

Relationships between testate amoeba communities and water quality in Lake Donghu, a large alkaline lake in Wuhan, China

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Abstract The middle Yangtze Reach is one of the most developed regions of China. As a result, most lakes in this area have suffered from eutrophication and serious environmental pollution during recent decades. The aquatic biodiversity in the lakes of the area is thus currently under significant threat from continuous human activities. Testate amoebae (TA) are benthic (rarely planktonic) microorganisms characterized by an agglutinated or autogenous shell. Owing to their high abundance, preservation potential in lacustrine sediments, and distinct response to environmental stress, they are increasingly used as indicators for monitoring water quality and reconstructing palaeoenvironmental changes. However this approach has not yet been developed in China. This study presents an initial assessment of benthic TA assemblages in eight lakes of Lake Donghu in the region of Wuhan, China. Testate amoeba community structure was most strongly correlated to water pH. In more alkaline conditions, communities were dominated by *Centropyxis aculeata*, *Diffugia oblonga*, *Pontigulasia compressa*, *Pon. elisa* and *Lesquereusia modesta*. These results are consistent with previous studies and show that TA could be useful for reconstructing past water pH fluctuations in China. To achieve this, the next step will be to expand the database and build transfer function models.

Keywords testate amoebae (TA), water quality, water pH, Lake Donghu, China

1 Introduction

The middle Yangtze Reach is known as the country of a thousand lakes and is one of a few (17) regions recognized both as a Global Biodiversity Hotspot and a Global 200 Priority Ecoregion in China (Olson and Dinerstein, 1998). However, it is also one of the most developed regions of China. With the development of extensive agriculture, fishing, farming and urbanization during recent decades, most lakes in this area have suffered from eutrophication and serious environmental pollution (Xie et al., 2000; Jiang et al., 2007; Qin et al., 2009; Gu et al., 2012). Large amounts of wastewater containing different kinds of toxic chemicals are continuously released into the lakes. This has had serious detrimental impact on aquatic ecosystems, and resulted in a decrease of biodiversity in lakes of the region during the last few decades (Xie et al., 2000; Jiang et al., 2007; Wang et al., 2010; Hu et al., 2012). The aquatic biodiversity in the lakes of the area is thus currently under significant threat from eutrophication and environmental pollution and there is an urgent need for limnological data to aid future long-term watershed management and planning (Gu et al., 2008, 2012).

Assessment of ecosystem responses to environmental stresses is a new branch of biomonitoring in China (Xu et al., 2005; Jiang et al., 2007). An increasing body of evidence suggests that microorganisms respond faster to environmental pollution (Xu et al., 2005; Wang et al., 2010) and environmental changes (Xu et al., 2005; Qin et al., 2009) than larger organisms such as animals or plants growing in the same region because of their rapid population growth rates. Among potential microbial bioindicators in freshwater systems, testate amoebae have

been repeatedly shown to be good indicators of eutrophication and acidification of lake waters and sediments, caused by human activities, including mining and tourism (Scott and Medioli, 1983; Medioli and Scott, 1988; Reinhardt et al., 1998; Patterson et al., 2002; Escobar et al., 2008; Qin et al., 2009; Roe et al., 2010).

Testate amoebae (TA) are benthic (rarely planktonic) microorganisms characterized by an agglutinated or autogenous generally vase-shaped shell (Scott and Medioli, 1983; Medioli and Scott, 1988; Bobrov et al., 1999, 2012; Mitchell et al., 2008). Previous studies have examined and modeled the relationship between TA diversity, community structure and environmental conditions (especially water chemistry) in various lakes of the world. Owing to their high abundance, preservation potential in lacustrine sediments, and distinct response to environmental stress, they are increasingly recognized as good indicators for monitoring water quality and reconstructing palaeoenvironmental changes (Scott and Medioli, 1983; Medioli and Scott, 1988; Reinhardt et al., 1998; Bobrov et al., 1999, 2012; Patterson et al., 2002; Escobar et al., 2008; Qin et al., 2009, 2011, 2012; Roe et al., 2010).

This paper presents an initial assessment of benthic TA assemblages in eight small lakes of Lake Donghu, Wuhan, China. Our main aim is to evaluate the relationships between TA diversity and community structure and hydrochemical variables in the context of alkaline lakes ecosystems and assess the potential use of TA analysis as a tool for monitoring the present and past trophic state in these lakes.

