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Diversity and distribution of testate amoebae in freshwater ecoregions of Rio de Janeiro State, Brazil

Diversidade e distribuição de amebas testáceas nas ecorregiões de água doce do estado do Rio de Janeiro, Brasil

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Abstract: Aim: Testate amoebae are a group that remains understudied. The present study conducted a survey on the testaceans' records in several biotopes in ecoregions of the State of Rio de Janeiro, exploring differences in the occurrence of taxa. **Methods:** A literature survey was carried out in the Scopus, Web of Science, Google Scholar and Scielo databases using the keywords in English and Portuguese "Testate Amoebae", "Thecamoebian", "Zooplankton" and accompanied by "Rio de Janeiro". Further, samples (collected by plankton mesh nets from 50 to 68 µm; bucket and bottle) from 12 locations were analyzed. The identification of taxa was conducted using an optical microscope with digital camera and Toup-View software for morphological measurements and with specific bibliography. All the records were georeferenced across the freshwater ecoregions of Rio de Janeiro. Statistical analyses were performed using R 4.3.3 Software (R Core Team 2024) through the Vegan package**,** a species accumulation curve (specaccum function), non-metric multidimensional scaling (nMDS) analysis based on 'Jaccard' dissimilarity and the PERMANOVA test performed. **Results:** The bibliographic survey resulted in 35 publications from 1910 to 2023. The sum of records from the literature and from the samples analyzed comprised 37 localities and 250 taxa, including new records for the State of Rio de Janeiro. *Centropyxis aculeata* was the most frequent species (63.7%), and *Difflugia, Centropyxis* and *Arcella* were the most diverse genera. The species accumulation curve indicated that testaceans' richness is underestimated, evidencing insufficient sampling. There was no significant difference between testaceans' assemblages from different biotopes. **Conclusions:** The records in Rio de Janeiro are underestimated due to insufficient sampling in some ecoregions. Future studies are needed to produce a taxonomic inventory more faithful real diversity.

Keywords: testaceans; zooplankton; protozooplankton; biogeography; *Centropyxis aculeata.*

Resumo: Objetivo: Amebas testáceas representam um grupo com carência de estudos. O presente estudo realizou um levantamento dos registros de tecamebas em diversos biótopos das ecorregiões do Estado do Rio de Janeiro, explorando diferenças na ocorrência dos táxons. **Métodos:** Foi realizado um levantamento bibliográfico nas bases de dados Scopus, Web of Science, Google Acadêmico e Scielo

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utilizando as palavras-chave em inglês e português "Testate Amoebae", "Thecamoebian", "Zooplankton" e acompanhadas de "Rio de Janeiro". Posteriormente, foram analisadas amostras (coletadas por redes de plâncton de 50 a 68 µm; balde e garrafa) de 12 localidades. A identificação dos táxons foi realizada utilizando microscópio óptico com câmera digital e software Toup-View para medidas morfológicas e com bibliografia específica. Todos os registros foram georreferenciados nas ecorregiões de água doce do Rio de Janeiro. As análises estatísticas foram realizadas no software R 4.3.3 (R Core Team 2024) por meio do pacote Vegan, produzindo uma curva de acumulação de espécies (função specaccum), análise de escalonamento multidimensional não métrico (nMDS) baseada na dissimilaridade de 'Jaccard' e teste PERMANOVA. **Resultados:** O levantamento bibliográfico resultou em 35 publicações de 1910 a 2023. A soma dos registros da literatura e das amostras analisadas compreendeu 37 localidades e 250 táxons, incluindo novos registros para a área de estudo. *Centropyxis aculeata* foi a espécie mais frequente (63,7%), e *Difflugia*, *Centropyxis* e *Arcella* foram os gêneros mais diversos. A curva de acumulação de espécies indicou que a riqueza de testáceos está subestimada, evidenciando amostragem insuficiente. Não houve diferença significativa entre as assembleias de tecamebas de diferentes biótopos. **Conclusões:** Os registros no Rio de Janeiro estão subestimados devido à amostragem insuficiente em algumas ecorregiões. Estudos futuros são necessários para produzir um inventário taxonômico mais fiel à diversidade real.

Palavras-chave: tecamebas; zooplâncton; protozooplâncton; biogeografia; *Centropyxis aculeata*.

