

Article

Ecological Study of the Active Ciliate Community from Bromeliads during the Humid Season in Different Types of Forests of the Mexican Neotropics

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Abstract: Bromeliads are a Neotropical family of monocots, colonized by several families of ciliates, with some species that inhabit only this type of microecosystems. Ecological factors such as the presence of water, plant volume, seasonality and predators, have been recognized as important factors, which play a role in the ciliate community structure in tank bromeliads. The objective of this study is to describe different communities of active ciliates from epiphytic and terrestrial bromeliads that inhabit the dry tropical forest, montane cloud forest, oak forest and semideciduous tropical forest in the Mexican Neotropics during the humid season of the year 2016. We found 22 species of active ciliates. A higher richness of ciliate species was found in the bromeliads of the montane cloud forest. Based on statistical tests, we determine that the pH and water temperature, plant diameter, its distance above the ground and altitude above sea level in relation to ciliate abundance explain <50% of the variation; however, the combination of plant diameter and its distance above the ground shows a positive effect in relation to ciliate abundance. We provide new evidence that bromeliads that inhabit mountains and lowland forests in a larger geographic area host ciliate communities with different species composition.

Keywords: abundance; Ciliophora; Bromeliaceae; diversity; Neotropics; phytotelma



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1. Introduction

Bromeliads comprise a family of flowering plants widely adapted to tropical, subtropical and mountain ecosystems, with more than 3140 species distributed almost exclusively in the Neotropics [1]. Since Nadkarni [2] referred bromeliads as a keystone resource in Neotropical ecosystems, increasing literature has been published about their associated biota and their participation in the nutrient cycling. Bromeliads are also important models to study processes related to the ecology of islands [3] and food webs [4]. For nutrient acquirement, the foliar architecture of many species of bromeliads has evolved to enhance the formation of a tank as a result of the coalescence of their leaves. The accumulation of rainwater and litter in this tank results in the formation of a phytotelm [5].

Ciliates are heterotrophic protists included in the supergroup Alveolata [6]. More than 170 species have been recorded in bromeliad phytotelmata [7], especially from the classes Oligohymenophorea, Colpodea and Spirotrichea. Ciliate communities that colonize this type of microhabitats are composed of cosmopolitan ciliates (planktonic and soil autochthonous) and the so-called endemic ciliates, inhabiting along the water column, the bottom of the tank or as epibionts of invertebrates [8,9].

Bromeliads can host ciliate communities of active and encysted ciliates [10,11]. An active ciliate can be defined as a trophont, which is the non-cystic form recognized by its

interfissional or feeding and growing stage in the life cycle of any ciliate [12]. How ciliates do colonize bromeliads is an unsolved question, but a passive dispersion [13], frogs [14] and mites [15] could play an important role in bromeliad colonization by ciliates.

Carrias et al. [16] provided evidence that ciliates act as important consumers of bacteria in tank bromeliads and established that they appear to be one of the groups of protists of major importance within the aquatic community of tank bromeliads, with similar densities to those reported for freshwater ponds and productive lakes. Kratina et al. [17] recognized the effect of vertical height above the ground and canopy openness on amoebae and flagellates from bromeliads in Costa Rica. However, ecological aspects of ciliates communities that inhabit bromeliads still remain poorly understood.

Some studies that analyzed the influence of biotic and abiotic factors on ciliate communities in tank bromeliads have taken place in small geographic areas and only considered one or a few bromeliad species. Buosi et al. [18,19] analyzed the ciliate community in *Aechmea distichantha* along the margin of the Paraná River in South Brazil, emphasizing the importance of water for the presence of ciliates in bromeliads and the influence of proximity to freshwater bodies as a factor that enhances the colonization of ciliates to bromeliads. In relation to the community composition and structure, they found that species' richness was higher during the dry season in contrast to the abundance, which was higher in the humid season [18,19].

Durán-Ramírez et al. [20] and Malfatti et al. [21] recognized altitude as an important factor for ciliate diversity in bromeliads and the results of both studies supported the idea that ciliate diversity in bromeliads is higher at intermediate elevations. In Mexico, some studies regarding ciliates in bromeliads have been published under a perspective of alpha taxonomy in agroecosystems, montane cloud forests and different types of tropical vegetation in small areas of the country [7,11,20]. Other ecological factors such as the plant volume [16], canopy cover [22] and predators [23] were identified to play a role on the ciliate community structure in phytotelmata; however, it is more likely that stochastic processes are responsible for the ciliate community structure and composition in tank bromeliads.

More than 422 species of bromeliads have been recorded in Mexico [24] within different ecosystems, especially in forests of the Neotropical region. The aim of this study is to compare the communities of active ciliates from epiphytic and terrestrial tank and tank-less bromeliads, and to test if there is any effect of some environmental factors in four types of Neotropical forests in Mexico during the humid season of the year 2016. Our results represent the first ecological work about ciliates in bromeliads that considers a wider geographic area in the Mexican Neotropics.

2. Materials and Methods

2.1. Area of Study

We collected water samples and detritus from 60 individuals of bromeliads during the humid season of year 2016 (June to November) from six localities (Figure 1) selected as representative ecosystems of the Neotropical region of Mexico *sensu* Morrone [25], where bromeliads are common floristic elements. Ten bromeliads (belonging to 12 species, epiphytic and terrestrial) were sampled from each locality (Table 1).

2.2. Sample Collection

We collected samples of 10 mL of water volume and/or detritus from the rosettes of each bromeliad (Table 1). None of the plants were removed from their habitat. Leaves that impounded water were considered for sampling, and pipettes were introduced to the bottom of the leaf axil to obtain water with some detritus. Samples of water and detritus were collected by using a sterile plastic transfer pipette for each plant and placed into sterilized Falcon tubes. For bromeliads that did not have water accumulated in their leaves, we only collected the detritus by using a fine spatula to carefully remove them over a tray. Sessile species of ciliates were not collected by the application of this sampling method. Water temperature impounded for each sampled plant was measured in situ using

a thermometer Taylor-5984; if water was absent inside the plant, temperature inside the tank was measured. Due to the small size of many of the sampled bromeliads, we used pH indicator stripes 0–14 Hydriion[®] to record pH values in situ of the water. To obtain an indirect value of plant volume, we measured the diameter considering both extremes of the leaves in the widest portion of the plants. For epiphytic plants, we also measured the distance above the ground to understand if there was any influence on ciliate community related to soil proximity. Box-plotted graphics were performed for the following variables: water temperature, pH, plant diameter and its distance above the ground. All variables, including the altitude above sea level, were considered to analyze their effect on ciliate community of bromeliads. Samples were maintained at room temperature during their transportation and in the laboratory. Bromeliads were identified by photoidentification according to the study of Espejo-Serna and López-Ferrari [24].

