

## Environmental patterns structure of testate amoebae assemblages in Neotropical Conservation Units

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### ABSTRACT

#### Environmental patterns structure of testate amoebae assemblages in Neotropical Conservation Units

Testate amoebae are effective bioindicators of environmental changes in aquatic ecosystems. This study assessed the influence of environmental heterogeneity and seasonality on the structure of testate amoebae assemblages in two Conservation Units in northeastern Brazil: the National Park of Boa Nova (PARNA) and the Wildlife Sanctuary (REVIS), Bahia. Samples were collected from 12 sites during the rainy and dry seasons, alongside abiotic data. We identified 83 *taxa*, including 26 new records for Bahia. The families Euglyphidae and Hyalospheniidae were the most species-rich. *Arcella hemisphaerica* Perty, 1852, *Centropyxis aculeata* (Ehrenberg, 1838), and *Euglypha tuberculata* Dujardin, 1841 occurred in all samples, with the latter two, along with *Cyphoderia ampulla* (Ehrenberg, 1840), being the most abundant. Both environmental heterogeneity and season significantly shaped amoebae assemblages, with greater abundance and diversity in the dry season. Rarefaction curves indicated adequate sampling. Cluster analysis distinguished assemblages by rainfall patterns and highlighted the distinctiveness of samples from the Caatinga (P12) compared to ecotonal sites. Redundancy analysis showed that water temperature, transparency, depth, conductivity, dissolved solids, and salinity were key environmental predictors. These findings underscore the importance of considering seasonal and spatial variability in conservation strategies and highlight the relevance of testate amoebae as bioindicators in Neotropical protected areas.

**KEY WORDS:** environmental heterogeneity, seasonality, environmental predictors, semiarid.

### RESUMO

#### Padrões Ambientais na Estrutura de Assembleias de Amebas Testáceas em Unidades de Conservação Neotropicalis.

As amebas testáceas são bioindicadores eficazes de alterações ambientais em ecossistemas aquáticos. Este estudo avaliou a influência da heterogeneidade ambiental e da sazonalidade sobre a estrutura das assembleias de amebas testáceas em duas Unidades de Conservação no nordeste do Brasil: o Parque Nacional de Boa Nova (PARNA) e o Refúgio de Vida Silvestre (REVIS), ambos localizados na Bahia. As amostras foram coletadas em 12 locais durante as estações chuvosa e seca, simultaneamente com dados abióticos. Foram identificados 83 *taxa*, incluindo 26 novos registros para o estado da Bahia. As famílias Euglyphidae e Hyalospheniidae foram as mais ricas em espécies. *Arcella hemisphaerica* Perty, 1852, *Centropyxis aculeata* (Ehrenberg, 1838) e *Euglypha tuberculata* Dujardin, 1841 ocorreram em todas as amostras, sendo estas duas últimas, juntamente com *Cyphoderia ampulla* (Ehrenberg, 1840), as mais abundantes. Tanto a heterogeneidade ambiental quanto a sazonalidade influenciaram significativamente as assembleias de amebas, com maior abundância e diversidade observadas na estação seca. As curvas de

rarefação indicaram esforço amostral adequado. A análise de agrupamento separou as assembleias de acordo com os padrões de pluviosidade e revelou a distinção das amostras da Caatinga (P12) em relação aos locais ecotonais. A análise de redundância identificou a temperatura da água, transparência, profundidade, condutividade, sólidos dissolvidos e salinidade como principais preditores ambientais. Esses resultados reforçam a importância de se considerar a variabilidade sazonal e espacial em estratégias de conservação e destacam o papel das amebas testáceas como bioindicadores relevantes em áreas protegidas neotropicais.

**PALAVRAS CHAVE:** heterogeneidade ambiental, sazonalidade, preditores ambientais, semiárido..

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## INTRODUCTION

Testate amoebae are a polyphyletic group of free-living unicellular amoeboid protists, characterized by the presence of a shell with an aperture through which pseudopods are projected for locomotion and feeding (Smith *et al.*, 2009). These organisms have been investigated, for the most part, in lentic freshwater ecosystems located in Neotropical regions, although they also occur in several other biotopes, such as peatlands, periphyton, sediments and riparian vegetation (Lansac-Tôha *et al.*, 2014; Miranda & Mazzoni, 2015).

Because of their diversity and widespread distribution (Foissner, 1999; Lansac-Tôha *et al.*, 2007), the testate amoebae are considered efficient bioindicators of aquatic systems (Lamentowicz *et al.*, 2010; Freitas *et al.*, 2022; Silva *et al.*, 2022). Furthermore, these organisms are extremely sensitive to variations in environmental conditions (Marcisz *et al.*, 2020), and their short life cycles allow the detection of rapid responses to ecological disturbances (Foissner, 1999). These characteristics make testate amoebae excellent bioindicators, as changes in their composition, abundance, or diversity can immediately reflect changes in water quality and ecosystem integrity. Despite their potential, the use of these testate amoebae in ecological analyses of aquatic ecosystems is still largely neglected, which represents an opportunity to expand understanding of environmental health and the impacts of anthropogenic pressures on aquatic environments.

Accordingly, most reports about the assemblages of testate amoebae in Brazil refer to aquatic habitats in southern, southeastern and central regions (see Lansac-Tôha *et al.*, 2007), while similar studies in northern and northeastern Brazil have been neglected (Silva *et al.*, 2009). In spite of these biased data, a total of 240 species

of testate amoebae have been recorded in Brazil, where the Upper Paraná stands out as the richest hydrographic system in protozooplankton, followed by Southeastern Atlantic, Tocantins-Araguaia and Amazon basins, respectively (Elmoor-Loureiro *et al.*, 2023).

