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RESEARCH ARTICLE

Exploring uncharted waters: insights into groundwater zooplankton of the Brazilian semiarid region

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Abstract

The subterranean environments of the Caatinga drylands (Brazilian biome) host a diverse array of aquatic systems and diversity, comprising both lentic and lotic ecosystems. Species diversity in these environmnts has been overlooked and remains poorly understood, especially regarding zooplankton. We studied the species richness and composition of zooplankton living in groundwater of the Caatinga drylands and explored how they vary in relation to habitat type (lentic and lotic) by testing two hypotheses: (1) species richness, gamma diversity, and zooplankton density are higher in lentic environments and (2) zooplankton species composition differs between habitat types. We sampled 12 lentic and lotic groundwater environments quarterly for 2 years, and identified 100 species of zooplankton in groundwater environments, including testate amoebae (50 species), Rotifera (25 species), Copepoda (16 species), and Cladocera (9 species). Rotifer species richness and copepod density differed between lentic and lotic habitats, as did zooplankton composition. Additionally, each habitat was found to harbor distinct indicator species based on their ecology and morphological characteristics. These findings contribute to current understanding of groundwater biodiversity and ecology, providing support for freshwater and speleological management and conservation programs.

Keywords Subterranean biodiversity · Caves · Springs · Lentic and lotic habitats · Caatinga

Introduction

Hydrological subterranean environments are primarily influenced by precipitation and surface watercourses, which infiltrate the underground system via gravity through small pores and fractures, forming complex pathways known as epikarst (Ford and Williams 2007; Bonacci et al. 2009; Audra and Palmer 2011; Culver and Pipan 2019). These flowing waters can penetrate the phreatic zone (i.e., saturated zone), either fully or partially replenishing subterranean water bodies. Such bodies may also originate from phreatic conduits regulated by elevation or pressure differentials (Gibert et al. 1994; Williams 2008; White 2019; Audra and Palmer 2011; Culver and Pipan 2019). Within caves, a variety of aquatic habitats, including those with lentic (e.g., lakes and pools) and lotic (e.g., rivers) features, are formed under the influence of both saturated and/or unsaturated zones (Bonacci et al. 2009; Souza-Silva et al. 2012; Pipan et al. 2020). The vadose zone (i.e., region from land surface to the regional groundwater table) hosts formations such as epikarst structures, percolating water, and drip pools. Transitioning to the

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epiphreatic zone, cave streams characterized by riffles and pools become prominent. Ultimately, in the phreatic zone, the prevailing feature is the presence of lakes (Bonacci et al. 2009; Robertson et al. 2023). Springs and sinks, which are considered to be ecotones between surface and subsurface waters, also serve as conduits for accessing groundwater, with a biotic composition that is usually distinct from surface and subterranean habitats (Culver and Pipan 2019).

Subterranean environments are typically aphotic, with the exception of areas near cave entrances, lacking photoautotrophic primary producers and potentially harboring chemoautotrophic organisms (Sarbu et al. 1996; Ferreira et al. 2007). Consequently, the majority of organic matter in these environments originates from external sources, such as precipitation, percolating water, transportation by surface-dwelling animals, or the input of detritus (e.g., feces and carcasses) (Gibert et al. 1994; Trajano 2000; Ferreira et al. 2007; Bento et al. 2016; Saccò et al. 2022a). Roots also serve as important habitats and food resources for sustaining subterranean fauna in aquatic and terrestrial habitats (Jasinska et al. 1996; Saccò et al. 2022b). Despite being less common, subterranean habitats can be nutrient-rich environments capable of supporting substantial populations of organisms, particularly in tropical regions (Romero 2012).

The structure of cave food webs heavily relies on the input of organic matter from surface ecosystems (Simon and Benfield 2001; Simon et al. 2007; Venarsky and Huntsman 2018). The rate at which this organic matter is supplied plays a crucial role in shaping cave communities. Caves maintaining strong connections to surface ecosystems receive significant inputs of coarse particulate organic matter (Souza-Silva et al. 2012; Venarsky et al. 2018), influencing the composition of invertebrate communities, and favoring the presence of distinct functional and taxonomic taxa. However, in scenarios where the input of coarse particulate organic matter is absent, the energy base for higher trophic levels is established by heterotrophic microbial biofilms that colonize inorganic substrates (Venarsky et al. 2023). These biofilms are sustained by dissolved organic matter (Simon and Benfield 2001), or by chemolithoautotrophic biofilms (Engel 2019).

Zooplankton are renowned for their high diversity in aquatic ecosystems and assume a pivotal role as a food web link, significantly contributing to material and energy cycling (Litchman et al. 2013). They are considered to be a reliable indicator of water quality and environmental shifts, as both biotic and abiotic factors can influence the structure and composition of zooplankton communities (Deharveng and Bedos 2019; Shen and Liu 2021). Groundwater zooplankton have been extensively studied in Europe, Asia, and Oceania with research predominantly focused on the taxonomy and ecology of stygobiotic zooplankton, especially Copepoda (De Laurentiis et al. 1999; Pipan and Culver 2007; Galassi et al. 2009a, b; Karavonik et al. 2015, Hose et al. 2016, Cavite et al. 2017; Culver and Pipan 2019; Bozkurt 2022; Guo et al. 2022). However, in Brazil, understanding of the diversity and ecology of subterranean zooplankton remains relatively limited, with only a handful of studies conducted thus far (e.g., Simões et al. 2013; Souza-Silva et al. 2017). In this context, the present study represents a pioneering effort in the Caatinga dryland biome domain, Northeast Brazil.

Lentic and lotic habitats exhibit inherently distinct physical, chemical, and biological attributes (Diniz et al. 2020, Eramma et al. 2023; Park et al. 2023). Lentic environments are characterized by low water flow rates, prolonged water retention periods, elevated nutrient concentrations, and heightened decomposition activity within the sediment bed and at the bottom of the water column. Conversely, lotic environments are typified by continuous water flow, shorter and variable water retention periods influenced by rainfall, and typically lower nutrient levels (Eramma et al. 2023). These unique conditions give rise to specific patterns of habitat functionality and resource availability, requiring species adaptability and shaping zooplankton communities to comprise species adapted to either lentic or lotic environments (Shen and Liu 2021; Eramma et al. 2023). Lentic environments, marked by higher food availability, offer favorable conditions for the development, reproduction, and growth of zooplankton populations.

