LEAF POSITION AND LEAF AREA EFFECTS ON THE DIVERSITY OF FOLIICOLOUS LICHENS ASSOCIATED WITH *STENANONA FLAGELLIFLORA* (ANNONACEAE)

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Abstract. Foliicolous lichens grow on the surface of alive leaves of vascular plants and are highly diverse in tropical habitats. Their taxonomy and ecology are relatively well known; however, there are no studies on the within-individual variation in the diversity and composition of the communities growing on single leaves. We describe the diversity of foliicolous lichens associated with leaves of *Stenanona flagelliflora* (Annonaceae) of different sizes and within-canopy position at Los Tuxtlas, Veracruz, Mexico. Species composition, richness, cover, and the Shannon-Wiener diversity index of the lichen communities growing on each leaf were determined. The community of foliicolous lichens associated with *S. flagelliflora* is highly diverse: 94 foliicolous lichen species from 12 families were recorded. *Porina karnatakensis* (Porinaceae) was the dominant species across leaves at different positions. Species richness, lichen cover, and diversity index were not significantly affected by leaf area nor by leaf position, but both of these factors influenced community composition. We discuss the importance of within-phorophyte microclimate variation, competitive interactions, and environmental requirements of each lichen species as the main drivers of the differences found in the assemblage of lichens. More studies on the factors that determine the diversity of the communities of organisms within the phylloplane are needed.
Keywords: *phylloplane, phyllosphere, Shannon-Wiener diversity index, Sörensen similarity index, species composition, species richness – area relationship*

Introduction

Plant leaves are a harsh habitat exposed to diverse sources of environmental stress (Lindow and Brandl, 2003). Leaves are an ephemeral habitat exposed to UV radiation, desiccation, rainfall, nutrient limitation, and frequent fluctuations in temperature and relative humidity (Gomes et al., 2018; Hirano and Upper, 2000; Lindow and Brandl, 2003; Thapa and Prasanna, 2018). In spite of this, numerous epiphyte microorganisms such as bacteria, fungi, protozoa, yeasts, algae, and nematodes (Lindow and Brandl, 2003; Thapa and Prasanna, 2018), as well as foliicolous lichens (Lücking, 2001; Lücking and Cáceres, 2002) colonize and establish successfully on the phylloplane.

Diverse studies have documented the existence of spatial variation in the communities of phylloplane organisms within the phorophyte and the factors that might explain such variation. These studies have shown that the diversity, abundance, and composition of phylloplane bacteria, fungi, and yeast communities vary within a single host according to canopy position (Andrews et al., 1980; Carroll, 1979; Harrison et al., 2016; Hermann et al., 2021; Izuno et al., 2016; Laforest-Lapointe et al., 2016; Leff et al., 2015; Nguyen, 2017; Stone and Jackson, 2019), leaf age, leaf region (Carroll, 1979) tissue and organ type (Leff et al., 2015), leaf orientation (Andrews et al., 1980; Nguyen, 2017), and microclimate (temperature, vapor pressure deficit, humidity and precipitation; Al-Ashhab et al., 2021).

In contrast, only one study has documented the existence of within-individual variation in the diversity of the communities of foliicolous lichens (Sipman, 1997). This study shows that lichen diversity decreases from the top to the bottom part of the phorophyte, such that the lowest lichen diversity is found in the most shaded region of the phorophyte. This pattern of vertical variation on the diversity of foliicolous lichens was explained by the differential incidence of light along the phorophyte (Sipman, 1997).

In this study, we describe the intra-individual variation in the diversity and composition of the communities of foliicolous lichens associated with the phorophyte *Stenanona flagelliflora* T. Wendt & G. E. Schatz (Annonaceae). Particularly, we were interested in finding out if the communities of lichens vary as a function of leaf area and leaf position within the phorophyte. We hypothesized that: i) the diversity of lichens will increase with leaf area, as it has been demonstrated for numerous groups of organisms (Brunet and Medellín, 2001; Feinstein and Blackwood, 2012; Flores-Palacios and García-Franco, 2006; Kohn and Walsh, 1994; Löfgren and Jerling, 2002; Lyons et al., 2010), and ii) the composition, abundance and diversity of the communities of lichens will vary across leaves with different positions within an individual phorophyte as it has been documented for different groups of organisms within the phylloplane (Andrews et al., 1980; Carroll, 1979; Harrison et al., 2016; Hermann et al., 2021; Izuno et al., 2016; Laforest-Lapointe et al., 2016; Leff et al., 2015; Nguyen, 2017; Sipman, 1997).

Methods

Phorophyte species

Stenanona flagelliflora (Annonaceae) is a tree of 1–4.5 m in height, that produces inflorescences on specialized branches (i.e., flagelliflorous) with up to 3 m in length on the surface of the ground. Leaves are membranaceous, bright medium glossy green above and paler beneath when fresh, elliptic, 9-18 cm in length and 2.6-6.5 cm width, getting narrower towards the tip (Schatz and Wendt, 2004). *Stenanona flagelliflora* has been recorded in the southern part of the Uxpanapa region of extreme southern Veracruz and the adjacent part of the Chimalapa region on eastern Oaxaca (Schatz and Wendt, 2004). A population at Los Tuxtlas, Veracruz, where the present study took place, has also been recorded (Xicohténcatl-Lara et al., 2016).

Study site

The study took place at the Los Tuxtlas Biosphere Reserve, in the Lic. Adolfo López Mateos locality (18°26'19.60"N, 94°57'53.16"; 219 m a.s.l.; Morteo, 2011), in the State of Veracruz, Mexico (*Fig. 1*). Vegetation at the locality is an evergreen rainforest (Miranda and Hernández, 1963). Climate is hot and humid, with an average annual temperature of 22-26 °C (Cruz, 2009) and a mean annual precipitation of 2000-2500 mm (Cruz, 2009; Guevara et al., 1999).

