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Effects of urbanization on the diversity of testate amoebae (Protist, Rhizopoda) in a stream of the southwestern Amazon basin (Igarapé São Francisco in Acre state, Brazil)

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Abstract. We investigated the diversity of testate amoebae in an urban stream located within the Igarapé São Francisco Environmental Protection Area in Acre, northern Brazil, during the dry season, and evaluated the factors mediating the structure of this protist community. We collected 108 water samples for the analysis of the testate amoeba community and the limnological variables at six sampling points on the stream, between July and September 2018. We used a Redundancy Analysis (RDA) to verify the influence of environmental variables on the protist community. We recorded 76 species of testate amoebae from eight families, with most records from the families Difflugiidae, Arcellidae, Centropydae and Netzeliidae. More than half (49) of the species were recorded in Acre for the first time. The abundance of the amoebae of the family Trigonopiridae was regulated by the dissolved oxygen concentrations and the pH, while that of the Netzeliidae, Difflugiidae and Lesquereusidae was influenced by the pH, chloride concentrations, and the depth and transparency of the water. In the case of the family Arcellidae, abundance was determined by the turbidity and transparency of the water and the nitrate concentrations, while that of *Centropyxis* sp. was associated with the concentrations of thermotolerant coliforms. These findings indicate that, while the São Francisco stream is subject to anthropogenic impacts, it still presents adequate conditions for these organisms in some of its stretches. The abundance of these amoebae was influenced primarily by the productivity of the system, as indicated by the high protist densities recorded in the areas in which primary productivity was highest. These findings support the use of these protists in studies that investigate the most appropriate indicator organisms that respond to anthropogenic impacts and shifts in environmental quality. The results of the present study demonstrated the importance of this aquatic ecosystem for the biodiversity of the study area, and the need to further expand our knowledge on the adaptations and interactions of the aquatic communities of the Amazon region.

Keywords: protists, plankton, abundance, biodiversity, community

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INTRODUCTION

As the characteristics of biological communities tend to vary systematically in response to shifts in environmental quality, they have long been used for the assessment of the impact of urban development on the environment (Munn et al. 2002). Communities of testate amoebae, a polyphyletic group of free-living protozoa, provide a particularly valuable model for this type of assessment (Smith et al. 2008; Rossi et al. 2016). These amoebae have shells and pseudopods and tend to be present in the plankton at very high densities (Lansac-Tôha et al. 2014). They are involved in a number of roles in different ecological processes, at varying scales, in the most diverse types of habitats, in which they occupy multiple trophic levels, and exploit different types of food, being related directly to the processes of energy flow and nutrient cycling (Mattheeussen et al. 2005; Adl and Gupta 2006; Gimenes et al. 2004; Jassey et al. 2013).

Several studies have described the effects of urbanization on aquatic systems, including changes in hydrography, increased concentrations of both nutrients and contaminants, and shifts in the morphology and stability of channels (Paul and Meyer 2001; Cunico et al. 2012; Harfuch et al. 2019; Barrios and Mello, 2022). To best understand the quality of an aquatic ecosystem, any analysis must consider the physical, chemical and biological characteristics of the water (Lobo et al. 2004; Lippert et al. 2020), which are linked directly to the structural patterns of the aquatic biota. Any such analysis can provide important insights into ecosystem function, which can be applied to the development of effective conservation and management strategies.

Due to their unique characteristics, streams are important ecosystems for the understanding of the mechanisms that determine the species richness and diversity of aquatic environments in the Amazon (Chaparro et al. 2014), and in particular, their vulnerability to impacts. Streams are highly susceptible to shifts in the hydrological regime and variations in current velocity, and changes in their connectivity with adjacent terrestrial systems, which define the patterns of exchange between flooded areas and the stream channel. Streams are also important ecosystems for the modelling of energy flow and nutrient cycling, through shifts in the abundance, composition and diversity of their biological communities, which create marked structural heterogeneity (Wantzen et al. 2011).

The diversity of testate amoebae may be influenced by the intrinsic characteristics of the local environment, related to the variation in environmental factors and the intensity of biotic interactions, which influence the physiology and behaviour of the organisms (Gering et al. 2003). These factors determine species richness and composition (Simões et al. 2013). The environmental characteristics of a stream can thus serve as a reliable predictor of the structure of aquatic communities, including those of the testate amoebae (Neiff 1996). Testate amoebae also respond rapidly to changes in environmental conditions, including anthropogenic impacts, reflecting the influence of environmental variables on community structure (Schonborn 1992).

Despite their importance for the structure and functioning of aquatic ecosystems, studies of testate amoebae in the Amazon region are still in their infancy, even though these organisms are among the most abundant in aquatic environments (Araujo et al. 2020; Arrieira et al. 2016; Laut et al. 2010, 2016). In particular, there are very few taxonomic inventories on the testate amoebae of this region, due to the lack of specialists in the taxonomy of the group (Debastiani et al. 2016). Even so, the available studies indicate the presence of a high diversity of species in the environments that have been surveyed.

In this context, any study that focuses on the testate amoebae of urban streams may not only contribute to the resolution of taxonomic questions, but may also help to define the principal predictors of the characteristics of these communities, given that urbanisation typically impacts environments significantly, often by reducing species diversity while also favouring the increasing dominance of the taxa that are more tolerant of the new environmental conditions (Ferraro et al. 2006). Given the importance of the testate amoebae in the planktonic communities of lotic environments and the lack of data on these protists, we investigated the diversity of these organisms in an urban stream in the southwestern Brazilian Amazon during the dry season. The results include 49 new species records for the Brazilian state of Acre.

MATERIAL AND METHODS

Study area

The samples were collected in the São Francisco stream, belonging to the Environmental Preservation Area (APA) of the São

Francisco stream, located in the state of Acre (Figure 1), in the western portion of the Amazon, it is one of the tributaries of the Acre river basin and encompasses part of the municipalities of Rio Branco and Bujari (09º55' S, 68º10' W to 10º00' S, 68º00' W). The stream has a total length of approximately 53.5 km and an area of approximately 44,767 hectares (Nascimento et al. 2013), about 10 % of its area integrates the urban area of the city of Rio Branco and 90 % the rural area of the cited municipalities.

