ORIGINAL ARTICLE

First ecological analysis of lacustrine testate amoebae in Guatemala: A case study from the highland Lake Chichoj

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ABSTRACT

Freshwater quality represents a central issue for human populations and the conservation of aquatic communities. In this sense, freshwater reservoirs, such as lakes, require proper management and monitoring plans to avoid their deterioration and pollution. Bioindicators, such as testate amoebae, are an excellent tool increasingly utilized for limnology and paleolimnology to assess the trophic status of lacustrine environments. However, despite their potential as bioindicators, the ecological research status of testate amoebae in Central American lakes remains poor. We conducted our research at highland Lake Chichoj, Alta Verapaz, Guatemala, which has become increasingly eutrophic since the 1980s. This study contributes to fill the knowledge gap about neotropical testate amoebae, parallel to testing their utility as bioindicators of lacustrine conditions. From a collection of 12 surface sediment samples (associated with different land uses), we found 19 testate amoebae taxa, and for the first time in Guatemala, we recorded *Arcella megastoma*, *Arcella gibbosa*, *Cucurbitella tricuspis*, *Difflugia protaeiformis* strain "acuminata", *Difflugia urceolata* strain "elongata", *Lesquereusia spiralis*, *Lesquereusia modesta*, and *Mediolus corona*. Our cluster analyses revealed three testate amoebae assemblages in connection to trophic conditions: 1) Stressed Conditions (SC), 2) Lowest Contamination Conditions (LC), and 3) Deep Transitional Conditions Assemblage (DT). After performing a transformation-based redundancy analysis (tb-RDA), we found total organic carbon as the only significant environmental parameter associated with testate amoebae assemblages ($p<0.004$). Our indicator species analysis (IndVal) confirms the eutrophic regime of Lake Chichoj in connection to the presence of *Cucurbitella tricuspis* and *Centropyxis aculeata* strain "aculeata" as indicators of nutrient enrichment and stressful conditions. The testate amoebae assemblages identified in Lake Chichoj represent a critical baseline for future studies of Guatemalan lakes, strengthening our understanding of the causal factors behind water quality in neotropical regions.

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INTRODUCTION

Montane ecosystems deserve special attention due to their significant contribution to biodiversity despite their significantly smaller terrestrial surface area (24%) compared to lowland ecosystems (Rahbek *et al.*, 2019). From this perspective, conservation biology and restoration ecology of montane areas become even more relevant in the face of global environmental change (Watts and Jump, 2022) and thus should be studied and understood from the short and long-term temporal ecological perspectives (Lamorgese *et al.*, 2015). Lake Chichoj is the only deep permanent freshwater system in the Guatemalan Central Highlands in Las Verapaces Region (Brocard *et al.*, 2014), which makes it a unique montane ecosystem in Mesoamerica. The trophic conditions of the lake have been influenced primarily by industrial development in the neighbouring town of San Cristobal Verapaz town and secondly due to the associated accelerated urbanization, particularly since the 1970s, including greywater release and deforestation (Albizurez-Palma, 1978; Tejada, 2011; Brocard *et al.*, 2016a, 2016b). Previous and parallel to the environmental deterioration of the last decades, heavy metal pollution (particularly chromium) has resulted from the leather tanning of a neighboring local shoe factory operating since 1914 (Brocard *et al.*, 2016a). Even more, the whole lake surface has been covered on many occasions by floating plants, such as invasive water hyacinth (*Eichhornia crassipes*) and *Hydrilla verticillate* (Tejada, 2011), as a response to decreased dissolved oxygen concentration (Brocard *et al.*, 2016a). The historical deterioration of Lake Chichoj demands an understanding of the ecological impacts in connection to human activities, and thus, we believe that bioindicators become valuable tools to guide water management and ecological restoration planning.

Testate amoebae are a group of valuable protists bioindicators (Payne *et al.*, 2006), mainly due to their sensitivity to environmental changes (Alves *et al.*, 2010; Neville *et al.*, 2010) and rapid response to contamination effects (Reinhardt *et al.*, 2005; Neville *et al.*, 2010; Kihlman and Kauppila, 2012; Patterson *et al.*, 2013; Nasser *et al.*, 2020). Besides being a well-known polyphyletic group of shelled protists, testate amoebae are easy to identify due to their usual well-preserved test (Medioli and Scott, 1988; Asioli *et al.*, 1996; Roe and Patterson, 2006; Escobar *et al.*, 2008). Furthermore, testate amoebae are increasingly studied in limnology and paleolimnology (Patterson and Kumar, 2000a; Roe *et al.*, 2010; Nasser *et al.*, 2020) because of their wide spatial distribution from aquatic (i.e., planktonic and benthic habitats) to soil environments (Kornecki *et al.*, 2020; Marcisz *et al.,* 2020). Testate amoebae assemblages have been noticed to change in response to several environmental parameters such as land-use change (Patterson *et al.*, 2002), heavy metal pollution (Asioli *et al.*, 1996; Patterson *et al.*, 1996; Neville *et al.*, 2014; Gavel *et al.*, 2018; Nasser *et al.*, 2020), pH variability and oxygen concentration (Dalby *et al.*, 2000; Escobar *et al.*, 2008; Charqueño-Celis *et al.*, 2020), phosphorus flows (Roe *et al.*, 2010; Patterson *et al.*, 2012), road salt concentration (Roe and Patterson, 2014; Cockburn *et al.*, 2020), water quality (Roe *et al.*, 2010; Qin *et al.*, 2013), and thermocline dynamics (Steele *et al.*, 2018).

