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Testate amoebae (Protozoa Rhizopoda) in two biotopes of Ubatiba stream, Maricá, Rio de Janeiro State

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ABSTRACT. Four samplings were carried out during the dry and rainy seasons in 2014, in two biotopes (plankton and aquatic macrophytes) to assess the composition and species richness of testate amoebae community in a coastal stream in the state of Rio de Janeiro, Brazil. Results showed great representation of Difflugiidae, Centropyxidae, Lesquereusiidae and Arcellidae families. Higher richness was observed in the plankton samples and higher densities of testate amoebae were reported among the aquatic vegetation during the dry season. Current investigation is a pioneer study conducted in the Ubatiba stream. Further researches on these protists, especially in Rio de Janeiro, should be undertaken.

Keywords: tropical waters, biodiversity, protozooplankton.

Amebas testáceas (Protozoa Rhizopoda) de dois biótopos do riacho Ubatiba, Maricá, Estado do Rio de Janeiro

RESUMO. Com o intuito de avaliar a composição e a riqueza de espécies da comunidade de amebas testáceas de um riacho costeiro, localizado no estado do Rio de Janeiro, foram realizadas quatro campanhas em 2014 (estação seca e estação chuvosa), em dois biótopos (plâncton e macrófitas aquáticas). Os resultados mostraram maior representatividade das famílias Difflugiidae, Centropyxidae, Lesquereusiidae e Arcellidae. Maiores riquezas foram registradas no plâncton e maiores densidades de amebas testáceas entre a vegetação aquática, ambos no período seco. O estudo foi pioneiro no riacho Ubatiba e evidencia a necessidade de intensificar as pesquisas sobre estes protistas, sobretudo no Rio de Janeiro.

Palavras-chave: águas tropicais, biodiversidade, protozooplâncton.

Introduction

Testate amoebae is a term used to designate amoeboid protozoa organisms whose protoplasm is inserted into a teak (shell). They present an oral opening from where pseudopods protrude during feeding and locomotion (SMIT et al., 2008). They are considered a polyphyletic group whose species inhabit mainly aquatic environments associated with marginal vegetation and sediments (MATTHEUSSEN et al., 2005; ALVES et al., 2012). However, several studies have recorded high densities of these organisms in lakes, reservoirs and peatlands (ALVES et al., 2007, 2012; LANSAC-TÔHA et al., 2009), swamps, rivers and streams (MATTHEEUSSEN et al., 2005), estuaries and, more rarely, in marine environment (GOLEMANSKY et al., 2006).

Although testate amoebae have been studied in different biotopes of freshwater ecosystems of Brazil (LANSAC-TÔHA et al., 2008; TODOROV et al., 2009), there are few studies focusing exclusively on testate amoebae in coastal environments, especially in coastal streams of Rio de Janeiro, Brazil. Studies on amoeba in association with foraminifera from myxohyaline environments are still the most common studies for the above mentioned region (LEÃO et al., 2012).

The abundance and distribution of planktonic forms depend on specific adaptations to the local aspects of biotic and abiotic characteristics of the environment. Therefore, it is important to recognize all types of environmental variations in coastal ecosystems. Species diversity is one of the most important attributes of a biological system. Although studies on testate amoebae in streams are on the increase (FULONE et al., 2008; COSTA et al., 2011; MAZEI; BELYAKOVA, 2011), little is known on the diversity of these organisms in the coastal streams of Rio de Janeiro. Considering the importance of testate amoebae in the various biotopes and the lack of studies related to these protozoa, especially in the state of Rio de Janeiro, current study evaluates the composition and species richness of testate amoebae in a coastal stream in the southeastern region of Rio de Janeiro. Current investigation provides in-depth additional knowledge on the biodiversity of these organisms in Brazil.

