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ORIGINAL RESEARCH

Biodiversity in Pristine Wetlands of Central Brazil: a Multi-Taxonomic Approach

Bárbara Medeiros Fonseca[1] · Luciana de Mendonça-Galvão[1] · Francisco Diogo Rocha Sousa[1] · Lourdes Maria Abdu Elmoor-Loureiro[1] · Maria Beatriz Gomes-e-Souza[2] · Ricardo Lourenço Pinto[3] · Paula Petracco[4] · Regina Célia de Oliveira[3] · Elizângela de Jesus Lima[5]

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Abstract This study main goal was to characterize biodiversity in tropical pristine wetlands of two protected areas from central Brazil under a multi-taxonomic approach, with special interest on the distribution and identity of those taxa that most represent these reference conditions. Samplings were conducted during dry and rainy seasons (2009), in nine wetlands. We reported 114 herbaceous macrophytes, 334 algae/cyanobacteria, 45 microcrustaceans and 139 testate amoebae. Macrophytes presented the highest beta diversity compared to the other groups ($\beta w = 3.2$), while microcrustaceans showed the lowest one ($\beta w = 1.4$). The microorganism diversity associated with the dominating emergent macrophytes (e.g., Poaceae and Cyperaceae) was mainly composed of zygnematophycean algae, branched heterocytous cyanobacteria, along with Chydoridae (Cladocera) and Cyclopoida (Copepoda) microcrustaceans and Lesquereusiidae testate amoebae. Many tropical rare/endemic taxa were reported, e.g., among genera *Parallela* (green algae), *Placocista* and *Quadrulella* (testate amoebae), *Celsinotum, Ephemeroporus, Metacyclops* and *Paracyclops* (microcrustaceans); also, the genus *Paralimnetis* (Laevicaudata) is cited for the first time in Brazil. These findings reinforce these small wetlands as potential biodiversity hotspots and stepping stones for dispersing organisms. Finally, the presence of temporary environments among Cerrado wetlands may contribute as an additional beta diversity driver that deserves more attention in future studies.

Keywords Beta diversity · Cerrado · Ponds · Species richness · Wet grasslands

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✉ Bárbara Medeiros Fonseca
barbara.fonseca0603@gmail.com

1 Grupo de Estudos de Ecossistemas Aquáticos, Laboratório de Biodiversidade Aquática, Catholic University of Brasília, Sala M-204, QS 07, Lote 01, EPCT, Taguatinga, DF 71966-700, Brazil

2 Keratella Estudos e Projetos Ambientais Ltda, Belo Horizonte, MG, Brazil

3 University of Brasília, Brasília, DF, Brazil

4 Federal Institute of Brasília, Brasília, DF, Brazil

5 Serviço Social do Comércio, Brasília, DF, Brazil

Introduction

Wetlands as ecotones are unique environments with remarkable potential for biodiversity (Junk et al. 2006a). Their importance concerning ecological functions has been recognized worldwide, simultaneously with alerts towards actions in order to improve their best management and protection (Junk 2013; Richardson et al. 2015).

Wetland characterizations or classifications in tropical regions have been carried out in East Africa (Sakané et al. 2011), Central America (Pérez et al. 2011) and South America (Ricaurte et al. 2012). Recently Junk et al. (2014) organized the available information about Brazilian wetlands. In general, larger wetlands such as the Amazon river floodplain (Junk et al. 2011, 2012, 2015) and the Pantanal (Junk et al. 2006b) have received long-term and intensive research. In central Brazil, covered by the Cerrado phytogeographical domain (Brazilian savanna), some studies have been concentrated on the Araguaia river floodplain (e.g., Irion et al. 2016).

Assuming the broad definition of wetlands adopted by the Ramsar Convention (Ramsar 2016), Cerrado domain presents a wide variety of natural sites, highlighting here the several wet grasslands and ponds. In the recent classification system for Brazilian wetlands proposed by Junk et al. (2014), Cerrado wetlands are only generally mentioned as *veredas*, which are defined as "inland waters with relatively stable water level, with multi-species herbaceous vegetation". Hydrology and vegetation cover were used as the main criteria to differentiate Brazilian wetlands. These same authors, however, recognized local specificities that might be hidden among their general classification.

Wet grasslands are found when soils are hydromorphic, in poorly drained bottomlands or depressed regions with slow and ill-defined drainage, where the water table is near the surface for most or all of the year (Cianciaruso and Batalha 2008). These herbaceous ecosystems are frequently established around *veredas*, a phytophysiognomy characterized by the presence of the palm *Mauritia flexuosa* Mart. (Ribeiro and Walter 2008). They can also occur as a belt between the Cerrado's savanna vegetation and gallery forest. Cerrado wet grasslands have been also referred to in the literature as wet campo, wet campo marshes (Reid 1984, 1987) or swamp grasslands (Padovesi-Fonseca et al. 2015). When small hills are present all over the ground, they are known as hummock grasslands (Walter et al. 2008) or mound fields (Silva et al. 2010) (Portuguese *campo de murundus*). Sometimes a pond can be present, surrounded by wet grasslands or by Cerrado's savanna vegetation, in general with permanent hydroperiod, although they can also eventually dry out during years of severe drought.

Habitat fragmentation and land conversion for agriculture have been constant threats to Cerrado conservation (Carvalho et al. 2009; de Marco Jr et al. 2014). Pristine environments have been generally restricted to protected areas such as conservation units defined by law. The literature with limnological data about pristine aquatic ecosystems that could be used as a reference in restoration efforts in this domain has covered, in general, abiotic variables in lotic systems (Wantzen 2003; Fonseca et al. 2014; Fonseca and Mendonça-Galvão 2014). Moreover, studies that address wetland biodiversity considering simultaneously different biological groups are quite scarce, especially those microscopic organisms whose identification to species level is very time consuming (Junk et al. 2006a).

de Marco Jr et al. (2014) had already discussed patterns in the organization of Cerrado aquatic biodiversity (alpha, beta and gamma), but focused on ponds with different sizes and trophic states located in agriculture dominated landscapes. Our study, on the other hand, assesses aquatic biodiversity in pristine wetlands including both ponds and wet grasslands, in order to incorporate the natural landscape heterogeneity generally reported for Cerrado domain (Ribeiro and Walter 2008), with special interest on the distribution and identity of those taxa that better describe these reference conditions.