1. Introduction

Testate amoebae comprise a polyphyletic group of amoeboid protozoa, whose main characteristic is the presence of a carapace (or theca). Testaceans are easily identified, which can be done almost exclusively by the features of the theca, which may be of endogenous or exogenous origin and presents spines, siliceous plates, and other differentiations. Through an opening in their theca, called pseudostoma, pseudopods are emitted during feeding and/or locomotion (Ogden & Hedley, 1980; Smith et al., 2008, Kosakyan et al., 2020). In addition, these organisms have a short life cycle and are generalized as asexual, although there is evidence of more complex forms of reproduction (Bonnet, 1975; Mignot & Raikov, 1992; Foissner, 1999; Lahr et al., 2011).

Abundant and widely distributed, some testaceans taxa are sometimes considered cosmopolitan, such as *Centropyxis aculeata* (Ehrenberg, 1838) and *Arcella vulgaris* (Ehrenberg, 1830) (Wilkinson, 2001; Fernández, et al., 2015). These protozoa can be found from plankton of rivers and reservoirs, clayey sediments to interstices of sandy beaches, in mosses, phytotelms of bromeliads and in ecosystems with marine influence, such as estuaries, mangroves and others (Eichler & Bonetti, 1995; Golemansky, 2000; Golemansky & Todorov, 2004; Cruz, 2012; Wailes, 1913; Sampaio et al., 2021).

Testate amoebae have several valuable characteristics associated with abiotic conditions that allow their use as bioindicators of environmental conditions, monitors of recovery phases of degraded environments, presence of pollutants, and can be use in palaeoecological studies due to the preservation of their teaks (Regalado et al., 2018; Marcisz et al., 2020; Freitas, et al., 2022; Sysoev et al., 2024). From an ecological perspective, testate amoebae are also extremely important for the cycling of nutrients between trophic levels, since they feed on a wide variety of organisms including fungi, bacteria, microalgae, other protozoa, and even metazoans such as rotifers and nematodes (Yeates & Foissner, 1995; Hardoim, 1997; Gilbert et al., 2000; Han et al., 2008).

However, the knowledge on diversity and ecology of testate amoebae remains geographically restricted, and aquatic environments in large areas of the global Earth's surface not yet received any studies on this group (Payne et al., 2020). Therefore, testate amoebae, like other free-living protozoans are less studied in tropical areas, when compared to other members of zooplankton, thus representing a gap in scientific knowledge (Miranda et al., 2020; Castilho-Noll et al., 2023). The scarcity of research on this ubiquitous group of protists in most inland waters is mainly due to the absence of a specific methodology for collecting these organisms and the lack of specialists on the group (Esteves, 2011; Regali-Seleghim et al., 2011). Despite the scarcity of studies, comprehensive surveys exist in some Brazilian states, such as Mato Grosso do Sul and São Paulo. (Regali-Seleghim et al., 2011; Rosa et al., 2017). Besides Brazil, the lack of studies on tecamebas is also reported in other Latin American countries such as Chile (Madrid, 2006), Ecuador (Krashevska et al., 2010), and Peru (Bobrov et al., 2019).

Given this lack, the present study aims to increase the knowledge on testate amoebae collection in Brazil conducting a survey on the testaceans recorded in the State of Rio de Janeiro.

The present study targets to reveal the biodiversity of testaceans in the ecoregions of the State of Rio de Janeiro from previous records reported in the literature and, including new records from the analysis of samples collected in 12 locations and not yet published. Differences in the occurrence of taxa according to biotope and ecoregion were explored.

2. Material and Methods

2.1. Freshwater ecoregions of the State of Rio de Janeiro

Species records were organized according to the world's freshwater ecoregions (Abell et al., 2008). The freshwater ecoregions on the map are based on the biogeography of freshwater fishes, covering almost all freshwater ecosystems on Earth. Following the criteria proposed by Abell et al. (2008), the State of Rio de Janeiro was divided into four ecoregions: Ribeira de Iguape (RIG), Paraíba do Sul (PBS), Fluminense (FLU) and Northeast Atlantic Forest (MAN) (Figure 1).

2.2. Data on the occurrence of testate amoebae

The literature review was conducted in the Scopus, Web of Science, Google Scholar, and Scielo research databases using the keyword combinations of "Testate Amoebae", "Thecamoebian", "Zooplankton" and in Portuguese "Ameba Testácea", "Amebas Testáceas", "Tecameba" and "Zooplâncton" and accompanied by the keyword "Rio de Janeiro". Then, results were organized according to type (article, Master dissertation or doctorate thesis), with no time frame applied to the acquired data. The review articles were not used, since the data contained in them referred to other articles. When the manuscript did not include the geographic coordinates of the samples explored by the study, this information was obtained through contact with the respective authors; specimens of uncertain location were not considered.