Most of the global testate amoebae research has been carried out in temperate lakes (Patterson and Kumar, 2002), while research in the tropics has been limited to a few countries (e.g., Brazil, India, Mexico, the Democratic Republic of the Congo, Republic of the Congo and Vietnam) (Velho *et al.*, 2004; Kosakyan *et al.*, 2016; Sigala *et al.*, 2016; Farooqui *et al.*, 2020; Tran, 2020; Tran *et al.*, 2021), and even lesser in Mesoamerica (Laminger, 1973; Heger *et al.*, 2011; Betancur and Acevedo, 2016), with little notice to assess short-term and long-term historical records (Patterson *et al.*, 2015; Sigala *et al.*, 2018). The first study of testate amoebae in Guatemala was completed in several highlands lakes (Chicabal, Burra, and El Pino) (Laminger, 1973), reporting *Euglypha rotunda*, *Trinema lineare*, *Trinema enchelys*, *Nebela collaris*, *Nebela wailesi*, *Corithion dubium*, *Assulina seminulum*, *Assulina muscorum*, *Nebela lageniformis*, and *Phryganella acropodia*. However, we do not have studies on the relationship between testate amoebae and environmental parameters. The investigation at Lake Chichoj aims to fill this information gap; our main goals are to present the first ecological analysis of testate amoebae in Guatemala and explore the utility of testate amoebae assemblages as bioindicators in Neotropical conditions.

METHODS

Study area

Lake Chichoj is located in the jurisdiction of San Cristobal Verapaz (68,819 inhabitants), Alta Verapaz (15°21”30’N, 90°28”40’W) in the Guatemala central highlands (Fig. 1). The lake has a surface area of 500,000 m^2 and a volume of about 100,000 m^3, and is found at an elevation of 1,440 m with a low montane climate (i.e., annual temperature of 16 to 25°C, and mean annual precipitation of approximately 2,284 mm). The underlying geology of the lake’s catchment is unique due to the Jurassic fluvial deposits and Cretaceous and Permian carbonates, covered by thick, clay-rich tropical soils from Los Chocoyos Formation (84±5 kya extensive rhyolitic pumice) (Drexler *et al.*, 1980; Brocard *et al.*, 2016b). In addition, the lake occupies aligned coalescent dolines that probably resulted from a dissolved gypsum body which has resulted in the only deep permanent lake in the central highlands (Brocard *et al.*, 2014). Lake Chichoj has three basins separated by shallow sills (Brocard *et al.*, 2014), two outflows (Chijulja River and El Desague), and is partially surrounded by a wetland (Brocard *et al.*, 2016b) and montane forests (Albizurez-Palma, 1978).

Field sampling

In February 2016, we collected twelve surface sediment samples from Lake Chichoj (Fig. 1c) and preserved them with absolute ethanol (100%): Five samples from the littoral zone (L1T1, L1T2, L1W, L1C, L2F), and seven from the pelagic zone across the three basins (P11, P12, P13, P21, P22, P31, P32). Two of the littoral samples come from near the shore of the adjacent town (codes L1T1, L1T2), and three from the surrounding wetland neighboring different land uses: Forest cover (L2F), invasive water hyacinth at the lakeshore (L1W), and cow pasture (L1C). The littoral samples were collected with a spatula, and the pelagic sediment with an Ekman dredge to remove only the top sediment layer. Pelagic samples were collected at different water depths (3-24.7 m) using a graduated-sounding line with a lead weight. Environmental parameters were measured *in situ* in 12 samples with a portable HQ40D (Hach) water property probe, including dissolved oxygen concentration (DO) (mg L^{-1}), redox potential (RP) (mV), pH, salinity (ppm), total dissolved solids (TDS) (ppm), and temperature (°C). In the laboratory, heavy metal concentrations (chromium and arsenic) were measured by atomic absorption techniques with an S4 VP100 Thermo Fisher Scientific following the procedures given in the Standard Methods (American Public Health Association *et al.*, 1998). Total organic carbon (TOC) (wt.%) was quantified according to the method presented by Heiri *et al.*, 2001.

A 1 cm^3 wet volume sample was used for testate amoebae analysis and sieved through a 53-µm screen to retain the tests, that was examined in a Petri dish at a magnification ranging from 40 to 80X using a stereomicroscope (Kyowa Optical *SD-2PL*), and tests were extracted using a fine brush (Ellison and Ogden, 1987). Some species were scanned through the use of a Jeol scanning electron microscope at the Universidad Nacional Autónoma de México. Ecological studies of testate amoebae usually aim to reach at least 150 individuals count per sample, which in some instances, can be challenging to reach in tropical lakes (Patterson *et al.,* 2015). Therefore, two samples containing less than 50 individuals (L1T1 and P32) were excluded from the statistical analysis to avoid underestimation (Payne and Mitchell, 2009). Testate amoebae identification was made using taxonomic keys from Ogden and Hedley (1980), Kumar and Dalby (1998), and Sigala *et al.* (2016). Testate amoebae species present eco-phenotypically infraspecific morphological variability (Medioli and Scott, 1988), and thus informal 'strain' names are used in this study to avoid the possible inadvertent of unjustified new species (Patterson and Kumar, 2000b) and distinguish environmentally significant populations (Patterson *et al.*, 2012), even when not recognized in the International Commission on Zoological Nomenclature (1999).

Statistical analyses

To assess environmental health conditions and the diversity of lacustrine testate amoebae, we used the Shannon diversity index (SDI) (R version 1.1.383; R Core Team, 2020) according to the method proposed by Patterson and Kumar (2002) in temperate lakes, which has been applied in the tropics (Sigala *et al.*, 2018; Charqueño-Celis *et al.*, 2020). SDI values below 1.5 are associated with stressed environments where few taxa are dominant.