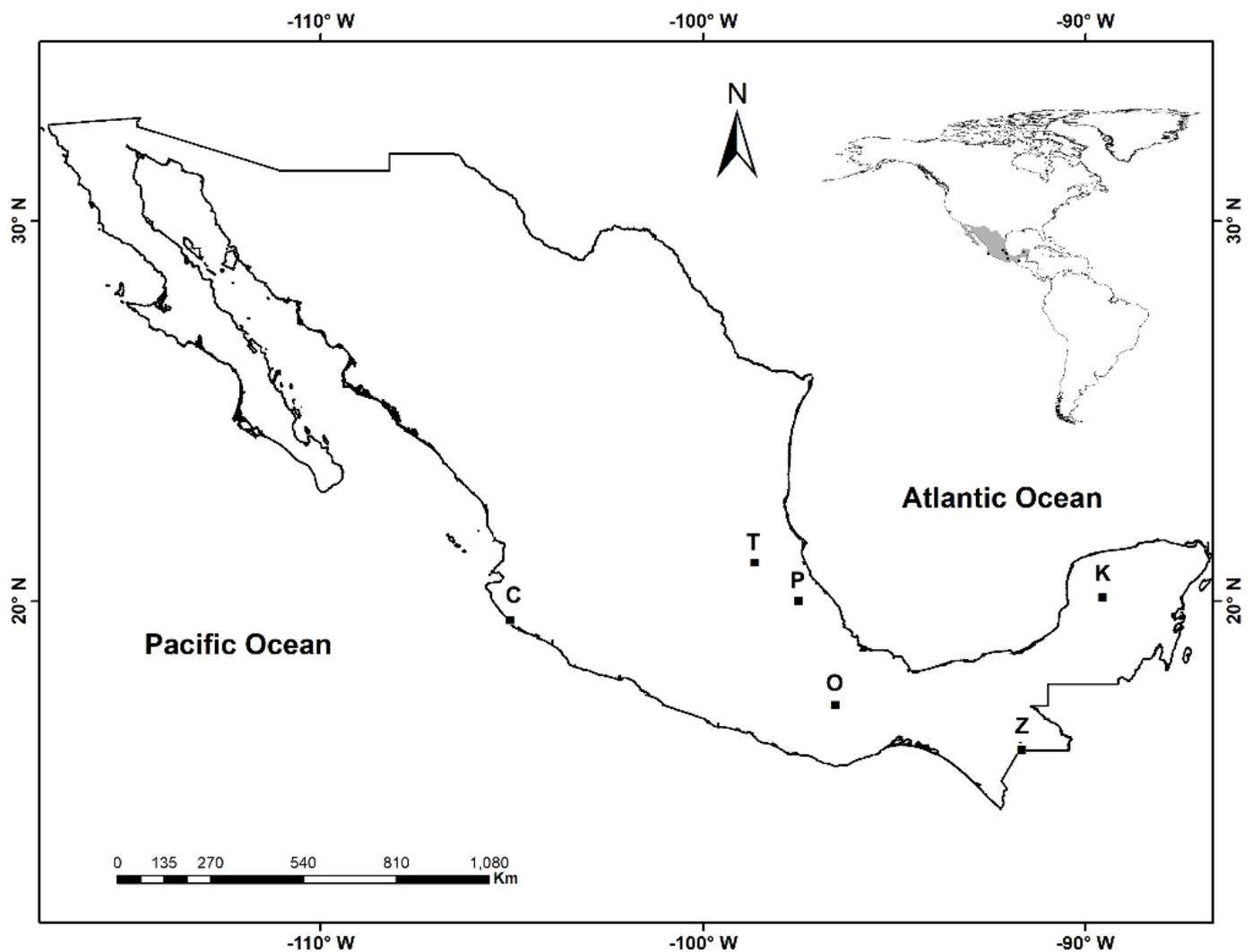


Figure 1. Map of the six localities of study. For the locality names, see Table 1.

2.3. Ciliate Identification

We analyzed each one of the 60 samples 48 h after sampling and at intervals of two days through the next two weeks. We observed cytological characters of taxonomical importance in vivo (e.g., cell size and shape, position and shape of the cytostome, position and number of the contractile vacuoles) using bright field and differential interference contrast microscopy with a Nikon Labophot-2 microscope equipped (Tokyo, Japan) with a Nikon Digital Sight DS-2Mv camera (Tokyo, Japan). Additionally, we established cul-

tures in Petri dishes using some drops of the original sample, table water Evian® (Haute Savoie, France) and wheat grains to stimulate bacterial growth [9]. We performed silver impregnation techniques following the protocols of Foissner [26] to reveal oral and somatic infraciliature, number and disposition of cirri, the Silverline pattern and nuclear apparatus. We followed Foissner [27–32], Foissner et al. [33], Guggiari and Peck [34], Lee et al. [35], Omar and Foissner [36,37] and Penard’s studies [38] for species identification. Systematics was according to Lynn [12].

Table 1. Description of studied localities and bromeliad species in the Mexican Neotropics.

Locality Identifier	Locality	Type of Vegetation	Geographic Coordinates	Altitude (m asl)	Bromeliad Species
Locality C	Surroundings of the Estación de Biología Chamela-UNAM, inside the Reserve of the Biosphere Chamela-Cuixmala, municipality of La Huerta, Jalisco.	Dry tropical forest	19°29′54.82″ N, 105°02′45.07″ W	51	<i>Bromelia karatas</i> L., <i>Tillandsia makoyana</i> Baker, <i>T. rothii</i> Rauh
Locality Z	Forest around Lake Tziscaco and Lake Dos Lagunas, located at National Park Lagos de Montebello, municipality of La Independencia and Trinitaria, Chiapas.	Montane cloud forest	16°05′47.43″ N, 91°41′04.77″ W	1444	<i>T. guatemalensis</i> L. B. Sm., <i>T. multicaulis</i> Steudel, <i>Werauhia</i> sp.
Locality O	Sierra Juárez, Km 175 of the road Oaxaca–Tuxtepec, near the village El Cerezal, municipality of Santa Catarina Ixtepeji, Oaxaca.	Oak forest	17°15′33.87″ N, 96°32′28.70″ W	2377	<i>T. prodigiosa</i> Baker
Locality T	Forest area for conservation El Tegolome, near the town of Tlanchinol, municipality of Tlanchinol, Hidalgo.	Montane cloud forest	21°01′19″ N, 98°38′45″ W	1533	<i>T. imperialis</i> E. Morren ex Mez, <i>T. multicaulis</i> Steudel, <i>Tillandsia</i> sp.
Locality P	Near the town of Cuetzalan, municipality of Cuetzalan del Progreso, Puebla.	Montane cloud forest	20°00′21.438″ N, 97°30′30.56″ W	983	<i>Catopsis sessiliflora</i> (Ruiz and Pavón) Mez, <i>T. heterophylla</i> E. Morren
Locality K	Biocultural Reserve Kaxil Kiuic, located 27 KM southern of the town of Oxkutzcab, municipality of Oxkutzcab, Yucatán.	Semideciduous tropical forest	20°06′10.8″ N, 89°33′43.2″ W	92	<i>Aechmea bracteata</i> (Sw.) Grisebach

2.4. Laboratory Analysis

Trophonts of both free-swimming and sessile individuals were considered as the active component of the community [12]; any cyst was considered. We calculated the abundance

of active ciliate species from all the samples after they were transported to the laboratory, following the direct count method of Foissner [39] and Lüftenegger et al. [40], with some modifications described here briefly. Each one of the field water samples was manually homogenized by gentle manual agitation and dropped 1 mL of the sample on a sterile cell culture Sarstedt®, Nümbrecht, Germany plate of 60 mm size by using an Eppendorf micropipette. Then, we placed a line of five separated droplets of 10 µL each (50 µL) of the sample on a single slide; by using the objective of 10× of a bright field microscope, we counted the number of cells in each droplet with a Counter Clay Adams (New York, NY, USA) For each one of the samples, the abundance for each ciliate species expressed in individuals/mL corresponded to the average of the number of individuals in each droplet extrapolated to 1 mL of water volume.