The general goal of the present study was to characterize biodiversity in pristine small wetlands in two protected areas from central Brazil under a multi-taxonomic approach. Our specific objectives were: 1) to describe abiotic variables of water; 2) to compare biodiversity (alfa, beta and gamma) for different taxonomic groups (macrophytes, algae/cyanobacteria, microcrustaceans and testate amoebae), 3) to highlight the classes or families that contribute most to species richness, with special emphasis on rare and/or endemic taxa.

Methods

Study Area

This study was carried out in two protected areas of central Brazil, the Brasília National Park (*Parque Nacional de Brasília*) and the Formosa Instructional Camp (*Campo de Instrução de Formosa*) (Fig. 1). The distance between them is around 60 km, and they are located in two different great Brazilian hydrographic basins (Paraná and São Francisco, respectively).

The Cerrado domain (Brazilian savanna) predominates in the region. Climate is Aw (rainy tropical, according to Köppen classification), marked by strong seasonality, with a rainy season from October to April concentrating up to 80% of annual precipitation (mean temperature around 29 °C), and a dry season from May to September when precipitation ranges from zero to below 50 mm (mean temperature around 18 °C). Annual mean precipitation is around 1500 mm, ranging from 750 mm to 2000 mm (Silva et al. 2008). Soils in the Cerrado domain are mostly *Latossolos* (46%), according to the Brazilian Soil Classification, which corresponds to Oxisols in the US soil classification. Near streams *Gleissolos* (there is no analogous order in US Soil Taxonomy, although the Aquox suborder would be similar) are also common (Silva et al. 2011). These are reduced hydromorphic soils generally occupying landscape depressions frequently flooded (Reatto and Martins 2005).

The wetlands focused in this study were initially identified using Landsat satellite images, and varied from typical wet grasslands where the water flows from the soil, without a delimited water body, to areas where a pond was present (Table 1). In order to facilitate comparisons, from now on they will be referred only as wet grasslands and ponds, respectively. They are relatively small systems, most of them with less than 30 ha. Precipitation and groundwater are the main water inflows.

The Brasília National Park (hereafter BNP) is a conservation unit (42,355 ha, 930 m.a.s.l.) located in the Federal District of Brazil, surrounded by intense urban occupation. Five wetlands were sampled in this park: the ponds Henrique (H) and Exército (E), and the wet grasslands Peito de Moça (PM), Meandros (M) and Murundus (MU) (Table 1).

The Formosa Instructional Camp (hereafter FIC) is a preserved area (~88,212 ha; 1100 m.a.s.l.) belonging to the Brazilian Army, located between the Federal District of

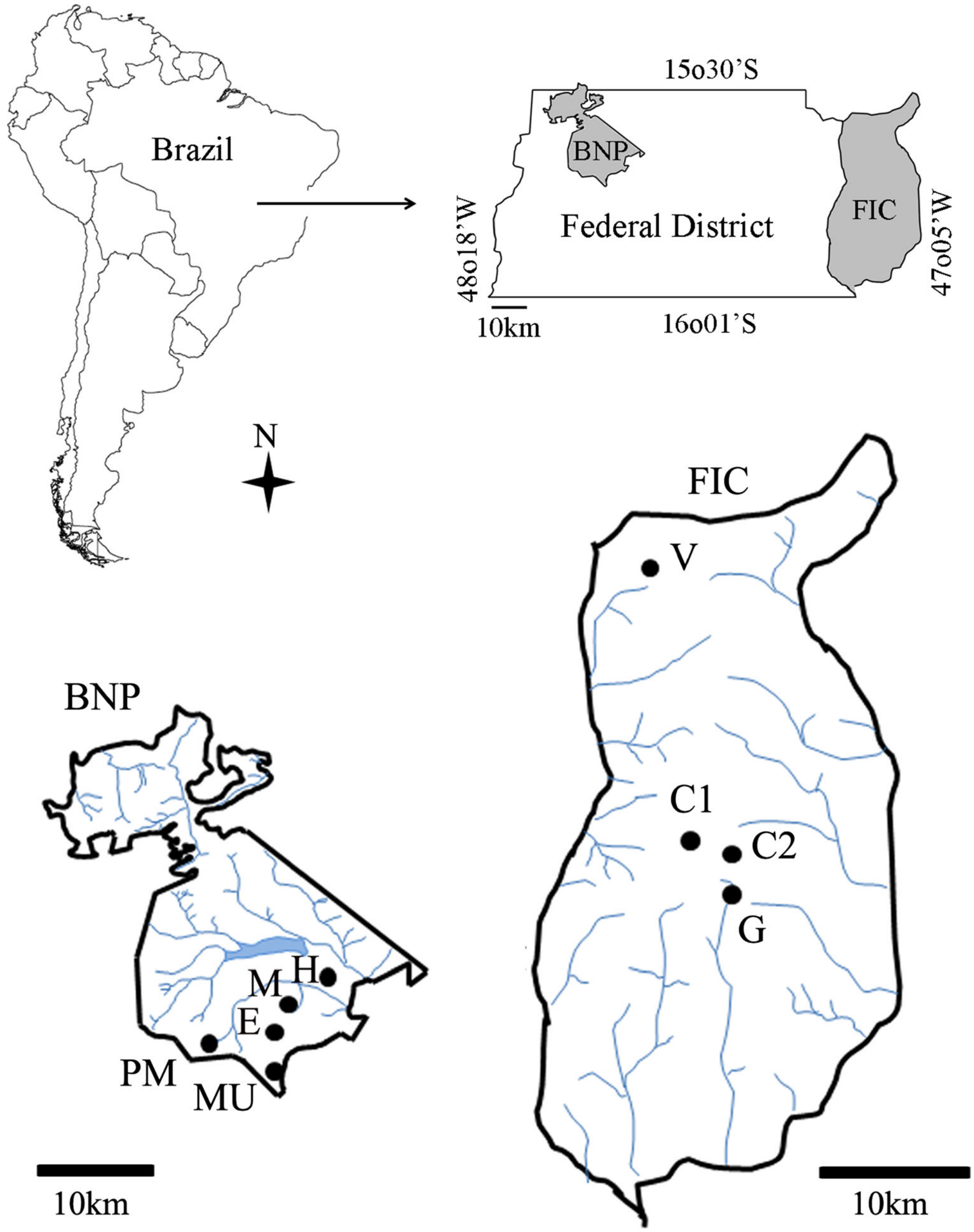

Fig. 1 Localization of the studied wetlands (BNP = Brasília National Park; FIC = Formosa Instructional Camp; PM = Peito de Moça; MU = Murundus; H = Henrique Pond; M = Meandros; E = Exército Pond; V = Veado Pond; C1 = Cabocla 1 Pond; C2 = Cabocla 2 Pond; G = Grande Pond)

Brazil and the State of Goiás. It is surrounded by agricultural activities, mainly corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). Four wetlands were sampled in this area: Cabocla 1 Pond (C1), Cabocla 2 Pond (C2), Grande Pond (G) and Veado Pond (V) (Table 1). Pictures from all sites are provided in Online Resource (fig. S1 and S2).