Despite the establishment of conservation units aimed at safeguarding the speleological heritage within the Caatinga biome, a significant number of caves remain devoid of environmental protection measures, rendering them vulnerable to anthropogenic impacts, such as lime extraction, rock drilling for oil extraction, and tourism (Ferreira et al. 2010; Bento et al. 2021). Understanding the diversity of zooplankton in subterranean environments is crucial for the conservation of groundwater in the Caatinga drylands, alongside other taxa, particularly those that have been understudied. This study aims to access the structure and composition of the zooplankton community in different subterranean environments of the Caatinga drylands. We compared different aspects of both total zooplankton diversity and that of the main four zooplankton groups (species richness, gamma diversity, composition, and density) between lentic and lotic subterranean environments, assuming that: (1) higher species richness, gamma diversity, and density of zooplankton are found in lentic environments due to the greater resource availability, more stable hydrological conditions, and reduced organism transport by water currents; and (2) distinct zooplankton species compositions exist between types of lentic and lotic habitats. In this regard, our study will shed new light on the ecological aspects of the zooplankton community in Caatinga groundwater ecosystems, providing valuable insights for discussions concerning biodiversity hotspots and priority conservation areas in the region.

Materials and methods

Study area

We conducted our study in seven lentic and five lotic subterranean aquatic environments situated within the Jandaíra Formation (municipalities of Felipe Guerra, Baraúna, Mossoró, and Governador Dix-Sept Rosado), located in the semiarid region of Rio Grande do Norte State, Brazil (Fig. 1; Table 1). These environments encompass a variety of lentic and lotic aquatic systems, including pools, lakes, springs, rivers, and streams, which receive water inputs from phreatic and/or epikarst sources. Rio Grande do Norte ranks as the fourth state in Brazil in terms of cave abundance, with a total of 1372 cavities, predominantly within the Jandaíra Formation (Bento et al. 2017; CANIE/CECAV 2023). The semiarid region is characterized by elevated air temperatures (20–35 °C) and low precipitation levels (300–1000 mm/year), with rainfall concentrated within a few months (January– May) (Sampaio 1995; Araújo and Medeiros 2013; De Jesus and Santana 2017; Silva et al. 2018). Geographically, the region lies within the boundaries of the Caatinga biome, a seasonal dry forest dominated by small-leaved deciduous trees, succulents, and drought-resistant herbs (de Queiroz et al. 2017). The Jandaíra Formation constitutes the largest expanse of Phanerozoic carbonate outcrops in Brazil, giving rise to rocky plateaus, locally called as "*lajedos*", which harbor the majority of limestone caves in the state of Rio Grande do Norte (CANIE/CECAV 2011; Bento et al. 2016).

Field sampling and laboratory analysis

Samples were collected from lentic and lotic groundwater environments during the dry (October 2021 and December 2022) and wet (July 2021 and May 2022) seasons. In the field, various limnological parameters were assessed using a

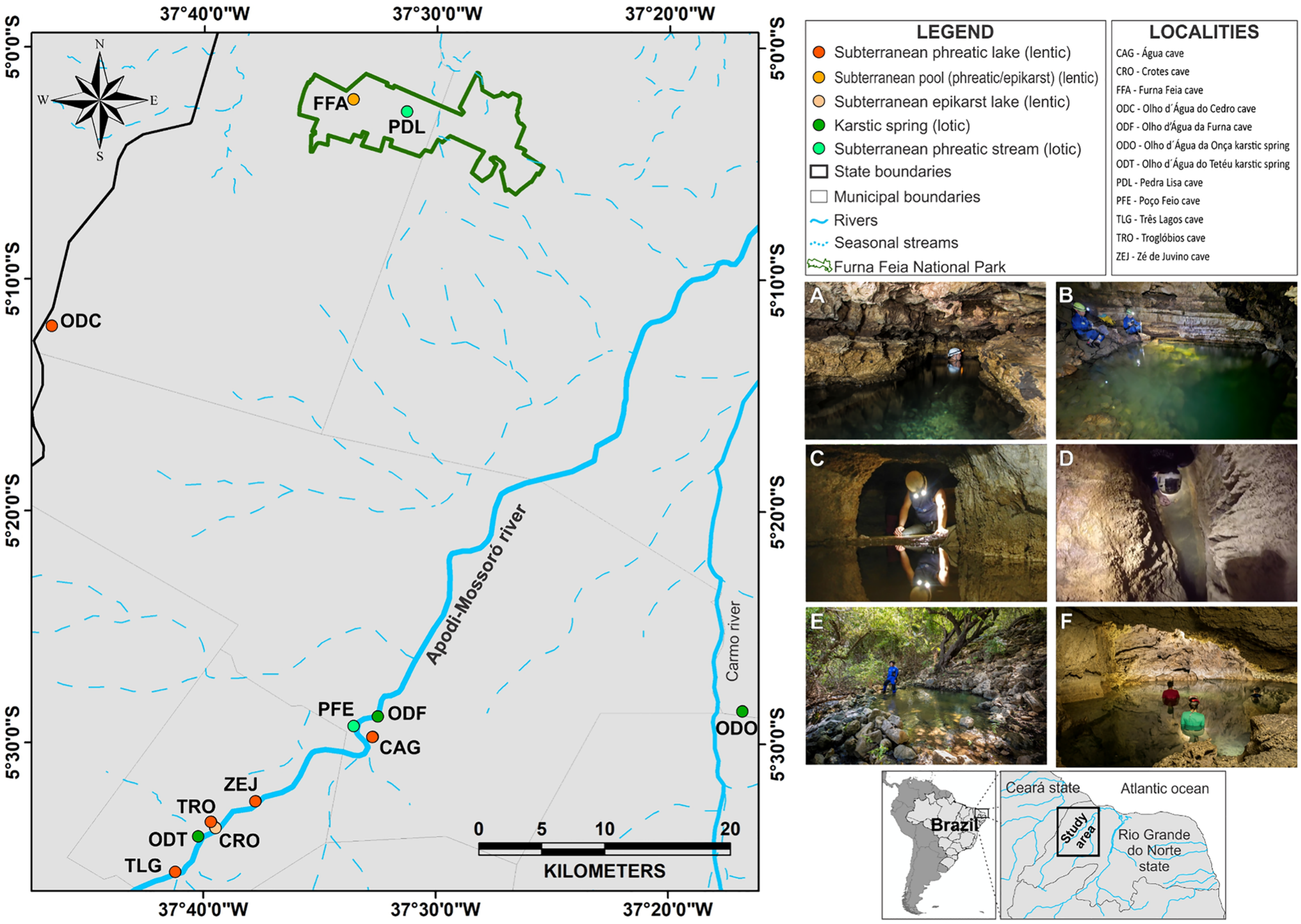

Fig. 1 Distribution of the 12 sampled subterranean aquatic environments located in the semiarid region of Rio Grande do Norte, Brazil. **a**–**f** Photographs of subterranean environments: Poço Feio cave (**a**), Zé do Juvino cave (**b**), Crotes cave (**c**), Troglóbios cave (**d**), Olho d'água do Tetéu Spring (**e**), and Três Lagos cave (**f**)

