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ECOSYSTEMS

Testate amoebae (Arcellinida and Euglyphida) from the state of Bahia: a synthesis of 108 years of scientific discoveries and contributions

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Abstract: Testate amoebae are widely distributed organisms, playing essential roles in several aquatic and terrestrial ecosystems. This study presents an overview of the biodiversity of Testate amoebae (Arcellinida and Euglyphida) in the state of Bahia, Brazil, over the last 108 years, based on unpublished data and information from the literature. The records covered 19 municipalities and seven river basins. A total of 172 infrageneric *taxa* were identified, with Difflugiidae and Arcellidae standing out as the most representative and *Centropyxis aculeata* as the only constant species (FO = 100%). We observed a total of 25 occurrences that were new to the state. The study considered ecological and methodological aspects, including biomes, types of aquatic bodies, ecosystems, collection methods, mesh sizes, and compartments. Methods such as washing macrophyte rhizospheres and using 20 µm nets have demonstrated greater efficiency in estimating richness. The analysis of accumulation curves indicated that the sampling method recorded approximately 76.3% of the estimated biodiversity. Despite the advances, gaps remain, *e.g.,* in the Cerrado biome, in temporary lakes, and in sediments. This study reinforces the importance of methodological standardization and investment in studies in the Bahia state to improve knowledge about regional and global biodiversity.

Key words: Biodiversity, Limnology, Thecamoebians, Northeast, Brazil.

INTRODUCTION

Testate amoebae belong to the domains Amorphea (Amoebozoa-Tubulinea-Arcellinida) and Diaphoretickes (Rhizaria-Cercozoa-Euglyphida), according to the updated classification of Adl et al. (2019). This heterogeneous and polyphyletic group of free-living protists is characterized by the presence of a carapace that encloses the cytoplasm, with a single opening (pseudostome) through which the pseudopods project (Gilbert et al. 2000, Ogden & Hedley 1980). The composition of the carapace, the presence of vacuoles, and the size and shape of the pseudostome are essential morphological attributes for species identification (Bonnet 1975, Smith et al. 2008). The shells can be formed by internal secretion (endogenous) or by agglutination of external particles (exogenous) (Beyens & Meisterfeld 2006).

These heterotrophic organisms have a diverse diet, consuming organic matter, microalgae, protists, microfungi, plant cells, and metazoans. Polyphagy is common, although some species have distinct dietary preferences. Testate amoebae inhabit a variety of environments, such as soil, moss and water bodies. In aquatic environments, occurrence is strongly influenced by environmental variables, such as primary productivity, humidity levels, light, temperature, oxygen, and pH (Schwind et al. 2019, Arrieira et al. 2017, Mitchell et al. 1999).

In plankton, these amoebae can directly and indirectly impact other aquatic organisms (Tito & Schwind 2021). Their short life cycle allows them to respond quickly to environmental changes (Foissner 1999), making them useful tools in studies on the restoration of aquatic ecosystems. Using amoebae in ecological monitoring helps to assess the effectiveness of recovery actions and preserve the health of ecosystems. In addition, studies involving these organisms contribute to predicting the impacts of future environmental changes and to the management and conservation of local biodiversity (Tito & Schwind 2021, Arrieira et al. 2017).

The first records of Testate amoebae in Brazil date back to the 19th century, with important contributions from researchers such as Ehrenberg (1838), Schewiakoff (1893), Daday (1905), Aragão (1909, 1910), Prowazek (1910), Hartmann & Chagas (1910) and Cunha (1916). These pioneers described several species in different regions of the country. Recently, 240 species of Testate amoebae were catalogued for Brazil (Elmoor-Loureiro et al. 2023).

Among Brazilian hydrographic regions, the greatest richness of protozooplankton species was recorded in the Upper Paraná River region, followed by the Southeast Atlantic, Tocantins-Araguaia, and Amazon regions. These significant geographic variations in protozooplankton diversity appear to be more related to sampling effort than to established biogeographic patterns (Elmoor-Loureiro et al. 2023).

In the state of Bahia, the first investigations on Testate amoebae date back to the beginning of the 20th century, with pioneering records by Cunha (1916) and Neumann-Leitão & Nogueira-Paranhos (1987). However, studies on this group of organisms are still insufficient and underestimated. This knowledge gap can be largely attributed to the scarcity of specialists in the area and the concentration of research efforts in other regions of Brazil, as highlighted by Lansac-Tôha et al. (2007).

This study aims to expand the knowledge about the biodiversity of Testate amoebae in the state of Bahia, gathering information from the last 108 years available in the literature about the occurrence and distribution of species belonging to the Arcellinida and Euglyphida groups in the region. In addition, the study presents unpublished data on the occurrence of these amoebae. The research includes the preparation of a list of species for the state and seeks to provide a comprehensive overview of the number of studies carried out and the richness of *taxa* recorded, considering ecological and methodological aspects. Thus, the aim is to better understand the distribution of these organisms and contribute to the expansion of the knowledge about their biodiversity in the state.

MATERIALS AND METHODS

The literature review of this study was conducted through a systematic search of the literature on the occurrence of Testate amoebae in the state of Bahia, Brazil. For this purpose, databases such as Scopus, SciELO and the Google Scholar search engine were consulted. The search included several combinations of keywords, such as “Testacea”, “Testáceas”, “Testáceos”, “Tecamebas”, “Tecamoebas”, “Amebas testáceas”, “Testate amoebae”, associated with the terms “Bahia”, “Brasil”, “Brazil”, “Protozoa” and “Thecamoebians”.

To compile the list of occurrences of Testate amoebae in Bahia, data from studies published by Cunha (1916), Neumann-Leitão & Nogueira-Paranhos (1987), Silva et al. (2009, 2020), Cruz (2012), Araújo & Nogueira (2016) and Rocha et al. (2021) were gathered (Table I, Supplementary Material - Table SI).

In addition, data from samplings carried out in lentic and lotic systems from several locations were incorporated, including Senhor do Bonfim, Andorinha, São Domingos, Vitória da Conquista, Floresta Azul, Ibicaraí, Itaju do Colônia, Itororó, Itapé, Itabuna, Ilhéus and Poções, from 2008 to 2019 (Table I, Table SI). This information was provided by those responsible for the Limnology and Biomonitoring Laboratory, with the corresponding samples deposited in the Scientific Limnological Collection of the Federal University of Bahia, Anísio Teixeira Campus (CLCAT) and by the Plankton Ecology Laboratory of the State University of Santa Cruz (UESC).

Considering the total of 19 sampling sites investigated for the occurrence of Testate amoebae in the state of Bahia, 52.63% of the

Table I. Sampling locations of testate amoebae in the state of Bahia, with number of sampling sites, code, location, municipality, geographic coordinates and research references. Sampling sites 1 to 10 refer to data available in the literature, while sites 11 to 19 refer to data from the present study.

Sampling sites	Code	Locality	Municipality	Coordinates (S)	Coordinates (W)	References
1	PE	Peixe	Remanso	9°36'43"	42°05'08"	Cunha (1916)
2	SFRr	São Francisco River	Rodelas	8°59'14.64"	38°39'32.78"	Neumann-Leitão & Nogueira-Paranhos (1987)
3	SFRi	São Francisco River	Itaparica	9°01'16"	38°25'59"	Neumann-Leitão & Nogueira-Paranhos (1987)
4	SFRs	São Francisco River	Sobradinho	9°26'31.36"	40°47'57.05"	Neumann-Leitão & Nogueira-Paranhos (1987)
5	CRBa	Cachoeira River Basin	Itabuna	14°48'2.1"	39°16'47.0"	Silva et al. (2009)
6	CRBb	Cachoeira River Basin	Ilhéus	14°48'27.3"	39°8'49.6"	Silva et al. (2009)
7	IB	Iguape Bay	Cachoeira	12° 00'	38° 30'	Cruz (2012)
8	NR	Natural Reservoir	Povoado Olhos D'água do Souza - Glória	9°21'55''	38°12'51''	Araújo & Nogueira (2016)
9	PF	Periperi Farm	Vitória da Conquista	14°48.178'	40°48.376'	Silva et al. (2020)
10	MP	Marimbús Pantanal	Andaraí	12°39'14"	41°17'00"	Rocha et al. (2021)
11	QFp	Quicé Farm pond	Senhor do Bonfim	10°28'58.80''	40°00'33.20''	Date 2008 - 2009
12	ADII	Andorinha II Dam	Andorinha	10°18'01.6"	39°47'05.6"	Date 2009 - 2010
13	SAD	Santo Antônio Dam	São Domingos	11°29'13''	39°38'09,32''	Date 2010
14	BL	Bateias Lake	Vitória da Conquista	14°51'15''	40°52'11''	Date 2010 - 2011
15	RFBF	Reservoir at Beija-flor farm	Vitória da Conquista	14°56'48''	40°50'06''	Date 2012
16	SR	Salgado River	Floresta Azul and Ibicaraí	14°53'56.47"	39°42'21.53"	Date 2014 - 2015
17	CoR	Colônia River	Itaju do Colônia and Itororó	15°06'18.19"	39°56'12.04"	Date 2014 - 2015
18	CaR	Cachoeira River	Itapé, Itabuna and Ilhéus	14°54'44.16'	39°23'15.74"	Date 2014 - 2015
19	MD	Morrinhos Dam	Poções	14°34'17"	40°18'04"	Date 2019

