



# Soft-bodied benthic algae in pristine Brazilian streams: from acid to karst<sup>1</sup>

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## ABSTRACT

This study evaluated soft-bodied benthic algae from seven streams located in protected areas of central Brazil (Parque Nacional de Brasília, Parque Nacional da Chapada dos Veadeiros and Parque Estadual de Terra Ronca), and related them to abiotic factors. We addressed the following questions: A) how algal community structure in Cerrado streams is influenced by the marked seasonality typical of the region; and B) how karst geology influences algal communities in Parque Estadual de Terra Ronca, as compared to streams in other Cerrado areas. Samples were collected between 2012 and 2013 from a single site in each stream, and upstream and downstream of caves present in Parque Estadual de Terra Ronca. Altogether, 159 taxa were recorded. Algal community structure varied significantly among sites, but not between seasons, despite relatively lower abundances during the rainy season, likely due to a negative influence of the faster currents in streams during this period. Some alkaliphilic species were observed only at sites near caves (e.g. the cyanobacterium *Tolypothrix distorta*), while acidophilic taxa were more frequent in the other streams (e.g. desmids and Cyanobacteria such as *Scytonema caldarium*, *Hapalosiphon stuhlmannii* and *Stigonema ocellatum*). We observed a rare green alga, *Microthamnion kuetzingianum*, and reported the cyanobacterium *Rivularia aquatica* for the first time in Brazil.

**Keywords:** abiotic variables, biodiversity, Cerrado, Cyanobacteria, lotic systems, periphyton, soft-bodied algae, pristine streams, Zygnematophyceae

## Introduction

All over the world, anthropogenic influence on natural environments is increasing, and pristine aquatic systems are becoming scarce (Lindstrøm *et al.* 2004). Running waters unaffected by human impact are frequently restricted to areas protected by law (Fonseca *et al.* 2014; Fonseca & Mendonça-Galvão 2014), and are thus very precious, as they can provide basic knowledge on biological reference conditions (Schneider 2015).

Soft-bodied benthic (SB) algae have been defined as non-diatom algae, including cyanobacteria, attached to substrata in lotic environments (Rott & Schneider 2014).

Studies on SB algae in reference conditions have been mainly carried out in streams from temperate regions of North America (Thomas *et al.* 2015; Stancheva & Sheath 2016) and northern Europe (Lindstrøm *et al.* 2004). In Brazil, this terminology has not been widely used; instead, the literature about benthic or periphytic algae in lotic systems mostly refers to diatoms (e.g. Salomoni *et al.* 2011; França *et al.* 2017) or macroalgal communities (e.g. Branco *et al.* 2001; 2008a; b; Necchi Jr. *et al.* 2008; Tonetto *et al.* 2015; 2018), and the majority of them are concentrated in the southeastern/southern regions. Microscopic attached algae from other taxonomical groups are less known, which may lead to underestimated numbers for general phycological diversity in such environments.

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Soft-bodied benthic algae have already provided information for the development of ecological indexes used for monitoring ecological changes or spatial patterns (Schneider & Lindström 2009; 2011; Stancheva *et al.* 2013; Fetscher *et al.* 2014; Stancheva & Sheath 2016). Although diatoms have been more studied under this approach and have often been considered the best indicator to reflect environmental conditions when compared to non-diatoms (Kelly *et al.* 2008), in some temperate countries the study of SB algae alongside diatoms is also mandatory for ecological quality analysis (Poikane *et al.* 2016).

Besides trophic status, other variables such as rainfall, current velocity and turbidity can be important environmental factors that influence periphytic and macroalgal communities in lotic environments, especially in nutrient-poor waters (Branco & Necchi Jr. 1997; Branco *et al.* 2008b). Sometimes, despite regional variations, particular features of a stream can influence species richness on a local scale, promoting low inter-stream similarities and consequently increasing beta diversity (Rott & Wehr 2016). Such spatial differences have been observed in several studies carried out in the southeastern/southern regions of Brazil (*e.g.* Branco *et al.* 2008b; 2009; Tonetto *et al.* 2015).

In central Brazil, a region covered by the Cerrado Domain (Brazilian savanna), seasonality is typically marked by the alternation between dry and rainy seasons (Silva *et al.* 2008), with consequences for both terrestrial and aquatic ecosystems (Fonseca & Mendonça-Galvão 2014). Cerrado pristine waters generally have a slightly acidic pH, with low electrical conductivity and low nutrient concentrations (Markewitz *et al.* 2006; Fonseca *et al.* 2014). However, some limestone outcrops with karst geology are also found in the region, generally belonging to the Brazilian sedimentary unit known as the Bambuí Group (Faquim *et al.* 2017), promoting alkaline waters and higher electrical conductivity (Righi-Cavallaro *et al.* 2010). Variation in these chemical variables (pH and electrical conductivity) can result in marked differences in algal community structure (Mutinová *et al.* 2016).

The Parque Estadual de Terra Ronca (hereafter PETeR), one of the three protected areas considered in the present study, comprises the biggest South American cave complex,

and its karst geology contrasts with the other two areas, Parque Nacional de Brasília (hereafter PNB) and Parque Nacional da Chapada dos Veadeiros (hereafter PNCV). These three protected areas are located in the core of the Cerrado Domain, at a distance of 160 km to 250 km from one another. Ecological studies on benthic or periphytic algae in lotic systems in central Brazil, especially unpolluted ones, are very scarce (*e.g.* França *et al.* 2017), despite their importance as primary producers and their strategic position at the bottom of trophic cascades (Necchi Jr. 2016).

For this study, our aim was to address two main questions: A) how algal community structure in Cerrado streams is influenced by the marked seasonality typical of the region; and B) how karst geology influences the algal communities in Parque Estadual de Terra Ronca, as compared to streams in other Cerrado areas. Special attention was also given to identifying rare taxa, or those that are characteristic of particular habitats.

