Assemblage of testate amoebae (Protozoa, Rhizopoda) associated to aquatic macrophytes stands in a marginal lake of the São Francisco river floodplain, Brazil

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ABSTRACT. The composition and abundance of testate amoebae fauna in the littoral region of Cajueiro lake were studied during two years. The lake, marginal to São Francisco river, has a great quantity of aquatic plants and low depth. High richness and abundance of Protozoa-Rhizopoda among the macrophytes roots and leafs were reported. Some species were more abundant during distinct periods. In a total of 51 taxa five are new registers for Brazil.

Key words: aquatic Protozoa, testate amoebae, marginal lakes, São Francisco river, Southeastern Brazil.

RESUMO. Tecamebas (Protozoa, Rhizopoda) associadas a macrófitas aquáticas em uma lagoa marginal da planícies de inundação do rio São Francisco, Brasil. A composição e abundância da fauna de tecamebas foi estudada na região litorânea da lagoa Cajueiro durante dois anos. A lagoa é marginal ao rio São Francisco e possui grande quantidade de plantas aquáticas e pouca profundidade. Foi constatada uma alta abundância e riqueza de espécies de tecamebas entre as raízes e folhas das macrófitas. Algumas espécies atingiram seus ótimos de abundância em distintos períodos sazonais de coleta. Do total de 51 táxons identificados, cinco correspondem a novos registros para o Brasil.

Palavras-chave: protozoários aquáticos, tecamebas, lagoas marginais, planície de inundação, rio São Francisco, Sudeste do Brasil.

Marginal lakes, which occur on the floodplains of large tropical rivers, are common in the middle São Francisco river. The volume of these lagoons varies significantly during the year due to the flooded area and depth, both depending on the season, and to the intensity of the hydrological cycle in the drainage basin. During the rainy season (November to March) the lagoons receive water from temporary streams and channels connected to the São Francisco river. As the dry period in the studied area is long, lasting generally from April to October, and temperatures are high, many lagoons dry up.

A characteristic of these ecosystems is the constant presence and abundance of emergent floating and rooted aquatic plants. The development of these aquatic plants accompanies the hydrological cycle imposed on the environment. Thus a senescent phase during the dry season and rapid growth during the flood period occur. Macrophytes are very efficient and well adapted to the environment since they present a high production of biomes during favorable periods and become sources of organic material during the dry season (Esteves, 1988). The availability of plant detritus, ecological niches and high temperatures cause a high aquatic metabolism which characterizes the littoral zone as the richest in species and the most productive region of these water bodies (Wetzel, 1990).

The characteristic morphology and structures of these plants include the development of stems, leaves and roots that form multi-niche mats appropriate for the colonization of micro fauna and flora (Dioni, 1974). The testate amoebae take advantage of these microhabitats formed by the macrophyte stands. It is here that the decomposition process of detritus is intense, periphyton flourishes, small invertebrates and invertebrates find shelter and other decomposers such as bacteria, ciliates and fungi proliferate (Hardoim, 1997).

The free-living protozoa community plays a functional role in the aquatic biotopes (Finlay and Esteban, 1998). Since protozoa are small and have

accelerated growth rates, they control the growth of bacteria and fungi, besides being a prey to invertebrates and even to some aquatic vertebrates. According to analyses of stomach contents, Hardoim and Heckman (1996) report that testate amoebae were the preferred item in the diet of several fish species in the Pantanal.

Although there is a growing number of studies on the ecology, occurrence and geographical distribution of testate amoebae, they are still scarce in Brazil. Further, according to Lansac-Tôha *et al.* (2000) and Velho *et al.* (2000), these studies tend to be restricted to only a few geographical regions. In floodplain systems studies on testate amoebae are restricted to those of Dabés (1995), who examined plankton samples from marginal lagoons in the São Francisco river valley; Lansac-Toha *et al.* (1997), Velho and Lansac-Toha (1996), Velho *et al.* (1996, 1999) who examined plankton samples from lakes in the floodplain of the Upper Paraná River, and those of Hardoim and Heckman (1996) and Hardoim (1997), who examined plankton samples, benthic samples and samples of fauna associated with macrophytes in the Pantanal of Poconé, state of Mato Grosso.

Given the important role that testate amoebae may play in the metabolism of inland waters and the scarcity of taxonomical and ecological studies in Brazil, the object of this study was to characterize the composition and abundance of assemblages of testate amoebae associated with aquatic macrophytes stands and to evaluate the relative importance of the group compared to other invertebrates associated with aquatic macrophytes, in a marginal lake of the middle São Francisco river floodplain, state of Minas Gerais, Brazil.

