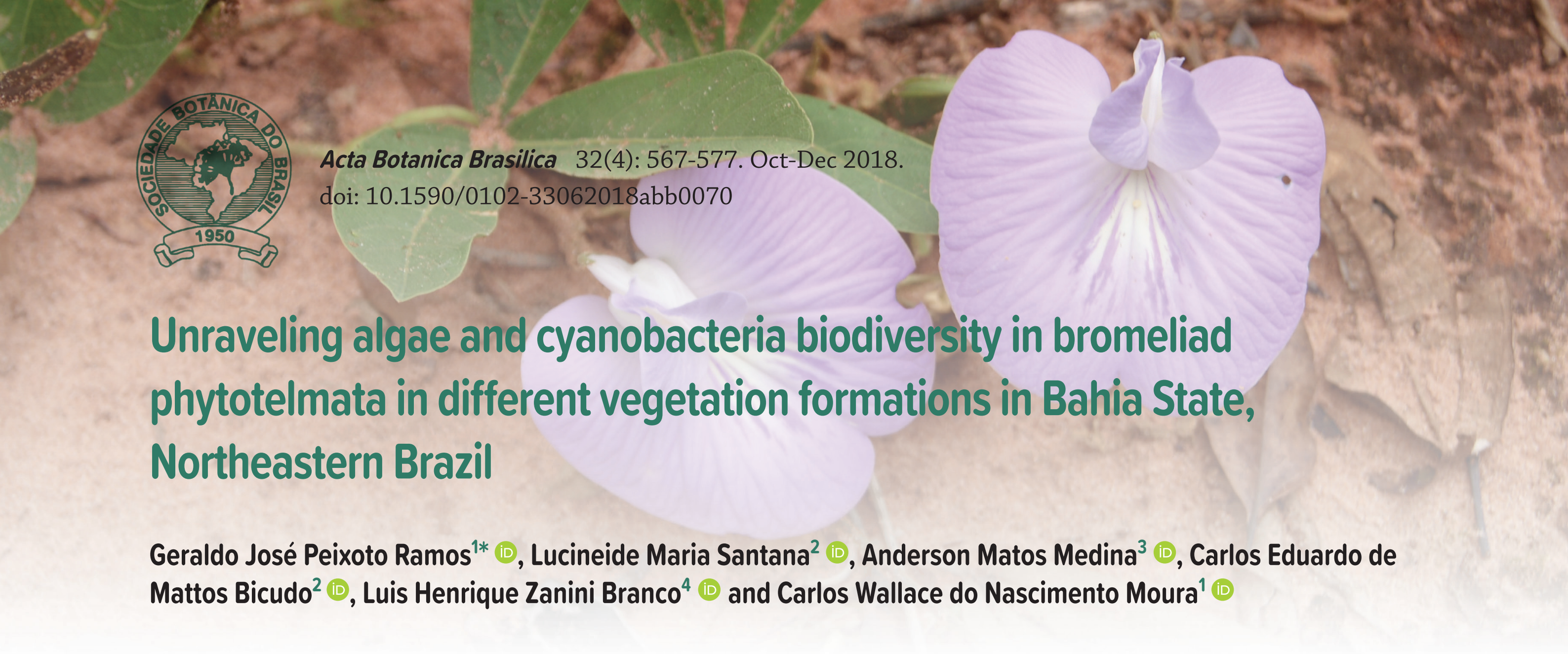

Acta Botanica Brasilica 32(4): 567-577. Oct-Dec 2018.
doi: 10.1590/0102-33062018abb0070

Unraveling algae and cyanobacteria biodiversity in bromeliad phytotelmata in different vegetation formations in Bahia State, Northeastern Brazil

Geraldo José Peixoto Ramos[1*], Lucineide Maria Santana[2], Anderson Matos Medina[3], Carlos Eduardo de Mattos Bicudo[2], Luis Henrique Zanini Branco[4] and Carlos Wallace do Nascimento Moura[1]

Received: February 23, 2018
Accepted: April 17, 2018

ABSTRACT

Knowledge of algal and cyanobacterial diversity of phytotelmata remains poorly-known, especially for bromeliads from different vegetation formations. We investigated the microalgae communities of four species of tank bromeliads from different vegetation formations in Bahia State, Northeast Brazil, highlighting the composition, richness and diversity of taxa. Sampling of water stored in bromeliads was carried out quarterly between 2014 and 2016, and abiotic variables and morphometric attributes of bromeliads were measured. A total of 89 taxa of algae and cyanobacteria were recorded for the four bromeliad species studied. The microalgae communities of the phytotelmata varied among vegetation formations, with one tank bromeliad, *Alcantarea nahoumii*, with more complex architecture (higher number of leaves and thus more cavities), being distinguished by its high species richness (73 taxa). The bromeliads exhibited little similarity in species composition, with only one species (*Phacus polytrophos*) occurring in all four species. Throughout the entire sampling period, classes with higher species richness, especially due to *A. nahoumii*, were Zygnematophyceae, Cyanophyceae and Chlorophyceae, which accounted for about 80 % of all species inventoried. Our results contribute to the knowledge of microalga communities of bromeliad phytotelmata in Brazil with regard to species richness and composition, as well as significant environmental characteristics.

Keywords: diversity, ecology, microalgae, microhabitat, phytotelm, richness, tank bromeliads

Introduction

The term phytotelm (φυτόν, phyton = plant; τέλμα, telm = pool) refers to the small amounts of water that accumulate in plant structures, such as leaves, flowers, or tree trunk and maintain an associated biota (Varga 1928; Maguire 1971). Phytotelmata occur in almost all regions of the world, although they are most common and most diversified in the tropical region, mainly due to the high rainfall there and the large numbers of plants capable of accumulating water (Fish 1983).

Little is currently known about the diversity, distribution patterns, and compositions of phytotelmata microalgae communities. Among the few published studies are those of Gebühr *et al*. (2006) with allochthonous populations of *Sarracenia purpurea* (Sarraceniaceae) in Germany, and Hernández-Rodríguez *et al*. (2014) with *Tillandsia multicaulis* (Bromeliaceae) in Mexico. Studies of phytotelmata algae began in Brazil in the 1970s, but have only recently become

1 Laboratório de Ficologia, Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, 44036-900, Feira de Santana, BA, Brazil
2 Núcleo de Pesquisa em Ecologia, Instituto de Botânica, 04301-902, São Paulo, SP, Brazil
3 Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal de Goiás, 74690-900 Goiânia, GO, Brazil
4 Departamento de Zoologia e Botânica, Instituto de Biociências, Universidade Estadual Paulista, 15054-000, São José do Rio Preto, SP, Brazil

* Corresponding author: geraldojpr@gmail.com

more frequent, especially in Bahia State, where several taxa have been recorded for the first time for that country, including a number of species new to science (Ramos *et al*. 2017a; b; c; d; 2018a; b).