Table 1 Details on the subterranean aquatic environments sampled in the Caatinga of Rio Grande do Norte State, Brazil

Sampling site	Site abbreviation	Latitude (S)	Longitude (W)	Ecosystem type
Água Cave	CAG	05°29′43.55″	37°32′43.46″	Subterranean phreatic lake (lentic)
Crotes Cave	CRO	05°33′37.92″	37°39′30.89″	Subterranean epikarst lake (lentic)
Furna Feia Cave	FFA	05°02′12.76″	37°33′36.64″	Subterranean pond (phreatic/epikarst) (lentic)
Olho d'água do Cedro Cave	ODC	05°12′01.13″	37°46′34.45″	Subterranean phreatic lake (lentic)
Olho d'água da Furna Cave	ODF	05°28′50.54″	37°32′29.93″	Karstic spring (lotic)
Olho d'Água da Onça	ODO	05°28′34.18″	37°16′48.73″	Karstic spring (lotic)
Olho d'Água do Tetéu	ODT	05°34′02.63″	37°40′13.88″	Karstic spring (lotic)
Pedra Lisa Cave	PDL	05°02′43.84″	37°31′18.85″	Subterranean phreatic stream (lotic)
Poço Feio Cave	PFE	05°29′15.68″	37°33′33.46″	Subterranean phreatic stream (lotic)
Três Lagos Cave	TLG	05°35′34.40″	37°41′12.70″	Subterranean phreatic lake (lentic)
Troglóbios Cave	TRO	05°33′24.26″	37°39′40.57″	Subterranean phreatic lake (lentic)
Zé de Juvino Cave	ZEJ	05°32′30.63″	37°37′44.70″	Subterranean phreatic lake (lentic)

Horiba-U50 multiparameter water quality probe (HORIBA, Kyoto, Japan), including temperature (°C) pH, specific conductivity (mS/cm), turbidity (nephelometric turbidity unit [NTU]), total dissolved solids (g/L), and salinity (ppt). Water samples were also collected for laboratory analysis of total phosphorus concentration (μg/L) (Valderrama 1981), total organic and inorganic carbon content (μg/L; Xylem Analytics analyzer; Xylem Analytics, Washington DC, USA), and total nitrogen content (μg/L; Xylem Analytics analyzer; Xylem Analytics). Sediment samples were also obtained for the determination of organic matter content (%) through loss by ignition, achieved by combusting the sediment in a muffle furnace at 550 °C, with the organic matter value calculated as the difference between the initial and post-ignition weights.

Zooplankton samples were collected at the surface of groundwater environments using a 8-L graduated bucket and a conical plankton net (pore mesh size: 68 μm). Due to spatial heterogeneity among underground environments and challenges in accessing water, it was not feasible to sample an identical volume of water across all sites. Nevertheless, efforts were made to standardize the volume collected as much as possible. Water volumes ranged from 3 to 400 L (mean ± standard deviation: 105 ± 115 L) and from 0.28 to 400 L (190 ± 159 L) in lentic and lotic environments, respectively. Subsequently, all samples were fixed with 4% formaldehyde buffered with calcium carbonate.

Zooplankton taxa (copepods, cladocerans, rotifers, and testate amoeba) were identified and quantified using an optical microscope, glass slides, and a Sedgewick-Rafter chamber for counting. Organisms were identified to the lowest taxonomic level possible based on specialized bibliography (e.g., Deflandre 1928, 1929; Gauthier-Lièvre and Thomas 1958, 1960; Voight and Koste 1978; Ogden and Hedley 1980; Reid 1985; Velho and Lansac-Tôha 1996; Elmoor-Loureiro 1997; Rocha 1994; Rocha & Botelho 1998; Karaytug 1999; Silva 2003; Ueda and Reid 2003; Souza 2008; Neves 2011). A minimum of three subsamples of 2 ml each, captured using a Hensen–Stempell pipette, were counted from the concentrated sample until a minimum of 50 individuals was reached for each taxonomic group. Densities were standardized as the number of individuals per liter (ind./L).

Data analysis

A principal component analysis (PCA) was conducted to examine the ordination of environmental variables (temperature, pH, specific conductivity, turbidity, total dissolved solids, salinity, nutrients, and sediment organic matter) across habitat types (lentic vs. lotic). All abiotic data underwent *Z*-score standardization prior to analysis. To address multicollinearity among environmental variables, variance inflation factors (VIFs) were computed, and a threshold of 3 was applied in the PCA. Additionally, differences in environmental variables between lentic and lotic habitats were accessed using a permutational multivariate analysis of variance (PERMANOVA).

Sample-based rarefaction curves (Gotelli and Colwell 2001) were constructed to compare the richness of total and main zooplankton groups (i.e., Copepoda, Cladocera, Rotifera, and testate amoebae) across lentic and lotic habitats. Those curves were employed to mitigate the influence of varying sample volumes on species richness. A Wilcoxon rank-sum test was used to test differences in density of total and main zooplankton groups between habitat types. Immature forms of copepods (nauplii and copepodites) were included in the density analysis.

PERMANOVA was employed to evaluate the differences in community composition, taking into consideration zooplankton density (i.e., Bray-Cutis distance) and occurrence (i.e., presence/absence data, Jaccard distance) between lentic and lotic habitats. This analysis was conducted with 999 permutations. Dissimilarities in community composition between habitat types were visualized using a non-metric multi-dimensional scaling (NMDS) plot. To identify the key contributors to dissimilarities in community composition between habitats (Bray–Curtis distance), a similarity percentages (SIMPER) analysis was conducted. Furthermore, potential indicator species associated with specific habitats were identified using the Indicator Value (IndVal) approach for both density and occurrence data (Jaccard and Bray-Cutis distances).

All statistical analyses and graphs were performed using RStudio ® Core Team 2022), using the libraries "vegan" (Oksanen et al. 2022), "ggplot2" (Wickham 2016), "indcspecies" (De Cáceres and Legendre 2009) and "usdm" (Naimi et al. 2014).

Results

Principal component analysis axis 1 (Dim1) ordered the sampled units based on temperature, specific conductivity, and total inorganic carbon, the three variables which made the highest contributions, explaining up to 33% of the data variability (Fig. 2). PCA axis 2 (Dim2) arranged the sampled units according to sediment organic matter, total nitrogen, and phosphorus, which accounted for 26% of the variation. There were significant differences in environmental variables between the lentic and lotic habitats (PERMANOVA $F_{1,40}=4.8$; $r^2=0.11$, $p=0.002$). Lentic habitats exhibited higher mean values of total phosphorus and nitrogen, while lotic environments displayed higher mean values of temperature, electric conductivity, and total inorganic carbon (Table 2).