Material and methods

Sampling was undertaken in a lagoon situated in the Parque Estadual da Lagoa do Cajueiro (State Park of Cajueiro Lake), inaugurated on October 8, 1998, under the jurisdiction of the Instituto Estadual de Florestas (IEF/MG)(Forests State Institute), in the state of Minas Gerais. The park spans an area of 20,500 hectares and includes eleven marginal lagoons on the floodplain of the middle São Francisco river in the municipality of Matias Cardoso, in the same state (Figure 1). The perennial Cajueiro lake (44º 00' 16"W; 15º 02'13" S) is the largest, about 3 kilometers long and 150 meters wide. Area varies between 0.22 to 0.48 km². While maximum depth is 3.5 m, depth at the littoral zone varies between 0.30 e 1.80 m.

Figure 1. Map of the area under analysis

There are aquatic macrophytes at all the margins of the lake. *Eicchornia azurea is* predominant, followed by *Egeria* sp. and *Nymphoides humboldtiana*. *Najas conferta,* a rooted and submersed species, occurs in the limnetic region.

Samples were collected to evaluate the "effects" of different seasons on the testate amoebae assemblages. Two samples were taken during the dry season, in September 1995 and October 1996, and two samples during the rainy season, in March 1996 and March 1997. On each sampling date, samples were taken from two sites in the littoral region of the lagoon on opposite margins.

Plastic trays measuring 42.0 x 26.5 x 8.0 cm were placed under or among the aquatic macrophytes, as described by Dioni (1967), and filled with water. Water collected in 8 - 10 full trays was filtered through a 35 μ mesh net and collected in a graduated pail to measure the sampled volume. The material concentrated in the mesh was fixed in 5% formaldehyde for further examination on the assemblage of testate amoebae.

At least two 1ml sub-samples were counted in a Sedgwick-Rafter counting chamber and the mean number of individuals was expressed per cubic meter. All larger organisms, such as crustaceans, gastrotriches and nematodes, were placed in 5ml chambers and counted under a stereomicroscope.

Testate amoebae were divided into dominant, frequent and rare categories according to the frequency and abundance of the different taxa:

Dominant (***) are taxa which were very abundant and present in all samples; mean densities equivalent to more than 10.000 ind/m³;

Frequent (**) are taxa which were frequent but not very abundant; their mean densities was less than 10.000 ind/m³;

Rare (*) are taxa which are sporadic and not abundant; their mean densities was less than 5.000 ind/m^3.

Results and Discussion

Testate amoebae formed the most abundant group of invertebrates in the littoral region of Cajueiro Lake. The mean density was 1,687 x 1,000 ind/m^3, which represents 40% of the sampled community (Figure 2). Hardoim and Heckmann (1996) discuss the results of studies undertaken in tropical biotopes, in Minas Gerais and Mato Grosso, where testate amoebae were predominant over ciliates, as a contrast to what occurs in temperate regions. In samples of the littoral zone in floodplain lakes and lagoons of the upper Paraná river, Velho *et al.* (1999) found that testate amoebae were often more frequent than the typically zooplanktonic groups such as rotifers and microcrustaceans.

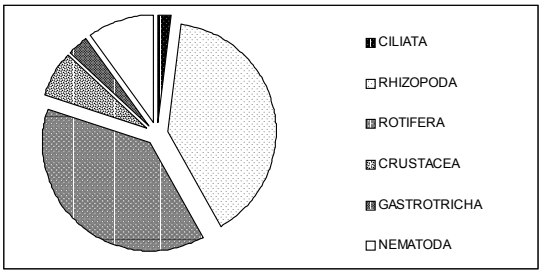

Figure 2. Mean relative abundance of microfauna groups in the littoral region of the Cajueiro lake from September 1995 to March 1997

A total of 51 taxa, or 48 species and 2 subspecies, were registered in the littoral region of Cajueiro lagoon. These represent 6 families and 12 genera of the class Lobosia, 2 families and 2 genera of the class Filosia. The family Difflugiidae was most numerous with 23 taxa. (18 were of the genus *Difflugia*), followed by Centropyxidae (8 taxa) and Arcellidae and Lesquereusiidae with 6 taxa each. According to several studies the first three families are frequently the most specious and abundant in plankton samples (Lansac-Tôha *et al.,*1997; Velho *et al.,* 1999).