Material and methods

Study area

The coastal stream Ubatiba (22º55'10" S; 42º49'04" W) makes up a small coastal system that drains the western slope of the Atlantic Rain Forest (Serra do Mar) in the state of Rio de Janeiro and flows into the Maricá Lagoon system (Figure 1). The stream's water level in the region is solely regulated by, and fluctuates according to, rainfall (~1500 mmyr^{-1}) and run-off with abundant summer rain (November–January), enhancing water fluctuations (November to January) (MAZZONI; LOBÓN-CERVIÀ, 2000). Riparian vegetation is quite changed in some sections of this stream, mainly due to human activity (agriculture and livestock), and sewage dump outbreaks. However, original remnants of the Atlantic Rain Forest are still found at the headwaters.

Sampling design

Testate amoebae and environmental samples were retrieved during four sampling events, between May and December 2014, or rather, during the dry (May and August) and the rainy (September and December) seasons. Two biotopes, aquatic macrophytes and plankton, were sampled at five sites (Figure 2) along the Ubatiba stream. Environmental characterization was based on *in situ* analyses of water temperature (°C), dissolved oxygen (DO; mg L^{-1}), electrical conductivity (μS cm^{-1}), turbidity (N.T.U.) and pH, by multiparameter probes.

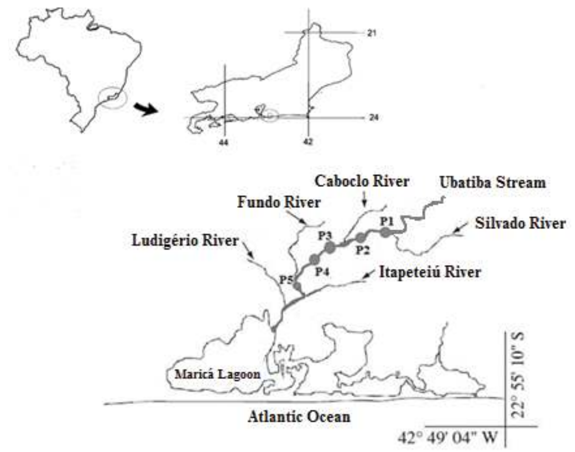

Figure 1. Fluvial System of Ubatiba stream, with the location of the study area.

Sampling of Testate Amoebae

The amoebae testate were obtained with different techniques according to the biotope. Amoebae testate associated with aquatic macrophytes samples were obtained by dragging graduate bucket (13 L) in the rooted marginal vegetation (five times), totaling 65 L, and then filtered in a 20 μm-mesh plankton net. Plankton samples were collected with graduate bucket (13 L) dragged at the center of the stream channel (10 times), totaling 130 L, and then filtered in a 20 μm-mesh plankton net. The amoebae testate and plankton concentrated samples were stored in 200 mL-bottles and fixed with formaldehyde 4% solution neutralized with borax.

