



# Influence of environmental and morphological parameters on the microfauna community present in phytotelmata of a bromeliad in a fragment of Atlantic Forest, southern Brazil

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## Abstract

Bromeliads are important epiphytes due to their abundance in the Neotropical region and morphological complexity. Their compact and imbricated leaf bases form water storage cisterns that promote important resources for colonization by several prokaryotic and eukaryotic microorganisms. Due to the lack of knowledge about these environments, the objective of the study was to investigate which physical-chemical and ecological parameters exert effects on the biological richness present in the cisterns of *Vriesea platynema*. The study was carried out in the Center for Research and Nature Conservation (CPCN – Pró-Mata), in the Serra Geral plateau, northeastern Rio Grande do Sul, Brazil. Active searches were performed for 10 trees with bromeliads fixed at two heights (< 1.5 m and > 2.5 m). For each bromeliad individual, the height in relation to the ground, the diameter and depth of the central cistern, water temperature, number of lateral cisterns and number of adjacent bromeliads, were measured. A total of 23 taxa were identified in the phytotelmata of *V. platynema*, with *Philodina*, *Lambornella*, *Paramecium*, *Tetrahymena* and Diptera larvae being the most representative groups. The richness of organisms in the phytotelmata presented a positive correlation with water temperature ( $p = 0.01$ ), and the number of adjacent bromeliads ( $p = 0.05$ ), indicating that physicochemical and ecological factors could influence the richness of bromeliad biota.

## Keywords

Bromeliaceae, cisterns, ecological factors, eukaryotes microorganisms, temperature

## Introduction

In southern Brazil, most remnants of the Atlantic Forest are located in the hillside areas of the Serra Geral mountain range, saved by acclivity and access difficulty (Kageyama and Gandara 2000). These Atlantic Forest remnants are one of the largest biodiversity hotspots in the world (Myers et al. 2000). Bromeliads are one of the most important groups of plants in this biome due to their strictly Neotropical distribution, their morphological complexity and epiphytic habit. In terms of occurrence of bromeliads, Brazil is the country with the highest degree of endemism and diversity of this group (Martinelli et al. 2008). Their leaves are arranged in rosettes, and on its bases, the accumulation of water and organic matter, promotes an environment for several organisms (Goffferdi et al. 2015), emphasizing the importance of these plants in the expansion of biological diversity (Tews et al. 2004). The wide geographic distribution and ecological success of this group in the neotropics can be explained by the epiphytic habit, the presence of absorbent trichomes and CAM physiology (crassulaceous acid metabolism) (Leroy et al. 2015). Some bromeliad species are also able to hold water in their leaves forming cisterns that can accumulate up to 50 liters of suspended water per hectare, promoting important resources for the community living in these environments (Goffferdi et al. 2015; Simão et al. 2017).

The process of colonization of bromeliad tanks seems to be complex, involving a series of interconnected events, such as active and passive dispersion, arrival and the process of establishment of the organism (Maguire 1971). The dispersion could be driven by wind and rain (passive), flight (active) and phoresia (Maguire 1963) and it will depend on the distance traveled and on the type of barriers crossed during the process (Maguire 1971). The process of colonization of phytotelmata could be compared, as a whole, with that of islands and swamps, with organisms that are phytotelma-specialists (highly selective colonization) and those that are non-specialists (random colonization) (Frank and Lounibos 1987; Richardson 1999). In addition, the colonization process of bromeliad tanks may be related to the Island Biogeography Theory, in which they can be seen as islands of aquatic habitat, stating the establishment between observed richness in the cisterns and size of the area in which they are established, as well as the wealth ratio and the distance that propagule sources meet (Jocque and Field 2014).

Components of the microfauna are important for the metabolism of the host plant. For example, Giongo et al. (2019) found that the genera *Pseudomonas* and *Enterobacter* are the dominant bacteria in *Vriesea platynema* Gaudich. and *Aechmea gamosepala* Wittm., which include species that are plant-beneficial, producing molecules that enhance plant health, also helping with the stability of phosphorus levels in the bromeliad (Bodenhausen et al. 2013; Jain and Das 2016).

The aquatic microfauna present in phytotelmata is composed of a mixture of species that inhabit soil and freshwater (Maguire 1971; Richardson 1999). Some species could be cosmopolitans and others could be endemics, as a result of speciation inside a very peculiar habitat. For example, Dunthorn et al. (2012) found 28 of

45 ciliate species endemics of bromeliad tanks. Other studies on microfauna present in bromeliad phytotelmata have shown that diversity is high on epiphytes or ground dwelling plants (Simão et al. 2017).

