

Testate amoebae (Amoebozoa: Arcellinidae) as indicators of dissolved oxygen concentration and water depth in lakes of the Lacandón Forest, southern Mexico

Norma Fernanda Charqueño-Celis,^{1*} Martin Garibay,² Itzel Sigala,^{2,3} Mark Brenner,⁴ Paula Echeverría-Galindo,⁵ Socorro Lozano-García,² Julieta Massafferro,¹ Liseth Pérez⁵

¹CENAC-PNNH-CONICET, Bariloche, Argentina; ²Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México; ³Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad de México, México; ⁴Department of Geological Sciences and Land Use and Environmental Change Institute (LUECI), University of Florida, Gainesville, FL, USA; ⁵Institut für Geosysteme und Bioindikation, Technische Universität Braunschweig, Germany

ABSTRACT

The ecology of aquatic protists such as testate amoebae is poorly known worldwide, but is almost completely unknown in lakes of the northern Neotropics. To address this knowledge gap, we analyzed testate amoebae (Amoebozoa: Arcellinidae) in lakes of the Lacandón Forest, one of the most biodiverse parts of southern México. We set out to evaluate the diversity of testate amoebae communities and assess whether testate amoebae taxa are reliable indicators of environmental variables dissolved oxygen and water depth. We collected 17 surface sediment samples from a range of water depths in six lakes across the Naha-Metzabok Biosphere Reserve, northeastern Chiapas state. We identified 15 testate amoebae taxa distributed across seven genera. Eleven were identified to species level and four to strain (infra-subspecific level), and taxa were distributed unevenly among samples. Distribution of taxa in samples was related to dissolved oxygen (DO) concentration in the water measured near the sediment surface. *Arcella discoides* and *Centropyxis aculeata* strain “aculeata” were the most tolerant of low oxygen concentrations, whereas the other taxa require higher DO levels. The influence of oxygen was also seen at the assemblage level. Sites with low DO concentrations had Shannon Diversity Index (SDI) values <1.5, an indication of stressful ambient conditions. We identified two species assemblage types, which are distinguished by their oxygen concentration requirements. Assemblage 1 was more diverse and possessed species that are intolerant of low oxygen concentrations, whereas Assemblage 2 possessed fewer, rarer, opportunistic species that tolerate stressful conditions. Low oxygen concentrations are related to water depth and the combination of these two variables is important in determining the composition of testate amoebae assemblages in Lacandón Forest lakes. Quantitative relationships between testate amoebae assemblages and water depth will enable use of sedimented amoebae remains for paleolimnological inference of past water level changes in lakes of the Lacandón Forest.

INTRODUCTION

Aquatic protists in biodiverse ecosystems of the Neotropics have received little attention. For example, little

is known of these organisms in lakes of the Lacandón Forest (16°45'0" N, 91°30'0" W) in northeastern Chiapas state, México. This forest is a biodiversity hotspot that hosts a vast amount of Mexican biodiversity with respect to vertebrates (46%), butterflies (60%) and plants (50%) (Castillo-Campos and Narave, 1992; Lazcano-Barrero *et al.*, 1992; Conanp, 2006b). In 2010 UNESCO declared two of its five natural protected areas (NPAs), Metzabok and Naha, a Biosphere Reserve. Although there are numerous lacustrine systems in the area, knowledge of the aquatic biota in the region is limited to fish, amphibian and reptile inventories (Conanp, 2006a). More recently, Díaz *et al.* (2017) and Vázquez-Molina *et al.* (2016) published paleolimnological studies that employed sedimented ostracods and cladocerans, respectively. Whereas the primary goal of those studies was paleoenvironmental inference, they contributed to knowledge about the biota of these lakes and highlighted the importance of modern ecological studies in this poorly studied area of México.

One goal of neoecological studies is to understand the relation between communities and environmental variables and assess the sensitivity of taxa to climate and environmental changes (McGill *et al.*, 2006). Communities and subfossil assemblages are useful bioindicators of modern and past conditions, respectively (Smol *et al.*, 2001). Testate amoebae are abundant

Corresponding author: fcharquenocelis@comahue-conicet.gob.ar

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components of benthic communities and are highly sensitive to environmental conditions (Yeates and Foissner, 1995; Neville *et al.*, 2010; Han *et al.*, 2011). They are a polyphyletic group of free-living protists, whose main character is a test (“shell”) that protects the cytoplasm. They possess a simple aperture for extrusion of pseudopods (Medioli and Scott, 1988; Beyens and Meisterfeld, 2006). Testate amoebae are present in all kinds of lakes, from the littoral to the profundal zone (Sigala *et al.*, 2016). A number of studies have shown strong relationships between lacustrine testate amoebae community composition and variables such as salinity, conductivity, pH and human impacts (Patterson *et al.*, 1996; Roe *et al.*, 2010; Patterson *et al.*, 2012), suggesting that these protists are useful modern and paleo-environmental bioindicators.