The community of foliicolous lichens associated with Stenanona flagelliflora

In March 2013, we collected 63 leaves from 12 individuals of *Stenanona flagelliflora* [Annonaceae; 1–7 leaves per individual plant, depending upon the availability of leaves without (or very low) damage by herbivores]. Although leaves were collected on a specific time of the year, community patterns of foliicolous lichens might be similar at other times because: (i) the lifespan of leaves in tropical forests is between 1-3 years;

(ii) within few months after their establishment, foliicolous lichens reproduce assuring their prevalence in the habitat (Lücking 2008a); (iii) *Stenanona flagelliflora* is a perennial plant, its leaves are available for colonization throughout the year. We chose *S. flagelliflora* to conduct the study because most of the foliar surface is covered by foliicolous lichens and because it has a relatively short height [1–4.5 m according with Schatz and Wendt (2004); but a maximum of 2.2 m in the sampled population), which facilitates the collection of leaves at different heights within each individual. At the locality of study, the species is distributed close to the edge of the forest, but where a canopy layer is well developed. Leaves were collected from individual phorophytes selected randomly but at a distance of 1.5-2 m from each other. In order to have represented foliicolous species from different positions within the phorophyte, leaves were collected from the top (>150 cm in height; N = 21), base (<70 cm in height; N = 21) and middle (70 – 150 cm in height; N = 21) regions of each individual plant. Collected leaves were pressed and dried using standard herbarization procedures. Specimens were deposited in the Lichen collection of the Biological Sciences Department at Autonomous University of Puebla. Identification of the foliicolous lichen species growing on the adaxial surface of each leaf was conducted under a NIKON SMZ645 stereoscopic microscope. Species identification at the lowest taxonomic level was conducted using Lücking and Cáceres (2002) guide and Lücking (2008b).
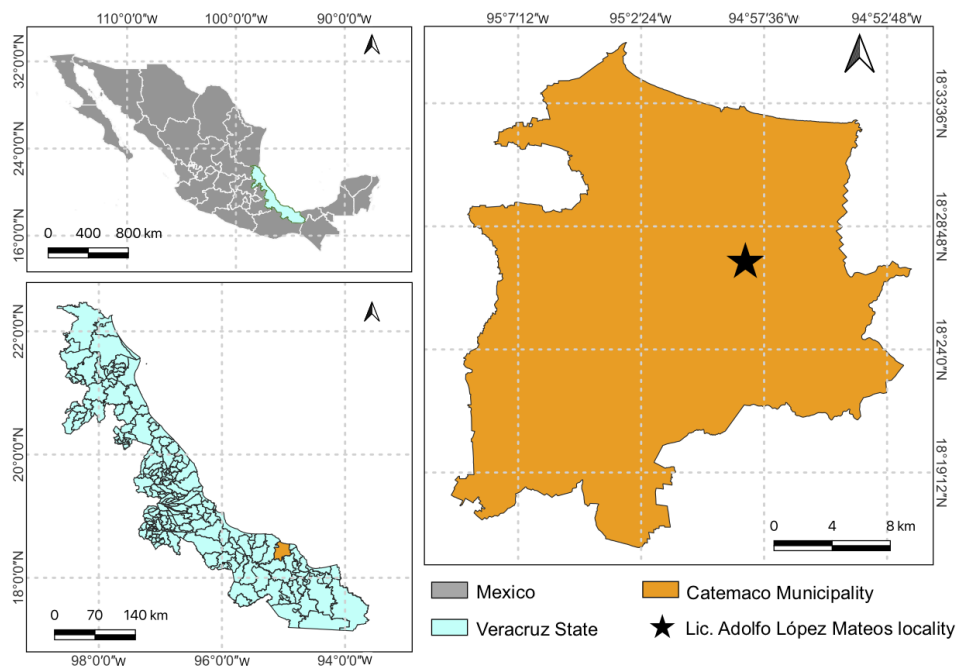

Figure 1. Location of the area of study

Lichen abundance was estimated as the area covered by each lichen species within each leaf (Lõhmus and Lõhmus, 2001). To do this, dry leaves were scanned in a HP Scanjet G2410 at 300 pixels per inch. Then, using the free software ImageJ (Rasband, 1997-2018), we determined the total area covered by each lichen species within each leaf as well as the total area of each collected leaf. Shannon-Wiener (H') diversity index was estimated as $H' = -\sum p_i \ln p_i$, where p_i is the relative cover of each foliicolous

species in relation to the total cover of foliicolous lichens growing on each leaf (Lõhmus and Lõhmus, 2001). The importance of leaf position (i.e., top, middle, and base) on species richness and relative lichen cover were analyzed with one-way ANOVA tests. Species richness was transformed logarithmically, whereas relative lichen cover was transformed as arcsine $(p)^{1/2}$ (Zar, 2010). The effect of leaf position on the diversity index was tested with a non-parametric Kruskal-Wallis analysis. Finally, Spearman correlation analyses were used to test the relationship between leaf area and the community parameters estimated (species richness, relative lichen cover, and diversity index). Analyses were conducted in PAST v. 2.17 (Hammer et al., 2001).

Rank-abundance curves (James and Rathbun, 1981) were constructed to compare the dominant foliicolous lichen species among leaves with different positions. Relative abundance was estimated as n_i/N, where n_i = relative cover of the ith foliicolous lichen species, and N = relative cover from all the lichen species including all leaf positions. Relative abundance was plotted on a log_{10} scale against the rank from the most to the least common species.

Differences in species composition among leaves of different size and position were analyzed with two ordination methods. Using a matrix of species relative cover on each collected leaf, we first conducted a non-metric multidimensional scale (NMDS) analysis to determine if the communities of foliicolous lichens grouped together according with leaf position. The NMDS analysis is widely used in community studies (Arcos-Pulido and Gómez-Prieto, 2006; Ruíz-Pineda et al., 2016). The stress value obtained in the analysis is a measure of fit of the similarity among samples in the two-dimensional space (Clarke et al., 2014). In addition, an analysis of similarities (ANOSIM) was conducted. The ANOSIM test shows the existence of differences among groups according to the value of the statistic R. If R = 1 there is low similarity among groups, whereas if R = 0, the groups are rather similar (Clarke et al., 2014). The NMDS analysis was conducted using the Bray-Curtis similarity index and the foliicolous lichen species collected in at least five sampled leaves. The analysis was performed in the program PAST v. 4.03 (Hammer et al., 2001). In addition, we conducted two canonical correspondence analyses (CCA) to evaluate the effects of both leaf area and leaf position on the composition of the communities of foliicolous lichens. Leaves collected in different positions were classified as 1 for those collected on the base, 2 on the middle, and 3 when they were collected from the top part of the tree. A first CCA analysis was conducted at the species level (using relative cover data of the lichen species found in at least five sampled leaves), whereas the second one was conducted at the family level (using relative cover data pooled across all lichen species within each family). CCA analyses were conducted in the software MVSP v. 3.21 (Kovach, 2004).

