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Structure and Dynamics of Periphyton in a Neotropical Freshwater Lake, with Emphasis on Ciliates and Their Relationships with Bacterial Taxa

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Abstract
Periphyton communities in freshwater systems play an essential role in biogeochemical processes, but knowledge of their structure and dynamics lags far behind other environments. We used eDNA metabarcoding of 16S and 18S rRNA markers to investigate the formation and establishment of a periphytic community, in addition to a morphology-based approach for peritrich ciliate determinations, its most abundant group. We sampled two nearby sites within a large Neotropical lake at four time points, aiming to assess whether periphyton establishment can be replicated on this local scale. Producers and denitrifiers were abundant in the community, illustrating the relevant role of biofilms in freshwater nutrient recycling. Among microeukaryotes, peritrich ciliates dominated the community, with genera *Epistylis* and *Vorticella* being the most abundant and showing a clear succession at both sites. Other ciliates were morphologically identified and, in some cases, their occurrence was strongly related to bacterial abundance. The structure of both prokaryotic and eukaryotic components of periphyton was not different, while the turnover dynamics differed between the two sites, in spite of their adjacent locations and similar abiotic properties. This indicates that the establishment of these communities can vary even on a local scale within a lake ecosystem.

Keywords Biofilm · eDNA · Lake · Ciliophora · Peritrichia · Amplicon sequencing

Introduction

Periphyton or biofilms in freshwater systems are complex communities of prokaryotes, algae, protozoa, fungi, and small metazoans that live on submerged surfaces [1]. Such communities can represent a significant portion of the bacterial biomass in natural and artificial environments [2]. Accordingly, it is estimated that periphytic production could contribute to over 80% of the primary production in some lakes [3]. Moreover, periphyton plays an essential role in biogeochemical processes such as nitrification, denitrification, and nutrient cycling, including mercury methylation and hydrogen sulfide production [4]. Despite their ecological relevance, knowledge about the structure and dynamics of these communities, including the interactions among their component microorganisms and their role in food webs, lags far behind other aquatic environments [5].

A wide range of prokaryotes and eukaryotes is an essential component of the periphyton, although the factors driving its species richness variation remain incompletely understood [6]. Among eukaryotic microorganisms in periphytic communities, the most abundant are sessile and free-living ciliates [7]. Ciliates, in general, participate in a broad range of metabolic pathways, selectively preying on bacteria as well as on small eukaryotes, and play a leading role in nutrient recycling that supports the trophic cascade in aquatic environments [5]. Several studies have

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pointed out ciliates as a one of the most abundant groups in marine and freshwater periphytic communities. For example, 29 species of periphytic ciliates were identified and their occurrence correlated with water quality in a coastal area of Korea [8]. Mieczan [9] investigated the periphytic community in lakes dominated by phytoplankton or macrophytes and observed that ciliates were a major component of periphyton. Sikder et al. [10] investigated the composition of periphyton at different depths in a bay near Qingdao (China) and observed 92 ciliate species and a high degree of community succession.

Within ciliates, the subclass Peritrichia comprises sessile species that colonize both living and non-living substrates [11] and tend to be a major component of periphytic communities. Several studies have shown that peritrich ciliates are often dominant in freshwater periphyton, regardless of whether the community was sampled using artificial surfaces [10, 12] or living substrates [9].

Most studies on periphyton diversity have been conducted using morphology-based species identification, which limits the ability to characterize these communities as a whole. A more comprehensive assessment of their composition can be achieved with molecular approaches such as environmental DNA (eDNA) metabarcoding, which allows a standardized survey of all taxonomic groups present in a sample [13]. In addition, eDNA metabarcoding is also efficient at retrieving sequences from low-abundance taxa, allowing them to be included more effectively in community composition studies. Despite its power, few studies have used eDNA metabarcoding to characterize periphyton communities in marine or freshwater environments [14–17]. None of these studies was conducted in the Neotropics, highlighting the lack of information on these aquatic communities in this highly biodiverse region. Moreover, previous studies have focused mainly on the bacterial or algal components of periphyton, without exploring other groups that may play critical roles in the dynamics of these communities.

To address these issues, in the present study, we employed ribosomal DNA amplicon sequencing to investigate the formation and establishment of the periphytic community of a large Neotropical lake. Given the critical role of peritrich ciliates in periphyton dynamics, we focused particular attention on this group by combining morphological and molecular data to characterize their species composition and succession process as this community is assembled. Specifically, we aimed to test the following hypotheses: (i) periphytic communities comprise high diversity of prokaryotic and eukaryotic taxa, whose presence and abundance vary over time; (ii) the composition and turnover process of the periphytic community is similar at two sampling sites within the same lake; (iii) morphological and molecular surveys of peritrich ciliates within the periphyton retrieve the same taxon composition, at similar levels of abundance.

Material and Methods

Study Area, Experimental Design, and Sampling

The study was conducted in Guaíba lake, Porto Alegre municipality, Rio Grande do Sul state, southernmost Brazil. The lake has a surface area of 496 km^2 and a length of approximately 50 km, ranging in width from 0.9 to 19 km. Its average depth is 2 m, reaching 12 m in the navigation channel [18]. The lake is used for water supply, irrigation, fishing, recreation, and shipping. Large amounts of domestic, agricultural, and industrial wastewater are drained into the lake, which contributes to the system classification as eutrophic body of water [19, 20].

We sampled periphyton communities between June and September 2012 at two locations within Guaíba lake: Jangadeiros (J) private pier (30°6′38″S; 51°15′38″W) and Veleiros (V) private pier (30°5′40″S; 51°15′22″W), ca. 3.5 km apart (Fig. 1). These two sites were expected to have similar ecological features, despite differences in the water current [21]. Jangadeiros receives inputs from the predominant north-to-south flow direction, while Veleiros is more sheltered.

To sample periphyton, we used paired glass microscope slides pressed against each other, suspended from a buoyant device at a depth of 10 cm below the waterline. We set 12 pairs of slides at each sampling site, comprising four sampling units (each of which was retrieved at a different time point) containing three pairs each. We identified the time-based sampling units as T1, T2, T3, and T4 (Table 1). At each time point, three slide pairs from each site were removed from the water, placed in a jar with lake water, and taken to the laboratory. Temperature, pH, and conductivity measurements were performed in the field using a multiparamenter equipment (Sanxin, SX751 model). The Research Center of the Municipal Department of Water and Sewage (DMAE) provided total solids and dissolved oxygen values from Veleiros and from a site in the vicinity of Jangadeiros. These water parameters were measured monthly. Standard *t*-test was used to compare the water parameters observed in each sampling site.