research was conducted by Dr. Márcio Borba da Silva, based at the Multidisciplinary Institute of Health, Anísio Teixeira Campus, of the Federal University of Bahia, who has been dedicating to the study of Testate amoebae in the state of Bahia since 2008. These studies were carried out in collaboration with undergraduate students from the Biological Sciences courses at the Federal University of Bahia, Anísio Teixeira campus, and the State University of Bahia, Senhor do Bonfim campus, in addition to projects linked to postgraduate programs, with the collaboration of students from the Postgraduate Program in Tropical Aquatic Systems at the State University of Santa Cruz (UESC) and the Postgraduate Program in Genetics, Biodiversity and Conservation at the State University of Bahia (UESB).

The research carried out between 2008 and 2019 used plankton nets with a mesh size of 20 µm to collect specimens, which were fixed in formaldehyde at a final concentration of 4%. The samples were obtained by means of horizontal subsurface drag using a 30x70 cm conical plankton net for five minutes at each collection point, in addition to washing the rhizospheres of aquatic macrophytes and collecting water in volumes of 40, 100 and 400 L, using graduated buckets (Table SI).

The samples were sorted in Sedgewick-Rafter chambers, and the analysis was performed using an Olympus CX31 optical microscope equipped with a camera for photomicrography of the specimens. For taxonomic identification, the specimens were mounted on slides with glycerin and analyzed based on specialized literature, including Deflandre (1928, 1929), Gauthier Liévre & Thomaz (1958), Decloitre (1962), Vucetich (1973), Ogden & Zivkovic (1983), Ogden (1983, 1984), Velho et al. (1996, 2000), Lansac-Tôha et al. (2000, 2001), Dabés & Velho (2001), Fulone et al. (2005), Mazei & Warren (2012), Reczuga et al. (2015), Kosakyan et al. (2013, 2016) and Gonzaléz-Miguéns et al. (2021).

After compiling the data from the studies into a table, the taxonomic nomenclature was verified using the GBIF (Global Biodiversity Information Facility) and Microworld (World of Amoeboid Organisms) databases, platforms specialized in sharing information on biodiversity.

In addition, a map was created containing the sampling sites and locations of the studies related to Testate amoebae in Bahia. For this, the QGIS software was used, a free, open-source tool widely used for creating, analyzing, and visualizing georeferenced data.

The frequency of occurrence (FO%) was calculated by dividing the number of articles in which each species was present by the total number of articles. Based on this calculation, the species were classified into four categories: constant (present in more than 80% of the samples), frequent (present in 50% to 80%), common (20% to 50%) and rare (less than 20%), the Dajoz Consistency Index (1983) was used, as presented in Castilho et al. (2016). It is worth mentioning that here, each article was considered a sample.

The number of studies and the rate of Testate amoebae recorded in the state of Bahia were analyzed, considering ecological and methodological factors. Of the aspects evaluated, the following stand out: biomes (Caatinga, Cerrado and Atlantic Forest), type of aquatic body (river, dam, farm pond, reservoir, lagoon, estuary, temporary lagoon and weir), type of freshwater ecosystem (lentic or lotic), collection methods (washing of rhizospheres of aquatic macrophytes, horizontal drags of 5 and 10 minutes, collection using graduated buckets in volumes of 20, 40, 100 and 400 liters, and the Van Veen sediment sampler), sampling net mesh sizes (20 µm, 65 µm, 64 µm, and 62 µm mesh sieve), and compartment (plankton, periphyton, combination of plankton and periphyton, and surface layer of the sediment) (Table SI).

These criteria were fundamental to understanding the diversity and distribution patterns of Testate amoebae in the region. The species richness of Testate amoebae was estimated using the Jackknife 2 estimator on the EstimateS 9.1 software. This analysis was based on the presence and absence of the species data in the samples. A species accumulation curve was constructed to assess whether the number of samples was sufficient to capture the total diversity of Testate amoebae. The curve was complemented by an asymptotic behavior analysis, according to the methods described by Santos et al. (2019).

RESULTS

In the state of Bahia, 172 infrageneric *taxa* of Testate amoebae were recorded, distributed in 12 families and 21 genera (Table II, Figure 1).

Among these, 25 *taxa* were reported as new occurrences for the state (*Arcella crenulata* (Deflandre, 1928), *Arcella hemisphaerica hemisphaerica* (Perty, 1852), *Arcella mitrata* (Leidy, 1879), *Galeripora dentata cashiana* (Deflandre, 1928), *Centropyxis globulosa* (Bonnet & Thomas, 1955), *Centropyxis platystoma armata* (Pénard, 1890), *Cylindrifflugia acuminata inflata* (Pénard, 1899), *Cylindrifflugia acuminata magna* (Deflandre, 1926) *Difflugia acutissima* (Deflandre, 1931), *Difflugia kempnyi* (Stepanek, 1953), *Difflugia linearis* (Penard, 1890), *Difflugia mammillaris* (Penard, 1893), *Difflugia molesta* (Penard, 1902), *Difflugia pyriformis* (Perty, 1849), *Difflugia scalpellum* (Penard, 1899), *Difflugia ventricosa* (Deflandre, 1926), *Zivkovicia compressa* (Carter, 1864), *Quadrulella tubulata* (Gauthier-Lièvre, 1953), *Lesquereusia modesta minima* (Van Oye, 1956), *Lesquereusia spiralis caudata* (Playfair, 1917), *Cucurbitella megastoma* (Gauthier-Lièvre & Thomas, 1960), *Cyclopyxis aplanata* (Penard, 1911), *Cyclopyxis impressa* (Daday, 1905), *Netzelia muriformis* (Gauthier-Lièvre & Thomas, 1958), *Pseudonebela africana* (Gauthier-Lièvre, 1953) (Table II).

Spatially, Testate amoebae were identified in 19 municipalities (Table I) and in seven of the 13 hydrographic basins present in the state (Figure 2). The Leste and Pardo basins stand out, with five and four sampling sites, respectively (Figure 2).

The taxonomic richness of the Testate amoebae families recorded in Bahia highlighted Difflugiidae as the most representative, with 38 *taxa*, followed by Arcellidae (34 *taxa*), Netzeliidae (23 *taxa*), Centropyxidae (19 *taxa*), Euglyphidae (14 *taxa*), Lesquereusiidae (13 *taxa*), Hyalospheniidae (10 *taxa*), Cryptodifflugiidae (6 *taxa*), Cylindriflugiidae (6 *taxa*), Trinematidae (3 *taxa*), Phryganellidae (2 *taxa*), Cyphoderiidae (1 *taxa*) and Incertae sedis (3 *taxa*) (Table II, Figure 3a).

Considering the richness of Testate amoebae in the sampling sites, the Cachoeira River Basin (CRBa) Itabuna and Cachoeira River Basin CRBb (Ilhéus) sites stood out with 98 and 97 *taxa*, respectively. On the other hand, the lowest number of *taxa* was recorded in São Francisco River (SFRs) Sobradinho, with only one taxon (Figure 3b).