Sampling

Samplings were conducted in 2009, during the dry (July–August) and rainy (December) seasons. Air temperature and precipitation during the study period were obtained from the Meteorological Institute (INMET) (Formosa and Brasília stations) (Online Resource, fig. S3). Except by Veado Pond, which had no water during the dry season, all the other wetlands could be sampled in both periods.

The number of sampling points varied from one to five in each wetland (altogether 60, 31 in the BNP and 29 in the FIC) (Table 1). At the ponds, each sampling point was located on a depth gradient over transects about 50 m long marked with a wooden stake and georeferenced (UTM, WGS 84) using a standardized handheld global positioning system (Garmin eTrex Vista HCx). This systematic distribution of sampling points was adopted assuming that differences in water level could influence both macrophytes and their associated microbiological community (e.g., Munhoz and Felfili 2008; Eugênio et al. 2011; Sousa et al. 2013). At the wet grasslands, sampling points were located randomly. Sampling procedures

Table 1 Geographic coordinates, area, mean water level relative to the soil surface and number of samples collected in the studied wetlands in 2009 (BNP = Brasília National Park; FIC = Formosa Instructional Camp; * = the two numbers are the mean water level during the dry and rainy seasons, respectively; ** = in parenthesis, the number of samples during the rainy season, when it was different from the dry season)

	Wetland	Code	Description	Proximate area (ha)	Geographic coordinates	Water level* (cm)	Number of samples**
BNP	Henrique	H	Pond (diameter ~200 m) surrounded by Cerrado sensu stricto vegetation	2.9	15°41′18.00″S 47°56′26.10″W	59–73	5
	Exército	E	Pond (diameter ~15 m) surrounded by wet grassland	0.4	15°44′44.30″S 47°58′49.10″W	20–34	5
	Peito de Moça	PM	Wet grassland with a water table outcropping on a hill ~1.5 m high	0.1	15°45′05.08″S 48°01′33.20″W	5–5	3
	Meandros	M	Wet grassland (hummock grassland)	15.9	15°43′29.80″S 47°58′08.90″W	7–18	1(2)
	Murundus	UM	Wet grassland (hummock grassland)	29.2	15°46′48.10″S 47°58′42.20″W	5–5	1
FIC	Cabocla 1	C1	Pond (length ~1000 m) surrounded by hummock grassland	113.7	15°48′15.00″S 47°14′57.50″W	33–47	4
	Cabocla 2	C2	Pond (diameter ~200 m) surrounded by hummock grassland	18.9	15°48′21.00″S 47°14′09.20″W	51–66	5
	Grande	G	Pond (diameter ~2000 m) surrounded by hummock grassland	326.7	15°49′35.70″S 47°13′49.40″W	61–83	4
	Veado	V	Pond (diameter ~50 m); surrounded by wet grassland there was no water in the dry season	0.9	15°36′19.40″S 47°16′32.70″W	0–32	0(3)

around each stake respected a sequence in order that water and sediment revolving could not impair the subsequent samples. In this sense, water for abiotic variables was always the first to be collected, sometimes with the use of a syringe to avoid artificial turbidity (Online Resource, fig. S4). Water level in the sampling points varied from 0.01 to 1.20 m.

Water temperature, dissolved oxygen, oxygen percentage saturation, pH and electrical conductivity were measured in the field using standard electrodes (Yellow Spring Instruments). Water samples were collected in previously acid-washed polypropylene bottles and kept in a cooler until returned to the laboratory. Dissolved nutrients were determined in water filtered through combusted fiberglass filters GF-5 (0.7 μm pore size). Ammonium (Nessler Method), nitrate (chromotropic acid and phenoldissulfonic acid methods), nitrite (colorimetric method) and soluble reactive phosphorus (Stannous Chlorid Method) were analyzed after water filtration. Unfiltered samples were used for total nitrogen and total phosphorus determinations (Valderrama 1981). Nutrients along with turbidity and total solids were analyzed according to Standard Methods (APHA 2005).

Biotic Variables

Macrophytes (herbaceous) were collected over 10 m transects perpendicularly oriented to the left of the main transect, at each sampling point. **Algae** and **cyanobacteria** were collected from metaphyton and periphyton by macrophyte squeezing (every plant present) in a 20 cm ratio around each sampling point; samples were preserved in 4% formaldehyde. **Microcrustaceans** were collected by dragging an 80 μm plankton net among aquatic vegetation, exploring the macrophyte physical structure; samples were preserved in 75% alcohol. **Testate amoebae** were collected after filtering 5 L of water in 20 μm plankton net and preserved in 75% alcohol. These groups were all sampled simultaneously in each sampling point, first the ones demanding more delicate procedures, such as testate amoebae and algae, and then macrophytes and microcrustaceans.

Macrophyte specimens and algae/cyanobacteria samples were included in the Brasília University Herbarium (UB). The other samples are deposited in the Laboratory of Aquatic Biodiversity, at the Catholic University of Brasília. Identifications were based on specialized literature and comparisons in herbarium.

Data Analysis

For abiotic data, medians (me) were used as measures of central tendency for all samples ($n = 60$). Multivariate descriptive analysis was carried out by applying Principal Component Analysis (PCA) to the abiotic data using a covariance matrix with data transformed by ranging $[(x\text{-}min_x)/ (max_x\text{-}min_x)]$. The following 10 variables were included in the PCA: water temperature, pH, electrical conductivity, dissolved oxygen, total phosphorus, soluble reactive phosphorus, ammonium, nitrate, total nitrogen, and TN:TP molar ratio.