A few studies in the Neotropics identified the effect of dissolved oxygen on the structure of testate amoebae communities (Dalby *et al.*, 2000; Roe *et al.*, 2010; Sigala *et al.*, 2018). Our goal was to evaluate the role of dissolved oxygen concentration in determining the structure and diversity of testate amoebae communities in lakes of the Lacandón Forest, Chiapas, México. We hypothesized that testate amoeba diversity would be directly correlated with oxygen concentration, which in turn, might be inversely related to lake water depth. We sought to gather fundamental data about the ecology of these tropical protists, and to explore their potential for use as bioindicators.

METHODS

This study was carried out in the Naha-Metzabok Biosphere Reserve, in the Lacandón Forest of northeastern Chiapas State (16°95'0" N, 90° 78'0" W). Mean annual temperature in the area is 23.6°C and the mean annual precipitation is ~1860 mm. Regional vegetation is a mosaic of tropical rain forest, cloud forest, and coniferous forest elements, mixed with large patches of agriculture and pasture (Rzedowski, 2006). The area is a karstic platform of Cretaceous marine origin (Padilla, 2007) and is characterized by calcareous hills and valleys with altitudes that range from 580 to 1800 m asl. Two major rivers in the region, the Lacanjá and Lacantún, feed 21 lakes that differ with respect to their geomorphological characteristics (Conabio, 2013a, 2013b). An important hydrological feature of this karst region is the groundwater, which influences lake water levels and governs hydrologic connectivity between the lakes.

We collected 17 surface sediment samples from different water depths in six lakes across the Lacandón Forest (Fig. 1), Chiapas México, in March 2015 and June 2016. Samples were collected with an Ekman grab (Tab. 1). Water temperature, dissolved oxygen, pH and conductivity were measured *in situ* near the sediment surface using a YSI EX01 Multi-parameter probe (Yellow Springs, OH, USA). Sediment samples were preserved in absolute ethanol and subsamples of 1 cm³ wet volume were