The frequency of occurrence (FO) analysis revealed a community structure characterized by the dominance of *Centropyxis aculeata* (Ehrenberg, 1838) as the only constant species,

Table II. List of occurrences and frequency of occurrence (FO%) of testate amoebae in the state of Bahia, Brazil. *New occurrences for the state. Sampling sites 1 to 10 refer to data available in the literature, while sites 11 to 19 refer to data from the present study. The meanings of the codes are in Table I.

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
ARCELLINIDA Kent, 1880																				
Arcellidae Ehrenberg, 1843																				
Arcella brasiliensis Cunha, 1913					x	x				x	x									21.05
Arcella conica (Playfair, 1918)					x	x				x							x		x	26.32
Arcella costata Ehrenberg, 1847									x	x				x	x		x	x		31.58
Arcella costata angulosa (Perty, 1852)										x				x						10.53
Arcella crenulata Deflandre, 1928 *														x						5.26
Arcella gibbosa Penard, 1890					x	x								x	x	x	x	x	x	42.11
Arcella gibbosa laevis Deflandre, 1928					x	x														10.53
Arcella gibbosa mitriformis Deflandre, 1928					x	x			x					x						21.05
Arcella hemisphaerica Perty, 1852					x	x			x	x				x	x	x	x	x	x	52.63
Arcella hemisphaerica gibba Deflandre, 1928					x	x										x	x			21.05
Arcella hemisphaerica hemisphaerica Perty, 1852 *														x	x					10.53
Arcella hemisphaerica undulata Deflandre, 1928					x	x				x	x	x		x	x	x	x	x		52.63
Arcella intermedia (Deflandre, 1928)					x	x								x			x			21.05
Arcella lobostoma Deflandre, 1928					x	x					x			x						21.05
Arcella mitrata Leidy, 1879 *											x			x						10.53
Arcella nordestina Vucetich, 1973					x	x														10.53
Arcella peruviana Reczuga et al., 2015										x										5.26
Arcella rotundata Playfair, 1918					x	x					x			x		x	x	x	x	42.11
Arcella rotundata alta Playfair, 1918														x		x		x		15.79
Arcella rotundata aplanata Deflandre, 1928											x		x	x	x	x	x	x		36.84
Arcella rotundata stenostoma Deflandre, 1928 *											x									5.26

Table II. Continuation.

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
Arcellidae Ehrenberg 1843																				
Arcella vulgaris Ehrenberg, 1830		x	x		x	x				x	x	x	x	x		x	x	x		63.16
Arcella vulgaris undulata Deflandre, 1928										x	x		x	x	x	x	x	x		42.11
Arcella vulgaris wailesi Deflandre, 1928										x		x								10.53
Galeripora arenaria (Greeff, 1866)						x														5.26
Galeripora bathystoma (Deflandre, 1928)					x															5.26
Galeripora catinus (Penard 1890)						x					x	x	x							21.05
Galeripora dentata (Ehrenberg, 1830)		x	x		x	x														21.05
Galeripora dentata cashiana (Deflandre, 1928) *											x									5.26
Galeripora discoides (Ehrenberg, 1871)					x	x				x	x	x	x	x	x	x	x	x	x	63.16
Galeripora discoides pseudovulgaris (Deflandre, 1928)					x	x				x	x			x					x	31.58
Galeripora megastoma (Penard, 1902)					x	x				x	x	x	x	x	x	x	x	x		57.89
Galeripora polypora (Penard, 1890)					x	x														10.53
Galeripora rota (Daday, 1905)										x										5.26
Centropyxidae Jung, 1942																				
Centropyxis aculeata (Ehrenberg, 1838)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	100.00
Centropyxis aculeata minima Van Oye, 1956										x					x					10.53
Centropyxis aculeata oblonga Deflandre, 1929										x	x	x	x	x	x	x	x	x		47.37
Centropyxis aculeata tropica Deflandre, 1929										x			x							10.53
Centropyxis aerophila Deflandre, 1929					x						x	x	x	x		x	x	x	x	47.37
Centropyxis cassis (Wallich, 1864) Deflandre, 1929					x	x			x	x	x		x	x	x	x	x	x	x	63.16
Centropyxis cassis spinifera Decloitre, 1954					x	x				x	x									21.05
Centropyxis constricta (Ehrenberg, 1841)					x	x				x	x		x			x			x	36.84
Centropyxis discoides (Penard, 1902)					x	x				x	x	x	x	x	x	x	x	x		57.89
Centropyxis ecornis (Ehrenberg, 1841)					x	x				x	x	x	x	x	x	x	x	x	x	63.16
Centropyxis gibba Deflandre, 1929										x			x	x	x					21.05

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
Centropyxidae Jung, 1942																				
Centropyxis globulosa (Bonnet & Thomas, 1955) *																		x		5.26
Centropyxis hemisphaerica (Barnard, 1875)					x	x								x						15.79
Centropyxis hirsuta Deflandre, 1929					x	x			x	x	x		x		x					36.84
Centropyxis marsupiformis (Wallich, 1864)						x					x			x		x	x	x	x	36.84
Centropyxis minuta Deflandre, 1929					x	x					x		x	x	x			x		36.84
Centropyxis platystoma (Penard, 1890)					x	x			x	x	x	x	x			x	x	x	x	57.89

Table II. Continuation.

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
Centropyxis platystoma armata Pénard, 1890 *												x	x							10.53
Centropyxis spinosa (Cash, 1905)												x	x	x	x	x	x	x		36.84
Cryptodifflugiidae Jung, 1942																				
Cryptodifflugia angusta (Schönborn, 1965)					x	x														10.53
Cryptodiffflugia crenulata Playfair, 1917					x	x														10.53
Cryptodifflugia horrida Page, 1966					x	x														10.53
Cryptodifflugia oviformis Penard, 1902					x	x			x											15.79
Cryptodifflugia patinata (Schönborn, 1965)					x	x						x								15.79
Cryptodifflugia voigti (Golemansky, 1970)					x	x					x									15.79
Cylindriflugiidae González-Miguéns et al., 2022																				
Cylindrifflugia acuminata (Ehrenberg, 1838)					x	x			x	x	x	x	x	x	x	x	x	x	x	68.42
Cylindrifflugia acuminata inflata (Pénard, 1899) *															x					5.26
Cylindrifflugia acuminata magna (Deflandre, 1926) *														x						5.26
Cylindrifflugia elegans (Penard, 1890)					x	x			x	x	x	x	x	x	x	x	x	x	x	68.42
Cylindrifflugia elegans teres (Penard, 1890)						x					x									10.53
Cylindrifflugia lanceolata (Penard, 1890)					x	x			x		x	x			x	x	x	x		47.37
Difflugiidae Awerintzew, 1906																				
Difflugia acutissima Deflandre, 1931 *																	x	x		10.53
Difflugia angulostoma Gauthier-Lièvre & Thomas, 1958					x	x														10.53
Difflugia angustata (Deflandre, 1926)					x	x														10.53
Difflugia avellana Penard, 1890					x	x					x								x	21.05
Difflugia bacillariarum Perty, 1849					x															5.26
Difflugia brevicolla Cash & Hopkinson, 1909					x	x					x									15.79
Difflugia corona Wallich, 1864					x	x					x	x	x	x	x	x	x	x		52.63
Difflugia corona ecornis Gauthier-Lièvre & Thomas, 1958					x	x			x			x		x		x	x	x		42.11
Difflugia corona tuberculata Vucetich, 1973					x	x					x			x						21.05
Difflugia decloitrei Godeanu, 1972					x	x														10.53
Difflugia difficilis Thomas, 1955					x	x					x		x	x	x		x	x	x	47.37
Difflugia dragana Ogden & Živković, 1983									x											5.26
Difflugia gigantea Schumlemberg, 1845									x											5.26

Table II. Continuation.

