



Testate amoebae (Protozoa) in lakes of the Qinghai-Tibet Plateau: Biodiversity, community structures, and protozoic biosilicification in relation to environmental properties and climate warming

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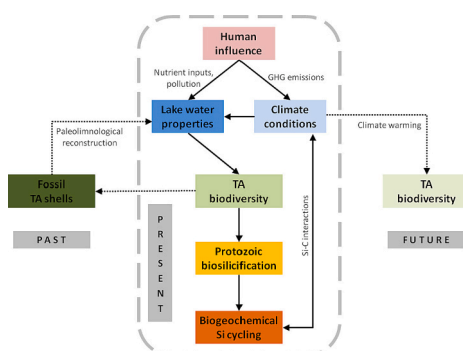
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HIGHLIGHTS

- Sediment sampling in the Qinghai-Tibet Plateau (QTP), the “Asian Water Tower”
- Testate amoebae (TA) as bio-indicators of environmental properties were analyzed.
- In total we found 102 TA taxa including some rare and one unknown TA species.
- TA community composition was significantly correlated with water temperature and pH.
- Increasing protozoic biosilicification in the near future due to climate warming.

GRAPHICAL ABSTRACT



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ABSTRACT

The Qinghai-Tibet Plateau (QTP) is characterized by a vast number of frozen and unfrozen freshwater reservoirs, which is why it is also called “the third pole” of the Earth or “Asian Water Tower”. We analyzed testate amoeba (TA) biodiversity and corresponding protozoic biosilicification in lake sediments of the QTP in relation to environmental properties (freshwater conditions, elevation, and climate). As TA are known as excellent bio-indicators, our results allowed us to derive conclusions about the influence of climate warming on TA communities and microbial biogeochemical silicon (Si) cycling. We found a total of 113 TA taxa including some rare and one unknown species in the analyzed lake sediments of the QTP highlighting the potential of this remote region for TA biodiversity. >1/3 of the identified TA taxa were relatively small (<30 μm) reflecting the relatively

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harsh environmental conditions in the examined lakes. TA communities were strongly affected by physico-chemical properties of the lakes, especially water temperature and pH, but also elevation and climate conditions (temperature, precipitation). Our study reveals climate-related changes in TA biodiversity with consequences for protozoic biosilicification. As the warming trend in the QTP is two to three times faster compared to the global average, our results provide not only deeper insights into the relations between TA biodiversity and environmental properties, but also predictions of future developments in other regions of the world. Moreover, our results provide fundamental data for paleolimnological reconstructions. Thus, examining the QTP is helpful to understand microbial biogeochemical Si cycling in the past, present, and future.

1. Introduction

The Qinghai-Tibet Plateau (QTP) is known as “Asian Water Tower” or the Earth’s “third pole” due to huge amounts of water stored in wetlands, lakes, rivers, glaciates, and the underground in this region (Lutz et al., 2014; Qiu, 2008). Nearly one-third of the lake water in China is stored in about 1500 lakes in the QTP (Wang and Dou, 1998). Around 1200 of these lakes are larger than 1 km² with a total area of 46,000 km² (Zhang et al., 2021). The wide distribution of lakes in the QTP is important for the water security, maintenance of biodiversity, and ecological stability in this region (Wu et al., 2021). However, water resources (including glaciers, rivers, and lakes) and hydrological processes have been affected dramatically by global warming (degradation, eutrophication; Wu et al., 2021), as the warming trend in the QTP is two to three times faster compared to the global average (Li et al., 2014). Nevertheless, the lakes in the QTP are still comparably undisturbed (Mao et al., 2018), making the QTP an ideal model region for observations of ecosystems under the influence of global change.

Microbial organisms (e.g., diatoms, testate amoebae, and ostracods) are abundant and sensitive to environmental changes in many ecosystems, especially in lakes. Testate amoebae (TA) are a group of microorganisms (Protozoa) characterized by an agglutinated or autogenous shell, which can be found in terrestrial and aquatic ecosystems worldwide (e.g., Bobrov and Wetterich, 2012; Booth, 2002; Ehrmann et al., 2012). Their rich diversity and functional traits, high abundance, rapid responses to environmental change, and cosmopolitan distribution have made TA widely used as bio-indicators in various habitats (Mitchell et al., 2008; Qin et al., 2011). The significance of TA for nutrient (Schröter et al., 2003) and silicon (Si) cycling (Puppe, 2020) in terrestrial ecosystems has been emphasized since the beginning of the 21st century. Annual protozoic biosilicification, i.e., the incorporation of inorganic Si into TA shells per year, by TA that synthesize idiosomes (the single building blocks of siliceous TA shells) has been found to be comparable or even exceed the annual biosilicification by trees in forest ecosystems (Puppe et al., 2015). Most recently Qin et al. (2022) showed that protozoic biosilicification in Asian peatlands is strongly affected by humans. They found protozoic biosilicification to increase with an increasing grade of human impact, while TA biodiversity decreased. From their results Qin et al. (2022) concluded that changes in TA community structures were responsible for their observations.

However, there is no information on interactions between TA biodiversity and protozoic biosilicification in aquatic ecosystems until now. In general, TA are highly abundant in freshwater lakes, brackish waters, lagoons, and other waterbodies (Escobar et al., 2008; Roe et al., 2010; Patterson and Kumar, 2000; Patterson et al., 2012), even in stressed environments like acidified and contaminated lakes (Kauppila et al., 2006; Kihlman and Kauppila, 2009; Patterson et al. Payne, 2013; Roe and Patterson, 2014; Qin et al., 2013, 2016). Rapid response to environmental changes and well-preserved TA shells in lacustrine sediments proved TA to represent well-suited proxies for the reconstruction of environmental conditions and changes in the past (Patterson et al., 2002; Payne, 2013; Macumber et al., 2020; Nasser et al., 2020; Qin et al., 2021a, 2021b).

TA in the QTP have been subject to investigations since the 1970s, whereby nearly 200 TA species were found and described in

corresponding articles (e.g., Wang, 1974, 1977; Shen, 1983). However, these studies mainly focused on TA taxonomy and most sampling sites were in the near of human settlements (Wang, 1977). No studies on TA biodiversity and community structures in the lakes of the QTP and corresponding relations to environmental properties and climate warming have been carried out so far.

We hypothesized the relatively undisturbed lakes in the QTP to represent hot spots of TA biodiversity and corresponding protozoic biosilicification. To test our hypothesis, we analyzed a total of 74 sediment samples of lakes in the QTP for TA biodiversity, community structures, and protozoic biosilicification. The corresponding results were linked to lake water properties (water temperature, pH, conductivity, and depth), geographic positions (longitude, latitude), site elevation, and climate conditions (temperature, precipitation). Our results will be helpful (i) to better understand the interactions between TA biodiversity, TA community structures, and environmental properties in lake sediments, (ii) to provide modern evidence for paleoenvironmental reconstructions based on fossil TA records in the future, (iii) to assess the biosilicification potential of TA in aquatic ecosystems, and (iv) to derive predictions for future directions of TA communities and protozoic Si cycling in lake sediments under climate warming.