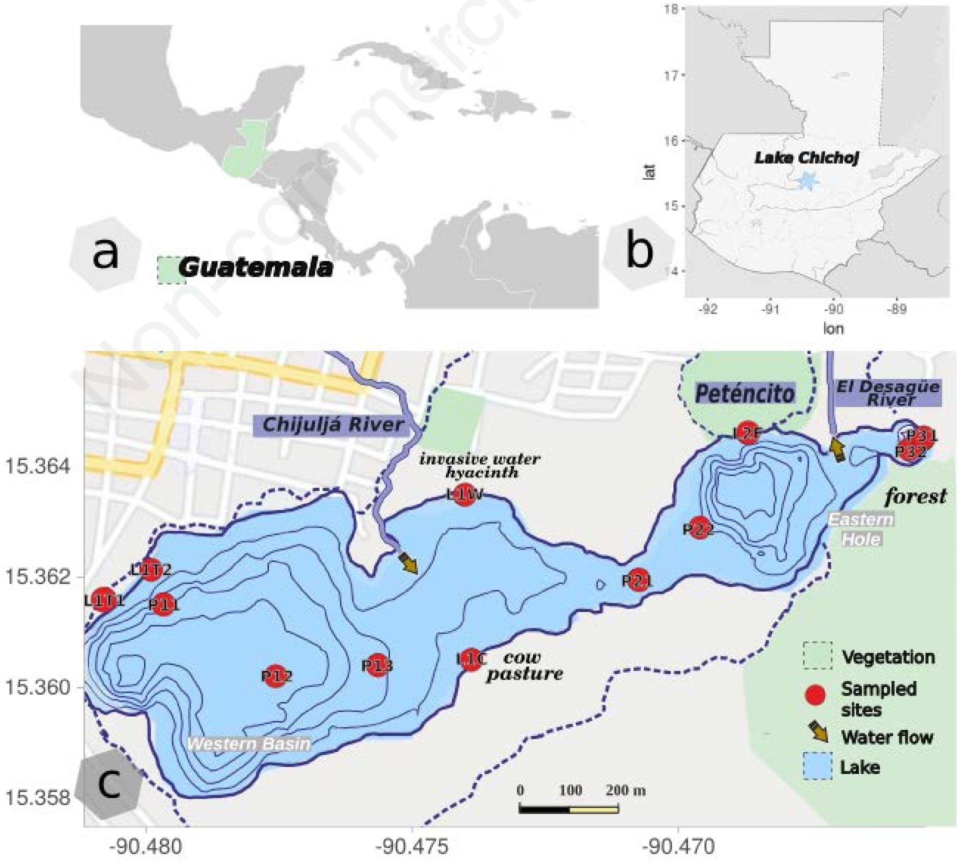


Fig. 1. Locations of the twelve sediment samples on Lake Chichoj in Guatemala. Insert map shows the location of the Lake in the central highlands of Guatemala.

In contrast, values between 2.5 and 3.5 suggest healthy environments with a more even abundance between taxa. SDI values between 1.5 and 2.5 show a transitionary stage. In this study, we used these categories for SDI values, aware of the need for the relative interpretation of heterogeneity indexes that measure entropy (Jost, 2006). The standard error (Sxi) was calculated to remove non-statistically significant species as bioindicators (Payne *et al.*, 2006, Patterson and Fishbein, 1989). If the Sxi in all samples is greater than the relative fractional abundance in each sample, the taxon is excluded because it is statistically non-significant. The Sxi is defined as:

$$Sxi = 1.96\sqrt{\frac{Fi(1-Fi)}{Ni}} \qquad \text{(eq. 1)}$$

where Fi is the relative fractional abundance per taxon, and Ni is the species total counts per sample (Patterson *et al.*, 2012). Based on this selection, we used ten samples and 17 statistically significant taxa (species and strains) for the rest of the statistical analyses.

Previous to multivariate analysis, the species data was transformed through the Hellinger distance, which is appropriate for community composition data containing many zeros (Legendre and Gallagher, 2001). In addition, this transformation makes data suitable for Euclidean-based ordination methods, such as redundancy analysis (Rao, 1995). We used cluster analysis to identify samples containing similar testate amoebae assemblages and establish species association (Q-and R-modes, and Ward's minimum variance method) (Ward, 1963), which were named regarding Lake Chichoj environmental conditions (Roe *et al.*, 2010; Cockburn *et al.*, 2020). To evaluate testate amoebae indicator species identified in the cluster analysis, we employed *indicspecies* R package with an indicator value analysis (IndVal) using Monte Carlo permutation tests (n=999) (de Cáceres *et al.*, 2016).

Transformation-based Redundancy analysis (RDA) helped identify the relationship between the measured environmental parameters and testate amoebae assemblages. We used Nasser *et al.* (2016) and Reimann *et al.* (2008) protocols to remove environmental parameters with more than 25% of their values below detection (Tab. 1). A scatter plot of matrices (SPLOM) (psych package) was used to remove highly correlated parameters (Pearson's r>0.75) with no influence on testate amoebae assemblages. The variance inflation factor (VIF) was critical to check for no co-linearity in the final environmental parameters. Partial RDA analysis was used on the selected environmental parameters to determine the variance percentage explained by each parameter. The final RDA model was selected with the four environmental parameters that showed the highest variance percentage (>10%). A permutation test was performed to recognize the significance of the species-environment parameter relationship. All multivariate analyses were generated with the R packages vegan, psych, ggplot2 and indicspecies (de Cáceres and Legendre, 2009; Wickham, 2016; Oksanen *et al.*, 2022; Revelle, 2022); while aesthetic editions were performed in Inkscape (version 1.0.2, GNU/Linux OS).

RESULTS

New data of testate amoebae from Guatemala

We collected 1,250 specimens, which included 19 taxa, out of which five were assigned to ecophenotypes "strains" (Tab. 2; Fig. 2). The range of species richness across samples was from five to eleven. The three dominant genera were *Difflugia* (seven taxa, 37%), *Centropyxis* (four taxa, 21%), and *Arcella* (three taxa, 16%, please note, *recently Arcella megastoma and Arcella discoides*

Tab. 1. Environmental parameters of sampled sites at Lake Chichoj, Guatemala.