## Materials and methods

### Study area

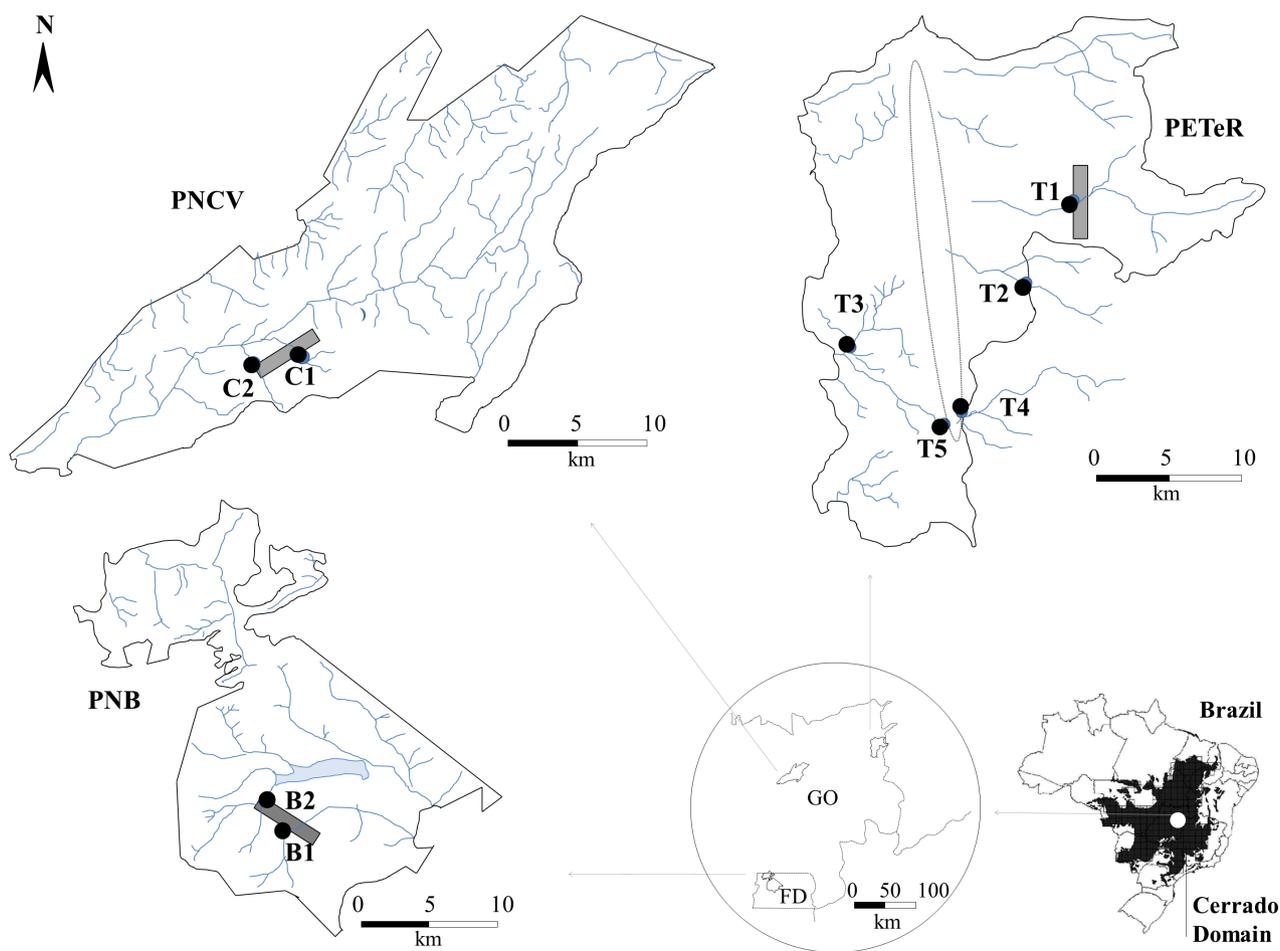
This study was carried out in seven pristine streams located in three protected areas in the Cerrado Domain, within the coordinates 13°–15°S 46°–48°W: Parque Nacional de Brasília, Parque Nacional da Chapada dos Veadeiros and Parque Estadual de Terra Ronca (Tab. 1, Fig. 1). The climate in the region is rainy tropical, *i.e.* “Aw” according to Köppen’s classification (Alvares *et al.* 2014), marked by strong seasonality, with a dry/cool season from April to September (precipitation 30 mm; air temperature 18 °C) and a rainy/warm season from October to March (precipitation 202 mm; air temperature 29 °C). The mean annual air temperature is around 22 °C (Silva *et al.* 2008).

The PNB covers 42,355 ha and is located in the Federal District of Brazil, within the large Brazilian hydrographic basin of Paraná. The other two protected areas are located in the State of Goiás, within the hydrographic basin of Tocantins-Araguaia. The PNCV spans an area of 64,727 ha, and the PETeR, 57,000 ha (ICMBIO 2016; SEMAD 2018).

**Table 1.** Geographical coordinates of nine sampling sites located in three protected areas in central Brazil. PNB = Parque Nacional de Brasília, PNCV = Parque Nacional da Chapada dos Veadeiros, PETeR = Parque Estadual de Terra Ronca. S = substrata.

Protected area	Stream	Code	Geographical coordinates	Altitude (m)	Order	Description
PNB	Bananal	B1	15°42'50.23"S 48°00'45.03"W	1082	2	Shaded. S: leaf litter, macrophytes ( <i>Sphagnum</i> ), roots
	Santa Maria	B2	15°41'46.10"S 48°01'37.50"W	1096	2	Partially shaded; substrata: clay, sand, macrophytes
PNCV	Córrego 1	C1	14°06'13.50"S 47°42'19.50"W	1172	3	Partially shaded. S: sand, clay, pebbles, trunks, leaf litter, macrophytes, roots
	Estiva	C2	14°06'40.30"S 47°44'02.20"W	1162	2	Unshaded. S: macrophytes, pebbles, trunks, sand
PETeR	São Vicente	T1	13°36'03.72"S 46°17'21.27"W	733	5	Unshaded. S: sand, leaf litter
	São Mateus S1	T2	13°39'20.86"S 46°18'58.78"W	712	2	Unshaded. S: pebbles, leaf litter, macrophytes, sand
	São Mateus S2	T3	13°41'47.78"S 46°25'43.91"W	512	3	Unshaded. S: pebbles, sand, leaf litter, clay
	Lapa S1	T4	13°44'04.38"S 46°21'24.38"W	602	5	Unshaded. S: sand, pebbles, leaf litter, trunks
	Lapa S2	T5	13°44'14.91"S 46°21'57.91"W	613	5	Partially shaded. S: pebbles, sand, leaf litter





**Figure 1.** Location of the nine sampling sites within their respective protected area in the Cerrado Domain. PNB = Parque Nacional de Brasília, PNCV = Parque Nacional da Chapada dos Veadeiros, PETeR = Parque Estadual de Terra Ronca. Rectangles represent permanent grids (5 x 1 km) for integrated inventories delimited by the ComCerrado Network; the dotted area in PETeR marks the location of caves. GO = State of Goiás; FD = Federal District of Brazil.

The approximate distances between the parks vary from 160 km (PNCV and PETeR) to 250 km (PNB and PETeR).

A permanent grid (5 x 1 km) for integrated inventories had been previously delimited by the ComCerrado Network in each protected area, as part of a Brazilian research program (SISBIOTA/PPBio) whose main goal was to survey biodiversity in the Cerrado Domain, including different groups of organisms (e.g. França *et al.* 2017; Teixeira *et al.* 2017). Concerning aquatic ecosystems, every stream intersecting the aforementioned permanent grids was supposed to be sampled. So, in the present study, the following streams within the grids were examined: Bananal (B1) and Santa Maria (B2) in the PNB, Córrego 1 (C1) and Estiva (C2) in the PNCV, and São Vicente (T1) in the PETeR (Fig. 1). In the PETeR, besides São Vicente (T1), two other streams located outside the permanent grid were also sampled. The São Mateus and Lapa streams sink underground and then come up again downstream of caves and, considering this peculiarity, two sampling sites were located in each

of them, upstream (sites T2 and T4) and downstream (sites T3 and T5) of their respective caves (Tab. 1). Altogether, nine sampling sites were considered in this study (one site per stream, except in the case of São Mateus and Lapa, where two sites were sampled).

### Fieldwork and laboratory analyses

Sampling was conducted between 2012 and 2013, during the dry (July-August) and rainy (February-March) seasons, between 9 a.m. and 3 p.m. Each site was visited once in each season (exact sampling dates available as Tab. S1 in supplementary material). A 50 m transect was delimited on the stream edge, following its curves along the longitudinal axis. A suite of physical variables was measured for each site, including depth (maximum depth within the channel), wetted width and water velocity; for all variables, we considered the mean of six measures over the 50 m transect. For depth, a ruler was used; for width, a 20-m

tape; and for water velocity, a flowmeter model FLK1 (JDC Instruments, Switzerland). Geographical coordinates and altitude were measured with a Garmin eTrex Vista HCx GPS.