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
Difflugia globularis (Wallich, 1864)					x	x					x	x				x	x	x		36.84
Difflugia globulosa (Dujardin, 1837)					x	x					x				x				x	26.32
Difflugia helvetica Heuscher, 1885									x		x									10.53
Difflugia kabylica Gauthier-Lièvre et Thomas, 1958									x											5.26
Difflugia kempnyi (Stepanek, 1953) *													x			x	x	x		21.05
Difflugia limnetica (Levander, 1900)								x	x			x	x	x	x	x			x	42.11
Difflugia linearis (Penard, 1890) *											x				x	x	x			21.05
Difflugia mammillaris Penard, 1893 *											x				x	x	x	x		26.32
Difflugia microclaviformis (Kourov, 1925)										x										5.26
Difflugia minuta Rampi, 1950					x	x						x	x				x		x	31.58
Difflugia mitriformis Wallich, 1864					x	x						x								15.79
Difflugiidae Awerintzew, 1906																				
Difflugia molesta (Penard, 1902) *																x			x	10.53
Difflugia oblonga Ehrenberg, 1838					x	x				x	x	x	x	x	x	x	x	x	x	63.16
Difflugia penardi (Penard, 1980)					x	x					x		x	x		x	x	x		42.11
Difflugia pleustonica Dioni, 1970					x	x				x				x						21.05
Difflugia pristis Penard, 1902					x	x														10.53
Difflugia pyriformis Perty, 1849 *												x								5.26
Difflugia scalpellum Penard, 1899 *											x				x					10.53
Difflugia schuurmani Van Oye, 1932					x	x			x	x	x	x	x		x	x	x	x		57.89
Difflugia soudanensis Gauthier-Lièvre et Thomas, 1958 *												x								5.26
Difflugia urceolata Carter, 1864					x									x						10.53
Difflugia urceolata lageniformis Wallich, 1864					x	x														10.53
Difflugia urceolata minor Deflandre, 1926					x	x														10.53
Difflugia ventricosa Deflandre, 1926 *																			x	5.26
Zivkovicia compressa (Carter, 1864) *																x	x			10.53
Hyalospheniidae Schultze, 1877																				
Gibbocarina galeata (Penard, 1890)										x										5.26
Longinebela penardiana (Deflandre, 1936)										x										5.26
Longinebela tubulosa (Penard, 1890)										x										5.26
Nebela barbata Leidy, 1874										x										5.26
Nebela collaris (Ehrenberg, 1848)										x										5.26
Padaungiella lageniformis (Penard, 1890)						x														5.26
Padaungiella tubulata (Brown, 1910)						x				x										10.53
Planocarina carinata (Archer, 1867)										x										5.26

Table II. Continuation.

Quadrulella symmetrica (Wallich, 1863)										x										5.26
Quadrulella tubulata Gauthier-Lièvre, 1953 *																			x	5.26

Taxa	**Published studies**										**Sampling in this study**									**FO%**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	
	PE	**SFRr**	**SFRi**	**SFRs**	**CRBa**	**CRBb**	**IB**	**NR**	**PF**	**MP**	**QFp**	**ADII**	**SAD**	**BL**	**RFBF**	**SR**	**CoR**	**CaR**	**MD**	
Lesquereusiidae Jung, 1942																				
Lesquereusia epistomium Penard, 1902					x	x														10.53
Lesquereusia gibbosa Thomas et Gauthier-Lièvre, 1959					x							x							x	15.79
Lesquereusia globulosa Thomas et Gauthier-Lièvre, 1959										x		x		x		x				21.05
Lesquereusia minor Walton, 1930					x	x			x	x	x									26.32
Lesquereusia modesta caudata (Playfair, 1917)					x	x					x									15.79
Lesquereusia modesta minima Van Oye, 1956 *											x									5.26
Lesquereusia modesta Rhumbler, 1896					x	x				x	x			x		x	x	x	x	47.37
Lesquereusia ovalis Gauthier-Lièvre & Thomas, 1960					x	x														10.53
Lesquereusia parva (Thomas et Gauthier-Lièvre, 1959)																			x	5.26
Lesquereusia spiralis (Ehrenberg, 1840)					x	x			x	x	x			x	x	x	x	x	x	57.89
Lesquereusia spiralis caudata Playfair, 1917 *											x									5.26
Lesquereusia spiralis hirsuta Thomas & Gauthier-Lievrè, 1960										x										5.26
Lesquereusia spiralis arcuata Gauthier- Lièvre & Thomas, 1960					x	x			x											15.79
Netzeliidae Kosakyan et al., 2016																				
Cucurbitella madagascariensis Gauthier-Lièvre & Thomas, 1960					x	x			x			x								21.05
Cucurbitella megastoma Gauthier-Lièvre & Thomas, 1960 *											x			x						10.53
Cucurbitella mespiliformis Penard, 1902					x	x					x								x	21.05
Cucurbitella obturata G.L &. Thomas, 1958																x				5.26
Cyclopyxis aplanata (Penard, 1911) *																		x		5.26
Cyclopyxis arcelloides (Penard, 1902)									x		x		x	x	x		x			31.58
Cyclopyxis eurystoma (Deflandre, 1929)					x	x					x	x	x		x					31.58

Table II. Continuation.

Taxa	Published studies										Sampling in this study								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
	PE	**SFRr**	**SFRi**	**SFRs**	**CRBa**	**CRBb**	**IB**	**NR**	**PF**	**MP**	**QFp**	**ADII**	**SAD**	**BL**	**RFBF**	**SR**	**CoR**	**CaR**	**MD**
Netzeliidae Kosakyan et al., 2016																			
Cyclopyxis impressa (Daday, 1905) *													x						x
Cyclopyxis kahli (Deflandre, 1929)					x	x					x	x	x	x		x			
Netzelia achlora (Penard, 1902)					x	x					x		x						x
Netzelia corona (Wallich, 1864)					x	x	x		x	x	x	x	x	x				x	x
Netzelia gramen (Penard, 1902)					x	x			x	x	x	x	x	x	x	x	x	x	x
Netzelia gramen caudata (Gauthier-Lièvre & Thomas, 1960)					x	x													
Netzelia lithophila (Penard, 1902)					x	x			x		x	x	x						x
Netzelia lobostoma (Leidy, 1874)					x	x	x		x	x	x	x	x	x	x	x	x		x
Netzelia lobostoma cornuta (Gauthier-Lièvre & Thomas, 1958)					x					x									
Netzelia lobostoma globulus (Playfair, 1917)										x				x					
Netzelia lobostoma multilobata (Gauthier-Lièvre & Thomas, 1958)					x	x					x		x	x	x				x
Netzelia lobostoma tuberosa (Gauthier-Lièvre & Thomas, 1958)					x							x							
Netzelia muriformis (Gauthier-Lièvre & Thomas, 1958) *											x	x	x	x		x	x	x	
Netzelia oviformis (Cash, 1909)									x	x	x	x		x	x	x	x	x	x
Netzelia tuberculata (Wallich, 1864)					x	x						x	x	x	x	x		x	x
Netzelia wailesi (Ogden, 1980)					x	x			x			x		x	x	x	x	x	x
Phryganellidae Jung, 1942																			
Phryganella hemisphaerica Penard, 1902													x			x	x	x	
Phryganella nidulus Penard, 1902							x												
Taxa	**Published studies**										**Sampling in this study**								FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD
EUGLYPHIDA Copeland 1956																			
Cyphoderiidae De Saedeleer 1934																			
Cyphoderia ampulla (Ehrenberg, 1840)							x											x	10.53
Euglyphidae Wallich 1864																			
Euglypha acanthophora (Ehrenberg, 1841)					x		x		x	x			x	x	x	x	x	x	52.63
Euglypha acanthophora brevispina Penard, 1902										x									5.26
Euglypha ciliata (Ehrenberg, 1848)					x														5.26
Euglypha cristata Leidy, 1874							x												5.26
Euglypha cristata major Wailes, 1912					x		x												10.53
Euglypha denticulata Brown, 1912													x	x	x	x	x		26.32
Euglypha filifera Penard, 1880					x		x			x			x	x	x	x	x	x	47.37
Euglypha filifera magna Van Oye, 1958					x				x										10.53
Euglypha laevis (Ehrenberg, 1845)					x								x	x	x	x		x	36.84
Euglypha mucronata Leidy, 1879					x														10.53
Euglypha rotunda Wailes & Penard, 1911					x					x	x							x	26.32
Euglypha strigosa (Ehrenberg, 1848)					x														10.53

Table II. Continuation.