Code	As	TOC	Cr	DO	SAL	T	TDS	ph	redox	z
L1T1	673	7.31	-	4.92	0.1	19.6	144.7	7.64	-66.6	0
P11	1333	15.38	-	0.25	0.1	19.1	144.7	6.89	-23.7	7
P12	1631	15.61	612	0.25	0.1	19.9	143	7.14	-37.3	24.7
L1T2	1171	14.83	-	8.8	0	20.8	2.4	7.73	-71.5	0
P13	3215	15.13	526	05.02	0.1	19.8	111.3	7.54	-60.7	15
L1W	15398	34.68	169	04.04	0.1	20.5	124.3	7.23	-42.9	0
L1C	18110	62.67	78	7.13	0.11	19.7	145.5	7.65	-66.6	0
P21	2611	33.25	758	04.03	0.1	19.3	281	7.35	-48.2	3
P22	13079	16.73	357	1.71	0.1	19.2	151.7	7.26	-44.6	4.97
L2F	13902	46.95	8	2.88	0.2	19.6	154.8	6.93	-26.3	0
P31	2734	30.43	-	0.19	0.2	19.1	152.7	7.18	-39.7	3.5
P32	2713	17.69	-	0.47	0.1	19.1	73.7	7.29	-46.7	14

Arsenic concentration (As, mg L^{-1}*), total organic carbon (TOC, wt.%), chromium concentration (Cr, mg* L^{-1}*), dissolved oxygen concentration (DO, mg* L^{-1}*), salinity (SAL, ppm), temperature (T, °C), total dissolved solids (TDS, ppm), ph, redox potential (redox, mV), depth (z, m).*

has transferred to the genus Galeripora; González-Miguéns et al., 2022). *Centropyxis aculeata* strain “aculeata” was found in all 12 samples, followed by *Difflugia protaeiformis* strain “acuminata” found in 10 samples. *Cucurbitella tricuspis* and *Cyclopyxis kahli* were also relatively abundant as we found them in 9 samples. In contrast, *Difflugia urceolata* strain “urceolata” and *Lesquereusia modesta* were found only in one sample (S3P and L2B, respectively). *Lesquereusia spiralis* and *L. modesta* were found statistically insignificant as bioindicators according to the Sxi calculation. In this study, we are recording for the first time in Guatemala the following species: *Arcella megastoma* (Recently *A. megastoma* has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022), *Arcella gibbosa*, *C. tricuspis*, *D. protaeiformis* strain “acuminata,” *Difflugia urceolata* strain “elongata,” *L. spiralis*, *L. modesta*, and *Mediolus corona*. Testate amoebae were present in all surface sediment samples, showing differences in abundance and richness across the collected taxa (Tab. 2). The L1T2, L2F, P21, and P31 samples had the highest species richness (n=11), and in contrast, two littoral zones showed the lowest species richness (L1T1 and L1C, 5 and 6, respectively). In the pelagic zone, species richness ranged from eight to eleven.

Testate amoebae assemblages as bioindicators of environmental conditions

The Q-mode cluster analysis showed testate amoebae assemblages in three conditions (Fig. 3): i) stressed conditions assemblage (SC); ii) lowest contamination conditions assemblage (LC); iii) deep transitional conditions assemblage (DT). The RDA analysis was consistent with the cluster analysis by showing a clear separation between SC, LC and DT (Axes 1 and 2 show 42.6% of the variance and 58.24% of the species-environment parameter relationship) (Fig. 4). According to the RDA, only TOC influences significantly the testate amoebae assemblages (p=0.004) (Fig. 4).

Stressed conditions assemblage

This assemblage is associated with the littoral zone samples, which are linked to forest cover (L2F), invasive water hyacinth (L1W), and cow pasture (L1C). The SC has the highest TOC (range:34.68-62.67 wt.%, mean:48.1 wt.%, SD:14.03 wt.%) and DO (range:2.88-7.13 mg L^{-1}, mean:4.68 mg L^{-1}), and a low TDS (range:124.3-154.8 ppm, mean:141.5 ppm). The L1C and L2F samples show SDIs lower than 1.5, suggesting a stressful environment. The L1W has a transitory SDI value of 1.88 and shows

Tab. 2. Relative abundances (percentage) of testate amoebae in Lake Chichoj, Guatemala.