2 Materials and methods

2.1 Study site

Lake Donghu (also named East Lake, 30°33'N, 114°23'E) is a large, shallow alkaline lake, located about 5 km away from the Yangtze River, in the north-east part of Wuhan City, the capital of Hubei Province (Jiang et al., 2007; Gu et al., 2008; Wang et al., 2010). The current lake surface area is 33.6 km² and its maximum depth is 4.75 m (Fig. 1).

As one of the largest urban lakes in China, Lake Donghu is extremely and increasingly polluted by industrial products, pesticides, and other chemicals from agriculture and sewage beings drained into the aquatic ecosystems resulting from the increasing population in the region and the associated expansion of agricultural, fisheries, and industrial activities (Xie et al., 2000; Gu et al., 2008). Pollutants including heavy metals (e.g., Pb, Hg, Cr, Cd, As, Cu) from coal dust, industry sewage with electroplate and press, and tail gas (Liu et al., 2006), higher concentrations of nitrogen and phosphorus (Meng and Zhao, 2008), organic pollutants like alkylbenzene, alkylphenol, isophorone and polycyclic aromatic hydrocarbon (PAH), and pesticide related pollution like pyrethrin (Hu et

al., 2012). The watershed pollutants have become a great environmental concern with their toxicity, persistence, bioaccumulation, and biomagnification in the food chain (Liu et al., 2006; Wang et al., 2010).

Human related eutrophication, watershed pollution and hydrological modification have thus become a serious and recognized environmental problem in many lakes in the Middle Yangtze reach of China, including the Lake Donghu. In recent years increasing human pressure has caused a decrease of biodiversity, changes of vegetation, blooms of cyanobacteria (Xie et al., 2000; Gu et al., 2008), and even “red tide” caused by dinoflagellate and saprophytic bacterium released from an ocean museum near Lake Donghu (Qin et al., 2008b). Like many other lakes in this region, Lake Donghu was formerly connected to the Yangtze River, but due to the construction of dams and dikes between the later Qing Dynasty (1900s) and the 1950s, this connection was lost and it became an isolated controlled urban lake (Xie et al., 2000; Gu et al., 2008). Then, in the late 1960s, the lake was divided into several parts by artificial dikes (Xie et al., 2000; Liu et al., 2006). We selected eight of these lakes as study sites (Fig. 1). Depending on the size of the lakes, two to three surface sedimentary samples (upper 3 cm) were collected from each lake in September 2009, and pooled into a composite sample representing the lake, resulting in a total of eight samples.

2.2 Water chemistry

Environmental variables including water depth, pH, conductivity, total nitrogen (TN), total phosphorus (TP), chlorophyll-*a*, and water transparency (expressed as Secchi depth, Zsd) were obtained from a former study of Lake Donghu (Wang et al., 2010, Table 1). The water chemical data used in this study were the averaged values of four seasons in 2008 (Wang et al., 2010).

2.3 Testate amoeba analysis

Samples of known weight (about 1g) were sieved through 300 μm and 35 μm meshes. Testate amoebae were collected from the 35–300 μm fraction, and counted under light microscope at 200–400X magnification (Scott and Medioli, 1983; Patterson et al., 2002). Ecological and palaeoecological studies of TA usually aim to reach at least 150 specimens per sample (Payne and Mitchell, 2009), however, in some lake sediments, this number can be hard to reach within reasonable time. In this study, the total count in most samples reach 70 to 100 shells, excluding two lakes for which 44 shells were counted. Species identification and taxonomy follows, Ogden and Hedley (1980), Ogden (1983), Mazei and Tsyganov (2006), Meisterfeld (2002) using morphology, composition, size and color of shells to distinguish among taxa. However benthic TA are dominated by agglutinated