Euglypha strigosa glabra Wailes, 1898										x									5.26
Euglypha tuberculata Dujardin, 1841					x			x		x			x		x	x	x	x	47.37

Taxa	Published studies										Sampling in this study									FO%
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
	PE	SFRr	SFRi	SFRs	CRBa	CRBb	IB	NR	PF	MP	QFp	ADII	SAD	BL	RFBF	SR	CoR	CaR	MD	
Trinematidae Hoogenraad & De Groot, 1940																				
Trinema complanatum Penard, 1890					x	x					x									15.79
Trinema enchelys (Ehrenberg, 1838)	x				x	x				x				x	x				x	36.84
Trinema lineare Penard, 1890					x	x					x			x	x	x	x	x		42.11
Insertae sedis																				
Argynnia caudata (Leidy, 1879)										x										5.26
Pentagonia marrocana Gauthier-Lièvre & Thomas, 1958					x	x			x											15.79
Pseudonebela africana Gauthier-Lièvre, 1953 *															x					5.26

followed by a set of 21 frequent *taxa*, 58 common and 92 rare (Table II).

When analyzing the number of studies on Testate amoebae in the state of Bahia in different ecological and methodological contexts, it was observed that the Caatinga biome (n=13) showed greater number of studies compared to the Atlantic Forest (n=6), evidencing greater interest and direction of research in the Caatinga (Figure 4a). Regarding the types of aquatic bodies, rivers concentrated the largest number of studies (n=8), while the smallest number was recorded in estuaries, temporary lagoons and dams, with only one study each (Figure 4b). In aquatic systems, the lentic environment recorded the highest frequency of studies (n=10), followed by lotic systems (n=8) (Figure 4c).

Regarding the collection methods, the use of eight different approaches was identified, with emphasis on washing the rhizospheres of aquatic macrophytes (n=7), species such as *Eichhornia crassipes*, and the genera *Pistia* sp. and *Salvinia* sp., were investigated. On the other hand, the methods using the sampling of 40 L and 20 L of water with a graduated bucket, as well as the Van Veen sampler, were the least frequent, with only one study each (Figure 4d). Considering the compartments studied, the largest number of studies was observed in plankton (n=10), only one study was carried out in the surface layer of the sediment (Figure 4e). Regarding the mesh sizes of the nets used, the 20 µm net was the most used (n=13), while the 64 µm net and the 62 µm mesh sieve were the least used, both with only one study each (Figure 4f).

Finally, it was found that, of the three biomes present in the state of Bahia (Caatinga, Cerrado and Atlantic Forest), only the Cerrado biome was not investigated, indicating a significant gap in studies for this biome (Figure 4g).

When analyzing the number of testate amoebae taxa recorded in the state of Bahia, we found that the observed patterns, from the largest number of studies to the ecological and methodological contexts investigated (Figure 4), are the same for the number of *taxa*, except for the compartment criterion, although plankton presented the largest number of studies,

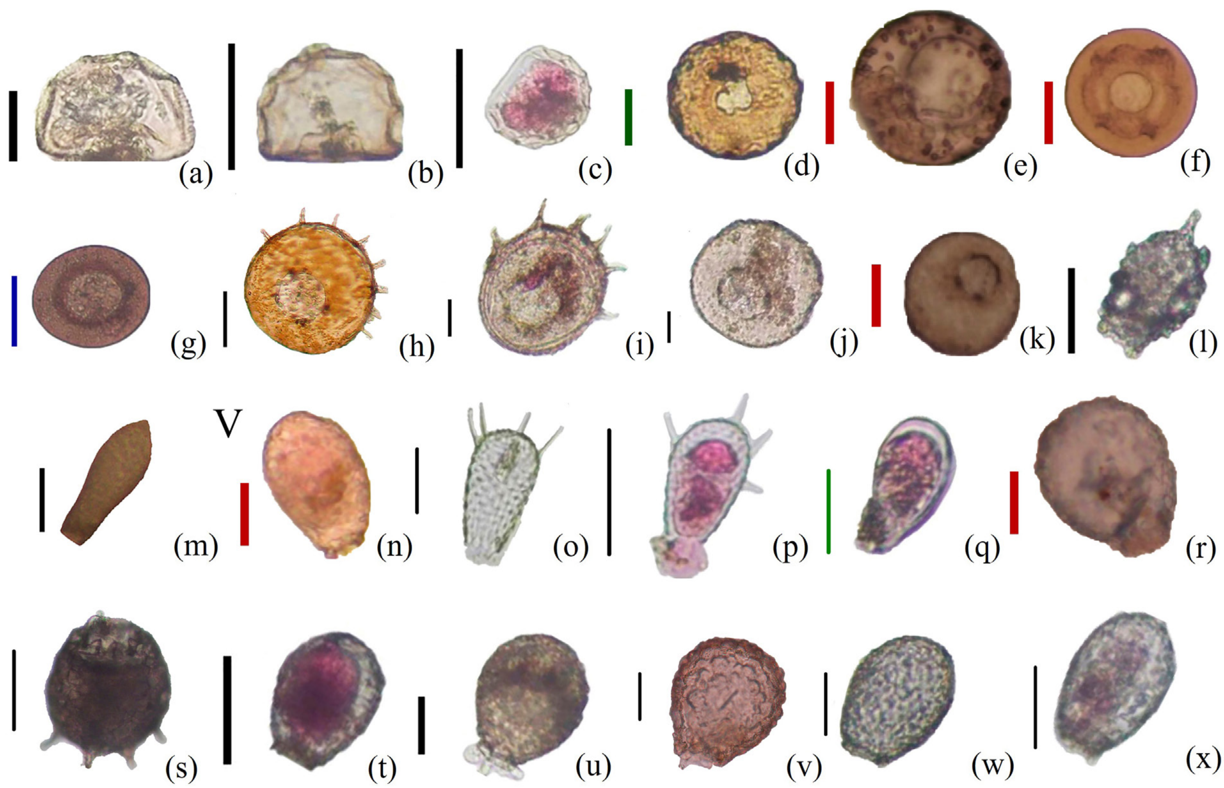

Figure 1. **Testate amoebae from the state of Bahia. (a)** ***Arcella costata,*** **(b)** ***Arcella gibbosa,*** **(c)** ***Arcella hemisphaerica undulata,*** **(d)** ***Arcella lobostoma,*** **(e)** ***Arcella mitrata,*** **(f)** ***Galeripora discoides,*** **(g)** ***Galeripora megastoma,*** **(h)** ***Centropyxis aculeata,*** **(i)** ***Centropyxis discoides,*** **(j)** ***Centropyxis ecornis,*** **(k)** ***Centropyxis ecornis,*** **(l)** ***Cylindrifflugia elegans,*** **(m)** ***Difflugia acuminata,*** **(n)** ***Difflugia schuurmani,*** **(o)** ***Euglypha acantophora,*** **(p)** ***Euglypha filifera,*** **(q)** ***Euglypha tuberculata,*** **(r)** ***Lesqueuresia modesta,*** **(s)** ***Netzelia corona,*** **(t)** ***Netzelia gramen,*** **(u)** ***Netzelia. lobostoma,*** **(v)** ***Netzelia.muriformis,*** **(w)** ***Netzelia oviformis,*** **(x)** ***Netzelia wailesi.*** **Bar scales, Black: 50 µm, blue: 100 µm, green: 20 µm and red: 10 µm.**

periphyton stood out with the largest number of recorded *taxa* (n=124) (Figure 4e). The greatest richness of *taxa* was observed in the Caatinga (n=139) (Figure 5a), in the rivers (n=127) (Figure 4b), in the lentic system (n=141) (Figure 4c), and in the washing of rhizospheres of aquatic macrophytes (n=148) (Figure 4d). Furthermore, all *taxa* recorded in the studies were observed using the 20 µm net (n=172) (Figure 5f).

The rarefaction curve (Figure 5) demonstrates that the sampling of Testate amoebae in Bahia was sufficient to define an asymptotic trend, suggesting that the data obtained allow estimating approximately 76.3% of the total biodiversity of these organisms. The high diversity recorded reflects the heterogeneity of the aquatic bodies and biomes present in the state, highlighting the environmental richness of the region.

