forming morphotype (*Dictyonema sericeum*), a crustose form (*Dictyonema* sp.), and a microsquamulose species (*Acantholichen pannarioides*). Specimens collected until recently were therefore assigned to one of these taxa based on their morphology. In addition, *Dictyonema moorei* (Nyl.) A. Henss. and *D. membranaceum* C. Agardh. have

been used as working names for specimens collected in the islands, but these names have now been added to the list of rejected taxa for the archipelago (Bungartz et al. 2013a) after reexamination of the material.

Yánez et al. (2012) was the first study to emphasize the diversity of basidiolichens in the Galapagos using revised genus and species concepts first proposed by Lawrey et al. (2009), who for the first time recognized four genera within the *Dictyonema* clade, *Dictyonema, Cora, Cyphellostereum* and *Acantholichen.* Yánez et al. (2012) used a conservative approach when identifying these lichens, only publishing new species where no other available name could be applied to the observed material. This led to the report of eight species in the *Dictyonema* clade for the islands: *Acantholichen pannarioides, Cora glabrata, Cyphellostereum imperfectum, Cyphellostereum* sp.*, Dictyonema galapagoense, Dictyonema pectinatum, Dictyonema sericeum,* and *Dictyonema schenkianum.* Of these, *D. galapagoense and D. pectinatum* were proposed as new to science based on Galapagos specimens and *C. imperfectum* was also described as new, based on material from Guatemala, and included in the taxa identified from the Galapagos.

Recently, Dal-Forno et al. (Chapter 3) discovered, based on molecular, anatomical and morphological evidence, that the species previously identified as the widespread neotropical *Acantholichen pannarioides* in the Galapagos Islands is genetically and morphologically distinct and appears to be endemic to the archipelago. Galapagean material previously identified as *A. pannarioides* should therefore now be referred to as *A. galapagoensis.*

Given the extensive collections representing the *Dictyonema* clade gathered by us recently in the Galapagos Islands and throughout the Neotropics and other regions of the world, and reports of high levels of endemism in this clade from other Neotropical areas (Dal-Forno et al., 2013; Lücking et al., 2013a, 2014b, 2014c; Vargas et al., 2014), here we wanted to test: (1) the hypothesis that many or all species of the *Dictyonema* clade found in the Galapagos Islands are potentially endemic to the archipelago; (2) whether these species are the result of island radiations or separate colonization events and, in the latter case, identify their closest relatives. To accomplish these objectives, we added to our morphological and anatomical data extensive phylogenetic information using two nuclear markers of the rDNA cistron.

Materials and methods

Taxon sampling

As mentioned in Yánez et al. (2012), this work is part of the Galapagos Lichen Inventory, in which focused collecting took place on the following islands: Española, Floreana, Isabela, Pinta, Santa Cruz, Santiago, and San Cristóbal (**Figure 11**).

We also added to our dataset historical collections available at the Charles Darwin Research Station (CDS) herbarium. Our specimens are distributed as follows: Floreana (10), Isabela (20), Pinta (1), San Cristóbal (4), Santa Cruz (52), and Santiago (3), for a total of 90 specimens (**Table 6**). No specimens belonging to the *Dictyonema* clade were found in Española. We restricted our study to specimens for which we had molecular data

available, and we include here abbreviated collection information, including the holotypes (marked with * in **Table 6**). Further details concerning these collections can be found at the Charles Darwin Foundation (CDF) Collections Database online at http://www.darwinfoundation.org/datazone/collections/. In addition to the Galapagos material, several specimens mainly from the Neotropics belonging to the *Dictyonema* clade were used to augment our dataset and to test our hypotheses concerning relationships within species. Information for all of these additional taxa can be found in **Table 7** (Subset 1 – 161 specimens belonging to 13 localities), and **Table 13** (Subset 2 – 399 specimens belonging to 18 localities).

Morphological and anatomical studies

All morphological measurements are taken from dry and/or rehydrated specimens, unless fresh specimens were photographed with a scale, in which case these are also considered. No major differences were measured in dry versus wet state regarding structural details; however, color was observed (when possible) in both states, as well as texture (when applicable), since these can be substantially different. For standard descriptions and observation of characteristics, procedures following the protocols of Lücking et al. (2013a) were adopted (also see **Appendix 1**).

Specimens were examined with a LEICA MS5, an OLYMPUS SZX12 and a Zeiss Stemi DV4 dissecting microscope, and a ZEISS Axioskop 2, an OLYMPUS BH-2, and Zeiss Imager A1 compound microscope.

Macrophotos were taken with a Nikon Camera Control Pro 2, Sony Alfa 33

DSLR, a CANON Powershot SX20IS, NIKON F301, a Nikon D300 and/or D7000, 62 mm Nikkor Micro Lens and R1C1 macro flash directly in the field, or using a Novoflex macro-table to take images of herbarium specimens; for photographic magnifications higher than 1:1 an extension tube or Novoflex bellows was used.

For microphotos, the microscopes above were equipped with a DAGE MTI DC-330 3CCD, JENOPTIK ProgRes C3 and C5, a 1401KEM 10x Eyepiece 2.07 Megapixel PupilCam, or a Nikon DSLR phototube. All photos were processed with Photoshop CS4.

Secondary metabolites were not examined, since Dal-Forno et al. (2013) reported total absence of acetone-soluble compounds through thin-layer chromatography (TLC) from 64 specimens; we therefore considered the results from Piovano et al. (1995), who reported TLC chemistry in these species, as artifactual.

Molecular data

DNA was extracted from lichenized thalli using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogene, Illkirch, France) according to the manufacturer's protocol with slight modifications (**Appendix 2**). Of the final 150 μL volume, dilutions were prepared in new tubes for a volume of 50 μL in the 1:10 proportion, as follows: 5 μL DNA: 45 μL DEPC. 2 μL of this DNA dilution were subjected to a standard PCR in a 20 μL reaction volume using Taq Gold polymerase (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocols (**Appendix 3**). Sequence data were obtained from nuclear ribosomal ITS (ITS1, 5.8S, and ITS2) and nuLSU. PCR products were purified with magnetic beads (Agencourt Bioscience, Beverly, MA, USA) and then used in

standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The primers used were LR0R, LR3R, LR7, LR16, ITS4, ITS1F and ITS5 (http://www.biology.duke.edu/ fungi/mycolab/primers.htm, Gardes and Bruns, 1993; White et al., 1990). The sequencing reactions were purified using Sephadex G-50 (SigmaeAldrich, St. Louis, MO, USA), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed using ABI software. Individual sequences were assembled with Sequencher version 5.0 (Gene Codes, Ann Arbor, MI, USA) for visual assessment in base calling and to make contiguous alignments of overlapping fragments. When high quality sequence from the ITS could not be generated through Sanger sequencing, the pyrosequencing method using the 454 platform was adopted (Lücking et al., 2014d).

Datasets

Two subsets of data were generated for the phylogenetic analyses as described in detail below. The first (Subset 1) is for general placement of the species using two markers (ITS and nuLSU) and the second (Subset 2) for mainly species delimitation with several hundred species in which we used ITS only, the barcoding locus for Fungi (Schoch et al., 2012).

Subset 1: This subset includes 189 specimens (**Table 7**). The sequences are from material from 13 localities, and are distributed by geographic regions according to **Table 8**. All sequences correspond to different specimens (including the outgroup), with a

single sequence per specimen used. For the material where more than one ITS sequence was available (around 19), only the longest one was chosen, since they were identical (generated by 454 and Sanger sequencing). Out of the total number of 357 sequences (189 nuLSU + 168 ITS), 170 are new sequences (127 nuLSU + 43 ITS), and four have been recently updated.

Subset 2: This subset corresponds to a large number of sequences in which we mainly had ITS available. This set contains 497 sequences, including eight outgroup sequences. Due to the large number of taxa included, the table with specimen information can be found in the end of this chapter (**Table 13)**. Below it is shown the distribution of these ITS sequences by country (**Table 9)** and by genus in the *Dictyonema* clade **(Table 10)**.

Sequence alignment and phylogenetic analysis

All sequences were handled in Geneious (http://www.geneious.com). Individual nuLSU and ITS fasta files, as well as the concatenated dataset, were subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al., 2010a, 2010b), using MAFFT (Katoh and Toh, 2010; Katoh et al., 2005) as the alignment option, 100 bootstrap iterations and calculating Guidance scores.

Multiple treatments regarding different removal of regions in the alignment with low score were performed to test alternative tree topologies with the inclusion or exclusion of these regions. These alignments were saved separately in individual ITS and nuLSU sequence files, as well as the concatenated data sequence file representing Subset 1. These were: (1) Total evidence: with the entire multiple sequence alignment (MSA);

(2) Medium stringency: the MSA after the removal of unreliable columns below 0.93; and (3) High stringency: the MSA after the removal of unreliable columns below 1.00.

For Subset 2, multiple files were saved as well, but the treatments were slightly different: (1) Total evidence: with the entire multiple sequence alignment (MSA); (2) High stringency: the MSA after the removal of unreliable columns below 0.93; (3) Medium stringency: the MSA after the removal of unreliable columns below 0.90; and (4) Low stringency: the MSA after the removal of unreliable columns below 0.70.

It was originally intended that the same treatments be used for Subsets 1 and 2, but since ITS alone is much more variable, retaining columns with a score of 1.00 only provides little phylogenetic information, for example, artificial groups may be recovered.

Alignments (including all of the different treatments from Guidance) were subjected to ML search using RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2005), with nonparametric bootstrapping using 500 replicates under the GTRGAMMA model in the CIPRES Science Gateway V. 3.3 (Miller et al., 2010).

For **Subset 1**, each gene region was first analyzed separately, and then combined (**Concatenated dataset Subset 1**) after analysis for potential conflict in the individual gene trees. This analysis evaluates the nonparametric bootstrap values of the same clades in each tree and if strongly supported clades (BS higher than 70%) are in disagreement, significant conflict is assumed to exist that precludes combination of the datasets. This method is widely used in phylogenetic studies using multiple markers (Mason-Gamer and Kellogg, 1996). Since no conflict was detected in our datasets, we combined them and subjected the combined dataset to maximum likelihood analysis (ML) as described above

and Bayesian inference (BI). For the latter, the **Concatenated dataset Subset 1** was analyzed using MrBAYES 3.2.3 (Huelsenbeck and Ronquist, 2001), with two independent runs, a total chain length of five million generations, and four separate chains each, resampling every 200 trees and generating a 50% majority rule consensus tree from the sampled trees after discarding 25% burnin to obtain posterior probability estimates.

All trees were visualized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator CS5.1.

Results

For Subset 1 and 2, the alignment lengths and the Guidance Scores can be found in the tables below (**Tables 11** and **12**, respectively).

Here are displayed trees only for Subset 1 – Treatment 1 (ITS: **Figure 12**, nuLSU: **Figure 13**, Combined: **Figures 14 and 15**) and Subset 2 – Treatment 1 (**Figure 16**), since these were generated with alignments without regions removed. Additional phylogenetic trees generated for tree topology comparison are assembled in **Appendix 10**.

For the MrBayes run, 2248 trees were generated, and a 50% Majority-rule consensus of 1683 trees (565 removed after the 25% burnin) was used to recover the Bayes tree. Since there were no differences in tree topology for the Bayesian and the ML analysis, the posterior probabilities were plotted into the ML tree (**Figure 14** with details shown in **Figure 15)**.

For both subsets, well-supported clades were recovered to indicate the presence of ten species of *Dictyonema* s.l. in Galapagos. Several backbone relationships remained unresolved in some parts of the tree. For example, species relationships within the *Dictyonema* s.str. paraphyletic grade were poorly resolved. The genus *Cora* also appears to be polyphyletic in the nuLSU only tree (**Figure 13**); however it is monophyletic in the ITS (**Figure 12**) and in the combined dataset (**Figure 14**) trees.

Based on Subset 1 (**Figures 12, 13, 14 and** 1**5**), one would infer that all species of the target group in Galapagos are endemic. However, with the addition of many further ITS sequences in Subset 2, two of these species also occur on the Ecuador mainland (**Figure 16**). These two species, *D. subobscuratum* and *D. pectinatum*, have distinctive characteristics that can be observed in all specimens. Specimens of *D. subobscuratum* have a muriform aspect of the photobiont cells, which can be also observed in *D. obscuratum*, a species from Brazil, but have less wavy cells composing the fungal sheath surrounding the cyanobacteria. These two taxa (*D. obscuratum* and *D. subobscuratum*) are sister clades in the ITS trees and trees based on the concatenated dataset (ITS+nuLSU) as shown in **Figures 12** and 1**4**; in the nuLSU trees, they are recovered as sister clades. In all cases, however, they are closely related (**Figure 13**). *Dictyonema pectinatum* is another species rather easy to identify, even in the field, due to the "combed" appearance of the fibrils. Even if this characteristic is sometimes not observed in large thalli, for example for the Ecuadorian material, the fungal sheath shows the papillae characteristic of the species, which are especially papillose towards the tips. In

all phylogenetic trees, the relationships and origins of this taxon are unclear and we believe that its closest relatives are yet to be sampled.

Discussion

In comparison to our previous assessment of the Galapagos basidiolichens (Yánez et al., 2012), there is only a small increase in overall number of taxa, from eight to ten species found in the islands. This is still a far lower *Dictyonema* s.l. diversity than is observed in comparable areas in the Neotropics. For example, unpublished data collected by our working group has accounted for at least 30 species in Brazil and 50 for Colombia, mostly regional or local endemics. Ecuador and Costa Rica also have a high number of species (Lücking et al., 2014c), although further studies are necessary to assess the actual diversity. Peru, Bolivia and Venezuela are also expected to bear high levels of species diversity (Lücking et al., 2014c); however, further collecting is needed.

Nonetheless, if we considered that classically only four species have been used to to identify members of the *Dictyonema* clade in the Galapagos (Dodge, 1935; Elix and McCarthy, 1998; Jørgensen, 1998; Linder, 1934; Weber, 1966, 1986, 1993), our first assessment doubled this number (Yánez et al., 2012). Now with phylogenetic data included, a 25% increase in species number in shown, as well as more evidence to report high levels of endemism in basidiolichens within the islands. Based on the present study, we estimate that 80% of the basidiolichens in the Galapagos are endemic. It should be emphasized that this endemism is supported by comparisons with numerous specimens mainly from the Neotropics, since our results are settled within a very broad phylogenetic

framework, similar to the study on *Roccella* (Tehler et al., 2009). Therefore, we predict that conclusions about Galapagos endemism in other lichen groups will change with molecular studies. The two studies available so far that show the highest levels of species endemism in Galapagos lichens (Tehler et al., 2009, and this study, both with 80%) include molecular data, while others with lower numbers of lichens endemics, between 3–26% (Aptroot and Bungartz, 2007; Bungartz et al., 2010, 2013b; Bungartz, 2008; Yánez et al., 2013) do not. In the case of *Dictyonema* s.l., species and genus concepts have been altered drastically since molecular data began to be used to study the group. In the recent past, concepts based only on morphology and anatomy would lead to erroneous conclusions about species diversity and endemism. The 12.5% level of endemism calculated by Yánez et al. (2012) for the *Dictyonema* clade, based on morphological concepts, is very different from the 80% based on molecular and morphological evidence combined. We now know that *Cora glabrata* is not a widespread taxon also found in the Galapagos Islands, but instead the genus is represented by two endemic species, *C. galapagoensis* and *C. santacruzensis*, which were identified as the supposedly widespread *C. glabrata* and *C. pavonia* in the past. Similarly, lichens identified as *Dictyonema schenkianum* are now recognized as *D. bungartziana*, *D. subobscuratum* and *D. ramificans*. Other species (*Acantholichen pannarioides = A. galapagoensis, Dictyonema sericeum = D. barbartum, D. pectinatum)* were recognized by Yánez et al. (2012) as distinct taxa, but it was not known at the time that they were probably endemic, nor was it known that high levels of endemism existed in the clade generally. This did not become apparent until recently, when molecular data were used to assess the phylogeny

of large numbers of specimens of *Cora* and *Corella* from throughout the Neotropics (Lücking et al., 2014c).

Compared to other lichen groups found in the Galapagos, basidiolichens exhibit relatively low species diversity, possibly reflecting recent colonization of the Islands or unusual limitations on dispersal. Weber (1966) was the first to discuss endemism in the Galapagos lichens, hypothesizing that lichens might exhibit relatively low levels since adaptive radiation there might be more constrained and slower in symbiotic associations. Weber's (1986) original checklist gives the estimated percentage of endemic lichens as 8–10%, which is the number also given by Yánez et al. (2013), even though the most recent checklist of Bungartz et al. (2013a) has three times the number of species as Weber's (1986). As pointed out by Yanéz et al. (2013), levels of endemism in lichens vary substantially across groups, with the highest being in *Roccella*, where 80% of the species are endemic (Tehler et al., 2009), and the lowest in Cladoniaceae, with only 3% of the species being endemic to the Islands; however, no phylogenetic analyses were included in the last study (Yánez et al., 2013). Our results show a relatively high percentage of lichens endemics, a level much higher than Weber (1986) estimated originally, but identical to the *Rocella* findings of Tehler et al. (2009).

Based on our phylogenies, the ten species of basidiolichens recognized now for the Galapagos did not all descend from a single common ancestor. All *Dictyonema* s.l. species in the Galapagos arrived independently by multiple colonization events. Also, because the closest relatives of many of the Galapagos basidiolichens are found on the mainland, we assume that diversity in the Islands is not the consequence of adaptive

radiations, as is known to happen with many plants, such as the genus *Scalesia* (Asteraceae), and animals, such as the Darwin's finches, the giant tortoises and snails of the family Bulimulidae (Darwin, 1859; Losos and Ricklefs, 2009; Tye et al., 2002). Regarding species diversification in different groups of organisms in the Galapagos, radition within the islands seems to be more frequent than speciation without subsequent diversification. Nonetheless, Parent et al. (2008) states that animal species that descended from a non-Galapagos relative but have not diversified within the Galapagos Islands (single-endemic species) may occur. For example, out of the 30 land birds, five are indigenous (16.66%), seven are single-species endemics (23.33%) and 18 are multiple-species endemics (60%), the later forming two independent lineages.

It is not known with certainty the origin of lichens in the Galapagos Islands, but most hypotheses involve some form of transport from the mainland. There is no possibility of land dispersal per se, since the islands are of volcanic origin, extremely geographically isolated (ca. 1000 km from Ecuadorian coast), and have never been connected to the continent. Transport to the Islands could be accomplished by rafting on vegetation islands, wind and birds, all of which can bring either vegetative dispores (soredia, isidia and thallus fragments that contain both lichen partners) or fungal spores. For the later case, after germination, the mycobiont would require a suitable photosynthetic partner to successfully colonize, considered by Weber (1966) to limit lichens dependent on this mode of dispersal to arrive and survive in the islands. It is now recognized that lichen fungal partners, mycobionts, can share or even steal photobionts from other lichenized fungi (Goward, 1994; Lücking et al., 2009a; Piercey-Normore and

DePriest, 2001), so dispersal by fungal spores alone is not necessarily an ecological constraint on colonization.

Tye et al. (2002) state that "*filtering of species by the barriers to arrival and establishment results in an unusual selection of species reaching the islands, compared with the range of species available in the continental source areas.*" For the *Dictyonema* clade, our data suggest that the barriers to colonization and establishment are not restricted to any single genus. Out of the five genera in the *Dictyonema* clade, four occur in the Galapagos, *Acantholichen, Cora, Cyphellostereum*, and *Dictyonema* s.str. The genus *Corella* has not yet been found in the Galapagos, but this is a small genus and not many collectors are aware of it. The only countries up to now to have representatives of all *Dictyonema* s.l. genera are Brazil, Colombia and Costa Rica, countries from which most collections have been made. No *Cyphellostereum* species are reported from Venezuela, while Ecuador, like the Galapagos, has no reports for *Corella*. However, all of these observations are expected to change as further collections are made and herbarium collections are revised.

Given the small number of species and high levels of endemism of Galapagos basidiolichens, it is interesting to speculate on what may have made the particular collection of ten species successful colonists of the islands and not others. The point of origin of most of the species appears to be mainland Ecuador, according to the phylogenetic trees shown here. This assessment is based only on current mainly Neotropical collections, but not all Galapagos species have Neotropical relatives as of now. For example, one species, *Cyphellostereum floreanum,* appears in all phylogenetic

trees to be most closely related to *C. phyllogenum,* a species from Fiji, with 100% bootstrap support (BS) and 1.0 posterior probability (PP); these species are also similar morphologically and anatomically. This could be partially explained because the genus *Cyphellostereum* is not commonly collected due to its unsual lichen appearance, and likely there is a closer relative in the Neotropics. Most of the remaining species are closely related to species from Ecuador, Brazil and Costa Rica, which could represent a bias regarding the areas of our focused collecting. Nevertheless, our data is showing strong evidence that Galapagos species have originated from independent colonizations from Central and South America, especially Ecuador. Although some conclusions might change in the future with the addition of more specimens, the overall pattern is most likely to remain the same.

Other archipelagos and oceanic island groups have not been so well studied for basidiolichens as the Galapagos, but information available from Hawaii, another isolated oceanic island system, suggests that basidiolichen diversity in these situations is unusually low. Recently collected specimens representing the *Dictyonema* clade in Hawaii, in combination with phylogenetic data, indicate that there is only one species of *Dictyonema* there, *D. moorei* (Nyl.) Henss., and five undescribed *Cyphellostereum* species (unpublished data), a number slightly lower than the ten species of the clade in the Galapagos. *Dictyonema moorei* has been cited for Hawaii in Elix and McCarthy (1998) and in the current Checklist of Pacific Island Lichens: Hawaiian Islands (https://www.anbg.gov.au/abrs/lichenlist/HAWAIIAN_ISLANDS_lichen_list.html). Interestingly, we also found *D. moorei* from Korea and Brazil, indicating that this species

is possibly subcosmopolitan, arriving in Hawaii relatively recently. Similar results have been observed in *Crocodia aurata* (Moncada et al., 2014; Lücking unpublished data), in which material from Hawaii fits phylogenetically with Neotropical specimens; however, the material from Galapagos is a separate species.

Comparing basidiolichen diversity in the Galapagos with that of Hawaii may help to shed light on the causes of diversity and endemism in oceanic islands. A hypothetical scenario would be the that islands that are far in distance from the main land have a lower probability of colonization, hence low probability of gene exchange; this could result in early colonizers being more likely to radiate. But many groups take a long time until they successfully colonize, and a mix of radiations and late colonizers would therefore be present. Accordingly, an island that has a shorter distance from the main land would have more frequent colonization events and gene exchange, and hence low probability of separate evolution. A mid distance from the main land would present a balance between probability of colonization and isolation, thus likely a higher rate of colonization events, but too low to establish frequent gene exchange, which could lead to frequently separate species, but low incidence of radiation.

The Hawaiian Islands are ca. 4000 km from continental North America, far more distant from the mainland than the Galapagos, which are ca. 1000 km away from South America. We would expect that the Galapagos, being closer to the mainland, would have experienced colonization events more frequently than Hawaii. Furthermore, successful colonization of the Galapagos would have taken place more quickly after their origin than in Hawaii, leading to subsequent divergence of many colonizing lineages there. In

Hawaii, colonizing lineages would be fewer and younger, which provides less opportunity for evolutionary divergence. This hypothesis appears to be supported in part by our present data, since we see a higher frequency of colonization in Galapagos than in Hawaii for the *Dictyonema* group, with at least ten separate events for Galapagos and only three in the Hawaiian Islands. We also see higher levels of endemism in the Galapagos than in Hawaii, suggesting a longer period of divergence from colonizing ancestors there. In Hawaii, the presence of *D. moorei*, a relatively widespread and cosmopolitan species, is an indication of a recent colonization with little or no divergence from the ancestral condition. Nevertheless, there are what appear to be endemic species of *Cyphellostereum* in Hawaii that may have diverged from a common ancestor that arrived successfully in the islands a long time ago. *Cyphellostereum* is a basal, much older genus, sister to all other genera in *Dictyonema* (Dal-Forno et al., 2013; Lawrey et al., 2009; Lücking, 2012). In one of these two colonization events of *Cyphellostereum*, the close relationship of an entire lineage of Hawaiian *Cyphellostereum* species to a single species (DIC 333 from Costa Rica; **Figure 17**) provides some support for this hypothesis. By the branching pattern, it seems that a single species perhaps arrived to Hawaii a long time ago and radiated into multiple species.

The hypothesis by Lücking (unpublished data), which our data seems to corroborate, implies that isolation (i.e., distance) itself is not the reason for phylogenetic distinctiveness, but the time of isolation of a lineage after it arrived on an island. If one takes into consideration that a very distant island is much less likely to be colonized, since it would take much longer for chance dispersal to occur than a less distant

archipelago, this far away island would have less colonization events of a certain group when compared to other islands.

Tye et al. (2002) mention that evolution of endemism may happen in one of two ways: (1) evolution of a single endemic species from each colonizing ancestor, which results in no changes in diversity but high levels of endemism; and (2) evolution involving a radiation, with one original colonizing species giving rise to several new endemic species; this would lead to higher diversity as well as endemism. The low levels of diversity of basiodiolichens of the *Dictyonema* clade in both the Galapagos and Hawaiian Islands, but the apparently higher levels of endemism generally in the Galapagos, suggest that the former process (many colonizations with less diversification) may have happened there whereas the latter (fewer colonizatios with only rare radiations) happened in Hawaii. Some evidence if this pattern may also be observed in *Pseudocyphellaria* (Moncada et al., 2014).

The low diversity and high endemism in Galapagos basidiolichens may also have to do with the age of the Islands there. According to the most recent estimates (Geist et al., 2014), San Cristóbal is the oldest island, with the maximum emergence age, the time the island first surfaced above sea level, estimated to be 4 million years ago (mya). The next oldest is Espanola at 3.5 mya. Geist et al. (2014) also pointed out that for modeling dispersal, colonization, speciation and radiation of species that involves island geography more that 20,000 years ago, the current map of the Galapagos Islands is completely irrelevant due to the many it have occurred over time. This all means that the islands may not be old enough to have permitted much diversification of colonizing basidiolichens

there. The Hawaiian Islands, in comparison, are thought to be much older; however, extant islands from which we have collections (Kauai, Oahu, and Maui) are ca. of 0.4–5.7 mya (Macdonald et al., 1983), an age estimate comparable to the Galapagos Islands.

The *Cora* clade is a good illustration of this. It is represented in the Galapagos by two endemic species, *Cora galapagoensis* and *Cora santacruzensis*. They can be easily distinguished by the frequently subimbricate, mostly greyish white lobes of *C. galapagoensis* compared to the entire and usually darker lobes of *Cora santacruzensis* (**Figures 20 and 21**). This relatively low diversity in the Galapagos contrasts sharply with the remarkable diversity of the genus elsewhere in the Neotropics, with up to 30 species or more within an area comparable to the size of the Galapagos Islands. Studies by our working group suggest that *Cora* may be the most speciose genus in the *Dictyonema* clade, and it is certainly one of the most speciose of basidiolichen genera known, with also high levels of endemism (Dal-Forno et al., 2013; Lawrey et al., 2009; Lücking et al., 2014c). The crown age of the *Cora* clade is estimated by molecular clock modeling to be 13–15 mya (Lücking et al., 2013b; Lücking, 2012), and the remarkable diversity of the group is assumed to have resulted from recent radiations taking place in the paramos during the initial uplift of the northern Andes (Gregory-Wodzicki, 2002; Meade and Conrad, 2008). The two *Cora* species in the Galapagos are not phylogenetically closely related to each other (**Figures 16 and 17**) and morphologically distinct. The closest relative of *Cora santacruzensis* is *Cora* sp. from Ecuador (MDF474), and the closest relative of *C. galapagoensis* is *Cora* sp. from Brazil (MDF110) and *Cora* sp. from Ecuador (MDF421) (**Figure 18**), indicating at the present time probably two

colonization events followed by subsequent evolution. Furthermore, our observations indicate that evolution in each lineage took place in less than 4 million years, perhaps representating early stages of a larger radiation similar to that seen in the northern Andes, which apparently took place over a time span of at least 10 mya (Lücking et al., 2013b, 2014c; Lücking 2012). If this is the case, we would argue that one of the reasons for low diversity and high endemism in Galapagos *Cora* species is a lack of time for a fully developed radiation to take place.

Taxonomic Treatment

Key to species of the *Dictyonema* clade in the Galapagos Islands

1. Thallus filamentous ……..…. 2

Thallus squamulose/foliose …… 8

2. Fibrils thin, cyanobacterial cells usually up to 10 µm broad, mostly squared ……..… 3

Fibrils thicker, cyanobacterial cells usually broader than 10 µm, mostly flattened 4

3. Fibrils mostly erect, hyphal sheath composed of tightly packed cells with almost a jigsaw pattern, sheath (similar to that of *Dictyonema*) completely covering the cyanobacterial fibrils inside ……..……***Cyphellostereum galapagoense***

Fibrils not distinctly erect (thallus mostly horizontally orientated), hyphal sheath composed of sinuous cells, typically leaving at least a few spaces, thus not completely covering the fibrils inside..... ***Cyphellostereum floreanum***

4. Thallus forming shelves ……...…….…….. ***Dictyonema barbatum***

Thallus not forming shelves, instead, forming a more or less continuous mat growing closely attached to the substrate …….……...…….…..... 5

5. Thallus fibrils mostly parallel, appearing as if the thallus has been combed …….….

……..... ***Dictyonema pectinatum***

Thallus not as above, fibrils arranged irregularly or in different patterns …….…… 6

6. Fibrils connecting towards the tips, forming vertical structures net-like, anatomically fibrils with ramification …….…...…… ***Dictyonema ramificans***

Thallus not as above, fibrils independent ……..…… 7

7. Cyanobacterial cells often longitudinally divided, hyphae from fungal sheath angular, not wavy …….…..…... ***Dictyonema subobscuratum***

Cyanobacterial cells rarely longitudinally divided, hyphae from fungal sheath wavy ……. …….. ……..…….. ***Dictyonema bungartziana***

8. Thallus microsquamulose, with acanthohyphidia …...… ***Acantholichen galapagoensis***

Thallus foliose, without acanthohyphidia ...…...…….…… 9

9. Lobes imbricated, forming up to 1 m thalli, very common ……... ***Cora galapagoensis***

Lobes not imbricated (no sutures), small thalli, uncommon ….....…. ***Cora santacruzensis***

The Species

Acantholichen galapagoensis Dal-Forno, Bungartz & Lücking sp. nov.

Figures 7 and 19

Type: Ecuador, Galapagos Islands, Santa Cruz Island, along trail from Bellavista to El Puntudo, upper *Cinchona* forest, 0°39′002″S, 90°20′42″W, alt. 684 m, dense forest of *Cinchona pubescens*, some live trees but mostly dead trees due to management control of the invasive trees, on bryophyte, growing over *Frullania* sp., 23 Jun 2010, *M. Dal-Forno 1205* (CDS 44756, holotype; GMUF, F, isotypes).

Description. Thallus microsquamulose; squamules thin and elongated, attached basally to the substrate and proliferating from the tips, moderately swollen, 0.1–0.2(–0.3) mm broad, up to 2(–3) mm long, abundantly branched and typically intricately tiled, many growing together, occasionally thus shading one another, light blue grey to olivaceous when fresh, darker olivaceous grey when dry, the shaded parts becoming necrotic and pale beige to orange. Overall uniform pruinose appearance. Soredia absent. Thallus in section shows that the photobiont layer makes up most of the thallus; however, there is a thin cortex and a medulla formed by more or less dense irregular to rounded or elongated hyphae. Thallus 130–160 µm thick, cortex paraplectenchymatous 14–18 µm

thick, photobiont layer 85–92 µm thick, medulla 30–50 µm thick. Photobiont *Rhizonema*, forming clusters of 8–16 µm diam. of coiled cyanobacterial filaments wrapped in a dense hyphal sheath formed by jigsaw puzzle-shaped cells (*Cora*-type). Acanthohyphidia small to rarely medium, 12–16(–20) × 6–10 µm, subglobose to pyriform, as a spiny apical cell, present on upper and lower surfaces, however more abundant on lower surface. Thalli without sexual reproductive structures. No clamps observed.

Distribution and ecology. All *Acantholichen* specimens collected in Galapagos belong to a single, endemic species. In general, specimens do not grow directly on trees or shrubs, but typically on epiphytic liverworts and mosses, on introduced (*Cinchona pubescens*, *Psidium guava*), native (*Zanthoxylon fagara*) and endemic trees (*Scalesia pedunculata, Psychotria* spp.); one specimen was even collected on a soil inhabiting bryophyte (*Campylopus* sp.). Populations on *Frullania* (Jubulaceae, Marchantiophyta, overgrowing *Cinchona* in Santa Cruz represent not only the best developed material, such as the type specimen, but where the species is also most abundant. In this location, individual thalli are large and could cover areas of up to 1 m, indicating that the species thrives particularly in the humid highlands around El Puntudo and Cerro Crocker. Collections from other islands are mostly minute, not well developed thalli, with the exception of collections from Cerro Azúl (one of the highest and the southernmost volcano of Isabela), where specimens grow abundantly on the dead basal sheaths of fronds of the endemic Galapagos tree fern (*Cyathea weatherbyana*).

Remarks. The phylogeny places the species as a sister of the clade containing *A. campestris* and *A. variabilis* (**Chapter 3**). Its diagnostic characteristics are the olivaceous

thalli of elongated and intricate, often overlapping or "tiled" squamules; this structural arrangement of the squamules immediately differentiates *A. galapagoenis* from other *Acantholichen* species. Another characteristic of this taxon is that, when well developed, the thallus can get unusually large, occasionally covering bryophytes on tree trunks up to 1 m in length. Additionally, in large specimens, the thallus center squamules often become necrotic. Because of the tiled, overlapping growth of the squamules, squamules in the thallus center regularly become shaded and are then unable to photosynthesize; these areas then lose the characteristic pigmentation of the photobiont and become beige (**Figure 19**). Squamules of other *Acantholichen* species are mostly broader and rarely grow overlapping each other, thus typically do not become necrotic. The species was first reported from the archipelago as *Acantholichen pannarioides* (Jørgensen, 1998; Yánez et al., 2012), but the molecular data presented here and a thorough morphological and anatomical analysis of all material clearly indicates that all reports are based on one and the same species, which is endemic to the Galapagos. Throughout the archipelago no other lichen closely resembles *A. galapagoensis* (Bungartz et al., 2013a).

Additional material examined: Ecuador, Galapagos: — Isabela Island, Volcán Alcedo, alt. 1100 m, on bryophytes, growing over hepatics on *Zanthoxylum fagara*, 07-Mar-2006, *A. Aptroot 65187* (CDS 31771, GMUF); alt. 1066 m, on bark of *Zanthoxylum fagara*, 06-Mar-2006, *F. Bungartz 4125* (CDS 28152, GMUF); –San Crisóbal Island, Cerro San Joaquín, alt. 771 m, on bark, branches and twigs of *Miconia robinsoniana*, 24-Aug-2008, *F. Bungartz 8577* (CDS 41223, GMUF, F). –Santa Cruz Island, along trail from Bellavista to El Puntudo, alt. 684 m, on bryophyte, growing over *Frullania* sp., 23-

Jun-2010, *M. Dal-Forno 1202* (CDS 44753, GMUF), *M. Dal-Forno 1205* (CDS 44756 - TYPE, GMUF), *M. Dal-Forno 1204* (CDS 44755, GMUF, F); alt. 733 m, on bark, trunk of *Cinchona pubescens*, 08-Feb-2007, *F. Bungartz 5593* (CDS 33035, GMUF, F, B); close to El Puntudo, alt. 735 m, on bark of *Scalesia pedunculata*, 23-Feb-2007, *F. Nugra 400* (CDS35155, GMUF, F); eastern slope below the summit of El Puntudo, alt. 780 m, on bark of *Cinchona*, 28-Feb-2006, *A. Aptroot 64679* (CDS 31253, GMUF, F); NE-slope of El Puntudo, alt. 813 m, on bark of *Cinchona pubescens*, 10-Aug-2008, *F. Bungartz 8152* (CDS 40798, GMUF, F); behind El Puntudo, previously farm of Don Benito, alt. 732 m, on bark of *Cinchona pubescens*, 03-Feb-2007, *F. Nugra 379* (CDS 35134, GMUF, F);– Santiago Island, permanent plot # 11 Pampa dentro, alt. 870 m, on bark of *Zanthoxylum fagara*, 24-Mar-2006, *A. Aptroot* 65554 (CDS 32142, GMUF, F)

Cora galapagoensis Dal-Forno, Bungartz & Lücking sp. nov.

Figure 20

Type: Ecuador: Galapagos: Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park, 0°39′56″ S, 98°19′31″ W, alt. 502 m, *Miconia robinsoniana* shrubland, on bryophyte, growing on *Frullania* sp., 23-Jun-2010, *M. Dal-Forno 1223* (CDS 44748, holotype; GMUF, isotype).

Description. Thallus epiphytic, growing on bryophytes over branches and trunks, with other lichens and bryophytes, foliose, parallel to the substrate when on branches to completely perpendicular to the substrate when on trunks; lobes 0.5–1.5(–2) cm wide (delimited by sutures) and 1–3 cm long, densely branched, mostly white to light grey

when found dry in nature, grey, light green or olive when found wet in nature, to bluish green to grey when rehydrated (4.5 years after collecting), becoming white to light grey in the herbarium (similarly when found dry in nature), with concolorous margins, thin with a papery texture. Upper surface glabrous, with pronounced and shallow ridges, 3–5 per cm^2 and 10–12 per cm^2, respectively; lower surface ecorticate, mostly glabrous, rarely with few hairs where the thallus attaches to its substrate or along sutures, hairs white when fresh, not darkening with storage in the herbarium. Margins involute, indistinct, thin. Thallus in section 220–320(–360) μm thick, with distinct upper cortex, photobiont layer, and medulla; upper cortex *roof-like* formed by a 10–25 μm thick layer of loosely woven, irregularly arranged, 4–6 μm thick hyphae supported by a 50-105 μm high 'medullary' layer of irregularly arranged to anticlinal, 4–6 μm thick hyphae; photobiont layer 50–160(–200) μm thick, irregular, composed of clusters of short, coiled cyanobacterial filaments wrapped in a dense, paraplectenchymatous hyphal sheath formed by jigsaw puzzle-shaped cells, clusters 15–35 μm diam., individual photobiont cells 5–8 μm broad and 7–12 μm long, blue-green, penetrated by tubular fungal hyphae; heterocytes sparse, pale yellow, 5–7 μm diam; cells of hyphal sheath wavy in lateral outline, 5–6 μm thick; medulla 25–50(–70) μm thick, composed of loosely woven, irregularly arranged to more or less periclinal hyphae 3–5 μm thick; clamp connections not observed.

Hymenophore developed as linear to reticulate, large, steroid patches dispersed on the underside, patches 0.5–2 mm long and 0.5–3 mm broad, pale yellow, smooth surface and strongly involute, smooth margins; hymenophore in section 80–100 μm thick,

composed of a paraplectenchymatous layer resting on loose, 4–6 µm thick, generative medullary hyphae and supporting the hymenium; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles 10–15 × 5–6 µm; basidia 20–30 × 6–8 µm, basidiospores ellipsoid to fusiform, non-septate, hyaline, 5–7 × 4–7 µm.

Distribution and ecology. This species is known from multiple collections in Galapagos, from three islands lichenologists have visited: Isabela, Santa Cruz and Santiago. It is a very common basidiolichen on the islands. There are multiple habitats where this species grows, and most specimens, unless stated otherwise below, are growing on bryophytes on branches and tree trunks.

Etymology. The epithet refers to the whole archipelago, since this is the most common *Cora* found across the islands.

Remarks. This is a new but well known species found across the Galapagos Islands in the genus *Cora*. It is very easily recognized by its light grey to almost white color, growing mostly on bryophytes over trees. It is not the only *Cora* on the islands, but certainly the more common and widespread. This species can form up to 1 m broad thalli, and its most recognizable characteristic is the subimbricate lobes (closely adjoining, fused lobes), which appear as if "sewn together" by sutures. Among the species sampled by us, it is more closely related to yet undescribed species from Brazil, Ecuador and Colombia, which differ morphologically by not having the characteristic subimbricate lobes. The species was previously identified as *Cora glabrata* (Bungartz et al., 2013a; Yánez et al., 2012), *Cora pavonia* (Dodge, 1935) and *Dictyonema montanum* (Weber,

1986), the latter being a homotypic synonym of *Cora pavonia*. However, these names now apply to species probably endemic to the Caribbean Islands.

Additional material examined (20): Ecuador: Galapagos: Santiago Island (2), along the trail from Cerro Gavilan to La Central, alt. 890 m, on soil, 24-Mar-2006, *F. Bungartz 4831* (CDS 29005, GMUF); near permanent plot # 11 Pampa, alt. 870 m, on soil, 24-Mar-2006, *A. Aptroot 65557* (CDS 32145, GMUF). –Isabela Island (4), Volcán Sierra Negra, alt. 580 m, on bark, 14-Aug-2008, Herrera-Campos 10546 (CDS 40282, GMUF); Volcán Cerro Azul, alt. 456 m, 3-May-2012, *F. Nugra 1034* (CDS 52198, GMUF), alt. 655 m, on rock, 7-May-2012, *F. Nugra 1098* (CDS 52261, GMUF), alt. 767 m., 3-May-2012, *F. Bungartz 10325* (CDS 52298, GMUF). – Santa Cruz Island (14), along trail from Bellavista to El Puntudo, alt. 469 m, 23-June-2010, *M. Dal-Forno 1180a* (CDS 44714, GMUF), alt. 502 m, 23-June-2010, *M. Dal-Forno 1187A* (CDS 47764, GMUF), *M. Dal-Forno 1192* (GMUF), *M. Dal-Forno 1196* (CDS 44728, GMUF), *M. Dal-Forno 1206* (GMUF), *M. Dal-Forno 1218* (CDS 44741), alt. 684 m, *M. Dal-Forno 1199a* (CDS 44752, GMUF); N of Bellavista, alt. 555 m, 28-Oct-2010, *A. Yánez 1508* (CDS 44999), *A. Yánez 1509* (CDS 45000), *A. Yánez 1513* (CDS 45004); below El Puntudo, alt. 762 m, 28-Oct-2010, growing over *Cladonia confusa* on the ground, *A. Yánez 1538* (CDS 45031), growing over *Campylopus anderssonii* on front of boulder, *A. Yánez 1540* (CDS 45033); vía Media Luna, lindero del del Parque Nacional Galapagos, alt. 500 m, 23-Aug-2007, *F. Nugra 437* (CDS 36189); on the northwestern fork of the way from the parking lot to Caseta, near Media Luna, alt. 600 m, on rock, 28-Dec-2005, *F. Bungartz 3322* (CDS 26988).

Cora santacruzensis Dal-Forno, Bungartz & Lücking sp. nov.

Figure 21

Type: Ecuador: Galapagos: Santa Cruz Island, abandoned farm behind El Puntudo, 0°38′25″ S, 90°19′57″ W, alt. 729 m, tall forest of *Persea americana*, *Cinchona pubescens* and *Scalesia pedunculata*, on bryophyte, growing over hepatics on branch of *Persea americana*; semi-shaded, 28-Oct-2010, *A. Yánez 1547* (CDS 45041, holotype; GMUF, isotype).

Description. Thallus epithytic, growing on bryophytes over branches, foliose, up to 3 cm across, composed of 1–3 semicircular lobes per thallus; parallel to the substrate; lobes 0.8–1.3 cm wide and 1.7–2.6 cm long, unbranched (no sutures), yellowish green when rehydrated (4 years after collecting), with same color margins, grey in the herbarium (unknown color in nature), slightly varying in concentric zones. Upper surface glabrous, with shallow ridges, 12–13 per cm^2; lower surface ecorticate, white in the herbarium. Margins involute, indistinct, thin. Thallus in section 200–220 µm thick, with upper cortex, photobiont layer, and medulla; upper cortex formed by a 5–25 µm thick layer of rather loosely woven, irregularly arranged, 2–3 µm thick hyphae supported by a 25–28 µm high 'medullary' layer of irregularly arranged to anticlinal, 3–5 µm thick hyphae; photobiont layer 100–105 µm thick, irregular, composed of clusters of short, coiled cyanobacterial filaments wrapped in a dense, paraplectenchymatous hyphal sheath formed by jigsaw puzzle-shaped cells, clusters 25–30 µm diam., individual photobiont cells 10–15 µm broad and 6–8 µm long, blue-green to yellow-orange in upper portions, penetrated by tubular fungal hyphae; heterocytes sparse, pale yellow, 5–7 µm diam; cells

of hyphal sheath wavy in lateral outline, 3–5 μm thick; medulla 62–68 μm thick, composed of loosely woven, irregularly arranged to more or less periclinal hyphae 4–5 μm thick; clamp connections not observed.

Hymenophore developed as resupinate, linear to rarely reticulate patches dispersed on the underside, patches 0.02–7.5 mm long and 0.2–0.4 mm broad, with pale yellow, smooth surface and flat, smooth margins; hymenophore in section 70–80 μm thick, composed of a paraplectenchymatous layer resting on loose, 4–6 μm thick, generative medullary hyphae and supporting the hymenium; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles 12–30 × 4–5 μm; basidia 20–35 × 5–7 μm, 4-sterigmate; basidiospores 6–8 × 3–5 μm.

Distribution and ecology. This species is known from a couple of specimens in what seems to be a single population in Santa Cruz. The unique habitat around an old farm with a plantation of many invasive species (for example, avocado trees, yucca, pinapple), and restricted distribution makes us wonder if this is truly an endemic species or an introduced species that came along with introduced plant species. We cannot reject either hypothesis since the type specimen was growing on an avocado tree, but other specimens grow on an endemic guava (*Psidium galapageium*) in a forest of *Scalesia pedunculata*, another plant endemic to the Galapagos.

Etymology. The epithet refers to the locality type, the island where it is found in the Galapagos.

Remarks. This is another, but rather unexpected new species of *Cora* that can be found in the Galapagos. It has no unique characteristic when compared to other Coras;

however, it can be differentiated by the other Galapagos species by its darker thallus in the herbarium, and the absence of the typical sutured/subimbricated lobes of *C. galapagoensis.* In addition, *C. santacruzensis* is so far known from a single population in Santa Cruz, located north from many collections of *Cora galapagoensis* in the area.

In addition to the two species of *Cora* for which we have morphological, anatomical, ecological and molecular data, another different looking specimen (*F. Bungartz 3983*) was encountered while reviewing samples and images from the islands. This had been identified by us under the name *Cora glabrata* (Yánez et al., 2012). Unfortunately, this sample was not included in our molecular analysis; however, with a better understanding of ecological characteristics of the individual species, we are open to the possibility of a third species occurring in the islands, distributed among the already surveyed areas. This specimen grows over exposed basaltic rocks, in a very interesting geological site with giant lava bubbles, some collapsed, leaving rims of thin basaltic rock upon which the specimens grow relatively abundantly. We look forward to testing this hypothesis further using molecular markers.

Additional material examined (1): Ecuador: Galapagos: Santa Cruz Island, along trail from El Puntudo to abandoned farm of Don Benito, on bark, 08-Feb- 2007, *F. Bungartz 5594* (CDS 33039).

Cyphellostereum floreanum Dal-Forno, Bungartz & Lücking sp. nov.

Figure 22

Type: Ecuador: Galapagos: Floreana Island, on top of Cerro Asilo de la Paz, permanent plot 25, 1°19’1.7” S, 90°27'7.5" W, 531 m, humid zone, mixed dense *Scalesia pedunculata* forest with trees of *Zanthoxylum fagara* and *Psidium guajava*, *Tournefortia rufo-sericea* and *Lantana camara* in shrub layer; trees and shrubs covered with large curtains of bryophytes, growing on bryophytes over bark of branches of *Psidium guajava*; shaded, wind- and rain-sheltered, 13-Jan-2011, *F. Bungartz 9475* (CDS 46556, isotype GMUF).

Description. Thallus growing on and among bryophytes, epiphytic on tree branches, loose filamentous, in a confluent patch, forming a loose mat of irregular and more or less individual to slightly interwoven, green fibrils. Prothallus and hypothallus absent. Thallus lacking discernible layers; photobiont composed of numerous *Rhizonema* cyanobacterial filaments wrapped in an almost entirely closed hyphal sheath formed by sinuous cells; cyanobacterial filaments composed of 7.5–11 μm wide and 5–7 μm high, green cells; heterocytes frequent, pale to bright yellow, 7–10μm wide and 5–7.5 μm high; cells of hyphal sheath sinuous and irregular, 2–3 (–4) μm thick; no additional hyphae associated, lacking clamp connections.

Hymenophores not observed.

Distribution and ecology. This species is known from a single collection from the humid zone in Floreana.

Etymology. This species is named after the island Floreana, so far the only one in the archipelago where the species has been found.

Remarks. This specimen had been previously identified by us as *D. galapagoense* (= *Cyphellostereum galapagoense*) in Bungartz et al. (2013), due to its being one of the few *Cyphellostereum* collections in Galapagos. The differences lay mainly in the much denser fibrils and more bluish hue of *C. galapagoense* in comparison to this new species. In addition, *C. floreanum* also does not present the somewhat erect arrangement of the fibrils as *C. galapagoense. Cyphellostereum floreanum* is more closely related to, and morphologically similar to, *C. phyllogenum.*

No additional material examined.

Cyphellostereum galapagoense Dal-Forno, Bungartz & Lücking, comb. nov.

Figure 23

= *Dictyonema galapagoense* Yánez, Dal Forno & Bungartz, Fungal Diversity 52: 234 (2012)

Type: Ecuador: Galapagos: San Crisóbal Island, trail from Cerro Pelado to El Ripioso, 0°51′41″ S, 89°27′39″ W, alt. 392 m, *Psidium guajava* forest with some old *Hippomane mancinella* trees and dense understory of *Rubus niveus*, *Tournefortia rufo-sericea* and *Zanthoxylum fagara*, on bryophytes, growing over mosses on bark of *Hippomane mancinella*, upper side of inclined branch (ca. 20 cm in diam.), SW-exposed; semi-shaded, wind- and rainsheltered, 23-Aug-2008, *F. Bungartz 8517* (CDS 41163 holotype, isotype GMUF)

Description. Thallus growing on and among bryophytes, epiphytic on tree branches, filamentous, in a more or less confluent patch, forming a moderately dense mat

of interwoven green fibrils, forming irregularly erect compacted fibrils. Prothallus absent; hypothallus rarely present, formed by very a thin layer of white hyphae, not easily visible. Thallus lacking discernible layers; photobiont composed of numerous *Rhizonema* cyanobacterial filaments wrapped in an closed hyphal sheath; cyanobacterial filaments composed of 7–10 (–12) μm wide and 5–10 μm high, uniseriate, green cells, mostly square to elongate-cylindrical, in chains, penetrated by tubular fungal hyphae; heterocytes frequent, pale yellow, 7–10 μm wide and 5–10 μm high; cells of hyphal sheath with jigsaw pattern, somehow resembling those of *Dictyonema*, but thinner and less sinuous on the margins, 2.5–3.5 μm thick; with some occasional 3μm thick hyaline additional hyphae associated, lacking clamp connections.

Hymenophores not observed.

Distribution and ecology. This species is known now from an additional specimen from Santa Cruz, a different Island than the one the type if from, San Cristóbal. Both grow in humid environments, as do all basidiolichens in the archipelago.

Remarks. Aside from sharing characteristics with the genus *Dictyonema*, such as the similar patterns of hyphae composing the fungal sheath around the cyanobacteria and haustoria, the reason that led us to originally describe it in that genus, molecular data indicates that this species belongs to *Cyphellostereum*. As a typical *Cyphellostereum*, the cyanobacterial cells are squarer in shape, and much thinner, usually 10 μm broad or less. Both specimens of this species show a rather unique growth habit with fibrils forming a filamentous mat that also proliferates vertically, giving an erect-suberect aspect to the thallus. Anatomically, these erected compacted fibrils resemble hairs of *Cora* species.

Additional material examined (1): Ecuador: Galapagos: Santa Cruz Island, abandoned farm behind El Puntudo, alt. 729 m, on bryophyte, 28-Oct-2010, *A. Yánez 1545* (CDS 45039, GMUF).

In our previous paper (Yánez et al. 2012) we included a possible additional species of *Cyphellostereum*, referred as *Cyphellostereum* sp. A couple of specimens under this identification were revised and one (*A. Yánez 1545*) is now included in *Cyphellostereum galapagoense,* while others (*M. Dal-Forno 1180b* and *1190*) were not successfully amplified with regular PCR with the ITS or nuLSU primers commonly used. We also performed PCR with MTPS primers for ITS1F and ITS2 and despite effective amplification, the fungal reads came up as uncultured fungi in the Helotiales (Ascomycota) in BLAST (Altschul et al., 1990). We also amplified 16S, and the bacteria we amplified did not belong to *Rhizonema* or any other group of cyanobacteria based on searches in the RDP10 database (Cole et al., 2014). We therefore believe that this taxon needs to be further sampled and analyzed to establish its identity.

Furthermore, we are now rejecting the presence of *Cyphellostereum imperfectum*, a species from Guatemala included in our previous assessment of the island basidiolichens (Yánez et al., 2012). We believe that the intermixed filaments of *Cyphellostereum* found with *Dictyonema pectinatum*, originally identified as *C. imperfectum*, may potentially be one of the two species now confirmed with molecular data to be endemic of the archipelago. However, they have not been observed again and therefore cannot be further identified.

Dictyonema barbatum Dal-Forno, Bungartz & Lücking sp. nov.

Figure 24

Type: Ecuador, Galapagos, Isabela, Volcán Sierra Negra, close to the southern crater rim, along the trail to Alemania, 0°51′12″ S, 91°8′40.5″ W, alt. 1,055 m, pampa of *Pteridium arachnoideum*, *Pernettya howellii*, *Lycopodium* sp., and with occasional tree ferns (*Cyathaea weatherbyana*) and *Psidium guajava* shrubs, on bark, branch of *Psidium guajava*; semi- shaded, wind-andrain-sheltered,16-Aug-2008, *F. Bungartz 8363* (CDS 41009, holotype; GMUF, isotype).

Description. Thallus epiphytic on bark of branches and trunks, shelf-like filamentous, up to 60 cm across, with single lobes up to 8 cm wide, composed of loosely interwoven, dark green to bluish green fibrils leaving interspaces and bordered by a broad, irregularly interwoven, white to pale beige margin (prothallus). Fibrils arranged more or less horizontally, but also vertically. Thallus in section from 0.8 up to 5 mm thick, composed of a thick photobiont layer and a thick medulla forming a white hypothallus, this latter structure also with some green fibrils (with photobiont) on the under side; photobiont layer composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells; cyanobacterial filaments composed of 15–20 (–22) µm wide and 3–4(–8) µm high, bluish green cells penetrated by tubular fungal hyphae; heterocytes frequent, pale to slightly bright yellow, 9–15 µm wide and 2–5(–7) µm high; cells of hyphal sheath wavy in lateral outline, 3–4 µm thick, center hyphae reaching 9 µm thick; hyphae associated with hyphal sheath straight, hyaline, 4–7

μm thick, lacking clamp connections; hypothallus and prothallus formed by interwoven, strongly agglutinate, generative hyphae.

Hymenophores developed and frequently present, corticioid-steroid, forming irregular, reticulated, resupinate patches dispersed on the underside, patches up to 1.5 (–2) mm broad and 6 mm long, with white (when fresh) to pale yellow (in herbarium), smooth surface and sometimes minutely tomentose-fuzzy involute margins; hymenophore in section 80–130 μm thick, composed of a paraplectenchymatous layer resting on strongly agglutinated, 4–6 μm thick, generative hyphae emerging from the supporting thallus; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles 15–21 × 5–6 μm; basidia 20–30(–35) × 5–6 μm, 4-sterigmate; basidiospores ellipsoid to fusiform, non-septate, hyaline, 7–8 × 3–4 μm.

Distribution and ecology. This species is rather conspicuous and common in introduced substrates such as guava and avocado trees. This morphotype is usually present in areas with sun exposure, and it is still an important component in *Zanthoxylum* forests.

Etymology. The epithet meaning “bearded” or “having a beard” is a reference to the fuzzy white to beige appearance of the shelf-like morphology of this lichen, each shelf resembling a bearded chin.

Remarks. This species is represented by many specimens, uniform overall, but with minor variation in the thickness of the thallus and color of the prothallus (old specimens with a darkened hue, yellow to light brown). Aside from horizontal fibrils, there are also vertical fibrils compacted becoming erect structures; these can, but not

necessarily always, form a prothallus too. All specimens form filamentous shelves, a morphotype unique among *Dictyonema* s.str. species in Galapagos. The new species is difficult to separate from other species in the *Dictyonema sericeum* s.l. group, which does represent multiple distinct species (Dal-Forno et al., 2013; Lücking et al., 2013a, 2014b, 2014c; Vargas et al., 2014). *Dictyonema barbatum*, however, present a mostly continuous hymenophore, very different from *D. giganteum*, *D. discocarpum* and *D. hapteriferum*, all new species recently segregated from *D. sericeum*. *Dictyonema giganteum* (Vargas et al., 2014) present very small individual hymenophores, *D. discocarpum* has more or less disc-shaped hymenophores and *D. hapteriferum* has hymenophores that resemble hapteres found in some lichens (Lücking et al., 2014b). The species appears to have arrived to the islands independently from other *Dictyonema* species. *Cora galapagoensis* and *C. santacruzensis* also may resemble shelf-like fungi, but these have compacted foliose thalli, not filamenous, in which cyanobacterial cells are chroococcoid.

Additional material examined (10): Ecuador, Galapagos. — San Crisóbal Island (2), Cerro San Joaquín, alt. 681 m, on bark, among mosses on branches of *Psidium guajava* shrubs, 24-Aug-2008, *C. Truong 1533* (CDS 39844, GMUF), alt. 771 m, on bark of branches and twigs of *Miconia robinsoniana*, 24-Aug-2008, *F. Bungartz 8576* (CDS 41222, F); —Isabela Island (6), Volcán Sierra Negra, alt. 924 m, on *Polypodium* stems among mosses on the ground, 16-Aug-2008, *C. Truong 1259* (CDS 39570, GMUF), alt. 939 m, on *Frullania* sp. on *Psidium guajava* branch; 08-Sep-2007, *F. Bungartz 6849* (CDS 36297, GMUF), alt. 550 m, on branches of *Inga* sp., 09-Sep-2007, *F. Bungartz 6852* (CDS 36301, F), alt. 579 m, on bark of *Psidium guajava* branches, 09-Sep-2007, *F.*

Bungartz 6906 (CDS 36398, F), alt. 580 m, on bark of branches of *Psidium guajava* with mosses (together with *Cora galapagoensis*), 14-Aug-2008, *C. Truong 1275* (CDS 39586, F); Volcán Alcedo, alt. 1,100 m, on bark of *Zanthoxylum*, 07-Mar-2006, *A. Aptroot 65186* (CDS 31770, F); —Santa Cruz Island (2), Bellavista, alt. 400 m, on bark of *Miconia*, 27-May-2005, *A. Aptroot 63148* (CDS 29878, GMUF); abandoned farm behind El Puntudo, alt. 729 m, on bryophyte, growing over hepatics on branch of *Persea americana*; 28-Oct-2010, *A. Yánez 1550* (CDS no. 45044, GMUF).

Dictyonema bungartziana Dal-Forno, Yánez & Lücking sp. nov.

Figure 25

Type: Ecuador, Galapagos, Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park, 0°39'56.8" S 98°19'31.4" W, alt. 502 m, *Miconia robinsoniana* shrubland, growing over *Frullania* sp. and fern fronds, 23-Jun-2010, *M. Dal-Forno 1209* (CDS 44733, holotype; GMUF, isotype).

Description. Thallus epiphytic on tree trunks and branches, mostly on bryophytes, but also on detritus growing surrounding bryophytes, slightly appressed filamentous, in irregular, confluent patches, up to many cm across and entire thallus eventually covering larger areas of the substrate(largest specimen we found around 10 cm), forming a strongly compressed mat of mainly horizontal, loosely interwoven, mostly dark blue green fibrils sitting on top of a white hypothallus and a discrete but discernible prothallus. Thallus with a photobiont layer and a medulla (hypothallus); photobiont

composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells, sometimes thinner towards the tips; cyanobacterial filaments composed of (12–)15–20 µm wide and 4–8(–9) µm high, bluish green cells penetrated by tubular fungal hyphae, uniseriate (rarely biseriate); heterocytes sparse, pale yellow, 8–16 µm wide and 4–8 µm high; cells of hyphal sheath variably wavy in lateral outline, but showing the very typical jigsaw pattern characteristic of *Dictyonema*, 5–8 µm thick, very rarely branching; hyphae associated with hyphal sheath straight, 5–7 µm thick, lacking clamp connections.

Hymenophore developed as bulging, stereoid patches from the underside of the thallus margins, white patches up to 1.2 mm broad and 1 cm long, with white (when fresh) to pale yellow (in herbarium), smooth surface; hymenophore in section 50–85 µm thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of numerous, palisade-like basidioles and basidia; basidioles 20–30 × 5–7 µm; basidia 25–35 × 5–8 µm, 4-sterigmate; basidiospores ellipsoid to narrowly drop-shaped, non-septate, hyaline, 5–7 × 2–3 µm.

Distribution and ecology. This represents the most common *Dictyonema* species in the Islands, being represented by many collections from Santa Cruz and Isabela, as well as one from Pinta.

Etymology. This species is named after our esteemed colleague and lichenologist Frank Bungartz, who has lived, collected and studied lichens in Galapagos for several years.

Remarks. This is species is a rather typical *Dictyonema*, with wavy fungal sheath, mainly uniseriate cyanobacterial cells and without any very distinctive characteristic. The easiest way to recognize this species is by observing the fibrils, mostly erect and individual, much denser than *D. subobscuratum*, which also differs by the usually muriform aspect of the photobiont cells. *D. bungartziana* do not have net-like or combed fibrils, such as *D. ramificans* and *D. pectinatum*, respectively. This species also does not form bracket-like thalli, as it is characteristic of *D. barbatum.*

Additional material examined (28): Ecuador: Galapagos: — Pinta Island (1), on top of the highest point of the island, alt. 625 m, on plant debris and bryophytes, 26-Feb-2007, *F. Bungartz 5746* (CDS 33400, GMUF). −Santa Cruz Island (16), along the trail to El Puntudo, alt. 698 m, on bryophytes (*Frullania aculeate*) growing epiphytically on *Cinchona pubescens* branches, 28-Dec-2005, *F. Bungartz 3276* (CDS 26918, GMUF); alt. 469 m, growing on *Frullania* sp., 23-Jun-2010, *M. Dal- Forno 1177* (CDS 44711, GMUF); alt. 502 m, on bryophytes, 23-Jun-2010, *M. Dal-Forno 1182* (CDS 44717, GMUF), *M. Dal-Forno. 1183* (CDS 44718, GMUF), *M. Dal-Forno 1191* (CDS 44724, GMUF), *M. Dal-Forno 1211* (CDS 44735, GMUF); alt. 684 m, *A.A. Spielmann 8249* (CDS 44757, GMUF); along the road from Bellavista to Los Gemelos, alt. 574 m, on bryophytes over bark, 23-Jun-2010, *M. Dal-Forno 1171* (CDS 44706, GMUF), *M. Dal-Forno 1174* (CDS 44709, GMUF), *M. Dal-Forno* 1178 (CDS 44712, GMUF), *M. Dal-Forno 1179* (CDS 44713, GMUF); between El Puntudo and Cerro Crocker, alt. 760 m, growing on *Frullania* sp. on Cinchona trunk, 28-Oct-2010, *A. Yánez 1541* (CDS 45035, GMUF); N of Bellavista, alt. 555 m, *Miconia* shrubland, growing on *Frullania* sp. on

branches of *Miconia robinsoniana*, 28-Oct-2010, *A. Yánez 1507* (CDS no. 44998), Steve Divine's Farm at the end of Tortoise Road, alt. 364 m, Feb. 23, 2006, *A. Aptroot 64519* (CDS 31091, GMUF), *F. Bungartz 3956* (CDS 27838, GMUF). — Isabela Island (7), Volcán Alcedo, alt. 1,089 m, on bark of *Tournefortia*, 05-Mar-2006, *A. Aptroot 65037a* (CDS 31619, GMUF); Volcán Sierra Negra, alt. 550 m, growing over hepatics on top of *Psidium guajava* branches, 09-Sep-2007, *F. Bungartz 6883* (CDS 36362, GMUF); alt. 980 m, on bryophytes on branches of *Psidium guajava*, 14-Aug-2008, *M.A. Herrera-Campos 10560* (CDS 40297, GMUF); alt. 1,055 m, growing over dead plant material and *Lycopodium* and fern stems near the ground; 16-Aug-2008, *F. Bungartz 8350* (CDS 40996, GMUF); Volcán Cerro Azul, 917 m, on basaltic rock, 7-May-2012, *F. Nugra 1096* (CDS 52259, GMUF), alt. 767 m, on soil, May 3, 2012, *F. Nugra 1051* (CDS 52215, GMUF), alt. 902 m, on bryophytes on fern stems, 7-May-2012, *A.A. Spielmann 10621* (CDS 51988, GMUF). — Floreana Island (4), on top of Cerro Asilo de la Paz, permanent plot 25, alt. 531 m, on bryophytes, Jan. 13, 2011, *A. Yánez-Ayabaca 1828* (CDS 46566, GMUF), *A. Yánez-Ayabaca 1842* (CDS 46565, GMUF), *F. Bungartz 9476* (CDS 46557, GMUF); lava flow behind beach, alt. 4 m, Jan. 19, 2011, on bryophytes, *A. Yánez-Ayabaca 2041* (GMUF).

Dictyonema pectinatum Dal Forno, Yánez-Ayabaca & Lücking, Fungal Diversity 52: 234 (2012)

Figure 26

Type: Ecuador: Galapagos: Santa Cruz Island, along trail from Bellavista to El Puntudo, behind parking lot, 0°40′48″ S, 90°19′26″ W, alt. 469 m, secondary forest of *Cinchona pubescens* and *Psidium guajava*, on bark of *Psidium guajava*, 23-Jun-2010, *M. Dal-Forno 1170* (CDS 44705, holotype; GMUF, isotype).

Description. Thallus epiphytic on tree trunks and branches, slightly appressed filamentous, in irregular, confluent patches, up to 15 cm across and entire thallus covering larger areas of the substrate, forming a mat of horizontally arranged, as if "combed," loosely interwoven, dark green fibrils sitting on top of a white indistinct hypothallus, sometimes extending to form a discrete prothallus. Fibrils that do not get overly long to form the characteristic "combed" aspect of the thallus, are of arachnoid appearance, but still show principles of elongated fibrils. Thallus with a thick photobiont layer and a thin medulla; photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells; cyanobacterial filaments composed of 16–21 μm wide and 5–9 μm high, blue green cells penetrated by tubular fungal hyphae; heterocytes sparse to frequent, yellow, 12–13 μm wide and 2–3 μm high; cells of hyphal sheath wavy in lateral outline, 5–9 μm thick; hyphae associated with hyphal sheath straight, 4–6 μm thick, lacking clamp connections.

Hymenophore not observed.

Distribution and ecology. This is the only *Dictyonema* in Galapagos growing directly on bark, as previously mentioned by Yánez et al. (2012); all specimens from the islands grow on the introduced *Psidium guajava*, the common guava. Its known distribution for the Galapagos Islands is from a small population in Santa Cruz, but we

can now also expand the distribution of the species to Ecuador, where two specimens where collected in Mindo, a high altitude humid zone in the Pinchincha Province.

Remarks. The appearance of the species with the parallel-horizontal long arrangement of the fibrils is quite unique within the genus. Beyond that, most of the fibrils also have lighter tips and the fungal sheath has "papillae," a very discernible characteristic. These papillae might be the natural wavy outline of the fungal sheath with protuberances.

We thought we saw hymenophores in one of the specimens (M. Dal-Forno 1221); however, the structures resembling resupinate patches of hymenophore turned out to be an old decomposing specimen of a chlorolichen, evidenced by the many green algae around the hyphae in cross section.

The ITS sequences from Galapagos and mainland Ecuador differ in seven base pairs, and among these is an insertion of cytosine in the mainland specimens.

Additional material examined (3): Ecuador — Galapagos, Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park, 0°39′56.8″ S, 98°19′31.4″ W, alt. 502 m, *Miconia robinsoniana* shrubland, on bark of *Psidium guajava*, 23-Jun-2010, *M. Dal-Forno 1221* (CDS 44744, GMUF). — Pinchincha, Andes, Cantón San Miguel de Los Bancos, Mindo, Yellow House Trail, alt. 1,279 m, 3-Jul-2012, *M. Dal-Forno 1981c* (GMUF), *M. Dal-Forno 1986* (GMUF).

Dictyonema ramificans Dal-Forno, Yánez & Lücking sp. nov.

Figure 27

Type: Santa Cruz Island; along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park 0°39′56″ S, 98°19′31″ W, alt. 502 m, *Miconia robinsoniana* shrubland, on bryophyte, growing over *Frullania* sp., 23-Jun-2010, *M. Dal-Forno 1214* (CDS 44738, holotype; GMUF, isotype).

Description. Thallus epiphytic on bryophytes, but also on leaves, filamentous, in irregular, confluent patches, each up to 10 cm across, forming a dense mat of more of less horizontal but also vertical, loosely interwoven, dark green fibrils, sitting on top of a white hypothallus extending to form a prothallus. Fibrils forming an arachnoid pattern, formed by connecting fibrils in a more or less erect structure. Thallus organized in photobiont layer and medulla (hypothallus), photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath; cyanobacterial filaments uni- or biseriate, composed of up to 15 μm wide and up to 10 μm high, thinner towards the tips, bluish green cells, often longitudinally divided, penetrated by tubular fungal hyphae; heterocytes sparse to frequent, mostly central, rarely lateral, pale yellow, 10–15 μm wide and 5–10 μm high; cells of hyphal sheath highly variable in shape and size, angular to wavy in lateral outline, highly ornamented and sinuous towards the tips of the fibrils, up to 15 μm thick, ramification present and frequent, connecting different fibrils; hyphae associated with hyphal sheath straight, 5–7 μm thick, lacking clamp connections.

Hymenophore developed as bulging, stereoid, resupinate patches, 0.1–1 (–2) mm broad and up to 6 mm long, with white (when fresh) to slightly yellowish white (in

herbarium), smooth surface; hymenophore in section 62–75 μm thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of scattered, palisade-like basidioles and basidia; basidioles 15–20 × 4–6 μm; basidia 20–25 × 5–7 μm, basidiospores ellipsoid to narrowly drop-shaped, non-septate, hyaline, 7–8 × 2–4 μm.

Distribution and ecology. All specimens of this species grow on bryophytes, so far only found in the humid zone of Santa Cruz.

Etymology. This epithet refers to the branching pattern of the fibrils.

Remarks. This species is characterized by the formation of an arachnoid pattern on top of a white hypothallus; this aspect is due to the fibrils with ramification. Cyanobacterial cells are organized inside the fungal sheath as a single chain; however, fungal sheath branches and eventually forms a second chain. The fungal sheath hyphae is overall ornamented (sinuous and "papillae"-like), but has this ornamentation especially prominent in the tips.

Additional material examined (5): Ecuador, Galapagos, Santa Cruz Island, path from Media Luna to El Puntudo, alt. 684 m, on *Frullania* sp. on branch of *Cinchona pubescens*, 28-Oct-2010, *A. Yánez 1517* (CDS 45008, GMUF), *A. Yánez 1518* (CDS 45009, GMUF), *A. Yánez 1521* (CDS 45012, GMUF), alt. 684 m, on bryophyte on trunk of *Cinchona pubescens*, 28-Oct-2010, *A. Yánez 1534* (CDS 45027, GMUF), alt. 762 m, on *Frullania* sp. on the ground, 28-Oct-2010, *A. Yánez 1539* (CDS 45032, GMUF).

Dictyonema subobscuratum Dal-Forno, Bungartz & Lücking sp. nov.

Figure 28

Type: Ecuador, Imbabura, Andes, Cantón Cotacachi, near Intag, alt. 2,053 m, 26-Jun-2012, *M. Dal-Forno 1803* (GMUF).

Description. Thallus epiphytic on mostly bryophytes, but also on branches, filamentous, in irregular, confluent patches, each up to 5 cm across, forming a mat of more of less irregular, loosely interwoven, mostly individual, not dense, dark blue green to dark green fibrils, sitting on top of a white hypothallus forming sometimes a discrete prothallus. Thallus organized in photobiont layer and medulla (hypothallus), photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath; cyanobacterial filaments composed of up to 24 μm wide and up to 10 μm high, green bluish cells, often longitudinally divided, penetrated by tubular fungal hyphae; heterocytes frequent, central or lateral, pale yellow, 6–17 μm wide and 5–10 μm high; cells of hyphal sheath highly variable in shape and size, mostly angular and straight in lateral outline, not wavy typical of *Dictyonema*, 5–20 μm thick; hyphae associated with hyphal sheath straight, 5–7 μm thick but also angular, similar to the hyphal sheath, forming almost a connecting tissue between fibrils, lacking clamp connections.