Field sampling

Based on the length of the stream, six sampling stations were established equidistantly along its longitudinal axis, at intervals of approximately 10 km. These points were selected because they represent a gradient of land use, ranging from well-preserved areas with native riparian forest in the western headwaters (point 1) to highly impacted sites, with exposed soil (Figure 2). The points were sampled in July, August and September 2018 using horizontal and vertical hauls (Lampert 1989). A graduated bucket was used to filter 200 litres of water per sample through a 50 µm-mesh plankton net. The organisms collected in this way were preserved in 4 % formaldehyde buffered with calcium carbonate (Lampert 1989).

We measured the temperature of the water (°C), the concentration of dissolved oxygen (mg/L), the hydrogen-ion potential (pH), electrical conductivity (S.cm^{-1}), turbidity (NTU) and total dissolved solids (mg/L) using a multi-parameter probe (AHROM, model KR405). We also measured the transparency of the water using a Secchi disk, the depth (m), width (m) and flow rate (m/t) of the stream, and evaluated the vegetation cover, bottom substrate and the complexity of submerged and non-submerged habitats. The sinuosity of the channel and the leaf litter were evaluated using the integrity assessment protocol of Calisto et al. (2002). The concentrations of nitrate (mg/L), total nitrogen (mg/L), ammonia (mg/L), total phosphorus (mg/L), organic carbon total (mg/L) and total coliforms (NPM/100ml) were determined in a Flow Injection Apparatus (FossTecator, model FIASTAR 5000) using the standard multiple tubes method (APHA 1995). Based on these data, the sampling points were classified based on the protocol of Calisto et al. (2002) for the assessment of environmental integrity, following the gradient of increasing human interference from the source of the stream to its mouth.

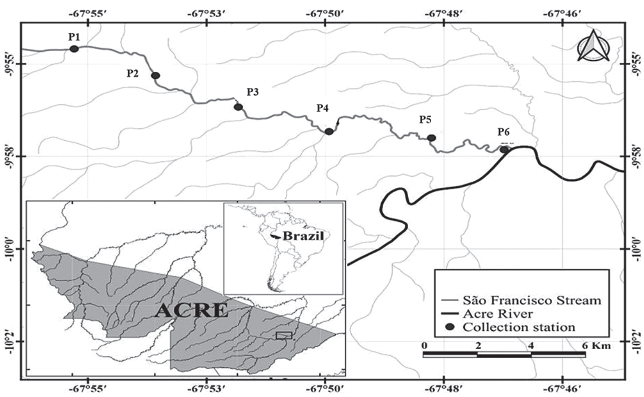

Fig. 1. Location of sampling points on the São Francisco stream in the municipalities of Rio Branco and Bujari, in Acre state, northern Brazil.

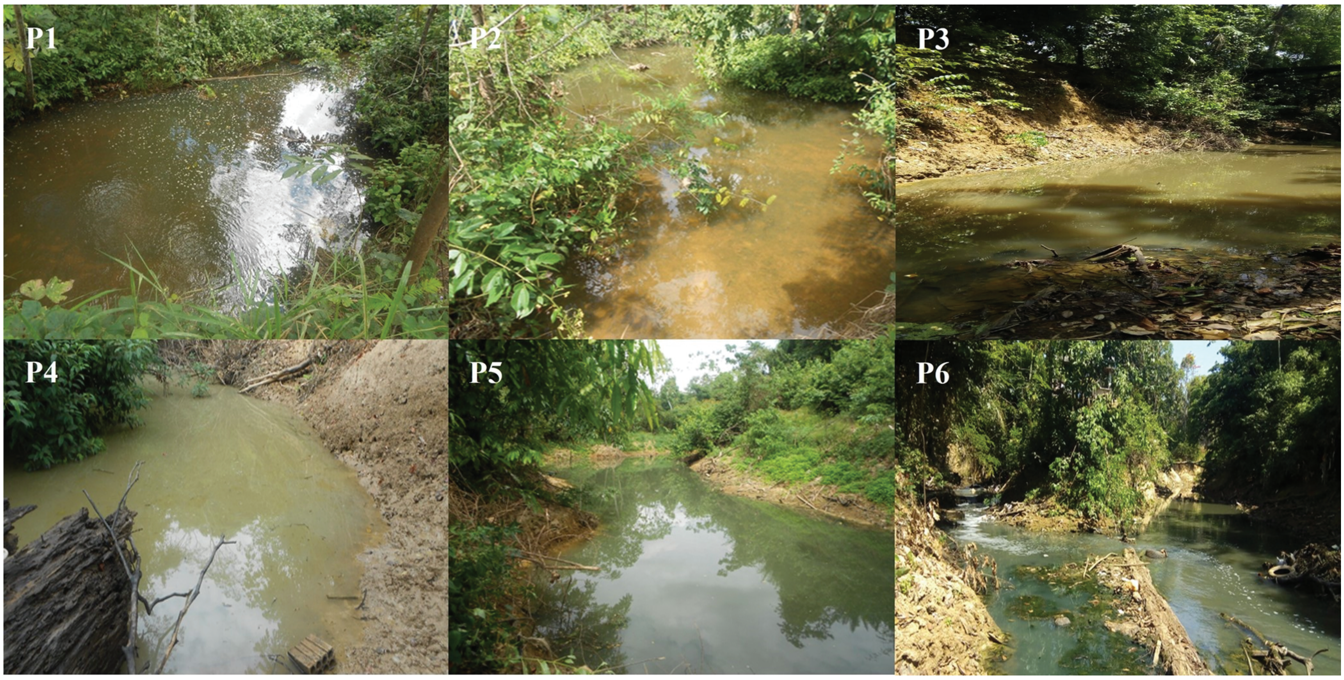

Fig. 2. Phytophysiognomy of sampling points in the São Francisco stream in the state of Acre, Brazil according to the degree of urbanization.