Samples from 12 water bodies were analyzed, including different biotopes (artificial pond, shallow lakes, coastal lagoons, and reservoirs) in both the Fluminense and Paraíba do Sul ecoregions, which were sampled in the activities of several research projects between 1996 and 2018. Qualitative analyses were performed using an optical microscope (Olympus BX-41 and BX-51) with a digital camera and Toup-View software to conduct morphological measurements (largest diameter). The taxonomic identification was performed according to specific bibliography for the group. Different sampling methods were used in several previous research projects. The samples were taken with graduated buckets, Van Dorn bottles or conducting horizontal or vertical hauls with plankton nets at different depths in the water column (Past studies of testate amoeba in the state of Rio de Janeiro; available in https://doi.org/10.48331/scielodata.X69ODT). The mesh size of the plankton nets varied between 50 and 68 µm and in general the samples were fixed with 4% formalin.

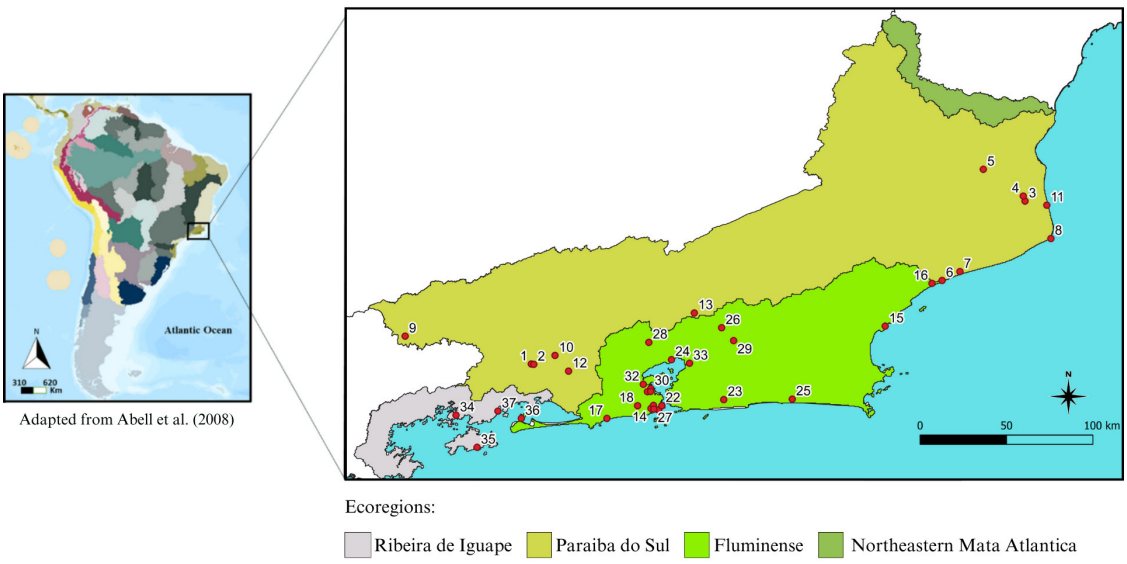

Figure 1. Map of the distribution of the 37 locations in the freshwater ecoregions of the State of Rio de Janeiro in which samples of the studies including testate amoebae were taken.

2.3. Statistical analyses

The data of taxa recorded at each biotope by considering literature data and samples analyzed was organized by ecoregion of the State of Rio de Janeiro. The frequency of taxa was calculated considering the total number of studied sites. From these data, species accumulation curve was plotted to estimate the total species richness in the State of Rio de Janeiro (specaccum function). To estimate the total richness of testacean species, the Chao2 estimator was used (Chao & Jost 2015). The analysis was performed using the Vegan package in the R software (Kindt & Coe, 2005) and an accumulation curve was produced for the data set of all ecoregions. To explore the differences in the testate amoebae assemblage among biotopes, we used nonmetric multidimensional scaling (nMDS) analysis based on 'Jaccard' dissimilarity from the species of testate amoebae. The objective of nMDS is to visualize and interpret information from several dimensions in the two-dimensional plane. To understand how well the ordination represents the divergences observed between samples, we adopted the stress value: a stress <0.05 means an excellent representation in reduced dimensions, <0.1 is excellent, <0.2 is good, and a stress of <0.3 provides a poor representation (Kruskal & Wish, 1978; Legendre & Legendre, 1998). Finally, a PERMANOVA test was performed to evaluate the differences between the testate amoeba assemblages present in the studied biotopes (Anderson & Walsh 2013). The PERMANOVA analysis performs a permutational test (999 permutations) using distance matrices of the variables (presence/absence). We verified the assumption of homogeneity of multivariate dispersion (Anderson 2004). Articles that did not inform the type of biotope studied or that produced data for more than one biotope without separation of the sampling sites were not included. All analyses were performed in R Software R 4.3.3 (R Core Team, 2024) using the Vegan package and the ggplot2 package to produce the graphs.