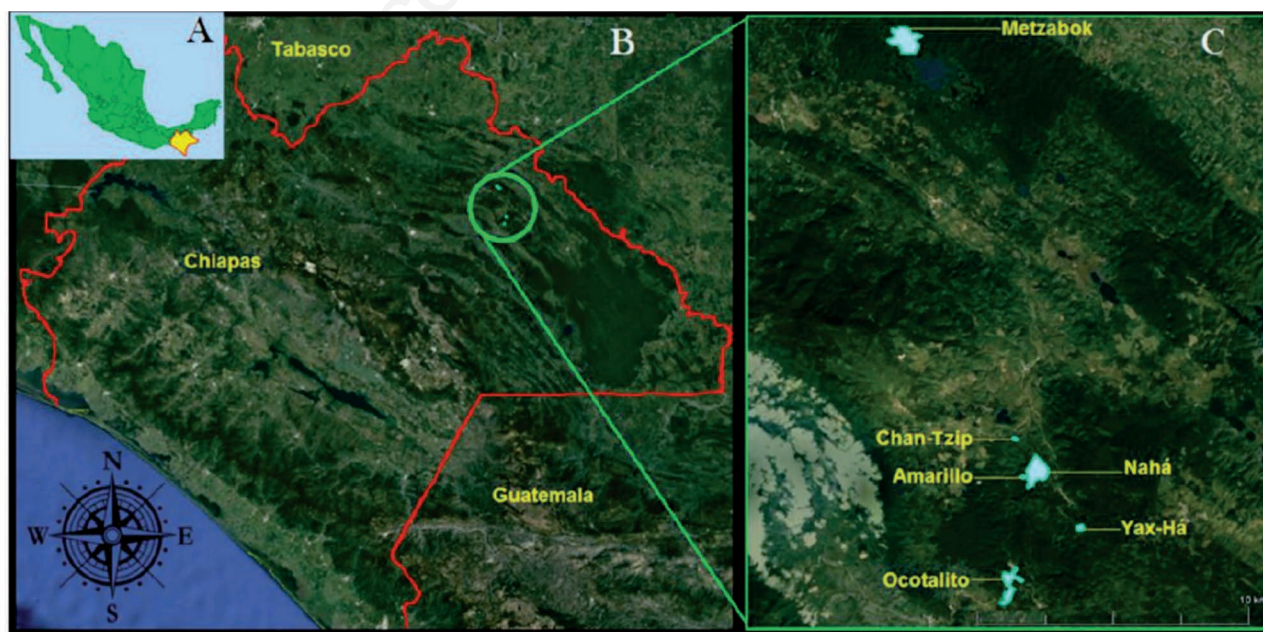


Fig. 1. Map showing the locations of the six lakes sampled in the Lacandón Forest, Chiapas, Mexico. Inset map (A) shows the location of the state of Chiapas in southern Mexico.

removed for analysis. In the lab, each subsample was examined under a ZEISS Stemi 508 stereomicroscope and testate amoebae were extracted using a fine brush (Ellison and Ogden, 1987). Pre-identification was done with an optical microscope, using the descriptions of Sigala *et al.* (2016) and confirmed with photographs taken using a ZEISS EVO MA10 scanning electron microscope (SEM) and comparison with the taxonomic keys of Ogden and Hedley (1980), Kumar and Dalby (1998) and Lee *et al.* (2000). Lacustrine arcellacean species can display broad eco-phenotypically controlled morphological variability. Therefore, it is common to assign informal, infra-subspecific ‘strain’ names to eco-phenotypes to avoid inadvertent unwarranted description of new species. Although the International Code of Zoological Nomenclature stipulates that infra-subspecific-level designations have no status (ICZN, 1999), in testate amoebae studies they are useful for distinguishing environmentally distinct populations in lacustrine environments (Patterson *et al.*, 2012).

Statistical analyses

Statistical analyses were carried out in the R environment version 3.5.3 (R Core Team, 2019), specifically with the Vegan package (Oksanen *et al.*, 2019). To visualize testate amoebae distributions along the dissolved oxygen gradient, we constructed an

INKSPOT-plot. That is a two-way, ordered “bubble” plot of testate amoebae by sites, with sites arrayed along the x-axis, species on the y-axis, and abundance indicated by the symbol (“bubble”) size.

Testate amoebae assemblage diversity is described using the Shannon Diversity Index (SDI). The SDI is defined in the equation below, with X_i = abundance of individuals of a species (i) in the sample; N_i = total number of individuals in the sample; S = species richness (*i.e.* number of species) in the sample:

$$SDI = \sum_{i=1}^S \left(\frac{X_i}{N_i} \right) \times \ln \left(\frac{X_i}{N_i} \right)$$

According to Patterson and Kumar (2002), the index value is an indication of the stress condition at the site where testate amoebae were collected. An SDI value between 2.5 and 3.5 indicates “stress-free” conditions for testate amoebae. If the site does not possess optimal conditions for the organisms, values of the index are between 1.5 and 2.5. In contrast, sites with poor environmental conditions for testate amoebae have low SDI values, between 0.1 and 1.5.

To test if the number of species present in each sample was statistically significant for analysis, we calculated the standard error (S_{xi}) associated with each taxon using the following formula:

Tab. 1. Environmental variables for sampled study lakes in the Lacandón Forest, Chiapas, Mexico. Reported values reflect those measured in the water column, just above the sediment surface.

