AN ABSTRACT OF THE DISSERTATION OF

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Title: Lichen Studies of Tropical Dry Forest: A Systematic and Ecological Approach

Abstract approved:

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Bruce McCune

In recent years, our ecological knowledge of tropical dry forests has increased dramatically. However, whole components of the ecosystem, like lichenized fungi, remain mostly unknown. Crustose lichens in these forests are so abundant, that they are responsible for the characteristic appearance of a "white bark forest" during the dry season. The aim of this dissertation is to incorporate lichens into our understanding of the functioning of tropical dry forests. Prior to this work, lichens in this ecosystem were not considered at all in ecological studies and only in recent years we started having a better understanding of what species are present. The thesis is divided in two sections: Chapters 2 and 3 deal with particular cases of lichen systematics, while chapters 4, 5 and 6 deal with ecological studies of lichens at the ecosystem level and how they interact with other organisms. All the chapters revolve around lichens of the tropical dry forest of the Chamela-Cuixmala Biosphere Reserve in Mexico.

In Chapter 2, new collections of the supposedly extinct and doubtfully lichenized fungus genus *Polypyrenula* were found. Given that anatomical studies of the fresh collections were not congruent with its current systematic position, a molecular approach was followed using the genes ITS, mtSSU and nuLSU. Our molecular analysis demonstrated that the monospecific genus, previously included in the family Pyrenulaceae, belongs instead in the Trypetheliaceae, but outside the core genera in the family. We extend the distribution of *Polypyrenula* to South America, provided new information on its phorophytic associations, corroborated that it is a facultatively

lichenized fungus, and reinstated the name *Polypyrenula sexlocularis* as the correct name for the species.

In Chapter 3, one new genus and two new species of lichens in the family Graphidaceae were described based on morphological, chemical and molecular data of the genes ITS, mtSSU, and nuLSU. The new genus *Jocatoa* in the subfamily Graphidoideae is described to accommodate the orphan species *Medusulina texana*. While the new species *Gymnographopsis corticola* and *Redonographa parvispora* are described in the subfamily Redonographoideae, together being the only two known corticolous species in the subfamily. A phylogenetic analysis including all the genera in the family Graphidaceae, with available sequences, is presented to accommodate the new genus and to validate for the first time the position of *Gymnographopsis*. Diagnostic anatomical and ecological characters are discussed for Redonographoideae. *Gymnographopsis* is newly reported to the Northern Hemisphere.

In Chapter 4, we estimate the total lichen biomass at the ecosystem level. Calculations were based on the bark area of trees, density of different sizes of trees per hectare, dry mass of lichens per unit area, and the percentage of lichen cover per tree. The epiphytic lichen biomass in the forest was 1.30 to 1.92 t/ha, of which 180 kg per hectare were located on the lowest 2.5 m of the main trunk of the trees. Lichen biomass represents 59 percent of the foliar biomass in the system, suggesting a significant ecological role that so far is unexplored. To our knowledge, this is the first time that a lichen biomass estimate is provided for an ecosystem in which crustose lichens are the dominant growth form.

In Chapter 5, the lichen consumption component of herbivory in the tropical dry forest was analyzed and compared to the leaf herbivory component. Lichen herbivory rates were calculated using high definition photographs of permanent microplots across a four-year period. The annual rate of lichen consumption was 11.5%, with no significant difference between years, even in the presence of catastrophic events like the category 4 hurricane Patricia. Lichen biomass annual consumption per hectare represents 28.5% of

the biomass lost to total herbivory when considering leaf folivory (chewing) and lichenivory together. The results show that lichen consumption is an established and regular process in the forest dynamics of the tropical dry forest. A discussion on the animals responsible for lichen herbivory is presented.

In Chapter 6, caterpillars of a moth species of the family Psychidae were discovered living inside mobile bags made from silk and completely covered with small pieces of lichens. The lichens used as construction material for the caterpillar bags were identified with molecular techniques and compared to a newly generated database of genetic barcodes for the lichens in the area. Of the approximately 300 lichen species expected to occur in the area, only five of them were used by the caterpillars. There was a strong selectivity for micro-foliose lichens of the family Physciaceae, even though they represent a small fraction of the mostly crustose lichens present in the forest.

In this dissertation new aspects in the study of tropical dry forest were revealed. Lichens that were previously ignored were shown to be diverse, abundant and key components in the dynamics of the ecosystem. Lichens revealed levels of biomass comparable with the biomass of leaves in the forest and were consumed at similar rates. Preliminary data from this dissertation points towards a major component of the trophic web of the ecosystem that is sustained by lichens. Of particular importance is the potential of lichens to maintain the functionality of the ecosystem during the extended dry seasons. We suggest that the crustose lichen component should not be underestimated *a priori* in ecological studies, especially in areas with significant lichen cover.

Lichen Studies of Tropical Dry Forest: A Systematic and Ecological Approach

by
Ricardo Miranda-González

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APPROVED:

Major Professor, representing Botany & Plant Pathology

Head of the Department of Botany & Plant Pathology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Ricardo Miranda-González, Author

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CONTRIBUTION OF AUTHORS

Chapter 2: All authors participated in preliminary discussions and reviewed the manuscript. André Aptroot found the samples belong to the genus *Polypyrenula* and reviewed the type collection. Adam Flakus contributed with specimens and data from Bolivia.

Chapter 3: All authors participated in preliminary discussions, contributed herbarium collections, and reviewed the manuscript. Robert Lücking found the link between samples of the study area and *Medusulina texana*.

Chapters 4 and 5: Bruce McCune participated with long discussions, analysis of data and reviewed the manuscripts.

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CHAPTER 1. INTRODUCTION

Ricardo Miranda-González

Ecological studies of lichenized fungi at the ecosystem scale are rare for tropical ecosystems. However, data from temperate and desert areas have demonstrated the large-scale ecological importance of lichens. Lichens are the main food for several animal groups, including cervids (Ward & Marcum 2005), snails (Asplund et al. 2010) and other invertebrates (Gerson & Seaward 1977, Krantz & Walter 2009). Given their high abundance in some areas (Boucher & Stone 1992, Berryman & McCune 2006, Nelson et al. 2013), lichens not only support an extensive trophic network (Gerson & Seaward 1977), but also contribute in mineral cycling (Pike 1978), nitrogen input (Jones & Shachak 1990) and habitat for other organisms (Gerson & Seaward 1977) at the ecosystem scale.

Given historical reasons and the higher complexity of tropical ecosystems, studies of tropical lichens are still dominated by a much-needed inventory and systematic approach. The hundreds of lichen species described in recent years and the development of high-quality monographic treatments (Aptroot et al. 2008, Lücking 2008, Herrera-Campos et al. 2016), are still a drop in the bucket when compared to the complexity and diversity of tropical lichens (Lücking et al. 2014). Nonetheless, these continuous advances are the framework that allows the study of novel ecological interactions, and eventually, a better understanding of the ecological role of lichens in the ecosystem functioning of the tropics.

Among tropical ecosystems, the tropical dry forests (TDF) are widespread and represents 42% of the forested tropical land in the world (Murphy & Lugo 1986, 1995). They are characterized by an extended dry season of three to eight months in which more than 50% of the trees lose their leaves completely. Tropical dry forests have a mean annual precipitation of 400–2000 mm, a mean annual temperature above 25 °C, and an elevation from sea level to 2000 m (Trejo & Dirzo 2000, Sánchez-Azofeifa et al. 2005, Portillo-Quintero & Sánchez-Azofeifa 2010).

However, compared to tropical rain forests, TDFs are well behind in terms of scientific research, conservation strategies, and public awareness (Miles et al. 2006,

Sánchez-Azofeifa et al. 2013a). Fortunately, in recent years, several publications have improved our understanding of these ecosystems (Bullock et al. 1995, Noguera et al. 2002, Dirzo et al. 2011, Sánchez-Azofeifa et al. 2013b). In particular, the creation of the Chamela-Cuixmala Biosphere Reserve (CCBR) in the Pacific Coast of Mexico has contributed extensively to the current knowledge of TDFs. The CCBR is the most intensively studied TDF in the Neotropics (Jaramillo et al. 2011) and its scientific productivity includes more than 600 scientific papers and more than 350 research theses since its creation in 1971 (Ayala 2011, Miranda-González 2012).

The Reserve sustains 1149 species of plants (Lott & Atkinson 2006, Sánchez-Azofeifa et al. 2013a) and more than 2200 species of arthropods (Rodríguez-Palafox & Corona 2002, García Aldrete & Anaya 2004). However, by the year 2010, the only lichen species recorded for the Reserve was *Cresponea leprieurii*, collected in 1985 and studied by Egea & Torrente (1993). During the last decade and in collaboration with other researches from Mexico and Germany, the number of known lichen species for the Reserve has increase to about 300 (Miranda-González 2012, Barcenas-Peña 2016, Herrera-Campos et al. 2017). A high proportion of those species are new to science or are in need of deep systematic treatments (Herrera-Campos et al. 2019, see Chapters 2 and 3 of this dissertation).

Although our recent efforts provide a better understanding of the lichen component of TDFs, we known relatively little about their ecology or their interactions with other organisms. This is surprising given that lichens in these forests are so abundant that crustose lichens are responsible for the characteristic appearance of a “white bark forest” during the dry season.

In this dissertation, I explore some of the ecological functions of crustose lichens at the ecosystem scale. Particularly, I quantify lichen biomass in the forest (Chapter 4), and annual rates of lichen consumption by invertebrates (Chapter 5), and compare these to the total biomass and herbivory of leaves per hectare of forest. Lastly, I describe the

highly specific use of lichens by caterpillars in the construction of mobile bag-shaped domiciles (Chapter 6).

CHAPTER 2. THE IDENTITY, ECOLOGY AND DISTRIBUTION OF *POLYPYRENULA* (ASCOMYCOTA: DOTHIDEOMYCETES): A NEW MEMBER OF TRYPETHELIACEAE REVEALED BY MOLECULAR AND ANATOMICAL DATA

Ricardo Miranda-González, André Aptroot, Robert Lücking, Adam Flakus, Alejandrina Barcenas-Peña & María de los Angeles Herrera-Campos

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Abstract

We report several new collections of the monospecific genus *Polypyrenula*, a supposedly extinct and doubtfully lichenized fungus, currently classified in the Pyrenulaceae. Our anatomical studies reveal that it is facultatively lichenized. Morphologically, the structure of its hamathecium suggests a closer relation with Dothideomycetes than with Eurotiomycetes. Our molecular analysis demonstrated its inclusion in Trypetheliaceae, but outside the core genera in the family. We extend the distribution of *Polypyrenula* to South America, provide new information on its phorophytes associations, and reinstate the name *Polypyrenula sexlocularis* as the current correct name for this species.
Key words: *albissima*, Bolivia, lichen, Mexico, tropical dry forest.

Introduction

Recent studies in the family Pyrenulaceae have shown a strong need of revision at the genus level; for instance, the genus *Pyrenula* (with the largest number of species in the family) is not monophyletic, while most of the other sequenced genera in the family are nested within *Pyrenula s.l.* (Gueidan et al. 2008, 2016; Aptroot, 2012; Weerakoon et al. 2012). One of the genera without DNA data is the monospecific *Polypyrenula* which is placed only provisionally in Pyrenulaceae (Hawksworth, 1983, 1985; Harris, 1995; Lumbsch & Huhnford, 2010).

Polypyrenula albissima (A. Massal.) Aptroot is only known from its type collection that consists of two small pieces collected by Fée almost 200 years ago. The taxon was believed to be extinct and doubtfully lichenized (Aptroot, 1991; Gueidan et al. 2016), although Hawksworth (1983) suggested it might have been associated with *Trentepohlia*. The species is unique in having ascospores with a pronounced basal euseptum (formed from the septal plate) followed by 3-4 distosepta (lacking a septal plate and formed from the endospore), but given the bad state of the type collection, its hamathecium has not been studied in detail (Hawksworth, 1983). This combination of septa, together with the poorly preserved type collection, has provoked several nomenclatural changes over the

years. As a result, *P. albissima* currently has six synonyms within five different genera (Aptroot, 1991).

Usually each type of ascospore septation is diagnostic at the genus, family or even order level (Hawksworth, 1983; Aptroot, 2012; Sweetwood, 2012,). Some families like Pyrenulaceae and Trypetheliaceae have species with both eusepta and distosepta but the eusepta are reduced instead of pronounced (Aptroot, 1991, 2008; Sweetwood, 2012). The ascospores of *P. albissima* resemble some species of *Splanchnonema* (Pleosporales: Pleomassariaceae) that have a pronounced submedium euseptum in addition to distosepta (Barr, 1982).

In this study we found several new collections of *Polypyrenula* which allowed us to study the material in detail. Given our findings in the hamathecium structure, we considered it more likely for *Polypyrenula* to belong to Trypetheliaceae in Dothideomycetes than to Pyrenulaceae in Eurotiomycetes. Our objectives were to provide a phylogenetic placement of this taxon using molecular data and to fill the gaps in our understanding of its nomenclature, anatomy and ecology.

Materials and Methods

Anatomical studies

Specimens were studied using standard techniques in an Olympus SZ61 dissecting microscope and an Olympus BX41 compound microscope, both connected to a NIKON D5300 digital camera. Sections were mounted and measured in tap water. KOH and IKI reagents were used at 10% and 0.3% respectively following Bungartz (2002).

Taxon sampling

New sequence data of the genes ITS, mtSSU and nuLSU were obtained from two samples of *Polypyrenula albissima* collected from Mexico. Initial BLAST showed members of Trypetheliaceae as the closest relatives. Two analyses were performed: 1) We included our nuLSU sequences in the analysis of Wijayawardene et al. (2014) to

place the new sequences within Dothideomycetes and within Trypetheliaceae (data not shown). 2) We based the final analysis on sequences from recent papers of Trypetheliaceae (Nelsen et al. 2014; Hyde et al. 2016; Lücking et al. 2016) with an emphasis on the basal lineages of the family. We included a total of 171 sequences, 85 of mtSSU and 86 of nuLSU, for 82 ingroup species, including representatives from all the genera of Trypetheliaceae currently published in GenBank (Appendix 2.1). The *Cladosporium cladosporoides* group was selected as outgroup following Nelsen et al. (2014) and Lücking et al. (2016).

DNA extraction, PCR, and sequencing

Total DNA was isolated from the new collections using the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) following the manufacturer's instructions, except only two perithecia per sample were used in 15 µl of extraction buffer followed by 15 µl of dilution buffer. The whole ITS and portions of mtSSU and nuLSU were amplified and sequenced using the following primers: ITS1F/ITS4 (Gardes & Bruns 1993; White et al. 1990), mrSSU1/mrSSU3R (Zoller et al. 1999), and AL2R/LR6 (Mangold et al. 2008; Vilgalys & Hester, 1990) respectively.

Each 10 µl PCR reaction consisted of 5 µl R4775 Sigma-Aldrich REDExtract-n-Amp PCR Ready Mix, 0.5 µl of each primer (10 µM), 3 µl water, and 1 µl undiluted DNA. The PCR cycling conditions for ITS were: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 45 s, and 72 °C for 105 s, followed by 72 °C for 5 min. The PCR cycling conditions for mtSSU and nuLSU were: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C (for mtSSU) or 57 °C (for nuLSU) for 1 min, and 72 °C for 105 s, followed by 72 °C for 10 min. 2 µl of each PCR products were visualized on 1.5% TBA agarose gel stained with GelRed (Biotium). Single bands were cleaned directly from PCR products with ExoSAP-IT ® for PCR product cleanup (Affymetrix, Santa Clara, CA, USA). If double bands appeared the rest of the PCR product was gel-extracted and cleaned with GELase (Epicentre Biotechnologies, Madison, Wisconsin, U.S.A.) following manufacturer's instructions.

Samples were sent to be sequenced at Eurofins MWG Operon LLC (Louisville, KY). Each 12 µl reaction consisted of 2.4 µl primer (at 10 µM), 2 µl undiluted PCR product cleaned with ExoSAP and 7.6 µl water or 2.4 µl primer (at 10 µM) and 9.6 µl DNA cleaned with GELase.

Phylogenetic analysis

New sequences were edited in Geneious v.8.1.9 (Kearse et al., 2012). All sequences of mtSSU and nuLSU were aligned independently using the multiple sequence alignment algorithm MAFFT (Katoh et al. 2005). Ambiguously aligned columns were removed using trimAl v1.2 (Capella-Gutierrez et al., 2009) with automatic settings. A maximum likelihood (ML) analysis of all genes partitioned by locus was performed in the RAxML-HPC BlackBox 8.2.10 (Stamatakis, 2014), with 552 bootstrapping replicates as automatically determined by RAxML using a saturation criterion. Furthermore, a Bayesian analysis was performed in MrBayes v.3.2.6 (Huelsenbeck & Ronquist, 2001), with two independent runs of two million generations each, resampling every 1000 trees, 25% burn-in, and heated chains of 0.2. Both analyses were done with the GTRGAMMA model and run on the Cipres Gateway server (Miller et al. 2010). Single locus analyses were performed to visually test for topological incongruence. The final Bayesian tree was plotted using Geneious and edited in Photoshop CS6.

Results

Taxonomy

Polypyrenula sexlocularis (Müll. Arg.) D. Hawksw. (see Typification remarks below) (Fig. 2.1)

=*Microthelia sexlocularis* Müll. Arg., *Mém. Soc. Phys. Hist. nat. Genève* 30(3): 38 (1888).

=*Polythelis sexlocularis* (Müll. Arg.) Clem., Gen. fung. (Minneapolis): 173 (1909).

Non *Verrucaria epidermidis* var. *albissima* Ach. (1809); sometimes as *Verrucaria epidermidis* var. *albissima* Fée, *Essai crypt. écorc*.: 84 (1824);

Sagedia albissima (Fée) A. Massal., *Ric. auton. Lich. Crost.*: 161 (1852);

Pyrenula albissima (Fée) Trevis., *Spighe e Paglie.*: 18 (1853);
Polypyrenula albissima (A. Massal.) Aptroot, *Bibliotheca Lichenologica* 44: 102 (1991); sometimes wrong as *Polypyrenula albissima* (Fée) Aptroot.

Type. WEST INDIES. On *Croton cf. linearis* ("Crotonis Cascarillae"). Fée s.n. (holotype, G).

Thallus ecorticate, endoperidermal, thin, whitish grey to brownish, without pseudocyphellae, black hypothallus sometimes present at contact points with other lichens. *Photobiont* trentepohlioid, but not always present. *Perithecia* solitary, erumpent from the substratum, sometimes partly covered by bark cells, 0.2–0.35 mm wide; usually with a well-defined involucrellum and then 0.35–0.55(0.65) mm in total. Ostiole apical, brownish-black up to 0.06 mm wide. Perithecial wall without crystals, proper exciple apically and laterally carbonized but basally only reddish brown. *Hamathecium* not inspersed, IKI-, pseudoparaphyses branched and anastomosed, 0.5–1.4 µm in diameter, no septation seen in water at 400×, embedded in a gelatinous matrix. *Asci* bitunicate, subcylindrical, tholus not amyloid, ocular chamber wide and rounded, (6)8 spored, 62–70×15 µm, not seen after discharge. *Ascospores* biseriate in the asci, elongate-ellipsoid, with rounded ends, reddish brown (greyish in KOH) but basal cell frequently paler, 20–30(35) × 5.8–8.7 (means = 24.58, 7.29; standard deviations = 3.1, 0.65; n = 45), with 1(2) pronounced and transverse basal euseptum that may constrict the cell, 3–5 transversal distosepta; basal euseptum forming first, followed by the distosepta, then later the pigmentation, endospore thick up to 1.3 µm, lumina rounded to angular but not astrothelioid, spore wall smooth, gelatinous sheath not seen. *Anamorph* not seen.

Chemistry. UV-, no substances detected by TLC.

Remarks. Most of the ascospores had a pronounced basal euseptum, nonetheless, it was common to see ascospores from the same perithecium with the euseptum reduced (Fig. 2.1H). Two ascospores of different thalli had two pronounced basal eusepta instead of one (Fig. 2.1D). Previously, Hawksworth (1983) found 4–6 spores per ascus, but our

samples agreed with Müller Argoviensis' (1888) description in having (6)8 spores per ascus.

Distribution and ecology. Previously only known for the type collection which was stated as "in America" and growing on the tree *Croton cascarilla* (L.) L. (Fée, 1837). Hawksworth (1983) proposed the name *C. cascarilla* was misapplied to *C. linearis* Jacq., he used the distribution of the phorophyte as well as the work of Fée at that time to suggest the West Indies as the origin of the type collection. In the present work new collections of *P. sexlocularis* were found in the Pacific Coast of México and in Bolivia.

All the new samples were associated to some degree with dry areas, especially with the tropical dry forests. This ecosystem typically consists of more than 50% of deciduous trees, an extended dry season of three to eight months, mean annual precipitation between 400–2000 mm, mean annual temperature above 25 °C, and an elevation from sea level to 2000 m (Trejo & Dirzo, 2000; Sánchez-Azofeifa et al. 2005; Portillo-Quintero & Sánchez-Azofeifa, 2010). Interestingly, most of this ecosystem in the Neotropics happens to be in Mexico followed by Bolivia, with important areas in the West Indies as well (Portillo-Quintero & Sánchez-Azofeifa, 2010).

In Mexico, *P. sexlocularis* is a rare species mostly found in secondary forests, with only one out of seven samples found in pristine forest. It was associated with the following phorophytes: *Apoplanesia paniculata* C. Presl, *Caesalpinia caladenia* Standl., *Gliricidia sepium* (Jacq.) Walp., *Heliocarpus pallidus* Rose, and *Leucaena lanceolata* S. Watson. Most samples were found at elevations below 340 m, but one Bolivian sample (M. Kukwa 11367) was found at 1500 m.

Typification remarks. Over the years this taxon has accumulated a series of misconceptions that probably started by misapplying Fée's work. Fée (1824) mentions the taxon *Verrucaria epidermidis* var. *albissima* Ach., not as a new variety but simply as a new record for America (eventually it was found to be a misidentification). Later, he mentioned the species again, saying asci were not present in his collection and suggesting

it might be just an immature state of his new species *Verrucaria cascarilla* Fée; nonetheless, he kept both species as separate entities in his work (Fée, 1837).

Massalongo (1852) studied Fée's sample of *V. epidermidis* var. *albissima* and made the new combination *Sagedia albissima* (Ach.) A. Massal. (he made no connection with *V. cascarilla* though). Interestingly he found spores in Fée's sample and provided illustrations in his Figure 316. The spores were fusiform with pointed ends, with three distosepta and without euseptum, the hamathecium was shown as not anastomosed and mostly unbranched. This description is not conspecific with *V. epidermidis* var. *albissima* Ach. nor with *P. sexlocularis*. Therefore, his new combination should not be applied to either species. In fact, the description does not fit Fée's either, which suggests a mixed collection. The description of *S. albissima* resembles a species of *Pyrenula* and in fact was recombined into *Pyrenula albissima* (Ach.) Trevis. (Trevisan, 1953). Currently the identity of this taxon remains unknown, but it should be considered as described by Massalongo with the current name *Pyrenula albissima* (A. Massal.) Trevis.

On the other hand, Müller (1888) found that the type collection of *V. cascarilla* was a mix of seven species, three already known: *Pseudopyrenula diluta* (Fée) Müll. Arg., *Arthopyrenia cinchonae* (Ach.) Müll. Arg. (now *Constrictolumina cinchonae* (Ach.) Lücking, M.P. Nelsen & Aptroot) and *Pyrenula guayaci* (Fée) Müll. Arg. (now *Parapyrenis guayaci* (Fée) Aptroot); and four new species: *Microthelia dominans* Müll. Arg., *Arthopyrenia feeana* Müll. Arg. (now *Anisomeridium feeanum* (Müll. Arg.) R.C. Harris), *Porina cascarilla* Müll. Arg. and *Microthelia sexlocularis* Müll. Arg. (now *Polypyrenula sexlocularis*).

Müller Argoviensis separated each species from the type collection of *V. cascarilla* and designated two pieces of bark as the type collection of *M. sexlocularis*. Unfortunately, he chose Fée's immature collection of *V. epidermidis* var. *albissima* as the anamorph state of *M. sexlocularis*. Müller Argoviensis was aware of Fée's misidentification and proceeded to use the name *V. epidermidis* var. *albissima* Fée to differentiate it from the Acharius species. It is beyond our knowledge how he decided the

anamorph was related to *M. sexlocularis* versus any of the other species in the *V. cascarilla* mix collection, or if he was aware of Massalongo's description of *S. albissima*. The fact is that the name *V. epidermidis* var. *albissima* Fée is illegitimate, both because it was not Fée's intention to describe a new variety and because the name was already in use by Acharius. In the unlikely case that an anamorph piece of that collection is conspecific with *P. sexlocularis*, the epithet *albissima* should not take priority over *sexlocularis*.

Clements (1909) made the new combination *Polythelis sexlocularis* (Müll. Arg.) Clem., which was not accepted by Zahlbruckner (1922); instead he accepted *M. sexlocularis* and included in the synonymies the illegitimate *V. epidermidis* var. *albissima* Fée (citing an illustration of *V. epidermidis* var. *quassiaecola* Fée) along with the unrelated *S. albissima* and *Pyrenula albissima*. This contribution perpetuated the confusion that lead to the combination of *Polypyrenula albissima* (A. Massal.) Aptroot (Aptroot, 1991). The current name of this taxon should be *P. sexlocularis* as described by Hawksworth (1985).

Specimens studied. **Mexico**: *Jalisco*: La Huerta, Chamela Biological Station (CBS), 300m W of Tejón trail 600 m, 19°30'11''N, 105°2'52''W, pristine tropical dry forest, May 2010, elev. 44 m, *Miranda 1791* (MEXU). Surrounding areas of CBS: Ejido Santa Cruz, 19°35'57''N, 105°2'55'W, secondary tropical dry forest, Oct 2010, elev. 118 m, *Miranda 2736* (MEXU); ibid., very disturbed tropical dry forest, 19°35'22''N, 105°2'4'W, elev. 144 m, *Miranda 3823, 3828, 3829, 3886* (MEXU); Ejido Caimán, secondary tropical dry forest, Oct 2010, 19°28'3''N, 105°56'11'W, elev. 54 m, *Miranda 2539* (MEXU). **Bolivia**: *Dept. Santa Cruz*: Prov. Cordillera, PNANMI Kaa-Iya del Gran Chaco, near Peto Blanco, park guard's station, 18°56'26"S, 60°22'39"W, Chiquitano forest, 5 Dec. 2011, elev. 340 m, *A. Flakus 23655* (LPB, KRAM); Prov. Guarayos, RN de Vida Silvestre Ríos Blanco y Negro, Plan de Manejo AISU, 15°09'13"S, 62°47'57"W, lowland Amazon forest, elev. 240 m, 24 July 2009, leg. *A. Flakus 13730 & P. Rodriguez* (LPB, KRAM); *Dept. Tarija*: Prov. Burnet O'Connor, 28 km from Entre Ríos, near

Soledad, 21°41’00”S, 64°07’29”W, Tucumano-Boliviano montano forest, elev. 1500 m, 11 Aug. 2012, *M. Kukwa 11367*.

Phylogenetic analysis

New sequences recovered in this study are two of ITS, two of mtSSU and two of nuLSU (Table 2.1). The combined data set consisted of 82 ingroup species (Appendix 2.1) and 838 unambiguously aligned characters (357 from mtSSU and 481 from nuLSU). The final topology (Fig. 2.2) was consistent with previous works (Nelsen et al. 2014; Lücking et al. 2016).

Our analysis showed that *P. sexlocularis* belongs in the Trypetheliaceae, in the same basal part of the family as the recently included species of *Bogoriella, Constrictolumina*, *Julella* and *Novomicrothelia*. *Polypyrenula* and *Alloarthopyrenia italica* formed a sister loosely supported clade, nonetheless, we consider their relation as tentative given that they were united by a long branch and showed conflict among loci. Using only nuLSU data *Polypyrenula* was instead closer to *Nigrovothelium* but without support.

Discussion

An excellent description of *Polypyrenula sexlocularis* (as *Polythelis sexlocularis*) was provided by Hawksworth (1983), but unfortunately the sample was so damaged that he could not study in detail the hamathecium. He suggested that the delicate tapering of the interascal filaments was very similar to the true paraphyses of *Pyrenula* and placed the genus in Pyrenulaceae. Based on Hawksworth’s description Harris (1989) and Aptroot (1991) thought that the hamathecium was better described as cellular pseudoparaphyses and moved the genus to Requienellaceae. Finally, Harris (1995) decided to restrict Requienellaceae to *Requienella*, which is currently in Sordariomycetes: Xylariales (Jaklitsch et al. 2016), and moved *Polypyrenula* back to the Pyrenulaceae.

Our observations showed that the hamathecium of the new collections of *P. sexlocularis* is more consistent with traberculate pseudoparaphyses (thin interascal filaments that are anastomosed, branched and without visible septation at 400×) which supports its inclusion in Trypetheliaceae. Even though is no longer wise to cut the type collection to compare, the description by Müller Argoviensis (1888, as *Microthelia sexlocularis*) states that the interascal filaments were anastomosed ("*connexæ*").

We have no doubt that the new specimens belong to *Polypyrenula sexlocularis* and that the type was collected in a community similar to the new specimens. The unique ascospores are diagnostic. Furthermore, Fée's type collection of *V. cascarilla* was a mix of several species in the genera *Constrictolumina*, *Anisomeridium*, *Porina*, *Parapyrenis, Pseudopyrenula*, and *Polypyrenula* (Müll. Arg., 1888), which, with the exception of *Parapyrenis*, are part of the core community of lichens surrounding the new collections of *P. sexlocularis* in Mexico.

In our phylogenetic analysis *P. sexlocularis* is not part of what was historically known as Trypetheliaceae, instead it is positioned at the base of the family. Interestingly, *P. sexlocularis* shares with these basal lineages an ecorticate thallus, exposed black perithecia, euseptate spores (in part) that are not astrothelioid, and often a weakly to non-lichenized thalli (Nelsen et al. 2014; Aptroot & Lücking 2016; Hyde et al. 2016; Lücking et al. 2016). Nonetheless, *Polypyrenula* is the only genus in the family with pronounced eusepta in combination with distosepta.