Recent studies indicate that these communities fit at least one well-established biogeographic pattern for islands: the species (richness)–area relationship. In Jabiol et al. (2009), aquatic insect richness and abundance were positively associated with water volume. For bromeliad invertebrate communities, some studies have shown the importance of light and organic material (proxy to productivity) in influencing community (Srivastava et al. 2008; Dézerald et al. 2014). Habitat complexity (number of leaves) may affect the invertebrate system, as observed by results combining terrestrial and aquatic components (Armbruster et al. 2002). In addition, the number of adjacent bromeliads enables the process of propagation and movement of microfauna, and the greater number of lateral cisterns provides different conditions and habitats for microfauna colonization (Marques and Forattini 2008).

Tropical forests with high canopies offer interesting environments to evaluate vertical differences in physical and chemical factors. Bromeliads and other vascular epiphytes are interesting models for studying the influence of these differences, once they are present in the dark and humid understory and in the sunny and dry outer canopy (Petter et al. 2015).

Regarding the aspects of the community that inhabits bromeliad tanks, some studies point out the importance of light and organic material, as well as the physico-chemical characteristics of water, such as pH and dissolved particle content influencing the bromeliad aquatic fauna (Srivastava et al. 2008; Dézerald et al. 2014). Jabiol et al. (2009) found that aquatic insect richness and abundance were positively associated with water volume, a proxy for island/habitat size. Habitat complexity, as measured by the number of lateral cisterns and adjacent bromeliads, can also influence system composition (Armbruster et al. 2002). According to Jocque and Field (2014), bromeliads with more leaves contain more species, based on habitat heterogeneity hypothesis. Adjacent bromeliads are related to the island's theory, which could increase diversity and the exchange of species (Frank and Lounibos 1987; Little and Hebert 1996).

The present study focused on environmental factors that may be influencing the richness of the phytotelmata community of *V. platynema* Gaudich, a common bromeliad species from the Atlantic Forest in southern Brazil. The main goal was to investigate which factors exert effects on the richness of microfauna in the bromeliad phytotelmata, evaluating physico-chemical parameters (relative height, water temperature) and ecological parameters (number of lateral cisterns and adjacent bromeliads) of the plant. We hypothesized that the richness of microfauna would be higher in bromeliads of lower strata due to the greater proximity to the soil; with higher water temperatures in their reservoirs, due to increased productivity; and, in bromeliads with a greater number of lateral cisterns and close to other bromeliads specimens, which could allow exchange of colonizers. On the contrary, the richness would be lower in bromeliads fixed at greater heights due to the decrease in contact with different niches.

## Material and methods

### Study area

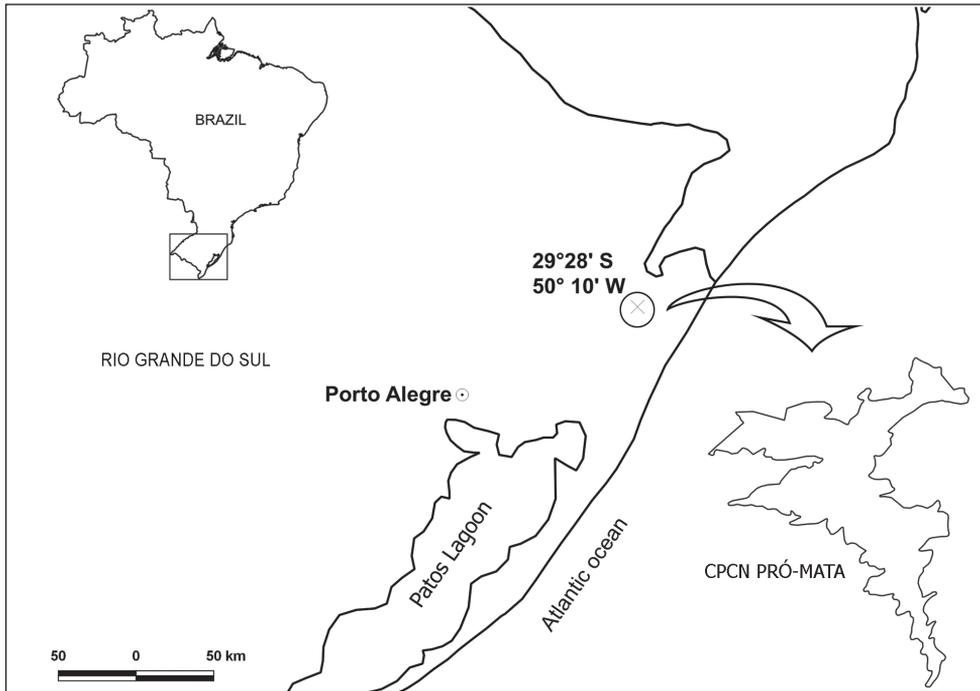
The study was carried out at the Center for Research and Nature Conservation (CPCN – Pró-Mata), located in the northeastern region of Rio Grande do Sul, in the border of three municipalities: São Francisco de Paula, Itati and Maquiné, in southern Brazil (Fig. 1). In a biogeographic context, the CPCN is framed in the Neotropical Phytogeographic Region (Cabrera and Willink 1973). This area is constituted by a mosaic of ecosystems, such as the Dense Ombrophilous Forest (DOF) and Mixed Ombrophilous Forest (MOF), and the top fields of the mountain range, including expressive biological diversity (IBGE – Instituto Brasileiro de Geografia e Estatística 1992). The altitude of the region varies between 900 and 1100 meters, with humid subtropical climate and average annual precipitation of 1200 mm (Pró-Mata Management Plan 2011).