One slide of each slide pair had its attached periphyton scraped with a scalpel into a microcentrifuge tube containing 1 mL of TES lysis buffer (1 mM Tris, 1 mM EDTA, 2% SDS) to undergo eDNA metabarcoding (see below). The other member was analyzed morphologically (genera or, when possible, species identification), focusing only on peritrich ciliates, using a CH30 RF100 Olympus light microscope. Since peritrichs could colonize the whole slide, we placed a glass coverslip (22 × 22 mm) in its center to delimit the analyzed area, in which we counted the total number of peritrich colonies (counted as

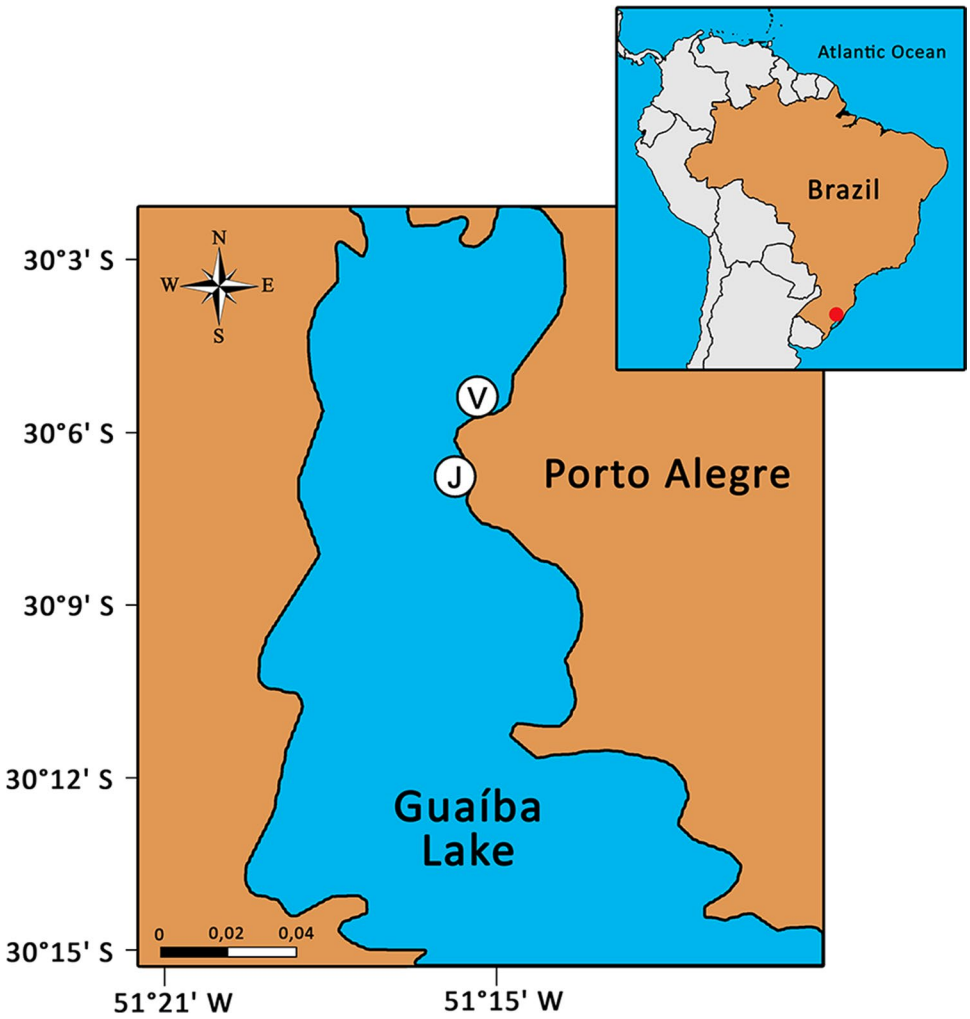

Fig. 1 Sampling sites at Guaíba Lake, Brazil. Samples were collected in triplicates at four time points (see Table 1) for each sampling site: Jangadeiros (J) and Veleiros (V) boat piers

Table 1 Sampling strategy and physical–chemical analysis of the water collected at Guaíba Lake, Brazil. Samples were collected in triplicates at four time points for each sampling site: Jangadeiros (J) and Veleiros (V) boat piers

Sampling site	Time-based sampling	Sample ID	Days after starting	Water parameters				
				pH	Total solids (mg mL^{-1})	Conductivity (µS cm^{-1})	Temperature (°C)	Dissolved O_2 (mg L^{-1})
Jangadeiros	T1	J1	15	6.1	0.15	77	11	5.9
	T2	J2	20	6.3	0.011	88.3	13.4	8.2
	T3	J3	30	6.4	0.13	74.7	14.4	6.9
	T4	J4	55	6.3	15	83.5	15.4	7.2
Veleiros	T1	V1	15	6.4	0.02	82.9	16.9	6.6
	T2	V2	25	6.6	0.04	88.4	17.9	6.6
	T3	V3	35	6.3	3	79.4	18.5	5.9
	T4	V4	45	6.2	2	78	18	6

one individual) or single zooids. Species abundance was calculated using the mean of the three replicates analyzed at each time point and was expressed as individuals per mm^2. Genera and species were identified using specialized literature [7, 22–24]. Photomicrographs of representative microorganisms were taken from live specimens using a digital camera mounted onto an optical Olympus BX50 microscope.

DNA Extraction and Amplicon Sequencing

The total DNA was extracted from a 250-µL aliquot of the TES-preserved sample using the DNeasy UltraClean Microbial Kit (Qiagen) following the manufacturer's instructions. For the prokaryotic community identification, a 291-bp of the V4 region of the 16S rRNA gene was amplified using the primers 515F and 806R [25]. Amplification was performed in a 50-µL reaction, containing 2 mM $MgCl_2$, 2 µM of each primer, 2 mM of each dNTP, 1U Platinum Taq DNA polymerase, 1X PCR reaction buffer, and approximately 10 ng of genomic DNA. PCR cycling conditions comprised one initial denaturation step at 95 °C for 3 min, 35 cycles including denaturation for 30 s at 95 °C, annealing for 1 min at 50 °C, and extension for 1 min at 72 °C, and one final extension step for 7 min at 72 °C. For eukaryotic identification, a 200-bp fragment of the 18S rRNA gene that includes the V3 region was amplified using primers fw and rv [26]. The PCR was also performed in a 50-µL reaction using the same conditions described above, with one initial cycle of denaturation at 94 °C for 4 min, 30 cycles including denaturation for 45 s at 94 °C, annealing for 30 s at 50 °C, and extension for 1 min at 72 °C, followed by one cycle of final extension of 7 min at 72 °C. Negative controls were included for the extraction and PCR amplification procedures. PCR amplicons were purified using the Agencourt AMPure Beads kit (Beckman Coulter), and libraries were constructed using the Ion Plus Fragment Library kit (Thermo Fisher) from an initial amount of 100 ng of DNA. Since all samples were sequenced in a multiplexed run, barcode sequences were used to identify each sample from the total sequencing output. Sequencing was conducted on an Ion PGM System (Thermo Fisher) using an Ion 316 chip, following the manufacturer's instructions. The barcodes identified after alignment were trimmed by Torrent Suite Software (Thermo Fisher). Sequences were deposited in the National Center for Biotechnology Information (NCBI) under BioProject PRJNA736224.