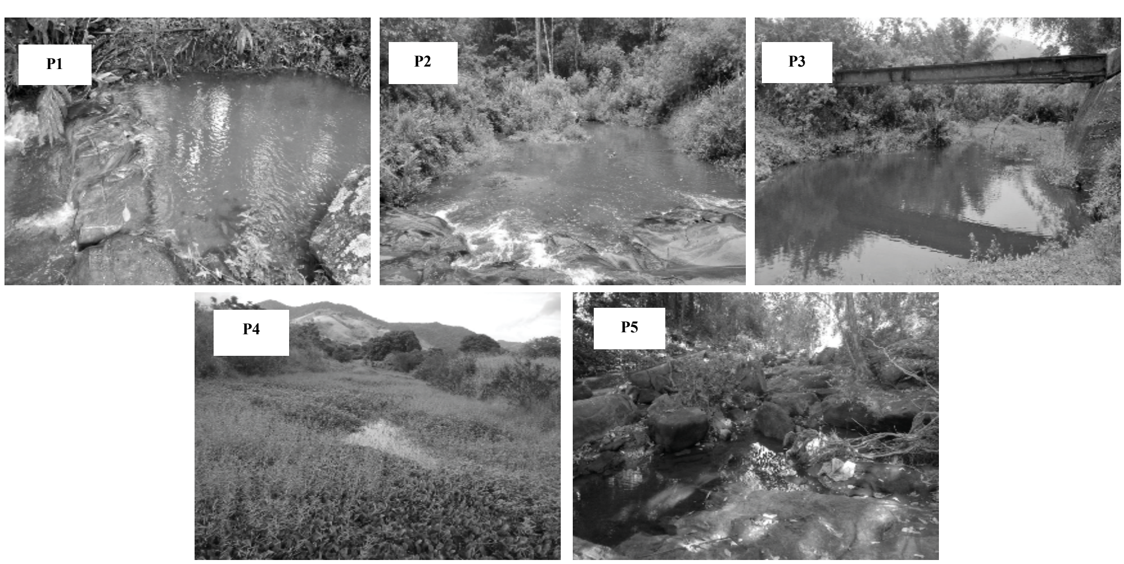

Figure 2. Study sites at Ubatiba Stream, P1, P3, and P5 show sites without vegetal cover (Open sites) and P2 and P4) show sites with vegetal cover (Close sites).

The testate amoebae composition of the two biotopes was determined by 10 sub-samples of 0.5 mL, with a Pasteur pipette. Analyses were done on a Sedgwick blade-beam on an optical microscope (Olympus). The taxonomic identification was based on the descriptions found in Adl et al. (2005). Adl et al. (2012), Velho et al. (1999), Alves et al. (2012) and Souza (2008) were used for species identification.

Data analyses

Data were processed to analyze the composition and spatial distribution of the testate amoebae and species richness at the different sampling sites of the Ubatiba stream. Densities of testate amoebae recorded in the plankton and aquatic macrophytes were estimated in ind m^{-3}. Individual Abundance (number of individuals per species) and species richness (total number of species in a community) were calculated according to Odum (1988). The Constancy Index of each taxon was calculated according to Dajoz (1973): 'constant' taxa were those that comprised more than 50% of samples; 'accessory' taxa comprised between 25 and 50% of the samples; 'rare' taxa were those present in less than 25% of the samples.

Results and discussion

Environmental variables

Results of the environmental variables showed that the Ubatiba stream proved to be a shallow and hot water stream, with high oxygenation, basic pH, high electric conductivity and low turbidity (Table 1).

In the dry season, the water temperature was stable, varying between 20 and 21°C. Dissolved oxygen (DO) concentrations were high, though they tended to decrease with the proximity of the rainy season. In the dry season, there was a decrease in pH and turbidity, whereas electrical conductivity increased with the proximity of the rainy season (Table 1).

During the rainy season, the Ubatiba stream had warm water (~ 22°C) and dissolved oxygen was low when compared to the rates registered during the dry season, with the lowest rate recorded in December. Further, pH was stable and remained slightly basic during all the study period, whereas turbidity increased during the rainy season, with the highest average rates registered in December. A similar pattern was observed for electrical conductivity, with higher rates in December and throughout the rainy season (Table 3). All these characteristics and mainly those related to mean water temperature (22.5ºC) indicated that the Ubatiba stream constituted a tropical system (ALLAN, 1995).

Dissolved oxygen is one of the most important physical aspects of the biological demand in aquatic ecosystem dynamics due to its centrality in the biota. Mean rate of dissolved oxygen in Ubatiba was 6.1 mg L^{-1} in 2014, within the standards recommended by Conama (2005).

According to Stumm and Morgan (2012), the pH of natural waters varies between 6.5 and 8.5, whereas rates between 6 and 9 are considered compatible in the long term for most organisms. However, rates outside this range of tolerance may be lethal. Although the Ubatiba stream presented a slight pH rise during the months under analysis (from the dry to the rainy season), this increase lay within the optimal tolerance for the maintenance of aquatic biota and complied with acceptable limits by Conama (2005).The pH in the dry season suggested the accumulation and decomposition of allochthonous and autochthonous material in the sediment, leading to the production of CO_2, with the acidification of the medium due to the formation of carbonic acid (H_2CO_3) (CUNHA-SANTINO, 2003). On the other hand, the slight basicity recorded during the rainy season indicated that the water input buffered the water cycle during which sampling occurred (CAMARGO et al., 1996).