### Data collection

Sampling took place along the “Vale das Bananeiras” trail, located at CPCN Pró-Mata, in January 2018. Along the trail, active searches were made for *Vriesea platynema*. The choice of the specimens was based on the presence of at least two individuals per tree, with one occurring at less than 1.5 meters above the ground, and the other occurring above 2.5 meters from the ground. The established height limits took into consideration the bromeliad individuals available in the sample area and their access, requiring standardization for sampling. The presence of water also influenced the choice of individuals to be sampled. When more than one individual was found in the same height stratum, one was randomly chosen to be sampled.

A total of 20 specimens of bromeliads were sampled, divided into 10 trees, comprising both height strata. The exact height in relation to the ground for each individual sampled was measured (using a tape measure), as well as the diameter and depth of the central cistern (using a caliper); and the water temperature (using a hygrometer). Approximately 10 ml of water from the cisterns were collected using sterile pipettes. After that, the samples were placed in Falcon tubes for further qualitative analysis in the laboratory. The number of adjacent bromeliads in a 1-meter radius was counted in the vicinity of the specimens collected. For this, both bromeliads present in the same tree trunk and those fixed in nearby trees were counted less than 1-meter.

*In vivo* samples were screened using a Zeiss stereomicroscope, with a standard time of 15 minutes per sample. A simple scan was performed throughout the sample, with 100× magnification. Eukaryotic organisms collected from the phytotohelm of each bromeliad were identified to the lowest possible taxonomic level using appropriate bibliography (Brinkhurst and Wetzel 1984; Patterson 1996; Foissner 2003; Foissner et al. 2003; Pinho 2008; Mendes et al. 2011).



**Figure 1.** Location of the Center for Research and Conservation of Nature (CPCN) Pró-Mata, Rio Grande do Sul, Brazil.

## Data analysis

The species richness was determined for the phytotelmata collected at different height strata. The frequency of occurrence (%) was calculated considering the more frequent taxa with occurrence greater than 70%. Possible differences in richness were compared using t-test. A relationship between water temperature and height was assessed using linear regression (Past 3.0 software).

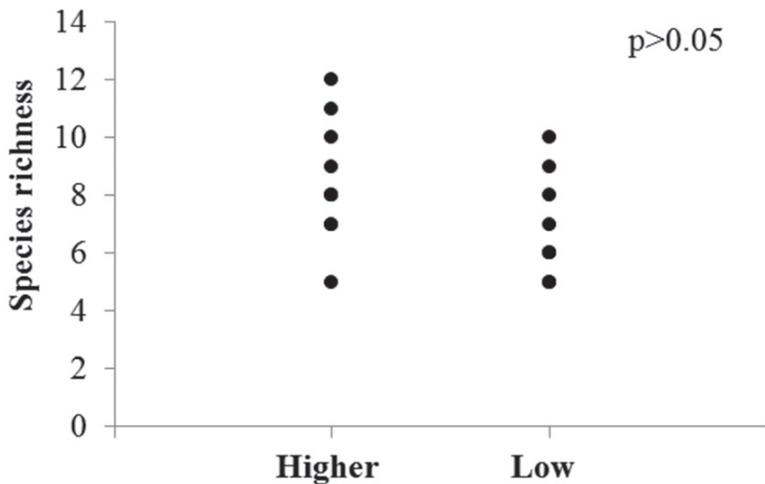
The relationship of community richness and height, water temperature, tank volume, number of lateral cisterns, and number of adjacent bromeliads was tested by GLM (general linear model), using the statistical package R (R Core Team 2018).