Hymenophore developed as bulging, stereoid patches, patches up to 1 mm broad and 3.5 mm long, with white (when fresh) to yellowish white (in herbarium), smooth surface; hymenophore in section 75–100 μm thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of scattered, palisade-like basidioles; basidioles 20–30 × 5–7 μm; basidia and basidiospores not observed.

Dictyonema subobscuratum* subsp. *galapagoensis Dal-Forno, Bungartz & Lücking subsp. nov.

Type: Galapagos, Floreana Island; along rim trail of Cerro Pajas, eastern part of rim, 1°17'44.8" S 90°27'15.6" W, alt. 442 m, humid zone, low dense vegetation of *Zanthoxylum fagara*, *Scalesia pedunculata*, *Macraea laricifolia*, *Croton scouleri* and ferns (*Polypodium tridens*) over lava boulders; bryophytes hanging in curtains from trees, growing over hepatics (*Frullania* sp.) hanging from *Zanthoxylum* twigs; semi-shaded, wind- and rain-exposed, on bryophytes, 26-Jan-2011, *F. Bungartz 9549* (CDS 46559, holotype; GMUF, isotype).

Morphologically identical to *Dictyonema subobscuratum subobscuratum*, but genetically different and geographically distributed in the Galapagos Islands.

Distribution and ecology. The species grow mostly on bryophytes over branches and trunks in humid zones of Santa Cruz and Floreana in the Galapagos Islands. The type of the species was collected in mainland Ecuador, also in a high altitude humid zone.

Etymology. The epithet refers to the similarity to another species described recently, *Dictyonema obscuratum*.

Remarks. Widened tips or other areas of the fibril are common in this species, with a muriform aspect also, similar to *Dictyonema obscuratum* from Brazil, mostly on tips. This species also has its fungal sheath with more angular cells, without the typical jigsaw puzzle aspect, characteristic of *Dictyonema*; however, the cells are still quite wide. This is the species observed by us to have more heterocyst, that is, the highest frequency

(one every 3–7 cyanobacterial cells). One specimen has many of what seems to be parasites, with many cyanobacterial cells completely brown and dead (*A. Yánez 2058*).

Additional material examined (6): Ecuador, Galapagos, —Floreana Island (4), along rim trail of Cerro Pajas, alt. 504 m, on bryophytes, 26-Jan-2011, *F. Bungartz 9550* (CDS 46560, GMUF), *F. Bungartz 9551* (CDS 46561, GMUF), *F. Bungartz 9552* (CDS 46560, GMUF); at SE-base of Cerro de los Suspiros, permanent plot 22, alt. 342 m, 22-Jan-2011, *A. Yánez 2058* (CDS 48407, GMUF). —Santa Cruz Island (1), along trail from Bellavista to El Puntudo, growing over *Frullania* sp., 23-Jun-2010, *M. Dal-Forno 1181* (CDS 44716, GMUF).

Figure 11 - Map of the Galapagos Islands showing the islands where focused collecting occurred

Table 6 - Specimens from Galapagos used in this chapter, including the island they came from

Species	Control#	Collector	Island	ITS	LSU
Acantholichen galapagoensis	DIC064	F. Bungartz 5593	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF057	M. Dal-Forno 1204	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF058	M. Dal-Forno 1205*	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF089	A. Aptroot 64679	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF090	F. Bungartz 4125	Galapagos, ISABELA	1	1
Acantholichen galapagoensis	MDF091	A. Aptroot 65187	Galapagos, ISABELA	1	0
Acantholichen galapagoensis	MDF092	A. Aptroot 65554	Galapagos, SANTIAGO	1	0
Acantholichen galapagoensis	MDF093	F. Nugra 400	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF094	F. Nugra 379	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF100	F. Bungartz 8152	Galapagos, SANTA CRUZ	1	1
Acantholichen galapagoensis	MDF101	F. Bungartz 8577	Galapagos, SAN CRISTÓBAL	1	0
Cora galapagoensis	DIC343	A. Aptroot 65557	Galapagos, SANTIAGO	1	1
Cora galapagoensis	DIC345	F. Bungartz 4831	Galapagos, SANTIAGO	1	1
Cora galapagoensis	MDF033a	M. Dal-Forno 1180a	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF040a	M. Dal-Forno 1187a	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF045	M. Dal-Forno 1192	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF049	M. Dal-Forno 1196	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF052	M. Dal-Forno 1199a	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF059	M. Dal-Forno 1206	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF068	M. Dal-Forno 1218	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF073	M. Dal-Forno 1223*	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF124	A. Yánez 1509	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF139	A. Yánez 1508	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF140	A. Yánez 1513	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF142	A. Yánez 1538	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF143	A. Yánez 1540	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF147	F. Nugra 437	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF148	F. Bungartz 3322	Galapagos, SANTA CRUZ	1	0
Cora galapagoensis	MDF149	M. Herrera-Campos 10546	Galapagos, ISABELA	1	0
Cora galapagoensis	MDF405	F. Nugra 1034	Galapagos, ISABELA	1	0
Cora galapagoensis	MDF406	F. Nugra 1098	Galapagos, ISABELA	1	0
Cora galapagoensis	MDF407	F. Bungartz 10325	Galapagos, ISABELA	1	0
Cora santacruzensis	DIC348	F. Bungartz 5594	Galapagos, SANTA CRUZ	1	1
Cora santacruzensis	MDF144	A. Yánez 1547*	Galapagos, SANTA CRUZ	1	0
Cyphellostereum floreanum	MDF176	F. Bungartz 9475*	Galapagos, FLOREANA	1	1
Cyphellostereum galapagoense	MDF120	F. Bungartz 8517*	Galapagos, SAN CRISTÓBAL	1	1
Cyphellostereum galapagoense	MDF126	A. Yánez 1545	Galapagos, SANTA CRUZ	1	0
Dictyonema barbatum	DIC341	F. Bungartz 8363*	Galapagos, ISABELA	1	1
Dictyonema barbatum	DIC342	F. Bungartz 6852	Galapagos, ISABELA	1	1
Dictyonema barbatum	DIC344	F. Bungartz 8576	Galapagos, SAN CRISTÓBAL	1	1
Dictyonema barbatum	DIC346	A. Aptroot 65186	Galapagos, ISABELA	1	1
Dictyonema barbatum	DIC349	C. Truong 1275	Galapagos, ISABELA	1	1
Dictyonema barbatum	DIC350	F. Bungartz 6906	Galapagos, ISABELA	1	1
Dictyonema barbatum	MDF131	C. Truong 1259	Galapagos, ISABELA	1	0
Dictyonema barbatum	MDF132	C. Truong 1533	Galapagos, SAN CRISTÓBAL	1	0
Dictyonema barbatum	MDF133	A. Aptroot 63148	Galapagos, SANTA CRUZ	1	0
Dictyonema barbatum	MDF136	F. Bungartz 6849	Galapagos, ISABELA	1	0
Dictyonema barbatum	MDF138	A. Yánez 1550	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	DIC347	M. Herrera-Campos 10560	Galapagos, ISABELA	1	0
Dictyonema bungartziana	MDF026	M. Dal-Forno 1171	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF028	M. Dal-Forno 1174	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF030	M. Dal-Forno 1177	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF031	M. Dal-Forno 1178	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF032	M. Dal-Forno 1179	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF035	M. Dal-Forno 1182	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF044	M. Dal-Forno 1191	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF062	M. Dal-Forno 1209*	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF064	M. Dal-Forno 1211	Galapagos, SANTA CRUZ	1	1
Dictyonema bungartziana	MDF076	A. A. Spielmann 8249	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF086	M. Dal-Forno 1183	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF156	A. Yánez 1828	Galapagos, FLOREANA	1	0
Dictyonema bungartziana	MDF157	A. Yánez 1842	Galapagos, FLOREANA	1	0

Dictyonema bungartziana	MDF159	A. Yánez 2041	Galapagos, FLOREANA	1	0
Dictyonema bungartziana	MDF168	A. Aptroot 64519	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF169	A. Aptroot 65037a	Galapagos, ISABELA	1	0
Dictyonema bungartziana	MDF171	F. Bungartz 3276	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF172	F. Bungartz 3956	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF173	F. Bungartz 5746	Galapagos, PINTA	1	0
Dictyonema bungartziana	MDF174	F. Bungartz 6883	Galapagos, ISABELA	1	0
Dictyonema bungartziana	MDF175	F. Bungartz 8350	Galapagos, ISABELA	1	0
Dictyonema bungartziana	MDF177	F. Bungartz 9476	Galapagos, FLOREANA	1	0
Dictyonema bungartziana	MDF184	A. Yánez 1507	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF195	A. Yánez 1541	Galapagos, SANTA CRUZ	1	0
Dictyonema bungartziana	MDF409	F. Nugra 1051	Galapagos, ISABELA	1	0
Dictyonema bungartziana	MDF410	A. A. Spielmann 10621	Galapagos, ISABELA	1	1
Dictyonema bungartziana	MDF411	F. Nugra 1096	Galapagos, ISABELA	1	0
Dictyonema pectinatum	MDF025	M. Dal-Forno 1170*	Galapagos, SANTA CRUZ	1	1
Dictyonema pectinatum	MDF071	M. Dal-Forno 1221	Galapagos, SANTA CRUZ	1	1
Dictyonema ramificans	MDF066	M.Dal-Forno1214*	Galapagos, SANTA CRUZ	1	1
Dictyonema ramificans	MDF187	A. Yánez 1517	Galapagos, SANTA CRUZ	1	0
Dictyonema ramificans	MDF188	A. Yánez 1518	Galapagos, SANTA CRUZ	1	0
Dictyonema ramificans	MDF190	A. Yánez 1521	Galapagos, SANTA CRUZ	1	0
Dictyonema ramificans	MDF193	A. Yánez 1534	Galapagos, SANTA CRUZ	1	1
Dictyonema ramificans	MDF194	A. Yánez 1539	Galapagos, SANTA CRUZ	1	0
Dictyonema subobscuratum	MDF034	M. Dal-Forno 1181	Galapagos, SANTA CRUZ	1	0
Dictyonema subobscuratum	MDF179	F. Bungartz 9549*	Galapagos, FLOREANA	1	1
Dictyonema subobscuratum	MDF180	F. Bungartz 9550	Galapagos, FLOREANA	1	0
Dictyonema subobscuratum	MDF181	F. Bungartz 9551	Galapagos, FLOREANA	1	0
Dictyonema subobscuratum	MDF182	F. Bungartz 9552	Galapagos, FLOREANA	1	0
Dictyonema subobscuratum	MDF196	A. Yánez 2058	Galapagos, FLOREANA	1	1
			Total=	90	27

Table 7 - Specimens included in Subset 1
Country abbreviation key available in Table 8

Control #	Genus	epithet	Country*	Collector	Collector #	GenBank # ITS	GenBank # nuLSU
DIC050	*Cyphellostereum*	*nitidum*	BOLI	RivasPlata	1130	--	EU825970
DIC051	*Cora*	*squamiformis*	BOLI	Luecking	23500	--	EU825969
DIC052	*Cora*	*spec*	BOLI	Luecking	23564	--	EU825964
DIC053	*Dictyonema*	*aeruginosulum*	CORI	Nelsen	3754	EU825955	EU825955
DIC054	*Dictyonema*	*aeruginosulum*	CORI	WillWolf	12733	--	EU825954
DIC055	*Dictyonema*	*schenkianum*	CORI	Luecking	15340	EU825978	EU825978
DIC056	*Cora*	*byssoidea*	CORI	Luecking	15581	EU825958	EU825958
DIC057	*Dictyonema*	*sericeum4*	CORI	Luecking	16561	EU825975	EU825975
DIC058	*Cora*	*spec*	CORI	Luecking	16563	EU825956	EU825956
DIC059	*Dictyonema*	*subschenkianum*	CORI	Luecking	17200	EU825972	EU825972
DIC060	*Acantholichen*	*sorediatus*	CORI	Sipman	48329	--	EU825952.2
DIC061	*Cyphellostereum*	*phyllogenum*	CORI	Luecking	15207a	--	EU825971
DIC062	*Dictyonema*	*hernandezii*	CORI	Luecking	15243a	EU825966	EU825966
DIC063	*Cora*	*minor*	CORI	unkn	isotype	EU825968.2	EU825968
DIC064	*Acantholichen*	*galapagoensis*	GALA	Bungartz	5593	EU825953.2	EU825953.2
DIC065	*Dictyonema*	*interruptum*	MADE	unkn	10475	--	EU825967
DIC066	*Cora*	*arachnoidea*	MEXI	Luecking	17538	--	EU825957
DIC100	*Dictyonema*	*sericeum1*	PERU	unkn	sn	NEW	NEW
DIC104	*Cora*	*aspera*	PERU	unkn	sn	KJ802408	NEW
DIC105	*Cora*	*strigosa*	PERU	unkn	sn	KJ802410	NEW
DIC106	*Cora*	*glabrata3*	PERU	unkn	sn	KJ780364	NEW
DIC107	*Cora*	*strigosa*	PERU	Paz	3	KF443241	KF443274
DIC108	*Cora*	*squamiformis*	PERU	unkn	sn	KJ780365.2	NEW
DIC109	*Cora*	*boliviensis*	BOLI	Luecking	29363	KJ780367	NEW
DIC110	*Cora*	*aspera*	BOLI	Luecking	29128	KF443230	KF443267
DIC111	*Cora*	*glabrata*	BOLI	Luecking	29364	KJ780368	NEW
DIC112	*Cora*	*glabrata*	BOLI	Luecking	29356	KJ780369	NEW
DIC113	*Dictyonema*	*schenkianum*	BRAZ	Luecking	30062	KF443225	KF443251

DIC114	*Dictyonema*	*thelephora*	BRAZ	Luecking	30060	NEW	NEW
DIC115	*Cyphellostereum*	*imperfectum*	GUAT	Luecking	25588	KF443218	KF443243
DIC116	*Dictyonema*	*spongiosum*	GUAT	Luecking	25551b	KF443228	KF443254
DIC118	*Dictyonema*	*metallicum*	ECUA	Luecking	26203	--	NEW
DIC119	*Cora*	*subreticulifera*	ECUA	Luecking	26201	KF443239	KF443269
DIC120	*Dictyonema*	*spongiosum*	ECUA	Luecking	26200	--	NEW
DIC121	*Dictyonema*	*spongiosum*	GUAT	Luecking	25561	NEW	NEW
DIC122	*Dictyonema*	*immersum*	ECUA	Luecking	26258	KF443221	KF443246
DIC123	*Dictyonema*	*metallicum*	ECUA	Luecking	26255	KF443222	KF443248
DIC125	*Dictyonema*	*irpicinum*	FIJI	Lumbsch	19837e	NEW	KF443247
DIC126	*Dictyonema*	*obscuratum*	BRAZ	Luecking	23025	KF443223	KF443249
DIC127	*Cyphellostereum*	*membranaceoides*	PHIL	RivasPlata	2138	NEW	NEW
DIC128	*Cyphellostereum*	*membranaceum*	PHIL	RivasPlata	2129	--	NEW
DIC129	*Dictyonema*	*irpicinellum*	PHIL	RivasPlata	2143	NEW	NEW
DIC130a	*Cyphellostereum*	*membranaceum*	PHIL	RivasPlata	2183	NEW	KF443245
DIC131	*Dictyonema*	*obscuratum*	BRAZ	Luecking	23041	NEW	NEW
DIC132	*Dictyonema*	*obscuratum*	BRAZ	Luecking	23204	NEW	NEW
DIC133	*Dictyonema*	*sericeum2*	BOLI	Wilk	9327	NEW	NEW
DIC135	*Cora*	*squamiformis*	BOLI	unkn	2607	KJ780370	NEW
DIC136	*Dictyonema*	*discocarpum*	BOLI	unkn	4788	KF443227	KF443253
DIC138	*Dictyonema*	*hapteriferum*	BOLI	Wilk	8868	KF443226	KF443252
DIC140	*Cora*	*subarachnoidea*	BOLI	Luecking	2780a	KJ802420	NEW
DIC141	*Cora*	*aspera*	BOLI	Luecking	2780b	KF443231	NEW
DIC144	*Cora*	*squamiformis*	BOLI	Wilk	7434	KJ780371	NEW
DIC145	*Cora*	*squamiformis*	BOLI	Wilk	7562	KJ780372	NEW
DIC146	*Cora*	*squamiformis*	BOLI	Wilk	7577	KF443240	KF443273
DIC147	*Cora*	*squamiformis*	BOLI	Wilk	7587	KJ802423	NEW
DIC148	*Cora*	*squamiformis*	BOLI	Wilk	7446	KJ780373	NEW
DIC149	*Cora*	*inversa*	COLO	Luecking	sn	KF443236	KF443260
DIC150	*Cora*	*inversa*	COLO	Luecking	sn	KJ780374	NEW
DIC151	*Cora*	*byssoidea*	COLO	Luecking	25901	KF443234	KF443268
DIC152	*Cora*	*hirsuta*	COLO	Luecking	sn	KF443235	KF443270
DIC154	*Cora*	*inversa*	COLO	Luecking	sn	KF443237	NEW
DIC156	*Dictyonema*	*phyllophilum*	FIJI	Lumbsch	19812	KF443224	KF443250
DIC157	*Cyphellostereum*	*membranaceum*	FIJI	Lumbsch	19811b	--	NEW
DIC158	*Cyphellostereum*	*phyllogenum*	FIJI	Lumbsch	sn	KF443219	KF443244
DIC200	*Cora*	*squamiformis*	ECUA	Luecking	sn	KJ780376	NEW
DIC201	*Cora*	*squamiformis*	ECUA	Luecking	sn	KJ780377	NEW
DIC202	*Cora*	*squamiformis*	ECUA	Luecking	sn	KJ780378	NEW
DIC203	*Cora*	*squamiformis*	ECUA	Luecking	sn	KJ780379	NEW
DIC204	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780380	NEW
DIC205	*Cora*	*sp*	ECUA	Luecking	sn	KJ780381	NEW
DIC209	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780382	NEW
DIC210	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780383	NEW
DIC213	*Cora*	*undulata*	ECUA	Luecking	sn	NEW	NEW
DIC214	*Cora*	*undulata*	ECUA	Luecking	sn	NEW	NEW
DIC215	*Cora*	*undulata*	ECUA	Luecking	sn	KF443238	KF443275
DIC216	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780384	NEW
DIC217	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780385	NEW
DIC218	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780386	NEW
DIC219	*Cora*	*undulata*	ECUA	Luecking	sn	KJ780387	NEW
DIC234	*Cora*	*undulata*	ECUA	Paredes	653	KJ780389	NEW
DIC236	*Cora*	*undulata*	ECUA	Ceron	36059	KJ780390	NEW
DIC237	*Cora*	*squamiformis*	ECUA	Paredes	62	KJ780391	NEW
DIC238	*Cora*	*sp*	ECUA	Ceron	38530	KJ780392	NEW
DIC241	*Cora*	*undulata*	ECUA	Paredes	41	KJ780393	NEW
DIC250	*Cora*	*sp*	ECUA	Nugra	867	KJ780394	NEW
DIC251	*Cora*	*sp*	ECUA	Nugra	866	KJ780395	NEW
DIC252	*Cora*	*sp*	ECUA	Nugra	865	KJ780396	NEW
DIC253	*Cora*	*sp*	ECUA	Nugra	864	KJ780397	NEW
DIC254	*Cora*	*sp*	ECUA	Nugra	863	KJ780398	NEW
DIC255	*Cora*	*sp*	ECUA	Nugra	862	KJ780399	NEW
DIC256	*Cora*	*sp*	ECUA	Nugra	818	KJ780400	NEW
DIC263	*Dictyonema*	*sp*	BRAZ	Luecking	31306	NEW	NEW
DIC265	*Corella*	*brasiliensis*	BRAZ	Luecking	31330	--	NEW
DIC266	*Cora*	*sp*	BRAZ	Luecking	31351a	KJ780401	NEW

DIC268	*Cora*	*sp*	BRAZ	Luecking	31353	--	NEW
DIC277	*Cora*	*sorediata*	VENE	Hernandez	1777	--	NEW
DIC278	*Cora*	*undulata*	VENE	Hernandez	1778	KJ780402	NEW
DIC279	*Cora*	*arachnoidea*	VENE	Hernandez	1779	KF443232	KF443266
DIC280	*Cora*	*arachnoidea*	VENE	Hernandez	1780	KF443233	NEW
DIC282	*Cora*	*arachnoidea*	VENE	Hernandez	1782	KJ780403	NEW
DIC283	*Cora*	*hirsuta*	VENE	Hernandez	1783	KJ780404	NEW
DIC300	*Cora*	*arachnoidea*	COLO	Luecking	32700	KJ780405	NEW
DIC301	*Cora*	*arachnoidea*	COLO	Luecking	32701	--	NEW
DIC302	*Cora*	*sp*	COLO	Luecking	32702	KJ780406	NEW
DIC303	*Cora*	*sp*	COLO	Luecking	32703	KJ780407	NEW
DIC304	*Cora*	*sp*	COLO	Luecking	32704	KJ780408	NEW
DIC305	*Cora*	*sp*	COLO	Luecking	32705	KJ780409	NEW
DIC307	*Cora*	*sp*	COLO	Luecking	32707	KJ780410	NEW
DIC308	*Cora*	*sp*	COLO	Luecking	32708	KJ780411	NEW
DIC309	*Cora*	*sp*	COLO	Luecking	32709	--	NEW
DIC310	*Cora*	*sp*	COLO	Luecking	32710	KJ780412	NEW
DIC311	*Cora*	*sp*	COLO	Luecking	32711	KJ780413	NEW
DIC312	*Cora*	*sp*	COLO	Luecking	32712	KJ780414	NEW
DIC313	*Cora*	*sp*	COLO	Luecking	32713	KJ780415	NEW
DIC314	*Cora*	*sp*	COLO	Luecking	32714	KJ780416	NEW
DIC315	*Cora*	*sp*	COLO	Luecking	32715	KJ780417	NEW
DIC316	*Cora*	*sp*	COLO	Luecking	32716	KJ780418	NEW
DIC317	*Cora*	*sp*	COLO	Luecking	32717	KJ780419	NEW
DIC318	*Cora*	*sp*	COLO	Luecking	32718	KJ780420	NEW
DIC319	*Cora*	*sp*	COLO	Luecking	32719	KJ780421	NEW
DIC320	*Cora*	*sp*	COLO	Luecking	32720	KJ780422	NEW
DIC321	*Cora*	*sp*	COLO	Luecking	32721	KJ780423	NEW
DIC322	*Cora*	*sp*	COLO	Luecking	32722	KJ780424	NEW
DIC323	*Cora*	*sp*	COLO	Luecking	32723	KJ780425	NEW
DIC324	*Cora*	*sp*	COLO	Luecking	32724	KJ780426	NEW
DIC330	*Dictyonema*	*sp*	CORI	Luecking	17252i	NEW	NEW
DIC331	*Dictyonema*	*aeruginosulum*	CORI	Trest	1569	NEW	NEW
DIC333	*Dictyonema*	*phyllogenum*	CORI	Luecking	17013	NEW	NEW
DIC334	*Dictyonema*	*Luecking*	CORI	Luecking	15327	NEW	NEW
DIC335	*Acantholichen*	*sorediatus*	CORI	Luecking	sn	Chapter 5	Chapter 5
DIC336	*Dictyonema*	*sp*	CORI	Amtoft	3095	NEW	NEW
DIC337	*Dictyonema*	*Luecking*	CORI	Luecking	18008	NEW	NEW
DIC338	*Dictyonema*	*Luecking*	CORI	Luecking	15353	NEW	NEW
DIC339	*Dictyonema*	*Luecking*	CORI	Luecking	18053	NEW	NEW
DIC341	*Dictyonema*	*barbatum*	GALA	Bungartz	8363	NEW	NEW
DIC342	*Dictyonema*	*barbatum*	GALA	Bungartz	6852	NEW	NEW
DIC343	*Cora*	*galapagoensis*	GALA	Aptroot	65557	NEW	NEW
DIC344	*Dictyonema*	*barbatum*	GALA	Bungartz	8576	NEW	NEW
DIC345	*Cora*	*galapagoensis*	GALA	Bungartz	4831	NEW	NEW
DIC346	*Dictyonema*	*barbatum*	GALA	Aptroot	65186	NEW	NEW
DIC348	*Cora*	*santacruzensis*	GALA	Bungartz	5594	NEW	NEW
DIC349	*Dictyonema*	*barbatum*	GALA	Truong	1275	NEW	NEW
DIC350	*Dictyonema*	*barbatum*	GALA	Bungartz	6906	NEW	NEW
DIC522	*Cora*	*sp*	COLO	Luecking	33347	NEW	NEW
DIC595b	*Acantholichen*	*campestris*	BRAZ	Spielmann	10243b	Chapter 5	Chapter 5
MDF017	*Corella*	*brasiliensis*	BRAZ	DalForno	1271	KF443229	KF443255
MDF018	*Corella*	*sp*	BRAZ	DalForno	1272	NEW	NEW
MDF019	*Corella*	*brasiliensis*	BRAZ	DalForno	1280	KJ802432	NEW
MDF020	*Corella*	*brasiliensis*	BRAZ	DalForno	1281	KJ802435	NEW
MDF021	*Corella*	*brasiliensis*	BRAZ	DalForno	1282	KJ802436	NEW
MDF022	*Corella*	*brasiliensis*	BRAZ	DalForno	1283	KJ802439	NEW
MDF023	*Corella*	*brasiliensis*	BRAZ	DalForno	1284	KJ802441	NEW
MDF024	*Corella*	*brasiliensis*	BRAZ	DalForno	1285	KJ802443	NEW
MDF025	*Dictyonema*	*pectinatum*	GALA	DalForno	1170	NEW	NEW
MDF057	*Acantholichen*	*galapagoensis*	GALA	DalForno	1204	Chapter 5	Chapter 5
MDF058	*Acantholichen*	*galapagoensis*	GALA	DalForno	1205	Chapter 5	Chapter 5
MDF064	*Dictyonema*	*bungartziana*	GALA	DalForno	1211	NEW	NEW
MDF066	*Dictyonema*	*ramificans*	GALA	DalForno	1214	NEW	NEW
MDF071	*Dictyonema*	*pectinatum*	GALA	DalForno	1221	NEW	NEW
MDF089	*Acantholichen*	*galapagoensis*	GALA	Aptroot	64679	Chapter 5	Chapter 5

MDF090	*Acantholichen*	*galapagoensis*	GALA	Bungartz	4125	Chapter 5	Chapter 5
MDF093	*Acantholichen*	*galapagoensis*	GALA	Nugra	400	Chapter 5	Chapter 5
MDF094	*Acantholichen*	*galapagoensis*	GALA	Nugra	379	Chapter 5	Chapter 5
MDF100	*Acantholichen*	*galapagoensis*	GALA	Bungartz	8152	Chapter 5	Chapter 5
MDF120	*Cyphellostereum*	*galapagoense*	GALA	Bungartz	8517	NEW	NEW
MDF176	*Cyphellostereum*	*floreanum*	GALA	Bungartz	9475	NEW	NEW
MDF179	*Dictyonema*	*subobscuratum*	GALA	Bungartz	9549	NEW	NEW
MDF193	*Dictyonema*	*ramificans*	GALA	Yánez	1534	NEW	NEW
MDF196	*Dictyonema*	*subobscuratum*	GALA	Yánez	2058	NEW	NEW
MDF200	*Corella*	*lobulifera*	BRAZ	Eliasaro	5006	KJ780569	NEW
MDF202	*Corella*	*affzahlbruckneri*	BRAZ	Beilke	623	KJ780570	NEW
MDF352	*Acantholichen*	*pannarioides*	CORI	DalForno	1752	Chapter 5	Chapter 5
MDF410	*Dictyonema*	*bungartziana*	GALA	Spielmann	10621	NEW	NEW
MDF543	*Acantholichen*	*albomarginatus*	BRAZ	DalForno	2043	Chapter 5	Chapter 5
MDF643	*Cora*	*itabaiana*	BRAZ	DalForno	2138	NEW	NEW
MDF679	*Acantholichen*	*variabilis*	COLO	Coca	5209	Chapter 5	Chapter 5
OUTGROUP	*Eonema*	*pyriformis*	SWED	Hjm	18581	EU118605	EU118605
R01	*Dictyonema*	*thelephora*	CORI	Luecking	sn	EU825973	EU825973
R02	*Dictyonema*	*thelephora*	CORI	Luecking	sn	EU825974	EU825974
R04	*Cyphellostereum*	*pusiolum*	CORI	Luecking	sn	EU825976	EU825976
R06	*Cora*	*hymenocarpa*	CORI	Luecking	sn	EU825959	EU825959
R07	*Dictyonema*	*schenkianoides*	CORI	Luecking	sn	--	NEW
R08	*Dictyonema*	*hernandezii*	CORI	Luecking	sn	--	EU825965
R10	*Dictyonema*	*hernandezii*	CORI	Luecking	sn	EU825977	EU825977
R11	*Cora*	*asperoides*	CORI	Luecking	21016	EU825960	EU825960
R14	*Dictyonema*	*hernandezii*	CORI	Luecking	sn	--	NEW
R18	*Cora*	*wilsoniorum*	CORI	Luecking	sn	--	EU825961
R19	*Cora*	*arachnoidea*	CORI	Luecking	sn	EU825961	EU825962
R20	*Cora*	*undulata*	CORI	Luecking	sn	EU825963	EU825963

Table 8 - Subset 1, sequences distributed by country

Country	Abbreviation	LSU	ITS
Bolivia	BOLI	18	15
Brazil	BRAZ	22	20
Colombia	COLO	31	29
Costa Rica	CORI	33	26
Ecuador (continental)	ECUA	32	30
Fiji	FIJI	4	3
Galapagos Islands (Ecuador)	GALA	27	27
Guatemala	GUAT	3	3
Madeira (Portugal)	MADE	1	0
Mexico	MEXI	1	0
Peru	PERU	6	6
Philippines	PHIL	4	3
Venezuela	VENE	6	5
	OUTGROUP	1	1
Total = 13		189	168

Table 9 - Subset 2, sequences distributed by country

Country	Abbreviation	ITS
Bolivia	BOLI	14
Brazil	BRAZ	115
Colombia	COLO	53
Costa Rica	CORI	66
Chile	CHILE	10
Ecuador (continental)	ECUA	76
Fiji	FIJI	3
Galapagos Islands (Ecuador)	GALA	90
Guatemala	GUAT	3
Hawaii	HAWA	25
Korea	KOREA	1
Mascarene Islands (France)	MASC	4
Mexico	MEXI	1
New Zealand	NEWZE	1
Peru	PERU	8
Philippines	PHIL	1
Puerto Rico	PUERI	8
United States	USA	4
Venezuela	VENE	6
	OUTGROUP	8
Total = 19		497

Table 10 - Number of ITS sequences per genus (and outgroup)

	ITS
Acantholichen	16
Cora	222
Corella	14
Cyphellostereum	38
Dictyonema	199
Outgroup	8
Total =	497

Table 11 - Summary of information gathered for each marker and combined dataset (concatenated) for Subset 1
The nuLSU and the ITS sequences overlap (see http://sites.biology.duke.edu/fungi/mycolab/primers.htm), and therefore the number of base pairs in the concatenated data set is less than the individual markers combined. Bold text represents phylogenetic trees displayed in this chapter. For all other trees, please see Appendix 10

	ITS	nuLSU	Concatenated
Subset 1 – Guidance Score	0.870305	0.996101	0.962072
Subset 1 – Treatment 1 (full MSA)	**963 bp**	**1472 bp**	**2388 bp**
Subset 1 – Treatment 2 (columns with score below 0.93 removed)	515 bp (448 columns removed)	1436 bp (36 columns removed)	1890 bp (498 columns removed)
Subset 1 – Treatment 3 (columns with score below 1.00 removed)	348 bp (615 columns removed)	1407 bp (65 columns removed)	1504 bp (884 columns removed)
Subset 1 – Total ML trees	3	3	3

Table 12 - Summary of information gathered for the ITS marker for Subset 2
Bold text represents the only phylogenetic trees displayed in this chapter. For all other trees, please see Appendix 10

	ITS
Subset 2 – Guidance Score	0.831294
Subset 2 – **Treatment 1 (full MSA)**	**1208 bp**
Subset 2 – Treatment 2 (columns with score below 0.93 removed)	521 bp
Subset 2 – Treatment 3 (columns with score below 0.90 removed)	553 bp
Subset 2 – Treatment 4 (columns with score below 0.70 removed)	661 bp
Subset 2 – Total ML trees	4

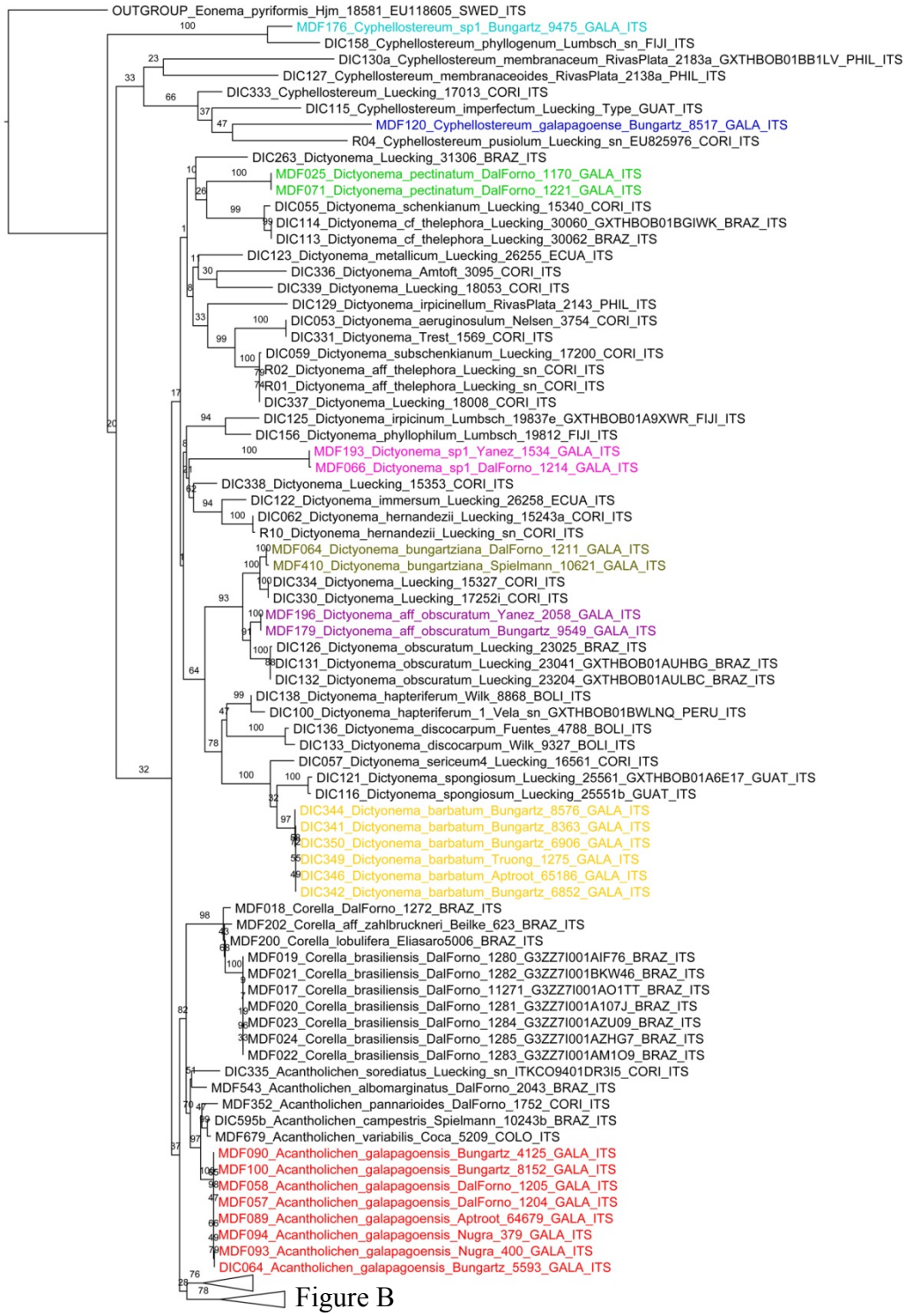

OUTGROUP_Eonema_pyriformis_Hjm_18581_EU118605_SWED_ITS
MDF176_Cyphellostereum_sp1_Bungartz_9475_GALA_ITS
DIC158_Cyphellostereum_phyllogenum_Lumbsch_sn_FIJI_ITS
DIC130a_Cyphellostereum_membranaceum_RivasPlata_2183a_GXTHBOB01BB1LV_PHIL_ITS
DIC127_Cyphellostereum_membranaceoides_RivasPlata_2138a_PHIL_ITS
DIC333_Cyphellostereum_Luecking_17013_CORI_ITS
DIC115_Cyphellostereum_imperfectum_Luecking_Type_GUAT_ITS
MDF120_Cyphellostereum_galapagoense_Bungartz_8517_GALA_ITS
R04_Cyphellostereum_pusiolum_Luecking_sn_EU825976_CORI_ITS
DIC263_Dictyonema_Luecking_31306_BRAZ_ITS
MDF025_Dictyonema_pectinatum_DalForno_1170_GALA_ITS
MDF071_Dictyonema_pectinatum_DalForno_1221_GALA_ITS
DIC055_Dictyonema_schenkianum_Luecking_15340_CORI_ITS
DIC114_Dictyonema_cf_thelephora_Luecking_30060_GXTHBOB01BGIWK_BRAZ_ITS
DIC113_Dictyonema_cf_thelephora_Luecking_30062_BRAZ_ITS
DIC123_Dictyonema_metallicum_Luecking_26255_ECUA_ITS
DIC336_Dictyonema_Amtoft_3095_CORI_ITS
DIC339_Dictyonema_Luecking_18053_CORI_ITS
DIC129_Dictyonema_irpicinellum_RivasPlata_2143_PHIL_ITS
DIC053_Dictyonema_aeruginosulum_Nelsen_3754_CORI_ITS
DIC331_Dictyonema_Trest_1569_CORI_ITS
DIC059_Dictyonema_subschenkianum_Luecking_17200_CORI_ITS
R02_Dictyonema_aff_thelephora_Luecking_sn_CORI_ITS
R01_Dictyonema_aff_thelephora_Luecking_sn_CORI_ITS
DIC337_Dictyonema_Luecking_18008_CORI_ITS
DIC125_Dictyonema_irpicinum_Lumbsch_19837e_GXTHBOB01A9XWR_FIJI_ITS
DIC156_Dictyonema_phyllophilum_Lumbsch_19812_FIJI_ITS
MDF193_Dictyonema_sp1_Yanez_1534_GALA_ITS
MDF066_Dictyonema_sp1_DalForno_1214_GALA_ITS
DIC338_Dictyonema_Luecking_15353_CORI_ITS
DIC122_Dictyonema_immersum_Luecking_26258_ECUA_ITS
DIC062_Dictyonema_hernandezii_Luecking_15243a_CORI_ITS
R10_Dictyonema_hernandezii_Luecking_sn_CORI_ITS
MDF064_Dictyonema_bungartziana_DalForno_1211_GALA_ITS
MDF410_Dictyonema_bungartziana_Spielmann_10621_GALA_ITS
DIC334_Dictyonema_Luecking_15327_CORI_ITS
DIC330_Dictyonema_Luecking_17252i_CORI_ITS
MDF196_Dictyonema_aff_obscuratum_Yanez_2058_GALA_ITS
MDF179_Dictyonema_aff_obscuratum_Bungartz_9549_GALA_ITS
DIC126_Dictyonema_obscuratum_Luecking_23025_BRAZ_ITS
DIC131_Dictyonema_obscuratum_Luecking_23041_GXTHBOB01AUHBG_BRAZ_ITS
DIC132_Dictyonema_obscuratum_Luecking_23204_GXTHBOB01AULBC_BRAZ_ITS
DIC138_Dictyonema_hapteriferum_Wilk_8868_BOLI_ITS
DIC100_Dictyonema_hapteriferum_1_Vela_sn_GXTHBOB01BWLNQ_PERU_ITS
DIC136_Dictyonema_discocarpum_Fuentes_4788_BOLI_ITS
DIC133_Dictyonema_discocarpum_Wilk_9327_BOLI_ITS
DIC057_Dictyonema_sericeum4_Luecking_16561_CORI_ITS
DIC121_Dictyonema_spongiosum_Luecking_25561_GXTHBOB01A6E17_GUAT_ITS
DIC116_Dictyonema_spongiosum_Luecking_25551b_GUAT_ITS
DIC344_Dictyonema_barbatum_Bungartz_8576_GALA_ITS
DIC341_Dictyonema_barbatum_Bungartz_8363_GALA_ITS
DIC350_Dictyonema_barbatum_Bungartz_6906_GALA_ITS
DIC349_Dictyonema_barbatum_Truong_1275_GALA_ITS
DIC346_Dictyonema_barbatum_Aptroot_65186_GALA_ITS
DIC342_Dictyonema_barbatum_Bungartz_6852_GALA_ITS
MDF018_Corella_DalForno_1272_BRAZ_ITS
MDF202_Corella_aff_zahlbruckneri_Beilke_623_BRAZ_ITS
MDF200_Corella_lobulifera_Eliasaro5006_BRAZ_ITS
MDF019_Corella_brasiliensis_DalForno_1280_G3ZZ7I001AIF76_BRAZ_ITS
MDF021_Corella_brasiliensis_DalForno_1282_G3ZZ7I001BKW46_BRAZ_ITS
MDF017_Corella_brasiliensis_DalForno_11271_G3ZZ7I001AO1TT_BRAZ_ITS
MDF020_Corella_brasiliensis_DalForno_1281_G3ZZ7I001A107J_BRAZ_ITS
MDF023_Corella_brasiliensis_DalForno_1284_G3ZZ7I001AZU09_BRAZ_ITS
MDF024_Corella_brasiliensis_DalForno_1285_G3ZZ7I001AZHG7_BRAZ_ITS
MDF022_Corella_brasiliensis_DalForno_1283_G3ZZ7I001AM1O9_BRAZ_ITS
DIC335_Acantholichen_sorediatus_Luecking_sn_ITKCO9401DR3I5_CORI_ITS
MDF543_Acantholichen_albomarginatus_DalForno_2043_BRAZ_ITS
MDF352_Acantholichen_pannarioides_DalForno_1752_CORI_ITS
DIC595b_Acantholichen_campestris_Spielmann_10243b_BRAZ_ITS
MDF679_Acantholichen_variabilis_Coca_5209_COLO_ITS
MDF090_Acantholichen_galapagoensis_Bungartz_4125_GALA_ITS
MDF100_Acantholichen_galapagoensis_Bungartz_8152_GALA_ITS
MDF058_Acantholichen_galapagoensis_DalForno_1205_GALA_ITS
MDF057_Acantholichen_galapagoensis_DalForno_1204_GALA_ITS
MDF089_Acantholichen_galapagoensis_Aptroot_64679_GALA_ITS
MDF094_Acantholichen_galapagoensis_Nugra_379_GALA_ITS
MDF093_Acantholichen_galapagoensis_Nugra_400_GALA_ITS
DIC064_Acantholichen_galapagoensis_Bungartz_5593_GALA_ITS
0.2

Figure B

A

B

Figure 12 - Phylogenetic tree inferred by ML with RAxML for the ITS marker with the Subset 1 Treatment 1


OUTGROUP_Eonema_pyriformis_EU118605_SWED_unkn_18581_LSU
DIC115_Cyphellostereum_imperfectum_GUAT_Luecking_25588_LSU
DIC130a_Cyphellostereum_membranaceum_PHIL_RivasPlata_2183_LSU
DIC127_Cyphellostereum_membranaceoides_PHIL_RivasPlata_2138_LSU
R04_Cyphellostereum_pusiolum_EU825976_CORI_Luecking_LSU
MDF120_Cyphellostereum_galapagoense_Bungartz_8517_GALA_LSU
DIC061_Cyphellostereum_phyllogenum_EU825971_CORI_Luecking_15207a_LSU
DIC158_Cyphellostereum_phyllogenum_FIJI_Lumbsch_sn_LSU
MDF176_Cyphellostereum_Bungartz_9475_GALA_LSU
DIC128_Cyphellostereum_membranaceum_PHIL_RivasPlata_2129_LSU
DIC050_Cyphellostereum_nitidum_EU825970_BOLI_RivasPlata_1130_LSU
DIC157_Cyphellostereum_membranaceum_FIJI_Lumbsch_19811b_LSU
R10_Dictyonema_hernandezii_EU825977_CORI_Luecking_LSU
R08_Dictyonema_hernandezii_EU825965_CORI_Hernandez_holotype_LSU
DIC062_Dictyonema_hernandezii_EU825966_CORI_Luecking_15243a_LSU
R14_Dictyonema_hernandezii_CORI_Hernandez_holotype_LSU
DIC136_Dictyonema_discocarpum_BOLI_unkn_4788_LSU
DIC133_Dictyonema_sericeum2_BOLI_Wilk_9327_LSU
DIC156_Dictyonema_phyllophilum_FIJI_Lumbsch_19812_LSU
DIC125_Dictyonema_irpicinum_FIJI_Lumbsch_19837e_LSU
DIC129_Dictyonema_irpicinellum_PHIL_RivasPlata_2143_LSU
MDF066_Dictyonema_ramificans_DalForno_1214_GALA_LSU
MDF193_Dictyonema_ramificans_Yanez_1534_GALA_LSU
MDF643_Cora_itabaiana_DalForno_2138_BRAZ_LSU
DIC105_Cora_strigosa_PERU_unkn_sn_LSU
DIC107_Cora_strigosa_PERU_unkn_3_LSU
DIC056_Cora_byssoidea_EU825958_CORI_Luecking_15581_LSU
DIC151_Cora_byssoidea_COLO_Luecking_25901_LSU
DIC058_Cora_spec_EU825956_CORI_Luecking_16563_LSU
DIC149_Cora_inversa_COLO_Luecking_sn_LSU
DIC150_Cora_inversa_COLO_Luecking_sn_LSU
DIC154_Cora_inversa_COLO_Luecking_sn_LSU
DIC250_Cora_Nugra_867_ECUA_LSU
DIC205_Cora_ECUA_Luecking_sn_LSU
DIC255_Cora_Nugra_862_ECUA_LSU
DIC110_Cora_subaspera_BOLI_Luecking_29128_LSU
DIC112_Cora_aff_glabrata_BOLI_Luecking_29356_LSU
DIC141_Cora_aspera_BOLI_Luecking_2780b_LSU
DIC052_Cora_spec_EU825964_BOLI_Luecking_23564_LSU
R11_Cora_asperoides_EU825960_CORI_Luecking_LSU
DIC104_Cora_aspera_PERU_unkn_sn_LSU
DIC345_Cora_galapagoensis_Bungartz_4831_GALA_LSU
DIC343_Cora_galapagoensis_GALA_Aptroot_65557_LSU
DIC111_Cora_aff_glabrata_BOLI_Luecking_29364_LSU
DIC348_Cora_santacruzensis_Bungartz_5594_GALA_LSU
DIC266_Cora_Luecking_31351a_BRAZ_LSU
DIC106_Cora_glabrata3_PERU_unkn_sn_LSU
R06_Cora_hymenocarpaEU825959_CORI_Luecking_LSU
DIC253_Cora_Nugra_864_ECUA_LSU
DIC238_Cora_Ceron_38530_ECUA_LSU
DIC119_Cora_subreticulifera_ECUA_Luecking_26201_LSU
DIC254_Cora_Nugra_863_ECUA_LSU
DIC148_Cora_squamiformis_BOLI_Wilk_7446_LSU
DIC237_Cora_squamiformis_Paredes_62_ECUA_LSU
DIC147_Cora_squamiformis_BOLI_Wilk_7587_LSU
DIC146_Cora_squamiformis_BOLI_Wilk_7577_LSU
DIC200_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC144_Cora_squamiformis_BOLI_Wilk_7434_LSU
DIC201_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC202_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC051_Cora_squamiformis_EU825969_BOLI_Luecking_23500_LSU
DIC203_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC108_Cora_squamiformis_PERU_unkn_sn_LSU
DIC135_Cora_squamiformis_BOLI_unkn_2607_LSU
DIC236_Cora_undulata_Ceron_36059_ECUA_LSU
DIC213_Cora_undulata_ECUA_Luecking_sn_LSU
DIC204_Cora_undulata_ECUA_Luecking_sn_LSU
DIC209_Cora_undulata_ECUA_Luecking_sn_LSU
DIC210_Cora_undulata_ECUA_Luecking_sn_LSU
DIC214_Cora_undulata_ECUA_Luecking_sn_LSU
DIC215_Cora_undulata_ECUA_Luecking_sn_LSU
DIC216_Cora_undulata_ECUA_Luecking_sn_LSU
DIC219_Cora_undulata_ECUA_Luecking_sn_LSU
DIC218_Cora_undulata_ECUA_Luecking_sn_LSU
DIC217_Cora_undulata_ECUA_Luecking_sn_LSU
DIC234_Cora_undulata_Paredes_653_ECUA_LSU
DIC241_Cora_undulata_Paredes_41_ECUA_LSU
R20_Cora_undulata_EU825963_CORI_Luecking_sn_LSU
DIC278_Cora_undulata_VENE_Hernandez_1778_LSU
DIC319_Cora_undulata_Luecking_32719_COLO_LSU
DIC321_Cora_undulata_Luecking_32721_COLO_LSU
0.02

Figure B

A

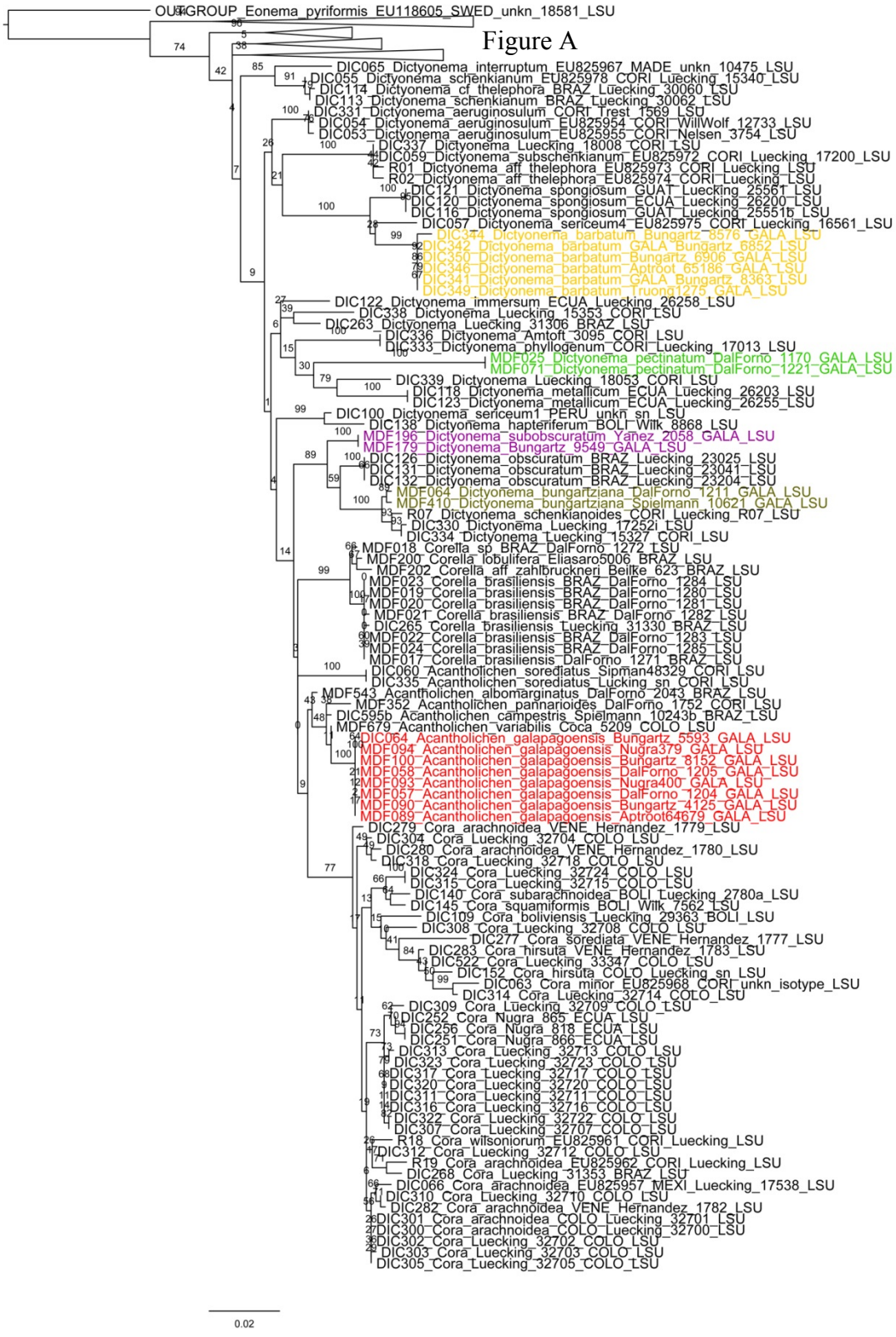


Figure A

B

Figure 13 - Phylogenetic tree inferred by ML with RAxML for the nuLSU marker with the Subset 1 Treatment 1

A

Cont. to Figure B

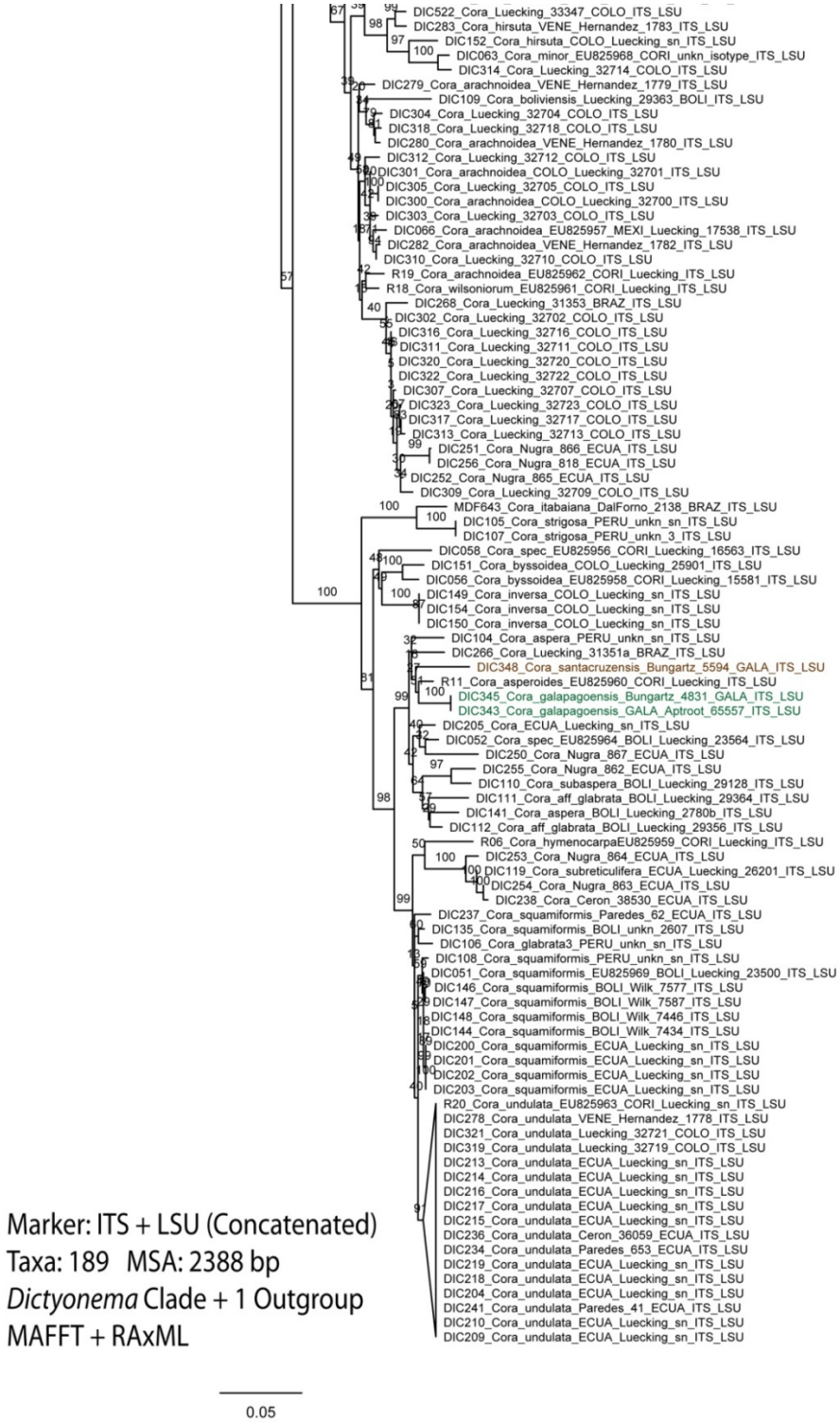


Figure 14 - Phylogeny (ITS + nuLSU) of *Dictyonema* obtained under ML with the Subset 1 Treatment 1 Branches are thickened for all bootstrap (BS) values ≥ 70. Branches involving Galapagos specimens have their exact BS value (if ≥ 70) displayed as well as Bayesian posterior probabilities ≥ 0.95

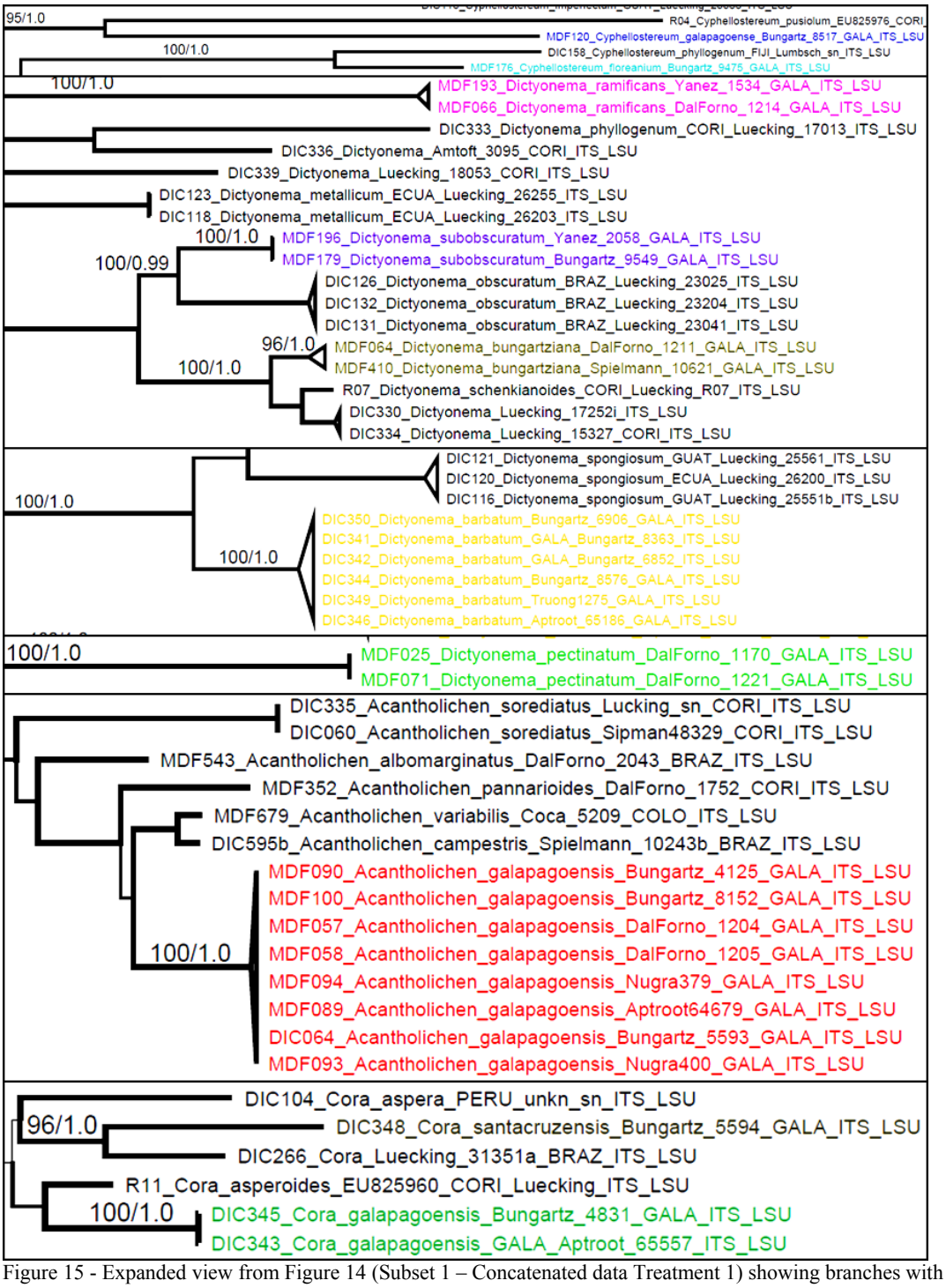
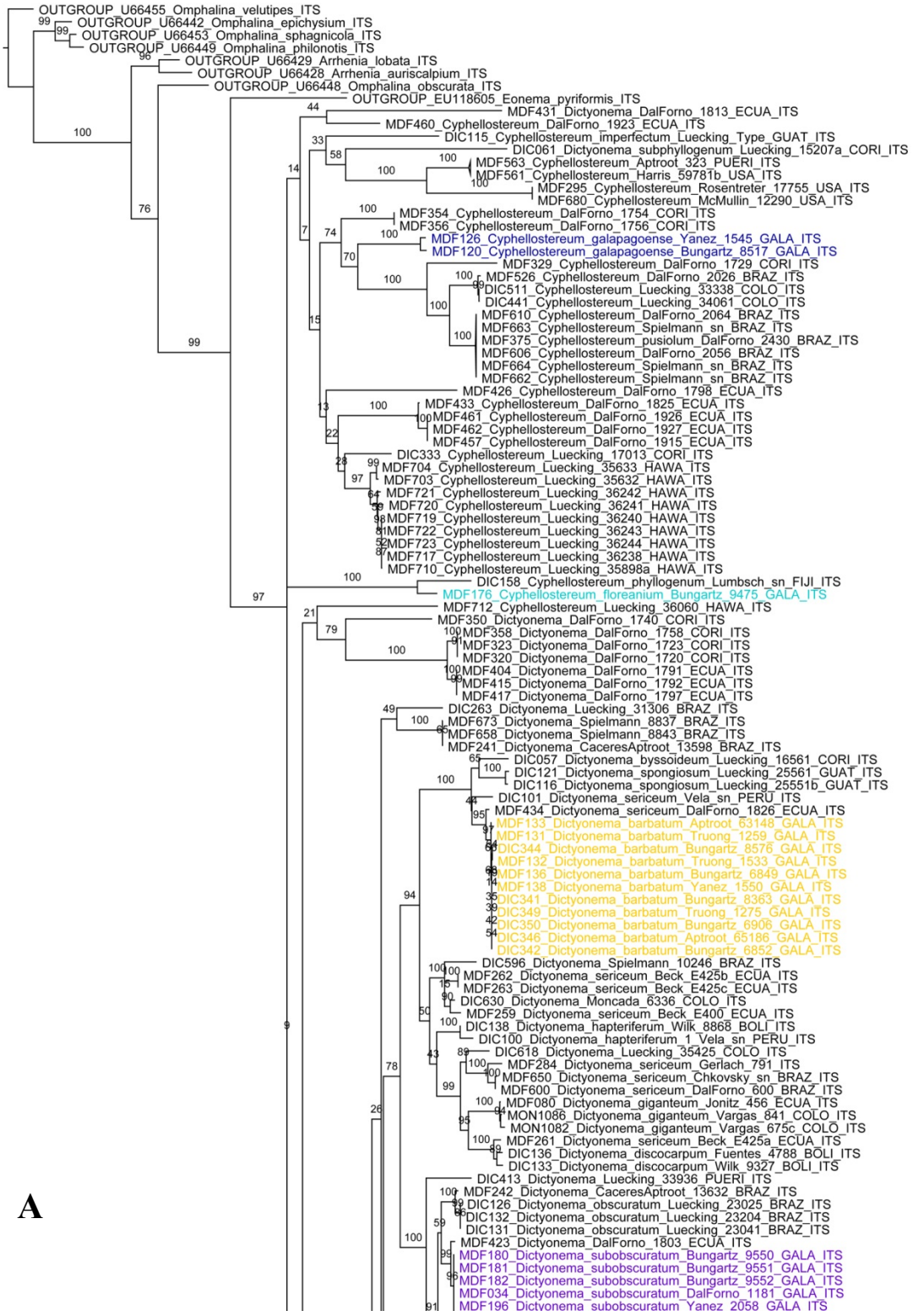


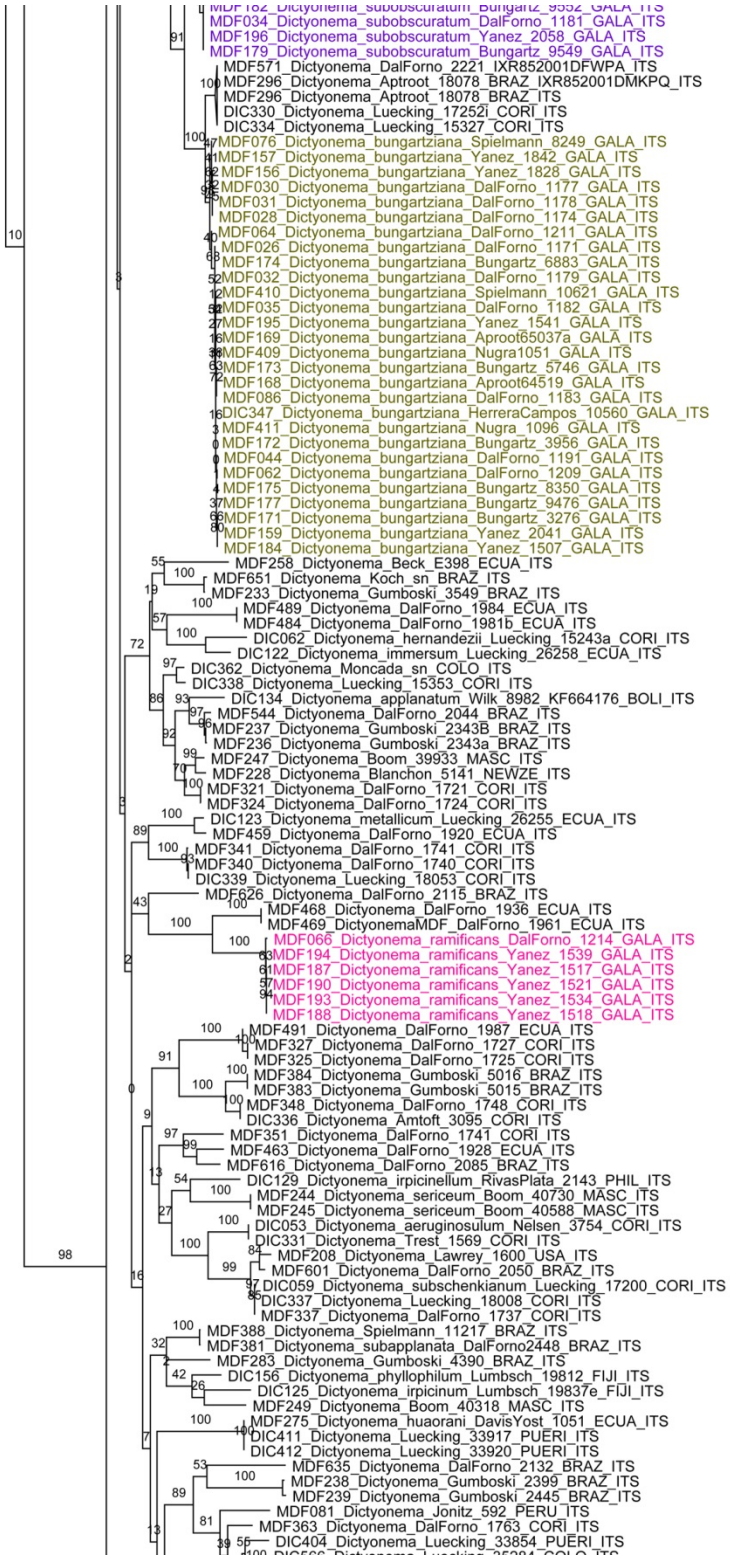
Figure 15 - Expanded view from Figure 14 (Subset 1 – Concatenated data Treatment 1) showing branches with Galapagos specimens

Branches are thickened for all bootstrap (BS) values ≥ 70. Branches involving Galapagos specimens have their exact BS value (if ≥ 70) displayed as well as Bayesian posterior probabilities ≥ 0.95 (ML tree)