A total of 100 zooplankton species were recorded in the subterranean environments of the Caatinga (Electronic Supplementary Material S1). Testate amoebae accounted for nearly half of the zooplankton richness (50%) and density (45.7%), with the most abundant testate amoebae species being *Cyclopyxis* sp. (11%), *Centropyxis aculeata* (Ehrenberg, 1838) (5.7%), and *Galeripora* sp. (3.5%). There were

Table 2 Environmental variables registered in lentic and lotic subterranean environments of the Caatinga drylands

Environmental variables	Lentic	Lotic
Temperature (°C)	28.7 ± 1.8	31.2 ± 1.0
pH	7.1 ± 0.9	6.8 ± 0.8
Specific conductivity (mS/cm)	0.7 ± 0.3	1.0 ± 0.3
Turbidity (nephelometric turbidity unit [NTU])	14.2 ± 25.1	16.6 ± 37.1
Total dissolved solid (g/L)	0.4 ± 0.2	0.6 ± 0.2
Total phosphorous (µg/L)	117.6 ± 193.2	53.5 ± 15.7
Total carbon (mg/L)	24.2 ± 13.5	47.2 ± 30.5
Total inorganic carbon (mg/L)	24.8 ± 13.6	41.3 ± 17.6
Total organic carbon (mg/L)	1.4 ± 3.3	10.6 ± 26.9
Total nitrogen (mg/L)	16.2 ± 13.1	13.5 ± 6.3
Sediment organic matter (%)	7.0 ± 6.5	7.9 ± 6.9

Values in table are presented as the mean ± standard deviation

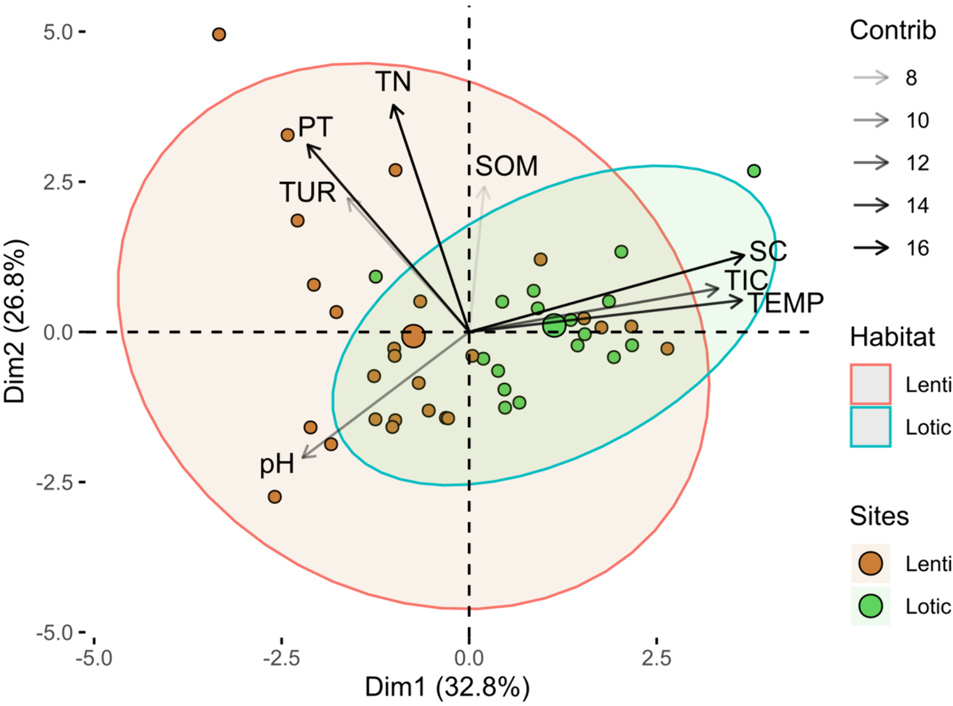

Fig. 2 Principal component analysis (PCA) of the environmental variables studied in lentic and lotic subterranean environments of the Caatinga drylands. The strength of the contribution of environmental variables is indicated by the shade of gray of the arrows, as shown to right of the PCA under heading 'Contrib'. *SC* Specific conductivity (mS/cm), *SOM* sediment organic matter in sediment (%), *TEMP* temperature (°C), *TIC* total inorganic carbon (mg/L), *TN* total nitrogen (mg/L), *TP* total phosphorus (µg/L), *TUR* turbidity (nephelometric turbidity unit [NTU])

25 species of Rotifera recorded (25% of the zooplankton richness), contributing 14.5% to the zooplankton density. Rotifers of class Bdelloidea were the most abundant rotifer in the subterranean community (12.2%). A total of 16 species of Copepoda were recorded (16% of the zooplankton richness), contributing 39.5% to the relative density of zooplankton, including immature stages. Among the Copepoda, larval stages were predominant, constituting 33.2% of the zooplankton community and representing 84% of all copepods. Cyclopoids were the dominant group within Copepoda, comprising 99.5% of the group. Calanoida and Harpacticoida represented 0.3 and 0.2% of Copepoda density, respectively. The most abundant copepod species were *Thermocyclops decipens* (Kiefer, 1929) (1.6%) and *Mesocyclops aspericornis* (Daday, 1906) (1.3%). Nine species of Cladocera were noted (9%); Cladocera exhibited the lowest relative contribution to hypogean communities (0.3%), with *Moina minuta* Hansen (1899) (0.1%) being the most abundant species.

A total of 85 taxa were observed in lentic habitats versus 61 in lotic habitats (Table 3). The rarefaction curves showed that lentic environments contributed more to the overall richness and that Rotifera was the only group that differed in species richness across the habitats (Fig. 3c). However, the curves displayed overlapping standard deviations, suggesting no significant disparities in gamma diversity between the habitats (Fig. 3a). In addition, as no curves reached an asymptote, there is a great potential for new records in both habitats with increasing sampling efforts. In terms of density, copepods and rotifers displayed higher density ($W = 403.5$, $p = 0.004$ and $W = 357$, $p = 0.05$, respectively) in lentic habitats (Fig. 4).

The PERMANOVA analysis revealed significant differences in zooplankton community structure (i.e., accessed by Bray-Cutis distance) between lentic and lotic habitats (pseudo-$F_{1,45} = 2.16$, $p = 0.004$), as well as in species composition (i.e., Jaccard distance) (pseudo-$F_{1,45} = 2.10$, $p = 0.006$) (Fig. 5). SIMPER analysis identified 13 pivotal zooplankton species contributing to dissimilarities between the habitats, accounting for 71% of the total dissimilarity. Bdelloidea and *C. aculeata* densities collectively accounted for 30% of the cumulative contribution for dissimilarities between the habitats (Table 4).