Subsurface water samples for abiotic analyses were collected in a midstream location. The following abiotic variables were measured in the field, using standard electrodes (Yellow Spring Instruments, USA): water temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), oxygen percentage saturation (%), pH and electrical conductivity (µS cm<sup>-1</sup>). Each variable was measured three times, also in a midstream location, and then the mean value for that site was calculated. Water samples for nutrient, solid and turbidity analyses were also collected and kept in a cooler until they were returned to the laboratory. Nitrate (N-NO<sub>3</sub>) (chromotropic acid and phenoldisulfonic acid methods), ammonium (N-NH<sub>4</sub>) (Nessler Method), soluble reactive phosphorus (SRP) (Stannous Chlorid Method) and silicate (Si) (Molybdosilicate Method) were analyzed after water filtration through combusted glass fiber filters with 0.7 µm pore size (Merck Millipore, Ireland). Unfiltered samples were used for total nitrogen (TN) and total phosphorus (TP) determinations (Valderrama 1981). Samples from PNB were processed in 24 h after the fieldwork. In the case of PNCV and PETeR, samples were preserved with sulfuric acid at pH <2 (N-NO<sub>3</sub>, N-NH<sub>4</sub>, TP, TN) or kept under 4 °C (SRP and silicate), and processed after no more than five days. Nitrate was analyzed according to Taras (1950); the other nutrients, along with turbidity and solids, were analyzed according to Standard Methods (APHA 2005).

Benthic algae were sampled over the 50 m transect from the following substrata, whenever they were present: leaf litter, macrophytes, roots, trunks, pebbles, sand and clay. After an initial visual inspection of the transect, sampling of all possible substrata was carried out at both marginal and central positions and gathered within a tray. The number of substrata in each site varied from two (at T1) to seven (at C1) (Tab. 1). Macrophytes were squeezed and the other substrates were scraped with a toothbrush (leaf litter, roots, trunks, pebbles) using gentle jets of distilled water, or collected with the use of a syringe (sand and clay), and combined into a composite sample (150 mL). Samples were preserved in 3-5% formaldehyde solution and deposited in the Laboratório de Biodiversidade Aquática, at the Universidade Católica de Brasília (collection number available as Tab. S2 in supplementary material).

We are aware of the weakness in making comparisons among sites without a measurement of the sampled area. However, this method was used because substrata were very heterogeneous among streams; all systems were being sampled for the first time, and the priority was to access as many SB species as possible, in order to capture the best picture of local biodiversity. Richness was then estimated and compared among sites, after imposing uniformity on the number of individuals counted. In each sample (N = 18, i.e. nine sampling sites, two seasons), 400 individuals

were counted in the course of a systematic inspection of the slides at 400x magnification, using an Olympus BX41 light microscope. In most of our samples, 400 individuals were found within the second inspected slide; however, in some of them densities were very low (<30 individuals). In such cases (B2d, B2r, T1r and T5r; letters “d” and “r” represent dry and rainy seasons, respectively), at least four slides were inspected for each sample. This counting procedure was preceded by additional qualitative analyses at 100-1000x magnification, when at least four additional slides were evaluated for taxonomic identification using traditional literature (e.g. Förster 1964; Ramanathan 1964; Růžička 1981; Kadlubowska 1984; Lenzenweger 1996; Komárek & Anagnostidis 2005; Komárek 2013). The number of species reported only in this qualitative assessment was insignificant, representing less than 2% of the total richness registered during the counting of 400 individuals. For filamentous green algae that cannot be identified to species level in their vegetative forms (e.g. *Spirogyra*, *Mougeotia*, *Oedogonium*), categories based on filamentous width and/or chloroplast features were used to distinguish morphospecies.

### Data analysis

Multivariate descriptive analysis was carried out by applying Principal Component Analysis (PCA) to explore spatial and temporal variability, using a covariance matrix with data of eight abiotic variables transformed by their amplitude of variation [“ranging” = (x-minx)/(maxx-minx)]. The number of abiotic variables considered in the analysis was reduced after excluding the ones with missing values (problems with equipment or analytical analyses). This was the case of dissolved oxygen, nitrate, TN and turbidity. Abiotic variables (and also species richness) were compared between dry and rainy seasons using the non-parametric, paired difference, Wilcoxon signed rank test ( $\alpha = 0.05$ ).

Species were classified into four categories based on their relative abundance: “rare” = RA ≤ 5%; “common” = 5% < RA ≤ 40%; “very common” = 40% < RA ≤ 70%; “abundant” = RA > 70%. We used the Whittaker Index ( $\beta_w$ ) to estimate beta diversity, dividing the overall richness (gamma diversity) by the average richness per sample (alpha diversity) ( $\beta_w = (\gamma/\alpha) - 1$ ) (McCune & Grace 2002). The one in the equation is subtracted to make zero beta diversity correspond to zero variation in species presence. Non-Metric Multidimensional Scaling (NMDS) analysis was also performed in order to make inferences on similarity among sampling sites using the Dice Similarity Index, which is calculated on the presence/absence matrix (Hammer *et al.* 2001; Garraza *et al.* 2019). This index was chosen after some tests with other ones (e.g. Bray-Curtis) because it resulted in a lower stress value (<0.20) (Clarke 1993). The samples with very low densities previously cited (B2d, B2r, T1r and T5r) were excluded from this analysis because their



presence also increased the NMDS stress to values above 0.20. The abiotic variables pH and electrical conductivity were also considered in the NMDS. The SB community structure was then compared by means of a non-parametric ANOSIM test (Analysis of Similarity), with the Dice Index, run independently for the following null hypothesis: a) no difference between the distribution of taxa between rainy and dry seasons; b) no difference between the sites grouped by NMDS. Analyses were all run in the free software PAST ver. 2.17c (Hammer *et al.* 2001).

## Results

### Abiotic data

The majority of streams had slightly acidic water (pH 4.78 – 6.82) and extremely low nutrient concentrations, usually under the detection limit (Tab. 2). Sites downstream of caves (T3 and T5) had higher pH (ranging from 7.25 to 7.82) and higher electrical conductivity (ranging from 38.3 to 263.4  $\mu\text{S cm}^{-1}$ ) when compared to the others. Site T4, while upstream of Lapa cave, had relatively high pH (7.70). We recorded pH = 7.24 and electrical conductivity = 717  $\mu\text{S cm}^{-1}$  in water infiltrating through the inner wall of Lapa cave during the rainy season.

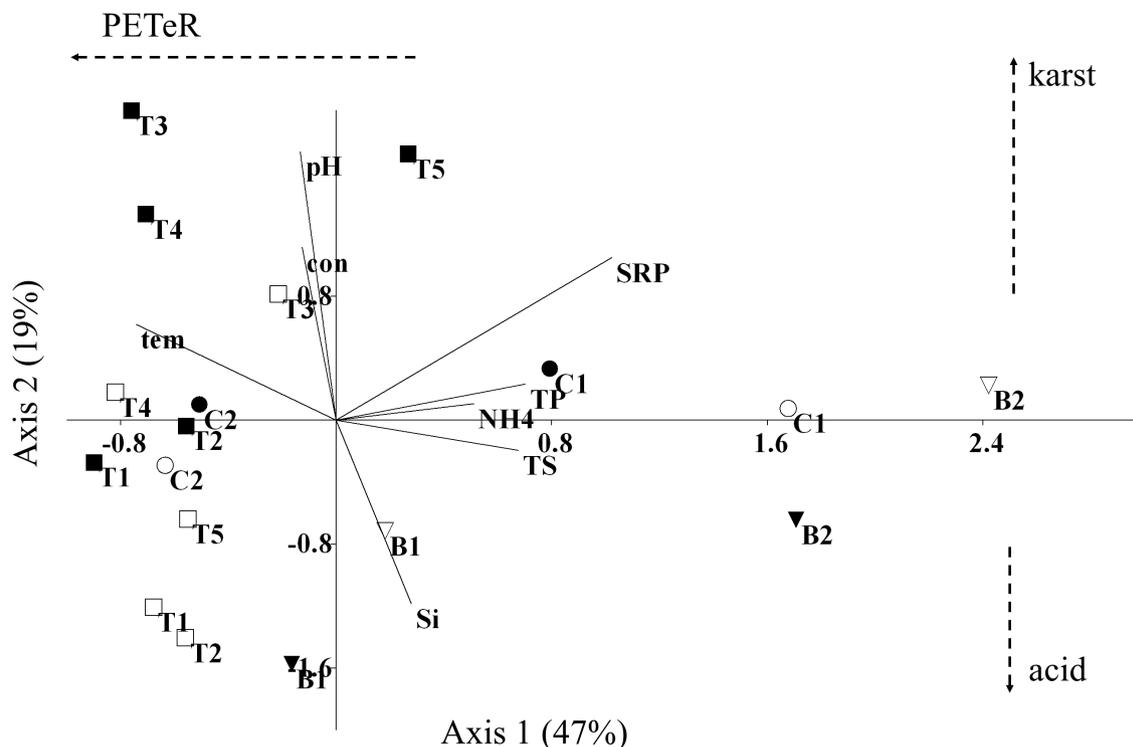
The first two PCA axes explained 66 % of the variation in the abiotic data (Fig. 2). The main environmental gradient