3. Results

The bibliographic survey resulted in 35 published studies, of which 23 were articles, seven were review articles, four were master's dissertations, and one was a doctoral thesis. The studies ranged from 1910 to 2023. Only eight of them focused exclusively on testate amoebae. The institutions that published the most on testaceans were UNIRIO (Federal University of the State of Rio de Janeiro) and UEM (State University of Maringá) with 11 and 7 studies, respectively. Testate amoebae were recorded in reservoirs, temporary water bodies, lakes, ponds, rivers and streams, artificial lakes, mosses, sandy beach sediments, tanks of bromeliads, estuaries, sewage treatment plants (STPs), and in the digestive tract of anurans of the State of Rio de Janeiro. The studies employed different sampling methodologies, including sieves and plankton nets with different mesh sizes and followed by fixation with 5% or 10% formaldehyde.

The ecoregions Paraíba do Sul, Fluminense, and Ribeira de Iguape had 13, 21, and 3 biotopes studied respectively. In the Northeast Atlantic Forest, no studies were. The results of the analyzed samples were added to the records collected in specific literature, comprising a total of 37 biotopes studied, in three ecoregions of the State of Rio de Janeiro (Table 1).

Table 1. Sample locations with geographic coordinates.

Biotope and locality	Latitude	Longitude	Reference
Ecorregion Paraíba do Sul			
1. Ribeirão das Lajes Reservoir /Piraí e Rio Claro [A]	-22.719	-43.896	* Lopes et al. (2017, 2018)
2. Temporary ponds (near the Ribeirão das Lajes Reservoir/Piraí e Rio Claro)	-22.721	-43.882	Cordioli (2013)
3. Pipeiras Lake /Campos [A]	-21.806	-41.128	*
4. Palacete Lake /Campos [A]	-21.778	-41.138	*
5. Limpa Lake /Campos [A]	-21.628	-41.362	*
6. Carapebus Lagoon /Carabebus [A]	-22.251	-41.593	*

*Results of the present study. A = Light Energia S.A. Research & Development Projects; B = SALGA Project (South American Lake Gradient Analysis); C = UNIRIO Institutional Projects. D = BIOTA FAPERJ Project.

Table 1. Continued...

Biotope and locality	Latitude	Longitude	Reference
Ecorregion Paraíba do Sul			
7. Temporary and permanent ponds in National Park of Restinga de Jurubatiba /Macaé	-22.201	-41.493	Silva (2018), Nova et al. (2021)
8. Paraíba do Sul River/ São Francisco de Itabapoana	-21.466	-41.033	Laut et al. (2011, 2016)
9. Funil Reservoir/ Resende [C]	−22.563	−44.603	* Silva et al. (2014)
10. Paracambi Reservoir/ Paracambi [B]	-22.676	-43.763	* Branco et al. (2019)
11. Veiga Lagoon/ São João da Barra	-21.829	-41.006	Féres et al. (2016)
12. Artificial Lake UFRRJ/ Seropédica [D]	-22.759	-43.687	Silva-Neto et al. (2015)
13. Small ponds and edaphic environments of the Atlantic Forest in the National Park of Serra dos Órgãos/ Teresópolis [D]	-22.433	-42.982	Silva-Neto et al. (2015)
Ecorregion Fluminense			
14. Artificial Lake Vitória Régia (Botanical Garden) /Rio de Janeiro [D]	-22.968	-43.225	* Silva-Neto et al. (2015)
15. Coca-Cola Lake/Rio das Ostras [A]	-22.507	-41.912	*
16. Comprida Lagoon /Macaé [AD]	-22.267	-41.650	* Silva-Neto et al. (2015)
17. Tachas Lake/Rio de Janeiro [C]	-23.024	-43.472	*
18. Mosses in the Bico do Papagaio Hills / Rio de Janeiro	-22.953	-43.300	Wailes (1913)
19. Mosses in the Corcovado Hills / Rio de Janeiro	-22.951	-43.211	Wailes (1913)
20. Temporary ponds in Manguinhos (FIOCRUZ) /Rio de Janeiro	-22.874	-43.245	von Prowazek (1910), Cunha (1913, 1916)
21. Sediment from Copacabana Beach / Rio de Janeiro	-22.975	-43.181	Golemansky (2000), Golemansky & Todorov (2004)
22. Bromeliad tanks at Cláudio Coutinho Track in Urca/ Rio de Janeiro	-22.953	-43.164	Sampaio et al. (2021)
23. Ubatiba River/ Rio de Janeiro	-22.919	-42.817	Miranda & Mazzoni (2015), Miranda (2018); Miranda et al. (2020)
24. Estuary of the Suruí River/ Magé	-22.695	-43.110	Laut et al. (2016)
25. Jacarepiá Lagoon/ Saquarema	-22.916	-42.433	Barbosa et al. (2005)
26. Guapiaçu River/ Itaboraí and Magé	-22.516	-42.829	Pereira (2012)
27. Rodrigo de Freitas Lagoon/ Rio de Janeiro [C]	−22.972	−43.209	*
28. Taquara River/ Duque de Caxias	-22.598	-43.237	Miranda & Gomes (2013)
29. Macacu River/ Rio de Janeiro [D]	-22.588	-42.762	Silva-Neto et al. (2015)
30. Bromeliad tanks and artificial lake at UFRJ/Rio de Janeiro [D]	-22.857	-43.226	Silva-Neto et al. (2015)
31. Sewage Treatment Plant of Alegria/ Rio de Janeiro [D]	-22.873	-43.227	Silva-Neto et al. (2015)
32. Sewage Treatment Plant of Penha/ Rio de Janeiro [D]	-22.833	-43.268	Silva-Neto et al. (2015)
33. Caceribu River/ Rio de Janeiro [D]	-22.715	-43.009	Silva-Neto et al. (2015)
34. Andorinhas Creek (Island Grande) /Angra dos Reis	-23.186	-44.200	Miranda (2013)
Ecorregion Ribeira de Iguape			
35. Temporary ponds in Angra dos Reis	-23.006	-44.318	Cunha (1916)
36. Sediments of the Bernardo Island /Mangaratiba	-23.024	-43.952	Golemansky (2000)
37. Borboleta River / Private Natural Heritage Reserve / Mangaratiba	-22.983	-44.083	Sousa Filho et al. (2007)