Most phytotelmata algal studies have taken floristic approaches (morphospecies), usually addressing specific groups (Lyra 1971; Sophia 1999; Ramos *et al*. 2011), or ecological approaches (Brouard *et al*. 2011; Carrias *et al*. 2014), although they have often been published without reliable taxonomic support at the species level. Studies comparing microalgae communities from bromeliad phytotelmata found in different vegetation formations, however, have not yet been undertaken.

The principal environmental variables that regulate which algal groups will dominate in the bromeliad tanks are: light intensity (Laessle 1961; Sophia *et al*. 2004; Brouard *et al*. 2011), plant size (Marino *et al*. 2011) and plant architecture (Carrias *et al*. 2014), rainfall (Pires *et al*. 2017), and certain limnological characteristics (Sophia *et al*. 2004; Gebühr *et al*. 2006; Marino *et al*. 2011; Killick *et al*. 2014).

We investigated the algae and cyanobacteria communities present in four species of tank bromeliads in different vegetation formations in northeastern Brazil, emphasizing the composition, richness, and diversity of the species in those environments in order to: (1) evaluate the influence of morphometric attributes of the bromeliad tanks and the abiotic variables of the water retained in them on the richness of the algal and cyanobacterial communities; and, (2) determine the species richness and similarities of the algal and cyanobacterial communities in four tank-bromeliad species.

Materials and methods

Study sites and the bromeliads sampled

The present study was conducted in four areas with different vegetation formations in Bahia State in northeastern Brazil (Fig. 1A): (1) Fazenda Itaberaba (12°30'S, 40°04'W), in the municipality of Itaberaba, an area of caatinga (dryland) vegetation with bromeliads (*Aechmea* cf. *lingulatoides* Leme & H.E.Luther) growing on arid soils and fully exposed to the sun light; (2) Serra da Jiboia (12°51'S, 39°28'W), in the municipality of Santa Teresinha, an area of granitic rock outcrops, with bromeliads [*Alcantarea nahoumii* (Leme) J.R.Grant] growing at 850 m a.s.l. and fully exposed to sun light; (3) Parque das Dunas (12°55'S, 38°19'W), in the municipality of Salvador, a restinga (sandy shoreline) area with bromeliads (*Hohenbergia littoralis* L.B.Sm.) growing on sand dunes 600 m from ocean and fully exposed to sun light; and (4) Reserva Sapiranga (12°33'S, 38°02'W), in the municipality of Mata de São João, an area of Atlantic Forest with bromeliads (*Hohenbergia stellata* Schult. & Schult.f.) growing mainly in shaded forest sites (Fig. 1). Some climatic data, such as the rainfall in each study area (municipality), were obtained from the National Institute of Meteorology (INMET 2018); air temperatures were obtained using portable probes during collections. Those data are presented in Table 1.

Sampling

Study material was gathered from the water retained in the cavities (tanks) formed by the leaves of the bromeliads

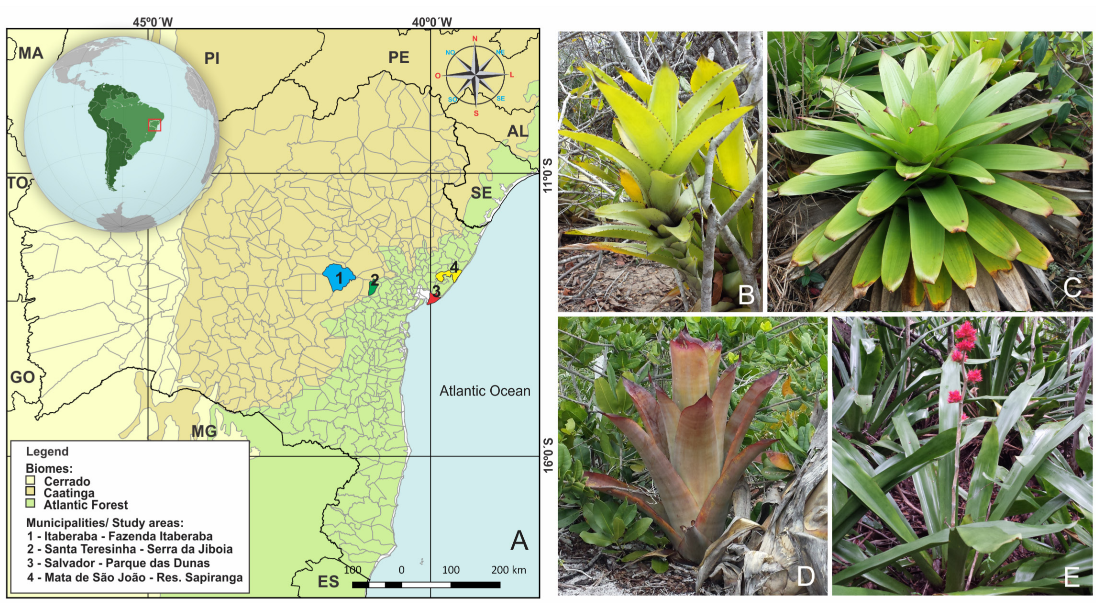

Figure 1. Map of study area, showing the four municipalities in Bahia State, Brazil (**A**) and the bromeliads studied: *Aechmea* cf. *lingulatoides* (**B**), *Alcantarea nahoumii* (**C**), *Hohenbergia littoralis* (**D**), *Hohenbergia stellata* (**E**). Please see the PDF version for color reference.

Table 1. Rainfall data of the four study areas (by municipality; INMET 2018) between December/2014 and February/2016 and air temperature during the samplings. Values are represented by mean.