For biotic data, a presence-absence matrix was developed with the species for each wetland, after considering all local sampling points in a unique list ($n = 9$). The overall species richness of the studied wetlands was considered as gamma diversity. The first-order jackknife estimator for species richness was also used. Rarefaction curves based on the number of wetlands were used to compare species richness (alfa diversity), considered separately for each taxonomic group. Beta diversity was estimated dividing the overall diversity (gamma) by the average alpha diversity ($\beta w = (\gamma/\alpha)$ -1) (McCune and Grace 2002). The one in the equation is subtracted to make zero beta diversity correspond to zero variation in species presence. Cluster analysis was obtained using the Simpson pairwise dissimilarity, which is independent of differences in local richness (Lopes et al. 2014). The free software PAST ver. 2.17c was used for all analyses (Hammer et al. 2001).

Results

Abiotic Data

In general, the wetlands were all characterized by slightly acidic waters, with electrical conductivity below 10 μS cm^{-1} and relatively low nutrient concentrations (TP = 13 μg L-1; TN = 138 μg L-1) (Table 2). Data for each wetland is available in Online Resource (Tables S5 and S6).

The PCA explained 46% of data variability in the first two ordination axes (axis 1: 26%; axis 2: 20%) (Fig. 2). The most important variables influencing sampling units' distribution on the graph were TN ($r = 0.91$) and ammonium ($r = 0.75$) for axis 1, and dissolved oxygen ($r = 0.66$), TP ($r = -0.64$) and TN:TP ($r = 0.61$) for axis 2. A few samples from both protected areas with relatively higher nitrogen concentrations drove this ordination, positioned to the positive side of axis one, while most of the samples were concentrated to the negative side. Concerning axis 2, samples from BNP were generally associated to low TP and higher TN:TP molar ratio and dissolved oxygen.

Table 2 Median (me), minimum (min) and maximum (max) values of limnological variables in the studied wetlands ($n = 60$) during dry and rainy seasons in 2009

Variables	me	min	max
Water temperature (oC)	**25.9**	17.0	31.4
pH	**5.6**	3.9	7.0
Dissolved oxygen (mg L-1)	**4.4**	2.7	7.0
Oxygen saturation (%)	**58.2**	31.9	88.3
Electrical conductivity (μS cm-1)	**4.9**	2.5	39.3
Total phosphorus (μg L-1)	**13.0**	<10.0	120.0
Soluble reactive phosphorus (μg L-1)	**<10.0**	<10.0	34.0
Ammonium (μg L-1)	**45.0**	10.0	185.0
Nitrite (μg L-1)	**<20.0**	<20.0	32.0
Nitrate (μg L-1)	**45.5**	14.3	308.7
Total nitrogen (μg L-1)	**137.9**	33.4	412.2
TN/TP molar ratio	**22.6**	2.0	713.2
Turbidity (UT)	**1.4**	0.3	57.3
Total solids (mg L-1)	**3.9**	0.2	45.0
Suspended solids (mg L-1)	**1.5**	0.1	33.9
Dissolved solids (mg L-1)	**1.5**	0.1	36.0

Species Richness and Composition

This study reported 632 taxa, considering all taxonomic groups (Table 3; Online Resource, Tables S7-S10). However, according to Jackknife-1 estimator, 236 taxa could still be found, especially among algae and cyanobacteria. Rarefaction curves for microcrustaceans showed a slight trend to stabilization, differently from the other groups, whose curves were further from the asymptote (Fig. 3).

Among **macrophytes** 114 herbaceous taxa belonging to 27 families were reported (Table 3). Emergent macrophyte belonging to the families Poaceae (e.g., *Andropogon virgatus* Desv. ex Ham.), Cyperaceae (e.g., *Rhynchospora globosa* (Kunth) Roem. & Schult., *Cyperus* spp.), Eriocaulaceae (e.g., *Paepalanthus lundii* Körn.), Melastomataceae (e.g., *Acisanthera divaricata* Cogn., *Microlicia* spp.) and Xyridaceae (e.g., *Xyris laxifolia* Mart., *X. jupicai* Rich.) were the most representative in species richness (Fig. 4).

Some species reported by this study have not yet been cited for the region (Federal District or the State of Goiás). This was the case among the families Poaceae (*Paspalum morichalense* Davidse, Zuloaga & Filg., *P. denticulatum* Trin., *Luziola fragilis* Swallen, *Ichnanthus panicoides* P. Beauv., *Andropogon durifolius* Renvoize), Eriocaulaceae (*Paepalanthus lundii* Körn.), Lycopodiaceae (*Lycopodiella geometra* B. Øllg. & P.G. Windisch) and Polygalaceae (*Polygala molluginifolia* St.-Hil.).

Altogether, 334 taxa of **algae** (except diatoms) **and cyanobacteria** were reported, distributed among nine taxonomical classes. Zygnematophyceae was by far the most representative class, contributing with 64% of the algal richness (Table 3, Fig. 4). Filamentous green algae belonging to genera *Spirogyra* Link, *Zygnema* C.Agardh, *Mougeotia* C.Agardh, and *Temnogametum* West & G.S.West were found in more than 90% of the samples. The same happened with species of branched heterocytous cyanobacteria such as *Scytonema* Agardh ex Bornet et Flahault, *Hapalosiphon* Nägeli in Kützing ex Bornet et Flahault and *Stigonema* Agardh ex Bornet et Flahault.