*Results of the present study. A = Light Energia S.A. Research & Development Projects; B = SALGA Project (South American Lake Gradient Analysis); C = UNIRIO Institutional Projects. D = BIOTA FAPERJ Project.

The data reported from the 37 studied locations resulted in a total of 250 taxa from 22 families of testate amoebae including Arcellidae (34 spp.), Centropyxidae (38 spp.), Difflugiidae (51 spp.), Hyalospheniidae (30 spp.), Netzeliidae (20 spp.) as the most diverse (List of testaceans found in Rio de Janeiro, Brazil – available in https://doi.org/10.48331/scielodata.X69ODT). The samples analyzed resulted in 13 new records for the Paraíba do Sul ecoregion (*Plagiopyxis callida* Penard, 1910; *Difflugia avellana* Gauthier-Lièvre & Thomas, 1958; *Difflugia muriformes* Gautier-Lièvre & Thomas, 1958; *Cylindrifflugia acuminata magna* Deflandre, 1926; *Cylindrifflugia lanceolata* Penard, 1890; *Hyalosphenia papilio* Leidy, 1874; *Cyclopyxis eurystoma* Deflandre, 1929; *Netzelia lithophila* Penard, 1902; *Netzelia achlora* Penard, 1902; *Netzelia lobostoma cornuta* Gauthier-Lievre & Thomas,1958; *Netzelia wailesi* Ogden, 1980; *Trinema enchelys* Ehrenberg, 1838; *Cyphoderia ampulla* Ehrenberg, 1840) and 16 new records for the Fluminense ecoregion *(Arcella crenulata* Deflandre, 1928; *Arcella gandalfi* Féres et al., 2016; *Galeripora dentata* Ehrenberg, 1830; *Galeripora catinus* Penard, 1890; *Galeripora scutelliformis* Playfair, 1918; *Centropyxis dentistoma* Decloitre, 1949; *Centropyxis tropica* Deflandre, 1929; *Difflugia distenda* Penard, 1899; *Difflugia dragana* Ogden & Zivkovic, 1983; *Difflugia levanderi* Playfair, 1917; *Difflugia nodosa* Leidy, 1879; *Difflugia umbilicata* Penard, 1902; *Padaungiella tubulata* Brown, 1911; *Lesquereusia mimetica* Pénard, 1911; *Euglypha rotunda* Ehrenberg, 1845; *Centropyxiella lucida* Golemansky, 1971).