Electrical conductivity is the total concentration of dissolved ionized substances in the water column (MIRANDA; GOMES, 2013).Streams from areas with little or no influence of urbanization demonstrated low rates of electrical conductivity, normally between 4 and 50 μS cm^{-1}, reaching up to 100 μS cm^{-1}downstreamand/or under the influence of recent rains (SÚAREZ; LIMA-JÚNIOR, 2009). Rates higher than those cited indicate urbanized waters.

Table 1. Mean and standard deviation (SD) values for data of water temperature (WT – °C); dissolved oxygen (DO - mg L^{-1}); hydrogen potential (pH); electrical conductivity (EC - μS cm^{-1}); turbidity (TU – N.T.U.), registered for each season (dry and rainy seasons) for the Ubatiba stream, in May, August, September and December 2014.

Seasons	Dry				Rainy			
Variables	May - 2014		Aug - 2014		Sep - 2014		Dec - 2014	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
WT (°C)	20.8	21.0	20.1	0.7	22.9	3.1	26.2	1.1
DO (mg L^{-1})	7.2	1.5	6.7	1.4	5.6	2.4	4.9	1.8
pH	6.8	1.1	6.9	2.0	7.3	0.6	7.4	0.4
EC (μS cm^{-1})	77.4	23.4	107.3	28.1	100.0	23.4	123.4	46.7
TU (N.T.U.)	1.6	0.7	0.6	0.4	1.4	1.1	1.6	1.2

Although the higher conductivity of the Ubatiba stream occurred during the rainy season, this parameter was higher during one month of the dry season (August = 107.3 µS cm^{-1}) when compared to the rainy season, which registered the highest conductivity (123.4 µS cm^{-1}). It may be inferred that intense degradation of organic matter of biological processes in May and August decreased salt and nutrient loads which, consequently, reduced electrical conductivity. Studies by Vercellino and Bicudo (2006) also identified this pattern of reduced conductivity during the dry season. In fact, August was not a typical dry season and conductivity did not follow the same expected pattern. High rates of electrical conductivity in the rainy season suggest that the stream showed anthropic influence, intensified by rainfall, typical of the season (FELIPE; SÚAREZ, 2010). Similar results were registered by Alves et al. (2012) who studied streams from the upper river Paraná, Brazil.

The Ubatiba stream presented high water turbidity with rates below those suggested by N.T.U. Although the Ubatiba stream featured low turbidity during the rainys season, higher rates were registered when compared to those in the dry season. This may be attributed to the production of sediments associated with margin erosion and to the variations in rainfall that occurred during the study period (MALUTTA et al., 2013).

Thecamoebians

Eighty-two taxa of testate amoebae were reported, belonging to 10 families: 33 taxa of Difflugiidae, 13 of Centropyxidae, 9 of Arcellidae, 9 of Lesquereusiidae, 7 of Euglyphidae, 5 of Trigonopyxidae, 2 of Cyphoderiidae, 2 of Trinematidae and 1 each of Hyalospheniidae and Nebelidae (Figure 3). The most representative families of testate amoebae registered at Ubatiba stream, namely, Difflugiidae, Centropyxidae, Lesquereusiidae and Arcellidae, have been highlighted as the most diverse in several studies developed in freshwater plankton (LANSAC-TÔHA et al., 2008).

Twenty-two out of the 82 taxa registered in Ubatiba were exclusively planktonic, distributed between nine genera. Fourteen taxa were exclusively associated to aquatic macrophytes and were distributed between seven genera. Forty-six taxa were present in the two biotopes under analysis and were distributed in 12 genera. Four exclusive genera, namely, *Apodera, Lagenodifflugia, Nebela* and *Trigonopyxis* were registered in the plankton (Figure 4).