Table 3 Gamma diversity and frequency of occurrence (%) of the zooplankton species classified according to main zooplankton groups in lentic and lotic subterranean environments of the Caatinga

Zooplankton group	Lentic environment	Lotic environment
Testate amoebae	46 (54.1%)	39 (63.9%)
Rotifera	23 (27.0%)	8 (13.1%)
Copepoda	12 (14.1%)	11 (18.0%)
Cladocera	4 (4.7%)	3 (4.9%)
Total	85	61

Values in table are presented as the frequency (*n*) with the percentage in parentheses

In terms of zooplankton density, the IndVal analysis highlighted distinct species-specific associations with each habitat. In lentic environments, the rotifer Bdelloidea ($p = 0.03$, indicator value = 0.60), the copepod *Metacyclops* cf. *oraemaris* Rocha 1994 ($p = 0.009$, indicator value = 0.38), and the testate amoebae *Bullinularia indica* (Penard, 1907) ($p = 0.03$, indicator value = 0.35) were identified as significant indicators. Conversely, in lotic environments, the analysis identified the testate amoebae *C. acuelata* ($p = 0.04$, indicator value = 0.51), *Difflugia lacustris* (Penard, 1899) ($p = 0.01$, indicator value = 0.29), and *Centropyxis discoides* (Penard, 1890) ($p = 0.009$, indicator value = 0.24) as indicative species. Regarding zooplankton occurrence (i.e., presence/absence), IndVal indicated the copepod *M.* cf. *oraemaris* ($p = 0.04$, indicator value = 0.33) and the testate amoebae *Galeripora* sp. ($p = 0.02$, indicator value = 0.36) and *B. indica* ($p = 0.01$, indicator value = 0.35) as indicators for lentic habitats. For lotic habitats, the testate amoebae *D. lacustris* (p = 0.01, indicator value = 0.32), *Difflugia* sp2 ($p = 0.01$, indicator value = 0.32), and *C. discoides* ($p = 0.04$, indicator value = 0.22) were significant indicators.

Discussion

In this study, we investigated different aspects of zooplankton diversity (gamma diversity, species richness, species composition, and density) within subterranean ecosystems (springs, rivers, pools, and lakes) of the Caatinga domain in Brazil, focusing on variations between habitat types (lentic and lotic). We identified a total of 100 zooplankton species, with testate amoebae exhibiting the highest species richness and dominance. The observed number of zooplankton species in Caatinga subterranean environments was comparable to that found in water wells from Turkey (51 spp. excluding testate amoebae; Bozkurt & Bozça 2019) and higher than that reported in various studies conducted in Asia (13 spp. [Cavite et al. 2017]; 20 spp. [Singh and Jatav 2017]; 62 spp. [Guo et al. 2022]), Central America (12 spp. [Hendus et al. 2019]), and Central Brazil (36 spp. [Simões et al. 2013]). The higher species richness of zooplankton in Caatinga subterranean environments compared to other cave systems worldwide is a significant finding, as it reflects favorable climatic stability and resource availability in a semi-arid climate, which is a relatively understudied area (Culver et al. 2021; Guo et al. 2022). Moreover, it underscores the substantial potential for biodiversity, given that the subterranean fauna of the Caatinga remains largely under-sampled, with limited knowledge of its biological diversity and environmental dynamics (Bento et al. 2017, 2021). The richness

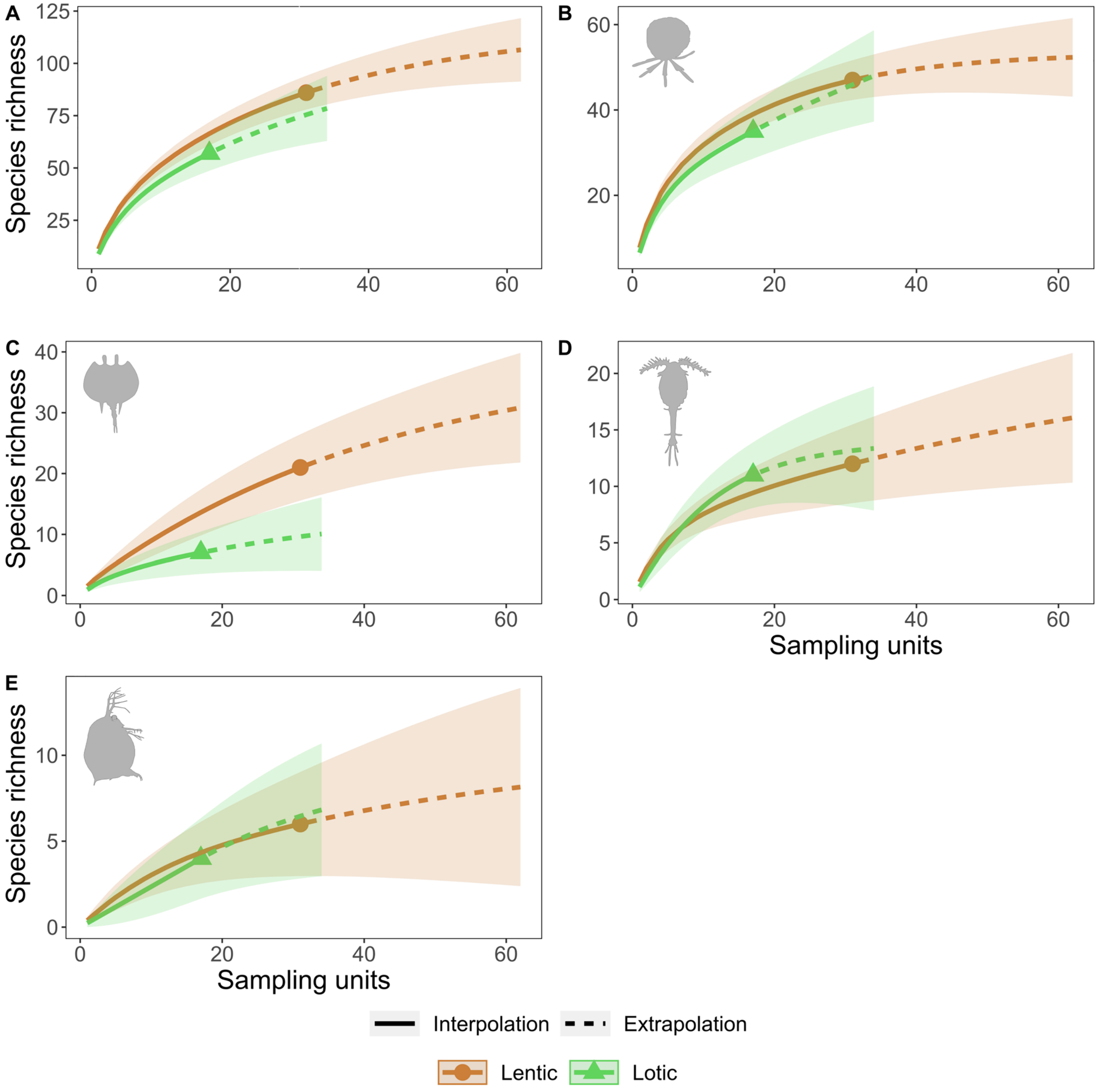

Fig. 3 Sample-based rarefaction curve for zooplankton richness overall (total, **a**) and for testate amoebae (**b**), Rotifera (**c**), Copepoda (**d**), and Cladocera (**e**) in lentic and lotic ecosystems of subterranean environments of the Caatinga