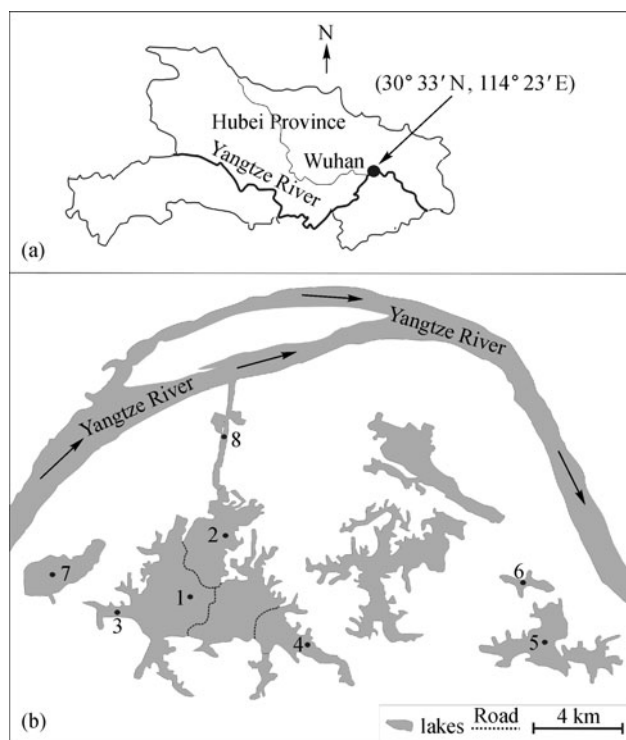


Fig. 1 Location of the eight studied lakes of Lake Donghu. (a) Location of Wuhan City near the middle Yangtze Reach of Central China, (b) Eight studied lakes in the Lake Donghu of Wuhan, see Table 1 for name of lakes

Table 1 Environmental variables measured in Lake Donghu (Wuhan, China) (From Wang et al., 2010)

Sample No	Name of lake	Depth/m	pH	Cond/($\mu\text{S}\cdot\text{cm}^{-1}$)	TN/($\text{mg}\cdot\text{L}^{-1}$)	TP/($\text{mg}\cdot\text{L}^{-1}$)	Chlorophyll- <i>a</i> /($\mu\text{g}\cdot\text{L}^{-1}$)	Zsd/cm
1	Guozhenghu (GZ)	3.7	9.1	411	0.225	0.133	25.70	60
2	Tanglinhu (TL)	3.1	9.0	404	0.229	0.140	11.80	60
3	Shuiguohu (SG)	2.5	8.7	393	0.693	0.188	21.14	55
4	Houhu (HH)	3.6	8.8	368	0.382	0.086	18.91	75
5	Yandonghu (YD)	1.6	8.6	410	0.246	0.017	3.18	162
6	Qingtianhu (QT)	1.4	8.5	401	0.203	0.036	17.56	65
7	Shahu (SH)	0.5	8.0	559	1.465	0.311	6.06	33
8	Qingshangang (QSG)	2.0	8.1	453	0.626	0.098	11.18	60

taxa of genera *Diffugia* and *Centropyxis* for which the taxonomy is confusing and debated. We therefore used a pragmatic and conservative morpho-type approach in this study and we do not claim to reach the finest level of taxonomic identification, for example, *D. globulosa*-type may include other similar taxa such as *D. gramen* (illustrated in Fig. 2).

2.4 Numerical analyses

The testate amoeba data consisted in a site per species

matrix of relative abundance (absolute counts). These data were Hellinger transformed (Legendre and Gallagher, 2001) prior to analyses, and environmental variables were scaled. Correlations between water chemical variables were computed in SPSS version 15.0.

In addition to species richness, we calculated the Shannon–Wiener diversity index (SDI), as follow:

$$S.I. = - \sum_{i=1}^S \left(\frac{X_i}{N_i} \right) \times \ln \left(\frac{X_i}{N_i} \right), \quad (1)$$

where X_i is the abundance of each taxon in a sample, N_i is the total abundance of the sample, and S is equal to the species richness of the sample (Shannon and Weaver, 1949). The SDI values usually fall between 1.5 and 3.5 (Margalef, 1972). To assess the relationship between environment and TA diversity and species richness, we used the diversity and species richness values as response variables in a linear model including the environmental variables as predictors. Each model was tested using ANOVA.

To quantify the relationship between the measured environmental variables and testate amoeba community structure, we computed a redundancy analysis (RDA), using the R software for statistical analyses (R Development core team, 2010).