2.5. Data Analysis

To compare ciliate species richness in all the bromeliads of the six studied localities, we obtained α -diversity Menhinick index ($D_{Mn} = S/\sqrt{N}$), where S is the number of species of a sample and N is the total number of individuals in the sample, considering the analyzed water volume as the sample. The index expresses the relation between the total number of species and the total number of observed individuals from a sample. To evaluate community structure through the proportional abundance of ciliates, we applied Simpson's index ($\lambda = \sum p_i^2$), where p_i is the proportional abundance of the species i , which is the number of individuals of species i divided into the total number of individuals of the sample. Shannon–Wiener index ($H' = -\sum p_i \ln p_i$) was also applied to express the uniformity of the importance values through all the species in the samples to measure the average degree of uncertainty in predicting which species would belong to an individual randomly selected [41,42]. Box-plotted graphics of α -diversity values for each index of all the bromeliads in all the localities were performed, and a Tukey's test was applied to compare if there were differences between the means of α -diversity values for all the localities. To test differences in mean values of α -diversity indexes in the six localities, we carried out a one-way analysis of variance (ANOVA) for data with a normal distribution. A simple linear regression was performed using locality C as a reference of basal level due to its low diversity of active ciliates in the sampled bromeliads; the simple linear regression was applied to know if there were differences in the values of the α -diversity indexes in relation to the other five localities as input predictor variables. To show if there was any correlation between abundance of ciliates, values of α diversity indexes and environmental variables, we applied the Pearson correlation coefficient using the function *GGally*. A generalized linear mixed model (GLMM) was performed to find out the effect and influence by considering response variables without normal distributions, including both fixed and random effects; this model provided a more flexible approach for analyzing non-normal data that involve random effects [43]. By applying the function *glmmTMB* and the package *MuMIn*, the localities were considered as the random effect to carry out the modeling of abundance (response variable) in relation to altitude above sea level, pH and water temperature, plant diameter and its distance above the ground as the set of environmental variables. By using the information criteria function of the program, the most informative resulting model was selected. A factor analysis of mixed data (FAMD), which considers both quantitative and qualitative variables [44], was employed as a principal component method to assess differences in the occurrence and abundance of ciliates, in relation to environmental variables (altitude above sea level, distance above the ground, plant diameter and water pH and temperature) in the four types of vegetation. Graphics and data analytics were carried out by using the function 'plot_model', Package *sjPlot* of RStudio 4.0.3.

3. Results

3.1. Microhabitat Characterization

All sampled bromeliad species were epiphytic with the exception of *Bromelia karatas* in locality C as the only terrestrial and tank-less bromeliad. Epiphytic bromeliads were located from 0.2 to 3.5 m above the ground ($mean = 1.64$). Some individuals of *Werauhia* sp. in locality Z were epiphytic on tree branches above the Tzisco Lake surface. Bromeliads were adult plants with diameters from 0.12 to 1.52 m ($mean = 0.54$). In almost all the bromeliads of locality C, water impoundments were absent and temperature values inside the plants ranged from 24 to 41.8 °C. In the plants of the rest of the localities, water was present with temperature ranging from 14.8 °C to 29 °C ($mean = 22.43$). A trend to acidity is usually common in the bromeliad microecosystem. We obtained water samples with pH values ranging from 3.5 to 6.7 ($mean = 4.59$) (Figure 2).

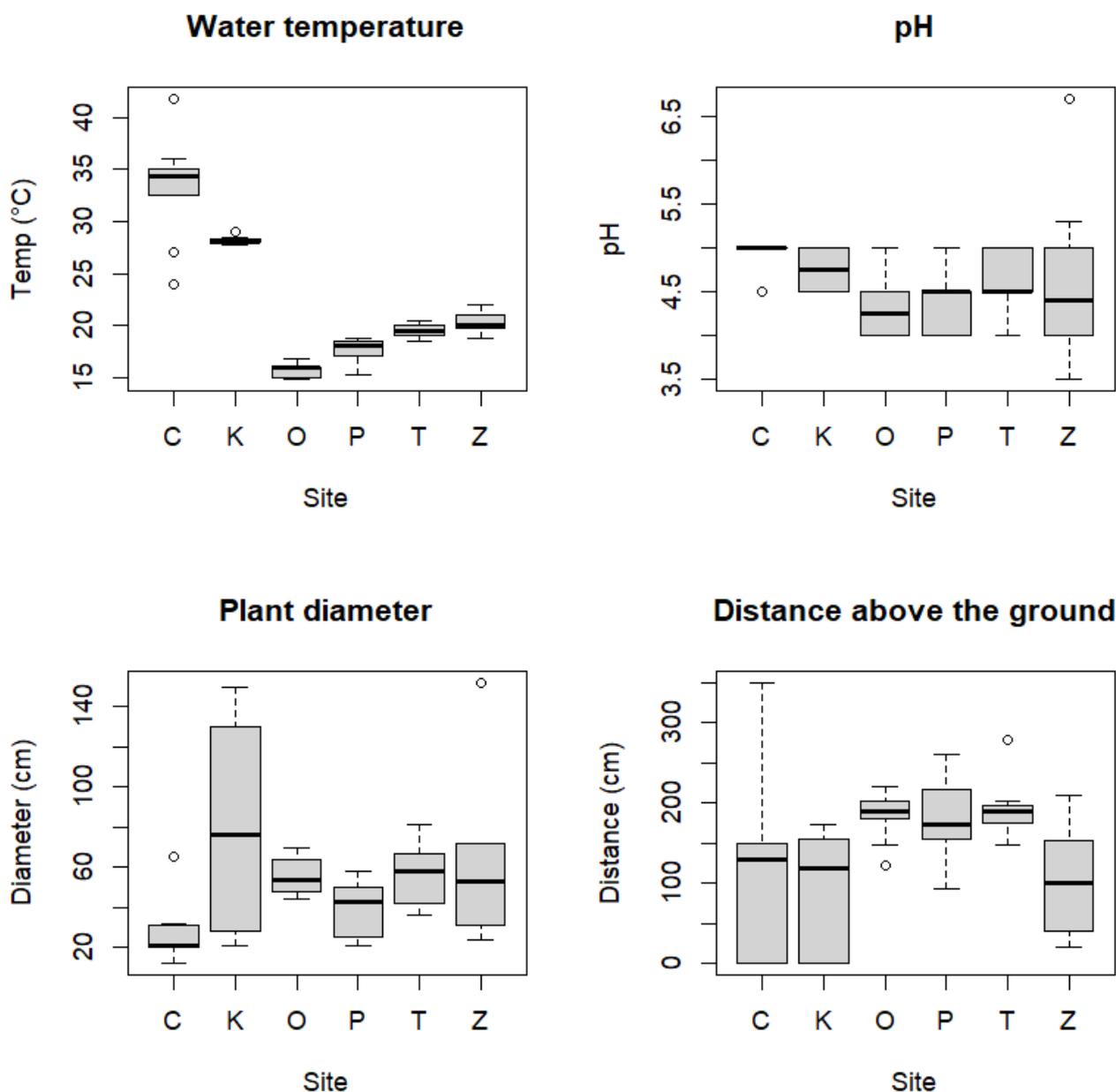


Figure 2. Boxplots of environmental factors related to bromeliads in the Mexican Neotropics. Dots are the outliers from the upper and the lower quartiles. See Table 1 for the names of the localities in capital letters.