DNA Metabarcoding Data Analyses

Sequences from 16S or 18S rRNA datasets were preprocessed and classified using the DADA2 (Divisive Amplicon Denoising Algorithm) v.1.12.1 pipeline [27] in R version 4.1.0 [28]. Quality-trimming and filtering steps were performed using the "FilterAndTrimmed" function. On both datasets, reads shorter than 100 bp were removed, and a maximum of 2 expected errors per read was allowed. The subsequent steps included error inference, denoising, and chimera removal (Table S1). The resulted amplicon sequencing variants (ASVs) with lengths between 100 and 297 bp (16S avg. 240 bp; 18S avg, 198 bp) were taxonomically assigned using the SILVA database v.138 [29]. Particularly, the ASVs belonging to Ciliophora were manually curated at genus level with a Megablast nucleotide search against the NCBI non-redundant nucleotide database. Sequences with > 97% identity to a known taxon were considered a potential match. When more than one taxon was retrieved above this threshold, the one with the highest identity and lowest e-value was considered the most likely match.

The microbial composition of samples was assessed after transformation to relative abundance. Heatmaps were constructed using *phyloseq* [30], and Venn diagrams were drawn using the *VennDiagram* [31]. Alpha- and Beta-diversity was assessed for bacterial ASVs using the *phyloseq*, *vegan*, and *microbiome* packages [30–33]. Bacterial sequences were rarefied considering the lowest number of sequences identified among all samples. Due to the rarefaction, one sample from T4 (Jangadeiros) and one from T2 (Veleiros) with less than 4200 reads were not included in the analysis. Shannon biological diversity index, Chao1 richness index, and Pielou's evenness index were tested for the different time points using Kruskal–Wallis and Wilcoxon signed rank tests, which were also used to compare the sampling sites. Beta-diversity for the square root transformed ASV count data was assessed with a Bray–Curtis dissimilarity index followed by a multidimensional scaling (MDS) analysis. Permutation analysis of variance (PERMANOVA) with 10,000 permutations was used to assess the statistical significance of these comparisons. We also performed a Spearman correlation analysis between the abundance levels of bacteria and ciliates ($n = 12$, assuming independence, with respect to this co-occurrence, of the four time points and the three replicates per time point), with significance inferred when $p < 0.05$, using ANOVA with a false discovery rate (FDR) correction.

Finally, we tested whether morphology-based and molecular surveys retrieved a similar community composition (including diversity and relative abundance) for peritrich ciliates. This was assessed with a Pearson correlation analysis, followed by a two-tailed test with 2 degrees of freedom.

Results

Physical–Chemical Parameters

Most water quality parameters showed no statistically significant differences between the two sampling sites when a simple *t*-test was run (pH, $p = 0.4683$; total solids, $p = 0.5458$; conductivity, $p = 0.7483$; dissolved oxygen, $p = 0.1792$). Water temperature (which ranged from 11 to 18 °C throughout the study) was the only parameter that was significantly different ($p = 0.0052$) between the sampling sites, being higher in Veleiros (Table 1).

Periphytic Bacterial Community

The bacterial diversity (Shannon; $p=0.52$), richness (Chao1; $p=0.11$), and evenness (Pielou; $p=0.95$) indices were not significantly different between the two sites (Fig. 2A). The same was observed when the indices were used to assess the diversity of the community across time points, except for evenness (Pielou; $p=0.027$). After the initial formation of a microbial biofilm (T1), the periphytic community changed, and a significant difference ($p<0.05$) was observed in evenness between T1 and T2, and T1 and T3, but not between T1 and T4 ($p>0.05$). Over time, the number of representatives of the species that constitute the microbial community of the periphyton became more equivalent. No significant differences were observed between other time point combinations.

A MDS analysis was performed for both sampling sites (Fig. 2B). The distance between groups was assessed for the variable time (PERMANOVA; $R^2=0.19$; $p=8.9\times10^{-4}$), and for the variable site (PERMANOVA; $R^2=0.12$; $p=9.9\times10^{-5}$). For Jangadeiros, the bacterial periphytic community presented a more similar composition in T1, T2, and T3, differing from T4. Surprisingly, in Veleiros, T1, T3, and T4 clustered together and T2 was more dissimilar to the other time points. This implies that, in Veleiros, a disturbance of periphytic succession occurred between T1 and T2, and then the bacterial composition was re-established at T3. These observations are also discerned in the Venn diagrams. The periphytic succession in Jangadeiros includes an increase in the number of taxa commonly present in T1 to T3 (T1–T2, $n=72$ and T1–T3, $n=89$), but this is reduced between T1 and T4 ($n=53$) (Fig. 2C). In Veleiros, the number of common bacterial taxa for T1 and T2 ($n=54$) was similar to that for T1–T3 ($n=48$), but higher than T2–T3 ($n=36$) and lower than T1–T4 ($n=65$) (Fig. 2D).

A total of 28 bacterial phyla were identified during the study at the two sites. Of these, 11 presented a relative abundance higher than 1% at one or more sampling times. Proteobacteria dominated both sampling sites, with abundances ranging from 62.1 to 77.3% of the total community (Table S2). Bacteroidota was the second most abundant at both sites, followed by Nitrospira and Cyanobacteria. Acidobacteriota, Actinobacteriota, and Planctomycetota were also present at abundances higher than 1%, with Planctomycetota presenting an abundance higher than 6% at T4 in Veleiros.