A

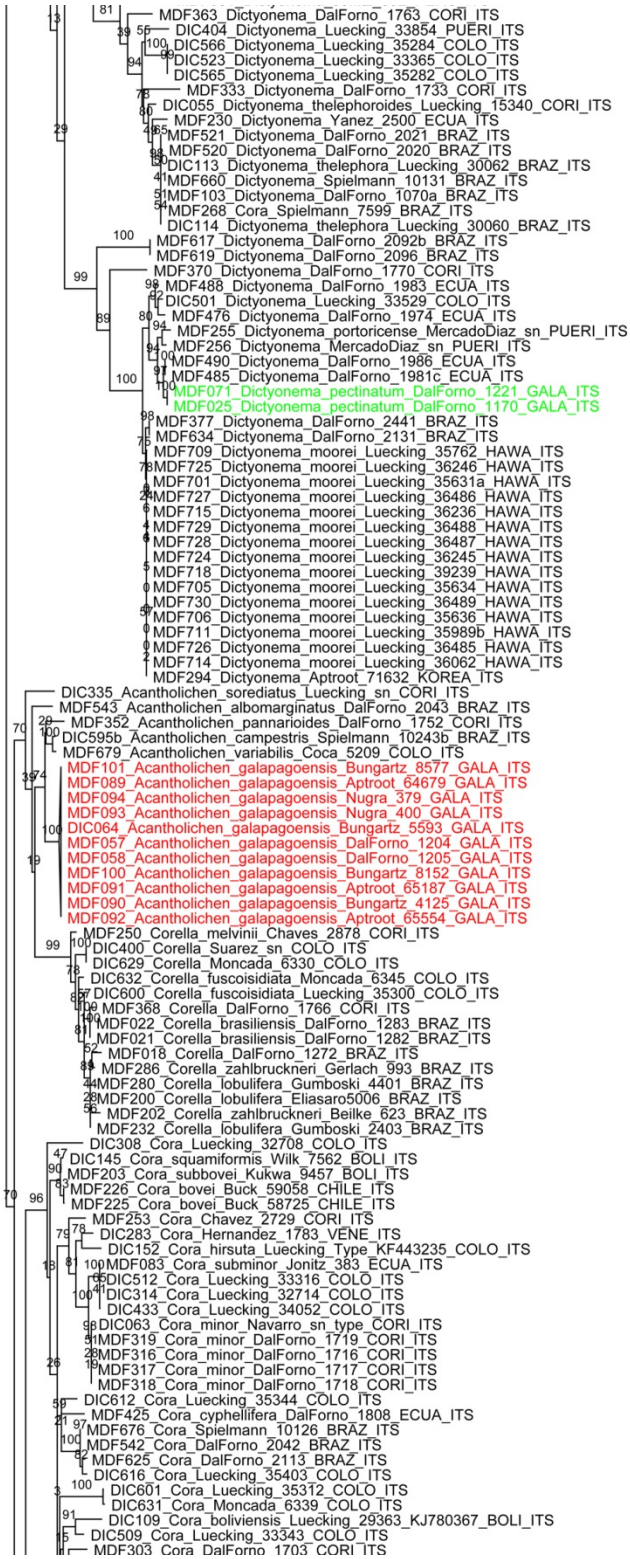
Cont. to Figure B



B

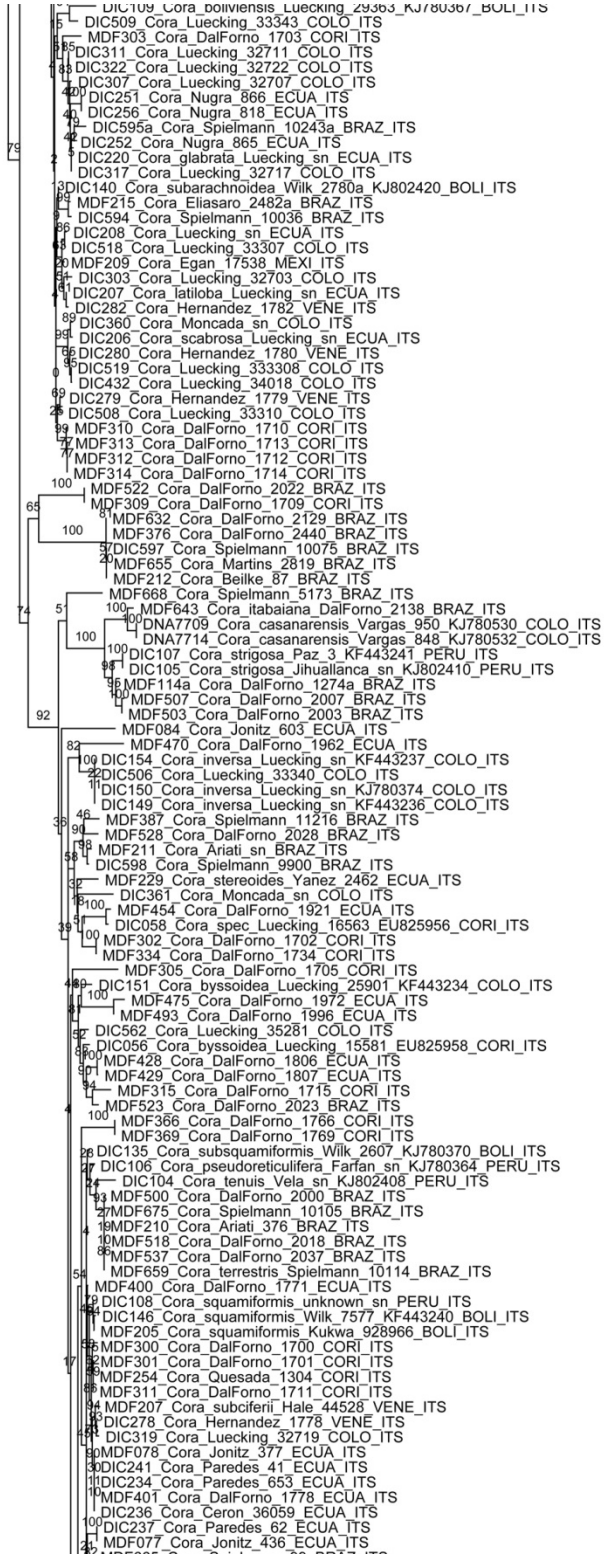
Cont. to Figure C

C

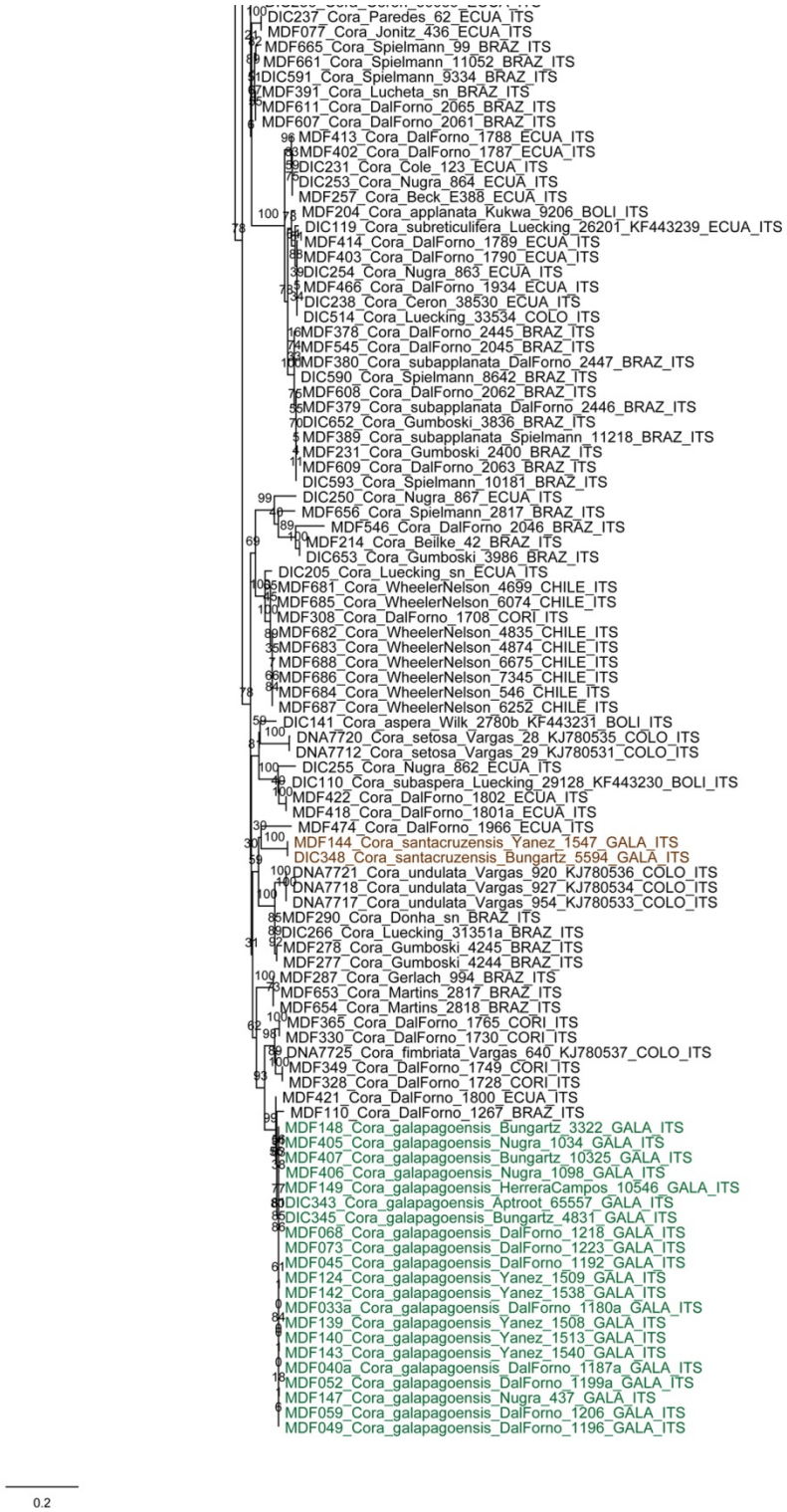


Cont. to Figure D

D



Cont. to Figure E



E

Figure 16 - Phylogenetic tree of *Dictyonema* obtained under ML with the Subset 2 (Treatement 1)

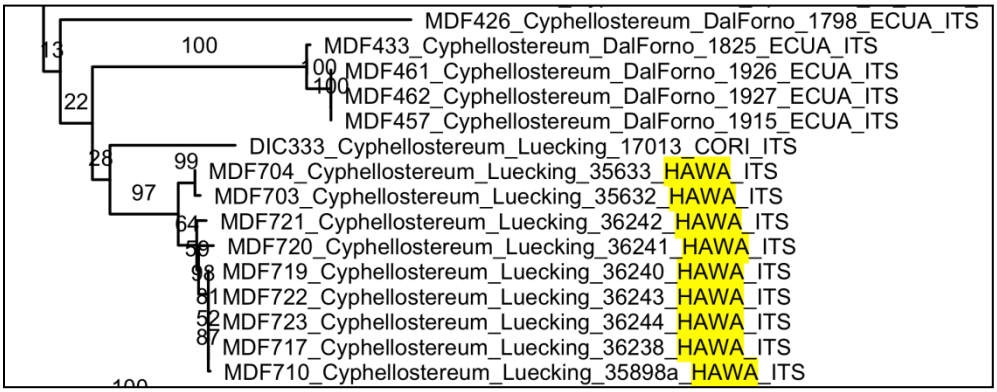


Figure 17 - Branching pattern of an undescribed species of *Cyphellostereum* from Hawaii, indicating a single colonization event and subsequent possible radiation

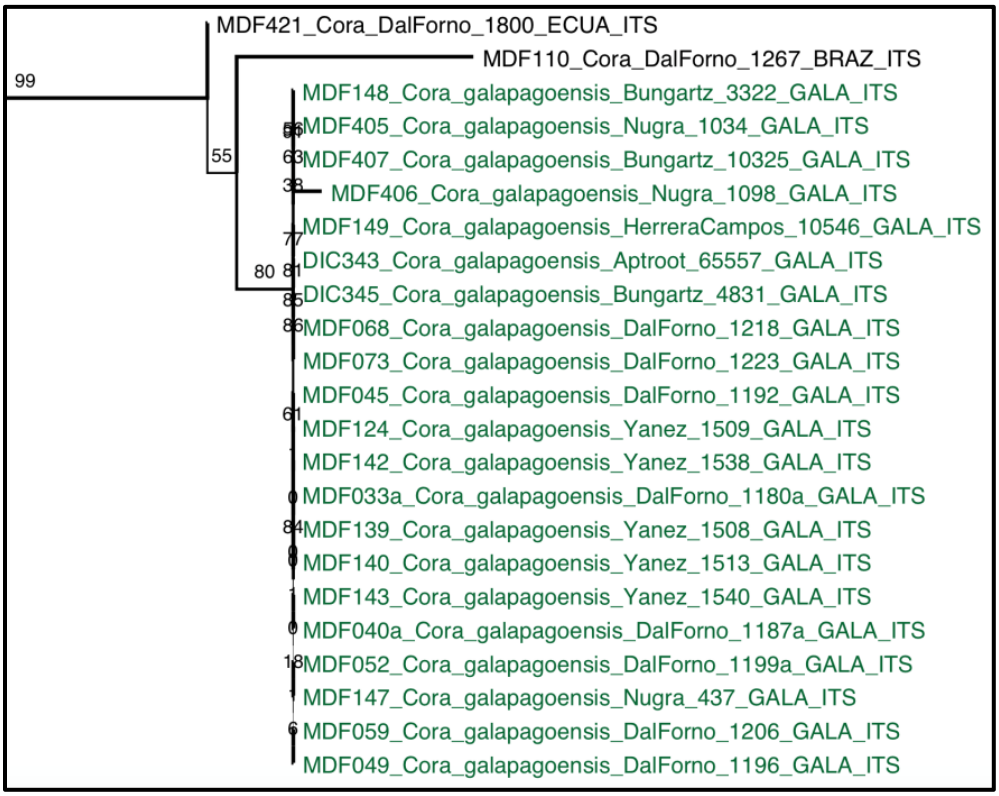


Figure 18 - An additional close up in *Cora galapagoensis*, showing a more clear separation from *Cora* sp. 1 from Brazil (MDF110) and *Cora* sp. 2 from Ecuador (MDF421) in Subset 2

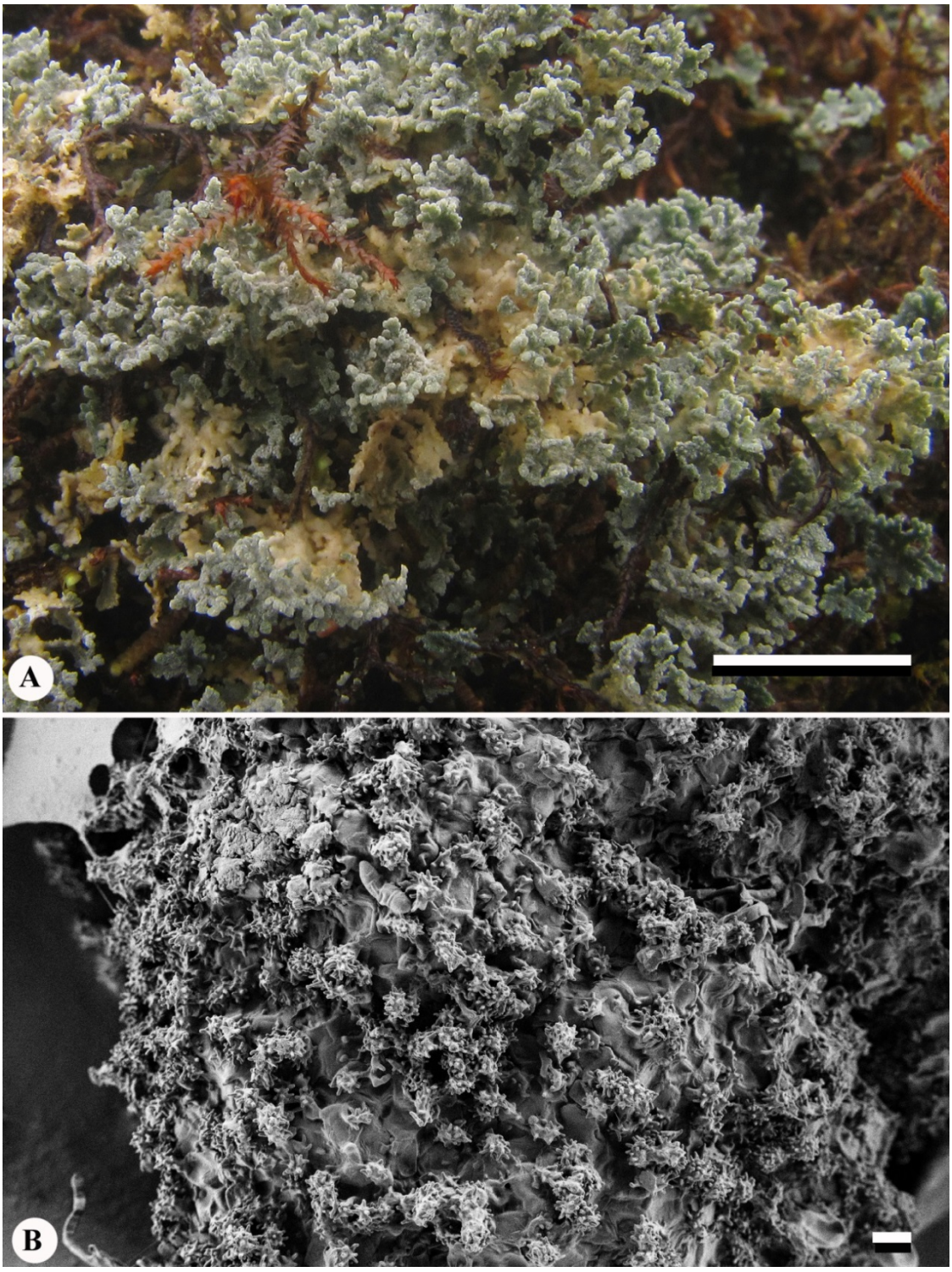


Figure 19 - *Acantholichen galapagoensis* (holotype, M. Dal-Forno 1205)
A- Habit of the species (scale bar = 1 cm); B- SEM image showing thallus surface covered with acanthohyphidia (scale bar = 10 µm). Photo A by Robert Lücking, photo B by Morgan Gostel

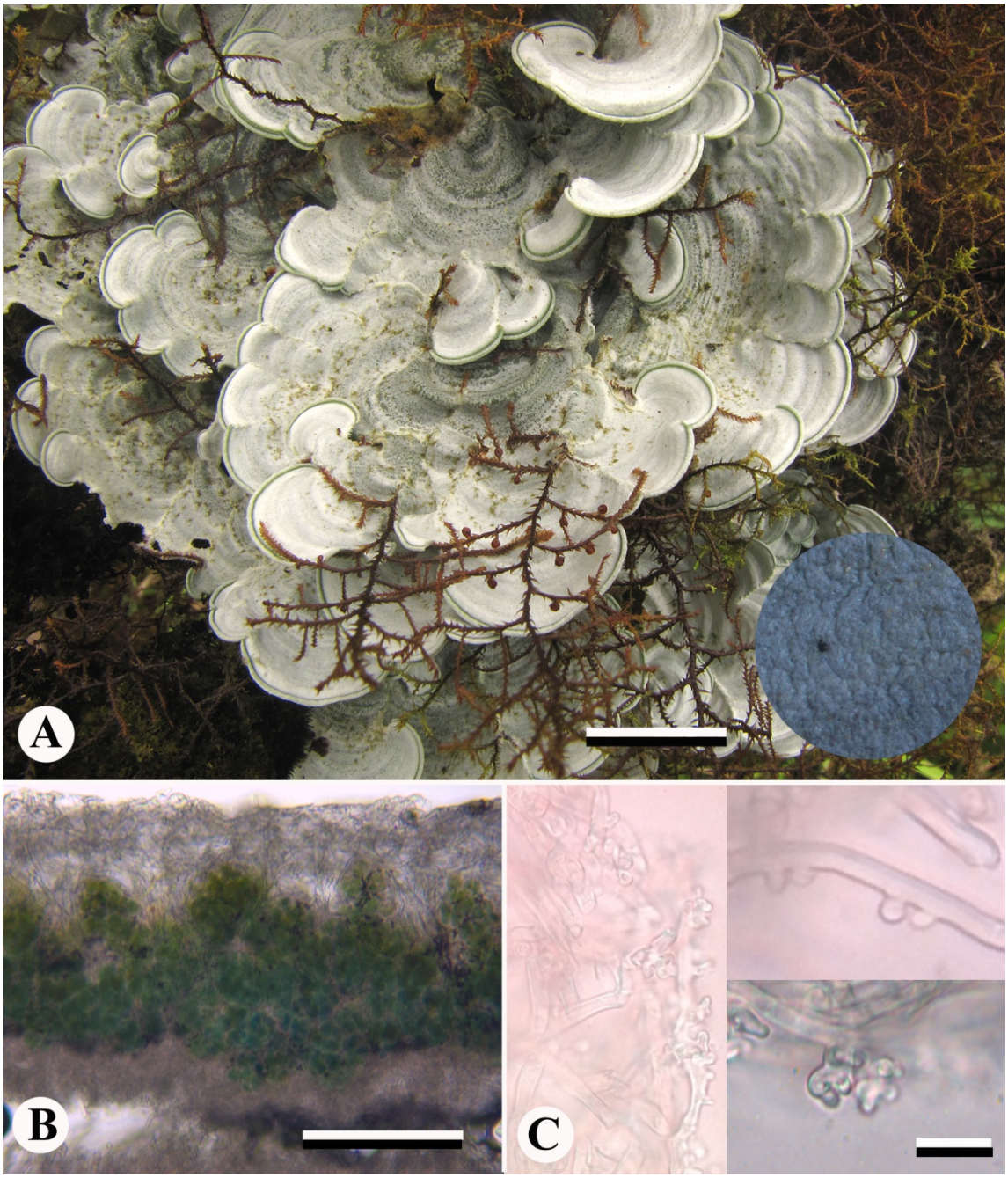


Figure 20 - *Cora galapagoensis* (multiple specimens)
A- Habit of the species (M. Dal-Forno 1180a, scale bar = 2 cm), inlet showing the texture of the upper surface (elephant skin). B- Cross section showing the thallus layers, note the lose hyphae from the cortex (F. Nugra 1034, scale bar = 100 µm) . C- Montage showing the different hyphae "sprouting", multiple specimens (scale = 6 µm). Photo A by Robert Lücking

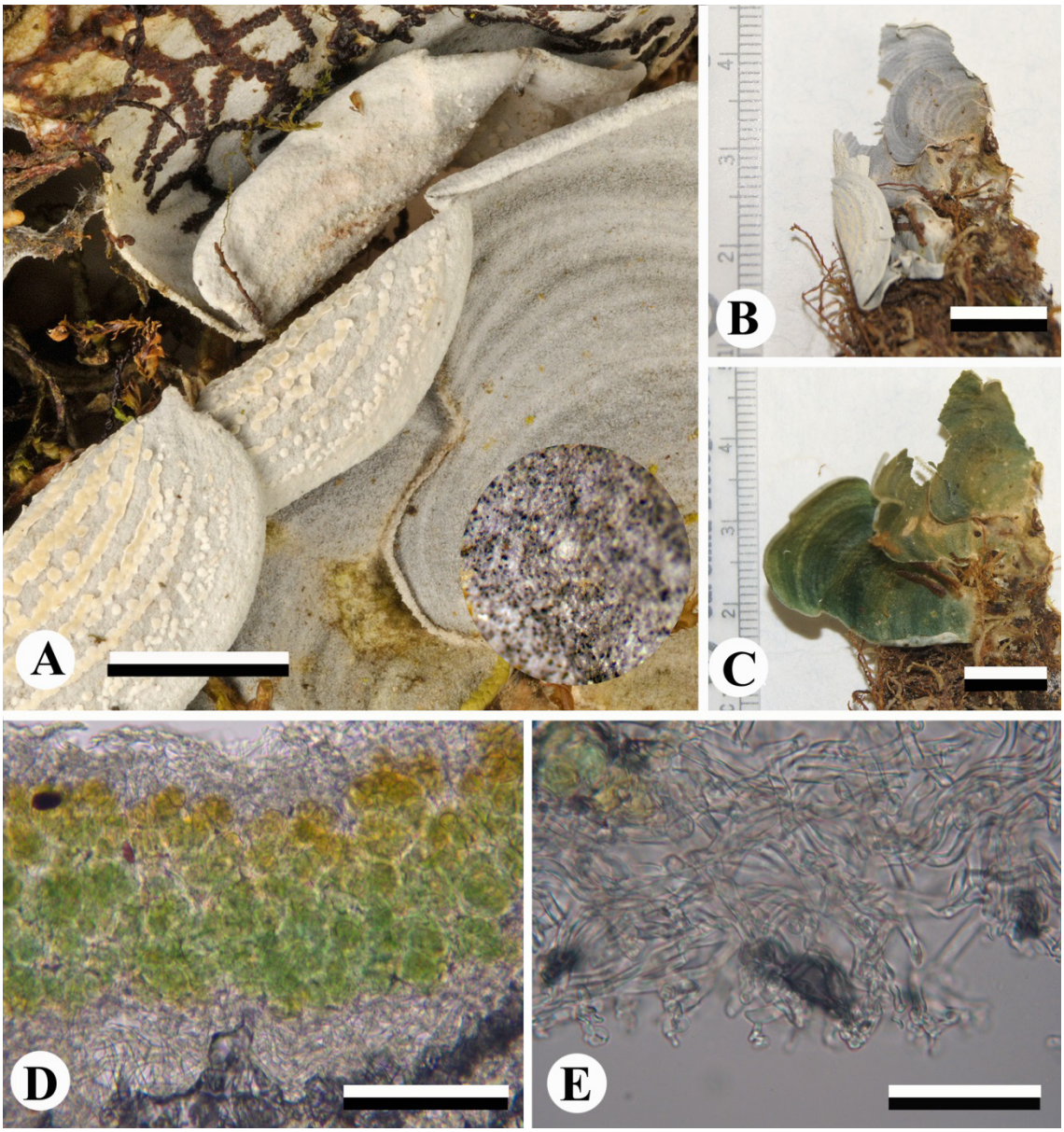


Figure 21 - *Cora santacruzensis* (all A. Yánez 1547)
A- Close up showing upper and lower sides, the later with hymenophores, inlet showing the texture of the upper surface (scale bar = 1cm). B- Isotype dry (scale bar = 1cm). C- Isotype wetted in the laboratory after 4 years of collecting (scale bar = 1cm). D- Cross section showing the layers, note the thinner cortex (scale bar = 100 µm). E- Medullar hyphae "sprouting" (scale bar = 25 µm). Photo A by Frank Bungartz

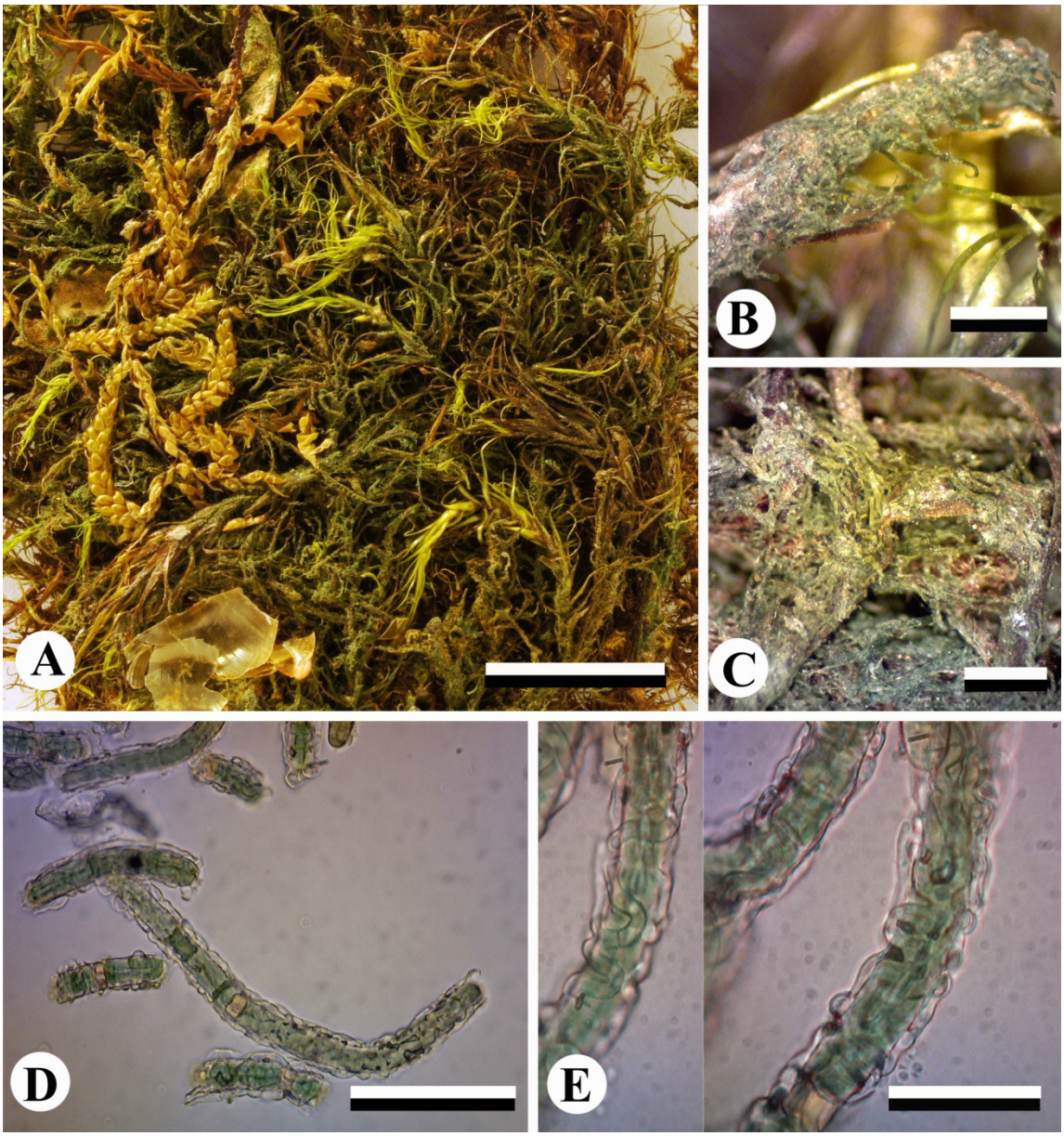


Figure 22 - *Cyphellostereum floreanum* (holotype, F. Bungartz 9475)
A- Habit of the species, growing on bryophytes (scale bar = 1cm). B, C- Close up of the thallus, showing fibrils loosely attached and not very dense (scale bar = 1mm). D- Fibrils general appearance 40× (scale bar = 60 µm). E- Fibrils showing the fungal sheath around the cyanobacteria, note that despite dense, fungal sheath do not completely cover the cyanobacterial cells 100× (scale bar = 40 µm)

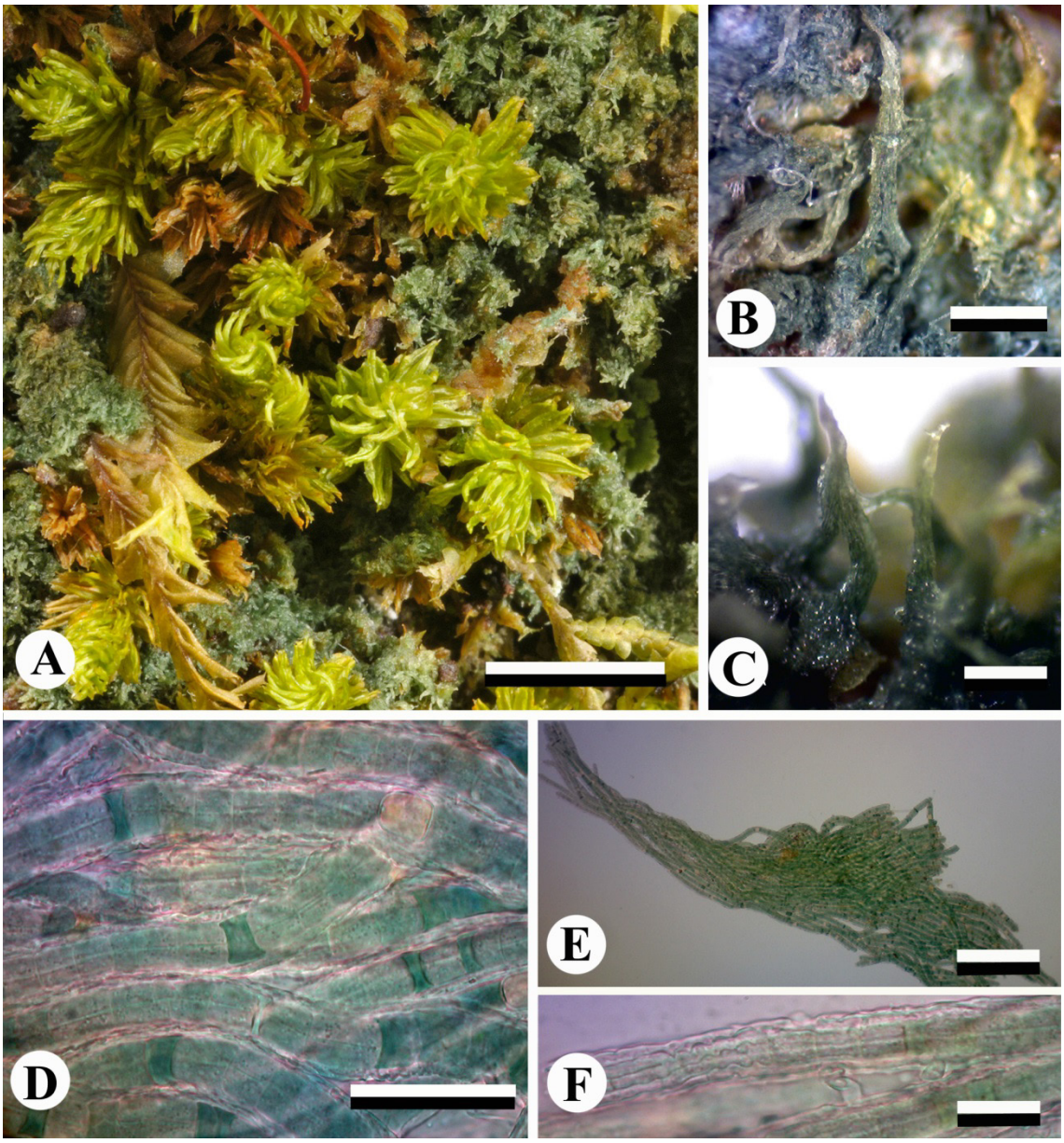


Figure 23 - *Cyphellostereum galapagoense* (all F. Bungartz 8517)
A- Habit of the species (scale bar = 5 mm). B,C- Dry and wetted specimen, respectively, showing fibrils condensed (scale bar = 0,5 mm). D- Arrangement of the fibrils 40× (scale bar = 60 µm). E- Arrangement of the fibrils 10× (scale bar = 200 µm). F- Close up showing fungal sheath (scale bar = 20 µm). Photo A by Frank Bungartz

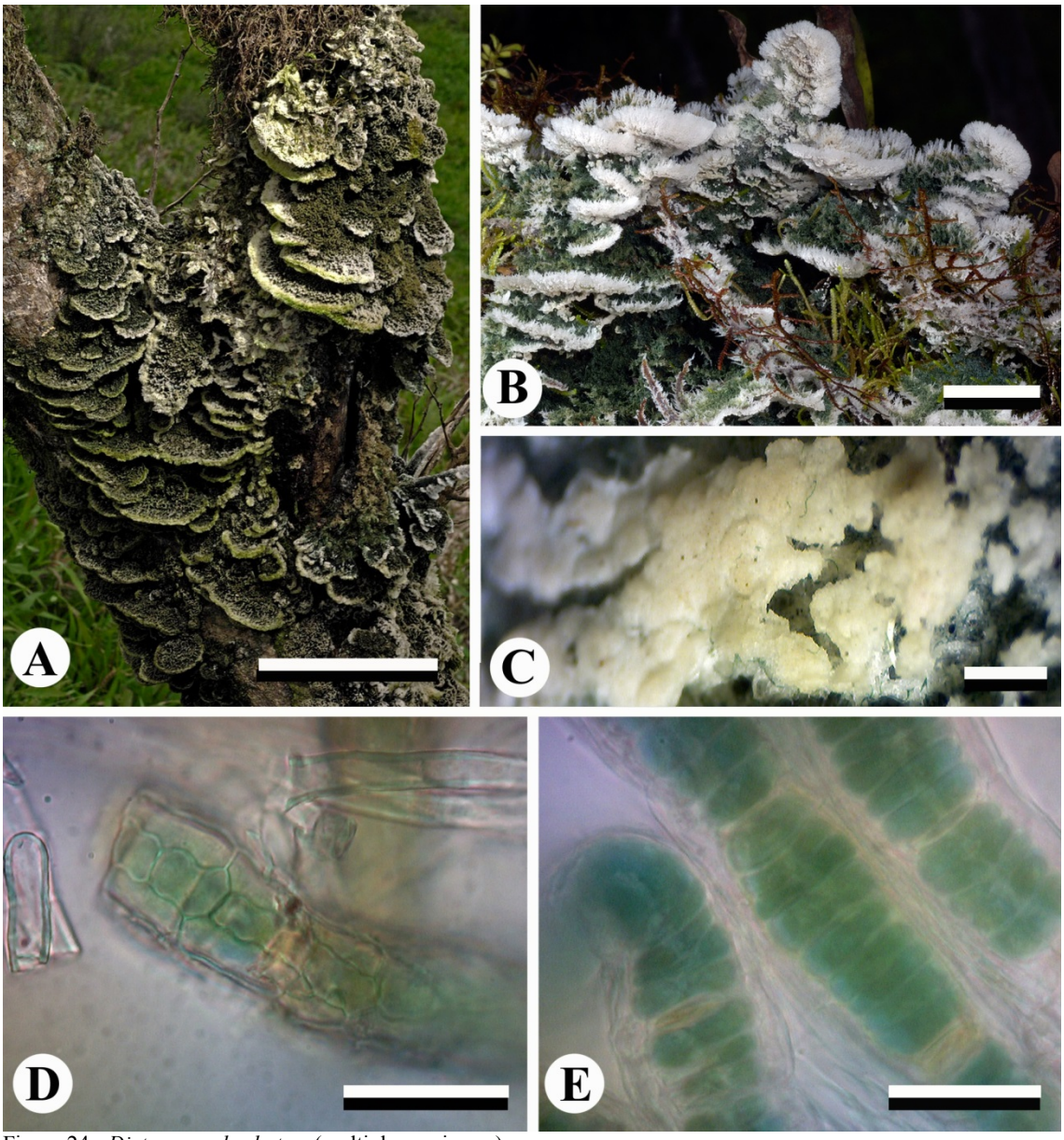


Figure 24 - *Dictyonema barbatum* (multiple specimens)
A- Specimen growing conspicuously in Isabela (F. Bungartz 4127, scale bar = 15 cm). B- General aspect of shelves of the species (F. Bungartz 6906, scale bar = 3cm). C- Close up showing the white to beige continuous hymenophore (C. Truong 1259, scale bar = 1 mm). D- Loose hyphae and fungal sheath hyphae, note the different shapes 100× (F. Bungartz 6849, scale bar = 30 µm). E- Cyanobacterial cells involved in fungal sheath 100× (F. Bungartz 6849, scale bar = 25 µm). Photos A and B by Frank Bungartz

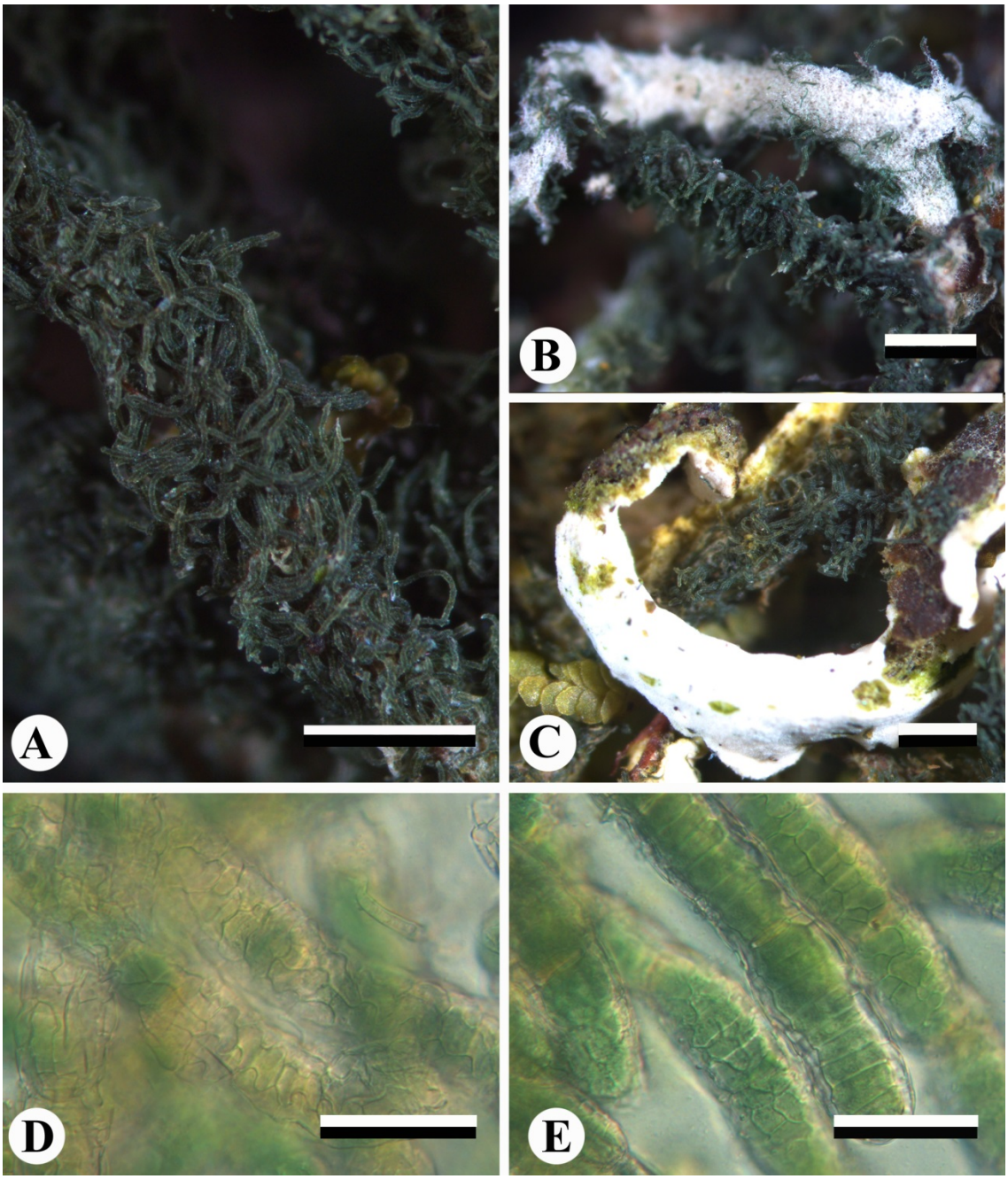


Figure 25 - *Dictyonema bungartziana* (multiple specimens)
A, B- General aspect of the erect fibrils of the species (A - M. Dal-Forno 1182, scale bar = 1 mm, B - M. Dal-Forno 1183, scale bar = 2 mm) C- Stereoid hymenophore (M. Dal-Forno 1209, scale bar = 2 mm). D, E- Cyanobacterial cells involved in fungal sheath 40× (M. Dal-Forno 1174, scale bar = 50 μm)

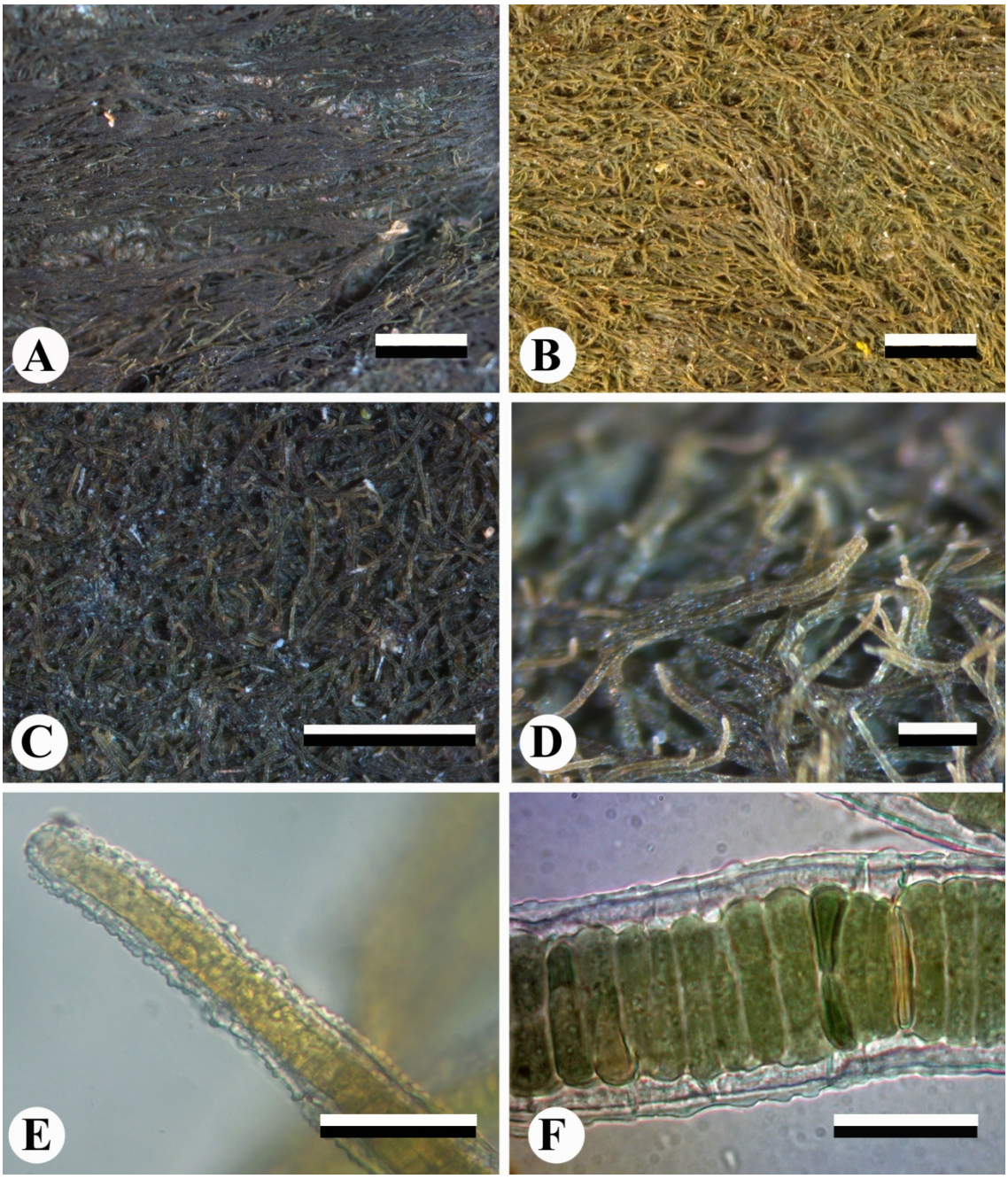


Figure 26 - *Dictyonema pectinatum* (multiple specimens)
A,B – General aspect of combed fibrils of the species in two different lighting conditions (M. Dal-Forno 1170, scale bar = 4 mm). C- General aspect of the fibrils in parts of the thallus where the fibrils do not have the combed appearance, note the lighter tips (M. Dal-Forno 1221, scale bar = 2 mm). D- Close up showing the lighter tips of the fibrils in stereoscope (M. Dal-Forno 1170, scale bar = 0.7 mm). E- Close up showing the lighter tips of the fibrils in light microscope (M. Dal-Forno 1170, scale bar = 25 µm). E- Cyanobacterial cells involved in fungal sheath 100× (M. Dal-Forno 1221, scale bar = 30 µm). Photo B by Frank Bungartz

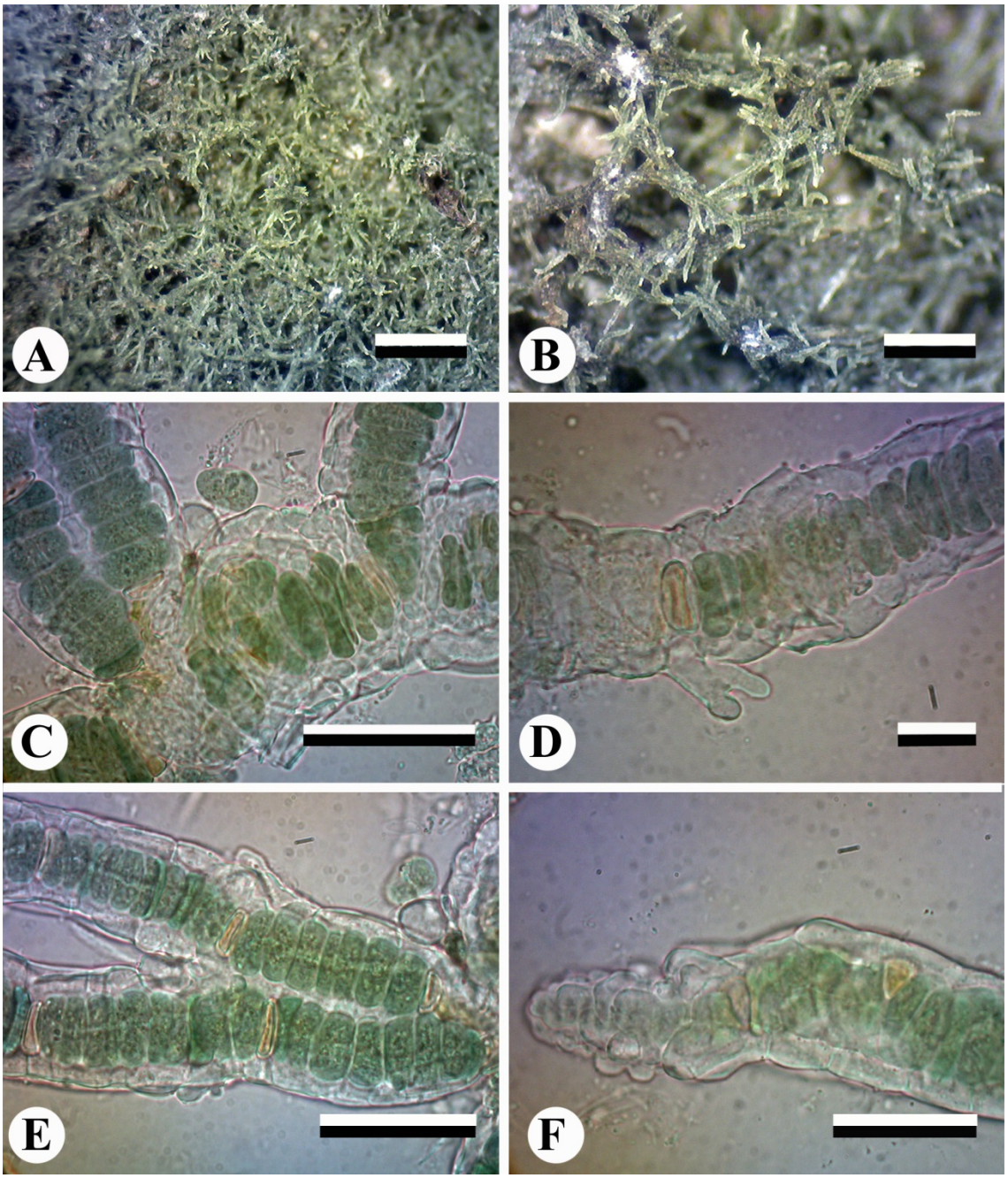


Figure 27 - *Dictyonema ramificans* (M. Dal-Forno 1214)
A - General aspect of fibrils (scale bar = 1 mm). B - Arachnoid arrangement of the fibrils (scale bar = 0,5 mm). C - Many fibrils growing together 100× (scale bar = 50 µm). D - The beginning of a lateral ramification 100× (scale bar = 15 µm). E - Two fibrils connected by fungal sheath, also note the haustoria 100× (scale bar = 50 µm). F- Ornamented hyphae from fungal sheath towards the tip of a fibril 100× (scale bar = 25 µm)

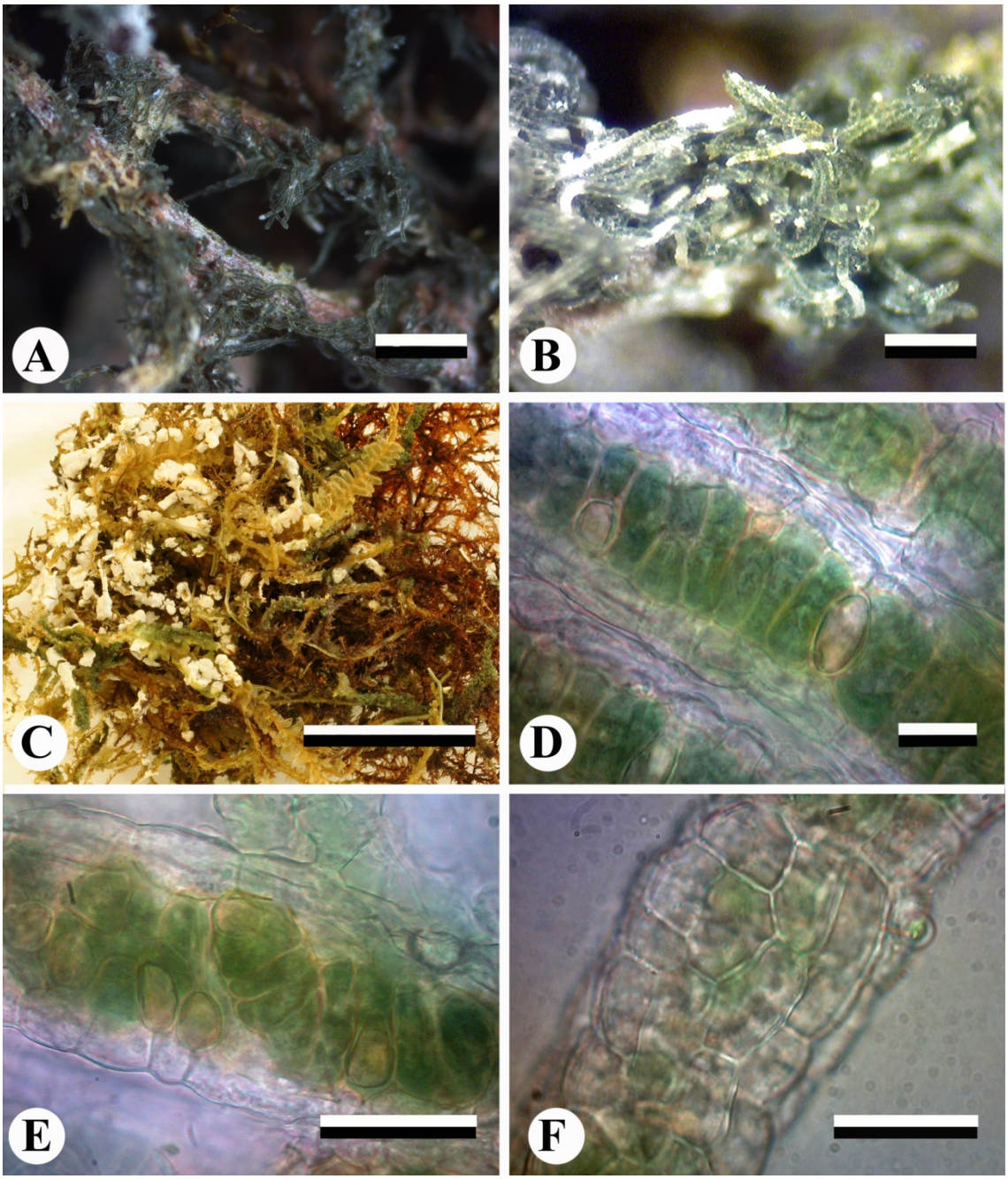


Figure 28 - *Dictyonema subobscuratum* (multiple specimens)
A, B - General aspect of fibrils (M. Dal-Forno 1181, scale bar = 1 mm, and 0.25 mm, respectively). C- Thallus growing on bryophytes showing abundant white hymenophore (F. Bungartz 9550, scale bar = 1.5 cm). D, E - Close up showing the longitudinally divided cells 100× (F. Bungartz 9551, scale bar = 15 µm, and 25 µm, respectively). F- Angular variable hyphae from fungal sheath 100× (F. Bungartz 9551, scale bar = 25 µm). Photo B by Frank Bungartz

Table 13 - Specimens from Subset 2

Control #	Genus	epithet	Collector #	Collector # or Strain	Country	GB # ITS
DIC053	*Dictyonema*	*aeruginosulum*	Nelsen	3754	CORI	
DIC055	*Dictyonema*	*thelephoroides*	Luecking	15340	CORI	
DIC056	*Cora*	*byssoidea*	Luecking	15581	CORI	EU825958
DIC057	*Dictyonema*	*byssoideum*	Luecking	16561	CORI	
DIC058	*Cora*	*spec*	Luecking	16563	CORI	EU825956
DIC059	*Dictyonema*	*subschenkianum*	Luecking	17200	CORI	
DIC061	*Dictyonema*	*subphyllogenum*	Luecking	15207a	CORI	
DIC062	*Dictyonema*	*hernandezii*	Luecking	15243a	CORI	
DIC063	*Cora*	*minor*	Navarro	sn	CORI	
DIC064	*Acantholichen*	*galapagoensis*	Bungartz	5593	GALA	
DIC100	*Dictyonema*	*hapteriferum*	Vela	sn	PERU	
DIC101	*Dictyonema*	*sericeum*	Vela	sn	PERU	
DIC104	*Cora*	*tenuis*	Vela	sn	PERU	KJ802408
DIC105	*Cora*	*strigosa*	Jihuallanca	sn	PERU	KJ802410
DIC106	*Cora*	*pseudoreticulifera*	Farfan	sn	PERU	KJ780364
DIC107	*Cora*	*strigosa*	Paz	3	PERU	KF443241
DIC108	*Cora*	*squamiformis*	unknown	sn	PERU	
DIC109	*Cora*	*boliviensis*	Luecking	29363	BOLI	KJ780367
DIC110	*Cora*	*subaspera*	Luecking	29128	BOLI	KF443230
DIC113	*Dictyonema*	*thelephora*	Luecking	30062	BRAZ	
DIC114	*Dictyonema*	*thelephora*	Luecking	30060	BRAZ	
DIC115	*Cyphellostereum*	*imperfectum*	Luecking	Type	GUAT	
DIC116	*Dictyonema*	*spongiosum*	Luecking	25551b	GUAT	
DIC119	*Cora*	*subreticulifera*	Luecking	26201	ECUA	KF443239
DIC121	*Dictyonema*	*spongiosum*	Luecking	25561	GUAT	
DIC122	*Dictyonema*	*immersum*	Luecking	26258	ECUA	
DIC123	*Dictyonema*	*metallicum*	Luecking	26255	ECUA	
DIC125	*Dictyonema*	*irpicinum*	Lumbsch	19837e	FIJI	
DIC126	*Dictyonema*	*obscuratum*	Luecking	23025	BRAZ	
DIC129	*Dictyonema*	*irpicinellum*	RivasPlata	2143	PHIL	
DIC131	*Dictyonema*	*obscuratum*	Luecking	23041	BRAZ	
DIC132	*Dictyonema*	*obscuratum*	Luecking	23204	BRAZ	
DIC133	*Dictyonema*	*discocarpum*	Wilk	9327	BOLI	
DIC134	*Dictyonema*	*applanatum*	Wilk	8982	BOLI	KF664176
DIC135	*Cora*	*subsquamiformis*	Wilk	2607	BOLI	KJ780370
DIC136	*Dictyonema*	*discocarpum*	Fuentes	4788	BOLI	
DIC138	*Dictyonema*	*hapteriferum*	Wilk	8868	BOLI	
DIC140	*Cora*	*subarachnoidea*	Wilk	2780a	BOLI	KJ802420
DIC141	*Cora*	*aspera*	Wilk	2780b	BOLI	KF443231
DIC145	*Cora*	*squamiformis*	Wilk	7562	BOLI	
DIC146	*Cora*	*squamiformis*	Wilk	7577	BOLI	KF443240
DIC149	*Cora*	*inversa*	Luecking	sn	COLO	KF443236
DIC150	*Cora*	*inversa*	Luecking	sn	COLO	KJ780374
DIC151	*Cora*	*byssoidea*	Luecking	25901	COLO	KF443234
DIC152	*Cora*	*hirsuta*	Luecking	Type	COLO	KF443235
DIC154	*Cora*	*inversa*	Luecking	sn	COLO	KF443237
DIC156	*Dictyonema*	*phyllophilum*	Lumbsch	19812	FIJI	
DIC158	*Cyphellostereum*	*phyllogenum*	Lumbsch	sn	FIJI	
DIC205	*Cora*		Luecking	sn	ECUA	
DIC206	*Cora*	*scabrosa*	Luecking	sn	ECUA	
DIC207	*Cora*	*latiloba*	Luecking	sn	ECUA	
DIC208	*Cora*		Luecking	sn	ECUA	
DIC220	*Cora*	*glabrata*	Luecking	sn	ECUA	
DIC231	*Cora*		Cole	123	ECUA	
DIC234	*Cora*		Paredes	653	ECUA	
DIC236	*Cora*		Ceron	36059	ECUA	
DIC237	*Cora*		Paredes	62	ECUA	
DIC238	*Cora*		Ceron	38530	ECUA	
DIC241	*Cora*		Paredes	41	ECUA	
DIC250	*Cora*		Nugra	867	ECUA	
DIC251	*Cora*		Nugra	866	ECUA	
DIC252	*Cora*		Nugra	865	ECUA	

DIC253	*Cora*		Nugra	864	ECUA
DIC254	*Cora*		Nugra	863	ECUA
DIC255	*Cora*		Nugra	862	ECUA
DIC256	*Cora*		Nugra	818	ECUA
DIC263	*Dictyonema*		Luecking	31306	BRAZ
DIC266	*Cora*		Luecking	31351a	BRAZ
DIC278	*Cora*		Hernandez	1778	VENE
DIC279	*Cora*		Hernandez	1779	VENE
DIC280	*Cora*		Hernandez	1780	VENE
DIC282	*Cora*		Hernandez	1782	VENE
DIC283	*Cora*		Hernandez	1783	VENE
DIC303	*Cora*		Luecking	32703	COLO
DIC307	*Cora*		Luecking	32707	COLO
DIC308	*Cora*		Luecking	32708	COLO
DIC311	*Cora*		Luecking	32711	COLO
DIC314	*Cora*		Luecking	32714	COLO
DIC317	*Cora*		Luecking	32717	COLO
DIC319	*Cora*		Luecking	32719	COLO
DIC322	*Cora*		Luecking	32722	COLO
DIC330	*Dictyonema*		Luecking	17252i	CORI
DIC331	*Dictyonema*		Trest	1569	CORI
DIC333	*Cyphellostereum*		Luecking	17013	CORI
DIC334	*Dictyonema*		Luecking	15327	CORI
DIC335	*Acantholichen*	*sorediatus*	Luecking	sn	CORI
DIC336	*Dictyonema*		Amtoft	3095	CORI
DIC337	*Dictyonema*		Luecking	18008	CORI
DIC338	*Dictyonema*		Luecking	15353	CORI
DIC339	*Dictyonema*		Luecking	18053	CORI
DIC341	*Dictyonema*	*barbatum*	Bungartz	8363	GALA
DIC342	*Dictyonema*	*barbatum*	Bungartz	6852	GALA
DIC343	*Cora*	*galapagoensis*	Aptroot	65557	GALA
DIC344	*Dictyonema*	*barbatum*	Bungartz	8576	GALA
DIC345	*Cora*	*galapagoensis*	Bungartz	4831	GALA
DIC346	*Dictyonema*	*barbatum*	Aptroot	65186	GALA
DIC347	*Dictyonema*	*bungartziana*	HerreraCampos	10560	GALA
DIC348	*Cora*	*santacruzensis*	Bungartz	5594	GALA
DIC349	*Dictyonema*	*barbatum*	Truong	1275	GALA
DIC350	*Dictyonema*	*barbatum*	Bungartz	6906	GALA
DIC360	*Cora*		Moncada	sn	COLO
DIC361	*Cora*		Moncada	sn	COLO
DIC362	*Dictyonema*		Moncada	sn	COLO
DIC400	*Corella*		Suarez	sn	COLO
DIC404	*Dictyonema*		Luecking	33854	PUERI
DIC411	*Dictyonema*		Luecking	33917	PUERI
DIC412	*Dictyonema*		Luecking	33920	PUERI
DIC413	*Dictyonema*		Luecking	33936	PUERI
DIC432	*Cora*		Luecking	34018	COLO
DIC433	*Cora*		Luecking	34052	COLO
DIC441	*Cyphellostereum*		Luecking	34061	COLO
DIC501	*Dictyonema*		Luecking	33529	COLO
DIC506	*Cora*		Luecking	33340	COLO
DIC508	*Cora*		Luecking	33310	COLO
DIC509	*Cora*		Luecking	33343	COLO
DIC511	*Cyphellostereum*		Luecking	33338	COLO
DIC512	*Cora*		Luecking	33316	COLO
DIC514	*Cora*		Luecking	33534	COLO
DIC518	*Cora*		Luecking	33307	COLO
DIC519	*Cora*		Luecking	333308	COLO
DIC523	*Dictyonema*		Luecking	33365	COLO
DIC562	*Cora*		Luecking	35281	COLO
DIC565	*Dictyonema*		Luecking	35282	COLO
DIC566	*Dictyonema*		Luecking	35284	COLO
DIC590	*Cora*		Spielmann	8642	BRAZ
DIC591	*Cora*		Spielmann	9334	BRAZ
DIC593	*Cora*		Spielmann	10181	BRAZ
DIC594	*Cora*		Spielmann	10036	BRAZ

DIC595a	*Cora*		Spielmann	10243a	BRAZ	
DIC595b	*Acantholichen*	*campestris*	Spielmann	10243b	BRAZ	
DIC596	*Dictyonema*		Spielmann	10246	BRAZ	
DIC597	*Cora*		Spielmann	10075	BRAZ	
DIC598	*Cora*		Spielmann	9900	BRAZ	
DIC600	*Corella*	*fuscoisidiata*	Luecking	35300	COLO	
DIC601	*Cora*		Luecking	35312	COLO	
DIC612	*Cora*		Luecking	35344	COLO	
DIC616	*Cora*		Luecking	35403	COLO	
DIC618	*Dictyonema*		Luecking	35425	COLO	
DIC629	*Corella*		Moncada	6330	COLO	
DIC630	*Dictyonema*		Moncada	6336	COLO	
DIC631	*Cora*		Moncada	6339	COLO	
DIC632	*Corella*	*fuscoisidiata*	Moncada	6345	COLO	
DIC652	*Cora*		Gumboski	3836	BRAZ	
DIC653	*Cora*		Gumboski	3986	BRAZ	
DNA7709	*Cora*	*casanarensis*	Vargas	950	COLO	KJ780530
DNA7712	*Cora*	*setosa*	Vargas	29	COLO	KJ780531
DNA7714	*Cora*	*casanarensis*	Vargas	848	COLO	KJ780532
DNA7717	*Cora*	*undulata*	Vargas	954	COLO	KJ780533
DNA7718	*Cora*	*undulata*	Vargas	927	COLO	KJ780534
DNA7720	*Cora*	*setosa*	Vargas	28	COLO	KJ780535
DNA7721	*Cora*	*undulata*	Vargas	920	COLO	KJ780536
DNA7725	*Cora*	*fimbriata*	Vargas	640	COLO	KJ780537
MDF018	*Corella*	*aff. bra*	DalForno	1272	BRAZ	
MDF021	*Corella*	*brasiliensis*	DalForno	1282	BRAZ	
MDF022	*Corella*	*brasiliensis*	DalForno	1283	BRAZ	
MDF025	*Dictyonema*	*pectinatum*	DalForno	1170	GALA	
MDF026	*Dictyonema*	*bungartziana*	DalForno	1171	GALA	
MDF028	*Dictyonema*	*bungartziana*	DalForno	1174	GALA	
MDF030	*Dictyonema*	*bungartziana*	DalForno	1177	GALA	
MDF031	*Dictyonema*	*bungartziana*	DalForno	1178	GALA	
MDF032	*Dictyonema*	*bungartziana*	DalForno	1179	GALA	
MDF033a	*Cora*	*galapagoensis*	DalForno	1180a	GALA	
MDF034	*Dictyonema*	*subobscuratum*	DalForno	1181	GALA	
MDF035	*Dictyonema*	*bungartziana*	DalForno	1182	GALA	
MDF040a	*Cora*	*galapagoensis*	DalForno	1187a	GALA	
MDF044	*Dictyonema*	*bungartziana*	DalForno	1191	GALA	
MDF045	*Cora*	*galapagoensis*	DalForno	1192	GALA	
MDF049	*Cora*	*galapagoensis*	DalForno	1196	GALA	
MDF052	*Cora*	*galapagoensis*	DalForno	1199a	GALA	
MDF057	*Acantholichen*	*galapagoensis*	DalForno	1204	GALA	
MDF058	*Acantholichen*	*galapagoensis*	DalForno	1205	GALA	
MDF059	*Cora*	*galapagoensis*	DalForno	1206	GALA	
MDF062	*Dictyonema*	*bungartziana*	DalForno	1209	GALA	
MDF064	*Dictyonema*	*bungartziana*	DalForno	1211	GALA	
MDF066	*Dictyonema*	*ramificans*	DalForno	1214	GALA	
MDF068	*Cora*	*galapagoensis*	DalForno	1218	GALA	
MDF071	*Dictyonema*	*pectinatum*	DalForno	1221	GALA	
MDF073	*Cora*	*galapagoensis*	DalForno	1223	GALA	
MDF076	*Dictyonema*	*bungartziana*	Spielmann	8249	GALA	
MDF077	*Cora*		Jonitz	436	ECUA	
MDF078	*Cora*		Jonitz	377	ECUA	
MDF080	*Dictyonema*	*giganteum*	Jonitz	456	ECUA	
MDF081	*Dictyonema*		Jonitz	592	PERU	
MDF083	*Cora*	*subminor*	Jonitz	383	ECUA	
MDF084	*Cora*		Jonitz	603	ECUA	
MDF086	*Dictyonema*	*bungartziana*	DalForno	1183	GALA	
MDF089	*Acantholichen*	*galapagoensis*	Aptroot	64679	GALA	
MDF090	*Acantholichen*	*galapagoensis*	Bungartz	4125	GALA	
MDF091	*Acantholichen*	*galapagoensis*	Aptroot	65187	GALA	
MDF092	*Acantholichen*	*galapagoensis*	Aptroot	65554	GALA	
MDF093	*Acantholichen*	*galapagoensis*	Nugra	400	GALA	
MDF094	*Acantholichen*	*galapagoensis*	Nugra	379	GALA	
MDF100	*Acantholichen*	*galapagoensis*	Bungartz	8152	GALA	
MDF101	*Acantholichen*	*galapagoensis*	Bungartz	8577	GALA	

MDF103	*Dictyonema*		DalForno	1070a	BRAZ
MDF110	*Cora*		DalForno	1267	BRAZ
MDF114a	*Cora*		DalForno	1274a	BRAZ
MDF120	*Cyphellostereum*	*galapagoense*	Bungartz	8517	GALA
MDF124	*Cora*	*galapagoensis*	Yánez	1509	GALA
MDF126	*Cyphellostereum*	*galapagoense*	Yánez	1545	GALA
MDF131	*Dictyonema*	*barbatum*	Truong	1259	GALA
MDF132	*Dictyonema*	*barbatum*	Truong	1533	GALA
MDF133	*Dictyonema*	*barbatum*	Aptroot	63148	GALA
MDF136	*Dictyonema*	*barbatum*	Bungartz	6849	GALA
MDF138	*Dictyonema*	*barbatum*	Yánez	1550	GALA
MDF139	*Cora*	*galapagoensis*	Yánez	1508	GALA
MDF140	*Cora*	*galapagoensis*	Yánez	1513	GALA
MDF142	*Cora*	*galapagoensis*	Yánez	1538	GALA
MDF143	*Cora*	*galapagoensis*	Yánez	1540	GALA
MDF144	*Cora*	*santacruzensis*	Yánez	1547	GALA
MDF147	*Cora*	*galapagoensis*	Nugra	437	GALA
MDF148	*Cora*	*galapagoensis*	Bungartz	3322	GALA
MDF149	*Cora*	*galapagoensis*	HerreraCampos	10546	GALA
MDF156	*Dictyonema*	*bungartziana*	Yánez	1828	GALA
MDF157	*Dictyonema*	*bungartziana*	Yánez	1842	GALA
MDF159	*Dictyonema*	*bungartziana*	Yánez	2041	GALA
MDF168	*Dictyonema*	*bungartziana*	Aproot64519		GALA
MDF169	*Dictyonema*	*bungartziana*	Aproot65037a		GALA
MDF171	*Dictyonema*	*bungartziana*	Bungartz	3276	GALA
MDF172	*Dictyonema*	*bungartziana*	Bungartz	3956	GALA
MDF173	*Dictyonema*	*bungartziana*	Bungartz	5746	GALA
MDF174	*Dictyonema*	*bungartziana*	Bungartz	6883	GALA
MDF175	*Dictyonema*	*bungartziana*	Bungartz	8350	GALA
MDF176	*Cyphellostereum*	*floreanum*	Bungartz	9475	GALA
MDF177	*Dictyonema*	*bungartziana*	Bungartz	9476	GALA
MDF179	*Dictyonema*	*subobscuratum*	Bungartz	9549	GALA
MDF180	*Dictyonema*	*subobscuratum*	Bungartz	9550	GALA
MDF181	*Dictyonema*	*subobscuratum*	Bungartz	9551	GALA
MDF182	*Dictyonema*	*subobscuratum*	Bungartz	9552	GALA
MDF184	*Dictyonema*	*bungartziana*	Yánez	1507	GALA
MDF187	*Dictyonema*	*ramificans*	Yánez	1517	GALA
MDF188	*Dictyonema*	*ramificans*	Yánez	1518	GALA
MDF190	*Dictyonema*	*ramificans*	Yánez	1521	GALA
MDF193	*Dictyonema*	*ramificans*	Yánez	1534	GALA
MDF194	*Dictyonema*	*ramificans*	Yánez	1539	GALA
MDF195	*Dictyonema*	*bungartziana*	Yánez	1541	GALA
MDF196	*Dictyonema*	*subobscuratum*	Yánez	2058	GALA
MDF200	*Corella*	*lobulifera*	Eliasaro5006		BRAZ
MDF202	*Corella*	*zahlbruckneri*	Beilke	623	BRAZ
MDF203	*Cora*	*subbovei*	Kukwa	9457	BOLI
MDF204	*Cora*	*applanata*	Kukwa	9206	BOLI
MDF205	*Cora*	*squamiformis*	Kukwa	928966	BOLI
MDF207	*Cora*	*subciferii*	Hale	44528	VENE
MDF208	*Dictyonema*		Lawrey	1600	USA
MDF209	*Cora*		Egan	17538	MEXI
MDF210	*Cora*		Ariati	376	BRAZ
MDF211	*Cora*		Ariati	sn	BRAZ
MDF212	*Cora*		Beilke	87	BRAZ
MDF214	*Cora*		Beilke	42	BRAZ
MDF215	*Cora*		Eliasaro	2482a	BRAZ
MDF225	*Cora*	*bovei*	Buck	58725	CHILE
MDF226	*Cora*	*bovei*	Buck	59058	CHILE
MDF228	*Dictyonema*		Blanchon	5141	NEWZE
MDF229	*Cora*	*stereoides*	Yánez	2462	ECUA
MDF230	*Dictyonema*		Yánez	2500	ECUA
MDF231	*Cora*		Gumboski	2400	BRAZ
MDF232	*Corella*	*lobulifera*	Gumboski	2403	BRAZ
MDF233	*Dictyonema*		Gumboski	3549	BRAZ
MDF236	*Dictyonema*		Gumboski	2343a	BRAZ
MDF237	*Dictyonema*		Gumboski	2343B	BRAZ