Laboratory analyses

The plankton samples were analyzed and deposited in the Limnology Laboratory of the Federal University of Acre in Rio Branco. The composition and density of the testate amoebae in each sample were determined using a Sedgewick-Rafter counting chamber and an optical microscope, considering subsamples of 6 mL collected using a Hensen-Stempell pipette. The density of the amoebae was expressed as individuals/m^{-3}. The taxa were identified based on Ogden and Hedley (1980), Velho et al. (1996), Velho and Lansac-Tôha (1996), Souza (2008) and Lansac-Tôha et al. (2008, 2014).

Data analysis

We applied a Principal Components Analysis (PCA) to summarize the data on the physical and chemical parameters of the water, that is, its temperature, dissolved oxygen concentrations, pH, electrical conductivity, turbidity, nitrate and ammonia, and the total nitrogen, phosphorus, organic carbon, dissolved solids and coliforms. With this, we reduced the volume of data to a set of uncorrelated orthogonal axes. We used the Broken-Stick criterion to select the most significant PCA axes (Jackson 1993), for which the variables were ln (x + 1) transformed.

The frequency of occurrence (Fo%) of the testate amoeba species was calculated by dividing the number of samples in which the species was identified by the total number of samples collected. Three categories of occurrence were considered here, based on the constancy index of Dajoz (1973): constant (> 50 % of the samples), accessory (25–50 %) and accidental (< 25 %).

We also calculated the species richness and mean density of amoebae for each sampling point. We evaluated the variation in species richness and density among the sampling points using the Kruskal-Wallis nonparametric analysis of variance (Kruskal and Wallis 1952). We then analyzed the Indication Values (Indvals) of the species to determine which species or genera were potential indicators of the different environments formed along the stream according to the level of anthropogenic impact (Dufrêne and Legendre 1997).

We used a Redundancy Analysis (RDA) to verify the relationship between the testate amoeba community and environmental variables (Legendre and Legendre 1998). For this, we compiled a matrix of the densities of the different taxa of amoebae and a second matrix of the abiotic variables (dissolved oxygen, pH, electrical conductivity, turbidity, nitrate, ammonia, and the total nitrogen, phosphorus). We ran all the analyses in the R software (R Core Team, 2022; version 4.1.2).

RESULTS

Limnological variables

We applied the rapid protocol for the assessment of habitat diversity, which allowed us to identify a gradient in the parameters of water quality between the sampling points located in the most preserved environments and those in the most impacted areas. The sampling points were classified as natural, altered or impacted according to the results of the protocol proposed by Calisto et al. (2002). Three of the six points (4, 5 and 6) were classified as impacted due to their low scores for the parameters analyzed (< 40), while two (2 and 3) were identified as altered environments, given their intermediate scores (41–60), and point 1 was considered natural, based on its score of over 60.

The environmental variables shifted within the study area in response to the level of urbanization. The waters of the least urbanized points (1, 2 and 3) had the lowest electrical conductivity, temperature, turbidity and transparency, and the lowest concentrations of ammonia, thermotolerant coliforms, nitrate, nitrite, phosphorus and chlorine in comparison with the most urbanized points (4, 5 and 6).

The Principal Components Analysis (PCA) segregated the sampling points based on their environmental variables (Figure 2). The first and second PCA axes were distinguished significantly ($p < 0.05$). The parameters of points 1–3 were thus correlated positively with the first PCA axis, associated primarily with conductivity, transparency, chloride, total phosphorus and ammonia, which explained 35.0 % of the variation. The second axis explained 17.2 % of the variation, with points 4 and 5 being correlated positively with this axis, and nitrate and nitrite contributing 52.2 % of all the variation in the data (Figure 3).

Species composition

We identified 76 taxa of testate amoeba in the 108 samples analyzed in the present study, representing eight families and 10 genera. The families Difflugiidae (28 taxa), Arcellidae (18 taxa) and Centropyxidae (10 taxa) were the most taxonomically diverse. Only 27 of the taxa identified here had been recorded previously in the state of Acre (Araújo et al. 2020), while 49 taxa were recorded in this state for the first time (Table 2, Figure 4).

Overall, 75 % of the species identified in the present study were classified as accidental (rare), based on the constancy index, with 15 % being considered accessory and 10 % constant (Table 1). Although the Difflugiidae is the family represented by the largest number of species, all the taxa of this family were classified as accessory or accidental. The families Centropyxidae, Arcellidae and Netzeliidae had many constant species, in particular *Arcella vulgaris undulata*, *Acella mitrata*, *Centropyxis aculeata*, *Netzelia corona* and *Netzelia gramen* (Table 1). Some of the taxa, such as *Arcella brasiliensis*, *A. discoide*, *A. megastoma*, *A. vulgaris* and *Centropyxis aculeata*, were recorded at all the sampling points. No

Table 1. Species of testate amoeba recorded in the present study and their frequency of occurrence at each sampling point, according to the Dajoz index (xxx = constant; xx = accessory; x = accidental). Species indicated with an asterisk (*) were recorded in the state of Acre for the first time.