On the other hand, few bioecological studies in testate amoebae are available for the state of Bahia, even though it represents one fifth of the Brazilian territory, encompassing an area of 564 760 429 km<sup>2</sup> (IBGE, 2024). The first report in this region was carried out by Cunha (1916) in the municipality of Remanso, who identified two species: *Centropyxis aculeata* (Ehrenberg, 1988) and *Trinema enchelys* (Ehrenberg, 1938). Recently, Silva *et al.* (2025) conducted a comprehensive inventory of testate amoebae in Bahia, gathering comprehensive information on the groups occurrence throughout the state. The study, based on samples from 19 collection sites, resulted in the registration of 170 *taxa*, distributed across 12 families and 21 genera. This survey represents a milestone in the understanding of regional diversity and reinforces the need for expanded similar studies, given the relevance of testate amoebae in ecological processes and as environmental bioindicators.

Moreover, there is a remarkable gap in analyses of testate amoebae assemblages in Conservation Units (CUs), once most of reports refer to unprotected natural areas (Silva *et al.*, 2025). On the other hand, the National Park of Boa Nova (PARNA) and the Wildlife Sanctuary (REVIS) located in the state of Bahia, Brazil, represent strategic conservation areas located in a transition zone (ecotone) between Caatinga and Atlantic Forest biomes (ICMBio, 2014), thus being particularly relevant to biodiversity studies. In addition, both CUs are within the Brazilian semiarid region, determining a remarkable hydrologic variation

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characterized by long droughts interspersed with rainy periods and unique ecological dynamic features. Consequently, studying the seasonality of testate amoeba communities in these CUs is crucial, as these water variations can directly alter the availability of nutrients, oxygen, and microhabitats in aquatic ecosystems, impacting the abundance, diversity, and composition of these organisms' communities. Thus, understanding how seasonal changes influence testate amoeba communities provides valuable information about the dynamics and functionality of these ecosystems, reinforcing the importance of monitoring them to assess the environmental health of these protected areas.

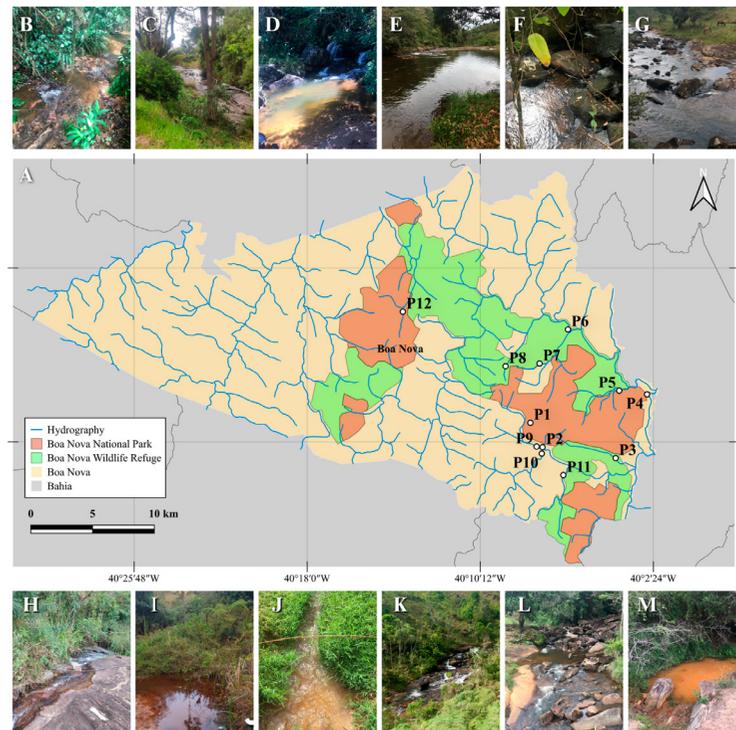
Therefore, the goal of this study was to characterize the structure of the testate amoebae assemblages in the aquatic habitats from PARNA and

REVIS in Bahia, northeastern Brazil. Analyses of abundance, frequency of occurrence and diversity indices were carried out in 12 localities from both regions during two distinct hydrological periods (low and high precipitation). Furthermore, the possible relationships between these biological variables and abiotic factors were investigated in order to infer the spatial and seasonal dynamics of these aquatic ecosystems.

## METHODS

### Study area

The PARNA and REVIS refer to two Neotropical Conservation Units (CUs) located in the municipalities of Boa Nova and Manoel Vitorino, state of Bahia, northeastern Brazil, respectively



**Figure 1.** (A) Location of the Neotropical Conservation Units (PARNA and REVIS, Boa Nova – Bahia, Brazil) and collection sites (P1 to P12). Images of collection sites during low precipitation periods: (B) P1 "José Miguel Stream", (C) P2 "Three Waterfalls", (D) P3 "Timorante River", (E) P4 "Uruba River", (F) P5 "Piabanha Waterfall Stream", (G) P6 "Vazante", (H) P7 "Traíras Stream", (I) P8 "Inglês Hills", (J) P9 "Alba Waterfall", (K) P10 "Three Waterfalls – Embasa Catchment", (L) P11 "7 de setembro Waterfall" and (M) P12 "Pé da Ladeira Stream". (A) *Localização das Unidades de Conservação Neotropicals (PARNA e REVIS, Boa Nova – Bahia, Brasil) e dos locais de coleta (P1 a P12). Imagens dos locais de coleta durante os períodos de baixa precipitação: (B) P1 "Riacho José Miguel", (C) P2 "Três Cachoeiras", (D) P3 "Rio Timorante", (E) P4 "Rio Uruba", (F) P5 "Riacho da Cachoeira do Piabanha", (G) P6 "Vazante", (H) P7 "Riacho das Traíras", (I) P8 "Morro dos Ingleses", (J) P9 "Cachoeira da Alba", (K) P10 "Três Cachoeiras – Captação da Embasa", (L) P11 "Cachoeira 7 de Setembro" e (M) P12 "Riacho Pé da Ladeira".*

(Fig. 1). This territory is separated by the Ouricana Hills, acting as a natural barrier to the humid winds from the Atlantic Ocean. These areas encompass two biomes (Caatinga and Atlantic Forest) separated by a narrow transition zone of about 15 km, representing a distinctive regional feature (ICMBio, 2014).