	Climatic conditions							
	Itaberaba		Santa Teresinha		Mata de São João		Salvador	
Period	Rainfall (mm)	Air temperature (°C)	Rainfall (mm)	Air temperature (°C)	Rainfall (mm)	Air temperature (°C)	Rainfall (mm)	Air temperature (°C)
Dec/14	214.48	-	151.06	-	146.38*	28.12*	149.04	-
Jan/15	7.08*	31.29*	151.04*	28.66*	146.24	-	149.03*	30.89*
Feb/15	88.52	-	150.78	-	145.88	-	148.72	-
Mar/15	30.4	-	150.61	-	145.25	-	148.23	-
Apr/15	79.38	-	150	-	143.91	-	147	-
May15	49.26*	28.37*	139.38	-	143.92*	26.23*	147.29*	29.88*
Jun/15	48.21	-	138.58	-	143.85	-	149.19	-
Jul/15	56.44	-	128.64*	23.39*	137.07	-	152.22	-
Aug/15	35.44*	26.76*	129.37	-	140.74*	25.59*	159.58	-
Sep/15	4.16	-	132.4*	26.28*	151.35	-	174.85*	27.28*
Oct/15	19.62	-	135.62	-	151.11	-	177.17	-
Nov/15	47.19	-	132.37*	25.14*	142.9	-	168.45	-
Dec/15	3.98	-	129.19	-	133.18	-	160.43	-
Jan/16	407.67*	26.82*	126.14	-	132.92	-	151.62*	29.13*
Feb/16	41.79	-	125.33	-	132.74*	28.67*	150.91	-

*Sampling periods

(four species, Figs. 1B-E) in the four different vegetation formations. The accumulated water was collected using a plastic hose coupled to a 50 ml syringe. To ensure greater efficiency in collecting all biological material, that procedure was repeated several times in each tank. The water in some bromeliads was available in two kinds of phytotelmata (central and lateral), but the same collection procedures were used in both situations. Water from distinct tanks (if present), but from the same bromeliad specimen, were mixed so that each bromeliad represented a single sampling unit.

Quarterly excursions were carried out in the four study areas during 14 months, between 2014 and 2016, totaling 320 sampling units (80 bromeliads from each study area). Water from 20 tank-bromeliads were collected during each sampling period; the bromeliads sampled were randomly chosen based on the throw of a die (1 = North; 2 = East; 3 = West; 4 = South; 5 and 6 =, irrelevant), indicating the direction to follow to the next bromeliad. The distance between a sampled bromeliad and the next was always at least 5 m.

The collected material was held in properly labeled plastic containers (50 ml) and transported in a portable cooler to the Phycology Laboratory (UEFS); all collected materials were subsequently fixed in Transeau solution (Bicudo & Menezes 2017).

Environmental variables

Abiotic information concerning the tank water, such as temperature, pH, electrical conductivity, and total dissolved solids (TDS) was measured using a Hanna multiparameter probe; dissolved oxygen was measured using a portable digital Instrutherm (MO-910). All limnological variables were measured immediately after harvesting the bromeliad tank water samples. The morphometric characteristics of the bromeliads, such as height, width, and numbers of leaves were also recorded.

Identification of the microalgae and the attributes analyzed

All of the microalgae material was examined under an Olympus LX35 Optical Microscope. Taxonomic identifications were carried out to the infrageneric level whenever possible, and were mainly based on the morphological and metric characteristics of the population, consulting the specialized literature (Huber-Pestalozzi 1955; Prescott *et al*. 1975; Komárek & Fott 1983; Komárek & Anagnostidis 1998; 2005; John *et al*. 2011; Wołowski 2011; Komárek 2013; Carty 2014).

Taxonomic richness was calculated based on the total number of species sampled in each bromeliad tank (Brower *et al*. 1998). The frequency of occurrence of each algae species (in each bromeliad species) was calculated considering the number of samples in which the taxa occurred in relation to the total number of samples collected. Categories of frequency followed Matteucci & Colma (1982): > 70 % (quite frequent, VF); ≤ 70 % and > 40 % (frequent, F); ≤ 40 % and > 10 % (occasional, O); and ≤ 10 % (rare, R).

Microalgae community

Algae and cyanobacteria were classified according to their size class (nanoplankton: 2-20 µm, microplankton: > 20-200 µm, mesoplankton: > 200 µm-2 mm) (Reynolds 2006); and life form: unicellular flagellate (UF), colonial flagellate (CF), unicellular non-flagellated (UNF), colonial non-flagellated (including coenobia) (CNF), and filaments (Fil) (Crossetti & Bicudo 2008).

Statistical analyses

One-way analysis of variance (ANOVA) was used to detect differences in the morphological characteristics of the bromeliads, the abiotic variables of water, and microalgae richness (dependent variables), among the different bromeliad species (independent factor). We also tested for pairwise differences, employing the Tukey post-hoc test using the multcomp package (Hothorn *et al*. 2008). For this analysis, the data were log10 (x + 1) transformed to fit the assumptions of normality and homoscedasticity.

Principal Component Analysis (PCA) was performed using a variance-covariance matrix; the data was transformed by Z-score to reduce the dimensionality of the morphological and abiotic water parameters of the different bromeliads tanks sampled. All analysis were performed in R environment (R Core Team 2017).The similarities of the algal and cyanobacterial compositions between the four bromeliad species studied were determined by using the Sørensen similarity index (Muller-Dombois, 1981): $2c/2c + A + B \times 100$, where A and B represent the number of species in areas A and B; and c corresponds to the number of species held in common in both areas.

A Venn diagram was prepared to illustrate the distribution of algal and cyanobacterial species richness among the different bromeliads, using software available at the Bioinformatics & Evolutionary Genomics (2017) site.

Results

Morphological attributes of the bromeliads, limnological conditions, and microalgae richness

In terms of the characteristics of the bromeliads sampled, *Hohenbergia stellata* had high mean diameter and height values while *Hohenbergia littoralis* had low mean values for those parameters; the other two bromeliad species showed similar measures of those same morphological characteristics. Significant differences were found in the mean numbers of leaves among the bromeliad species, especially between *Alcantarea nahoumii* (37.8 leaves) and *H. littoralis* (14.9 leaves) (Tab. 2). The waters in the bromeliad tanks of the four species were predominantly acidic, but with other significant differences between them. *Aechmea* cf. *lingulatoides* and *A. nahoumii* showed high mean electrical conductivities, and their total dissolved solids were two to three times greater than those observed in *H. littoralis* and *H. stellata*. The greatest mean dissolved oxygen concentration was found in *A. nahoumii* (7.8 mg L^{-1}), followed by *H. littoralis* (6.8 mg L^{-1}). That parameter displayed considerable variation among the different bromeliad species.