Results

The community of foliicolous lichens

A total of 94 foliicolous lichen taxa were identified growing on the adaxial surface of the leaves of *Stenanona flagelliflora* (*Table 1; Fig. 2*). Most taxa (90, 95.7%) were identified to the genus (51 species, 54.3%) or the species level (39 species, 41.5%). The remaining 4 taxa (4.3%) were distinguished only as morphospecies, determination at family level was not even possible for them. Porinaceae was the richest family (41 species, 43.6%; *Table 1*). The other 11 families were represented by 1–8 foliicolous lichen species: Gomphillaceae (8), Arthoniaceae, Pilocarpaceae, Roccellaceae (7

species each), Strigulaceae (6), Microthelipsidaceae (4), Coenogoniaceae, Lyrommataceae (3 species each), Monoblastiaceae (2), Ramalinaceae, and Thelotremataceae (1 species each; *Table 1; Fig. 2*). Three species, *Coenogonium luteum* (Dicks.) Kalb & Lücking, *Trichotelium pauciseptatum* Vězda, and *Strigula obducta* (Müll. Arg.) R.C. Harris represent new records for Mexico.

Table 1. *List of foliicolous lichens growing on the adaxial surface of the leaves of Stenanona flagelliflora (Annonaceae) at Los Tuxtlas, Veracruz, Mexico. Relative cover (mean ± s.e.) across the 63 sampled leaves of Stenanona flagelliflora and number of leaves with different position on which each lichen species was registered are shown*

Family Species	Lichen cover (%)	Top	Middle	Base
Arthoniaceae				
Arthonia accolens Stirt.	0.46 ± 0.3	1	3	3
Arthonia lecythidicola (Bat. & H. Maia) Lücking & Sérus	1.75 ± 0.88	1	3	3
Arthonia sp. 1	2.15 ± 0.91	8	9	10
Arthonia sp. 2	0.05 ± 0.04	1	1	0
Arthonia sp. 3	0.098 ± 0.098	1	0	0
Arthonia sp. 4	0.012 ± 0.012	1	0	0
Arthonia sp. 5	0.25 ± 0.2	0	3	0
Coenogoniaceae				
Coenogonium luteum (Dicks.) Kalb & Lücking	0.92 ± 0.92	1	0	0
Coenogonium sp. 1	2.63 ± 1.6	3	2	5
Coenogonium sp. 2	0.73 ± 0.73	0	0	1
Gomphillaceae				
Aulaxina microphana (Vain.) R. Sant.	0.024 ± 0.017	0	0	2
Aulaxina sp. 1	0.31 ± 0.16	6	6	4
Aulaxina sp. 2	0.001 ± 0.001	1	0	0
Gyalectidium filicinum Müll. Arg.	0.04 ± 0.03	1	1	0
Gyalectidium sp. 1	0.003 ± 0.002	0	0	2
Gyalectidium sp. 2	0.009 ± 0.009	1	0	0
Tricharia sp. 1	0.002 ± 0.001	0	2	0
Tricharia sp. 2	0.001 ± 0.001	1	0	0
Lyrommataceae				
Lyromma nectandrae Bat. & H. Maia	0.01 ± 0.01	0	1	0
Lyromma sp. 1	0.22 ± 0.15	0	1	3
Lyromma sp. 2	0.22 ± 0.22	0	0	1
Microthelipsidaceae				
Microtheliopsis sp. 1	0.41 ± 0.25	1	1	3
Microtheliopsis sp. 2	0.2 ± 0.2	0	0	1
Microtheliopsis uleana Müll. Arg.	0.24 ± 0.14	0	3	1
Microtheliopsis uniseptata Herrera-Campos & Lücking	1.18 ± 1.18	0	0	1
Monoblastiaceae				
Anisomeridium foliicola R. Sant. &Tibell	0.065 ± 0.065	1	0	0
Anisomeridium sp. 1	0.89 ± 0.89	0	0	1
Not determined family				
Sp. 1	8.53 ± 2.66	13	12	13
Sp. 2	0.64 ± 0.64	1	0	0
Sp. 3	1.79 ± 1.24	2	3	5
Sp. 4	0.69 ± 0.63	1	0	2