3.2. Species Richness

We identified 22 active ciliates species (Table 2) from the 12 bromeliad species across the six localities in the Mexican Neotropics. Ciliate species were included in the classes Colpodea (*Bromeliothrix metopoides*, *Colpoda cavicola*, *C. cucullus*, *C. maupasi*, *C. lucida* and *Paracolpoda lajacola*), Nassophorea (*Drepanomonas sphagni*, *Leptopharynx bromelicola*, *L. bromeliophilus* and *L. costatus*), Oligohymenophorea (*Bromeliophrya brasiliensis*, *Cyclidium glaucoma*, *Glaucomides bromelicola*, two hymenostomatids, *Lambornella* sp., three peritrichs and *Tetrahymena* sp.) and two unidentified species of spirotricheans. From each sampled bromeliad, a number of one to four active ciliate species ($mean = 1.43$) were observed and 20 of the 60 plants did not have any active ciliate species. A single ciliate species was recorded in 14 individuals of bromeliads. Due to the lack of water, active ciliates were absent in almost all bromeliads of the tropical dry forest (locality C), with the exception of one individual of *Tillandsia makoyana*. *Glaucomides bromelicola*, which is a polymorphic endemic ciliate in bromeliads, was the most frequent species in the study, being present in one third of all the plants and observed in all the localities, with the exception of the dry and semideciduous tropical forests (localities C and K). None of the ciliate species were present in all the localities and bromeliads. Bromeliads from the montane cloud forest of locality Z showed the higher species richness (eleven species), followed by locality P (nine species), locality O (eight species), locality K (six species) and localities C and T with three species each. With the exception of samples without active ciliates, α -diversity indexes showed differences between the sampled plants of the six localities (Figure 3). Values of Menhinick index were from 0.01 to 0.22 ($mean = 0.103$), which expressed the low richness of the active ciliate community in bromeliads of this Neotropical region. Simpson's index values were from 0.38 to 1 ($mean = 0.49$), and the Shannon–Wiener index provided values from 0 to 1.07 ($mean = 0.288$). For the three α -diversity indexes, differences of the mean values between localities were indicated according to groups *a*, *b* and *ab* (Figure 3) at the top of each box plot, where *a* expressed significant differences in relation to *b*, and group *ab* was not significantly different in relation to *a* and *b*; for Menhinick index (one way ANOVA: $F = 4.292$; $p = 0.00233$); for Shannon–Wiener index (one way ANOVA: $F = 2.474$; $p = 0.043$); for Simpson index (one way ANOVA: $F = 4.805$; $p = 0.00105$). According to the data obtained from the simple linear regression and *p* values (Table 3), the richness and community structure of the bromeliads from the dry tropical forest of Chamela-Cuixmala (locality C) was different in comparison to the other five localities.

3.3. Abundance

We estimated and extrapolated a total count of 32,260 individuals of all the active ciliate species from the 60 sampled bromeliads, and ciliate densities were obtained only in 40 of them due to the absence of trophonts when samples were analyzed. Ciliate species densities ranged from 20 to 6220 individuals \times mL⁻¹. *Bromeliophrya brasiliensis* and *Glaucomides bromelicola* had the highest densities values (Table 2), both species inhabiting only bromeliads of the montane cloud forest and oak forest. It was common to observe that microstome forms of *G. bromelicola* and *B. brasiliensis* frequently coexisted in the same bromeliad, especially in localities O and Z, and sometimes they were observed as the only two active species in bromeliads (pers. obs.). Only some of the bromeliads from the oak forest, located above 2377 m asl (locality O), and the montane cloud forest (locality Z) showed the highest densities of active ciliates. Other species endemic to bromeliads, such as *Leptopharynx bromelicola* and *L. bromeliophilus*, were observed in low densities in a few bromeliads too. Genus *Colpoda* was considered as one of the most common groups of ciliates in bromeliads and was the genus with more species in this study, but with the exception of two plants, the abundance values of species such as *Colpoda cucullus* and *C. mupasi* were also low.

Table 2. Mean densities (individuals per milliliter) of ciliate species in bromeliads recorded during the humid season of year 2016 in the Mexican Neotropics.

Ciliate species	Locality C										Locality Z									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
<i>Bromeliophrya brasiliensis</i> Foissner, 2003	-	-	-	-	-	-	-	-	-	-	-	-	-	5240	-	20	3900	-	-	-
<i>Bromeliothrix metopoides</i> Foissner, 2010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda cavicola</i> Kahl, 1935	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda cucullus</i> (Müller, 1773)	-	-	-	-	-	-	-	-	-	-	580	-	-	-	-	-	-	-	-	-
<i>Colpoda lucida</i> Greeff, 1888	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda maupasi</i> Enriques, 1908	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cyclidium glaucoma</i> Müller, 1773	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Drepanomonas sphagni</i> Kahl, 1931	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Glaucomides bromelicola</i> Foissner, 2013	-	-	-	-	-	-	-	-	-	-	1800	-	1860	-	-	180	-	-	260	-
Hymenostomatia 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	180	-	-
Hymenostomatia 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lambornella</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-
<i>Leptopharynx bromelicola</i> Foissner et al., 2011	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	20	-	-	-	-
<i>Leptopharynx bromeliophilus</i> Omar and Foissner, 2011	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	80	-
<i>Leptopharynx costatus</i> Mermod, 1914	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Operculariidae	-	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paracolpoda lajacola</i> Foissner, 2016	-	-	-	-	-	-	-	-	-	-	-	80	-	-	-	-	-	-	-	-
Peritrichia 1	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-
Peritrichia 2	-	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporadotrichida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	-	-
Stichotrichida	-	-	-	-	-	-	-	-	-	-	-	80	-	-	-	-	-	-	-	-
<i>Tetrahymena</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of species per bromeliad	0	3	0	0	0	0	0	0	0	0	3	4	1	1	0	3	2	2	2	0
Total abundance of active species	0	140	0	0	0	0	0	0	0	0	1840	760	1860	5240	0	220	3920	480	340	0
Bromeliad species	Bk	Tk	Tr	Tr	Tr	Bk	Bk	Tk	Tr	Tk	Tg	Tg	Tm	Tg	Ws	Tg	Tm	Ws	WS	Ws

Table 2. Cont.