TAXON	FREQUENCY OF OCCURRENCE AT SITE					
	1	2	3	4	5	6
ARCELLIDAE Ehrenberg, 1830						
**Galeripora artocrea* (González-Miguéns et al. 2021)	x	x	x			
Arcella brasiliensis (Cunha, 1913)	xx	xx	xx	xx	x	x
* *Galeripora catinus* (González-Miguéns et al. 2021)						
**Arcella crenulata* (Deflandre, 1928)		x	x			
**Galeripora dentata* (González-Miguéns et al. 2021)	x	x	x			
* *Galeripora discoide* (González-Miguéns et al. 2021)	xx	xx	x	x	x	x
**Arcella excavata* (Cunningham, 1919)			x			
**Arcella formosa* (Nicholls, 2005)		x		x		
**Arcella gandalfi* (Féres et al. 2016)	x	x				
**Arcella hemisphaerica* (Perty, 1852)	x	x	x	x	x	x
**Arcella hemisphaerica undulata* (Deflandre, 1928)	x			x	x	
Galeripora megastoma (González-Miguéns et al. 2021)	x	x	x	x	x	x
Arcella mitrata (Leidy, 1876)			xxx			
**Arcella roduntata aplanata* (Deflandre, 1928)			x			x
Galeripora rota (González-Miguéns et al. 2021)	x					
Arcella vulgaris (Ehrenberg, 1830)	x	x	x	x	x	x
**Arcella vulgaris undulata* (Deflandre, 1928)	x	x	xxx	x		
**Arcella vulgaris wailesi* (Deflandre, 1928)	x	x		x		
CENTROPYXIDAE Deflandre 1953						
Centropyxis aculeata (Ehrenberg, 1838)	xxx	xx	xx	x	x	x
Centropyxis aerophila (Deflandre, 1929)		x				
**Centropyxis carinata* (Chardez, 1964)				x	x	
Centropyxis discoides (Penard, 1902)			x		x	x
Centropyxis ecornis (Ehrenberg, 1841)	x	x	x		x	x
Centropyxis gibba (Deflandre, 1929)			x			
**Centropyxis hemisphaerica* (Barnard, 1875)			x			
Centropyxis marsupiformis (Wallich, 1864)	x		x			
Centropyxis spinosa (Cash, 1905)			x			
**Centropyxis sylvatica* (Deflandre, 1929)			x	x		
DIFLLUGIIDAE Awerintzew, 1906						
Protocucurbitella coroniformis (Gauthier-Lièvre and Thomas, 1960)		x				
**Pontigulasia bigibbosan* (Penard, 1902)				x	x	
**Difflugia acutissima* (Deflandre, 1931)	x	x				
**Difflugia biconcava* (Ertl, 1965)		x				
**Difflugia bidens* (Penard, 1902)	x		x			
Difflugia campreolata (Pénard, 1902)	x	x	x			
**Difflugia cylindrus* (Thomas, 1953)	x	x	x			
**Difflugia diafana* (Vucetich, 1987)	xx		x			
**Difflugia distenda* (Ogden, 1983)	x					
**Difflugia labiosa* (Wailes, 1919)	x	x	x			

**Difflugia lemani* (Blanc, 1892)	x					
**Difflugia limnetica* (Levander, 1900)	x					
Difflugia lithofila (Penard, 1902)	x	x	x			
**Difflugia lithoplites* (Penard, 1902)			xx	x		
**Difflugia lobostoma* (Leidy, 1879)	x		x			
**Difflugia longum* (Chardez, 1987)			x	x		
**Difflugia lucida* (Penard, 1890)	x	x	x			
Difflugia mamilaris (Pénard, 1899)	x	x	x	x		
**Difflugia oblonga* (Ehrenberg, 1838)	xx	xx	x			
Difflugia pyriformis (Perty, 1849)	x	x	x			
**Difflugia rubescens* (Penard, 1891)	x					
**Difflugia scalpellum* (Penard, 1899)	x		x			
**Difflugia schizocaulis* (Stepanek, 1952)	x					
**Difflugia sinuata* (Gauthier-Lièvre and Thomas, 1958)	x	x	x			
**Difflugia urceolata* (Carter, 1864)	x	x	x			
**Difflugia varians* (Penard, 1902)			xx			
**Difflugia venusta* (Penard, 1902)	x					
EUGLYPHIDAE Ogden, 1981						
**Euglypha strigosa* (Ehrenberg, 1871)	x					
LESQUEREUSIIDAE Jung, 1942						
Lesquereusia epistomium (Ehrenberg, Butschli, 1880)		x	X			
**Lesquereusia globulosa* (Gauthier-Lièvre and Thomas, 1959)	x					
Lesquereusia mimetica (Penard, 1911)	x	x	x			
Lesquereusia modesta (Rhumbler, 1895)	x	x	x			
**Lesquereusia modesta minima* (Van Oye, 1953)	x					
Lesquereusia spiralis (Ehrenberg, 1840)	x	x	x			
**Lesquereusia spiralis caudata* (Playfair, 1917)	x					
NETZELIIDAE Ogden, 1979						
Netzelia corona (Wallich, 1864)	xxx	xx	xx	x	x	x
**Netzelia danubialis* (Zivkovich, 1975)	x	x	x	x	x	
Netzelia gramen (Penard, 1902)	xx	xxx	xx	x	x	
**Netzelia mulanensis* (Yang et al. 2005)			xx	x	x	x
**Netzelia muriformis* (Gauthier-Lièvre and Thomas, 1958)	xx	x				
Netzelia oviformis (Cash, 1909)	xx	xx	x			x
Netzelia tuberculata (Wallich, 1864)	x	x	xx	xx	x	
Netzelia wailesi (Ogden, 1980)		x	xx	xx	x	
PHRYGANELLIDAE Jung, 1942						
Phryganella hemisphaerica (Penard, 1902)	x			x	x	x
TRIGONOPYXIDAE Loeblich and Tappan, 1964						
**Cyclopyxis aplanata* (Penard, 1911)		x	x			x
**Cyclopyxis lithostoma* (Bonnet, 1974)	x		x	x	x	x
**Cyclopyxis impressa* (Daday, 1905)	x	x				x
**Cyclopyxis intermedia* (Kufferath, 1932)	x		x			