MDF238	*Dictyonema*		Gumboski	2399	BRAZ
MDF239	*Dictyonema*		Gumboski	2445	BRAZ
MDF241	*Dictyonema*		Caceres & Aptroot	13598	BRAZ
MDF242	*Dictyonema*		Caceres & Aptroot	13632	BRAZ
MDF244	*Dictyonema*	*sericeum*	Boom	40730	MASC
MDF245	*Dictyonema*	*sericeum*	Boom	40588	MASC
MDF247	*Dictyonema*		Boom	39933	MASC
MDF249	*Dictyonema*		Boom	40318	MASC
MDF250	*Corella*	*melvinii*	Chaves	2878	CORI
MDF253	*Cora*		Chavez	2729	CORI
MDF254	*Cora*		Quesada	1304	CORI
MDF255	*Dictyonema*	*portoricense*	Mercado-Diaz	sn	PUERI
MDF256	*Dictyonema*		Mercado-Diaz	sn	PUERI
MDF257	*Cora*		Beck	E388	ECUA
MDF258	*Dictyonema*		Beck	E398	ECUA
MDF259	*Dictyonema*	*sericeum*	Beck	E400	ECUA
MDF261	*Dictyonema*	*sericeum*	Beck	E425a	ECUA
MDF262	*Dictyonema*	*sericeum*	Beck	E425b	ECUA
MDF263	*Dictyonema*	*sericeum*	Beck	E425c	ECUA
MDF268	*Cora*		Spielmann	7599	BRAZ
MDF275	*Dictyonema*	*huaorani*	Davis & Yost	1051	ECUA
MDF277	*Cora*		Gumboski	4244	BRAZ
MDF278	*Cora*		Gumboski	4245	BRAZ
MDF280	*Corella*	*lobulifera*	Gumboski	4401	BRAZ
MDF283	*Dictyonema*		Gumboski	4390	BRAZ
MDF284	*Dictyonema*	*sericeum*	Gerlach	791	BRAZ
MDF286	*Corella*	*zahlbruckneri*	Gerlach	993	BRAZ
MDF287	*Cora*		Gerlach	994	BRAZ
MDF290	*Cora*		Donha	sn	BRAZ
MDF294	*Dictyonema*		Aptroot	71632	KOREA
MDF295	*Cyphellostereum*		Rosentreter	17755	USA
MDF296	*Dictyonema*		Aptroot	18078	BRAZ
MDF296	*Dictyonema*		Aptroot	18078	BRAZ
MDF300	*Cora*		DalForno	1700	CORI
MDF301	*Cora*		DalForno	1701	CORI
MDF302	*Cora*		DalForno	1702	CORI
MDF303	*Cora*		DalForno	1703	CORI
MDF305	*Cora*		DalForno	1705	CORI
MDF308	*Cora*		DalForno	1708	CORI
MDF309	*Cora*		DalForno	1709	CORI
MDF310	*Cora*		DalForno	1710	CORI
MDF311	*Cora*		DalForno	1711	CORI
MDF312	*Cora*		DalForno	1712	CORI
MDF313	*Cora*		DalForno	1713	CORI
MDF314	*Cora*		DalForno	1714	CORI
MDF315	*Cora*		DalForno	1715	CORI
MDF316	*Cora*	*minor*	DalForno	1716	CORI
MDF317	*Cora*	*minor*	DalForno	1717	CORI
MDF318	*Cora*	*minor*	DalForno	1718	CORI
MDF319	*Cora*	*minor*	DalForno	1719	CORI
MDF320	*Dictyonema*		DalForno	1720	CORI
MDF321	*Dictyonema*		DalForno	1721	CORI
MDF323	*Dictyonema*		DalForno	1723	CORI
MDF324	*Dictyonema*		DalForno	1724	CORI
MDF325	*Dictyonema*		DalForno	1725	CORI
MDF327	*Dictyonema*		DalForno	1727	CORI
MDF328	*Cora*		DalForno	1728	CORI
MDF329	*Cyphellostereum*		DalForno	1729	CORI
MDF330	*Cora*		DalForno	1730	CORI
MDF333	*Dictyonema*		DalForno	1733	CORI
MDF334	*Cora*		DalForno	1734	CORI
MDF337	*Dictyonema*		DalForno	1737	CORI
MDF340	*Dictyonema*		DalForno	1740	CORI
MDF341	*Dictyonema*		DalForno	1741	CORI
MDF348	*Dictyonema*		DalForno	1748	CORI
MDF349	*Cora*		DalForno	1749	CORI

MDF350	*Dictyonema*		DalForno	1740	CORI
MDF351	*Dictyonema*		DalForno	1741	CORI
MDF352	*Acantholichen*	*pannarioides*	DalForno	1752	CORI
MDF354	*Cyphellostereum*		DalForno	1754	CORI
MDF356	*Cyphellostereum*		DalForno	1756	CORI
MDF358	*Dictyonema*		DalForno	1758	CORI
MDF363	*Dictyonema*		DalForno	1763	CORI
MDF365	*Cora*		DalForno	1765	CORI
MDF366	*Cora*		DalForno	1766	CORI
MDF368	*Corella*		DalForno	1766	CORI
MDF369	*Cora*		DalForno	1769	CORI
MDF370	*Dictyonema*		DalForno	1770	CORI
MDF375	*Cyphellostereum*	*pusiolum*	DalForno	2430	BRAZ
MDF376	*Cora*		DalForno	2440	BRAZ
MDF377	*Dictyonema*		DalForno	2441	BRAZ
MDF378	*Cora*		DalForno	2445	BRAZ
MDF379	*Cora*	*subapplanata*	DalForno	2446	BRAZ
MDF380	*Cora*	*subapplanata*	DalForno	2447	BRAZ
MDF381	*Dictyonema*	*subapplanata*	DalForno	2448	BRAZ
MDF383	*Dictyonema*		Gumboski	5015	BRAZ
MDF384	*Dictyonema*		Gumboski	5016	BRAZ
MDF387	*Cora*		Spielmann	11216	BRAZ
MDF388	*Dictyonema*		Spielmann	11217	BRAZ
MDF389	*Cora*	*subapplanata*	Spielmann	11218	BRAZ
MDF391	*Cora*		Lucheta	sn	BRAZ
MDF400	*Cora*		DalForno	1771	ECUA
MDF401	*Cora*		DalForno	1778	ECUA
MDF402	*Cora*		DalForno	1787	ECUA
MDF403	*Cora*		DalForno	1790	ECUA
MDF404	*Dictyonema*		DalForno	1791	ECUA
MDF405	*Cora*	*galapagoensis*	Nugra	1034	GALA
MDF406	*Cora*	*galapagoensis*	Nugra	1098	GALA
MDF407	*Cora*	*galapagoensis*	Bungartz	10325	GALA
MDF409	*Dictyonema*	*bungartziana*	Nugra	1051	GALA
MDF410	*Dictyonema*	*bungartziana*	Spielmann	10621	GALA
MDF411	*Dictyonema*	*bungartziana*	Nugra	1096	GALA
MDF413	*Cora*		DalForno	1788	ECUA
MDF414	*Cora*		DalForno	1789	ECUA
MDF415	*Dictyonema*		DalForno	1792	ECUA
MDF417	*Dictyonema*		DalForno	1797	ECUA
MDF418	*Cora*		DalForno	1801a	ECUA
MDF421	*Cora*		DalForno	1800	ECUA
MDF422	*Cora*		DalForno	1802	ECUA
MDF423	*Dictyonema*		DalForno	1803	ECUA
MDF425	*Cora*	*cyphellifera*	DalForno	1808	ECUA
MDF426	*Cyphellostereum*		DalForno	1798	ECUA
MDF428	*Cora*		DalForno	1806	ECUA
MDF429	*Cora*		DalForno	1807	ECUA
MDF431	*Dictyonema*		DalForno	1813	ECUA
MDF433	*Cyphellostereum*		DalForno	1825	ECUA
MDF434	*Dictyonema*	*sericeum*	DalForno	1826	ECUA
MDF454	*Cora*		DalForno	1921	ECUA
MDF457	*Cyphellostereum*		DalForno	1915	ECUA
MDF459	*Dictyonema*		DalForno	1920	ECUA
MDF460	*Cyphellostereum*		DalForno	1923	ECUA
MDF461	*Cyphellostereum*		DalForno	1926	ECUA
MDF462	*Cyphellostereum*		DalForno	1927	ECUA
MDF463	*Dictyonema*		DalForno	1928	ECUA
MDF466	*Cora*		DalForno	1934	ECUA
MDF468	*Cora*		DalForno	1936	ECUA
MDF469	*Cora*		DalForno	1961	ECUA
MDF470	*Cora*		DalForno	1962	ECUA
MDF474	*Cora*		DalForno	1966	ECUA
MDF475	*Cora*		DalForno	1972	ECUA
MDF476	*Dictyonema*		DalForno	1974	ECUA
MDF484	*Dictyonema*		DalForno	1981b	ECUA

MDF485	*Dictyonema*		DalForno	1981c	ECUA
MDF488	*Dictyonema*		DalForno	1983	ECUA
MDF489	*Dictyonema*		DalForno	1984	ECUA
MDF490	*Dictyonema*		DalForno	1986	ECUA
MDF491	*Dictyonema*		DalForno	1987	ECUA
MDF493	*Cora*		DalForno	1996	ECUA
MDF500	*Cora*		DalForno	2000	BRAZ
MDF503	*Cora*		DalForno	2003	BRAZ
MDF507	*Cora*		DalForno	2007	BRAZ
MDF518	*Cora*		DalForno	2018	BRAZ
MDF520	*Dictyonema*		DalForno	2020	BRAZ
MDF521	*Dictyonema*		DalForno	2021	BRAZ
MDF522	*Cora*		DalForno	2022	BRAZ
MDF523	*Cora*		DalForno	2023	BRAZ
MDF526	*Cyphellostereum*		DalForno	2026	BRAZ
MDF528	*Cora*		DalForno	2028	BRAZ
MDF537	*Cora*		DalForno	2037	BRAZ
MDF542	*Cora*		DalForno	2042	BRAZ
MDF543	*Acantholichen*	*albomarginatus*	DalForno	2043	BRAZ
MDF544	*Dictyonema*		DalForno	2044	BRAZ
MDF545	*Cora*		DalForno	2045	BRAZ
MDF546	*Cora*		DalForno	2046	BRAZ
MDF561	*Cyphellostereum*		Harris	59781b	USA
MDF563	*Cyphellostereum*		Aptroot	323	PUERI
MDF571	*Dictyonema*		DalForno	2221	PUERI
MDF600	*Dictyonema*	*sericeum*	DalForno	600	BRAZ
MDF601	*Dictyonema*		DalForno	2050	BRAZ
MDF606	*Cyphellostereum*		DalForno	2056	BRAZ
MDF607	*Cora*		DalForno	2061	BRAZ
MDF608	*Cora*		DalForno	2062	BRAZ
MDF609	*Cora*		DalForno	2063	BRAZ
MDF610	*Cyphellostereum*		DalForno	2064	BRAZ
MDF611	*Cora*		DalForno	2065	BRAZ
MDF616	*Dictyonema*		DalForno	2085	BRAZ
MDF617	*Dictyonema*		DalForno	2092b	BRAZ
MDF619	*Dictyonema*		DalForno	2096	BRAZ
MDF625	*Cora*		DalForno	2113	BRAZ
MDF626	*Dictyonema*		DalForno	2115	BRAZ
MDF632	*Cora*		DalForno	2129	BRAZ
MDF634	*Dictyonema*		DalForno	2131	BRAZ
MDF635	*Dictyonema*		DalForno	2132	BRAZ
MDF643	*Cora*	*itabaiana*	DalForno	2138	BRAZ
MDF650	*Dictyonema*	*sericeum*	Chkovsky	sn	BRAZ
MDF651	*Dictyonema*		Koch	sn	BRAZ
MDF653	*Cora*		Martins	2817	BRAZ
MDF654	*Cora*		Martins	2818	BRAZ
MDF655	*Cora*		Martins	2819	BRAZ
MDF656	*Cora*		Spielmann	2817	BRAZ
MDF658	*Dictyonema*		Spielmann	8843	BRAZ
MDF659	*Cora*	*terrestris*	Spielmann	10114	BRAZ
MDF660	*Dictyonema*		Spielmann	10131	BRAZ
MDF661	*Cora*		Spielmann	11052	BRAZ
MDF662	*Cyphellostereum*		Spielmann	sn	BRAZ
MDF663	*Cyphellostereum*		Spielmann	sn	BRAZ
MDF664	*Cyphellostereum*		Spielmann	sn	BRAZ
MDF665	*Cora*		Spielmann	99	BRAZ
MDF668	*Cora*		Spielmann	5173	BRAZ
MDF673	*Dictyonema*		Spielmann	8837	BRAZ
MDF675	*Cora*		Spielmann	10105	BRAZ
MDF676	*Cora*		Spielmann	10126	BRAZ
MDF679	*Acantholichen*	*variabilis*	Coca	5209	COLO
MDF680	*Cyphellostereum*		McMullin	12290	USA
MDF681	*Cora*		Wheeler & Nelson	4699	CHILE
MDF682	*Cora*		Wheeler & Nelson	4835	CHILE
MDF683	*Cora*		Wheeler & Nelson	4874	CHILE
MDF684	*Cora*		Wheeler & Nelson	546	CHILE

MDF685	*Cora*		Wheeler & Nelson	6074	CHILE	
MDF686	*Cora*		Wheeler & Nelson	7345	CHILE	
MDF687	*Cora*		Wheeler & Nelson	6252	CHILE	
MDF688	*Cora*		Wheeler & Nelson	6675	CHILE	
MDF701	*Dictyonema*	*moorei*	Luecking	35631a	HAWA	
MDF703	*Cyphellostereum*		Luecking	35632	HAWA	
MDF704	*Cyphellostereum*		Luecking	35633	HAWA	
MDF705	*Dictyonema*	*moorei*	Luecking	35634	HAWA	
MDF706	*Dictyonema*	*moorei*	Luecking	35636	HAWA	
MDF709	*Dictyonema*	*moorei*	Luecking	35762	HAWA	
MDF710	*Cyphellostereum*		Luecking	35898a	HAWA	
MDF711	*Dictyonema*	*moorei*	Luecking	35989b	HAWA	
MDF712	*Cyphellostereum*		Luecking	36060	HAWA	
MDF714	*Dictyonema*	*moorei*	Luecking	36062	HAWA	
MDF715	*Dictyonema*	*moorei*	Luecking	36236	HAWA	
MDF717	*Cyphellostereum*		Luecking	36238	HAWA	
MDF718	*Dictyonema*	*moorei*	Luecking	39239	HAWA	
MDF719	*Cyphellostereum*		Luecking	36240	HAWA	
MDF720	*Cyphellostereum*		Luecking	36241	HAWA	
MDF721	*Cyphellostereum*		Luecking	36242	HAWA	
MDF722	*Cyphellostereum*		Luecking	36243	HAWA	
MDF723	*Cyphellostereum*		Luecking	36244	HAWA	
MDF724	*Dictyonema*	*moorei*	Luecking	36245	HAWA	
MDF725	*Dictyonema*	*moorei*	Luecking	36246	HAWA	
MDF726	*Dictyonema*	*moorei*	Luecking	36485	HAWA	
MDF727	*Dictyonema*	*moorei*	Luecking	36486	HAWA	
MDF728	*Dictyonema*	*moorei*	Luecking	36487	HAWA	
MDF729	*Dictyonema*	*moorei*	Luecking	36488	HAWA	
MDF730	*Dictyonema*	*moorei*	Luecking	36489	HAWA	
MON1082	*Dictyonema*	*giganteum*	Vargas	675c	COLO	
MON1086	*Dictyonema*	*giganteum*	Vargas	841	COLO	
OUTGROUP	*Arrhenia*	*auriscalpium*	Lutzoni	930731-3	N/A	U66428
OUTGROUP	*Omphalina*	*epichysium*	Redhead	5223	N/A	U66442
OUTGROUP	*Arrhenia*	*lobata*	Lutzoni & Lamoure	910825	N/A	U66429
OUTGROUP	*Omphalina*	*obscurata*	Lamoure	L73-101	N/A	U66448
OUTGROUP	*Omphalina*	*philonotis*	Lutzoni	930804-5	N/A	U66449
OUTGROUP	*Eonema*	*pyriformis*	Hjm	18581	N/A	EU118605
OUTGROUP	*Omphalina*	*sphagnicola*	Lutzoni	930810-1	N/A	U66453
OUTGROUP	*Omphalina*	*velutipes*	Lamoure	L77-166h11Xh4	N/A	U66455

Chapter 5 – Photobiont versus mycobiont diversity: A case study of the lichen symbiosis in the *Dictyonema* clade

Abstract

Based on recent phylogenetic studies of the basidiolichen clade *Dictyonema* s.l., 63 species are now accepted in five genera, with hundreds of species remaining to be described. The identified photobionts of these species are members of a remarkable clade of cyanobacteria called *Rhizonema*, which may be entirely lichenized. To test this hypothesis, we obtained 16S rDNA sequences generated through multi-tag pyrosequencing (MTPS) from 560 specimens representing all major clades within *Dictyonema* s.l., and an additional 21 ascolichens suspected to contain *Rhizonema*, collected from Bolivia, Brazil, Canary Islands (Tenerife), Chile, Colombia, Costa Rica, Ecuador (continental), Fiji, Galapagos Islands, Guatemala, Mascarene Islands (La Reunión), Mexico, New Zealand, Panama, Peru, Philippines, Puerto Rico, Thailand, and Venezuela. All of the samples were found to harbor the *Rhizonema* photobiont and most contained more than one haplotype of *Rhizonema*. Initial alignments of the sequences suggested two main clusters of *Rhizonema* across all specimens, and maximum likelihood and TCS network analyses confirmed this result. We are interpreting these as two separate lineages of *Rhizonema*, and each is represented by a relatively large number of

haplotypes. There is no indication of photobiont-mycobiont co-evolution at the species level in any of the clades of *Dictyonema* s.l. However, one of the two lineages of *Rhizonema* (Lineage 2) appears to partner primarily with one of the *Cora* clades collected from northern Andean locations. The other *Rhizonema* lineage (1) is represented by a much larger number of haplotypes and these appear to partner with mycobionts from many *Dictyonema* clades, likely representing the ancestral *Rhizonema* clade within these lichens. Overall, our results show that the diversity of photobionts is far lower than that of the mycobionts, a view that lends support to a previously published hypothesis concerning photobiont sharing in the clade.

Introduction

Lichens are the result of a symbiotic interaction between a fungus (mycobiont) and one or more photosynthetic components (photobiont), which can be green algae and/or cyanobacteria (Ahmadjian, 1993; Nash, 1996; Purvis, 2000). Lichens are a neither a biological nor a systematic group, but an artificial classification based on a nutritional strategy (Kirk et al., 2008). For nomenclatural purposes, names given to lichens apply to the fungal component, this according to the International Code of Nomenclature for algae, fungi, and plants (Chapter II, Section 4, Article 13.1(d) 2011).

Far more research has been done on the phylogenetic relationships of lichen fungi than lichen photobionts (review of early research by DePriest, 2004; some examples: Ertz et al., 2008; Hodkinson et al., 2014; Lawrey et al., 2009; Lutzoni et al., 2001; Nelsen et

al., 2009; Redhead et al., 2002), and even less on the combined analysis of both partners together. Among the studies that have analyzed both components is the pioneering work of Piercey-Normore and DePriest (2001), who for the first time reconstructed the evolutionary histories of both partners of a number of *Cladonia* lichens to see if there was evidence for co-speciation in these associations. Although they found no evidence for co-speciation, they did find evidence for switching of algal genotypes among the *Cladonia* mycobionts.

Additional studies done since then (Nelsen and Gargas, 2008; Piercey-Normore, 2006; Zoller and Lutzoni, 2003) have also generally failed to indicate any co-speciation, but all show evidence of photobiont switching or sharing among mycobionts, an idea made earlier by Trevor Goward (1994), who stated that "*lichens are fungi that have taken up agriculture*". His idea is that despite the thousands of species of lichen-forming fungi known, ca. 18,000 (Lücking et al., 2009b), there are far fewer species of photobionts (Ahmadjian, 1967; Tschermak-Woess, 1988), and that the fungal partners cultivate and share only the most effective photobionts, a process not too different from agriculture (Goward, 1994; Lücking et al., 2009a). So one would expect that, of the many potential photobiont species and genotypes within species (Robertson and Piercey-Normore, 2007) available for exploitation by fungi, only certain ones will be used the most frequently and these will tend to become widely distributed among many populations and species of lichens.

Most studies of lichen photobionts have focused on chlorolichens, those that have green algae as their photobiont; studies of cyanolichens that contain cyanobacteria have

been far more limited. Rikkinen (2003) estimates that approximately 12% of lichen-forming fungal species have cyanobacteria as the primary photobiont, most of them in the fungal order Peltigerales and associating with the cyanobacterial genus *Nostoc*. *Nostoc* can be involved in bi- and tripartite lichens. Bipartite lichens are stable symbioses between one type of lichen-forming fungus and one type of photobiont, while in tripartite lichens one lichen-symbiotic fungus associates simultaneously with both green algal and cyanobacterial symbionts (Rikkinen, 2015). A recent example of study involving bi- and tripartite lichens is the one of Elvebakk et al. (2008), who showed that within an individual thallus of *Pannaria,* distinct *Nostoc* strains may be present in different cephalodia.

Research performed mainly by investigators at Duke University led by François Lutzoni and Jolanta Miadliskowska focuses primarily on members of the lichen order Peltigerales, lichenized with cyanobacteria in the Nostocales. For example, O'Brien et al. (2005), found that lichenized *Nostoc* are closely related to *Nostoc* associated with other symbiotic relationships, such as the cycad *Cycas* and the liverwort *Blasia*. In addition, their results also indicate that photobiont sharing is common among lichen species in the Peltigerales.

Dictyonema s.l. is the largest group of basidiolichens, with 63 species currently accepted and well over hundred yet to be validly described (**Appendix 10**). The photobionts of all members of the clade are now known to be in the genus *Rhizonema* (Lücking et al., 2009a, 2014a), a novel lineage recently discovered and found to be entirely lichenized. Historically, the photobiont of *Dictyonema* s.l. was thought to be

either species of *Scytonema* in filamentous forms (*Dictyonema, Cyphellostereum)* or of *Chroococcus* in foliose and squamulose forms (as in *Cora* and *Corella*; summaries in Oberwinkler, 1970; Parmasto, 1978). This interpretation is understandable since free-living *Scytonema* and lichenized *Rhizonema* (e.g. in the lichen *Cyphellostereum*) are very similar morphologically (**Figure 29**). While certain species of *Scytonema* can easily be distinghuished by their lighter color and their filament morphology, others require microscopical examination to separate them from lichenized *Rhizonema* (free-living *Scytonema* lacks fungal sheath).

Earlier studies (Lücking et al., 2009a) suggested that *Rhizonema* is not exclusive to *Dictyonema*; it is also the photobiont of ascolichens such as *Coccocarpia, Leptogidium,* and *Stereocaulon*, and possibly others that have not yet been studied (Lücking et al., 2009a, 2014a; Muggia et al., 2011). There was also an indication that *Rhizonema* clades were shared among various lineages of *Dictyonema* s.l. The nature of these associations, including the specificity and diversity of the *Rhizonema* photobionts used by all of these lichens, has not yet been thoroughly investigated, however.

Lichenization, especially by basidiolichen fungi, may constrain the evolution of photobionts when lichenized. Zoller and Lutzoni (2003) compared DNA substitution rates of fungal and algal internal transcribed spacer (ITS) in the lichenized basidiomycete *Omphalina* (= *Lichenomphalia* Redhead, Lutzoni, Moncalvo & Vilgalys) and its green algal photobiont *Coccomyxa*, and found that fungal substitutions rates are much higher than those of the algae. They also found higher substitution rates for free living *Coccomyxa*, and for lineages in symbiotic associations with ascolichens in the

Peltigerales, suggesting that lichenization by itself does not necessarily constrain speciation the algae, but somehow only the ones partnering with the basidiomycete *Lichenomphalia*, indicating a strong selection for particular photobiont strains by these fungi-forming basidiomycete. This study sets the precedent for testing possible comparison between diversity of basidiolichens and their photobionts.

Given the limited amount of information about the distribution, diversity and symbiont specificity of *Rhizonema* generally, and the possibility that an association between *Dictyonema* and *Rhizonema* would result in especially low diversity of the *Rhizonema* photobiont, we set out to augment the photobiont sequence dataset of the previous work of Lücking et al. (2009a) to: (1) generate phylogenetic trees and networks to represent the quantity and frequency of haplotypes of the cyanobacterial genus *Rhizonema* of *Dictyonema* s.l., including some additional ascolichens; (2) discuss the possible reasons for differences in diversity between both partners; and (3) discuss the possibility of co-speciation, domestication and photobiont sharing, as has been done by previous authors.

Materials and methods

Taxon sampling

The dataset consisted of 560 specimens of *Acantholichen*, *Cyphellostereum*, *Cora, Corella* and *Dictyonema* species and 21 additional ascolichens containing *Rhizonema*, for a total of 581 specimens collected from 19 pantropical localities (**Tables 14** and **15**; for

detailed list of specimens included, please see **Table 21** at the end of the chapter). All of these were sequenced for both the mycobiont [M; nuclear ribosomal internal transcribed spacer (ITS)] and the photobiont [P; 16S ribosomal RNA (16S)] to add to existing sequence information from Lücking et al. (2009a), representing the diversity of the symbiotic relationships of these lichens. Our much enhanced sampling, 35 times more samples, was expected to better resolve the diversity and the phylogenetic relationships of the mycobiont (M) and the photobiont (P) for the clade. Sequences representing *Eonema pyriforme* were included as the fungal outgroup based on previous studies by Dal-Forno et al. (2013) and Lawrey et al. (2009).

Molecular Data

Genomic DNA was extracted from a piece of lichenized thallus using the Bio 101 Fast DNA Spin Kit for tissue (Qbiogen, Illkirch, France) according to the manufacturer's protocol with slight modifications (**Appendix 2**). PCR, clean up and Sanger sequencing methods are those of Dal-Forno et al. (2013) (also Chapter 2 of this dissertation). Most fungal sequences of the nuclear ribosomal internal transcribed spacer (ITS) were generated using Sanger sequencing. For that, the primers ITS5 and ITS 4 were used (White et al., 1990) (sequences of these primers in **Table 23** in **Appendix 3**), which represented the entire ITS region (ITS1, 5.8S and ITS2).

To obtain 16S data from the photobionts and also ITS from the mycobiont in cases where high quality sequences were not obtained through Sanger sequencing, we employed the pyrosequencing method (Gillevet and BioSpherex, 2006). We generated

additional representative ITS sequences for 44 fungal samples and a total of 1157 representative sequences for the photobiont 16S with a Roche 454 GS Junior following the methods of Lücking et al. (2014d) (**Appendix 7**), out of a total of approximately 750,000 reads (= sequences) obtained for all samples. The universal bacterial primers L27F and 355R were used for the 16S (Lane, 1991), which covers the initial, more variable region of the primer combination used by Lücking et al (2009a) for the photobiont. For the mycobiont, the primers ITS1F and ITS2 were used (Gardes and Bruns, 1993; White et al., 1990), which covers only the ITS1 and partial 5.8S of the ITS region. Both 454 sequencing runs (ITS and 16S) were usually performed separately to yield a higher number of reads, but since barcodes are different for the fungal and bacterial primer sets, we could also combine samples if needed into a single emulsion PCR and downstream 454 steps.

Data were analyzed by GS Junior System Software Package (Roche) with signal processing for amplicon option. Sequencing data from each pooled sample were sorted based on the barcodes (Gillevet and BioSpherex, 2006) using a custom PERL script (Gillevet et al., 2010). Thus, we were able to normalize each sample by the total number of reads from each barcode.

The 454 sequence data were then analyzed in Geneious 7.1.7 (http://www.geneious.com; Kearse et al., 2012). Each lichen specimen had hundreds to thousands of reads for each marker and the following protocol was applied to each set of sequences (ITS and 16S separately per lichen specimen): (1) Each file containing all ITS or 16S reads for one single lichen sample was dragged into Geneious and “Create

Sequence List" was selected. (2) A De Novo Assemble with High Sensitivity/slow option was performed generating a contig (ITS) or contigs (16S), which were then visualized overall. (3) A BLAST search was performed for the consensus sequence for each contig generated to confirm their identity as main mycobiont/photobiont. (4) When more than one contig was generated (mainly for the photobiont), comparisons of consensus sequences were made by a pairwise alignment. (5) A single sequence was selected that was identical to the consensus sequence for the contig with the most number of reads (contig 1, for photobiont called "primary"), which still represents a real read (= sequence), since GenBank repository do not allow for contig consensus sequences to be deposited. If a second contig was present with a high number of reads (varies according to each sample), a second sequence representing a real read was also selected for use (which we called "secondary"). (6) These DNA sequences were assembled in an Excel Spread sheet, where original barcodes are still available if necessary. An additional spread sheet is created with specimen information and the sequences to be used in down stream phylogenetic analyses.

This Geneious process allows for manual inspection of sequences representing the fungal or cyanobacterial taxa that is the most frequent read of that lichen specimen. When partial Sanger sequences were available, we compare both in Geneious and Sequencher, the later checking the chromatographs (only from Sanger sequencing).

For 16S bacterial sequences, before importing files into Geneious, we previously had subselected for cyanobacterial sequences only through a PERL script (Gillevet, unpublished data), which pulls information from the RDP10 database (Cole et al., 2014)

to select for cyanobacteria taxa only, with the objective to disregard non photobiont bacteria present in the sample.

For fungal ITS, a single most abundant sequence was apparent for each lichen sample. On the other hand, for most cyanobacterial 16S, two main contigs were apparent for each lichen sample most of the time. We therefore thought it was prudent to use both cyanobacterial sequences in phylogenetic analyses to acknowledge that there was natural variation captured by the 454 sequencing method. The most frequent cyanobacterial read was given the name "primary", while the second most frequent cyanobacterial read was given the name "secondary". For 38 lichen samples, however, a similar number of reads (±10 reads) were present in both contigs (**Table 16**).

Sequence alignment and phylogenetic analysis

New sequences from Sanger and 454 sequencing were aligned using MAFFT in GUIDANCE for the fungal ITS (Penn et al., 2010a, 2010b) and MAFFT for the bacterial 16S (Katoh et al., 2005 -- auto option, output sorted). The ITS dataset was broken down into three sub-datasets to improve alignment accuracy and make use of as many alignment columns as possible: (1) *Cyphellostereum,* (2) *Dictyonema* s.str. and (3) *Cora-Corella-Acantholichen* (**Tables 19** and **21**).

To assess the global phylogeny of the *Rhizonema* photobionts newly sequenced based on the 16S dataset, we downloaded 77 cyanobacterial 16S sequences from GenBank (numbers in the tree), including all specimens harboring *Rhizonema* from the study of Lücking et al. (2009a), and added 63 newly generated sequences (**Table 22**), for

a total of 140 samples. For this phylogenetic analysis, *Gloeobacter violaceus* and *G. kilaueensis* were used as outgroups following Miadlikowska et al. (2014).

The 16S data were arranged into two alignments, both with sequences generated by Sanger and 454 sequencing included, one in which all representative sequences were used in their complete length (= full-length alignment), and one in which the length used was representing only the shorter length of the 454 reads (= trimmed alignment). This separation was aimed to assess values support for a larger versus a smaller partial 16S rDNA and how that affects bootstrap values in the phylogenetic trees.

All alignments were subjected to maximum likelihood (ML) searches using RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2005) with nonparametric bootstrapping using 500 replicates under the GTRGAMMA model. RAxML was run in CIPRES Science Gateway V. 3.3 on XSEDE (Miller et al., 2010, 2011).

Phylogenetic trees were visualized, colored and saved as a PDF on FigTree (http://tree.bio.ed.ac.uk/software/figtree/), then edited in Adobe Illustrator and Photoshop CS5.1.

Mapping 16S as characters into an ITS tree

Once all newly generated 16S sequences were aligned in MAFFT as described above the sorted output file was first visualized on BioEdit (Hall, 1999) and then in Geneious (Kearse et al., 2012). Each sequence of the partial 16S in the alignment was given a haplotype number (1–132) (**Table 21**). This number was then added to the ITS name string in Excel, for the primary and secondary haplotypes, for example:

>MDF086_Dictyonema_bungartziana_Galapagos_pri_13_sec_30_ITS

This name string states that the lichen specimen with control number MDF086 is a *Dictyonema bungartziana* from Galapagos and it has as primary photobiont haplotype 13 and secondary haplotype 30. Of the 560 *Dictyonema* samples from which we generated 16S data, 495 had a corresponding ITS sequence available from the mycobiont. This same string was generated for each one of these 495 samples and the ITS sequence was added as well. The Excel spreadsheet was then transformed into a fasta file and an outgroup sequence was added. Three datasets with mycobiont sequences recognizing photobiont haplotypes were generated in total [(1) *Cyphellostereum,* (2) *Dictyonema*, and (3) *Cora-Corella-Acantholichen*], and subjected to alignment and phylogenetic reconstruction as described above.

Networks

In addition to manually creating and visualizing haplotypes in Geneious, networks were created in PopART (http://popart.otago.ac.nz) under a TCS network (Clement et al., 2000). The TCS software was created to estimate genealogical relationships among sequences using the method of Templeton et al. (1992). Networks were used in our dataset since sequences had very low levels of divergence. Two datasets were inserted into the program as a phylip file (Felsenstein, 1991). The first dataset had all 1157 sequences (**Table 15**), allowing us to assess not only the diversity of haplotypes but also their relative frequency. The 1157 correspond to 560 primary and 556 secondary photobiont sequences from *Dictyonema*, and 21 primary and 20 secondary photobiont sequences from non-*Dictyonema* samples (**Table 14**). Once 12 main haplotypes were

identified, we selected those and re-did the analysis to better visualize the relationships between them (second dataset). Network graphics were saved as a PDF and edited in Adobe Photoshop CS5.1.

Results

Photobiont Diversity

Our results demonstrate that all photobionts from specimens of *Dictyonema* s.l. and the 21 ascolichens included in this analysis belong to the cyanobacterial genus *Rhizonema.* Remarkably, all haplotypes recovered from our specimens (primary and secondary) fit phylogenetically into one of two clusters, which we are referring to as lineages. Since we used a limited, small region of the 16S, we opted for the term "lineages" instead of "species" since they could represent species complexes. Of these two lineages, *Rhizonema* 1 (royal blue in the trees) is by far the most common and *Rhizonema* 2 (brown in the trees) is second most common (**Figure 30A**).

Out of the 581 lichens sampled containing *Rhizonema*, 576 (~99.14%) contained a primary and a secondary photobiont haplotype with high numbers of reads. The two haplotypes usually found within a single lichen thallus only differed by a single nucleotide in the base pair number (bp#) 12 in the sequences gathered (base pair number 19 in *E. coli* counting, GenBank J01859, Ehresmann et al., 1972), which corresponds to the base ambiguity from the 27F primer (AGAGTTTGATC**M**TGGCTCAG, Lane, 1991). This nucleotide substitution is observed as a cytosine in one haplotype and an adenine in

another. This single difference indicated that haplotypes could be identical aside from bp#12, so we identified them as "combinations". Although these bp#12 variants do not represent different taxonomic units, we treated them as different haplotype combinations to demonstrate the actual sequence diversity in the dataset.

The frequencies of primary and secondary photobionts appear not to have any significance since they differ only by this single nucleotide substitution. Indeed, several specimens showed a similar number of reads of each haplotype (± 10 reads); however, we still referred to one as primary and the other as secondary for consistency in the dataset (**Table 16**). In all cases, primary and secondary belong to the same *Rhizonema* lineage (1 or 2), and both of them show this single nucleotide polymorphism (SNP) variation at the same location. This could have two possible explanations: (1) a systematic sequencing error or (2) a universal polymorphism in the *Rhizonema* across all species. A sequencing error is not likely since only a specific position is affected; the hypothesis of a universal polymorphism could be tested by looking at chromatograms of the Sanger sequences generated for the *Rhizonema* 16S; unfortunately, however, the sequences from Lücking et al. (2009a) do not cover that region. Nonetheless, *Rhizonema* sequences generated by Sanger sequencing by Lücking et al. (2009a) showed many base pairs ambiguities in several regions of the 16S rDNA, which may demonstrate the presence of multiple haplotypes within a single lichen thallus. Direct Sanger sequencing without cloning may hide this diversity, unlike pyrosequencing methods.

The 16S dataset generated by our 454 sequences (**Figure 30**) had an alignment of 317 bp length. Of these base pairs, 37 were variable sites, including 3 insertions, probably

due to sequencing errors since they followed a repeated base. In addition, 25 sites were unique substitutions observed only in a single sequence; and nine sites had frequent base pair substitutions. Substitution patterns at these nine sites were highly correlated with the species delimitation of the two lineages of *Rhizonema* (lineage 1 and lineage 2).

A total of 132 photobiont haplotypes were identified, with a clear pattern demonstrating that there are 12 most frequent haplotypes (**Table 17**), which we are calling "main" haplotypes. As outlined above, each haplotype had a corresponding "sister" haplotype (= combination) in which the only difference was the bp#12 position. We therefore considered them to be related and colored them the same shade. Altogether, therefore, we recognized 6 main combinations (= 12 haplotypes).

Within each lineage of *Rhizonema*, 1 and 2, many haplotype combinations were observed (**Figure 30B**). However, haplotypes representing both *Rhizonema* lineages were never found together within the same lichen thallus. Similarly, a lichen mycobiont (= fungal species) was never found to associate with photobionts representing both *Rhizonema* 1 and 2. This possibility could only be evaluated when there are several specimens available for the same lichen species, which is the case for 20 species represented by five or more specimens.

Combination 3 (purple) is the most frequent haplotype combination, with almost one quarter of all sequences belonging to it (23.34%), representing either haplotype 14 (12.27%) or 31 (11.06%). The differences among the combinations within lineage 1 is denoted by a star (*) in comparison to Combination 3 (**Table 18**), in the means of base pair substitutions. All together, they account for nearly half of all sequences (49.18%).

Rhizonema 2 is clearly less frequent than *Rhizonema* 1, accounting for only 22.9% of all sequences. Lineage 2 has a very unique, constant pattern in base pair substitutions across all haplotypes, which are the base pairs highlighted in bold in **Table 18** (bp#119 = T, bp#154 = A, bp#156 = A). This means that all haplotypes within lineage 2, 22 in total (**Table 17**), have those three base pairs different if compared to lineage 1. This consistency of base pair differences suggests that lineage 2 is a separate lineage of *Rhizonema.* Those three unique base pair substitutions are marked in **Figure 31B** as the differences (line marks) between both lineages.

16S haplotype networks

Our TCS network analyses (**Figures 31A** and **31B**) confirmed the possibility of two separated lineages of *Rhizonema*, first observed in Geneious and in the ML inferred 16S phylogenetic tree (**Figure 30A**). It is possible to see that all haplotypes are connected, but there are only four connections between both clusters (= lineages 1 and 2), with all four separated by two base differences, whereas almost all haplotypes within a cluster (= same lineage) have 1 base pair difference, with only a few unique haplotypes having more base differences (**Figure 31A**). The frequency of the haplotypes is directly related to the size of the circles (**Figure 31A**). It is possible to observe that *Rhizonema* 1 and 2 are two separate clusters, each with main haplotypes shown as larger circles, with several less frequent haplotypes shown as smaller circles connected. *Rhizonema* 1 has many more haplotypes than *Rhizonema* 2. The distribution of these haplotypes is based on a single haplotype difference in the main cluster region of lineage 1, containing all

major haplotypes within this lineage (towards the right in the image), while it has up to three base pair differences in the region without a main haplotype (towards the lower left portion in the image, all in royal blue). After identifying the main haplotypes, we ran again the network with only those 12 major haplotype sequences, to better visualize the number of base pairs differences (**Figure 31B**). The two *Rhizonema* lineages remain well separated, with all haplotypes within lineage 1 differering by a single nucleotide, while in lineage 2 they may also vary by two base pairs among them. Finally, the separation between both *Rhizonema* lineages can be visualized by the three line marks, which denotes the three consistent differences in base pairs mentioned above (**Table 18).** Since a single sequence was used for each haplotype in this second analysis, the size of the circles do not denote frequency in this network.

Cyanobacterial 16S phylogenetic trees

Cyanobacterial 16S phylogenetic trees were produced using two alignments, one with a full length of 768 bp and 140 sequences (called afterwards a full-length dataset) and a trimmed one with a length of 326 bp (corresponding to the length of our 454 reads) and 123 sequences (called afterwards a trimmed dataset). Since some sequences downloaded from GenBank included little or none of the 16S gene region recovered from the 454 dataset, 17 taxa were removed from the trimmed dataset, generating a slightly different number of sequences in the two trees. The phylogenies generated from these 16S datasets showed that all lichenized *Rhizonema* belong to a single monophyletic clade, with high support (92%) in the full-length dataset (**Figure 32A**), but with medium

support (68%) in the trimmed dataset (**Figure 32B)**. The tree recovered using the full-length dataset is more informative than the trimmed one, with generally higher support values. For example, the tree generated using the trimmed alignment recovered the clade containing *Fischerella* within *Nostoc,* making *Nostoc* paraphyletic, but it is monophyletic in the full-length dataset (without high support, however). The two lineages of *Rhizonema* do not form supported separate clades (**Figures 33A** and **33B**); nonetheless, lineage 2 is still recovered as a separate group with 49% and 61% bootstrap values in the full-length alignment and in the trimmed dataset, respectively. Haplotypes of both lineages cluster together with previously deposited sequences from Lücking et al. (2009a).

The various haplotypes and haplotype combinations observed earlier do not seem to be highly supported in either analysis (**Figures 33A** and **33B**). However, as observed in the networks (**Figure 33B)**, all haplotypes are separated by a single SNP, and the GTR model of substitution we used will not place much weight on a single base pair difference, especially since some of these substitutions are identical, differing only in position within the sequence.

In our efforts to test whether all *Rhizonema* species are entirely lichenized or not, an hypothesis first proposed by Lücking et al. (2009a), we attempted in our collecting trips to include any examples of free-living cyanobacteria that were morphologically similar to *Rhizonema* (**Figure 29**). There were seven specimens collected and these were included in the 16S sequencing and phylogenetic reconstructions. Of all free-living cyanobacteria collected, none was found to be *Rhizonema.* Instead, our phylogenies

indicated that five cluster with sequences of *Scytonema*, while two others seemed to form sister clades with either a lichenized *Stigonema* from *Ephebe* sp. or with *Gloeothece* and *Gloeocapsa* (**Figure 32A**).

16S haplotypes mapped into fungal ITS phylogenetic trees

The 16S photobiont haplotypes were mapped onto ITS phylogenies (one for each of the major clades in the *Dictyonema* s.l. phylogeny) obtained for the lichen mycobionts of 495 specimens. The *Cora* clade (containing sequences of *Cora*, *Corella* and *Acantholichen*) includes by far the most sequences, but has the shortest alignment length (882 bp) and highest GUIDANCE score (0.940855), suggesting less interspecific variation as compared to the other genus-level groups (**Table 19**). ITS phylogenetic trees (**Figures 34, 35**, **36A, 36B, 36C** and **37**) inferred by ML for the *Dictyonema* clade with the 16S haplotypes mapped into them show that most of the species across all three phylogenetic trees have lineage 1 of *Rhizonema* as the photobiont.

The *Cyphellostereum* specimens included in our analysis all have *Rhizonema* 1 as the photobiont (**Figure 34A**). The majority of the specimens within this genus seem to lack a dominant haplotype or haplotype combination (**Figure 34B)**, and the six specimens with one of the dominant haplotype combinations show only two of the possible six combinations. Two specimens have Comb. 3, three have Comb. 4, and one sample has haplotype 35 as primary with no secondary haplotype.

In the *Dictyonema* s.str. tree (**Figure 35**), both species of *Rhizonema* are represented, with lineage 1 being far more common than lineage 2. *Rhizonema* 2 appears

mostly in the *sericeum*-clade (in **Figure 35** as the top clade), in specimens collected in Bolivia, Ecuador, Colombia and Guatemala. Outside of the *D. sericeum* clade, only one specimen also harbors lineage 2, and this is *Dictyonema huaorani* Dal-Forno et al., a new species from Amazonian Ecuador which presumably has hallucinogenic properties (Schmull et al., 2014). This species is very closely related to the Puerto Rican species *D. pallidoschenkianum* (ined.) in the tree, but *D. pallidoschenkianum* has *Rhizonema* 1 and is morphologically very different from *D. huaorani.* For the *Cora*-clade (dataset for specimens of *Acantholichen*-*Cora*-*Corella*), there is much greater diversity of haplotypes representing both *Rhizonema* species (**Figures 36A, B, C and 37**). There is, however, a pattern demonstrating that the majority of specimens in one of the *Cora* clades (in **Figure 37** as Clade 1) have a photobiont belonging to *Rhizonema* lineage 2. Indeed, out of the 115 specimens of *Cora* in Clade 1, only five specimens (4.35%) do not have *Rhizonema* 2 as photobiont. These five specimens account for only two species (4.35%) out of the approximately 46 found in the clade. This indicates that *Cora* clade 1 has almost exclusively (over 95%) *Rhizonema* 2 as photobiont.

Similarly, specimens in the other major clade of *Cora* in the ITS tree (**Figure 37** as Clade 2) generally have a photobiont assignable to *Rhizonema* 1. Out of 173 specimens of *Cora* in Clade 2, only seven specimens (4.05%), representing four species (5.97%), have lineage 2 of *Rhizonema* as photobiont.

While *Cora* species from Clade 1 are mainly from the northern Andean region in Colombia, which corresponds to the “wet” paramos in our sampling, the *Cora* species

from Clade 2 are more spread out thorough the Neotropics, mainly in countries such as Brazil, Costa Rica, and Ecuador (mainland and the Galapagos Islands), among others.

Corella also shows interesting patterns of photobiont diversity, with a much more balanced number between *Rhizonema* lineages (**Figure 36A**). Out of the 30 specimens (ten species) included, 18 specimens (six species) have *Rhizonema* 2 and 12 specimens (four species) have *Rhizonema* 1. In terms of geographic distribution, two species from Costa Rica have lineage 1, three undescribed species from Colombia have lineage 2, and from the five species from Brazil, two have *Rhizonema* 1 and three have *Rhizonema* 2. Among these ten *Corella* species, three that harbor *Rhizonema* 2 have a single haplotype combination (Comb. 6). Remaining seven species harbor less frequent haplotypes.

We have only three species representing the genus *Acantholichen,* and two of them, both from Costa Rica, harbor *Rhizonema* 2. The other species, from the Galapagos, has *Rhizonema* 1 (**Figure 36A**).

An analysis of the distribution of the *Rhizonema* species by country (**Table 20**) shows that all ten species from Galapagos (all *Dictyonema* species known to occur in the islands, represented by 95 specimens) have lineage 1 as a photobiont. This lineage is also found in collections from Peru (9), Puerto Rico (8), Fiji (3), Canary Islands (1), Mascarene Islands (5), New Zealand (1) and the Philippines (4). This would suggest a much wider geographic distribution of *Rhizonema* 1 than *Rhizonema* 2, but it should be mentioned that we have fewer collections from most of these countries in our dataset, so our results could change with the incorporation of additional collections. Collections from Brazil, Colombia, Costa Rica, Ecuador, and Venezuela are relatively well-

represented and these have both photobiont species. Of these, only collections from Colombia and Venezuela, mainly northern Andean locations, have specimens predominantly with lineage 2, while the others have mainly lineage 1 as the photobiont.

It is notable that some fungal species are restricted to a single haplotype combination (information in **Table 21**). For example, all three specimens we have of *Dictyonema obscuratum* Lücking, Spielmann & Marcelli from Brazil (**Figure 35**) have Comb. 3 (haps. 14 and 31) as photobiont. However, most fungal species have more than one haplotype or haplotype combination as the photobiont. For example, *Dictyonema barbatum* Dal-Forno, Bungartz & Lücking from Galapagos has one of the highest numbers of different haplotypes, with 9 different haplotypes in 10 specimens sampled.

Discussion

The occurrence of possibly only two distinct *Rhizonema* lineages in the entire *Dictyonema* clade suggests strongly that mycobiont diversity in this group is far higher than photobiont diversity globally. This genus of cyanobacteria was first discovered by Lücking et al. (2009a), who included in their analysis 16 specimens of *Dictyonema* s.l. along with specimens representing various ascolichens which had previously been thought to contain photobionts of the genera *Chroococcus* and *Scytonema*. Since all non-lichenized samples with *Scytonema* morphology were found to belong to *Scytonema* or another cyanobacterial genus, as also confirmed here, Lücking et al. (2009a) speculated on the uniquely lichenized habit of *Rhizonema* to cause the relatively low genetic diversity of these cyanobacteria, which represents photobionts of all genera in

Dictyonema s.l. and in various ascolichens. Our study confirms their results with a far greater sampling of specimens. All of our 581 lichen specimens with suspected *Rhizonema* photobiont newly sampled (560 *Dictyonema* and 21 non-*Dictyonema*) were found to contain *Rhizonema* photobionts. Based on the limited resolution of the short 16S fragment sequenced, these denote only two major lineages representing either putative species with allelic variation or species complexes, a strikingly low diversity considering that the lichens they associate with are distributed among several continents and many clades, not necessarily closely related, including hundreds of species.

Due to the limited resolution of the sequenced fragment of the 16S, we are unable at this point to formally recognize actual species among *Rhizonema*, apart from the type species, *Rhizonema interruptum*, which is the photobiont of the European lichen fungus *Dictyonema coppinsii* (Lücking et al., 2014a). The full length phylogeny of *Rhizonema* based mostly on Sanger sequences obtained for the previous study Lücking et al. (2009a) suggests that more than two species might be involved, with our lineage 1 forming a paraphyletic grade composed of possibly several species including *R. interruptum* and also where our lineage 2 is nested. It should be noted that cyanobacteria can currently be described formally both under the ICN (International Code of Nomenclature for algae, fungi, and plants) and the bacterial code and particularly under the latter, species delimitation and nomenclature may be challenging and require additional studies, including culturing and physiological characterization. Prokaryote taxonomy under the bacterial code was initially based on morphological comparisons of axenical cultures with reference strains, but modern techniques have added molecular information to that

concept (Castenholz et al., 2001; Rippka et al., 1979). Hence, reliably delimiting species based on phylogeny requires preferably a multigene approach combined with a species-recognition method. This poses a challenge in this case since traditional Sanger sequencing often fails to obtain good sequence data especially from filamentous *Dictyonema* lichens containing several cyanobacterial strains and other bacteria, and next-generation sequencing makes a multigene approach difficult at this point. O'Brien et al. (2005) and Yahr et al. (2004) use the term "clade" to refer to groups of *Nostoc* and *Asterochloris*, respectively, found in lichen thalli (and free living in the first case). We opted to use the term "lineage", since these lineages (here called 1 and 2) could possibly be species complexes, clades, subspecies, etc.; in addition, lineage 1 forms a paraphyletic grade wheres lineage 2 is monophyletic.

As mentioned above, the low photobiont diversity observed here is in part a result of the short 16S fragment (ca. 314 bp of the 1,522 bp on average for the full length of this gene) we obtained for a marker that is considered to be relatively conserved (Woese and Fox, 1977). While this fragment is ideal for initial characterization of broad photobiont identity, as it allows for accurate alignment across many phylogenetic groups, it provides limited resolution at the species level within the target genus. It is therefore our goal to investigate additional markers used for cyanobacterial phylogenies, for example *rbcLX* (Miadlikowska et al., 2014; O'Brien, 2013a; O'Brien et al., 2005). However, as discussed above, this is likely to fail for many filamentous forms of *Dictyonema* s.l. while it will work better for foliose representatives of *Cora* and *Corella*. Another alternative is to amplify longer pieces of 16S, which remains the main genetic marker to infer

cyanobacterial phylogenies (Elvebakk et al., 2008; Miadlikowska et al., 2014; O'Brien, 2013; O'Brien et al., 2005; Svenning et al., 2005). Nevertheless, the fragment of 16S used here provided sufficient resolution to observe distinct phylogenetic structure in *Rhizonema*, allowing us to test various hypotheses on mycobiont-photobiont associations among lichens.

One of these hypotheses, now supported by results from many studies, is that lichen fungi exhibit extensive photobiont switching and sharing, not unlike the cultivation or domestication of crops by farmers. This has been observed frequently in both chlorolichens (Piercey-Normore and Deduke, 2011; Piercey-Normore and DePriest, 2001; Yahr et al., 2004, 2006), and cyanolichens (Lücking et al., 2009a; O'Brien et al., 2005). In all of these studies, different species of fungi, whether closely or only distantly related or not related at all, were observed to use the same strains of cyanobacteria or green algae, presumably by "sharing" or "stealing" them from other lichens in the same habitat. In most cases, this "sharing" will happen by photobionts becoming liberated from their original thallus by accidental fragmentation and then entering a new symbiosis with a different mycobiont. Such horizontal movement of photobionts among mycobionts results in phylogenies for the two symbionts that bear no obvious resemblance to each other, very different from cospeciation reported for other symbiotic systems, which yield congruent phylogenies, such as gophers and parasitic lice (Hafner and Page, 1995), figs and wasp pollinators (Weiblen and Bush, 2002) , gut bacteria of the acanthosomatid stinkbugs (Kikuchi et al., 2009), among others (Brooks, 1979; Downie and Gullan, 2005; Kawakita et al., 2004; Mueller and Gerardo, 2002; Weiblen and Bush, 2002).

The pioneering work of Piercey-Normore and DePriest (2001) that provided early phylogenetic evidence demonstrating that only a few algal genotypes were shared among different *Cladonia* species, implied that selective forces are very different for the two symbionts. The fungus may be selective about particular photobiont strains it partners with, whereas the photobiont may be selective at a much broader level (Piercey-Normore and DePriest, 2001). This could be reflecting the fact that the mycobiont may be the dominant partner of the relationship, and may be able to force the photobiont into the symbiosis. If the photobiont were highly selective as the mycobiont, such a biological system would not work.

Yahr et al. (2004) investigated photobiont diversity within eight co-occurring *Cladonia* species in rosemary scrubs in Florida. They found that there were three clades of *Asterochloris* (green algae) in the community, with several genotypes represented in each. They found some evidence for fungal selectivity, with six *Cladonia* species partnering with only a single clade (including several genotypes), and two species partnering with two clades. Their conclusion was that the *Cladonia* lichens they sampled had high specificity for algae in the genus *Asterochloris*, but they have a variety of selectivity patterns regarding the algal clades and genotypes they select, perhaps reflecting factors that may increase fitness of the lichen symbiosis. Low and high selectivity patterns by the fungus have been observed in many different groups of lichens (Lücking et al., 2009a; Magain and Sérusiaux, 2014; Miadlikowska et al., 2014; Muggia et al., 2011, 2013, 2014; Nelsen and Gargas, 2008; O'Brien et al., 2013; Piercey-Normore, 2004, 2006; Piercey-Normore and DePriest, 2001; Yahr et al., 2004).

Our results for the cyanolichen *Dictyonema* s.l. support the idea that lichen species vary in their photobiont selectivity. Some fungi are relatively generalized with regard to photobiont choices, others are intermediate and some appear to be relatively specialized, at least as defined by Yahr et al. (2004). All of our specimens formed associations with a single genus of photobiont, *Rhizonema*, with two lineages to choose from, *Rhizonema* 1 and *Rhizonema* 2 in cases where the two lineages are actually present in a geographic area or habitat, such as the Colombian paramo. No mycobiont associates with more than one lineage of photobiont, so each mycobiont species can be called a lineage specialist in terms of photobiont selection.

Certain species of *Dictyonema* s.l. species have a single haplotype combination as photobiont; i.e., the Brazilian *Cora terrestris* (ined., nine specimens, Combination 1), and the Colombian *Cora elephas* (ined., seven specimens, Combination 5). These would therefore be considered photobiont specialists according to Yahr et al. (2004). Among the intermediates, there is *Acantholichen galapagoensis* Dal-Forno, Bungartz & Lücking, represented by ten specimens, in which the main photobiont appears to be the Combination 3 (= haplotypes 14 and 31), but there are also two other haplotypes present (haplotypes 22 and 38). In the photobiont generalist category, there is *Dictyonema bungartziana* Dal-Forno, Yánez & Lücking, where the main photobiont found is Combination 2 (= haplotypes 13 and 30), but many more haplotypes can also be present (haplotypes 80, 82, 86, 88, 95, 102, and 112), for a total of nine different haplotypes in 29 specimens. While it is possible all of this is caused by differences in sample sizes representing these species, we have several examples of species represented by small

sample sizes that exhibit high diversity of haplotypes. For example, *D. barbatum* has the same number of haplotypes (9) than *D. bungartziana* in a much smaller sample size (10).

Mycobiont selectivity may be constrained by the pool of photobionts available at any given site. Yahr et al. (2004) measured the photobiont pool for each site they sampled, and all but one had all three clades of *Asterochloris* present. Nevertheless, some *Cladonia* species associate with only haplotypes from a single *Asterochloris* clade. This led them to conclude that, despite the availability of photobionts in the habitat, the *Cladonia* species were selecting haplotypes specifically from one clade and discriminating against the others. In our study, we did not thoroughly analyze all available photobiont pools at all locations, but we do have indications that selectivity occurs in *Dicytonema* lichens and that this varies from group to group. We have evidence that some locations harbor both lineages of *Rhizonema*, lineages 1 and 2, but lichens show evidence of associating with only one. For instance, Itatiaia National Park in Brazil has fungal species that associate mainly with *Rhizonema* 1; out of the 29 specimens collected from that locality, a single *Coccocarpia* thallus has *Rhizonema* 2 as photobiont, with all *Dictyonema* with *Rhizonema* 1. This may indicate a low availability in the photobiont pool of *Rhizonema* 2, which explain its absence in most lichens there. However, since we have not systematically collected other non-*Dictyonema* in that locality, it is hard to assess whether this is a sampling artifact or reflects the real photobiont diversity. Another *Coccocarpia* thallus was collected in the area and it has *Rhizonema* 1, which at least discards the possibility of genus specificity there. However, the Santuário do Caraça, another locality we sampled within the same Brazilian state

(Minas Gerais), harbors two co-occurring *Corella* species, one with *Rhizonema* 1 and one with *Rhizonema* 2. This would suggest that both photobiont species are available there but that the fungal species select one photobiont over the other.

Further evidence for selectivity comes from an analysis of the distribution of unique photobionts among lichens in the dataset. For example, all ten specimens of *Corella fuscoisidiata* (ined.) from Colombia have only Combination 6 (= haplotypes 52 and 61, *Rhizonema* lineage 2). On the other hand, *Corella brasiliensis* Vain. has four different haplotypes (haplotypes 22, 23, 38, and 39), none of which is a main haplotype, in seven specimens, but still all represent *Rhizonema* lineage 1. *Corella carassensis* (ined.) was collected at the type locality of *C. brasiliensis*, but has lineage 2 of *Rhizonema* (haplotypes 60 and 51). Notably, *C. carassensis* is morphologically practically indistinguishable from *C. brasiliensis*, but represents a phylogenetically distinct clade based on both Sanger and 454 sequences. In this case, cryptic speciation is supported by different photobiont selectivity for either lineage 1 or lineage 2 of *Rhizonema*. It is often argued that associated photobiont identity cannot be used to distinguish lichen fungal species since it is not a mycological character. However, the case of *C. brasiliensis* vs. *C. carassensis* highlights that photobiont selectivity is in fact a mycological character, likely depending on molecular wall receptors developed on the fungal hyphae.

In the genus *Cora*, photobiont specificity is even developed at a much higher level above species. *Cora* is the largest genus of basidiolichens, with over 100 species currently distinguished and over 400 predicted in total (Lücking et al., 2014c). The genus

is mostly neotropical, with one reported species from the Island of St. Helena, between Brazil and west Africa (Angola and Namibia) (Lücking et al., 2014e; Parmasto, 1978) and another, *Cora gyrolophia*, reported from Mauritius (east from Madagascar, Africa); however, there is some doubt about the latter since no additional or recent records of its presence have been reported since its discovery by Fries (1838; Lücking et al., 2013a).

While *Cora* is monophyletic, there is a distinct split into two major clades in multigene phylogenies which is also reflected by single-marker ITS phylogenies (Dal-Forno et al., 2013; Lawrey et al., 2009; Lücking et al., 2014c). Due to the lack of morphological and anatomical data to separate the two clades taxonomically, they are considered a single genus. One of these clades, here named Clade 1, is almost exclusively lichenized with *Rhizonema* lineage 2, whereas the other almost exclusively associates with lineage 1. In Clade 1, over 95% of the species harbor *Rhizonema* 2 as photobiont. In Clade 2, the opposite is true, with over 95% of the species having *Rhizonema* 1 as photobiont. This selection appears to be mostly driven by geography since the *Cora* Clade 1 associating with lineage 2 is largely restricted to the northern Andes in Colombia (with few species from other regions); however, several species from the same area but belonging to *Cora* Clade 2, which is otherwise widespread in the Neotropics, associate with *Rhizonema* lineage 1.

The high level of diversification in *Cora* Clade 1 could be associated with the fact that in Colombia, the Andes divide into three separate Cordilleras, thus providing more possibilities for allopatric speciation during their orogenesis. Instead, this new find suggests that actually the evolution of a novel photobiont strain could have triggered this

radiation. The northern Andes in Colombia, northern Ecuador, and western Venezuela are mainly characterize by wet paramo vegetation, whereas the central Andes in southern Ecuador, Peru and Bolivia harbor drier puna associations. Thus, a novel *Rhizonema* photobiont specifically adapted to this climate, together with the fragmented nature of paramo vegetation, could have triggered this regional radiation. This is also supported by the fact that *Rhizonema* 2 is nested within a paraphyletic *Rhizonema* 1 grade and hence clearly derived.

Divergence dating analyses show crown and stem ages for the *Cora* at approximately 10–15 million years ago (mya) (Lücking, 2012; Lücking et al., 2013b, 2014c), which corresponds with the range suggested for the initial uplift of the Andes (Gregory-Wodzicki, 2002; Meade and Conrad, 2008). If a recent radiation of *Cora* in the wet northern Andes was triggered by the evolution of a novel lineage of *Rhizonema*, one that was uniquely adapted to the new habitats being created there, the fungal partners would have subsequently diversified while the photobiont did not, since it was already evolved as optimal photobiont, resulting in less haplotypes of *Rhizonema* lineage 2 in comparison to lineage 1. This is indeed what we see in this clade: a lower diversity of mostly *Rhizonema* lineage 2 haplotypes associated with highly diverse *Cora* lichens from the northern Andes.

Remarkably, all seven *Cora* specimens in Clade 2 with *Rhizonema* lineage 2 are from Colombia (5), Costa Rica (1) and Ecuador (1) and are phylogenetically and morphologically distinct from their closest fungal relatives with *Rhizonema* lineage 1. We attribute this to photobiont sharing (= cyanobacterial switching) within the same

geographic region and habitat across the wet paramos of the northern Andes, suggesting that these *Cora* lineages from clade 2 colonized this habitat subsequently to clade 1 and successfully established due to their ability to switch their main photobiont type from lineage 1 to lineage 2.

Overall our results suggest a low photobiont lineage diversity within *Rhizonema* photobionts associated with *Dictyonema* s.lat. lichenized fungi. *Rhizonema* 1 is widely distributed among all mycobiont clades and is universally found in *Cyphellostereum* species and almost all *Dictyonema* species, and *Rhizonema* 2 is found scattered in *Dictyonema* and frequently in *Cora* and *Corella*. Due to the low sampling of *Acantholichen*, it is hard to assess its photobiont lineage distribution. These trends lend support to our hypothesis of *Rhizonema* 2 being a derived lineage, since between *Cyphellostereum* and *Cora* there is a clear pattern from phylogenetically and morphologically primitive to advanced lichens lineages (Dal-Forno et al., 2013). Haplotype diversity is also greater in *Rhizonema* lineage 1 than in *Rhizonema* lineage 2, which would again suggest more recent origin of *Rhizonema* lineage 2, as also supported by the nested position of lineage 2 within lineage 1. Further evidence comes from the fact that *Rhizonema* 1 is found in ascolichens and in lichens outside of the Neotropics, whereas *Rhizonema* 2 seems absent from these lichens. For example, *Rhizonema* is commonly found in ascolichens such as *Coccocarpia*, *Erioderma* and *Stereocaulon* (Lücking et al., 2009a) suggesting the possibility that many more cyanolichens, especially those thought previously to associate with *Scytonema* photobionts, could potentially harbor *Rhizonema.* Muggia et al. (2011) demonstrated that the ascolichen

genus *Polychidium* (Ach.) S. F. Gray was polyphyletic and resurrected the genus *Leptogidium* Nyl. to include species previously identified as *Polychidium* that supposedly have a *Scytonema* photobiont. However, the three specimens of *Leptogidium* we included in our analyses contain a *Rhizonema* photobiont belonging to lineage 1 and not *Scytonema* as presumed (Henssen, 1963; Muggia et al., 2011). Notably, in *Leptogidium* the fungal cells are shaped like interlocking puzzle pieces (Muggia et al., 2011), strongly resembling cells of the fungal sheath in *Dictyonema* s.l. (Dal-Forno et al., 2013; Lücking et al., 2013a; Oberwinkler, 1970, 1984; Parmasto, 1978), which explains why these entirely unrelated lichens are often confused in the field.

The genus name *Rhizonema* and its family, Rhizonemataceae, were only recently validly published to address the need for a name for this apparently widespread but previously unrecognized photobiont. The type species, *R. interrumptum*, is from Ireland (Lücking et al., 2014a). We do not have sequence data for the type material (photo- or mycobiont), but we do have ITS and 16S data for a specimen listed as a paratype (*Ertz 10475*), a specimen from Madeira. The sequence was generated with Sanger sequencing (Lücking et al., 2009a) and it is much longer than the ones we generated with the 454 GS Junior. However, we are able to establish that *Dictyonema coppinsii*, the mycobiont of *R. interruptum*, has *Rhizonema* 1.

Given that the fungal partners in *Dictyonema* s.l. represent hundreds of species and that *Rhizonema* is also the photobiont of many species of ascolichens, the overall genetic diversity of the photobionts is orders of magnitude lower than the diversity of the fungal partners. The remarkable difference suggests that photobiont species may be under

strong selection caused by lichenization. Unlike *Nostoc* and other cyanobacteria that can be found both in lichens and in a free-living state, *Rhizonema* is thus far only known to be lichenized. This could explain the observed low genetic diversity as a result of domestication, retaining over evolutionary time only a narrow range of physiologically optimized strains that live in the contained environment of a lichen thallus. Cyanobacteria that are frequently found in the free-living state, however, are expected to adapt to a much broader range of habitats and hence should be genetically more diverse. Thus, as with highly domesticated crops, *Rhizonema* depends ultimately on another species to survive and persist, and strong selection by this other species determines what evolutionary trajectory it can take. Trevor Goward (1994) has made this point before, saying that "*lichens are fungi that have taken up agriculture*", and the idea has also been applied to the *Rhizonema* lineage by Lücking et al. (2009a). Our data provides further support for this hypothesis, which appears the best explanation we can give for the extraordinarily low diversity of the *Rhizonema* lineage, while on the other hand representing the most successful cyanobacterial photobiont in lichens in terms of the phylogenetic diversity of its associated mycobionts.