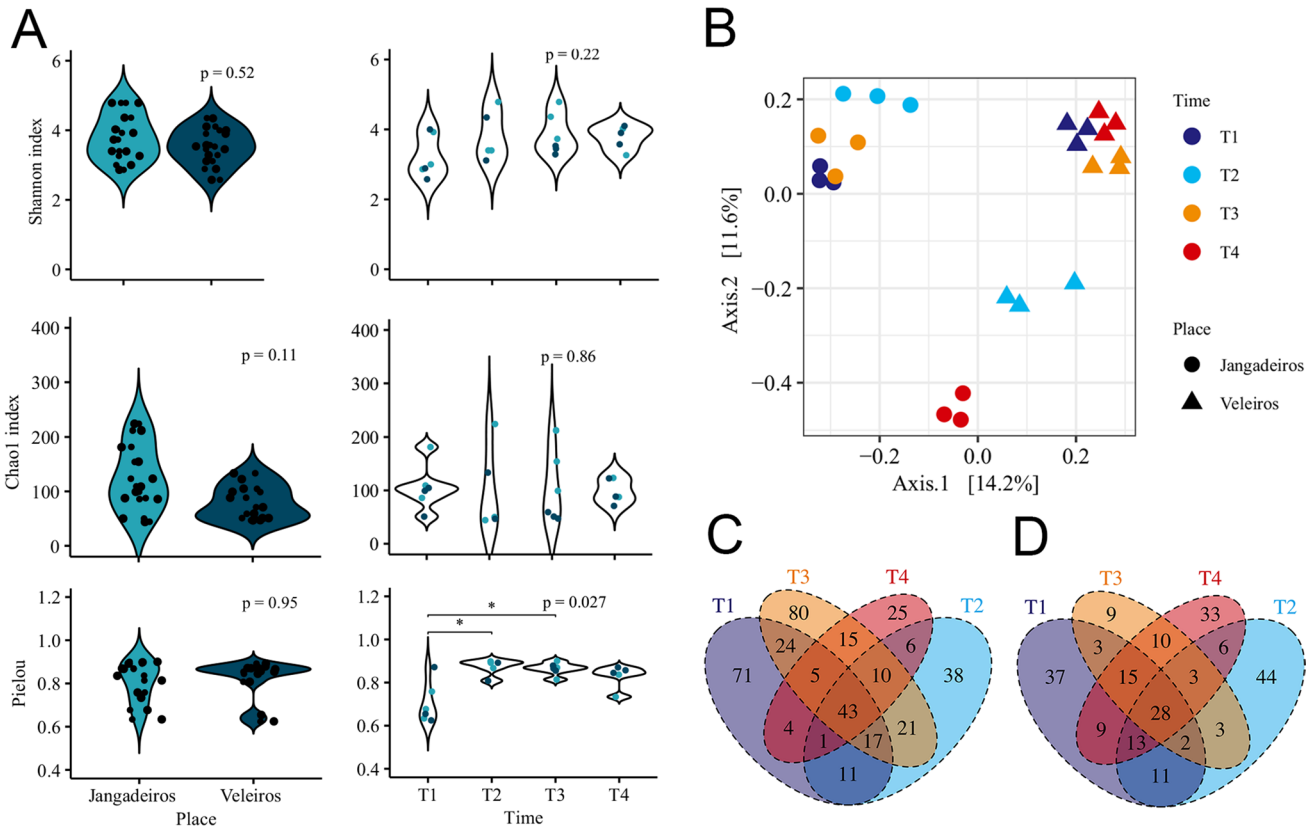

Fig. 2 Bacterial diversity of the periphytic communities at the two sampling sites, Jangadeiros and Veleiros boat piers, in Guaíba Lake, Brazil. **A** Alpha diversity index of sample groups. **B** Multidimensional scaling (MDS) ordination of Bray–Curtis dissimilarity matrix. Different dot colors represent time points and different shapes represent sample sites. **C**, **D** Venn diagrams showing unique and shared bacterial taxa observed at **C** Jangadeiros and **D** Veleiros in each time points

Among the most abundant ASVs in Jangadeiros, *Citrobacter*, a gram-negative coliform bacteria from the family Enterobacteriaceae, was the most abundant genus at T1 (up to 43% of the total sequences), disappearing at the other time points (Fig. 3A; Table S3). In Veleiros, *Serratia*, a gram-negative coliform (Fig. 3B; Table S3) was highly abundant at T1 (up to 44% of the total sequences), along with *Pseudomonas*, which appeared mainly at the first time point. *Nitrosomonas*, *Candidatus* Nitrotoga, *Nitrosospira*, and *Flavobacterium* were constantly present at the four time points.

Periphytic Eukaryotic Communities

Among eukaryotes, the most abundant group found at both sites was Alveolata, followed by "Excavates", Diatomea, and other Stramenopila (Fig. 4A). Chlorophyta showed peaks of high abundance in Veleiros, but the same pattern was not observed in Jangadeiros. Ciliophora was the most abundant group within the Alveolata at both sites, representing more than 75% of the sequences, followed by Dinoflagellata, with up to 25% relative abundance (Fig. 4B). A Venn diagram of the distribution of Ciliophora taxa at different time points in Jangadeiros revealed that T1 harbored three unique taxa, while T2 and T3 presented five, and T4 had four unique taxa (Fig. 4C; Table S4). In Veleiros, T1 harbored only two unique taxa, while T2, T3, and T4 harbor six, one, and four, respectively (Fig. 4D; Table S4).

Among ciliates, the periphytic community at both sites was primarily composed by peritrichs, with the colonial genus *Epistylis* being the most abundant taxon (Fig. 4E).