About **microcrustaceans**, this study focused on the taxa Cladocera and Copepoda. We reported 33 taxa of Cladocera. The family Chydoridae was the most representative (Table 3, Fig. 4). *Alona iheringula* Sars, 1901, *A. ossiani* Sinev, 1998, *Ephemeroporus barroisi* (Richard, 1984) and *Ilyocryptus spinifer* Herrick, 1882 were present

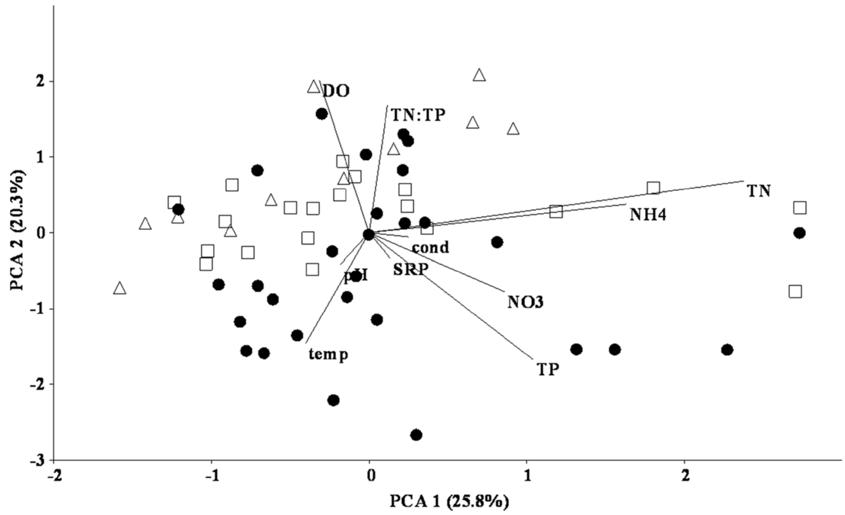

Fig. 2 Biplot of Principal Component Analysis for 10 abiotic variables. White and dark symbols represent, respectively, samples from Brasília National Park (triangles for wet grasslands and squares for ponds) and Formosa Instructional Camp. Legend for abiotic variables: TN = total nitrogen, cond = electrical conductivity, TP = total phosphorus, SRP = soluble reactive phosphorus, temp = water temperature, DO = dissolved oxygen

in 90% of the samples. Considering Copepoda, Cyclopoida was the most representative order among the 11 taxa reported (Table 3, Fig. 4). *Paracyclops chiltoni* (Thomson, 1882) was present in all wetlands, except Veado Pond. In general, besides the low Copepoda density, a common feature among samples was the rare occurrence of adult individuals, especially ovigerous females. It decreased the number of individuals that could be used for taxonomic identifications, since for some species the presence of adults is required. This study also reported a single species belonging to the genus *Paralimnetis* Gurney, 1931 (Laevicaudata). It was represented by a relatively high number of individuals, without any males, which hampered the identification to species level. This taxon was present only at Veado Pond, associated with underwater sediments.

Considering **testate amoebae**, 139 taxa were reported, distributed in 13 families. The families Lesquereusiidae (e.g., *Lesquereusia* Schlumberger, 1845, *Quadrulella* Cockerell, 1909), Difflugiidae (e.g., *Difflugia* Leclerc, 1815) and Arcellidae (*Arcella* Ehrenberg, 1832) presented the highest richness (Table 3, Fig. 4). *Pseudonebela africana* Gauthier-Lièvre, 1953 and *Centropyxis gibba* Deflandre, 1929 were present in all wetlands sampled (except Veado pond).

Beta Diversity

Macrophytes presented the highest beta diversity compared to the other groups, while microcrustaceans showed the lowest one (Table 3). According to cluster analysis, Veado pond (code V) was the most dissimilar among the wetlands (Fig. 5).

Discussion

Considering abiotic variables, our results confirmed the nutrient poorness so common in pristine environments from the Cerrado region and already reported in previous works for wet grasslands (Reid 1984), *veredas* (Ramos et al. 2006) and especially small streams (Wantzen 2003; Fonseca et al. 2014; Fonseca and Mendonça-Galvão 2014). Such geochemical features resemble the clear waters described for the central Amazon, characterized by tertiary sediments originating from Precambrian shields, which are highly lixiviated and geochemically very poor, according to a review in Rodrigues et al. (2000). All the wetlands sampled were generally in the oligotrophic range of the trophic spectrum (total phosphorus medians <30 μg L-1), according to general classification trophic schemes for tropical lakes (e.g., Salas and Martino 1991),

Table 3 Alfa (mean species richness), beta (βw index) and gamma (observed overall species richness and Jackknife-1 estimator with standard deviations) diversity for different taxonomic groups in the studied wetlands during dry and rainy seasons in 2009

	α	β	γ	Jackknife	Difference between estimated and observed (γ) richness
Macrophytes	27	3.2	114	166.4 (13.80)	52
Algae and cyanobacteria	86	2.9	334	456.7 (32.06)	123
Microcrustaceans	19	1.4	45	55.6 (4.24)	11
Testate amoebae	38	2.7	139	194.1 (22.41)	55
Total	165	2.8	632	867.7 (42.35)	236

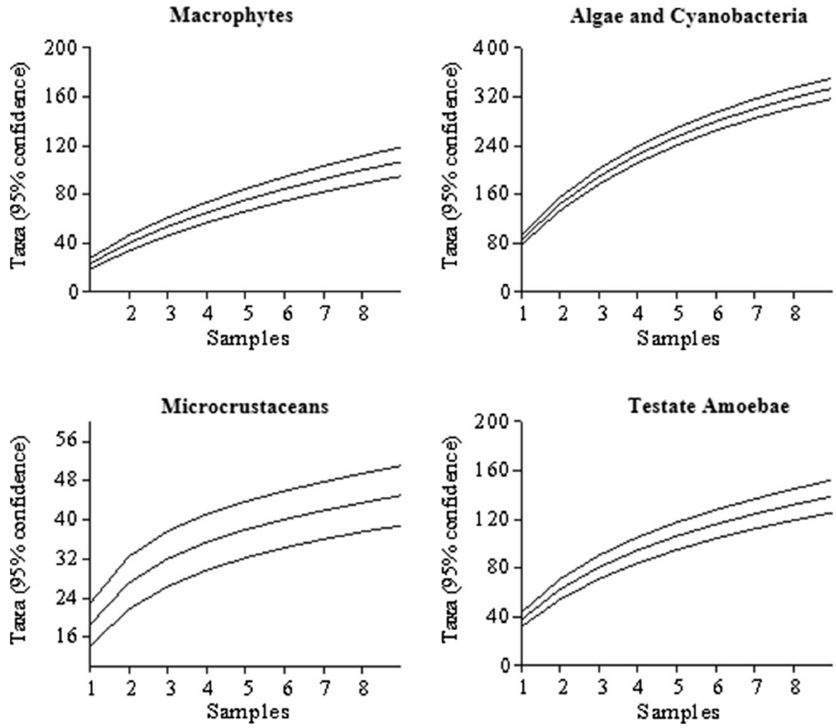

Fig. 3 Rarefaction curves for different taxonomic groups in the studied wetlands during dry and rainy seasons in 2009

although the PCA had indicated relatively higher phosphorus concentrations at FIC compared to BNP.