3 Results

3.1 Testate amoeba community structure and diversity

Testate amoebae occurred in all samples, although their abundance and diversity differed. A total of 23 morpho-taxa belonging to six genera were identified in the eight lakes of Lake Donghu (Fig. 2, Table 2). The dominant genus was *Diffflugia* (15 morpho-taxa, 65%). Other represented genera were *Centropyxis* (3 morpho-taxa, 13%), *Pontigulasia* (2 morpho-taxa, 9%), *Cyclopyxis*, *Phryganella*, and *Lesquereusia* (each 1 morpho-taxon, 4.3%). The most abundant morpho-taxa, *Centropyxis aculeata*, *C. ecornis*, *C. cassis*, *Diffflugia acuminata*, *D. oblonga*, *Pontigulasia compressa*, and *Pon. elisa* occurred

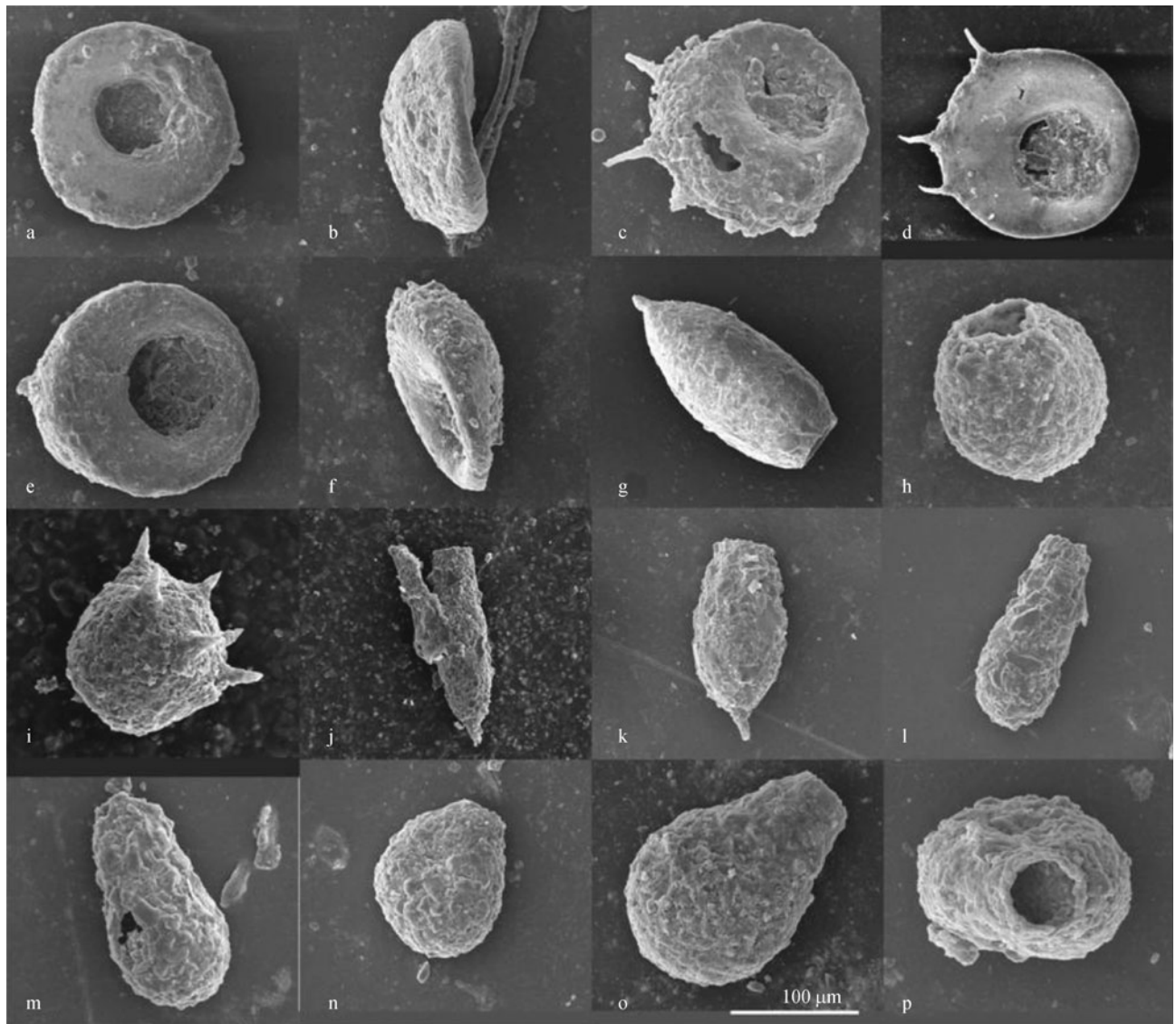


Fig. 2 SEM photos of testate amoebae from sediments of Lake Donghu, Wuhan, China. a–b) *Cyclopyxis* cf. *aplanata*, c–d) *Centropyxis aculeata*, e–f) *C. ecornis*, g) *Diffflugia accutissima*, h) *Diffflugia globulosa*-type, here *D. cf. gramen*, i) *D. corona*, j) *D. acuminata*, k) *D. elegans*, l) *D. lanceolata*, m) *D. oblonga*, n) *Lesquereusia modesta*, o–p) *Pontigulasia compressa*

Table 2 Relative abundance (percentage of total count) of testate amoeba morpho-taxa in eight lakes of Lake Donghu (Wuhan, China)

	GZ	TL	SG	HH	YD	QT	SH	QSG
<i>Cyclopyxis</i> cf. <i>aplanata</i>	0.00	0.00	8.33	1.65	6.42	0.00	15.38	0.00
<i>Centropyxis aculeata</i>	23.44	25.71	16.67	47.93	69.72	32.08	34.62	52.13
<i>C. ecornis</i>	7.03	7.14	8.33	5.79	2.75	13.21	9.62	2.13
<i>C. cassis</i>	3.91	7.14	41.67	10.74	7.34	0.00	19.23	23.40
<i>Diffugia acuminata</i>	5.47	2.86	6.25	6.61	6.42	0.00	0.00	0.00
<i>D. accutissima</i>	2.34	4.29	0.00	0.83	0.00	1.89	0.00	0.00