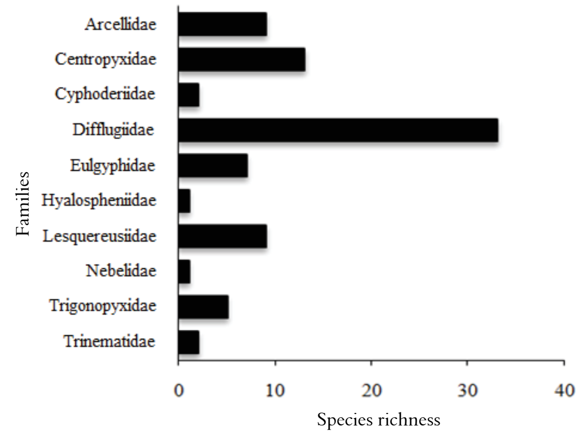

Figure 3. Species richness and families identified in Ubatiba stream.

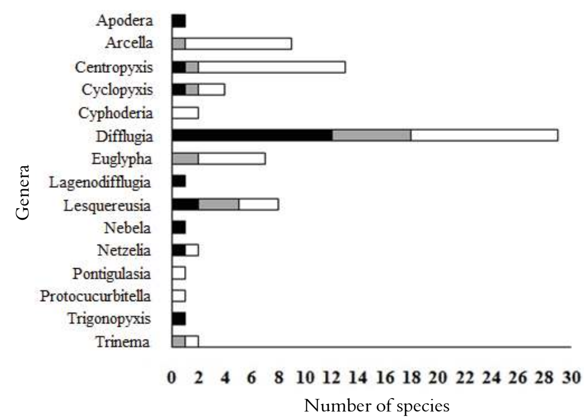

Figure 4. Richness of testate amoebae in distinct biotopes from the Ubatiba stream. Black bars = Plankton; Dark gray bars: Aquatic macrophytes; White bars = plankton and macrophytes.

The high species diversity of testate amoebae, observed in plankton and in aquatic macrophytes, suggests their adaptability to different aquatic environments favoring their movement between and establishment in the two biotopes. Differences in the occurrence of mesohabitats (lotic *vs.* lentic)in the Ubatiba stream have also contributed to the success of testate amoebae, corroborating studies by Lansac-Tôha et al. (2008), even if their studies were developed in large aquatic systems. According to these authors, the stream flow works as a collecting mechanism carrying the plankton and organisms associated with the marginal vegetation to the water column. According to Velho et al. (1999), it should be highlighted that the abundance of testate amoebae in planktonic samples is not only due to stochastic processes. In fact, these organisms produce gas vacuoles to fluctuation (STEPANEK; JIRI, 1958) and some species have special shapes with low-densities hells. These characteristics may allow access to these water bodies and to the planktonic environment.

The species diversity of planktonic testate amoebae from tropical streams may also be attributed to the lotic features of the systems. Streams generate a dynamic of nutrients and organic matter, determining trophic conditions and favoring the development of testate amoebae (PEREIRA et al., 2011). It has also been suggested that the teak of testate amoebae living in the plankton is composed of particles similar to grains of sand and frustules of diatoms to better adapt themselves to environmental dynamics and protection to re-suspension promoted by the lotic flow (MIRANDA; GOMES, 2013).

Lentic stretches or pools with reduced turbulence and abundant aquatic macrophytes provide a favorable environment to the development of testate amoebae to cope with a high heterogeneity of space and food, favored by reduced turbulence and increased accumulation of decomposed allochthonous material (VAN ONSEM et al., 2010).

The temporal results of testate amoebae diversity were influenced by differences in rainfall, i.e. the highest rates were observed during the dry season (Figure 5). Higher species diversity during the rainy season is the most common pattern in rivers, due to the increase of flow regime and re-suspension of sediments that increase the availability of nutrients from the margin region to the water column (COSTA, 2011). Nonetheless, in the Ubatiba stream, higher rates of species diversity were reported during the dry season (May and August), following decrease in rainfall and reduced water volume in the stream, generating a lentic regime that favored the development of testate amoebae.