Many species registered in this study have been previously reported in Brazil (Green, 1975; Dabés,1995, 1999a and b; Velho *et al*., 1996; Velho and Lansac-Tôha, 1996; Lansac-Tôha *et al.,* 1997 and Hardoim, 1997). However *Centropyxis* sp 1, *Cyclopyxis* sp 1 and *Difflugiella* sp 1 (Table 1) showed different morphological and anatomical structures from those found in the literature and, thus, deserve further study. Five other species which have not been previously reported in Brazil were identified: *Difflugia bicornis, D. hydrostatica, D. microclaviformis, D. microstoma* and *Protocucurbitella coriniformis* (Figure 3).

Table 1. List of tecamoebae species registered in Cajueiro lake and their relative frequency (*** abundant, ** frequent, * rare)

FAMILY ARCELLIDAE
Arcella conica (Playfair 1917)**
Arcella costata Ehrenberg 1847 *
Arcella discoides Ehrenberg 1843***
Arcella hemisphaerica f. *undulata* Deflandre 1928*
Arcella megastoma Pénard 1902*
Arcella vulgaris Ehrenberg 1830*
FAMILY CENTROPYXIDAE
Centropyxis aculeata (Ehrenberg, 1838) ***
Centropyxis aculeata var. *tropica* Deflandre, 1929 **
Centropyxis discoides (Pénard, 1890) ***
Centropyxis ecornis (Ehrenberg, 1841)**
Centropyxis hirsuta Deflandre, 1929 **
Centropyxis marsupiformis (Wallich, 1864) *
Centropyxis spinosa (Cash, 1905) **
Centropyxis sp *1****
FAMILY TRIGONOPYXIDAE
Cyclopyxis kahli (Deflandre, 1929) **
FAMILY DIFFLUGIIDAE
Cucurbitella madagascariensis Gauthier-Lievre and Thomas, 1960 *
Difflugia acuminata Ehrenberg, 1938 **
Difflugia acutissima Deflandre, 1931 *
Difflugia angulostoma Gauthier-Lievre and Thomas 1958**
Difflugia bicornis Pénard, 1980*
Difflugia capreolata Pénard, 1902 **
Difflugia corona Wallich, 1964 ***
Difflugia elegans Pénard, 1890 ***
Difflugia gramen Pénard, 1902 ***
Difflugia hydrostatica Zacharias1897 **
Difflugia lithophila Penard, 1902 **
Difflugia lobostoma Leidy, 1879 ***
Difflugia lobostoma multilobata Gauthier-Lievre and Thomas 1958***
Difflugia microstoma (Thomas, 1954)**
Difflugia microclaviformis (Kourov, 1925) *
Difflugia muriformis Gauthier-Lievre and Thomas, 1958*
Difflugia oblonga Ehrenberg, 1838*
Difflugia pseudogramen Gauthier-Lievre and Thomas 1958***
Difflugia urceolata Carter, 1864 **
Pentagonia maroccana Gauthier-Lievre and Thomas 1958*
Pontigulasia compressa (Carter, 1864) *
Protocucurbitella coroniformis Gauthier-Lievre and Thomas 1958***
Suiadifflugia multipora Green, 1975**
FAMILY LESQUEREUSIIDAE
Lesquereusia epistomium Penard, 1902 **
Lesquereusia modesta Rhumbler, 1896 *
Lesquereusia spiralis (Ehrenberg, 1840) ***
Netzelia oviformis (Cash, 1909) ***
Netzelia tuberculata (Wallich, 1964) ***
Netzelia wailesi (Ogden, 1980) **
FAMILY CRYPTODIFFLUGIIDAE
Difflugiella sp*1**
FAMILY EUGLYPHIDAE
Euglypha acanthophora (Ehrenberg, 1841) ***
Euglypha denticulata Brown, 1912 *
Euglypha filifera Penard, 1890**
Euglypha tuberculata Dujardin, 1841 *
FAMILY TRINEMATIDAE
Trinema enchelys ((Ehrenberg, 1838) ***
Trinema lineare Penard, 1890 *

The taxonomy of some species of testate amoebae with agglutinated test is still problematic (Ogden and Meisterfeld, 1989) due to the fact that these species have a variable morphology and biometrics. We will therefore provide a description of the structure of the tests, a description of aperture

and measurements of several of these problematic species.