Name	ID	L1T1	P11	P12	L1T2	P13	L1W	L1C	P21	P22	L2F	P31	P32
*Arcella megastoma** Penard, 1902	AM	9.53	0.00	1.85	0.00	0.00	0.00	0.00	0.75	0.00	4.21	0.00	0.00
Arcella gibbosa Penard, 1902	AG	0.00	0.00	20.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.00
*Arcella discoides** Ehrenberg, 1843	GD	0.00	5.24	11.11	0.97	0.00	6.41	0.00	0.00	2.44	7.00	2.08	3.21
Centropyxis aculeata Ehrenberg, 1832 strain “aculeata”	CAA	11.90	27.75	27.78	8.74	27.78	38.46	86.17	22.39	15.85	62.15	39.58	16.03
Centropyxis aculeata Ehrenberg, 1832 strain “discoides”	CAD	0.00	0.00	0.00	5.83	0.00	0.00	0.00	4.48	2.44	7.48	0.00	0.00
Centropyxis ecornis Ehrenberg, 1841	CE	11.90	0.00	3.70	0.97	0.00	6.41	0.00	2.99	2.44	0.00	0.00	0.00
Centropyxis constricta Ehrenberg, 1843 strain “aerophila”	CCA	42.86	0.00	0.00	0.00	9.26	15.39	2.13	0.00	0.00	0.00	0.00	4.49
Cucurbitella tricuspis Carter 1856	CUT	0.00	14.14	20.38	6.79	25.93	0.00	1.06	32.84	6.09	0.00	16.67	33.96
Cyclopyxis kahli Deflandre, 1929	CYK	23.81	1.05	0.00	0.97	5.56	11.54	0.00	0.75	1.22	0.47	0.00	0.64
Difflugia protaeiformis Lamarck, 1816 strain “acuminata”	DPA	0.00	38.22	3.70	6.80	14.81	6.41	0.00	15.67	59.76	3.74	20.83	14.74
Difflugia urceolata Carter, 1864 strain “elongata”	DUE	0.00	0.52	3.70	0.00	3.70	0.00	0.00	7.46	7.32	2.34	10.42	0.00
Difflugia oblonga Ehrenberg, 1838 strain “linearis”	DOL	0.00	0.00	0.00	16.50	0.00	1.28	3.19	0.00	0.00	7.00	4.17	3.21
Difflugia oblonga Ehrenberg, 1838 strain “byrophila”	DOB	0.00	0.00	0.00	22.33	0.00	0.00	3.19	0.00	0.00	0.00	0.00	0.00
Difflugia urceolata Carter, 1864 strain “urceolata”	DUU	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.13
Difflugia oblonga Ehrenberg, 1838 strain “oblonga”	DOO	0.00	0.00	0.00	10.68	0.00	3.85	0.00	8.21	0.00	4.67	4.17	0.64
Difflugia oblonga Ehrenberg, 1838 strain “lanceolata”	DOLA	0.00	10.47	0.00	0.00	1.85	8.97	0.00	0.00	0.00	0.00	0.00	0.00
Lesquereusia spiralis Ehrenberg, 1840	LS	0.00	0.52	5.56	0.00	3.70	1.28	0.00	2.23	0.00	0.47	0.00	1.92
Lesquereusia modesta Rhumbler, 1895	LM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.00
Mediolus corona (Wallich, 1864)	MC	0.00	2.09	1.85	19.42	7.41	0.00	4.26	2.23	2.44	0.00	0.00	16.03
Abundance		42	191	54	103	54	78	94	134	82	214	48	156
Richness		5	9	10	11	9	10	6	11	9	11	8	11
SDI		1.43	1.57	1.92	2.07	1.88	1.89	0.61	1.88	1.38	1.42	1.65	1.89

**Recently,* Arcella megastoma *and* Arcella discoides *has transferred to the genus* Galeripora*; González-Miguéns et al., 2022.*

the lowest TOC and DO in the SC. Through IndVal we identified *C. aculeata* strain “aculeata” (p=0.001) as the SC indicator species.

Lowest contamination conditions assemblage

This assemblage found at a single littoral zone sample (L1T2) adjacent to the town shore (Fig. 4), is dominated by *Difflugia oblonga* strain “bryophila” (22%), *M. corona* (19%), *Difflugia oblonga* strain “linearis” (16%), and *Difflugia oblonga* strain “oblonga” (11%). The sample registered the highest temperature (20.8°C) and DO (8.80 mg L^{-1}) in contrast with the lowest TOC (14.83 wt.%) and TDS (2.4 ppm). The proportion of *C. aculeata* strain “aculeata” (9%) was the lowest across all samples, showing the highest SDI value (2.07), suggesting transitional environmental conditions. We did not identify any indicator species through the use of IndVal.

Deep transitional conditions assemblage

The DT is found across the six pelagic samples along the different water depths in the lake. This group does not differ across the lake’s three basins despite being separated by shallow sills. DT is dominated by *C. aculeata* strain “aculeata” (range: 16-29%), *D. protaeiformis* strain “acuminata” (range: 4-60%), *C. tricuspis* (range: 6-35%), and *M. corona* (range: 6-16%). Five samples showed SDI values (1.57-1.92) typical of transitional environmental conditions. The P22 SDI value (1.38) indicates stressed environmental conditions. The Q-mode cluster showed a subgroup (P11, P22) with lower DO (range:0.25-1.71 µg L^{-1}, mean:0.98 µg L^{-1}) and TOC (range:15.38-16.73 wt.%, mean:16.05 wt.%, SD:0.95 wt.%) and, on the other hand, a subgroup (P32, P13, P21) with higher DO (range:0.47-5.02 µg L^{-1}, mean:3.17 µg L^{-1}) and TOC (15.13-33.25 wt.%, mean:22.02 wt.%, SD:9.81 wt.%). The RDA analy-