Currently, the family Trypetheliaceae has 17 recognized genera (Hyde et al. 2016; Lücking et al. 2016; this paper), more than 400 species and c. 800 predicted species, making it the second largest family of tropical corticolous lichens (Aptroot et al. 2016). In particular, the basal lineages in the family are poorly understood and have few DNA sequences available, which partly explain the lack of support in the base of the phylogeny. For instance, more species of *Constrictolumina* are needed to establish the monophyly of the genus, that consists of at least two unresolved groups: the species

closer to *Constrictolumina cinchonae* and the ones closer to *Constrictolumina malaccitula* (previously known as *Arthopyrenia bifera*).

Our current analysis was not able to replicate the results of Ertz et al. (2015) that proposed the lichenicolous family Polycoccaceae as sister to Trypetheliaceae in the order Trypetheliales. Instead, the sequences of Polycoccaceae clustered as a group inside Trypetheliaceae. We excluded those sequences in this paper as the result was sensitive to the number of species of Trypetheliaceae included in the analysis and showed incongruence when comparing single locus vs. combined loci analyses. The only combination that resulted in a separation of both families was when we included few species of Trypetheliaceae and only nuLSU sequences. Given that only nuLSU sequences are available for members of Polycoccaceae, it is possible that a future analysis with more loci and better representation of both the Polycoccaceae and the basal lineages of Trypetheliaceae would show them as separate families.

For this paper we decided to include in our analyses the ITS sequences of *P. sexlocularis,* regardless of the infrequent use of the ITS region in this family. Even though the ITS is the official genetic barcode of fungi (Schoch et al. 2012), the number of sequences for pyrenocarpous lichens is extremely under-represented. The use of ITS in broad phylogenies may represent a problem because its high variability makes unambiguous alignments difficult, which might be the reason why recent works in Dothideomycetes did not include this gene (Hyde, 2013; Nelsen et al. 2009, 2011, 2014; Wijayawardene et al. 2014). Regardless of this, the ITS region appears to be quite informative, and a database with ITS sequences would allow the inclusion of these species in floristic, environmental and ecological studies as well. We strongly encourage other researchers to include the ITS gene in their studies of pyrenolichens.

With the recent collections, *P. sexlocularis* should no longer be considered extinct. Most of the samples in Mexico were found in disturbed forests, suggesting that this particular species might be able to adapt to the current conditions of tropical dry forest: small relicts of pristine areas surrounded by a majority of secondary forests (Quesada et

al. 2009). Contrary to the ecosystem as a whole (Janzen, 1988; Portillo-Quintero & Sánchez-Azofeifa, 2010), this lichen may not be particularly endangered. *Polypyrenula sexlocularis* should be considered a rare species, but given that it was found in Mexico, West Indies and Bolivia it is expected to occur throughout the Neotropics in forested ecosystems with a dry season.

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Table 2.1 GenBank accession numbers of new sequences generated in this study. All samples from Mexico.

Species name	Voucher	DNA number	ITS	mtSSU	nuLSU
Polypyrenula sexlocularis	*Miranda 1791*	RMG057	GB	GB	GB
P. sexlocularis	*Miranda 3886*	RMG058	GB	GB	GB

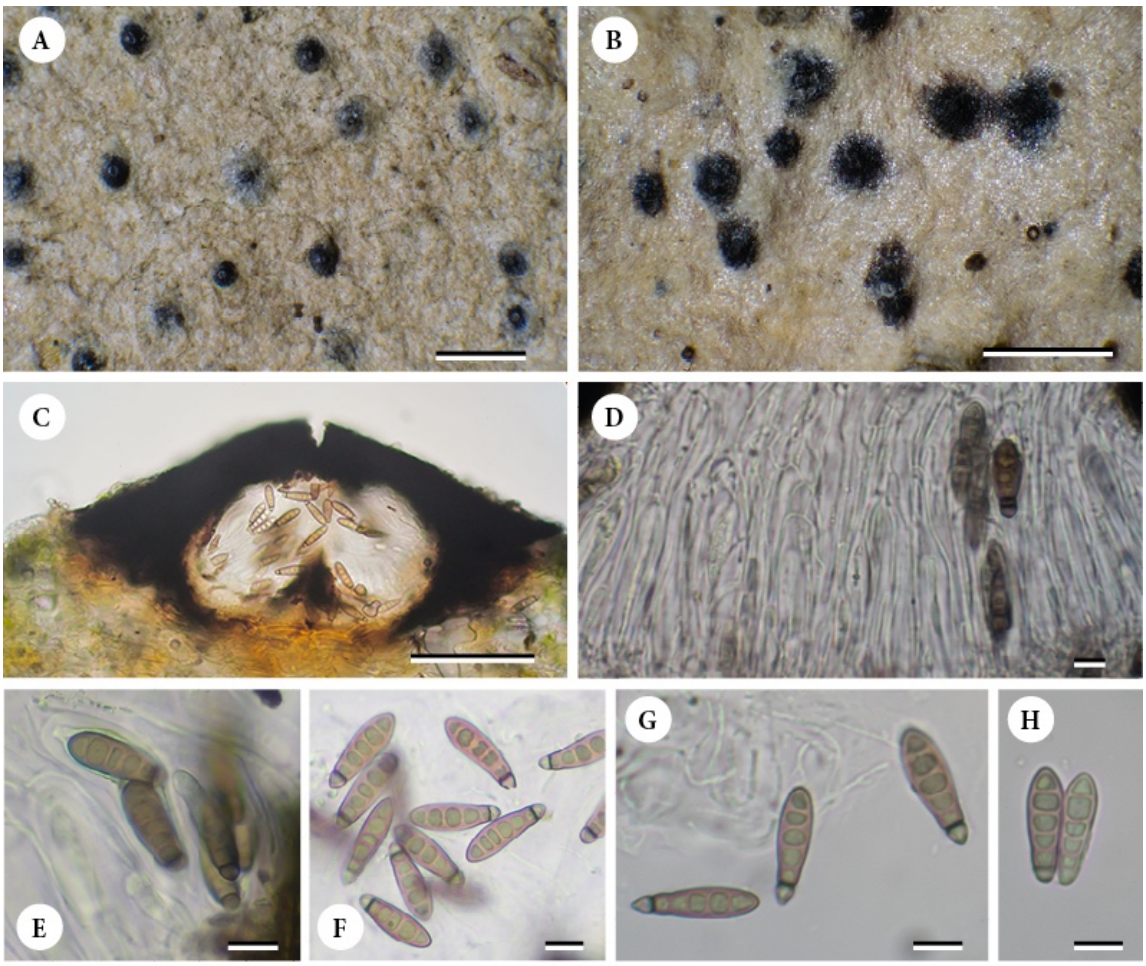

Figure 2.1 *Polypyrenula sexlocularis*. A, B) thallus. C) section of perithecia showing Trentepohlioid algae. D) hamathecium showing anastomosed pseudoparaphyses and spore with two eusepta. E) ascus. F, G) spores. H) spore with reduced euseptum. Scale bars: A, B = 1 mm; C = 100 µm; D–H = 10 µm. Collection numbers: A, C, E, F, G *Miranda 2736*; B, D *Miranda 1791*; H *Miranda 2539*.

Figure 2.2 Phylogeny of the family Trypetheliaceae based on a Bayesian analysis of the genes mtSSU and nuLSU. Support values are shown as numbers if Maximum Likelihood bootstrap values ≥ 75 and as bold branches if Bayesian posterior probabilities ≥ 0.95. Bold names and arrow show the position of *Polypyrenula sexlocularis*.

Appendix 2.1 GenBank accession numbers of all sequences used in this study.

Taxon	Country	Voucher	DNA number	nuLSU	mtSSU	Group
Alloarthopyrenia italica Phukhams., Camporesi, Ariyaw. & K.D. Hyde	Italy	E. Camporesi IT122 (MFLU)	MFLU 15-0399	KX655550	KX655555	ingroup
Aptrootia elatior (Stirt.) Aptroot	New Zealand	Knight O61815 (OTA)	MPN560B	KM453754	KM453821	ingroup
Aptrootia robusta (P.M.McCarthy & Kantvilas) Aptroot	Australia	Lumbsch 20012 (F)	MPN235B	KM453755	KM453822	ingroup
Aptrootia terricola (Aptroot) Lücking, Umaña & Chaves	Costa Rica	Lücking 17211 (F)	HTL1501	KM453756	DQ328995	ingroup
Architrypethelium nitens (Fée) Aptroot	Panama	Lücking 27038 (F)	MPN257	KM453757	KM453823	ingroup
Architrypethelium uberinum (Fée) Aptroot	Brazil	Nelsen s.n. (F)	MPN489	KM453758	-	ingroup
Astrothelium aff. *crassum* (Fée) Aptroot	Brazil	Cáceres 6011 (F)	MPN335	KM453761	KM453827	ingroup
Astrothelium aff. *megaspermum* (Mont.) Aptroot & Lücking	Philippines	Rivas Plata 2093 (F)	MPN190	KM453787	KM453852	ingroup
Astrothelium aff. *sepultum* Mont.	Peru	Nelsen 4001a (F)	MPN63C	GU327714	GU327690	ingroup
Astrothelium carassense Lücking, M.P. Nelsen & Marcelli	Brazil	Lücking 31004 (F)	MPN438	KM453784	KM453849	ingroup
Astrothelium cinereorosellum (Kremp.) Aptroot & Lücking	Philippines	Rivas Plata 2110 (F)	MPN191	KM453809	KM453873	ingroup
Astrothelium cinnamomeum (Eschw.) Müll. Arg.	Costa Rica	Lücking 15322b (DUKE)	AFTOL110	AY584652	AY584632	ingroup
Astrothelium crassum (Fée) Aptroot	Peru	Nelsen s.n. (F)	MPN98	GU327710	GU327685	ingroup
Astrothelium degenerans (Vain.) Aptroot & Lücking	Ecuador	Rivas Plata 4065 (F)	MPN397	KM453773	KM453838	ingroup
Astrothelium degenerans (Vain.) Aptroot & Lücking	Panama	Lücking 27109 (F)	MPN267	KM453770	KM453835	ingroup
Astrothelium diplocarpum Nyl.	Nicaragua	Lücking 28529 (F)	MPN210	KM453781	KM453846	ingroup
Astrothelium endochryseum (Vain.) Aptroot & Lücking	Brazil	Lücking 31088 (F)	MPN436	KM453772	KM453837	ingroup
Astrothelium floridanum Zahlbr. ex M. Choisy	Panama	Lücking 27131a (F)	MPN304	KM453811	KM453876	ingroup
Astrothelium gigantosporum (Müll. Arg.) Aptroot & Lücking	Panama	Lücking 33037 (F)	MPN590	KM453786	KM453851	ingroup
Astrothelium grossum Müll. Arg.	Panama	Lücking 27045 (F)	MPN259	KM453769	KM453834	ingroup
Astrothelium grossum Müll. Arg.	Brazil	Cáceres & Aptroot 11137 (F)	MPN703	KM453765	-	ingroup
Astrothelium grossum Müll. Arg.	Fiji	Lumbsch 20556h (F)	MPN770	KM453766	KM453831	ingroup
Astrothelium laevithallinum Lücking, M.P. Nelsen & Marcelli	Brazil	Lücking 31061 (F, SP)	MPN442	KM453771	KM453836	ingroup
Astrothelium leucoconicum Nyl.	Peru	Nelsen 4000c (F)	MPN42	KM453764	KM453830	ingroup
Astrothelium leucosessile Lücking, M.P. Nelsen & Aptroot	Panama	Lücking 27059 (F)	MPN258	KM453762	KM453828	ingroup
Astrothelium leucosessile Lücking, M.P. Nelsen & Aptroot	Brazil	Cáceres & Aptroot 11201 (F)	MPN713	KM453805	KM453869	ingroup
Astrothelium macrocarpum (Fée) Aptroot & Lücking	Panama	Lücking 27077 (F)	MPN260	KM453763	KM453829	ingroup
Astrothelium meristosporum (Mont. & Bosch) Aptroot & Lücking	Philippines	Rivas Plata 2108 (F)	MPN189	KM453785	KM453850	ingroup
Astrothelium neogalbineum (R. C. Harris) Aptroot & Lücking	Brazil	Cáceres & Aptroot 11100 (F)	MPN711	KM453812	KM453877	ingroup
Astrothelium neoinspersum Aptroot	Peru	Nelsen s.n. (F)	MPN62	KM453802	KM453866	ingroup
Astrothelium nitidiusculum (Nyl.) Aptroot & Lücking	Brazil	Cáceres & Aptroot 11297 (F)	MPN704	KM453804	KM453868	ingroup
Astrothelium norisianum Lücking, M.P. Nelsen & Aptroot	Peru	Nelsen 4000d (F)	MPN52C	KM453783	KM453848	ingroup
Astrothelium obtectum Lücking, M.P. Nelsen & Benatti	Brazil	Lücking 31242 (F)	MPN422	KM453767	KM453832	ingroup
Astrothelium pulcherrimum (Fée) Aptroot & Lücking	Panama	Lücking 27046 (F)	MPN313	KM453814	KM453879	ingroup
Astrothelium pupula (Ach.) Aptroot & Lücking	Colombia	Lücking 26305 (F)	MPN224	KM453815	KM453880	ingroup
Astrothelium purpurascens (Müll. Arg.) Aptroot & Lücking	Peru	Nelsen s.n. (F)	MPN53C	KM453782	KM453847	ingroup
Astrothelium robustum Müll. Arg.	Costa Rica	Mercado-Díaz 586 (F)	MPN754	KM453760	KM453826	ingroup
Astrothelium sanguinarium (Malme) Aptroot & Lücking	Brazil	Cañez 3133 (CGMS, F)	MPN765	KM453788	KM453853	ingroup
Astrothelium scorizum (Müll. Arg.) Aptroot & Lücking	Brazil	Lücking 29814 (F)	MPN336	KM453808	KM453872	ingroup
Astrothelium subcatervarium (Malme) Aptroot & Lücking	Peru	Nelsen 4009a (F)	MPN97	GU327729	GU327707	ingroup
Astrothelium subscoria Flakus & Aptroot	Nicaragua	Lücking 28640 (F)	MPN217	KM453813	KM453878	ingroup
Astrothelium variolosum (Ach.) Müll. Arg.	Peru	Nelsen s.n. (F)	MPN43	KM453768	KM453833	ingroup
Bathelium lineare (C.W.Dodge) R.C.Harris	Vietnam	Gueidan 2078 (F)	MPN741	KM453774	KM453839	ingroup
Bathelium madreporiforme (Eschw.) Trevis.	Brazil	Lücking 23290 (F)	MPN354	KM453775	KM453840	ingroup
Bathelium porinosporum Lücking, M.P. Nelsen & Gueidan	Vietnam	Gueidan 3040 (F)	MPN743	KM453776	KM453841	ingroup
Bathelium tuberculosum (Makhija & Patw.) R.C.Harris	India	Lumbsch 19739z (F)	MPN81	KM453777	KM453842	ingroup
Bogoriella hemisphaerica (Müll. Arg.) Aptroot & Lücking	Nicaragua	Lücking 28641 (F)	MPN102	GU327719	GU327695	ingroup
Bogoriella miculiformis (Nyl. ex Müll. Arg.) Aptroot & Lücking	Nicaragua	Lücking 28637 (F)	MPN101B	GU327720	GU327696	ingroup
Bogoriella minutula (Zahlbr.) Aptroot & Lücking	Thailand	Nelsen s.n. (F)	MPN567	-	KM453856	ingroup
Constrictolumina cinchonae (Ach.) Lücking, M.P. Nelsen & Aptroot	Brazil	Lücking 29583 (F)	MPN333	JN872351	JN872349	ingroup
Constrictolumina cinchonae (Ach.) Lücking, M.P. Nelsen & Aptroot	Brazil	Lücking s.n. (F)	MPN417	KM453759	KM453825	ingroup
Constrictolumina malaccitula (Nyl.) Lücking, M.P. Nelsen & Aptroot	Thailand	Nelsen s.n. (F)	MPN574	-	KM453824	ingroup
Constrictolumina planorbis (Ach.) Lücking, M.P. Nelsen & Aptroot	Brazil	Lücking 29584 (F)	MPN334	JN872352	JN872350	ingroup
Dictyomeridium proponens (Nyl.) Aptroot, M.P. Nelsen & Lücking	Venezuela	Lücking 26103 (F)	MPN359	JN887403	KM453860	ingroup
Julella fallaciosa (Stizenb. ex Arnold) R.C.Harris	U.S.A.	Nelsen s.n. (F)	MPN547	JN887400	JN887412	ingroup
Marcelaria cumingii (Mont.) Aptroot, Nelsen & Parnmen	Thailand	Parnmen s.n. (F)	MPN552	KM453789	KM453854	ingroup
Marcelaria purpurina (Nyl.) Aptroot, Nelsen & Parnmen	Brazil	Cáceres 2009	MPN323A	KM453790	KM453855	ingroup
Nigrovothelium tropicum (Ach.) Lücking, M.P. Nelsen & Aptroot	U.S.A.	Nelsen s.n. (F)	MPN130	KM453819	KM453883	ingroup
Novomicrothelia oleosa (Aptroot) Aptroot, M.P. Nelsen & Lücking	Brazil	Cáceres & Aptroot 11821 (F)	MPN700	KM453794	KM453857	ingroup
Novomicrothelia oleosa (Aptroot) Aptroot, M.P. Nelsen & Lücking	Peru	Nelsen 4007a (F)	MPN95	GU327721	GU327697	ingroup
Polymeridium albocinereum (Kremp.) R.C.Harris	Brazil	Lücking s.n. (F)	MPN439	KM453795	KM453858	ingroup
Polymeridium catapastum (Nyl.) R.C.Harris	Venezuela	Lücking 26052 (F)	MPN358	JN887402	KM453859	ingroup
Polypyrenula sexlocularis (Müll. Arg.) D. Hawksw.	Mexico	Miranda 1791 (MEXU)	RMG57	GB	GB	ingroup
Polypyrenula sexlocularis (Müll. Arg.) D. Hawksw.	Mexico	Miranda 3886 (MEXU)	RMG58	GB	GB	ingroup
Pseudopyrenula diluta (Fée) Müll. Arg.	Venezuela	Lücking 26062 (F)	MPN362	KM453797	KM453861	ingroup
Pseudopyrenula diluta (Fée) Müll. Arg.	Brazil	Lücking 31068 (F)	MPN697	KM453798	KM453862	ingroup
Pseudopyrenula endoxanthoides Vain.	Thailand	Lücking 24079 (F)	MPN106	GU327724	GU327699	ingroup
Pseudopyrenula subgregaria Müll. Arg.	U.S.A.	Nelsen 4082b (F)	MPN391	KM453799	KM453863	ingroup
Pseudopyrenula subgregaria Müll. Arg.	Panama	Lücking 27053 (F)	MPN292	KM453800	KM453864	ingroup
Pseudopyrenula subnudata Müll. Arg.	Panama	Lücking 27014r1 (F)	MPN293	KM453801	KM453865	ingroup

Appendix 2.1 (Continued).

Taxon	Country	Voucher	DNA number	nuLSU	mtSSU	Group
Trypethelium eluteriae Spreng.	India	Lumbsch 19701a (F)	MPN111	GU327726	KM453874	ingroup
Trypethelium foveolatum Müll. Arg.	Argentina	Lücking 30515 (F)	MPN351	KM453816	KM453881	ingroup
Trypethelium inamoenum Müll. Arg.	Thailand	Lücking 24125 (F)	MPN228	KM453810	KM453875	ingroup
Trypethelium platyleucostomum Makhija & Patw.	Argentina	Lücking 30512 (F)	MPN349	KM453806	KM453870	ingroup
Trypethelium rubroplatystomum ined.	Peru	Nelsen s.n. (F)	MPN54	KM453807	KM453871	ingroup
Trypethelium sprengelii Ach	U.S.A.	Nelsen 4169 (F)	MPN382	KM453803	KM453867	ingroup
Trypethelium subeluteriae Makhija & Patw.	Peru	Nelsen s.n. (F)	MPN49C	KM453818	KM453882	ingroup
Viridothelium tricolor Lücking, M.P. Nelsen & N. Salazar	Panama	Lücking 27125 (F)	MPN268	KM453778	KM453843	ingroup
Viridothelium tricolor Lücking, M.P. Nelsen & N. Salazar	Venezuela	Lücking 32241 (F)	MPN399	KM453779	KM453844	ingroup
Viridothelium tricolor Lücking, M.P. Nelsen & N. Salazar	Panama	Nelsen s.n. (F)	MPN646	KM453780	KM453845	ingroup
Viridothelium virens (Tuck. ex Michener) Lücking et al.	U.S.A.	Nelsen s.n. (F)	MPN497	KM453820	KM453884	ingroup
Viridothelium vonkonratii Lücking, Naksuwankul & Lumbsch	Fiji	Lumbsch 20551a (F)	MPN764	KM453817	-	ingroup
Cladosporium cladosporioides (Fresen.) G.A.de Vries				DQ678057	FJ190628	outgroup
Dothiora cannabinae Froid.				DQ470984	FJ190636	outgroup
Hortaea werneckii (Horta) Nishim. & Miyaji				GU301818	GU561844	outgroup
Macrophomina phaseolina (Tassi) Goid.				DQ678088	FJ190645	outgroup
Mycosphaerella punctiformis (Pers.) Starbäck				DQ470968	FJ190611	outgroup
Myriangium duriaei Mont. & Berk.				DQ678059	AY571389	outgroup

CHAPTER 3. THE NEW GENUS *JOCATOA* (GRAPHIDACEAE) AND NEW INSIGHTS INTO SUBFAMILY REDONOGRAPHOIDEAE

Ricardo Miranda-González, Robert Lücking, Alejandrina Barcenas-Peña & María de los Angeles Herrera-Campos

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Abstract

One new genus and two new species of Graphidaceae are described from the tropical dry forests of Mexico, based on morphological and molecular data of the mtSSU, nuLSU and ITS regions. The new genus *Jocatoa* in the subfamily Graphidoideae is described to accommodate the species *Medusulina texana*. The new genus resembles *Diorygma* but differs by having simple paraphyses tips that do not form an epithecium. The new combination *Jocatoa texana* is similar to *Diorygma monophorum* but differs by having larger ascospores, hypostictic and stictic acids and by the type of paraphyses tips. In the subfamily Redonographoideae, the two new species *Gymnographopsis corticola* and *Redonographa parvispora* are described, together being the only corticolous species in the subfamily. *Gymnographopsis corticola* sp. nov. is characterized by the smallest spores in the genus, the presence of norstictic acid, and a rectangular perispore that appears to be a newly recognized character state in fungi. *Redonographa parvispora* sp. nov. is characterized by warty periphysoids, small spores with 3 transverse septa, and norstictic acid. It also frequently develops a rectangular perispore. We present a phylogenetic analysis including all the genera in the family Graphidaceae, with available sequences, to accommodate the new genus and to validate for the first time the position of *Gymnographopsis*. Diagnostic anatomical and ecological characters are discussed for Redonographoideae. *Gymnographopsis* is newly reported for the Northern Hemisphere.
Keywords: Tropical Dry Forest, Mexico, rectangular perispore.

Introduction

Graphidaceae is the second largest family of lichenized fungi, after Parmeliaceae (Lücking et al. 2017). The family contains only crustose lichens, is predominantly subtropical and tropical, and associates with trentepohlioid or very rarely trebouxioid algae (Kraichak et al. 2015). Staiger (2002), Frisch et al. (2006), and Mangold et al. (2008) laid the bases for the current classification of the family and triggered several new studies. Recent molecular work showed that Graphidaceae includes three core subfamilies: Fissurinoideae, Graphidoideae, and Redonographoideae (Mangold et al.

2008; Rivas Plata et al. 2013; Lücking et al. 2013; Lumbsch et al. 2014a); a fourth subfamily, Gomphillaceae (Rivas Plata et al. 2012) has been shown to be sister to Graphidaceae (Jaklitsch et al. 2016; Lücking & Lumbsch, in prep.). Of the three subfamilies, Graphidoideae is further divided in seven tribes that contain most of the species of Graphidaceae and the previously separated Thelotremataceae (Rivas Plata et al. 2012; Lumbsch et al. 2014a).

Presently, Graphidaceae includes c. 2100 known species in 79 genera (Lücking et al. 2017), and it is expected to have another 1500 undescribed species (Lücking et al. 2014). The relations within the family are relatively well known and only nine of the accepted genera have not yet been sequenced (*Amazonotrema*, *Anomalographis*, *Anomomorpha*, *Byssotrema*, *Diaphorographis*, *Gymnographopsis*, *Kalbographa*, *Polistroma*, and *Thecographa*; nomenclature follows Lücking et al. 2017, except as noted). Nonetheless, the position of many small genera like *Aggregatorygma* or *Schistophoron* are not resolved with support, some of the larger genera like *Acanthothecis*, *Fissurina,* and *Phaeographis*, are not monophyletic, and some species like *Medusulina texana* are of unknown generic affinity (Rivas Plata et al. 2013; Lumbsch et al. 2014a; Lücking et al. 2017b).

Lücking et al. (2014) predicted that just the tropical part of Mexico would have 429 species of Graphidaceae. Herrera-Campos et al. (2014) counted 175 species registered for the whole country, including both published and unpublished data. Mexico is currently considered to be one of the world hotspots for undescribed species of Graphidaceae, with recent papers (Barcenas-Peña et al. 2014, 2015) marking the start of modern studies in this group in Mexico.

In this paper we describe a new genus in the subfamily Graphidoideae and two new species in the subfamily Redonographoideae from the tropical dry forests of Mexico. We present, for the first time, sequences of the genus *Gymnographopsis*. To accommodate the new genus and to test the inclusion of *Gymnographopsis* in the subfamily Redonographoideae, we present a three-gene phylogenetic reconstruction of

Graphidaceae that includes representatives of all the genera in the family with available sequences.

Materials and Methods

Study area

All new taxa and new sequences were obtained from samples collected in or around the Chamela-Cuixmala Biosphere Reserve near the Pacific Coast of Mexico. All samples were found in the tropical dry forest, an ecosystem characterized by a warm sub-humid climate with summer rains (Garcia, 2004), and a dry season of about six continuous months in which more than 95% of the plant individuals lose their leaves completely. The remaining months of the year are marked by a fast greening of the canopy which is the product of few scattered rains intercalated with dry periods. The area has a strong oceanic influence that maintains mean monthly values of relative humidity above 75% all year around, with mean annual temperature of 24.6°C and mean annual precipitation of 788 mm (Garcia-Oliva et al. 2002; Maass et al. 2002). Lichen communities cover most of the bark of most trees and are mostly represented by crustose groups in the families Arthoniaceae, Graphidaceae and Pyrenulaceae, while species of macrolichens are few, rare, and usually limited to the canopy (Miranda-González 2012).

Anatomical studies

Specimens were studied using standard techniques in an Olympus SZ61 dissecting microscope and an Olympus BX41 compound microscope, both connected to a NIKON D5300 digital camera. Sections were mounted in tap water. KOH and IKI reagents were used at 10% and 0.3% respectively following Bungartz (2002). All anatomical measurements were made in tap water. Morphological characters of lirellae follow terminology in Lücking (2009). Thin layer chromatography (TLC) was performed with solvents A and C using the standard techniques in Culberson & Johnson (1982) and Orange et al. (2010).

Taxon sampling

The phylogenetic analysis was based on data from Lumbsch et al. (2014a) and supplemented with sequences from Kalb et al. (2004), Staiger et al. (2006), Rivas Plata et al. (2013), Kraichak et al. (2013), Lücking et al. (2013), Lumbsch et al. (2014b), and new sequences generated in this study. We included a total 273 sequences of mtSSU (122), nuLSU (99) and RPB2 (52) for 122 ingroup species, with representatives from all the genera of Graphidaceae currently published in GenBank, as well as all the available species of *Diorygma* (Appendix 3.1).

DNA extraction, PCR, and sequencing

One or two lirellae per sample were detached and washed in acetone for five minutes. Total DNA was isolated using the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) following the manufacturer's instructions, except only 15 µL of extraction buffer and 15 µL of dilution buffer were used per sample. The whole ITS and portions of mtSSU and nuLSU were amplified and sequenced using the following primers: ITS1F/ITS4 (Gardes & Bruns 1993; White et al. 1990), mrSSU1/mrSSU3R (Zoller et al. 1999), and AL2R/LR6 (Mangold et al. 2008; Vilgalys and Hester, 1990) respectively. If samples were old or the PCR was problematic the following primer combinations were used: ITS1F/ITS86R for ITS1, ITS86F/ITS4 for ITS2 (Gardes & Bruns 1993; Op De Beeck et al. 2014; Turenne et al. 1999; White et al. 1990), and mrSSU1/mrSSU2R for mtSSU (Zoller et al. 1999).

Each 10 µL PCR reaction consisted of 5 µL R4775 Sigma-Aldrich REDExtract-n-Amp PCR Ready Mix, 0.5 µL of each primer (10 µM), 3 µL water, and 1 µL undiluted DNA. The PCR cycling conditions for ITS were: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 52 °C for 45 s, and 72 °C for 105 s, followed by 72 °C for 5 min. The PCR cycling conditions for mtSSU and nuLSU were: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C (for mtSSU) or 57 °C (for nuLSU) for 1 min, and 72 °C for 105 s, followed by 72 °C for 10 min. 2 µL of each PCR products were visualized on 1.5% TBA agarose gel stained with GelRed (Biotium). Single bands were cleaned directly from PCR products with ExoSAP-IT ® for PCR product cleanup (Affymetrix,

Santa Clara, CA, USA). If double bands appeared the rest of the PCR product was gel-extracted and cleaned with GELase (Epicentre Biotechnologies, Madison, Wisconsin, U.S.A.) following manufacturer's instructions.

Samples were sent to be sequenced at Eurofins MWG Operon LLC (Louisville, KY). Each 12 µL reaction consisted of 2.4 µL primer (at 10 µM), 2 µL undiluted PCR product cleaned with ExoSAP and 7.6 µL water or 2.4 µL primer (at 10 µM) and 9.6 µL DNA cleaned with GELase.