The altitude of PARNA and REVIS ranges from 440 and 1111 m above sea level while the annual temperature varies between 14°C and 26°C, with a mean value of 23°C (Rêgo, 2017). This region is characterized by a remarkable climate variation across a relatively short extension, divided into: humid tropical areas covered by forests and presenting high temperature and rainfall (1500 mm/year); humid and hot climate with a dry season during winter and vegetation adapted to this seasonal variation; and semiarid (Caatinga biome), with reduced rainfall (less than 700 mm/year) (Simões, 2020).

### Sampling Strategy

The hydrological periods were determined based on the accumulated data of rainfall (in mm) over 30 days before the collection of samples obtained from the National Institute of Meteorology (INMET). Since there is no meteorological station in Boa Nova, these data were derived from the facility available in Vitória da Conquista, a municipality close to PARNA and REVIS showing similar climate conditions in relation to both CUs.

The sampling was carried out in 12 sites (P1 to P12) (Fig. 1) during two diurnal field expeditions: C1 (October/2022; low precipitation: LP < 10 mm) and C2 (April/2023; high precipitation: HP ≥ 40 mm), totalling 72 samples. Of the 12 sampling points analyzed in our study, the majority are in the Atlantic Forest (P1 to P11), while only one point (P12) is located in the Caatinga. Each sample was collected in triplicates by filtering 100 l of water using a graded bucket and a plankton net (pore net of 20 µm), followed by fixation in 4% formalin.

Simultaneously, several abiotic parameters were measured in situ, as follows: water temperature (°C), conductivity (µS/cm), total dissolved solids (TDS in ppm), and salinity (%) levels were recorded using a multi-parameter probe

(AKSO®). The water transparency (cm) was determined using a Secchi disk while the depth (cm) was determined with a 3-meter-long tape measure.

### Laboratory Procedures

For the qualitative and quantitative analyses of testate amoebae, the collected samples were stained in Rose Bengal and sorted in Sedgewick-Rafter chambers using an optical microscope (Opton, model TNB 41). Only the test with stained protozoa were taken into account for counts.

The species were identified according to Ehrenberg (1830), Dujardin (1841), Leidy (1874), Penard (1890), Deflandre (1928, 1929), Gassowsky (1936), Gauthier-Liévre (1953), Gauthier Lièvre & Thomaz (1958) Decloitre (1962), and Todorov & Bankov (2019). In the case of new occurrences for the state of Bahia, we relied on the report by Silva *et al.* (2025). It should be pointed out that *taxa* identified only at the genus level were not interpreted as new occurrences.

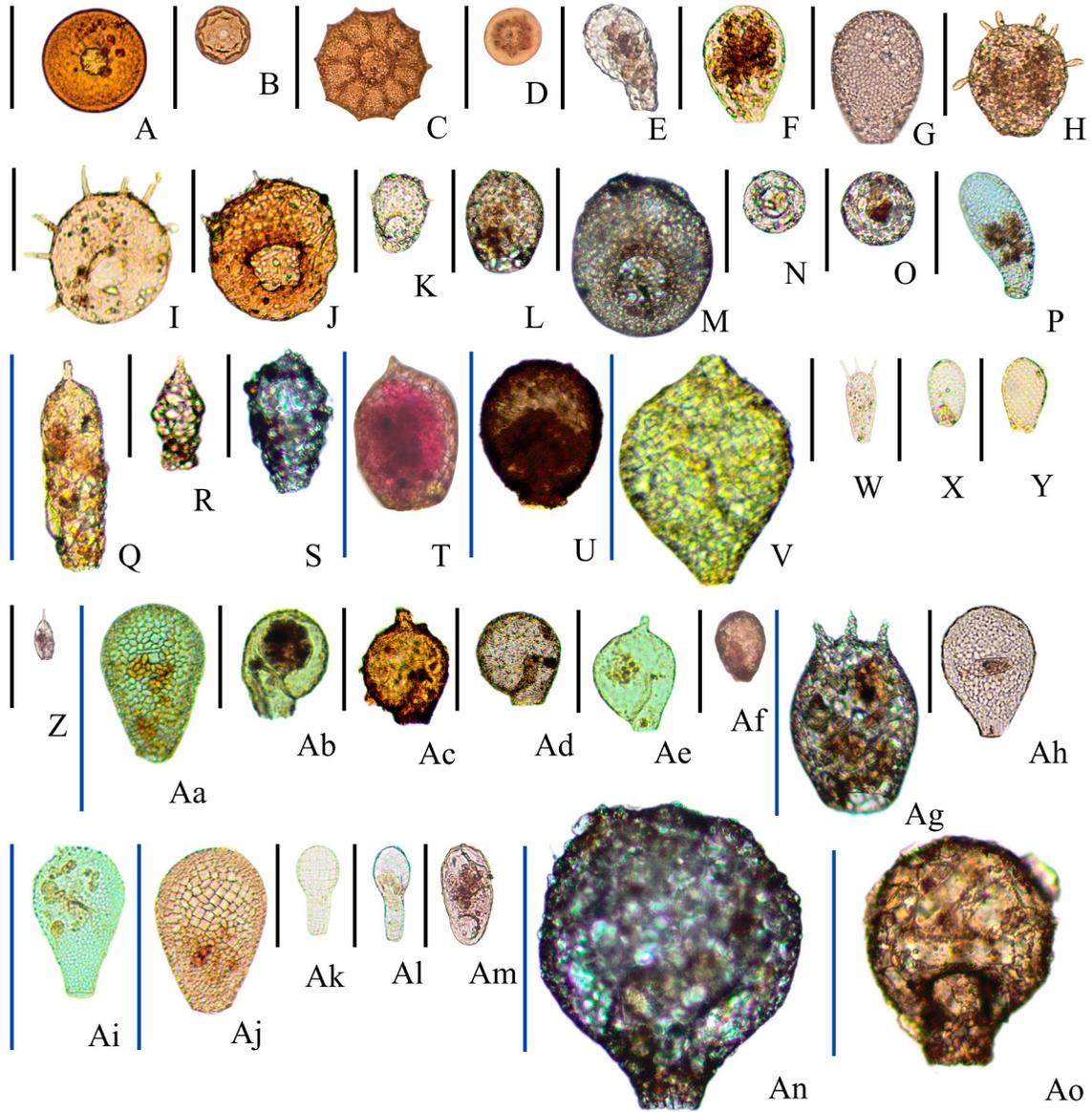
All samples were stored as vouchers in the Coleção Limnológica Científica (CLCAT) from the Laboratório de Limnologia e Biomonitoramento do Instituto Multidisciplinar de Saúde da Universidade Federal da Bahia (UFBA), Campus Anísio Teixeira.