was represented by spatial differences among the protected areas, with samples from PETeR associated with lower nutrient concentrations and higher temperature. On the other hand, most samples from PNB were located on the opposite side of axis 1. Samples from PNCV were on both sides of axis 1; stream C2 was associated with relatively higher temperatures, while C1 grouped along with samples from PNB. On axis 1, the variables with the highest correlation were SRP ( $r = 0.86$ ), TP ( $r = 0.84$ ) and water temperature ( $r = -0.84$ ). PCA axis 2 was primarily a pH gradient, with all sampling sites connected with caves plotting with higher pH ( $r = 0.79$ ) and electrical conductivity ( $r = 0.61$ ).

When each abiotic variable was compared between rainy and dry seasons ( $N = 9$ ), there were significant differences for electrical conductivity ( $W = 41$ ,  $p = 0.03$ ), water velocity ( $W = 27$ ,  $p = 0.04$ ) and depth ( $W = 40$ ,  $p = 0.04$ ), all them with higher values during the rainy season.

### Soft-bodied benthic algae

Altogether, 159 taxa were reported, consisting of 105 Zygnematophyceae, 30 Cyanobacteria, 13 Chlorophyceae, two Chrysophyceae, five Euglenophyceae, one Rhodophyceae, one Xanthophyceae, one Cryptophyceae, and one Dinophyceae (Tab. 3). Individuals of *Chara* sp. (Charophyceae) were observed in Lapa stream, downstream of Lapa cave (T5); as this macroscopic taxon was not counted in the slide inspection, it was not included in the species list.



**Figure 2.** Principal Component Analysis (PCA) biplot with eight abiotic variables (pH, tem = temperature, con = electrical conductivity, Si = silicate, NH4 = ammonium, TS = total solids, TP = total phosphorus, SRP = soluble reactive phosphorus) and 18 sampling units (pristine stream sites in protected areas in central Brazil). Inverted triangles = Parque Nacional de Brasília; circles = Parque Nacional da Chapada dos Veadeiros; squares = Parque Estadual de Terra Ronca. Open and filled symbols represent dry and rainy seasons, respectively.

**Table 2.** Abiotic variables in streams in three protected areas in central Brazil during dry and rainy seasons (2012-2013). PNB = Parque Nacional de Brasília, PNCV = Parque Nacional da Chapada dos Veadeiros, PETeR = Parque Estadual de Terra Ronca. (\* = missing values).

Variables	PNB				PNCV				PETeR									
	B1		B2		C1		C2		T1		T2		T3		T4		T5	
	dry	rainy																
Depth (m)	0.51	0.54	0.5	0.64	0.51	0.95	0.52	0.78	>2	>2	0.59	0.57	0.48	0.57	0.72	0.72	0.52	0.42
Width (m)	1.02	0.9	1.7	1.94	3.64	4.52	5.46	5.46	7.33	8.33	5.07	5.88	9.47	11.04	10.44	12.0	15.92	16.76
Water velocity (m s <sup>-1</sup> )	0.1	0.0	0.4	0.4	0.1	0.5	0.1	0.5	0.6	0.6	0.6	0.9	0.3	0.5	0.6	1.0	1.1	1.9
Air temperature (°C)	35.1	30.7	26.7	26.4	28.5	26.5	27.2	28.4	27.8	24.0	36.7	25.5	32	27.5	29.8	25.0	28.3	24.8
Water temperature (°C)	20.2	21.5	17.9	21.4	18.0	21.9	25.0	22.1	24.2	23.8	24.4	25.1	24.2	25.7	26.4	26.4	24	24.3
pH	5.57	5.19	6.6	5.19	6.65	6.53	6.54	6.82	4.78	6.13	5.73	5.72	7.25	7.47	7.53	7.82	7.35	7.37
Electrical conductivity (µS cm <sup>-1</sup> )	5.4	13.5	4.2	23.6	13.0	8.6	3.0	4.8	7.5	8.4	4.5	5.5	124.4	236.3	8.1	26.4	13.4	38.3
Dissolved oxygen (mg L <sup>-1</sup> )	*	2.44	*	4.56	*	6.93	*	7.60	6.48	6.32	6.63	6.51	7.26	7.02	6.96	7.82	7.81	7.91
Oxygen (% saturation)	*	27.7	*	51	*	79	*	84	76.9	75.0	76.8	78.6	85.7	86.2	86.7	94.8	91.3	94.8
Total phosphorus (µg L <sup>-1</sup> )	4.0	3.0	9.0	7.0	7.0	6.0	2.0	3.0	<1	<1	<1	2	<1	2	<1	3	<1	4
SRP (P-PO <sub>4</sub> ) (µg L <sup>-1</sup> )	2.0	<1	4.0	4.0	4.0	3.0	<1	1.3	<1	<1	<1	2	<1	2	<1	2.0	<1	4.0
Ammonium (N-NH <sub>4</sub> <sup>+</sup> ) (µg L <sup>-1</sup> )	<1	<1	4.0	<1	2.0	<1	<1	<1	<1	<1	<1	<1	1.4	<1	<1	<1	<1	<1
Nitrate (N-NO <sub>3</sub> ) (µg L <sup>-1</sup> )	6.0	5.0	9.0	8.0	10.0	3.0	2.0	2.0	<1	3.0	*	3.0	*	4.0	*	5.0	*	7.0
Total nitrogen (µg L <sup>-1</sup> )	8.0	8.0	14.0	11.0	12.0	7.0	2.0	2.0	*	2.0	*	6.0	*	7.0	*	6.0	*	9.0
Turbidity (NTU)	0.23	0.88	4.39	4.58	0.67	7.63	0.72	3.16	*	1.02	*	0.93	*	1.42	*	0.73	*	1.14
Total solids (mg L <sup>-1</sup> )	4.0	7.0	16.0	22.0	8.0	13.2	9.0	0.9	0.3	1.7	0.3	2.0	6.2	3.0	0.1	1.0	0.7	1.0
Suspended solids (mg L <sup>-1</sup> )	3.0	5.0	14.0	18.0	3.0	4.0	8.0	1.0	*	2.0	*	1.0	*	1.0	*	1.0	*	1.0
Dissolved solids (mg L <sup>-1</sup> )	1.0	2.1	2.0	2.4	5.0	3.6	1.0	1.0	*	1.0	*	1.0	*	2.0	*	1.0	*	1.0
Silicate (mg L <sup>-1</sup> )	1.066	1.893	1.801	2.181	2.179	1.015	1.123	0.613	1.074	0.478	2.682	0.478	1.141	0.677	1.893	0.603	3.078	0.582

Species richness ranged from five (B2) to 61 (C2) (Tab. 3) and did not show a significant difference between rainy and dry seasons ( $W = 37, p = 0.08, N = 9$ ) (Fig. 3). Considering the total of occurrences, 85 % of the taxa were classified as “rare”, contributing less than 5 % of total abundance in their respective sample (Tab. 3). Some green algal taxa, notably *Mougeotia* and *Spirogyra* species, were classified as “very common”.