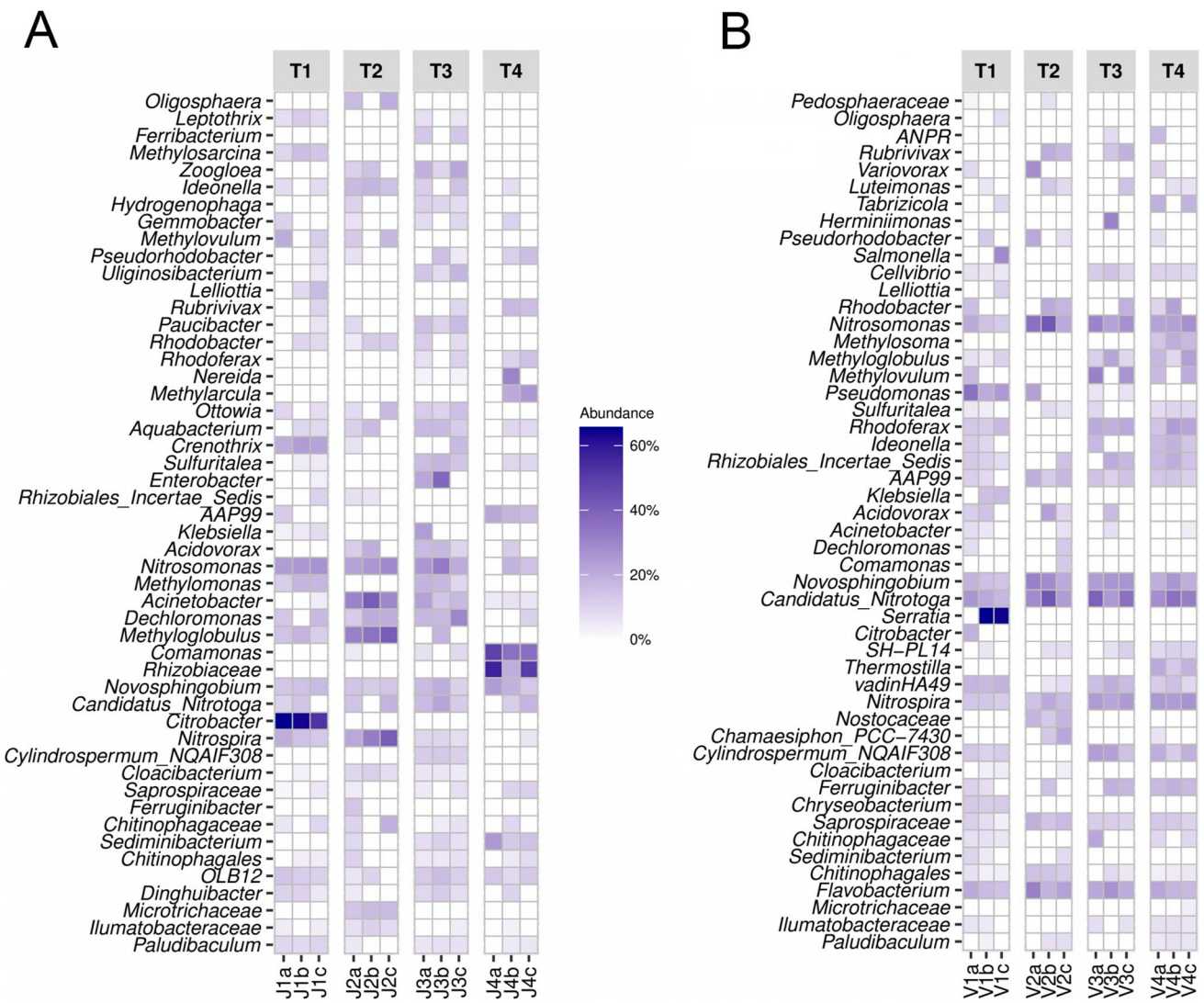

Fig. 3 Relative abundance of the bacterial community composition at the two sampling sites, Jangadeiros and Veleiros boat piers, in Guaíba Lake, Brazil. The fifty most abundant genera (or taxonomic annotation) in **A** Jangadeiros and **B** Veleiros samples are separated by time points. ANPR, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium

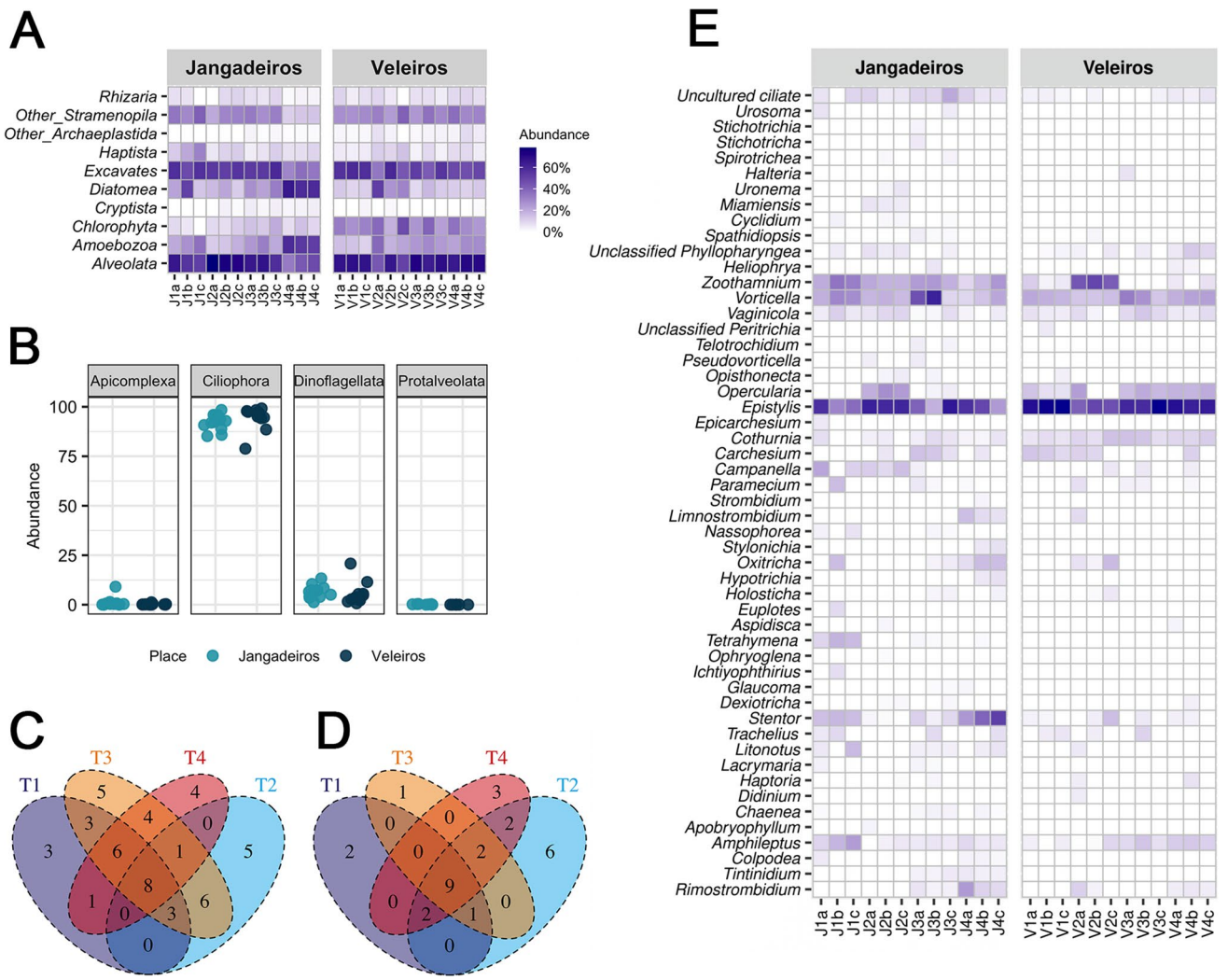

Fig. 4 Eukaryotic community composition at the two sampling sites, Jangadeiros and Veleiros boat piers, in Guaíba Lake, Brazil. **A** Main eukaryotic clades; **B** phyla within Alveolata for each on the samples analyzed; **C**, **D** Venn Diagrams showing unique and shared taxa belonging to the phylum Ciliophora found in **C** Jangadeiros and **D** Veleiros; **E** relative abundance (square root transformed) of Ciliophora taxa

Its highest relative abundance was observed at T2 in Jangadeiros and at T1, T3, and T4 in Veleiros. The genera *Vorticella* and *Zoothamnium* also exhibited high abundance. *Vorticella* reached a peak in Jangadeiros at T3, while *Zoothamnium* had its highest abundance in Veleiros at T2. Other peritrich genera were also present in the community, but at low abundance at both sites.

With regard to other ciliate genera, the heterotrich *Stentor* had a high relative abundance at T4 in Jangadeiros, but in Veleiros, it was present at very low abundance along the microbial succession. Among predators, *Amphileptus* had an abundance of up to 12% at T1 in Jangadeiros. In Veleiros, the same genus showed a slightly lower abundance at T3 and T4 (Fig. 4E).