### Data analysis

The abundance (Ind/L) and the frequency of occurrence (FO%) of each taxon were estimated. The FO was calculated as the percentage of samples presenting each taxon in relation to the total of samples, following the categorization proposed by Dajoz (1983).

The abundance values were submitted to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests. Putative significant differences in the abundance of testate amoebae assemblages among the collection sites (P1 to P12) were evaluated using the analysis of variance (ANOVA) followed by the Tukey's test. The t test was used to verify possible differences in the abundance according to precipitation index (low and high precipitation).

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**Figure 2.** Photomicrograph of some *taxa* of testate amoebae from two Neotropical Conservation Units (PARNA and REVIS) in northeastern Brazil. Black scale bars = 100 μm; blue = 200 μm. *Fotomicrografia de alguns taxa de Amebas testáceas das duas Unidades de Conservação Neotropicals (PARNA e REVIS) no nordeste do Brasil. Barras de escala pretas = 100 μm; azuis = 200 μm.* A - *Arcella multilobata* (Golemsky, 1964); B - *Arcella hemisphaerica undulata* Deflandre, 1928; C - *Galeripora dentata* (Ehrenberg, 1830); D - *Galeripora discoides* (Ehrenberg, 1871); E - *Apodera* sp1; F - *Argynnia dentistoma* (Penard, 1890); G - *Argynnia dentistoma hesperia* (Wailes, 1913); H - *Argynnia spicata* (Wailes, 1913); I - *Centropyxis aculeata* (Ehrenberg, 1838); J - *Centropyxis cassis* (Wallich, 1864) Deflandre, 1929; K - *Centropyxis cassis spinifera* Decloitre, 1954; L - *Centropyxis platystoma* (Penard, 1890); M - *Centropyxis discoides* (Penard, 1902); N - *Cyclopyxis arcelloides* (Penard, 1902); O - *Cyclopyxis kahli* (Deflandre, 1929); P - *Cyphoderia* sp2; Q - *Cylindridifflugia acuminata* (Ehrenberg, 1838); R - *Cylindridifflugia elegans* (Penard, 1890); S - *Difflugia oblonga* Ehrenberg, 1838; T - *Difflugia sinuata* (Gauthier-Lievre & Thomas, 1958); U - *Difflugia globulosa* (Dujardin, 1837); V - *Difflugia lingula* (Penard, 1911); W - *Euglypha acantophora* (Ehrenberg, 1841); X - *Euglypha rotunda* Wailes & Penard, 1911; Y - *Euglypha tuberculata* Dujardin, 1841; Z - *Pareuglypha reticulata* Penard, 1902; Aa - *Jungia* cf. *sundanensis* (Van Oye, 1949); Ab - *Lesquereusia modesta* Rhumbler, 1896; Ac - *Lesquereusia modesta caudata* (Playfair, 1917); Ad - *Lesquereusia spiralis* (Ehrenberg, 1840); Ae - *Lesquereusia spiralis caudata* Playfair, 1917; Af - *Netzelia oviformis* (Cash, 1909); Ag - *Netzelia corona* (Wallich, 1864); Ah - *Gibbocarina galeata* (Penard, 1890); Ai - *Padaungiella tubulata* (Brown, 1910); Aj - *Quadrullella quadrigerata* (Deflandre, 1936); Ak - *Quadrullella tubulata* Gauthier-Lievre, 1953; Al - *Quadrullella tubulata cylindrica* Deflandre, 1929; Am - *Trinema enchelys* (Ehrenberg, 1838); An - *Zivkovicia* sp1; Ao - *Zivkovicia compressa* (Carter, 1864).

The species richness was determined using Jackknife 2 estimator. In addition, the Shannon (H') diversity, Pielou (J') evenness and Berger-Parker (D) dominance indexes were also measured to evaluate the assemblage structure. A species-accumulation curve was generated to verify if the sampling efforts were sufficient to represent the studied communities as described by Santos et al. (2019).

A cluster analysis based on the Morisita-Horn index was carried out to verify the similarities among the collection sites in relation to the composition of testate amoebae assemblages. The influence of abiotic parameters over the abundance of the testate amoebae was inferred from the Analysis of Redundancy (RDA). All statistical analyses were performed in R 4.0.5 (R Development Core Team, 2021), using the following packages: stats (Shapiro-Wilk test, ANOVA, and Tukey's test), lawstat (Levene test), diverse (ecological indices of diversity, evenness, and dominance), and vegan (species accumulation curves and redundancy analysis). Cluster analysis based on the Morisita-Horn index was performed in Past 4.03 software.