The Whittaker Index ( $\beta_w$ ) for beta diversity was 5.3. The NMDS revealed two groups of samples: group 1, formed by sites with low pH and low electrical conductivity (B1, C2, T1, T2); and group 2, with site C1 and the ones with relatively higher pH and electrical conductivity (T3, T4 and T5) (Fig. 4). The ANOSIM showed significant differences between groups 1 and 2 ( $R = 0.70; p < 0.001$ ), but not between rainy and dry seasons ( $p > 0.05$ ). Group 1 comprised sites located in the three protected areas. The cyanobacteria *Scytonema caldarium* and *Hapalosiphon stuhlmannii* were common in this group, especially in the sites with macrophytes as substrates (B1 and C2). Other exclusive taxa were the chlorophyte *Microthamnion kuetzingianum* (B1) and desmid species in the genera *Micrasterias* and *Euastrum*. Group 2 corresponded to sites with higher pH and electrical conductivity in Parque Estadual de Terra Ronca (T3, T4 and T5). The only exception was site C1. Although it had moderately acidic waters, it had some species in common with the other streams. Some cyanobacterial taxa were reported exclusively at T3, such as *Lynghya martensiana* and *Oscillatoria princeps* (Tab. 3).

We highlight below some taxa of special importance, in general cited for the first time for the country or for central

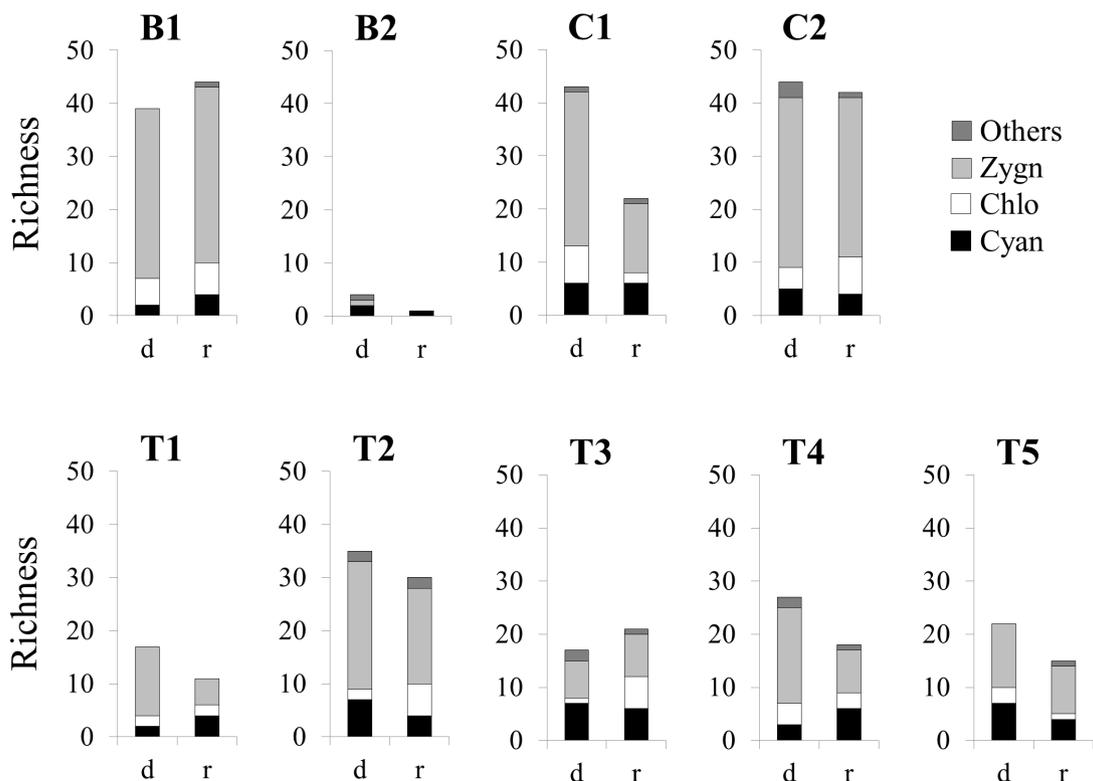
Brazil. This was the case of the cyanobacteria *Rivularia aquatica* and *Tolypothrix distorta* and the chlorophyte *Microthamnion kuetzingianum*.

*Rivularia aquatica* was observed only in São Mateus stream (T2), upstream of its cave, at pH = 5.5 and electrical conductivity = 5.7 µS cm<sup>-1</sup> (mean values), during both dry and rainy seasons. It formed conspicuous olive-green (sometimes darker) gelatinous hemispherical to spherical colonies, encrusted on pebbles, with a smooth slimy surface, up to 10 mm. Trichomes were gradually attenuated towards ends, 8-9 µm at the base, slightly constricted at cross-walls, attenuated into a long hair at the end, blue-green. Heterocytes were observed at the base of trichomes, spherical, solitary, and wider than trichomes.

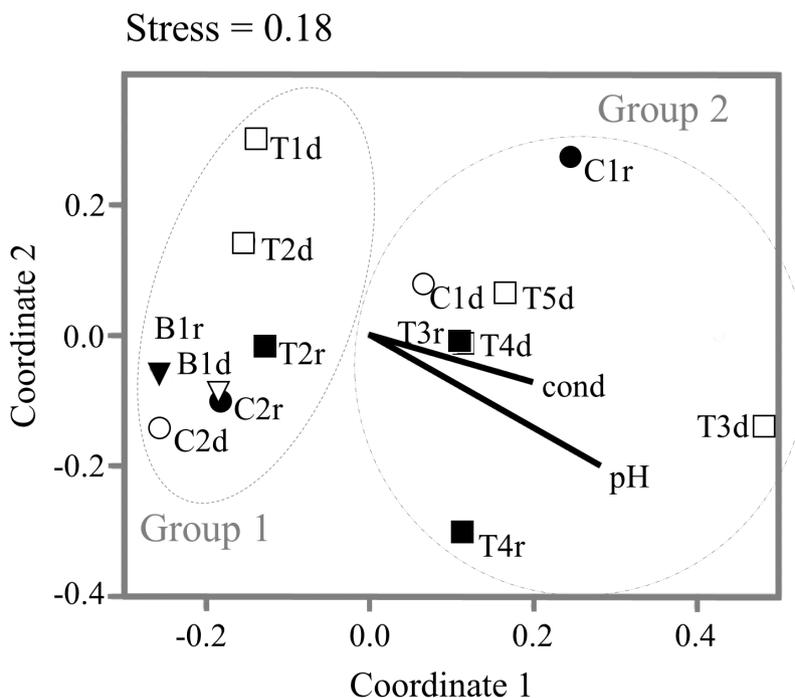
*Tolypothrix distorta* was observed only in Lapa stream (T4 and T5), at pH = 7.6 and electrical conductivity = 21.6 µS cm<sup>-1</sup> (mean values), during both dry and rainy seasons. Trichomes were cylindrical, slightly constricted at cell walls, width 6.9-7.6(8.6) µm, length 3.6-5.7 µm, blue-green or olive-green. Heterocytes were spherical, 8.7-9.4 µm. Filaments were 12-13 µm (-44.5 when fasciculate), colorless or sometimes yellowish-brown.