Correlation Between Bacterial and Ciliate Communities

Spearman's correlation between Ciliophora and Bacteria taxa performed at genus level (or corresponding annotation) demonstrated potential ecological relationships among these groups along the periphytic microbial succession. A larger number of significant correlations were found in Jangadeiros compared to Veleiros (Fig. 5). In Jangadeiros, genera associated with methanotrophy and methylotrophy, along with the nitrite-oxidasing bacterium *Nitrospira*, presented significant correlations with ciliates. Considering the Ciliophora community, five genera showed positive correlations with a broad range of bacterial taxa (Fig. 5A). In Veleiros,

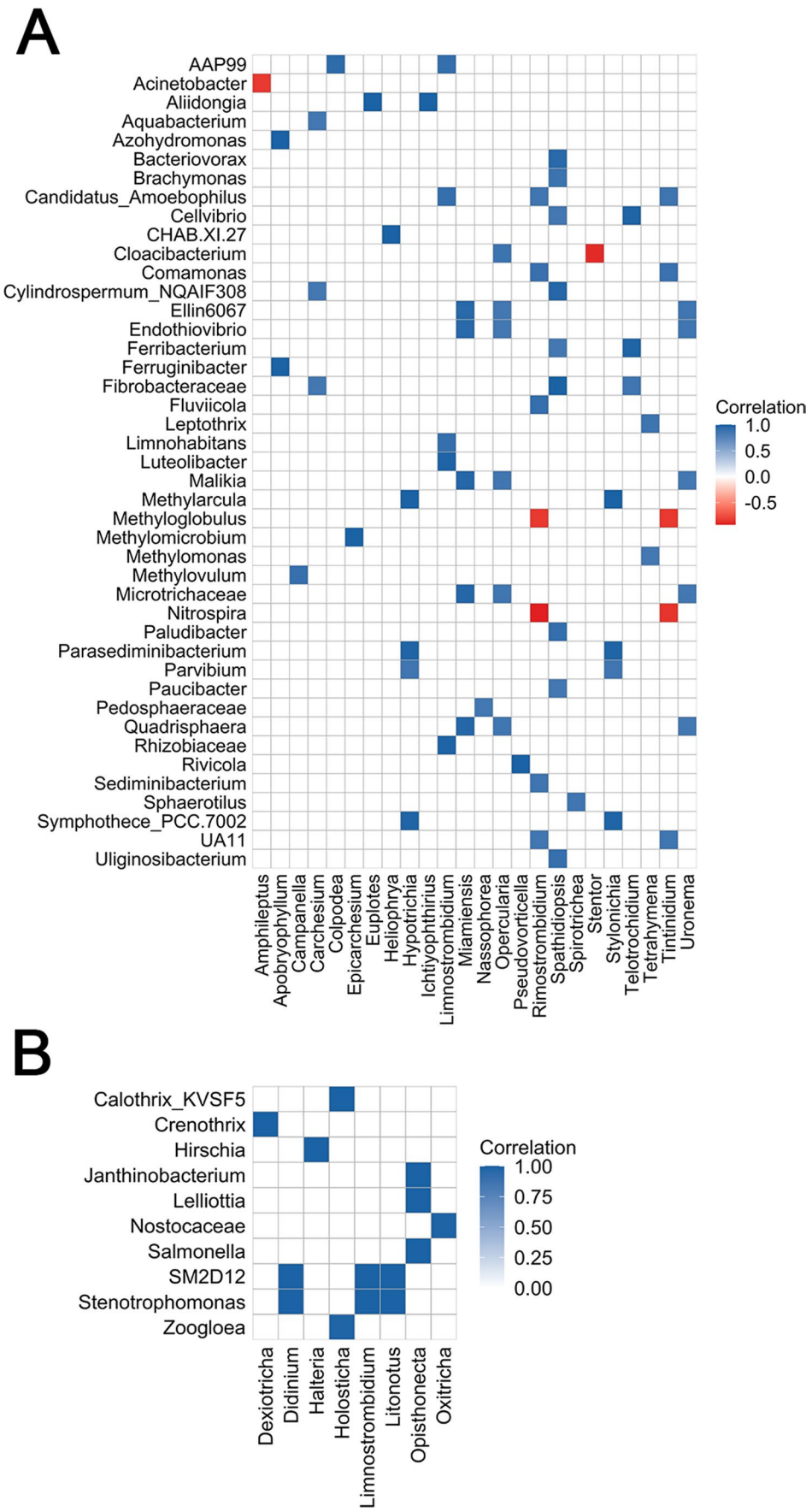

Fig. 5 Correlation analysis between the bacterial taxa with abundance ≥ 1% and Ciliophora taxa at the two sampling sites: **A** Jangadeiros and **B** Veleiros boat piers. Spearman correlation was computed, and statistical significance was defined for all pairwise comparisons. Only significant correlations, either positive (blue squares) or negative (red squares) (p-value < 0.05), are shown

the methane oxidizer *Crenothrix* presented a positive correlation with three ciliate genera, and no significant negative correlations were observed at this site (Fig. 5B).

Ciliate Morphology-Based Analyses

The peritrich ciliate assemblage was composed of seven genera and nine morphologically identified species. All genera and species were recorded at both sites, except for *Mioschiston duplicatum* and *Vaginicola tincta*, recorded only in Jangadeiros (Fig. 6). *Epistylis* was the most abundant peritrich genus in Jangadeiros at T1, with *E. portoalegrensis* reaching a peak of abundance, followed by *E. smalli* and *E. plicatilis* (Fig. 6A). In Veleiros at T1, the most abundant genus was *Vorticella*, with *V. convallaria* reaching a high peak of abundance (Fig. 6B).

At T2 in Jangadeiros, different species of *Epistylis* were still abundant, but *Vorticella* spp. showed an increase in abundance, reaching more than 1000 inds/mm^2 (Table S5). A similar situation was observed at T2 in Veleiros, where *Vorticella* represented more than 45% of the peritrichs present in the samples (Fig. 6A). At T3, both sites presented a dominance of genus *Vorticella*. Jangadeiros had a low abundance of peritrichs at T3, with *Vorticella* reaching an abundance of 150 inds/mm^2. In Veleiros, the highest abundance of peritrichs during the study was observed at T3, with *Vorticella* reaching more than 90% of the peritrich records observed in the samples.

The lowest abundance of peritrichs in Jangadeiros was observed at T4. Species of *Vorticella* accounted for more than 80% of the peritrichs observed in the samples. In Veleiros at T4, a similar result was observed for *Vorticella*, with other genera presenting very low abundances (Fig. 6B).