The bromeliads studied here were organized according to their morphological characteristics and abiotic water variables in the PCA, with the first two axes explaining 55.8 % of the data variability (eigenvalues: axis 1 = 2.35, axis 2 = 2.03, permutation test = 0.009). Most individuals of *H. stellata* were grouped on the negative side of axis 1, and were mainly correlated with higher plant diameter (r = -0.76) and height (r = -0.61) values; most individuals of *A. nahoumii* were also grouped there, correlated to pH (r = -0.65) and numbers of leaves (r = -0.58). Individuals of *H. littoralis* were grouped on the positive side of axis 1, correlated with high water temperature (r = 0.52). Most individuals of *A. nahoumii* and some of *A.* cf. *lingulatoides* (mainly the ones sampled in Jan/2015) were grouped on the negative side of axis 2, and were correlated with the highest TDS (r = -0.82) and conductivity values (r = -0.81) (Fig. 2).

The phytotelmata algae and cyanobacteria communities were represented by a total of 89 taxa in the four bromeliads species studied, distributed among 54 genera

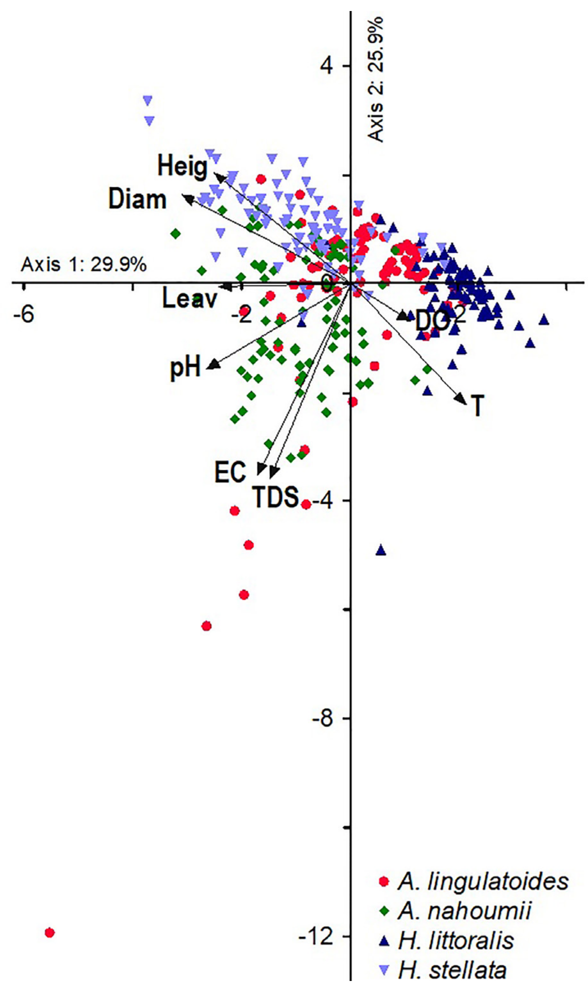

Figure 2. Principal component analysis based on morphological characteristics and abiotic water variables of four bromeliad species (Diam: Diameter, Heig: Height, Leav: number of leaves, T: water temperature, pH, EC: electrical conductivity, TDS: total dissolved solids, DO: dissolved oxygen).

Table 2. Mean and standard deviation values (n = 80) of the morphological characteristics, abiotic water conditions, and algae richness sampled in four different bromeliads species (Superscript letter represents the Tukey test results: different letters indicate significant differences, $p < 0.05$).

Variables		*Aechmea* cf. *lingulatoides*	*Alcantarea nahoumii*	*Hohenbergia littoralis*	*Hohenbergia stellata*	ANOVA F	P
Morphological characteristics	Diameter (cm)	77.6^b (± 18.8)	79.3^b (± 18.1)	41.5^c (± 11.9)	115.1^a (± 33.2)	200.7	<0.001
	Height (cm)	69.6^b (± 11.6)	65.9^b (± 13.4)	58.2^c (± 12.6)	86.7^a (± 24.4)	34.2	<0.001
	Leaves number	23.2^b (± 6.6)	37.8^a (± 10.6)	14.9^c (± 3.5)	22.5^b (± 5.6)	169.1	<0.001
Abiotic water conditions	T (°C)	28.3^b (± 3.2)	27.1^c (± 3.0)	30.3^a (± 2.2)	26.1^c (± 1.8)	35.1	<0.001
	pH	5.0^c(± 1.1)	5.9^a (± 0.6)	4.4^d (± 0.6)	5.2^b (± 0.7)	56.5	<0.001
	EC (mS cm^{-1})	0.21^a (± 0.35)	0.23^a (± 0.17)	0.07^b (± 0.09)	0.09^b (± 0.09)	18.4	<0.001
	TDS (ppt)	0.11^a (± 0.18)	0.12^a (± 0.10)	0.05^b (± 0.11)	0.04^b (± 0.04)	13.8	<0.001
	DO (mg L^{-1})	5.1^c (± 2.4)	7.8^a (± 3.7)	6.8^b (± 3.9)	4.2^d (± 1.3)	29.3	<0.001
Algae community	Richness per individual bromeliad	1.1^b (± 0.7)	15.9^a (± 5.5)	1.3^b (±0.8)	0.2^c (± 0.5)	949.5	<0.001

and nine classes: Zygnematophyceae (27 % of the species), Cyanobacteria (24 %), Chlorophyceae (21 %), Euglenophyceae (12 %), Trebouxiophyceae (7 %), Bacillariophyceae (4 %), Chrysophyceae (2 %), Dinophyceae (1 %), and Klebsormidiophyceae (1 %). Of the 320 bromeliad specimens studied, 240 individuals contained representatives of microalgae and/or cyanobacteria; all of the samples collected from *Alcantarea nahoumii* (80 bromeliad tanks) had microalgae; *H. stellata* had the lowest number of algal and cyanobacterial species, and they occurred in only 13 of the 80 bromeliads sampled.