<i>D. corona</i>	0.00	0.00	2.08	0.00	0.00	0.00	0.00	2.13
<i>D. elegans</i>	0.00	1.43	0.00	0.00	0.00	0.00	0.00	0.00
<i>D. glans</i>	4.69	5.71	0.00	0.00	0.92	5.66	1.92	1.06
<i>D. globulosa</i>	0.00	4.29	4.17	0.00	0.00	0.00	7.69	18.09
<i>D. labiosa</i>	0.78	0.00	4.17	2.48	2.75	0.00	0.00	0.00
<i>D. lithophila</i>	3.91	1.43	0.00	0.00	0.00	7.55	1.92	0.00
<i>D. lanceolata</i>	1.56	2.86	0.00	0.00	0.00	5.66	1.92	0.00
<i>D. lacustris</i>	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.00
<i>D. oblonga</i>	25.78	11.43	4.17	16.53	1.83	18.87	7.69	0.00
<i>D. lobostoma</i>	0.00	0.00	0.00	0.00	0.92	1.89	0.00	0.00
<i>D. longum</i>	0.00	0.00	0.00	0.00	0.00	1.89	0.00	0.00
<i>D. smilion</i>	2.34	1.43	4.17	0.83	0.92	3.77	0.00	0.00
<i>D. urceolata</i>	2.34	2.86	0.00	0.00	0.00	0.00	0.00	0.00
<i>Phryganella nidulus</i>	2.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Pontigulasia elisa</i>	8.59	11.43	0.00	2.48	0.00	1.89	0.00	0.00
<i>Pon. compressa</i>	5.47	1.43	0.00	2.48	0.00	3.77	0.00	0.00
<i>Lesquereusia modesta</i>	0.00	8.57	0.00	0.83	0.00	1.89	0.00	1.06
SDI	2.26	2.41	1.86	1.74	1.20	2.09	1.82	1.25
Richness value	16	17	11	14	10	14	10	8

preferentially in alkaline pH conditions.

3.2 Water chemistry in Lake Donghu

Most water chemic variables varied considerably among lakes (Table 1, Wang et al., 2010). Water pH was overall alkaline and ranged from 8.0 to 9.1. Correlations between water chemical variables are shown in Table 3. Total P, TN, conductivity, and pH were significantly correlated to each other ($p < 0.05$). Water pH was positively correlated with water depth, and negatively with TN, conductivity and TP. In addition, TN was correlated with TP and conductivity (Table 3).