observed in our study may also be attributed to the habitat heterogeneity that we explored (i.e., lakes, springs, streams, pools, and rivers), which fosters environmental and biological diversity (Chaparro et al. 2015; Schuler et al. 2017). Our findings regarding copepods and rotifers align with our hypothesis, with copepods showing higher density and rotifers exhibiting greater richness in lentic environments. Zooplankton composition varied between habitat types, and indicator species reflected biological and ecological adaptation to specific environmental conditions in each habitat. It is noteworthy that the distribution of the most diverse groups from our study (Protozoa, Rotifera, Copepoda, and Cladocera, respectively) differs from that observed in surface rivers and lakes (e.g., Lansac-Tôha et al. 2009), where Cladocera typically outnumber Copepoda due to the successful adaptation of copepods to subterranean environments.

Testate amoebae play a pivotal role in subterranean food webs as they feed on bacteria and organic compounds while

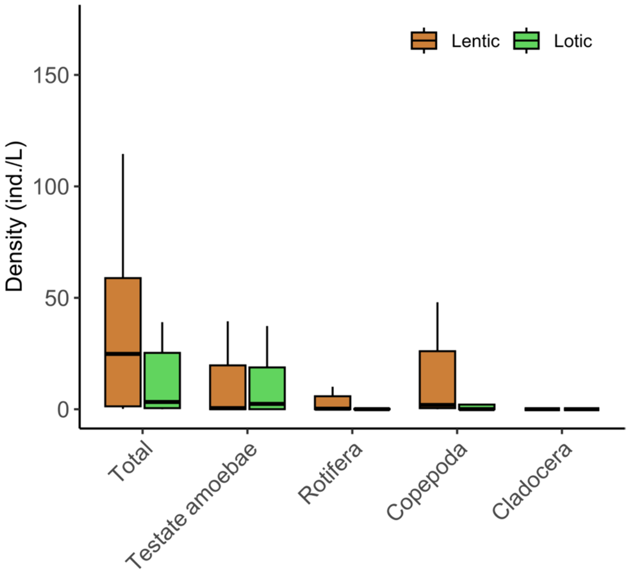

Fig. 4 Zooplankton density of total zooplankton and of main zooplankton groups in lentic and lotic ecosystems of subterranean environments of Caatinga

also serving as a food source for many other organisms (Mazei et al. 2012; Bakóvic et al. 2019). Most species of protozoans found in hypogean habitats are also commonly found in surface habitats and are transported by percolating and flowing water into the underground environment (Mazei et al. 2012; Bakóvic et al. 2019; Culver and Pipan 2019). Therefore, the subterranean protozoan community primarily consists of eurybiont stygoxenes species, with no confirmed stygobiotic species (Mazei et al. 2012, Bakóvic et al. 2019, 2022). Nevertheless, the observed binary division reproduction of testate amoebae in the Caatinga subterranean environments suggests their independence from surface populations (Bakóvic et al. 2019).

Rotifers are recognized as opportunistic R-strategists, characterized by parthenogenetic reproduction and rapid population growth (Allan 1976; Matsumura-Tundisi 1999). They also exhibit a remarkable tolerance for unfavorable environments, including the ability to enter the dormancy stage and produce eggs resistant to desiccation (Allan 1976; Matsumura-Tundisi 1999; Maia-Barbosa 2014). However, the dominance of Copepoda over Rotifera in zooplankton density suggests a more stable environmental condition and lower trophic conditions of the water bodies (Nogueira 2001; Matsumura-Tundisi and Tundisi 2005; Neto et al. 2014). Rotifers play a crucial role in the food web, serving as intermediaries for transferring energy from pico- and nanoplankton to higher-level consumers, such as microzooplankton. Additionally, they contribute to the recycling of degraded organic compounds within the microbial web (Arndt 1993; Phan et al. 2021).

Copepods, particularly those of the order Cyclopoida, exhibited the second-highest density in our study, following testate amoebae. These microcrustaceans dominate especially within hyporheic and epikarst environments (Culver and Pipan 2019). Copepods consume microplankton (such as protozoans, algae, rotifers, bacteria, and microcrustaceans) and serve as food source for meio- and macrofauna. Additionally, they contribute to the decomposition of organic compounds by consuming biofilms and organic matter, facilitating nutrient exchange between hypogean and epigean ecosystems (Schmid-Araya and Schmid 2000; Benincà et al. 2008; Galassi et al. 2009a; Neto et al. 2014).

The colonization success of copepods in groundwater can be attributed to pre-adaptations in the body plans of their ancestors. Stygobiotic species display high morphological variation, often linked to heterochrony, which involves alterations in the development of ontogenetic characteristics, such as the reduction of appendages and miniaturization (Galassi et al. 2009a). Until now, no stygobiotic copepod has been recorded in subterranean Caatinga habitats, but some individuals (herein classified as *Microcylops* sp1, *Microcyclops* sp2, *Diacyclops* sp. and *Paracyclops* sp1) exhibited some morphological traits that warrant further investigation. The Caatinga region holds significant potential for the discovery of stygobiotic species, as evidenced by the prior identification of 21 such species of Amphipoda, Isopoda and Platyhelminthes (Leal-Zanchet et al. 2014; Bento et al. 2021). Among these, 13 species are considered to be oceanic relicts, having evolved from marine ancestors trapped in isolated subterranean areas during events of ocean introgression and regression (Ferreira et al. 2010). Additionally, a novel cyclopoid, *Paracyclops* sp. nov., was discovered in a water spring and will be described in a future publication. Another interesting record on subterranean habitats of Caatinga is the cyclopoid *Metacyclops* cf. *oraemaris*, a species observed only in Atlantic Forest remnants in Southeast Brazil, including in the entrance of a cave near the sea (Rocha 1994; Silva and Matsumara-Tundisi 2011).