Ciliate species	Locality O										Locality T									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
<i>Bromeliophrya brasiliensis</i> Foissner, 2003	400	-	20	-	1100	20	-	60	-	1750	-	-	-	-	-	-	-	-	-	-
<i>Bromeliothrix metopoides</i> Foissner, 2010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda cavicola</i> Kahl, 1935	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda cucullus</i> (Müller, 1773)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda lucida</i> Greeff, 1888	-	-	-	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda maupasi</i> Enriques, 1908	-	-	20	-	-	-	1520	20	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cyclidium glaucoma</i> Müller, 1773	-	-	-	540	-	-	-	-	-	-	-	-	60	-	-	-	-	-	-	-
<i>Drepanomonas sphagnii</i> Kahl, 1931	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Glaucomides bromelicola</i> Foissner, 2013	740	-	140	-	260	-	-	-	620	6220	-	-	20	40	80	40	40	20	20	40
Hymenostomatia 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hymenostomatia 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lambornella</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leptopharynx bromelicola</i> Foissner et al., 2011	-	-	-	-	-	-	-	-	-	-	-	-	-	40	100	-	-	-	-	-
<i>Leptopharynx bromeliophilus</i> Omar and Foissner, 2011	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leptopharynx costatus</i> Mermod, 1914	-	-	-	240	60	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Operculariidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paracolpoda lajacola</i> Foissner, 2016	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peritrichia 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peritrichia 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporadotrichida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stichotrichida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tetrahymena</i> sp.	-	-	80	20	-	420	-	60	-	-	-	-	-	-	-	-	-	-	-	-
Number of species per bromeliad	2	0	4	4	4	3	1	3	1	2	0	0	1	2	2	2	1	1	1	1
Total abundance of active species	1140	0	260	840	1520	480	1520	140	620	7970	0	0	20	100	120	140	40	20	20	40
Bromeliad species	To	To	To	To	To	To	To	To	To	To	Ti	Ti	Ti	T1	Ti	Ti	Tm	Tm	T1	T1

Table 2. Cont.

Ciliate species	Locality P										Locality K									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
<i>Bromeliophrya brasiliensis</i> Foissner, 2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bromeliothrix metopoides</i> Foissner, 2010	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda cavicola</i> Kahl, 1935	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	280	-	-
<i>Colpoda cucullus</i> (Müller, 1773)	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda lucida</i> Greeff, 1888	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Colpoda maupasi</i> Enriques, 1908	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-
<i>Cyclidium glaucoma</i> Müller, 1773	-	-	-	20	-	360	-	-	-	-	-	20	-	-	-	-	-	120	-	-
<i>Drepanomonas sphagnii</i> Kahl, 1931	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Glaucomides bromelicola</i> Foissner, 2013	-	-	20	-	-	20	-	-	40	240	-	-	-	-	-	-	-	-	-	-
Hymenostomatia 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hymenostomatia 2	-	-	-	-	-	20	20	80	120	-	-	-	-	-	-	-	-	-	-	-
<i>Lambornella</i> sp.	-	-	-	-	-	-	-	-	180	200	-	60	-	-	-	-	-	-	-	-
<i>Leptopharynx bromelicola</i> Foissner et al., 2011	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leptopharynx bromeliophilus</i> Omar and Foissner, 2011	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	60	-	-	-
<i>Leptopharynx costatus</i> Mermod, 1914	20	-	200	-	-	20	40	-	-	40	-	-	-	-	-	-	-	-	-	-
Operculariidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paracolpoda lajacola</i> Foissner, 2016	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peritrichia 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peritrichia 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporadotrichida	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
Stichotrichida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tetrahymena</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of species per bromeliad	3	1	2	1	0	4	2	2	3	3	0	2	1	0	0	1	1	4	0	0
Total abundance of active species	140	20	220	20	0	420	60	100	340	480	0	100	20	0	0	50	20	480	0	0
Bromeliad species	Th	Th	Cs	Th	Th	Th	Th	Th	Th	Th	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab

Bromeliad species: Ab—*Aechmea bracteata*; Bk—*Bromelia karatas*; Cs—*Catopsis sessiliflora*; Tg—*T. guatemalensis*; Th—*T. heterophylla*; Ti—*T. imperialis*; Tk—*Tillandsia makoyana*; Tm—*T. multicaulis*; Tr—*T. rothii*; To—*T. prodigiosa*; T1—*Tillandsia* sp.; Ws—*Werauhia* sp. Samples are indicated with the numbers 1 to 10 for each locality (capital letters, see Table 1 for names).

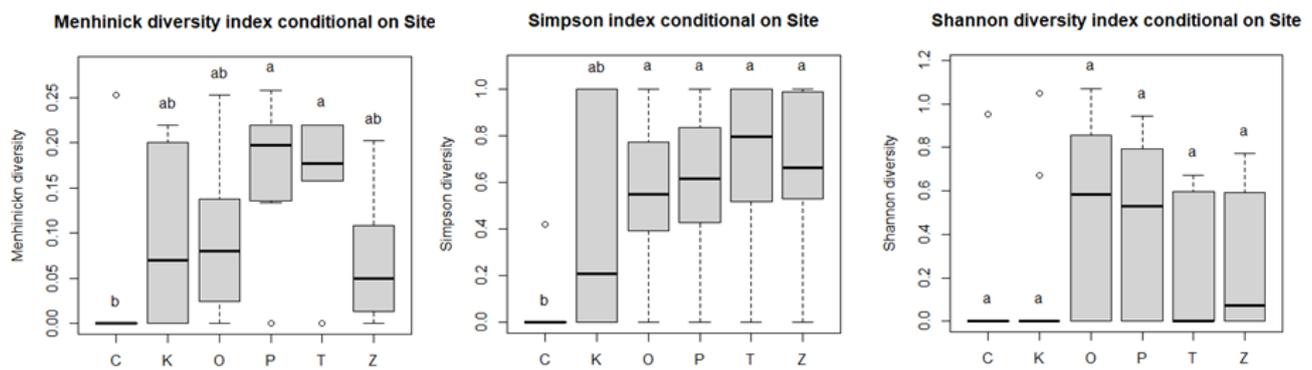


Figure 3. Boxplots of α -diversity indexes values of ciliates inhabiting bromeliads in the Mexican Neotropics. Dots are the outliers from the upper and the lower quartiles. Significant differences for each locality are indicated in groups by the use of letter *a*, *b* or *ab*. See Table 1 for the names of the localities in capital letters.

Table 3. Simple linear regression of α -diversity indexes values of ciliates inhabiting bromeliads in the Mexican Neotropics. See Table 1 for the names of the localities. CI: confidence interval; *p*: *p*-value.