*Microthamnion kuetzingianum* was observed only in Bananal stream (B1), at pH = 5.4 and electrical conductivity = 9.4 µS cm<sup>-1</sup> (mean values), during both dry and rainy seasons, associated with *Sphagnum* L. banks. Filaments were often densely and irregularly branched, branches spreading or slightly curved; cells 2.6 µm wide, up to 40 µm long, cylindrical, each cell with parietal chloroplast, without pyrenoids.





**Figure 3.** Species richness of soft-bodied benthic algae from streams in three protected areas in central Brazil, during dry (d) and rainy (r) seasons. The letters B, C and T represent, respectively, sites in Parque Nacional de Brasília, Parque Nacional da Chapada dos Veadeiros and Parque Estadual de Terra Ronca. Cyan = Cyanobacteria, Chlo = Chlorophyceae, Zygn = Zygnematophyceae, Others = other algal groups.



**Figure 4.** NMDS plot for soft-bodied benthic algae from streams in three protected areas in central Brazil (Dice Index). Inverted triangles = Parque Nacional de Brasília; circles = Parque Nacional da Chapada dos Veadeiros; squares = Parque Estadual de Terra Ronca. Open and filled symbols represent dry and rainy seasons, respectively.



**Table 3.** Species list of soft-bodied benthic algae from streams, reported in three protected areas in central Brazil, during dry (white circles) and rainy seasons (black circles) (2012-2013). The letters B, C and T represent, respectively, sites in Parque Nacional de Brasília, Parque Nacional da Chapada dos Veadeiros and Parque Estadual de Terra Ronca. The numbers 1, 2, 3 and 4 mean, respectively, “rare”, “common”, “frequent” and “abundant”.

Taxa	Sites								
	B1	B2	C1	C2	T1	T2	T3	T4	T5
CYANOBACTERIA									
Aff. <i>Geitlerinema</i> sp.	①	④	①	①	②	①①	①		②①
<i>Chamaesiphon</i> sp.								②	
<i>Chroococcus</i> sp.				①					
<i>Hapalosiphon stuhlmannii</i> Hieronymus	②②			②②		①②			
<i>Johanseninema constrictum</i> (Szafer) Hasler, Dvorák & Poulícková							①		①
<i>Lyngbya martensiana</i> Meneguini ex Gomont							①		
<i>Lyngbya</i> sp. 1					②		①		
<i>Lyngbya</i> sp. 2							①		
<i>Lyngbya</i> sp. 3					②				
<i>Lyngbya</i> sp. 4					②				
<i>Lyngbya</i> sp. 5									①
<i>Merismopedia glauca</i> (Ehrenberg) Kützing							①		
Nostocaceae sp. 1								①	②
Nostocaceae sp. 2									②
Nostocaceae sp. 3	①		①			①			
Nostocaceae sp. 4				①					
<i>Oscillatoria princeps</i> Vaucher ex Gomont							①②		
Phormidioideae sp. 1							①		
<i>Phormidium</i> sp. 1			①①		②	①	①①	①①	
<i>Phormidium</i> sp. 2			①						②
<i>Phormidium</i> sp. 3								①	②
<i>Pseudanabaena</i> sp. 1						②			
Pseudanabaenaceae sp. 1		③	②②		①		①①	①	
Pseudanabaenaceae sp. 2			①						
<i>Rivularia aquatica</i> De-Wildeman						②①			
<i>Scytonema caldarium</i> Setchell	②①	②		②②		②②			
<i>Scytonema stuposum</i> (Kützing) Bornet ex Bornet et Flahault			①①					②①	①①
<i>Stigonema ocellatum</i> Thuret ex Bornet & Flahault				②②					
<i>Tapinothrix</i> sp.			①③						
<i>Tolypothrix distorta</i> Kützing ex Bornet et Flahault								②	①
CHLOROPHYCEAE									
<i>Acutodesmus</i> sp.								①	
<i>Botryococcus</i> sp.				①					
<i>Bulbochaete</i> sp.	②①		①	①②	①②	①①	①		
<i>Chaetophora</i> sp.			②				①①	①①	②
Chlorophyta sp.	①			①					
<i>Klebsormidium scopulinum</i> (Hazen) Ettl & Gartner	①①		①	①①		①	①	①	
<i>Klebsormidium</i> cf. <i>subtile</i> (Kützing) Mikhailyuk, Glaser, Holzinger & Karsten					①	①			
<i>Microspora</i> sp.	①①		①①	①		①			
<i>Microthamnion kuetzingianum</i> Nägeli in Kützing	①①								
<i>Oedogonium</i> sp. 1					②	①②	①		①②
<i>Oedogonium</i> sp. 2	②①		①	①①		①	①	②②	①
<i>Oedogonium</i> sp. 3			②				①	①	
<i>Oocystis</i> sp.			①	①①					
CHRYSOPHYCEAE									
<i>Dinobryon</i> sp.				①					
<i>Lagynion</i> sp.				①					
CRYPTOPHYCEAE									
<i>Cryptomonas</i> sp.						①			
DINOPHYCEAE									
Dinophyta sp. 1				①					



Table 3. Cont.

Taxa	Sites									
	B1	B2	C1	C2	T1	T2	T3	T4	T5	
EUGLENOPHYCEAE										
<i>Trachelomonas</i> sp. 1								①		
<i>Euglena</i> sp. 1						① ①				①
<i>Euglena</i> sp. 2							①	①		
<i>Euglena</i> sp. 3							①			
<i>Phacus</i> sp.			①							
RHODOPHYTA										
<i>Batrachospermum</i> sp.		②	①				①	②		
XANTHOPHYCEAE										
<i>Heterothrix</i> sp.	①			②		①				
ZYGNEMATOPHYCEAE										
<i>Actinotaenium cucurbita</i> var. <i>cucurbita</i> f. <i>minus</i> (West et West) Teiling ex Croasdale	① ②			① ①	②	① ①	①	①	①	①
<i>Actinotaenium cucurbita</i> var. <i>cucurbita</i> f. <i>latius</i> (West et West) Teiling				① ①						
<i>Actinotaenium cucurbita</i> var. <i>rotundatum</i> (Krieger) Teiling			①	①						②
<i>Actinotaenium cucurbitinum</i> (Bisset) Teiling			①		①	①				
<i>Actinotaenium curtum</i> (Brébisson ex Ralfs) Teiling ex Růžička & Pouzar										①
<i>Actinotaenium elongatum</i> (Raciborski) Teiling	①		①	①						
<i>Actinotaenium globosum</i> (Bulnheim) Kurt Förster ex Compère	① ①		①	① ①				①		
<i>Actinotaenium wollei</i> (West & G.S.West) Teiling			① ①	①	①			①		
<i>Bambusina brebissonii</i> Kützing ex Kützing var. <i>brasiliensis</i> Kurt Förster	①			① ①						
<i>Closterium closterioides</i> (Ralfs) A.Louis & Peeters	①		① ①		①					①
<i>Closterium diana</i> Ehrenberg ex Ralfs			①							
<i>Closterium gracile</i> Brébisson ex Ralfs	① ①					①		①		
<i>Closterium jenneri</i> Ralfs			① ①		①	①		①	①	
<i>Closterium kuetzingii</i> Brébisson					①					
<i>Closterium moniliferum</i> (Bory) Ehrenberg ex Ralfs			①				① ①			①
<i>Closterium navicula</i> (Brébisson) Lütkemüller		②			①	①				
<i>Closterium parvulum</i> Nägeli	①		①							
<i>Closterium pusillum</i> Hantzsch			①			①				①
<i>Closterium regulare</i> Brébisson			①							
<i>Closterium setaceum</i> Ehrenberg ex Ralfs			①							
<i>Closterium cf. striolatum</i> Ehrenberg ex Ralfs					①					
<i>Closterium subulatum</i> (Kützing) Brébisson					①	①				
<i>Cosmarium abbreviatum</i> Raciborski var. <i>minus</i> (West & G.S.West) Willi Krieger & Gerloff	① ①		①	① ①		①		①	①	① ①
<i>Cosmarium decoratum</i> West & G.S.West				① ①						
<i>Cosmarium cf. decussiferum</i> var. <i>mediogranulatum</i> Kurt Förster & Eckert			①							
<i>Cosmarium cf. luetzelburgii</i> Kurt Förster	① ①									
<i>Cosmarium cf. subundulatum</i> Wille							①	①	①	
<i>Cosmarium cf. taxichondrum</i> P.Lundell			①							
<i>Cosmarium contractum</i> O.Kirchner var. <i>minutum</i> (Delponte) Coesel		①	①		①		①			
<i>Cosmarium depressum</i> (Nägeli) Lundell var. <i>depressum</i> f. <i>minutum</i> Heimerl		①	① ①		①	①		①		
<i>Cosmarium dimaziforme</i> (Grönbl.) Scott & Grönbl. var. <i>undulatum</i> Kurt Förster			①							
<i>Cosmarium dimaziforme</i> (Grönbl.) Scott & Grönbl. var. <i>concauum</i> Kurt Förster				①						
<i>Cosmarium granatum</i> Brébisson ex Ralfs							①	①	②	
<i>Cosmarium intermedium</i> Delponte	① ①									
<i>Cosmarium laeve</i> Rabenhorst var. <i>acervatum</i> Kurt Förster				① ①		①				
<i>Cosmarium lundelli</i> var. <i>borgei</i>								①	①	
<i>Cosmarium moniliforme</i> f. <i>elongatum</i> West & G.S.West						①				
<i>Cosmarium pseudopyramidatum</i> P.Lundell	① ①					①		①	①	
<i>Cosmarium punctulatum</i> Brébisson				① ①		①				
<i>Cosmarium quadratum</i> P.Lundell var. <i>minus</i> Nordstedt							①	①	①	
<i>Cosmarium quinarium</i> P.Lundell var. <i>brasiliense</i> Kurt Förster	①									
<i>Cosmarium regnesi</i> Reinsch			①	①						①
<i>Cosmarium</i> sp. 1				①						
<i>Cosmarium</i> sp. 2			②							