The general low nutrient status described above, however, did not seem to affect species richness negatively. The overall species richness reported in these pristine wetlands (632) for the four taxonomic groups (macrophytes, algae/cyanobacteria, microcrustaceans and testate amoebae) can be considered relatively high, bearing in mind that in the wet grasslands the water depth was no more than 5 cm. de Marco Jr et al. (2014) recorded 661 taxa in Cerrado ponds located in agriculture landscapes, distributed in seven taxonomic groups (algae, macrophytes, water beetles, water bugs, Odonata, fish, amphibians and birds). Junk et al. (2006a) had already mentioned this low relationship between nutrients in the water and food web structure, after analyzing species richness in seven wetlands around the world.

The beta diversity expressed by the Whittaker index (βw) has shown relatively intermediary values. According to McCune and Grace (2002, p. 31), "values of βw < 1 are rather low and βw > 5 can be considered high". The highest βw (3.2) was reported for macrophytes, with communities located in each protected areas (BNP and FIC) forming two distinct clusters. On the other hand, the microscopic organism distribution among the wetlands was relatively more homogenous, with the overall βw ranging from 1.4 (Cladocera) to 2.9 (algae/cyanobacteria).

These results (higher beta diversity among macrophytes, compared to microorganisms) come across with the literature about biogeography and dispersal of microorganisms and the paradigm of "everything is everywhere, but the environment selects", so often discussed in this context (e.g., Finlay 2002; Foissner 2006; Wit and Bouvier 2006; Incagnone et al. 2015). Microorganisms tend to have broader geographic ranges than large ones because they produce resting stages which facilitate dispersal by wind or migrating animals. About macrophytes, it is important do not neglect, however, the potential role of waterbirds also dispersing plant seeds (e.g., Charalambidou and Santamaría 2005; Green et al. 2008).

Nevertheless, as was pointed out by Foissner (2006), as more new species are described, the possibility of restricted distribution increases. In this sense, Cerrado wetlands as a whole seem to have a great potential for endemic and/or rare species. It was probably highlighted in this study for two reasons. First, it explored microhabitats usually neglected in most of previous works that focused on planktonic communities (e.g., de Marco Jr et al. 2014). Second, information on Cerrado pristine wetlands biodiversity is quite scarce.

Cantonati et al. (2015) emphasized the low "substitutability" among freshwater environments as a consequence of diversification at small geographic scales, species with restricted distribution and endemisms. These authors recommended that larger areas with a good representativeness of different kinds of wetlands are considered. In this sense, BNP and FIC comprise within their respective borders a great variety of natural wetlands, besides the fact of being located in two different great hydrographic basins. These factors, together, probably enhanced beta diversity in the regional scale.

Another additional beta diversity driver would be the presence of temporary aquatic systems, such as Veado Pond (FIC). It had no water during the dry season in the present study, and was the most dissimilar wetland, for all taxonomic groups. According to a review in Brendonck et al. (2016), temporary

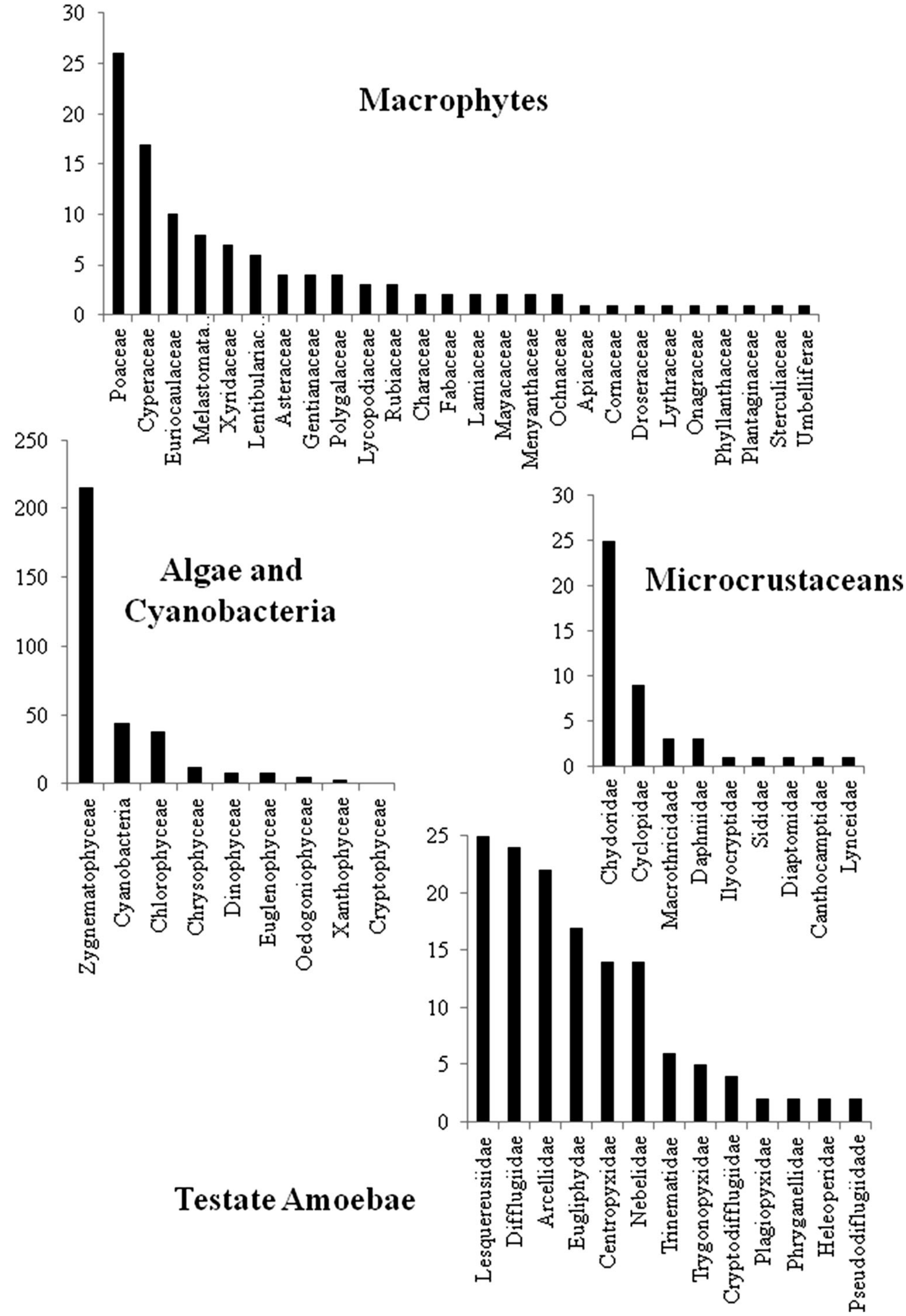

Fig. 4 Species richness among families (macrophytes, microcrustaceans and testate amoebae) and classes (algae and cyanobacteria, except diatoms) in the studied wetlands during dry and rainy seasons in 2009