Table 1. Continued

Family Species	Lichen cover (%)	Top	Middle	Base
Pilocarpaceae				
Bapalmuia sp. 1	0.24 ± 0.22	0	1	1
Byssolecania fumosonigricans (Müll. Arg.) R. Sant.	0.26 ± 0.16	1	2	0
Byssolecania sp. 1	0.87 ± 0.87	0	1	0
Byssoloma sp. 1	1.95 ± 1.35	1	2	1
Calopadia sp. 1	1.45 ± 1.13	0	0	2
Lasioloma sp. 1	0.04 ± 0.04	0	1	0
Sporopodium sp. 1	0.18 ± 0.17	1	0	0
Porinaceae				
Porina alba (R. Sant.) Lücking	4.44 ± 2.31	9	10	7
Porina atrocoerulea Müll. Arg.	0.11 ± 0.07	2	0	2
Porina atropunctata Lücking & Vezda	0.61 ± 0.61	0	0	1
Porina epiphylla (Fée) Fée	2.65 ± 1.56	4	10	2
Porina karnatakensis Makhija, Adawadkar & Patwardhan	51.82 ± 4.48	18	20	20
Porina limbulata (Kremp.) Vain.	0.02 ± 0.01	0	2	0
Porina monocarpa (Kremp.) F. Schill.	0.02 ± 0.02	1	0	0
Porina nitidula Müll. Arg.	0.04 ± 0.04	1	0	0
Porina octomera (Müll. Arg.) F. Schil	0.07 ± 0.04	3	1	1
Porina rubentior (Stirt.) Müll. Arg.	2.32 ± 0.47	14	18	13
Porina rubescens (Lücking) Hafellner & Kalb	0.04 ± 0.03	3	0	1
Porina rufula (Kremp.) Vain.	0.04 ± 0.03	2	0	0
Porina sp. 1	1.19 ± 1.04	2	2	1
Porina sp. 2	0.02 ± 0.02	0	1	0
Porina sp. 3	0.11 ± 0.11	0	1	0
Porina sp. 4	0.19 ± 0.19	1	0	0
Porina sp. 5	0.05 ± 0.05	1	0	0
Porina sp. 6	0.06 ± 0.06	1	0	0
Porina sp. 7	0.04 ± 0.04	1	0	0
Porina sp. 8	0.02 ± 0.02	1	0	0
Porina sp. 9	0.003 ± 0.003	0	1	0
Porina sp. 10	0.002 ± 0.002	1	0	0
Porina sp. 11	0.1 ± 0.096	0	2	1
Porina sp. 12	0.006 ± 0.006	0	1	0
Porina sp. 13	0.06 ± 0.06	0	1	0
Porina sp. 14	0.34 ± 0.22	2	0	1
Porina sp. 15	0.04 ± 0.04	1	0	0
Porina sp. 16	0.002 ± 0.002	0	1	0
Porina sp. 17	0.03 ± 0.03	1	0	0
Porina tetramera (Malme) R. Sant.	2.11 ± 1.03	5	8	5
Trichothelium epiphyllum Müll. Arg.	0.33 ± 0.17	3	4	5
Trichothelium intermedium Herrera-Campos & Lücking	0.01 ± 0.01	1	0	0
Trichothelium longisporum Lücking	0.009 ± 0.009	1	0	0
Trichothelium minus Vain.	0.07 ± 0.04	3	2	0

Table 1. *Continued*

Family Species	Lichen cover (%)	Top	Middle	Base
Trichothelium mirum Lücking	0.1 ± 0.04	7	4	2
Trichothelium pallidesetum Lücking	0.004 ± 0.004	1	0	0
Trichothelium pauciseptatum Vézda	0.09 ± 0.04	1	4	1
Trichothelium sp. 1	0.16 ± 0.06	4	5	6
Trichothelium sp. 2	0.04 ± 0.03	0	0	2
Trichothelium sp. 3	0.01 ± 0.01	1	0	0
Trichothelium sp. 4	0.009 ± 0.009	1	0	0
Ramalinaceae				
Bacidina sp. 1	0.005 ± 0.004	0	1	1
Roccellaceae				
Enterographa sp. 1	0.01 ± 0.01	1	0	0
Mazosia melanopthalma (Müll. Arg.) R. Sant.	0.29 ± 0.21	4	2	2
Mazosia phyllosema (Nyl.) Zahlbr.	0.02 ± 0.02	1	0	0
Mazosia rotula (Mont.) A. Massal.	0.14 ± 0.06	5	2	4
Mazosia sp. 1	0.05 ± 0.03	1	3	2
Mazosia sp. 2	0.03 ± 0.03	1	0	0
Opegrapha sp. 1	0.12 ± 0.05	0	3	5
Strigulaceae				
Phyllobathelium firmum (Stirt.) Vézda	0.02 ± 0.02	1	0	0
Phyllobathelium sp. 1	0.01 ± 0.01	1	0	0
Strigula nitidula Mont.	0.18 ± 0.09	1	1	4
Strigula obducta (Müll. Arg.) R.C. Harris	0.44 ± 0.27	2	0	1
Strigula phyllogena (Müll. Arg.) R.C. Harris	0.35 ± 0.21	1	2	1
Strigula platypoda (Müll. Arg.) R.C. Harris	1.46 ± 0.66	2	3	3
Thelotremataceae				
Chroodiscus coccineus (Leight.) Müll. Arg.	0.006 ± 0.006	1	0	0

A single leaf of *S. flagelliflora* had a mean area of 44.99 ± 2.01 cm^2 (range: 19.82–94.26 cm^2) and supported a community of $7.94 \pm$ s.e. 0.44 species of foliicolous lichens (range: 1–17 species) in average. Likewise, mean lichen cover on a single leaf was 11.75 ± 0.81 cm^2 (range: 0.04–30.5 cm^2). Mean diversity index on the leaves of *S. flagelliflora* was 0.83 ± 0.06. The highest diversity index across all sampled leaves was 1.89; whereas the lowest one was 0, on a leaf on which a single lichen species was growing.