## Results

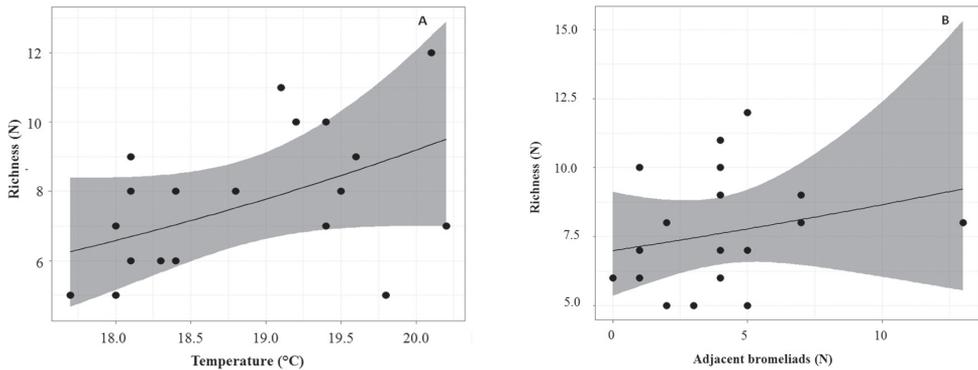
A total of 23 taxa were identified in samples collected from phytotelmata of *Vriesea platynema* (Table 1). The genera most frequently observed were the ciliate *Paramecium* (95%), the rotifer *Philodina* (90%), and the ciliate *Lambornella* (80%). The ciliates *Rhabdostyla* and *Spirostomum* were the least representative, occurring in only one and two samples, respectively. Other groups such as rotifers in the genus *Philodina* and some oligochaetes were present in high abundance in the analyzed samples.

**Table 1.** Distribution of the taxa sampled in *Vriesea platynema* phytotelmata in the two strata analyzed: low (< 1.5 m) and high (> 2.5 m) along the Bananeiras trail in the CPCN Pro-Mata.

Morphospecies	Sampled strata	
	< 1.5 m	> 2.5 m
<b>Ciliophora</b>		
<i>Colpidium</i> sp.	x	x
<i>Colpoda</i> sp.	x	x
<i>Euplotes</i> sp.	x	x
<i>Halteria</i> sp.	x	x
<i>Lambornella trichoglossa</i>	x	x
<i>Paramecium</i> sp.	x	x
<i>Rhabdostyla</i> sp.		x
<i>Spirostomum</i> sp.	x	x
<i>Vorticella</i> sp.	x	x
Hipotrichia	x	x
Unidentified ciliate (Tetrahymenidae)	x	x
Not Identified I	x	x
Not identified II	x	x
Not identified III	x	x
Not identified IV		x
<b>Arthropoda</b>		
Hydrachnidia (Acari)		x
Culicidae larvae (Diptera)	x	x
Chironomidae larvae (larvae)	x	x
Harpacticoid copepod (Crustacea)	x	x
Ostracoda (Crustacea)	x	x
<b>Nematoda</b>		
Nematode	x	x
<b>Rotifer</b>		
<i>Philodina</i> sp.	x	x
<b>Annelida</b>		
Oligochaeta		x



**Figure 2.** Distribution of the taxa richness in the phytotelmata of *Vriesea platynema* in the two height strata analyzed: low (< 1.5 m) and high (> 2.5 m), along the Bananeiras Trail, in CPCN Pró-Mata, southern Brazil.



**Figure 3.** Variation of the richness of taxa present in the phytotelmata of *Vriesea platynema* in relation to water temperature (A) and to the number of adjacent bromeliads (B).

Although the upper stratum presented a higher species number (23) when compared to the lower stratum (19), the richness between the two sampled strata (upper and lower) did not show a significant difference ( $p = 0.67$ ) (Fig. 2). Also, there was no difference in water temperature in the cisterns of bromeliads from different strata ( $p = 0.35$ ).