It is worth noting that, when analyzing all available studies, including both scientific publications and gray literature, it was observed that most studies follow an ecological approach. These studies predominantly focus on describing the taxonomic composition of Testate amoebae species present in different aquatic ecosystems, as well as on analyzing the influences exerted by environmental variables on the structure and dynamics of these communities. However, the pioneering study by Cunha (1916) stands out as an exception, which stood out for being

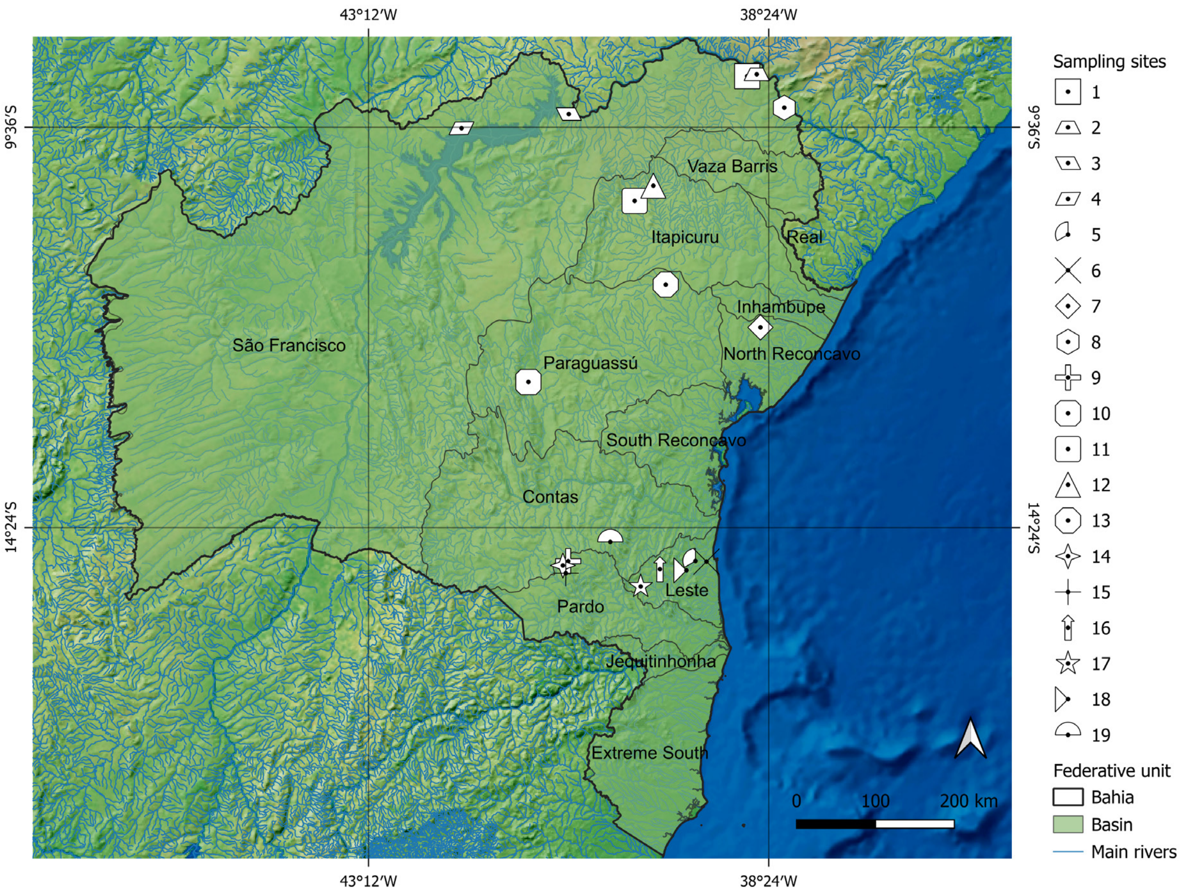

Figure 2. Map of the state of Bahia, Brazil, with the 19 sampling points of testate amoebae as described in Table I, highlighting the hydrographic basins of the region.

a general survey, contributing to consolidating initial knowledge about the group.

DISCUSSION

Taxonomic diversity

The record of 172 infrageneric *taxa* of Testate amoebae for the state of Bahia reveals a significant richness in its continental aquatic environments, representing 71.67% of the 240 species listed for Brazil (Elmoor-Loureiro et al. 2023). This richness was reflected in the species accumulation curve, which indicated a tendency to approach the asymptote. Additionally, according to the criteria of Heck Junior et al. (1975) on the species accumulation curve, inventories that record between 50% and 75% of common species can be considered satisfactory, which highlights the relevance of the data obtained. Furthermore, the taxonomic richness of the state surpassed that recorded in Mato Grosso do Sul (141 *taxa*), according to a survey carried out by Rosa et al. (2017), based on an extensive compilation of data from 26 studies, seven more than those analyzed in the present research.

The richness of Testate amoebae in Bahia includes periphytic, planktonic and benthic forms. The families Difflugiidae and Arcellidae stood out as the most representative among Testate amoebae in the state, a pattern also observed in other regions of Brazil (Lansac-Tôha 2007, Pereira et al. 2011). In a study carried out by Elmoor-Loureiro et al. (2023) which also addresses Testate amoebae in Brazil,

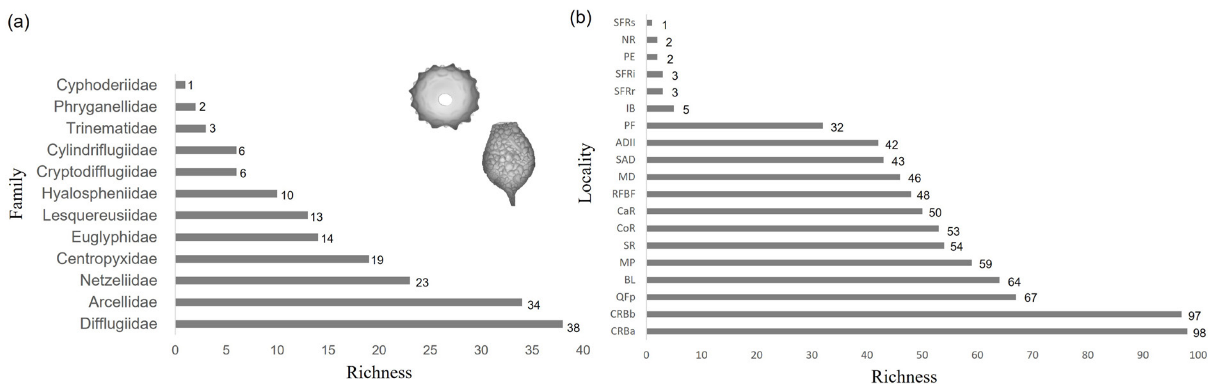

Figure 3. Occurrence of testate amoebae in the state of Bahia. (a) Richness by family of testate amoebae in the state of Bahia, (b) Richness of testate amoebae by location in the state of Bahia. The meanings of the codes are in Table I.

these families were highlighted as the most representative, with 101 and 27 *taxa*, respectively.

The dominance of the Difflugiidae family suggests that its members have greater adaptive capacity, possibly due to morphological and physiological characteristics that give them evolutionary advantages (Ogden 1983). The genus *Difflugia* is the most frequently found in the studies, which reflects its wide distribution and abundance.

On the other hand, the families Cyphoderiidae and Phryganellidae had the lowest richness, reflecting the low diversity recorded for these families (5 and 4 *taxa* described, respectively). According to Lansac-Tôha et al. (2001), the families Cyphoderiidae and Phryganellidae are generally very frequent and present greater richness in moss and sphagnum samples. Thus, the scarcity of studies on testate amoebae in these habitats in Brazil reflects the restricted distribution and low occurrence of these families in the country. Schwind et al. (2017), when studying the testate amoebae in the Paraná River Floodplain, recorded only one species belonging to this family. Understanding the factors that influence the distribution and abundance of the different families of thecamoeba is essential to understand the structure and functioning of aquatic ecosystems (Schwind et al. 2019, Arrieira et al. 2017). These factors are not yet sufficiently researched, especially in tropical and subtropical regions. More investment in this type of research is needed, both in Brazil and on a regional scale, *e.g.* in the state of Bahia.

In addition, some *taxa* were recorded, such as *Argynnia caudata* (Leidy, 1879), *Pentagonia maroccana* (Gauthier-Lièvre & Thomas, 1958) and *Pseudonebela africana* (Gauthier-Lièvre, 1953), whose taxonomic position is still uncertain (*Insertae sedis*), requiring more in-depth studies to concretely define the phylogenetic position of these *taxa*.

Differences in sampling efforts make it difficult to identify patterns in the richness of Testate amoebae in Bahia, considering the various ecological and methodological aspects evaluated, such as biome, type of environment, ecosystem, compartment, and the methods and mesh sizes used. It is important to highlight that nets with mesh sizes greater than 20 μm tend to underestimate the richness of these organisms (Silva et al. 2020), while the 20 μm mesh proved to be adequate for recording the 172 *taxa* observed in this study.