Phylogenetic analysis

New sequences were edited in Geneious v.8.1.9 (Kearse et al. 2012). All sequences of mtSSU, nuLSU, and RPB2 were aligned independently using the GUIDANCE2 server (Sela et al. 2015) with the multiple sequence alignment algorithm MAFFT (Katoh et al. 2005). Unreliable columns were removed using a cutoff of 0.6. Introns were visually identified and removed. A maximum likelihood (ML) analysis of all genes partitioned by locus was performed using the RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014), with 450 bootstrapping replicates as automatically determined by RAxML using a saturation criterion. Furthermore, a Bayesian analysis was performed using MrBayes v.3.2.6 (Huelsenbeck & Ronquist 2001), with two independent runs of three million generations each, resampling every 1000 trees, 25% burn-in, and heated chains of 0.2. Both analyses were done with the GTR GAMMA model and run on the Cipres Gateway server (Miller et al. 2010). Single locus analyses were also performed to visually test for topological incongruence. The final ML tree was plotted using FigTree v1.4 (Rambaut & Drummond 2012) and edited in Photoshop CS6.

Results

Phylogenetic analysis

A total of 23 new sequences were generated for this study (Table 3.1). The combined data set consisted of 124 OTU's and 2864 unambiguously aligned characters, 939 from mtSSU, 982 from nuLSU and 943 for RPB2, of which 789, 812, and 673 respectively

were phylogenetically informative. The combined Maximum Likelihood and Bayesian analysis (Fig. 3.1, Appendix 3.2) recovered the three subfamilies and 7 tribes as supported branches presented in Lumbsch et al. (2014a), with the exception of the tribe Graphideae that did not reach support values. The new sequences of *Gymnographopsis* were positioned as sister to *Redonographa* in the subfamily Redonographoideae, which was anticipated by Lücking et al. (2013), although the type species has not yet been sequenced. The new genus and combination *Jocatoa texana* clustered with high support as the sister group to *Schistophoron* and unrelated to the monophyletic *Diorygma* clade. Sequences of the ITS gene were obtained for all new specimens; unfortunately, we could not include them in the analysis because sequences of Graphidaceae for this locus are extremely underrepresented in GenBank. The ITS is the official genetic barcode of fungi (Schoch et al. 2012) and is useful for phylogenetic, environmental, floristic and ecological studies, therefore we encourage other researchers to include it in their studies.

Taxonomy

Gymnographopsis corticola R. Miranda, Herrera-Campos & Lücking sp. nov. Fig. 3.2

MycoBank MB XXXXXX

Differing from other species of Gymnographopsis in being corticolous, having smaller ascospores (15–18 × 3–5 µm), and containing norstictic acid.

Type: Mexico. Jalisco, La Huerta, Chamela-Cuixmala Biosphere Reserve, Estación de Biología Chamela, pristine tropical deciduous dry forest, 19°29'53”N, 105°2'33”W, 107 m, between trails Chachalaca and Camino Antiguo del Sur, on bark of *Loncharpus* sp., June 2009, *Miranda 1158* (MEXU–holotype).

Thallus crustose, corticolous, epiperidermal, continuous to rimose, ecorticate, whitish green to pale grey, with a black prothallus present at contact lines with other lichens. *Photobiont* trentepohlioid, in a continuous layer surrounded by small crystals POL+ beige to salmon that dissolve in KOH. *Ascocarps* abundant, lirelliform, immersed to

erumpent, straight to curved, rarely branched, 0.2–1.2 × 0.1–0.2 mm; thalline margin raised above disc, complete to lateral, whitish grey; disc initially concealed, but in mature lirellae exposed and open (Fig. 3.2B), black to light brown. *Exciple* not striate, laterally light brownish, apically brownish to black and sometimes appearing carbonized, POL-, 10–30 µm wide, in young ascocarps forming an open roof on top of the hymenium (Fig. 3.2F) that later recedes as the ascocarps mature; hymenium hyaline, not inspersed with oil droplets, embedded in a gelatinous matrix, sparsely anastomosed, 45–75 µm high, I-; periphysoids short, smooth to slightly verrucose, originating in the inner exciple from about the upper third of the hymenium to the inner tip of the exciple, embedded in a gelatinous matrix, 7.5–16 µm long; epihymenium not differentiated; epithecium absent; hypothecium hyaline yellowish, 12–24 µm deep. Ascospores 8 per ascus, hyaline, mostly with 3 transverse septa but some with up to 5 septa, very rarely with 1 longitudinal septum, oblong, 12.5–18 × 3–5 µm, I-, frequently with a rectangular gelatinous perispore of up to 3(5) µm thick.

Chemistry. Norstictic (major) and connorstictic (minor) acids.

Etymology. The epithet refers to the substrate as it is so far the first species in the subfamily Redonographoideae known to grow on bark.

Ecology and distribution. *Gymnographopsis corticola* has only been found in the mature tropical deciduous dry forests of the Chamela-Cuixmala Biosphere Reserve. It is a frequent species in the study area, generally associated with the main trunk of trees of the genus *Lonchocarpus* and less frequently with *Cordia alliodora*, *Erythrina lanata*, *Forchhammeria pallida*, and *Heliocarpus pallidus*.

Remarks. *Gymnographopsis corticola* is superficially similar to species of the genera *Diorygma* and *Thalloloma*, but these genera have I+ violet spores and lack periphysoids. The closely related genus *Redonographa* differs by its complete to laterally carbonized exciple and by the nature of the periphysoids, which grow from the inside of the excipulum in *Gymnographopsis* and from the outside of the excipulum in *Redonographa*.

Only two other species are known in the genus *Gymnographopsis*: *G. chilena* Dodge (1966) and *G. latispora* Egea & Torrente (1996), both from the Southern Hemisphere, saxicolous, with much larger ascospores, longer lirellae, and without norstictic acid. The new species is therefore tentatively assigned to the genus *Gymnographopsis*, since the differences with the other two species, which have not been sequenced, suggest that an unrecognized genus might be involved.

Additional specimens examined. **Mexico**, Jalisco: La Huerta, Chamela-Cuixmala Biosphere Reserve, Estación de Biología Chamela, pristine tropical deciduous dry forest: 19°29'47"N, 105°2'24"W, 60 m, on trail Chachalaca, on bark of unknown tree, June 2008, *Lücking 25090*; 19°29'53"N, 105°2'33"W, 107 m, between trails Chachalaca and Camino Antiguo del Sur, on bark of *Erythrina lanata*, *Lonchocarpus* spp., and an unknown tree, June 2009, *Miranda 1105, 1121, 1680, 1681, 1685, 4365, 4366, 4367*; 19°30'55"N, 105°2'7"W, 73 m, near Hornitos stream, 250 NE of the end of Eje Central road, on bark of *Cordia alliodora*, *Forchhammeria pallida*, *Lonchocarpus* sp., June 2009, and an unknown tree, *Miranda 795, 1570, 1574, 1581, 4369*; 19°30'11"N, 105°2'50"W, 57 m, 300 m W of trail Tejón 600 m, on bark of *Bursera* cf. *exelsa*, April 2010, *Miranda 2103, 2122*; 19°30'34"N, 105°2'22"W, 98 m, near Tejón trail, on bark of unknown tree, September 4, 2011, *Barcenas Peña 4616, 4618*; 19°29'54"N, 105°2'34"W, 82 m, on trail Chachalaca, June 2014, *Miranda 4567;* 19°30'17''N, 105°2'49''W, 56 m, on trail Tejón 800 m, on bark of unknown tree, August 2014, *Miranda* 4729. (MEXU).

Redonographa parvispora R. Miranda, Barcenas-Peña & Lücking sp. nov. Fig. 3.3
MycoBank No.: XXXXXX

Similar to Redonographa galapagoensis but with smaller, 3-septate ascospores, longer lirellae, lateral to complete excipular carbonization, and corticolous habit.

Type: **Mexico**, Jalisco: La Huerta, Chamela-Cuixmala Biosphere Reserve, Estación de Biología Chamela, pristine tropical deciduous dry forest, 19°29'51''N, 105°2'30''W, 136

m, between trails Chachalaca and Camino Antiguo del Sur, on bark of *Piptadenia constricta*, June 2009, *Miranda* 1128 (MEXU–holotype).

Thallus crustose, corticolous, epiperidermal, rimose, ecorticate to weakly corticate, whitish grey, with a black prothallus present at contact lines with other lichens. *Photobiont* trentepohlioid, in a continuous layer surrounded by small crystals POL+ beige that dissolve in KOH, as well as insoluble coarse crystals. *Ascocarps* abundant, lirelliform, erumpent to prominent, mostly curved to sinuous, sparsely branched, 1–7 × 0.2–0.3 mm; thalline margin complete but thin above and giving the impression of pruinose discs, concolorous with the thallus; disc concealed, black. *Exciple* laterally to completely carbonized, POL-, 20–50 µm wide, not striate, covering most of the hymenium, with short and warty periphysoids originated from the carbonized exciple and specially abundant towards the outside of it; hymenium hyaline, not inspersed with oil droplets, embedded in a gelatinous matrix, 60–90 µm high, I-; paraphyses simple to anastomosed near the excipulum; epihymenium not differentiated; epithecium absent; hypothecium hyaline, 45–90 µm deep. *Ascospores* 8 per ascus, ellipsoid, hyaline, with 3 transverse septa, 10–15 × 2.5–4.5 µm, I-, frequently with a rectangular gelatinous perispore of up to 3 µm thick.

Chemistry. Norstictic (major) and connorstictic (minor) acids.

Etymology. The epithet refers to the ascospores, which are the smallest among the species of this genus with transverse septation.

Ecology and distribution. Redonographa parvispora has only been found in the mature tropical deciduous dry forests of the Chamela-Cuixmala Biosphere Reserve. It is a rare species in the study area. Most specimens were found on the main trunk of trees of *Piptadenia constricta*.

Remarks. The corticolous *R. parvispora* is characterized by having warty periphysoids, small and narrow spores with three transverse septa, and norstictic acid. The only other

species in the genus with warty to verrucose periphysoids is the saxicolous *Redonographa galapagoensis* Bungartz & Lücking, which has larger submuriform ascospores, and mostly rounded lirellae. The only species in the remarkably similar genus, *Carbacanthographis,* with norstictic acid as major secondary metabolite is *Carbacanthographis induta* (Müll. Arg.) Lücking, but this species has ascospores up to 70 µm long (Lücking et al. 2009). *Carbacanthographis marcescens* (Fée) Staiger & Kalb may have traces of norstictic acid but its major secondary metabolite is salazinic acid, and the species further differs in its muriform ascospores (Staiger 2002).

Redonographa parvispora shares the warty periphysoids and the corticolous habit with the genus *Carbacanthographis* in the Graphidoideae. As these two characters are traditionally used to distinguish between these genera, the new species would appear to belong in *Carbacanthographis*, however, we described it in *Redonographa* for two reasons: First, the available sequences of ITS and mtSSU support its position in Redonographoideae (Fig. 3.1), of the two genera available in the subfamily, *Redonographa* shares with the new species the excipular carbonization and the periphysoids type. Second, the rectangular perispore is similar to the one of *Gymnographopsis corticola* in the same subfamily, a character that to our knowledge has never been reported for fungi. Perispores are common in some genera of lichenized fungi, but typically their outline follows that of the spore wall.

Unfortunately, the samples of *R. parvispora* were difficult to sequence and only three sequences of ITS and one short sequence of mtSSU were successful. From the available sequences of *Redonographa* in GenBank there is none of ITS and only one of mtSSU from *R. chilensis*. This resulted in few phylogenetically informative characters and lack of molecular support for the inclusion of *R. parvispora* in *Redonographa*. To avoid unnecessary taxonomic changes, we refrained from describing a new genus and tentatively assigned the new species to *Redonographa*. A future analysis with more sequences will be needed to solve the problem.

A Brazilian collection referred as “KALB 28829 (systematic position unclear)” in Staiger & Kalb (1999) is remarkably similar to *R. parvispora*, sharing the lateral to completely carbonized exciple, warty and short periphysoids, ascospores size and septation pattern, and norstictic acid as major secondary metabolite. It only differs in the spore’s cell lumina, which are somehow interconnected in Kalb’s sample, and in the form of the perispore, which is a typical oval shape (Fig 3.4 in Staiger & Kalb 1999). Nonetheless, given their scarce material these differences may be just an artifact. As we did not examine Kalb’s collection, we are not sure if *R. parvispora* and KALB 28829 are conspecific. Therefore, we refrain from extending the distribution of *R. parvispora* to Brazil.

Additional specimens examined. **Mexico**, Jalisco: La Huerta, Chamela-Cuixmala Biosphere Reserve, Estación de Biología Chamela, pristine tropical deciduous dry forest: near Búho trail: 19°29'57.2 N, 105°2'14.2 W, 83 m, on bark of unknown tree, November 9, 2008, *Barcenas Peña 2006, 2007, 2008, 2011*; 19°29’51’’N, 105°2’30’’W, 136 m, between trails Chachalacas and Camino Antiguo del Sur, on bark of *Piptadenia constricta*, June 2009, *Miranda 1099, 1134, 1135*; 19°29’46’’N, 105°2’28’’W, 98 m, on trail Chachalacas, on bark of unknown tree, June 2014, *Miranda 4558*. (MEXU).

Worldwide key to the known species of subfamily Redonographoideae in Graphidaceae.

1a Exciple laterally to completely carbonized; periphysoids mostly forming from the top external area of the carbonized exciple and not embedded in a gelatinous matrix; norstictic acid present..... (*Redonographa*) 2

1b Exciple hyaline to brownish, sometimes apically brownish black and appearing carbonized; periphysoids mostly forming from the inner part of the exciple and embedded in a gelatinous matrix; norstictic acid present or not... (*Gymnographopsis*) 6

2a Periphysoids verrucose to warty; ascospores less than 6 µm wide 3

2b Periphysoids smooth; ascospores more than 7 µm wide 4

3a Ascospores submuriform, 5–6 transverse and 1–2 longitudinal septa, 15–20 × 4–5 µm; exciple completely carbonized; lirellae short to rounded; disc often partially open; Galapagos ..... *R. galapagoensis*

3b Ascospores with 3 transverse septa, 10–15 × 2.4–4.5 µm; exciple lateral to completely carbonized; lirellae elongated; disc concealed; western Mexico ..... *R. parvispora*

4a Ascospores with 3–7 transverse septa, 20–28 × 8–11 µm; lirellae unbranched to sparsely branched; exciple lateral to completely carbonized; Californian peninsula (USA, Mexico) and Galapagos ..... *R. saxorum*

4b Ascospores submuriform, 18–25 × 8–11 µm; lirellae frequently branched to stellate; exciple lateral or completely carbonized; Chile 5

5a Lirellae stellate-branched to pseudostromatic; exciple completely carbonized; ascospores 18–25 × 8–10 µm, 3–5 transverse and 0–2 longitudinal septa, with regular cell lumina (graphidoid) ..... *R. chilensis*

5b Lirellae irregularly branched and bent; exciple laterally carbonized; ascospores 18–22 × 9–11 µm, 4–5 transverse and 1–2 longitudinal septa, with irregular cell lumina (astrothelioid) ..... *R. saxiseda*

6a Ascospores 15–18 × 3–5 µm; lirellae to 1 mm long; norstictic acid present in thallus; corticolous habit; western Mexico ..... *G. corticola*

6b Ascospores larger than 20 × 8 µm; lirellae longer than 1 mm; norstictic acid lacking; saxicolous habit; Southern Hemisphere 7

7a Ascospores 20–26 × 10–12 µm; lirellae 2–3 mm long; with an unknown substance of the stictic acid complex; northern Chile ..... *G. chilena*

7b Ascospores 20–31(–37) × 12–16(–18) µm; lirellae 0.8–1.5 mm long; no substances detected by TLC; South Africa ..... *G. latispora*

Jocatoa R. Miranda gen. nov. Fig. 3.4

MycoBank MB XXXXXX

A new genus in the family Graphidaceae, subfamily Graphidoideae, tribe Graphideae, differing from Diorygma in that the paraphyses tips are simple, thin and do not form an

epithecium. Thallus ecorticate; ascocarps solitary to pseudostromatic; excipulum not carbonized; spores muriform, I+ strongly violet; chemistry of the stictic acid complex.

Type species: *Jocatoa texana* (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda.

Etymology: The genus is named in honor of the late Prof. José Castillo Tovar, for educating the current generation of Mexican mycologists and for introducing the first author to the study of lichens.

Remarks. The new monospecific genus strongly resembles species of *Diorygma* in the ecorticate thallus, spore type, chemistry, and in the laterally branched and anastomosed paraphyses that are embedded in a thick gelatinous matrix. Nonetheless, in *Diorygma* the paraphyses tips are reticulately branched, anastomosed and thickened, which form a clear epithecium (Kalb et al. 2004), while in the new genus the paraphyses tips are simple, thin and do not form an epithecium. The genus *Glyphis* differs by having a heavily carbonized exciple with dark brown paraphyses tips intermingled with brown granules.

The younger names of genera that are synonyms with *Diorygma* (Type *Diorygma hieroglyphicum (Pers.) Staiger & Kalb*) are: *Solenographa* (Type *Diorygma confluens* (Fée) Kalb, Staiger & Elix), *Glaucinaria* (Type *Diorygma poitaei* (Fée) A. Massal), and *Cyclographina* (Type *Diorygma pruinosum* (Eschw.) Kalb, Staiger & Elix). Of these, *D. poitaei* and *D. pruinosum* cluster in a monophyletic group with *D. hieroglyphicum*. *Diorygma confluens* has not been sequenced yet, but it differs from the new genus in the carbonized exciple and in the presence of the epithecium. As the genus *Jocatoa* is outside the *Diorygma* clade (Fig. 3.1) none of those names are available.

They type species of *Jocatoa* was previously included in *Medusulina*, a polyphyletic genus no longer recognized and loosely characterized by having aggregate lirellae, carbonized excipulum and muriform ascospores (Zahlbruckner 1926; Lücking 2013). However, the name is no longer available because its type species, *Medusulina nitida,* belongs in *Fissurina* (Staiger 2002). *Medusulina* was previously believed to be the

hyaline ascospore counterpart of *Sarcographa* (Müller 1894) or very close to *Glyphis* but with muriform ascospores (Zahlbruckner 1926). Besides *Fissurina* and the new genus *Jocatoa*, another species previously described in *Medusulina* is now a member of *Redonographa* (Lücking et al. 2013), none of these genera is particularly close to *Sarcographa* or *Glyphis*, however, these last two genera together with *Jocatoa* belong in the tribe Graphideae.

Jocatoa texana (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda comb. nov.
Fig. 3.4
MycoBank MB XXXXXX

Medusulina texana Müll. Arg., Bull. Herb. Boissier 2: 93 (1894); Type: **USA**, Eckfeldt 56A (G—holotype!).

Thallus crustose, corticolous, epiperidermal, ecorticate, white to whitish green, sometimes not continuous and then with endoperidermal parts but always well-developed near the ascocarps, UV-, with a black prothallus present at contact lines with other lichens. *Photobiont* trentepohlioid, in a continuous layer surrounded by small crystals POL+ beige that dissolve in KOH, as well as insoluble coarse crystals. *Ascocarps* abundant, lirelliform to rounded, erumpent, straight to curved, unbranched when young to stellate or in groups similar to a white pseudostroma, individual lirellae 0.2–1.5 × 0.2–0.25 mm, in groups up to 1 cm long; thalline margin lateral, raised above disc, concolorous with the thallus; disc black to brown black, but sometimes with remnants of thallus that gives the impression of coarse pruina, immersed, exposed when mature but sometimes partly cover by the thalline margin. *Exciple* not striate, hyaline to light brown, with small crystals and a granular appearance POL+ beige originated from the thallus margin, 22–30 µm wide; hypothecium hyaline 30–50 µm deep; hymenium hyaline, not inspersed with oil droplets, embedded in a strong gelatin matrix, 175–250 µm high, I-, epihymenium golden brown, with a granulose appearance; paraphyses simple to anastomosed specially towards the exciple, tips simple and not swollen, periphysoids absent. Epithecium absent. *Spores* 1 per ascus, hyaline, strongly muriform, ellipsoid,

inner cells larger than peripheral cells, 150–192(217) × 50–70(85) µm, I+ strongly violet, frequently with a hyaline gelatinous halo.

Chemistry. Hypostictic, stictic, cryptostictic, and constictic acids, plus three unknown substances with Rf 5–6 (solvent A) and Rf 5 (solvent C) that react UV+ red and white on TLC plates before the acid+heat treatment.

Ecology and distribution. In the study area this species is mostly found in the mature tropical deciduous dry forests of the Chamela-Cuixmala Biosphere Reserve, but one depauperate sample was found in a secondary forest in an area surrounding the Reserve. It is a rare species in the study area, found on the main trunk of the phorophytes *Apoplanesia paniculata*, *Cordia alliodora*, *Thouinia paucidentata*, and in the canopy of *Amphipterygium adstringens*. The species was previously known from its type locality in Brownsville, Texas (Müller 1984) and collections (not seen by us) are registered in the Consortium of North American Lichen Herbaria (CNALH) from Louisiana (USA) and Tamaulipas (Mexico) (Accessed through Consortium of North American Lichen Herbaria (CNALH) Data Portal, http//:lichenportal.org/portal/index.php, 2018-07-16).

Remarks. *Jocatoa texana* is similar to *Diorygma monophorum* (Nyl.) Kalb, Staiger & Elix, which has smaller spores (105–165 × 35–60 µm), anastomosing paraphyses tips, lacks stictic acid and does not have aggregate ascocarps. Interestingly, *D. monophorum* was described as having a slightly different nature of paraphyses, which are hardly anastomosed, thinner towards the tips and forming a mostly distinctly epithecium (Kalb et al. 2004). The original description of Müller (1894) and the one in Fink (1935) mention that *J*. (*Medusulina*) *texana* has soredia; however, neither the type collection nor our samples have soredia, so it is possible they were referring to the granular remnants of thallus present on the lirellae.

Additional specimens examined. **Mexico**, Jalisco: La Huerta, Chamela-Cuixmala Biosphere Reserve, Estación de Biología Chamela, mature tropical deciduous dry forest: 19°30’11’’N, 105°2’50’’W, 57 m, 300 m W of trail Tejón 600 m, on bark of *Apoplanesia*

paniculata, June 2009, *Miranda 1451, 2040*; 19°30’12’’N, 105°2’47’’W, 60 m, on trail Tejón 480 m, on bark of *Thouinia paucidentata*, May 2015, *Miranda 5004*; 19°30’24’’N, 105°2’57’’W, 60 m, on trail Tejón 1150 m, on bark of *Apoplanesia paucidentata*, June 2015, *Miranda 5005*; 19°30’1’’N, 105°2’33’’W, 62 m, on road Eje Central 430 m, on bark of fallen branches from the canopy of *Amphipterygium adstringens*, December 2015, *Miranda 4744*; 19°30’21’’N, 105°2’55’’W, 58 m, on trail Tejón 1050 m, on bark fallen branch from canopy of unknown tree, December 2015, *Miranda 4745*. Surrounding areas of the Chamela-Cuixmala Biosphere Reserve, Ejido Caimán, secondary tropical dry forest, 19°28’39’’N, 104°56’6’’W, 72 m, on bark of *Cordia alliodora*, September 2010, *Miranda 3080* (all specimens in MEXU).

Discussion

Subfamily Redonographoideae

Our phylogenetic analysis includes for the first time sequences of a species that can be assigned to the genus *Gymnographopsis*, supporting its inclusion in subfamily Redonographoideae. Traditionally, the only two genera in this subfamily were separated by the presence of norstictic acid and lateral to complete excipular carbonization in *Redonographa* versus absence of norstictic acid and an uncarbonized exciple in *Gymnographopsis* (Lücking et al. 2013). With the description of *G. corticola* in this paper, the presence of norstictic acid is no longer a good character to distinguish these genera. We propose instead to emphasize the nature of the periphysoids and excipular carbonization. In *Gymnographopsis*, the periphysoids are embedded in a gelatinous matrix and originate from the inner part of the exciple; in the case of *G. corticola*, the periphysoids start to appear at the upper third of the hymenium and extend towards the tip of the exciple. In *Redonographa* the periphysoids are not embedded in a gelatinous matrix and mostly originate from the top and outer part of the carbonized exciple, similar to *Carbacanthographis*.

This is the first report of the genus *Gymnographopsis* for the Northern Hemisphere, with previous localities being in northern Chile (Dodge 1966) and South

Africa (Egea & Torrente 1996). The whole subfamily Redonographoideae is known to occur only on subtropical and tropical coastal areas with a dry season. Except for the South African *G. latispora*, all species occur along the coast of the Pacific Ocean (Lücking et al. 2013). Our study area near the Pacific Coast of Mexico fits the known ecology of the subfamily.

Interestingly, the new species *G. corticola* and *R. parvispora* are the only species in Redonographoideae that are corticolous. It was hypothesized by Lücking et al. (2013) that the common ancestor of Graphidaceae was corticolous and from the wet tropics, and thus, that the peculiar saxicolous ecology and subtropical-dry habitat of the subfamily Redonographoideae evolved secondarily within the subfamily. The inclusion of corticolous species in both genera further supports the idea of a derived association with rock substrates in Redonographoideae. However, these new species represent a problem for the previous concept of the subfamily, because being saxicolous was considered an important diagnostic character for the subfamily. Currently Redonographoideae is only separated from other subfamilies by its tendency to occupy subtropical and tropical coastal dry habitats and by molecular data.

Our samples of both *G. corticola* and *R. parvispora* have a peculiar rectangular shaped perispore with apparent folds at both ends (Figs. 3.2D and 3.3F-G). In some spores the ends of the perispore are reduced and the tips of the spore protrude from the perispore, perhaps to start germination. Even though the presence of this perispore is frequent, not all spores show it, but this variability is typical of other perispore-producing species, as in *Opegrapha* and *Rhizocarpon*. We currently do not know if the perispore is correlated with the maturity of the spores. This type of perispore has not been reported for fungi before and may represent a unique character of Redonographoideae. If this is the case, it could be useful as a diagnostic character of the subfamily, as well as to distinguish *Redonographa* form the very similar but unrelated genus *Carbacanthographis*.

Subfamily Graphidoideae

Our phylogenetic analysis agrees with Kalb et al. (2004) in that *Diorygma* forms a monophyletic clade, when *Thalloloma* is included (Rivas Plata et al. 2013). Nonetheless, of the 71 species of *Diorygma* (Lücking et al. 2016), only a handful have been sequenced, including the type species of both genera. Our analysis clearly shows that the new genus *Jocatoa* does not belong in the *Diorygma* clade. Given that *Diorygma* is a large genus and that both *G. corticola* and *J. texana* could easily pass as *Diorygma* species, we expect some of the current species of *Diorygma* to fall outside the *Diorygma* clade. We also expect that the genus *Jocatoa* will remain monospecific for only a short period of time.

The new genus *Jocatoa* is here recovered as sister to *Schistophoron*, a mazaediate genus that was isolated within the tribe Graphideae. *Nadvornikia* is the only other truly mazaediate genus in Graphidaceae, where it originated independently of its occurrence in *Schistophoron* (Lumbsch et al. 2014b). *Nadvornikia* was recently found to include two non-mazaediate species (Medeiros et al. 2017). However, the differences between *Schistophoron* and *Jocatoa* go beyond the mazaediate ascocarp and they should not be considered congeneric. *Schistophoron* is distinguished by prominent to sessile lirellae and ascospores that are brown, with submuriform to transverse septation, up to 15 × 10 µm, and non-amyloid. *Jocatoa* on the other hand, has immersed to erumpent lirellae and ascospores that are hyaline, strongly muriform, larger than 150 × 50 µm, and strongly amyloid (violet). This last character, amyloid ascospores, is particularly important and was found to be conserved at the generic and even tribe level within the tribe Graphideae and most of the subfamily Graphidoideae (Lumbsch et al. 2014b).

The two new species in this paper depend strongly on undisturbed tropical dry forests. This ecosystem suffers from constant anthropogenic pressures and it is considered among the most threatened in the world (Janzen 1988; Portillo-Quintero & Sánchez-Azofeifa 2010). In Mexico, tropical dry forests have a high conversion rate to use in agriculture, and most of the forested area in the country is heavily fragmented and disturbed (Trejo & Dirzo 2000; Portillo-Quintero & Sánchez-Azofeifa 2010). Given that only 1.1% of the total extent of this ecosystem in Mexico is under protection (Sánchez-

Azofeifa et al. 2009), we consider the two new species described here to be vulnerable, especially *R. parvispora,* which was found to be a rare species with a very limited distribution inside the study area.

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Table 3.1 GenBank accession numbers of the new sequences generated in this study. – indicates missing data. * indicates holotypes. All samples from Mexico.