Lake name	Code	Latitude (N)	Longitude (W)	Year	Depth (m)	Altitude (m asl)	Dissolved oxygen (mg L ⁻¹)	pH	Temperature (°C)	Electrical conductivity (μS cm ⁻¹)
Metzabok	M510	17°07'54.18"	91°38'37.65"	2015	10.57	550	4.21	7.75	21.60	390
Metzabok	M607	17°07'53.89"	91°38'23.93"	2016	6.50	550	6.94	8.12	29.10	289
Metzabok	M610	17°07'45.15"	91°38'08.27"	2016	9.90	550	6.28	7.80	27.70	287
Metzabok	M613	17°07'52.27"	91°38'03.56"	2016	13	550	4.83	7.63	26.80	294
Nahá	N517	16°59'24.10"	91°35'56.70"	2015	17.66	832	4.90	7.69	20.60	495
Nahá	N522	16°59'39.43"	91°35'23.51"	2015	22.09	832	1.29	7.49	20.60	496
Nahá	N624	16°59'08.68"	91°35'38.45"	2016	23.50	832	0.52	7.47	20.60	348
Amarillo	A510	16°58'56.45"	91°30'31.52"	2015	10.35	867	0.32	7.40	20.70	346
Amarillo	A610	16°58'57.17"	91°30'31.74"	2016	9.37	867	0.92	7.10	21.10	283
Yaxhá	Y529	16°57'53.48"	91°35'42.70"	2015	28.74	930	1.05	7.36	21.30	296
Yaxhá	Y625	16°57'57.98"	91°35'55.58"	2016	25.04	930	3.50	7.58	21.40	200
Yaxhá	Y630	16°57'57.08"	91°35'23.87"	2016	30	930	0.21	7.36	21.40	227
Ocotálito	O508	16°56'34.27"	91°36'04.76"	2015	7.57	946	5.63	7.93	21.30	313
Ocotálito	O521	16°56'40.21"	91°36'48.54"	2015	21.01	946	0.11	7.28	20.20	341
Ocotálito	O605	16°57'36.07"	90°56'44.34"	2016	5.02	946	5.16	8.22	26.90	234
Ocotálito	O619	16°59'05.08"	91°35'31.32"	2016	19.10	946	0.22	7.44	20.10	231
Chan-Tzip	C508	16°58'47.73"	91°37'56.75"	2015	7.50	950	1.15	7.96	20.60	425

$$S_{XI} = 1.96 \sqrt{\frac{F_i(1 - F_i)}{N_i}}$$

where F_i is the relative fractional abundance of each taxon and N_i is the total number of individuals of all species in each sample. If the S_{xi} value is greater than the relative fractional abundance, then it is not statistically significant and cannot be included in the multivariate analysis (Patterson and Fishbein, 1989; Patterson *et al.*, 2012). In this study, all species abundances were statistically significant and included in the analysis. We then organized species and sample data into a hierarchical diagram using Q-mode and R-mode cluster analysis and Wards minimum variance method, using Ward distance (Patterson and Kumar, 2002; Roe and Patterson, 2014). Lastly, we applied a permutational multivariate analysis of variance (PERMANOVA) with two variables (dissolved oxygen and water depth) to assess the statistical significance of the effects of the environmental variables on testate amoebae assemblages, accomplished with the hierarchical diagram and non-metric multi-dimensional scaling (NMDS) (Anderson, 2017). This was used to describe patterns in testate amoebae assemblages and assess relationships among testate amoebae communities and dissolved oxygen and water depth. NMDS is a robust non-parametric ordination method for analyzing ecological community data, which does not make assumptions regarding the underlying species distribution patterns.

RESULTS

Testate amoebae were present at all sites sampled. A total of 15 taxa distributed across seven genera were identified. Eleven were identified to species level and four were assigned to an infra-subspecific level, hereafter referred to as “strain” (Tab. 2). Abundance of taxa was plotted against dissolved oxygen concentration values (Fig. 2) and shows that the diversity of testate amoebae changes in accordance with oxygen concentrations. *Centropyxis aculeata* strain “aculeata” and *Arcella discoides* were the most common and abundant taxa in the samples. Remaining taxa displayed preferences for higher concentrations of dissolved oxygen (Fig. 2). Sites with the highest oxygen concentrations (>4 mg L⁻¹) yielded SDI values >1.5 , whereas samples from sites with relatively low oxygen concentrations (<4 mg L⁻¹) had SDI values between 0 and 1.5 (Fig. 3).

Statistical analysis revealed the effect of dissolved oxygen concentration on testate amoebae diversity. The Q-mode and R-mode cluster analysis and NMDS, based on the abundance and distribution of testate amoebae, enabled identification of two main assemblage types (Fig. 4), which are related to the tolerance of taxa to oxygen

concentrations, and the water depth at the sampling site (Fig. 5). PERMANOVA results showed there is a stronger correlation between testate amoebae distribution and dissolved oxygen ($F=3.4$, $\text{Pr}(>F)=0.01$) than between the protists and water depth ($F=0.68$, $\text{Pr}(>F)=0.64$). Nevertheless, the interaction between the two variables also had a significant effect on the rhizopod assemblages ($F=3.01$, $\text{Pr}(>F)=0.03$) (Tab. 3, Fig. 3).