The taxonomic richness of microalgae and cyanobacteria was highest in *Alcantarea nahoumii* (Serra da Jiboia – rock outcrops) with 73 taxa, followed by *Hohenbergia littoralis* (Parque das Dunas – restinga) (nine), *Aechmea* cf. *lingulatoides* (Fazenda Itaberaba – caatinga) (seven), and *Hohenbergia stellata* (Reserva Sapiranga – Atlantic Forest) (six). In terms of the species richness in the different bromeliads in the four vegetation formations studied, those on the rock outcrops containing 69 exclusive taxa, followed by restinga and caatinga vegetation (six each) and Atlantic Forest (four) (Fig. 3). Only one species, *Phacus polytrophos* Pochmann, occurred in all four bromeliad species studied. The most representative genus was *Cosmarium* (nine taxa), and it was the only desmid occurring in more than one bromeliad species.

The mean species richness per individual bromeliad was significantly higher (> 12 times) in *Alcantarea nahoumii* (mean: 15.9) than in the other three bromeliads (mean: 1.3-0.2 species/ind.). Individual specimens of *H. stellata* contain the lowest mean number of species (0.2) (Tab. 2).

In terms of the entire sampling period, the microalgae classes with the highest species richness in *A. nahoumii* were Zygnematophyceae, Cyanophyceae, and Chlorophyceae, which contributed approximately 80 % of the total number of species inventoried. Among the bromeliads species with the lowest richness, *A.* cf. *lingulatoides* contained mainly by diatoms, whereas the most representative class in *H. littoralis* was Euglenophyceae (Fig. 4).

Only four microalgae species (4 % of the total were considered very frequent: *Enallax costatus*, *Parvodinium umbonatum*, and P*leurotaenium trabecula*, which were found in the bromeliad *A. nahoumii*; *Oedogonium pulchrum* was identified in the bromeliad *H. littoralis*). Most of microalgae species where considered rare (46 %), followed by occasional (35 %), and frequent (15 %). In terms of life forms, the microalgae community was dominated by unicellular non-flagellated taxa (45 %); the most common size class of organisms was microplankton (75 %) (Tab. 3).

The species compositions of the microalgae communities showed low similarities among the different bromeliad species (Tab. 4). The species composition in *A. nahoumii* was only 3 % similar to that in *A.* cf. *lingulatoides*, 5 % similar to that in *H. stellata*, and 7 % similar to that in *H. littoralis*. The species composition in *A.* cf. *lingulatoides* was most similar to that in *H. littoralis* (13 %) and *H. stellata* (15 %).

Taxonomic groups - richness and main representatives

Zygnematophyceae - Representatives were distributed among 24 taxa, with desmids being the dominant group (19 taxa). Among the most notable representatives were *Cosmarium amoenum* var. *jiboensis*, *C. bahianum*, *C. oliveirae*, *Spirotaenia filiformis*, and *Staurastrum pseudoteliferum*, which were recently described as new to science (Ramos *et al*. 2017a; b; 2018a). *Pleurotaenium trabecula* was the most frequent desmid in Serra da Jiboia (F = 78 %).

Chlorophyta - Green algae were one of the most diverse groups occurring in phytotelmata in Bahia State, being represented in the present study by two classes: Chlorophyceae (19 taxa) and Trebouxiophyceae (six taxa), which occurred in all four areas, especially Serra da Jiboia (20 taxa); *Monoraphidium* was the most representative genus (four species). Some taxa, such as *Enallax costatus*, were widely distributed in the bromeliad tanks of *A. nahoumii*; *Oedogonium pulchrum* was the only species consistently encountered, often forming large and dense populations in *H. littoralis* tanks.

Cyanobacteria - Cyanobacteria displayed the greatest species richness in the phytotelmata at Serra da Jiboia (21 taxa), especially *Hapalosiphon stuhlmannii*, which was the principal species forming gelatinous masses located in the central rosette of the bromeliads. Those masses contained numerous cyanobacteria representatives including coccoid and colonial, but mainly filamentous organisms.

Bacillariophyta - Diatoms were not very common in the phytotelmata studied, with only four taxa occurring in a small number of bromeliads in Serra da Jiboia and Fazenda Itaberaba.

Dinophyta - Dinoflagellates were represented by a single species (*Parvodinium umbonatum*) that was restricted to bromeliads at Serra da Jiboia. Nonetheless, that species showed the greatest frequency of occurrence (F = 79 %) among all taxa encountered at Serra da Jiboia.

Euglenophyta - Euglenophytes were represented by 11 taxa; *Euglena mutabilis* was the most frequent species (F=56 %) of that group in the bromeliads studied.

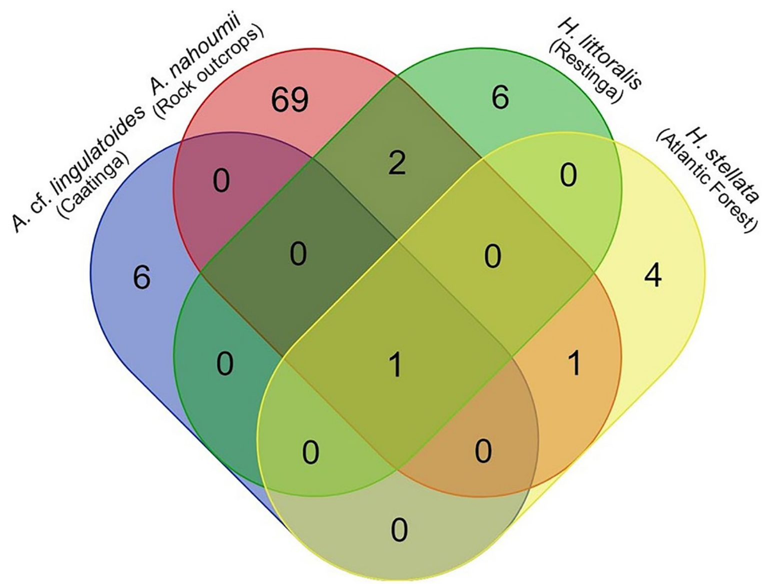

Figure. 3 - Venn diagram showing the numbers of microalgae taxa found in the phytotelmata of different bromeliad species in four vegetal formations in Bahia, Brazil.