3.3 Relationships between testate amoeba communities and environmental variables

The SDI value ranged from 1.20 (YD lake) to 2.41 (TL lake) (Table 2). To assess the relationship between testate amoeba diversity and environmental conditions, several linear models were developed between species richness,

diversity, and environmental variables. No linear model was significant for SDI. The best model with species richness was obtained using pH alone ($p = 0.007$, adjusted- $R^2 = 0.69$, Fig. 3).

The RDA ordination showed that the first two axes explained 65.7% of environmental information, with axis 1 explaining 47.9% (Table 4, Fig. 4). This suggests that the measured environmental variables include the main hydrochemical factors to which TA respond. However, although pH explained 26% of variance of TA data, it remained only marginally significant ($p = 0.09$). Moreover, none of the environmental variables had a significant relationship with TA communities. A possible reason for this may be the limited number of samples. In the RDA ordination map, species distributions differ greatly along the pH gradient. For example, most *Centropyxis* species reach highest abundance in relative low pH conditions, while other species such as *Pontigulasia elisa*, *Pon. compressa* and *Lesquereusia modesta* are more abundant in higher pH conditions (Fig. 4).

Table 3 Correlations between water chemical variables measured in Lake Donghu (Wuhan, China)

	Depth	pH	Cond	TN	TP
pH	0.842**				
Cond	-0.714*	-0.751*			
TN	-0.600	-0.728*	0.863**		
TP	-0.252	-0.315	0.719*	0.856**	
Chlorophyll- <i>a</i>	0.687	0.589	-0.530	-0.352	-0.047
Zsd	-0.029	0.172	-0.373	-0.490	-0.696

Notes: ** Correlation is significant at the 0.01 level, * correlation is significant at the 0.05 level (2-tailed)

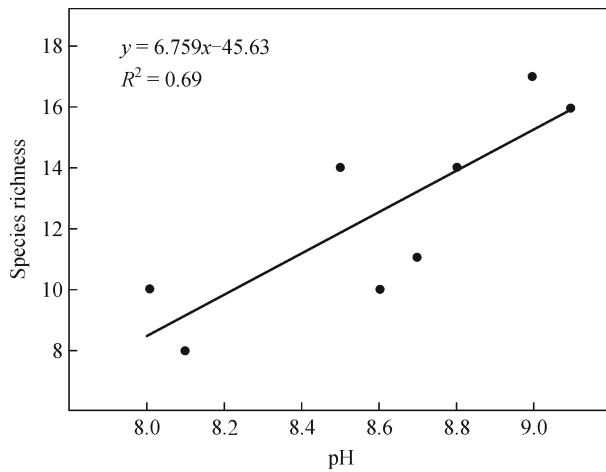


Fig. 3 Scatter plot of pH versus testate amoeba species richness in eight lakes of Lake Donghu (Wuhan, China). The solid line represents a linear regression function (adjusted- $R^2 = 0.69$)

4 Discussion

In this study, the clearest relationship we observed was between species richness and pH (Fig. 3). By contrast, no significant correlation was found for SDI. The SDI of TA in Lake Donghu was relatively high by comparison with other studies. For example, in Lake Donghu, the SDI values ranged from 1.20 (Yandonghu) to 2.41 (Tanglinhu) (Table 3), but from 0.65 to 1.44 in Lake Sentani, Indonesia (Dalby et al., 2000), from 0 to 1.4 in ponds in Barbados (Roe and Patterson, (2006), and from 0.37 to 2.37 in Florida lakes (Escobar et al., 2008). In this study, lakes with the highest SDI value (2.41) occurred at high pH values (8–9) and mesotrophic to eutrophic conditions (TP 0.14 mg/L, TN 0.23 mg/L, Table 1). Although there were no strictly acid lakes in this study, our results indicate that the SDI of TA is generally higher in more alkaline waters. These results are consistent with other studies in James Lake (Kumar and Patterson, 2000), Florida lakes (Escobar et al., 2008), and some lakes in Canada (Roe et al., 2010).

The RDA showed that TA communities were most strongly correlated with pH. This is consistent with the results of Escobar et al., (2008) from Florida lakes, where pH explained the highest fraction of the community data in

a canonical correspondence analysis (12.97%, $p < 0.005$). However, investigations on lacustrine testate amoebae in some lakes suggested that TA also respond strongly to the phosphorus concentration of sediments (Roe et al., 2010). Total P concentrations in sediments are much higher than that in water and this difference may explain the stronger correlation with TA communities. As most TA are benthic microorganisms, they may be more sensitive to micro-environmental conditions in the sediments than to the chemistry of the water column (Wall et al., 2010). However there is also evidence for a strong influence of water-column conditions on TA communities (Escobar et al., 2008). Clearly further studies, ideally combining analyses of both water and sediments, will be needed to clarify the factors controlling the diversity and community structure of benthic lake TA.