Frequency of each foliicolous lichen species on the sampled leaves of *S. flagelliflora* was highly variable (range: 1–58 leaves). *Porina karnatakensis* Makhija, Adawadkar & Patwardhan (Porinaceae) was the most frequent species, being identified in 92.1% of the leaves. Other species with high frequency were *P. rubentior* (Stirt.) Müll. Arg. (45 leaves, 71.43%), *P. alba* (R. Sant.) Lücking (Porinaceae) (26 leaves, 41.27%), *Arthonia* sp. 1 (Arthoniaceae) (27 leaves, 42.86%), and the unidentified morphospecies 1 (38 leaves, 60.32%). In contrast, 45 species were present in only one out of the 63 leaves sampled (*Table 1*).
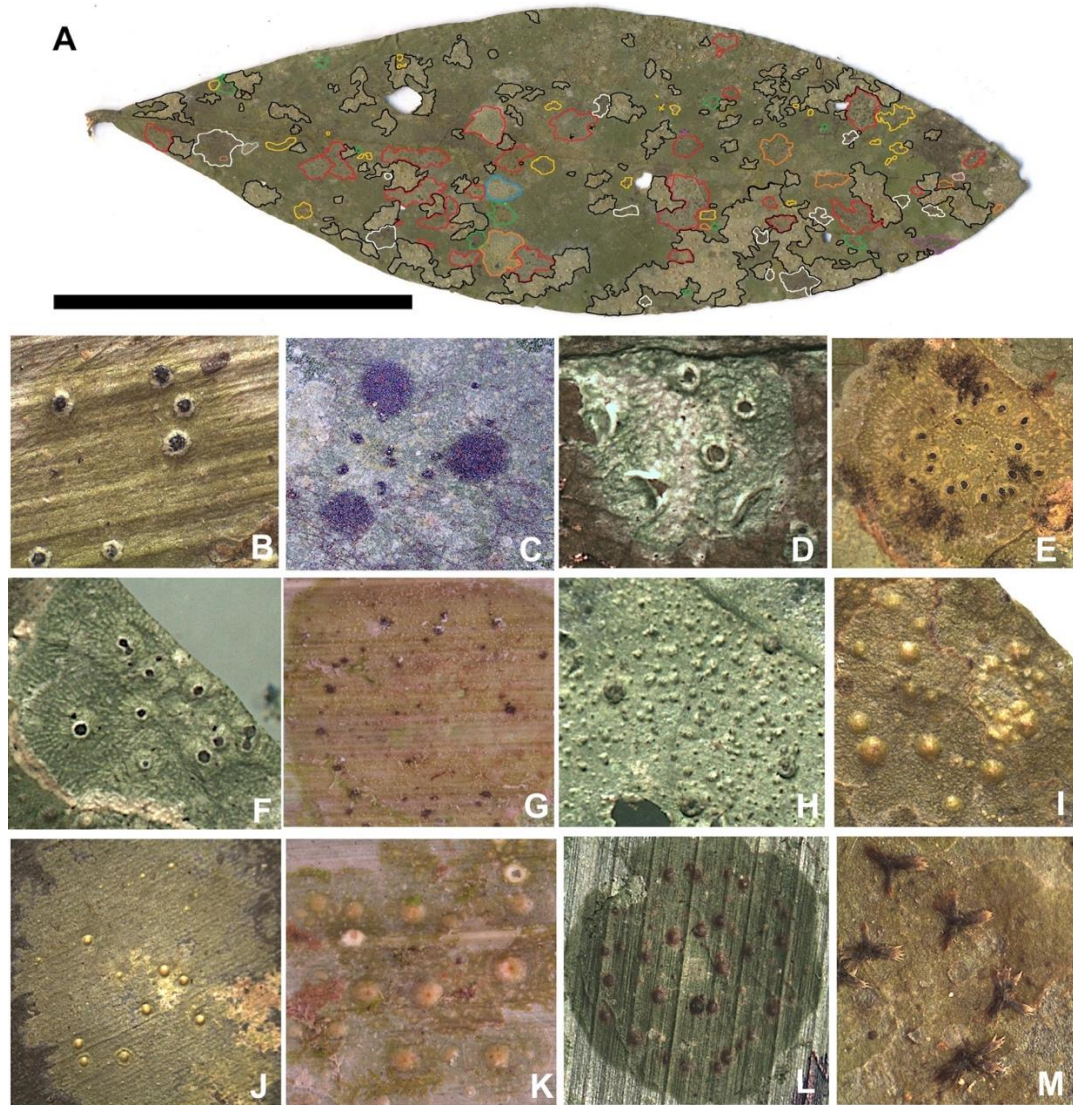
Figure 2. *Foliicolous lichens growing on the leaves of Stenanona flagelliflora. (A) The community of foliicolous lichens on a single leaf. Scale bar = 5 cm. (B) Anisomeridium foliicola (Monoblastiaceae), (C) Arthonia lecythidicola (Arthoniaceae), (D) Gyalectidium filicinum (Gomphillaceae), (E) Mazosia melanopthalma (Roccellaceae), (F) Mazosia rotula (Roccellaceae), (G) Microtheliopsis uniseptata (Microthelipsidaceae), (H) Phyllobathelium firmum (Strigulaceae), (I) Porina alba (Porinaceae), (J) Porina epiphylla (Porinaceae), (K) Porina karnatakensis (Porinaceae), (L) Porina rubentior (Porinaceae), (M) Trichothelium epiphyllum (Porinaceae). B-M photographs were taken with a microscope Nikon DXM 1200*

Mean relative cover of lichen species per leaf varied between $0.001 \pm 0.001\%$ and $51.82 \pm 4.48\%$ (*Table 1*). Lichen species with the highest relative cover per leaf were *Porina karnatakensis* ($51.82 \pm 4.48\%$), the unidentified morphospecies 1 ($8.53 \pm 2.66\%$), *P. alba* ($4.44 \pm 2.31\%$), *P. epiphylla* (Fée) Fée ($2.65 \pm 1.56\%$), *Coenogonium* sp. 1 ($2.63 \pm 1.6\%$), *P. rubentior* ($2.32 \pm 0.47\%$), *Arthonia* sp. 1 ($2.15 \pm 0.91\%$), and *P. tetramera* (Malme) R. Sant. ($2.11 \pm 1.03\%$; *Table 1*). In contrast, *Tricharia* sp. 2, and *Aulaxina* sp. 2 had the lowest relative cover per leaf ($<0.0016\%$).

Effect of leaf area and leaf position on the community of foliicolous lichens

Overall, top leaves were colonized by a total of 66 foliicolous lichen species; whereas middle and basal leaves represented a substrate for 48 and 47 species, respectively (*Table 1*). Leaf-area was not significantly different among leaves from different positions (base: 44.71 ± 3.16 cm^2, middle: 49.02 ± 3.74 cm^2, top: 41.25 ± 3.49 cm^2; $F_{2, 60} = 1.26$, $P = 0.291$).