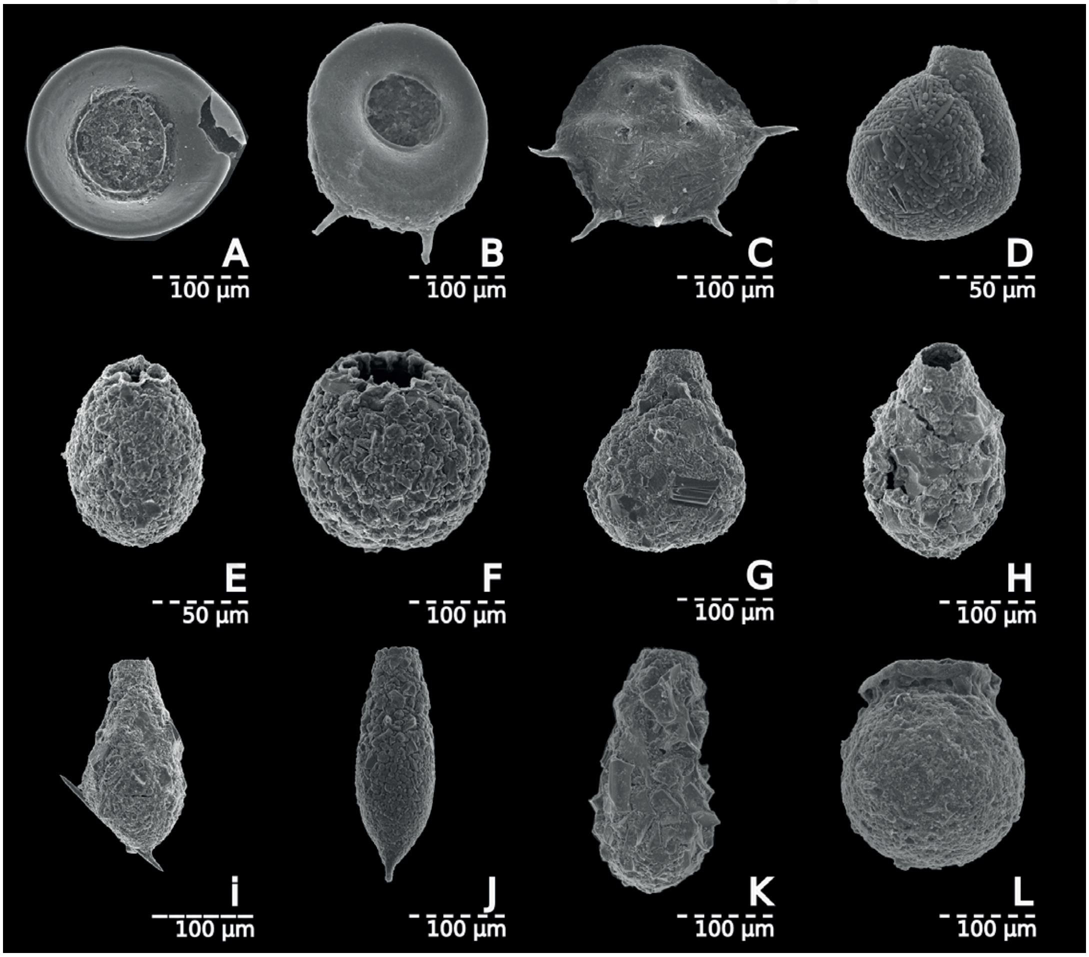


Fig. 2. Scanning electron microscopy of selected testate amoebae from Lake Chichoj, Guatemala. *Arcella discoides* (recently, *A. discoides* has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022), ventral view (A), *Centropyxis aculeata* strain “aculeata” (B) ventral view (C) dorsal view, *Lesquereusia spiralis*, lateral view (D), *Curcurbitella tricuspis* lateral view (E, F), *Difflugia oblonga* strain “oblonga,” lateral view (G), *Difflugia oblonga* strain “linearis,” lateral view (H,I), *Difflugia protaeiformis* strain “acuminata,” lateral view (J), *Difflugia oblonga* strain “bryophila,” lateral view (K), *Difflugia urceolata* strain “urceolata,” lateral view (L).

sis indicates that DT samples are generally a cohesive group, except for sample P12, the deepest lake sample, which is positively correlated with TDS and z. IndVal identified *C. tricuspis* (p=0.02) as a DT indicator species.

DISCUSSION

New data on Arcellinida from Guatemala

As expected, the main taxa recorded in this study were Difflugiidae, Centropyxidae, and Arcellidae; because these families are typical of freshwater environments across the neotropics, as registered in Mexico (Sigala *et al.*, 2016), the Upper Paraná River floodplain (Alves *et al.*, 2010), and Brazil (Ferreira *et al.*, 2007). The different richness between the littoral and pelagic zones of Lake Chichoj is the same pattern observed in other studies (Sigala *et al.*, 2016; Mousinho *et al.*, 2018), which seems to be related to variability in food resources, macrophytes, and plankton (Bastidas-Navarro and Modenutti, 2007; Lansac-Tôha *et al.*, 2009). In general, tropical and subtropical regions show large testate amoebae abundance variability (Patterson *et al.*, 2015; Sigala *et al.*, 2016), which was the case for Lake Chichoj. As testate amoebae ecological drivers remain largely unexplored in Guatemala, there is a need to explore the role of variables such as nutrient concentrations, climate variability, and sediment type.

Testate amoebae assemblages as bioindicators

Through the IndVal analysis (p=0.001), we identified *C. aculeata* strain "aculeata" as an indicator species of the SC (i.e., dominant), where organic content was high (TOC, range:38-63 wt.%). This opportunistic strain has been reported to reflect locally stressed conditions (Roe and Patterson, 2006), such as acidic-mine contexts (Patterson *et al.*, 2013; Reinhardt *et al.*, 2005), heavy metal pollution (Patterson *et al.*, 1996), eutrophication (Sigala *et al.*, 2018), low DO levels (Charqueño-Celis *et al.*, 2020), and low salinity levels (van Hengstum *et al.*, 2008). Furthermore, the relationship between *C. aculeata* strain "aculeata" and TOC indicates accelerated eutrophication at Lake Chichoj, suggested to have intensified