Assemblage 1

This group was dominant primarily in samples from Lakes Metzabok, Yaxhá and Naha, and generally had high abundances of *C. constricta* strain “aerophila” (Fig. 3), as well as relatively high SDI values (>1.4) (Fig. 3). This assemblage is associated with the positive ordination NMDS axis 1 (Fig. 5), with high abundances of *D. oblonga* strain “oblonga”, *D. bidens* and *M. corona*. *Diffflugia protaeiformis* strain “acuminata”, *L. spiralis*, *D. protaeiformis* strain “amphoralis”, *L. vas*, and *C. constricta* strain “spinosa” were encountered only at these sites. Assemblage 1 was also related to high values of dissolved oxygen, as well as to intermediate water depths (Fig. 5).

Assemblage 2

In general, sites characterized by Assemblage 2 had low SDI value (<1.5) (Fig. 3) because of the strong dominance of *C. aculeata* strain “aculeata” and *A. discoides*. The rest of the species were absent or present at low abundances (Fig. 4). Assemblage 2 samples came

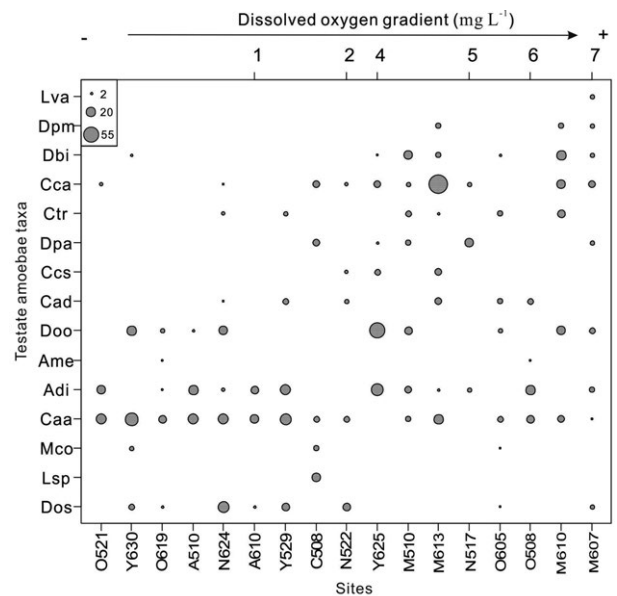


Fig. 2. INKSPOT plot of the abundance of testate amoebae along a dissolved oxygen gradient. The code for sample sites is in Tab.1 and the codes for testate amoebae species are in Tab. 2.

1, Assemblage 2 is associated with low values of dissolved oxygen, (Fig. 5), and is found in samples from relatively deep water, dominated by *C. aculeata* strain “aculeata” and *A. discoides*. The rest of the species were absent or present at low abundances (Fig. 5).

Tab. 2. Relative abundances of testate amoebae in lakes of the Lacandón Forest. See Tab. 1 for ID codes associated with each lake.

[illegible]

DISCUSSION

Abundances and distributions of testate amoebae taxa in Lacandón Forest lakes are related strongly to dissolved oxygen concentration in the bottom waters (Fig. 6). These protists are well represented in lakes of the Lacandón Forest, however distribution of taxa among the water bodies is heterogenous (Fig. 2). This is reflected in the Shannon Diversity Index values from these sites, which indicate low alpha diversity in sites with low dissolved oxygen. Strong dominance of a few tolerant species in sites with low oxygen concentrations results in low SDI values (Fig. 3). According to Patterson and Kumar (2002), SDI values <1.5 reflect a stressful environment for testate amoebae. We concluded that low oxygen concentrations in these Neotropical lakes constitute unfavorable environmental conditions for these protists.

Species less tolerant of low oxygen concentration were clustered in Assemblage 1, whereas species more tolerant of low dissolved oxygen were present in Assemblage 2. In the first assemblage, *Centropyxis constricta* strain “aerophila” was the most common and abundant taxon, however it displayed low representation in samples of Assemblage 2 (Fig. 4). *Centropyxis constricta* strain “aerophila” has been reported in both central México and the Yucatan Peninsula (Van Hengstum *et al.*, 2008; Sigala *et al.*, 2016). Those studies related the distribution of *C. constricta* “aerophila” to salinity and conductivity and the authors considered this taxon to be euryhaline. In our study, distribution of this taxon appears to have been determined by the amount of dissolved oxygen in the water, as we saw little difference in conductivity values across sampling sites (Tab. 1).

C. aculeata strain “aculeata” and *A. discoides* displayed high relative abundance, mainly in Assemblage 2 samples (Fig. 3). Both testate amoebae are typical opportunistic species, recorded in hostile conditions in tropical, subtropical and temperate environments with very low pH and high salinity, and even in badly polluted waters (Dalby *et al.*, 2000; Patterson and Kumar, 2000; Escobar *et al.*, 2005; Sigala *et al.*, 2018). Our results confirm this interpretation, as these two taxa were found

in sites with low dissolved oxygen concentrations (Fig. 2).