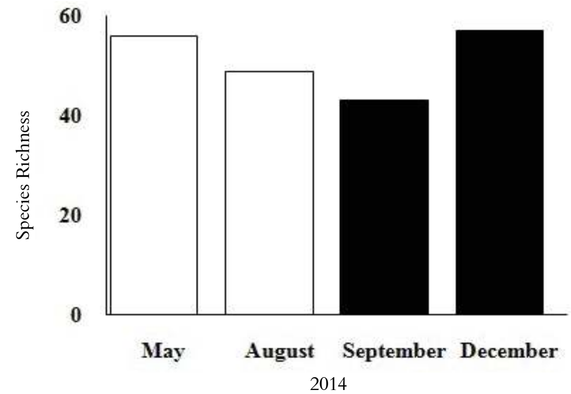

Figure 5. Testate amoebae richness in the Ubatiba stream between May and December 2014. Legend: White = dry season; Dark Gray = rainy season.

The species *Arcella vulgaris*, *Centropyxis aculeata*, *Euglypha rounda* and *Trinema enchelys* were recorded in all samples and thus 'constant' (100% of occurrence) in the two biotopes (macrophytes and plankton) (Table 4).The species *Arcella vulgaris* and *Centropyxis aculeata* are among the most widely distributed species in Brazil, recorded in different continental and coastal environments, in samples of plankton, stream sediment and associated with aquatic macrophytes (VELHO et al., 2000; LANSAC-TÔHA et al., 2008). According to Bento et al. (2005), *Euglypha rounda* and *Trinema enchelys* revealed a high degree of biological stability which allowed distribution and adaptation in different aquatic environments. It has been observed that the genus *Difflugia* was the most evident among the rare species recorded in the planktonic environment. On the other hand, *Euglypha* and *Lesquereusia* were the rare species in the aquatic macrophytes.

Table 4. Species list of testate amoebae registered in the Ubatiba stream and their rate constancy estimated by the Dajoz method (Dajoz, 1973) for the year 2014. Taxa were recorded associated to aquatic macrophytes (M), to plankton (P) and to the two biotopes (MP). Taxa were considered constant (+ +) when present in more than 50% of the samples; accessory (+) when they occurred between 50 and 25%, and rare (+) when present in less than 25% of the samples. (*)Taxa constant (100%) in the two biotopes.

Taxa	Ubatiba stream - 2014			
	Dry season		Rainy season	
	May	August	September	December
Hyalospheniidae family				
Apodera vas				P + +
Arcellidae family				
Arcella artocrea				M + + /P + +
Arcella conica	P + +	M + + + / P + +	M + / P + +	M + + +
Arcella costata			P +	M + + +
Arcella discoides	P +	MP +	MP + +	
Arcella gibbosa	MP +	M + + + / P + +	MP + +	M + + + / P + +
Arcella hemisphaerica undulata	M +	M +		
Arcella megastoma	MP + +	MP + + +	M + + / P +	MP + + +
Arcella rotundata alta	P +	MP +		M + +
*Arcella vulgaris**	MP + + +	MP + + +	MP + + +	MP + + +
Centropyxidae family				
*Centropyxis aculeata**	MP + + +	MP + + +	MP + + +	MP + + +
Centropyxisa culeata oblonga	P + + +	M + / P + +	M + / P + + +	M + + + / P + +
Centropyxis aerophila		P +	M + + / P +	MP + +
Centropyxis cassis	P + + +	M +		

Continue...