2. Material and methods

2.1. Study area and sampling

Sampling sites covered most regions of the QTP with some sites located near or in the margin of the Yunnan-Guizhou plateau (Fig. 1A). While some lakes were characterized by relatively large areas (e.g., Erhai Lake), other sampling spots were in quite small and unnamed lakes in the near of cirques and/or streams (Table 1). In total we analyzed 74 sediment samples, which were originally taken in the summers of 2002 (northeastern QTP), 2003 (southeastern QTP), and 2004 (central QTP) for pollen analyses (for details see Herzschuh (2006) and Herzschuh et al. (2010)). Most of the sampling sites were in the middle to east plateau, which is characterized by a large number of wetlands and rivers. Lakes with an elevation lower than 4000 m are more located in the eastern and northern to middle regions of the plateau, while higher lakes are more distributed in the middle to western regions of the QTP. The climate is relatively cold and dry in the north and in the west, while the southern and eastern regions are relatively wet and warm (Table 1).

In the field 3–4 sediment samples were collected from each lake and water depth and water temperature were measured during sample collection (details can be found in Herzschuh (2006) and Herzschuh et al. (2010)). Depending on time, road and weather conditions, water pH and conductivity were measured either directly in the field or subsequently in the laboratory. Samples were stored in plastic bags or tubers and taken back to the laboratory, where they were stored at 4 °C until analyses.

In our study we used published (geographical positions, elevation, and climate characteristics) and previously unpublished (water pH, conductivity, depth, and temperature) data collected within the framework of the studies of Herzschuh (2006) and Herzschuh et al. (2010) and complemented it with the results of detailed TA analyses (see Section 2.2). Samples with low TA abundances (<25 individuals) were excluded

from statistical analyses (see Section 2.3) resulting in a data set comprising 32 sampling sites in the QTP (Table 1, Fig. 1B, C).

2.2. Testate amoeba analyses

About 1 g of each sediment sample was analyzed for TA. TA isolation followed the method described in Patterson and Kumar (2000). In brief, samples were washed through 500 μm and 15 μm sieves into Petri dishes to retain the TA shells. TA species identification and counting was performed using an Olympus BX-53 microscope (magnification 70–200 \times), which was also used to directly take micrographs of selected TA taxa (magnification 200 \times). All occurring TA shells in a sample were identified and enumerated until a quantity of 100 to 150 individuals was reached. Due to the age of the examined sediment samples (sampling took place in the summers of 2002, 2003, and 2004), a differentiation between full (living and encysted TA) and empty (dead TA) shells was not possible. TA species identification referred to Ogden (1983), Shen

(1983), Mazei and Tsyganov (2006), and Meisterfeld (2002). For a convenient readability of this article, TA taxon names without author citations were used only. However, a full list of all identified TA taxa including information on authorities and years of publication is provided in the Supplement (Supplementary Table 1).

The Shannon diversity index (H') was used to quantify TA diversity in each sample. We assumed H' values higher than 2.5 to characterize a relatively healthy environment, while H' values below 1.5 indicated a stressed environment (Patterson and Kumar, 2000). The H' value for each sample was calculated as follows:

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

where X_i is the abundance of each TA taxon in a sample, N_i is the total TA abundance in the same sample, and S is the species richness in this sample (Shannon and Weaver, 1949). Shannon's equitability index (E)

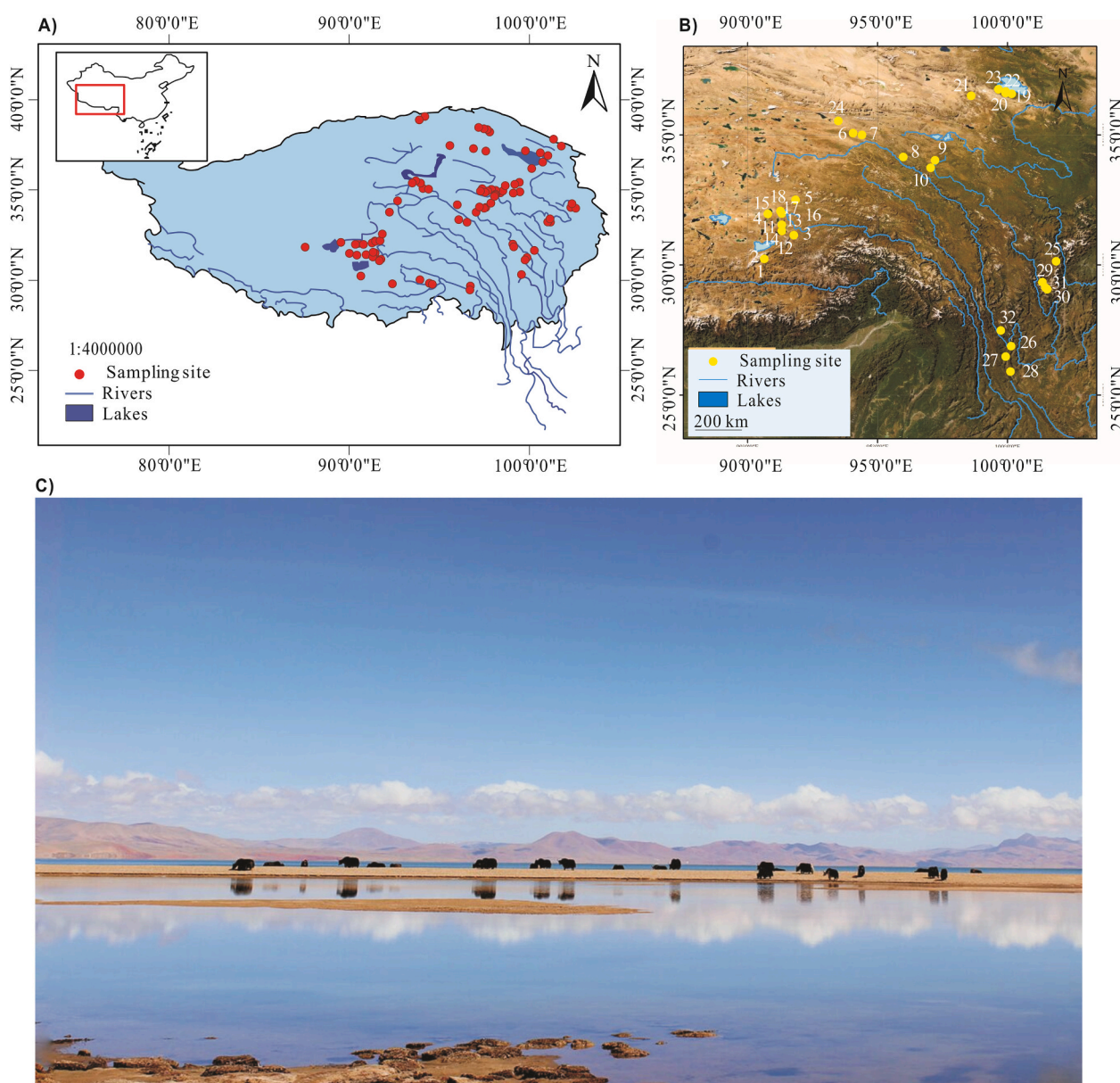


Fig. 1. Overview of the sampling sites in the Qinghai-Tibet Plateau (QTP). (A) Locations of all sampled sites in previous pollen studies (modified from Herzsuh, 2006 and Herzsuh et al., 2010), (B) the selected 32 sites used for statistical analyses in our study, and (C) exemplary view onto a lake in the QTP (lake Co Na, site nos. 15–18). The site numbers in (B) and (C) are identical to the ones used in Table 1.