Species name	Voucher	DNA number	ITS	mtSSU	nuLSU
Gymnographopsis corticola*	Miranda 1158	RMG338	–	GB	–
G. corticola	Miranda 4567	RMG012	GB	GB	GB
G. corticola	Miranda 4729	RMG053	GB	GB	GB
Jocatoa texana	Miranda 2040	RMG031	GB	GB	–
J. texana	Miranda 3080	RMG065	GB	GB	–
J. texana	Miranda 4744	RMG305	GB	GB	GB
J. texana	Miranda 4745	RMG315	GB	GB	GB
Redonographa parvispora	Miranda 1099	RMG029	GB	–	–
R. parvispora*	Miranda 1128	RMG030	GB	–	–
R. parvispora	Miranda 4558	RMG242b	GB	GB	–
Schistophoron tenue	Herrera-Campos et al. 77	RMG265	GB	GB	–

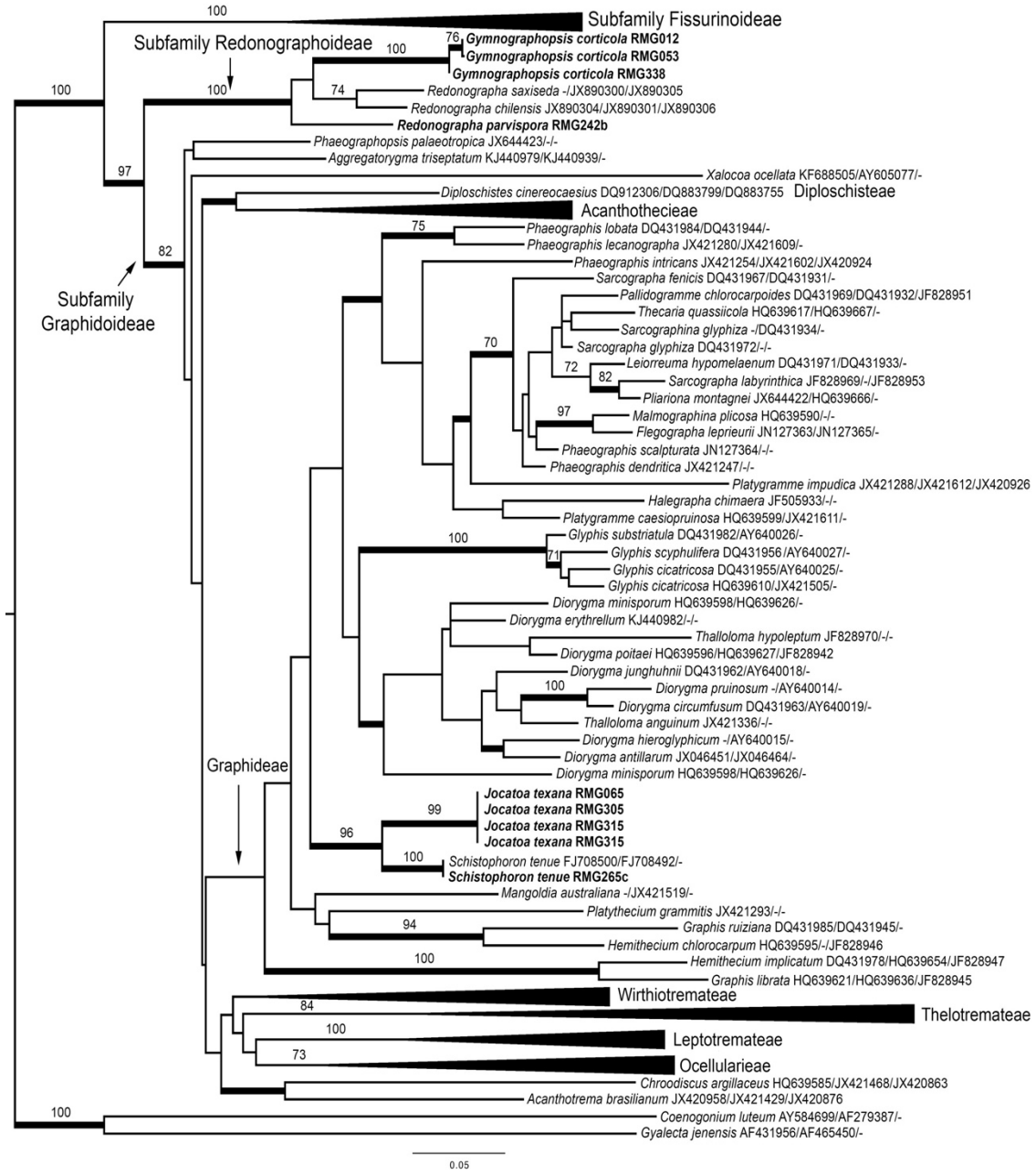

Figure 3.1 Phylogeny of the family Graphidaceae based on a Maximum Likelihood analysis of the genes mtSSU, nuLSU and RPB2. Support values are shown as numbers if Maximum Likelihood bootstrap values ≥ 70 and as bold branches if Bayesian posterior probabilities ≥ 0.95. Bold names show new sequences from this study. For collapsed branches refer to Appendix 3.2.

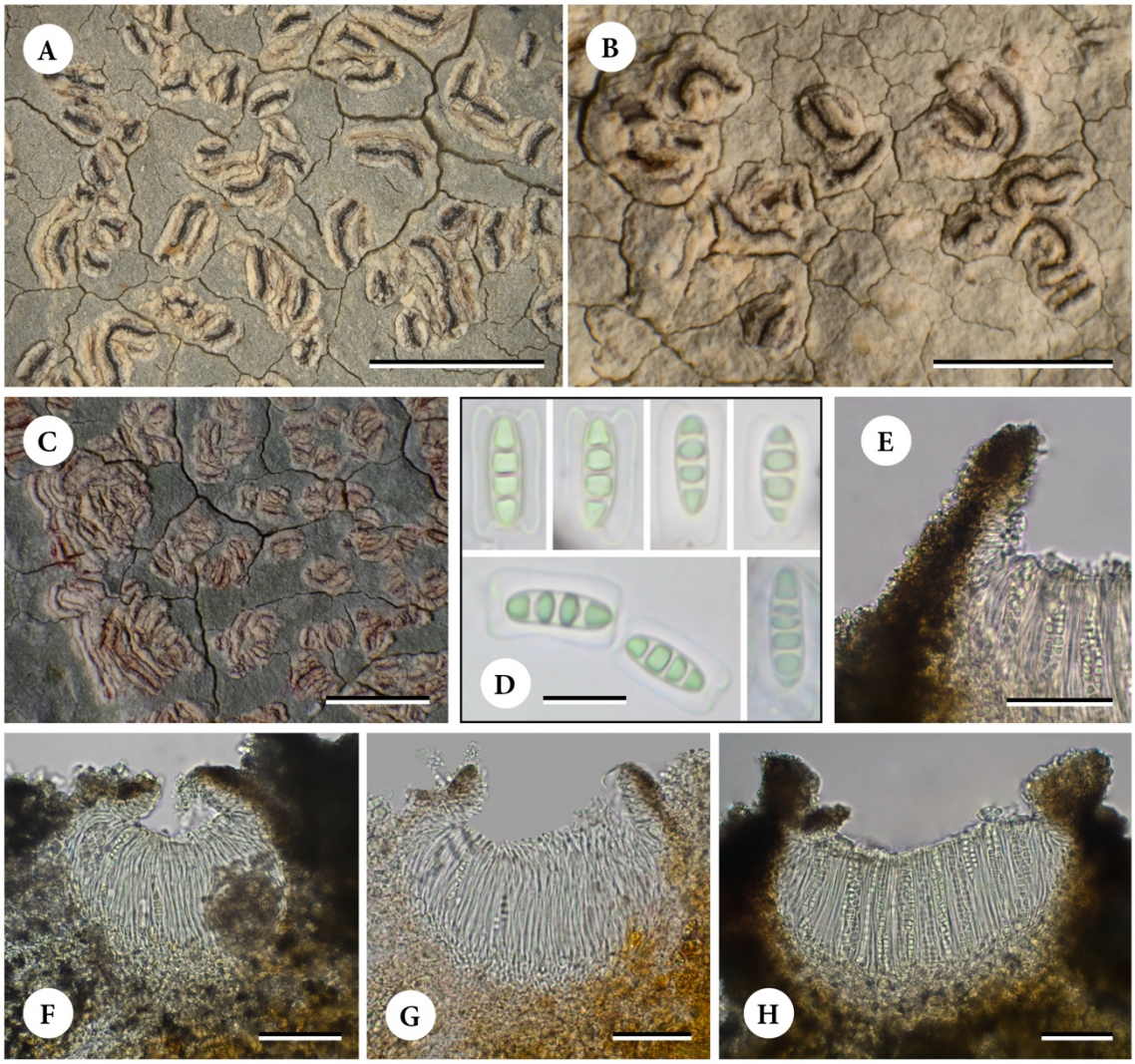

Figure 3.2 *Gymnographopsis corticola*. A-C) Habit showing lirellae, note open discs in B and aggregate lirellae in C; D) Ascospores with rectangular halo and apical folds; E) Section of a mature lirella showing periphysoids; F-G) Section of an immature lirella in water and KOH respectively; H) Section of a mature lirella. Scale: A-C) 1 mm; D) 10 µm; E-H) 40 µm. Specimens: A) *Lücking 25090*; B, E, H*) Miranda 4365*; C) *Miranda 4729*; D, F, G) *Miranda 4367*.

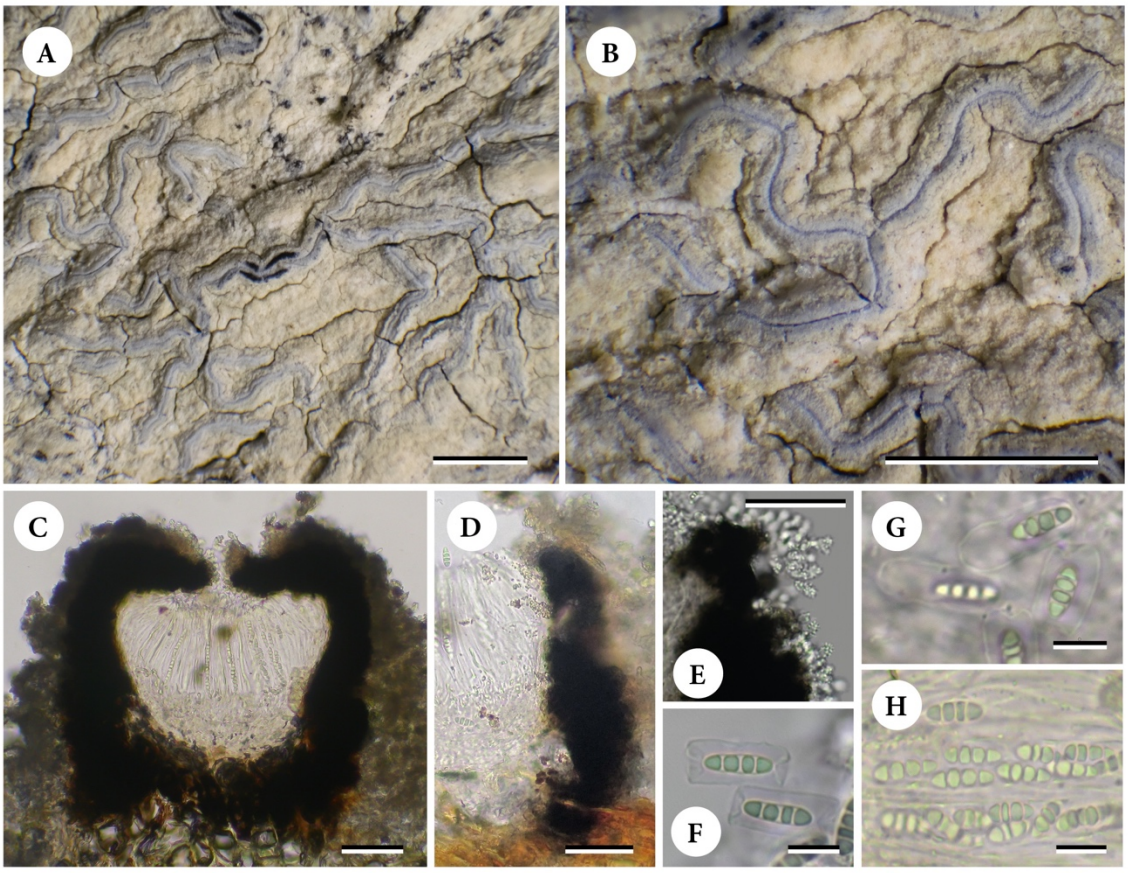

Figure 3.3 Holotype of *Redonographa parvispora* (*Miranda 1128*). A-B) Habit showing lirellae; C) Section of lirella showing complete excipular carbonization; D) Section of lirella showing lateral excipular carbonization; E) Verrucose periphysoids in KOH; F) Ascospores with rectangular halo; G) Ascospores with halo pushed towards one side; H) Biseriate ascospores in asci. Scale: A-B) 1 mm; C-E) 40 µm; F-H) 10 µm.

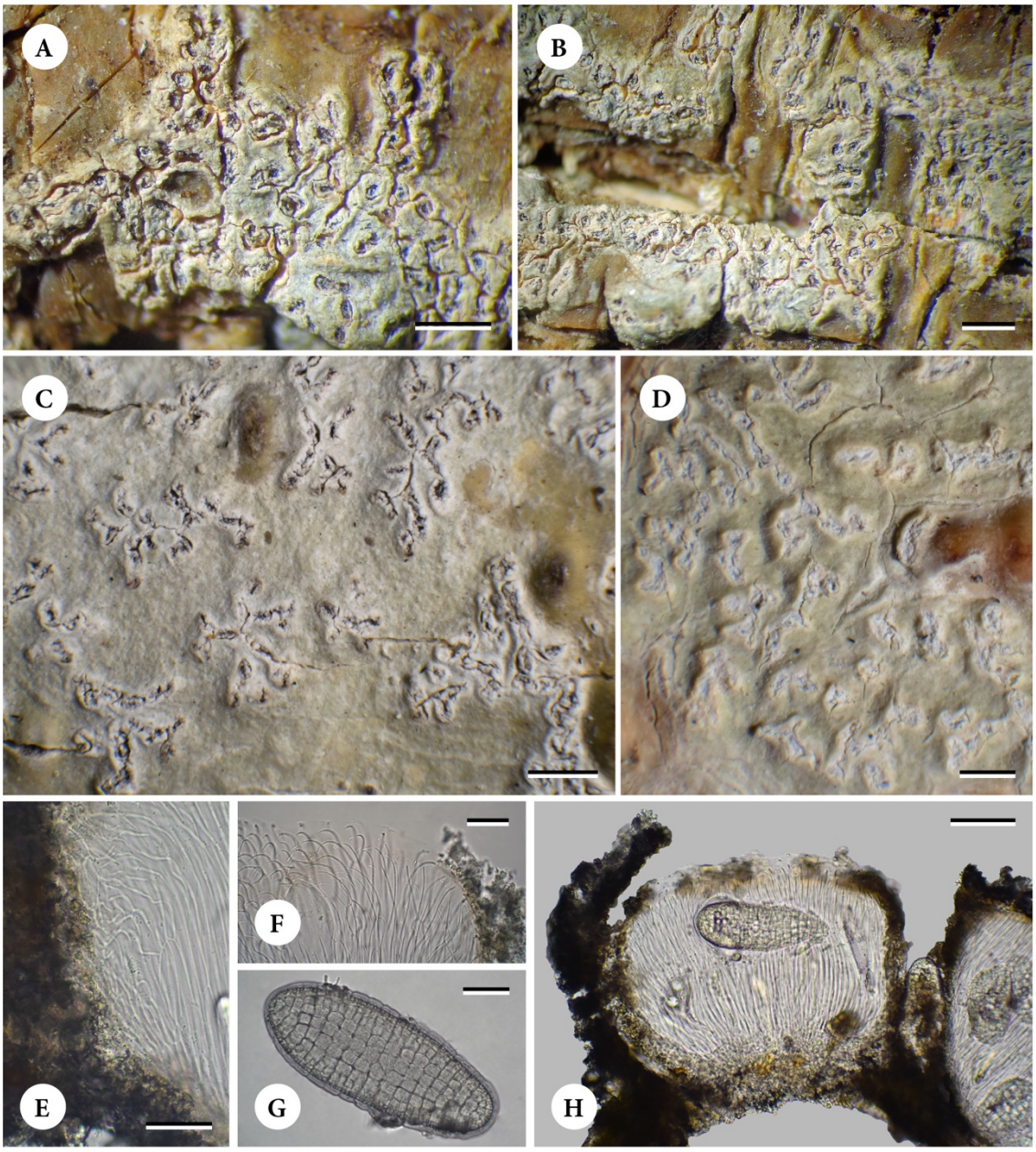

Figure 3.4 *Jocatoa texana*. A-D) Habit showing lirellae; E) Section of lirella showing anastomosed paraphyses towards the exciple; F) Section of lirella showing tips of paraphyses in KOH; G) Muriform ascospore; H) Section of lirellae showing uncarbonized excipulum. Scale: A-D) 1 mm; E-G) 40 µm; H) 100 µm. Specimens: A, B) *Eckfeldt 56A* (Holotype of *Medusulina texana*); C, F) *Miranda 2040*; D) *Miranda 5005*; E, G, H) *Miranda 5004*.

Appendix 3.1 GenBank accession numbers of all sequences used in this study.

Taxon	mtSSU	nuLSU	RPB2
Acanthothecis hololeucoides (Nyl.) Staiger & Kalb	JX420952	JX421423	JX420938
Acanthothecis peplophora (M. Wirth & Hale) E.A. Tripp & Lendemer	JX420953	JX421424	-
Acanthotrema brasilianum (Hale) Frisch	JX420958	JX421429	JX420876
Aggregatorygma triseptatum M. Cáceres, Aptroot & Lücking	KJ440979	KJ440939	-
Ampliotrema amplius (Nyl.) Kalb ex Kalb	JF828958	JF828973	-
Ampliotrema sp.	JX420963	JX421432	JX420900
Asteristion leucophthalmum (Nyl.) I. Medeiros, Lücking & Lumbsch	JX421374	JX421658	JX420830
Asteristion platycarpum (Tuck.) I. Medeiros, Lücking & Lumbsch	JX421007	JX421460	-
Astrochapsa astroidea (Berk. & Broome) Parnmen, Lücking & Lumbsch	JX420974	JX421441	JX420859
Austrotrema bicinctulum (Nyl.) I. Medeiros, Lücking & Lumbsch	EU075598	EU075642	JF828955
Borinquenotrema soredicarpum Mercado-Díaz, Lücking & Parnmen	KJ440980	-	-
Carbacanthographis stictica Staiger & Kalb	-	JX421435	JX420875
Chapsa alborosella (Nyl.) Frisch	JX420972	JX421439	JX420936
Chroodiscus argillaceus (Müll. Arg.) Lücking & Papong	HQ639585	JX421468	JX420863
Clandestinotrema stylothecium (Vain.) Rivas Plata, Lücking & Lumbsch	HQ639597	JX421470	-
Coenogonium luteum (Dicks.) Kalb & Lücking	AY584699	AF279387	-
Compositrema cerebriforme J. Hern. & Lücking	JX421017	JX421471	JX420901
Corticorygma stellatum M. Cáceres, S.C. Feuerst., Aptroot & Lücking	KJ440981	KJ435136	-
Cruentotrema cruentatum (Mont.) Rivas Plata, Lumbsch & Lücking	HQ639587	HQ639660	-
Crutarndina petractoides (P.M. Jørg. & Brodo) Parnmen, Lücking & Lumbsch	JX421383	JX421664	JX420891
Diorygma antillarum (Vain.) Nelsen, Lücking & Rivas Plata	JX046451	JX046464	-
Diorygma circumfusum (Stirt.) Kalb, Staiger & Elix	DQ431963	AY640019	-
Diorygma erythrellum (Mont. & Bosch) Kalb, Staiger & Elix	KJ440982	-	-
Diorygma hieroglyphicum (Pers.) Staiger & Kalb	-	AY640015	-
Diorygma junghuhnii (Mont. & Bosch) Kalb, Staiger & Elix	DQ431962	AY640018	-
Diorygma microsporum M. Cáceres & Lücking	JX421024	-	-
Diorygma minisporum Kalb, Staiger & Elix	HQ639598	HQ639626	-
Diorygma poitaei (Fée) Kalb, Staiger & Elix	HQ639596	HQ639627	JF828942
Diorygma pruinosum (Eschw.) Kalb, Staiger & Elix	-	AY640014	-
Diploschistes cinereocaesius (Sw.) Vain.	DQ912306	DQ883799	DQ883755
Dyplolabia afzelii (Ach.) A. Massal.	JX421027	JX421483	-
Enigmotrema rubrum Lücking	JX421030	-	-
Fibrillithecis gibbosa (H. Magn.) Rivas Plata & Lücking	EU075573	EU075621	-
Fissurina aggregatula Common & Lücking	JX421036	JX421490	JX420871
Fissurina astroisidiata Herrera-Camp. & Lücking	JX421039	-	JX420843
Fissurina insidiosa C. Knight & Mitt.	DQ972995	DQ973045	DQ973083
Fissurina monilifera Mercado-Díaz, Lücking & Parnmen	KJ435167	KJ440941	-
Fissurina nigrolabiata Rivas Plata, Bawingan & Lücking	JF828961	JF828976	JF828943
Flegographa leprieurii (Mont.) A. Massal.	JN127363	JN127365	-
Gintarasia darlingtonii (Frisch & Kalb) Lumbsch, Kraichak & Luecking	DQ384924	-	-
Gintarasia megalophthalma (Müll. Arg.) Kraichak, Lücking & Lumbsch	-	JX421456	KF688538
Glaucotrema glaucophaenum (Kremp.) Rivas Plata & Lumbsch	JX421061	JX421501	JX420862
Glyphis cicatricosa Ach.	HQ639610	JX421505	-
Glyphis cicatricosa Ach.	DQ431955	AY640025	-
Glyphis scyphulifera (Ach.) Staiger	DQ431956	AY640027	-
Glyphis substriatula (Nyl.) Staiger	DQ431982	AY640026	-
Graphis librata C. Knight	HQ639621	HQ639636	JF828945
Graphis ruiziana (Fée) A. Massal.	DQ431985	DQ431945	-
Gyalecta jenensis (Batsch) Zahlbr.	AF431956	AF465450	-
Gymnographopsis corticola R. Miranda, Herrera-Campos & Lücking	GB	–	–
Gymnographopsis corticola R. Miranda, Herrera-Campos & Lücking	GB	GB	–
Gymnographopsis corticola R. Miranda, Herrera-Campos & Lücking	GB	GB	–
Gyrotrema wirthii Rivas Plata, Lücking & Lumbsch	JX421071	-	-
Halegrapha chimaera Rivas Plata & Lücking	JF505933	-	-
Heiomasia sipmanii (Aptroot, Lücking & Rivas Plata) Nelsen, Lücking & Rivas Plata	GU395552	-	-
Hemithecium chlorocarpum (Fée) Trevis.	HQ639595	-	JF828946
Hemithecium implicatum (Fée) Staiger	DQ431978	HQ639654	JF828947

Appendix 3.1 (Continued).

Taxon	mtSSU	nuLSU	RPB2
Jocatoa texana (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda	GB	–	–
Jocatoa texana (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda	GB	–	–
Jocatoa texana (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda	GB	GB	–
Jocatoa texana (Müll. Arg.) Lücking, Herrera-Campos & R. Miranda	GB	GB	–
Leiorreuma hypomelaenum (Müll. Arg.) Staiger	DQ431971	DQ431933	-
Leucodecton occultum (Eschw.) Frisch	HQ639611	HQ639657	JF828949
Malmographina plicosa (C.F.W. Meissn.) M. Cáceres, Rivas Plata & Lücking	HQ639590	-	-
Mangoldia australiana Lücking, Parnmen & Lumbsch	-	JX421519	-
Melanotopelia rugosa (Kantvilas & Vězda) Lumbsch & Mangold	HQ639615	-	-
Melanotrema lynceodes (Nyl.) Rivas Plata, Lücking & Lumbsch	JX421088	JX421520	JX420907
Myriochapsa psoromica (M. Cáceres, Santos de Jesus & Santos Vieira) M. Cáceres, Lücking & Lumbsch	JX421009	JX421461	JX420884
Myriotrema album Fée	JX421090	-	-
Myriotrema olivaceum Fée	JX421095	EU126645	-
Nadvornikia expallescens (Nyl.) I. Medeiros, Lücking & Lumbsch	-	AY605072	-
Nadvornikia hawaiiensis (Tuck.) Tibell	EU075581	JX421533	-
Nadvornikia peninsulae (R.C. Harris) I. Medeiros, Lücking & Lumbsch	HQ639616	-	JF828950
Nitidochapsa leprieurii (Mont.) Parnmen, Lücking & Lumbsch	JX420991	JX421451	JX420930
Ocellularia albocincta (Hale) Divakar & Mangold	JX421113	JX421543	JX420873
Ocellularia allosporoides (Nyl.) Patw. & C. Kulk.	JX421118	JX421544	JX420925
Ocellularia cavata (Ach.) Müll. Arg.	DQ384878	DQ431935	-
Ocellularia dolichotata (Nyl.) Zahlbr.	JX421146	JX421554	-
Ocellularia domingensis (Fée ex Nyl.) Müll. Arg.	JX421151	JX421560	JX420918
Ocellularia eumorpha (Stirt.) Hale	DQ384885	JX421561	-
Ocellularia inturgescens (Müll. Arg.) Mangold	EU075577	EU075625	-
Ocellularia laeviuscula (Nylander) Kraichak, Lücking & Lumbsch	JX421094	JX421528	JX420920
Ocellularia laeviusculoides Sipman & Lücking	JX421167	JX421569	JX420896
Ocellularia microstoma (Müll. Arg.) Hale	JX421140	JX421576	JX420823
Ocellularia percolumellata Sipman	JX421180	-	JX420888
Ocellularia praestans (Müll. Arg.) Hale	JX421192	JX421581	JX420892
Ocellularia profunda (Stirt.) Mangold, Elix & Lumbsch	JX421198	JX421585	JX420825
Ocellularia psorbarroensis Sipman	JX421202	JX421588	JX420874
Ocellularia pyrenuloides Zahlbr.	DQ384896	-	-
Ocellularia wirthii Mangold, Elix & Lumbsch	JX421228	JX421599	-
Pallidogramme chlorocarpoides (Nyl.) Staiger, Kalb & Lücking	DQ431969	DQ431932	JF828951
Paratopeliopsis caraibica Mercado-Díaz, Lücking & Parnmen	KJ440983	-	-
Phaeographis dendritica (Ach.) Müll. Arg.	JX421247	-	-
Phaeographis intricans (Nyl.) Staiger	JX421254	JX421602	JX420924
Phaeographis lecanographa (Nyl.) Staiger	JX421280	JX421609	-
Phaeographis lobata (Eschw.) Müll. Arg.	DQ431984	DQ431944	-
Phaeographis scalpturata (Ach.) Staiger	JN127364	-	-
Phaeographopsis palaeotropica Kalb & Frisch	JX644423	-	-
Platygramme caesiopruinosa (Fée) Fée	HQ639599	JX421611	-
Platygramme impudica (A. W. Archer) A. W. Archer	JX421288	JX421612	JX420926
Platythecium grammitis (Fée) Staiger	JX421293	-	-
Pliariona montagnei (Bosch) A. Massal.	JX644422	HQ639666	-
Pseudochapsa dilatata (Müll. Arg.) Parnmen, Lücking & Lumbsch	JX420981	JX421446	JX420906
Pseudoramonia richeae Kantvilas & Vězda	KF875555	KF875534	-
Pseudotopeliopsis laceratula (Müll. Arg.) Parnmen, Lücking & Lumbsch	JX420988	JX421448	JX420831
Pycnotrema pycnoporellum (Nyl.) Rivas Plata & Lücking	JX421295	JX421615	-
Redingeria desseiniana Van den Broeck, Lücking & Ertz	KJ145246	KJ145245	-
Redingeria glaucoglyphica (Sipman) Frisch	JX421296	JX421618	-
Redonographa chilensis (Zahlbr.) Lücking &Tehler	JX890304	JX890301	JX890306
Redonographa parvispora R. Miranda, Barcenas-Peña & Lücking	–	–	–
Redonographa parvispora R. Miranda, Barcenas-Peña & Lücking	–	–	–
Redonographa parvispora R. Miranda, Barcenas-Peña & Lücking	GB	–	–
Redonographa saxiseda (Zahlbr.) Lücking &Tehler	-	JX890300	JX890305
Reimnitzia santensis (Tuck.) Kalb	HQ639622	HQ639664	JF828952

Appendix 3.1 (Continued).