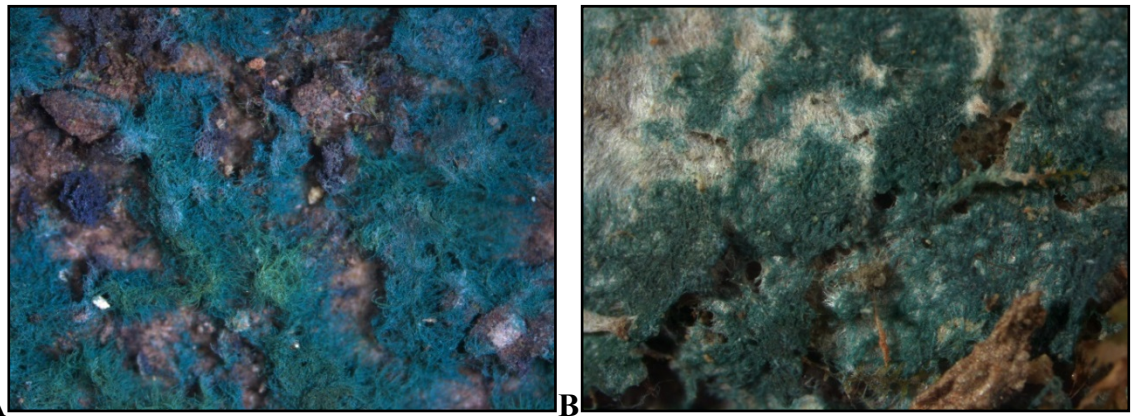


Figure 29 - Comparison of free living *Scytonema* MDF082 (A) and lichenized *Rhizonema* in *Cyphellostereum imperfectum* (B)

Table 14 - Summary of specimens, and ITS and 16S sequences by country (outgroups not included)
The number of 16S sequences should be approximately twice, since each specimen had a primary and a secondary photobiont 16S sequence (all, except five), in which we are calling haplotypes. Note that only *Dictyonema* samples are included in the ITS count. We did not generate ITS sequences for the ascolichens

Country	Lichen specimens	(M) Non *Dictyonema*	*Dictyonema* clade (M)	ITS (M) available	ITS (M) missing	16S (P) sequences
Bolivia	21	0	21	19	2	42
Brazil	99	10	89	70	19	196
Canary Islands (Tenerife)	1	0	1	1	0	2
Chile	1	0	1	1	0	2
Colombia	154	0	154	139	15	307
Costa Rica	61	2	59	56	3	121
Ecuador (continental)	104	6	98	90	8	208
Fiji	3	0	3	2	1	5
Galapagos Islands	98	3	95	85	10	196
Guatemala	2	0	2	2	0	4
Mascarene Islands (La Reunión)	5	0	5	5	0	10
Mexico	1	0	1	1	0	2
New Zealand	1	0	1	1	0	2
Panama	1	0	1	0	1	2
Peru	9	0	9	7	2	18
Philippines	4	0	4	3	1	8
Puerto Rico	8	0	8	7	1	16
Thailand	1	0	1	0	1	2
Venezuela	7	0	7	6	1	14
Total = 19	581	21	560	495	65	1157

Table 15 - Summary of sequences used per genus (outgroups not included). The number of 16S sequences should be approximately twice, since each specimen had a primary and a secondary photobiont 16S sequence (all, except five)

	ITS	16S primary	16S secondary
***Dictyonema* clade**	**495**	**560**	**556**
Acantholichen	12	14	14
Cora	288	314	313
Corella	30	35	35
Cyphellostereum	23	25	23
Dictyonema	142	172	171
Others	**N/A**	**21**	**20**
Coccocarpia	N/A	16	16
Erioderma	N/A	2	2
Leptogidium	N/A	3	2
Subtotal =	**495**	**581**	**576**
Total =	**495**	**1157**	

Table 16 - Samples with same or similar number of reads (± 10 reads) for primary secondary photobiont frequency Samples represented by their control number, which is listed in Table 21

DIC116, 126, 131, 134, 217, 349, 422, 470
MDF046a, 268, 283, 286, 290, 324, 325, 348, 350, 367, 415, 418, 423, 435, 443, 447, 450, 464, 465, 480, 510, 522, 526, 536, 543, 602, 617, 625, 630, 634

Table 17 - Haplotype diversity and frequency among the 581 lichens sampled
Hap = haplotype number. Spec = number of sequences having that haplotype as primary or secondary photobiont.
Royal blue refers to all less frequent haplotypes within lineage 1, while brown for less frequent haplotypes in lineage 2.
All additional colors represent six main haplotypes combinations

Hap	Spec
001	1
002	3
003	50
004	10
005	1
006	1
007	3
008	51
009	1
010	2
011	10
012	1
013	76
014	142
015	1
016	3
017	1
018	4
019	27
020	1
021	4
022	10
023	1
024	7
025	1
026	1
027	1
028	1
029	2
030	67
031	128
032	1
033	3
034	4
035	28
036	1
037	4
038	13
039	4
040	10
041	1
042	1
043	1
044	3
045	2
046	1
047	95
048	10
049	9
050	4
051	8
052	40
053	1
054	1
055	91
056	10
057	1
058	1
059	7
060	8
061	39
062	1
063	4
064	2
065	2
066	1
067	1
068	1
069	4
070	1
071	5
072	1
073	1
074	1
075	1
076	6
077	1
078	2
079	1
080	3
081	3
082	7
083	10
084	1
085	4
086	4
087	5
088	1
089	3
090	1
091	2
092	1
093	1
094	1
095	8
096	3
097	1
098	1
099	2
100	1
101	4
102	2
103	1
104	1
105	1
106	2
107	3
108	1
109	2
110	1
111	1
112	1
113	2
114	1
115	1
116	1
117	3
118	1
119	1
120	1
121	1
122	1
123	2
124	1
125	1
126	1
127	1
128	1
129	1
130	1
131	1
132	1
TOT=	1157

Table 18 - Summary of haplotypes present in the dataset
Comb = combination. Comb perc = combination percentage. Hap = Haplotype. # = number of sequences with that haplotype. Freq perc = Frequency of the haplotype, shown in percentage towards the entire dataset (n=1157). * denotes for differences in comparison to other combinations within the same lineage

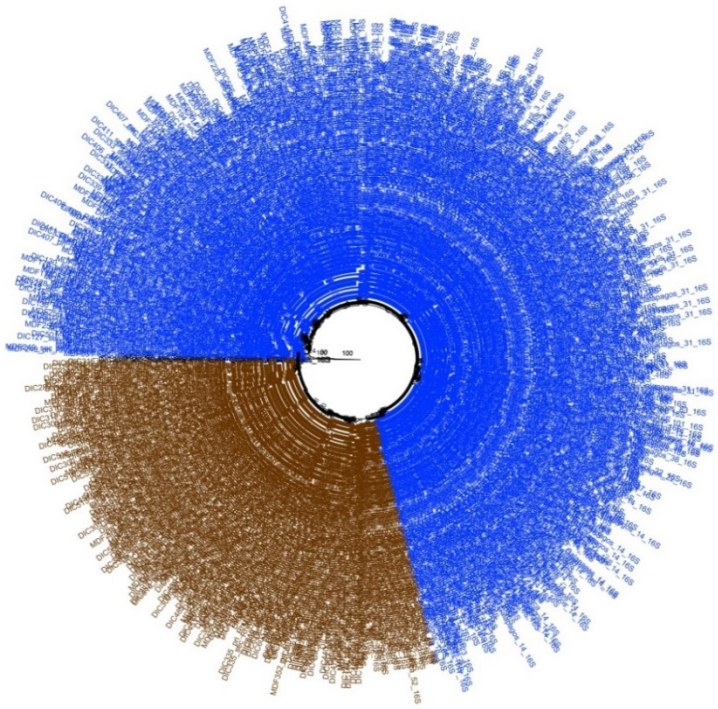
Comb	Comb perc	Hap	#	Freq perc	Differences in base pairs					
		Lineage 1 – main haplotypes = 49,18%								
Comb. 1	8,73%	003	50	4,32%	12 = A	69 = A*	224 = A	225 = A		
		008	51	4,41%	12 = C	69 = A*	224 = A	225 = A		
Comb. 2	12,36%	013	76	6,57%	12 = C	69 = C	224 = A	225 = G*		
		030	67	5,79%	12 = A	69 = C	224 = A	225 = G*		
Comb. 3	23,34%	014	142	12,27%	12 = C	69 = C	224 = A	225 = A		
		031	128	11,06%	12 = A	69 = C	224 = A	225 = A		
Comb. 4	4,75%	019	27	2,33%	12 = C	69 = C	224 = G*	225 = A		
		035	28	2,42%	12 = A	69 = C	224 = G*	225 = A		
		Lineage 2 – main haplotypes = 22,90%								
Comb. 5	16,08%	047	95	8,21%	12 = C	66 = T*	109 = A*	**119 = T**	**154 = A**	**156 = A**
		055	91	7,87%	12 = A	66 = T*	109 = A*	**119 = T**	**154 = A**	**156 = A**
Comb. 6	6,83%	052	40	3,46%	12 = C	66 = C	109 = G	**119 = T**	**154 = A**	**156 = A**
		061	39	3,37%	12 = A	66 = C	109 = G	**119 = T**	**154 = A**	**156 = A**

. Table 19 - Alignment information for each ITS datasets

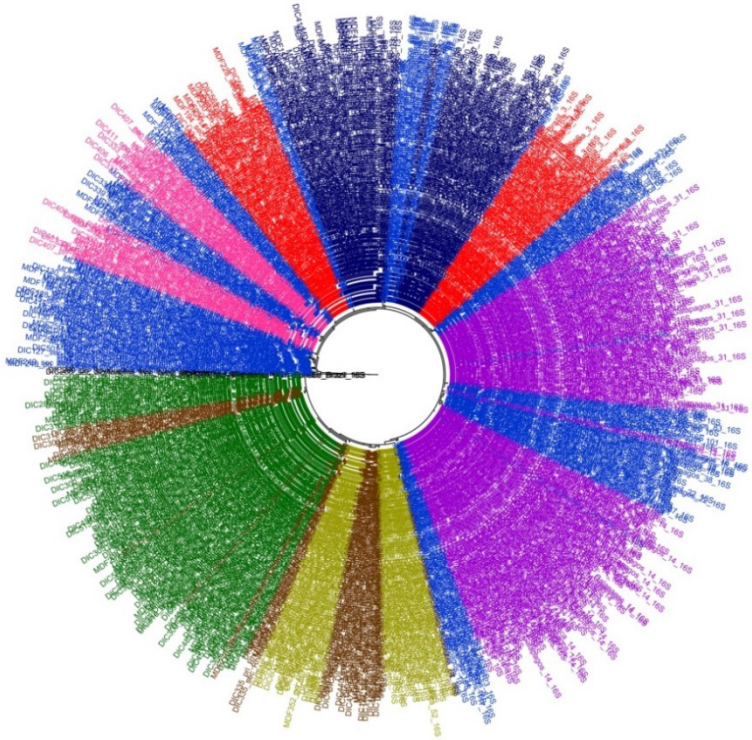
	# Specimens	MSA	Longer sequence	GUIDANCE score
Cora-clade	330	882	717	0.940855
Cyphellostereum	23	856	741	0.810703
Dictyonema s.str.	142	967	752	0.849136

Table 20 - Information about *Rhizonema* species present in each locality. Bold information denotes focused collecting

Country	Lichen specimens	*Rhizonema* present
Bolivia	21	Both
Brazil	**99**	**Both, mainly lineage 1**
Canary Islands	1	Lineage 1
Chile	1	Lineage 2
Colombia	**154**	**Both, mainly lineage 2**
Costa Rica	**61**	**Both, mainly lineage 1**
Ecuador (continental)	**104**	**Both, mainly lineage 1**
Fiji	3	Lineage 1
Galapagos Islands	**98**	**Lineage 1**
Guatemala	2	Lineage 2
Mascarene Islands	5	Lineage 1
Mexico	1	Lineage 2
New Zealand	1	Lineage 1
Panama	1	Lineage 1
Peru	9	Lineage 1
Philippines	4	Lineage 1
Puerto Rico	8	Lineage 1
Thailand	1	Lineage 1
Venezuela	7	Both, mainly lineage 2
Total = 19	581	



A



B

Figure 30 - 16S rDNA phylogenetic tree inferred by ML in RAxML. Polar view
A - Colors royal blue and brown showing the two lineages of *Rhizonema* (1and 2, respectively). B- The different combination of haplotypes within each lineage. The colors brown and royal blue here denote the lack of a dominant haplotype

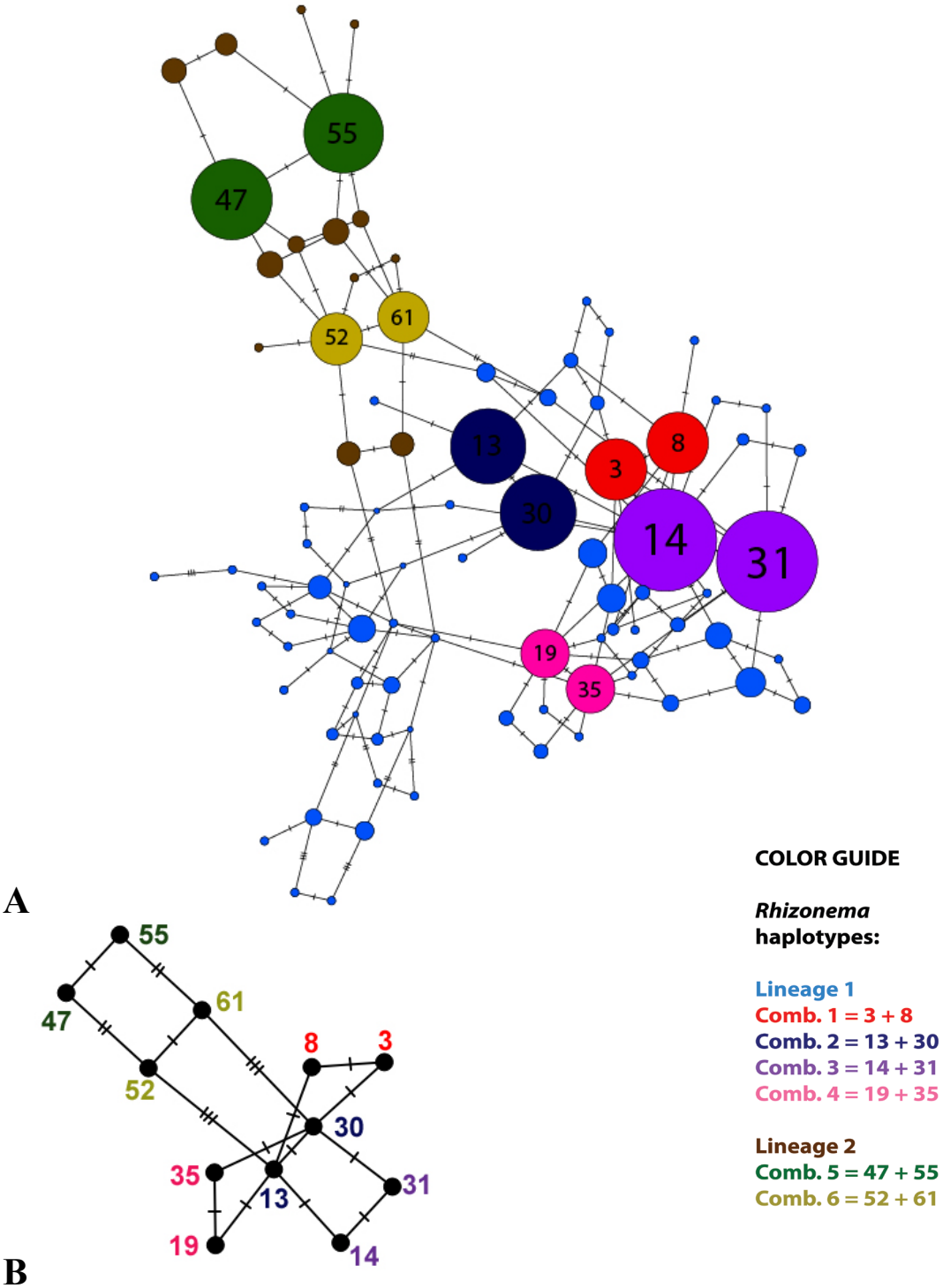
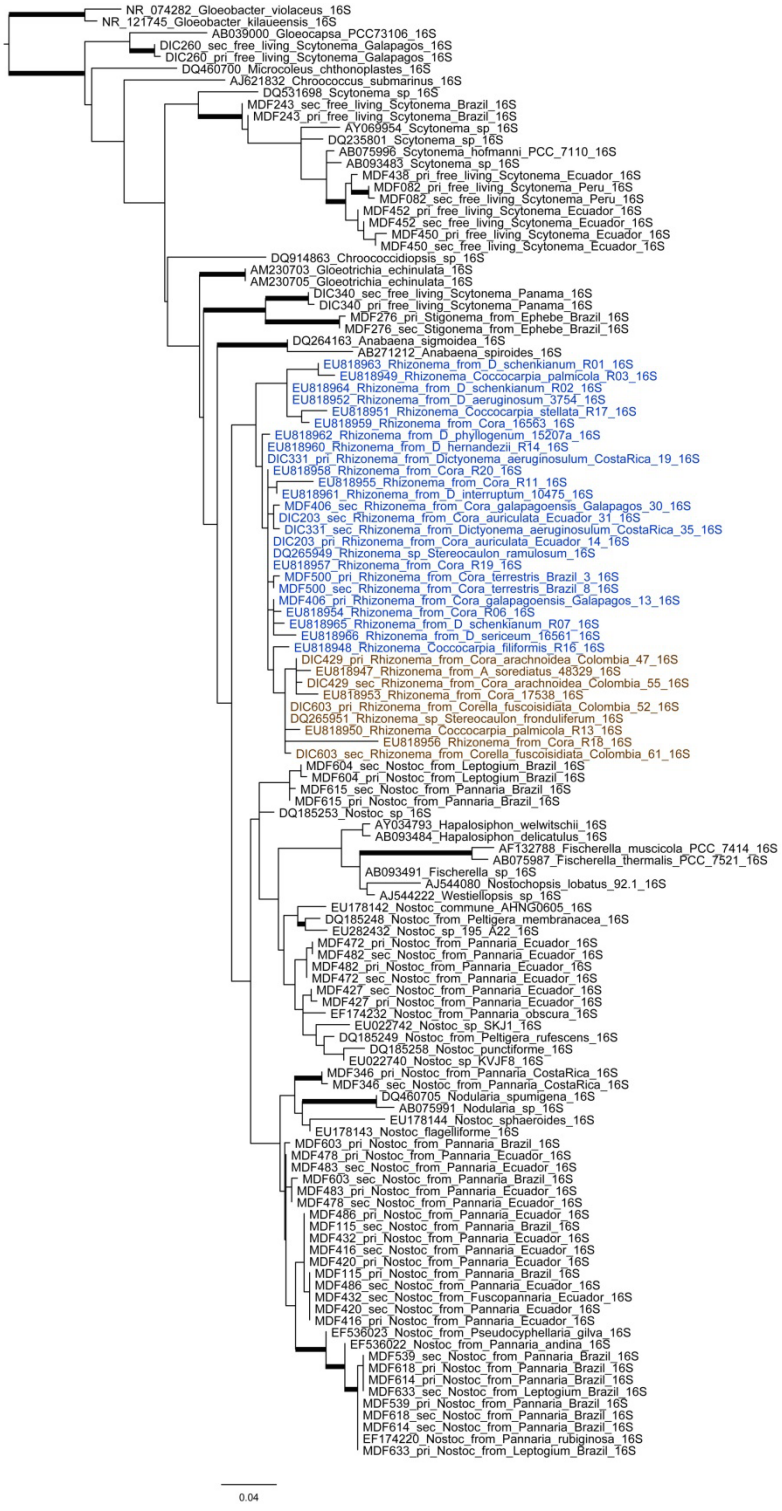


Figure 31 - TCS Networks showing the haplotypes of *Rhizonema* based on partial 16S rDNA
A- All haplotypes and their frequency on PopART. B- Simplified network ran on PopART showing only the main haplotypes relationship. All haplotypes in lineage 1 are only separated by a single base difference (line mark). All haplotypes combinations are separated by a SNP in bp#12 (in both lineages)


NR_074282_Gloeobacter_violaceus_16S
NR_121745_Gloeobacter_kilaueensis_16S
AJ871987_Microcoleus_steenstrupii_16S
DQ460700_Microcoleus_chthonoplastes_16S
DQ914863_Chroococcidiopsis_sp_16S
DQ072918_Gloeothece_sp_CrN_V_P4_16S
AJ621832_Chroococcus_submarinus_16S
AY422695_Gloeothece_sp_Bad_Sachsa_16S
AB039000_Gloeocapsa_PCC73106_16S
DIC260_sec_free_living_Scytonema_Galapagos_16S
DIC260_pri_free_living_Scytonema_Galapagos_16S
AF334698_Scytonema_hyalinum_clone_16S
AF334700_Scytonema_hyalinum_clone_16S
AY790835_Scytonema_sp_CCMEE5099_16S
DQ531698_Scytonema_sp_16S
MDF243_sec_free_living_Scytonema_Brazil_16S
MDF243_pri_free_living_Scytonema_Brazil_16S
EU818967_Scytonema_sp_Las_Cruces_R09_16S
AB075996_Scytonema_hofmanni_PCC_7110_16S
AB093483_Scytonema_sp_16S
AY069954_Scytonema_sp_16S
DQ235801_Scytonema_sp_16S
MDF438_pri_free_living_Scytonema_Ecuador_16S
MDF082_pri_free_living_Scytonema_Peru_16S
MDF082_sec_free_living_Scytonema_Peru_16S
MDF452_pri_free_living_Scytonema_Ecuador_16S
MDF452_sec_free_living_Scytonema_Ecuador_16S
MDF450_pri_free_living_Scytonema_Ecuador_16S
MDF450_sec_free_living_Scytonema_Ecuador_16S
EU818962_Rhizonema_from_D_phyllogenum_15207a_16S
EU818960_Rhizonema_from_D_hernandezii_R14_16S
DIC331_sec_Rhizonema_from_Dictyonema_aeruginosulum_CostaRica_35_16S
DIC331_pri_Rhizonema_from_Dictyonema_aeruginosulum_CostaRica_19_16S
EU818958_Rhizonema_from_Cora_R20_16S
MDF406_pri_Rhizonema_from_Cora_galapagoensis_Galapagos_13_16S
MDF500_sec_Rhizonema_from_Cora_terrestris_Brazil_8_16S
DQ265949_Rhizonema_sp_Stereocaulon_ramulosum_16S
DIC203_pri_Rhizonema_from_Cora_auriculata_Ecuador_14_16S
EU818950_Rhizonema_Coccocarpia_palmicola_R13_16S
DIC603_pri_Rhizonema_from_Corella_fuscoisidiata_Colombia_52_16S
EU818956_Rhizonema_from_Cora_R18_16S
DQ265951_Rhizonema_sp_Stereocaulon_fronduliferum_16S
DIC603_sec_Rhizonema_from_Corella_fuscoisidiata_Colombia_61_16S
EU818947_Rhizonema_from_A_sorediatus_48329_16S
DIC429_sec_Rhizonema_from_Cora_arachnoidea_Colombia_55_16S
DIC429_pri_Rhizonema_from_Cora_arachnoidea_Colombia_47_16S
EU818953_Rhizonema_from_Cora_17538_16S
EU818955_Rhizonema_from_Cora_R11_16S
DIC203_sec_Rhizonema_from_Cora_auriculata_Ecuador_31_16S
EU818957_Rhizonema_from_Cora_R19_16S
MDF500_pri_Rhizonema_from_Cora_terrestris_Brazil_3_16S
EU818948_Rhizonema_Coccocarpia_filiformis_R16_16S
EU818961_Rhizonema_from_D_interruptum_10475_16S
EU818966_Rhizonema_from_D_sericeum_16561_16S
MDF406_sec_Rhizonema_from_Cora_galapagoensis_Galapagos_30_16S
EU818954_Rhizonema_from_Cora_R06_16S
EU818965_Rhizonema_from_D_schenkianum_R07_16S
EU818959_Rhizonema_from_Cora_16563_16S
EU818951_Rhizonema_Coccocarpia_stellata_R17_16S
EU818963_Rhizonema_from_D_schenkianum_R01_16S
EU818949_Rhizonema_Coccocarpia_palmicola_R03_16S
EU818952_Rhizonema_from_D_aeruginosum_3754_16S
EU818964_Rhizonema_from_D_schenkianum_R02_16S
AY452533_Rexia_erecta_CAT_1M_16S
HE797727_Coleodesmium_sp_HINDAK_2000_24_16S
FR822754_Hassallia_antarctica_16S
MDF276_pri_Stigonema_from_Ephebe_Brazil_16S
MDF276_sec_Stigonema_from_Ephebe_Brazil_16S
DIC340_sec_free_living_Scytonema_Panama_16S
DIC340_pri_free_living_Scytonema_Panama_16S
AF132788_Fischerella_muscicola_PCC_7414_16S
AB075987_Fischerella_thermalis_PCC_7521_16S
EF545603_Mastigocladus_sp_CR_11M_16S
AY034793_Hapalosiphon_welwitschii_16S
AB093484_Hapalosiphon_delicatulus_16S
AB093491_Fischerella_sp_16S
AJ544080_Nostochopsis_lobatus_92.1_16S
AJ544222_Westiellopsis_sp_16S
AM230703_Gloeotrichia_echinulata_16S
AM230705_Gloeotrichia_echinulata_16S
AB075991_Nodularia_sp_16S
DQ460705_Nodularia_spumigena_16S
DQ264163_Anabaena_sigmoidea_16S
AB271212_Anabaena_spiroides_16S
KM005735_Nostoc_from_Peltigera_hydrothyria_P1492_16S
KM005733_Nostoc_from_Peltigera_gowardii_P1429_16S
KM005734_Nostoc_from_Peltigera_aquatica_P1430_16S
DQ185253_Nostoc_sp_16S
MDF615_pri_Nostoc_from_Pannaria_Brazil_16S
MDF615_sec_Nostoc_from_Pannaria_Brazil_16S
MDF604_pri_Nostoc_from_Leptogium_Brazil_16S
MDF604_sec_Nostoc_from_Leptogium_Brazil_16S
DQ185248_Nostoc_from_Peltigera_membranacea_16S
EU282432_Nostoc_sp_195_A22_16S
MDF482_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF472_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF472_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF482_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF427_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF427_sec_Nostoc_from_Pannaria_Ecuador_16S
EF174232_Nostoc_from_Pannaria_obscura_16S
AY577535_Nostoc_lichenoides_CNP_AK1_16S
AY577537_Nostoc_desertorum_CM1_VF14_16S
EU022740_Nostoc_sp_KVJF8_16S
DQ185258_Nostoc_punctiforme_16S
EU022742_Nostoc_sp_SKJ1_16S
EU178142_Nostoc_commune_AHNG0605_16S
DQ185249_Nostoc_from_Peltigera_rufescens_16S
MDF346_sec_Nostoc_from_Pannaria_CostaRica_16S
MDF346_pri_Nostoc_from_Pannaria_CostaRica_16S
EU178144_Nostoc_sphaeroides_16S
EU087570_Nostoc_sp_CCMEE_6160_16S
EU178143_Nostoc_flagelliforme_16S
MDF603_pri_Nostoc_from_Pannaria_Brazil_16S
MDF483_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF478_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF603_sec_Nostoc_from_Pannaria_Brazil_16S
MDF483_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF478_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF115_pri_Nostoc_from_Pannaria_Brazil_16S
MDF486_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF432_sec_Nostoc_from_Fuscopannaria_Ecuador_16S
MDF420_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF416_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF486_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF115_sec_Nostoc_from_Pannaria_Brazil_16S
MDF420_pri_Nostoc_from_Pannaria_Ecuador_16S
MDF416_sec_Nostoc_from_Pannaria_Ecuador_16S
MDF432_pri_Nostoc_from_Pannaria_Ecuador_16S
EF536023_Nostoc_from_Pseudocyphellaria_gilva_16S
EF536022_Nostoc_from_Pannaria_andina_16S
EF174220_Nostoc_from_Pannaria_rubiginosa_16S
MDF539_sec_Nostoc_from_Pannaria_Brazil_16S
MDF633_sec_Nostoc_from_Leptogium_Brazil_16S
MDF614_pri_Nostoc_from_Pannaria_Brazil_16S
MDF618_pri_Nostoc_from_Pannaria_Brazil_16S
MDF633_pri_Nostoc_from_Leptogium_Brazil_16S
MDF614_sec_Nostoc_from_Pannaria_Brazil_16S
MDF618_sec_Nostoc_from_Pannaria_Brazil_16S
MDF539_pri_Nostoc_from_Pannaria_Brazil_16S
0.04

A



B

Figure 32 - 16S rDNA phylogenetic trees inferred by ML in RAxML
A- Full lenght alignment (768bp and 140 specimens). B- Alignment shortened to match only the partial region of 16S rDNA we had sequence from 454 (326bp and 123 specimens). Both *Rhizonema* lineages are presented by the colors royal blue (1) and brown (2)

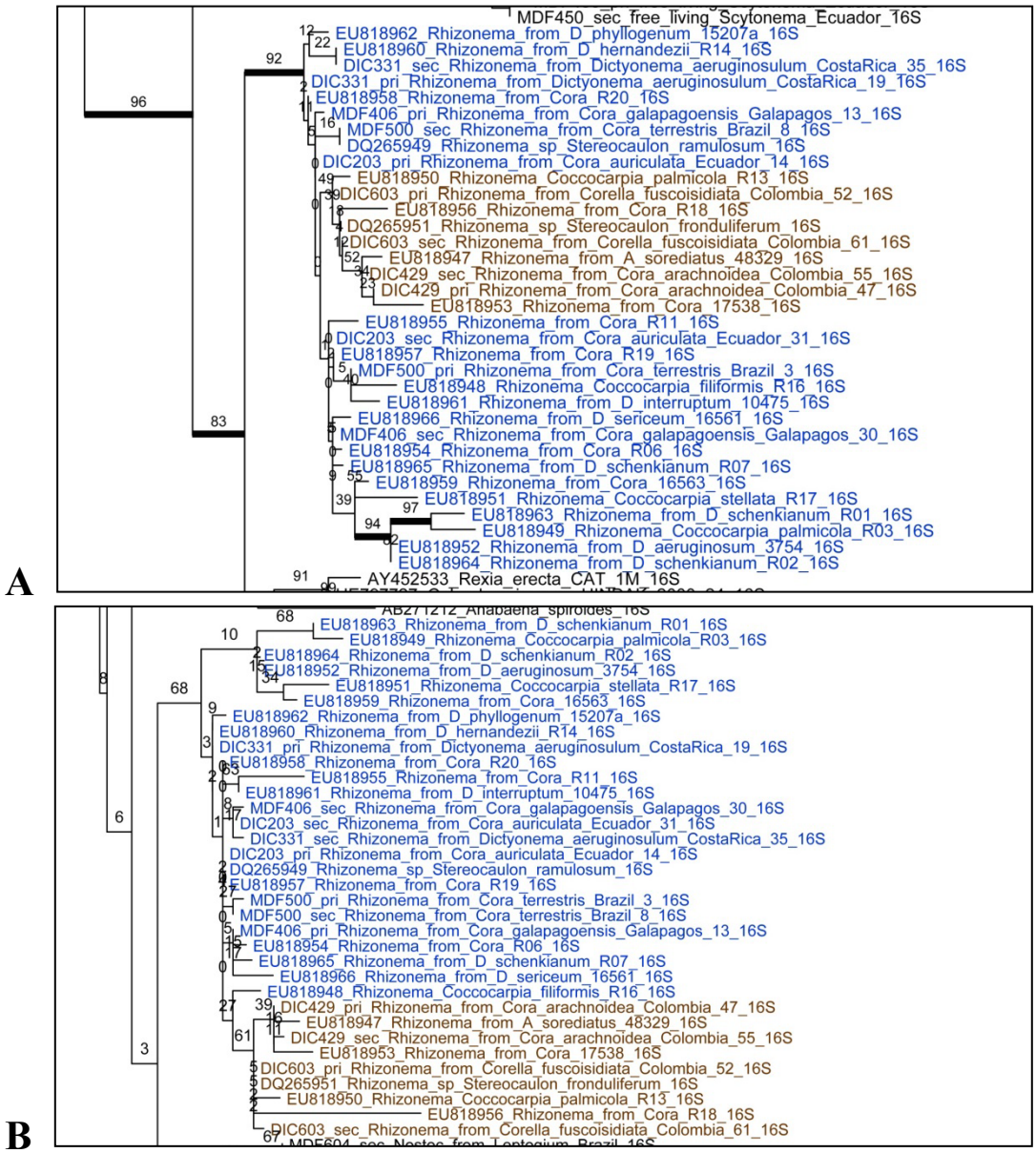


Figure 33 - Close up from the *Rhizonema* clade from Figure 32, but showing all bootstrap values
16S rDNA phylogenetic trees inferred by ML in RAxML. A- Full-length alignment (768bp and 140 specimens). B- Alignment shortened to match the length of the partial region of the 16S rDNA we had sequence from 454 (326bp, 123 specimens, including 34 *Rhizonema*). Bootstrap values ≥ 70 are represented by thickened branches

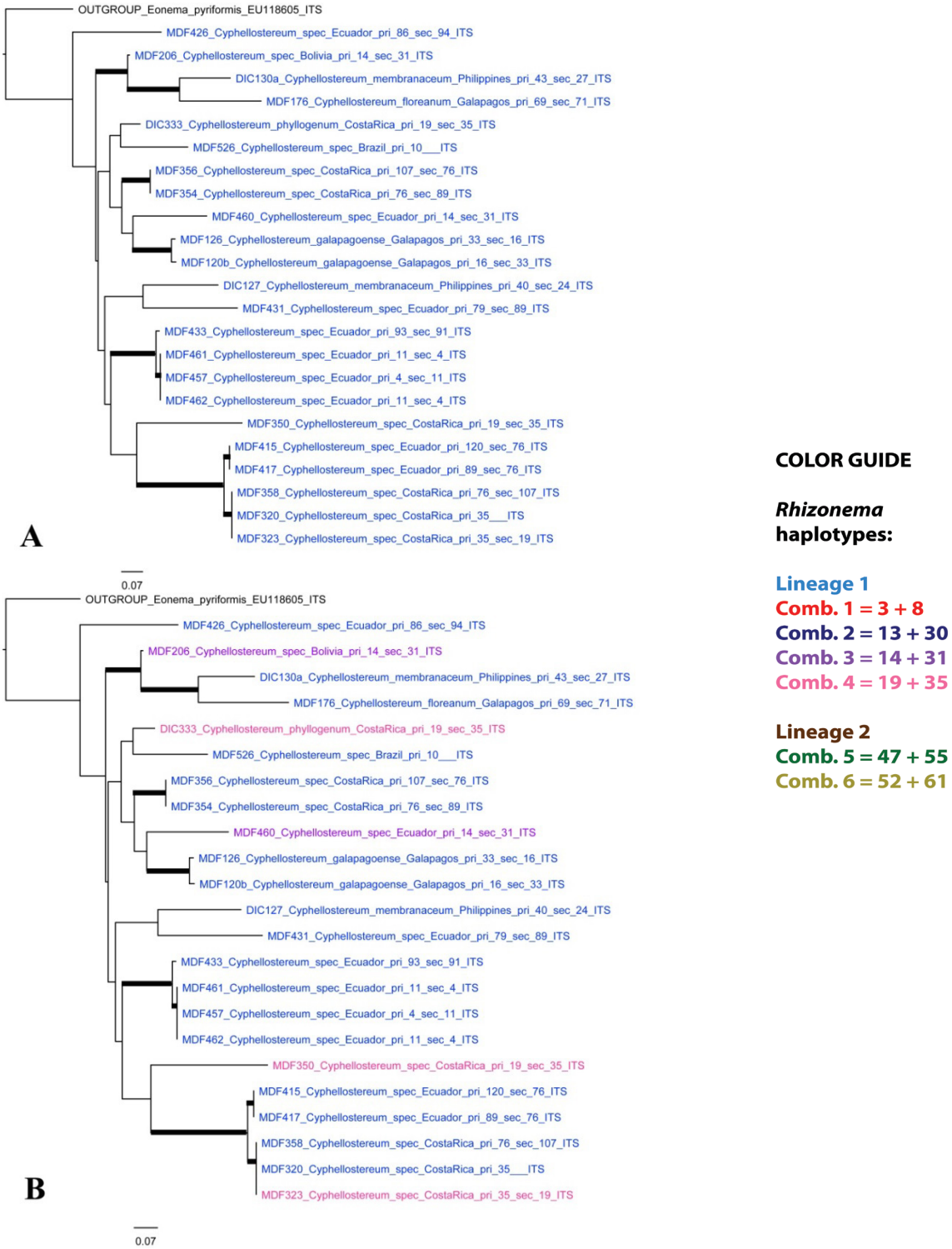


Figure 34 - Phylogenetic tree inferred by ML in RAxML for the ITS marker for *Cyphellostereum* species only
A - Colors showing only lineage 1 of *Rhizonema* for all *Cyphellostereum* species. B- Two combination of haplotypes are present within the genus (comb. 3 and comb. 4). 23 specimens plus one outgroup. The color royal blue denotes the lack of a dominant haplotype. Bootstrap values ≥ 70 are represented by thickened branches

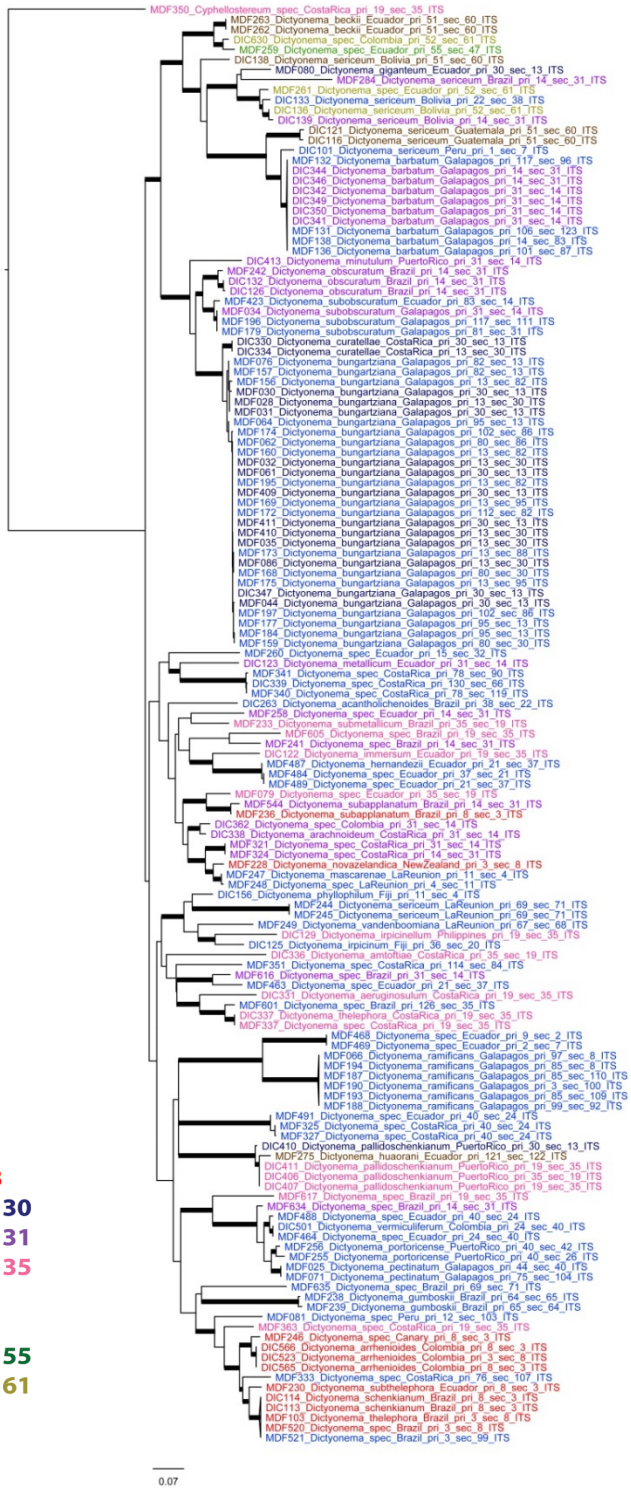
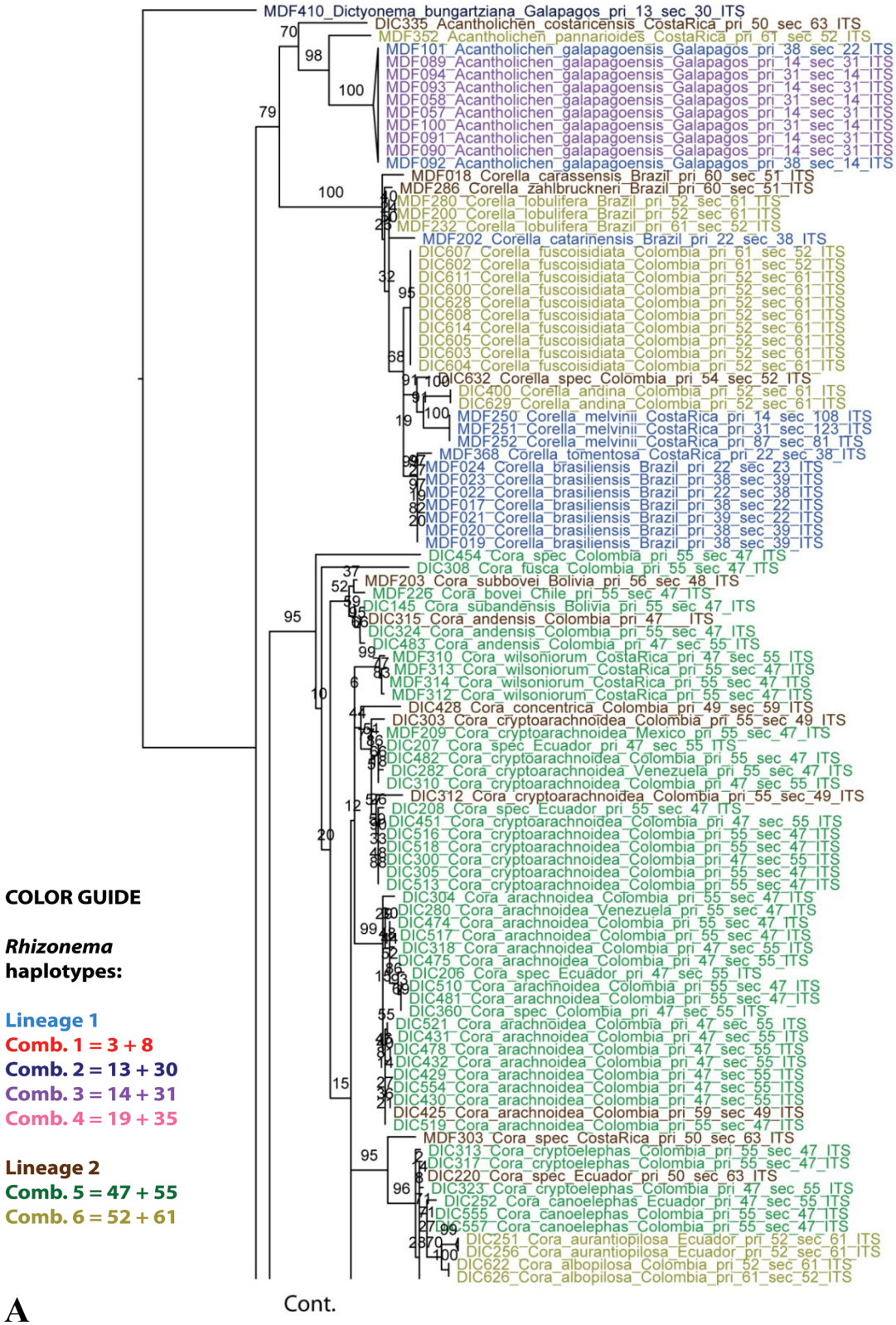
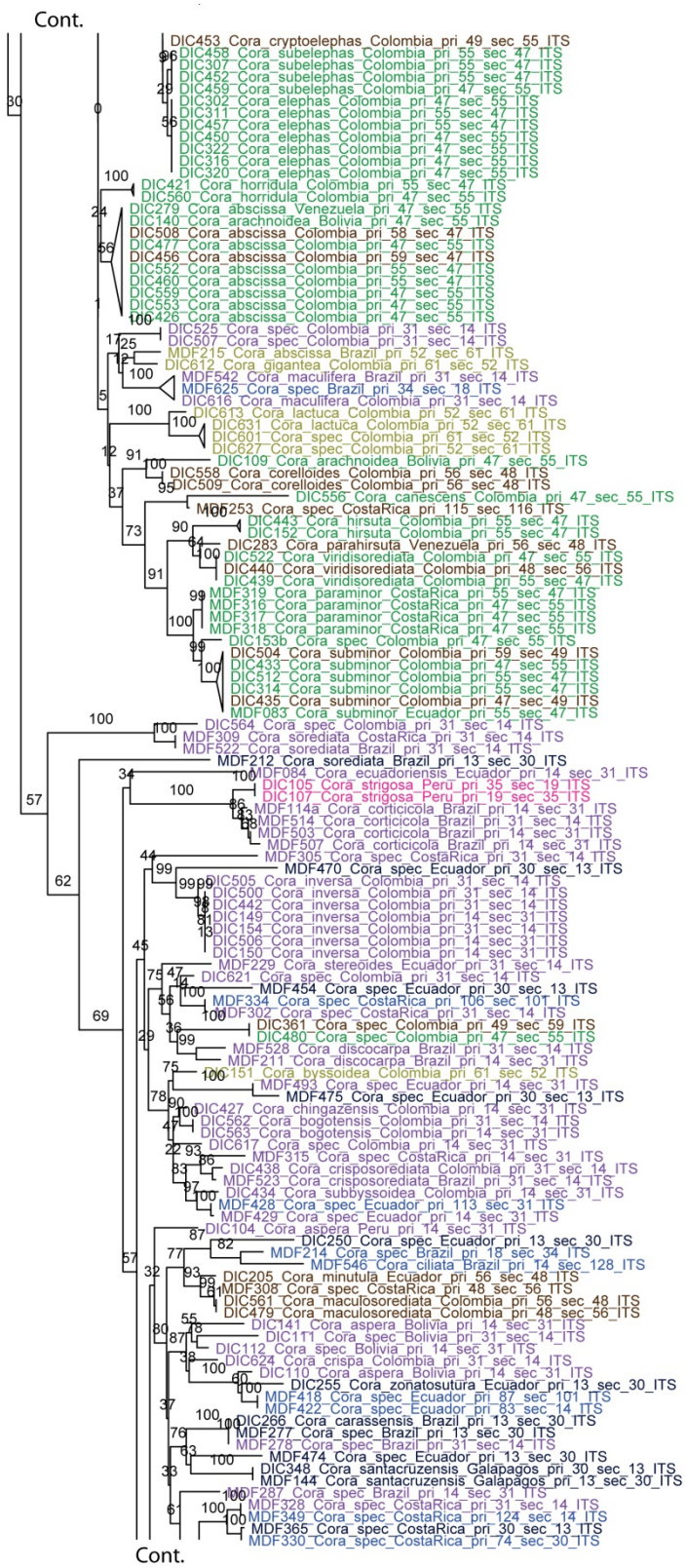


Figure 35 - Phylogenetic tree inferred by ML in RAxML for the ITS marker for *Dictyonema* species only Colors showing that the majority of *Dictyonema* s.s .species has lineage 1 of *Rhizonema*, however all 6 haplotype combinations are present. 142 specimens plus one outgroup. The colors brown and royal blue denote the lack of a dominant haplotype. Bootstrap values ≥ 70 are represented by thickened branches


MDF410_Dictyonema_bungartziana_Galapagos_pri_13_sec_30_ITS
DIC335_Acantholichen_costaricensis_CostaRica_pri_50_sec_63_ITS
MDF352_Acantholichen_pannarioides_CostaRica_pri_61_sec_52_ITS
MDF101_Acantholichen_galapagoensis_Galapagos_pri_38_sec_22_ITS
MDF089_Acantholichen_galapagoensis_Galapagos_pri_14_sec_31_ITS
MDF094_Acantholichen_galapagoensis_Galapagos_pri_31_sec_14_ITS
MDF093_Acantholichen_galapagoensis_Galapagos_pri_14_sec_31_ITS
MDF058_Acantholichen_galapagoensis_Galapagos_pri_31_sec_14_ITS
MDF057_Acantholichen_galapagoensis_Galapagos_pri_14_sec_31_ITS
MDF100_Acantholichen_galapagoensis_Galapagos_pri_31_sec_14_ITS
MDF091_Acantholichen_galapagoensis_Galapagos_pri_14_sec_31_ITS
MDF090_Acantholichen_galapagoensis_Galapagos_pri_14_sec_31_ITS
MDF092_Acantholichen_galapagoensis_Galapagos_pri_38_sec_14_ITS
MDF018_Corella_carassensis_Brazil_pri_60_sec_51_ITS
MDF286_Corella_zahlbruckneri_Brazil_pri_60_sec_51_ITS
MDF280_Corella_lobulifera_Brazil_pri_52_sec_61_ITS
MDF200_Corella_lobulifera_Brazil_pri_52_sec_61_ITS
MDF232_Corella_lobulifera_Brazil_pri_61_sec_52_ITS
MDF202_Corella_catarinensis_Brazil_pri_22_sec_38_ITS
DIC607_Corella_fuscoisidiata_Colombia_pri_61_sec_52_ITS
DIC602_Corella_fuscoisidiata_Colombia_pri_61_sec_52_ITS
DIC611_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC600_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC628_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC608_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC614_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC605_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC603_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC604_Corella_fuscoisidiata_Colombia_pri_52_sec_61_ITS
DIC632_Corella_spec_Colombia_pri_54_sec_52_ITS
DIC400_Corella_andina_Colombia_pri_52_sec_61_ITS
DIC629_Corella_andina_Colombia_pri_52_sec_61_ITS
MDF250_Corella_melvinii_CostaRica_pri_14_sec_108_ITS
MDF251_Corella_melvinii_CostaRica_pri_31_sec_123_ITS
MDF252_Corella_melvinii_CostaRica_pri_87_sec_81_ITS
MDF368_Corella_tomentosa_CostaRica_pri_22_sec_38_ITS
MDF024_Corella_brasiliensis_Brazil_pri_22_sec_23_ITS
MDF023_Corella_brasiliensis_Brazil_pri_38_sec_39_ITS
MDF022_Corella_brasiliensis_Brazil_pri_22_sec_38_ITS
MDF017_Corella_brasiliensis_Brazil_pri_38_sec_22_ITS
MDF021_Corella_brasiliensis_Brazil_pri_39_sec_22_ITS
MDF020_Corella_brasiliensis_Brazil_pri_38_sec_39_ITS
MDF019_Corella_brasiliensis_Brazil_pri_38_sec_39_ITS
DIC454_Cora_spec_Colombia_pri_55_sec_47_ITS
DIC308_Cora_fusca_Colombia_pri_55_sec_47_ITS
MDF203_Cora_subbovei_Bolivia_pri_56_sec_48_ITS
MDF226_Cora_bovei_Chile_pri_55_sec_47_ITS
DIC145_Cora_subandensis_Bolivia_pri_55_sec_47_ITS
DIC315_Cora_andensis_Colombia_pri_47___ITS
DIC324_Cora_andensis_Colombia_pri_55_sec_47_ITS
DIC483_Cora_andensis_Colombia_pri_47_sec_55_ITS
MDF310_Cora_wilsoniorum_CostaRica_pri_47_sec_55_ITS
MDF313_Cora_wilsoniorum_CostaRica_pri_55_sec_47_ITS
MDF314_Cora_wilsoniorum_CostaRica_pri_55_sec_47_ITS
MDF312_Cora_wilsoniorum_CostaRica_pri_55_sec_47_ITS
DIC428_Cora_concentrica_Colombia_pri_49_sec_59_ITS
DIC303_Cora_cryptoarachnoidea_Colombia_pri_55_sec_49_ITS
MDF209_Cora_cryptoarachnoidea_Mexico_pri_55_sec_47_ITS
DIC207_Cora_spec_Ecuador_pri_47_sec_55_ITS
DIC482_Cora_cryptoarachnoidea_Colombia_pri_55_sec_47_ITS
DIC282_Cora_cryptoarachnoidea_Venezuela_pri_55_sec_47_ITS
DIC310_Cora_cryptoarachnoidea_Colombia_pri_47_sec_55_ITS
DIC312_Cora_cryptoarachnoidea_Colombia_pri_55_sec_49_ITS
DIC208_Cora_spec_Ecuador_pri_55_sec_47_ITS
DIC451_Cora_cryptoarachnoidea_Colombia_pri_47_sec_55_ITS
DIC516_Cora_cryptoarachnoidea_Colombia_pri_55_sec_47_ITS
DIC518_Cora_cryptoarachnoidea_Colombia_pri_55_sec_47_ITS
DIC300_Cora_cryptoarachnoidea_Colombia_pri_47_sec_55_ITS
DIC305_Cora_cryptoarachnoidea_Colombia_pri_55_sec_47_ITS
DIC513_Cora_cryptoarachnoidea_Colombia_pri_55_sec_47_ITS
DIC304_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
DIC280_Cora_arachnoidea_Venezuela_pri_55_sec_47_ITS
DIC474_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
DIC517_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
DIC318_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC475_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC206_Cora_spec_Ecuador_pri_47_sec_55_ITS
DIC510_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
DIC481_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
DIC360_Cora_spec_Colombia_pri_47_sec_55_ITS
DIC521_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC431_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC478_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC432_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC429_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC554_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC430_Cora_arachnoidea_Colombia_pri_47_sec_55_ITS
DIC425_Cora_arachnoidea_Colombia_pri_59_sec_49_ITS
DIC519_Cora_arachnoidea_Colombia_pri_55_sec_47_ITS
MDF303_Cora_spec_CostaRica_pri_50_sec_63_ITS
DIC313_Cora_cryptoelephas_Colombia_pri_55_sec_47_ITS
DIC317_Cora_cryptoelephas_Colombia_pri_55_sec_47_ITS
DIC220_Cora_spec_Ecuador_pri_50_sec_63_ITS
DIC323_Cora_cryptoelephas_Colombia_pri_47_sec_55_ITS
DIC252_Cora_canoelephas_Ecuador_pri_47_sec_55_ITS
DIC555_Cora_canoelephas_Colombia_pri_55_sec_47_ITS
DIC557_Cora_canoelephas_Colombia_pri_55_sec_47_ITS
DIC251_Cora_aurantiopilosa_Ecuador_pri_52_sec_61_ITS
DIC256_Cora_aurantiopilosa_Ecuador_pri_52_sec_61_ITS
DIC622_Cora_albopilosa_Colombia_pri_52_sec_61_ITS
DIC626_Cora_albopilosa_Colombia_pri_61_sec_52_ITS
COLOR GUIDE
Rhizonema haplotypes:
Lineage 1
Comb. 1 = 3 + 8
Comb. 2 = 13 + 30
Comb. 3 = 14 + 31
Comb. 4 = 19 + 35
Lineage 2
Comb. 5 = 47 + 55
Comb. 6 = 52 + 61
Cont.

A



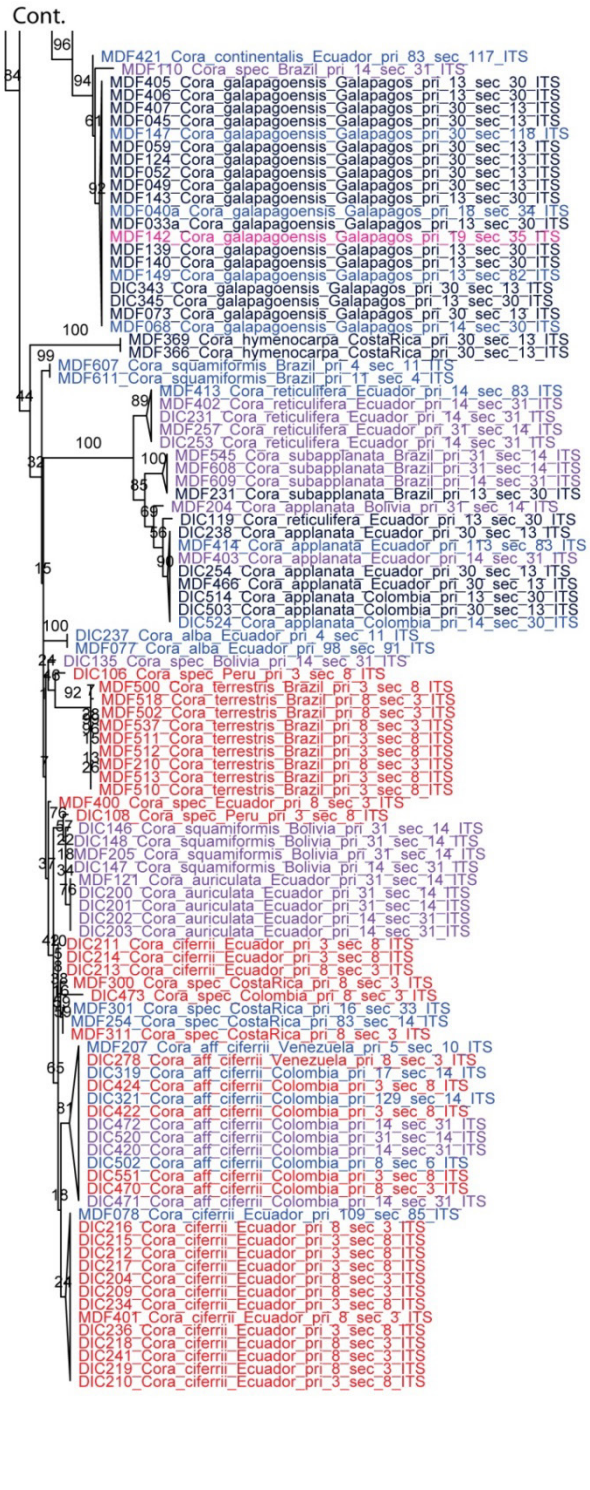
COLOR GUIDE

***Rhizonema* haplotypes:**

Lineage 1
Comb. 1 = 3 + 8
Comb. 2 = 13 + 30
Comb. 3 = 14 + 31
Comb. 4 = 19 + 35

Lineage 2
Comb. 5 = 47 + 55
Comb. 6 = 52 + 61

B



C

Figure 36 - Phylogenetic tree inferred by ML in RAxML for the ITS marker for the *Acantholichen-Corella-Cora* clade Colors show that the majority of species has lineage 1 of *Rhizonema*, however all 6 haplotype combinations are present. *Cora* clade 1 seems to have lineage 2 of *Rhizonema* as the predominant photobiont. Bootstrap values are shown. 330 specimens plus one outgroup

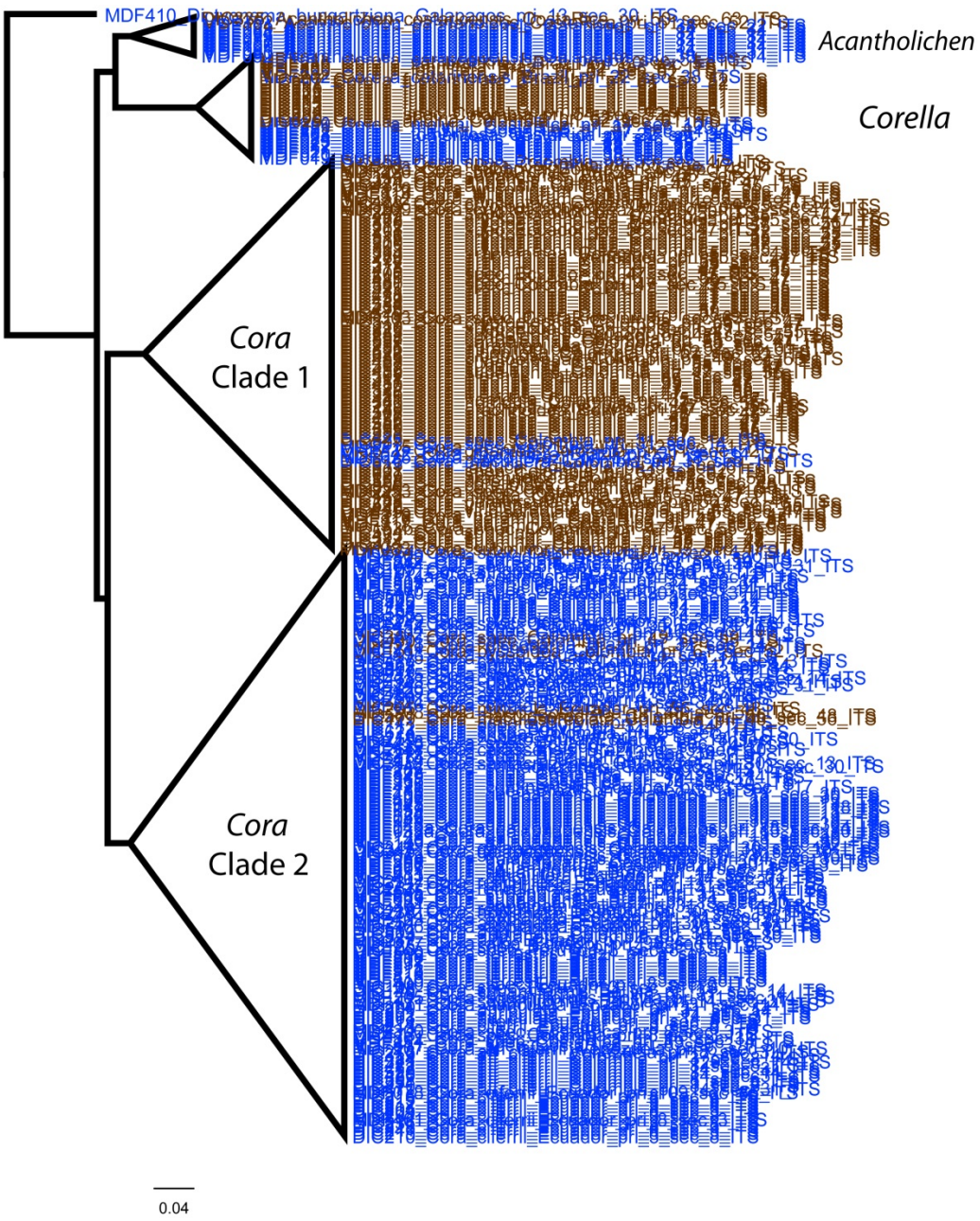


Figure 37 - Cartooned and compacted phylogenetic tree inferred by ML in RAxML for the ITS marker for the *Acantholichen-Corella-Cora* clade to show distribution of *Rhizonema* lineages (same data as Figure 36)
For names, please consult previous figure. Colors show that the majority of species has lineage 1 of *Rhizonema*. *Cora* clade 1 seems to have lineage 2 of *Rhizonema* as the predominant photobiont, while *Cora* clade 2 has *Rhizonema* lineage 1 almost exclusively. *Corella* and *Acantholichen* species seem to have both species of *Rhizonema* as photobiont. Bootstrap values ≥ 70 are represented by thickened branches

Table 21 - Specimens, including collection information used in this study
X denotes for ITS not available. Rhiz = *Rhizonema* lineage present (lineage 1 or lineage 2)

Control	Genus	epithet	Voucher	Country	ITS	Pri	Sec	Rhiz
DIC335	*Acantholichen*	*costaricensis*	*Lücking sn*	Costa Rica		50	63	2
MDF057	*Acantholichen*	*galapagoensis*	*DalForno 1204*	Galapagos		14	31	1
MDF058	*Acantholichen*	*galapagoensis*	*DalForno 1205*	Galapagos		31	14	1
MDF088	*Acantholichen*	*galapagoensis*	*Aptroot 63215*	Galapagos	X	14	31	1
MDF089	*Acantholichen*	*galapagoensis*	*Aptroot 64679*	Galapagos		14	31	1
MDF090	*Acantholichen*	*galapagoensis*	*Bungartz 4125*	Galapagos		14	31	1
MDF091	*Acantholichen*	*galapagoensis*	*Aptroot 65187*	Galapagos		14	31	1
MDF092	*Acantholichen*	*galapagoensis*	*Aptroot 65554*	Galapagos		38	14	1
MDF093	*Acantholichen*	*galapagoensis*	*Nugra 400*	Galapagos		14	31	1
MDF094	*Acantholichen*	*galapagoensis*	*Nugra 379*	Galapagos		31	14	1
MDF099	*Acantholichen*	*galapagoensis*	*Truong 1532*	Galapagos	X	38	14	1
MDF100	*Acantholichen*	*galapagoensis*	*Bungartz 8152*	Galapagos		31	14	1
MDF101	*Acantholichen*	*galapagoensis*	*Bungartz 8577*	Galapagos		38	22	1
MDF352	*Acantholichen*	*pannarioides*	*DalForno 1752*	Costa Rica		61	52	2
DIC104	*Cora*	*aspera*	*Vela sn*	Peru		14	31	1
DIC105	*Cora*	*strigosa*	*Jihuallanca sn*	Peru		35	19	1
DIC106	*Cora*	sp.	*Farfan sn*	Peru		3	8	1
DIC107	*Cora*	*strigosa*	*Paz 3*	Peru		19	35	1
DIC108	*Cora*	sp.	*unknown sn*	Peru		3	8	1
DIC109	*Cora*	*arachnoidea*	*Lücking 29363*	Bolivia		47	55	2
DIC110	*Cora*	*aspera*	*Lücking 29128*	Bolivia		14	31	1
DIC111	*Cora*	sp.	*Lücking 29364*	Bolivia		31	14	1
DIC112	*Cora*	sp.	*Lücking 29356*	Bolivia		14	31	1
DIC119	*Cora*	*reticulifera*	*Lücking 26201*	Ecuador		13	30	1
DIC135	*Cora*	sp.	*Wilk 2607*	Bolivia		14	31	1
DIC140	*Cora*	*arachnoidea*	*Wilk 2780a*	Bolivia		47	55	2
DIC141	*Cora*	*aspera*	*Wilk 2780b*	Bolivia		14	31	1
DIC142	*Cora*	sp.	*Wilk 7979*	Bolivia	X	56	48	2
DIC145	*Cora*	*squamiformis*	*Wilk 7562*	Bolivia		55	47	2
DIC146	*Cora*	*squamiformis*	*Wilk 7577*	Bolivia		31	14	1
DIC147	*Cora*	*squamiformis*	*Wilk 7587*	Bolivia		14	31	1
DIC148	*Cora*	*squamiformis*	*Wilk 7446*	Bolivia		31	14	1
DIC149	*Cora*	*inversa*	*Lücking sn*	Colombia		14	31	1
DIC150	*Cora*	*inversa*	*Lücking sn*	Colombia		14	31	1
DIC151	*Cora*	*byssoidea*	*Lücking sn*	Colombia		61	52	2
DIC152	*Cora*	*hirsuta*	*Lücking Type*	Colombia		55	47	2
DIC153b	*Cora*	sp.	*Lücking sn*	Colombia		47	55	2
DIC154	*Cora*	*inversa*	*Lücking sn*	Colombia		31	14	1
DIC200	*Cora*	*auriculata*	*Lücking sn*	Ecuador		31	14	1
DIC201	*Cora*	*auriculata*	*Lücking sn*	Ecuador		31	14	1
DIC202	*Cora*	*auriculata*	*Lücking sn*	Ecuador		14	31	1
DIC203	*Cora*	*auriculata*	*Lücking sn*	Ecuador		14	31	1
DIC204	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		8	3	1
DIC205	*Cora*	*minutula*	*Lücking sn*	Ecuador		56	48	2
DIC206	*Cora*	sp.	*Lücking sn*	Ecuador		47	55	2
DIC207	*Cora*	sp.	*Lücking sn*	Ecuador		47	55	2
DIC208	*Cora*	sp.	*Lücking sn*	Ecuador		55	47	2
DIC209	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		8	3	1
DIC210	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		3	8	1
DIC211	*Cora*	sp.	*Lücking sn*	Ecuador		3	8	1
DIC212	*Cora*	sp.	*Lücking sn*	Ecuador		3	8	1
DIC213	*Cora*	sp.	*Lücking sn*	Ecuador		8	3	1
DIC214	*Cora*	sp.	*Lücking sn*	Ecuador		3	8	1
DIC215	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		3	8	1
DIC216	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		8	3	1
DIC217	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		3	8	1
DIC218	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		8	3	1
DIC219	*Cora*	*ciferrii*	*Lücking sn*	Ecuador		8	3	1
DIC220	*Cora*	sp.	*Lücking sn*	Ecuador		50	63	2
DIC231	*Cora*	*reticulifera*	*Cole 123*	Ecuador		14	31	1
DIC234	*Cora*	*ciferrii*	*Paredes 653*	Ecuador		3	8	1
DIC235	*Cora*	sp.	*Paredes 653*	Ecuador	X	14	31	1

DIC236	*Cora*	*ciferrii*	*Ceron 36059*	Ecuador		3	8	1
DIC237	*Cora*	*alba*	*Paredes 62*	Ecuador		4	11	1
DIC238	*Cora*	*applanata*	*Ceron 38530*	Ecuador		30	13	1
DIC241	*Cora*	*ciferrii*	*Paredes 41*	Ecuador		8	3	1
DIC250	*Cora*	sp.	*Nugra 867*	Ecuador		13	30	1
DIC251	*Cora*	*aurantiopilosa*	*Nugra 866*	Ecuador		52	61	2
DIC252	*Cora*	*canoelephas*	*Nugra 865*	Ecuador		47	55	2
DIC253	*Cora*	*reticulifera*	*Nugra 864*	Ecuador		14	31	1
DIC254	*Cora*	*applanata*	*Nugra 863*	Ecuador		30	13	1
DIC255	*Cora*	*zonatosutura*	*Nugra 862*	Ecuador		13	30	1
DIC256	*Cora*	*aurantiopilosa*	*Nugra 818*	Ecuador		52	61	2
DIC266	*Cora*	*carassensis*	*Lücking 31351a*	Brazil		13	30	1
DIC268	*Cora*	sp.	*Lücking 31353*	Brazil	X	30	13	1
DIC277	*Cora*	sp.	*Hernandez 1777*	Venezuela	X	47	55	2
DIC278	*Cora*	aff. *ciferrii*	*Hernandez 1778*	Venezuela		8	3	1
DIC279	*Cora*	*abscissa*	*Hernandez 1779*	Venezuela		47	55	2
DIC280	*Cora*	*arachnoidea*	*Hernandez 1780*	Venezuela		55	47	2
DIC282	*Cora*	*cryptoarachnoidea*	*Hernandez 1782*	Venezuela		55	47	2
DIC283	*Cora*	*parahirsuta*	*Hernandez 1783*	Venezuela		56	48	2
DIC300	*Cora*	*cryptoarachnoidea*	*Lücking 32700*	Colombia		47	55	2
DIC301	*Cora*	sp.	*Lücking 32700*	Colombia	X	47	55	2
DIC302	*Cora*	*elephas*	*Lücking 32702*	Colombia		47	55	2
DIC303	*Cora*	*cryptoarachnoidea*	*Lücking 32703*	Colombia		55	49	2
DIC304	*Cora*	*arachnoidea*	*Lücking 32704*	Colombia		55	47	2
DIC305	*Cora*	*cryptoarachnoidea*	*Lücking 32705*	Colombia		55	47	2
DIC306	*Cora*	sp.	*Lücking 32705*	Colombia	X	55	47	2
DIC307	*Cora*	*subelephas*	*Lücking 32707*	Colombia		55	47	2
DIC308	*Cora*	*fusca*	*Lücking 32708*	Colombia		55	47	2
DIC309	*Cora*	sp.	*Lücking 32709*	Colombia	X	47	55	2
DIC310	*Cora*	*cryptoarachnoidea*	*Lücking 32710*	Colombia		47	55	2
DIC311	*Cora*	*elephas*	*Lücking 32711*	Colombia		55	47	2
DIC312	*Cora*	*cryptoarachnoidea*	*Lücking 32712*	Colombia		55	49	2
DIC313	*Cora*	*cryptoelephas*	*Lücking 32713*	Colombia		55	47	2
DIC314	*Cora*	*subminor*	*Lücking 32714*	Colombia		55	47	2
DIC315	*Cora*	*andensis*	*Lücking 32715*	Colombia		47	X	2
DIC316	*Cora*	*elephas*	*Lücking 32716*	Colombia		47	55	2
DIC317	*Cora*	*cryptoelephas*	*Lücking 32717*	Colombia		55	47	2
DIC318	*Cora*	*arachnoidea*	*Lücking 32718*	Colombia		47	55	2
DIC319	*Cora*	aff. *ciferrii*	*Lücking 32719*	Colombia		17	14	1
DIC320	*Cora*	*elephas*	*Lücking 32720*	Colombia		47	55	2
DIC321	*Cora*	aff. *ciferrii*	*Lücking 32721*	Colombia		129	14	1
DIC322	*Cora*	*elephas*	*Lücking 32722*	Colombia		47	55	2
DIC323	*Cora*	*cryptoelephas*	*Lücking 32723*	Colombia		47	55	2
DIC324	*Cora*	*andensis*	*Lücking 32724*	Colombia		55	47	2
DIC343	*Cora*	*galapagoensis*	*Aptroot 65557*	Galapagos		30	13	1
DIC345	*Cora*	*galapagoensis*	*Bungartz 4831*	Galapagos		13	30	1
DIC348	*Cora*	*santacruzensis*	*Bungartz 5594*	Galapagos		30	13	1
DIC360	*Cora*	sp.	*Moncada sn*	Colombia		47	55	2
DIC361	*Cora*	sp.	*Moncada sn*	Colombia		49	59	2
DIC420	*Cora*	aff. *ciferrii*	*Lücking 34005*	Colombia		14	31	1
DIC421	*Cora*	*horridula*	*Lücking 34006*	Colombia		55	47	2
DIC422	*Cora*	*aspera*	*Lücking 34007*	Colombia		3	8	1
DIC424	*Cora*	aff. *ciferrii*	*Lücking 34009*	Colombia		3	8	1
DIC425	*Cora*	*arachnoidea*	*Lücking 34010*	Colombia		59	49	2
DIC426	*Cora*	*abscissa*	*Lücking 34011*	Colombia		47	55	2
DIC427	*Cora*	*chingazensis*	*Lücking 34012*	Colombia		14	31	1
DIC428	*Cora*	*concentrica*	*Lücking 34013*	Colombia		49	59	2
DIC429	*Cora*	*arachnoidea*	*Lücking 34014*	Colombia		47	55	2
DIC430	*Cora*	*arachnoidea*	*Lücking 34015*	Colombia		47	55	2
DIC431	*Cora*	*arachnoidea*	*Lücking 34016*	Colombia		47	55	2