The most frequent species in terms of sampling sites belong to the genera *Centropyxis* (3 spp.), *Netzelia* (3 spp.), *Arcella* (1 spp.), *Galeripora* (2 spp.), *Euglypha* (1 spp.), and *Difflugia* (1 spp.). Only one species occurred in more than 50% of the sites, that was (*Centropyxis aculeata* - 63.7%). It was followed by *Centropyxis constricta* (Ehrenberg, 1841): 46.7%, *Galeripora discoides* (Ehrenberg, 1871): 46.7%, *Difflugia oblonga* (Ehrenberg, 1838): 46.7%, *Netzelia corona* (Wallich, 1864): 43.3%, *Arcella vulgaris*: 40%, *Euglypha acanthophora* (Ehrenberg, 1841): 36.7%, *Centropyxis ecornis* (Ehrenberg, 1843): 33.3%, *Galeripora megastoma* (Penard, 1902): 33.3%, *Netzelia tuberculata* (Wallich, 1864): 33.3%, *Netzelia wailesi* (Ogden, 1980): 30%.

Despite this, there were no records of the species *Netzelia corona*, *N. tuberculata*, *N. wailesi*, and *Galeripora discoides* in the Ribeira do Iguape ecoregion. The genus *Difflugia* was the most speciose, with 43 taxa, followed by the genera *Centropyxis* and *Arcella* with 29 and 25 taxa, respectively. Considering all ecoregions of the Rio de Janeiro State, the species accumulation curve showed that the richness of testate amoebae recorded was lower than the estimated richness (Figure 2).

The ordination of taxa grouped by biotopes indicated the proximity of some ecosystems regarding the testaceans composition such as lagoons and estuaries, bromeliads and rivers, and streams, producing a stress value = 0.137 (Figure 3), which is a good representation of the data set (Kruskal & Wish, 1978; Legendre & Legendre, 1998). The

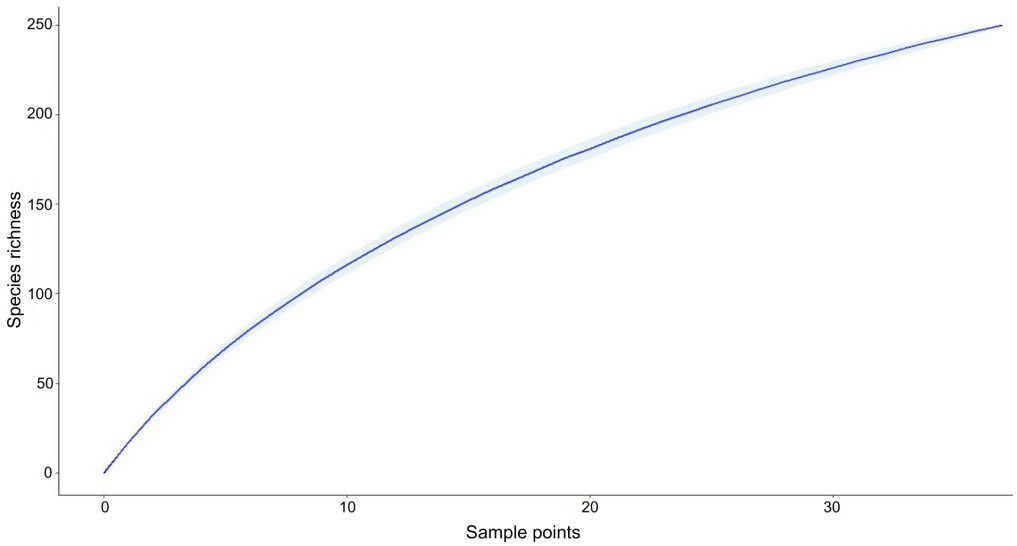

Figure 2. Accumulation curve of testate amoeba species observed in the state of Rio de Janeiro. (Chao = 337.1784; Chao.se = 21.61439).