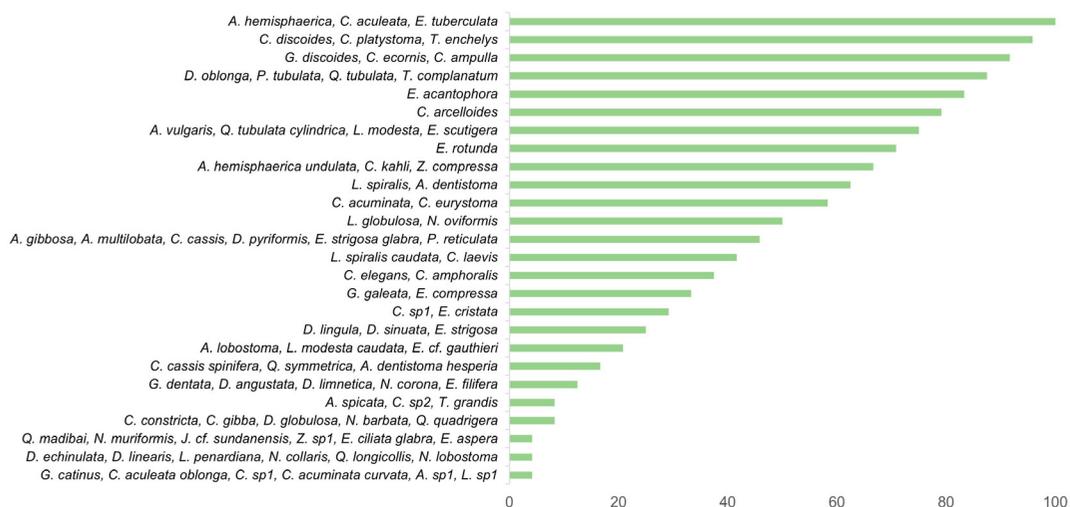
## RESULTS

The testate amoebae assemblages in PARNA and REVIS comprised 83 taxa, being 26 of them

referred to new occurrences for the state of Bahia, northeastern Brazil (Table S1, supplementary information, available at <https://www.limnetica.com/en/limnetica>) (Fig. 2). Most of the recorded taxa corresponded to the genus *Euglypha* Dujardin, 1841 (n = 5).

The testate amoebae species identified in this study were distributed into the following families: Euglyphidae (12 taxa), Hyalosphenidae (12 taxa), Diffflugidae (11 taxa), Centropyxidae (10 taxa), Arcellidae (9 taxa), Netzeiliidae (7 taxa), Lesquereusiidae (6 taxa), Cyphoderiidae (5 taxa), Cylindridiffugiidae (3 taxa), and Trinematiidae (3 taxa), besides five taxa classified as incertae sedis.

Following the scores in the frequency of occurrence (FO) proposed by Dajoz et al. (1983), 16.87% of the recorded taxa were classified as constant, while 15.66% of them were regarded as frequent, 26.51% represented common taxa and 40.69% were classified as rare. The species *Arcella hemisphaerica* Perty, 1852, *Centropyxis aculeata* (Ehrenberg, 1838) and *Euglypha tuberculata* Dujardin, 1841 are the most constant ones, being found in 100% of the collection sites (Fig. 3). On the other hand, the rarest taxa (FO of 4.17%) were represented by *Galeripora catinus* (Penard, 1890), *Centropyxis aculeata oblonga* Deflandre, 1929, *Centropyxis* sp1, *Cylindridiffugia acuminata curvata* (Leidy, 1874), *Arcella* sp1, *Lesquereusia* sp1, *Diffflugia*



**Figure 3.** Frequency of occurrence (FO%) in the testate amoebae assemblages from two Neotropical Conservation Units (PARNA and REVIS) in northeastern Brazil. *Frequência de ocorrência (FO%) nas assembleias de amebas testáceas de duas Unidades de Conservação Neotropicals (PARNA e REVIS) no nordeste do Brasil.*

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*linearis* (Penard, 1890), *Longinebela penardiana* (Deflandre, 1936), *Nebela collaris* (Ehrenberg, 1848), *Quadrullella longicollis* (Taranek, 1882), *Netzelia lobostoma* (Leidy, 1874), *Quadrullella madibai* (Kosakyan et al, 2016), *Netzelia muriformis* (Gauthier-Lièvre & Thomas, 1958), *Jungia* cf. *sundanensis* (Van Oye, 1949), *Zivkovicia* sp1, *Euglypha ciliata glabra* (Wailes, 1915), and *Euglypha aspera* (Penard, 1899) (Fig. 3).

The total abundance of testate amoebae in the collection sites from both CUs (PARNA and REVIS) ranged from 5814.81 Ind/L to 37.04 Ind/L (Fig. 3). The site identified as P8 showed the highest abundance, with a mean of  $692.99 \pm 1205.63$  Ind/L, while P12 recorded the lowest abundance, with a mean of  $66.04 \pm 204.34$  Ind/L. The ANOVA showed significant differences in abundance among the collection sites ( $F = 6.95$ ,  $p < 0.0001$ ). Likewise, the Tukey's test revealed statistically significant differences ( $p < 0.005$ ) in 14 pairwise comparisons (Fig. 4A).