... continuation

Taxa	Ubatiba stream - 2014			
	Dry season		Rainy season	
	May	August	September	December
Centropyxis constricta	MP + + +	MP + + +	M + + / P + + +	MP + + +
Centropyxis discoides	P + + +	MP + +	MP + +	M + / P + + +
Centropyxis ecornis	P + +	M + + / P + + +	M + +	M + + / P+ +
Centropyxis gibba	P + +	M + +		M + + +
Centropyxis hirsuta	M + + / P +	M + +		
Centropyxis minuta	M +			P +
Centropyxis platystoma	P + +	MP + + +	M + + / P + + +	M + + + / P + +
Centropyxis spinosa	M +	P +	P +	M +
Centropyxis sylvatica	P + +			
Trigonopyxidae family				
Cyclopyxis arcelloides	P +			
Cyclopyxis impressa			P +	M +
Cyclopyxis intermedia		M +		M +
Cycloyxis kahli	M + + / P + + +	MP + + +	M + + / P + + +	MP + + +
Trigonopyxis arcula	P +			
Cyphoderiidae family				
Cyphoderia ampulla	MP + +	M + + + / P + +	MP + +	M + +
Cyphoderia trochus	MP + + +	M + + / P + +	MP + + +	M + + + / P + +
Difflugiidae family				
Difflugia acuminata	P + +		MP +	P ++
Difflugia acutissima	P +			
Difflugia bacillifera	M +			
Difflugia bryophila	P +	P +		
Difflugia capreolata	P +	M + +	MP +	M + / P + +
Difflugia corona	P +	MP +	P +	P +
Difflugia cylindrus				P + + +
Difflugia difficilis	M + +	M +		
Difflugia distenda	MP +	M + +	M + + / P +	M + + / P + + +
Difflugia elegans	MP + + +	MP + + +	M + + + / P + +	M + + + / P +
Difflugia globulosa				P +
Difflugia gramen	P +			
Difflugia kempnyi	P +			
Difflugia lacustris				P + +
Difflugia lanceolata	MP +		MP +	M + + / P + ++
Difflugia lebes				M +
Difflugia limnetica		M ++	P ++	MP +
Difflugia litophila		P +		
Difflugia lobostoma	P ++			
Difflugia mamilaris	P +		P +	
Difflugia limnetica	M +	P + +		M +
Difflugia nebeloides	M + / P + +	M + + / P +	M + / P + +	M + + / P +
Difflugia oblonga	M + + + / P + +	M + + / P +	MP + +	MP + + +
Difflugia penardi		M +		
Difflugia praestans				P +
Difflugia tenuis	M + +			
Difflugia urceolata		P +		P +
Difflugia venusta			M + + / P +	M + +/ P +
Lagenodifflugia vas				P +
Netzelia oviformis	MP +	M + + / P +	MP + +	MP + +
Netzelia wailesi			P +	
Pontigulasia compressa	M + + + / P +		P +	
Protocucurbitella coroniformis		P +	M + / P + +	M + + / P +
Eulgyphidae family				
Euglypha acanthophpra	MP + +	M + + + / P + +	M + + / P + + +	M + + +
Euglypha cristata	M + +	P + +	P +	M + + / P +
Euglypha denticulata	M + +	M + +		M +
Euglypha filifera	M + + / P + + +	MP + +	P + +	M + + +
Euglypha laevis				M +
Euglypha rotunda★	MP + + +	MP + + +	MP + + +	MP + + +
Euglypha tuberculata	P + +	M + + / P +	MP +	M +
Lesquereusiidae family				
Lesquereusia epistomium			M +	M +
Lesquereusia globulosa		P +		
Lesquereusia minor			M +	
Lesquereusia modesta	M + + + / P + +	M + + / P +	MP + + +	MP + +
Lesquereusia modesta minima				P + +
Lesquereusia ovalis	M +			
Lesquereusia spiralis	P +	M +		
Lesquereusia spiralis caudata				P + +
Quadrulella symetrica	M +	M +		M + / P +
Nebelidae family				
Nebela militaris			P + +	
Trinematidae family				
Trinema complanatum	M +	M +		M +
Trinema enchelys★	MP + + +	MP + + +	MP + + +	MP + + +

Testate amoebae densities were higher in aquatic macrophytes. The dry season revealed high densities for this biotope during May and August, although they tended to decrease with the proximity of the rainy season (Figure 6). Site P5 had the highest densities of testate amoebae (50 10^3 ind m^{-3}, in May) during the dry season, while P4 exhibited the lowest density in the rainy season (5 10^3 ind m^{-3}, in December).