Taxon	mtSSU	nuLSU	RPB2
Rhabdodiscus isidiifer (Hale) Rivas Plata, Lücking & Lumbsch	JX421302	JX421623	JX420908
Sanguinotrema wightii (Taylor) Lücking	EU075574	JF828977	JF828948
Sarcographa fenicis (Vain.) Zahlbr.	DQ431967	DQ431931	-
Sarcographa glyphiza (Nyl.) Kr.P. Singh & G.P. Sinha	DQ431972	DQ431934	-
Sarcographa labyrinthica (Ach.) Müll. Arg.	JF828969	-	JF828953
Sarcographina cyclospora Müll. Arg.	KJ435230	-	-
Schistophoron tenue Stirt.	GB	–	–
Schistophoron tenue Stirt.	EU544933	EU544932	-
Schizotrema schizolomum (Müll. Arg.) Mangold & Lumbsch	FJ708500	FJ708492	-
Stegobolus wrightii (Tuck.) Frisch	JX421334	JX421636	JX420913
Thalloloma anguinum (Mont.) Trevis.	JX421336	-	-
Thalloloma hypoleptum (Nyl.) Staiger	JF828970	-	-
Thecaria quassiicola Fée	HQ639617	HQ639667	-
Thelotrema lepadinum (Ach.) Ach.	JX421366	JX421653	JX420934
Topeliopsis muscigena (Stizenb.) Kalb	EU075611	EU126655	JF828957
Wirthiotrema trypaneoides (Nyl.) Rivas Plata & Lücking	JX421422	JX421681	JX420916
Xalocoa ocellata (Vill.) Kraichak, Lücking & Lumbsch	KF688505	AY605077	-

Appendix 3.2 Phylogeny of the family Graphidaceae based on a Maximum Likelihood analysis of the genes mtSSU, nuLSU and RPB2. Support values are shown as numbers if Maximum Likelihood bootstrap values ≥ 70 and as bold branches if Bayesian posterior probabilities ≥ 0.95

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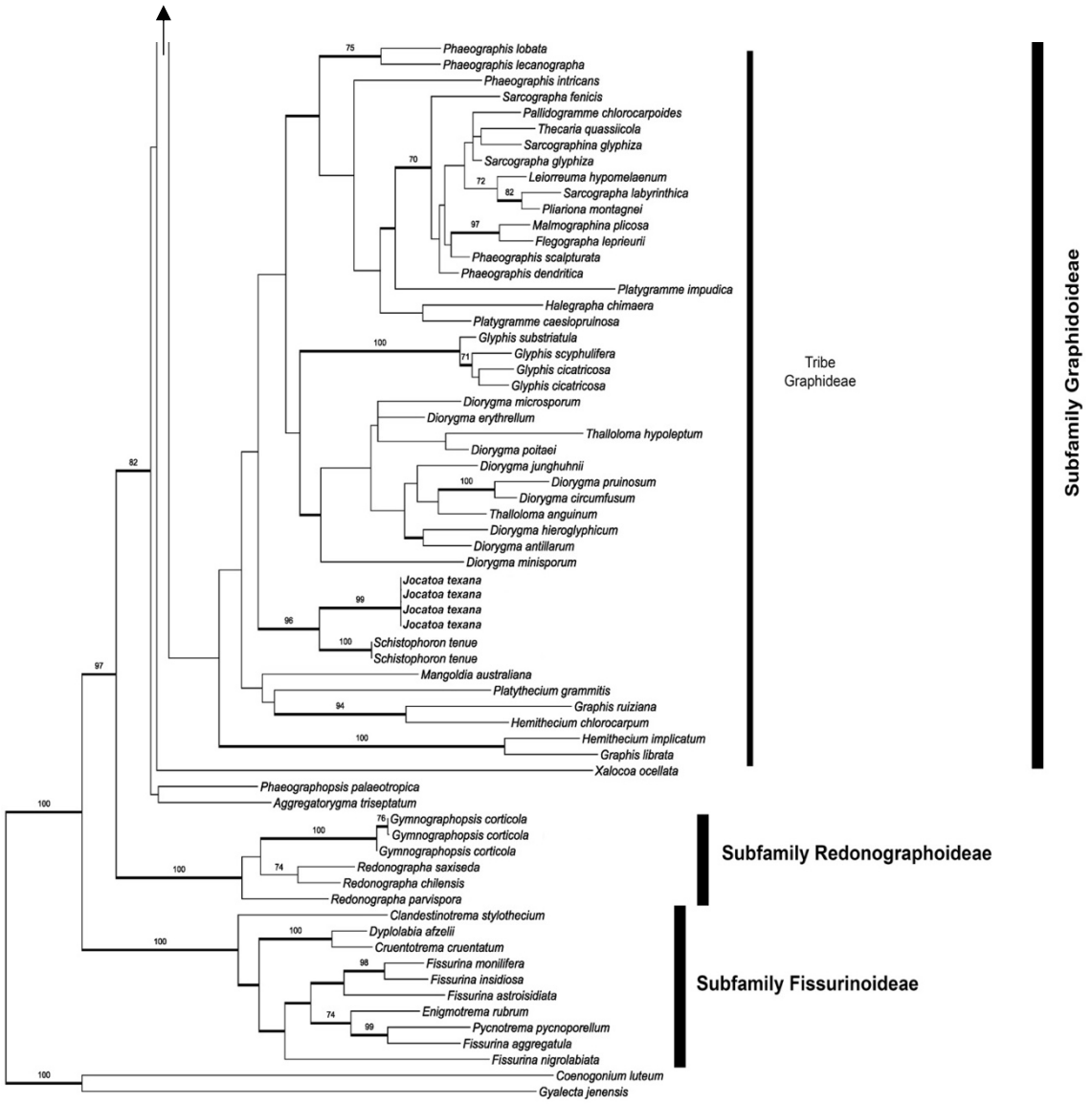
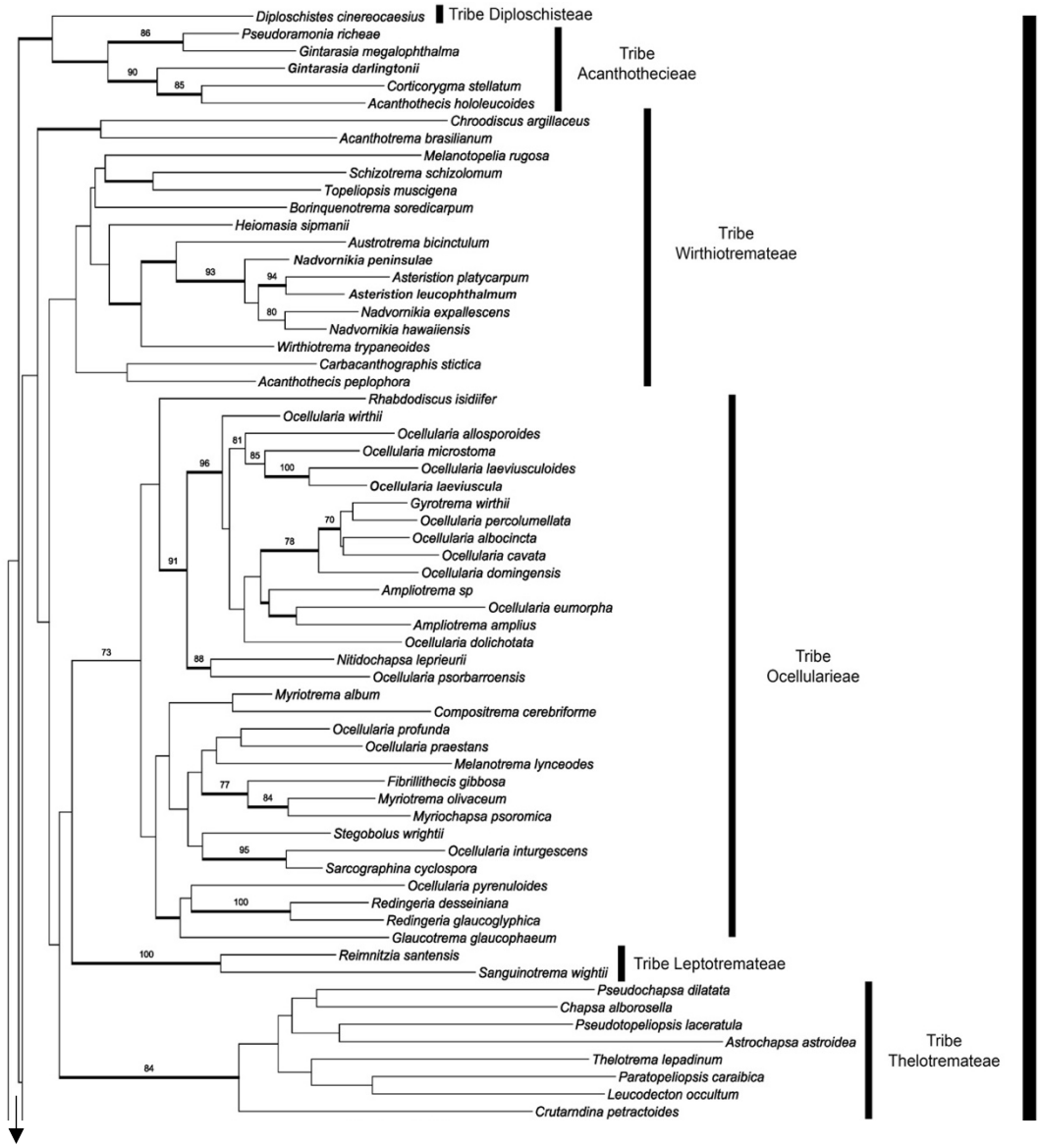


Appendix 3.2 (Continued).



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CHAPTER 4. THE WEIGHT OF THE CRUST: LICHEN BIOMASS ESTIMATION FOR TROPICAL DRY FORESTS

Ricardo Miranda-González & Bruce McCune

Target journal: *Biotropica*

Abstract

In recent years, our ecological knowledge of tropical dry forests has increased dramatically. However, whole components of the ecosystem, like lichenized fungi, remain mostly unknown. Crustose lichens in these forests are so abundant, that they are responsible for the characteristic appearance of a white bark forest during the dry season. We estimated the lichen biomass at the ecosystem-level by calculating the bark area of trees, the dry mass of lichens per unit area of bark, and the percentage of lichen cover of different trees sizes. Lichen biomass values per hectare were determined by extrapolating the above measurements using known tree densities for the study area. The mean coverage of lichens per tree was 85 percent of the available bark area for trees smaller than 12 cm of DBH, which represent most of the trees in this ecosystem. The epiphytic lichen biomass in the forest was 1.30 to 1.92 t/ha, of which 180 kg per hectare were located on the lowest 2.5 m of the main trunk of the trees. Lichen biomass represented 59 percent of the foliar biomass in the system. To our knowledge, this is the first time that a lichen biomass estimate is provided for an ecosystem in which crustose lichens are the dominant growth form. In other ecosystems and related studies, crustose lichens are considered to contribute little to the total lichen biomass and to be too difficult to include in an analysis. The high values of lichen biomass in this forest suggest a significant ecological role that so far is unexplored. We suggest that the crustose lichen component should not be underestimated *a priori* in ecological studies, especially in areas with significant lichen cover.
Key words: Mexico, Chamela, ecosystem scale.

Introduction

The tropical dry forest (TDF) (Holdridge 1967) is the most extensive ecosystem in the tropics and represents 42% of the forested tropical land in the world (Murphy & Lugo 1986a, 1995). Its original distribution in Americas was continuous from north of Mexico to north of Argentina, but due to human impacts only 66% of it remains (Trejo & Dirzo

2000, Portillo-Quintero & Sánchez-Azofeifa 2010). At a global scale, it is estimated that 97% of the remaining TDF is at high risk of disappearance (Miles et al. 2006). Despite this, TDF has been a neglected ecosystem in terms of research priorities, especially when compared to more charismatic ecosystems the tropical rain forests. In a literature search of the years 1945 through 2005, Sánchez-Azofeifa et al. (2013a) found a ratio of 1 published paper about TDF for every 300 published papers about tropical rain forest.

In recent years however, several publications have improved our understanding of this ecosystem (Bullock et al. 1995, Noguera et al. 2002, Dirzo et al. 2011, Sánchez-Azofeifa et al. 2013b). Tropical dry forests are characterized by an extended dry season of three to eight months in which more than 50% of the trees lose their leaves completely. Their mean annual precipitation is 400–2000 mm, mean annual temperature is above 25 °C, and elevation ranges from sea level to 2000 m (Trejo & Dirzo 2000, Sánchez-Azofeifa et al. 2005, Portillo-Quintero & Sánchez-Azofeifa 2010). Within areas with TDF, Kalacska et al. (2004) found that drier areas tend to have higher species richness of vascular plants per unit area than wetter areas.

In an effort to understand forest structure and functionality, some papers have calculated the biomass of plant material and the total net primary productivity at the ecosystem scale. For TDF the aboveground annual net primary productivity was estimated to be 4.5 t/ha in Puerto Rico (Clark et al. 2001) and from 6.1 to 8.1 t/ha in Mexico, of which the leaf component represents 2.4-3.1 t/ha (Vizcaíno 1983, Martínez-Yrízar & Sarukhán 1990, Martínez-Yrízar et al. 1996). For aboveground plant biomass the estimated mean value is 77.4 ± 30.5 t/ha (Jaramillo et al. 2011).

Some TDFs have an abundant and diverse epiphyte community (Lott et al. 1987) that not only contributes to the total plant biomass in the system (Martínez-Yrízar et al. 1992) but also provide ecosystem services like water availability and food for other organism (Andrade 2003, Reyes-García et al. 2008, Sáyago et al. 2013). Nonetheless, from all studies dealing with above ground biomass of TDF, only one paper from Puerto Rico included epiphytes (Murphy & Lugo 1986). They found that of the total 44.9 t/ha of

above ground plant biomass, the epiphyte component contributed only 0.14 t/ha; unfortunately, they did not provide information regarding the type of epiphytes in their study site, nor whether they included non-vascular groups.

Among the epiphytes of TDF, the lichen component is virtually absent in the scientific literature. However, preliminary studies in Mexico show more than 386 species of lichens from seasonally dry forests (Herrera-Campos et al. 2014). In the case of the Chamela-Cuixmala Biosphere Reserve, which is the most intensively studied TDF in the Neotropics (Jaramillo et al. 2011), lichens were shown to be abundant and to cover the bark of most of the trees (Miranda-González 2012). In other ecosystems, lichens contribute to the nutrient cycle by incorporating nutrients from the atmosphere, some of which may come from outside the system (Pike 1978). As photosynthetic and primary colonizers, lichens support an extensive network of microorganisms and invertebrates by providing habitat, water and nutrients (Gerson & Seaward 1977). In turn, this creates a cascading effect in which an increase in the biomass of lichens in the system, contributes to the abundance of spiders (Gunnarsson et al. 2004) and of passerine birds (Pettersson et al. 1995) by increasing the availability of prey. It is expected that some of these contributions from lichens to ecosystem dynamics are present in the TDF as well. In particular, the Chamela-Cuixmala Biosphere Reserve is known to be an important habitat for its 83 species of winter migratory birds (Hutto 1987, Arizmendi et al. 2002). We expect that a high abundance of lichens in the reserve could increase the resources available for those birds and for other animals that are active during the dry season.

In this study we aim to provide the first estimate of epiphytic lichen biomass of the TDF. This is a necessary step in order to incorporate lichens into our understanding of the ecosystem processes. In particular we: 1) estimated the amount of lichens present in the lowest 2.5 m of trees of different sizes; 2) estimated the total lichen biomass per ha of TDF; and 3) discussed the implications of abundant lichen resources at the ecosystem level.

Methods

Study area

The study was conducted in the Chamela-Cuixmala Biosphere Reserve, located 2 km inland from the Pacific Coast of Mexico. The tropical dry forest component of the Reserve is characterized by a warm sub-humid climate with summer rains (Garcia 2004) and a dry season (Fig. 4.1A) of six continuous months in which more than 95% of the plants lose their leaves completely. The remaining six months of the year are marked by a fast greening of the canopy, which is the product of short and intense rains intercalated with dry periods. The area has a strong oceanic influence that maintains mean monthly values of relative humidity above 75% year-round, with mean annual temperature of 24.6°C and mean annual precipitation of 763 mm (1977-2006 period), of which 80% falls between the months of July and October (Garcia-Oliva et al. 2002, Maass et al. 2002, Sánchez-Azofeifa et al. 2013).

The mature forest consists of trees 4-15 m tall in a dense pattern of up to 4500 trees per ha, with more than 50% of the stems having a diameter at breast height (DBH) smaller than 5 cm (Lott et al. 1987). The reserve sustains 1149 species of plants of which 229 are trees. The most representative families are Leguminosae (including Caesalpinioideae, Mimosoideae, and Papilionoideae) and Euphorbiaceae (Lott & Atkinson 2006, Sánchez-Azofeifa et al. 2013). Lichen communities cover most of the bark of most trees. These lichens are predominantly crustose species in the families Arthoniaceae, Graphidaceae and Pyrenulaceae. Macrolichens are few and usually limited to the canopy (Miranda-González 2012).

The dry forest in the Reserve is considered well conserved and is immediately surrounded by a matrix of well conserved and secondary forest outside the Reserve (Sánchez-Azofeifa et al. 2009). Nonetheless, the area was severely damaged by hurricane Jova in 2011 and especially by hurricane Patricia in 2015. Most of the damage was concentrated in the forest canopy, which reduced its mean tree height from 6.8 m to only 3.5 m (Parker et al. 2018). As a consequence, and to avoid the hurricane effect, our lichen

biomass estimation was focus on the lowest 2.5 m of the main trunk of the trees. Using available studies, we extrapolated the lichen biomass estimate to include the canopy strata.

Lichen dry mass per unit area

To calculate lichen dry mass per unit area, a 2 cm^2 area with lichens present was carefully scratched from ten previously collected pieces of bark that represent common lichen species in the forest. To avoid loss of material, the 2 cm^2 area was moisturized before scratching it with a single razor blade under the stereoscope. Special care was taken to avoid removing the bark layer. All the collected lichen tissue was heated for 24 hrs. at 60°C and weighted immediately to the nearest 0.1 mg with an Ohaus Analytical Plus balance. All measurements were transformed to g/m^2.

Lichen biomass and cover estimations per tree

The lichen biomass of the lowest 2.5 m of each analyzed tree was calculated as the product of the lateral surface area in m^2, the percentage of lichen cover in each tree, and the mean lichen dry mass in g/m^2. Data were taken from 62 randomly selected trees of varying diameter at breast height (DBH), however, trees were selected only if the main trunk did not branch out below 2.5 m. For each tree, we measured the lateral surface area of the main trunk using the formula of a truncated cone with diameter measurements at 0.3 m and 2.5 m high. The lichen cover of the measured area was visually estimated as a percentage.

We used linear regression to analyze the relationship between DBH and lichen cover on the lowest 2.5 m of the 62 trees. After examining the residuals, we divided the trees in two groups: DBH smaller than 12 cm and DBH larger than 12 cm. Given the approximately constant values within groups, the mean lichen cover on trees in each of the two groups was used for the analysis of biomass at the plot level.

Lichen biomass estimation of the lowest 2.5 m of all trees per ha

To extrapolate the lichen biomass of the lowest 2.5 m of individual trees to the trees present in one hectare, we used the study of Lott et al. (1987) that provides the density of trees per ha at different categories of DBH for the Chamela-Cuixmala Biosphere Reserve. Using a linear regression with a logarithmic scale in the *Y* axis, we transform the tree density in the five DBH categories of Lott et al. (1987) into tree density at increments of 1 cm and a range from 3 to 32 cm of DBH. This upper limit of DBH accounts for the majority of individuals and species in the study area (Lott et al. 1987, Bullock 2000). To maintain the structure of the generated forest in the regression, we conserved the rate of trees from each of Lott's DBH categories with respect to the total amount of trees. To avoid overestimating the number of trees, we constrained the analysis to fit the basal area of the real forest provided by Lott et al. (1987).

Using the number of trees per hectare in each of the new DBH categories, we calculated the lateral surface area of the lowest 2.5 m of the trees in one hectare. Our 62 measured trees did not include samples from all the DBH categories in the range of 3 to 32 cm. To solve this, we estimated the lateral surface area of the trees in each DBH category using a linear regression from the 62 measured trees. Lichen biomass estimations for each DBH category was calculated as the product of the newly generated lateral surface area per hectare, and the previously generated percent of lichen cover and lichen dry mass per unit area.

To calculate a range of values for the lichen biomass estimate, we generated simulations of 1000 samples size, that followed a normal distribution based on standard deviation and mean for each of the following variables: lichen cover percent, lichen dry mass per unit area, and basal area of the forest. This last variable, basal area, was obtained from the work of Lott et al. (1987), Martínez-Yrízar et al. (1992) and Jaramillo et al. (2003). Lichen biomass estimates for each DBH categorie were calculated using the 1000 sample size simulation.

To estimate the kg of lichen biomass per ha in the lowest 2.5 m of the forest we used the following formula:

$$Lichen\ biomass = \frac{\sum_{i=3}^{32} (surface\ area_i \times lichen\ dry\ mass \times lichen\ cover_i)}{1000}$$

Where *Lichen biomass* is the dry mass of lichens in kg per ha, *surface area* is the lateral surface area of the lowest 2.5 m of all trees in m^2/ha at each DBH category, *lichen dry mass* is the estimated dry mass constant in g/m^2, *Lichen cover* is the percent of bark covered by lichens at each DBH category, and *i* is the DBH category ranging from 3 to 32 cm.

Lichen biomass estimation for all tree strata per ha

To account for the missing canopy values of surface area of bark we applied the ratios developed by Whittaker (1966) and Whittaker & Woodwell (1967) for temperate deciduous forests. These ratios provide a way to estimate the surface area of bark in the canopy, based on the surface area of the main trunk (tree level), or based on the surface area of the ground (stand level). Trees in tropical dry forests tend to be smaller than trees in temperate deciduous forest, but with a higher density per ha. To reduce a possible overestimation, we applied the ratio values in the lower range of those provided by Whittaker (1966) and Whittaker & Woodwell (1967). To estimate the bark surface area of trees in the canopy, we calculated the lateral surface area of the main trunk of trees in each of our DBH size classes using the formula of a cylinder with radius at breast height and a total height of 4 m. We then applied a ratio of 1:5 (area on the main trunk to area in the canopy). To estimate the bark surface area in the canopy based on the surface area of the ground we applied a ratio of 1.19:1.

These two approaches provided a value of bark surface area in the canopy. To get the value of bark for whole trees, we add the canopy value to the main trunk value. To obtain the lichen biomass estimates for the whole stand, we multiply the new surface area values by the mean percentage of lichen cover, the mean lichen dry mass per unit area, and the tree densities from our study area at each respective DBH category. Range values for the lichen biomass estimate of the whole stand were calculated using a simulation of

1000 sample size in the same way as the estimation for the lowest 2.5 m of the forest. Because even small branches are soon colonized by lichens, we made the simplifying assumption that the bark is uniformly covered by crustose lichens. Because some unknown portion of the growing twigs is inevitably bare, this procedure would overestimate the lichenized area, but this would be compensated to an unknown degree by the presence of macrolichens in the upper branches with higher mass per unit area than what we measured for crustose lichens.

Results

Most of the trees in the study area had high cover values of crustose lichens in the lowest 2.5 m of the main trunk (Fig. 4.1B), with few exceptions like some species of *Bursera* that support lichens only on scar tissue. The mean lichen cover was 85 percent (n = 51, SD of 13.1) of the available bark area for trees smaller than 12 cm of DBH. Using a regression (Fig. 4.2A), no relation was detected between lichen cover percent and DBH for trees in the range of 1 to 12 cm of DBH ($R^2 < 0.001$, $p = 0.97$); which represent more than 85 percent of the total trees in a mature forest for the study area (Lott et al. 1987). This lack of relation was the result of constant and high values of lichen cover percent from trees throughout the DBH range. Larger trees (DBH range of 12 to 37 cm) on the other hand, were rare and had a mean lichen cover of 38 percent (n = 11, SD of 24.5). Similar to smaller trees, a regression (Fig. 4.2B) found no relation between lichen cover and DBH for trees in the range of 12 to 37 cm of DBH ($R^2 = 0.003$, $p = 0.85$).

Lichen dry mass per unit area was estimated as 103 g/m^2 (n = 10, SD of 44.16), from a taxonomically diverse set of lichens in six different families (Appendix 4.1). Given that most trees had similarly high values of lichen percent cover, the lichen biomass per tree was strongly dependent on the DBH (Table 4.1). Due to their higher density, trees in the range of 3–7 cm of DBH had around 50 percent of the total lichen biomass in the forest (Table 4.1).

The lichen biomass in the lowest 2.5 m of the forest was estimated to be 180.9 kg/ha (155.5–206.2 kg/ha using ± 1 SD). This value was obtained by the product of the lateral surface area of all trees per ha, the percentage of bark cover with lichens, and the lichen dry mass per unit area. To obtain the lateral surface area of tree per ha, we used a linear regression ($R^2 = 0.98$, $p < 0.001$) to transform the five DBH categories of tree density per ha from Lott et al. (1987) into tree density for each of our 30 DBH size classes (Fig. 4.2C, Table 4.1). Then, with another regression ($R^2 = 0.99$, $p < 0.001$) from our 62 sampled trees, we calculated the lateral surface area of the bark for the lowest 2.5 m of trees at each DBH category (Fig. 4.2D). The small error in this relationship derives from the rather limited variation in the taper of the truncated cone representing the lower trunk of trees of a given DBH.

The lichen biomass at the stand level (main trunks and canopy) was estimated as 1.92 t/ha (1.69–2.15 t/ha using ± 1 SD) after the ratio of 1:5 (trunk to canopy area) developed by Whittaker & Woodwell (1967). If we used instead the ratio of 1.19:1 (canopy to ground area) developed by Whittaker (1966) and Whittaker & Woodwell (1967), then, the lichen biomass estimation would be 1.30 t/ha (0.86–1.74 t/ha using ± 1 SD).

Discussion

Lichen biomass estimate at the stand level

This is the first time that the biomass of the epiphytic lichen component of a tropical dry forest has been estimated. In contrast to the 85 t/ha of above ground plant biomass for the same study area (Martínez-Yrízar et al. 1992), our lichen biomass estimate represents a small fraction. Nonetheless, the above ground plant biomass is mostly constituted by non-labile woody components, like branches and trunks, that make it harder for nutrients to move to a different trophic level (Nadkarni 1984). Foliage on the other hand, has similar turnover rates as lichens (Pike 1978), and together they are the primary photosynthetic components of the ecosystem. In the study area, the mean foliage biomass was estimated to be 2.72 t/ha (Vizcaíno 1983, Martínez-Yrízar & Sarukhán 1990, Martínez-Yrízar et al.

1996). Our results showed that lichen biomass in the lowest 2.5 m of the trees represents 6.6 percent of the total foliage biomass of the forest and 59 percent of the total foliage biomass when the whole canopy is included (using the mean lichen biomass at the stand level from the two approaches in this study). An important difference between foliage and lichen biomass is, however, that lichen biomass remains relatively constant and available as a trophic and habitat resources throughout the year, whereas foliage biomass is almost completely lost and renewed every year in these seasonally deciduous forests.

The 1.30–1.92 t/ha of lichen biomass was calculated to represent a mature dry forest from the Chamela-Cuixmala Biosphere Reserve. As expected for all estimations, ours include components that overestimate and ones that underestimate the lichen biomass. The main sources of overestimation are the fraction of recent canopy branches that are bare of lichens and the presence in the forest of a few species of trees that have few to no lichens. The main sources of underestimation are the following three: 1) Trees smaller than 3 cm of DBH, although abundant, were not included because their density was not known. 2) The few foliose and fruticose lichens in the canopy were not measured. Even though they are infrequent, their dry mass per unit area is higher than that for crustose lichens. 3) Our method only takes into account the lichen component that grows outside the bark and not the endoperidermic component present in crustose lichens (Tucker et al. 1991). We consider that these two types of components compensate each other to some extent and leave us with a useful and reasonable estimate of lichen biomass for the study area.

Two sources of error require further discussion. Canopy destruction by recent hurricanes prevented us from measuring the percent of lichen cover in the tree branches of the upper canopy. We extrapolated the percent values from the main trunk following earlier, pre-hurricane observations by the first author that show a high percentage of lichen cover in the canopy (around 95 percent). High cover in the upper canopy is particularly noticeable in larger trees that have a depauperate lichen cover in the lower part of the main trunk but an abundant lichen cover in the canopy.

A second source of error was that the ratios to estimate the surface area of the canopy were developed by Whittaker (1966) using regressions on a series of temperate deciduous forests. Extrapolation to tropical deciduous forest is, of course, not ideal. However, in all their differences, both ecosystems share many useful metrics. For instance, the tropical dry forest of our study area has values of basal area, aboveground plant biomass, and foliage productivity per year that are well in the range of the values present in the temperate deciduous forests used by Whittaker in his regressions. For example, the ratio of surface area of the main trunks to the surface area of the ground was 0.4:1 for the forest in Chamela, while the range present in temperate deciduous forests was 0.2:1 to 1:1 (Whittaker and Woodwell 1967). We consider that by using the lower range of ratios developed for temperate deciduous forests, we can provide a conservative estimate of lichen biomass for the tropical dry forests. The fact that the lichen biomass estimates based on two independent ratios (surface area of trunk to canopy and surface area of canopy to ground) provided similar results, further supports the validity of our adopting of Whittaker's ratios.

Ecosystem implications of a high lichen biomass

If we consider lichens as resources in the TDF, then the amounts of nutrients, minerals and water they contain are not trivial at the ecosystem scale. Furthermore, a high abundance of lichens suggests the possibility of them creating specialized niches that are being filled by other organisms in the same ecosystem. For example, a high proportion of caterpillars that feed on lichens, bryophytes and/or dead leaves, were found in montane rain forests of Ecuador (Bodner et al. 2015).

High cover values of lichens on trees could be especially important during the long dry season, because in the absence of leaves the amount of light reaching the bark of the trees increases substantially (Barradas & Adem 1992, Parker et al. 2005). As lichens effectively replace the bark color from brown to different tones of gray in most of the trees (Fig. 4.1B), they must increase the albedo of the forest, therefore, a reduction in temperature is expected both for the surface of the trunks and for the animals that live on them.

The poikilohydric water relations of lichens allows them to obtain water directly from the humidity of the air (Green et al. 2008) and to remain metabolically active even during the harsh conditions of the dry season. This continuous stability in resources can benefit the animals that live in this environment. Even though many of these animals enter a diapause to avoid the lack of liquid water in the dry months, at least 231 species of arthropods in the study area are known to occur only during the dry season (Pescador-Rubio et al. 2002, Rodríguez-Palafox & Corona 2002). Some of these arthropods like species of mites and of insect orders like Collembola and Psocoptera, depend on lichen resources to survive (Laundon 1971, Gerson & Seaward 1977, García-Aldrete 2014, Krantz & Walter 2009). Furthermore, some of the resident (Vega Rivera & Lobato García 2002) and migratory (Arizmendi et al. 2002) insectivorous birds that arrive in the study area during the dry season would depend on some of those arthropods. Throughout the wet season, lichens could also provide resources to a subset of the invertebrates of the TDF, but currently, there is no information regarding how big this subset could be.

Comparison to other ecosystems

The lichen biomass estimate presented here is particularly unexpected because the lichens of the study area are mostly crustose forms that are inconspicuous to the untrained eye. As most of the trees are heavily covered with lichens, it is easy to confuse them with the bark itself and completely miss them (Barajas Morales & Pérez Jiménez 1990). To our knowledge, this is the first time that a lichen biomass estimate is provided for an ecosystem in which crustose lichens are the dominant growth form. In other ecosystems and studies, crustose lichens are considered to contribute little to the total lichen biomass and are also considered too difficult to include in the analysis (Esseen et al. 1996), but most of the time they are not considered at all. However, the crustose form is the most abundant and diverse growth form among tropical lichens (Lücking *et* al. 2009) and represents a high proportion of the around 20,000 species of lichens known in the world (Lücking et al. 2017).

Other lichen growth forms like foliose or fruticose tend to be more conspicuous, three-dimensional and larger. In temperate forests their biomass is usually more than 1 t/ha (Rhoades 1981, McCune 1993, Arseneau et al. 1997), but it can reach from 0.1 to 3.2 t/ha (Boucher & Stone 1992) as a function of elevation, stand age, and presence of remnant trees (Berryman & McCune 2006). Our total lichen biomass estimate for the tropical dry forest is within the low to medium range of the estimated biomass for temperate forests; this seems reasonable given the dominance of crustose lichens and the smaller trees present in tropical dry forests. On the other hand, lichen biomass in temperate forests of the Pacific Northwest represent 6-15 percent of the leaf biomass in the forest (Boucher & Stone 1992), while our tropical dry forest site has 6.6 percent of the leaf biomass in just the lowest 2.5 m of the trees and 59 percent when considering all tree strata. These high values suggest that similar to moist temperate forests, lichens in tropical dry forests do play significant roles in the ecosystem.

Not surprisingly, most studies of lichen biomass are from temperate forests. We could only find four studies from tropical areas that provided a specific estimate for lichen biomass at the stand level, none of which included crustose lichens (Table 4.2). Interestingly, all of them estimated lower values of lichen biomass than in the temperate forests: 0.006 t/ha for a montane rain forest in Colombia (Forman 1975), 0.1 t/ha for an upper montane forest in Uganda (Pentecost 1998), 0.14 t/ha for a montane moist evergreen broad leave forest in China (Li et al. 2011), and 0.16 to 0.20 t/ha for a montane moist forest in Ecuador (Werner et al. 2012). Note that one challenge in interpreting the literature is that some other studies dealing with epiphyte biomass in the tropics have included lichens, but did not provide a separate number for each epiphyte group and instead, mixed bryophytes, lichens and vascular plants into a single value (Edwards & Grubb 1977, Wolf 1993, Hofstede et al. 2001, Nadkarni et al. 2004, Gehrig-Downie et al. 2011).