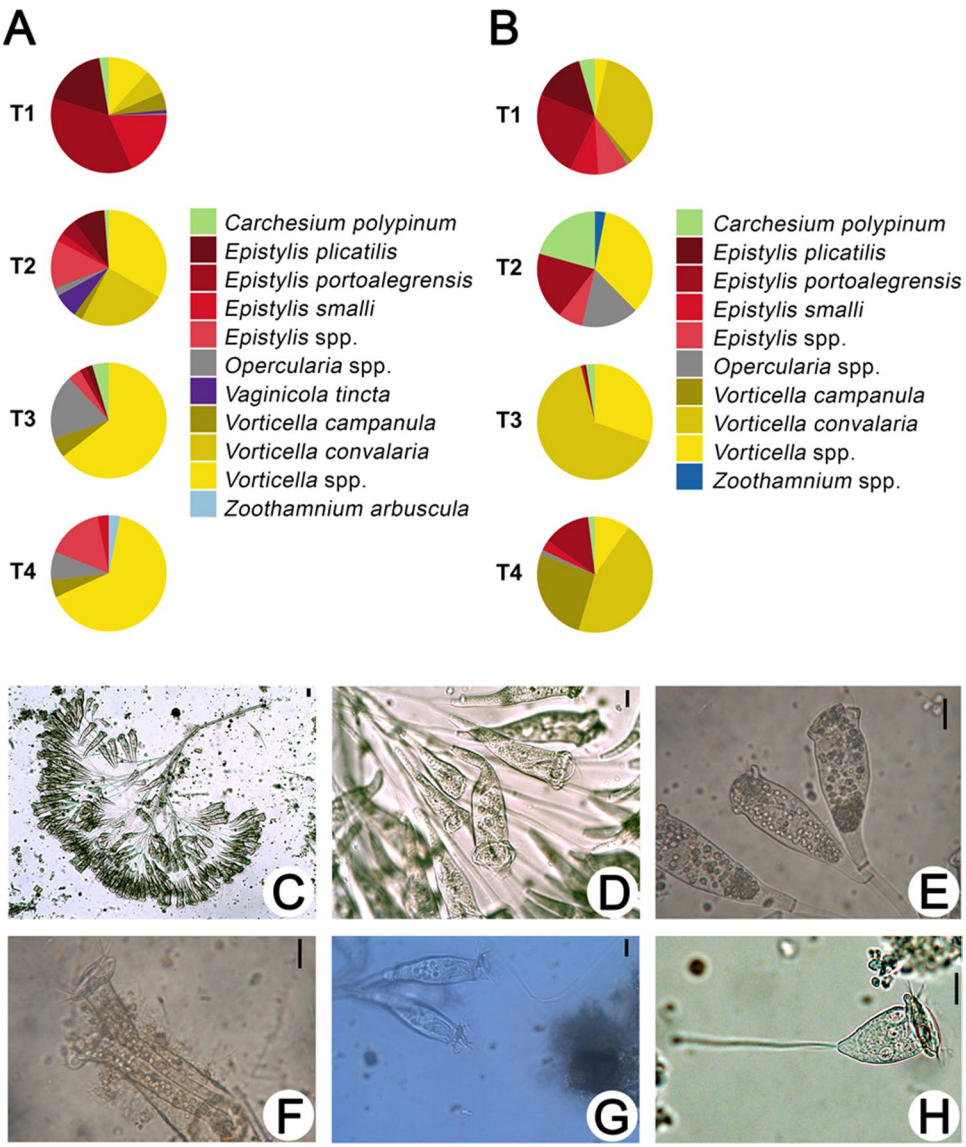

Fig. 6 Morphology-based characterization of the peritrich ciliate community present at the two sampling sites, **A** Jangadeiros and **B** Veleiros boat piers, at four time points (T1-T4; see Table 1). **C**–**H** Photomicrographs of the observed genera: **C** colony of *Epistylis riograndensis*; **D–H** detailed view of the zooids of: **D** *Epistylis riograndensis*; **E** *Epistylis plicatilis*; F *Vaginicola* sp.; **G** *Opercularia* sp.; and **H** *Vorticella* sp. Scale bars are 20 µm

When we compared the peritrich community composition as assessed by molecular and morphological methods, we found no statistically significant correlation ($r = 0.4$; $p = 0.3$) between their diversity estimates. On the other hand, when the relative abundance of peritrich genera was compared, we observed a highly significant correlation ($r = 0.6$; $p = 1.3 \times 10^{-12}$) between the two methods.

Discussion

Biofilm communities have been explored in several freshwater environments due to their importance in aquatic food webs [5, 32] and as water quality indicators [7, 17, 35, 36]. To better understand the dynamics of periphytic communities, it is important to analyze the bacterial and microeukaryotic components jointly, since together, they participate in production and recycling processes [37]. In this study, the bacterial and ciliate communities and their dynamics in a eutrophic lake in southern Brazil were explored using eDNA metabarcoding. Direct morphological identification was also applied to Peritrichia, the most abundant ciliate group during the surveyed period. To our knowledge, this is the first molecular study to analyze bacteria and microeukaryotes of periphyton in a Neotropical environment. We observed that, although our two sampling sites were adjacent within a larger lake and quite similar in terms of their abiotic properties, their periphyton composition and succession processes presented detectable differences.

The succession of the periphyton was discerned through the four sampling times. The diversity between the time points showed no differences, but a tendency of the community establishment could be observed. As expected, the colonization and periphytic establishment occur fast in eutrophicated lakes. For further studies, starting sampling points earlier than fifteen days would provide more information on the first stages of the periphytic succession.

With respect to functional assessments, it has been reported that most bacteria found in stream and river biofilms and revealed by amplicon sequencing belong to the phyla Proteobacteria, Bacteroidota, Acidobacteriota, Verrucomicrobiota, and Cyanobacteria [37, 38]. Proteobacteria was the dominant phylum in Veleiros and Jangadeiros during the periphytic succession. The peaks of high abundance of Proteobacteria at T1 observed for both sites are probably related to these bacteria's ability to first colonize rigid substrates. Moreover, sewage input contributes to this high abundance of enterobacteria since genera since genera *Citrobacter* and *Serratia*, all associated with humans, presented a high relative abundance in Jangadeiros and Veleiros, respectively. Other studies have also reported the presence of enterobacteria in the periphyton, suggesting that these communities could act as reservoirs of these bacteria [39].

Cyanobacteria were present at both sampling sites, but at higher abundances in Veleiros than Jangadeiros. In Veleiros, water temperature and total solids were higher, probably having an important role in the increase of primary production of the periphytic community [40]. Primary producers are generally abundant, with Cyanobacteria reported in different studies [e.g. 37, 41]. For example, Zancarini et al. [37] found this group as the second most abundant prokaryotic phylum in a river in France, in addition to diatoms and chlorophytes. In marine environments, Cyanobacteria have also been reported as highly abundant in communities sampled at the Great Barrier Reef [42].