As many variables may influence the distribution of testate amoebae taxa, it can be difficult or impossible to isolate the effect of a single variable such as dissolved oxygen from other variables in complex aquatic ecosystems (Legendre and Anderson, 1999). Our PERMANOVA results indicate that even though testate amoebae distribution is related to oxygen concentration, the organisms may in fact be responding to the interacting effects of oxygen concentration and water depth (Tab. 3). Dalby *et al.* (2000) identified a strong relationship between dissolved oxygen concentration and these protists in a thermally stratified lake in Indonesia. Lake

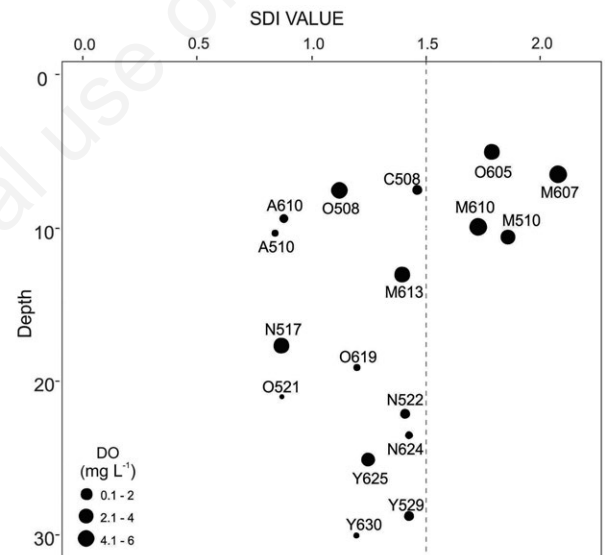


Fig. 3. Relationship between Shannon Diversity Index (SDI) value and water depth of each sample. The bubbles in the code lakes represent the dissolved oxygen concentration at each sample site. The red dashed line represents the limit between stressful conditions (SDI = 0.1-1.5) and transition conditions (SDI = 1.5-2.5) according to Patterson and Kumar (2002). Codes for each lake are given in Tab. 1.

Tab. 3. Results of PERMANOVA analysis to assess the effects of single and interacting factors (dissolved oxygen and water depth) on testate amoebae distribution in Lacandón Forest lakes.

	Df	SS	R ²	F	Pr(>F)
Dissolved oxygen	1	0.52	0.17	3.44	0.01
Water depth	1	0.10	0.04	0.68	0.64
Dissolved oxygen + water depth	1	0.46	0.15	3.01	0.03
Residual	13	1.97	0.65		
Total	16	3.05	1.00		

stratification results in persistent isolation of bottom waters and progressive reduction of oxygen in the hypolimnion. This, in turn, can have a negative impact on the survival of benthic organisms, as indicated by the stressed arcellacean fauna in this study. Limnological investigations in several lakes close to the Lacandón Forest showed that most stratify and develop hypolimnetic anoxia (Alcocer *et al.*, 2016). Although we did not do a detailed characterization of limnological conditions in our study lakes, we did observe a decrease in dissolved oxygen concentration with increasing water depth in the lakes (Fig. 3). We note, however, that other factors, not measured in this study, may influence the concentration of dissolved oxygen and thereby affect the testate amoebae community. Roe *et al.* (2010) reported low oxygen associated with polluted sites in temperate lakes. With respect to our lakes, it is unlikely that pollutants play a role in influencing arcellacean distributions, as the water bodies are far from industrial pollutant sources and mines. Low oxygen concentrations in some of the lakes, however, may be related to lake productivity, which in turn, is related to the anthropogenic history of the area, i.e. land use changes around the lakes (Trench, 2005).

Our preliminary study suggests that further efforts should be directed at identifying the relation between dissolved oxygen concentrations and testate amoebae assemblages. In particular, it would be worthwhile to determine the optima and ranges of oxygen concentration for individual taxa. Additionally, a more rigorous study of testate amoebae distributions along a water depth gradient could better define the influence of this variable on distributions of taxa. In this study, we deliberately did not sample the littoral zone of the lakes to avoid the potential effect of macrophytes, which have been reported as important determinants of testate amoebae presence in some studies (Alves *et al.*, 2010; Neville *et al.*, 2010). Future analysis of littoral zone samples from Lacandón Forest lakes will almost certainly result in an increase in the number of taxa recorded and better knowledge of the ecology of testate amoebae in these tropical lakes. Acquisition of additional ecological information about testate amoebae in tropical regions will enable the use of these protists as bioindicators of modern water quality conditions, and as variables in paleolimnological studies for inference of past changes in water level, lacustrine productivity, oxygen concentration, conductivity and pH.