ponds can host many specialist species, which require some type of drought-resistant dormant life stage, usually in the pool sediment, to bridge recurrent dry periods. The other wetlands in this study did not dry up in 2009, but are susceptible to that, especially the wet grasslands. It happened, for example, in the dry season of 2016 (June–September), when all wetlands focused in this study were totally dried up as a consequence of a severe drought phase (El Niño year) in the Cerrado region.

The next paragraphs discuss in details the biodiversity reported for each taxonomic group focused in this study.

Macrophytes

Macrophytes are key components in any wetland, providing architecture and different microhabitats for the development of an intricate microbiota. In terms of ecological groups, the

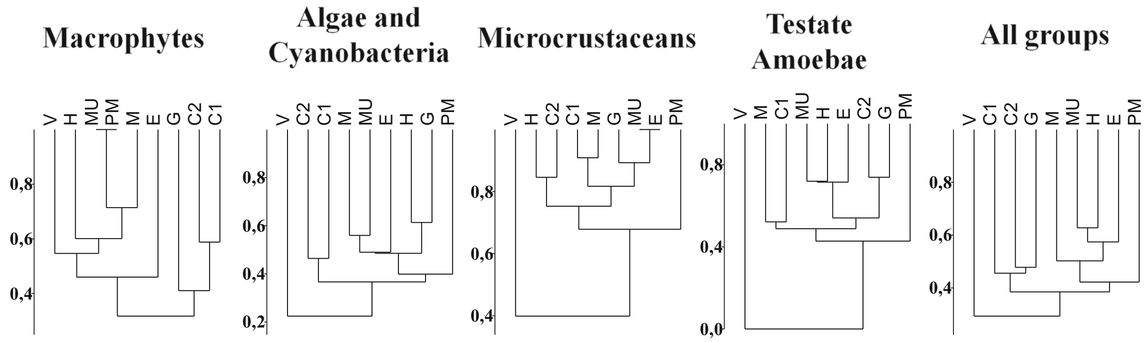

Fig. 5 Cluster analysis for different taxonomic groups in the studied wetlands (see codes in Table 1) during dry and rainy seasons in 2009. Cophenetic coefficient: macrophytes = 0.78; algae and cyanobacteria = 0.87; microcrustaceans = 0.89; testate amoebae = 0.92; all groups = 0.79

dominance of species with roots in the sediments and the absence of floating ones would be expected, considering both the shallowness of the wetlands sampled and the low nutrient availability in the water.

According to a review in Junk et al. (2006a), herbaceous richness in wetlands around the world has ranged from 280 (Canadian Peatlands) to 1148 species (Pantanal, Brazil). In the Cerrado region, floristic studies on aquatic macrophyte are relatively recent and scarce. A general estimation comprising at the same time wet grasslands, *veredas,* ponds and even riparian forests will still demand a lot of work. The few studies in the Cerrado have mainly focused on herb-subshrub layer in wet grasslands, highlighting here contributions such as Munhoz and Felfili (2007, 2008) and Eugênio et al. (2011). The families Poaceae and Cyperaceae have been among the most representative in terms of both species number and coverage, which is in agreement with present work.

In general, the floristic studies in Cerrado wetlands have carried out sampling in drier areas, without going into the ponds. This may explain the fact that some species seen in this study had not been yet reported in official plant flora lists for the region (Reflora 2016), especially at the Formosa Instructional Camp. High beta diversity has already been described for Cerrado wet grasslands by Munhoz and Felfili (2008) as a consequence of groundwater level variations. In this sense, the heterogeneity related to water level among Cerrado wetlands when wet grasslands and ponds are considered together probably makes the beta diversity even stronger. Moreover, something not to be disregarded is the eventual geochemical differences in the hydrographic basins where the two protected areas are located and its influence on soil properties.

Algae and Cyanobacteria

Dunck et al. (2013) studied periphyton in 23 *veredas* under different anthropogenic impacts and reported 200 taxa (against 334 in the present study, which did not consider diatoms). Their diversity number is probably underestimated as a consequence of the method used in quantitative samples for periphyton, which demands the control of the area scraped. The present study lacks abundance data, gaining in the overall diversity estimation nevertheless.

According to the authors cited above, diatoms had the highest density, but zygnematophyceans predominated in terms of richness (S = 79; against 215 here) and biovolume, followed by chlorophyceans (S = 26; against 38 here) and cyanobacteria (S = 25; against 44 here). The acidic waters in Cerrado wetlands are suitable environments for zygnematophyceans, which are recognized by their general preference for oligotrophic environments with low pH (Gerrath 2003). Rodríguez et al. (2011) reported a relatively low epiphytic algal richness (S = 105) in Argentinian humic wetlands (pH 7.0–8.3; conductivity 880–6843 μS cm-1), relating that to the low light penetration. Diatoms represented 48%, Cyanobacteria 22% and Chlorophyta 18%; zygnematophyceans were not even specifically cited in this study.

Another group that deserves attention is branched heterocytous cyanobacteria. It was constantly present in all wetlands sampled, forming filamentous mats along with zygnematophyceans. Rejmánková and Komárková (2000) studied mats (periphyton, metaphyton, epiphyton and epipelon) in oligotrophic wetlands from limestone-based regions of the Caribbean and reported the dominance of cyanobacteria. The abundance of heterocyte-forming cyanobacteria decreased with an increase in water conductivity, which was generally three orders of magnitude higher than the other ones described here. In the present study, it may have relevant ecological implications in the nitrogen cycle in such oligotrophic systems (Cantonati et al. 2015).