Table 1
 Overview of geographical positions, elevation, water properties, and climate characteristics of the sampled lakes in the QTP. Additionally, the Shannon diversity index (H'), Shannon's equitability index (E), the mean size of TA, and protozoic biosilicification are stated for each lake. The site numbers (1–32) are identical to the ones in Fig. 1B. T = temperature, P = precipitation.

Site no.	Sample code	Site name/description	Latitude (°)	Longitude (°)	Elevation (m)	Water depth (m)	Conductivity (S/m)	pH	Water T (°C)	Jan. P (mm)	Jul. P (mm)	Annual P (mm)	Jan. T (°C)	Jul. T (°C)	Mean T (°C)	H'	E	Protozoic Si (ng Si per 150 shells)	Mean size (µm)
1	04-CTP-01	Unnamed lake	30.23	90.63	4607	0.3	0.4	9.9	11.5	8	210	857	-11.1	6.4	-1.2	0.0	0.06	0.0	54
2	04-CTP-03	Small lake	30.22	90.63	4607	0.5	0.3	10.3	13.6	8	210	857	-11.0	6.4	-1.2	1.4	0.10	0.0	61
3	04-CTP-05	Small lake	31.17	91.76	4650	NA	NA	NA	NA	11	203	890	-15.4	7.7	-2.2	0.0	0.06	0.0	110
4	04-CTP-12	Small lake	31.97	90.79	4646	0.6	0.8	10.3	17.3	7	150	653	-12.9	8.5	-0.7	0.0	0.06	0.0	15
5	04-CTP-23	Small lake	32.54	91.82	5002	0.4	0.2	9.1	6.2	11	165	792	-17.4	5.3	-5.2	0.0	0.06	0.0	48
6	04-CTP-32	stream	35.06	94.08	4540	0.1	14.1	8.6	18.4	8	103	568	-16.5	6.4	-4.0	1.6	0.10	0.0	19
7	04-CTP-34	Small lake	35.03	94.39	4367	0.5	0.6	8.0	11.2	10	105	594	-17.1	6.4	-4.3	1.7	0.11	0.0	83
8	04-CTP-35	Ice lake	34.16	95.98	4701	NA	NA	NA	NA	14	116	667	-19.7	5.4	-5.9	1.9	0.11	0.0	74
9	04-CTP-39	Small lake	34.04	97.22	4545	0.3	0.4	8.4	8.4	14	116	688	-20.9	6.1	-6.0	2.3	0.11	0.0	86
10	04-CTR-36	Unnamed lake	33.75	97.04	4552	0.3	0.2	9.5	14.9	15	136	780	-20.4	6.0	-5.7	2.2	0.11	0.0	95
11	CE-04	Co Er Lake	31.28	91.30	4562	NA	NA	NA	NA	9	172	763	-13.4	8.6	-0.8	0.0	0.06	0.0	133
12	CE-3	Co Er Lake	31.28	91.31	4562	7.4	6.9	9.5	14.1	9	172	763	-13.4	8.6	-0.8	2.5	0.12	0.0	112
13	CE-6	Co Er Lake	31.30	91.32	4562	7.0	9.1	9.6	9.6	9	172	763	-13.4	8.6	-0.8	0.7	0.08	0.0	100
14	CE-7	Co Er Lake	31.29	91.30	4562	7.5	8.5	9.6	8.6	9	172	763	-13.4	8.6	-0.8	1.4	0.10	0.0	55
15	CN-1	Co Na Lake	32.01	91.30	4800	19.1	0.6	9.7	7.4	8	165	769	-14.5	7.6	-1.9	0.0	0.06	0.0	32
16	CN-5	Co Na Lake	31.56	91.30	4800	5.5	0.6	9.8	7.7	9	173	821	-14.7	6.8	-2.4	1.0	0.09	0.0	131
17	CN-8	Co Na Lake	32.04	91.27	4800	18.3	0.6	9.5	8.7	8	165	769	-14.4	7.6	-1.9	2.4	0.12	4.3	63
18	CN-9	Co Na Lake	32.05	91.26	4800	5.1	0.6	9.3	6.9	8	155	727	-14.0	7.7	-1.8	1.8	0.11	5.4	103
19	KPW-10	Unnamed lake	36.68	99.92	3234	0.2	0.4	6.9	16.8	4	165	726	-11.5	13.2	2.4	1.9	0.11	0.0	67
20	KPW-15	Unnamed lake	36.63	100.10	3205	0.2	0.7	8.9	19.5	4	153	690	-11.4	12.6	2.0	0.0	0.06	0.0	48
21	KPW-2	Unnamed lake	36.52	98.61	3526	0.3	0.7	8.3	10.5	10	91	427	-13.7	11.9	0.9	1.8	0.11	0.0	101
22	KPW-DE	Unnamed lake	36.78	99.68	3382	NA	NA	NA	NA	4	163	705	-11.4	12.7	2.4	0.0	0.06	0.0	48
23	KPW-K	Unnamed lake	36.78	99.68	3382	0.3	0.6	8.6	17.9	4	163	705	-11.4	12.7	2.4	1.7	0.11	0.0	88
24	KX-20	Salt lake	35.52	93.47	4441	0.7	191.4	8.0	12.7	6	76	418	-16.6	6.7	-3.6	0.0	0.06	0.0	48
25	Set-1	Muge Co	30.15	101.86	3780	31.0	0.0	7.3	16.1	14	221	1372	-8.8	9.1	1.6	2.6	0.12	5.3	113
26	Set-13	Lashihai	26.88	100.14	2441	1.9	0.2	9.8	21.7	19	292	1392	4.7	16.6	11.2	2.3	0.12	7.4	107
27	Set-15	Jianhu	26.49	99.92	2189	3.2	0.2	8.6	26	19	251	1171	4.9	16.7	11.4	1.7	0.11	0.0	141
28	Set-16	Erhai	25.92	100.12	1967	5.0	0.2	9.0	27.7	25	248	1295	6.3	17.2	12.3	1.9	0.12	1.4	116
29	Set-26	Cirque lake	29.15	101.41	3705	29.8	0.0	7.7	16.5	23	233	1450	-9.4	8.3	1.3	3.1	0.12	5.9	127
30	Set-28	Cirque lake	29.14	101.44	4408	7.4	0.1	7.5	10.4	23	233	1450	-9.4	8.3	1.3	2.5	0.12	12.0	101
31	Set-32	Cirque lake	29.33	101.33	3603	0.4	0.1	7.0	15	19	232	1415	-12.9	5.6	-1.6	2.1	0.11	0.0	109
32	Set-9	Unnamed lake	27.50	99.74	3939	4.8	0.0	7.3	17	44	249	1315	-2.7	12.7	6.1	2.1	0.12	7.2	54

was calculated as a measure of the commonness or rarity of TA species by dividing H' by H'_{\max} . E can reach values between 0 and 1 with 1 indicating complete evenness.