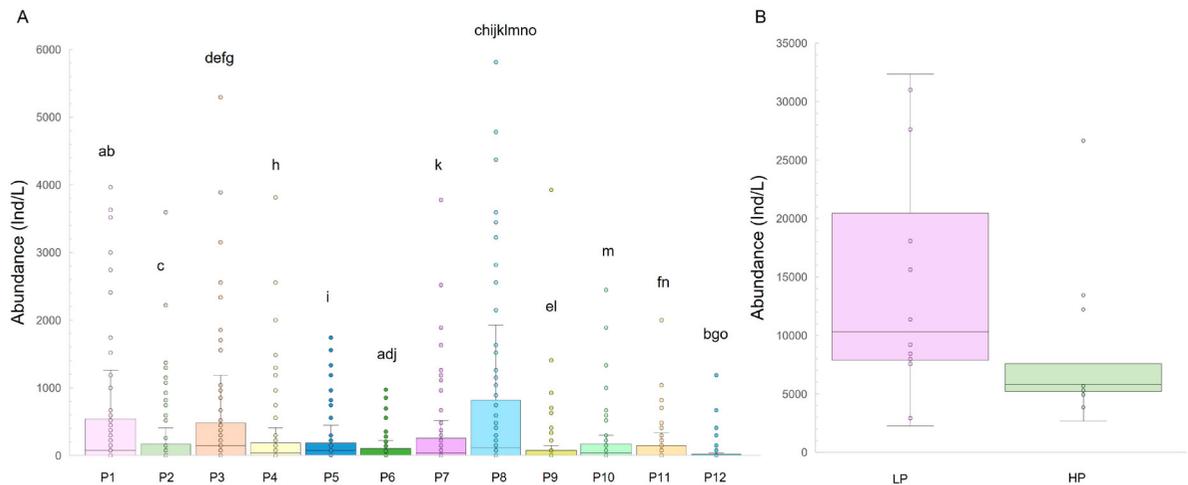
In relation to the precipitation index, we observed a higher abundance during the low precipitation (mean of  $14\ 511.57 \pm 10\ 531.39$  Ind/L)

when compared to the high precipitation (mean of  $8\ 188.28 \pm 6\ 620.18$  Ind/L), with significant differences between both periods according to the t test ( $p = 0.0299$ ) (Fig. 4B).

The species with the highest mean abundance values were *Centropyxis platystoma* (Penard, 1890), *Euglypha tuberculata* Dujardin, 1841, *Cyphoderia ampulla* (Ehrenberg, 1840), *Centropyxis aculeata* (Ehrenberg, 1838), and *Arcella hemisphaerica* Perty, 1852, respectively (Table S1).

The highest and the lowest values of species richness were observed in P8 (57) and P12 (21), respectively (Fig. 4A). The diversity index ( $H'$ ) followed the same pattern, being higher in P8 (3.33) and lower in P12 (2.45). The evenness ( $J'$ ) was higher in P6 (0.88) and lower in P9 (0.74). On the other hand, the highest value of dominance (Berger-Parker index) was recorded in P9 (0.31), while the lowest value was observed in P5 (0.09) (Fig. 5A).

As for the seasonal differences, the highest values in species richness (73) and species diversity ( $H'$ ) (3.42) were observed in periods of low precipitation (LP). In turn, the evenness ( $J'$ ) and



**Figure 4.** Abundance (Ind/L) of testate amoebae in two Neotropical Conservation Units (PARNA and REVIS) from Bahia, northeastern Brazil. (A) Abundance (Ind/L) per collection site (P1 to P12) and (B) precipitation index (LP: low precipitation and HP: high precipitation). The line inside the boxplot indicates the median value. Statistical comparisons were performed between the collection sites and superscript letters indicate significant differences (Tukey's test,  $p < 0.05$ ). *Abundância (Ind/L) de amebas testáceas em duas Unidades de Conservação Neotropicals (PARNA e REVIS), na Bahia, nordeste do Brasil. (A) Abundância (Ind/L) por local de coleta (P1 a P12) e (B) índice de precipitação (LP: baixa precipitação e HP: alta precipitação). A linha dentro do boxplot indica o valor da mediana. Comparações estatísticas foram realizadas entre os locais de coleta, e letras sobrescritas indicam diferenças significativas (teste de Tukey,  $p < 0.05$ ).*

dominance (Berger-Parker) estimates were higher during the high precipitation (HP), reaching values of 0.80 and 0.10, respectively (Fig. 5B).

The species-accumulation curve indicated that our sampling efforts were sufficient to reach an asymptotic trend (Fig. 5C). By extrapolating the mean richness percentage, the present data allowed evaluating 78.91% of the testate amoebae *taxa*.

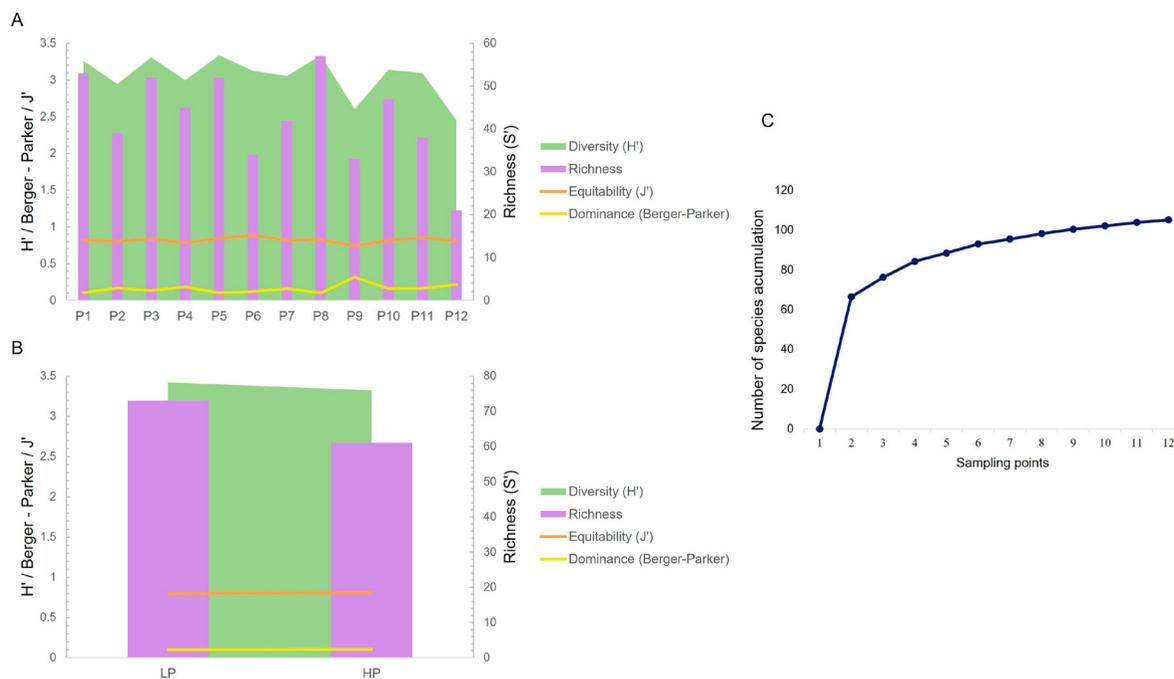
The similarity analysis using the Morisita-Horn index revealed that the collection site identified as P12 was the most divergent one in relation to the other localities, independently on the season (LP or HP) (see Fig. 6, yellow box).