Species of genus *Centropyxis* are known to tolerate hostile conditions (Scott and Medioli, 1983; Patterson and Kumar, 2000; Roe et al., 2010). Previous studies have shown that *Centropyxis aculeata* can tolerate both eutrophic (Escobar et al., 2008) and oligotrophic environments. In this study, *C. aculeata* was observed in each lake but reached highest abundance both in an oligotrophic lake (YD, 74% with TP 0.017 mg/L) and hypereutrophic lake (QSG, 52% with TP 0.098 mg/L) (Tables 1–2, Fig. 4). These results and previous associated studies (Patterson and Kumar, 2000; Escobar et al., 2008; Roe et al., 2010) suggest that *C. aculeata* has a wide tolerance to phosphorous. Alternatively these results may be explained by the existence of several morphologically similar cryptic taxa but with different ecological optima within the *C. aculeata* species complex. Interestingly, the pH of the two lakes with highest relative abundance of *C. aculeata* (YD and QSG) was lower than 8.6 (Tables 1–2). This is in agreement with results from ecological studies in Florida lakes, where Escobar et al. (2008) reported highest abundance (56%–93%) of *C. aculeata* in Panasoffkee Lake with pH 8.3. In addition, investigations in James Lake of Canada (Kumar and Patterson, 2000) indicated that the abundance of Centropixids increased with increasing pH.

Furthermore, the highest abundance of *Pontigulasia elisa*, *Pon. compressa* and *Lesquereusia modesta* was recorded in the most alkaline lakes suggesting that these

Table 4 Redundancy analyses (RDA) results showing the percentage variance in the testate amoeba data set from in eight lakes of Lake Donghu (Wuhan, China) explained by the measured environmental variables (separate analysis for each variable)

Individual RDA	Explained variance/%	<i>p</i>
Depth	17	0.31
pH	26	0.09
Cond.	15	0.42
TN	23	0.10
TP	14	0.36
Chlorophyll- <i>a</i>	17	0.30
Water transparency	12	0.66