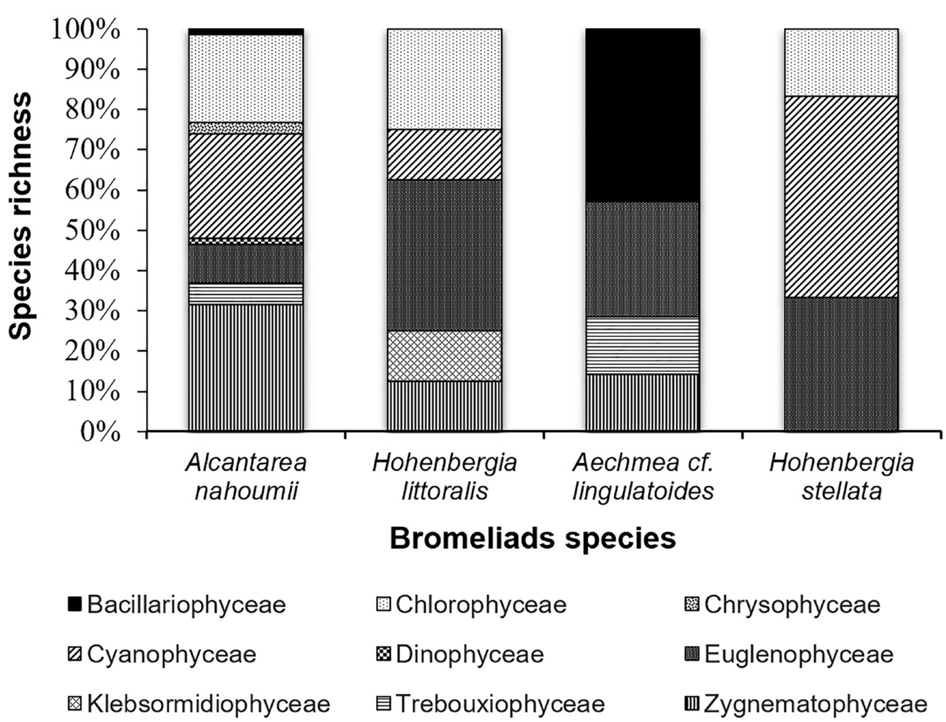

Figure 4. Relative species richness of the taxonomic classes of the microalgae communities in four bromeliad species.

Table 3. List of microalgae taxa and their classification by size class, life form, and frequency of occurrence in each bromeliad species. Size class: nano (nanoplankton), micro (microplankton), meso (mesoplankton). Life form: UF (unicellular flagellate), CF (colonial flagellate), UNF (unicellular non-flagellated), CNF (colonial non-flagellated, including coenobia), Fil (filaments). Areas: SB (Serra da Jiboia), PD (Parque das Dunas), Sap (Reserva Sapiranga), Ita (Itaberaba). Bromeliad species: A. nah (*Alcantarea nahoumii*), H. lit (*Hohenbergia littoralis*), H.ste (*Hohenbergia stellata*), A. ling (*Aechmea* cf. *lingutaloides*).

Taxa	Size class	Life form	SB	PD	Sap	Ita
			A. nah	H. litt	H. ste	A. ling
			Frequency (%)			
Bacillariophyceae						
Gomphonema gracile Ehrenberg	micro	UNF				1
Pinnularia gibba Ehrenberg	micro	UNF	3			
P. latarea Krammer	micro	UNF				1
Staurosirella leptostauron var. *dubia* (Grunow) M.B.Edlund	micro	UNF				1
Chlorophyceae						
Ankistrodesmus falcatus (Corda) Ralfs	micro	CNF	1			
A. fusiformis Corda	micro	CNF	20			
Asterococcus superbus (Cienkowski) Scherffel	nano	CNF	4			
Coelastrum indicum W.B.Turner	micro	CNF	43			
Crucigenia quadrata Morren	nano	CNF	1			
Enallax costatus (Schmidle) Pascher	micro	CNF	70			
Gongrosira papuasica (Borzì) Tupa	micro	Fil		4		
Monoraphidium caribeum Hindák	micro	UNF	6			
M. contortum (Thuret) Komárková-Legnerová	nano	UNF	3			
M. griffithii (Berkeley) Komárková-Legnerová	micro	UNF	31			
M. komarkovae Nygaard	micro	UNF	5			
M. subclavatum Nygaard	nano	UNF	3			
Oedogonium areschougii Wittrock ex Hirn	meso	Fil	28			
O. pulchrum Nordstedt & Hirn	meso	Fil		85		
Oedogonium sp.	meso	Fil			3	
Scenedesmus ecornis (Ehrenberg) Chodat	micro	CNF	13			
S. obtusus Meyen	micro	CNF	20			
Sorastrum americanum (Bohlin) Schmidle	micro	CNF	4			
S. spinulosum Nägeli	micro	CNF	4			
Chrysophyceae						
Synura synuroidea (Prowse) Pusztai, Certnerová, Skaloudová & Skaloud	micro	CF	4			
Mallomonas sp.	micro	UF	6			
Cyanophyceae						
Aphanothece saxicola Nägeli	micro	CNF	18			
Chroococcus obliteratus Richter	nano	CNF	26			
Cyanobium eximium (J.J.Copeland) Komárek, Kopeck‡ & Cepák	nano	UNF	46			
Cylindrospermum licheniforme Kützing ex Bornet & Flahault	meso	Fil	4			
Hapalosiphon stuhlmannii Hieronymus	meso	Fil	49			
Leptolyngbya perelegans (Lemmermann) Anagnostidis & Komárek	micro	Fil	28			
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O.Komárek & Zapomelová	nano	CNF	4			
Oscillatoria subbrevis Schmidle	micro	Fil	26			
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová	micro	Fil	8			
Planktothrix isothrix (Skuja) Komárek & Komárková	micro	Fil	20			
Pseudanabaena acicularis (Nygaard) Anagnostidis & Komárek	nano	Fil	3			
P. catenata Lauterborn	micro	Fil	33			
P. mucicola (Naumann & Huber-Pestalozzi) Schwabe	nano	Fil	14		1	
Romeria okensis (C.Meyer) Hindák	nano	Fil	11			
Stigonema minutum Hassall ex Bornet & Flahault	micro	Fil	5	4		
S. crassivaginatum (Geltler) Sant'Anna, Kaštovský, Hentschke & Komárek	micro	Fil	3			
S. ocellatum Thuret ex Bornet & Flahault	micro	Fil	9			
Stigonema sp.	micro	Fil			3	

Table 3. Cont.