TA size data were referred and collected from original literatures (Ogden, 1983; Meisterfeld, 2002; Bobrov and Wetterich, 2012). The mean size for each sample was determined by averaging the length/diameter of taxa presented in the sample.

Protozoic biosilicification by idiosomic TA was quantified as follows: (i) multiplication of total individual numbers (living plus dead individuals) with species-specific silica contents and (ii) conversion of protozoic silica quantities into protozoic Si quantities using a silica to Si conversion factor of 28/60 (SiO_2 , $M = 60.08 \text{ g mol}^{-1}$; Si, $M = 28.085 \text{ g mol}^{-1}$) (Puppe et al., 2014, 2018). Finally, protozoic biosilicification was calculated based on 150 TA shells per sample (nanogram (ng) Si per 150 shells).

While TA taxa with very low abundances were grouped according to similarities in morphology, ecological characters, and habitats (see Supplementary Table 1) before running an ordination analysis (see Section 2.3), H' , E , mean TA size, and protozoic biosilicification were calculated without grouping of TA taxa.

2.3. Statistical analyses

We initially found 113 TA taxa in the 74 sediment samples. However, as some samples contained only very few TA shells and/or environmental variables were not available or determined for some samples, several samples were finally excluded from statistical analyses. After data set processing a total of 32 sediment samples including 32 TA groups (comprising 101 TA taxa) were finally included in our statistical analyses (Table 1, Supplementary Table 1). Correlations within the data set were analyzed using Spearman's rank correlation (r_s , monotonic relationships).

Canonical Correspondence Analysis (CCA) was performed to examine the relations between TA community composition and environmental variables (mean water temperature, water pH, water conductivity, depth of water, and site elevation a.s.l.). Samples with low TA abundances (<25 individuals in total) and TA taxa with low frequencies (<2 individuals) were deleted before running the CCA ordination. The counted species data were transformed in percentages (Supplementary Table 2). A forward-selection approach using the Monte Carlo permutation test (999 random permutations) was applied to reduce the variables to a manageable number and to test the significance ($\alpha \leq 0.05$) of these variables (Leps and Smilauer, 2003).

We used a classical multivariate cluster analysis (Ward's minimum variance method with squared Euclidean distances) to group the sampling sites according to water pH and temperature as these environmental properties were found in the CCA to have the most significant influence on TA community composition. Differences between means of water pH and temperature of the resulting clusters were tested using the Kruskal-Wallis analysis of variance (ANOVA) followed by pairwise multiple comparisons (Dunn's post-hoc test).

Detrended correspondence analysis (DCA) was used for arranging the sampling sites in relation to TA community composition. In contrast to the cluster analysis, which distributed the sampling sites in the single clusters entirely randomly, the DCA allowed an arrangement of the sampling sites along environmental gradients.

Computations of CCA and DCA ordinations were performed in CANOCO 4.5. Spearman's rank correlations, the cluster analysis, and the Kruskal-Wallis ANOVA were performed using the software package SPSS Statistics (version 22.0.0.0, IBM Corp.).

3. Results

3.1. Climate and environmental properties

Generally, higher air temperatures and precipitation were found in

the south-eastern and lower regions of the QTP, while lake sites with a colder and drier climate were mainly located in the northern and higher QTP regions (Fig. 1, Table 1). Mean air temperatures at the sampled lakes ranged from -20.4 to 6.3 °C, from 5.3 to 17.2 °C, and from -6 to 12.3 °C related to January, July, and one year, respectively. Precipitation ranged from 3.5 to 44.3 mm, 76.2 to 291.7 mm, and 418.1 to 1392 mm related to January, July, and one year, respectively (Table 1).

The sampled lakes were situated in a quite big area (latitude and longitude ranged from 25.92° to 36.78° N and 90.63° to 99.74° E) covering an elevation gradient of >3000 m (site elevation ranged from 1967 to 5002 m a.s.l.) with relatively large ranges in environmental properties (Table 1). Water depth varied between 0.1 and 31.0 m, water temperature ranged from 6.2 to 27.7 °C, water pH ranged from 6.9 to 10.3 , and conductivity showed values between 0.01 and 191.4 S/m.

3.2. Testate amoeba species diversity and community composition

We found TA diversity to be relatively high in lake sediments of the QTP with a total of 113 TA taxa including one unknown species (Supplementary Table 1). Species such as *Centropyxis aculeata*, *C. aerophila*, *Diffflugia globularis*, *D. minuta*, *D. pristis*, *D. nana*, and *D. penardi* were the most abundant TA taxa occurring in almost all sediment samples. Some species like *Arcella arenaria*, *A. megastoma*, *A. jurassica*, *Centropyxis hirsuta*, *Campascus minutus*, *Diffflugia nana*, and *Lagenodiffflugia bryophila* (Fig. 2, Supplementary Table 1), were absent from a Chinese TA checklist summarized in 2004 (Yang et al., 2004), but most of them (except *Diffflugia nana* and *Lagenodiffflugia bryophila*) were later found in lakes in the NW of the Yunnan province, which is close to the margin of the eastern QTP (Yang et al., 2005). It is noteworthy that we found TA taxa even under strongly alkaline ($\text{pH} > 10$) conditions in the lakes of the QTP, namely *Diffflugia geosphaerica*, *D. globulus*, *D. petricola*, *D. stechlinensis* (all found at site no. 2, see Table 1), and *Cryptodiffflugia cf. crenulata glabra* (found at site no. 4, Table 1) (Supplementary Tables 1 and 2).

In general, we found $H' > 1.5$ in lakes in the eastern (94.1 – 101.9 E) part of the QTP, while $H' < 1.5$ were characteristic for lakes further to the west with 2 exceptions (KPW-15 and KPW-DE). While only 4 out of 32 lakes showed H' values ≥ 2.5 indicating a relatively healthy environment, 13 lakes were characterized by H' values below 1.5 indicating a stressed environment. 15 lakes were characterized by intermediate H' values ranging between 1.5 and 2.4. As E values were mathematically derived from H' values, they indicated identical trends, with very low values ranging from 0.06 to 0.12.