Predictors	Menhinick Index (D_{Mn})			Simpson’s Index (λ)			Shannon–Wiener Index (H')		
	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>
Locality C	0.025	−0.028–0.079	0.349	0.042	−0.178–0.262	0.703	0.095	−0.128–0.319	0.396
Locality K	0.096	0.043–0.150	0.001	0.394	0.174–0.614	0.001	0.172	−0.051–0.395	0.128
Locality O	0.102	0.049–0.156	<0.001	0.581	0.361–0.800	<0.001	0.538	0.315–0.761	<0.001
Locality P	0.178	0.124–0.231	<0.001	0.621	0.401–0.840	<0.001	0.463	0.240–0.686	<0.001
Locality T	0.153	0.099–0.207	<0.001	0.666	0.446–0.886	<0.001	0.191	−0.033–0.414	0.093
Locality Z	0.068	0.015–0.122	0.014	0.639	0.419–0.859	<0.001	0.271	0.048–0.494	0.018

3.4. Environmental Variables and Their Effect on the Ciliate Community

The correlogram of Pearson correlation coefficient (Figure 4) summarized the degree of correlation between pairs of random continuous quantitative variables. Water temperature and altitude above the sea level of the studied localities showed a negative correlation ($r = -0.8$), and the proportional abundance of ciliates, expressed through the Simpson index, showed a negative trend correlated with water temperature too ($r = -0.5$). Values of both Simpson and Shannon–Wiener indexes, which express the uniformity of the importance value of the species, were positively correlated with Menhinick index ($r = 0.6$). The rest of the variables considered in the study showed low *r* values to establish if they could be positively or negatively correlated.

The selected resulting GLMM model included the ciliate abundance, altitude, distance above the ground, plant diameter, water temperature and the interaction of plant diameter and its distance above the ground (Table 4). We established that the abundance (response variable) fit a negative binomial distribution zero-inflated (nbinom2) as a direct consequence of the high frequency of the absence of ciliates (zeros) in the samples and samples with no ciliates, especially in locality C (Tables 2 and 4). To explain the variability of the abundance, the resulting model (Table 4) based on confidence intervals, considered the altitude above sea level (CI = 0.36–3454.15; $p = 0.129$), distance above the ground (CI = 2.02–20.47; $p = 0.002$), plant diameter (CI = 0.15–3.68; $p = 0.711$) and water temperature (CI = 0.00–46.41; $p = 0.713$). The only significant interaction of variables (Figure 5) was obtained between the plant diameter and distance above the ground (CI = 0.00–0.29; $p = 0.004$), with a positive influence on abundance in plants with diameters similar or lower to the mean as the distance above the ground increased. However, plants with diameters above the mean showed a negative influence on abundance as the distance above the ground increased (Figure 5). The intercept

of the zero-inflated model of GLMM was significant (CI = 0.12–0.62; $p = 0.002$), indicating that the type of forest in each locality influenced the minimum abundance of ciliate species observed in the samples. The statistical differences of the variables from the localities were explained only by the 28% (ICC = 0.28) of the variation (Table 4).

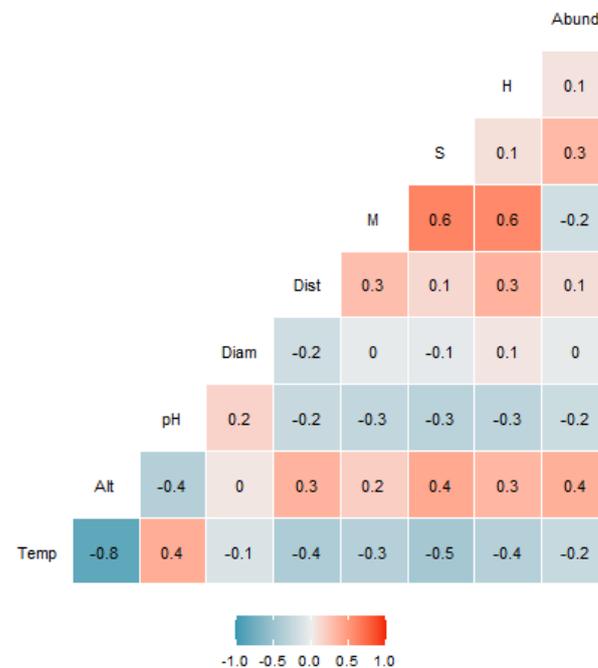


Figure 4. Correlogram of abundance, α -diversity indexes of ciliates and environmental factors related to bromeliads in the Mexican Neotropics. Abund—abundance; H—Shannon–Wiener index; S—Simpson index; M—Menhinick index; Dist—distance above the ground; Diam—plant diameter; pH—water pH; Alt—altitude asl; Temp—water temperature.

Table 4. Values of the generalized linear mixed model.

Predictors	Incidence Rate Ratios	Abundance	
		CI	p
Count model			
(Intercept)	87.75	23.54–327.19	<0.001
Plant diameter	0.74	0.15–3.68	0.711
Distance above the ground	6.42	2.02–20.47	0.002
Altitude (asl)	35.04	0.36–3454.15	0.129
Water temperature	0.41	0.00–46.41	0.713
Plant diameter, distance above the ground	0.02	0.00–0.29	0.004
(Intercept)	0.94		
Zero inflated model			
(Intercept)	0.28	0.12–0.62	0.002
Random effects			
σ^2	5.69		
$T_{00\ ST}$	2.17		
ICC	0.28		
N_{ST}	6		
Total observations	60		
Marginal R^2 /Conditional R^2	0.485/0.627		

σ^2 —locality variance; $T_{00\ ST}$ —variance between the localities; ICC—intraclass correlation coefficient. N_{ST} —number of localities; CI—confidence interval.

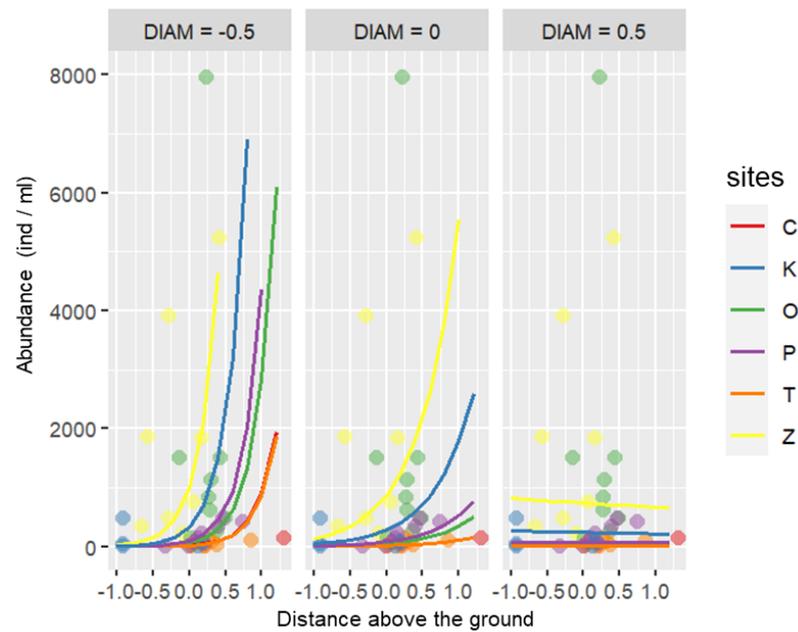


Figure 5. Generalized linear mixed model of the interaction between distance above the ground and DIAM (plant diameter). Total abundance of ciliates for each plant is indicated with color dots according to the localities. See Table 1 for the names of the localities in capital letters.