Table 3. Cont.

Taxa	Sites								
	B1	B2	C1	C2	T1	T2	T3	T4	T5
<i>Cosmarium</i> sp. 3				1					
<i>Cosmarium subspeciosum</i> var. <i>subspeciosum</i> Nordstedt							①	①	1
<i>Cosmarium subtumidum</i> Nordstedt	① 1		1		1	①			
<i>Cosmarium trilobulatum</i> Reinsch var. <i>abscissum</i> (Schmidle) Willi Krieger & Gerloff	① 1					① 1			
<i>Cosmarium vitiosum</i> Scott & Grönblad	① 1								
<i>Cylindrocystis brebissonii</i> (Ralfs) De Bary	① 1				①	0			
<i>Cylindrocystis obesa</i> West & G.S.West			1						
<i>Desmidium graciliceps</i> (Nordstedt) Lagerheim				①					
<i>Desmidium grevillei</i> (Kützing ex Ralfs) De Bary						2			
<i>Euastrum arciferum</i> Borge	① 1								
<i>Euastrum brasiliense</i> Borge	1			1					
<i>Euastrum</i> cf. <i>angolense</i> (West & G.S.West) Willi Krieger var. <i>brasiliense</i> Willi Krieger	① 1			①					
<i>Euastrum</i> cf. <i>bilobum</i> Lütkemüller	① 1								
<i>Euastrum</i> cf. <i>cornubiense</i> West & G.S.West					1				
<i>Euastrum crassangulatum</i> Børgesen				①					
<i>Euastrum croasdaleae</i> Gronblad var. <i>incrassatum</i> Kurt Förster	① 1			① 1		①			
<i>Euastrum evolutum</i> var. <i>trilobum</i> (W.R.Taylor) W.Krieger	①			1					
<i>Euastrum humbertii</i> P.Bourelly var. <i>brasiliense</i> W.Krieger	① 1			1					
<i>Euastrum inusitatum</i> Kurt Förster	①					①			
<i>Euastrum pirassunungae</i> Borge				①					
<i>Euastrum rectangulare</i> Fritsch & M.F.Rich				1					
<i>Euastrum sublobatum</i> Brébisson ex Ralfs				① 1					
<i>Euastrum subtrilobulatum</i> var. <i>gibbosum</i> Kurt Förster & F.Eckert	1			①					
<i>Euastrum</i> sp.1	① 1		①			①			
<i>Euastrum</i> sp. 2						1	1	1	
<i>Gonatozygon pilosum</i> Wolle	① 2			1					
<i>Haplotaenium minutum</i> (Ralfs) Bando	① 1		①	① 1	1			1	
<i>Hyalotheca dissiliens</i> Brébisson ex Ralfs			①	①					
<i>Mesotaenium</i> sp.				① 1		① 1	1		
<i>Micrasterias arcuata</i> Bailey var. <i>subpinatifida</i> West & G.S.West	① 1			①		1			
<i>Micrasterias arcuata</i> Bailey var. <i>robusta</i> Borge				1					
<i>Micrasterias depauperata</i> Nordstedt var. <i>kitchelii</i> (Wolle) West & West	①					①			
<i>Micrasterias rotata</i> Ralfs			①						
<i>Micrasterias truncata</i> Nordstedt var. <i>excavata</i> Nordstedt	①								
<i>Mougeotia</i> sp. 1			2 1	① 1		① 2	3	2	2 2
<i>Mougeotia</i> sp. 2			①		2	2			
<i>Mougeotia</i> sp. 3	2 1		①	① 1	3	2 1	1	2 1	①
<i>Netrium digitus</i> (Brébisson ex Ralfs) Itzigsohn & Rothe	① 1		①						
<i>Pleurotaenium ehrenbergii</i> (Ralfs) Delponte			①						
<i>Spirogyra</i> sp. 1							4 1	2	
<i>Spirogyra</i> sp. 2						①		2	
<i>Spirogyra</i> sp. 3	① 1								
<i>Spirotaenia</i> cf. <i>condensata</i> Brébisson			①						
<i>Spondyliosium desmidiforme</i> (Borge) West & West					①				
<i>Staurastrum hystrix</i> Ralfs var. <i>floridense</i> Scott & Gronblad	① 1			① 1					
<i>Staurastrum</i> cf. <i>margaritaceum</i> Meneghini ex Ralfs								①	①
<i>Staurastrum orbiculare</i> Meneghini ex Ralfs	① 1			① 1		1			
<i>Staurastrum</i> cf. <i>proboscideum</i> Archer	① 1								
<i>Staurastrum quadrangulare</i> Brébisson				①		①			
<i>Staurastrum</i> sp. 1			①						
<i>Staurastrum</i> sp. 2								1	
<i>Stauroidesmus convergens</i> (Ehrenberg ex Ralfs) Teiling var. <i>laportei</i> Teiling							①		
<i>Stauroidesmus incus</i> (Hassal ex Ralfs) Teiling				①					
<i>Stauroidesmus dickiei</i> (Ralfs) S.Lillieroth	1		①	①					
<i>Stauroidesmus spencerianus</i> (Nordstedt) Teiling						① 1			



Table 3. Cont.