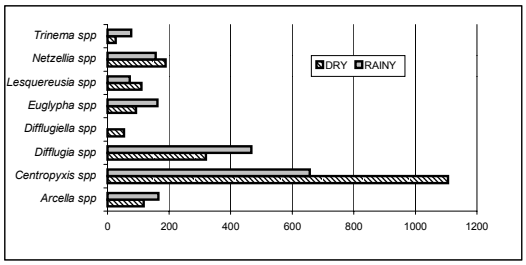

Figure 3. Mean densities of the Rhizopoda genus in the littoral region of the Cajueiro lake from September 1995 to March 1997

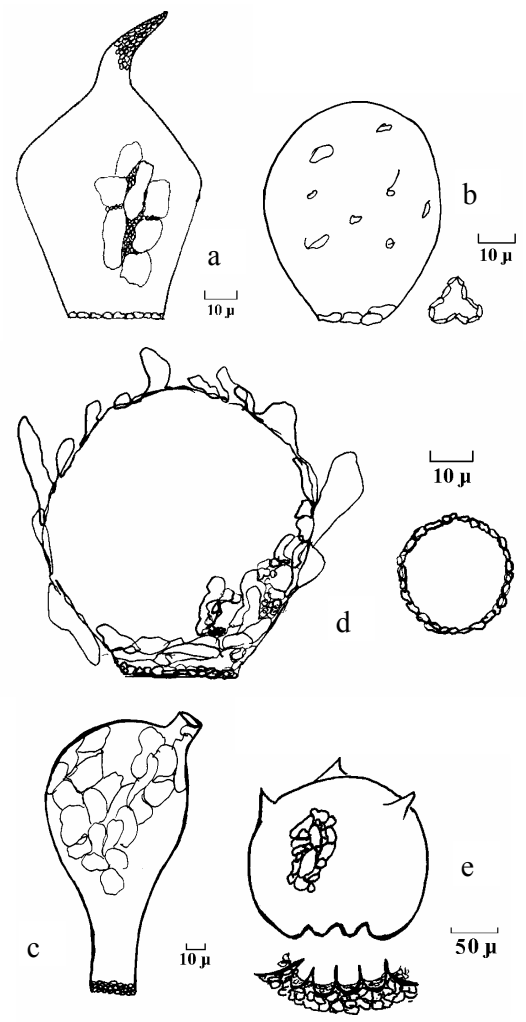

Figure 4. Species of testate amoebae recorded in the Cajueiro lake, which represent new records for Brazilian inland waters: *Difflugia bicornis* (a), *D. hydrostatica,* (b), *D. microclaviformis,* (c) *D. microstoma* (d) and *Protocucurbitella coriniformis* (e)

With the exception *Centropyxis* sp1, the list of species in Table 1 is made up of species of reported cosmopolitan distribution commonly cited in tropical environments. Those not yet cited for Brazil have, nevertheless, been cited for Africa and other countries in South America, such as Argentina (Vucetich, 1973, 1978).

The most abundant genera of Rhizopoda are shown in Figure 4. The genus *Centropyxis* was equally abundant in both the dry and the rainy season, followed by *Difflugia*. According to Bonetti (1995) and Oliveira (1999), *Centropyxis* species may be considered as tolerant to stressful environmental conditions, such as estuarine ecosystems. Some groups of species such as *Centropyxis* spp e *Difflugiella* sp. were more abundant in the dry season, while *Difflugia, Euglypha e Trinema* spp. were more abundant during the rainy season. The seasonality of species may also be related to food availability. It is common knowledge that smaller species such as *Difflugiella* e *Trinema* feed on bacteria, fungi and small protozoans (Laminger, 1973). On the other hand, Hardoim (1997) observed that the larger species consume filamentous algae, small rotifers and other testate amoebae. A marked seasonality in the abundance of some species in the Pantanal, such as *Difflugia corona* and *D. lobostoma,* with larger populations during the flood period, were also observed (Hardoim, 1997).

Conclusions

The fauna associated to the littoral region of the Cajueiro lake is characterized by the dominance of the Rhizopoda-Testacea group. Some testate amoebae taxa had greater abundance in different seasons, suggesting a different response of the community in the wake of environmental variations. Many species registered occur frequently in Brazilian waters or are distributed in the tropics. It is important to emphasize the need of good morphobiometric descriptions of the testate amoebae found in Brazilian inland waters to identify species with greater reliability.