Relative cover of foliicolous lichens in top, middle, and basal leaves was $24.5 \pm 3.3\%$, $30.78 \pm 2.22\%$, and $25.5 \pm 3.21\%$, respectively. Rank-cover curves showed a strong dominance of a few foliicolous lichen species within the communities growing on leaves from all positions (*Fig. 3*). *Porina karnatakensis* was the most dominant (*Fig. 3*) and frequent species in leaves of all positions (top: 18 leaves, middle and basal: 20 leaves each). Moreover, *P. karnatakensis* had the highest relative cover across all leaf positions (top: $14.8 \pm 0.03\%$, middle: $20.3 \pm 0.03\%$, basal: $12.8 \pm 0.03\%$). Leaf position did not have a significant effect on the relative cover of *P. karnatakensis* ($F_{2, 60} = 2.4$, $P = 0.099$). The unidentified morphospecies 1 was the second and third dominant species in leaves of all positions; whereas *P. alba* was the third and second dominant species in middle and top leaves. *Coenogonium* sp. 1 was the third most dominant species on basal leaves. In contrast, 42 (63.64%), 17 (35.42%), and 18 (38.3%) species were found in only one top, middle, and basal leaf, respectively. In top leaves, *Tricharia* sp. 2, and *Aulaxina* sp. 2 had the lowest mean relative cover per leaf (<0.01 cm^2, <0.005%). Likewise, *Porina octomera* (Müll. Arg.) F. Schil in middle leaves, as well as *Bacidina* sp. 1 and *Porina* sp. 14 in basal leaves, had the lowest mean relative cover per leaf (<0.004%).
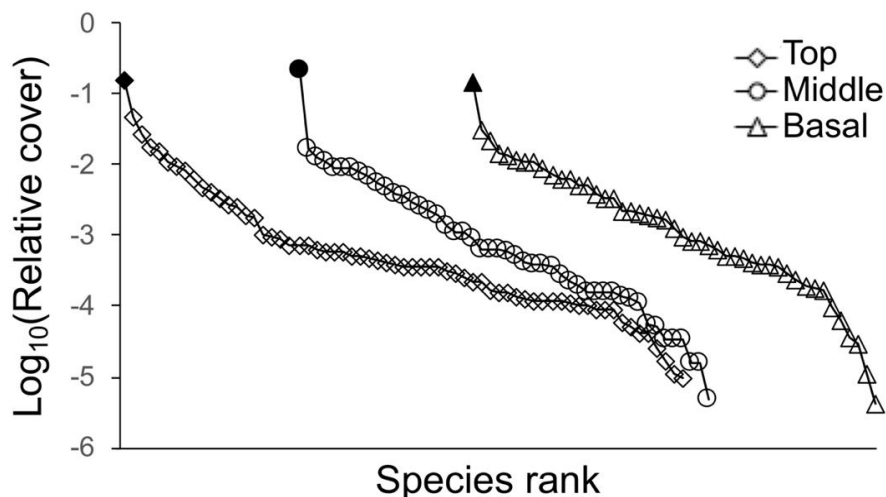

Figure 3. *Mean relative cover-rank curve of the foliicolous lichen species recorded in top, middle, and basal leaves of Stenanona flagelliflora. Relative cover is plotted on a log$_{10}$ scale and the horizontal axis corresponds to the rank from the most to the less common species (James and Rathbun, 1981). Black symbols correspond to the cover of Porina karnatakensis (Porinaceae)*

Lichen species richness, relative cover, and the Shannon-Wiener diversity index did not differ significantly among leaves with different position (*Table 2*). Likewise, none

of the three variables were significantly affected by leaf area (species richness: $r_s = 0.215$, df = 63, $P = 0.09$; lichen cover: $r_s = -0.16$, df = 63, $P = 0.21$; diversity: $r_s = 0.047$, df = 63, $P = 0.714$).

Table 2. *Species richness, relative cover, and Shannon-Wiener diversity index (mean ± s.e.) of the communities of foliicolous lichens associated to Stenanona flagelliflora leaves with different position. Results of statistical tests (ANOVA for species richness and relative cover; and Kruskal-Wallis for diversity) are shown. n = 63 leaves (21 from each position)*

Community parameter	Leaf position			Statistical results
	Base	**Middle**	**Top**	
Species richness	7.57 ± 0.65	8.24 ± 0.71	8.0 ± 2.56	$F_{2, 60} = 0.18, P = 0.83$
Relative cover (%)	25.5 ± 3.21	30.78 ± 2.22	24.5 ± 3.3	$F_{2, 60} = 1.672, P = 0.197$
Shannon diversity index	0.888 ± 0.102	0.825 ± 0.09	0.802 ± 0.37	$H_2 = 0.51, P = 0.774$

The communities of foliicolous lichens associated with leaves on different positions within the plant were fairly similar. The communities of middle and basal leaves had a similarity index of 67.37%. Similarity index between the communities of lichens on top and middle leaves was 52.63%, whereas between top and basal leaves it was 54.87%. Out of the 94 lichen species distinguished, 26 (27.6%) were recorded in all leaf positions (*Table 1*). In contrast, 31 (33% out of the total; 47% from the species recorded in top leaves), 12 (12.8% out of the total; 25% from the species recorded in the middle position), and 10 (10.6% out of the total; 21.3% from the species observed on basal leaves) taxa were unique to top, middle, and basal leaves, respectively (*Table 1*).

Porinaceae was the richest family at all leaf positions (top: 31; middle: 20; base: 17). Out of the 12 families of foliicolous lichens identified across all leaves, 10 were registered on top and middle leaves, and 11 in basal leaves. Species from the Lyrommataceae and Ramalinaceae families were not observed on top leaves (*Table 1*). Likewise, taxa from the Monoblastiaceae were not recorded in leaves from middle positions; whereas Thelotremataceae was not found in both middle and basal leaves. All other families were found in leaves from all positions (range: 1–6 lichen species per family; *Table 1*).

The stress value obtained in the NMDS analysis was 0.1091, indicating that the ordination is adequate for interpretation (Clarke et al., 2014). Results from the NMDS show that there is not a clear grouping of the communities of foliicolous lichens according with leaf position (*Fig. 4*). Results of the ANOSIM confirmed the lack of significant differences among lichen communities collected on leaves with different position ($R = 0.026$; $P = 0.067$).