CONCLUSIONS

This study was the first to explore testate amoebae in lakes of the Lacandón Forest, Chiapas state, southern Mexico. Testate amoebae in Lacandón water bodies display different tolerances to low dissolved oxygen

concentrations in the water column. Some species are less tolerant of low DO concentrations, such as *C. constricta* strain “aerophila”, whereas *C. aculeata* strain “aculeata”

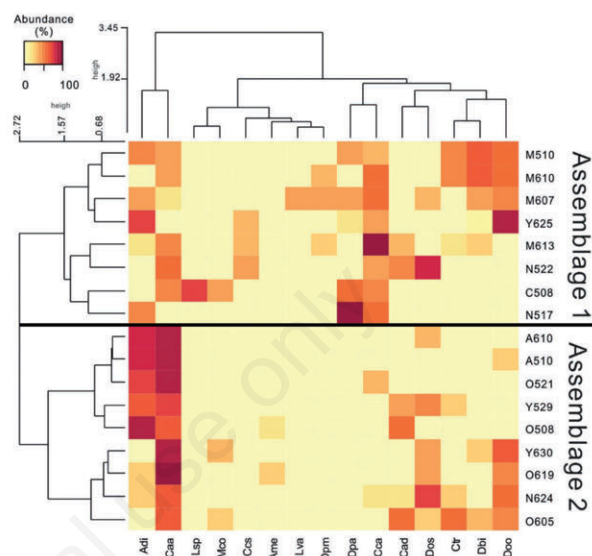


Fig. 4. Abundance of testate amoebae represented as a Q-mode and R mode cluster analyses. We included only statistically significant species and samples. According to the graph we differentiated two principal assemblages. Codes for samples are in Tab. 1 and codes for species are in Tab. 2.

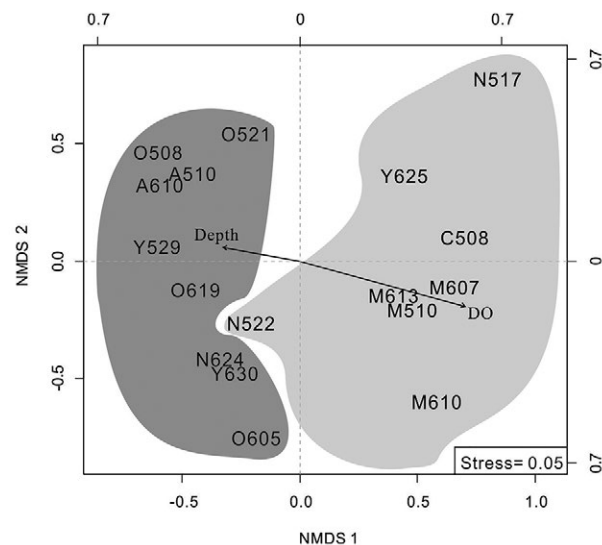


Fig. 5. Non-metric multidimensional scaling (NMDS), illustrating the relationship between distribution of testate amoebae assemblages identified in the Q-R plot (Fig. 4). Assemblage 1 in light gray; Assemblage 2 in dark gray. DO, dissolved oxygen concentration.