Taxa	Size class	Life form	SB	PD	Sap	Ita
			A. nah	H. litt	H. ste	A. ling
			Frequency (%)			
Scytonema sp. 1	micro	Fil	4			
Scytonema sp. 2	micro	Fil			4	
Synechococcus nidulans (Pringsheim) Komárek in Bourrelly	nano	UNF	40			
Dinophyceae						
Parvodinium umbonatum (Stein) S.Carty	micro	UF	79			
Euglenophyceae						
Astasia comma Pringsheim	micro	UF	1			
Euglena mutabilis F.Schmitz	micro	UF	56			
Euglena sp.	micro	UF	9			
Heteronema sp. 1	micro	UF	1			
Heteronema sp. 2	micro	UF				19
Peranema trichophorum (Ehrenberg) Stein	micro	UF	5			
Phacus ocellatus (Pringsheim) Marin & Melkonian	micro	UF			4	
P. orbicularis Hübner	micro	UF	6			
P. polytrophos Pochmann	micro	UF	26	21	8	21
P. wettsteinii Drezepolski	micro	UF				
Rhabdomonas incurva Fresenius	micro	UF				
Klebsormidiophyceae						
Klebsormidium sp.	micro	Fil		3		
Trebouxiophyceae						
Dispora speciosa Korshikov	micro	CNF	13			
Eremosphaera viridis De Bary	micro	UNF	54			
Lagerheimia chodatii C.Bernard	nano	UNF		1		
Oocystis borgei J.W.Snow	micro	UNF	15			
O. lacustris Chodat	micro	UNF	14			
Rhopalosolen cylindricus (F.Lambert) Fott	micro	UNF				68
Zygnematophyceae						
Actinotaenium mooreanum (W.Archer) Teiling	nano	UNF	5			
Closterium cornu var. *minus* Irénée-Marie	micro	UNF	29			
Cosmarium amoenum var. *jiboensis* G.J.P.Ramos, C.E.M. Bicudo & C.W.N.Moura	micro	UNF	43			
C. bahianum G.J.P.Ramos, C.E.M. Bicudo & C.W.N.Moura	micro	UNF	16	10		
C. elegantissimum Lundell	micro	UNF	60			
C. majae Strøm	nano	UNF	16			
C. oliveirae G.J.P.Ramos, C.E.M. Bicudo & C.W.N.Moura	micro	UNF	11			
C. pachydermum var. *aethiopicum* (West & G.S.West) West & G.S.West	micro	UNF	11			
C. pseudoconnatum Nordstedt	micro	UNF	29			
C. scrobiculosum Borge	micro	UNF	56			
Cosmarium sp.	micro	UNF	23			
Docidium baculum Brébisson ex Ralfs	micro	UNF	65			
Euastrum luetkemuelleri var. *carniolicum* (Lütkemüller) Willi Krieger	micro	UNF	24			
E. quadriceps Nordstedt	micro	UNF	4			
Micrasterias radians W.B.Turner	micro	UNF	65			
Mougeotia sp.	meso	Fil	26			
Netrium digitus (Brébisson ex Ralfs) Itzigsohn & Rothe	micro	UNF	48			
Pleurotaenium trabecula Nägeli	meso	UNF	78			
Spirotaenia closteridia (Kützing) Rabenhorst	micro	UNF	4			
S. endospira W. Archer	nano	UNF	4			
S. filiformis G.J.P.Ramos, C.E.M. Bicudo & C.W.N.Moura	micro	UNF	3			
Staurastrum pseudosebaldi var. *compactum* A.M.Scott & Grönblad	micro	UNF	4			
S. pseudoteliferum G.J.P.Ramos, C.E.M.Bicudo & C.W.N.Moura.	micro	UNF	44			
Xanthidium mamillosum var. *borgei* K.Förster	micro	UNF				1

Table 4. Sorensen's similarity index (expressed as %) applied to presence-absence matrix of microalgae species in water tanks of different bromeliad species.

	Alcantarea nahoumii	*Aechmea* cf. *lingulatoides*	*Hohenbergia littoralis*	*Hohenbergia stellata*
Alcantarea nahoumii	100			
Aechmea cf. *lingulatoides*	3	100		
Hohenbergia littoralis	7	13	100	
Hohenbergia stellata	5	15	13	100

Discussion

Microalgae richness and the influence of environmental factors

The high taxonomic richness of algae and cyanobacteria in the bromeliads at Serra da Jiboia was apparently related to the following factors: (1) plant architecture - *Alcantarea nahoumii* generally produces many (over 30) wide leaves that form large numbers of cavities (greater complexity) and consequently more places for water to accumulate; those leaves are also held at open angles that allow higher solar illumination; (2) the regional climate – the bromeliads grow on mountain tops, with forests on their slopes (distinct from the other three areas) – which favors the sites being constantly humid, so that the bromeliad tanks would not usually experience abrupt variations in water volumes (allowing greater community stability); (3) environmental data - the tank water was slightly acidic to neutral, with moderate conductivity and high TDS (indicative of high concentration of dissolved salts and nutrients) as compared to the other bromeliads; the usually high dissolved oxygen content probably contributed to a greater microalgae diversity.

In terms of plant architecture, Carrias *et al*. (2014) reported that greater bromeliad complexity (larger numbers of leaves – and consequently more sub-reservoirs) was associated with lower algal richness. The opposite was observed during the present study, however, mainly in *A. nahoumii* – the bromeliad species with the greatest number of leaves and the highest algal and cyanobacterial richness when compared to less complex bromeliads. Water volume may also directly influenced algal and cyanobacterial species richness and diversity, as the bromeliads at Serra da Jiboia usually contained large amounts of water well-distributed among the leaves in all four sampling periods.

Overall, the algal and cyanobacterial community similarities among the different bromeliads species were considered quite low (Tab. 4), and probably linked to the distinct environmental conditions in each of the four study areas. Additionally, different bromeliad species tend to host distinct algal communities (Carrias *et al*. 2014).

Recent studies have demonstrated that changes in rainfall distribution can reduce chlorophyll-a concentrations in bromeliad tanks and therefore significantly affect microalgae dominance (Pires *et al*. 2017). Although we did not measure chlorophyll-a concentrations in the water accumulated in each bromeliad tank, it was evident that the plants from Serra da Jiboia (*A. nahoumii*) and Parque das Dunas (*H. littoralis*) contained greater volumes of water than those at Reserva Sapiranga (*H. stellata*) and Fazenda Itaberaba (*A.* cf. *lingulatoides*) –and the bromeliads in the former two areas showed quite different richnesses. The unevenly distributed water storage by *H. littoralis* leaves (with large amounts of water accumulated in the central tank and considerably smaller volumes in the lateral tanks) may affect the low species richness in that bromeliad in Parque das Dunas. The leaves forming the central tank are usually long and perpendicular to the ground, which allows less light into the bottom of the central tank; the leaves forming the lateral tanks, however, are held at more open angles, permitting greater light penetration. Additionally, the very acidic waters and lower conductivity and TDS values measured in *H. littoralis* may depress algal and cyanobacterial richness.