The structure of the cyclopoid community (i.e., 99.5% of the copepods) was predominantly composed of larval and juvenile stages, including nauplii and copepodites, with a low density of adults. The high density of immature forms underscores the presence of favorable environmental conditions for copepod reproduction and may also be linked to the extended life stages observed in resource-limited hypogean environments (Mulec and Oarga 2014; Kur et al. 2020). In some environments, ovigerous females were scarce, suggesting an alternative breeding location apart from the surveyed water bodies (Brancelj 2002). However, due to the absence of sampling from the epikarst, definitive insights into the potential source of copepod populations remain elusive (Brancelj 2002; Pipan et al. 2010). The most frequent species among copepods in this study, *M. aspericornis* and *T.*

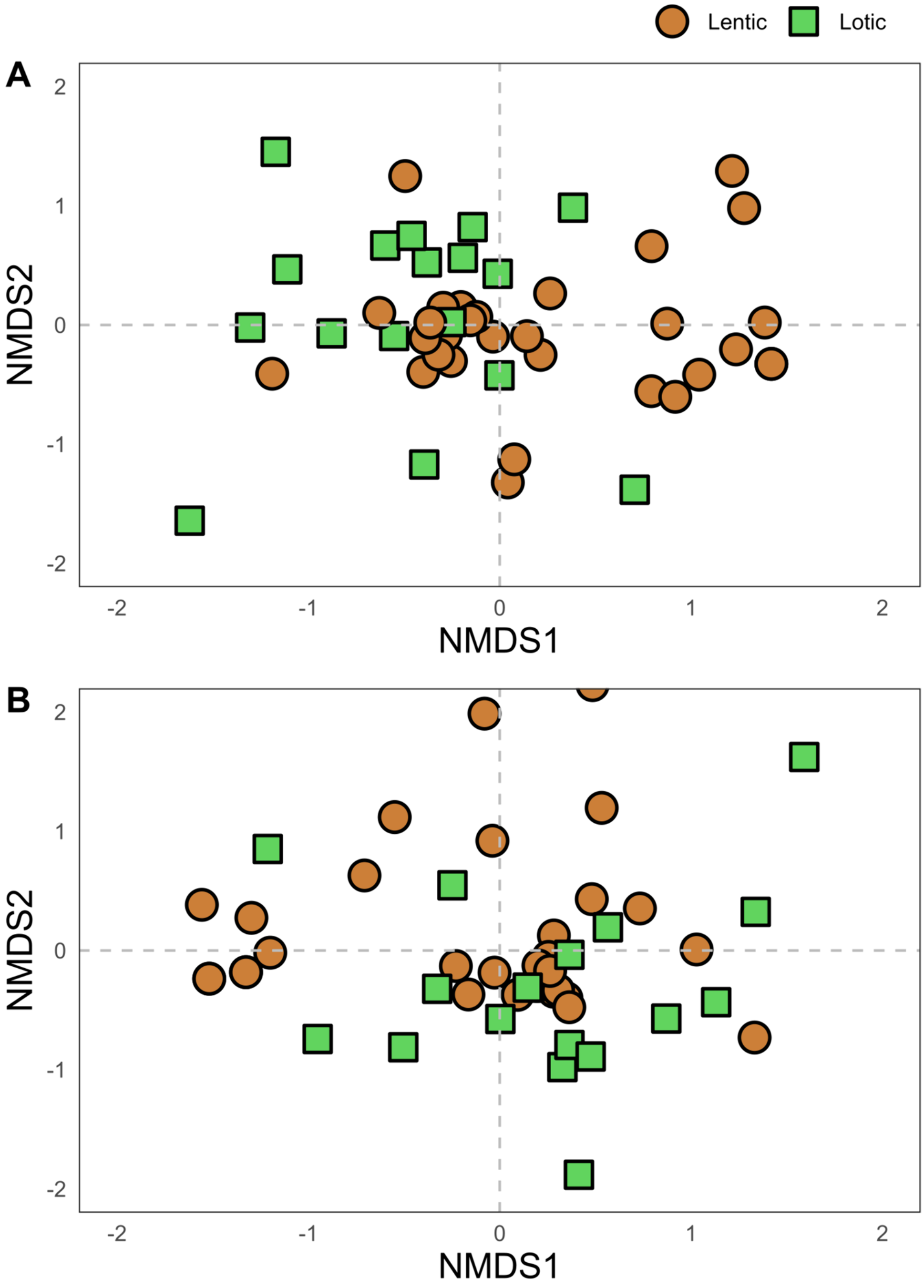

Fig. 5 Diagram illustrating non-metric multidimensional scaling (NMDS) of the zooplankton density (ind./L) (**a**) and zooplankton species composition (presence/absence data) (**b**) in lentic and lotic ecosystems of subterranean environments of the Caatinga

decipiens, are common in the surface environments of the Caatinga (Cabral et al. 2020). *Thermocyclops decipiens* is a dominant species in the tropics and is highly prevalent in lentic eutrophic environments (Sampaio et al. 2002), and *Mesocyclops aspericornis* is also widely distributed and is known for its ecological adaptability (Reid and Saunders 1986).

The Harpacticoida individuals were limited, and further identification at the species level is imperative to examine the potential presence of troglomorphic traits. This becomes particularly relevant as all individuals were retrieved from sites where prior studies have confirmed the existence of aquatic stygobiotic species (i.e., the CRO, ODO, ODT and PDL sites; see Fig. 1 and Table 1). The worm-shape body structure of harpacticoids are pre-adapted to benthic and interstitial lifestyle, making them the most abundant group among stygobiotic copepods (640 for harpacticoids and 330 for cyclopoids on a global scale) (Galassi et al. 2009a; Culver and Pipan 2019). Calanoida copepods were notably scarce, being observed exclusively in two caves (ZEJ and TLG) and identified as a common surface species (i.e., *Notodiaptomus iheringi*). The scarcity of calanoids at our

Table 4 List of zooplankton species selected in the similarity percentages (SIMPER) analysis performed based on density data in lentic and lotic subterranean environments of the Caatinga

Taxa	Cum. cont. (%)	Av. Lentic	Av. Lotic	*p*
Bdelloidea	0.16	5.95	0.41	0.88
Centropyxis aculeata (Ehrenberg, 1838)	0.30	0.36	4.46	0.01*
Cyclopyxis sp.	0.41	4.76	1.41	0.84
Mesocyclops aspericornis (Daday, 1906)	0.45	0.61	0.11	0.67
Thermocyclops decipens (Kiefer, 1929)	0.49	0.69	0.21	0.30
Galeripora sp.	0.52	1.28	0.88	0.64
Cyclopyxis kahli Deflandre 1929	0.55	0.49	1.82	0.13
Plagiopyxis callida Penard, 1910	0.58	0.48	0.97	0.22
Centropyxis constricta (Ehrenberg, 1841)	0.61	0.16	1.20	0.04*
Bullinularia indica (Penard, 1907)	0.64	1.09	1.25	0.56
Cyclopyxis sp1	0.67	0.08	0.71	0.07
Difflugia sp.	0.69	0.42	0.36	0.34
Plationus patulus var. *macracanthus* (Daday, 1905)	0.71	0.60	0.00	0.67