Considering not only the lowest 2.5 m of the trees, but also the total lichen biomass at the stand level, we report the highest recorded value of lichen biomass per hectare for a tropical ecosystem. Even though our study area harbors lichen communities

of great diversity and abundance, we believe this comparison is simply the consequence of smaller number of studies, and especially, of ignoring the crustose component of biomass. We also predict that other areas of tropical dry forest that lack a coastal influence will have lower values of lichen biomass than our study area.

We suggest that the crustose lichen component should not be underestimated *a priori* in ecological studies, especially in areas with a significant cover of crustose lichens. Such habitats include the neotropical lowland rainforests (Komposch & Hafellner 2002), leaves of tropical humid forests (Lücking 2008), temperate stands of red alder in the Pacific Northwest (Harrington 2006) or even coastal forests in Florida (Lücking et al. 2011). From the perspective of a tropical dry forests, lichens are abundant and diverse, but work is needed to understand their functional role, their interactions with other organisms (trophic or otherwise), and their variability at different localities within the same ecosystem. In this study we provided a first and essential step to include lichens as part of integrative studies of ecosystem functioning and trophic webs in tropical dry forests.

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Table 4.1 Estimated tree density and lichen biomass on trees per DBH category.

		lowest 2.5 m of the trees		All tree strata
DBH (cm) of trees	Tree density per ha	Lichen dry mass per tree (g)	Lichen biomass contribution (kg/ha)	Lichen biomass contribution (kg/ha)
3	1054.62	21.26	22.42	204.19
4	890.52	28.66	25.52	232.87
5	751.95	35.97	27.05	247.04
6	337.56	43.43	14.66	134.00
7	285.04	50.28	14.33	131.08
8	240.69	59.60	14.35	131.27
9	203.23	64.80	13.17	120.55
10	171.61	72.21	12.39	113.46
11	86.57	79.08	6.85	62.69
12	73.10	39.27	2.87	53.85
13	61.72	45.28	2.79	49.00
14	52.12	46.78	2.44	45.38
15	44.01	50.71	2.23	41.31
16	37.16	53.35	1.98	35.77
17	31.38	58.83	1.85	32.79
18	26.50	62.74	1.66	28.95
19	22.37	65.14	1.46	26.05
20	18.89	65.94	1.25	22.76
21	22.04	70.41	1.55	27.79
22	18.61	75.20	1.40	24.88
23	15.72	78.17	1.23	22.38
24	13.27	80.45	1.07	19.44
25	11.21	84.41	0.95	16.80
26	9.46	88.77	0.84	14.75
27	7.99	92.04	0.74	13.16
28	6.75	93.10	0.63	11.50
29	5.70	95.68	0.55	9.80
30	4.81	101.64	0.49	8.87
31	11.41	105.57	1.20	21.74
32	9.64	102.25	0.99	18.01
Total	**4525**		**180.88**	**1922.12**

Table 4.2 Lichen studies of biomass at the stand level with an emphasis in tropical areas.

Source	Vegetation type	Country	Measured trees	Lichen type	Lichen biomass (kg/ha)	Tree strata
Rhoades 1981	Temperate forest of *Abies lasiocarpa*	U.S.A.	10	Macrolichens	1427-2079	all
McCune 1993	Temperate forest in the Pacific Northwest	U.S.A.	42	Macrolichens	950-1870	all
This study	Tropical dry forest	Mexico	62	Microlichens	180 (155-206)	lowest 2.5 m
This study	Tropical dry forest	Mexico	62	Microlichens	1303-1922	all
Forman 1975	Tropical montane rain forest	Colombia	25	Macrolichens	6.9	all
Werner et al. 2012	Tropical montane forest	Ecuador	63	Macrolichens	162-204	all
Li 2011	Montane moist evergreen broad leave primary forest	China	n/a	Macrolichens	136	all
Pentecost 1998	Upper montane heath forest	Uganda	4	Macrolichens	100	all

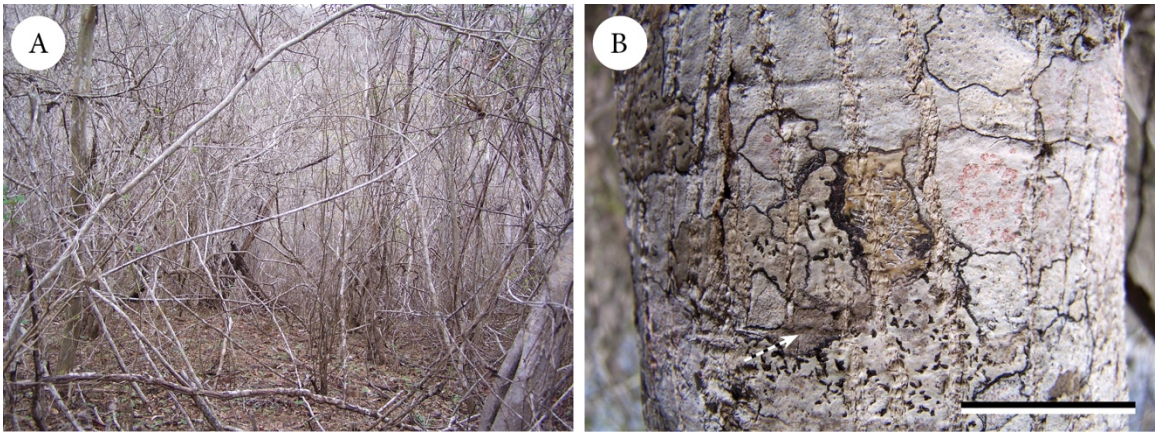

Figure 4.1 View of the study area A) Typical dense pattern of thin trees just before the start of the rainy season. B) Close up of the bark of *Helliocarpus pallidus* showing lichen cover close to 100%. Notice the small brown area (arrow) representing the original color of the bark, the rest of the bark is covered by grey to greenish-grey crustose lichens. Scale = 2 cm.

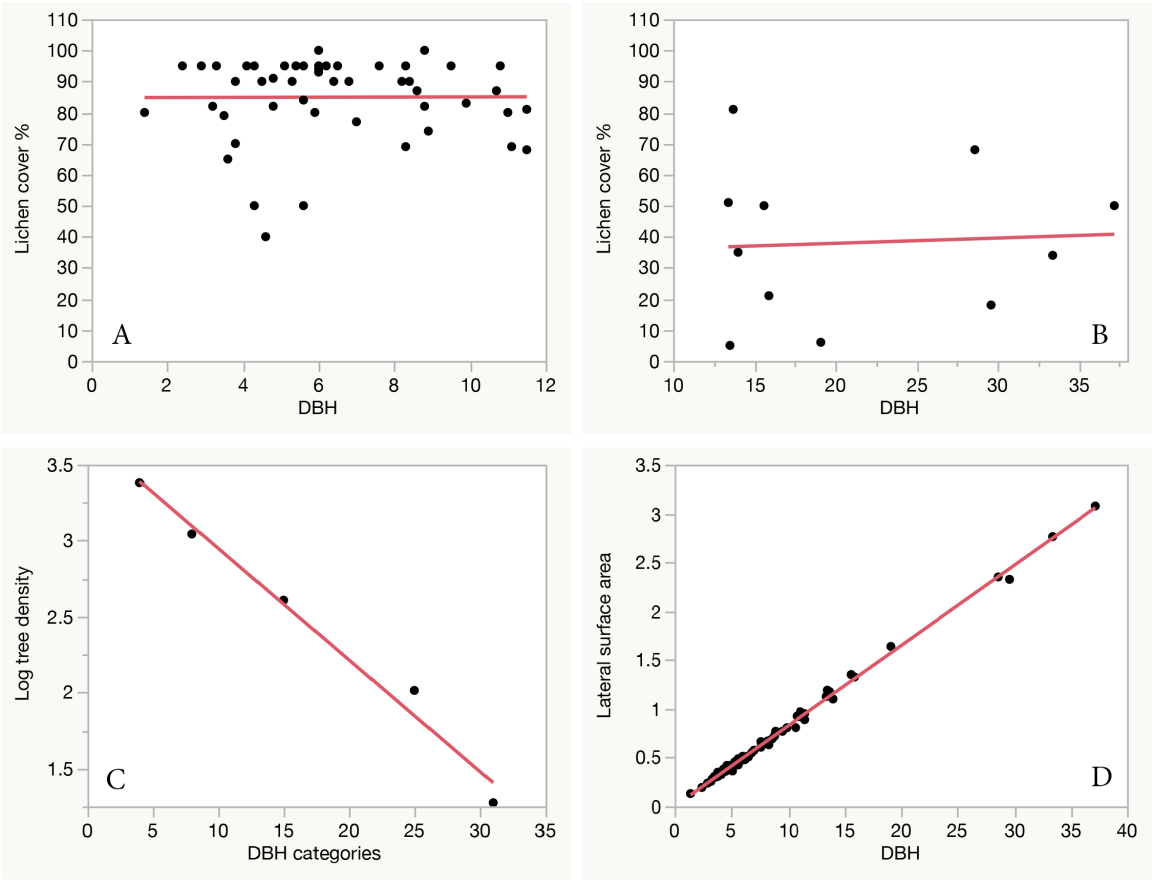

Figure 4.2 Linear regressions in this study. A, B) Relationship between diameter at breast height (DBH) and percentage of lichen cover on the lowest 2.5 m of the bark of trees. A) range of DBH from 3–11.9 cm ($R^2 < 0.001$, $p = 0.97$). B) range of DBH from 12–37 cm ($R^2 = 0.003$, $p = 0.85$). C) Relation between the five DBH categories of Lott et al. (1987) and the number of trees per hectare in a logaritmic scale ($R^2 = 0.98$, $p < 0.001$). D) Relation between DBH and the lateral surface area in m^2 for the lowest 2.5 m of the main trunk of trees ($R^2 = 0.99$, $p < 0.001$).

Appendix 4.1 Dry mass per unit area for common lichens in the study area.

Lichen sample	Dry mass (g/m2)
Arthonia sp 1	73.50000
Arthonia sp 2	32.50000
Bathelium sp 1	82.50000
Fissurina sp 1	136.00000
Gymnographopsis sp 1	71.00000
Lecanora sp 1	96.50000
Pertusaria sp 1	151.50000
Pyrenula sp 1	181.00000
Pyrenula sp 2	121.50000
Pyrenula sp 3	84.00000

CHAPTER 5. THE OVERLOOKED PRIMARY PRODUCERS: LICHEN HERBIVORY AS A MAJOR CARBON FLUX IN THE TROPICAL DRY FOREST

Ricardo Miranda-González & Bruce McCune

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Abstract

Measured herbivory rates in terrestrial ecosystems are usually limited to leaf consumption, however, less conventional nutrient sources may have large impacts as well. We studied the lichen consumption component of herbivory in a tropical dry forest of Mexico and compared it to the leaf herbivory rate. Using high definition photographs of permanent microplots, we calculated the area of lichens consumed by herbivory across a four-year study. Lichens were consumed regularly and at an annual rate of 11.5%, with no significant difference between years even in the presence of catastrophic events like the category 4 hurricane Patricia. Lichen biomass annual consumption per hectare represents 28.5% of the biomass lost to total herbivory when considering leaf folivory (chewing) and lichenivory together. This is the first study that calculates annual lichen herbivory rates at the ecosystem scale for a tropical area. Our results show that lichen consumption is an established and regular process in the forest dynamics of the tropical dry forest. The high and constant values of lichen consumption indicate that lichens have an unexpected but substantial functional role in the forest.
Key words: Mexico, Chamela, ecosystem scale, hurricane, invertebrates, lichenivory.

Introduction

Herbivory is a key component in nutrient cycling and in the availability of labile forms of nitrogen and phosphorous (Metcalfe et al. 2014). For many tropical ecosystems, invertebrates contribute more than vertebrates to the total amount of herbivory in the forests (Janzen 1981, Coley & Barone 1996, Dirzo & Domínguez 1995). Given its conspicuous nature, leaf and seed consumption are the most-studied types of herbivory (Dirzo & Domínguez 2002); however, primary consumers feed on a larger set of resources. Other plant parts like roots, stems or even phloem content are harder to quantify and are typically not considered at an ecosystem scale (Coley & Barone 1996). Even less studied is the contribution by feeding on fungi (Shaw 1992) or lichens (Gerson & Seward 1977). Nonetheless, the contribution of non-traditional herbivory can be of great importance. For instance, Bodner et al. (2015) showed that 22.5 percent of the

caterpillars in a montane forest in Ecuador do not feed on leaves from their host plant, but rather on dead leaves, lichens or other epiphylls.

A recent study showed that crustose lichens in a Mexican tropical dry forest (TDF) reached biomass levels per ha equivalent to 59 percent of the leaf biomass values (Miranda-González & McCune 2019). If this large amount of resources in the form of lichens is being consumed by herbivory, then lichens could strongly contribute to the carbon and nutrient mineralization in the forest. The strong seasonality of climate and foliage in the TDF forces its inhabitants to be adapted to withstand intermittent wet and dry periods (Maass et al. 2002). At a smaller temporal scale, and given the poikilohydric nature of lichens, adaptations for similar intermittent periods are common in the invertebrates that feed on lichens (Gerson & Seward 1977). This convergence in adaptations seems ideal to favor lichen consumption at a large scale. However, for the TDF ecosystem not a single study has dealt with lichenivory rates or even with whether lichens are being consumed or not.

Data from other ecosystems show that lichen consumption can have large scale implications. In boreal ecosystems, lichens are the main winter food for caribou and other cervids and are consumed at a rate of 7.91 kg per ha (Ward & Marcum 2005), which can constitute 30 percent of the energy in the cervids diet (Ditchkoff & Servello 1998). In the Negev desert of Israel, inconspicuous lichens that grow just under the surface of rocks are the main food for two species of snails. To reach the lichens, these snails bite the rocks and contribute up to 1.1 tons per year of rock weathering into soil. At the same time, lichens are the source of 11 percent of the total nitrogen input in that ecosystem (Shachak et al. 1987, Jones & Shachak 1990). For tropical areas, information on rates of lichenivory at the ecosystem scale are non-existent; however, Lücking & Bernecker-Lücking (2000) studied the lichens growing on leaves in a tropical rain forest in Costa Rica and found that 19.6 of the lichen thalli were affected by herbivory. This suggests that lichenivory might be high in tropical forests as well.

In order to incorporate the lichen component in ecological studies of tropical dry forests, we aimed to answer four questions. 1) Is lichen herbivory a widespread and common process in the TDF? 2) How much lichen biomass is consumed on a yearly basis and how does it compare to leaf herbivory? 3) How much do lichenivory rates change between and within years? 4) What is the effect of an extreme weather disturbance (hurricane) on the lichen consumption rates in the remaining forest?

Methods

Study area

The study was made in the Chamela-Cuixmala Biosphere Reserve, located 2 km inland from the Pacific Coast of Mexico (105°W, 20°N). The tropical dry forest component of the Reserve is characterized by a warm sub-humid climate with summer rains (Garcia 2004) and an extended dry season during which more than 95% of the plants lose their leaves completely. The wet season is marked by a fast greening of the canopy which is the product of short and intense rains intercalated with dry periods. The mean annual precipitation is highly variable, ranging from 340 to 1329 mm and with a mean of 800 mm (1983-2015 period), of which 86.8% falls between the months of June and October. The area has a strong oceanic influence that maintains mean monthly values of relative humidity above 75% year-round, with mean annual temperature of 24.6°C. (Garcia-Oliva et al. 2002, Maass et al. 2002, Sánchez-Azofeifa et al. 2013, Maass et al. 2018).

The mature forest consists of trees of 4-15 m tall, in a dense pattern of up to 4500 trees per ha, with more than 50% of the stems having a diameter at breast height (DBH) smaller than 5 cm (Lott et al. 1987). The Reserve sustains 1149 species of plants of which 229 are trees. The most representative families are Leguminosae (including Caesalpinioideae, Mimosoideae, and Papilionoideae) and Euphorbiaceae (Lott & Atkinson 2006, Sánchez-Azofeifa et al. 2013). Lichen communities are mostly of crustose growth form and cover more than 80% of the bark on most trees, while macrolichens are few and usually limited to the canopy (Miranda-González & McCune

2019). The most representative lichen families are Arthoniaceae, Graphidaceae and Pyrenulaceae (Miranda-González 2012).

The dry forest in the Reserve is considered well conserved and it is immediately surrounded by a matrix of well conserved and secondary forest outside the Reserve (Sánchez-Azofeifa et al. 2009). Nonetheless, the area was severely damaged by hurricane Patricia in October 2015. Most of the damage was concentrated in the forest canopy, decreasing its mean tree height from 6.8 m to only 3.5 m (Parker et al. 2018).

Microplot analysis

19 trees were randomly selected from among four different sites of the study area. For each tree we randomly selected an area on the main trunk and marked a permanent microplot of 3 by 5 cm using three pinheads attached to the bark of the tree. High definition photographs were taken for each microplot using a tripod and a Nikon 5300 camera with a flash ring attached to a macro lens. Each microplot was photographed twice a year, once in the summer and once at the end of the year from 2014 through 2017. To avoid inaccurate measurements, all photographs included a measuring tape for scale, and all were taken when lichens were dry.

Photographs were edited in Adobe Photoshop CS6. Using the pinheads as reference, an equal area was selected for all photographs from the same microplot. Herbivory marks were manually delineated using an Intuos Wacom tablet connected to the computer and their area was calculated in Photoshop to the nearest 0.01 mm^2. Herbivory measurements were transformed as percentage of the total area of each microplot to facilitate comparisons. Special care was taken to only include new herbivory events at each part of the temporal cycle and to distinguish between herbivory and other changes like bark exfoliation or lichen disease or parasitism.

Typically, herbivory by chewing insects is recognized by a contiguous area inside the lichen thallus with a different coloration and a smoother texture (Fig. 5.1A), sometimes the exposed area reaching the bark of the tree. Herbivory by molluscs appears

as zigzag deep cuts that sometimes are intercalated with thin uneaten areas (Fig. 5.1B); typically the disturbed area in the lichen thallus is wider than if done by chewing insects. Herbivory by mites tends to be less conspicuous but longer in duration, initially it appears as a discoloration or minute holes in the lichen thallus, eventually a network of tunnels or swollen and hollow areas that may flake out and become visible (Fig. 5.1C-E). In contrast, fungal infections start as small incongruences in the lichen thallus (e.g. a fruiting body that does not belong to the lichen) and then, a general decay of the lichen that gets worse over time (Fig. 5.1C-D). Finally, bark exfoliation appears as a piece of lichen suddenly disappearing (Fig. 5.1F-G), the missing area may be rectangular or irregular and may disappear after a previous rise in the bark. Bark exfoliation occurrence and appearance is related to the species of tree.

Herbivory between years and hurricane effect

As our data only included new herbivory in each photograph, it was possible to add the herbivory values both of summer and end of year into a single yearly value. We compared herbivory between years with a Repeated Measures Analysis of Variance. The response variable "Herbivory per year" measured as a proportion was arcsine square root transformed to achieve a normal distribution (Boege 2004). This transformation was preferred over the logit transformation as the latter one cannot handle zero values (Rao 1998). Each microplot was considered as a random effect and was nested within one of the four sites in the study area. The variable "year" was considered a fixed effect. In a second analysis, "year" was substituted by "hurricane effect" which was applied as a binary factor with a value of one assigned to the years 2015 and 2016 (hurricane hit on October 2015) and zero to the years 2014 and 2017.

Herbivory within years

Given that the intervals between photographs within each year were not the same (Appendix 5.2), we transformed the amount of total herbivory in each photograph into amount of herbivory per day. Because the lichen herbivory marks remain distinguishable for more than one photographic cycle (lichen regrowth was slower than the interval between photographs), it was not necessary to calculate proportionate herbivory rates

with a compound interest formula (McCune & Cottam 1985); therefore, the amount of herbivory per day was calculated by dividing total herbivory by the number of days present at each photographic interval.

Logistical difficulties in taking the photographs and the high variability in onset and duration of the rainy season caused the intervals between photographs to track imperfectly the differences between dry and wet seasons (Appendix 5.2). Therefore, our data were inadequate to compare dry and wet seasons. Instead, we divided the years into two parts, the first part includes the dry season and a part of the rainy season, while the second part includes the rest of the rainy season.

A Repeated Measures Analysis of Variance was performed using the response variable "Herbivory per day", which was arcsine square root transformed to achieve normality. Each microplot was considered as a random effect and was nested within one of the four sites in the study area. The variable "part of the year" was considered a fixed effect.

Stand level estimation of lichen herbivory

To extrapolate our microplot values of herbivory to the stand level (1 ha), we used the lichen biomass estimation of Miranda-González & McCune (2019) from the same study area. We applied the average of lichen herbivory, across our four-year study, to the lichen biomass estimation per hectare at two levels: all strata of trees and only the lowest 2.5 m of the trunks.

Our herbivory estimates are based only on microplots that were positioned on trunks at a height below 3 m. Typically, the arthropods in the canopy tend to be more abundant and diverse (Neves et al. 2014); however, in the same study area Vega-Badillo et al. (2018) found no difference in richness or diversity of Coleoptera between the canopy and the understory. Although it is possible that herbivory rates are higher in the canopy, we followed a conservative approach, using the herbivory rate from the understory as a proxy for herbivory in the canopy.

Results

We analyzed the amount of lichen herbivory from 19 microplots (Fig. 5.2) twice a year for a period of four years and with a total of 151 photographs with one missing photo (Appendix 5.2). Lichen herbivory was present in 97% of the individual photographs (n = 151, range 0–54% of the area) and in 98% of the yearly cycles (n = 75, range 0–56% of the area). The mean lichen herbivory throughout the four years of study was 11.5% of the analyzed area each year. A Repeated Measure Analysis of Variance on herbivory per year, showed no statistically significant differences between years and no random effect of the site or of the microplot (Table 5.1, Fig. 3A).

Of the four studied years, the highest herbivory value occurred in 2016, which was the year after the hurricane, followed by the year 2015. However, the herbivory values during those two years (hurricane effect) were not statistically different than in the other years of our study (Table 5.1, Fig. 3B). However, there was a tendency in the years with a hurricane effect to have more variance in the data (Levene's test, $F = 4.6$, $p = 0.03$) and an increase in isolated peaks of herbivory at the microplot level. Across the 19 microplots, the highest herbivory value occurred for seven of them in 2016, five in 2015, four in 2017, and three in 2014 (Appendix 5.2).

Herbivory was about twice as high during the second versus the first part of the year ($p < 0.01$), with an average of 0.020% and 0.044% of lichen cover consumed daily for the first and second part of the years respectively (Table 5.1, Fig. 5.3C). In particular, the difference was noticeable during the years 2017 and 2016.

The average annual lichen herbivory, together with the 1.30–1.92 t/ha of lichen biomass estimated at the stand level by Miranda-González & McCune (2019), suggest an annual lichen consumption of 149–220 kg of lichen dry weight per hectare of forest. In the same way, if only the lowest 2.5 m of the trunks in the forest are considered, the

expected annual lichen consumption is 20.8 kg of lichen dry weight per hectare (range 17.8–23.7 kg/ha using ± 1 SD).

Discussion

Lichen herbivory as a constant stochastic process

Lichen consumption is an established and regular process in the forest dynamics of the study area. Ninety seven percent of the 151 analyzed photographs had evidence of herbivory. Yet during the four years of our study, lichen herbivory rates on the main trunk of standing trees had no consistent differences across years, even when including drastic events like hurricane Patricia.

The observed patterns of lichen herbivory were remarkably similar to the ones of leaf herbivory. For instance, a three year-long study by Filip et al. (1995) found no differences in the yearly leaf herbivory of 16 species of trees in the same study area, even though their study happened to include both the wettest and the driest years in the recorded history of the Reserve at that time. Both lichen and leaf herbivory follow a pattern in which most individuals get low to medium levels of herbivory while few, apparently random individuals, experience disproportionally large herbivory (Dirzo & Domínguez 2002).

Leaf herbivory in the TDF is limited to a short amount of time per year. Even though the wet season lasts an average of 4.9 months per year (Maass et al. 2018), most of the herbivory is concentrated on the first weeks of the wet season (Filip et al. 1995). Two explanations for this are that the peak abundance of invertebrates likely occurs in the first half of the wet season (Janzen 1981), and that plants increase their defenses as the season advances (Boege 2004), while at the same time, losing nutritional value and water content (Janzen & Waterman 1985). Lichens on the other hand, are present regardless of the time of the year and can be considered as perennial resources for herbivores.

Our study was not designed to compare lichen herbivory between seasons, however, we found evidence of herbivory year-round with higher values during the wet season. This agrees with the abundance patterns of insects in the area, as well as with the activity of land snails that are restricted to the wet season (Smith & Temple 1982) and are generally known to be lichen feeders (Asplund et al. 2010, Miquel & Bungartz 2017). Nonetheless, the evidence of lichenivory during the dry season suggests the presence of animals that feed on them. Among the hundreds of invertebrate species that are active during the dry season in the Reserve, the order Psocoptera is of special interest. Insects in this order have high abundance and species richness during the dry season (Pescador-Rubio et al. 2002), and several of its species are well known as voracious lichen feeders (Laundon 1971, García-Aldrete 2014).

Lichens could also be especially important for invertebrates whose life cycle is longer than the wet season. Among the many species of caterpillars in the study area, Luviano et al. (2018) found that a species in the family Psychidae ("Psychidae sp. 1") is one of the most abundant in the forest; however, their efforts to rear adults by providing leaves from the host tree were unsuccessful (Ek del-Val personal communication, 2017). Our field observations showed that this species is actually a lichen feeder. This agrees with the literature, as many species in that family are known to feed on lichens, leaves or a combination of both (Sobczyk 2011). The Psychidae are also known for having a larval stage that typically requires several months in the Neotropics (Rhainds et al. 2009). Given the short duration of the wet season and the abundance of caterpillars of this family in the study area, lichens are expected to be a vital food resource for this group.

Hurricane effect on lichen herbivory

After a hurricane hits a forested area, most of the leaves get blown away (Lugo 2008). This has an immediate effect on the folivorous invertebrates that remain after the strong winds which can cause a period of greatly reduced herbivory in the forest (Koptur et al. 2002). The synchronized regrowth of leaves can happen in a few weeks, as in our study area (Parker et al. 2018), or in a few months as in other areas (Frangi & Lugo 1991, Koptur et al. 2002). But regardless of the massive input of new leaves, the herbivory

levels after a hurricane are context-dependent. In some cases, folivory values tend to dramatically decrease in the months after a hurricane, in part, because many folivore invertebrates such as beetles and caterpillars have complex and synchronized life cycles that stops them from taking advantage of the sudden increase of new leaves (Angulo-Sandoval et al. 2004, Koptur et al. 2002). In other cases, folivory values dramatically increase as a consequence of a surge in the abundance of invertebrate folivorous and resources (Torres 1992, Hunter & Forkner 1999, Spiller & Schoener 2007).

In contrast to leaves, lichens in our study area do not regrow quickly after a hurricane. The less intense hurricane Jova, which reached our study area in 2011, noticeably reduced the abundance in the canopy of the fruticose lichen genus *Roccella.* Furthermore*,* its abundance did not recover in the following years (RMG personal observation), however, hurricane Patricia not only detached epiphytes but actually took the canopy away (Parker et al. 2018), including its abundant crustose lichens. To fully recover lichen abundance in the upper canopy, it is first necessary that branches in the canopy grow back, only after that, would lichens have a chance to regrow.

On the other hand, the abundances of most of the lichens on the remaining branches and main trunks were not much affected by the hurricane, if the tree remained in place, so did the lichens. We did not observe *in-situ* lichen mortality in response to the hurricane. Lichen herbivory appeared to increase slightly after the hurricane, but the increase was not statistically significant (fig. 5.3B).

In the same study area, Novais et al. (2018) found that three months after hurricane Patricia, sap-sucking, predatory and xylophagous beetles increased in abundance, but chewing folivorous beetles remained constant. Similarly, abundance of Lepidoptera before and after the hurricane was within the typical year to year fluctuation for the study area (Luviano et al. 2018). Given the continuity of the lichen communities and chewing invertebrates after the hurricane, it is reasonable to expect similar levels of lichen herbivory before and after the hurricane.

However, our herbivory estimates did not include the abundant fallen trees and branches after the hurricane. It is quite plausible that as lichens start to decay on the fallen branches, they will be an easier target for herbivores and detritivores (Asplund & Wardle 2012), effectively increasing the lichen consumption in the forest after the hurricane. In temperate ecosystems, lichens on fallen branches are typically consumed within one year (McCune & Daly 1994), however, those rates are based on macrolichens that besides being consumed by mollusks, are also consumed by larger animals like elk or deer, especially when fallen branches make the macrolichens accessible on the forest floor. Currently, no information is available for microlichen litter decomposition or consumption in the TDF.

Lichen herbivory in the ecosystem context

Most of the invertebrate herbivory in the TDF is caused by sap-sucking, underground chewing, xylophagy, gall formation, and leaf chewing, but given methodological difficulties, the current knowledge focuses on leaf chewing events (Dirzo & Domínguez 2002). In our study area, Filip et al. (1995) used a long-term approach and found that 17% of the leaf area in the forest was consumed by leaf-chewing herbivores every year, whereas Cuevas-Reyes et al. (2006) found that 18.9 percent of the leaf area is consumed by gall-forming herbivores. Those values are very similar to the 11.5 percent lichen herbivory found in this study.

Based on the mean leaf (Vizcaíno 1983, Martínez-Yrízar et al. 1990, 1996) and lichen (Miranda-González & McCune 2019) biomass per hectare in the TDF of the Reserve, we calculated the proportion of the total consumed biomass that comes from lichens or leaves. We used the simplified assumption that percent of leaf area loss is equivalent to percent of leaf biomass consumed. When we include consumption of leaves by gall-formers (Cuevas-Reyes et al. 2006), leaf-chewing (Filip et al. 1995) and lichen-chewing invertebrates, 15.9 percent of the primary producer's biomass consumed in the forest corresponds to lichens. Given the lack of studies at the ecosystem scale about gall forming leaves in other regions (Grandez-Rios et al. 2015), we also calculated the proportion of lichen biomass consumed in relation to only leaf consumption by chewing.