The mean size of TA shells in the samples varied from 15 to 141 μm (Table 1) and was related to distinct environmental gradients, which were especially indicated by relatively large TA taxa, e.g., *Centropyxis constricta*, *C. ecornis*, *C. pontigulasiformis*, *Cyclopyxis arcelloides cf. gibbosa*, *Diffflugia globularis*, *D. oblonga*, *D. oblonga cf. angusticollis*, *D. pyriformis*, *D. petricola*, or *D. sarissa*. These TA taxa were more abundant in the South-East of the QTP and in low-altitude lakes (e.g., lakes 25–32, Table 1), with one exception (lake no. 12).

3.3. Statistical findings

The CCA on individual variables showed that the TA community composition was significantly correlated with water temperature ($p = 0.038$) and pH ($p = 0.032$) (Tables 2, Fig. 3). The first two axes of the CCA ordination diagram accounted for 22.1 % of the total variance and 70.3 % of the variance of species-environment relations (Table 3, Fig. 3).

Correlation analysis showed that species richness, H' , E and mean size were positively correlated with longitude, water temperature, and water depth, and negatively correlated with latitude, elevation, water pH, and conductivity (Table 4).

We found idiosomic TA, i.e., TA with self-secreted siliceous shells, only in 8 out of 32 sediment samples. Protozoic biosilicification ranged between 1.4 and 12 ng Si per 150 TA shells and was positively correlated

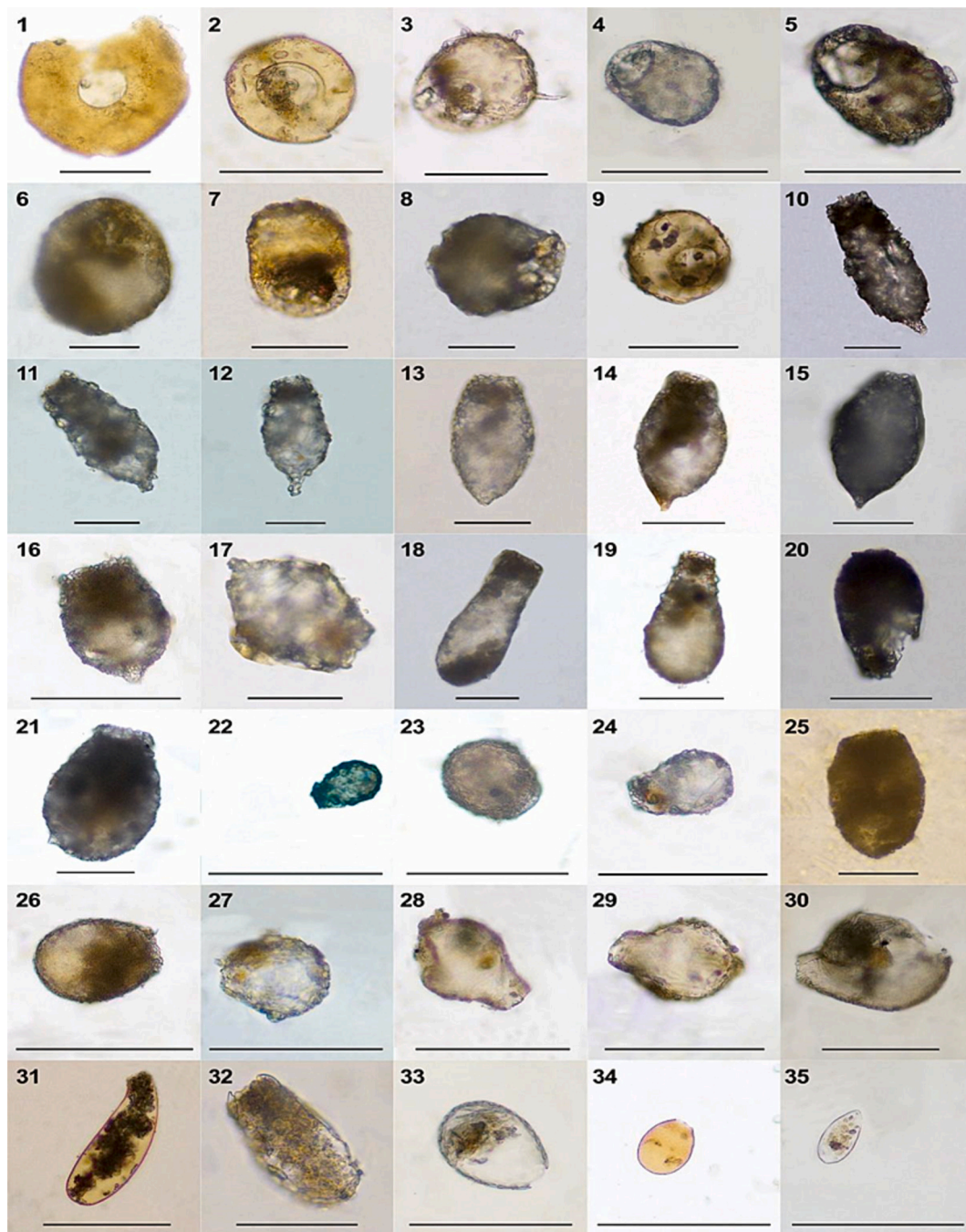


Fig. 2. Micrographs of some testate amoebae found in lake sediments of the QTP (cf. Supplementary Table 1). 1. *Arcella polypora*, 2. *A. megastoma* (first record for the QTP), 3. *Centropyxis aculeata*, 4. *C. cassis*, 5. *C. constricta*, 6. *C. penardi*, 7. *C. invaginata*, 8. *C. aerophila*, 9. *Cyclopyxis eurystoma*, 10/11. *Diffugia acuminata*, 12. *D. distenda*, 13. *D. sarissa*, 14/15. *D. difficilis*, 16/17. *D. elegans*, 18. *D. linearis*, 19. *D. oblonga*, 20. *D. penardi*, 21. *D. sphincta*, 22. *D. nana* (first record for the QTP and China), 23. *D. minuta*, 24. *D. rubescens*, 25. *D. perfilievi*. 26. *D. pristis*, 27. *D. pulex*, 28/29. *Diffugia* sp. 1 (unknown species), 30. *Lesquereusia spiralis*, 31. *Cyphoderia ampulla*, 32. *Euglypha tuberculata*, 33. *E. strigosa*, 34. *Assulina muscorum*, 35. *Trinema grandis*. Scale bars are 100 μm except for no. 6–15, 17, 18, 20, 22, 25, 27, and 32, where scale bars are 50 μm .

Table 2

CCA analysis results showing the variance (in %) in the TA data set of Tibetan lakes as explained by each measured environmental variable.

Individual CCA	Explained variance (%)	p-Value (999 permutations)
Water temperature	7.0	0.038
pH	6.0	0.032
Conductivity	5.0	0.150
Water depth	4.0	0.179
Elevation	0.07	0.255

with longitude, precipitation, and temperature, but negatively correlated with latitude and elevation (Table 4).