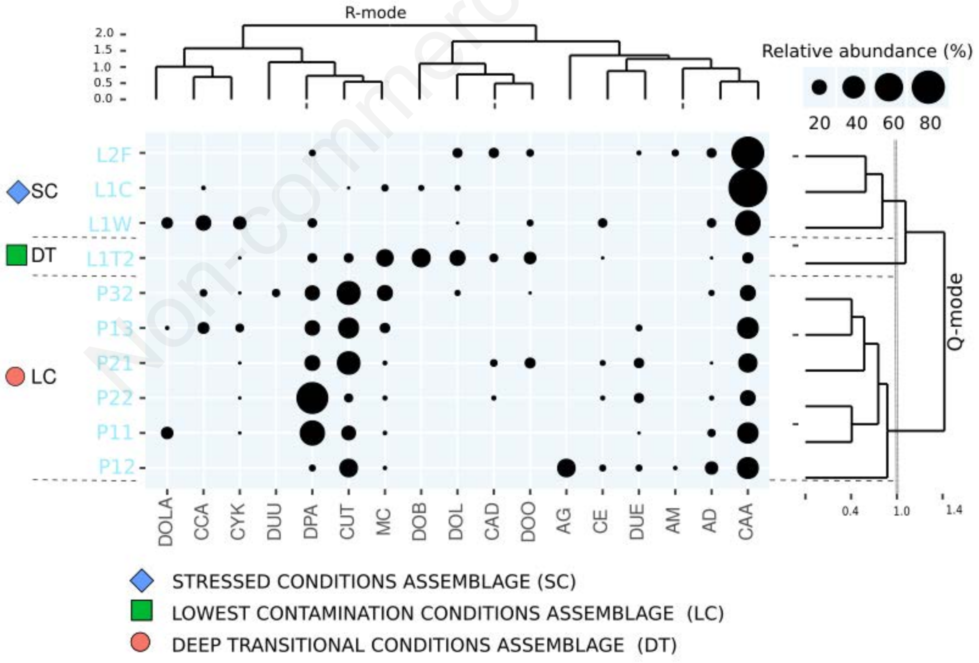
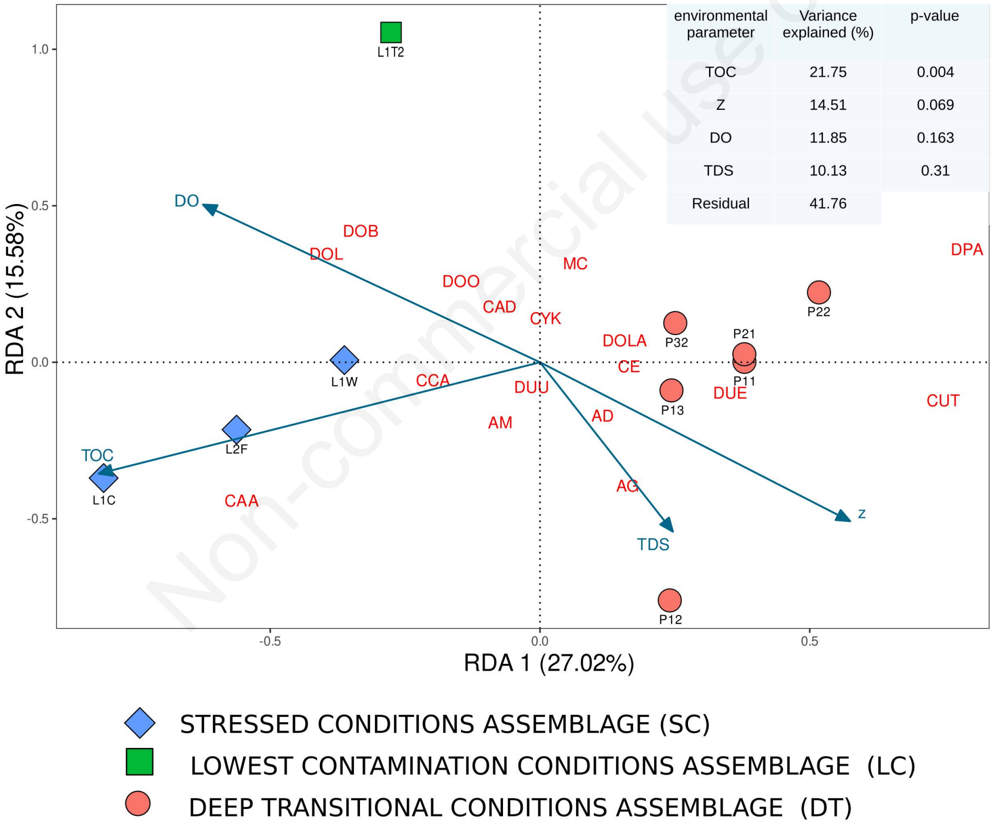


Fig. 3. The Q-mode and R-mode cluster analyses were represented as a bubble graph for ten samples and 14 statistically significant species and strains. Three faunal assemblages are indicated. Species noted: AM, *Arcella megastoma* (recently, AM has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022); AG, *Arcella gibbosa*; AD, *Arcella discoides* (recently, AD has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022); CAA, *Centropyxis aculeata* strain "aculeata"; CAD, *Centropyxis aculeata* strain "discoides"; CE, *Centropyxis ecornis*; CCA, *Centropyxis constricta* strain "aerophila"; CUT, *Cucurbitella tricuspis*; CYK, *Cyclopyxis kahli*; DPA, *Difflugia protaeiformis* strain "acuminata"; DUE, *Difflugia urceolata* strain "elongata"; DOL, *Difflugia oblonga* strain "linearis"; DOB, *Difflugia oblonga* strain "byrophila"; DUU, *Difflugia urceolata* strain "urceolata"; DOO, *Difflugia oblonga* strain "oblonga"; DOLA, *Difflugia oblonga* strain "lanceolata"; MC, *Mediolus corona*.

since the late 1970s (Albizurez-Palma, 1978). In addition, paleochemical studies in the lake have found that increasing TOC is connected historically to anthropogenic factors (Brocard *et al.*, 2016b). Our results are coherent with these findings as the indicator strain reflects stressful conditions caused by TOC at Lake Chichoj.

Although we removed arsenic from our statistical analysis to explain Arcellinida assemblages, the SC showed the highest levels due to its high collinearity with TOC. Elevated arsenic can cause a reduction in microbial biomass in lacustrine ecosystems (Gough and Stahl, 2011) which could affect food sources for Arcellinida assemblages and increase stressing conditions. Furthermore, the positive correlation between TOC, high organic matter concentration and heavy metals (positive correlation between TOC and As, r>0.75) at Lake Chichoj is typical of lakes contaminated due to mining (Nasser *et al.*, 2020; Riou *et al.*, 2021). Our results emphasize the utility of testate amoebae as indicators when studying the history of pollution in lakes where heavy metals have played a relevant role.