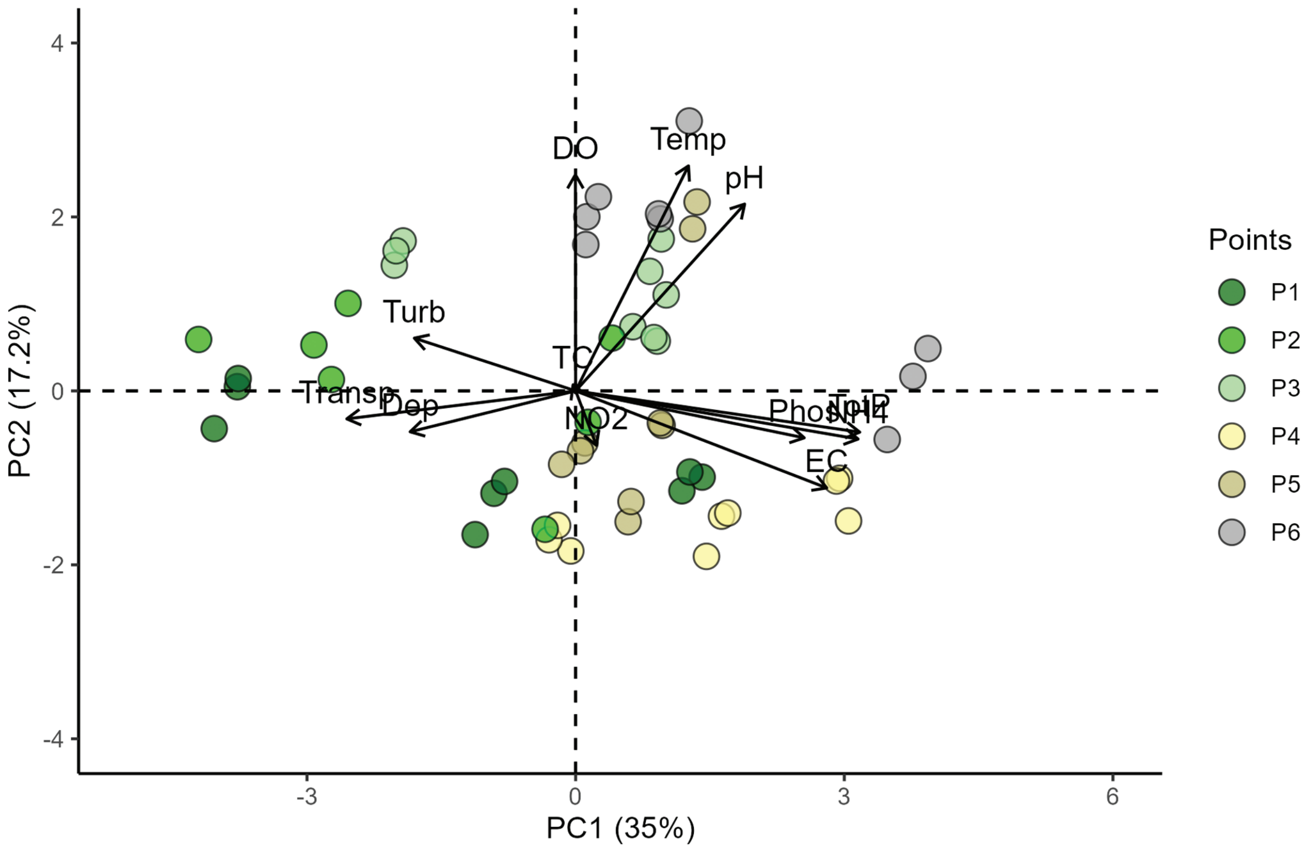

Fig. 3. Plot of the results of the Principal Components Analysis (PCA) of the environmental variables of the São Francisco stream in Rio Branco e Bujari, Acre (Brazil): TC = Thermotolerant Coliforms; EC = Electrical Conductivity; pH = Hydrogen potential; DO = Oxygen; TotP = Total Phosphorus; Phos = Phosphate; Temp = Temperature; Transp = Transparency; Turb = Turbidity; FR = Flow Rate; Dep = Depth; NH_4 = Ammonia; NO_3 = Nitrite; NO_2 = Nitrate.