The grouping of sampling sites according to water pH and temperature via cluster analysis resulted in 5 clusters (Fig. 4). Cluster 1 was characterized by a mean water pH of 9.2 and a mean water temperature of 19.0 °C. Cluster 2 comprised sites with a neutral pH (mean 7.2) and a mean water temperature of 16.3 °C. Cluster 3 grouped sites with the warmest waters (mean 26.9 °C) and an alkaline pH (mean 8.8). Cluster 4 showed a very similar water pH (mean 8.9), but distinctly colder water temperatures (mean 12.4 °C) compared to cluster 3. Cluster 5 comprised

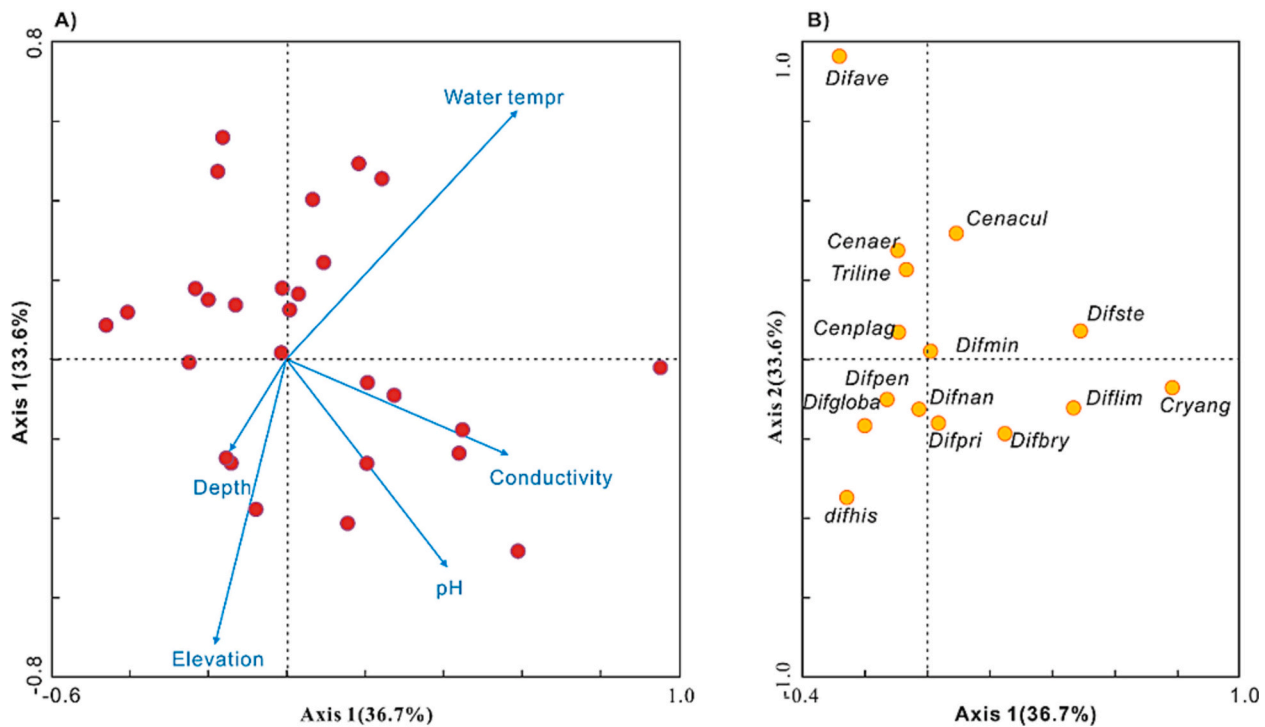


Fig. 3. CCA ordination showing the position of sampling sites (A) and TA taxa (B) in the ordination space. Water tempr = water temperature. TA taxon abbreviations are given in the Supplementary Table 1.

Table 3

CCA analysis results showing the relations between TA communities and environmental variance.

Axes	1	2	3	4
Eigenvalues	0.210	0.192	0.087	0.043
Species-environment correlations	0.679	0.838	0.711	0.456
Cumulative percentage variance of species data	11.0	21.1	25.7	27.9
Cumulative percentage variance of environment data	36.7	70.3	85.5	93.1

sites with the highest pH (mean 9.4 °C) and the lowest water temperatures (mean 7.9 °C). The different clusters were dominated by specific TA taxa as follows (top 3 each). Cluster 1: (i) *Diffflugia minuta*, (ii) *Cryptodiffflugia cf. crenulata glabra*, and (iii) *Centropyxis constricta minuta*; Cluster 2: (i) *Centropyxis constricta*, (ii) *Diffflugia minuta*, and (iii) *Centropyxis cassi*; Cluster 3: (i) *Centropyxis discoides*, (ii) *Cryptodiffflugia sacculus*, and (iii) *Diffflugia minuta*; Cluster 4: (i) *Diffflugia minuta*, (ii) *Diffflugia geosphaerica*, and (iii) *Diffflugia globulus*; Cluster 5: (i) *Diffflugia minuta minor*, (ii) *Diffflugia minuta*, and (iii) *Paraquadrula discoides*.

DCA ordination showed an arrangement of sampling sites and TA taxa along environmental gradients (water pH, temperature, conductivity, and depth), which are mainly controlled by climate and elevation (Fig. 5). Generally, the grouping of sampling sites obtained from cluster analysis (based on water pH and temperature) was only roughly reproduced in the DCA ordination (based on TA taxa) underlining the complex interactions between environmental properties, climate, and TA communities.

4. Discussion

4.1. Climate and environmental properties in the QTP

In general, climate data reflected the well-known warm-cold/wet-dry gradients in the QTP. While relatively high values of air temperature

and precipitation appeared in the lower altitudes in the south Himalayas, an extremely cold-dry climate was characteristic for the northern high-altitude regions of the QTP with consequences for biogeographical patterns of flora and fauna including TA (Herzschuh et al., 2010; Wang, 1974, 1977; Wang et al., 2022). As most samples were collected in the summer time (cf. Herzschuh, 2006; Herzschuh et al., 2010), the measured water temperatures were relatively high reaching even about 28 °C in Erhai Lake (see Table 1). We found water temperatures to be correlated to the geographic position, elevation, and climate (see Table 4). The Erhai Lake, for example, which is in the near of the margin of the QTP and the Yuanna-Guizhou plateau, is not only the lake with the lowest elevation (1967 m), but also one of the most southern lakes (25.92°N) in our study characterized by a warm monsoon climate (mean annual air temperature: 12.3 °C, annual precipitation: 1295 mm). Such extremely warm surface water was also observed during a monitoring study of Erhai Lake, which represents a typical shallow lake of the QTP (Zhu et al., 2017).

Moreover, we found some extreme values of water pH (even above 10) and conductivity in some of the lakes in our study. In general, alkalinity and salinity of the lakes in the QTP are controlled by topography, climate, and geological material. In most lakes of the QTP, which often formed in shallow topological depressions, the outflow of water is limited and evaporation-induced water losses are balanced by precipitation. These factors in combination with strong physicochemical weathering rates result in the observed high levels of salinity (caused by high concentrations of dissolved salts like sodium chloride) and alkaline water pH (caused by high concentrations of carbonate salts) (Yang et al., 2003; Wu et al., 2009). These extreme environmental conditions in the lakes of the QTP strongly affect the diversity and species composition of TA (see Section 4.2), as it is also the case for other aquatic microorganisms like phytoplankton (including diatoms, Yang et al., 2003; Li et al., 2021) or ostracods (Mischke et al., 2007; Han et al., 2022).