Studies have demonstrated that periphytic communities can accelerate nutrient removal from the water column [43], enhance denitrification processes, and participate in methanogenesis [e.g.14, 44, 45]. In Guaíba lake, bacteria involved in nitrogen fixation (Rhizobiaceae taxa) and nitrite oxidation, such as *Nitrosomonas* and *Nitrospira*, were present at high abundance along the periphytic succession in Jangadeiros. On the other hand, the genera *Nitrosomonas* and *Candidatus* Nitrotoga, involved in nitrification, were recorded in Veleiros at low to medium–high abundance along the periphytic succession. The relative high abundance of bacteria involved in nitrification processes suggests that denitrification occurred in the periphyton of this eutrophic lake. That has been detected by other studies [14, 43], pointing out the important role of periphyton in eutrophication processes.

A high abundance of methanotrophs and methylotrophs, specialized bacteria capable of using methane and methanol, respectively, as a sole carbon and energy source [46, 47], was observed during the periphytic succession. Type I methanotrophs have been shown as the main microorganisms responsible for active methane consumption in lakes [48]. In the periphytic community of Guaíba lake, Type I methanotrophs belonging to the order Methylococcales (Gammaproteobacteria) were identified as the most abundant.

Among heterotrophic microeukaryotes, one of the most abundant groups found in periphytic communities are ciliates. For example, Glud and Fenchel [49] observed extreme densities of ciliates in biofilms of marine environments, with the genus *Euplotes* reaching 20,000 inds/cm^2. Ackerman et al. [50] in a long-term study of the periphyton from the Rhine river observed that more than 50% of the biovolume composition of the biofilm was ciliates. In the present study, the clade Alveolata dominated the composition of the periphytic community, with ciliates reaching the highest abundance among alveolates. When the two sampling sites were compared, ciliates tended to be more diverse in Jangadeiros. This fact may be related to abiotic factors, such as water flow and temperature, or biotic factors, such as food availability and predation [51].

Several studies have focused on ciliates as a significant component of the periphyton in freshwater or marine

environments [9, 10, 52, 53]. Among ciliates, the most common groups found in biofilm communities are peritrichs, suctorians, and heterotrichs, since they encompass organisms that attach permanently to substrates or that could easily detach when the conditions are unfavorable. In addition, peritrich ciliates may contribute to constructing the biofilm architecture since their stalks can interconnect with filamentous bacteria forming a complex network that supports a multilayer organization of the whole community [52]. In Guaíba lake, peritrichs were the dominant ciliate taxon at both sites, which led us to perform in-depth analyses of this group.

Peritrich communities were surveyed using both molecular and morphology-based approaches, so we initially tested whether they produced congruent results. We observed some visible similarities between the two types of survey, such as the dominance of genera *Epistylis* and *Vorticella* at the four time points. On the other hand, there were differences between the two data sets, including taxa that were only recorded with the morphological survey (*Myoschiston*) or only with the molecular data (*Telotrochidium*, *Opisthonecta*, *Pseudovorticella*, *Epicarchesium*, *Cothurnia*, *Campanella*). We formally tested for correlation in genus diversity and relative abundance between the two methods. While the former was not significant, the latter did yield a very significant result, indicating that both methods do generate consistent results regarding peritrich community composition.

The dominance of *Epistylis* and *Vorticella* in Guaíba lake periphyton has been previously reported in a 1-year morphological survey [12]. In that study, *Epistylis* and *Vorticella* were the most abundant and species-rich genera found in the peritrich community, clearly showing a seasonal cycle. Although *Epistylis* and *Vorticella* have been recorded as constant genera in this study, other peritrich genera such as *Opercularia* and *Carchesium* were also abundant at specific time points. If we look at the overall diversity of peritrich taxa at both sites, Jangadeiros tended to be more diverse than Veleiros during the four time points. This difference could be related to water circulation, which is higher in Jangadeiros. At the same time, in contrast to metazoans, studies have revealed that peritrichs are highly abundant in environments that receive a considerable input of sewage [54, 55]. Although both sites are highly polluted, slight differences in dissolved oxygen or even the type of available food (possibly driven by the differences in water current and temperature) may have led to a more diverse community found in Jangadeiros. Further studies are required to assess if slight differences in dissolved oxygen or even food sources can explain the difference observed at both sites.

Ciliates are known to graze efficiently on planktonic or attached bacteria and algae. Studies of biofilms have shown that ciliates are usually the most easily recognizable grazers within the periphytic community. Specific groups of periphytic ciliates such as hypotrichs and stichotrichs could be observed dislodging attached bacteria with their cilia and collecting them with the adoral membranelles [5]. Predatory ciliates are also known to crawl upon biofilms, where they can prey on other unicellular organisms or even on metazoans [5]. In lakes saturated by organic matter, the predatory activity of ciliates releases nutrients held by a plethora of primary producers that anchor inorganic carbon inside an active microbial community [56]. On the other hand, peritrichs and heterotrichs are suspension filter feeders, consuming bacteria, algae, and other planktonic organisms present in the water column or suspended from the substrate [57]. Some genera of ciliates were abundant when specific bacterial taxa were present in the environment. For example, *Stylonichia*, *Euplotes*, and *Holosticha* were abundant and showed a strong correlation with specific bacterial groups (Fig. 5), suggesting that these ciliates are probably grazing upon these bacteria. In addition, there were also predatory ciliates that prey on unicellular eukaryotes and metazoans. Interestingly, the abundance of *Epistylis* and *Vorticella* was not correlated with the abundance of bacterial taxa, which may be related to the condition of suspension feeders of these two peritrich genera. Further studies are needed to dissect these trophic interactions in more detail.

Overall, our results demonstrated that Guaíba lake periphyton presented a high diversity and abundance of bacteria, and these parameters differed over time. In relation to eukaryotes, the periphytic community was dominated by peritrich ciliates, especially genera *Vorticella* and *Epistylis*. Their diversity and abundance followed the same pattern observed for bacteria. Regarding the two sampled sites, they were also very similar in the composition of the periphytic community, despite the distance between them. In addition, molecular- and morphology-based methods showed a highly significant correlation in relative abundance, but no correlation was observed when diversity of taxa was analyzed. This demonstrates, for this system, that both methods could complement each other depending on the analyzed parameter. Given the complexity of the assembled communities and the different succession processes observed at sampling sites with similar properties, we can conclude that additional studies focusing on the structure and dynamics of Guaíba Lake biofilms will be required to fully understand their diversity and ecological roles in this aquatic ecosystem.

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Author Contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Adriana Giongo, Taiz Leonor Lopes Simão, Luiz Gustavo dos Anjos Borges, Eduardo Eizirik, and Laura Roberta Pinto Utz. The first draft of the manuscript was written by Adriana Giongo and Laura Roberta Pinto Utz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval This is an observational study. The Pontifical Catholic University of Rio Grande do Sul Research Ethics Committee has confirmed that no ethical approval is required.

Conflict of Interest The authors declare no competing interests.

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