DIC432	*Cora*	*arachnoidea*	*Lücking 34018*	Colombia		47	55	2
DIC433	*Cora*	*subminor*	*Lücking 34052*	Colombia		47	55	2
DIC434	*Cora*	*subbyssoidea*	*Lücking 34053*	Colombia		14	31	1
DIC435	*Cora*	*subminor*	*Lücking 34054*	Colombia		47	49	2
DIC436	*Cora*	*viridisorediata*	*Lücking 34055*	Colombia	X	14	31	1
DIC438	*Cora*	*crisposorediata*	*Lücking 34057*	Colombia		31	14	1
DIC439	*Cora*	*viridisorediata*	*Lücking 34058*	Colombia		55	47	2
DIC440	*Cora*	*viridisorediata*	*Lücking 34060*	Colombia		48	56	2
DIC442	*Cora*	*inversa*	*Lücking 34062*	Colombia		31	14	1
DIC443	*Cora*	*hirsuta*	*Lücking 34069*	Colombia		55	47	2
DIC450	*Cora*	*elephas*	*Lücking 341*	Colombia		47	55	2
DIC451	*Cora*	*cryptoarachnoidea*	*Lücking 34107*	Colombia		47	55	2
DIC452	*Cora*	*subelephas*	*Lücking 34108*	Colombia		55	47	2
DIC453	*Cora*	*cryptoelephas*	*Lücking 34109*	Colombia		49	55	2
DIC454	*Cora*	sp.	*Lücking 34111*	Colombia		55	47	2
DIC455	*Cora*	*aspera*	*Lücking 34112*	Colombia	X	127	47	2
DIC456	*Cora*	*abscissa*	*Lücking 34113*	Colombia		59	47	2
DIC457	*Cora*	*elephas*	*Lücking 34114*	Colombia		55	47	2
DIC458	*Cora*	*subelephas*	*Lücking 34115*	Colombia		55	47	2
DIC459	*Cora*	*subelephas*	*Lücking 34116*	Colombia		47	55	2
DIC460	*Cora*	*abscissa*	*Lücking 34117a*	Colombia		55	47	2
DIC461	*Cora*	*squamiformis*	*Lücking 34117b*	Colombia	X	47	59	2
DIC470	*Cora*	aff. *ciferrii*	*Moncada 4596*	Colombia		8	3	1
DIC471	*Cora*	aff. *ciferrii*	*Moncada 4597*	Colombia		14	31	1
DIC472	*Cora*	aff. *ciferrii*	*Moncada 4598*	Colombia		14	31	1
DIC473	*Cora*	*glabrata*	*Moncada 4599*	Colombia		8	3	1
DIC474	*Cora*	*arachnoidea*	*Moncada 4600*	Colombia		55	47	2
DIC475	*Cora*	*arachnoidea*	*Moncada 4601a*	Colombia		47	55	2
DIC476	*Cora*	sp.	*Moncada 4601b*	Colombia	X	59	49	2
DIC477	*Cora*	*abscissa*	*Moncada 4602*	Colombia		47	55	2
DIC478	*Cora*	*arachnoidea*	*Moncada 4603*	Colombia		47	55	2
DIC479	*Cora*	*maculosorediata*	*Moncada 4604a*	Colombia		48	56	2
DIC480	*Cora*	sp.	*Moncada 4604b*	Colombia		47	55	2
DIC481	*Cora*	*arachnoidea*	*Moncada 4605*	Colombia		55	47	2
DIC482	*Cora*	*cryptoarachnoidea*	*Moncada 6301*	Colombia		55	47	2
DIC483	*Cora*	*andensis*	*Moncada 6340*	Colombia		47	55	2
DIC500	*Cora*	*inversa*	*Lücking 33362*	Colombia		31	14	1
DIC502	*Cora*	aff. *ciferrii*	*Lücking 33303*	Colombia		8	6	1
DIC503	*Cora*	*applanata*	*Lücking 33530*	Colombia		30	13	1
DIC504	*Cora*	*subminor*	*Lücking 33306*	Colombia		59	49	2
DIC505	*Cora*	*inversa*	*Lücking 33300*	Colombia		31	14	1
DIC506	*Cora*	*inversa*	*Lücking 33340*	Colombia		14	31	1
DIC507	*Cora*	sp.	*Lücking 33326*	Colombia		31	14	1
DIC508	*Cora*	*abscissa*	*Lücking 33310*	Colombia		58	47	2
DIC509	*Cora*	*corelloides*	*Lücking 33343*	Colombia		56	48	2
DIC510	*Cora*	*arachnoidea*	*Lücking 33392*	Colombia		55	47	2
DIC512	*Cora*	*subminor*	*Lücking 33316*	Colombia		55	47	2
DIC513	*Cora*	*cryptoarachnoidea*	*Lücking 33384*	Colombia		55	47	2
DIC514	*Cora*	*applanata*	*Lücking 33534*	Colombia		13	30	1
DIC516	*Cora*	*cryptoarachnoidea*	*Lücking 33349*	Colombia		55	47	2
DIC517	*Cora*	*arachnoidea*	*Lücking 33335*	Colombia		55	47	2
DIC518	*Cora*	*cryptoarachnoidea*	*Lücking 33307*	Colombia		55	47	2
DIC519	*Cora*	*arachnoidea*	*Lücking 333308*	Colombia		55	47	2
DIC520	*Cora*	aff. *ciferrii*	*Lücking 33388*	Colombia		31	14	1
DIC521	*Cora*	*arachnoidea*	*Lücking 33319*	Colombia		47	55	2
DIC522	*Cora*	*viridisorediata*	*Lücking 33347*	Colombia		47	55	2
DIC524	*Cora*	*applanata*	*Lücking 33533*	Colombia		14	30	1
DIC525	*Cora*	sp.	*Lücking 33382*	Colombia		31	14	1
DIC550	*Cora*	sp.	*Moncada 5425*	Colombia	X	57	47	2
DIC551	*Cora*	aff. *ciferrii*	*Moncada 5422*	Colombia		3	8	1
DIC552	*Cora*	*abscissa*	*Moncada 5473*	Colombia		55	47	2
DIC553	*Cora*	*abscissa*	*Moncada 5400*	Colombia		47	55	2
DIC554	*Cora*	*arachnoidea*	*Moncada 5444*	Colombia		47	55	2
DIC555	*Cora*	*canoelephas*	*Moncada 5421*	Colombia		55	47	2
DIC556	*Cora*	*canescens*	*Moncada 5404*	Colombia		47	55	2
DIC557	*Cora*	*canoelephas*	*Moncada 5418*	Colombia		55	47	2

DIC558	*Cora*	*corelloides*	*Moncada 5407*	Colombia		56	48	2
DIC559	*Cora*	*abscissa*	*Moncada 5412*	Colombia		47	55	2
DIC560	*Cora*	*horridula*	*Moncada 5469*	Colombia		47	55	2
DIC561	*Cora*	*maculosorediata*	*Moncada 5452*	Colombia		56	48	2
DIC562	*Cora*	*bogotensis*	*Lücking 35281*	Colombia		31	14	1
DIC563	*Cora*	*bogotensis*	*Lücking 35278*	Colombia		14	31	1
DIC564	*Cora*	sp.	*Lücking 35267*	Colombia		31	14	1
DIC601	*Cora*	sp.	*Lücking 35312*	Colombia		61	52	2
DIC606	*Cora*	sp.	*Lücking 35328*	Colombia	X	52	61	2
DIC609	*Cora*	sp.	*Lücking 35335*	Colombia	X	61	52	2
DIC612	*Cora*	*gigantea*	*Lücking 35344*	Colombia		61	52	2
DIC613	*Cora*	*lactuca*	*Lücking 35349*	Colombia		52	61	2
DIC615	*Cora*	sp.	*Lücking 35402*	Colombia	X	14	31	1
DIC616	*Cora*	*maculifera*	*Lücking 35403*	Colombia		31	14	1
DIC617	*Cora*	sp.	*Lücking 35424*	Colombia		14	31	1
DIC621	*Cora*	sp.	*Moncada 6158*	Colombia		31	14	1
DIC622	*Cora*	*albopilosa*	*Moncada 6160*	Colombia		52	61	2
DIC623	*Cora*	sp.	*Moncada 6199*	Colombia	X	61	52	2
DIC624	*Cora*	*crispa*	*Moncada 6308*	Colombia		31	14	1
DIC626	*Cora*	*albopilosa*	*Moncada 6326*	Colombia		61	52	2
DIC627	*Cora*	sp.	*Moncada 6327*	Colombia		52	61	2
DIC631	*Cora*	*lactuca*	*Moncada 6339*	Colombia		52	61	2
MDF033a	*Cora*	*galapagoensis*	*DalForno 1180a*	Galapagos		13	30	1
MDF040a	*Cora*	*galapagoensis*	*DalForno 1187a*	Galapagos		18	34	1
MDF045	*Cora*	*galapagoensis*	*DalForno 1192*	Galapagos		30	13	1
MDF049	*Cora*	*galapagoensis*	*DalForno 1196*	Galapagos		30	13	1
MDF052	*Cora*	*galapagoensis*	*DalForno 1199a*	Galapagos		30	13	1
MDF059	*Cora*	*galapagoensis*	*DalForno 1206*	Galapagos		30	13	1
MDF068	*Cora*	*galapagoensis*	*DalForno 1218*	Galapagos		14	30	1
MDF073	*Cora*	*galapagoensis*	*DalForno 1223*	Galapagos		30	13	1
MDF077	*Cora*	*alba*	*Jonitz 436*	Ecuador		98	91	1
MDF078	*Cora*	*ciferrii*	*Jonitz 377*	Ecuador		109	85	1
MDF083	*Cora*	*subminor*	*Jonitz 383*	Ecuador		55	47	2
MDF084	*Cora*	*ecuadoriensis*	*Jonitz 603*	Ecuador		14	31	1
MDF110	*Cora*	sp.	*DalForno 1267*	Brazil		14	31	1
MDF114a	*Cora*	*corticicola*	*DalForno 1274a*	Brazil		14	31	1
MDF121	*Cora*	*squamiformis*	*Aptroot 63657*	Galapagos		31	14	1
MDF124	*Cora*	*galapagoensis*	*Yanez 1509*	Galapagos		30	13	1
MDF139	*Cora*	*galapagoensis*	*Yanez 1508*	Galapagos		13	30	1
MDF140	*Cora*	*galapagoensis*	*Yanez 1513*	Galapagos		13	30	1
MDF142	*Cora*	*galapagoensis*	*Yanez 1538*	Galapagos		19	35	1
MDF143	*Cora*	*galapagoensis*	*Yanez 1540*	Galapagos		13	30	1
MDF144	*Cora*	*santacruzensis*	*Yanez 1547*	Galapagos		13	30	1
MDF147	*Cora*	*galapagoensis*	*Nugra 437*	Galapagos		30	118	1
MDF149	*Cora*	*galapagoensis*	*HerreraCampos 10546*	Galapagos		13	82	1
MDF203	*Cora*	*bovei*	*Kukwa 9457*	Bolivia		56	48	2
MDF204	*Cora*	*applanata*	*Kukwa 9206*	Bolivia		31	14	1
MDF205	*Cora*	*squamiformis*	*Kukwa 928966*	Bolivia		31	14	1
MDF207	*Cora*	aff. *ciferrii*	*Hale 44528*	Venezuela		5	10	1
MDF209	*Cora*	*cryptoarachnoidea*	*Egan 17538*	Mexico		55	47	2
MDF210	*Cora*	*terrestris*	*Ariati 376*	Brazil		8	3	1
MDF211	*Cora*	*discocarpa*	*Ariati sn*	Brazil		14	31	1
MDF212	*Cora*	*sorediata*	*Beilke 87*	Brazil		13	30	1
MDF214	*Cora*	sp.	*Beilke 42*	Brazil		18	34	1
MDF215	*Cora*	*abscissa*	*Eliasaro 2482a*	Brazil		52	61	2
MDF216	*Cora*	sp.	*Eliasaro 2482b*	Brazil	X	31	14	1
MDF226	*Cora*	*bovei*	*Buck 59058*	Chile		55	47	2
MDF229	*Cora*	*stereoides*	*Yanez 2462*	Ecuador		31	14	1
MDF231	*Cora*	*subapplanata*	*Gumboski 2400*	Brazil		13	30	1
MDF253	*Cora*	sp.	*Chavez 2729*	Costa Rica		115	116	2
MDF254	*Cora*	*glabrata*	*Quesada 1304*	Costa Rica		83	14	1

MDF257	*Cora*	*reticulifera*	*Beck E388*	Ecuador		31	14	1
MDF264	*Cora*	sp.	*Spielmann 3282*	Brazil	X	31	14	1
MDF266	*Cora*	sp.	*Spielmann 5042*	Brazil	X	14	31	1
MDF271	*Cora*	sp.	*Spielmann 2455*	Brazil	X	13	30	1
MDF272	*Cora*	sp.	*Spielmann 2932*	Brazil	X	13	30	1
MDF273	*Cora*	sp.	*Spielmann 2061*	Brazil	X	51	60	2
MDF277	*Cora*	sp.	*Gumboski 4244*	Brazil		13	30	1
MDF278	*Cora*	sp.	*Gumboski 4245*	Brazil		31	14	1
MDF287	*Cora*	sp.	*Gerlach 994*	Brazil		14	31	1
MDF300	*Cora*	*glabrata*	*DalForno 1700*	Costa Rica		8	3	1
MDF301	*Cora*	*glabrata*	*DalForno 1701*	Costa Rica		16	33	1
MDF302	*Cora*	sp.	*DalForno 1702*	Costa Rica		31	14	1
MDF303	*Cora*	sp.	*DalForno 1703*	Costa Rica		50	63	2
MDF304	*Cora*	sp.	*DalForno 1704*	Costa Rica	X	50	63	2
MDF305	*Cora*	sp.	*DalForno 1705*	Costa Rica		31	14	1
MDF308	*Cora*	sp.	*DalForno 1708*	Costa Rica		48	56	2
MDF309	*Cora*	*sorediata*	*DalForno 1709*	Costa Rica		31	14	1
MDF310	*Cora*	*wilsoniorum*	*DalForno 1710*	Costa Rica		47	55	2
MDF311	*Cora*	*glabrata*	*DalForno 1711*	Costa Rica		8	3	1
MDF312	*Cora*	*wilsoniorum*	*DalForno 1712*	Costa Rica		55	47	2
MDF313	*Cora*	*wilsoniorum*	*DalForno 1713*	Costa Rica		55	47	2
MDF314	*Cora*	*wilsoniorum*	*DalForno 1714*	Costa Rica		55	47	2
MDF315	*Cora*	sp.	*DalForno 1715*	Costa Rica		14	31	1
MDF316	*Cora*	*paraminor*	*DalForno 1716*	Costa Rica		47	55	2
MDF317	*Cora*	*paraminor*	*DalForno 1717*	Costa Rica		47	55	2
MDF318	*Cora*	*paraminor*	*DalForno 1718*	Costa Rica		47	55	2
MDF319	*Cora*	*paraminor*	*DalForno 1719*	Costa Rica		55	47	2
MDF328	*Cora*	sp.	*DalForno 1728*	Costa Rica		31	14	1
MDF330	*Cora*	sp.	*DalForno 1730*	Costa Rica		74	30	1
MDF334	*Cora*	sp.	*DalForno 1734*	Costa Rica		106	101	1
MDF349	*Cora*	sp.	*DalForno 1749*	Costa Rica		124	14	1
MDF365	*Cora*	sp.	*DalForno 1765*	Costa Rica		30	13	1
MDF366	*Cora*	*hymenocarpa*	*DalForno 1766*	Costa Rica		30	13	1
MDF369	*Cora*	*hymenocarpa*	*DalForno 1769*	Costa Rica		30	13	1
MDF400	*Cora*	sp.	*DalForno 1771*	Ecuador		8	3	1
MDF401	*Cora*	*ciferrii*	*DalForno 1778*	Ecuador		8	3	1
MDF402	*Cora*	*reticulifera*	*DalForno 1787*	Ecuador		14	31	1
MDF403	*Cora*	*applanata*	*DalForno 1790*	Ecuador		14	31	1
MDF405	*Cora*	*galapagoensis*	*Nugra 1034*	Galapagos		13	30	1
MDF406	*Cora*	*galapagoensis*	*Nugra 1098*	Galapagos		13	30	1
MDF407	*Cora*	*galapagoensis*	*Bungartz 10325*	Galapagos		30	13	1
MDF413	*Cora*	*reticulifera*	*DalForno 1788*	Ecuador		14	83	1
MDF414	*Cora*	*applanata*	*DalForno 1789*	Ecuador		113	83	1
MDF418	*Cora*	sp.	*DalForno 1801a*	Ecuador		87	101	1
MDF421	*Cora*	*continentalis*	*DalForno 1800*	Ecuador		83	117	1
MDF422	*Cora*	sp.	*DalForno 1802*	Ecuador		83	14	1
MDF428	*Cora*	sp.	*DalForno 1806*	Ecuador		113	31	1
MDF429	*Cora*	sp.	*DalForno 1807*	Ecuador		14	31	1
MDF430	*Cora*	sp.	*DalForno 1809*	Ecuador	X	14	96	1
MDF454	*Cora*	sp.	*DalForno 1921*	Ecuador		30	13	1
MDF466	*Cora*	*applanata*	*DalForno 1934*	Ecuador		30	13	1
MDF470	*Cora*	sp.	*DalForno 1962*	Ecuador		30	13	1
MDF474	*Cora*	sp.	*DalForno 1966*	Ecuador		13	30	1
MDF475	*Cora*	sp.	*DalForno 1972*	Ecuador		30	13	1
MDF493	*Cora*	sp.	*DalForno 1996*	Ecuador		14	31	1
MDF500	*Cora*	*terrestris*	*DalForno 2000*	Brazil		3	8	1
MDF502	*Cora*	*terrestris*	*DalForno 2002*	Brazil		8	3	1
MDF503	*Cora*	*corticicola*	*DalForno 2003*	Brazil		14	31	1
MDF507	*Cora*	*corticicola*	*DalForno 2007*	Brazil		14	31	1
MDF510	*Cora*	*terrestris*	*DalForno 2010*	Brazil		3	8	1
MDF511	*Cora*	*terrestris*	*DalForno 2011*	Brazil		3	8	1
MDF512	*Cora*	*terrestris*	*DalForno 2012*	Brazil		3	8	1
MDF513	*Cora*	*terrestris*	*DalForno 2013*	Brazil		3	8	1
MDF514	*Cora*	*corticicola*	*DalForno 2014*	Brazil		31	14	1
MDF518	*Cora*	*terrestris*	*DalForno 2018*	Brazil		8	3	1

MDF519	*Cora*	*sorediata*	*DalForno 2019*	Brazil	X	14	31	1
MDF522	*Cora*	*sorediata*	*DalForno 2022*	Brazil		31	14	1
MDF523	*Cora*	*crisposorediata*	*DalForno 2023*	Brazil		31	14	1
MDF528	*Cora*	*discocarpa*	*DalForno 2028*	Brazil		31	14	1
MDF537	*Cora*	*terrestris*	*DalForno 2037*	Brazil		8	3	1
MDF542	*Cora*	*maculifera*	*DalForno 2042*	Brazil		31	14	1
MDF545	*Cora*	*subapplanata*	*DalForno 2045*	Brazil		31	14	1
MDF546	*Cora*	*ciliata*	*DalForno 2046*	Brazil		14	128	1
MDF607	*Cora*	*squamiformis*	*DalForno 2061*	Brazil		4	11	1
MDF608	*Cora*	*subapplanata*	*DalForno 2062*	Brazil		31	14	1
MDF609	*Cora*	*subapplanata*	*DalForno 2063*	Brazil		14	31	1
MDF611	*Cora*	*squamiformis*	*DalForno 2065*	Brazil		11	4	1
MDF625	*Cora*	sp.	*DalForno 2113*	Brazil		34	18	1
MDF630	*Cora*	sp.	*DalForno 2122*	Brazil	X	14	31	1
DIC265	*Corella*	*brasiliensis*	*Lücking 31330*	Brazil	X	38	22	1
DIC400	*Corella*	*andina*	*Suarez sn*	Colombia		52	61	2
DIC600	*Corella*	*fuscoisidiata*	*Lücking 35300*	Colombia		52	61	2
DIC602	*Corella*	*fuscoisidiata*	*Lücking 35314*	Colombia		61	52	2
DIC603	*Corella*	*fuscoisidiata*	*Lücking 35315*	Colombia		52	61	2
DIC604	*Corella*	*fuscoisidiata*	*Lücking 35316*	Colombia		52	61	2
DIC605	*Corella*	*fuscoisidiata*	*Lücking 35327*	Colombia		52	61	2
DIC607	*Corella*	*fuscoisidiata*	*Lücking 35330*	Colombia		61	52	2
DIC608	*Corella*	*fuscoisidiata*	*Lücking 35332*	Colombia		52	61	2
DIC610	*Corella*	*fuscoisidiata*	*Lücking 35336*	Colombia	X	52	61	2
DIC611	*Corella*	*fuscoisidiata*	*Lücking 35341*	Colombia		52	61	2
DIC614	*Corella*	*fuscoisidiata*	*Lücking 35350*	Colombia		52	61	2
DIC619	*Corella*	sp.	*Moncada 6108*	Colombia	X	61	52	2
DIC620	*Corella*	sp.	*Moncada 6110a*	Colombia	X	61	52	2
DIC628	*Corella*	*fuscoisidiata*	*Moncada 6329*	Colombia		52	61	2
DIC629	*Corella*	*andina*	*Moncada 6330*	Colombia		52	61	2
DIC632	*Corella*	*fuscoisidiata*	*Moncada 6345*	Colombia		54	52	2
MDF017	*Corella*	*brasiliensis*	*DalForno 1271*	Brazil		38	22	1
MDF018	*Corella*	*carassensis*	*DalForno 1272*	Brazil		60	51	2
MDF019	*Corella*	*brasiliensis*	*DalForno 1280*	Brazil		38	39	1
MDF020	*Corella*	*brasiliensis*	*DalForno 1281*	Brazil		38	39	1
MDF021	*Corella*	*brasiliensis*	*DalForno 1282*	Brazil		39	22	1
MDF022	*Corella*	*brasiliensis*	*DalForno 1283*	Brazil		22	38	1
MDF023	*Corella*	*brasiliensis*	*DalForno 1284*	Brazil		38	39	1
MDF024	*Corella*	*brasiliensis*	*DalForno 1285*	Brazil		22	23	1
MDF200	*Corella*	*lobulifera*	*Eliasaro 5006*	Brazil		52	61	2
MDF201	*Corella*	sp.	*Donha 1759*	Brazil	X	61	52	2
MDF202	*Corella*	*catarinensis*	*Beilke 623*	Brazil		22	38	1
MDF232	*Corella*	*lobulifera*	*Gumboski 2403*	Brazil		61	52	2
MDF250	*Corella*	*melvinii*	*Chaves 2878*	Costa Rica		14	108	1
MDF251	*Corella*	*melvinii*	*Chaves 2437*	Costa Rica		31	123	1
MDF252	*Corella*	*melvinii*	*Chaves 122*	Costa Rica		87	81	1
MDF280	*Corella*	*lobulifera*	*Gumboski 4401*	Brazil		52	61	2
MDF286	*Corella*	*zahlbruckneri*	*Gerlach 993*	Brazil		60	51	2
MDF368	*Corella*	*tomentosa*	*DalForno 1766*	Costa Rica		22	38	1
DIC127	*Cyphellostereum*	*membranaceum*	*RivasPlata 2138a*	Philippines		40	24	1
DIC130a	*Cyphellostereum*	*membranaceum*	*RivasPlata 2183a*	Philippines		43	27	1
DIC333	*Cyphellostereum*	*phyllogenum*	*Lücking 17013*	Costa Rica		19	35	1
MDF120b	*Cyphellostereum*	*galapagoense*	*Bungartz 8517*	Galapagos		16	33	1
MDF126	*Cyphellostereum*	*galapagoense*	*Yanez 1545*	Galapagos		33	16	1
MDF176	*Cyphellostereum*	*floreanium*	*Bungartz 9475*	Galapagos		69	71	1
MDF206	*Cyphellostereum*	sp.	*Kukwa 8736*	Bolivia		14	31	1
MDF320	*Cyphellostereum*	sp.	*DalForno 1720*	Costa Rica		35	X	1
MDF323	*Cyphellostereum*	sp.	*DalForno 1723*	Costa Rica		35	19	1
MDF350	*Cyphellostereum*	sp.	*DalForno 1740*	Costa Rica		19	35	1
MDF354	*Cyphellostereum*	sp.	*DalForno 1754*	Costa Rica		76	89	1
MDF356	*Cyphellostereum*	sp.	*DalForno 1756*	Costa Rica		107	76	1
MDF358	*Cyphellostereum*	sp.	*DalForno 1758*	Costa Rica		76	107	1
MDF415	*Cyphellostereum*	sp.	*DalForno 1792*	Ecuador		120	76	1
MDF417	*Cyphellostereum*	sp.	*DalForno 1797*	Ecuador		89	76	1

MDF426	*Cyphellostereum*	sp.	*DalForno 1798*	Ecuador		86	94	1
MDF431	*Cyphellostereum*	sp.	*DalForno 1813*	Ecuador		79	89	1
MDF433	*Cyphellostereum*	sp.	*DalForno 1825*	Ecuador		93	91	1
MDF457	*Cyphellostereum*	sp.	*DalForno 1915*	Ecuador		4	11	1
MDF460	*Cyphellostereum*	sp.	*DalForno 1923*	Ecuador		14	31	1
MDF461	*Cyphellostereum*	sp.	*DalForno 1926*	Ecuador		11	4	1
MDF462	*Cyphellostereum*	sp.	*DalForno 1927*	Ecuador		11	4	1
MDF465	*Cyphellostereum*	sp.	*DalForno 1933b*	Ecuador	X	31	14	1
MDF526	*Cyphellostereum*	sp.	*DalForno 2026*	Brazil		10	X	1
DIC101	*Dictyonema*	*sericeum*	*Vela sn*	Peru		1	7	1
DIC102	*Dictyonema*	*sericeum*	*Vela sn*	Peru	X	30	13	1
DIC103	*Dictyonema*	*sericeum*	*Vela sn*	Peru	X	7	2	1
DIC113	*Dictyonema*	*schenkianum*	*Lücking 30062*	Brazil		8	3	1
DIC114	*Dictyonema*	*schenkianum*	*Lücking 30060*	Brazil		8	3	1
DIC116	*Dictyonema*	*sericeum*	*Lücking 25551b*	Guatemala		51	60	2
DIC117	*Dictyonema*	*phyllophilum*	*Lücking 26211*	Ecuador	X	14	31	1
DIC118	*Dictyonema*	*metallicum*	*Lücking 26203*	Ecuador	X	14	31	1
DIC121	*Dictyonema*	*sericeum*	*Lücking 25561*	Guatemala		51	60	2
DIC122	*Dictyonema*	*immersum*	*Lücking 26258*	Ecuador		19	35	1
DIC123	*Dictyonema*	*metallicum*	*Lücking 26255*	Ecuador		31	14	1
DIC125	*Dictyonema*	*irpicinum*	*Lumbsch 19837e*	Fiji		36	20	1
DIC126	*Dictyonema*	*obscuratum*	*Lücking 23025*	Brazil		14	31	1
DIC128	*Dictyonema*	*membranaceum*	*RivasPlata 2129a*	Philippines	X	72	73	1
DIC129	*Dictyonema*	*irpicinellum*	*RivasPlata 2143*	Philippines		19	35	1
DIC132	*Dictyonema*	*obscuratum*	*Lücking 23204*	Brazil		14	31	1
DIC133	*Dictyonema*	*sericeum*	*Wilk 9327*	Bolivia		22	38	1
DIC136	*Dictyonema*	*sericeum*	*Fuentes 4788*	Bolivia		52	61	2
DIC137	*Dictyonema*	*sericeum*	*Wilk 9269*	Bolivia	X	52	61	2
DIC138	*Dictyonema*	*sericeum*	*Wilk 8868*	Bolivia		51	60	2
DIC139	*Dictyonema*	*sericeum*	*WIlk 8886*	Bolivia		14	31	1
DIC156	*Dictyonema*	*phyllophilum*	*Lumbsch 19812*	Fiji		11	4	1
DIC157	*Dictyonema*	*membranaceum*	*Lumbsch 19811b*	Fiji	X	19	X	1
DIC263	*Dictyonema*	*acantholichenoides*	*Lücking 31306*	Brazil		38	22	1
DIC264	*Dictyonema*	sp.	*Lücking 31307*	Brazil	X	14	31	1
DIC330	*Dictyonema*	*curatellae*	*Lücking 17252i*	Costa Rica		30	13	1
DIC331	*Dictyonema*	*aeruginosulum*	*Trest 1569*	Costa Rica		19	35	1
DIC334	*Dictyonema*	*curatellae*	*Lücking 15327*	Costa Rica		13	30	1
DIC336	*Dictyonema*	*amtoftiae*	*Amtoft 3095*	Costa Rica		35	19	1
DIC337	*Dictyonema*	*thelephora*	*Lücking 18008*	Costa Rica		19	35	1
DIC338	*Dictyonema*	*arachnoideum*	*Lücking 15353*	Costa Rica		31	14	1
DIC339	*Dictyonema*	*gomezianum*	*Lücking 18053*	Costa Rica		130	66	1
DIC341	*Dictyonema*	*barbatum*	*Bungartz 8363*	Galapagos		31	14	1
DIC342	*Dictyonema*	sp.	*Bungartz 6852*	Galapagos		31	14	1
DIC344	*Dictyonema*	sp.	*Bungartz 8576*	Galapagos		14	31	1
DIC346	*Dictyonema*	sp.	*Aptroot 65186*	Galapagos		14	31	1
DIC347	*Dictyonema*	sp.	*HerreraCampos 10560*	Galapagos		30	13	1
DIC349	*Dictyonema*	sp.	*Truong 1275*	Galapagos		31	14	1
DIC350	*Dictyonema*	sp.	*Bungartz 6906*	Galapagos		31	14	1
DIC362	*Dictyonema*	sp.	*Moncada sn*	Colombia		31	14	1
DIC402	*Dictyonema*	sp.	*Lücking 33852*	Puerto Rico	X	19	35	1
DIC406	*Dictyonema*	*pallidoschenkianum*	*Lücking 33901*	Puerto Rico		35	19	1
DIC407	*Dictyonema*	*pallidoschenkianum*	*Lücking 33907*	Puerto Rico		19	35	1
DIC410	*Dictyonema*	*pallidoschenkianum*	*Lücking 33915*	Puerto Rico		30	13	1
DIC411	*Dictyonema*	*pallidoschenkianum*	*Lücking 33917*	Puerto Rico		19	35	1
DIC413	*Dictyonema*	*minutulum*	*Lücking 33936*	Puerto Rico		31	14	1
DIC414	*Dictyonema*	sp.	*Lücking 27018k*	Panama	X	30	13	1
DIC415	*Dictyonema*	*pallidoschenkianum*	*Lücking 34241*	Thailand	X	29	45	1
DIC501	*Dictyonema*	*vermiculiferum*	*Lücking 33529*	Colombia		24	40	1
DIC523	*Dictyonema*	*arrhenioides*	*Lücking 33365*	Colombia		3	8	1
DIC565	*Dictyonema*	*arrhenioides*	*Lücking 35282*	Colombia		8	3	1

DIC566	*Dictyonema*	*arrhenioides*	*Lücking 35284*	Colombia		8	3	1
DIC630	*Dictyonema*	Sp.	*Moncada 6336*	Colombia		52	61	2
MDF025	*Dictyonema*	*pectinatum*	*DalForno 1170*	Galapagos		44	40	1
MDF028	*Dictyonema*	*bungartziana*	*DalForno 1174*	Galapagos		13	30	1
MDF030	*Dictyonema*	*bungartziana*	*DalForno 1177*	Galapagos		30	13	1
MDF031	*Dictyonema*	*bungartziana*	*DalForno 1178*	Galapagos		30	13	1
MDF032	*Dictyonema*	*bungartziana*	*DalForno 1179*	Galapagos		13	30	1
MDF034	*Dictyonema*	*subobscuratum*	*DalForno 1181*	Galapagos		31	14	1
MDF035	*Dictyonema*	*bungartziana*	*DalForno 1182*	Galapagos		13	30	1
MDF044	*Dictyonema*	*bungartziana*	*DalForno 1191*	Galapagos		30	13	1
MDF061	*Dictyonema*	*bungartziana*	*DalForno 1208*	Galapagos		30	13	1
MDF062	*Dictyonema*	*bungartziana*	*DalForno 1209*	Galapagos		80	86	1
MDF064	*Dictyonema*	*bungartziana*	*DalForno 1211*	Galapagos		95	13	1
MDF066	*Dictyonema*	*ramificans*	*DalForno 1214*	Galapagos		97	8	1
MDF071	*Dictyonema*	*pectinatum*	*DalForno 1221*	Galapagos		75	104	1
MDF076	*Dictyonema*	*bungartziana*	*Spielmann 8249*	Galapagos		82	13	1
MDF079	*Dictyonema*	sp.	*Jonitz 655*	Ecuador		35	19	1
MDF080	*Dictyonema*	*giganteum*	*Jonitz 456*	Ecuador		30	13	1
MDF081	*Dictyonema*	sp.	*Jonitz 592*	Peru		12	103	1
MDF086	*Dictyonema*	*bungartziana*	*DalForno 1183*	Galapagos		13	30	1
MDF103	*Dictyonema*	*thelephora*	*DalForno 1070a*	Brazil		3	8	1
MDF131	*Dictyonema*	*barbatum*	*Truong 1259*	Galapagos		106	123	1
MDF132	*Dictyonema*	*barbatum*	*Truong 1533*	Galapagos		117	96	1
MDF134	*Dictyonema*	*barbatum*	*Aptroot 65523*	Galapagos	X	14	83	1
MDF136	*Dictyonema*	*barbatum*	*Bungartz 6849*	Galapagos		101	87	1
MDF138	*Dictyonema*	*barbatum*	*Yanez 1550*	Galapagos		14	83	1
MDF156	*Dictyonema*	*bungartziana*	*Yanez 1828*	Galapagos		13	82	1
MDF157	*Dictyonema*	*bungartziana*	*Yanez 1842*	Galapagos		82	13	1
MDF159	*Dictyonema*	*bungartziana*	*Yanez 2041*	Galapagos		80	30	1
MDF160	*Dictyonema*	*bungartziana*	*Yanez 2056*	Galapagos		13	82	1
MDF165	*Dictyonema*	sp.	*Aptroot 63192a*	Galapagos	X	13	95	1
MDF168	*Dictyonema*	*bungartziana*	*Aptroot 64519*	Galapagos		80	30	1
MDF169	*Dictyonema*	*bungartziana*	*Aptroot 65037a*	Galapagos		13	95	1
MDF170	*Dictyonema*	*bungartziana*	*Bungartz 3275*	Galapagos	X	95	13	1
MDF172	*Dictyonema*	*bungartziana*	*Bungartz 3956*	Galapagos		112	82	1
MDF173	*Dictyonema*	*bungartziana*	*Bungartz 5746*	Galapagos		13	88	1
MDF174	*Dictyonema*	*bungartziana*	*Bungartz 6883*	Galapagos		102	86	1
MDF175	*Dictyonema*	*bungartziana*	*Bungartz 8350*	Galapagos		13	95	1
MDF177	*Dictyonema*	*bungartziana*	*Bungartz 9476*	Galapagos		95	13	1
MDF179	*Dictyonema*	*subobscuratum*	*Bungartz 9549*	Galapagos		81	31	1
MDF180	*Dictyonema*	*subobscuratum*	*Bungartz 9550*	Galapagos	X	101	87	1
MDF181	*Dictyonema*	*subobscuratum*	*Bungartz 9551*	Galapagos	X	14	96	1
MDF182	*Dictyonema*	*subobscuratum*	*Bungartz 9552*	Galapagos	X	14	83	1
MDF184	*Dictyonema*	*bungartziana*	*Yanez 1507*	Galapagos		95	13	1
MDF185	*Dictyonema*	sp.	*Yanez 1514*	Galapagos	X	87	125	1
MDF187	*Dictyonema*	*ramificans*	*Yanez 1517*	Galapagos		85	110	1
MDF188	*Dictyonema*	*ramificans*	*Yanez 1518*	Galapagos		99	92	1
MDF190	*Dictyonema*	*ramificans*	*Yanez 1521*	Galapagos		3	100	1
MDF193	*Dictyonema*	*ramificans*	*Yanez 1534*	Galapagos		85	109	1
MDF194	*Dictyonema*	*ramificans*	*Yanez 1539*	Galapagos		85	8	1
MDF195	*Dictyonema*	*bungartziana*	*Yanez 1541*	Galapagos		13	82	1
MDF196	*Dictyonema*	*subobscuratum*	*Yanez 2058*	Galapagos		117	111	1
MDF197	*Dictyonema*	*bungartziana*	*Yanez 2162*	Galapagos		102	86	1
MDF218	*Dictyonema*	*sericeum*	*Donha 103*	Brazil	X	31	14	1
MDF228	*Dictyonema*	*novazelandica*	*Blanchon 5141*	New Zealand		3	8	1
MDF230	*Dictyonema*	*subthelephora*	*Yanez 2500*	Ecuador		8	3	1
MDF233	*Dictyonema*	*submetallicum*	*Gumboski 3549*	Brazil		35	19	1
MDF236	*Dictyonema*	*subapplanatum*	*Gumboski 2343a*	Brazil		8	3	1
MDF238	*Dictyonema*	*gumboskii*	*Gumboski 2399*	Brazil		64	65	1
MDF239	*Dictyonema*	*gumboskii*	*Gumboski 2445*	Brazil		65	64	1
MDF241	*Dictyonema*	sp.	*Caceres & Aptroot 13598*	Brazil		14	31	1
MDF242	*Dictyonema*	*subobscuratum*	*Caceres & Aptroot 13632*	Brazil		14	31	1

MDF244	*Dictyonema*	*sericeum*	*Boom 40730*	La Reunion		69	71	1
MDF245	*Dictyonema*	*sericeum*	*Boom 40588*	La Reunion		69	71	1
MDF246	*Dictyonema*	sp.	*Boom 45707*	Canary Islands		8	3	1
MDF247	*Dictyonema*	*mascarenae*	*Boom 39933*	La Reunion		11	4	1
MDF248	*Dictyonema*	sp.	*Boom 40086*	La Reunion		4	11	1
MDF249	*Dictyonema*	*vandenboomiana*	*Boom 40318*	La Reunion		67	68	1
MDF255	*Dictyonema*	*portoricense*	*Mercado-Diaz sn*	Puerto Rico		40	26	1
MDF256	*Dictyonema*	*portoricense*	*Mercado-Diaz sn*	Puerto Rico		40	42	1
MDF258	*Dictyonema*	sp.	*Beck E398*	Ecuador		14	31	1
MDF259	*Dictyonema*	sp.	*Beck E400*	Ecuador		55	47	2
MDF260	*Dictyonema*	sp.	*Beck E415*	Ecuador		15	32	1
MDF261	*Dictyonema*	sp.	*Beck E425a*	Ecuador		52	61	2
MDF262	*Dictyonema*	*beckii*	*Beck E425b*	Ecuador		51	60	2
MDF263	*Dictyonema*	*beckii*	*Beck E425c*	Ecuador		51	60	2
MDF275	*Dictyonema*	*huaorani*	*DavisYost 1051*	Ecuador		121	122	2
MDF281	*Dictyonema*	sp.	*Gumboski 4388*	Brazil	X	30	13	1
MDF284	*Dictyonema*	*sericeum*	*Gerlach 791*	Brazil		14	31	1
MDF285	*Dictyonema*	sp.	*Gerlach 985*	Brazil	X	19	35	1
MDF321	*Dictyonema*	sp.	*DalForno 1721*	Costa Rica		31	14	1
MDF322	*Dictyonema*	sp.	*DalForno 1722*	Costa Rica	X	31	14	1
MDF324	*Dictyonema*	sp.	*DalForno 1724*	Costa Rica		14	31	1
MDF325	*Dictyonema*	sp.	*DalForno 1725*	Costa Rica		40	24	1
MDF327	*Dictyonema*	sp.	*DalForno 1727*	Costa Rica		40	24	1
MDF333	*Dictyonema*	sp.	*DalForno 1733*	Costa Rica		76	107	1
MDF337	*Dictyonema*	sp.	*DalForno 1737*	Costa Rica		19	35	1
MDF339	*Dictyonema*	sp.	*DalForno 1739*	Costa Rica	X	131	132	1
MDF340	*Dictyonema*	sp.	*DalForno 1740*	Costa Rica		78	119	1
MDF341	*Dictyonema*	sp.	*DalForno 1741*	Costa Rica		78	90	1
MDF351	*Dictyonema*	sp.	*DalForno 1741*	Costa Rica		114	84	1
MDF363	*Dictyonema*	sp.	*DalForno 1763*	Costa Rica		19	35	1
MDF409	*Dictyonema*	*bungartziana*	*Nugra 1051*	Galapagos		30	13	1
MDF410	*Dictyonema*	*bungartziana*	*Spielmann 10621*	Galapagos		13	30	1
MDF411	*Dictyonema*	*bungartziana*	*Nugra 1096*	Galapagos		30	13	1
MDF423	*Dictyonema*	sp.	*DalForno 1803*	Ecuador		83	14	1
MDF444	*Dictyonema*	sp.	*DalForno 1861*	Ecuador	X	29	45	1
MDF463	*Dictyonema*	sp.	*DalForno 1928*	Ecuador		21	37	1
MDF464	*Dictyonema*	sp.	*DalForno 1933a*	Ecuador		24	40	1
MDF467	*Dictyonema*	sp.	*DalForno 1935*	Ecuador	X	25	41	1
MDF468	*Dictyonema*	sp.	*DalForno 1936*	Ecuador		9	2	1
MDF469	*Dictyonema*	sp.	*DalForno 1961*	Ecuador		2	7	1
MDF473	*Dictyonema*	sp.	*DalForno 1965*	Ecuador	X	70	71	1
MDF484	*Dictyonema*	sp.	*DalForno 1981b*	Ecuador		37	21	1
MDF487	*Dictyonema*	*hernandezii*	*DalForno 1982a*	Ecuador		21	37	1
MDF488	*Dictyonema*	sp.	*DalForno 1983*	Ecuador		40	24	1
MDF489	*Dictyonema*	sp.	*DalForno 1984*	Ecuador		21	37	1
MDF491	*Dictyonema*	sp.	*DalForno 1987*	Ecuador		40	24	1
MDF492	*Dictyonema*	sp.	*DalForno 1988*	Ecuador	X	13	30	1
MDF520	*Dictyonema*	sp.	*DalForno 2020*	Brazil		3	8	1
MDF521	*Dictyonema*	sp.	*DalForno 2021*	Brazil		3	99	1
MDF536	*Dictyonema*	sp.	*DalForno 2036*	Brazil	X	3	8	1
MDF541a	*Dictyonema*	sp.	*DalForno 2041a*	Brazil	X	14	31	1
MDF544	*Dictyonema*	*subapplanatum*	*DalForno 2044*	Brazil		14	31	1
MDF601	*Dictyonema*	sp.	*DalForno 2050*	Brazil		126	35	1
MDF602	*Dictyonema*	sp.	*DalForno 2051*	Brazil	X	19	35	1
MDF605	*Dictyonema*	sp.	*DalForno 2055*	Brazil		19	35	1
MDF616	*Dictyonema*	sp.	*DalForno 2085*	Brazil		31	14	1
MDF617	*Dictyonema*	sp.	*DalForno 2092b*	Brazil		19	35	1

MDF621	*Dictyonema*	sp.	*DalForno 2102*	Brazil	X	31	14	1
MDF634	*Dictyonema*	sp.	*DalForno 2131*	Brazil		14	31	1
MDF635	*Dictyonema*	sp.	*DalForno 2132*	Brazil		69	71	1
MDF027	*Coccocarpia*	*pellita*	*DalForno 1172*	Galapagos	N/A	44	46	1
MDF046a	*Coccocarpia*	sp.	*DalForno 1193*	Galapagos	N/A	28	44	1
MDF114b	*Coccocarpia*	sp.	*DalForno 1274b*	Brazil	N/A	35	19	1
MDF344	*Coccocarpia*	*microphyllidia*	*DalForno 1744*	Costa Rica	N/A	14	83	1
MDF345	*Coccocarpia*	*stellata*	*DalForno 1745*	Costa Rica	N/A	95	13	1
MDF419	*Coccocarpia*	sp.	*DalForno 1801b*	Ecuador	N/A	81	31	1
MDF437	*Coccocarpia*	sp.	*DalForno 1831*	Ecuador	N/A	77	105	1
MDF455	*Coccocarpia*	sp.	*DalForno 1922*	Ecuador	N/A	35	19	1
MDF471	*Coccocarpia*	sp.	*DalForno 1963*	Ecuador	N/A	30	13	1
MDF477	*Coccocarpia*	sp.	*DalForno 1975*	Ecuador	N/A	35	19	1
MDF479	*Coccocarpia*	sp.	*DalForno 1977*	Ecuador	N/A	13	30	1
MDF505	*Coccocarpia*	sp.	*DalForno 2005*	Brazil	N/A	31	14	1
MDF515	*Coccocarpia*	sp.	*DalForno 2015*	Brazil	N/A	53	62	2
MDF613	*Coccocarpia*	sp.	*DalForno 2074*	Brazil	N/A	14	31	1
MDF629	*Coccocarpia*	sp.	*DalForno 2120*	Brazil	N/A	61	52	2
MDF631	*Coccocarpia*	sp.	*DalForno 2123*	Brazil	N/A	11	4	1
MDF506	*Erioderma*	sp.	*DalForno 2006*	Brazil	N/A	14	31	1
MDF532	*Erioderma*	sp.	*DalForno 2032*	Brazil	N/A	31	14	1
MDF029	*Leptogidium*	*stipitatum*	*DalForno 1176*	Galapagos	N/A	34	18	1
MDF508	*Leptogidium*	sp.	*DalForno 2008*	Brazil	N/A	31	X	1
MDF541b	*Leptogidium*	sp.	*DalForno 2041b*	Brazil	N/A	14	31	1

Table 22 - Cyanobacteria used for the 16S analysis only

Control		**Genus**		**Habit**		**Country**	**Haplotype**
>MDF115	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF115	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF346	pri	*Nostoc*	from	*Pannaria*	sp.	CostaRica	N/A
>MDF346	sec	*Nostoc*	from	*Pannaria*	sp.	CostaRica	N/A
>MDF416	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF416	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF420	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF420	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF427	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF427	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF432	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF432	sec	*Nostoc*	from	*Fuscopannaria*	sp.	Ecuador	N/A
>MDF472	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF472	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF478	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF478	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF482	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF482	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF483	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF483	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF486	pri	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF486	sec	*Nostoc*	from	*Pannaria*	sp.	Ecuador	N/A
>MDF539	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF539	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF603	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF603	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF604	pri	*Nostoc*	from	*Leptogium*	sp.	Brazil	N/A
>MDF604	sec	*Nostoc*	from	*Leptogium*	sp.	Brazil	N/A
>MDF614	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF614	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A

>MDF615	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF615	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF618	pri	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF618	sec	*Nostoc*	from	*Pannaria*	sp.	Brazil	N/A
>MDF633	pri	*Nostoc*	from	*Leptogium*	sp.	Brazil	N/A
>MDF633	sec	*Nostoc*	from	*Leptogium*	sp.	Brazil	N/A
>DIC203	pri	*Rhizonema*	from	*Cora*	*auriculata*	Ecuador	14
>DIC203	sec	*Rhizonema*	from	*Cora*	*auriculata*	Ecuador	31
>DIC331	pri	*Rhizonema*	from	*Dictyonema*	*aeruginosulum*	CostaRica	19
>DIC331	sec	*Rhizonema*	from	*Dictyonema*	*aeruginosulum*	CostaRica	35
>DIC429	pri	*Rhizonema*	from	*Cora*	*arachnoidea*	Colombia	47
>DIC429	sec	*Rhizonema*	from	*Cora*	*arachnoidea*	Colombia	55
>DIC603	pri	*Rhizonema*	from	*Corella*	*fuscoisidiata*	Colombia	52
>DIC603	sec	*Rhizonema*	from	*Corella*	*fuscoisidiata*	Colombia	61
>MDF406	pri	*Rhizonema*	from	*Cora*	*galapagoensis*	Galapagos	13
>MDF406	sec	*Rhizonema*	from	*Cora*	*galapagoensis*	Galapagos	30
>MDF500	pri	*Rhizonema*	from	*Cora*	*terrestris*	Brazil	3
>MDF500	sec	*Rhizonema*	from	*Cora*	*terrestris*	Brazil	8
>DIC260	pri	*Scytonema*	sp.	free	living	Galapagos	N/A
>DIC260	sec	*Scytonema*	sp.	free	living	Galapagos	N/A
>DIC340	pri	*Scytonema*	sp.	free	living	Panama	N/A
>DIC340	sec	*Scytonema*	sp.	free	living	Panama	N/A
>MDF082	pri	*Scytonema*	sp.	free	living	Peru	N/A
>MDF082	sec	*Scytonema*	sp.	free	living	Peru	N/A
>MDF243	pri	*Scytonema*	sp.	free	living	Brazil	N/A
>MDF243	sec	*Scytonema*	sp.	free	living	Brazil	N/A
>MDF438	pri	*Scytonema*	sp.	free	living	Ecuador	N/A
>MDF450	pri	*Scytonema*	sp.	free	living	Ecuador	N/A
>MDF450	sec	*Scytonema*	sp.	free	living	Ecuador	N/A
>MDF452	pri	*Scytonema*	sp.	free	living	Ecuador	N/A
>MDF452	sec	*Scytonema*	sp.	free	living	Ecuador	N/A
>MDF276	pri	*Stigonema*	from	*Ephebe*	sp.	Brazil	N/A
>MDF276	sec	*Stigonema*	from	*Ephebe*	sp.	Brazil	N/A

Chapter 6 – Conclusions and Future Work

Conclusions

As one of the main outcomes of this dissertation work, we now are able to recognize 5 genera in *Dictyonema* s.l., accepting one of them as paraphyletic. We also show that *Dictyonema* s.l. can be used as a model of evolutionary transitions, since it presents a gradual incorporation of the basidiocarps into the lichen thallus. Overall, the clade includes a remarkable variety of morphologies and symbioses, with the basal genus *Cyphellostereum* probably representing a transitional group.

The species in *Cora* appear to be relatively young and shows that they present an extraordinary case of rapid radiation. This genus had been previously synonymized under *Dictyonema*, and our results not only demonstrate is a separate genus, but also represents over a hundred species in what used to be considered only one, *Dictyonema glabratum.* This study was also able to show *Corella* as a separate genus, and therefore should be resurrected. It also used to be part of *Dictyonema glabratum*, and now we are able to say it constitutes a distinct genus, apart from *Dictyonema* and *Cora* and sister to *Acantholichen*. The genus *Acantholichen* previously thought to be monotypic has currently six species and also represents the potential to be speciose and to present high levels of endemism.

Our focus collecting in the Galapagos Islands allowed us to revise species concepts of basidiolichens and we now recognize ten species in the archipelago. Our enhanced, pantropical sampling of the *Dictyonema* clade showed that all ten current species have arrived to the islands separately by multiple colonization events. At this moment, eight are considered endemic, while two can also be found on mainland Ecuador.

ITS has been used widely in the mycological community as a barcoding locus and we strongly believe that among the markers currently being used, ITS is the best option for species delimitation in the *Dictyonema* clade. This molecular marker provides very good resolution and support for many clades and our additional data, prevenient from anatomical, morphological and ecological data corroborates with tree topology, except perhaps in the paraphyletic genus *Dictyonema.* However, monophyly tests (Dal-Forno, unpublished data; Lawrey et al., 2009), have not been able to reject that hypothesis.

The photobiont of all *Dictyonema* is the cyanobacteria *Rhizonema* so far only found lichenized. This cyanobacterial genus can also be found on other ascolichens, such as *Coccocarpia, Erioderma, Leptogidium* and *Stereocaulon*. There seems to be most of the time a primary and a secondary haplotypes within the lichen thallus. This is actually attributed to a SNP (Cytosine or Adenine) in bp# 12 (bp# 19 in *E. coli* numbering, or the very beginning of the 16S gene), and not a real representation of two different OTUs.

There are 12 main haplotypes of *Rhizonema* in the partial 16 rDNA used, or six main combinations of haplotypes. These haplotypes are almost always together, with

only a few exceptions. These exceptions include two haplotypes (one main one and a less frequent one), but those two haplotypes are also different by one or a couple SNPs.

The photoniont *Rhizonema* appears to be biologically constrained by the fungi, and only two distinct lineages can be observed across all specimens sampled with the current 16S rDNA data. Many haplotypes within these two lineages can be found; however, the different haplotypes are always within the same species of *Rhizonema*. Haplotypes within a single lichen thallus can only be of lineage 1 or lineage 2, but never one of each.

Rhizonema lineage 2 is mainly found within one of the two clades of *Cora.* This clade has no morphological or anatomical differences from the other *Cora*, and therefore we still call both of them the same genus. However, the unique finding of a different species of *Rhizonema* has lend support to think that there is a relationship between the two. The evolution of a second species of *Rhizonema* seems to have triggered a split in *Cora.* The *Rhizonema* lineage 2 and *Cora* Clade 1 are represented mainly by specimens from the wet paramos in northern Colombia.

Overall, our results reinforce the idea, not prevalent in mycology, that species other than microfungi represent most of the undescribed species of Fungi. This group, previously known by a couple species in a single genus, shows a high diversity in multiple genera. These species are mostly not cryptic, they have distinct morphology, anatomy, substrate preference, ecology and distribution. Our sampling efforts had focus mainly in the Neotropics, but we extrapolate that the Paleotropics and temperate zones may also harbor unrecognized diversity.

We hypothesize that one of the reasons that the diversity of this group has not been recognized before is because foliose forms, such as *Cora* and *Corella*, loose diagnostic characteristics, such as thallus color, texture and arrangement of its lobes, shape and color of hymenophores, etc., when removed from their natural habitat. We emphasize again that these are basidiolichens belonging to the order Agaricales (Agaricomycetes, Basidiomycota), an order which contains some of the most charismatic and known mushrooms, such as the fly agaric (*Amanita muscaria*), but also the most commonly eaten mushroom, called button, cremini or portobella mushrooms [*Agaricus bisporus* (J.E. Lange) Imbach]. Long known by mycologists, these mushrooms are measured and studied in the field, and we advocate for similar approaches when studying *Dictyonema* s.l. and basidiolichens in general.

Data from this dissertation points to a very high diversity in the fungal partner and a very low diversity of the photobiont. Some haplotypes of *Rhizonema* are shared among many different clades within *Dictyonema* s.l., while some are shared mainly among lineages within a single clade. Since only data from a small region of the 16S gene was used for the cyanobacterial partner, one that is considered very conserved, there is need to further investigatethe distribution of photobionts within the *Dictyonema* clade using other genetic markers. However, our use of only a partial sequence belonging to the 16S rDNA was able to elucidate haplotype patterns in two distinct lineages of *Rhizonema.* This is still very low diversity is compared to the hundreds species of the fungal partner we are now able to recognize. Comparing this extraordinary different numbers of species within both bionts lend support to previously published theory about lichen photobiont

domestication, in which the photobiont is being constrained by the fungi and evolutionary changes are very limited. This supports the ideas of the Canadian lichenologist Trevor Goward (1994), who said "lichens are fungi that have taken up agriculture", since it seems like the mycobionts and not photobionts are doing the selecting and the associations are a reflection of the "choices" they make.

Future work

There are a number of new questions that have resulted from this dissertation and remain to be answered in the future. Our working group has now the enormous challenge of publishing hundreds of new species in the genera *Cora*, *Corella*, *Cyphellostereum* and *Dictyonema* in which species boundaries have been established. Although this dissertation is a beginning to start recording this unrecognized diversity, we still have many more to go. This is a long life project, considering that our samples are mainly focus in the Neotropics and the paleotropics remain to be collected comprehensively. For example, it is a main goal to work on a large project of species of the *Dictyonema* clade in Brazil, including a field guide with pictures and distribution of each one of the species.

Concerning the DNA sequence data used in this study, molecular phylogenies of *Dictyonema* have primarily utilized ITS, which has been adopted by the mycological community as barcoding locus (Schoch et al., 2012). However, some clades, particularly those that are shallow-rooted and of recent origin, are poorly resolved using only ITS. Therefore, the development of new genetic markers is in need for phylogenetic

reconstruction of these rapidly evolving or recently derived lineages. In combination with ITS, new markers may help to resolve relationships and strengthen support throughout the tree, increasing our knowledge of species diversification in this clade.

Lastly, another project in preparation is regarding the microbiome of the lichen symbiosis of the *Dictyonema* clade. Lichens are traditionally described as a symbiosis between a fungus and a green algae and/or cyanobacteria, but this is a rather simplistic definition since they are also known to harbor many microorganisms (fungi and bacteria primarily), some of which are obligately lichen-associated. While collecting data for this dissertation, data was also collected on the lichen microbiome using next-generation sequencing (Roche 454 GS Junior) for approximately 560 specimens of *Dictyonema*, and it is possible to observe bacterial community profiles associated with these lichens, especially regarding their morpho-type. We expect that these will vary in interesting ways, especially regarding the lichen morpho-type, but perhaps also with habitat type and geography.

Appendices

Appendix 1. Specimen protocol for the different forms.

Dictyonema/Cyphellostereum Genbank ITS: _____

Control Number: _____

Collector: Country: Pictures:

MORPHOLOGY

Thallus: epiphytic – terricolous – rock-inhabiting – on wood – bryophylous – lichenicolous.

associates with: lichens – bryophytes on bark: twigs – branches – lianas – trunk – bryophyte-laden

Growth form: shelf-like – forming a mat / filamentous – crustose / fibrils appressed: irregularly – strongly / completely embedded in matrix – intermediate – resting on / clustering of fibrils: more or less individual – interwoven / density of the fibrils: dense – medium – scarce/ circular orientation: present – absent / angle of fibrils: horizontal (flat) – obliquous – perpendicular (erect) / direction: going everywhere – intermediate – same (uniform) – combed – arachnoid / special characters: none – microfruticose – with projections – other: _____

Size: ___-___ cm diam. or ___-___ cm long, ___-___ cm wide / small – medium – large / continuous – more or less continuous – dispersed patches

Lobes: present – absent / __ to __ per thallus / ___-___ cm long, ___-___ cm wide

Color: dark –light / olive-green – green – greenish – aeruginous – bluish – blue / dull – shiny

Prothallus: absent – present / thin – distinct / white - _____/___–___ µm thick

Hypothallus: absent – present / visible – not visible / white - _____/

Hymenophore: marginal–submarginal–laminal / resupinate–steroid–cyphelloid to corticioid–steroid–cyphelloid/ rounded–angular–linear–irregular–elongate–reticulate patches/ separated–slightly fused–largely fused/ small–

medium–large/ ___–___mm long and ___–___ mm broad/dense–scattered–sparse /surface: smooth – byssoid / margins: smooth–byssoid/ involute–flat

color: fresh: white–pale yellow–yellow–orange–brown herbarium: white–pale yellow–yellow–orange–brown

---

ANATOMY

Thallus in section: ___–___ µm thick, with: photobiont layer– medulla

Photobiont layer: ___–___ µm thick / individual photobiont cells ___–___ µm broad and ___–___ µm long / uniseriate – biseriate – triseriate

color: dark –light / olive-green – green – greenish – aeruginous – bluish – blue/ tips: same color – yellow – orange

heterocytes: sparse –frequent/hyaline – pale yellow – bright yellow/___–___ µm wide and ___–___ µm long

cells of hyphal sheath: ___–___ µm thick / completely covering cyanobacteria (dense) – leaving spaces (loose) / wavy (normal) – less wavy – more angular / ornamentation: papillose – verrucose – other: _____

Medulla/hypothallus: ___–___ µm thick / hyphae ___–___ µm thick

Clamp connections: not observed – observed/ ___–___ µm thick /location:_____

Hymenophore in section: ___–___ µm thick/ ___–___ µm thick medullary hyphae

hymenium: numerous–scattered palisade-like basidioles / numerous–scattered basidia

basidioles: ___–___× ___–___µm, cylindrical – clavate/ rounded-irregular tips

basidia: ___–___× ___–___µm, cylindrical – clavate/ rounded-irregular tips

basidiospores: absent – present/ few – abundant /ellipsoid – lacrymoid – fusiform/ ___–___× ___–___ µm

REMARKS.

It is part of the complex:

1- This species is morphologically similar to

_____

Differing in

_____

Related phylogenetically: yes – no

2- This species is morphologically similar to

_____

Differing in

_____

Related phylogenetically: yes – no

DRAWINGS

Cora/Corella Genbank ITS: _____ Control

Number: _____

Collector: Country: Pictures:

MORPHOLOGY

Thallus: epiphytic – terricolous – rock-inhabiting – on wood – bryophylous – lichenicolous.

associates with: lichens – bryophytes on bark: twigs – branches – lianas – trunk – bryophyte-laden

Growth form: Foliose – macrosqualmulose – squamulose. **Size:** ___-___ cm diam. or ___-___ cm long, ___-___ cm wide

Lobes: __ to __ per thallus / ___-___ cm long, ___-___ cm wide / densely branched–sparsely branched–once branched–not branched

Radial branching sutures: present (rare – sparse – abundant) – absent

Texture: thick –thin / coriaceous – papyraceous – crassus – succulentus – other:_____/ wrinkle – straight / _____

Relief/direction of lobes: completely flat – parallel with the substrate – somewhat erect / ascending – descending /

Fresh color: Uniform – varying in concentric zones (slight – distinct) / dull-shiny

white – grey – brown –green – blue – yellow**: _____**

submarginal zone: lighter – darker – same / white – grey – brown –green – blue – yellow**:**

Herbarium color: Uniform – varying in concentric zones

white – grey – brown –green – blue – yellow**: _____**

submarginal zone: lighter – darker – same – different / white – grey – brown –green – blue – yellow**:**

Rehydrated color (in the lab): same – different / white – grey – brown –green – blue – yellow**:**

Upper surface: concentric ridges: with – without / ridges __ to __ per cm lobe length / pronounced – shallow

pits: with – without / dense – sparse / pronounced – shallow

hairs: with – without / coarse – fine / dense – sparse/ angle: erect – obliquous – flat / direction: going everywhere – intermediate – same (uniform) – combed – arachnoid / tips: individual – intertwined – base: individual – intertwined / color: white – yellowish / location: on entire surface – in concentric zones – towards the margins – in concentric zones and towards the margin / size: long – short /__ - __ mm long, __ - __ mm wide at the tip, __ - __ mm wide at the base/

Margin: involute – flat/ slight – distinct/ thick – thin / __ - __ mm thick / surface: even – granular – sorediate to even – granular – sorediate / glabrous – finely arachnoid – hairy / color: white – grey – brown –green – blue – yellow.

Soredia: present – absent / marginal – submarginal – laminal / white – grey – brown –green – blue – yellow.

Lower surface: corticated – ecorticate / glabrous – hairy – felty-arachnoid / coarse – fine / dense – sparse/ angle: erect – obliquous – flat / direction: going everywhere – intermediate – same (uniform) – irregular – combed – arachnoid / tips: individual – intertwined – base: individual – intertwined / color: white – yellowish / location: on entire surface – in concentric zones – towards the margins – in concentric zones and towards the margin / size: long – short /__ - __ mm long, __ - __ mm wide at the tip, __ - __ mm wide at the base

Fresh color: Uniform – varying in concentric zones / same as upper surface – differing from upper surface

white – grey – brown –green – blue – yellow**: _____**

Herbarium color: Uniform – varying in concentric zones / same as upper surface – differing from upper surface

white – grey – brown –green – blue – yellow**: _____**

submarginal zone: lighter – darker- same / white – grey – brown –green – blue – yellow**:_____**

Hymenophore: marginal–submarginal–laminal / resupinate–steroid–cyphelloid to corticioid–steroid–cyphelloid/ rounded–angular–linear–irregular–elongate–reticulate patches/ separated–slightly fused–largely fused/ small–medium–large/ ___–___mm long and ___–___ mm broad/dense–scattered–sparse /surface: smooth – byssoid / margins: smooth–byssoid/ involute–flat

color: fresh: white–pale yellow–yellow–orange–brown herbarium: white–pale yellow–yellow–orange–brown

---

ANATOMY

Thallus in section: ___–___ µm thick, with: upper cortex – photobiont layer– medulla

Upper cortex: roof-like – paraplectenchymatous

Upper layer: ___–___ µm thick /loosely woven – densely packed/ irregularly arranged – periclinal / ___–___ µm thick hyphae

'Medullary' layer: ___–___ µm high/irregularly arranged – anticlinal/ ___–___ µm thick hyphae

Towards the margin: same pattern – no such distinction visible (cortex entirely formed by loosely woven – densely packed hyphae).

Photobiont layer: ___–___ µm thick / cyanobacteria clusters ___–___ µm diam./ individual photobiont cells ___–___ µm broad and ___–___ µm long

color: dark–light/blue–green to brown–yellow–orange in upper portions

heterocytes: sparse –frequent/hyaline – pale yellow – bright yellow/___–___ µm wide and ___–___ µm long

cells of hyphal sheath: ___–___ µm thick

Medulla: ___–___ µm thick / hyphae ___–___ µm thick/ clamp connections not observed–observed/ ___–___ µm thick /location:_____.

Hymenophore in section: ___–___ µm thick/ ___–___ µm thick medullary hyphae

hymenium: numerous–scattered palisade-like basidioles / numerous–scattered basidia

basidioles: ___–___× ___–___µm, cylindrical – clavate/ rounded-irregular tips

basidia: ___–___× ___–___µm, cylindrical – clavate/ rounded-irregular tips

basidiospores: absent – present/ few – abundant /ellipsoid – lacrymoid – fusiform/ ___–___× ___–___ µm

---

REMARKS.

It is part of the complex:

1- This species is morphologically similar to

_____,

Differing in

_____

Related phylogenetically: yes – no

2- This species is morphologically similar to

_____

Differing in

_____

Related phylogenetically: yes – no

DRAWINGS

Appendix 2. Protocol for DNA Extraction followed for all samples.

BIO101-FastPrep-DNA Extraction (Protocol for Fungi and Lichens):

1) Add 1ml lysing buffer (CLS-Y) to the provided tubes with the sample and the Lysing Matrix.

2) Add an additional ceramic bead to the tubes.

3) Mix and Homogenize in FastPrep Instrument for 30 sec. at speed 5.5 × 2.

4) Centrifuge tubes for 10 min. at 14,000 × g to pellet debries.

5) Transfer the supernatant to a clean 1.5 mL centrifuge tube.

6) Add 250 µL **PPS** reagent and mix by inverting the tube by hand 10 times.

7) Centrifuge at 14,000 × g for 5 min. to pellet precipitate.

8) Transfer supernatant (600–800 µL) to a clean 2mL centrifuge tube.

9) Add equal volume of Binding Matrix (mix the binding matrix well before use).

10) Mix gently and incubate tubes with agitation (in a rotator) for 5 min. at room temperature.

11) Incubate tubes for 15–20 min. at room temperature to settle down the matrix.

12) Remove 600–650 µL of top layer of the supernatant being careful to avoid settled **Binding Matrix** and discard.

13) Repeat last step.

14) Mix the remainder (approximately 650 μL) and transfer of the mixture to a **Spin Filter** and centrifuge the spin filter and catch tube at 14,000 × g for 1 minute.

15) Empty the catch tube and add 500 μL **SEWS-M** (make sure that ETOH is added to **SEWS-M**) to the **Spin Filter** and centrifuge at 14,000 × g for 1 minute.

16) Repeat last step (add SEWS-M and centrifuge).

17) Centrifuge for 2 min. at 14000 × g to dry Binding Matrix/DNA complex.

18) Transfer the spin filter to a new catch tube.

19) Air dry the Spin Filter for 5 min. at room temp (in the hood).

20) Elute DNA by resuspending Binding Matrix/DNA complex in 150 μL DES or DEPC water (warmed at 65°C).

21) Incubate for 2 minutes at 55°C (in a heat block) or water bath.

22) Centrifuge for 1 minute at 14000 × g to transfer the DNA to the catch tube.

23) Store the DNA at –20°C.

The size of the thallus used for extraction usually varied from a 0.2–0.5 cm^2, however if the specimen was small only 0.1 cm^2 was used. The extractions were initially quantified in a 1% agarose gel with ethidium bromide; however, this procedure was stopped because strong bands were not obtained and still, the PCR would work.

The DNA extraction was diluted in DEPC-treated water (Deionized, diethylpyrocarbonate) in the following proportions:

- 1:10 dilution (5 μL DNA + 45 μL of DEPC) = 50 μL
- 1:5 dilution (5 μL DNA + 20 μL of DEPC) = 25 μL

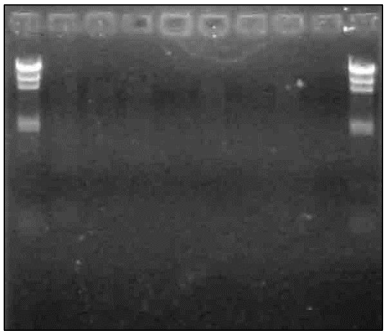


Figure 38 - Gel electrophoresis (1% agarose gel with ethidium bromide) with DNA extractions of *Corella*

Appendix 3 Polymerase Chain Reaction (PCR).

With the Polymerase Chain Reaction it is possible to selectively amplify a target region of genetic material. This process yields hundreds of thousands of copies of the target region of DNA, and involves three main steps: denaturation, annealing and extension. In the first step, the DNA solution is heated to separate the double helix into two separate strands. In the next step, the solution is cooled, allowing a set of primers to anneal. Primers are short segments of synthetic DNA which anneal to an area flanking the target region of the DNA. In the final step, extension, the DNA polymerase enzyme (Taq Gold polymerase - Applied Biosystems, Foster City, California, USA) synthesizes a complementary strand of DNA using synthetic nucleotide bases (dNTPs). This process is repeated in a thermal cycling machine for 40–45 cycles (the 40–45 cycles do not involve the extension or the initial long denaturation).

For a 20 μL reaction, we use:

- DEPC Water: 7.9 μL
- 10X buffer: 2 μL
- Mg mix: 2 μL
- dNTPs: 2 μL
- BSA: 2 μL
- Primer 1 (forward): 1 μL
- Primer 2 (reverse): 1 μL
- Taq Polymerase: 0.1 μL
- DNA: 2 μL

The PCR cycling program used is:

1- 95ºC for 11 min.
2- 95ºC for 30 sec.
3- 50ºC (ITS and *RPB2*) or 47 ºC (nuLSU) for 30 sec.
4- 72ºC for 2 min.
5- Repeat steps 2–4 39 times
6- 72ºC for 10 min.
7- ∞ at 4ºC

When amplification was not successful utilizing the regular PCR procedure (as described above), we performed a PCR using Bio-X-Act Long Mix (Bioline USA, Taunton, Massachusetts, USA).

For a 20 µL reaction, we use:

- DEPC Water: 10 µL
- 2X Mix: 6 µL
- Primer 1 (forward): 1 µL
- Primer 2 (reverse): 1 µL
- DNA: 2 µL

The PCR cycling program being used is shown below:

1. 95ºC for 5 min.
2. 95ºC for 30 sec.
3. 50ºC (ITS and *RPB2*) or 47 ºC (nuLSU) for 30 sec.
4. 72ºC for 2 min.
5. Repeat steps 2–4 34 times
6. 72ºC for 30 min.
7. ∞ at 4ºC

The PCR process is performed with primers to amplify three genes from the mycobiont (M) and photobiont (P) (**Table 23**):

- Nuclear Internal Transcriber Spacer rDNA (ITS, composed of ITS1, 5.8S and ITS2) (approximately 700 bp). Primers used ITS4 and ITS5.

- Nuclear Large Subunit rDNA (nuLSU) (approximately 1400 bp). Primers used: LR0R and LR7.

- RNA polymerase II subunit (*RPB2*) (approximately 600 bp). Primers used: b*RPB2*-6F or b*RPB2*-5F, and b*RPB2*-7R, b*RPB2*-7R2 or b*RPB2*-7.1R.

- 16S rRNA (approximately 355bp). Primers used: L27F and 355R.

Table 23 - Markers used for PCR and/or sequencing

	Primer	Primer sequence (5'–3')	References
M	ITS4	TCCTCCGCTTATTGATATGC	(White et al., 1990)
M	ITS5	GGAAGTAAAAGTCGTAACAAGG	(White et al., 1990)
M	ITS2	GCTGCGTTCTTCATCGATGC	(White et al., 1990)
M	ITS1F	CTTGGTCATTTAGAGGAAGTAA	(Gardes and Bruns, 1993)
M	LR0R	ACCCGCTGAACTTAAGC	http://www.biology.duke.edu/fungi/mycolab/primers.htm
M	LR7	ACCCGCTGAACTTAAGC	http://www.biology.duke.edu/fungi/mycolab/primers.htm
M	LR3R	GTCTTGAAACACGGACC	http://www.biology.duke.edu/fungi/mycolab/primers.htm
M	LR16	TTCCACCCAAACACTCG	http://www.biology.duke.edu/fungi/mycolab/primers.htm
M	LR5	TCCTGAGGGAAACTTCG	http://www.biology.duke.edu/fungi/mycolab/primers.htm
M	b*RPB2*-6F	TGGGGYATGGTNTGYCCYGC	(Matheny, 2005)
M	f*RPB2*-5F	GAYGAYMGWGATCAYTTYGG	(Liu et al., 1999)
M	b*RPB2*-7R	GAYTGRTTRTGRTCRGGGAAVGG	(Matheny, 2005)
M	b*RPB2*-7R2	ACYTGRTTRTGRTCNGGRAANGG	(Matheny et al., 2007)
M	b*RPB2*-7.1R	CCCATRGCYTGYTTMCCCATDGC	(Matheny, 2005)
P	L27F	AGAGTTTGATCMTGGCTCAG	(Lane, 1991)
P	355R	GCTGCCTCCCGTAGGAGT	(Lane, 1991)

PCR products are run in a 1% agarose gel with ethidium bromide to confirm the size, as well as the quantity and quality of the product.

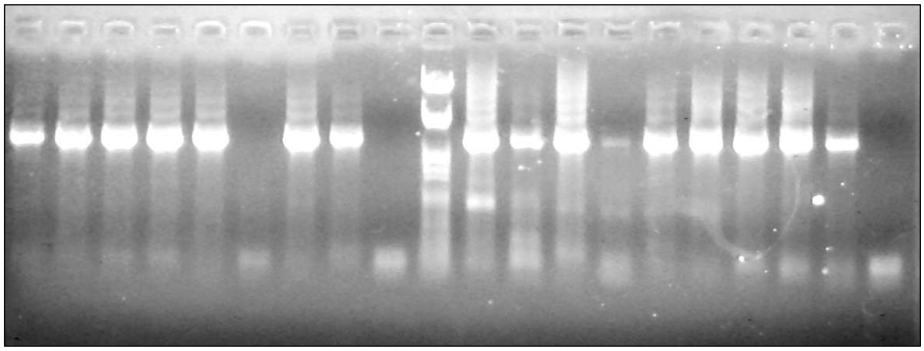


Figure 39 - Gel electrophoresis showing PCR products (bands)

Appendix 4. Clean up process using magnetic beads.

PCR cleanup is necessary to remove excess dNTPs and primers from the product solution before the product can be sequenced. The products are purified with magnetic beads (Agencourt Biosciences, Beverly, Massachusetts, USA), following the manufacturer's protocol below.