In this case, lichen consumption represents 28.5 percent of the biomass consumption in the forest.

These high and sustained values of lichen consumption indicate that lichens have an unexpectedly substantial role in the functionality of the ecosystem, specifically, as primary producers at the base of trophic networks throughout the year. Ecological studies about lichens at the ecosystem level are still rare in tropical areas, however, we expect similar values in areas with abundant lichen communities. It seems clear that including lichens in the research of TDF will contribute to understand a significant part of the ecosystem processes necessary for the functionality of the forest.

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Table 5.1 Results of the three independent Repeated Measures Analyses of Variance in this study.

Herbivory between years

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Site	0.04284	0.01428	3	0.3807	0.7684
Year (Fix)	0.02582	0.00861	3	0.2779	0.841
Plot[Site] (Random)	0.52514	0.03751	14	1.2115	0.2964

Herbivory and the hurricane effect

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Site	0.03712	0.01237	3	0.361	0.7821
Hurricane effect (Fix)	0.0157	0.0157	1	0.5258	0.4716
Plot[Site] (Random)	0.47982	0.03427	14	1.1479	0.3419

Herbivory within years

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Site	0.04712	0.01571	3	1.0213	0.411
Part of the year (Fix)	0.12736	0.12736	1	11.795	0.0008*
Plot[site] (Random)	0.2307	0.01538	15	1.4243	0.145

Figure 5.1 Herbivory signs on lichens and other common patterns. A) Chewing insects, notice the contiguous area inside the lichen thallus with a different coloration (white arrow) and the camouflaged caterpillar (striped arrows) of the bagworm family (Psychidae) responsible for the herbivory. B) Molluscs, notice the deep zigzag marks (striped arrow). C-D) Fungal infection, two photographs of the same area taken 1.5 years apart. Notice an initial decay of the lichen thallus (black arrow in C) and black fruiting bodies that do not belong to the lichen. In panel D a wide spread decay of the lichen thallus is shown with patches of brown bark visible. C-E) Mites, long term effect of subtle herbivory, notice in panel C a change in color and the start of longitudinal scars (striped arrows) in the lichen thallus. In panel E (close-up of panel D), notice the proliferation of scars (striped arrow) in the form of tunnels. F-G) Bark exfoliation, two photographs of the same area taken 6 months apart, notice in panel F the initial rise of a piece of bark (striped arrows) and its disappearance (striped arrows) in panel G. All scales equal 1 cm.

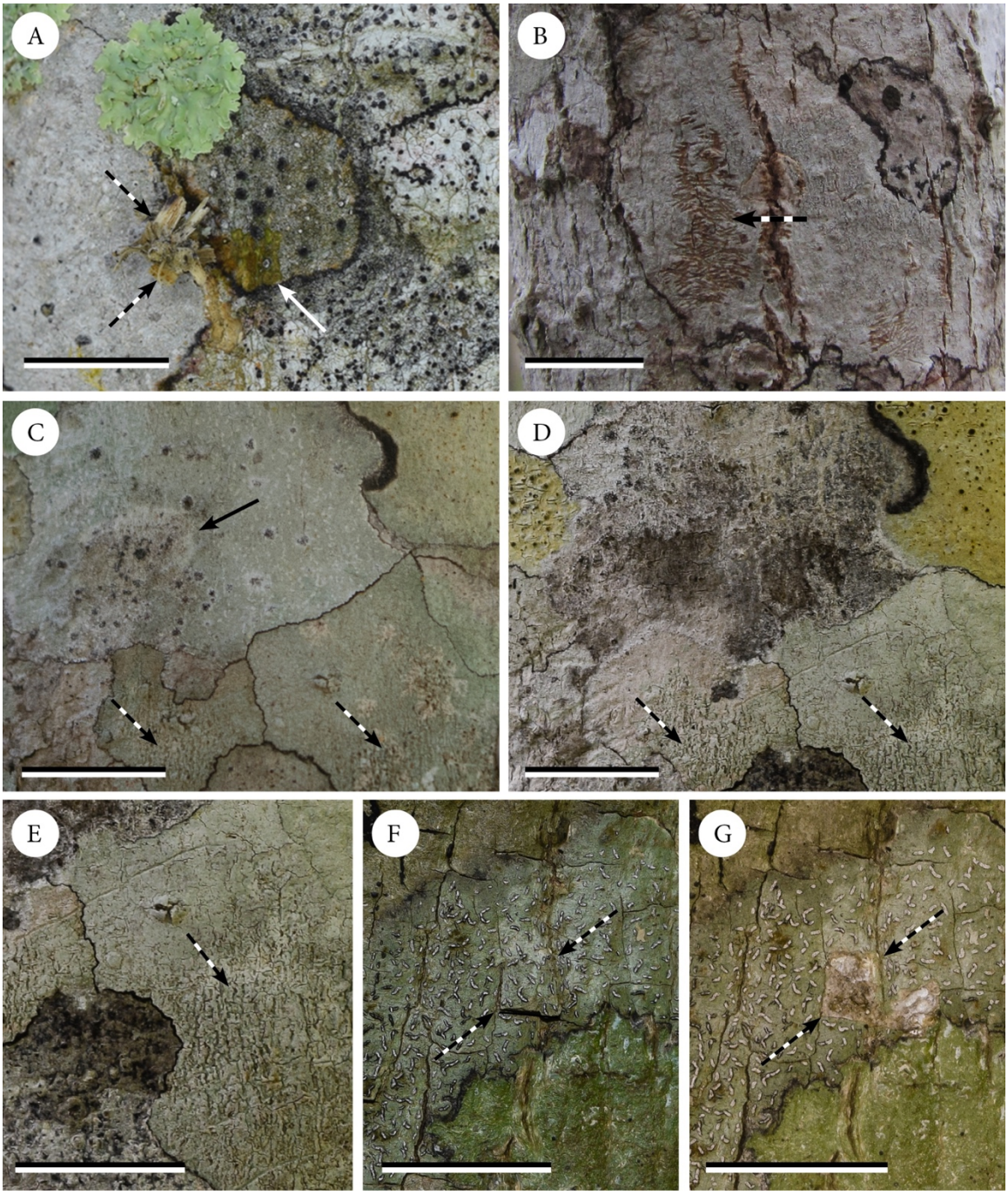


Figure 5.1 Herbivory signs on lichens and other common patterns.

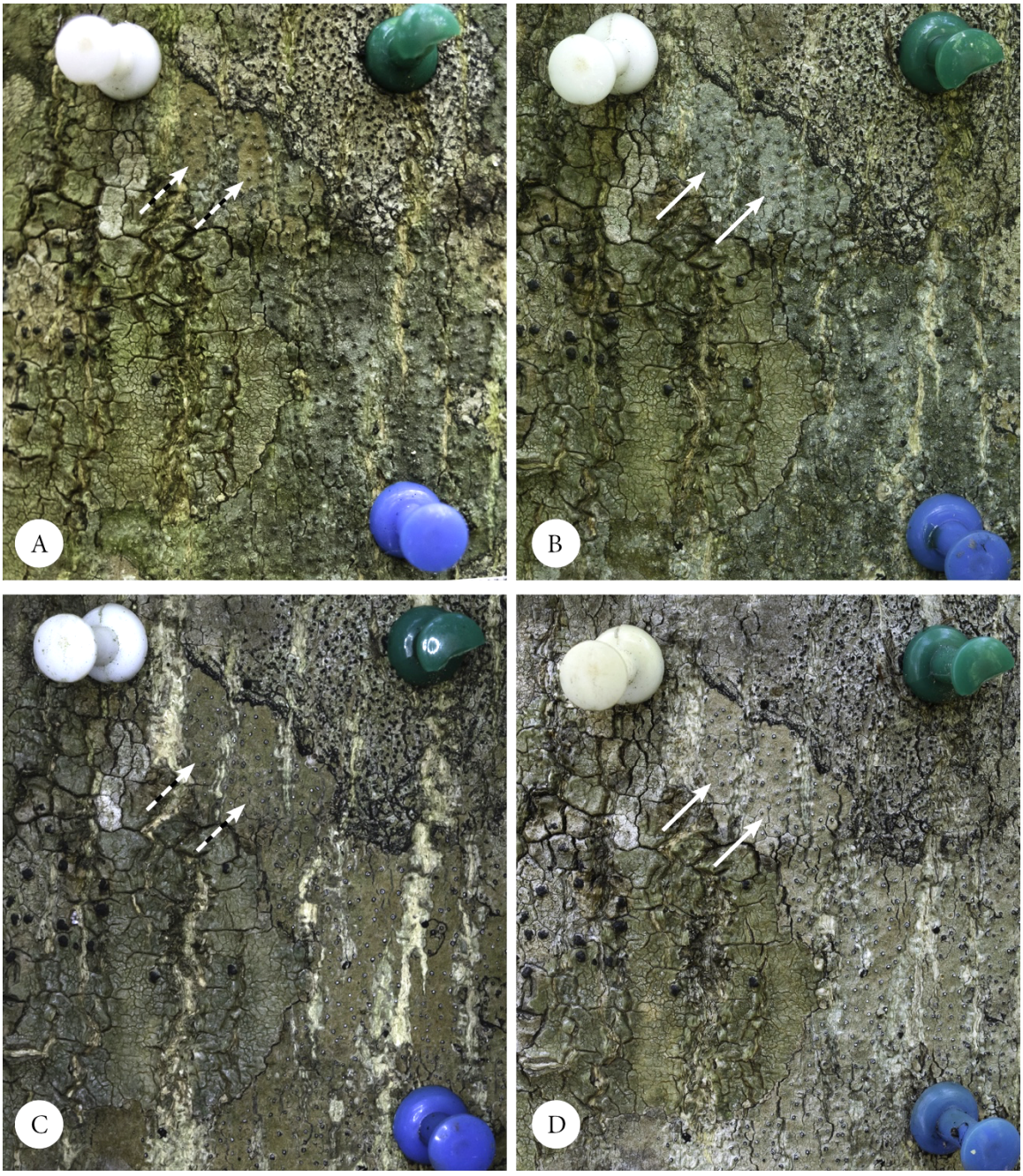


Figure 5.2 Subsample of a typical microplot. A) January 2015. B) August 2016. C) December 2016. D) August 2017. Notice the intercalate herbivory and regeneration. In panels A and C, the striped arrows show eaten parts of the lichen thallus exposing the brownish bark of the tree. In panels B and D, those same parts of the lichen thallus show a greyish color signaling the regeneration of the lichen. Scale, distance between top pinheads of each panel equals 2 cm.

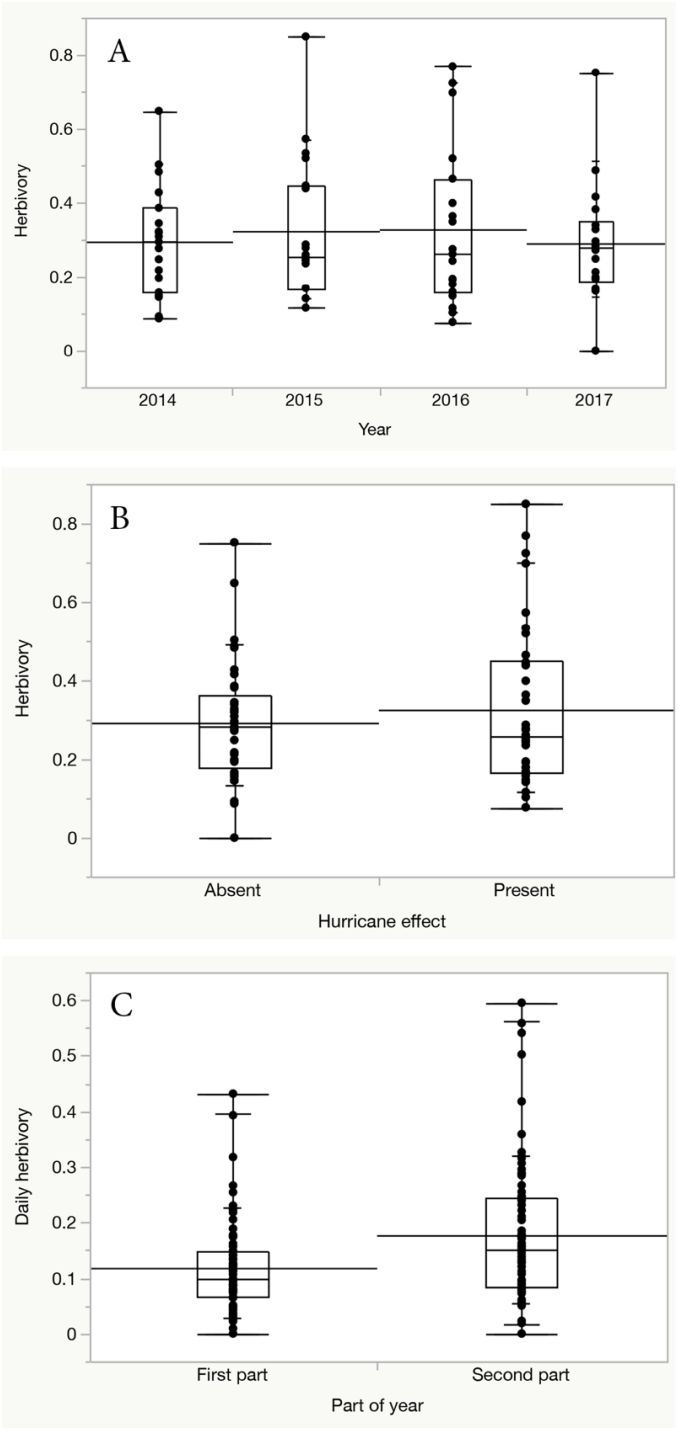
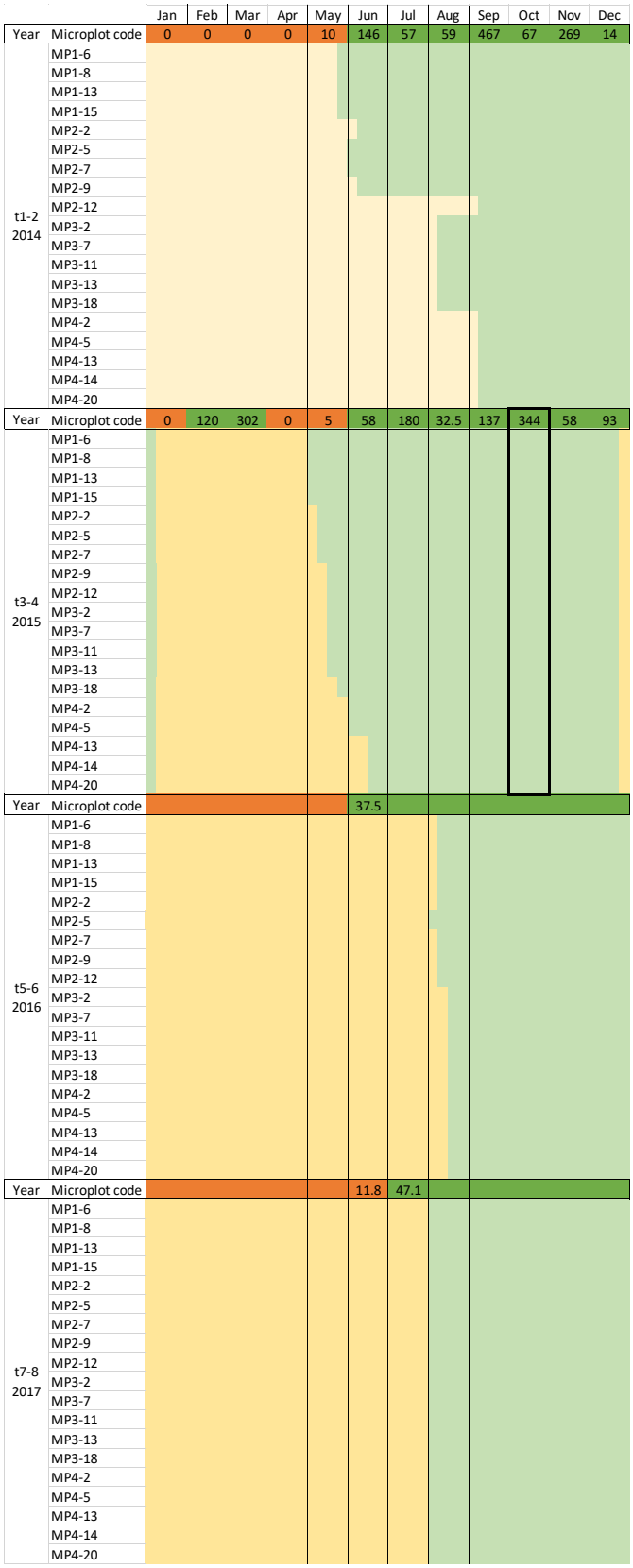


Figure 5.3 Graphical representation of the Repeated Measures Analyses of Variance in Table 5.3. The *Y*-axis of each panel was arcsine square root transformed. A) Annual rates of lichen herbivory across the four-year study. B) Annual rates of lichen herbivory and the hurricane effect. C) Daily rates of lichen herbivory within years. All panels show, for each value of *X,* a mean horizontal line (longest) and a quantile box plot which may include quantiles for 10% and 90% of the data (smallest lines), and 2% and 98% of the data (medium lines).

Appendix 5.1 Visual representation of the timing for each microplot's photograph. The figure is divided in four stacked rectangles that represents 1-year intervals. Each rectangle is further divided in two colors that signal the interval between two photographs: light yellow for the first part of the year (mostly dry season) and light green for the second part of the year (mostly rainy season). Numbers in dark orange or green, are mm of rain per month when available. Vertical lines in the center of the picture are there for guidance. Bold lines in the year 2015 represents the timing of hurricane Patricia.



Appendix 5.1 Visual representation of the timing for each microplot's photograph

Appendix 5.2 Area (percentage) of lichen herbivory per microplot. Each column represents the time interval between two photographs. Light orange columns are from the first part of the years (mostly dry season) and green columns are from the second part of the years (mostly rainy season). Hurricane Patricia hit the study area in late 2015 (bold column). The dash is a missing value.

microplot	t1 summer 14	t2 Jan 15	t3 summer 15	t4 Dec 15	t5 summer 16	t6 Dec 16	t7 summer 17	t8 Dec 17
MP1-6	0.88	22.29	0.96	1.86	11.00	13.57	1.58	2.13
MP1-8	2.42	1.41	2.40	53.99	6.92	13.10	5.31	2.47
MP1-13	1.09	1.36	0.67	6.86	1.34	1.17	1.58	1.01
MP1-15	0.00	0.75	0.46	0.87	0.62	12.02	0.00	2.76
MP2-2	5.25	8.89	8.39	17.41	4.22	1.56	3.02	0.88
MP2-5	15.21	6.34	17.73	11.63	0.11	0.94	0.38	10.69
MP2-7	2.03	34.37	5.90	18.73	15.00	0.07	–	1.46
MP2-9	3.63	6.24	1.86	4.48	5.24	6.42	1.72	4.33
MP2-12	6.38	2.85	1.09	17.48	3.45	3.23	1.17	20.70
MP3-2	4.58	1.40	2.02	4.56	2.65	1.00	6.68	9.61
MP3-7	1.71	0.37	0.18	1.79	1.68	5.68	0.21	4.25
MP3-11	1.34	3.32	1.94	3.52	4.69	36.52	4.27	4.22
MP3-13	4.59	2.87	0.08	5.75	1.78	0.39	1.42	1.15
MP3-18	5.23	4.78	0.76	1.21	40.95	7.40	0.02	13.86
MP4-2	16.87	0.29	7.34	0.67	1.03	2.17	6.80	0.42
MP4-5	0.18	2.00	5.87	12.12	0.54	0.05	0.00	0.00
MP4-13	3.05	8.34	2.73	3.27	2.31	41.48	0.52	9.81
MP4-14	0.51	0.34	1.47	4.31	0.40	0.92	0.44	9.93
MP4-20	4.35	4.06	1.35	1.43	0.28	3.46	2.11	44.55

CHAPTER 6. LICHENS USED AS CONSTRUCTION MATERIAL FOR LEPIDOPTERA'S HOUSING: A MOLECULAR APPROACH TO A WIDESPREAD AND HIGHLY SELECTIVE INTERACTION

Ricardo Miranda-González

Target journal: *Fungal Ecology*

Abstract

Interactions between invertebrates and lichens are widespread. Lichens usually participate as food, shelter, background for mimicry, or as a custom-made camouflage for animals to wear. This last case is particularly intriguing and has evolved independently in at least land snails, beetles, and immature stages of the insect orders Lepidoptera, Neuroptera and Psocoptera. Unfortunately, an invertebrate's behavior of attaching minute pieces of lichens onto its body makes identifying the lichens extremely difficult, which strongly limits our understanding of those interactions. During a study of lichens from tropical dry forest in Mexico, caterpillars of a moth species of the family Psychidae were discovered living inside mobile bag-like domiciles made from silk and completely covered with small pieces of lichens. For this study molecular techniques were used to identify the lichens used as construction material for the caterpillar bags and also to analyze the caterpillar selectivity for particular species of lichens. Nine caterpillar bags in good condition were selected and the ITS gene was recovered for every piece of lichen present that looked slightly different. A total of 33 lichen sequences were obtained and compared against a newly generated ITS database from the lichens in the study area. Of the around 300 species expected to occur in the area, only five of them were used by the caterpillars. Furthermore, there was a strong selectivity for micro-foliose lichens of the family Physciaceae, even though they represent a small fraction of the mostly crustose lichens present in the forest. Our results suggest that the caterpillars select particular species of lichens at a higher rate than what is expected by chance based on their local abundances. The methodological approach presented here provides an accessible way to study these widespread interactions, which hopefully will help to increase our understanding of the ecological roles that lichens play in ecosystems.
Keywords: Camouflage, Chamela, *Dirinaria*, Oiketicinae, Psychidae.

Introduction

Decorating behavior, or the attaching of environmental materials to the exterior of the body (Ruxton & Stevens 2015), is a common practice present to some degree in around

25 percent of the phyla in the animal kingdom (Berke et al. 2006). Among insects, fossil records show that decorating behavior was already present at least 130 million years ago (Pérez-de la Fuente et al. 2012, Wang et al. 2016). Currently, decorated species exist in six orders of insects: Coleoptera (Brown & Funk 2010), Hemiptera (Jackson & Pollard 2007), Lepidoptera (Rhainds et al. 2009), Neuroptera (Tauber et al. 2014), Psocoptera (Henderson & Hackett 1986), and Trichoptera (Ferry et al. 2013).

Decoration can theoretically provide several advantages, but its effect on the predator-prey interactions is the most common and best studied (Ruxton & Stevens 2015). Experimental studies concluded that decorated predators can capture prey without being noticed (Eisner et al. 1978) and, on the other hand, that decorated prey can avoid being attacked (Nakahira & Arakawa 2006). The mechanism to avoid being noticed consists of visually (Cott 1940) or chemically (Vencl et al. 1999) deceiving others by incorporating a layer of exogenous material like feces, dead insects, sand, rocks, lichens, or plant materials.

Among the materials for decorations, the use of lichens is well known in the scientific literature, with prominent examples like the Chrysopidae (Neuroptera) larvae of *Leucochrysa pavida* (Skorepa & Sharp 1971) and the Psychidae (Lepidoptera) caterpillars of *Luffia ferchaultella* (McDonogh 1939, Sims 1999). Other less known examples include larvae of the Neuroptera *Hemerobius* sp. (Sowerby 1806, Cott 1940), larvae of the Psocoptera *Trichadenotecnum fasciatum* (Henderson & Hackett 1986), caterpillars of the Lepidoptera *Eudarcia richardsonii* (Richardson 1974) and adults of several species in the Coleoptera genus *Gymnopholus* (Gressitt et al. 1965, Gressitt 1966). But by far most of the interactions with lichens occur with Lepidoptera (Richardson 1974, Weber 1974, Sigal 1984, Hawksworth 1991, Núñez Aguila 2006).

Most of our current knowledge is limited to simply describing that lichens have been used for camouflage, without information on the lichen species selection or any other ecological pattern (see exceptions in Gressitt 1966, Henderson & Hackett 1986, and Wilson & Methven 1997). Furthermore, the entomological and lichenological

communities are disconnected in the literature, with many cases of lichen use as camouflage by lepidopterans not reaching the lichenological community (Davis 1964, 1975, Núñez Aguila 2004). The limited knowledge is also a result of the difficulty, even by lichen specialists, of studying minute lichen fragments detached from the thallus.

In this study we found an unknown species of tropical caterpillar that lives inside a bag made out of silk and lichen fragments. The forest in which the caterpillars live sustains an abundant and diverse lichen community of around 300 species of lichens (Herrera-Campos et al. 2017) that cover most of the bark on most of the trees (Miranda-González & McCune 2019). Using molecular techniques, we identified the lichens present in the caterpillars bags and tested whether the caterpillars select lichens based on abundance or on a species-specific selection.

Methods

Study area

The study was made in the Chamela-Cuixmala Biosphere Reserve, located 2 km inland from the Pacific Coast of Mexico. All samples were found in the tropical dry forest component of the Reserve, which is characterized by a warm sub-humid climate with summer rains (Garcia 2004) and a dry season of six continuous months in which more than 95% of the plants lose their leaves completely. The remaining six months of the year are marked by a fast greening of the canopy which is the product of short and intense rains intercalated with dry periods. The area has a strong oceanic influence that maintains mean monthly values of relative humidity above 75% year-round, with mean annual temperature of 24.6°C and mean annual precipitation of 763 mm (1977-2006), of which 80% falls between the months of July and October (Garcia-Oliva et al. 2002, Maass et al. 2002, Sánchez-Azofeifa et al. 2013).

The mature forest consists of trees 4-15 m tall, in a dense pattern of up to 4500 trees per ha, with more than 50% of the stems having a diameter at breast height (DBH) smaller than 5 cm (Lott et al. 1987). Lichen communities are predominantly of crustose

growth form and cover most of the bark on most of the trees (Miranda-González & McCune 2019). Both plants and arthropods are highly diverse, with 1149 and more than 2200 species respectively (Lott & Atkinson 2006, Rodríguez-Palafox & Corona 2002, García Aldrete & Ayala 2004).

Field observations

Samples were found by walking through the forest and inspecting closely the bark of hundreds of trees during 7 trips to the study area from 2014 to 2017. Caterpillar bags were collected into plastic tubes and preliminarily analyzed at the Chamela Biological Field Station. Empty bags were kept for further study and living caterpillars were returned to the field.

Lichen analysis of bags

Most of the lichens present in the caterpillar bags were too small to identify with traditional methods, to solve the problem in those cases we used molecular techniques. We selected 9 well-conserved bags and carefully detached a small piece for every lichen that looked slightly different on each bag. For each lichen piece we applied a five-minute wash with acetone to elute secondary substances. Total DNA was extracted per cleaned lichen piece using the Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) following the manufacturer's instructions, except only 15 µl of extraction buffer and 15 µl of dilution buffer were used per sample. The whole ITS was amplified and sequenced using the primers ITS1F and ITS4 (Gardes & Bruns 1993; White et al. 1990).

Each 10 µl PCR reaction consisted of 5 µl R4775 Sigma-Aldrich REDExtract-n-Amp PCR Ready Mix, 0.5 µl of each primer (10 µM), 3 µl of water, and 1 µl of DNA template diluted at 1:10 in water. The PCR cycling conditions were: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 52 °C for 45 s, and 72 °C for 105 s, followed by 72 °C for 5 min. 2 µl of each PCR product were visualized on 1.5% TBA agarose gel stained with GelRed (Biotium). Single bands were cleaned directly from PCR products with ExoSAP-IT ® for PCR product cleanup (Affymetrix, Santa Clara, CA, USA). If

double bands appeared the rest of the PCR product was gel-extracted and cleaned with GELase (Epicentre Biotechnologies, Madison, Wisconsin, U.S.A.) following manufacturer's instructions. Samples were sequenced at Eurofins MWG Operon LLC (Louisville, KY). Each 12 µl reaction consisted of 2.4 µl primer (at 10 µM), 2 µl undiluted PCR product cleaned with ExoSAP and 7.6 µl water or 2.4 µl primer (at 10 µM) and 9.6 µl DNA cleaned with GELase.

To identify the species of lichens present in the caterpillars bags we performed a BLAST analysis against the GenBank database, however, none of the samples was successfully identified to the species level. We therefore generated our own database of ITS from lichens occurring in the study area. Lichen DNA was obtained from recent collections of the first author and from the lichen collection of the MEXU herbarium. Lichens were identified using standard techniques in lichenology (Bungartz 2002) with an Olympus SZ61 dissecting microscope and an Olympus BX41 compound microscope. Thin layer chromatography (TLC) was performed with solvents A and C using the standard techniques in Culberson & Johnson (1982) and Orange et al. (2010). Once each lichen fragment was identified to the species level in each bag, we calculated the cover value of each lichen species per bag. Given that some lichen pieces were detached for the molecular analysis, we calculated the cover values with a mix of pre-detaching photographs and post-detaching bags.

To calculate the level of specificity for lichen species selection in the construction of caterpillars bags, we compare the number of lichen species present in the study area against the lichen species used as material construction. Furthermore, we used herbarium material from the study area as a proxy to calculate natural species abundances for the two most common lichen species present in the bags. This was done by using the proportion of herbarium samples for each species of the genus *Dirinaria* as their expected abundances in the study area. Using a chi-square test, we test if the caterpillars select those lichen species in proportion to their abundance in the study area or not.

Caterpillar identification

Giving the lack of adult specimens we used the keys of Stehr (1987) for immature insects. We also generated a genetic barcode using the gene COI and the protocol mentioned above, except we used the Sigma-Aldrich REDExtract-N-Amp Tissue PCR Kit (St. Louis, Missouri, U.S.A.), the primers LepF and LepR (Hebert et al. 2004), and an annealing temperature of 45 °C.

Results

Caterpillar Biology

A species of caterpillar of the moth family Psychidae was found using lichens as building blocks for its bag (Fig. 6.1a). The bags are internally made of silk and have an outer layer of lichens that completely covers their surface. The lichens are attached to each bag following two patterns: 1) minute (0.05–0.4 mm) pieces of lichens of a rounded to irregular shape are cut and adhered to the bag with silk in a concentric pattern. As the caterpillar grows, more concentric rings are attached to the bag with the oldest rings in the caudal position. 2) on top of the first lichen layer, larger lichen pieces of 0.4–2 mm are secured. These larger pieces are composed of whole lichen lobes and are not completely appressed to the bag, sometimes giving the impression of lateral fins (Fig. 6.3a) or even a complete lichen thallus.