At the species level, the CCA analysis showed that axes 1 and 2 explained 52.3% and 33.1% of the total variation on lichen cover, respectively. Most of the lichen species were not associated with either leaf area or leaf position. However, *M. melanopthalma*, *P. tetramera*, *P alba*, *P. octomera*, *M. rotula*, and *P rubentior* showed some association with leaf position, in a gradient that goes from the top to the basal leaves within the tree (*Fig. 5A*). At the family level, axis 1 and 2 explained 46.1% and 19.7% respectively, of the total variation in lichen cover. Strigulaceae showed an association with small leaves (*Fig. 5B*). Porinaceae, Roccellaceae, and Thelotremataceae were associated with leaf position (from the base towards the top, respectively; *Fig. 5B*).
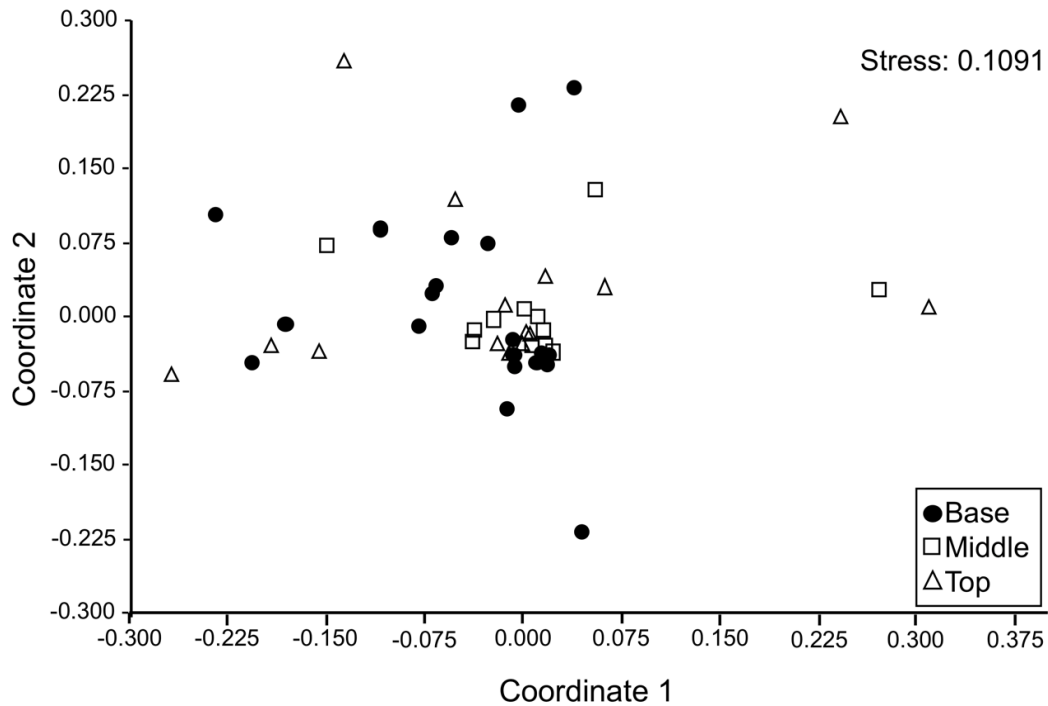
Figure 4. *Non-metric multidimensional scaling (NMDS) scatterplot of the communities of foliicolous lichens growing on leaves with different position within the phorophyte Stenanona flagelliflora*

Discussion

Our results showed that *Stenanona flagelliflora* supports a community of foliicolous lichens characterized by its high species richness and high dominance. Previous studies at Los Tuxtlas have recorded between 157 and 191 species of foliicolous lichen species (Bárcenas-Peña, 2007; Martínez-Colín, 2016). Accordingly, *Stenanona flagelliflora* supports between 32.6% and 59.8% of the total richness of foliicolous lichens recorded at the locality.

In contrast, species richness on a single leaf of *S. flagelliflora* was rather poor (1-17 species). Other studies have recorded a species richness of between 30 and 81 species per leaf (Lücking, 1995, 2008b; Lücking and Bernecker-Lücking, 2002; Lücking and Matzer, 2001). Thus, although *S. flagelliflora* supports a relatively rich community of foliicolous lichens, only 1 to 18% of the complete community is represented on each leaf. This suggests that there is a high variation in the composition of foliicolous lichens within each leaf (Lücking, 1995).

Leaf position and leaf area did not affect species richness, abundance (estimated as relative lichen cover) and diversity of the communities of foliicolous lichens, but they did have important effects on species composition. Likewise, the effect of leaf position on species composition has been documented for communities of other microorganisms of the phylloplane (Harrison et al., 2016; Hermann et al., 2021; Izuno et al., 2016; Laforest-Lapointe et al., 2016; Leff et al., 2015; Nguyen, 2017; Stone and Jackson, 2019). Vertical variation in species composition within a single tree might be determined by microclimate factors that influence the colonization and establishment of each species (Leff et al., 2015). Particularly, the colonization and establishment of lichens are influenced by fluctuations in UV radiation, desiccation, rainfall, nutrient