Purifying PCR products Using AMPure (Magnet Beads):

1) Transfer 10 μL PCR product to a 300 μL round bottom microtiter plate (you can save the pipette tips for pipetting at step 3).
2) Mix and add 18 μL AMPure PCR Bind buffer to PCR reaction (1.8 × Rxn. Vol.).
3) Mix the AMPure and PCR product by pipetting up and down thoroughly (DNA binds to the magnet beads).
4) Wait for about 10 minutes and repeat step 3.
5) Place the purification plate onto a SPRIplate to separate beads from solution.
6) Wait 15–20 minutes depend on Rxn. size volume (solution should be clear).
7) Aspirate cleared solution (~25 μL) from the purification plate situated on SPRIplate (care should be taken not to upset the ring of separated magnetic beads).

8) Dispense 200 μL of 70% ethanol (freshly made) to each well of purification plate.
9) Aspirate the wash solution after 30 sec. and discard (changing the pipette tips is not necessary).
10) Repeat steps 8 and 9.
11) Be sure to remove all of the ethanol from the bottom of the well or it may take a long time to dry (all of this should be done on the magnet plate).
12) Place the purification plate (while it's still on the magnet plate) on the bench top to air dry (dry completely: ~10 min.).
13) Add 40 μL of elution buffer (TE or water) to each well. Seal the plate and spin it down for 30 seconds (this should be done off of the magnet plate).
14) Mix samples by pipetting up and down 10 times.
15) Wait 10 minutes before next step to allow beads to hydrate.
16) Mix samples by pipetting up and down 10 times again.
17) Place the plate on magnet and allow solution to separate until it is clear (10–15 min.).

Optional:

18) Pipette DNA (~35 μL) from the plate while it is situated on the SPRIplate (you may run the remaining of the purified product on an agarose gel to quantify the product).
19) Purified PCR product is ready to use in downstream processes.

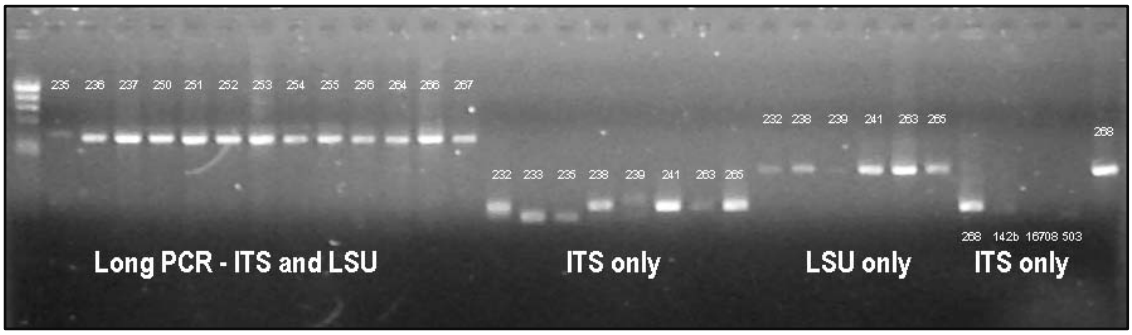


Figure 40 - Gel electrophoresis showing clean up products

Appendix 5. Sequencing reaction protocol.

The purified PCR products are used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems) as below:

Standard Sequencing Reaction Protocol using Big Dye Terminator:

Reagents	10 µL Rxn
1) Big Dye Terminator (Ready Reaction Mix)	1µL
2) 5× Reaction Buffer	3µL
4) Primer (final con.= 3.2 pmol)	1µL
5) diH2O+ DNA (Cleaned PCR product= 30–90 ng)	5 µL

1. Turn on the thermal cycler (it needs to reach the right temperature) and select the cycle, but do not start the cycle.
2. Keep and thaw all of the reagents on ice.
3. Label the tubes with ordered numbers (e.g., 1–32).
4. Add water to the tubes if needed (depend on the DNA concentration of the cleaned products).
5. Make a master mix of Big Dye and 5× Reaction buffer for the number of the reactions plus one extra, vortex and add 4 µL to each tube.

6. Add 1 μL of primer to the tubes (if different primers are being used, if only one, you may include in the master mix).

7. Add the DNA (cleaned PCR product) to the tubes and spin the tubes down.

8. Put the tubes in the thermal cycler, and start the proper cycle.

Note:

*Add the components in order: water, primer, master mix and DNA at the end.

*Put the tubes in thermal cycler immediately after adding the DNA and spinning.

Standard Rxn. Conditions for Sequencing:

1 cycle @ 96ºC for 1 min.

25–50 cycles@ 96ºC for 30 sec.

50ºC for 15 sec.*

60ºC for 4 min.

∞ @ 4ºC

*Note: Annealing temperature can be changed depend on the Tm of the primer used in the reaction.

The primers used were LR0R, LR3R, LR5, LR7, LR16, ITS4 and ITS5 (**Table 23**). *RPB2* sequences were obtained between conserved domains 6 and 7 of *RPB2* using basidiomycete specific primers b*RPB2*-6F or f*RPB2*-5F, and b*RPB2*-7R, b*RPB2*-7R2 or b*RPB2*-7.1R (**Table 23**).

Appendix 6. Sephadex protocol.

Sequencing Reaction Clean-up with Sephadex G-50:

1. Pour a small amount of Sephadex powder on the Multiscreen Filtration System (black plate) and spread powder into desired number of wells using flat glass plate. Once all desired wells are filled, put excess Sephadex powder back into Sephadex container.
2. Take a 96 well millipore plate and slide it onto the Multiscreen Filtration plate upside-down with the letters of the millipore plate against metal peg on the multiscreen filtration plate. Once it is in place, flip over so that the multiscreen filtration plate is now on top, allowing the Sephadex powder to go into the wells of your millipore plate. To ensure that all the Sephadex has gone into the wells of the millipore plate, lightly pound black plate with your fingertips before pulling it away.
3. Fill a container with diH_2O or DEPC water. Use a multi channel pipet, set to 150 μL and add a total of 300 μL of H_2O to each lane of the millipore plate containing the Sephadex powder.
4. Cover millipore plate with its plastic cap and allow the Sephadex to hydrate for 2–3 hours. *Note: The plate can be wrapped in saran rap and stored in the fridge overnight.

After hydrating the Sephadex for 2-3 hours:

1. Add an additional 50 μL of water to each well (changing pipet tips is **not** necessary for adding water to the wells).
2. Attach a clean 96 well reaction plate to bottom of millipore plate, being sure that the letters of each plate line up with each other. Place plate along with a balance plate and spin water out of Sephadex at 2000 rpm (=800 × g in CR 4-12 centrifuge) for 5 min. (make sure the columns are not cracked).
3. Use a new 96 well plate. This is the plate your DNA will be in. Only slightly empty out your balance plate, as you will need to re-balance it with your sample plate once you've put your DNA in.
4. Load your 10 uL sequencing reaction into the corresponding wells of the millipore filter plate (follow your sample sheet so you do not lose track of which well has which DNA sample). When loading your sequencing reaction samples into the millipore plate, be sure to load right into the middle of your Sephadex, as you want all of your sample to filter through the Sephadex. This will ensure that the terminators are removed.
5. Weigh your sample plate and the balance plate to be sure they are balanced and spin in CR 4-12 Centrifuge once again for 8 min. at 2000 rpm (= 800 × g).
6. Now your DNA will be in the 96 well reaction plate. Carefully take your sample plate (with the base) and dry the samples in Speed Vac. for about1 hour (or until dry) on medium heat. Be careful not to over dry your samples.

7. Once your samples are dried down, cover them and label it with your name and the date and what samples are in the plate (or project name) and place in freezer until ready to load into the DNA Sequencer.

*Note: Make sure to keep the balance when using centrifuge or Speed Vac.

The sequencing method used is fluorescent dye-labeled dideoxynucleotide DNA sequencing on an automated ABI sequencing machine. In this process, a one-sided PCR is run (meaning only one primer is added to the reaction, resulting in a single strand). In addition to dNTPs, fluorescent dye-labeled dideoxynucleotide bases are added to the solution. These bases result in the termination of the growing chain. Each nucleotide base (A, C, G and T) emits a different wavelength of light. As the PCR product flows through the polymer in the sequencing machine, each fragment migrates according to size – the smallest fragments move most rapidly, and pass by a laser on the sequencing machine. The wavelength emitted by each base is recorded by the machine, which then translates the wavelength into a 'base call'. In this way, the sequence of the DNA fragment is recorded.

Appendix 7. Multitag pyrosequencing for ITS and 16S.

When clean and reliable sequences could not be obtained by traditional Sanger sequencing for the fungal partner (which has only happened in the ITS marker), as well as for all photobiont sequences, we used a multitag pyrosequencing (MTPS) process on a Roche 454 GS Junior pyrosequencer (patent: Gillevet and BioSpherex, 2006; methodology: Gillevet et al., 2010).

For this purpose we develop an emulsion PCR fusion forward and reverse primers containing 454 emulsion PCR linkers and a unique 8 base barcode on the forward primer only. We use fusion primers and eliminate the ligation step that has been used by others (Binladen et al., 2007) to avoid distortion of the data.

For the initial PCR, each DNA extraction was amplified with the uniquely barcoded set of forward ITS1F (Gardes and Bruns, 1993) and a tagged reverse ITS2 (Lane, 1991), both universal fungal primers (Schoch et al., 2012); or 27F and 355R for universal bacterial primers (Lane, 1991) The PCR products are then purified and pooled and quantified with picogreen (Quant-iT PicoGreen assay, Invitrogen), on a Beckman Coulter Multimode DTX 880 instrument. An appropriate dilution of product is then made based on the picogreen result for emulsion PCR. Emulsion PCR is performed following manufacturer's protocol for the GS Junior emPCR Amplification Lib_L kit, but fewer cycles than suggested in the original protocol are performed on the thermal cycler based

on our experience. The resulting recovered beads are sequenced on a GS Junior sequencing instrument (Roche). All original kits and protocols from Roche are used for the sequencing process without modifications.

Appendix 8. List of currently accepted species.

Name Author	Type locality	Original publication	Current status
***Acantholichen* P. M. Jørg.**	**Total= 6**		
A. albomarginatus Dal-Forno, Marcelli & Lücking	Brazil	Dal-Forno et al., 2015*	-
A. campestris Dal-Forno, Spielmann & Lücking	Brazil	Dal-Forno et al., 2015*	-
A. galapagoensis Dal-Forno, Bungartz & Lücking	Galapagos	Dal-Forno et al., 2015*	-
A. pannarioides P. M. Jørg.	Costa Rica	Jørgensen, 1998	-
A. sorediatus Dal-Forno, Sipman & Lücking	Costa Rica	Dal-Forno et al., 2015*	-
A. variabilis Dal-Forno, Coca & Lücking	Colombia	Dal-Forno et al., 2015*	-
***Cora* Fr.**	**Total= 21**		
C. arachnoidea J. E. Hern. & Lücking	Venezuela	Lücking et al., 2013a	-
C. aspera Wilk, Lücking & E. Morales	Bolivia	Lücking et al., 2013a	-
C. bovei Speg.	Argentina	Spegazzini, 1888	-
C. byssoidea Lücking & Moncada	Colombia	Lücking et al., 2013a	-
C. casanarensis L. Vargas, Moncada & Lücking	Colombia	Vargas et al., 2014	-
C. ciferrii (Tomas.) Lücking, Grall & Thüs	Panama	Tomaselli, 1950	Lücking et al. 2014e
C. cyphellifera Dal-Forno, Bungartz & Lücking	Ecuador	Lücking et al., 2013a	-
C. fimbriata L. Vargas, Moncada & Lücking	Colombia	Vargas et al., 2014	-
C. galapagoensis Dal-Forno, Bungartz & Lücking	Galapagos	Dal-Forno et al., 2015*	-
C. glabrata (Spreng.) Fr.	Guadeloupe	Sprengel, 1820	Fries, 1838
C. gyrolophia Fr.	Mauritius	Fries, 1838	-
C. hirsuta (Moncada & Lücking) Moncada & Lücking	Colombia	Lumbsch et al., 2011	Lücking et al. 2013a
C. inversa Lücking & Moncada	Colombia	Lücking et al., 2013a	-
C. minor (Lücking, E. Navarro & Sipman) Lücking	Costa Rica	Chaves et al., 2004	Lücking et al., 2013a
C. pavonia (Sw.) Fr.	Jamaica	Swartz, 1788	Fries, 1838
C. reticulifera Vain.	Brazil	Vainio, 1890	-
C. santacruzensis Dal-Forno, Bungartz & Lücking	Galapagos	Dal-Forno et al., 2015*	-
C. setosa L. Vargas, Moncada & Lücking	Colombia	Vargas et al., 2014	-
C. squamiformis Wilk, Lücking & Yánez-Ayabaca	Bolivia	Lücking et al., 2013a	-
C. strigosa Lücking, E. Paz & L. Salcedo	Peru	Lücking et al., 2013a	-
C. undulata L. Vargas, Moncada & Lücking	Colombia	Vargas et al., 2014	-

***Corella* Vain.**	**Total= 4**		
C. brasiliensis Vain.	Brazil	Vainio, 1890	-
C. melvinii (Chaves, Lücking & Umaña) Lücking, Dal-Forno & Lawrey	Costa Rica	Chaves et al., 2004	Lücking et al., 2013a
C. tomentosa Vain.	Guadeloupe	Vainio, 1899	-
C. zahlbruckneri Schiff.	Brazil	Zahlbruckner, 1909	-
***Cyphellostereum* D. A. Reid**	**Total= 6**		
C. floreanum Dal-Forno, Bungartz & Lücking	Galapagos	Dal-Forno et al., 2015*	
C. galapagoense (Yánez, Dal Forno & Bungartz) Dal-Forno, Bungartz & Lücking	Galapagos	Yánez et al., 2012	Dal-Forno et al., 2015*
C. imperfectum Lücking, Barillas & Dal-Forno	Guatemala	Yánez et al., 2012	-
C. nitidum (Lücking) Lücking	Ecuador	Lücking, 2008	Yánez et al., 2012
C. phyllogenum (Müll. Arg.) Lücking, Dal-Forno & Lawrey	Borneo	Müller, 1883	Lücking et al., 2013a
C. pusiolum (Berk. & M. A. Curtis) D. A. Reid	Cuba	Berkeley and Curtis, 1868	Reid, 1965
***Dictyonema* C. Agardh ex Kunth**	**Total= 26**		
D. aeruginosulum Lücking, Nelsen & Will-Wolf	Costa Rica	Lücking et al., 2013a	-
D. applanatum Lücking, Dal-Forno & Wilk	Bolivia	Lücking et al., 2014b	-
D. barbatum Dal-Forno, Bungartz & Lücking	Galapagos	Dal-Forno et al., 2015*	-
D. bungartziana Dal-Forno, Yánez & Lücking	Galapagos	Dal-Forno et al., 2015*	-
D. caespitosum (Johow) Lücking	Brazil	Johow, 1884	Lücking et al., 2013a
D. coppinsii Lücking, Barrie & Genney	Ireland	Lücking et al., 2014a	-
D. diducens Nyl. ex Lücking	Peru	Nylander, 1885	Lücking et al., 2013a
D. discocarpum Lücking, Dal-Forno & Wilk	Bolivia	Lücking et al., 2014b	-
D. giganteum L. Vargas, Moncada & Lücking	Colombia	Vargas et al., 2014	-
D. hapteriferum Lücking, Dal-Forno & Wilk	Bolivia	Lücking et al., 2014b	-
D. hernandezii Lücking, Lawrey & Dal-Forno	Costa Rica	Lumbsch et al., 2011	-
D. huaorani Dal-Forno, Schmull, Lücking & Lawrey	Ecuador	Schmull et al., 2014	-
D. irpicinum Mont.	Taiti	Montagne, 1848	-
D. irrigatum (Berk. & M. A. Curtis) Lücking	China	Berkeley and Curtis, 1860	Lücking et al., 2013a
D. ligulatum (Kremp.) Zahlbr.	Papua New Guinea	Krempelhuber, 1875	Zahlbruckner, 1908
D. metallicum Lücking, Dal-Forno & Lawrey	Ecuador	Lücking et al., 2013a	-
D. moorei (Nyl.) Henssen	Japan	Nylander, 1890	Henssen, 1963
D. obscuratum Lücking, Spielmann & Marcelli	Brazil	Lücking et al., 2013a	-
D. pectinatum Dal Forno, Yánez & Lücking	Galapagos	Yánez et al., 2012	-
D. phyllophilum (Parmasto) Lücking, Dal-Forno & Lawrey	Borneo	Parmasto, 1978	Lücking et al., 2013a
D. ramificans Dal-Forno, Yánez & Lücking	Galapagos	Dal-Forno et al., 2015*	-

D. scabridum (Vain.) Lücking	Philippines	Vainio, 1923	Lücking et al., 2013a
D. schenckianum (Müll. Arg.) Zahlbr.	Brazil	Müller, 1891	Zahlbruckner, 1931
D. sericeum (Sw.) Berk.	Caribbean	Swartz, 1788	Berkeley, 1843
D. subobscuratum Dal-Forno, Bungartz & Lücking	Ecuador	Dal-Forno et al., 2015*	-
D. thelephora (Spreng.) Zahlbr.	Puerto Rico	Sprengel, 1820	Zahlbruckner, 1931
	Total= 63 species currently accepted		

- Marks species in which the current status is the same as in the original publication.

* When data from this dissertation is published validating the species.

Appendix 9. Key to currently accepted genera of *Dictyonema* s.l.
(adapted from Lücking et al., 2013a).

1. Thallus composed of distinct fibrils including cyanobacterial filaments, either appressed to substrate or forming horizontally projecting, semicircular lobes 2
- Thallus microsquamulose to foliose, no distinct fibrils visible, photobiont instead forming clusters of short, irregularly coiled threads inside the thallus..... 3

2. Photobiont cells narrow (5–7 µm broad), lacking haustoria; hyphal sheath around photobiont filaments composed of irregular hyphae leaving interspaces; basidiocarps (hymenophores) if present stipitate and erect (cyphelloid), only at the base connected to lichenized thallus ..... ***Cyphellostereum*** **D. A. Reid**
- Photobiont cells broad (7–20 µm broad), with tubular intracellular haustoria; hyphal sheath around photobiont filaments composed of paraplectenchymatous, jigsaw-puzzle-shaped cells forming a completely closed layer; basidiocarps (hymenophores) if present stereoid-corticioid, without stipe, their dorsal portion partially overgrown with the lichenized thallus or completely formed on the thallus underside..... ***Dictyonema*** **C. Agardh ex Kunth**

3. Thallus microsquamulose; surfaces in microscope view forming apically thickened, distinctly spiny hyphae (acanthohyphidia) ..... ***Acantholichen*** **P. M. Jørg.**
- Thallus macrosquamulose; acanthohyphidia absent..... 4

4. Upper cortex thin, distinctly paraplectenchymatous; upper surface dark blue-green, grey or olive-brown when dry; propagules sometimes present; hymenophores unknown .....
..... ***Corella*** **Vain.**
- Upper cortex thick, composed of an upper, periclinal layer of loosely packed hyphae supported by a layer of anticlinal hyphal bundles leaving large interspaces; sorediod propagules sometimes present; hymenophores mostly present ..... ***Cora*** **Fr.**

Appendix 10. Guidance Scores and additional phylogenetic trees from Chapter 4.

Subset 1 – nuLSU: 189 Taxa. Submitted to GUIDANCE, with MAFFT alignment option and 100 bootstrap. Saved 3 files:

- Treatment 1: Entire MSA (score = 0.996106) = 1472bp = 0 columns removed.
- Treatment 2: MSA with columns below 0.93 score removed = 1436bp = 36 columns removed.
- Treatment 3: MSA with columns below 1 removed = 1407 bp = 65 columns removed.

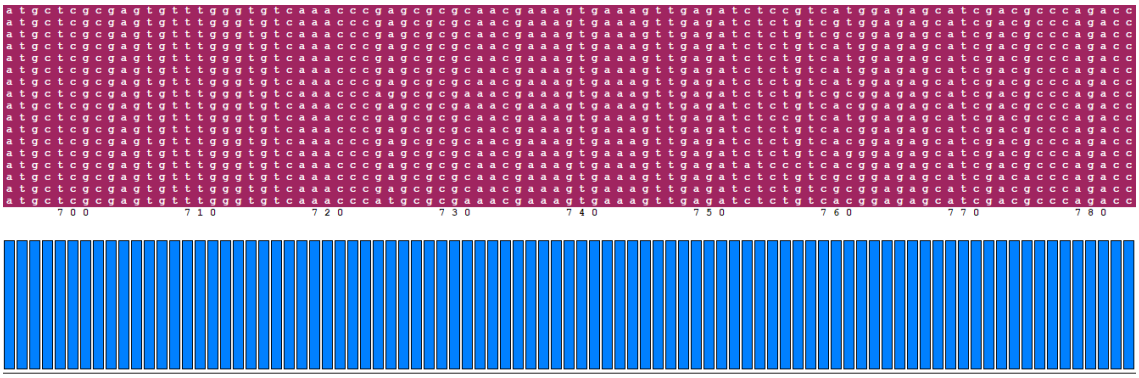


Figure 41 - Guidance visualization screen of conserved nuLSU region

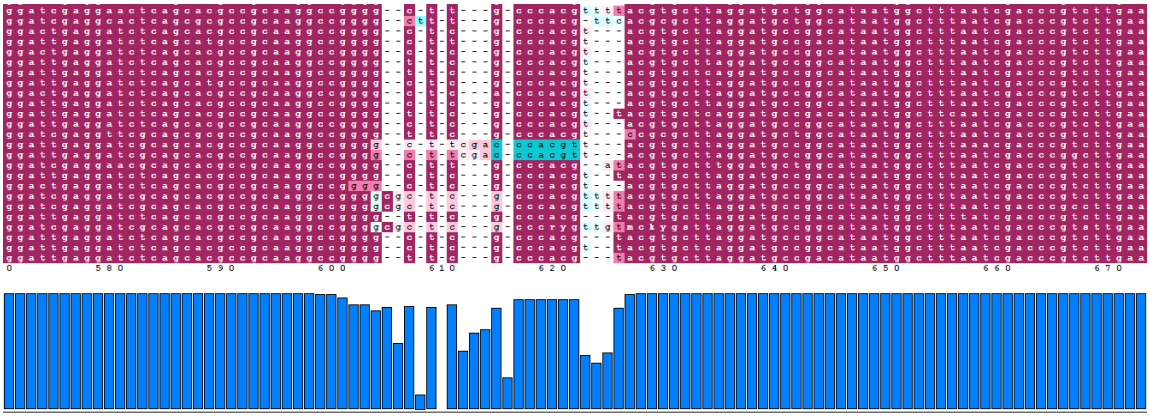


Figure 42 - Guidance visualization screen of nuLSU region with more variability

Subset 1 – ITS: 168 Taxa. Submitted to GUIDANCE, with MAFFT alignment option and 100 bootstrap. Saved 3 files:

Treatment 1 - Entire MSA (score = 0.870305)= 963bp = 0 columns removed.

Treatment 2 - MSA with columns below 0.93 score removed = 515bp = 448 columns removed.

Treatment 3 - MSA with columns below 1 removed = 348 bp = 615 columns removed.

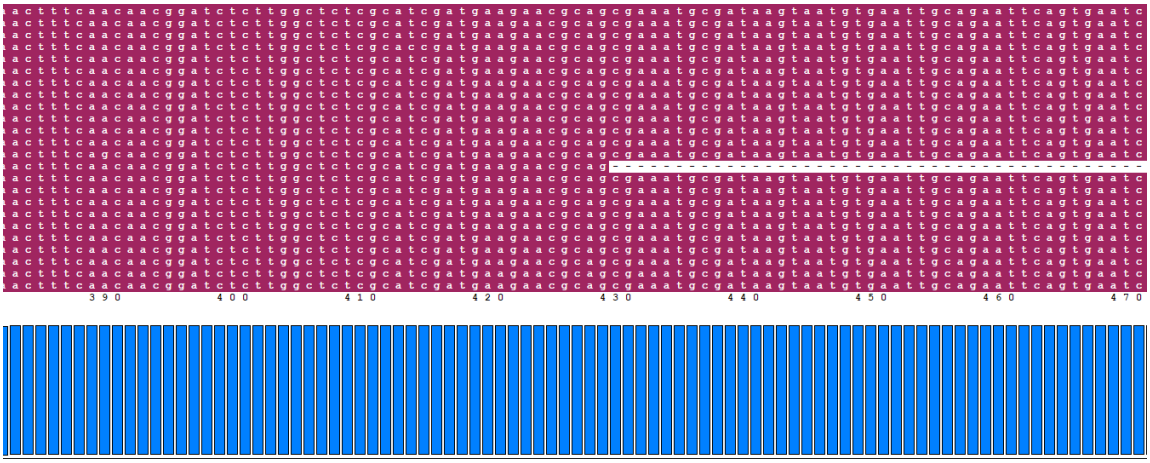


Figure 43 - Guidance visualization screen of 5.8S region

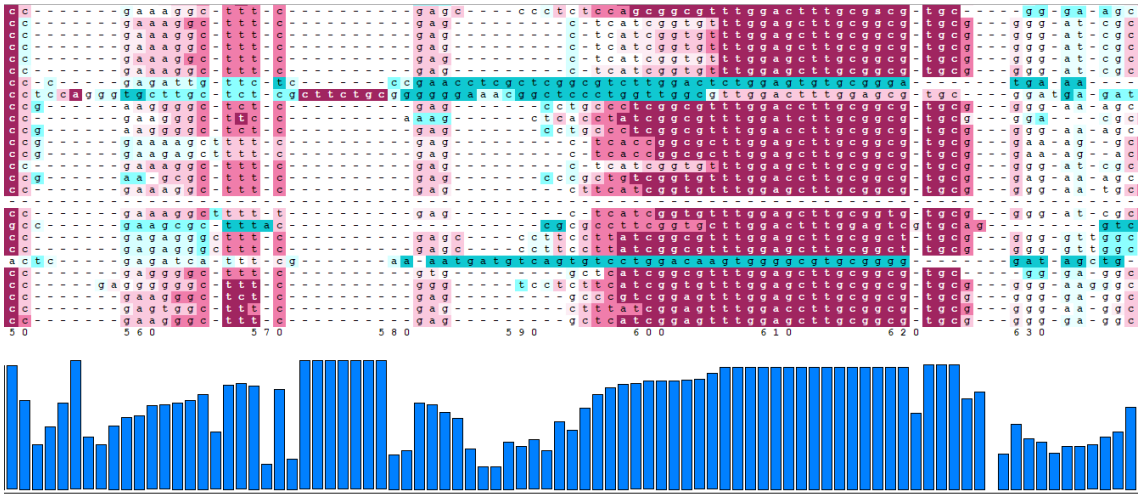
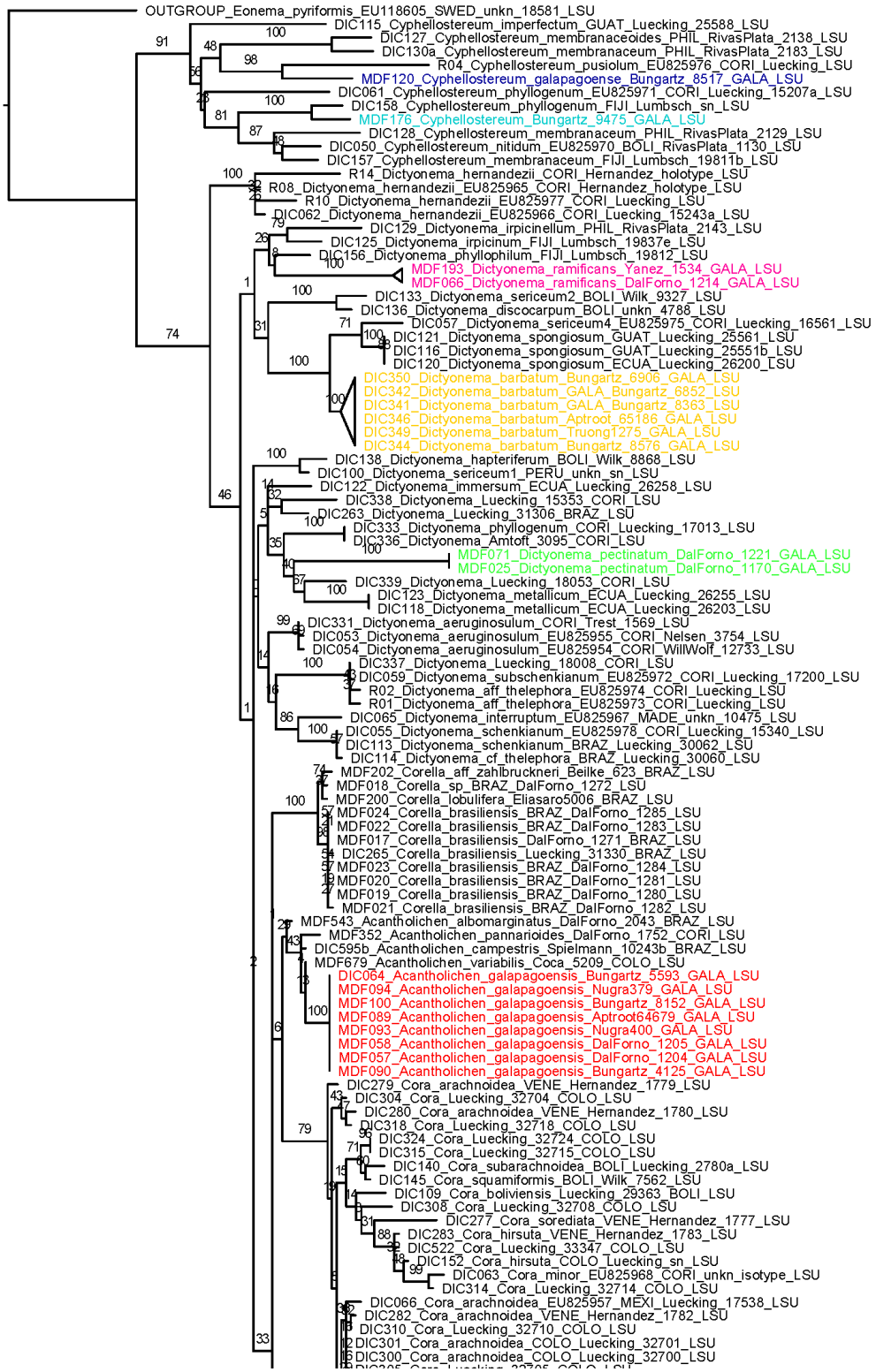
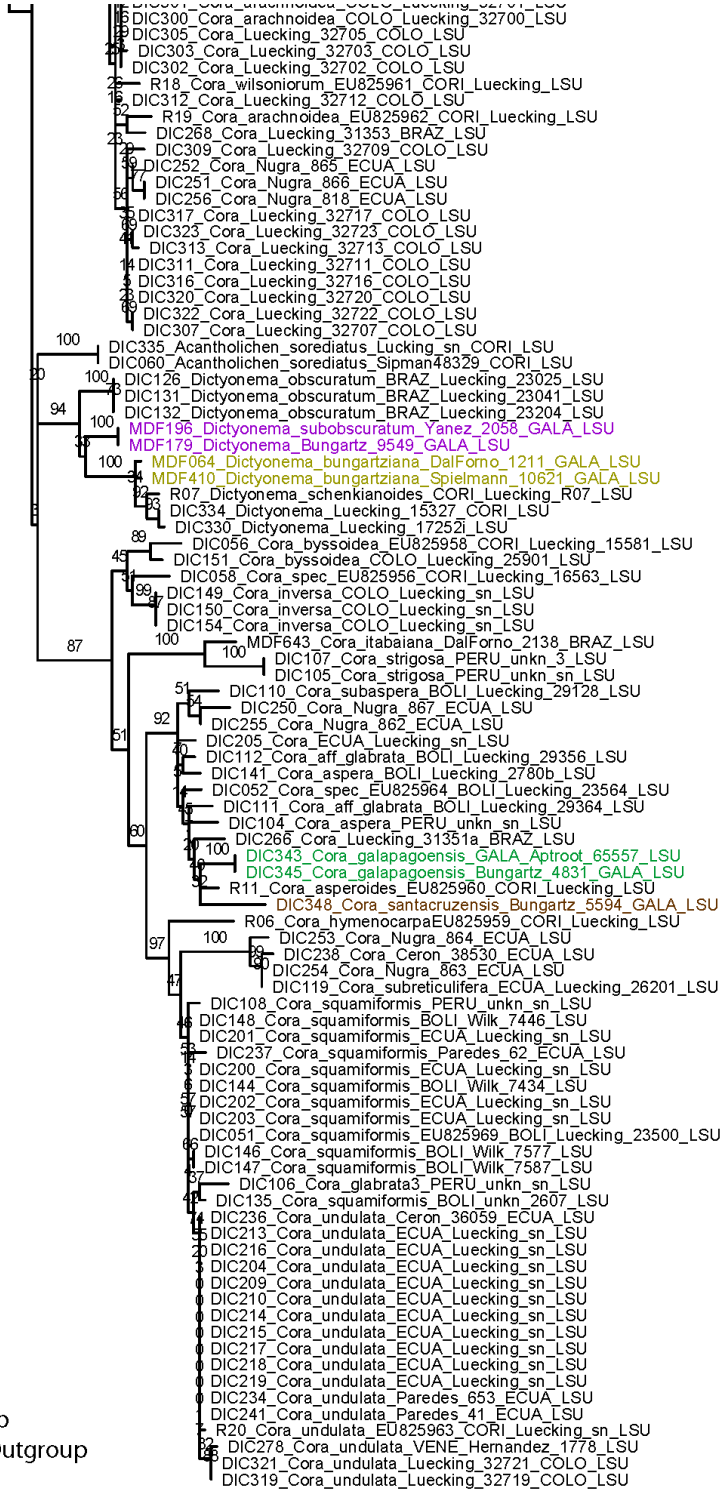


Figure 44 - Guidance visualization screen of ITS2 region

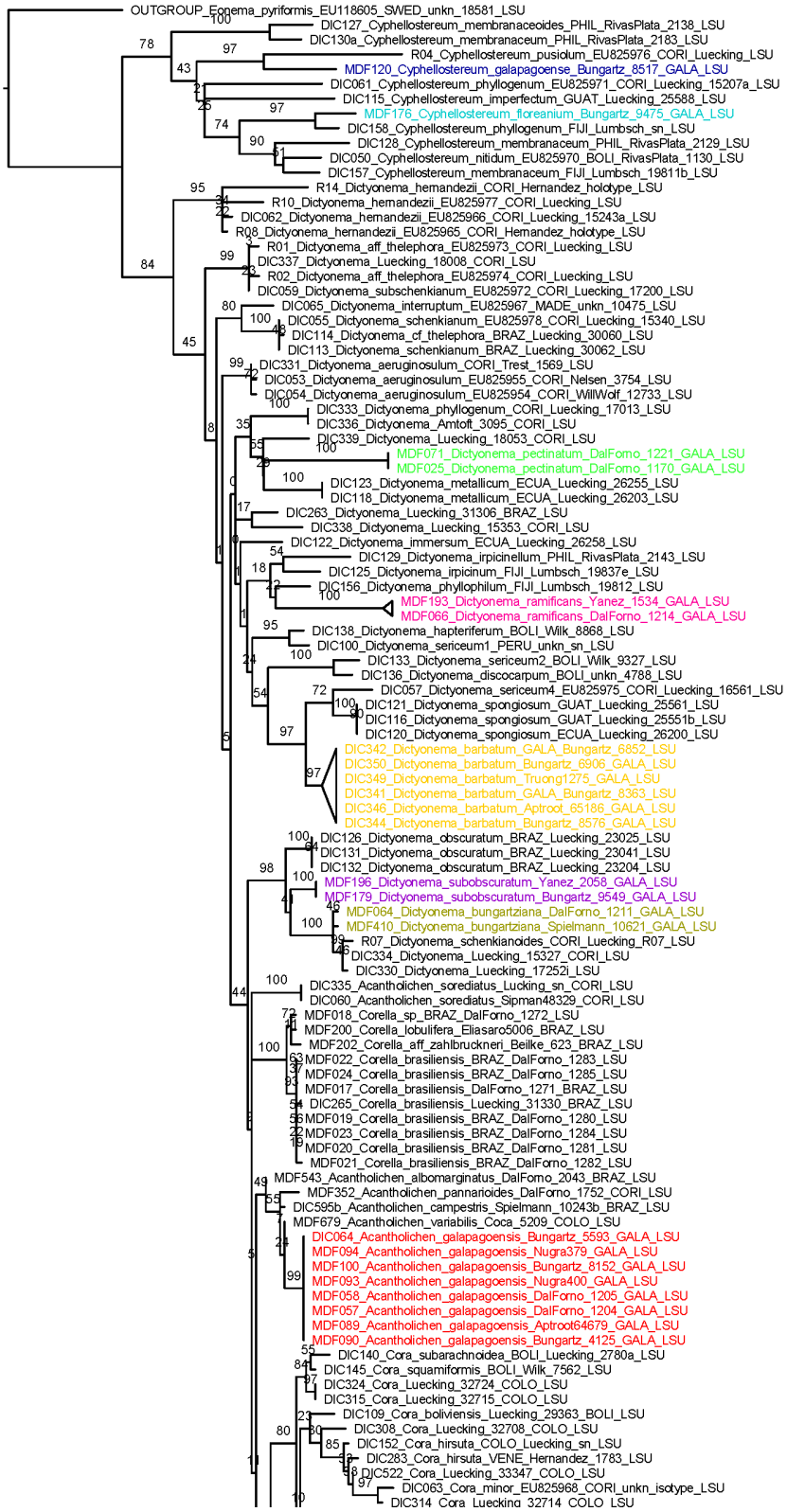


A

Cont. to Figure B

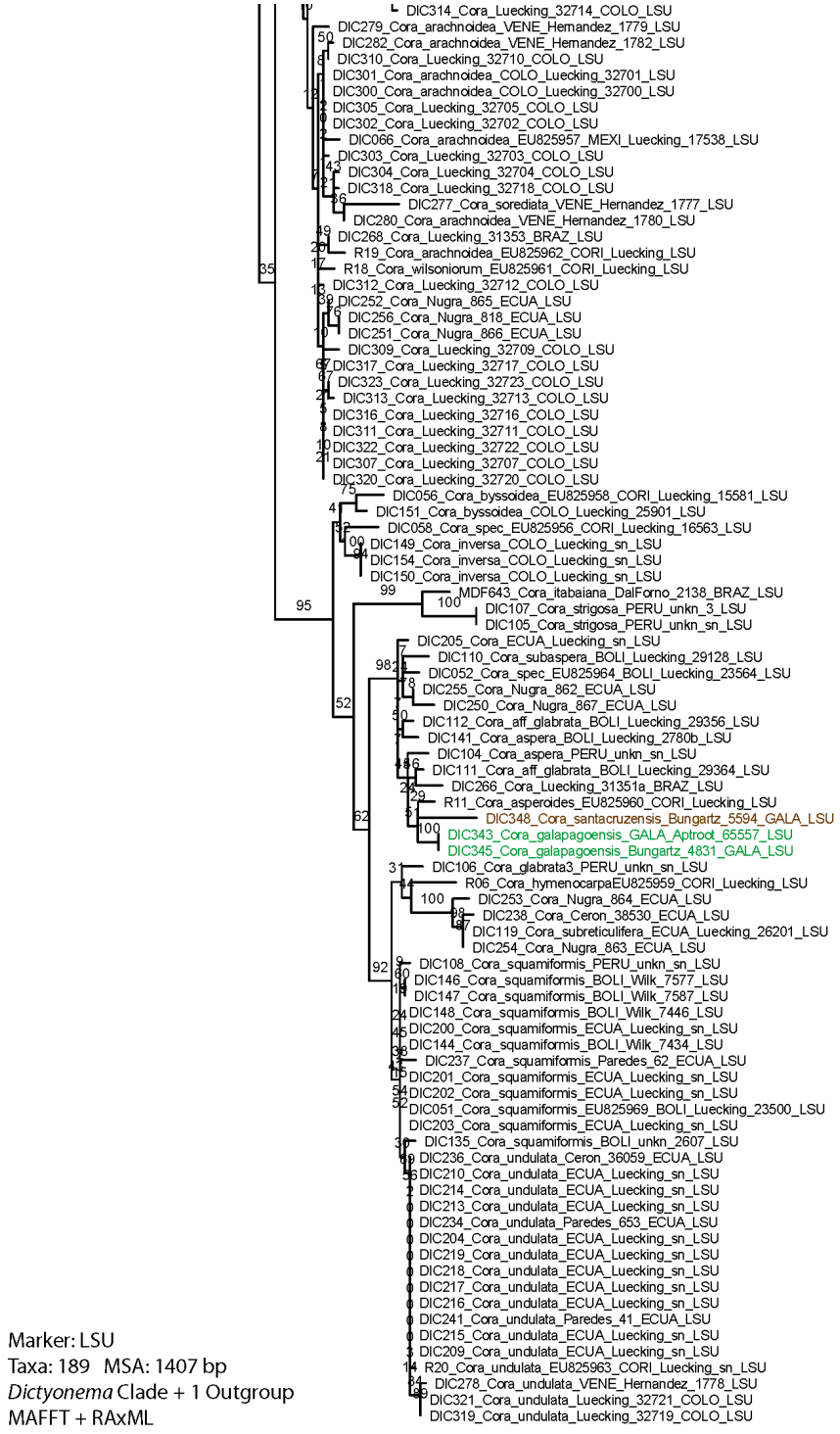

DIC300_Cora_arachnoidea_COLO_Luecking_32700_LSU
DIC305_Cora_Luecking_32705_COLO_LSU
DIC303_Cora_Luecking_32703_COLO_LSU
DIC302_Cora_Luecking_32702_COLO_LSU
R18_Cora_wilsoniorum_EU825961_CORI_Luecking_LSU
DIC312_Cora_Luecking_32712_COLO_LSU
R19_Cora_arachnoidea_EU825962_CORI_Luecking_LSU
DIC268_Cora_Luecking_31353_BRAZ_LSU
DIC309_Cora_Luecking_32709_COLO_LSU
DIC252_Cora_Nugra_865_ECUA_LSU
DIC251_Cora_Nugra_866_ECUA_LSU
DIC256_Cora_Nugra_818_ECUA_LSU
DIC317_Cora_Luecking_32717_COLO_LSU
DIC323_Cora_Luecking_32723_COLO_LSU
DIC313_Cora_Luecking_32713_COLO_LSU
DIC311_Cora_Luecking_32711_COLO_LSU
DIC316_Cora_Luecking_32716_COLO_LSU
DIC320_Cora_Luecking_32720_COLO_LSU
DIC322_Cora_Luecking_32722_COLO_LSU
DIC307_Cora_Luecking_32707_COLO_LSU
DIC335_Acantholichen_sorediatus_Lucking_sn_CORI_LSU
DIC060_Acantholichen_sorediatus_Sipman48329_CORI_LSU
DIC126_Dictyonema_obscuratum_BRAZ_Luecking_23025_LSU
DIC131_Dictyonema_obscuratum_BRAZ_Luecking_23041_LSU
DIC132_Dictyonema_obscuratum_BRAZ_Luecking_23204_LSU
MDF196_Dictyonema_subobscuratum_Yanez_2058_GALA_LSU
MDF179_Dictyonema_Bungartz_9549_GALA_LSU
MDF064_Dictyonema_bungartziana_DalForno_1211_GALA_LSU
MDF410_Dictyonema_bungartziana_Spielmann_10621_GALA_LSU
R07_Dictyonema_schenkianoides_CORI_Luecking_R07_LSU
DIC334_Dictyonema_Luecking_15327_CORI_LSU
DIC330_Dictyonema_Luecking_17252i_LSU
DIC056_Cora_byssoidea_EU825958_CORI_Luecking_15581_LSU
DIC151_Cora_byssoidea_COLO_Luecking_25901_LSU
DIC058_Cora_spec_EU825956_CORI_Luecking_16563_LSU
DIC149_Cora_inversa_COLO_Luecking_sn_LSU
DIC150_Cora_inversa_COLO_Luecking_sn_LSU
DIC154_Cora_inversa_COLO_Luecking_sn_LSU
MDF643_Cora_itabaiana_DalForno_2138_BRAZ_LSU
DIC107_Cora_strigosa_PERU_unkn_3_LSU
DIC105_Cora_strigosa_PERU_unkn_sn_LSU
DIC110_Cora_subaspera_BOLI_Luecking_29128_LSU
DIC250_Cora_Nugra_867_ECUA_LSU
DIC255_Cora_Nugra_862_ECUA_LSU
DIC205_Cora_ECUA_Luecking_sn_LSU
DIC112_Cora_aff_glabrata_BOLI_Luecking_29356_LSU
DIC141_Cora_aspera_BOLI_Luecking_2780b_LSU
DIC052_Cora_spec_EU825964_BOLI_Luecking_23564_LSU
DIC111_Cora_aff_glabrata_BOLI_Luecking_29364_LSU
DIC104_Cora_aspera_PERU_unkn_sn_LSU
DIC266_Cora_Luecking_31351a_BRAZ_LSU
DIC343_Cora_galapagoensis_GALA_Aptroot_65557_LSU
DIC345_Cora_galapagoensis_Bungartz_4831_GALA_LSU
R11_Cora_asperoides_EU825960_CORI_Luecking_LSU
DIC348_Cora_santacruzensis_Bungartz_5594_GALA_LSU
R06_Cora_hymenocarpaEU825959_CORI_Luecking_LSU
DIC253_Cora_Nugra_864_ECUA_LSU
DIC238_Cora_Ceron_38530_ECUA_LSU
DIC254_Cora_Nugra_863_ECUA_LSU
DIC119_Cora_subreticulifera_ECUA_Luecking_26201_LSU
DIC108_Cora_squamiformis_PERU_unkn_sn_LSU
DIC148_Cora_squamiformis_BOLI_Wilk_7446_LSU
DIC201_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC237_Cora_squamiformis_Paredes_62_ECUA_LSU
DIC200_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC144_Cora_squamiformis_BOLI_Wilk_7434_LSU
DIC202_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC203_Cora_squamiformis_ECUA_Luecking_sn_LSU
DIC051_Cora_squamiformis_EU825969_BOLI_Luecking_23500_LSU
DIC146_Cora_squamiformis_BOLI_Wilk_7577_LSU
DIC147_Cora_squamiformis_BOLI_Wilk_7587_LSU
DIC106_Cora_glabrata3_PERU_unkn_sn_LSU
DIC135_Cora_squamiformis_BOLI_unkn_2607_LSU
DIC236_Cora_undulata_Ceron_36059_ECUA_LSU
DIC213_Cora_undulata_ECUA_Luecking_sn_LSU
DIC216_Cora_undulata_ECUA_Luecking_sn_LSU
DIC204_Cora_undulata_ECUA_Luecking_sn_LSU
DIC209_Cora_undulata_ECUA_Luecking_sn_LSU
DIC210_Cora_undulata_ECUA_Luecking_sn_LSU
DIC214_Cora_undulata_ECUA_Luecking_sn_LSU
DIC215_Cora_undulata_ECUA_Luecking_sn_LSU
DIC217_Cora_undulata_ECUA_Luecking_sn_LSU
DIC218_Cora_undulata_ECUA_Luecking_sn_LSU
DIC219_Cora_undulata_ECUA_Luecking_sn_LSU
DIC234_Cora_undulata_Paredes_653_ECUA_LSU
DIC241_Cora_undulata_Paredes_41_ECUA_LSU
R20_Cora_undulata_EU825963_CORI_Luecking_sn_LSU
DIC278_Cora_undulata_VENE_Hernandez_1778_LSU
DIC321_Cora_undulata_Luecking_32721_COLO_LSU
DIC319_Cora_undulata_Luecking_32719_COLO_LSU
Marker: LSU
Taxa: 189 MSA: 1436 bp
Dictyonema Clade + 1 Outgroup
MAFFT + RAxML
0.02

B



C

Cont. to Figure D



D

Figure 45 - Phylogenetic tree inferred by ML with RAxML for the nuLSU marker with the Subset 1 A, B - Treatment 2. C, D - Treatment 3

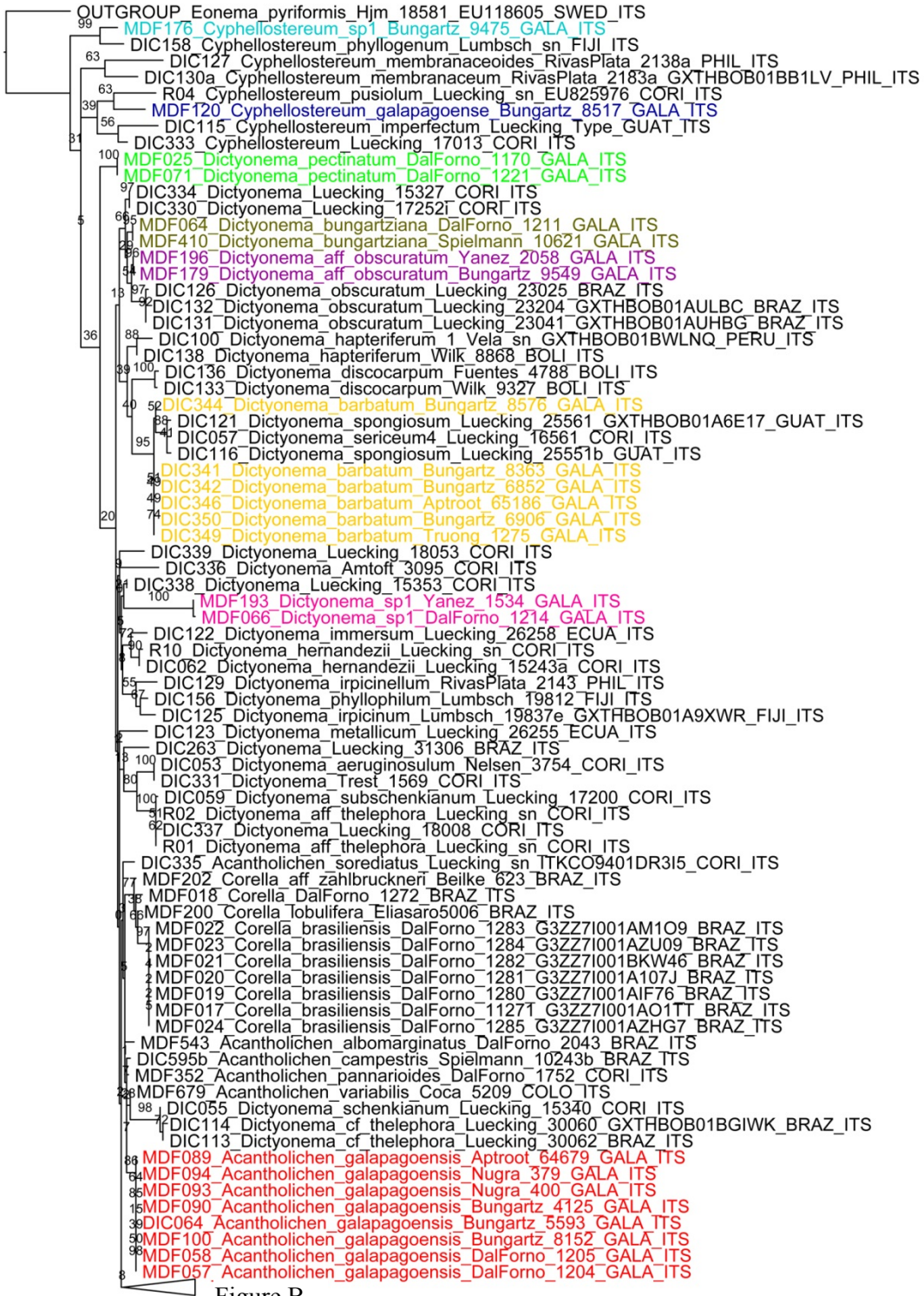

OUTGROUP_Eonema_pyriformis_Hjm_18581_EU118605_SWED_ITS
MDF176_Cyphellostereum_sp1_Bungartz_9475_GALA_ITS
DIC158_Cyphellostereum_phyllogenum_Lumbsch_sn_FIJI_ITS
DIC127_Cyphellostereum_membranaceoides_RivasPlata_2138a_PHIL_ITS
DIC130a_Cyphellostereum_membranaceum_RivasPlata_2183a_GXTHBOB01BB1LV_PHIL_ITS
R04_Cyphellostereum_pusiolum_Luecking_sn_EU825976_CORI_ITS
MDF120_Cyphellostereum_galapagoense_Bungartz_8517_GALA_ITS
DIC115_Cyphellostereum_imperfectum_Luecking_Type_GUAT_ITS
DIC333_Cyphellostereum_Luecking_17013_CORI_ITS
MDF025_Dictyonema_pectinatum_DalForno_1170_GALA_ITS
MDF071_Dictyonema_pectinatum_DalForno_1221_GALA_ITS
DIC334_Dictyonema_Luecking_15327_CORI_ITS
DIC330_Dictyonema_Luecking_17252i_CORI_ITS
MDF064_Dictyonema_bungartziana_DalForno_1211_GALA_ITS
MDF410_Dictyonema_bungartziana_Spielmann_10621_GALA_ITS
MDF196_Dictyonema_aff_obscuratum_Yanez_2058_GALA_ITS
MDF179_Dictyonema_aff_obscuratum_Bungartz_9549_GALA_ITS
DIC126_Dictyonema_obscuratum_Luecking_23025_BRAZ_ITS
DIC132_Dictyonema_obscuratum_Luecking_23204_GXTHBOB01AULBC_BRAZ_ITS
DIC131_Dictyonema_obscuratum_Luecking_23041_GXTHBOB01AUHBG_BRAZ_ITS
DIC100_Dictyonema_hapteriferum_1_Vela_sn_GXTHBOB01BWLNQ_PERU_ITS
DIC138_Dictyonema_hapteriferum_Wilk_8868_BOLI_ITS
DIC136_Dictyonema_discocarpum_Fuentes_4788_BOLI_ITS
DIC133_Dictyonema_discocarpum_Wilk_9327_BOLI_ITS
DIC344_Dictyonema_barbatum_Bungartz_8576_GALA_ITS
DIC121_Dictyonema_spongiosum_Luecking_25561_GXTHBOB01A6E17_GUAT_ITS
DIC057_Dictyonema_sericeum4_Luecking_16561_CORI_ITS
DIC116_Dictyonema_spongiosum_Luecking_25551b_GUAT_ITS
DIC341_Dictyonema_barbatum_Bungartz_8363_GALA_ITS
DIC342_Dictyonema_barbatum_Bungartz_6852_GALA_ITS
DIC346_Dictyonema_barbatum_Aptroot_65186_GALA_ITS
DIC350_Dictyonema_barbatum_Bungartz_6906_GALA_ITS
DIC349_Dictyonema_barbatum_Truong_1275_GALA_ITS
DIC339_Dictyonema_Luecking_18053_CORI_ITS
DIC336_Dictyonema_Amtoft_3095_CORI_ITS
DIC338_Dictyonema_Luecking_15353_CORI_ITS
MDF193_Dictyonema_sp1_Yanez_1534_GALA_ITS
MDF066_Dictyonema_sp1_DalForno_1214_GALA_ITS
DIC122_Dictyonema_immersum_Luecking_26258_ECUA_ITS
R10_Dictyonema_hernandezii_Luecking_sn_CORI_ITS
DIC062_Dictyonema_hernandezii_Luecking_15243a_CORI_ITS
DIC129_Dictyonema_irpicinellum_RivasPlata_2143_PHIL_ITS
DIC156_Dictyonema_phyllophilum_Lumbsch_19812_FIJI_ITS
DIC125_Dictyonema_irpicinum_Lumbsch_19837e_GXTHBOB01A9XWR_FIJI_ITS
DIC123_Dictyonema_metallicum_Luecking_26255_ECUA_ITS
DIC263_Dictyonema_Luecking_31306_BRAZ_ITS
DIC053_Dictyonema_aeruginosulum_Nelsen_3754_CORI_ITS
DIC331_Dictyonema_Trest_1569_CORI_ITS
DIC059_Dictyonema_subschenkianum_Luecking_17200_CORI_ITS
R02_Dictyonema_aff_thelephora_Luecking_sn_CORI_ITS
DIC337_Dictyonema_Luecking_18008_CORI_ITS
R01_Dictyonema_aff_thelephora_Luecking_sn_CORI_ITS
DIC335_Acantholichen_sorediatus_Luecking_sn_TTKCO9401DR3I5_CORI_ITS
MDF202_Corella_aff_zahlbruckneri_Beilke_623_BRAZ_ITS
MDF018_Corella_DalForno_1272_BRAZ_ITS
MDF200_Corella_tobulifera_Eliasaro5006_BRAZ_ITS
MDF022_Corella_brasiliensis_DalForno_1283_G3ZZ7I001AM1O9_BRAZ_ITS
MDF023_Corella_brasiliensis_DalForno_1284_G3ZZ7I001AZU09_BRAZ_ITS
MDF021_Corella_brasiliensis_DalForno_1282_G3ZZ7I001BKW46_BRAZ_ITS
MDF020_Corella_brasiliensis_DalForno_1281_G3ZZ7I001A107J_BRAZ_ITS
MDF019_Corella_brasiliensis_DalForno_1280_G3ZZ7I001AIF76_BRAZ_ITS
MDF017_Corella_brasiliensis_DalForno_11271_G3ZZ7I001AO1TT_BRAZ_ITS
MDF024_Corella_brasiliensis_DalForno_1285_G3ZZ7I001AZHG7_BRAZ_ITS
MDF543_Acantholichen_albomarginatus_DalForno_2043_BRAZ_ITS
DIC595b_Acantholichen_campestris_Spielmann_10243b_BRAZ_ITS
MDF352_Acantholichen_pannarioides_DalForno_1752_CORI_ITS
MDF679_Acantholichen_variabilis_Coca_5209_COLO_ITS
DIC055_Dictyonema_schenkianum_Luecking_15340_CORI_ITS
DIC114_Dictyonema_cf_thelephora_Luecking_30060_GXTHBOB01BGIWK_BRAZ_ITS
DIC113_Dictyonema_cf_thelephora_Luecking_30062_BRAZ_ITS
MDF089_Acantholichen_galapagoensis_Aptroot_64679_GALA_ITS
MDF094_Acantholichen_galapagoensis_Nugra_379_GALA_ITS
MDF093_Acantholichen_galapagoensis_Nugra_400_GALA_ITS
MDF090_Acantholichen_galapagoensis_Bungartz_4125_GALA_ITS
DIC064_Acantholichen_galapagoensis_Bungartz_5593_GALA_ITS
MDF100_Acantholichen_galapagoensis_Bungartz_8152_GALA_ITS
MDF058_Acantholichen_galapagoensis_DalForno_1205_GALA_ITS
MDF057_Acantholichen_galapagoensis_DalForno_1204_GALA_ITS
0.03

Figure B

A

Marker: ITS
Taxa: 168 MSA: 515 bp
Dictyonema Clade + 1 Outgroup
MAFFT + RAxML

B

Figure 46 - Phylogenetic tree inferred by ML with RAxML for the ITS marker with the Subset 1 Treatment 2

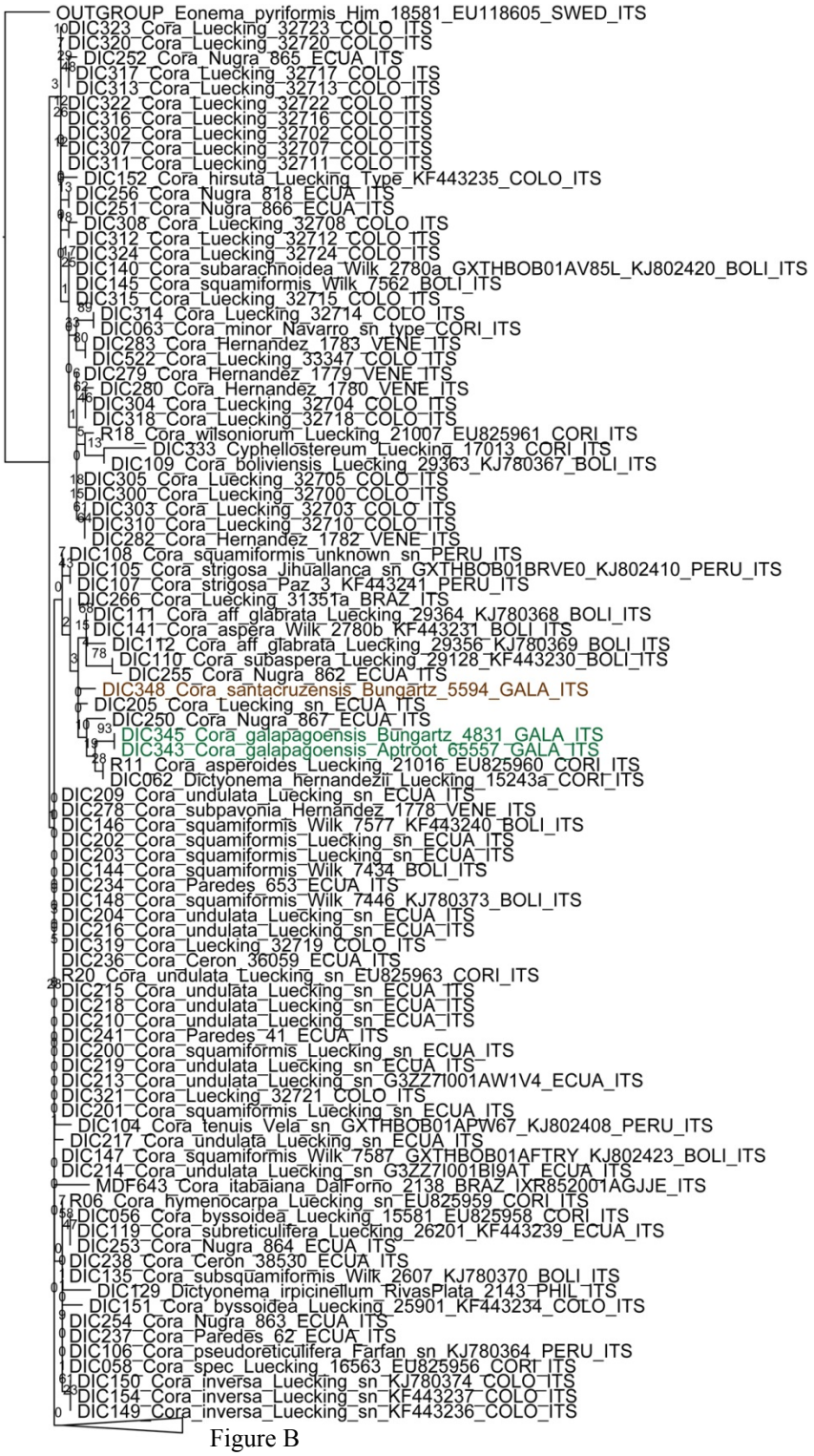
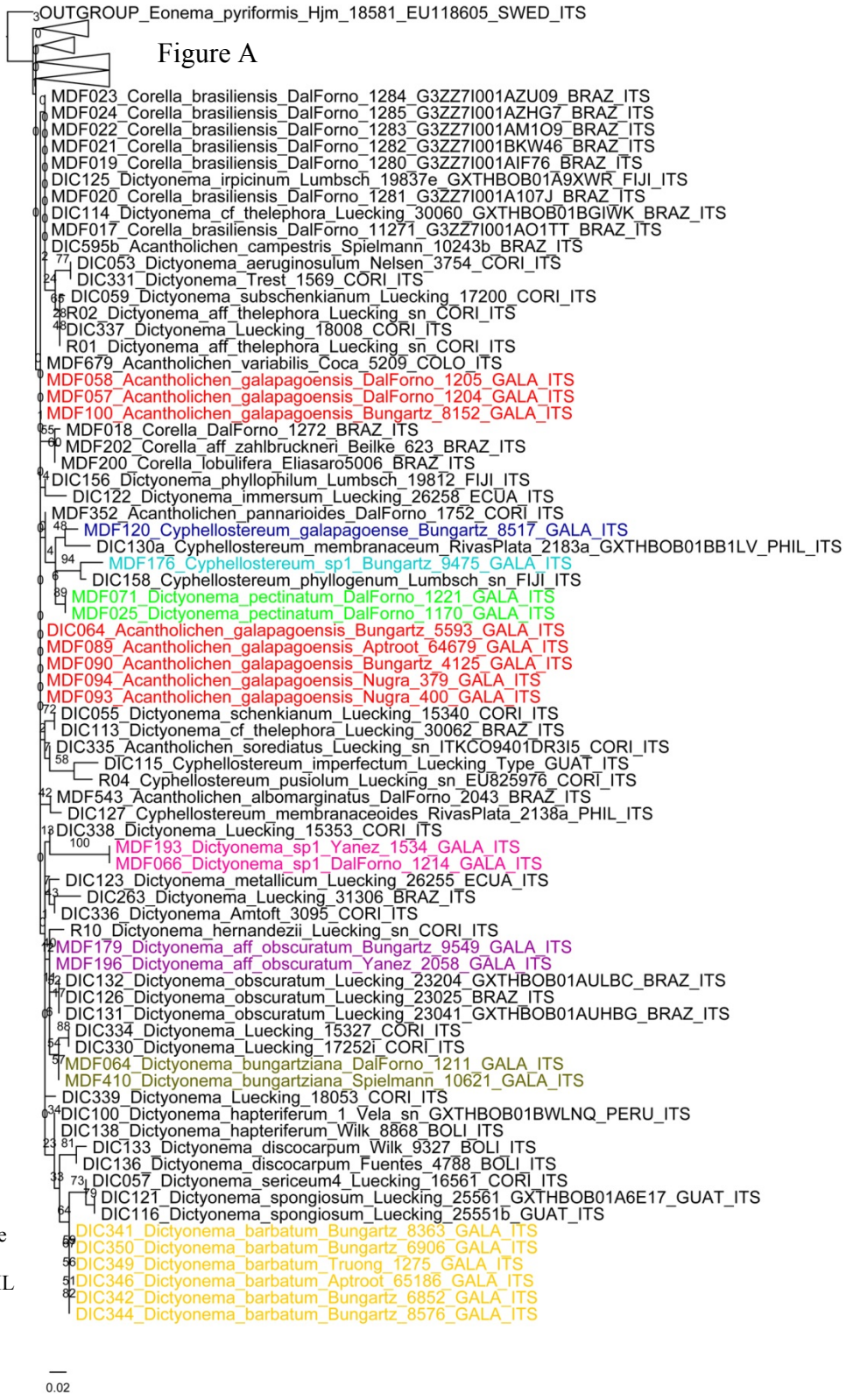

OUTGROUP_Eonema_pyriformis_Him_18581_EU118605_SWED_ITS
DIC323_Cora_Luecking_32723_COLO_ITS
DIC320_Cora_Luecking_32720_COLO_ITS
DIC252_Cora_Nugra_865_ECUA_ITS
DIC317_Cora_Luecking_32717_COLO_ITS
DIC313_Cora_Luecking_32713_COLO_ITS
DIC322_Cora_Luecking_32722_COLO_ITS
DIC316_Cora_Luecking_32716_COLO_ITS
DIC302_Cora_Luecking_32702_COLO_ITS
DIC307_Cora_Luecking_32707_COLO_ITS
DIC311_Cora_Luecking_32711_COLO_ITS
DIC152_Cora_hirsuta_Luecking_Type_KF443235_COLO_ITS
DIC256_Cora_Nugra_818_ECUA_ITS
DIC251_Cora_Nugra_866_ECUA_ITS
DIC308_Cora_Luecking_32708_COLO_ITS
DIC312_Cora_Luecking_32712_COLO_ITS
DIC324_Cora_Luecking_32724_COLO_ITS
DIC140_Cora_subarachnoidea_Wilk_2780a_GXTHBOB01AV85L_KJ802420_BOLI_ITS
DIC145_Cora_squamiformis_Wilk_7562_BOLI_ITS
DIC315_Cora_Luecking_32715_COLO_ITS
DIC314_Cora_Luecking_32714_COLO_ITS
DIC063_Cora_minor_Navarro_sn_type_CORI_ITS
DIC283_Cora_Hernandez_1783_VENE_ITS
DIC522_Cora_Luecking_33347_COLO_ITS
DIC279_Cora_Hernandez_1779_VENE_ITS
DIC280_Cora_Hernandez_1780_VENE_ITS
DIC304_Cora_Luecking_32704_COLO_ITS
DIC318_Cora_Luecking_32718_COLO_ITS
R18_Cora_wilsoniorum_Luecking_21007_EU825961_CORI_ITS
DIC333_Cyphellostereum_Luecking_17013_CORI_ITS
DIC109_Cora_boliviensis_Luecking_29363_KJ780367_BOLI_ITS
DIC305_Cora_Luecking_32705_COLO_ITS
DIC300_Cora_Luecking_32700_COLO_ITS
DIC303_Cora_Luecking_32703_COLO_ITS
DIC310_Cora_Luecking_32710_COLO_ITS
DIC282_Cora_Hernandez_1782_VENE_ITS
DIC108_Cora_squamiformis_unknown_sn_PERU_ITS
DIC105_Cora_strigosa_Jihuallanca_sn_GXTHBOB01BRVE0_KJ802410_PERU_ITS
DIC107_Cora_strigosa_Paz_3_KF443241_PERU_ITS
DIC266_Cora_Luecking_31351a_BRAZ_ITS
DIC111_Cora_aff_glabrata_Luecking_29364_KJ780368_BOLI_ITS
DIC141_Cora_aspera_Wilk_2780b_KF443231_BOLI_ITS
DIC112_Cora_aff_glabrata_Luecking_29356_KJ780369_BOLI_ITS
DIC110_Cora_subaspera_Luecking_29128_KF443230_BOLI_ITS
DIC255_Cora_Nugra_862_ECUA_ITS
DIC348_Cora_santacruzensis_Bungartz_5594_GALA_ITS
DIC205_Cora_Luecking_sn_ECUA_ITS
DIC250_Cora_Nugra_867_ECUA_ITS
DIC345_Cora_galapagoensis_Bungartz_4831_GALA_ITS
DIC343_Cora_galapagoensis_Aptroot_65557_GALA_ITS
R11_Cora_asperoides_Luecking_21016_EU825960_CORI_ITS
DIC062_Dictyonema_hernandezii_Luecking_15243a_CORI_ITS
DIC209_Cora_undulata_Luecking_sn_ECUA_ITS
DIC278_Cora_subpavonia_Hernandez_1778_VENE_ITS
DIC146_Cora_squamiformis_Wilk_7577_KF443240_BOLI_ITS
DIC202_Cora_squamiformis_Luecking_sn_ECUA_ITS
DIC203_Cora_squamiformis_Luecking_sn_ECUA_ITS
DIC144_Cora_squamiformis_Wilk_7434_BOLI_ITS
DIC234_Cora_Paredes_653_ECUA_ITS
DIC148_Cora_squamiformis_Wilk_7446_KJ780373_BOLI_ITS
DIC204_Cora_undulata_Luecking_sn_ECUA_ITS
DIC216_Cora_undulata_Luecking_sn_ECUA_ITS
DIC319_Cora_Luecking_32719_COLO_ITS
DIC236_Cora_Ceron_36059_ECUA_ITS
R20_Cora_undulata_Luecking_sn_EU825963_CORI_ITS
DIC215_Cora_undulata_Luecking_sn_ECUA_ITS
DIC218_Cora_undulata_Luecking_sn_ECUA_ITS
DIC210_Cora_undulata_Luecking_sn_ECUA_ITS
DIC241_Cora_Paredes_41_ECUA_ITS
DIC200_Cora_squamiformis_Luecking_sn_ECUA_ITS
DIC219_Cora_undulata_Luecking_sn_ECUA_ITS
DIC213_Cora_undulata_Luecking_sn_G3ZZ7I001AW1V4_ECUA_ITS
DIC321_Cora_Luecking_32721_COLO_ITS
DIC201_Cora_squamiformis_Luecking_sn_ECUA_ITS
DIC104_Cora_tenuis_Vela_sn_GXTHBOB01APW67_KJ802408_PERU_ITS
DIC217_Cora_undulata_Luecking_sn_ECUA_ITS
DIC147_Cora_squamiformis_Wilk_7587_GXTHBOB01AFTRY_KJ802423_BOLI_ITS
DIC214_Cora_undulata_Luecking_sn_G3ZZ7I001BI9AT_ECUA_ITS
MDF643_Cora_itabaiana_DalForno_2138_BRAZ_IXR852001AGJJE_ITS
R06_Cora_hymenocarpa_Luecking_sn_EU825959_CORI_ITS
DIC056_Cora_byssoidea_Luecking_15581_EU825958_CORI_ITS
DIC119_Cora_subreticulifera_Luecking_26201_KF443239_ECUA_ITS
DIC253_Cora_Nugra_864_ECUA_ITS
DIC238_Cora_Ceron_38530_ECUA_ITS
DIC135_Cora_subsquamiformis_Wilk_2607_KJ780370_BOLI_ITS
DIC129_Dictyonema_irpicinellum_RivasPlata_2143_PHIL_ITS
DIC151_Cora_byssoidea_Luecking_25901_KF443234_COLO_ITS
DIC254_Cora_Nugra_863_ECUA_ITS
DIC237_Cora_Paredes_62_ECUA_ITS
DIC106_Cora_pseudoreticulifera_Farfan_sn_KJ780364_PERU_ITS
DIC058_Cora_spec_Luecking_16563_EU825956_CORI_ITS
DIC150_Cora_inversa_Luecking_sn_KJ780374_COLO_ITS
DIC154_Cora_inversa_Luecking_sn_KF443237_COLO_ITS
DIC149_Cora_inversa_Luecking_sn_KF443236_COLO_ITS