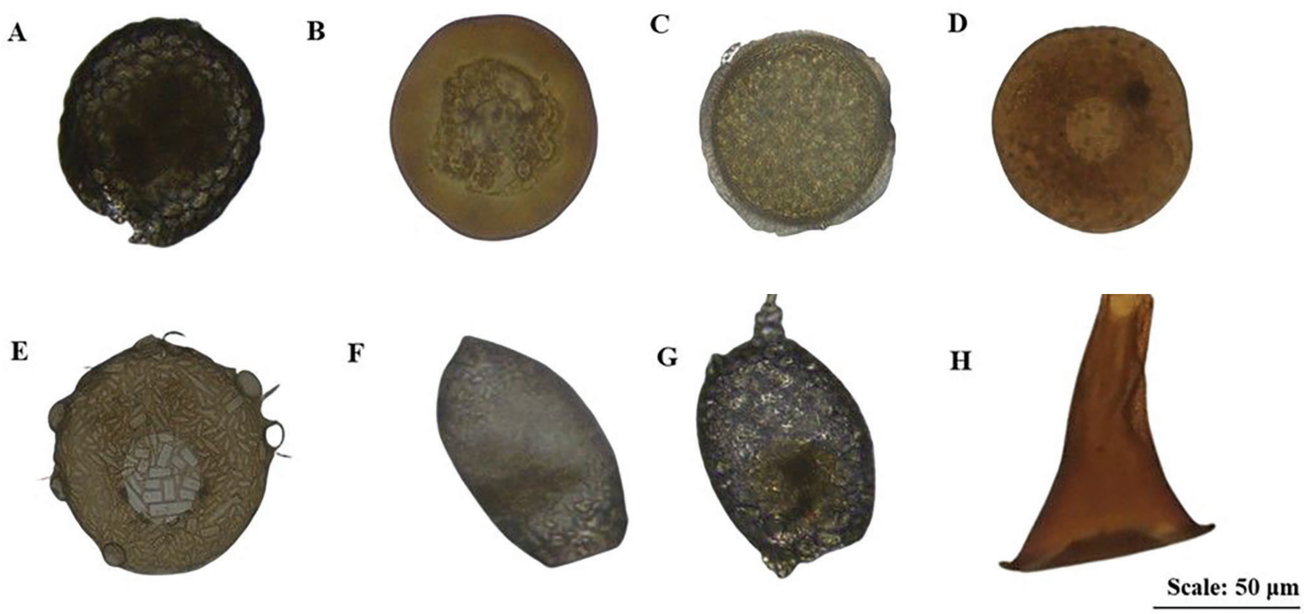

Fig. 4. The most abundant species of testate amoeba recorded in the present study: (A) *Netzelia corona*, (B) *Arcella vulgaris*, (C) *Arcella brasiliensis*, (D) *Arcella discoide*, (E) *Centropyxis aculeata*. Examples of the species of testate amoeba recorded in the state of Acre for the first time: (F) *Difflugia distenda*, (G) *Difflugia sinuata*, (H) *Arcella gandalfi.*

significant variation (Kruskal-Wallis: $H = 5.47$, df = 5, p = 0.360) was found in species richness among the sampling points, however (Figure 5).

The occurrence of the euglyphids and lesquereusids was restricted to the sampling points with the most preserved environments, that is, points 1–3, in the middle and upper stretches of the stream (Figure 1). Many taxa, including *Lesquereusia spiralis caudata*, *Euglypha strigosa*, *Arcella rota*, *Centropyxis aerophila*, *Protocucurbitella coroniformis* and *Difflugia venusta*, were recorded at only one sampling point (Table 1).

The mean density of the different amoeba species varied significantly among the sampling points (df = 5, p = 0.000). The highest densities were recorded at points 1, 2 and 3, in particular for *Arcella vulgaris*, *Netzelia corona*, *Netzelia gramen* and *Centropyxis aculeata*, whereas the lowest densities were recorded at points 4–6, with only two species – *Arcella megastoma* and *Difflugia urceolata* – being most abundant at these points. *Arcella discoides* was classified as abundant at all sampling points, while *Centropyxis aculeata* and *Netzelia corona*, were considered to be dominant at points 1–3, and abundant at all other points (Figure 5).

The Indication Value (IndVal) reflects the specificity of an amoeba for a given sampling point, that is, its propensity for specific types of environments. Significant values were recorded only at four points (1, 2, 3 and 6). The most relevant point was point 1, with eight indicator species, while the other three points each had five indicator species (Table 3).

Testaceous amoeba community composition varied significantly between sampling points (ANOVA: F = 3.11, Df = 0.13, P = 0.001). The RDA arranged the points according to their environmental parameters, which reflected the way in which species composition was related to these variables (Figure 6), with the same pattern observed in the PCA (Figure 3). Environmental variables explained 37 % of the variation in amoeba abundance, with RDA1 = 27.73 and RDA2 = 9.41.

The abundance of the trigonopirids was regulated by the concentration of dissolved oxygen and pH, while that of the netzeliids, diflugiids and lesquereusids was related to the chloride concentration, and the depth and transparency of the water. By contrast, the abundance of the arcellids was influenced by the nitrate concentrations, and the transparency and turbidity of the water, and the genus *Centropyxis* by the concentrations of thermotolerant coliforms.

According to these analyses, the density of testate amoebae recorded in the present study was lower when the total phosphorus and nitrogen concentrations were at their lowest (Figure 6). The nitrite and nitrate concentrations were the principal variables that explained the distribution of the genus *Arcella* in the present study. Increasing dissolved oxygen, conductivity and temperature had a negative effect on the density of the genera *Lesquereusia* and *Difflugia*. As dissolved oxygen is closely related to pH, it was assumed that these variables had a similar influence on the distribution of the amoebic taxa (genera or species) recorded in the present study.

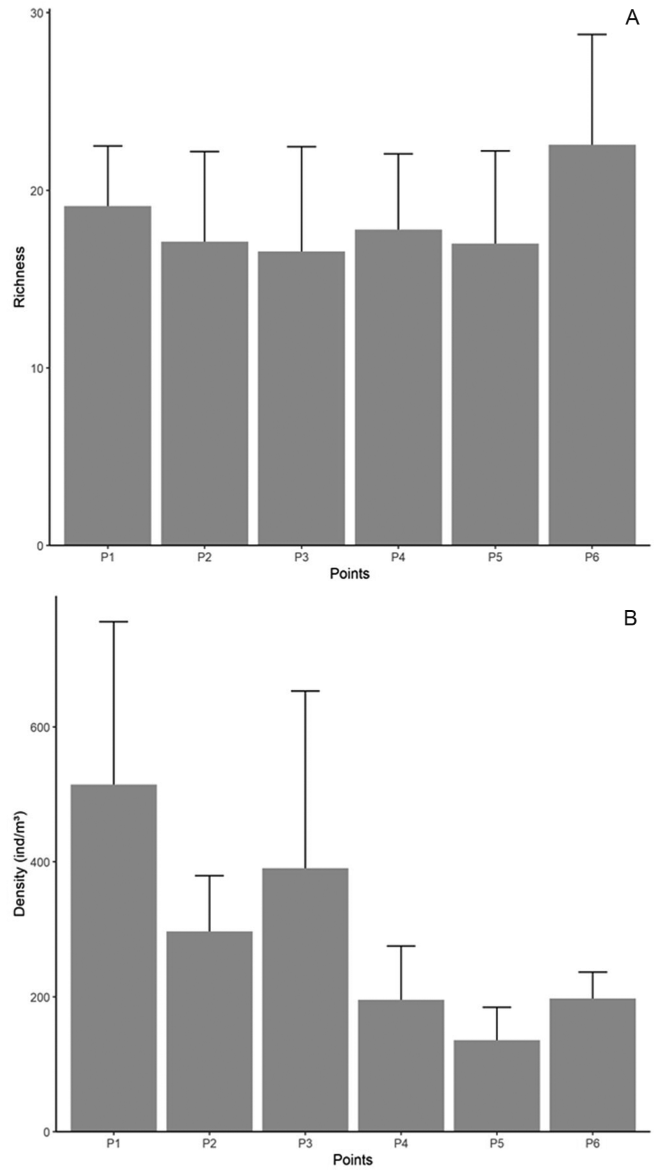

Fig. 5. Variation in the mean species richness (A) and density (B) of testate amoebae recorded among the different sampling points on the São Francisco stream in Acre state, northern Brazil.