Height ( $p = 0.14$ ), volume ( $p = 0.56$ ) and number of lateral cisterns ( $p = 0.20$ ) did not show a significant difference in community richness of the phytotelmata of *V. platynema*. In contrast, a positive effect of temperature on the eukaryotic richness of cisterns was observed ( $p = 0.01$ ) (Fig. 3A). The number of adjacent bromeliads also played a significant role in the increase of phytotelmata richness ( $p = 0.05$ ) (Fig. 3B).

## Discussion

The eukaryotic community of *Vriesea platynema* cistern, as evaluated in the present study, has a varied composition of organisms of different phyla (Ciliophora, Arthropoda, Rotifera, Nematoda and Annelida). The assembly of filtering ciliates is composed by genera previously reported as inhabitants of phytotelmata, such as *Tetrahymena*, *Euplotes*, *Colpoda*, *Halteria*, plus *Lambornella trichoglossa* (Foissner 2003; Foissner et al. 2007; Soto 2016). Ciliates in the genera *Vorticella* and *Rhabdostyla* were epibionts on mites and ostracods, a relationship that has been previously reported in phytotelmata of bromeliads (Houck and O'Connor 1991; Foissner et al. 2003; Sabagh et al. 2011).

Water temperature is an important environmental factor for many species and across all ecosystems (Dell et al. 2011). Kratina et al. (2017) investigated the relationship between temperature and richness of major taxonomic groups found in bromeliad tanks. The authors pointed out that an increase in temperature (max. of 25 °C) was correlated to an increase in richness of all investigated taxa, except flagellates. Moreover, small temperature increments could change thermal tolerance of the organisms. In our study, we also could detect changes in microfaunal richness

related to temperature. Our results demonstrated an increase in temperature correlated with an increase in richness of the investigated community.

The effects of water temperature fluctuation in bromeliad tanks are not only related to assemblage changes. Water temperature could influence physiological aspects of the organisms inhabiting bromeliad cisterns. For example, Hoekman (2010) demonstrated that an increase in temperature would directly influence the reproduction and growth rates of filtering ciliates, while increasing the metabolic and growth rates of Diptera larvae inhabiting *Sarracenia purpurea*. In the present study, the high representativeness of filter feeding ciliates may be related to the temperature. The occurrence of filter feeders may be providing trophic resources for the coexistence of different top predators, such as culicid larvae and chironomids, leading to an increase in the richness of organisms at higher trophic levels. The effects of top predator diversity on community structure are hard to evaluate due to a variety of responses (Bruno and O'Connor 2005; Bruno and Cardinale 2008). On one hand, greater predator richness could increase prey capture rates due to a complementarity in resource partition, where different predators consuming greater prey variability (Griffin et al. 2008). On the other hand, greater predator richness may also decrease prey consumption due to intra-guild interactions (Antiqueira et al. 2018a; Finke and Denno 2004).

The environment promoted in bromeliads, such as water and mud that accumulates in phytotelmata, allows the emergence of many niches for ciliate species, both due to the availability of food and the increase of other organisms that serve as fixation surface, promoting increased diversity of this species group (Foissner et al. 2003; Ngai and Srivastava 2006; Buosi et al. 2014). In addition to the ciliates, the cisterns presented a great diversity of arthropods, such as harpacticoid copepods and mosquito larvae of Culicidae and Chironomidae, which are important top predators in these ecosystems (Frank and Lounibos 2008). As highlighted by Antiqueira et al. (2018a), the occurrence of predators in the bromeliad cistern is important for maintaining this habitat and ecosystem functionality.

Contrary to what we expected, the number of cisterns does not influence the richness of organisms in the community. According to our results, phytotelmata richness is much more influenced by the number of adjacent bromeliads. This factor may be related to the variety of dispersion ways which the microorganisms experience, such as wind, rain (Maguire 1963), and other animals (Frank and Lounibos 1987). Since there are more bromeliads around, the distance ceases to become a limiting factor for dispersal, allowing organisms to be carried by a wider variety of dispersers (Benzing 2000; Leroy et al. 2015).

## Conclusion

We concluded that the bromeliad cisterns are environments where the eukaryotic community would be affected both by the temperature of the water, as well as by the number of bromeliads present in the environment. Considering the diversity

of microorganisms present in phytotelmata, and the relation of richness with the diversity of adjacent epiphytes, the role of bromeliads as enhancers of biological diversity is reinforced, making these plants important tools to assist in the conservation of the remaining ecosystems of the Atlantic Forest.

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