Figure B

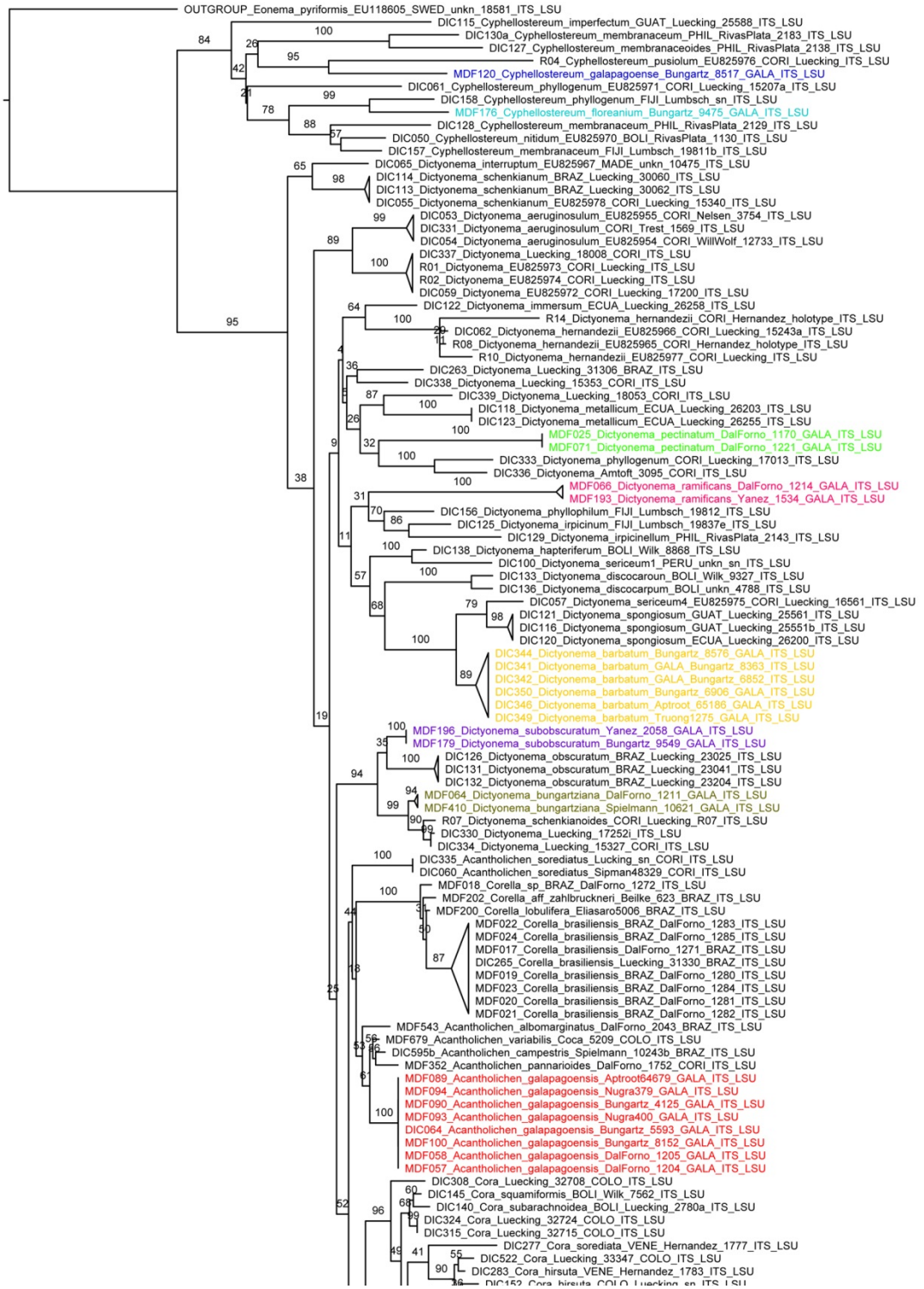
A



Marker: ITS
Taxa: 168
MSA: 348 bp
Dictyonema Clade
+ 1 Outgroup
MAFFT + RAxML

B

Figure 47 - Phylogenetic tree inferred by ML with RAxML for the ITS marker with the Subset 1 Treatment 3



A

Cont. to Figure B


DIC315_Cora_Luecking_32715_COLO_ITS_LSU
DIC277_Cora_sorediata_VENE_Hernandez_1777_ITS_LSU
DIC522_Cora_Luecking_33347_COLO_ITS_LSU
DIC283_Cora_hirsuta_VENE_Hernandez_1783_ITS_LSU
DIC152_Cora_hirsuta_COLO_Luecking_sn_ITS_LSU
DIC063_Cora_minor_EU825968_CORI_unkn_isotype_ITS_LSU
DIC314_Cora_Luecking_32714_COLO_ITS_LSU
DIC109_Cora_boliviensis_Luecking_29363_BOLI_ITS_LSU
DIC279_Cora_arachnoidea_VENE_Hernandez_1779_ITS_LSU
DIC304_Cora_Luecking_32704_COLO_ITS_LSU
DIC280_Cora_arachnoidea_VENE_Hernandez_1780_ITS_LSU
DIC318_Cora_Luecking_32718_COLO_ITS_LSU
R19_Cora_arachnoidea_EU825962_CORI_Luecking_ITS_LSU
DIC268_Cora_Luecking_31353_BRAZ_ITS_LSU
DIC301_Cora_arachnoidea_COLO_Luecking_32701_ITS_LSU
DIC300_Cora_arachnoidea_COLO_Luecking_32700_ITS_LSU
DIC305_Cora_Luecking_32705_COLO_ITS_LSU
DIC303_Cora_Luecking_32703_COLO_ITS_LSU
DIC066_Cora_arachnoidea_EU825957_MEXI_Luecking_17538_ITS_LSU
DIC282_Cora_arachnoidea_VENE_Hernandez_1782_ITS_LSU
DIC310_Cora_Luecking_32710_COLO_ITS_LSU
R18_Cora_wilsoniorum_EU825961_CORI_Luecking_ITS_LSU
DIC312_Cora_Luecking_32712_COLO_ITS_LSU
DIC302_Cora_Luecking_32702_COLO_ITS_LSU
DIC316_Cora_Luecking_32716_COLO_ITS_LSU
DIC311_Cora_Luecking_32711_COLO_ITS_LSU
DIC307_Cora_Luecking_32707_COLO_ITS_LSU
DIC322_Cora_Luecking_32722_COLO_ITS_LSU
DIC320_Cora_Luecking_32720_COLO_ITS_LSU
DIC309_Cora_Luecking_32709_COLO_ITS_LSU
DIC317_Cora_Luecking_32717_COLO_ITS_LSU
DIC323_Cora_Luecking_32723_COLO_ITS_LSU
DIC313_Cora_Luecking_32713_COLO_ITS_LSU
DIC252_Cora_Nugra_865_ECUA_ITS_LSU
DIC256_Cora_Nugra_818_ECUA_ITS_LSU
DIC251_Cora_Nugra_866_ECUA_ITS_LSU
DIC151_Cora_byssoidea_COLO_Luecking_25901_ITS_LSU
DIC056_Cora_byssoidea_EU825958_CORI_Luecking_15581_ITS_LSU
DIC058_Cora_spec_EU825956_CORI_Luecking_16563_ITS_LSU
DIC149_Cora_inversa_COLO_Luecking_sn_ITS_LSU
DIC150_Cora_inversa_COLO_Luecking_sn_ITS_LSU
DIC154_Cora_inversa_COLO_Luecking_sn_ITS_LSU
MDF643_Cora_itabaiana_DalForno_2138_BRAZ_ITS_LSU
DIC105_Cora_strigosa_PERU_unkn_sn_ITS_LSU
DIC107_Cora_strigosa_PERU_unkn_3_ITS_LSU
DIC250_Cora_Nugra_867_ECUA_ITS_LSU
DIC205_Cora_ECUA_Luecking_sn_ITS_LSU
DIC141_Cora_aspera_BOLI_Luecking_2780b_ITS_LSU
DIC112_Cora_aff_glabrata_BOLI_Luecking_29356_ITS_LSU
DIC052_Cora_spec_EU825964_BOLI_Luecking_23564_ITS_LSU
DIC255_Cora_Nugra_862_ECUA_ITS_LSU
DIC110_Cora_subaspera_BOLI_Luecking_29128_ITS_LSU
DIC104_Cora_aspera_PERU_unkn_sn_ITS_LSU
DIC111_Cora_aff_glabrata_BOLI_Luecking_29364_ITS_LSU
DIC348_Cora_santacruzensis_Bungartz_5594_GALA_ITS_LSU
DIC266_Cora_Luecking_31351a_BRAZ_ITS_LSU
R11_Cora_asperoides_EU825960_CORI_Luecking_ITS_LSU
DIC345_Cora_galapagoensis_Bungartz_4831_GALA_ITS_LSU
DIC343_Cora_galapagoensis_GALA_Aptroot_65557_ITS_LSU
DIC106_Cora_glabrata3_PERU_unkn_sn_ITS_LSU
R06_Cora_hymenocarpaEU825959_CORI_Luecking_ITS_LSU
DIC253_Cora_Nugra_864_ECUA_ITS_LSU
DIC119_Cora_subreticulifera_ECUA_Luecking_26201_ITS_LSU
DIC254_Cora_Nugra_863_ECUA_ITS_LSU
DIC238_Cora_Ceron_38530_ECUA_ITS_LSU
DIC135_Cora_squamiformis_BOLI_unkn_2607_ITS_LSU
DIC237_Cora_squamiformis_Paredes_62_ECUA_ITS_LSU
DIC051_Cora_squamiformis_EU825969_BOLI_Luecking_23500_ITS_LSU
DIC146_Cora_squamiformis_BOLI_Wilk_7577_ITS_LSU
DIC147_Cora_squamiformis_BOLI_Wilk_7587_ITS_LSU
DIC148_Cora_squamiformis_BOLI_Wilk_7446_ITS_LSU
DIC108_Cora_squamiformis_PERU_unkn_sn_ITS_LSU
DIC144_Cora_squamiformis_BOLI_Wilk_7434_ITS_LSU
DIC200_Cora_squamiformis_ECUA_Luecking_sn_ITS_LSU
DIC201_Cora_squamiformis_ECUA_Luecking_sn_ITS_LSU
DIC203_Cora_squamiformis_ECUA_Luecking_sn_ITS_LSU
DIC202_Cora_squamiformis_ECUA_Luecking_sn_ITS_LSU
R20_Cora_undulata_EU825963_CORI_Luecking_sn_ITS_LSU
DIC321_Cora_undulata_Luecking_32721_COLO_ITS_LSU
DIC278_Cora_undulata_VENE_Hernandez_1778_ITS_LSU
DIC319_Cora_undulata_Luecking_32719_COLO_ITS_LSU
DIC213_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC214_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC236_Cora_undulata_Ceron_36059_ECUA_ITS_LSU
DIC234_Cora_undulata_Paredes_653_ECUA_ITS_LSU
DIC218_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC216_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC217_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC219_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC204_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC215_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC241_Cora_undulata_Paredes_41_ECUA_ITS_LSU
DIC209_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
DIC210_Cora_undulata_ECUA_Luecking_sn_ITS_LSU
Marker: ITS + LSU (Concatenated)
Taxa: 189 MSA: 1890 bp
Dictyonema Clade + 1 Outgroup
MAFFT + RAxML
0.02

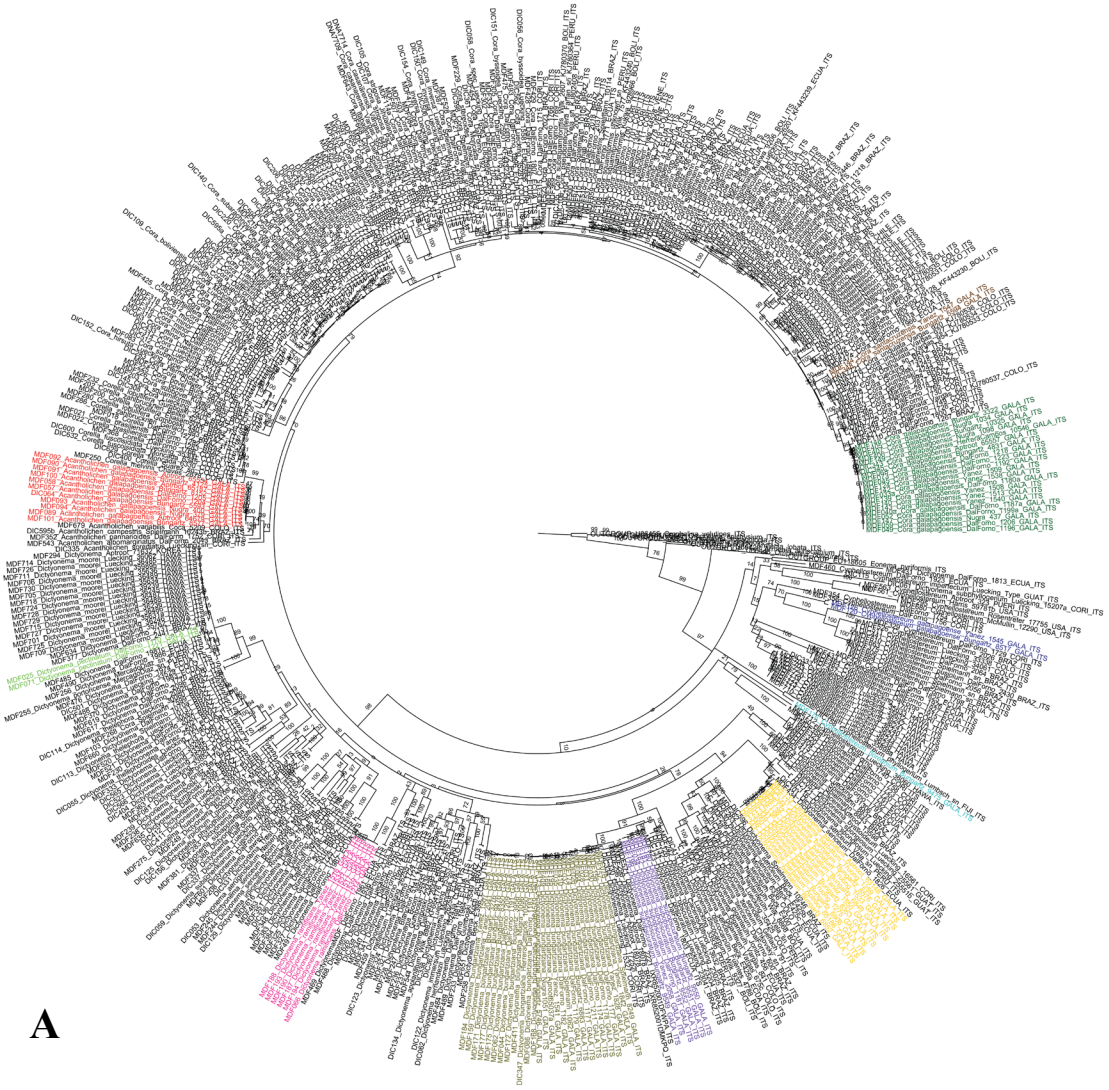
B

C

Cont. to Figure D

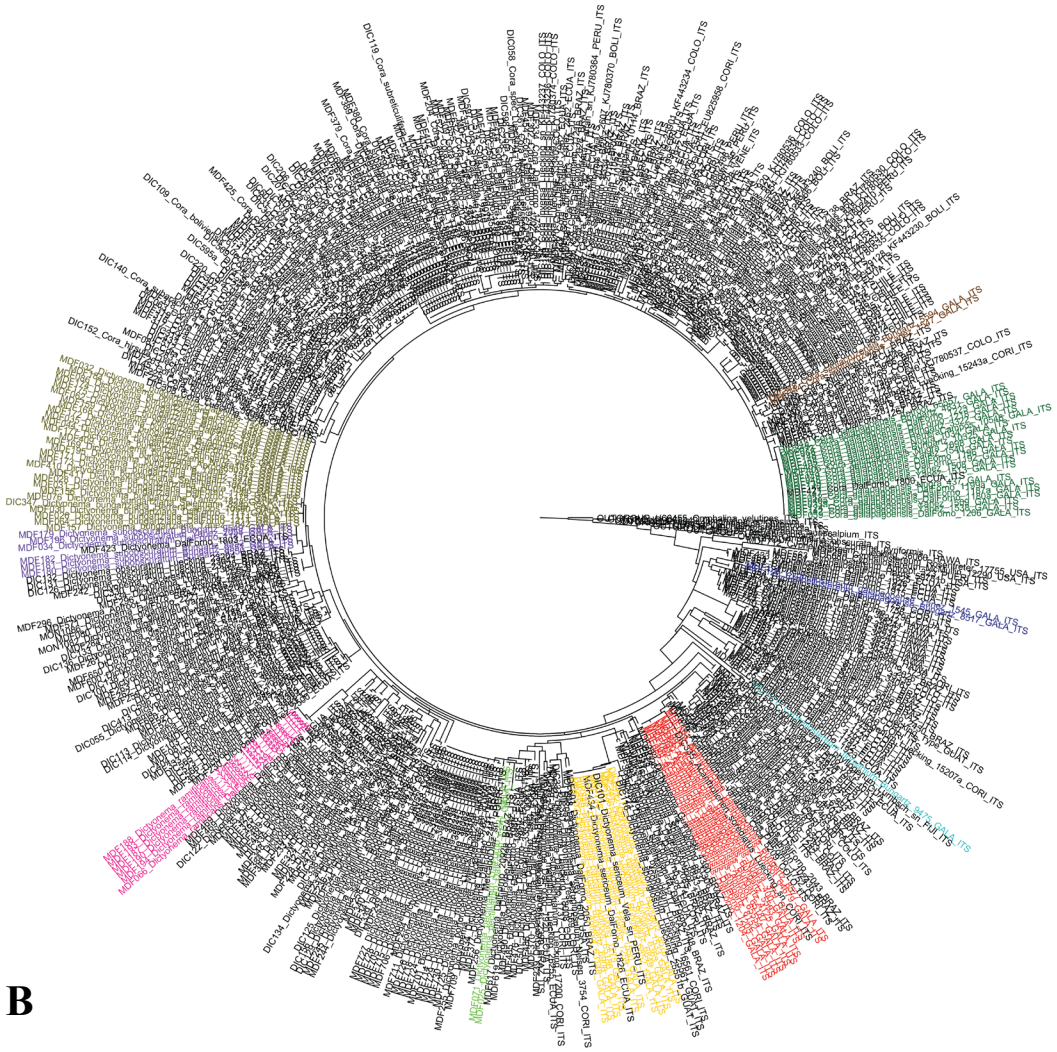
D

Figure 48 - Phylogeny (ITS + nuLSU) of *Dictyonema* obtained under ML with the Subset 1
A, B - Treatment 2. C, D - Treatment 3



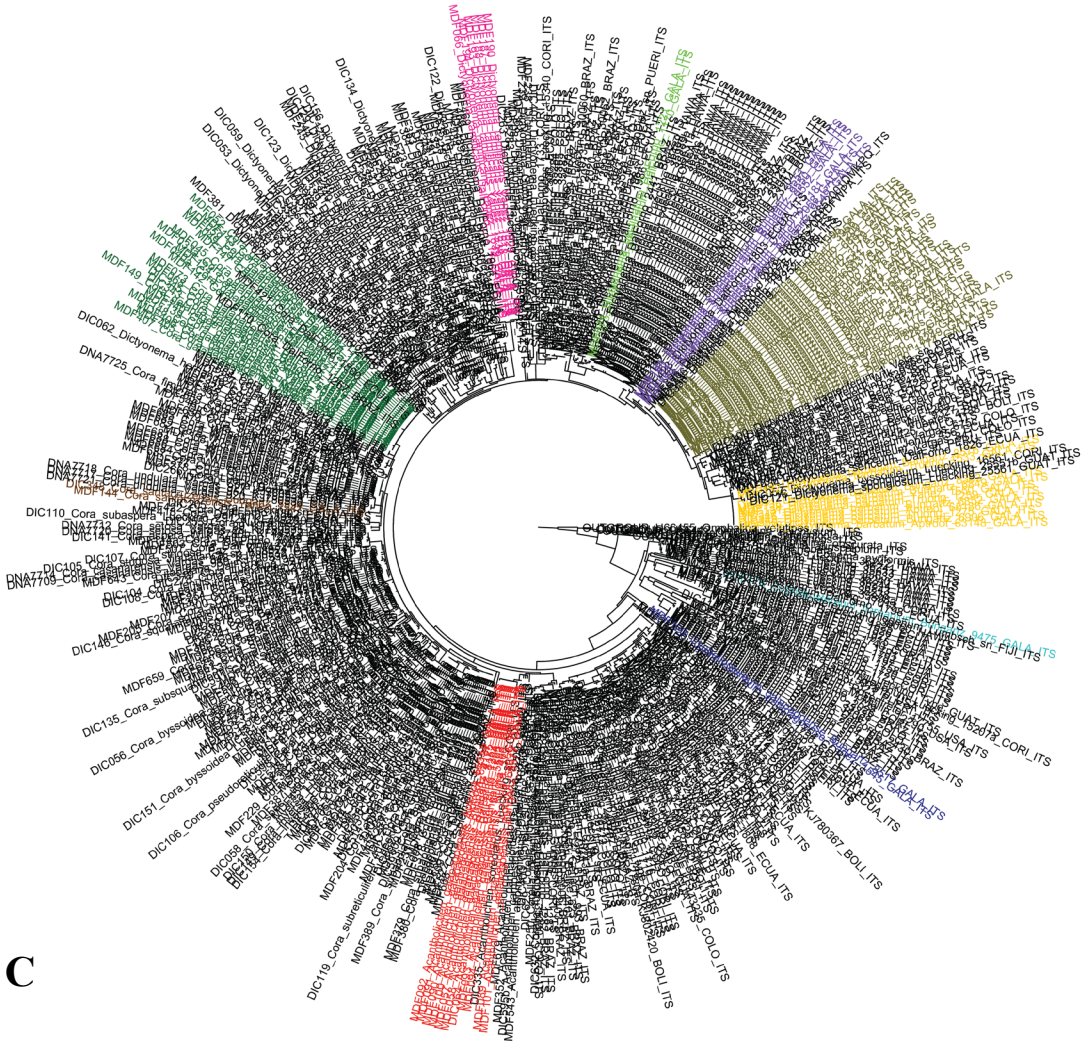
A

Marker: ITS (Treatment 1)
Taxa: 497 MSA: 1208 bp
Dictyonema Clade + 8 Outgroups
MAFFT + RAxML



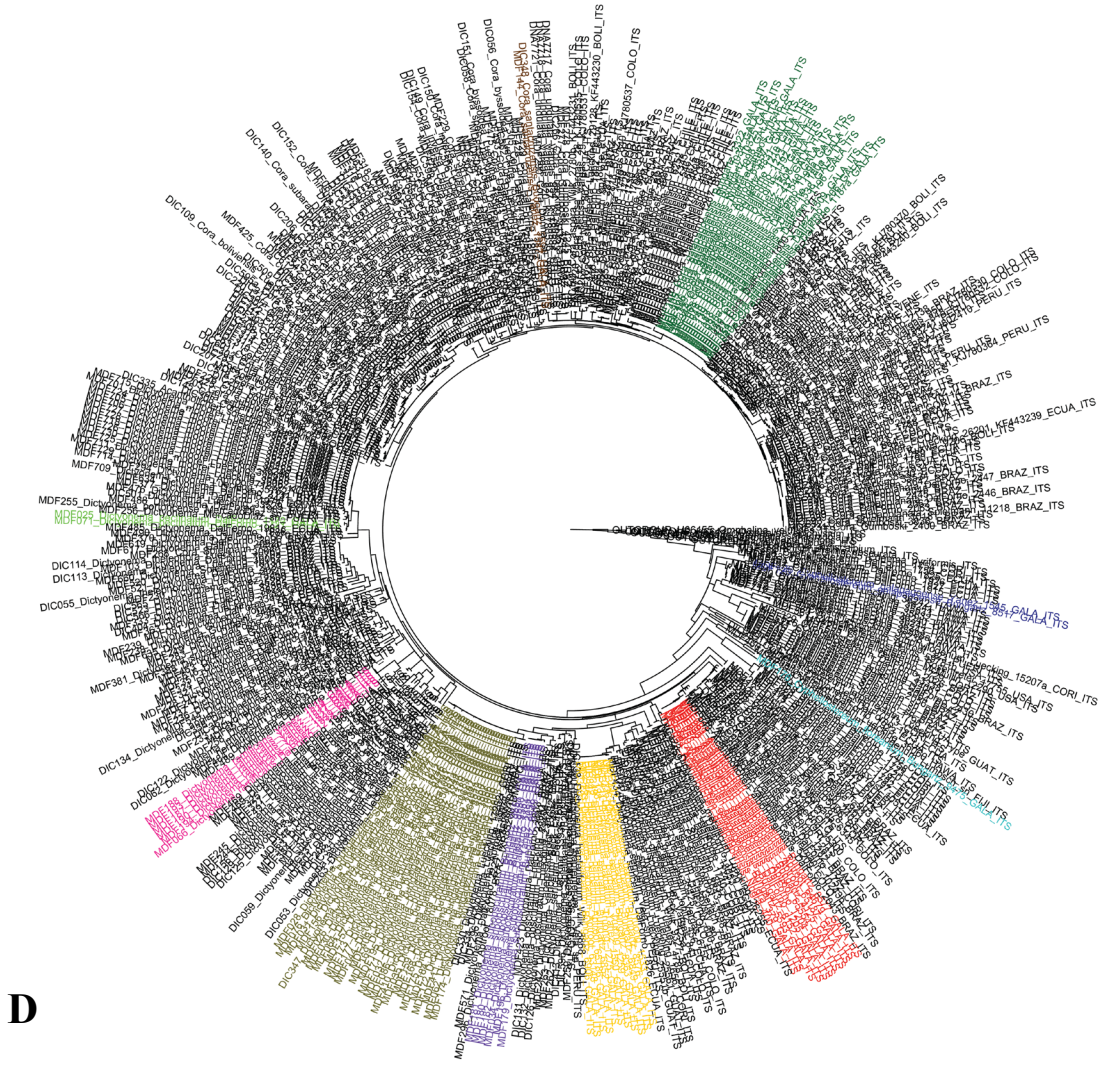
B

Marker: ITS (Treatment 2)
Taxa: 497 MSA: 521 bp
Dictyonema Clade + 8 Outgroups
MAFFT + RAxML



C

Marker: ITS (Treatment 3)
Taxa: 497 MSA: 553 bp
Dictyonema Clade + 8 Outgroups
MAFFT + RAxML



D

Marker: ITS (Treatment 4)
Taxa: 497 MSA: 661 bp
Dictyonema Clade + 8 Outgroups
MAFFT + RAxML

Figure 49 - Phylogenetic tree of *Dictyonema* obtained under ML with the Subset 2 to show rough tree topology under the different treatments
A- Treatment 1 (same data as Figure 16). B- Treatment 2. C- Treatment 3. D- Treatment 4

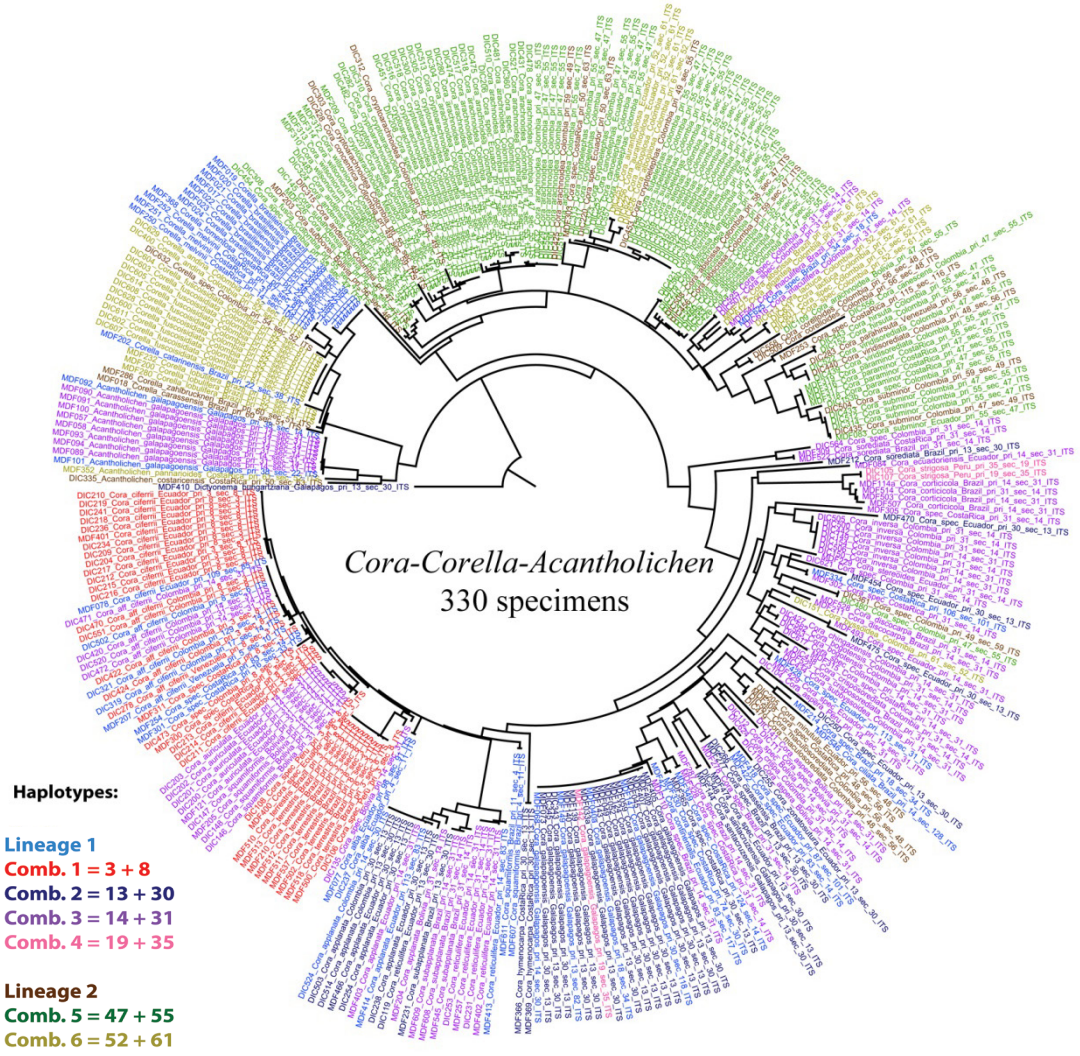


Figure 50 - Phylogenetic tree inferred by ML in RAxML for the ITS marker for the *Acantholichen-Corella-Cora* clade showing 16S rDNA haplotypes. Polar view
Colors show that the majority of species has *Rhizonema* 1, however all 6 haplotype combinations are present. *Cora* clade 1 seems to have *Rhizonema* 2 as the predominant photobiont. Legible names and bootstrap values are shown in Figure 36

References

Ahmadjian, V. (1967). A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology, and identification. Phycologia *6*, 127–160.

Ahmadjian, V. (1993). The lichen symbiosis (John Wiley & Sons).

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. *215*, 403–410.

Aptroot, A., and Bungartz, F. (2007). The lichen genus Ramalina on the Galapagos. The Lichenologist *39*.

Armaleo, D., and Clerc, P. (1991). Lichen chimeras: DNA analysis suggests that one fungus forms two morphotypes. Exp. Mycol. *15*, 1–10.

Barrasa, J.M., and Rico, V.J. (2003). The non-omphalinoid species of Arrhenia in the Iberian Peninsula. Mycologia *95*, 700–713.

Bensted-Smith, H. (2002). A biodiversity vision for the Galapagos Islands (Puerto Ayora, Ecuador: Charles Darwin Foundation and World Wildlife Fund).

Berkeley, M.J. (1843). Notices of some Brazilian fungi. Lond. J. Bot. *2*, 629–643.

Berkeley, M.J., and Curtis, M.A. (1860). Characters of New Fungi: Collected in the North Pacific Exploring Expedition by Charles Wright. Am. Acad. Arts Sci. *4*, 111–130.

Berkeley, M.J., and Curtis, M.A. (1868). Fungi Cubenses (Hymenomycetes). J. Linn. Soc. Lond. Bot. *10*, 280–320.

Bigelow, H.E. (1970). *Omphalina* in North America. Mycologia 1–32.

Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R., and Willerslev, E. (2007). The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. PloS One *2*, e197.

Bjerke, J.W., and Dahl, T. (2002). Distribution patterns of usnic acid-producing lichens along local radiation gradients in West Greenland. Nova Hedwig. *75*, 487–506.

Brodo, I., and Richardson, D. (1978). Chimeroid associations in the genus *Peltigera*. The Lichenologist *10*, 157–170.

Brodo, I.M., Sharnoff, S.D., and Sharnoff, S. (2001). Lichens of North America (Yale University Press).

Brooks, D.R. (1979). Testing the context and extent of host-parasite coevolution. Syst. Biol. *28*, 299–307.

Bungartz, F. (2008). Cyanolichens of the Galapagos Islands–the genera *Collema* and *Leptogium*. Sauteria 139–158.

Bungartz, F., Lücking, R., and Aptroot, A. (2010). The family Graphidaceae (Ostropales, Lecanoromycetes) in the Galapagos Islands. Nova Hedwig. *90*, 1–44.

Bungartz, F., Ziemmeck, F., Ayabaca, A.Y., Nugra, F., and Aptroot, A. (2013a). CDF checklist of Galapagos lichenized fungi. Charles Darwin Found. Galapagos Species Checkl. Charles Darwin Found. Galapagos Httpchecklists Datazone Darwinfoundation Orgtrue-Fungilichens Last Updat. *16*.

Bungartz, F., Benatti, M.N., and Spielmann, A.A. (2013b). The genus *Bulbothrix* (Parmeliaceae, Lecanoromycetes) in the Galapagos Islands: a case study of superficially similar, but overlooked macrolichens. The Bryologist *116*, 358–372.

Bungartz, F., Hillmann, G., Kalb, K., and Elix, J.A. (2013c). Leprose and leproid lichens of the Galapagos, with a particular focus on *Lepraria* (Stereocaulaceae) and *Septotrapelia* (Pilocarpaceae). Phytotaxa *150*, 1.

Carbonero, E.R., Sassaki, G.L., Gorin, P.A., and Iacomini, M. (2002). A (1→ 6)-linked β-mannopyrananan, pseudonigeran, and a (1→ 4)-linked β-xylan, isolated from the lichenised basidiomycete *Dictyonema glabratum*. FEMS Microbiol. Lett. *206*, 175–178.

Carlquist, S. (1974). Island biology. Columbia Univ. Press N. Y. Lond. 660pp *581*, 5279.

Castenholz, R.W., Wilmotte, A., Herdman, M., Rippka, R., Waterbury, J.B., Iteman, I., and Hoffmann, L. (2001). Phylum BX. Cyanobacteria (Springer).

Chaves, J.L., Lücking, R., Sipman, H.J., Umaña, L., and Navarro, E. (2004). A first assessment of the ticolichen biodiversity inventory in Costa Rica: the genus *Dictyonema* (Polyporales: Atheliaceae). The Bryologist *107*, 242–249.

Clémençon, H., Emmett, V., and Emmett, E.E. (2004). Cytology and plectology of the Hymenomycetes (J. Cramer Berlin/Stuttgart, Germany).

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

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., and Tiedje, J.M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. *42*, D633–D642.

Darwin, C. (1859). On the origins of species by means of natural selection (London, Murray).

Denton, A.L., McConaughy, B.L., and Hall, B.D. (1998). Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. Mol. Biol. Evol. *15*, 1082–1085.

DePriest, P.T. (2004). Early molecular investigations of lichen-forming symbionts: 1986-2001*. Annu Rev Microbiol *58*, 273–301.

Dodge, C. (1935). Lichenes. HK Svenson Plants Astor Exped. 1930 Galápagos Cocos Isl.

Downie, D., and Gullan, P. (2005). Phylogenetic congruence of mealybugs and their primary endosymbionts. J. Evol. Biol. *18*, 315–324.

Ehresmann, C., Stiegler, P., Fellner, P., and Ebel, J.-P. (1972). The determination of the primary structure of the 16S ribosomal RNA of *Escherichia coli*:(2) Nucleotide sequences of products from partial enzymatic hydrolysis. Biochimie *54*, 901–967.

Elifio, S.L., De Lourdes C. C. Da Silva, M., Iacomini, M., and Gorin, P. a. J. (2000). A lectin from the lichenized Basidiomycete *Dictyonema glabratum*. New Phytol. *148*, 327–334.

Elix, J.A., and McCarthy, P.M. (1998). Catalogue of the lichens of the smaller Pacific Islands. *70*, 1–361.

Elix, J., and McCarthy, P. (2008). Checklist of Pacific Island Lichens. Australian Biological Resources Study, Canberra. Version 21 August 2008.

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

Eriksson, O. (2005). Origin and evolution of Ascomycota—the protolichenes hypothesis. Sven. Mykol Tidskr *26*, 30–33.

Ertz, D., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Fischer, E., Killmann, D., and Serusiaux, E. (2008). A new lineage of lichenized basidiomycetes inferred from a two-gene phylogeny: The Lepidostromataceae with three species from the tropics. Am. J. Bot. *95*, 1548–1556.

Farlow, W. (1902). Lichens. BL Robinson Flora Galapagos Isl. Proc Amerc Acad Arts Sc *38*, 83–89.

Felsenstein, J. (1991). PHYLIP (Phylogenetic Inference Package).[3.6]. Univ. Wash. Seattle WA.

Fernández, E., Quilhot, W., González, I., Hidalgo, M., Molina, X., and Meneses, I. (1996). Lichen metabolites as UVB filters: Lichen metabolites show photoprotector capacity. Cosmet. Toilet. *111*, 69–74.

Fischer, E., Ertz, D., Killmann, D., and SERusiaux, E. (2007). Two new species of *Multiclavula* (lichenized basidiomycetes) from savanna soils in Rwanda (East Africa). Bot. J. Linn. Soc. *155*, 457–465.

Dal-Forno, M., Lawrey, J.D., Sikaroodi, M., Bhattarai, S., Gillevet, P.M., Sulzbacher, M., and Lücking, R. (2013). Starting from scratch: Evolution of the lichen thallus in the basidiolichen *Dictyonema* (Agaricales: Hygrophoraceae). Fungal Biol. *117*, 584–598.

Fries, E.M. (1838). Epicrisis systematis mycologici, seu synopsis Hymenomycetum.

Gardes, M., and Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. *2*, 113–118.

Gargas, A., DePriest, P.T., Grube, M., and Tehler, A. (1995). Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. Science *268*, 1492–1495.

Gauckler, K. (1960). Die Flaumflechten *Cystocoleus niger* und *Racodium rupestre* im fränkischen Schichtstufenland Nordbayerns.

Gauslaa, Y. (2009). Ökologische Funktionen von Flechtenstoffen. Rundgespräche Komm. Für Ökol. 95–108.

Gauslaa, Y., and Solhaug, K.A. (2001). Fungal melanins as a sun screen for symbiotic green algae in the lichen Lobaria pulmonaria. Oecologia *126*, 462–471.

Gaya, E., Högnabba, F., Holguin, Á., Molnar, K., Fernández-Brime, S., Stenroos, S., Arup, U., Søchting, U., Van den Boom, P., Lücking, R., et al. (2012). Implementing a cumulative supermatrix approach for a comprehensive phylogenetic study of the Teloschistales (Pezizomycotina, Ascomycota). Mol. Phylogenet. Evol. *63*, 374–387.

Geist, D., Snell, H., Snell, H., Goddard, C., and Kurz, M. (2014). A paleogeographic model of the Galápagos Islands and biogeographical and evolutionary implications. Galápagos Nat. Lab. Earth Sci. Am. Geophys. Union Wash. DC USA 145–166.

Gillevet, P., and BioSpherex, L. (2006). Multitag Sequencing and Ecogenomic Analysis, EPO 07871488.8 (PCT/US2007/084840. WPO: BioSpherex).

Gillevet, P., Sikaroodi, M., Keshavarzian, A., and Mutlu, E.A. (2010). Quantitative Assessment of the Human Gut Microbiome using Multitag Pyrosequencing. Chem. Biodivers. *7*, 1065–1075.

Goward, T. (1994). Living antiquities. Nat. Can. *1994*, 14–21.

Gregory-Wodzicki, K. (2002). Andean paleoelevation estimates: a review and critique. Geol. Soc. Am. Bull. *112*, 1091–1105.

Grube, M., and Hawksworth, D.L. (2007). Trouble with lichen: the re-evaluation and re-interpretation of thallus form and fruit body types in the molecular era. Mycol. Res. *111*, 1116–1132.

Gueidan, C., Villaseñor, C.R., De Hoog, G., Gorbushina, A., Untereiner, W., and Lutzoni, F. (2008). A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. Stud. Mycol. *61*, 111–119.

Hafner, M.S., and Page, R.D. (1995). Molecular phylogenies and host-parasite cospeciation: gophers and lice as a model system. Philos. Trans. R. Soc. B Biol. Sci. *349*, 77–83.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. *41*, 95–98.

Hariot, P. (1891). Observations sur les espèces du genre *Dictyonema*. Bull. Soci. Mycol. Fr. *7,* 32–41.

Hariot, P. (1892). Observations sur les espèces du genre *Dictyonema*. Beih. Zum Bot. Cent. *2*, 19.

Hawksworth, D. (1988). A new name for *Dictyonema pavonium* (Swartz) Parmasto. The Lichenologist *20*, 101–101.

Hawksworth, D.L. (2005). Life-style choices in lichen-forming and lichen-dwelling fungi. Mycol. Res. *109*, 135–136.

Hawksworth, D.L., Santesson, R., and Tibell, L. (2011). *Racoleus*, a new genus of sterile filamentous lichen-forming fungi from the tropics, with observations on the nomenclature and typification of Cystocoleus and Racodium. IMA Fungus Glob. Mycol. J. *2*, 71.

Henssen, A. (1963). Eine Revision der Flechtenfamilien Lichinaceae und Ephebaceae.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., et al. (2007). A higher-level phylogenetic classification of the Fungi. Mycol. Res. *111*, 509–547.

Hodkinson, B.P., and Lutzoni, F. (2009). A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. Symbiosis *49*, 163–180.

Hodkinson, B.P., Uehling, J.K., and Smith, M.E. (2012). *Lepidostroma vilgalysii*, a new basidiolichen from the New World. Mycol. Prog. *11*, 827–833.

Hodkinson, B.P., Moncada, B., and Lücking, R. (2014). Lepidostromatales, a new order of lichenized fungi (Basidiomycota, Agaricomycetes), with two new genera, *Ertzia* and *Sulzbacheromyces*, and one new species, *Lepidostroma winklerianum*. Fungal Divers. *64*, 165–179.

Honegger, R. (2001). The symbiotic phenotype of lichen-forming ascomycetes. In Fungal Associations, (Springer), pp. 165–188.

Hooker, J.D. (1847). IX. An Enumeration of the Plants of the Galapagos Archipelago; with Descriptions of those which are new. Trans. Linn. Soc. Lond. *20*, 163–233.

Howell, J.T. (1942). Up under the equator. Sierra Club Bull. *27*, 79–82.

Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics *17*, 754–755.

Huneck, S., and Yoshimura, I. (1996). Identification of lichen substances (Springer).

Jahns, H. (1988). The lichen thallus. CRC Handb. Lichenol. *1*, 95–143.

Jahns, H.M. (1970). Untersuchungen zur Entwicklungsgeschichte der Cladoniaceen unter besonderer Berucksichtigung des Podetien–Problems. Nova Hedwig *20*, 1–177.

James, P., and Henssen, A. (1976). morphological and taxonomic significance of cephalodia. In Lichenology: Progress and Problems; Proceedings of Aninternational Symposium.

Johow, F. (1884). Die Gruppe der Hymenolichenen: ein Beitrag zur Kenntniss basidiosporer Flechten. Pringsheims Jahrb. Für Wiss. Bot. *15*, 361–409.

Jørgensen, P.M. (1998). *Acantholichen pannarioides*, a new basidiolichen from South America. The Bryologist *101*, 444–447.

Katoh, K., and Toh, H. (2010). Parallelization of the MAFFT multiple sequence alignment program. Bioinformatics *26*, 1899–1900.

Katoh, K., Kuma, K., Toh, H., and Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. *33*, 511–518.

Kawakita, A., Takimura, A., Terachi, T., Sota, T., and Kato, M. (2004). Cospeciation analysis of an obligate pollination mutualism: Have *Glochidion* trees (Euphorbiaceae) and pollinating epicephala moths (gracillariidae) diversified in parallel? Evolution *58*, 2201–2214.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics *28*, 1647–1649.

Kikuchi, Y., Hosokawa, T., Nikoh, N., Meng, X.-Y., Kamagata, Y., and Fukatsu, T. (2009). Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. BMC Biol. *7*, 2.

Kirk, P.M., Cannon, P.F., Minter, D.W., and Stalpers, J.A. (2008). Ainsworth & Bisby's dictionary of the fungi (Wallingford, UK: Cabi).

Krempelhuber, A. von (1875). Lichenes quos legit O. Beccari in insulis Borneo et Singapore annis 1866 et 1867. N Giorn Bot Ital *7*, 5–67.

Lane, D. (1991). 16S/23S rRNA sequencing. Nucleic Acid Tech. Bact. Syst. 125–175.

Larcher, W., and Vareschi, V. (1988). Variation in morphology and functional traits of Dictyonema glabratum from contrasting habitats in the Venezuelan Andes. The Lichenologist *20*, 269–277.

Lawrey, J.D. (1986). Biological role of lichen substances. Bryologist 111–122.

Lawrey, J.D., Lücking, R., Sipman, H.J.M., Chaves, J.L., Redhead, S.A., Bungartz, F., Sikaroodi, M., and Gillevet, P.M. (2009). High concentration of basidiolichens in a single family of agaricoid mushrooms (Basidiomycota: Agaricales: Hygrophoraceae). Mycol. Res. *113*, 1154–1171.

Linder, D.H. (1934). The Templeton Crocker Expedition of the California Academy of Sciences, 1932. No. 18. Proc. Calif. Acad. Sci. Ser. IV *21*, 211–224.

Liu, Y.J., Whelen, S., and Hall, B.D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol. Biol. Evol. *16*, 1799–1808.

Lockhart, C., Rowell, P., and Stewart, W. (1978). Phytohaemagglutinins from the nitrogen-fixing lichens *Peltigera canina* and *P. polydactyla*. FEMS Microbiol. Lett. *3*, 127–130.

Lodge, D.J., Padamsee, M., Matheny, P.B., Aime, M.C., Cantrell, S.A., Boertmann, D., Kovalenko, A., Vizzini, A., Dentinger, B.T.M., Kirk, P.M., et al. (2014). Molecular phylogeny, morphology, pigment chemistry and ecology in Hygrophoraceae (Agaricales). Fungal Divers. *64*, 1–99.

Losos, J.B., and Ricklefs, R.E. (2009). Adaptation and diversification on islands. Nature *457*, 830–836.

Lücking, R. (2008). Foliicolous lichenized fungi. Flora Neotropica Monogr. *103*, 1–867.

Lücking, R. (2012). One species is many: Unrecognized levels of diversification of tropical macrolichens in neotropical paramos. In Proceedings of the 49th Annual Meeting of the Association for Tropical Biology and Conservation.

Lücking, R., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Chaves, J.L., Sipman, H.J.M., and Bungartz, F. (2009a). Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. Am. J. Bot. *96*, 1409–1418.

Lücking, R., Rivas Plata, E., Chaves, J., Umaña, L., Sipman, H., Thell, A., Seaward, M., and Feuerer, T. (2009b). How many tropical lichens are there... really. Bibl. Lichenol. *100*, 399–418.

Lücking, R., Dal-Forno, M., Lawrey, J.D., Bungartz, F., Rojas, M.E.H., Hernández M., J.E., Marcelli, M.P., Moncada, B., Morales, E.A., Nelsen, M.P., et al. (2013a). Ten new species of lichenized Basidiomycota in the genera *Dictyonema* and *Cora* (Agaricales: Hygrophoraceae), with a key to all accepted genera and species in the Dictyonema clade. Phytotaxa *139*, 1–38.

Lücking, R., Dal-Forno, M., and Lawrey, J.D. (2013b). Last but not least: Witnessing the "birth" of lichenization in the Basidiomycota. In Botany 2015.

Lücking, R., Barrie, F.R., and Genney, D. (2014a). *Dictyonema coppinsii*, a new name for the European species known as *Dictyonema interruptum* (Basidiomycota: Agaricales: Hygrophoraceae), with a validation of its photobiont *Rhizonema* (Cyanoprokaryota: Nostocales: Rhizonemataceae). The Lichenologist *46*, 261–267.

Lücking, R., Dal-Forno, M., Wilk, K., and Lawrey, J.D. (2014b). Three new species of *Dictyonema* (lichenized Basidiomycota: Hygrophoraceae) from Bolivia. Acta Nova *6*, 4–16.

Lücking, R., Dal-Forno, M., Sikaroodi, M., Gillevet, P.M., Bungartz, F., Moncada, B., Yanez-Ayabaca, A., Chaves, J.L., Coca, L.F., and Lawrey, J.D. (2014c). A single macrolichen constitutes hundreds of unrecognized species. Proc. Natl. Acad. Sci. *111*, 11091–11096.

Lücking, R., Lawrey, J.D., Gillevet, P.M., Sikaroodi, M., Dal-Forno, M., and Berger, S.A. (2014d). Multiple ITS Haplotypes in the Genome of the Lichenized Basidiomycete *Cora inversa* (Hygrophoraceae): Fact or Artifact? J. Mol. Evol. *78*, 148–162.

Lücking, R., Grall, A., and ThüS, H. (2014e). Typification of *Thelephora pavonia* Sw. and reinstatement of *Cora ciferrii* (Tomas.) comb. nov. The Lichenologist *46*, 825–828.

Lumbsch, H.T., and Leavitt, S.D. (2011). Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. Fungal Divers. *50*, 59–72.

Lumbsch, H.T., Chaves-Chaves, J.L., Umaña-Tenorio, L., Lücking, R., Dal-Forno, M., and others (2011). One hundred new species of lichenized fungi: a signature of undiscovered global diversity. Un centenar de nuevas especies de hongos liquenizados: una firma de la diversidad mundial por descubrir. Phytotaxa. 1–127.

Lutzoni, F.M. (1997). Phylogeny of lichen-and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Syst. Biol. *46*, 373–406.

Lutzoni, F., and Vilgalys, R. (1995). Integration of morphological and molecular data sets in estimating fungal phytogenies. Can. J. Bot. *73*, 649–659.

Lutzoni, F., Pagel, M., and Reeb, V. (2001). Major fungal lineages are derived from lichen symbiotic ancestors. Nature *411*, 937–940.

Macdonald, G.A., Abbott, A.T., and Peterson, F.L. (1983). Volcanoes in the sea: the geology of Hawaii (University of Hawaii Press).

Magain, N., and Sérusiaux, E. (2014). Do Photobiont Switch and Cephalodia Emancipation Act as Evolutionary Drivers in the Lichen Symbiosis? A Case Study in the Pannariaceae (Peltigerales). PLoS ONE *9*, e89876.

Mägdefrau, K., and Winkler, S. (1967). *Lepidostroma terricolens* n.g.n.sp., eine Basidiolicheneder Sierra Nevada de Santa Marta (Kolumbien). Mitteilungen des Instituto Colombo-Aleman de Investigaciones Científicas *1*, 11–17.

Mason-Gamer, R.J., and Kellogg, E.A. (1996). Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. *45*, 524–545.

Matheny, P.B. (2005). Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Mol. Phylogenet. Evol. *35*, 1–20.

Matheny, P.B., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Henrik Nilsson, R., Hughes, K.W., Hofstetter, V., Ammirati, J.F., and Schoch, C.L. (2007). Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol. Phylogenet. Evol. *43*, 430–451.

Meade, B.J., and Conrad, C.P. (2008). Andean growth and the deceleration of South American subduction: Time evolution of a coupled orogen-subduction system. Earth Planet. Sci. Lett. *275*, 93–101.

Metzner, P. (1934). Zur Kenntnis der Hymenolichenen. Berichte Dtsch. Bot. Ges. *51*, 231–240.

Miadlikowska, J., and Lutzoni, F. (2000). Phylogenetic Revision of the Genus Peltigera (Lichen-Forming Ascomycota) Based on Morphological, Chemical, and Large Subunit Nuclear Ribosomal DNA Data. Int. J. Plant Sci. *161*, 925–958.

Miadlikowska, J., Richardson, D., Magain, N., Ball, B., Anderson, F., Cameron, R., Lendemer, J., Truong, C., and Lutzoni, F. (2014). Phylogenetic placement, species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming Ascomycota, Lecanoromycetes). Am. J. Bot. *101*, 1141–1156.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Gateway Computing Environments Workshop (GCE), 2010, (IEEE), pp. 1–8.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2011). The CIPRES science gateway: a community resource for phylogenetic analyses. In Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery, (ACM), p. 41.

Möller, A. (1893). Ueber die eine Thelephoree, welche die Hymenolichenen: *Cora*, *Dictyonema* und *Laudatea* bildet. Flora *77*, 254–278.

Moncada, B., Luecking, R., and Betancourt-Macuase, L. (2013). Phylogeny of the Lobariaceae (lichenized Ascomycota: Peltigerales), with a reappraisal of the genus *Lobariella*. The Lichenologist *45*, 203–263.

Moncada, B., Reidy, B., and Lücking, R. (2014). A phylogenetic revision of Hawaiian *Pseudocyphellaria* sensu lato (lichenized Ascomycota: Lobariaceae) reveals eight new species and a high degree of inferred endemism. The Bryologist *117*, 119–160.

Montagne, C. (1848). Sixième centurie de plantes cellulaires exotiques nouvelles, Décades 1–2. Ann. Sci. Nat. Bot. Sér. *10*, 106–136.

Moser-Rohrhofer, M. (1969). Der vegetative Flechenthallus - ein Derivat des Ascophors. Anz. Math.- Naturwissenschaftlichen Kl. Österreichischen Akad. Wiss. 109–120.

Mueller, U.G., and Gerardo, N. (2002). Fungus-farming insects: multiple origins and diverse evolutionary histories. Proc. Natl. Acad. Sci. *99*, 15247–15249.

Muggia, L., Nelson, P., Wheeler, T., Yakovchenko, L.S., Tonsberg, T., and Spribille, T. (2011). Convergent evolution of a symbiotic duet: The case of the lichen genus *Polychidium* (Peltigerales, Ascomycota). Am. J. Bot. *98*, 1647–1656.

Muggia, L., Vancurova, L., Škaloud, P., Peksa, O., Wedin, M., and Grube, M. (2013). The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. FEMS Microbiol. Ecol. *85*, 313–323.

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G., and Grube, M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. Ann. Bot. *114*, 463–475.

Müller, J. (1883). Lichenologische Beiträge XVIII. Flora *66*, 344–354.

Müller, J. (1891). Lichenes Schenckiani. Hedwigia *30*, 216–234.

Nash, T.H. (1996). Lichen biology (Cambridge University Press).

Nelsen, M.P., and Gargas, A. (2008). Dissociation and horizontal transmission of codispersing lichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). New Phytol. *177*, 264–275.

Nelsen, M., Lücking, R., Grube, M., Mbatchou, J., Muggia, L., Plata, E.R., and Lumbsch, H. (2009). Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta. Stud. Mycol. *64*, 135–144.

Nelsen, M.P., Lücking, R., Umaña, L., Trest, M.T., Will-Wolf, S., Chaves, J.L., and Gargas, A. (2007). *Multiclavula ichthyiformis* (Fungi: Basidiomycota: Cantharellales: Clavulinaceae), a remarkable new basidiolichen from Costa Rica. Am. J. Bot. *94*, 1289–1296.

Nylander, W. (1885). Arthoniae novae America borealis, Continuatio. Flora *68*, 447–449.

Nylander, W. (1890). Lichenes Japoniae: accedunt observationibus lichenes insulae Labuan (E. typ. P. Schmidt).

Oberwinkler, F. (1970). Die Gattungen der basidiolichenen. Dt Bot Ges N. F. *4*, 139–169.

Oberwinkler, F. (1984). Fungus-alga interactions in basidiolichens. Beih. Zur Nova Hedwig. 739–774.

Oberwinkler, F. (2001). Basidiolichens. In Fungal Associations, (Springer), pp. 211–225.

Oberwinkler, F. (2012). Basidiolichens. In Fungal Associations, (Springer), pp. 341–362.

O'Brien, H. (2013). Another perspective on diversity of symbiotic cyanobacteria: 16S. Available at: https://photobiontdiversity.wordpress.com/2013/09/24/symbiotic-cyanobacteria-16s/

O'Brien, H.E., Miadlikowska, J., and Lutzoni, F. (2005). Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus *Peltigera*. Eur. J. Phycol. *40*, 363–378.

O'Brien, H.E., Miadlikowska, J., and Lutzoni, F. (2013). Assessing population structure and host specialization in lichenized cyanobacteria. New Phytol. *198*, 557–566.

Orange, A., James, P.W., and White, F. (2001). Microchemical methods for the identification of lichens (Twayne Publishers).

Ozenda, P. (1963). Lichens. In Handbuch Der Pflanzenanatomie, (Berlin: Borntraeger), p. p. 199 Teil 9.

Parent, C.E., Caccone, A., and Petren, K. (2008). Colonization and diversification of Galapagos terrestrial fauna: a phylogenetic and biogeographical synthesis. Philos. Trans. R. Soc. B Biol. Sci. *363*, 3347–3361.

Parmasto, E. (1978). The genus Dictyonema ("Thelephorolichenes"). Nova Hedwig. *29*, 99–144.

Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D., and Pupko, T. (2010a). GUIDANCE: a web server for assessing alignment confidence scores. Nucleic Acids Res. *38*, W23–W28.

Penn, O., Privman, E., Landan, G., Graur, D., and Pupko, T. (2010b). An Alignment Confidence Score Capturing Robustness to Guide Tree Uncertainty. Mol. Biol. Evol. *27*, 1759–1767.

Piercey-Normore, M.D. (2004). Selection of algal genotypes by three species of lichen fungi in the genus *Cladonia*. Can. J. Bot. *82*, 947–961.

Piercey-Normore, M.D. (2006). The lichen-forming ascomycete Evernia mesomorpha associates with multiple genotypes of *Trebouxia jamesii*. New Phytol. *169*, 331–344.

Piercey-Normore, M.D., and Deduke, C. (2011). Fungal farmers or algal escorts: lichen adaptation from the algal perspective. Mol. Ecol. *20*, 3708–3710.

Piercey-Normore, M.D., and DePriest, P.T. (2001). Algal switching among lichen symbioses. Am. J. Bot. *88*, 1490–1498.

Piovano, M., Chamy, M., Garbarino, J., and Quilhot, W. (1995). Studies on Chilean lichens. 24. Secondary products from *Dictyonema-glabratum* (Basidiomycotina). Boletin Soc. Chil. Quím. *40*, 163–165.

Poelt, J., and Wunder, H. (1967). Über biatorinische und lecanorinische Berandung von Flechtenapothecien untersucht am Beispiel der *Caloplaca ferruginea*-Gruppe.

Purvis, W. (2000). Lichens. Natural History Museum, London, UK.

Redhead, S.A., Lutzoni, F., Moncalvo, J.-M., and Vilgalys, R. (2002). Phylogeny of agarics: Partial systematics solutions for core omphalinoid genera in the Agaricales (Euagarics). Mycotaxon *83*, 19–57.

Reid, D.A. (1965). A monograph of the stipitate stereoid fungi. Beih. Zur Nova Hedwig. *18*, 1–388.

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. Symbiosis *34*, 99–110.

Rikkinen, J. (2015). Cyanolichens. Biodivers. Conserv. *24* (4), 973–993.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M., and Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. *111*, 1–61.

Rivas-Plata, E., and Lumbsch, H.T. (2011). Parallel evolution and phenotypic divergence in lichenized fungi: a case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). Mol. Phylogenet. Evol. *61*, 45–63.

Robertson, J., and Piercey-Normore, M.D. (2007). Gene flow in symbionts of Cladonia arbuscula. The Lichenologist *39*, 69.

Roskin, P.A. (1970). Ultrastructure of the host-parasite interaction in the basidiolichen *Cora pavonia* (Web.) E. Fries. Arch. Für Mikrobiol. *70*, 176–182.

Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A., Crous, P., Groenewald, J., Muggia, L., Grube, M., Isola, D., Schoch, C., et al. (2009). Phylogeny of rock-inhabiting fungi related to Dothideomycetes. Stud. Mycol. *64*, 123–133.

Rundel, P.W. (1978). The ecological role of secondary lichen substances. Biochem. Syst. Ecol. *6*, 157–170.

Ryan, B.D. (2001). *Dictyonema*. In Lichen Flora of the Greater Sonoran Desert Region., T.H. Nash, B.D. Ryan, C. Gries, and F. Bungartz, eds. (Tempe, AZ), pp. 169–170.

Sanders, W.B. (2001a). Composite lichen thalli of *Sticta* sp. from Brazil, with morphologically similar lobes containing either a chlorobiont or a cyanobiont layer. Symbiosis *31*, 47–55.

Sanders, W.B. (2001b). Lichens: The Interface between Mycology and Plant Morphology Whereas most other fungi live as an absorptive mycelium inside their food substrate, the lichen fungi construct a plant-like body within which photosynthetic algal symbionts are cultivated. Bioscience *51*, 1025–1035.

Sassaki, G.L., Gorin, P.A., and Iacomini, M. (2001). Characterization of lyso-galactolipids, C-2 and C-3 O-acyl trigalactosylglycerol isomers, obtained from the lichenized fungus *Dictyonema glabratum*. FEMS Microbiol. Lett. *194*, 155–158.

Schmull, M., Dal-Forno, M., Lücking, R., Cao, S., Clardy, J., and Lawrey, J.D. (2014). *Dictyonema huaorani* (Agaricales: Hygrophoraceae), a new lichenized basidiomycete from Amazonian Ecuador with presumed hallucinogenic properties. The Bryologist *117*, 386–394.

Schoch, C., Crous, P.W., Groenewald, J.Z., Boehm, E., Burgess, T.I., De Gruyter, J., De Hoog, G., Dixon, L., Grube, M., Gueidan, C., et al. (2009). A class-wide phylogenetic assessment of Dothideomycetes. Stud. Mycol. *64*, 1–15.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Consortium, F.B., List, F.B.C.A., Bolchacova, E., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. *109*, 6241–6246.

Slocum, R.D. (1980). Light and electron microscopic investigations in the Dictyonemataceae (basidiolichens). II. *Dictyonema irpicinum*. Can. J. Bot. *58*, 1005–1015.

Slocum, R.D., and Floyd, G.L. (1977). Light and electron microscopic investigations in the Dictyonemataceae (basidiolichens). Can. J. Bot. *55*, 2565–2573.

Smith, C.W. (2009). Lichens of Great Britain and Ireland (British Lichen Society).

Solhaug, K.A., and Gauslaa, Y. (1996). Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. Oecologia *108*, 412–418.

Spegazzini, C. (1888). Fungi fuegiani. Boletin Acad. Nac. Cienc. Córdoba *11*, 135–311.

Sprengel, K.P.J. (1820). Plantarum cryptogamicarum tropicarum pugillus. Kongliga Sven. Vetensk.-Akad. Handl. Stockh. 46–53.

Staiger, B. (2002). Die Flechtenfamilie Graphidaceae (Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung).Bibliotheca Lichenologica *185*, 1–526.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics *22*, 2688–2690.

Stamatakis, A., Ludwig, T., and Meier, H. (2005). RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics *21*, 456–463.

Stewart, A. (1912). Notes on the lichens of the Galápagos Islands (California Academy of Sciences).

Stocker-Wörgötter, E. (2002). Resynthesis of photosymbiodemes. In Protocols in Lichenology, (Springer), pp. 47–60.

Sulzbacher, M.A., Baseia, I.G., Lücking, R., Parnmen, S., and Moncada, B. (2012). Unexpected discovery of a novel basidiolichen in the threatened Caatinga biome of northeastern Brazil. The Bryologist *115*, 601–609.

Svenning, M.M., Eriksson, T., and Rasmussen, U. (2005). Phylogeny of symbiotic cyanobacteria within the genus Nostoc based on 16S rDNA sequence analyses. Arch. Microbiol. *183*, 19–26.

Swartz, O. (1788). Nova genera & species plantarum seu prodromus descriptionum vegetabilium maximam partem incognitorum: quae sub itinere in Indiam occidentalem annis 1783-87 (Swederus).

Takahashi, K., Wang, L.-S., Tsubota, H., Deguchi, H., and others (2006). Photosymbiodemes *Sticta wrightii* and *Dendriscocaulon* sp (lichenized Ascomycota) from Yunnan, China.

Tehler, A., Irestedt, M., Bungartz, F., and Wedin, M. (2009). Evolution and reproduction modes in the *Roccella galapagoensis* aggregate (Roccellaceae, Arthoniales). Taxon 438–456.

Templeton, A.R., Crandall, K.A., and Sing, C.F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics *132*, 619–633.

Tomaselli, R. (1950). Appunti sulla sistematica e distribuzione geografica dei Basidiolicheni. Arch. Bot *28*, 3–19.

Tomaselli, R. (1951). Notes sur les Basidiolichens. Rev. Bryol. Lichenol. *20*, 212–214.

Tomaselli, R., and Caretta, G. (1969). Morphological and anatomical observations on *Cora pavonia* (Basidiolichenes) and hypotheses of factors determining lichen symbiosis. Pavia Univ Lab Crittogam Atti *Serie 6*, 11–18.

Tønsberg, T., and Goward, T. (2001). *Sticta oroborealis* sp. nov., and other Pacific North American lichens forming dendriscocauloid cyanotypes. The Bryologist *104*, 12–23.

Trembley, M.L., Ringli, C., and Honegger, R. (2002). Differential expression of hydrophobins DGH1, DGH2 and DGH3 and immunolocalization of DGH1 in strata of the lichenized basidiocarp of Dictyonema glabratum. New Phytol. *154*, 185–195.

Tschermak-Woess, E. (1988). The algal partner. In CRC Handbook of Lichenology, M. Galun, ed. (Boca Raton, Florida, USA: CRC Press), pp. 39–92.

Tschermak-Woess, E. (1995). The taxonomic position of the green phycobiont of *Sticta canariensis* (Ach.) Bory ex Delise and its extraordinary modification in the lichenized state. Bibl. Lichenol. *58*, 433–438.

Tye, A., Snell, H., Peck, S., and Adsersen, H. (2002). Outstanding terrestrial features of the Galapagos archipelago. Biodivers. Vis. Galapagos Isl. Charles Darwin Found. World Wildl. Fund Puerto Ayora 25–35.

Vainio, E.A. (1890). Étude sur la classification naturelle et la morphologie des lichens du Brésil (Simelius).

Vainio, E.A. (1899). Lichenes novi rarioresque. Ser. III. Hedwigia *38*, 253–259.

Vainio, E.A. (1923). Lichenes in Insula Trinidad a Professore R. Thaxter collecti. In Proceedings of the American Academy of Arts and Sciences, (JSTOR), pp. 131–147.

Vargas, L.Y., Moncada, B., and Lücking, R. (2014). Five new species of *Cora* and *Dictyonema* (Basidiomycota: Hygrophoraceae) from Colombia: chipping away at cataloging hundreds of unrecognized taxa. The Bryologist *117*, 368–378.

Weber, W. (1981). Lichenes exsiccati distributed by the University of Colorado Museum, Boulder, fascicles 1-15, nos. 1-600, 1961-1979. Mycotaxon.

Weber, W.A. (1966). Lichenology and bryology in the Galapagos Islands, with check lists of the lichens and bryophytes thus far reported. In The Galapagos, R.I. Bowman, ed. (Berkeley, CA: University of California Press), pp. 190–200.

Weber, W.A. (1986). The lichen flora of the Galapagos Islands, Ecuador. Mycotaxon 451–497.

Weber, W.A. (1993). Additions to the Galápagos and Cocos Islands lichen and bryophyte floras. Bryologist 431–434.

Weber, W., and Beck, H. (1985). Effects on cryptogamic vegetation (lichens, mosses, and liverworts). El Niño Galápagos Isl. *1983*, 343–361.

Weber, W., Gradstein, S., Lanier, J., and Sipman, H. (1977). Bryophytes and lichens of the Galapagos Islands. Not. Galápagos *26*, 7–11.

Weiblen, G.D., and Bush, G.L. (2002). Speciation in fig pollinators and parasites. Mol. Ecol. *11*, 1573–1578.

White, T.J., Bruns, T., Lee, S., Taylor, J., and others (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc. Guide Methods Appl. *18*, 315–322.

Williamson, M. (1981). Island populations (Oxford: Oxford University Press xi, 286p.-illus., maps.. En Maps. Geog).

Woese, C.R., and Fox, G.E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. *74*, 5088–5090.

Xavier Filho, L., and Vicente, C. (1979). Observaciones morfológicas sobre *Corella*. Boletim da Sociedade Broteriana, Serie 2 *53*, 7–13.

Xavier-Filho, L., Mendes, L. da C.G., Vasconcelos, C.A.F., and Costa, A.C. (1980). Fitohemaglutininas (lectinas) em basidioliquens. Bol. Soc. Brotologia Bras. *2*, 41–46.

Yahr, R., Vilgalys, R., and Depriest, P.T. (2004). Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. Mol. Ecol. *13*, 3367–3378.

Yahr, R., Vilgalys, R., and DePriest, P.T. (2006). Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. New Phytol. *171*, 847–860.

Yánez, A., Dal-Forno, M., Bungartz, F., Lücking, R., and Lawrey, J.D. (2012). A first assessment of Galapagos basidiolichens. Fungal Divers. *52*, 225–244.

Yánez, A., Ahti, T., and Bungartz, F. (2013). The Family Cladoniaceae (Lecanorales) in the Galapagos Islands. Phytotaxa *129*, 1.

Zahlbruckner, A. (1908). Flechten (Lichenes). In Die Natürlichen Pflanzenfamilien, H.G. Engler, and K.A. Prantl, eds. p. 239.

Zahlbruckner, A. (1909). Lichenes (Flechten). Denkschr. Kais. Akad. Wiss. Math.-Naturwissenschaftliche Kl. *83*, 85–211.

Zahlbruckner, A. (1926). Lichenes (Flechten) B. Spezieller Teil. In A. Engler & K. Prantl (eds.), Die Natürlichen Pflanzenfamilien, (Engelmann, Leipzig), pp. pp. 61–270.

Zahlbruckner, A. (1931). Catalogus Lichenum Universalis (Borntraeger, Leipzig).

Zmitrovich, I., Malysheva, V., and Spirin, W. (2006). A new morphological arrangement of the Polyporales. I. Phanerochaetineae. Mycena *6*, 4–56.

Zoller, S., and Lutzoni, F. (2003). Slow algae, fast fungi: exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*. Mol. Phylogenet. Evol. *29*, 629–640.

Biography

Manuela Dal Forno received her Bachelor of Science from Universidade de Santa Cruz do Sul, RS, Brazil in 2006 and Master of Science from Universidade Federal do Paraná, PR, Brazil in 2009. Before applying for the PhD program of Environmental Sciences and Public Policy at George Mason University (GMU), she taught and interned at the Audubon Center of the North Woods in Sandstone, MN. While at GMU, Manuela held a four-year graduate research assistantship in the laboratories of Dr. James D. Lawrey and Dr. Patrick M. Gillevet, with Dr. Masoumeh Sikaroodi as the lab manager. In her last year she held a graduate teaching assistantship in the Department of Biology, being the instructor of record of 3 sessions of lab of BIO 103 for each semester. During her PhD she worked on projects funded by NSF and had opportunity to travel to several countries, including Ecuador (continental and the Galapagos Islands), Costa Rica, Thailand, Puerto Rico, and Brazil (where she is originally from).