For each species, respective cumulative contribution (Cum. cont.), average values (Av.), and *p*-value are shown for lentic and lotic habitats. Contribution cut-off was 70%

*Statistically significant difference at $p \leq 0.05$

study sites were likely due to their herbivorous feeding habits (Rietzler et al. 2002) as the availability of microalgae is primarily restricted to cave entrances and drastically diminishes in response to reduced or absence of light (Ferreira et al. 2007; Culver and Pipan 2019).

Cladocera exhibited lower density in the subterranean environments of Caatinga. Although some stygobiotic cladocerans have been previously documented (Brancelj and Dumont 2007), the species identified in our study are also prevalent in the surface habitats of the region (Cabral et al. 2020), indicating that some species are introduced in groundwater from surface habitats. Cladocera sporadically occurs in groundwater environments, with approximately 600 species documented worldwide, of which only 2% are classified as stygobiotic species (Dumont and Negrea 1996; Brancelj and Dumont 2007). However, *Macrotrix laticornis* and *Ceriodaphnia cornuta* (the 'hairy' one, which was not the case in this study) are classified as stygophiles. These species exhibit an ambivalent lifestyle and may engage in migrations between surface and subterranean environments (Dumont and Negrea 1996). Additionally, the benthic habits and body morphology of Chydoridae (e.g., herein represented as *Leydigia ipojucae*, *Anthalona verrucosa*, and *Coronatella monacantha*) are compatible with hypogean habitats, allowing the organisms to survive and even reproduce in such environments (Brancelj and Sket 1990). Moinidae cosmopolitan representatives (e.g., *Moina minuta*) are usually occasional or accidental in subterranean habitats (Brancelj and Sket 1990), likely transported to groundwater by percolating and flowing water.

Lentic and lotic groundwater environments exhibited distinct zooplankton communities, primarily shaped by variations in environmental conditions. These distinctions were particularly influenced by water flow velocity, a robust environmental filter known to influence nutrient dynamics, resource availability, and microhabitat features (Baranyi 2002; Larsen et al. 2019; Burgazzi et al. 2021; Dai et al. 2020). Among the zooplankton groups, copepods were the sole category that displayed significant variability between these habitats. The habitat preference of cyclopoids for slow water flow, sediments of medium-sized grains, nutrient and organic matter accumulation (Galassi et al. 2009a) aligns with the characteristics of lentic water bodies. These are characterized by longer water residence time, concentration of food resources, and greater environmental stability—all factors that also reduce the risk of organism displacement caused by water currents (Mwebaza-Ndawula et al. 2005; Galassi et al. 2009a; Jones et al. 2017).

The indicator species selected for lentic habitats include the rotifer Bdelloidea, the copepod *M.* cf. *oraemaris*, and the protozoan *B. indica*. Bdelloids are known for their broad ecological range, inhabiting sediment particles in various aquatic and terrestrial environments (Pejler and Bērziņš 1993; Ricci and Caprioli 2005). In lentic habitats, there is a tendency to accumulate microbial bottom matters and organic compounds, which serve as a nutritional supply for bdelloids (Lukashanets and Maisak 2023). Therefore, their significance in lentic groundwater habitats may stem from their adaptations to unfavorable and eutrophicated environments (Ricci 1998; Ricci and Caprioli 2005; Almeida et al. 2010; Andrade et al. 2022). This was corroborated in our study when we observed that lentic environments were separated from lotic environments by higher concentrations of nutrients (nitrogen, phosphorus, and carbon). Additionally, bdelloids are sensitive to water flow, with a decrease in species richness observed as flow rates increase (Ricci and Balsamo 2000).

The cyclopoid *Metacyclops* cf. *oraemaris* (still awaiting species confirmation) remains relatively under-studied in terms of its ecology. However, the species has been exclusively collected within the Atlantic Forest domain, including in moss near the sea and in a dam (Rocha 1994; Silva and Matsumara-Tundisi 2011). The dorsal position of the species' gonopore (i.e., in females) is consistent with life adaptation for reduced space, such as semiterrestrial, bromeliads, and groundwater habitats (Cuoc and Defaye 2011). Furthermore, *Metacyclops* are recognized for their ability to thrive and establish dominance in ephemeral habitats (Perbiche-Neves et al. 2013). Such habitats are frequently encountered in subterranean habitats of Caatinga, where both lentic and lotic systems may undergo a drying process during the dry season.

The selection of testate amoebae for lentic and lotic habitats adheres to the criteria established by Velho et al. (2003). According to their classification, the morphological characteristics of these organisms are adapted to the specific environment they inhabit, typically manifesting as spherical forms in lentic environments and adopting elongated or flattened shapes in lotic environments. *Bullinularia indica*, determined as an indicator for lentic environments, exhibits spherical morphology, whereas the flattened *C. aculeata*, *C. discoides*, *Galeripora* sp., and the elongated *D. lacustris* and *Difflugia* sp2 were selected for lotic habitats, whose morphologies are conducive to resisting water currents (Velho et al. 2003; Lansac-Tôha et al. 2014).

The groundwater ecosystem of Caatinga harbors a unique aquatic fauna, composed of endemic and stygobiotic species, including Brazil's sole known ocean relict (Leal-Zanchet et al. 2014; Bento et al. 2021). However, the groundwater and subterranean environments face numerous threats linked to anthropogenic activities and environmental changes, such as pollution, land use change, over-extraction, mining, climate change, deforestation, and regulatory gaps (Fried 1975; Ferreira et al. 2010; Minderhoud et al. 2017; Mammola et al. 2019, 2024; Bento et al. 2021; Ferreira et al. 2022). These external pressures substantially modify environmental conditions and hydrological connectivity, impacting the sensitive hypogean fauna (Boulton et al. 2023). Despite the existence of a conservation unit encompassing some caves in the Jandaíra Formation, most subterranean environments remain unprotected (Bento et al. 2021). Hence, the maim aim of this study was to draw attention to groundwater zooplankton diversity, with the goal of bridging knowledge gaps and providing valuable insights for conservation initiatives and the management of speleological and freshwater heritage in the Brazilian Caatinga.

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Data availability The datasets generated in this study are available on request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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