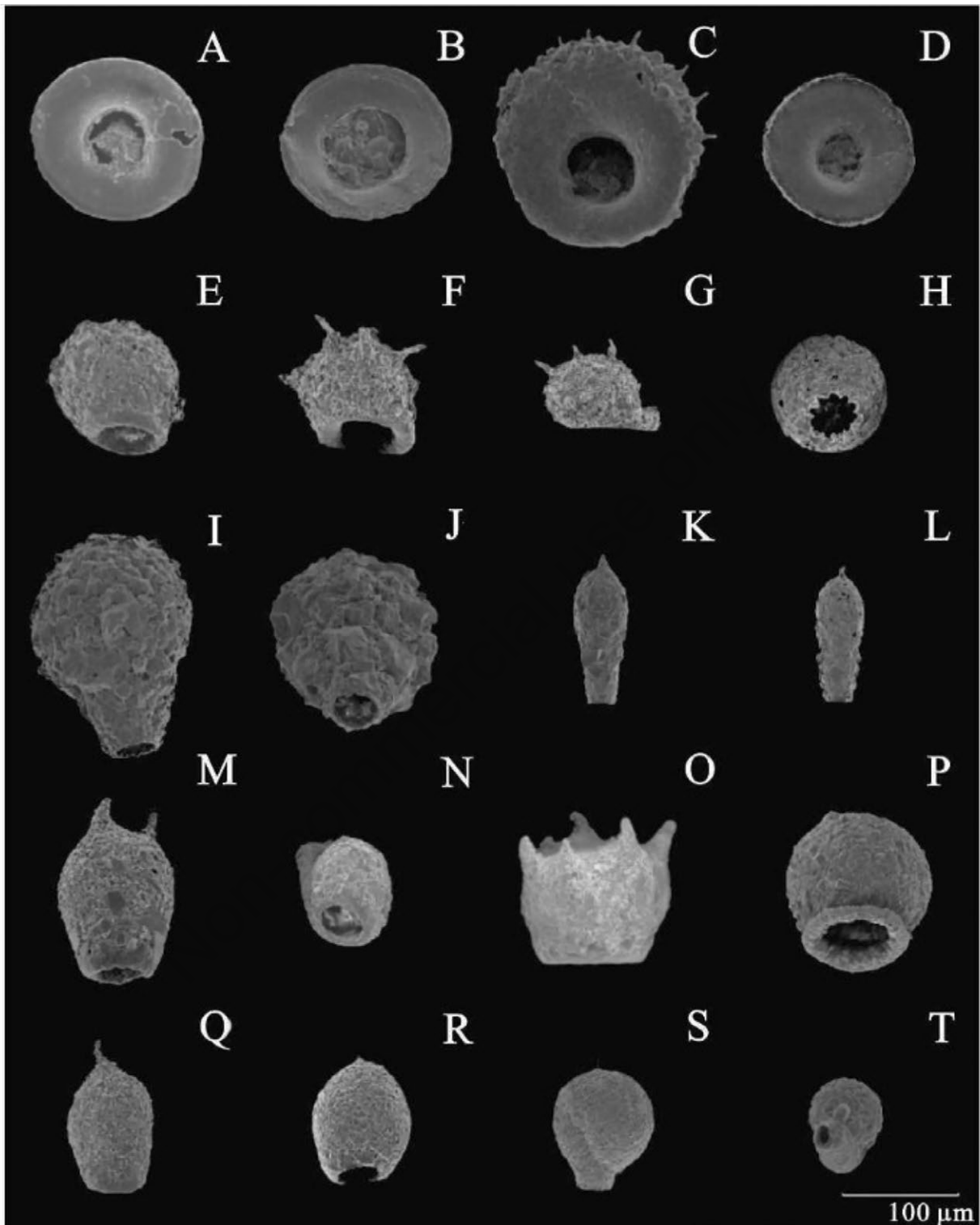


Fig. 6. Testate amoebae from Lacandón Forest lakes (A) ventral view *Arcella discoides*, (B) ventral view *Arcella megastoma*, (C) ventral view *Centropyxis aculeata* strain “aculeata”, (D) ventral view *Centropyxis aculeata* strain “discoides”, (E) ventral view *Centropyxis constricta* strain “aerophila”, (F) ventral view *Centropyxis constricta* strain “constricta”, (G) lateral view, (H) ventral view *Cucurbitella tricuspis*; (I) lateral view *Diffugia oblonga* strain “oblonga”, (J) ventral view, (K) lateral view *Diffugia oblonga* strain “spinosa”, (L) lateral view *Diffugia protaeiformis* strain “acuminata”, (M) lateral view *Diffugia bidens*, (N) ventral view *Diffugia globula*, (O) lateral view *Mediolus corona*, (P) ventral view *Diffugia urceolata* strain “urceolata”; (Q) lateral view *Diffugia protaeiformis* strain “amphoralis”, (R) ventral view, (S) lateral view *Lesquereusia spiralis*, (T) ventral view.

and *A. discoides* are dominant in low-DO conditions. At oxygen concentration values $<2 \text{ mg L}^{-1}$, SDI of testate amoebae assemblages is low and most species display low relative abundance. Testate amoebae assemblages are also influenced by water depth, which is correlated with dissolved oxygen concentration in these thermally stratified lakes. Our preliminary results indicate that dissolved oxygen concentration and water depth are two important variables that influence the composition of testate rhizopod assemblages in Lacandón Forest lakes, making these protists potentially useful bioindicators for studies of modern and paleoecology.

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