Variations in the algal and cyanobacterial communities in phytotelmata would be quite natural, with some well-established taxa and others more temporary. When comparing the desmids in *A. nahoumii* tanks (Serra da Jiboia) identified during this study with the 16 taxa previously identified for the same area (Ramos *et al*. 2011), nine continued to inhabit the tanks, whereas the others had disappeared. The nine taxa that persisted are widely distributed among bromeliads in the area, and well-adapted to local conditions, usually forming large populations, even with considerable external impacts (such as fires).

In terms of *H. stellata* (Reserva Sapiranga), only 13 out of 80 bromeliads sampled contained representatives of algae or cyanobacteria, and the numbers of species per sample were very low. Some contributing factors to that situation probably included: (1) luminosity - the bromeliads were largely shaded, but many of plants exposed to high sunlight (open areas in the forest) showed low diversity. Even in open areas, leaves from surrounding trees will fall into the tanks, often almost completely covering them; and, (2) dissolved oxygen - DO concentrations were commonly quite low (mean of 4.8 mg L^{-1}; near 2.5 mg L^{-1} in some samples). According to Laessle (1961), algae are usually abundant in bromeliads that have high DO concentrations – a condition rarely observed in *H. stellata* in the present study. Additionally, many of those bromeliads held only small amounts of water (or were completely dry during some sampling periods). Among the main components found in *H. stellata* tanks were fungi, pollen grains, protozoa and organic matter debris.

Taxonomic groups - richness and main representatives

Desmids are one of the main microalgae groups occurring in bromeliad phytotelmata in Brazil (Sophia 1999; Sophia *et al*. 2004; Ramos *et al*. 2011). Water conditions, such as low pH and low conductivity, are typical of phytotelmata environments and favor desmid development (Sophia *et al*. 2004). A number of desmid species were recently described for the first time from bromeliads in Bahia State, suggesting that those environments as important centers of desmid biodiversity (Ramos *et al*. 2018a).

In terms of the main Chlorophyta taxa, we highlight the genus *Rhopalosolen*, which was recently reported for the first time for Brazil (Ramos *et al*. 2017c). In the bromeliads with a predominance of filamentous green algae (such as the genus *Oedogonium*), common predators such as rotifers, cladocerans, and copepods could be influencing the low observed algal richness.

The cyanobacteria comprise one of the most important groups found in phytotelmata in terms of taxonomic diversity and their ecological roles (Bermudes & Benzing 1991). An interesting feature of the cyanobacteria identified in the bromeliad phytotelmata was their normal association with rivers, waterfalls, tree barks, rocks, even hot springs (Komárek & Anagnostidis 1998; 2005; Komárek 2013) – so that bromeliad phytotelmata are important environments for cyanobacteria diversity and bring together in just one place species otherwise known from very diverse habitats.

The low diversity of diatoms encountered in bromeliad tanks was not surprising. In a study of diatoms in bromeliads in Rio de Janeiro State, however, Lyra (1971) reported reduced numbers of those microalgae and attributed that result to their oligotrophic tank water conditions, as well as other environmental factors such as light intensity and temperature. According to that author, mineral element concentrations in those environments could considerably interfere with frustule development – although *Pinnularia* is one of the most common diatom genera inhabiting those microhabitats, and has been reported in a number of different bromeliad species (Lyra 1971; 1976).

Parvodonium umbonatum (the only dinoflagellate identified in the present study) have been reported as occurring in bromeliads in Rio de Janeiro State (as *Peridinium umbonatum*; Sophia 1999). During dry periods, spherical, reddish-brown cysts of *P. umbonatum* were often encountered in the bromeliads at Serra da Jiboia – possibly representing a reproductive strategy in response to unfavorable environmental conditions. That species was consistently observed forming large populations during all of the sampling periods (Ramos *et al*. 2016).

In terms of Euglenophytes, Carrias *et al*. (2014) reported that heterotrophic microalgae species were dominant in areas exposed to direct sunlight – but that tendency was precisely the opposite among bromeliads in Bahia – with euglenophytes appearing predominantly in shaded (or partially shaded) bromeliads. Colorless euglenophytes were encountered at Reserva Sapiranga, but chlorophyllous taxa were much more common in bromeliads exposed to direct sunlight, especially *Euglena mutabilis*, which commonly forms large populations (Ramos *et al*. 2017d)

Although a total of nine microalgae classes were encountered in the four bromeliad species, other microalgae groups may occur in those phytotelmata, such as Xanthophyceae (Sophia 1999) and Cryptophyceae (Hernandez-Rodriguez *et al*. 2014), although little is currently known about the diversity of those groups in those microhabitats, and what environmental conditions are ideal for their development.

Conclusion

The algae and cyanobacteria communities found in the phytotelmata of bromeliads growing in distinct vegetation formations were all very different, with one bromeliad species (*Alcantarea nahoumii*) being especially distinguished by its high taxonomic richness. We also observed low microalgae species similarity among the bromeliads studied, with only one species (*Phacus polytrophos*) occurring in all four bromeliad species.

Overall, the following conditions were found to be favorable to high algae and cyanobacteria richness in bromeliad phytotelmata: slightly acidic water at high temperatures, moderate conductivity, large numbers of leaves (with large amounts of water), high dissolved oxygen levels, and high environmental light intensities (Laessle 1961; Sophia *et al*. 2004; Brouard *et al*. 2011; Marino *et al*. 2011), high rainfall (Pires *et al*. 2017), and available nutrients (Laessle 1961; Marino *et al*. 2011).

Combined analyses of limnological variables, bromeliad position (shade or sun), and plant morphology are important to understanding the patterns of microalgae communities in phytotelmata environments, and more detailed studies will be needed to increase our knowledge of algae and cyanobacteria distributions and their ecological relationships within that interesting microhabitat.

Acknowledgements

The authors thank CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico and FAPESB – Fundação de Amparo à Pesquisa do Estado da Bahia (Project "Flora da Bahia", 483909/2012) for their financial support. GJPR thanks FAPESB for the doctoral fellowship (nº BOL0513/2014).

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