In addition, studies that do not focus on Testate amoebae, but rather on zooplankton, and therefore use nets with mesh sizes > 20 μm,

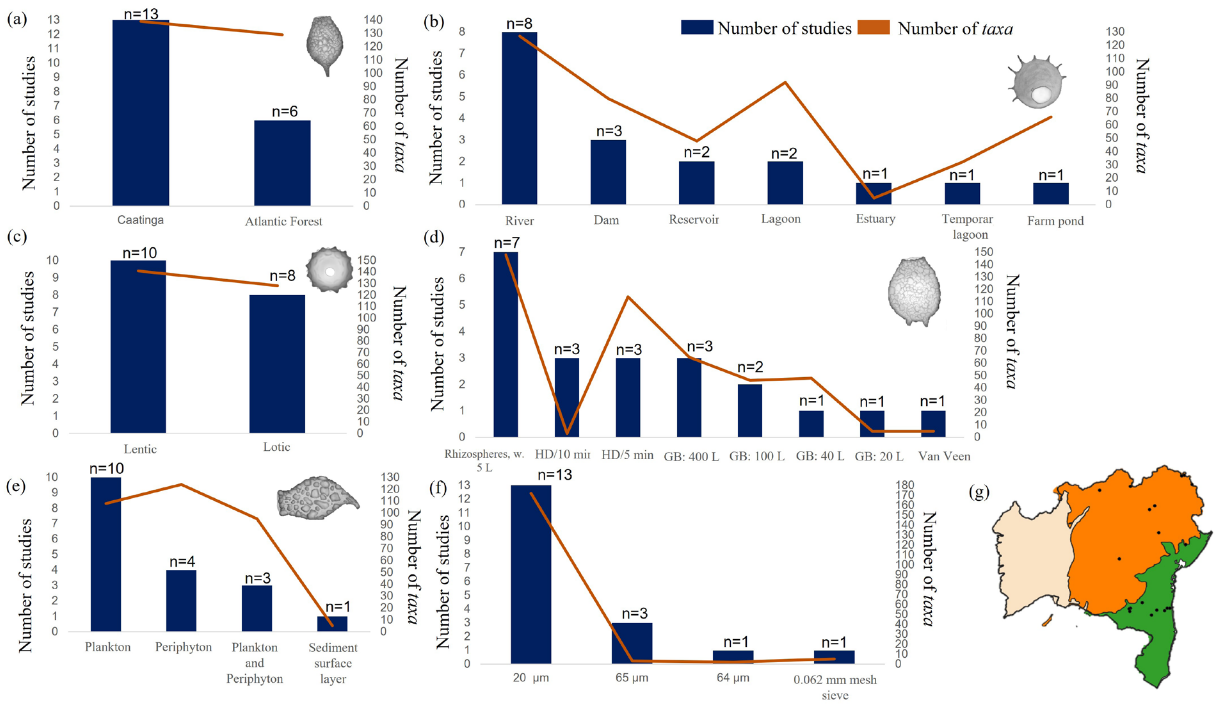

Figure 4. Number of studies and number of *taxa* of testate amoebae in the state of Bahia. (a) Biomes, (b) Type of aquatic bodies, (c) Type of ecosystem, (d) Collection methods, (e) Compartment, (f) Mesh sizes of sampling net, (g) Distribution of testate amoebae in the state of Bahia in relation to the biomes: green color: Atlantic Forest, orange color: Caatinga, beige color: Cerrado. AH: Horizontal drag, BG: Graduated bucket, l.: washed in 5 L, min.: minutes, n = number of studies.

often underestimate the richness of this group. This is evident in the works of Neumann-Leitão & Nogueira-Paranhos (1987), who recorded only 3 *taxa* (65 µm) (Table II, Figure 5f), and of Araújo & Nogueira (2016), with only 2 *taxa* (64 µm) (Table II, Figure 5f). Even in studies focusing on thecamoeba, such as that of Cruz (2012), the use of a62 µm mesh seems to have limited the detection of the group's richness, resulting in the recording of only 5 *taxa* (Table II, Figure 5f).

The heterogeneity in the choice of mesh size among published studies (RSF-65 µm, CRBa-20 µm, CRBb-20 µm, IB-62 µm, NR-64 µm, PF-20 µm, MP-20 µm) makes it difficult to compare results and identify distribution patterns of Testate amoebae. The variation in the number of *taxa* identified (SFR-7 *taxa*, CRBa-98 *taxa*, CRBb-97 *taxa*, IB-5 *taxa*, NR-2 *taxa*, PF-32 *taxa*, MP-59 *taxa*) suggests that the choice of mesh size can significantly influence the estimates of Testate amoebae richness. Although the 20 µm mesh retains a greater range of amoeboid organisms compared to larger mesh sizes, this single parameter is not sufficient to establish definitive patterns. Ecological, physical, and chemical factors of the environments may also contribute to the observed differences (Silva et al. 2020).

It is important to highlight that the greater number of *taxa* observed in the Caatinga biome, compared to the Atlantic Forest, may be associated with the greater number of studies carried out in the Caatinga, which reflects a more robust research pattern in this region. The scarcity of studies in several areas, including the absence of studies in the Cerrado, contributes

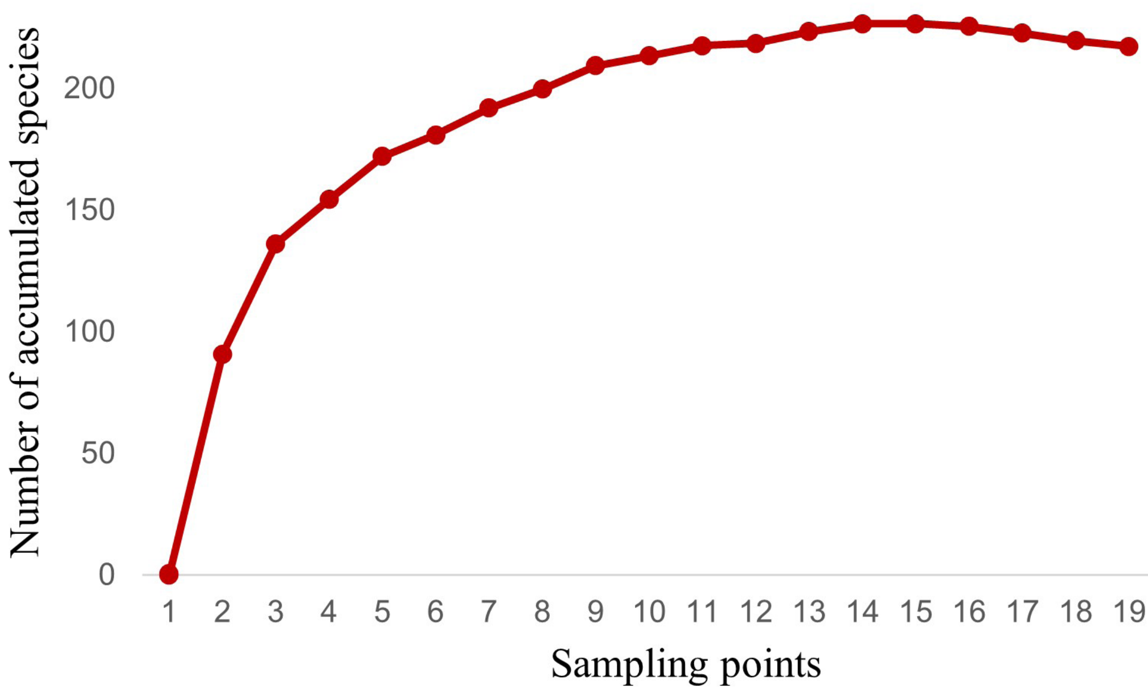

Figure 5. Accumulation curve of testate amoebae from the state of Bahia, Brazil, based on richness, sampled locations, and the Jeckknife 2 richness estimator.

to gaps in knowledge about the distribution of these Testate amoebae, making it difficult to fully understand the diversity of organisms in these biomes.