Among the rare taxa was the Chlorophyceae *Parallela novae-zelandiae* (Online Resource, fig. S11: A). It was described from New Zealand (Flint 1974), and so far, cited in Brazil only for two localities, the Serra do Cipó and the Itatiaia National Park (Sant'Anna et al. 1979), both protected areas in the Southeastern region. In the present study it was seen only at Cabocla 2 Pond (FIC).

Microcrustaceans

The Cladoceran richness observed (S = 33) might be considered high when compared to available data from other Brazilian wetlands, particularly bearing in mind differences in temporal and spatial scales. A rapid survey in the Paranã River valley, also in central Brazil, reported 39 species (Elmoor-Loureiro 2007). For Pantanal, although Junk et al. (2006b) had pointed to richness ranging from 22 to 81 taxa, some recent taxonomic studies (e.g., Sinev and Hollwedel 2005; Kotov and Štifter 2006) estimated 42 species, which is not much higher than the richness observed for BNP and FIC, if we consider the differences in spatial scale.

Regarding copepods, in their review about biodiversity in wetlands around the world, Junk et al. (2006a) highlighted the Pantanal with the highest richness, with 33 taxa, against 11 reported here. This number (11), however, is in accordance with other wetlands cited by the same authors, who mentioned copepods species richness ranging from seven to 33.

For both groups, Cladocera and Copepoda, shallow wetlands of Cerrado are remarkable for the occurrence of endemic species. The cladocerans *Celsinotum candango* and *Ephemeroporus quasimodo* (Online Resource, fig. S11: B-C) were originally described from BNP and, so far, not reported out of the Cerrado biome (Sinev and Elmoor-Loureiro 2010; Elmoor-Loureiro 2014). A new record of *C. candango* from a shallow pond in another region of the Cerrado might give support to the idea of associating them with a specific kind of habitat (Moreira et al. 2015). Also, the copepods *Metacyclops campestris* Reid 1987 and *Paracyclops carectum* Reid 1987, by now, are known only in small Cerrado wetlands.

This study contributes with the first record of the genus *Paralimnetis* in Brazil (Online Resource, fig. S11: D). It belongs to the family Lynceidae (Laevicaudata), which comprises two other genera, *Lynceus* Müller, 1776 and *Lynceiopsis* Daday, 1912. Up to now, there was only one Lynceidae species cited for Brazil, *Lynceus rotundirostris* (Daday, 1902), cited by Rocha and Guntzel (1999). The genus *Paralimnetis* had already been cited for Paraguay (Martin and Belk 1988) and Colombia (Roessler 1995a, b). This new record enlarges its geographical distribution, which is not a surprise, considering the absence of data about these animals in the Neotropical region. As is common among clam shrimps, *Paralimnetis* is usually found in temporary ponds, and individuals need to make the best use of water level fluctuations. In this sense, resting eggs produced in the last rainy season are used in order to recolonize the water body quickly and finish their life cycle. The Veado Pond, where it was found, fulfils its habitat requirements.

Testate Amoebae

Testate amoebae are rarely mentioned in biodiversity studies in wetlands, despite their relatively high contribution to microbial diversity. Shallow waters with abundant aquatic vegetation are particularly suitable habitats for these protozoans. Many of the species reported in the present study were also cited by Dabés and Velho (2001) and Gomes-e-Souza (2008) in similar biotopes in the hydrographic basin of the São Francisco river, especially the genera *Arcella* and *Difflugia*. The species richness reported by these authors was 45 and 51, respectively. In a marginal lagoon in the Paraná river basin (South Brazil), Alves et al. (2010) recorded 71 taxa among macrophytes, and Difflugidae was the richest family. The 139 testate amoebae taxa reported in the ponds and wet grasslands considered in this study, compared to these numbers, emphasize the biodiversity potential of Cerrado wetlands for these protozoans.

Regarding their worldwide distribution, this study reported many testate amoebae species initially described for the African continent. It is in agreement with Smith et al. (2008) about the existence of a “tropical Gondwana” group of species that seems not to have been affected by glaciations. In this sense, a relatively high species richness is highlighted here within the genera *Lesquereusia* and *Quadrulella,* which were described as restricted to the African continent (Thomas and Gauthier-Lièvre 1959; van Oye 1959; Gauthier-Lièvre and Thomas 1961). This study also contributed to update the geographical range of species such as *Pseudonebela africana* (Lahr and Gomes-e-Souza 2011). Further, *Placocista ventricosa* Thomas and Gauthier-Lièvre 1959*, Quadrulella alata* Gauthier-Lièvre, 1957 and *Q. debonti* van Oye 1959, by now reported only for Africa, are cited for the first time in Brazil.

Final Remarks

The acidic waters, with extremely low electrical conductivity and very low nutrient concentrations described for the small wetlands from BNP and FIC were, to a certain extent, already expected, based on previous works about other Cerrado pristine environments, especially streams. But going a step further, the present work held a magnifying glass over their microorganism diversity associated with the dominating emergent macrophytes (**Poaceae** and **Cyperaceae**), producing a pioneering integrated picture composed of **zygnematophycean algae** and **branched heterocytous cyanobacteria**, along with **Chydoridae** (Cladocera) and **Cyclopoida** (Copepoda) microcrustaceans and **Lesquereusiidae** testate amoebae. There was notably high richness and occurrence of many tropical rare and endemic taxa, e.g., among genera *Parallela* (green algae)*, Placocista* and *Quadrulella* (testate amoebae)*, Celsinotum, Ephemeroporus, Metacyclops*, *Paracyclops* (microcrustaceans); also, the genus *Paralimnetis*

(Laevicaudata) is cited for the first time in Brazil. These findings reinforce these small wetlands as potential biodiversity hotspots and stepping stones for dispersing organisms.

The qualitative knowledge about species composition presented here may subsidize quantitative approaches and/or hypothesis concerning, for instance, ecological preferences among each taxonomic group in relation to different types of wetlands (e.g., wet grasslands x ponds). Finally, the presence of temporary environments among the studied wetlands may contribute as an additional beta diversity driver that deserves more attention in future studies on Cerrado wetlands, especially considering the influence of climate changing scenarios on local hydroregimes in promoting prolonged drought episodes.

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