The application of FAMD at the community level explained a low proportion of the variation (48.3%) in both axes, one and two (Figure 6a,b). Even so, it was possible to determine in the diagram (Figure 6b,d) a negative correlation between the water temperature and altitude above the sea level, and the abundance of ciliates in bromeliads. By contrast, the distance above the ground by itself was apparently not important to explain the structure and composition of the ciliate community, same for the plant diameter and pH. In spite of the low percentage of explained variation by the FAMD, Figure 6a shows a clear separation between the ciliate species composition and their abundances in bromeliads of the dry tropical forest (locality C), semideciduous tropical forest (locality K) and oak forest (O) in relation to all bromeliad sampled in montane cloud forests (localities P, T and Z), but differences were not clear because of their representation in the center of the plot. Nevertheless, after the removal of the set of 30 samples from the contrasting localities C, O and K, the explained variation by axes one and two raised to 61.76% (Figure 6c) with a clear difference between the ciliate composition and abundances of the three montane cloud forests (localities P, T and Z).

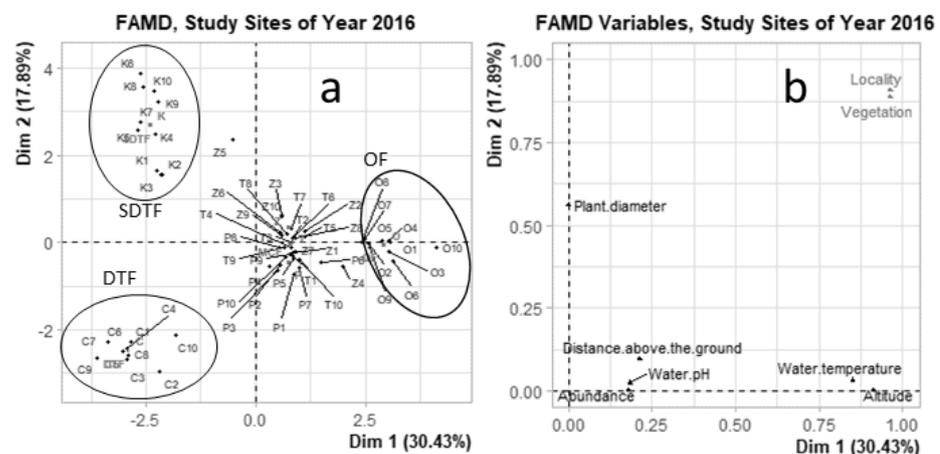


Figure 6. Cont.

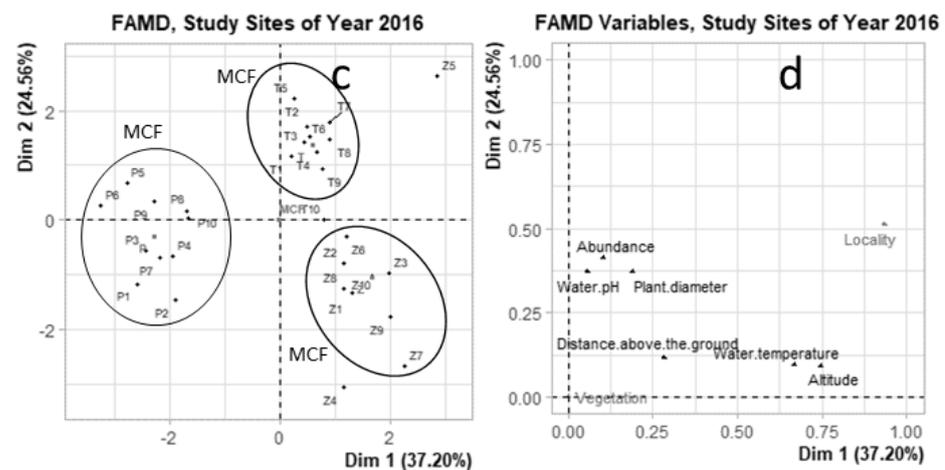


Figure 6. (a) FAMD biplot of ciliate abundance of all the sampled bromeliads during the humid season of year 2016 in the Mexican Neotropics; (b) Correlation diagram of the FAMD of the environmental variables and abundances of ciliates in bromeliads from all the localities during the humid season of year 2016. (c) FAMD biplot of ciliate abundance of sampled bromeliads of montane cloud forests during the humid season of year 2016; (d) Correlation diagram of the FAMD of the environmental variables and abundances of ciliates in bromeliads only from localities with montane cloud forest during the humid season of year 2016. See Table 1 for the names of the localities C, K, O, P, T and Z. MCF—montane cloud forest; OF—oak forest; DTF—dry tropical forest; SDTF—semideciduous tropical forest.

4. Discussion

Ecological traits of ciliate communities that inhabit bromeliads remain poorly understood. A very low number of contributions have been determined in the last ten years, comprising studies performed at the local scale, and including only a few bromeliad species during short periods of time. By considering only the humid season of the year 2016, the present work was not the exception in relation to the time constraint, but it represents the first study analyzing ciliate communities in a larger geographic area within the Neotropics.

There were three recognizable aspects about the distribution of bromeliads and ciliates: (i) the specificity of certain ciliates (the so-called endemics) to bromeliads is only at the biotope level, (ii) the taxonomical specificity is probably non-existent [7], which means that there was no evidence that certain ciliate species prefer to inhabit in a single bromeliad species, and (iii) almost all bromeliads have restricted patterns of geographical distribution along the Neotropics [1,24]; because of that, sampling in a wide geographic area caused it to be practically impossible to include a single bromeliad species. Based on the three previous aspects, the present study was conducted, including several bromeliad species whose distribution depended on the type of forest in each locality.

4.1. Species Richness

There are only two previous studies about ciliates and bromeliads, which used a similar dataset, considering an ecological and taxonomical approximation [18,19]. Not all the sampled bromeliads in the present study contained active ciliates. However, ciliate communities recorded in this study might not have included sessile or thigmotactic species as a result of the employed sampling methodology, the small plant sizes with low volumes of water and the impediment of plant removal from their habitats. The number of plants where active ciliates were absent was higher in comparison to the results of Buosi et al.'s work [18,19] in bromeliads along the margins of the Paraná River in South Brazil. In spite of the presence of impounded water in almost all the plants, with the exception of nine bromeliads in the dry tropical forest, the richness of active ciliates was lower, in comparison to the 92 species observed in *Aechmea distichantha* [18,19], with 13 species on average per plant, meanwhile only an average of 1.43 species per plant was recorded in the present

study. Lower richness values were recorded by Busse et al. [22] in *Quesnelia arvensis* Mez. near the coast of São Paulo, Brazil, where they only observed nine morphospecies of ciliates. Although encysted species of ciliates were not considered in the analysis of the present study, their presence was observed in bromeliads from a dry tropical forest in western Mexico [10].