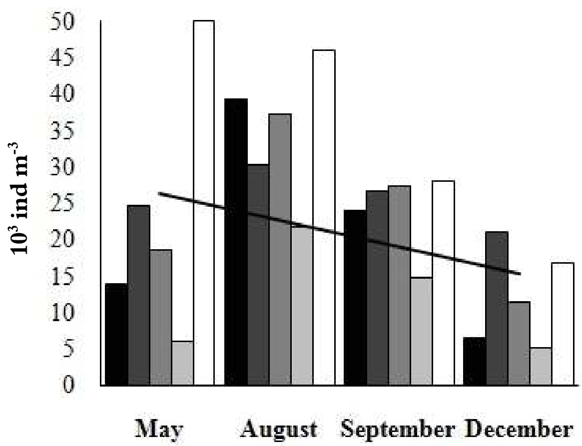

Figure 6. Densities of testate amoebae from Ubatiba stream registered in aquatic macrophytes, between May and December2014. May and August correspond to the dry season, while September and December correspond to the rainy season. Legend: sampling points; black = P1; dark gray = P2; gray: P3; light gray = P4; white = P5; The black line represents the linear trend.

Densities of testate amoebae registered in the plankton samples were lower than those in the aquatic macrophytes. The transition from the dry to the rainy season (August to September) was the period when densities varied markedly (Figure 7). However, similarly to aquatic macrophytes, there was a slight decreasing tendency in the density of testate amoebae during the rainy season. SiteP5 again showed the highest density rates (3510^3 ind m^{-3} in August) and site P1 showed the lowest density rates (3.5 10^3 ind m^{-3} in December).

Conclusion

The above is a pioneer study on the community of testate amoebae in the Ubatiba stream and on the organisms' relevance in the protozooplankton system.

Difflugiidae, Centropyxidae, Lesquereusiidae and Arcellidae were the most important families registered in the study. Only four species were registered in the two studied biotopes, macrophytes and plankton, or rather, *Arcella vulgaris*, *Centropyxis aculeata*, *Euglypha rounda* and *Trinema enchelys*.

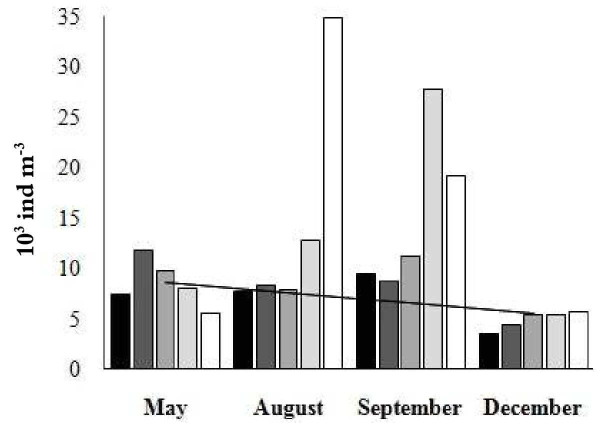

Figure 7. Densities of testate amoebae from Ubatiba stream registered in plankton, between May and December 2014. May and August correspond to the dry season while September and December correspond to the rainy season. Legend: sampling points; black = P1; dark gray = P2; gray: P3; light gray = P4; white = P5; The black line represents linear trend.

The highest diversity of the testate amoebae in the planktonic biotope was registered during the dry season. Four exclusive genera, *Apodera*, *Lagenodifflugia*, *Nebela* and *Trigonopyxis*, were registered during the dry season in this biotope. Following the pattern for the planktonic species, the testate amoebae from aquatic macrophytes presented the highest density during the dry season.

Results show the importance of this study to improve the knowledge on the diversity and geographical distribution of protists in Brazil, particularly in Rio de Janeiro, Brazil, where studies on these organisms in coastal environments are still underdeveloped.

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