4.2. Testate amoeba diversity and species composition in lake sediments

The relatively large ranges in latitude and longitude, the diverse

Table 4 Results of Spearman's rank correlation analysis ($n = 32$, * = $p < 0.05$, ** = $p < 0.01$). Jan. = January, Jul. = July, Annu = annual, T = temperature, P = precipitation.

	Mean size	H'	Richness	Protozoic Si	pH	Conductivity	Water depth	Water T	Latitude	Longitude	Elevation	Jan. P	Jul. P	Annu. P	Jan. T	Jul. T	Mean T
H'	0.489**																
Richness	0.533**	0.983**															
Protozoic Si	0.356**	0.626**	0.672**														
pH	-0.269	-0.462*	-0.487**	-0.224													
Conductivity	-0.453*	-0.552**	-0.601**	-0.590**	0.346												
Water depth	0.409*	0.329	0.365	0.519**	0.105	-0.159											
Water T	0.171	0.183	0.267	0.130	-0.199	-0.164	-0.263										
Latitude	0.580**	-0.350*	-0.433*	-0.528**	-0.113	0.582**	-0.559**	-0.225									
Longitude	0.363*	0.537**	0.572**	0.405*	-0.730**	-0.555**	0.002	0.521**	-0.167								
Elevation	-0.253	-0.311	-0.379*	-0.184	0.557**	0.271	0.237	-0.771**	0.074	-0.804**							
Jan. P	0.582**	0.599**	0.648**	0.480**	-0.309	-0.692**	0.318	0.135	-0.687**	0.524**	-0.209						
Jul. P	0.580**	0.319	0.406*	0.517**	0.000	-0.639**	0.465*	0.305	-0.886**	0.305	-0.242	0.520**					
Annu. P	0.548**	0.407*	0.461**	0.572**	-0.048	-0.810**	0.466*	0.146	-0.849**	0.338	-0.105	0.628**	0.917**				
Jan. T	0.331	0.238	0.347	0.501**	-0.111	-0.464*	0.242	0.638**	-0.592**	0.443*	-0.602**	0.201	0.763**	0.583**			
Jul. T	0.312	0.118	0.222	0.319	-0.140	-0.002	0.209	0.571**	-0.179	0.408*	-0.658**	-0.005	0.387*	0.123	0.706**		
Mean T	0.300	0.181	0.289	0.404*	-0.183	-0.262	0.168	0.672**	-0.349*	0.495**	-0.709**	0.080	0.576**	0.347	0.911**	0.916**	
E	0.536**	0.973*	0.999**	0.686**	-0.480*	-0.590**	0.369**	0.273	-0.480**	0.618**	-0.430*	0.678**	0.489**	0.523*	0.412*	0.324*	0.374*

topography, and the relatively big climate gradient covered in our study let us assume a micro-habitat-rich environment for TA in the QTP. However, the harsh conditions in the QTP generally pose a challenge for TA communities as indicated by the relatively low values of H' and E (see Table 1), especially in lakes on the colder and drier NW plateau or with extremely high pH (>9.5) and conductivity. The very low values of E indicated that for some TA taxa only very low individual numbers were recorded in a sample. However, it should be noted that these low values of E are not only indicating the extremely harsh environmental conditions in the QTP, but also result from the taxonomic approach applied in our study. We used a morphology based high resolution taxonomy including varieties and forms of TA taxa (see Supplementary Table 1), which provided detailed information on TA biodiversity in lakes of the QTP on the one hand. On the other hand, this high-resolution taxonomy resulted in relatively low individual numbers for many TA taxa, which is reflected in the values for H' and E. Interestingly, one strongly alkaline lake (no. 12, pH = 9.5, Table 1) was characterized by a quite high H'-value of 2.5 counterintuitively indicating a relatively healthy environment for TA. This surprising result might be explained by two aspects. Firstly, most TA are sediment-dwelling (benthic) microorganisms, and thus might be more affected by physicochemical sediment properties than water properties including pH (cf. Wall et al., 2010). Secondly, despite their extreme environmental conditions strongly alkaline lakes are often (but not always) characterized by extremely productive communities of prokaryotic and eukaryotic microorganisms (e.g., Lanzén et al., 2013). Unfortunately, examinations of TA in these extreme environments are very scarce and we hope that our study will initiate further research on this fascinating topic.

In contrast to our study, previous studies in the region of QTP reported a bigger abundance of species (131 TA species) with the genus *Centropyxis* as the dominant taxon (Wang, 1974, 1977). We assume that this discrepancy is caused by differences in sampling. While we analyzed samples from lake sediments, Wang (1974, 1977) used samples taken from small ponds, rivers, and soils. Most of the recorded TA species in our study are quite common and can be found in lakes all around the world, e.g., in the middle and lower Yangtze plain (Qin et al., 2009, 2013, 2021a, 2016), Canada (Patterson et al., 1996, 2002; Patterson and Kumar, 2000; Roe and Patterson, 2006, 2014; Roe et al., 2010; Nasser et al., 2016, 2020), Florida (Escobar et al., 2008), the West Indies (Roe and Patterson, 2006), or Finland (Kihlman and Kauppila, 2012). However, the seven species *Arcella arenaria*, *A. megastoma*, *A. jurassica*, *Centropyxis hirsuta*, *Campascus minutus*, *Diffflugia nana*, and *Lagenodifflugia bryophila* are new records for the QTP and two of them (i.e., *Diffflugia nana* and *Lagenodifflugia bryophila*) even for the Chinese TA checklist (Yang et al., 2004). Again, this might be caused by the fact that previous studies in China mainly focused on soils and ponds rather than lakes. This is underlined by another study of lakes of the northwest Yunnan province (near the southeast margin of the QTP), which also reported the occurrence of *Arcella arenaria*, *A. megastoma*, *A. jurassica*, *Centropyxis hirsuta*, and *Campascus minutus* (Yang et al., 2005). However, these authors did not find individuals of *Diffflugia nana* or *Lagenodifflugia bryophila*. Interestingly, *D. nana* was firstly observed in the Arctic tundra (Bobrov and Wetterich, 2012). In our study, we found *D. nana* in lakes of the QTP, which are located high in the mountains (above 4000 m), where temperatures are similar to the ones in Arctic regions, where the species was first observed (Bobrov and Wetterich, 2012).

A few Asian endemic TA taxa such as *Diffflugia biwae*, *D. mulanensis*, *Netzelia (Diffflugia) tuberspinifera*, and *Pentagonia zhangduensis* are abundant in lakes of subtropical China (especially near the middle and lower reaches of Yangtze River) and Japan, where most lakes are in mesotrophic to eutrophic states (Yang et al., 2004; Qin et al., 2011). However, none of these relatively large (100–200 μm) TA species have been recorded in lakes of the QTP in our study. The absence of these Asian endemic TA species in our samples might reflect their limited biogeographical distribution and narrow tolerance to harsh environmental conditions as they can be found in the QTP.