The LC was found at a single site (L1T2) with the lowest TOC, TDS, and the highest value of dissolved oxygen levels, dominated by *D. oblonga* strain "bryophila". This strain has been reported in temperate lakes (Patterson and Kumar, 2000a; Escobar *et al.*, 2008; Roe *et al.*, 2010), littoral zones (Sigala *et al.*, 2018) and deep sediments (Kornecki and Katz, 2020). Various *Difflugia oblonga* strains have been found to be dominant at high TOC values due to the high organic content required for their de-



environmental parameter	Variance explained (%)	p-value
TOC	21.75	0.004
Z	14.51	0.069
DO	11.85	0.163
TDS	10.13	0.31
Residual	41.76	

Fig. 4. Redundancy analysis (RDA) tri-plots, showing the relationship between testate amoebae species and the two environmental parameters and the sediments samples. The three arcellinid assemblages are represented by colored symbols. Parameters noted: Dissolved oxygen concentration, (DO, mg L^{-1}); total organic carbon, (TOC, wt.%); total dissolved solids (TDS, ppm); water depth (z, m). Species noted: *Arcella megastoma*, AM (Recently, AM has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022); *Arcella gibbosa*, AG; *Arcella discoides*, AD (Recently, AD has transferred to the genus *Galeripora*; González-Miguéns *et al.*, 2022); *Centropyxis aculeata* strain "aculeata," CAA; *Centropyxis aculeata* strain "discoides," CAD; *Centropyxis ecornis*, CE; *Centropyxis constricta* strain "aerophila," CCA; *Cucurbitella tricuspis*, CUT; *Cyclopyxis kahli*, CYK; *Difflugia protaeiformis* strain "acuminata," DPA; *Difflugia urceolata* strain "elongata," DUE; *Difflugia oblonga* strain "linearis," DOL; *Difflugia oblonga* strain "byrophila," DOB; *Difflugia urceolata* strain "urceolata," DUU; *Difflugia oblonga* strain "oblonga," DOO; *Difflugia oblonga* strain "lanceolata," DOLA; *Mediolus corona*, MC.

velopment (Patterson and Kumar, 2000b; Caffau *et al*., 2015). Nevertheless, at Lake Chichoj, the *D. oblonga* strain "bryophila" suggests the opposite scenario with low TOC. Kornecki *et al.* (2020) found that *D. oblonga* strain "bryophila" is tolerant of low food availability and positively correlated to low water turbidity in shallow waters. Sigala *et al.* (2018) found that *D. oblonga* strain "bryophila" is associated with warm water with basic pH and high sedimentation rates, consistent with LC's highest temperature and pH. Regarding land use, the LC location at the town's limit shows the highest SDI value. Our finding does not agree with Roe *et al.* (2010), who explain that lakes beyond urban limits have the highest SDI. Nevertheless, our RDA showed that high SDI values could be linked with higher dissolved oxygen and lower TOC. Our results suggest that the *D. oblonga* strain "bryophila" may prefer higher dissolved oxygen levels, shallow waters and low TOC.

Studies suggest that testate amoebae assemblages show a relationship between depth and lacustrine thermoclines (Patterson *et al*., 1996; Nasser *et al*., 2016; Cockburn *et al.,* 2020). Our cluster analysis and RDA (Figs. 3 and 4) suggested an association with depth, which at Lake Chichoj could reflect the separation between the three basins. The testate amoebae assemblage from the deepest sample taken from Lake Chichoj (P12) shows an association with four indicator taxa of eutrophic conditions and environmental stress: *Arcella* (33%), accompanied by *C. tricuspis* (6-35%), *D. protaeiformis* strain "acuminata" (4-60%) and *C. aculeata* strain "aculeata" (16-29%) (Patterson *et al*., 2002; Reinhardt *et al*., 2005; Prentice *et al*., 2008; Kihlman and Kauppila, 2012). IndVal aided us in selecting only *C. tricuspis* as a eutrophication indicator, which is similar to the findings in nutrient-enriched lakes in Scotland (Prentice *et al*., 2018), Finland (Kihlman and Kauppila, 2012) and Canada (Gavel *et al*., 2018). Furthermore, the known connection of *C. tricuspis* with *Spirogyra* algae (Patterson *et al*., 1996) and macrophytes (Gavel *et al*., 2018) can be linked to the extensive algal bloom at Lake Chichoj. Prentice *et al*. (2018) associated *C. tricuspis* with other eutrophic indicator taxa, although found in lower abundances, such as *M. corona*. Our results suggest that not only *C. tricuspis* indicates eutrophication but also the presence of *D. protaeiformis* strain "acuminata" which indicates low oxygen levels (Kihlman and Kauppila, 2012; Sigala *et al*., 2018). TOC is important in controlling and distributing sediment's organic and inorganic chemical components (Hall and Anderson, 2014). However, as TOC interacts with several autochthonous and allochthonous limnological parameters associated with lake morphology and depth (Yu *et al*., 2015), it remains critical to study other variables such as surrounding terrestrial ecosystems, incoming sediment type and organic carbon sources.

CONCLUSIONS

This study is the first to explore testate amobeae ecological assemblages at Lake Chichoj in the Guatemalan central highlands. We are providing new records of testate amoebae for Guatemala that show the need to fill in the information gap about lacustrine testate amoebae diversity. Our results show that testate amoebae assemblages are sensitive to various environmental parameters, particularly TOC, depth, DO and TDS. We are documenting *Centropyxis aculeata* strain "aculeata" as an indicator taxa of stressful conditions in Lake Chichoj. Also, we document how *Cucurbitella tricuspis* indicate nutrient enrichment and low dissolved oxygen concentration. Our results emphasize the need for further studies that include multiple biogeochemical parameters, such as particle size analysis, climatic variability and phosphorus concentrations, to understand better local limnological changes and testate amoebae assemblages responses.

Our study at Lake Chichoj demonstrates the utility of testate amoebae as bioindicators of water quality in a highland tropical region, which is an initial step to further the exploration of this indicator group. However, we need more ecological studies of testate amoebae in Central America to understand in a further scope the applications of using these protists as environmental indicators in neolimnology and paleolimnology.

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