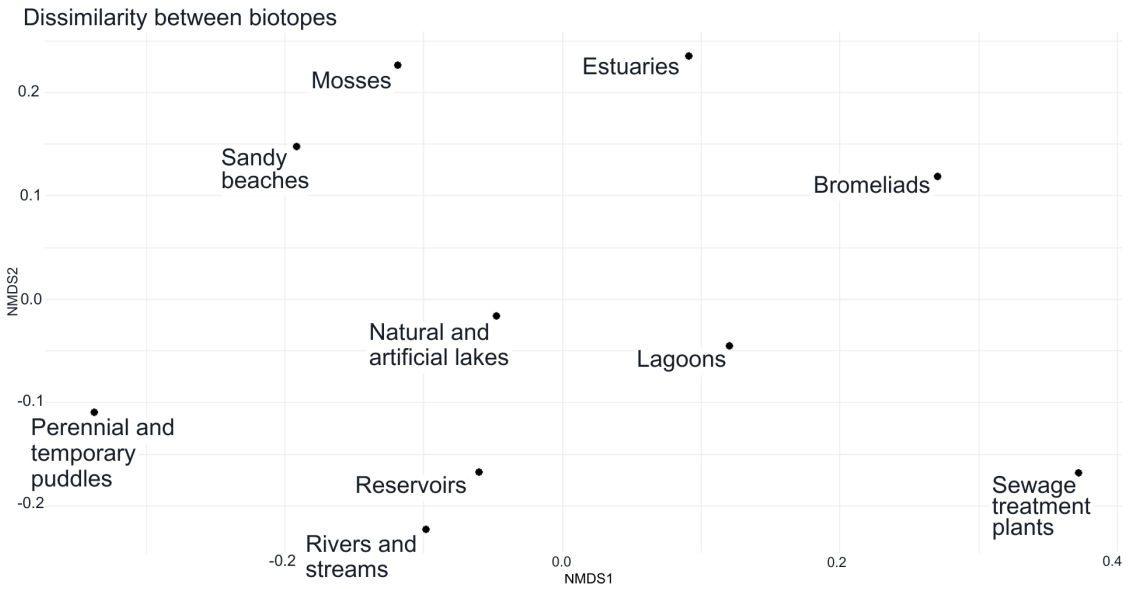

Figure 3. Dissimilarity between biotopes sampled in the state of Rio de Janeiro. Stress = 0.137.

opposite arrangement between samples from natural systems (temporary and permanent pools, estuaries and lagoons) and artificial systems (sewage treatment plants and reservoirs) stands out. The PERMANOVA test demonstrated that the assemblage of testaceans was significantly different between biotopes (Permanova; df = 9, sum of squares = 2.0078, R^2 = 1.00000, F = 1.227, p = 0.03).

3. Discussion

The richness of testate amoebae reported for the Rio de Janeiro State in the present study (250) was greater than that recorded for São Paulo and Mato Grosso do Sul states, 84 and 113 species, respectively (Regali-Seleghim et al., 2011; Rosa et al., 2017). However, greater diversities have already been recorded in the state of Mato Grosso do Sul by Lansac-Tôha et al. (2009), in which 120 species of testate amoebae were found in the Upper Paraná River floodplain. In addition, the richness found exceeded the expected for the entire southeast region according to the survey carried out by Lansac-Tôha et al. (2007), being only 92 taxa below the amount estimated for all the country in the same study. The comparatively increase in the number of recorded taxa by our study can be justified, firstly, by the methodology applied by Lansac-Tôha et al. (2007), which considered only articles. In the present study, we included Master dissertations and Doctorate theses as sources of testate amoebae records. In addition, we added new data from 12 water bodies. Likewise, the greater diversification of biotopes may have caused an expansion of the records. Another factor can be related to the increase in the number of zooplankton studies in Brazil in the last decade, mainly in the Southeast region as highlighted by Castilho-Noll et al. (2023). This, among the 35 studies resulting from the bibliographic survey, 23 were published from 2007 onwards.

It should be noted that the total number of testate amoebae taxa reported by us exceeded the number of other components of the zooplankton such as rotifers (238 taxa) and cladocerans (80 taxa) registered for the State of Rio de Janeiro (Costa et al., 2022; Macêdo et al., 2022). Unlike these groups, testate amoebae are less-studied components of lake plankton. The presence of testaceans is not caused solely by stochastic processes (Alves et al., 2010), and they are found in high diversity and abundance in planktonic environments, especially in coastal zones of lotic and lentic environments, in temporary ponds and as components of the phytotelm community in bromeliads tanks (Velho et al., 1999; Arrieira et al., 2016; Sampaio et al., 2021). Furthermore, some species are resistant to eutrophication processes and even feed on toxin-producing cyanobacteria such as those of the genus *Microcystis* (Mizuta et al., 2011; Prentice et al., 2018). This highlights the importance of more plankton studies that include testate amoebae.

Despite the significant number of species of testaceans recorded in the present study, a marked difference was observed between the number of studies conducted at each ecoregion. The Fluminense ecoregion, closer to research institutions, showed a

greater number of biotopes studied. However, the Paraíba do Sul ecoregion presented some sample clusters related to the existence of some freshwater ecosystems that present a long sampling effort on plankton such as the Ribeirão das Lajes Reservoir (Lopes et al., 2017, 2018) and the Paraíba do Sul River (Laut et al., 2011, 2016). On the other hand, differences between the number of studies carried out in the different ecoregions may explain the lack of records of species considered frequent as *Netzelia corona, N. tuberculata, N. wailesi,* and *Galeripora discoides* in the Ribeira do Iguape ecoregion in which only three studies were carried out. The influence of sampling bias on the biogeography of testate amoebae has already been raised in other regions of the world such as New Zealand and Chile (Bobrov et al., 2019; McKeown et al., 2021).