The bags have an oblanceolate shape with a posterior opening in which the fecal pellets are expelled and a wider anterior opening in which the head and thorax can protrude. The anterior opening can be rapidly closed by folding the upper part of the bag in what resembles a hood (Fig. 6.1b). The dimensions of the bags are approximately 10–16 mm long by 2–4 mm wide in the upper part and 1.5–3 mm wide in the basal part.

The species of caterpillar was not abundant in the study area; however, we were able to find 18 living individuals and 24 empty bags in the course of our study. Most of the individuals were found solitary or occasionally in groups of two. All individuals were found on the bark of trees and usually very well camouflaged against the lichens on the

trunk. The living caterpillars and empty bags were typically aligned parallel to the tree trunk or branch and with the head facing upwards.

No adult moths were found; nonetheless, some bags still contained the pupal exuvia that emerged from the posterior part of the bag (Fig. 6.1c). The exuvia showed the presence of wings, suggesting male moths (Rhainds et al. 2009) and consequently a non-parthenogenic species. Giving the lack of adult specimens, it was not possible to identify the caterpillars (Fig. 6.1d) to species or even to genus. Instead, we amplified the genetic barcode of the species using the gene COI (Appendix 6.1). The closest relatives in the GenBank databased were the genera *Lomera* and *Cladia*, both associated with the subfamily Oiketicinae, as well as an unknown species in Oiketicinae.

Lichen component of the bags

We successfully sequenced the ITS gene from 33 lichen pieces obtained from nine moth bags (Appendix 6.1). A preliminary maximum likelihood analysis in Appendix 6.2 (alignment length 728 bases of which 437 were variable positions), separated the sequences in only six operational taxonomic units (OTU's). The Blast analysis of the new generated sequences against the GenBank database showed a non-lichenized species close to *Fusarium equiseti* (Corda) Sacc. and five lichen species. None of the lichen sequences matched a species in the GenBank database, however, two OTU's were classified in the genus *Dirinaria*, one for each of the genera *Physcia* and *Chrysothrix*, and one in an unknown genus in the order Arthoniales.

To further identify the lichens used as construction material, we created an ITS database of foliose lichens from the study area with an emphasis on the genus *Dirinaria*. A further 50 ITS sequences were generated, belonging to 25 OTU's, including 10 putative species (or operational taxonomic units) of *Dirinaria* and 5 species of *Physcia* (data not shown). This allowed us to match the small pieces of lichens on each of the moth bags to actual herbarium lichen samples, which we then identified with standard techniques.

The three most important species in terms of biomass on the bags belonged to the family Physciaceae (Table 6.1). *Dirinaria aegialita s.l.* (Fig. 6.2a) was the most common lichen used as construction material. It was found in 77 percent of the bags, and when present, constituted a mean of 78 percent of the lichen material per bag. *Dirinaria leopoldii* (Fig. 6.2b) and *Chrysothrix* sp. (Fig. 6.2d) were present on 55 percent of the bags, *Physcia* sp. (Fig. 6.2c) on 33 percent, and both the unknown member of the Arthoniales (Fig. 6.2e) and the species of *Fusarium* (Fig. 6.3a) in only one occasion.

Of the two patterns of lichen attachment on the bags (Fig. 6.3a), the five lichen species were present as small pieces forming the first lichen layer. The second lichen layer, formed with larger pieces, only included the three micro-foliose species in the family Physciaceae. This second layer in particular was observed overgrowing the empty bags that remain adhered to the trees (Fig. 6.3b), while the lichens in the ventral part of those bags (towards the tree) were commonly dead.

Specificity of lichen species in the local lichenobiota context

There was a high level of specificity of species of lichens for the bag construction. Of the more than 300 species of lichens in the study area (Herrera-Campos et al. 2017), of which around 90 percent have a crustose growth form, only four species of lichens were used as bag material in more than one occasion, 75 percent of which had a micro-foliose growth form.

To test specificity for the genus *Dirinaria*, the main component of the bags, herbarium samples of *Dirinaria* were used as a proxy for species abundance in the study area. We found that the most collected species were *D. aegialita s.l.* and *D. leopoldii*, corresponding to 41 and 17 percent of the collected specimens respectively (n = 32 collections in total). Using a chi-square, we tested the null hypothesis that the caterpillars select the two species of *Dirinaria* in proportion to their abundance in the study area or, alternatively, that caterpillars preferred one species or the other. The results suggest that the caterpillars select for *D. aegialita s.l.* and *D. leopoldii* at a higher rate than what is expected by their abundance in the study area ($p < 0.001$; observed proportion of *D.*

aegialita = 0.77, expected = 0.37; observed proportion of *D. leopoldii* = 55.55, expected = 21.8). A similar pattern is expected with the only species of *Physcia* selected by the caterpillars, however, our sampling was insufficient to evaluate selection of this genus.

Discussion

Lichen specificity

The caterpillars studied in this paper were highly selective in the lichens they use to construct their bags. Out of around 300 species of lichens expected in the study area, only 5 of them were used as bag material. Interestingly, most of the lichen material belonged to micro-foliose lichens, which are a small fraction of the lichen communities in the study area (Miranda-Gonzalez & McCune 2019). We suggest that this can be explained by the utility of micro-foliose lichens vs. crustose lichens. Pieces of micro-foliose lichens can easily be cut, then attached to the bags. Micro-foliose lichens are also easer to separate from the bark and are positioned in a way that allows easier access to the mandibles of the caterpillars. Micro-foliose lichens retain their basic form when cut into small pieces, while crustose lichens are less easily removed as intact sheets.

Among the at least 25 species of (micro-)foliose lichens in the area, the caterpillar selectivity was again high, with only three species being selected. The most abundant species on the moth bags, *Dirinaria aegialita s.l.* and *Dirinaria leopoldii*, were also the most abundant in the study area, within the genus *Dirinaria*. Even though our study was not designed to test for differences in abundance between the local lichen community and the lichens used as material for construction, our results point towards preferential selection of lichens disproportionate to their abundance in the study area. Nonetheless, this hypothesis is based on using herbarium material as a proxy to lichen abundance in the forest. Given that sample collection is usually focused on obtaining higher diversity, instead of community structure, a future study specifically designed to test this hypothesis is needed to clarify its validity.

In the meantime, we propose that the same reasons that make a species abundant could make it a useful material for construction. All the moth bags containing living caterpillars were covered by lichens that appeared to be in good health. We do not know if the lichens are replaced constantly or if they survive for an extended time on the bags, but given that they tend to overgrow the bags when the caterpillars die, it is expected that at least some of the lichens are still viable while on the bags. Furthermore, Slocum & Lawrey (1976) found that the photosynthesis and respiration rates of lichens used as camouflage by the green lacewing *Leucochrysa pavida* in a temperate forest, were equal to the rates of the same lichen species when they were not being used as camouflage, suggesting they remain viable.

It is possible that caterpillar selection of lichens has evolved such that lichens are chosen when they survive a transient lifestyle: the construction process as well as the increased mobility and continuous change of microhabitat. The lichen species with that plasticity in their physiology would also be well equipped to do well on their own and henceforth be abundant. At the same time, the propagation of lichens by the empty moths bags could provide a positive feedback loop that increases the lichen abundance. Furthermore, the three micro-foliose lichens found in the moths bags were also found growing on other substrates beside tree bark: *D. aegialita s.l.* on roots of epiphytes, *D. leopoldii* on dead wood, and *Physcia* sp 1 on old flagging. This, in itself, suggests those species are ecologically flexible. On the other hand, the other common lichen on the moths bags, *Chrysothrix* sp 1, was typically found growing on top of *D. aegialita s.l.* This could provide easy access of this lichen for the caterpillar, and at the same time, a more similar appearance of the bag to the lichen *D. aegialita s.l.*

This is the first time molecular techniques were used to identify the lichens used by arthropods. Given the difficulty of identifying small fragments of lichens with traditional techniques, most of the previous work on this topic did not provide results on selectivity of the lichen-arthropod interaction. The only paper that previously studied the lichen-arthropod specificity was Wilson & Methven (1997). In their remarkable study, they compared the chemical profile of lichens used by the green lacewing *L. parvida*

against the chemical profile of the lichens in their locality. They found *L. parvida* selected lichens with the metabolites atranorin, usnic acid and/or zeorin. Of the 45 epiphyte lichens present in their study area only three (*Lecanora strobilina*, *Lepraria* sp. 1, and *Myelochroa aurulenta*) fit the chemical profile used by *L. parvida*. Their methodology could not distinguish lichens that do not produce metabolites, nor differences in chemical profiles within lichens (medulla vs. cortex), nor the presence/absence combinations for the three lichens that fit the chemical profile. However, their 3 out of 45 rate of specificity for lichen species follows a similar tendency of high selectivity as our rate of 5 out of 300 species of lichens used.

Benefits of living in a lichen bag

Animals that live inside a bag obtain advantages for their survival. Protection against physical attacks by other invertebrates have been demonstrated for larvae of caddisflies (Ferry et al. 2013), bagworm moths (Sugiura 2016), and leaf beetles (Brown & Funk 2010). Regardless of the material in the bags (leaves, minerals, sticks or feces) the rate of surviving a physical attack increased dramatically when the bag is present (Ferry et al. 2013, Sugiura 2016). Bags also increase the apparent size of the animals (Otto 2000) which can deter predators and offers extra protection against physical attacks (Sugiura 2016). The camouflage provided by bags or trash packets was also effective in reducing the rate of attack from visual predators by Reduviidae bugs (Jackson & Pollard 2007), Majid crabs (Thanh et al. 2003), green lacewings (Nakahira & Arakawa 2006), and bagworms moths (Rhainds et al. 2009).

Besides avoiding predator-prey interactions, bags also provide protection against weather conditions. In temperate ecosystems, internal temperature of bagworm bags increases with respect to the environment, accelerating development (Rhainds et al. 2009). However, in tropical ecosystems the bags could provide shelter against excessive direct sunlight and moderate fluctuations in humidity. Experimental studies by Kaufmann (1968) showed that if the bags are removed, the Psychidae larvae will dye of dehydration in a matter of days.

The microclimate advantages of living inside a bag could be particularly important to survive the harsh conditions of the tropical dry forest. Although there is virtually no information on most of the biology of the caterpillars found in this study, our molecular results showed it is a member of the Oiketicinae subfamily. Neotropical caterpillars in this group are known to have development times of 168-288 days (Rhainds et al. 2009). This period of time is much longer than the duration of the rainy season in the study area (Maass et al. 2018), which suggest the caterpillars might need to extend its development into the dry season. If this is the case, the internal microclimatic regulation of the bag might increase the survival rates.

A bag constructed from lichens might react differently to a bag constructed from leaves or sticks. Lichens can absorb water vapor directly from the air, even though humidity can accumulate in the surface of other biological materials, the lack of cuticle in lichens allows the water to penetrate the tissue, which leads to a larger amount of absorbed water. This humidity does not necessary reach the caterpillar inside the bag, but it could momentarily serve as a climatic barrier against extreme temperature. Lichens can also provide extra benefits by their high content of secondary metabolites (Lawrey 1986) which can deter predators by being unpalatable (Lawrey 1983, Asplund & Wardle 2013) or present antimicrobial properties (Ranković et al. 2008). Lastly, lichens cover approximately 80% of the bark of the trees (Miranda-González & McCune 2019) in which the caterpillar lives, conferring an excellent camouflage for the caterpillar bags.

Caterpillar and lichen ID

The taxonomy of the moth family Psychidae is based predominantly on characters of adult males (Davis 1964, Rhainds et al. 2009). This becomes a serious problem when the adult moth is not available, as is our case. However, by providing the genetic barcode of the species, we are confident that an appropriate species name for our samples will eventually be found. The CO1 gene we used is the official genetic barcode for animals and has been shown to be effective in delimiting species of Lepidoptera (Hajibabaei et al. 2006, Chevasco et al. 2014). Nonetheless, most species of Psychidae have no sequences available in GenBank and there are an estimated 500 undescribed species (Sobczyk

2011). Among the species of the family, those associated with lichens are less studied and it has been estimated that 80 percent of them in the Neotropics are undescribed (Davis 1975, Davis 2000).

Unsurprisingly, our sequences only showed that the caterpillars in this study belong to the subfamily Oiketicinae. This diverse subfamily has a worldwide distribution (except Antarctica), it can present high fecundity levels, it is associated with several families of host plants, and its larvae can be omnivorous scavengers (Rhainds et al. 2009, Sobczyk 2011). The position of the caterpillars in this study as members of the subfamily Oiketicinae was sufficient to preliminary rule out all the known examples of Psychidae that use lichens as material construction for their bags.

The bags in our study look very similar to those of *Luffia lapidella* and *Luffia ferchaultella*, which are already sequenced for CO1 (Mutanen et al. 2016). However, these two European species belong in the subfamily Psychinae (Sobczyk 2011) and are not known from America. Other species in the subfamily Psychinae that cover their bags with lichens include the European *Bacotia claustrella* and *Proutia betulina* (Richardson 1974). In the New World, *Prochalia licheniphilus* in Cuba (Nuñez Aguila 2004), and *Prochalia pygmaea* and *Zamopsyche commentella* in the USA (Davis 1964) also cover their bags with lichens.

One moth bag probably belonging to *Paucivena hispaniolae* in the subfamily Epichnopteriginae was found to use lichens in a similar way as the caterpillars in our study (Davis 1975, Fig. 188). Other bags decorated with lichens are reported for *Dahlica lichenella* (Hawksworth 1991) and *Narycia duplicella* (Richardson 1974) in the subfamily Naryciinae. In some cases (e.g., bags of *Lumacra brasiliensis*, *Naevipenna cruttwelli*, *Siederia walshella*, and *Taleporia tubulosa*), lichen material seems only secondary or fortuitous, instead, the main materials are sand, pieces of bark and leaves (Davis 1964, Davis 1975, Richardson 1974).

Problems derived from incomplete taxonomic knowledge also apply to the lichen component of the interaction. By using a genetic approach to identify the lichens on the bags we encountered a high level of polyphyletic species in the lichen genus *Dirinaria*. Our most abundant species, *Dirinaria aegialita*, is the only species in the genus that has isidia-like outgrowths that break into soredia; however, by analyzing the sequences from GenBank and from our study area, we found at least three independent, supported clades that share this diagnostic character. Further work is needed to delimit which of the clades corresponds to the true *D. aegialita*; meanwhile, we used the term *sensu lato* for the identity of our samples. Our preliminary analysis from personal and GenBank sequences also revealed that the common and widespread *Dirinaria applanata* is a mix of at least six different supported clades.

The high level of specialized methods and knowledge required to identify immature psychids and minute pieces of lichens has proven to be a barrier to study the ecological interactions of these organisms. Our knowledge of even worthy text-book examples like the lichen cover bags of *Luffia lapidella* and *Dahlica lichenella*, known since the 1700's, are limited to the following statement: they use lichens to construct their bags. Almost no information is available on which species of lichens are used, the trophic consequences to lichen use, the level of specificity in the selection, and how much the specificity of lichen selection differs among localities. We sincerely hope that our methodological approach will provide an accessible way to study these widespread interactions around the world.

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Table 6.1 Cover percent of lichen species used as material for each bag construction. At the bottom is the frequency of which each lichen species was found in the caterpillars bags. On the right is the number of DNA sequences obtained for each caterpillars bag.

Bag number	*Dirinaria aegialita s.l.*	*Dirinaria leopoldii*	*Chrysothrix sp. 1*	*Physcia sp. 1*	Arthonial	DNA samples
1	88	10	2	0	0	4
2	84	1	0	15	0	2
3	30	67	3	0	0	5
4	70	30	1	0	0	3
5	98	0	2	0	0	5
6	0	15	0	85	0	3
7	0	0	1	99	0	3
8	100	0	0	0	0	2
9	80	0	0	0	20	5
Frequency (percent)	77.8	55.6	55.6	33.4	11	

Figure 6.1 Caterpillar general biology. A) Bag against a lichen background on field conditions. B) Anterior opening of the bags: closed on left and opened on right. C) Exuvias attached to the posterior ends of the bags. D) General view of a caterpillar outside the bag. All scales equal 5 mm.

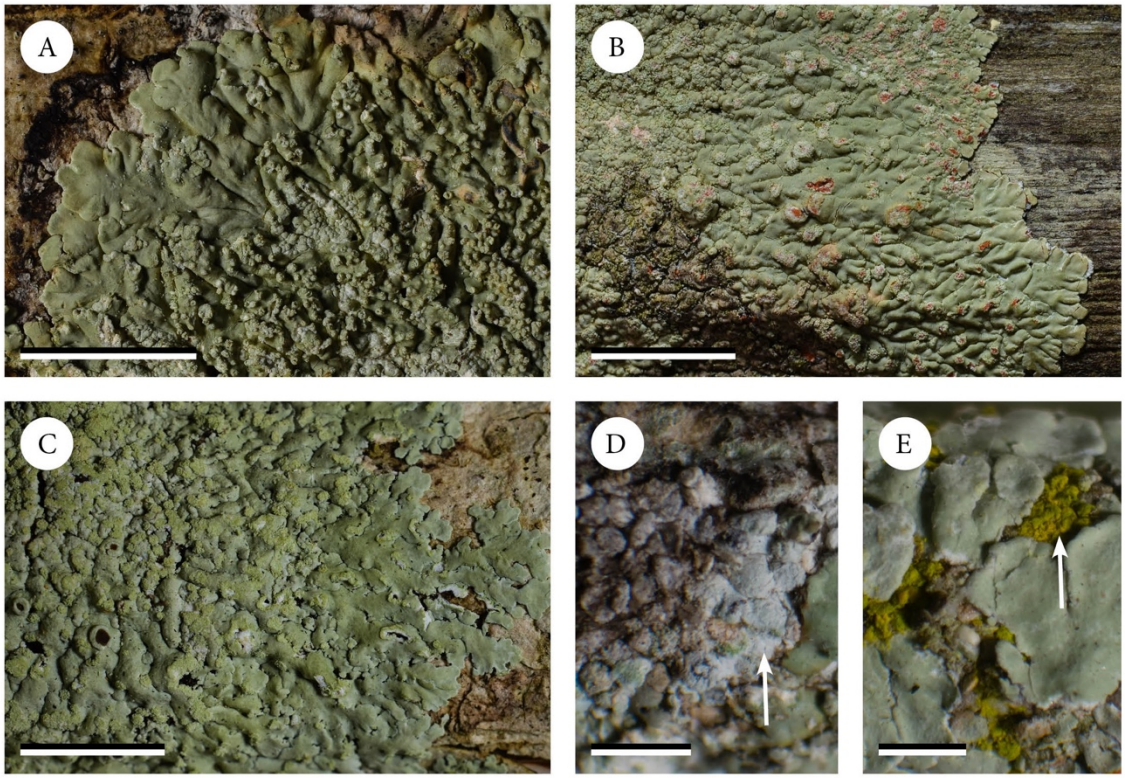


Figure 6.2 Lichen species used by caterpillars in bag construction. A) *Dirinaria aegialita s.l.* (*Miranda M354*) B) *Dirinaria leopoldii* (*Miranda M211*). C) *Physcia sp*. (*Miranda M353*) D) Unknown white lichen (arrow) in the order Arthoniales (bag B185). E) Yellow lichen (arrow) *Chrysothrix* sp. (bag A-004). A-C scales equal 5 mm, D-E scales equal 1 mm.

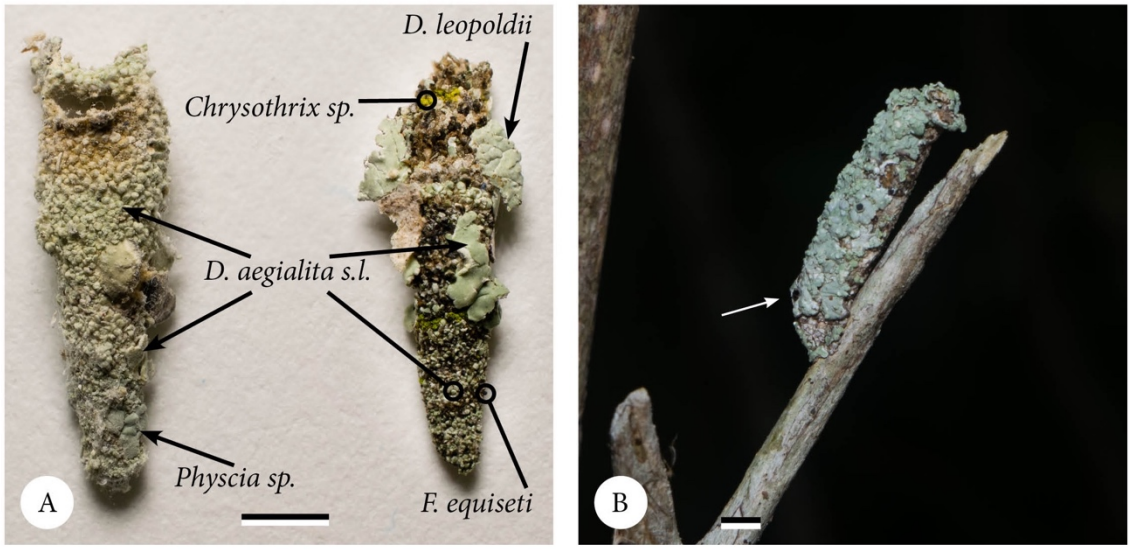
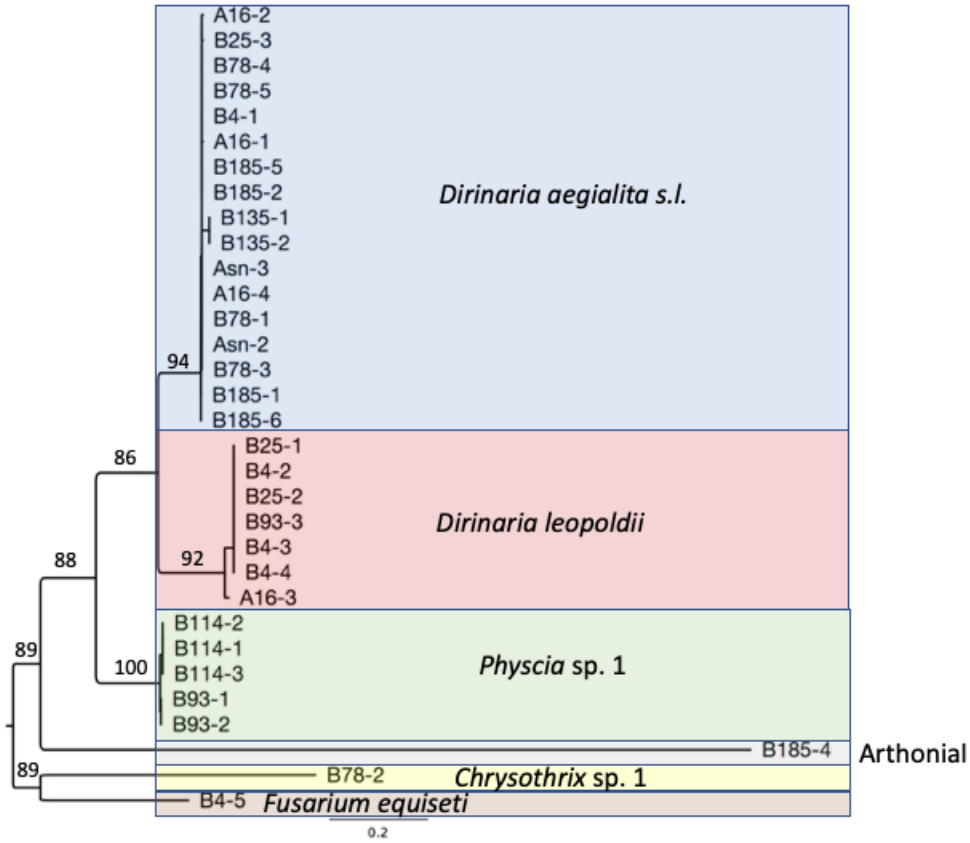


Figure 6.3 Lichen component of the bags. A) Lichen species and the fungus *F. equiseti* arrangement on the bags. Notice the first layer made from small lichen pieces and the second layer made from bigger pieces giving the impressions of fins. B) Lichen overgrowth after the bag is abandoned. Notice an opening (white arrow) made from a parasite that shows the caterpillar died. All scales equal 2 mm.

Appendix 6.1 New sequences generated in this study.

New sequences used as reference for this study				
Taxon	**Voucher**	**DNA number**	**ITS**	**CO1**
Lichenized fungi				
Dirinaria aegialita s.l. (Afzel. ex Ach.) B.J. Moore	Miranda 5025	RMG189	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	Miranda 5030	RMG354	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	Miranda 5028	RMG346	GB	
Dirinaria leopoldii (Stein) D.D. Awasthi	Miranda 5024	RMG178	GB	
D. leopoldii (Stein) D.D. Awasthi	Miranda 5027	RMG211	GB	
D. leopoldii (Stein) D.D. Awasthi	Miranda 5031	RMG360	GB	
Physcia sp.	Miranda 5026	RMG208	GB	
Physcia sp.	Miranda 5029	RMG353	GB	
Physcia sp.	Miranda 4539	RMG220	GB	
Lepidoptera				
Oiketicinae sp.	Miranda_Bsn			GB
Oiketicinae sp.	Miranda_B78			GB
New sequences obtained from caterpillars bags				
Taxon	**Voucher**	**DNA number**	**ITS**	
Arthonial	B185	B185-4	GB	
Chrysothrix sp	B078	B078-2	GB	
Dirinaria aegialita s.l. (Afzel. ex Ach.) B.J. Moore	A016	A016-1	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	A016	A016-2	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	A016	A016-4	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	Asn	Asn-2	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	Asn	Asn-3	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B004	B004-1	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B025	B025-3	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B078	B078-1	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B078	B078-3	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B078	B078-4	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B078	B078-5	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B135	B135-1	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B135	B135-2	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B185	B185-1	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B185	B185-2	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B185	B185-5	GB	
D. aegialita s.l. (Afzel. ex Ach.) B.J. Moore	B185	B185-6	GB	
Dirinaria leopoldii (Stein) D.D. Awasthi	A016	A016-3	GB	
D. leopoldii (Stein) D.D. Awasthi	B004	B004-2	GB	
D. leopoldii (Stein) D.D. Awasthi	B004	B004-3	GB	
D. leopoldii (Stein) D.D. Awasthi	B004	B004-4	GB	
D. leopoldii (Stein) D.D. Awasthi	B025	B025-1	GB	
D. leopoldii (Stein) D.D. Awasthi	B025	B025-2	GB	
D. leopoldii (Stein) D.D. Awasthi	B093	B093-3	GB	
Fusarium equiseti (Corda) Sacc.	B004	B004-5	GB	
Physcia sp.	B093	B093-1	GB	
Physcia sp.	B093	B093-2	GB	
Physcia sp.	B114	B114-1	GB	
Physcia sp.	B114	B114-2	GB	
Physcia sp.	B114	B114-3	GB	



Appendix 6.2 Operational taxonomic units of lichens used in bag construction. Tree shows a Maximum Likelihood analysis. Support values are shown as numbers if bootstrap values ≥ 75.

CHAPTER 7. CONCLUSIONS

Ricardo Miranda-González

The main purpose of this dissertation was to contribute towards a framework that could allow the incorporation of the study of lichens into the understanding of the functionality and dynamics of tropical dry forests (TDF). Given the scarce knowledge of lichens from this ecosystem, the dissertation was divided in two parts: one for systematics and one for ecology.

From the systematics point of view, our findings extended far beyond the lichens of TDF. We found new collections of the long believed to be extinct *Polypyrenula sexlocularis*, while at the same time updating its distribution from the Caribbean to the Neotropics, describing parts of its natural history and correcting its systematic position with the use of morphology and molecular data.

We also described the new genus *Jocatoa*, to accommodate an orphan lichen species, previously known as *Medusulina texana*, and described from the southeast USA. Finally, we described two new species in the subfamily Redonographoideae (Graphidaceae). This subfamily, with previously only six known species, is limited to dry regions with oceanic influence and was only known growing on rocks. Our two new species are the only instances in the family with a corticolous habit and one of them represents a new record at the genus level for the Northern Hemisphere. Furthermore, we provided diagnostic anatomical characters to distinguish the subfamily and the genera within.

We clearly need a systematic and taxonomic revisions for the lichens of the TDF. The results of such local studies will have a widespread positive effect in understanding the systematics and ecology of lichenized fungi around the world.

From an ecological point of view, we found that lichens not only give the impression of being abundant in the TDF, but that their biomass is equivalent to 59% of the leaf biomass in the forest. This amount of resources is so large, that neglecting to include lichens in future studies in the TDF at the ecosystem scale, should no longer be a possibility. We not only managed to characterize the amount of lichen as resources, but

also demonstrated they are being consumed in large quantities throughout the year. The stability of the annual lichen herbivory rates in our four-year study, suggest that lichens are an integral component of the ecosystem functioning. After our research, it is now possible to conclude that crustose lichens in the TDF should be considered as primary producers that support substantial parts of the trophic networks in the forest.

Besides trophic interactions, we found that lichens of the TDF are used by other organisms, specifically as construction material for bags of caterpillars in the family Psychidae. Although we described the interactions with only one species of caterpillar, at least four other species of insects in the study area were observed using lichens in a similar, though less charismatic way. For the first time, we managed to draw a clear understanding of the level of selectivity for lichens by these caterpillars. We also found these interactions to be widespread and in a strong need of even basic ecological information.

During this dissertation, we moved from an almost complete lack of ecological knowledge about lichens of TDF, to a significant understanding of their relevance and potential at the ecosystem scale. Further work is needed in several areas, but our results can be used as the basis for studies dealing with the continuity of the ecosystem functioning during the dry season, detailed trophic networks sustained by lichens, and mineral cycling at the ecosystem scale.

Finally, this dissertation is also a call to other researchers across the world, but in particular in the tropics. Lichens, although small and usually overlooked, can and should be included in integral ecosystem studies. We provide in here not only our results, but also methods and techniques that will allow ecological studies of lichens in tropical areas, as well as in the study of interactions with invertebrates around the world.

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