Table 3. Results of the IndVal analysis of the association of testate amoeba species with the sampling points, showing only the species with a significant ($p < 0.05$) association, as indicated by a run of 10,000 Monte Carlo permutations.

Species	Indication Value (IndVal)	Rank	p	Sampling points
Lesquereusia globulosa	1	0.751	0.00	1, 2
Netzelia mitrata	1	0.721	0.02	1, 2, 3
Arcella gandalfi	2	0.719	0.00	1, 6
Arcella rota	2	0.707	0.00	1, 6
Netzelia muriformis	2	0.703	0.00	2, 3
Arcella dentata	1	0.670	0.00	1, 2, 6
Centropyxis marsupiformis	2	0.617	0.01	1
Arcella crenulata	2	0.585	0.02	2, 3
Euglypha strigosa	1	0.577	0.02	1
Netzelia danubialis	2	0.577	0.02	3
Difflugia venusta	2	0.577	0.02	6
Difflugia cylindrus	2	0.544	0.04	6
Arcella artocrea	2	0.535	0.04	3

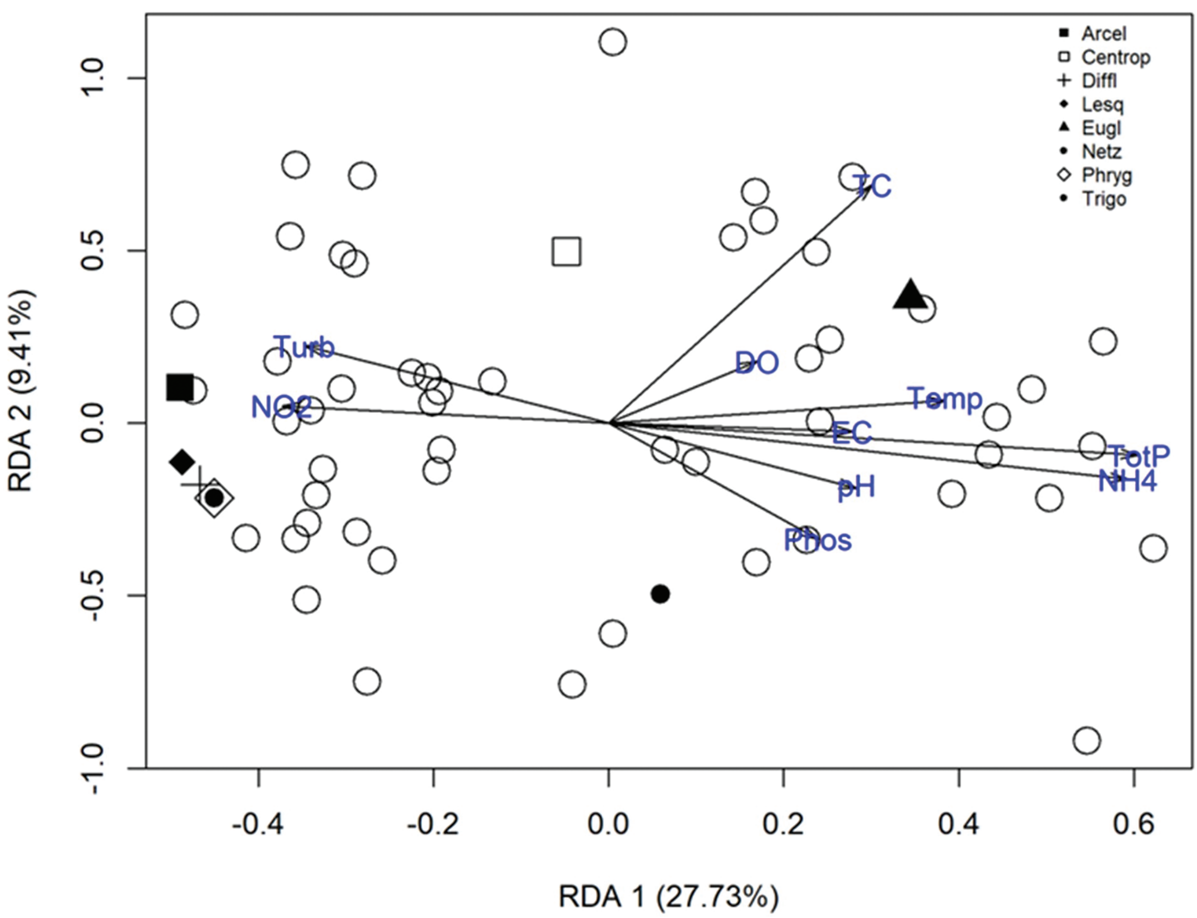

Fig. 6. Biplot of the first two axes of the Redundancy Analysis of the scores of the streams according to the abiotic variables (TC = Thermotolerant Coliforms; EC = Electrical Conductivity, DO = Oxygen; pH = Hydrogen potential; TotP = Total Phosphorus; Temp = Temperature; Turb = Turbidity; NH_4 = Ammonia; NO_2 = Nitrate), the legend explains the symbols corresponding to the family names.

DISCUSSION

The results of the present study indicate clearly that environmental conditions had a major influence on the structure of the testate amoeba community of the São Francisco stream. In general, community structure varied systematically among the different sampling points. The evidence clearly indicates the role of environmental conditions as a determinant of the characteristics of the amoebic community, including its species richness, diversity and abundance.