limitation, temperature and relative humidity (Gomes et al., 2018; Hirano and Upper, 2000; Lindow and Brandl, 2003; Thapa and Prasanna, 2018) commonly experienced by leaves (and the phylloplane communities on them; Lücking, 1998, 1999). Moreover, colonization and establishment of lichens on leaves might be determined by the dispersion patterns of each species (Stone and Jackson, 2019), leaf structure (Lücking, 1998, 1999); leaf age (Carroll, 1979) and the successional processes associated with it; interactions with other microorganisms, leaf orientation (Andrews et al., 1980; Nguyen, 2017), and horizontal intra-phorophyte position (Andrews et al., 1980; Stone and Jackson, 2019). Although *S. flagelliflora* is a short tree, vertical microclimate differences seem to explain the differences in species composition of foliicolous lichens among leaves. Undoubtedly, detailed studies at a small spatial scale are needed in order to determine the relative importance of each factor on the composition of the communities of foliicolous lichens.
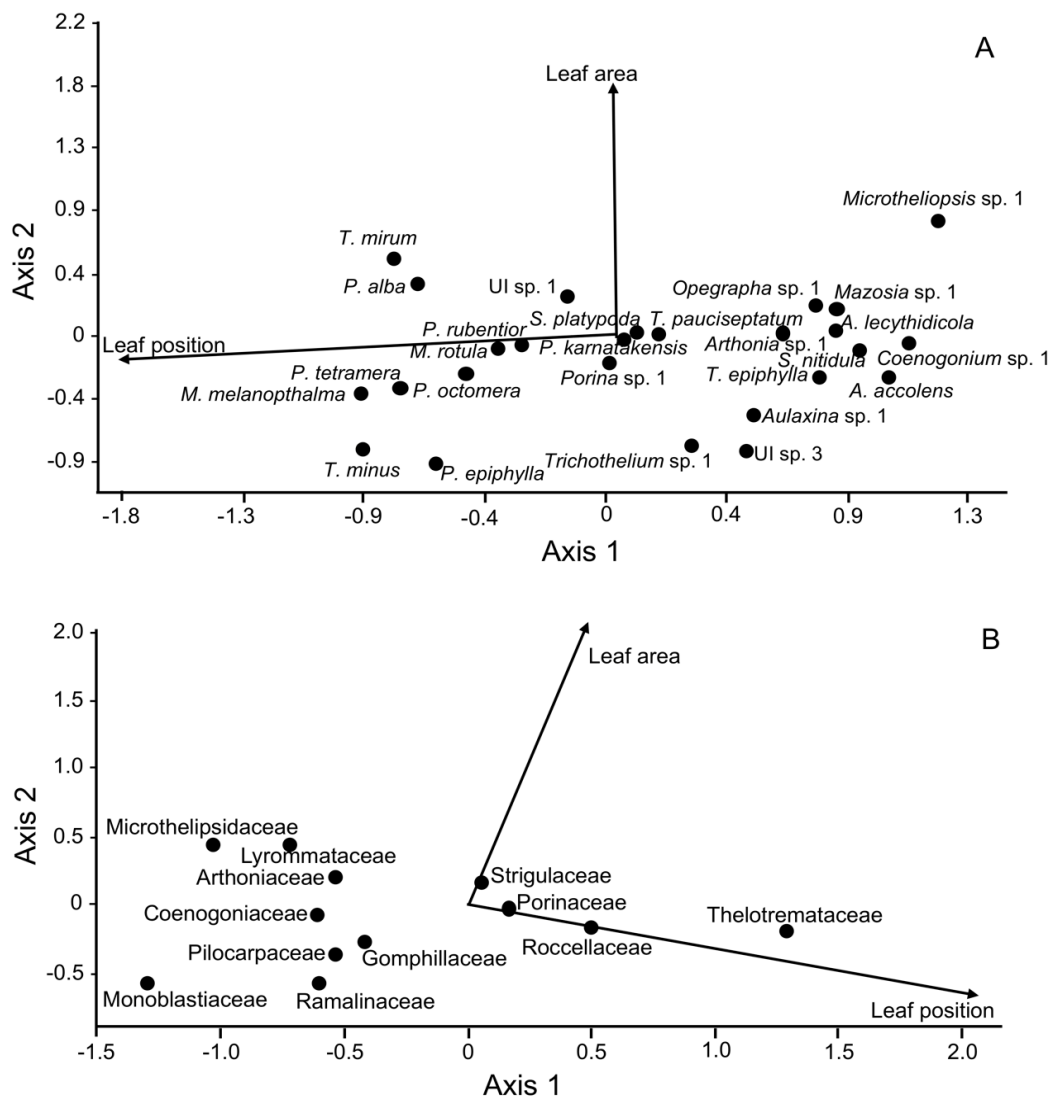

Figure 5. *Canonical correspondence analyses on the relative cover of foliicolous lichens in response to leaf area and leaf position (1 = base, 2 = middle, 3 = top) within individual plants of the phorophyte Stenanona flagelliflora. (A) Analysis at the species level, using the foliicolous species recorded in at least five leaves; (B) analysis at the family level*

Finally, three not mutually exclusive phenomena might explain the lack of significant effects of leaf position and leaf area on species richness, abundance, and diversity of foliicolous lichens. First, there might be a low microclimate variation across a short plant inhabiting the understory as *S. flagelliflora*, whose maximum height at the study site is 2.2 m. Significant vertical variation in communities of bacteria, fungi, microorganisms, and lichens of the phylloplane has been documented in phorophytes of between 4 - 108 m in height (Andrews et al., 1980; Carroll, 1979; Harrison et al., 2016; Hermann et al., 2021; Izuno et al., 2016; Leff et al., 2015; Nguyen, 2017; Sipman, 1997; Stone and Jackson, 2019). Second, *S. flagelliflora* leaf size (14.2 - 94.3 cm^2) might be too small to provide sufficient habitat heterogeneity that favors the establishment of a high number of species (Connor and McCoy, 2001; Kohn and Walsh, 1994). Last, the community dynamics within a single leaf (colonization and extinction) might be regulated by competitive interactions (Lindow and Brandl, 2003). In the leaves of *S. flagelliflora*, the communities of foliicolous lichens are strongly dominated by a few species (i.e., strong competitors), which might prevent the establishment of others (i.e., weaker competitors). Particularly, small leaf size and the existence of competitive interactions might be important in determining a not significant species richness-leaf area relationship in small-sized organisms such as molds, yeasts (Andrews et al., 1987), liverworts (Aranda et al., 2014), bryophytes (Kimmerer and Driscoll, 2000; Aranda et al., 2014), fungi (Kinkel et al., 1987; Feinstein and Blackwood, 2012), bacterioplankton (Logue et al., 2012), and foliicolous lichens (this study).

Conclusion

The leaves of *Stenanona flagelliflora* host a highly diverse community of lichens, whose richness, abundance (estimated as relative lichen cover) and diversity are not determined by both leaf area and leaf position. Instead, community composition seems to be associated with both of those factors. These patterns seem to be explained by the existence of microclimate variation within the canopy of *S. flagelliflora*, strong competitive interactions regulated by the dominant species within the communities, and particular environmental requirements needed for the colonization and establishment of each lichen species. Undoubtedly, more studies on the importance of organisms within the phylloplane and the factors that determine their diversity are needed.

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