Scope, reach, and accessibility of studies

Bahia, although rich in water resources, has a significant gap in knowledge about the diversity of Testate amoebae (Silva et al. 2009, 2020). The region showed great variation in *taxa* richness in different locations (Figure 3b). Among the biomes in the state, the Caatinga, with its unique characteristics of semiarid climate, xerophilous vegetation and high degree of endemism (Melo et al. 2023), concentrates a significantly higher number of studies (13) compared to the Atlantic Forest, which has only six studies. This disparity, combined with the lack of records in the Cerrado, compromises a comprehensive view of the geographic distribution of these organisms and highlights the need to expand research efforts in less explored biomes, such as the Atlantic Forest and the Cerrado.

Although most studies were carried out in rivers, with a total of eight studies, protozooplankton richness is not restricted to large ecosystems. These organisms can be found in a variety of habitats, including smaller ones with distinct characteristics. For example, the study by Silva et al. (2020), which recorded 32 *taxa* of Testate amoebae in a temporary and eutrophic water body, is the only study carried out in a temporary environment in the state.

In addition, the research by Silva et al. (2009), conducted in the Atlantic Forest in the regions of Itabuna and Ilhéus, identified a high species richness, with 115 *taxa* recorded. These results suggest that the richness of Testate amoebae may be closely related to the specific environmental characteristics of each biome, highlighting the importance of exploring a greater diversity of environments to better understand the distribution and diversity of these organisms.

Most studies on thecamoebae have focused on plankton (Schwind et al. 2015, 2017, Lansac-Tôha et al. 2000, Velho et al. 1996). However, the limited sampling in periphyton indicates the need to expand research efforts in this compartment, aiming at a more in-depth understanding of the dynamics of fixed communities, as presented by Lansac-Tôha et al. (2007). Although not being present in most studies, periphyton stood out for having the greatest richness of *taxa*, suggesting its importance for local biodiversity.

Conversely, the lack of more detailed investigations on the sediment reveals a significant gap in the understanding of the diversity of thecamoebae in this habitat. As one of the main ecological niches, the sediment offers fundamental conditions for the growth and reproduction of these amoebae, which reinforces the need for greater attention to this environment in future research (Lansac-Tôha et al. 2014).

The variability in collection methods, including the different volumes of water analyzed, may have influenced the number of Testate amoebae recorded. In general, larger volumes (such as 400 L) resulted in a slightly higher number of species compared to smaller volumes. However, sampling carried out in the rhizosphere demonstrated a greater richness of *taxa*. This trend was also observed by Miranda & Mazzoni (2015), who reported higher richness of Testate amoebae in environments with aquatic vegetation.

Aquatic macrophytes play an essential role in the structure and functioning of aquatic ecosystems, providing structural support and sustaining a high diversity of protozoa due to their high productivity (Barbosa et al. 2024, Silva et al. 2009, Zingel & Nõges 2008, Wetzel 1990). In this study, the results indicate that the rhizosphere washing method is highly efficient for sampling thecamoebae. However, it is essential to consider the limitations associated with the analyses, such as the increase in organic matter and, consequently, the difficulty in visualizing the specimens.

Perspective for the next 10 years

In addition to their relevance in understanding the biodiversity of Testate amoebae, their use as bioindicators has gained prominence, as demonstrated by the exponential growth of scientific publications on the subject on a global scale (Silva et al. 2022). Thanks to their versatility as indicators, these organisms can provide information on a wide range of environmental conditions, including pollution levels in aquatic, terrestrial and atmospheric systems, fluctuations in sea level, and even paleoclimatic reconstructions (Freitas et al. 2022).

Despite the abundance of river basins in Bahia (Brasil 2021), the scope of studies carried out in the state is still limited. Expanding investigations beyond the regions already explored, especially in the Far West, Northeast and Center-North areas, may reveal new records of Testate amoebae, highlighting the wealth of these organisms that is still unknown. Therefore, it is essential to intensify research in other continental aquatic ecosystems in Bahia, contributing to reducing the gaps in knowledge about the biogeographic distribution of Testate amoebae in the state.

The taxonomic identification of Testate amoebae presents significant challenges due to the variability in their morphological, morphometric, and compositional characteristics, which directly impacts the quality and accuracy of ecological studies (Silva et al. 2009). The lack of standardized and specific methodologies for Testate amoebae is a critical limitation that needs to be overcome in future regional studies (Rosa et al. 2017).

In addition, variable environmental conditions can induce morphological modifications in these organisms, making species identification even more difficult (Wanner 1995). In this context, the training of new taxonomists specialized in Testate amoebae is essential to overcome these barriers, allowing advances in the understanding of biodiversity, community dynamics and ecosystem functioning (Rosa et al. 2017, Silva et al. 2022).

This survey highlighted the significant taxonomic richness of Testate amoebae in Bahia but showed that the richness of this group in the Brazilian Northeast is still little explored. It is essential that new studies are conducted to expand the understanding of the distribution and ecology of these species, covering regions and environments that are still undersampled, such as wetlands, mangroves, soils, mosses, bromeliads and estuaries, among others.

The training of new taxonomists, combined with the standardization of protocols, the application of collection methods that are more appropriate for each type of environment and the use of molecular techniques for the identification and analysis of the genetic diversity of Testate amoebae, is essential to fill these gaps. These advances will allow for increased comparability between studies and the construction of a more comprehensive and detailed overview of the biodiversity of this group.

This approach not only seeks to expand taxonomic knowledge, but also to understand how these organisms respond to variations in environmental conditions and how they contribute to ecological processes in the ecosystems in which they are found.

Main study groups and collections of Testate amoebae in Bahia

The Dr. Márcio Borba da Silva in materials and methods, the aforementioned researcher, is the curator of the Scientific Limnological Collection of the Federal University of Bahia, Anísio Teixeira Campus (CLCAT), which contains approximately 3,100 samples (Figure S1). The CLCAT is a highly valuable collection, functioning as a robust database for limnological studies. This collection supports not only ongoing research but also future investigations, contributing significantly to the advancement of knowledge about aquatic ecosystems, playing a fundamental role in reducing research gaps in Bahia and encouraging the production of impactful scientific publications.

The other studies were carried out by researchers from Pernambuco and other municipalities in Bahia, such as Paulo Afonso, Ilhéus, and Salvador. Although there has been progress in research in recent years, there is still a significant gap in knowledge about Testate amoebae in the state. The concentration of studies in specific locations has left several regions without sampling, which limits the complete understanding of the distribution of these species.

However, the consolidation of research partnerships with other researchers in the state, such as teacher Dra. Patrícia Maria Mitsuka (State University of Bahia - UNEB, Caetité - Bahia) who has been carrying out limnological studies in the semiarid region since 2002, teacher Dr. Nadson Ressyé Simões da Silva (Federal University of Southern Bahia - UFSB, Porto Seguro - Bahia), teacher Dra. Luciana de Mendonça Galvão (State University of Southwest Bahia - UESB, Vitória da Conquista - Bahia) and Dra. Mariane Amorim Rocha (State University of Southwest Bahia - UESB, Jequié - Bahia) who carry out studies with zooplankton, especially microcrustaceans and Rotifera, can increase knowledge of the biodiversity of Testate amoebae in the state, exchanging samples and fostering scientific collaborations even in the capture of resources from development agencies.

CONCLUSIONS

Research conducted in Bahia has been essential for understanding the distribution of Testate amoebae in the state, revealing significant diversity. Compared to Mato Grosso do Sul, Bahia has the greatest species richness, although it is believed that this richness is even greater, with the possibility of new records from additional studies. These data can be enriched by integrating research already conducted in several locations, such as the São Francisco River, Colônia, Cachoeira, Salgado and other bodies of water that have already been sampled. However, the richness of Testate amoebae in the region may be underestimated, partly due to the limitation of research, which focuses on specific types of aquatic environments, in addition to the use of nets with mesh sizes larger than 20 µm, which do not retain most of the organisms. Additionally, the lack of resources contributes to the scarcity of in-depth studies. Despite the importance of these organisms for limnology, research on Testate amoebae in Bahia has a geographical distribution predominantly restricted to the Caatinga and Atlantic Forest biomes. This limitation results in significant gaps in knowledge about the seasonal, spatial and taxonomic distribution of these amoebae in the state, highlighting the urgent need to expand studies to other biomes and environments that are still little explored.

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SUPPLEMENTARY MATERIAL

Table SI.
Figure S1.

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