The application of α -diversity indexes was useful to compare species richness between localities, but unfortunately they were not very informative due to the low number of samples; although we obtained that there were no significant differences of α -diversity of ciliates between localities of the semideciduous tropical forest, oak and montane cloud forest (K, O, and Z) with the rest of the localities, the other two localities of montane cloud forests (P and T) were different to the α -diversity of ciliates of bromeliads from the dry tropical forest (locality C), because of its almost total absence of active ciliates. Buosi et al. [18] emphasized that water was the most important factor for the presence of ciliates in bromeliads; however, our results suggested that even if impounded water was present in bromeliads, other unknown factors could be restrictive for the excystment of ciliates to become active.

4.2. Abundance

Values of the abundance of the 22 ciliate species were highly variable, but only very few of them were more abundant in this type of microecosystems; by contrast, almost 40% of the species showed abundance values of <40 ind/mL and almost a quarter of the species were recorded only from one plant. Similar results were obtained by Busse et al. [22], who reported an average abundance of 40 ind/50 μ L of unidentified ciliates in *Q. arvensis*. It is common that ciliate communities are composed of a few dominant species contributing most to the total abundance and many less abundant species contributing to the rest, also depending on the effort, size and volume of the samples [45]. Regarding the species of peritrichs, the abundances of individuals of the unidentified operculariid and both Peritrichia included in our counting were likely swimmers or detached zooids at the moment of sampling from *Tillandsia guatemalensis* and *T. makoyana*, respectively.

Bromeliophrya brasiliensis and *Glaucomides bromelicola*, species that feed on bacteria and heterotrophic flagellates (Foissner 2013), were the two most abundant species in bromeliads of the montane cloud forests and oak forest. Although *G. bromelicola* is a hymenostomatid widely distributed in Neotropical bromeliads [7], there are no records of this species in Brazil. In this country, Buosi et al. [18,19] also reported highly variable values of abundance of free-living ciliates from *A. distichantha*, where *Colpoda steinii*, *Bromeliothrix metopoides* and *B. brasiliensis* were the three most abundant species, but they obtained lower abundance values of *B. brasiliensis* (238–604 ind/mL) in comparison to values obtained in the present study (20–5240 ind/mL). Although ciliate richness in this South American tank bromeliad was significantly higher, the lower abundance of species such as *B. brasiliensis* was possibly the result of different biotic and abiotic conditions, such as water temperature, plant architecture [46] and interspecific competition.

According to Caron [47], there are a considerable number of rare taxa-conforming protist communities, with unknown ecological roles, but remaining present most of the time. Low-abundant taxa in the context of the hypothesis of the rare biosphere can become more dominant when ecological conditions change [48], and those changes in abundances tend to be cyclical [45]. Caron et al. [49] argued that rare species are mostly affected by immigration and local extinction, which tend to be higher in microbial eukaryotes in comparison to prokaryotes [50]. Weisse [45] suggested that we are too far away from understanding the processes of the maintenance and functional roles of rare species among free-living ciliates, which is evident in the bromeliad phytotelmata.

4.3. Environmental Variables and Their Effect on the Ciliate Community

With the application of the GLMM, we discovered that the interaction between the plant diameter and its distance above the ground influenced ciliate abundance positively

and negatively. According to our results, plants with a small to median size showed higher abundances when they were located growing at larger distances above the ground, and such a combination of variables apparently promoted higher abundances only in some ciliate species. This pattern contrasted with the community of amoebae and flagellates found in *Vriesea* sp. of northwestern Costa Rica, where the distance above the ground was negatively related to species richness and abundance [17].

In the case of ciliates, a negative effect between the abundance and distance above the ground was only detected in plants with diameters above the mean ($mean = 0.54$ m); however, this could be an effect related to the sampling effort or as a consequence of distributional patterns of bromeliads in the forests. The rest of the variables considered in the study did not have a statistically important influence on the community. For the water pH and water temperature, similar values were reported in previous studies [5,11,20], and a negative relation between the altitude above sea level and water temperature showed the same pattern described in the bromeliads of eastern Mexico [20].

Our results, based on data of the FAMD analysis, confirmed the conclusions of Durán-Ramírez et al. [20] and Malfatti et al. [21] in the bromeliads of eastern Mexico and southern Brazil, respectively, where they found that the distributional patterns of ciliates communities were properly attributed to the influence of the altitude above sea level. According to both studies, bromeliads that inhabit intermediate altitudes, from 400 to 1300 m asl, hosted a higher diversity of ciliates.

The ciliate composition at the local level showed differences in relation to the type of forest where bromeliads grew [20], being now corroborated at the regional level based on data from the present study. Our results opened up the possibility to distinguish ciliates communities of bromeliads from tropical low-land forests, where soil ciliates are more frequent [10] but less abundant with many encysted species and, on the other hand, ciliate communities of bromeliads from high-land forests growing above 1000 m asl, such as those of the montane cloud forest and oak forest, where the so-called endemic ciliates to bromeliads were more common and abundant. A further evaluation of this pattern needs to consider the effect of the latitude, as Fernández et al. [51] found in soil communities of testate amoebae in Chile.

However, even if impounded water was present, other conditions such as the intra and interspecific competition for space and resources could trigger the encystment, as Foissner et al. [9] detected in tank bromeliads during the dry season, with a very low number of active ciliates. Future studies about ciliates in bromeliads need to incorporate the analysis of a mixture of several factors and conditions, such as the plant architecture, water characteristics, food availability, incidence of light, climatic variation and seasonality, biotic interactions, dispersal mechanisms, proximity to fresh-water bodies and stochastic processes.

5. Conclusions

Ciliates are a common group of heterotrophic protists that colonize epiphytic and terrestrial bromeliads in the Neotropics. In the present study, species from the classes Colpodea, Oligohymenophorea and Nassophorea were recorded from bromeliads in the Mexican Neotropics during the humid season of the year 2016. Not all the bromeliads hosted ciliates. Only one third of the sampled plants contained trophonts of at least one species. *Glaucomides bromelicola* was the most frequent species observed in one third of the plants.

According to our results, we recorded that the species richness and abundances of identified ciliates showed the trend to be higher in the bromeliads of the montane cloud forests and oak forest. However, α -diversity indexes of the active ciliate community showed low richness values in this part of the Neotropical region. It was outstanding that the richness and abundances of ciliates from the bromeliads of the dry tropical forest (locality C) were different compared to the other types of forests. In relation to their abundances, we found that *Bromeliophrya brasiliensis* and *Glaucomides bromelicola*, two commonly coexisting

species, showed the highest values in some bromeliads from the montane cloud forests (locality Z) and oak forest (locality O).

Based on statistical tests, we determined that the combination of the plant diameter and distance above the ground showed a positive effect in relation to ciliate abundance. We observed some species such as *B. brasiliensis*, *G. bromelicola*, *Lambornella* sp., *L. costatus* and *Tetrahymena* sp. showing higher abundances in plants with diameters similar or lower to the mean as the distance above the ground increased in the plants where they were recorded. By contrast, ciliates abundances were lower in plants with larger diameters. Our results provide new evidence in a wider geographic area suggesting that bromeliads that inhabit mountains and low-land forests host ciliate communities with different species compositions.

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