Table 2. Morphological features of some agglutinated species of testate amoebae from Cajueiro Lake

Species	Test: structure and size of particles	Test: aspects, surface and color	Aperture: form and structure	Measurements(μm) B=Breadth; H= height; L=length; PS=aperture
Centropyxis discoides	Chitinous with varied elements	robust spines on the margin; yellow and brown; translucid	circular-irregular, invaginated, smooth	L=300; PS= 120
Centropyxis sp1	Chitinous, some with many, some with few particles	with 2 fragile apical spine rare or more than 2; light and dark yellow; translucid	oval, invaginated, smooth	B=58-65; L=30-45; H=45-54; PS=24-33
Centropyxis spinosa	Chitinous with particles of various sizes	fragile spine on dorsal, light and dark yellow; translucid	Circular-irregular, with smooth inner membrane	Base=71; H=30; PS=30
Cyclopyxis sp1	irregularly arranged; medium and small particles	smooth; yellow and dark gray; translucid	Circular, little invaginated; with small particles	L=54; H=36; PS= 18
Cucurbitella madagas-cariensis	regularly arranged; medium and small particles	rough; dark brown; opaque	Trilobed, neck with small particles	H=81; L=75; PS=21
Difflugia acutissima	regularly arranged; medium and small particles	smooth; light gray; translucid	Circular-regular smooth	H=210; L=99; PS=51
Difflugia bicornis	regularly arranged; medium and small particles	smooth; fragile; light gray; translucid, with apical horn.	Circular with small regular particles	H=60-75; L=27-60; PS=24-30
Difflugia capreolata	particles of various sizes	smooth; brown; opaque.	Circular with regular particles	H=300; L=180; PS=105
Difflugia corona	regularly arranged; medium particles	Smooth; dark brown; opaque; with 0 - 4 short spines;	Denticulate lobed with regular neck	H=90; L=84; PS=30
Difflugia elegans	irregularly arranged, particles of various sizes	rough; light or dark gray; with 1 apical horn; translucid	circular; with neck of irregular particles	H=111-124; L=54-60; PS=12-33
Difflugia gramem	medium and small particles	rough; dark brown; opaque	trilobed as in clover	H=87-90 L=72-75 PS=27-30
Difflugia gramen var. globulosa	small particles well arranged	Smooth; yellow - gray; opaque	trilobed regular	H=L=60 PS=24
Difflugia hydrostatica	chitinous with small and sparse particles	Smooth; yellow; translucid	trilobed, with medium sized particles; without lip	H=60 L=45 PS=15
Difflugia lithophila	particles of various sizes overlapping	Smooth; dark brown; opaque	circular with apparent chiton	H=90-120 L=75-90 PS=36-45
Difflugia lobostoma	small particles and diatom frustules	Smooth; yellow-golden translucid	4 -5 lobes	H=88-165; L=72-75 PS=27-30
Difflugia microclaviformis	medium and big particles	Smooth; dark brown; opaque	circular, with small particles on margin	H=135-150 L=75 PS=24-27
Difflugia microstoma	particles of various sizes, irregularly arranged and jutting	rough; brown; opaque	circular with regular particles on margin	H=66 L=60 PS=22,5
Difflugia muriformis	particles regularly arranged	Smooth; undulate outline; Dark brown; Opaque	8 lobes and neck with small particles	H=105 L=99 PS=42
Difflugia pseudogramem	medium and small particles	Rough; Light to dark gray translucid	trilobed, with neck of small particles	H=96 L=66 PS=21
Difflugiella sp1	proteinaceous with small and sparse particles	Smooth; Translucid	circular, irregular	H=33-39 L=24-30 PS=12-15
Netzelia oviformis	chitinous with small and sparse particles	Smooth; Light yellow to golden Translucid	3-4 lobes with distinct lip (hyaline margin)	H=60-72 L=33-51 PS=9-21
Netzelia tuberculata	medium particles interspaced with porous matrix	Undulate outline or regular; yellow to brown; translucid	4 lobes with hyaline margin	H=84-99 L=66-72 PS=24-30
Netzelia wailesi	medium and small well arranged particles with shinny spots	Smooth; Yellow brown; Opaque	circular, denticulate, regular	H=70,5-78 L=42-58 PS=16,5-24
Pentagonia cf. maroccana	arms with smaller particles than on body	Smooth; 5 rounded arms; dark gray; translucid	circular with small particles and discrete neck	H=108-120 L=69 PS=36
Pontigulasia compressa	particles of various sizes	Rough; dark brown; Opaque	circular	H=120 L=90 PS=30
Protocucurbitella coriniformis	particles of various sizes well arranged	Smooth; with or without spines; Dark brown; opaque	Denticulate lobed with internal lip	H=180–210; L=180–282; PS=80-135

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