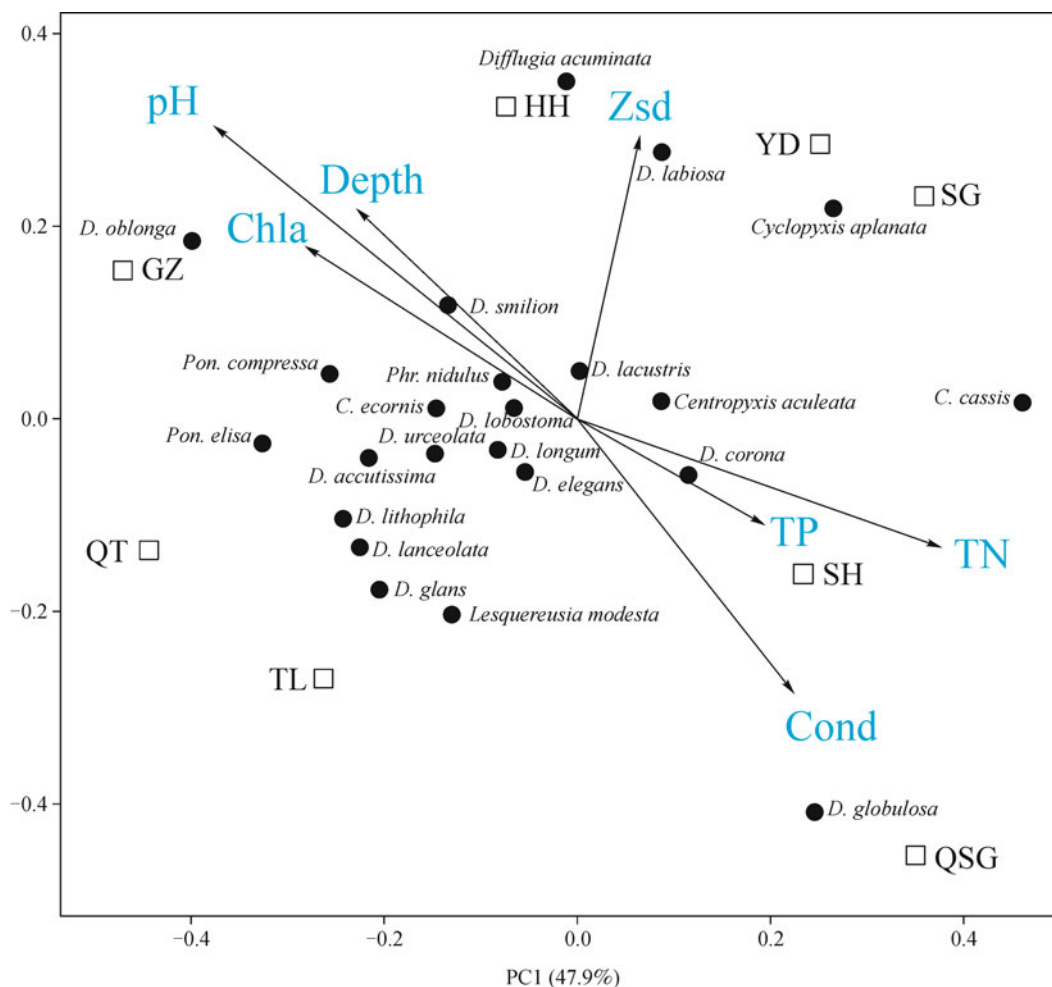


Fig. 4 RDA ordination showing the relationship between testate amoeba community structure in eight lakes of Lake Donghu (Wuhan, China) and the fitted environmental variables (circles are species, squares are eight small lakes of Lake Donghu)

three taxa could be indicators of higher pH. In addition, other species also show some trends response to the pH gradient in the Lake Donghu (Fig. 4). For example, the conditions of GZ and TL are quite similar, except the Chlorophyll-*a* in GZ (25.7 $\mu\text{g/L}$) is higher than TL (11.8 $\mu\text{g/L}$). Chlorophyll-*a* is generally interpreted as a measure of primary productivity in lakes (Kowalewska, 2005). The

relative abundance of *Diffflugia oblonga* was twice higher in GZ than in TL (Table 2), which suggests that *D. oblonga* might be a potential indicator for higher productivity. Previous studies also showed that *D. oblonga* tends to be more abundant in eutrophic environments (Reinhardt et al., 1998; Patterson and Kumar, 2000). *D. oblonga* was previously reported as abundant in organic-rich sediments

(Reinhardt et al., 1998; Patterson and Kumar, 2000).

Most studies reported significant correlations between TA community structure and environmental variables such as TOC, pH, total alkalinity, TP, TN, oxygen, mineral contents, and urban pollutions. However, TA responses to environmental variables are likely to be complex. For example, both YD Lake (present work) and Panasoffkee Lake (Escobar et al., 2008) are mesotrophic lakes, with TP 17 µg/L (Table 1) and 28 µg/L, respectively, but the TA communities in the two lakes are very different.

5 Conclusions

Our results provide initial data on the ecology of testate amoebae in alkaline conditions of a large urban lake in China. The results of this study suggest that pH is the main driver of testate amoeba community structure in Lake Donghu. Species with high abundance occurred in more alkaline conditions including *Centropyxis aculeata*, *Difflugia oblonga*, *Pontigulasia compressa*, *Pon. elisa* and *Lesquereusia modesta*. Such results are consistent with other testate amoeba studies in lakes and ponds elsewhere. TA may therefore be useful for environmental monitoring and ecological and palaeoecological studies in which pH reconstruction is required. Our studies suggested that testate amoebae could be used more widely as bio-indicators of water quality. However, more descriptive and experimental work is needed to better understand how TA respond to pH and other hydrochemical variables as well as sediment characteristics and to build transfer function models that can be used for biomonitoring and palaeoecology in the region.

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Author contributions: YQ designed this work, counted and identified the species under microscopy, with the help of EM and EL, and wrote the paper. BF conducted most statistical analyses. YQ, BF, EM and EL interpreted the data contributing to writing. YC and XZ helped with fieldwork, Prof. YG and HW oversaw the project and provided comments on the manuscript, YQ wrote the paper with input from all other authors.

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