In addition, two subsets of clusters were also formed, each one corresponding to the high (HP) and low (LP) precipitation (Fig. 5, purple and green boxes, respectively), showing a similarity value of nearly 65% between both groups. The only exception referred to P9, which behaved in

a discrepant manner in relation to the abovementioned pattern (Fig. 6, red dashed box).

The Redundancy Analysis (RDA) performed highlights the relationship between species composition and the environmental variables analyzed. Most species are clustered in the center of the graph, indicating a neutral or poorly explained response by the variables included in the model. However, some species have a more peripheral distribution and are clearly associated with specific environmental gradients (Fig. 7). The abiotic variables are properly described in Table S2 (supplementary information, available at <https://www.limnetica.com/en/limnetica>).

During the dry season (LP period), the first two axes of the redundancy analysis (RDA1 and RDA2) explained 83.69% of the total data variation (Fig. 7A). The environmental variables that had the greatest influence on species composition were depth, water transparency



**Figure 5.** Richness ( $S'$ ) and diversity measures ( $H' / \text{Berger-Parker} / J'$ ) in testate amoebae assemblages from two Neotropical Conservation Units (PARNA and REVIS) in Bahia, northeastern Brazil according to (A) collection sites (P1 to P12) and (B) precipitation index. In (C), the species-accumulation curve of testate amoebae from the collection sites based on richness based on the Jackknife 2 estimator (LP: low precipitation; HP: high precipitation). *Riqueza ( $S'$ ) e medidas de diversidade ( $H' / \text{Berger-Parker} / J'$ ) nas assembleias de amebas testáceas em duas Unidades de Conservação Neotropicals (PARNA e REVIS) na Bahia, nordeste do Brasil, de acordo com (A) os locais de coleta (P1 a P12) e (B) o índice de precipitação. Em (C), a curva de acumulação de espécies de amebas testáceas dos locais de coleta com base na riqueza estimada pelo estimador Jackknife 2 (LP: baixa precipitação; HP: alta precipitação).*



(Transp), temperature (Temp), total dissolved solids (TDS), electrical conductivity (EC), and salinity (Sal) (Fig. 7A). Depth and transparency were associated with the occurrence of *Cyclopyxis eurystoma* (Deflandre, 1929) (sp53) and *Cyphoderia* sp1 (sp66). Similarly, temperature and TDS contributed to the structure of the testate amoebae assemblage, influencing the presence of *Netzelia oviformis* (Cash, 1909) (sp58). In turn, *Diffflugia limnetica* (Levander, 1900) (sp26) was associated with higher values of electrical conductivity and salinity.

Regarding the high precipitation (HP period), the first two axes (RDA1 and RDA2) explained 76.60% of the total variation (Fig. 7B). The variables that influenced the species composition of testate amoebae were the same observed in the LP period. In this case, the occurrence of *Cyclopyxis eurystoma* (sp53), *Centropyxis ecornis* (Ehrenberg, 1841) (sp16), and *Cylindriefflugia elegans* (Penard, 1890) (sp22) was associated with water temperature (Temp), while the presence of *Galeripora discoides* (Ehrenberg, 1871) (sp9) was affected by transparency (transp) and depth (Depth). Furthermore, *Quadrullella tubulata cylindrica* Deflandre, 1929 (sp45), *Cylindriefflugia acuminata* (Ehrenberg, 1838) (sp20) and *Centropyxis aculeata* (sp10) stand out in the RDA for being placed apart from environmental vectors, which may indicate a preference for conditions not contemplated in the analyzed variables or low sensitivity of these species to the tested parameters.

At last, the *taxa* that remained close to the centre of the ordination graph suggest these species are more generalist or less affected to the measured environmental variables.

## DISCUSSION

The taxonomic composition of testate amoebae assemblages reported in this study, represented by 83 *taxa*, is typical of Neotropical freshwater regions (Morán, 2024; Picapedra *et al.*, 2022; Velho *et al.*, 2003). On the other hand, this diversity was higher than that described in Marimbus wetlands (65 *taxa*) (Rocha *et al.*, 2021) and temporary lakes (32 *taxa*) in the state of Bahia (Silva *et al.*, 2020). In fact, the richness herein recorded

represents 48.26% of the 172 *taxa* known to occur in Bahia (Silva *et al.*, 2025). Furthermore, the present study increases the knowledge about the regional diversity of testate amoebae by including 26 new occurrences.

These results are reinforced by the species accumulation curve, which reached 78.91% of the total estimated richness along the 12 collection sites, indicating a stabilization pattern. Therefore, the sampling efforts were highly adequate to represent most of the diversity in testate amoebae from aquatic environments in both Neotropical Conservation Units.