The most common families recorded in the present study were the Diflugiidae, Arcellidae, Centropyxidae and Netzeliidae. The Arcellidae and Centropyxidae were also the predominant families in previous studies of testate amoebae that focused on other aquatic environments in Brazil (Lansac-Tôha et al. 2009; Leão et al. 2009; Alves et al. 2012; Mansano et al. 2013; Arrieira et al. 2015; Arrieira et al. 2016; Silva et al. 2020; Lippert et al. 2020, Rocha et al. 2021). However, few studies of these organisms have been conducted in low-order streams and urban environments. In one of these studies, Fulone et al. (2005) identified 21 taxa in an urban river in southeastern Brazil. In the southern Brazilian state of Paraná, Lippert (2013) and Lippert et al. (2020) conducted comprehensive surveys of first-order streams under anthropogenic impact, and recorded 22 and 19 taxa of testate amoebae, respectively. These authors concluded that the low species richness recorded in these studies was the result of the negative impacts of human intervention in the study environments. However, this conclusion was not supported by the results of the present study, which recorded a total of 76 taxa in the São Francisco stream. This may be at least partly due to the fact that the dynamics of nutrient cycling and the response of streams to shifts in land use and occupation are still largely unknown in the Amazon region, in comparison with southern Brazil.

As shown in the present study, *C. aculeata*, *Centropyxis ecornis* and *A. discoides* are among the most common and widespread testate amoebae in Brazil, having been recorded in an ample range of freshwater environments, where they have been recorded in the plankton, sediments and in the fauna associated with aquatic macrophytes (Lansac-Toha et al. 2000, 2007, 2014; Velho et al. 2000; Picapedra et al. 2018, 2019; Silva et al. 2020; Rocha et al. 2021). The Arcellidae, Centropyxidae and Netzeliidae were the most abundant families in all samples. In addition to being abundant, *C. aculeata* and *N. corona* were the only dominant species. In fact, *C. aculeata* is an opportunistic species often found in impacted river systems (Patterson and Kumar 2000), and is tolerant of environments contaminated by industrial effluents. This tolerance may be attributed to the compression of its carapace, which is considered to be an adaptation to minimize resistance and facilitate floating in the water column (Lampert and Somer 1997). The numerical dominance of this species in the testate amoeba community has also been verified in other aquatic systems in Brazil (Lippert 2013; Lansac-Tôha et al. 2014, Silva et al. 2020).

The abundance and dominance of *N. corona* may be associated, in turn, with factors such as the depth, conductivity and pH of the water, and the presence of riparian vegetation and primary production (Ndayishimiye et al. 2019; Ndayishimiye et al. 2020). Laminger (1973) found that species with hemispherical or rounded xenosomal tests tend to be more abundant, at different depths, and that the presence of riparian vegetation supports the survival of the smaller testate amoebae. The abundance of the euglyphids and lesquereusids was related to low phosphorus and nitrogen concentrations, which may be related to nutritional limitations on carapace development and other functional traits. In fact, high phosphorus concentrations may restrict the occurrence of some species of testate amoeba (Mieczan 2012). Vogt et al. (2013) also demonstrated that phosphorus and nitrogen may function as limiting factors or environmental filters between different compartments of the plankton and may also influence the functional characteristics of the testate amoebae (Schwind et al. 2016).

The availability of nutrients is an important abiotic factor that controls the distribution of protist populations in different ecosystems. A number of studies have found a positive correlation between the abundance of testate amoebae and nutrient concentrations (Velho et al. 2005), which indicates that the availability of resources is a fundamental determinant of the distribution and abundance of these organisms in aquatic systems during periods of low water. This is consistent with the abundance parameters recorded in the present study, which were associated with the sampling points with the highest concentrations of nutrients, as highlighted by the RDA. This nutrient input results in an increase in the primary productivity of the plankton (Bonecker et al. 2013), which may have supported an increase in the biological diversity of the testate amoeba community identified during the present study.

The species-rich arcellids had the lowest scores on axis 2 of the RDA, which is related to phosphorus and nitrogen concentrations. A greater richness and abundance of species was recorded at the points with the highest concentrations of dissolved phosphate and nitrogen, which is consistent with the findings of Buosi et al. (2011), who demonstrated an increase in species richness in response to an increase in nutrient concentrations in an aquatic environment in southern Brazil. The results of the present study also revealed that dissolved oxygen concentrations, and the electrical conductivity and temperature of the water explain the variation in the abundance and composition of testate amoebae, given that the species richness and abundance of some genera, such as *Lesquereusia* and *Difflugia*, decreased as these environmental parameters increased. A similar pattern was observed by Ndayishimiye et al. (2019), who showed that the abundance and biomass of testate amoebae responded most decisively to shifts in water temperature, which reflects the potential of these amoebae for the quantitative reconstruction of variation in temperature.

Overall, then, the present study found systematic relationships between environmental conditions and the characteristics of the testate amoeba community in the study stream. A number of the factors assessed here, including conductivity, pH and temperature, dissolved oxygen, phosphorus, nitrogen and ammonia concentrations, and the depth of the stream and the presence of riparian vegetation, were identified as potential determinants of the diversity of amoebae in the sub-basin of the São Francisco stream. In particular, the abundance of testate amoebae was influenced strongly by the productivity of the system, given that the density was greatest at the points at which primary productivity was highest. In addition to the differential responses of the most common species or genera to environmental variables, the present study showed that, while all the sampling points were located within the same sub-basin, they presented major differences in species composition and abundance, reflecting the spatial variation in the nutrient concentrations in this body of water, and possibly also land use, given that we recorded reduced species richness in the urban environments, which implies that differences in the taxonomic structure of the communities can be predicted by land use and vegetation cover.

The findings of our study reinforce clearly the need for further research in well-preserved environments. The testate amoebae proved to be a potentially valuable indicator group for the assessment of anthropogenic impacts and environmental quality. The study also revealed the importance of the São Francisco stream for the aquatic biodiversity of the study area, and the need to further expand our understanding of the adaptations and interactions of aquatic communities in the Amazon region.

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