Taxa	Sites								
	B1	B2	C1	C2	T1	T2	T3	T4	T5
<i>Tetmemorus brebissonii</i> Ralfs	①			① ①		① ①			
<i>Tetmemorus laevis</i> Ralfs ex Ralfs	① ①			①		①			
<i>Xanthidium regulare</i> Nordstedt			①	①					
<i>Xanthidium</i> sp.	①								
<i>Zygnema</i> sp. 1	①					②	②	①	
<i>Zygnema</i> sp. 2						①			

## Discussion

This study provides new information about the limnological and algal properties of pristine streams in central Brazil. All streams have in common very low nutrient concentrations, in accordance with the literature about undisturbed Cerrado basins (e.g. Markewitz *et al.* 2006; Fonseca & Mendonça-Galvão 2014), despite the differences in their pH and electrical conductivity (EC). The relatively higher EC observed here for streams connected to caves (PETeR) indicates the presence of other dissolved ions in the water besides nutrients such as phosphorus and nitrogen, which were around the detection limits. Compared with pristine karst systems around the world, where EC is generally above 400  $\mu\text{S cm}^{-1}$  (e.g. Righi-Cavallaro *et al.* 2010; Vilenica *et al.* 2017), it is noticeable, however, that even the EC values in streams connected to caves were not very high. The headwaters of PETeR's streams are located in the hills of Serra Geral de Goiás. They flow around 20 km westwards through sandstone until they reach the limestone outcrop (Faquim *et al.* 2017). Such proximity may buffer the effects of rock dissolution on the water chemical variables. Sites T1 and T2, for instance, showed slightly acidic pH, with very low EC ( $<10 \mu\text{S cm}^{-1}$ ), similar to sites located in the other protected areas (PNB and PNCV).

The Cerrado region experiences strong seasonality (dry versus rainy season), and rainfall is a relevant water input to local lotic systems, especially low order ones (Markewitz *et al.* 2006). In the studied streams, seasonality significantly affected depth and water velocity, which are important physical variables influencing benthic communities, since physical disturbance promoted by higher flow velocity during the high rainfall phases may inhibit growth and maintenance for most taxa (Righi-Cavallaro *et al.* 2010). A similar pattern was reported for macroalgal communities in southeastern Brazil (Branco & Necchi Jr. 1997). In the present study, algal richness was not affected by seasonality; however, it was more difficult to reach the 400 individuals in most samples collected during the rainy season, suggesting that algal production is hindered during rainy periods.

Higher richness was reported in particular when macrophytes were present (e.g. B1, C1, C2 and T2). Aquatic plants, especially submerged ones with complex architecture (e.g. *Sphagnum*, *Utricularia*), contribute to habitat heterogeneity, which is an important factor for the

maintenance of periphyton richness (Algarde *et al.* 2017). In this context, site C2, the richest one, had the “best of all worlds”; it presented abundant macrophyte cover on the rocky bed, in an unshaded area of “crystal clear” waters. Since light availability is also recognized as a determinant abiotic factor for algal communities (Tonetto *et al.* 2015), algal development at site C2 was positively influenced by the combination of light and macrophyte cover in abundance.

The similarity among sites was relatively low, as indicated by the Whittaker Index of beta diversity ( $\beta_w = 5.33$ ). According to McCune & Grace (2002, p. 31), “values of  $\beta_w < 1$  are rather low and  $\beta_w > 5$  can be considered high”. Branco *et al.* (2008b) and Tonetto *et al.* (2015) also described low similarity in macroalgal communities from southeastern/southern Brazil, with most taxa restricted to a single sampling site. In a dataset composed of almost 500 temperate streams (New York State and Austria), Rott & Wehr (2016) also reported few widely distributed taxa, without any ubiquitous ones.

The species composition among SB algae ranged from typical acidophilic taxa to taxa associated with alkaline waters. The most common group in terms of species richness, Zygnematophyceae, is known for its ecological preference for oligo-mesotrophic waters, with slightly low pH (Coesel & Meesters 2007). For this reason, these algae are frequently observed in Cerrado waters (Estrela *et al.* 2011; Dunck *et al.* 2013). Fonseca *et al.* (2018) reported this group as the richest one among non-diatom periphytic algae in Cerrado wetlands. Some European floras have indicated many acidophilic taxa among Zygnematophyceae, and some of them were reported in our streams, highlighting here *Cylindrocystis brebissonii*, *Closterium closterioides*, *Tetmemorus laevis*, *Micrasterias* spp., *Euastrum* spp. (Štastný 2009). The presence of *Sphagnum* banks (e.g. site B1) as substrate, and the typical acidic environment created by this plant, certainly favored these occurrences (Coesel & Meesters 2007).

Cyanobacteria, the second group in terms of species richness, have also been reported as an important component in Cerrado acidic wetlands (Fonseca *et al.* 2018), represented mainly by branched heterocytous taxa (e.g. *Scytonema*, *Stigonema*, *Hapalosiphon*), which were commonly reported here as well, under similar ecological conditions. In the sites with relatively higher pH and electrical conductivity, however, there were some exclusive taxa belonging to



the orders Oscillatoriales (*Oscillatoria princeps*, *Lyngbya martesiana*) or Nostocales (*Tolypothrix distorta*) not usually reported in Cerrado waters.

### Some rare or interesting species

We reported the cyanobacterium *Rivularia aquatica* for the first time in Brazil (Menezes *et al.* 2015). The identification is according to Komárek (2013, p. 308), which describes this species as pantropical. Up to now, there were only two species cited in Brazil (State of São Paulo, southeastern region), *R. atra* Roth and *R. cf. beccariana* (De Notaris) Bornet et Flahault (Sant'Anna *et al.* 2011). The former, however, is a marine species, while the latter presents thinner trichomes when compared to *R. aquatica*.

*Tolypothrix distorta* (Cyanobacteria) had already been reported in Brazil only for the southeastern/southern regions (Branco *et al.* 2001; 2009; Krupek *et al.* 2013; Menezes *et al.* 2015), and was here cited for the first time in central Brazil. In Europe, Rott & Schneider (2014) reported the species at pH ranging from 6.91 (Norway) to 8.02 (Austria). According to Komárek (2013, p. 439), however, it is “usually in strictly alkaline, stagnant or slowly streaming waters”. This explains why this taxon has not been mentioned in previous studies on Cyanobacteria from Cerrado acidic environments, where genera such as *Stigonema*, *Hapalosiphon* and *Scytonema* are more common (Senna & Ferreira 1987; Fonseca *et al.* 2018). The environmental conditions found in PETeR, on the other hand, favor the occurrence of such alkaliphilic taxa. Branco *et al.* (2009) reported it at pH = 7.21-7.45, and electrical conductivity = 63.8-73.4  $\mu\text{S cm}^{-1}$ .

The genus *Microthamnion* is often reported from unpolluted, neutral to acidic fresh waters (Bicudo & Menezes 2006; Guiry 2017), sometimes with wet carpets of moss (John 2003). This agrees with conditions in Bananal stream (B1) in the present study, where it was recorded in both dry and rainy seasons. In Brazil, the only reference for the genus is Bicudo & Menezes (2006), in which the occurrence of *M. kuetzingianum* in the State of São Paulo is mentioned.

In summary, soft-bodied algal community structure varied significantly among sites, but not between dry and rainy seasons. There were somewhat lower abundances during the rainy season, likely due to faster currents during this period. The relatively higher pH and conductivity values in the sites connected to caves in Parque Estadual de Terra Ronca seemed to be an important factor driving differences in species composition among the studied sites. Some alkaliphilic taxa were reported only at sites near the caves, highlighting the cyanobacterium *Tolypothrix distorta*, while acidophilic taxa (e.g. Cyanobacteria *Scytonema caldarium*, *Stigonema ocellatum*, *Hapalosiphon stuhlmannii*; Zygnematophyceae *Cylindrocystis brebissonii*, *Closterium closterioides*, *Tetmemorus laevis*, *Micrasterias* spp., *Euastrum* spp.) were more frequent at the other sites, regardless of the protected area, especially when macrophytes were present.

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