The differences between ecoregions in the number of studies also reflected a lower sampling effort that was not sufficient to cover the diversity of testate amoebae in the state, which was shown by the species accumulation curve (Figure 2). The underestimation of taxa proven through the species accumulation curve was also observed for other zooplankton components such as cladocerans by Macêdo et al. (2022) and rotifers by Costa et al. (2022). Likewise, those studies (Macêdo et al., 2022; Costa et al., 2022) found a greater sampling effort for cladocerans and rotifers, respectively, in the ecoregions Paraíba do Sul and Fluminense. These results suggest insufficient sampling effort in the state of Rio de Janeiro as being a problem that encompasses several groups of zooplankton.

In this study, in contrast to the Fluminense ecoregion that presented the largest sample quantity, the Ribeira do Iguape ecoregion until now has not been reported a survey for testaceans. This ecoregion comprises a large number of preserved ecosystems such as the federal conservation units: Serra da Bocaina National Park and the Cairuçu Environmental Protection Area (ICMBio, 2024a, b). Therefore, with the appropriate sampling effort, this ecoregion should present diversity equal to or greater than the other ecoregions. Therefore, we suggest that more surveys should be conducted in areas where there are few or no studies, such as the central area of the Fluminense ecoregion, the Ribeira do Iguape ecoregion, and the Northeast Atlantic Forest. This effort would cover a variety of small and medium-sized aquatic ecosystems. Greater homogeneity in the number of sampling sites between ecoregions could provide a better understanding of the differences between testate amoeba assemblages.

Despite differences in sampling efforts across ecoregions, some species were conspicuous in all such as *Centropyxis aculeata*, *C. constricta* and *C. ecornis*. The genus *Centropyxis*, which is among the most diverse and has the most representative species in this study, has a worldwide distribution and is considered cosmopolitan. *Centropyxis aculeata* that attained the highest frequency of occurrence in this study, is among one of the most frequent species of testaceans in Brazil (Lansac-Tôha et al., 2007; Fernández et al., 2015; Bobrov, 2020). This species is found in all lacustrine biotopes and in humid terrestrial environments (Samant et al., 2020; Bobrov, 2023). *C. aculeata* is resistant to physical, chemical and biological environmental variations, such as increased acidity and salinity, from oligotrophic to eutrophic conditions, in addition to being present in lotic and lentic ecosystems (van Hengstum et al., 2008; Silva et al., 2012; Miranda & Gomes, 2013; Silva et al., 2020; Charqueño-Celis et al., 2022; Semensatto et al., 2023). Furthermore, *C. aculeata* is resilient to anthropogenic influences such as silting of water bodies, the entry of metallic contaminants and the presence of agricultural derivatives such as fertilizers (Silva et al., 2013; Alzeny et al., 2021; Tito & Schwind, 2021). Thus, the species is considered an indicator of environmental stress and depredated environments (Samant et al., 2020).

Besides *Centropyxis*, the predominance of the genera *Difflugia*, and *Arcella* recorded by us was also observed in surveys carried out for the states of São Paulo and Mato Grosso do Sul (Regali-Seleghim et al., 2011; Rosa et al., 2017). This pattern is found in studies throughout Brazil and can be justified by the fact that these genera are the most diverse in the country (Lansac-Tôha et al. 2000; Lansac-Tôha et al. 2007).

5. Conclusion

Based on past data and new records, this study produced an updated taxonomic list for the assembly of testate amoebae of the Rio de Janeiro State and contributed to the increase in knowledge of these organisms in Brazil. The number of taxa reported here (250) was greater than that recorded for testaceans in the states of Mato Grosso do Sul and São Paulo. The genera *Difflugia*, *Centropyxis,* and *Arcella* were the most specious, and *Centropyxis aculeata* was the most frequent species in the study area. According to our analyses, the records of testate amoebae are underestimated, and the real number of species

may be greater than the sampling effort allows us to glimpse. The presence of well-structured academic centers in the Fluminense ecoregion was a factor related to the larger sample size in contrast with the further away Ribeira do Iguape ecoregion. Future studies are needed to overcome the problem of the underreporting of this group of protozoa and thus, produce a taxonomic inventory more consistent with the real diversity of these organisms.

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Data availability

All research data from this study is available on SciELO Data. Access is free. It can be accessed at https://doi.org/10.48331/scielodata.X69ODT

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