The family Hyalospheniidae, one of the most species-rich groups in the present study, is frequently associated with oligotrophic environments (Mitchell *et al.*, 2000; Todorov, 2018). Accordingly, the species composition along with low dominance values per *taxa* and the increased values of water transparency indicate that the sampled environments should be classified as oligotrophic. These characteristics reflect the high-water quality in the PARNA and REVIS areas (Table S2). However, it is recognized that morphologically diverse genera, such as *Centropyxis* Stein, 1857 and *Diffflugia* Leclerc, 1815, include species with varying degrees of environmental specialization, which limits inferences based solely on morphology. Thus, the integration of molecular methods, such as environmental DNA, could improve taxonomic identification and strengthen the use of testate amoebae as bioindicators.

On the other hand, other abundant groups (e.g. genera *Diffflugia* and *Centropyxis*) are recognized as generalist and opportunistic *taxa*, being commonly found in aquatic ecosystems under distinct trophic conditions (Silva, 2020; Lansac-Tôha *et al.*, 2000). In particular, *Diffflugia* species are remarkably tolerant to environments with high concentrations of nutrients and heavy metals, representing a dominant genus in polluted areas (Qin *et al.*, 2016). Likewise, the representatives of *Centropyxis* show a wide ecological plasticity, being recorded in both oligotrophic and polluted water bodies (Fernández & Zapata, 2011; Qin, 2023).

The spatial and seasonal heterogeneity observed in the abundance, richness and diversity of testate amoebae assemblages can be related to several factors. In the present study, the highest

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values for these indexes were observed in P8 ("Ingês Hills") while the lowest values were recorded in P12 ("Pé da Ladeira stream"). It is worth noting that point P8 corresponds to a lentic environment, where genera such as *Nebela* (Leidy, 1874) and *Euglypha* Dujardin, 1841 are more frequent. These *taxa* tend to be rare in lotic environments, as their delicate thecae make it difficult for them to remain in areas with stronger currents, where the water flow can cause them to be swept away or damaged (Lansac-Tôha et al., 1997). In addition, the speed of the water flow would favor the resuspension of organic matter into the water column, thus causing disturbances in the community structure, as also pointed out by Lansac-Tôha et al. (2007). Indeed, a reduced species richness was usually observed in lotic collection sites, such as P6 ("Vazante"), which presented one of the lowest richness values, probably as a result of fast water flow and wide mouth area that could restrain the occurrence of some testate amoebae species.

In addition to the water flow, salinity might also have favored the low abundance, richness, and diversity of testate amoebae in P12 ("Pé da Ladeira stream"). This collection site is located in the Caatinga biome and presented the highest salinity values in both low (0.21%) and high (0.016%) precipitation (Table S2). In fact, previous reports showed that increased salinity can decrease the diversity of testate amoebae by selecting for species resistant to this salinity (Wanner, 2020).

The seasonal heterogeneity in the abundance, richness and diversity of testate amoebae among the collection sites was more pronounced during the periods of low rainfall. As expected, the shallow water bodies observed during the low precipitation could favor the accumulation of organic matter and increased nutrients in aquatic habitats (Carvalho et al., 2001), thus resulting in increased abundance levels (Schwind et al., 2013; Costa et al., 2011). In contrast, during the high precipitation, the dilution effects might reduce the diversity and abundance of planktonic organisms (Matsumura-Tundisi et al., 2015).

In general, some *taxa* of testate amoebae are affected by temperature, transparency and depth parameters. Pedrozo and Rocha (2005) also highlighted that luminosity and trophic conditions

should also play a key role in the seasonality of planktonic organisms in aquatic environments along subtropical regions. The RDA of the present study corroborated these inferences by revealing the influence of temperature, transparency and depth variables on the abundance of testate amoebae.

Similarly, the relationships between planktonic organisms and abiotic variables (e.g. temperature) have been reported in other studies in the Neotropical region. For instance, Borges and Pedrozo (2009) demonstrated that the abundance of planktonic organisms was strongly correlated with water temperature and transparency. Indeed, the temperature acts directly on the structure of zooplankton, affecting both demographic properties and body growth (Benke, 1993).

In summary, the assemblages of testate amoebae in Boa Nova National Park (PARNA) and Wildlife Refuge (REVIS) in Bahia proved to be diverse and varied significantly according to the spatial heterogeneity and season. It should be pointed out that studies about the testate amoebae in protected areas are essential since these organisms are sensitive bioindicators of environmental conditions and play key ecological roles in nutrient cycling and water quality parameters (Marcisz et al., 2020). Considering the present scenario of intensified anthropogenic pressures and climate change, the understanding about the biodiversity and ecology of plankton communities in Conservation Units is useful to design proper management policies and to preserve their ecosystem services.

## CONCLUSION

This report showed that the structure of testate amoebae assemblages in Neotropical Conservation Units is strongly determined by environmental heterogeneity and seasonality. The increased values of abundance and species richness during the low precipitation are likely to result from hydrological oscillations on the community dynamics. Abiotic variables such as water temperature, transparency, depth, conductivity, total dissolved solids and salinity were identified as main environmental predictors for some *taxa*, reinforcing the importance of spatial and temporal complex-

ity for the maintenance of diversity in aquatic habitats. The high representativeness of constant species and the new records of testate amoebae in the state of Bahia highlight the ecological relevance of these environments and the importance of monitoring studies and conservation efforts in semiarid ecotones. Furthermore, the results confirm the role of testate amoebae as effective bioindicators from overlooked Neotropical regions, contributing to the efficient management of water resources in protected areas. In spite of the significant result from this work, some gaps still remain to be investigated, such as the influence of biotic variables, such as predation and competitive interactions, and water quality analyses (e.g., concentration of nutrients) on the composition and abundance of testate amoebae species.

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#### AUTHOR CONTRIBUTIONS

F.H.A.M.: Data collection, Formal analysis, Data curation, Writing - Original draft; M.A.R.: Formal analysis, Data curation, Writing - Review & editing; M.B.D.S.: Conceptualization and methodology, Data collection, Formal analysis, Data curation, Writing - Original draft, Writing - Review & editing, Supervision and project administration.

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