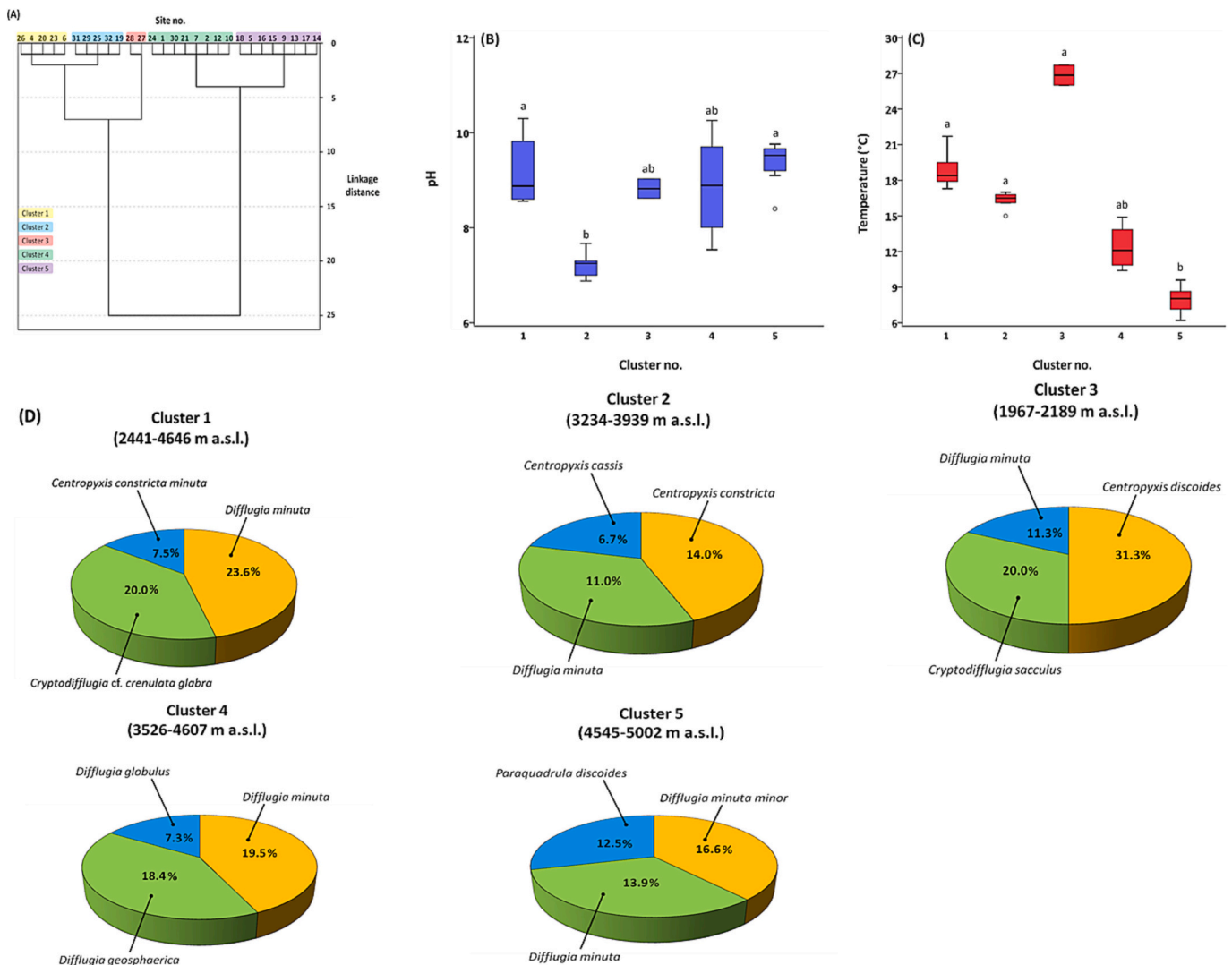


Fig. 4. Dendrogram showing the grouping of sampling sites ((A) the site numbers are identical to the ones in Table 1 and Fig. 1) according to water pH (B) and temperature (C). The top 3 dominant TA taxa per cluster are depicted in (D). The sites with the numbers 3, 8, 11, and 22 were excluded from cluster analysis as there were no data on water pH and temperature available (cf. Table 1). Different letters and circles in (B) and (C) indicate significant differences ($p < 0.05$, Kruskal-Wallis ANOVA) and outliers, respectively.

Furthermore, we frequently found individuals of *Cyphoderia ampulla*, *Plagiopyxis callida*, *P. declivis*, *P. minuta*, and *Cyclopyxis eurystoma*, which are known to be abundant in soils, moss, and peatlands (Meisterfeld, 2002). Their occurrence in our sediment samples might reflect an increased surface runoff in the QTP catchments caused by increased precipitation in this region in the last three decades due to climate change (Li et al., 2014; Zhang et al., 2021). However, as we did not analyze the potential sources of these shells, i.e., the surrounding catchments, in our study, this assumption remains speculative.

4.3. Factors controlling testate amoeba communities in lake sediments

The CCA showed that TA communities were most strongly correlated with water pH, which is consistent with the results of recent studies on lakes of northern America (Escobar et al., 2008), Canada (Patterson et al., 2012), Russia (Tsyganov et al., 2019), and China (Qin et al., 2013). Moreover, pH and water depth were found to represent significant controls of TA community composition and the abundance of platelets (idiosomes) of siliceous TA shells in sediments (Siver et al., 2020). In fact, TA are considered as excellent bio-indicators for water pH, and TA-based water pH transfer functions were thus developed for

paleoenvironmental reconstructions of lakes (Patterson et al., 2012). Additionally, some studies suggested that water chemistry (e.g., total phosphorus, total nitrogen, and metal concentrations) or air temperature are important factors controlling TA community composition in lakes (Escobar et al., 2008; Roe et al., 2010; Ju et al., 2014; Qin et al., 2016). However, most of these studies were conducted in lakes near human settlements, which have direct effects (e.g., nutrient inputs) on the surrounding lake catchments.

In the QTP the analyzed lakes comprise large-scale latitude, longitude, and elevation gradients, which is why we found water temperatures and pH to show relatively wide ranges. This might hamper interpretations of the relationships between TA community compositions and environmental properties because TA might be more sensitive to micro-environmental or micro-habitat conditions than to macro-environmental conditions (Yang et al., 2003; Qin et al., 2013; Tsyganov et al., 2019; Siver et al., 2020). In our study we found positive correlations between TA richness, H' , and mean shell size and climate factors (especially precipitation, see Table 4), but these relationships still need to be analyzed in more detail, because the underlying dataset is too small for deriving transfer functions. We see our study as a foundation for further studies that are needed to underline our

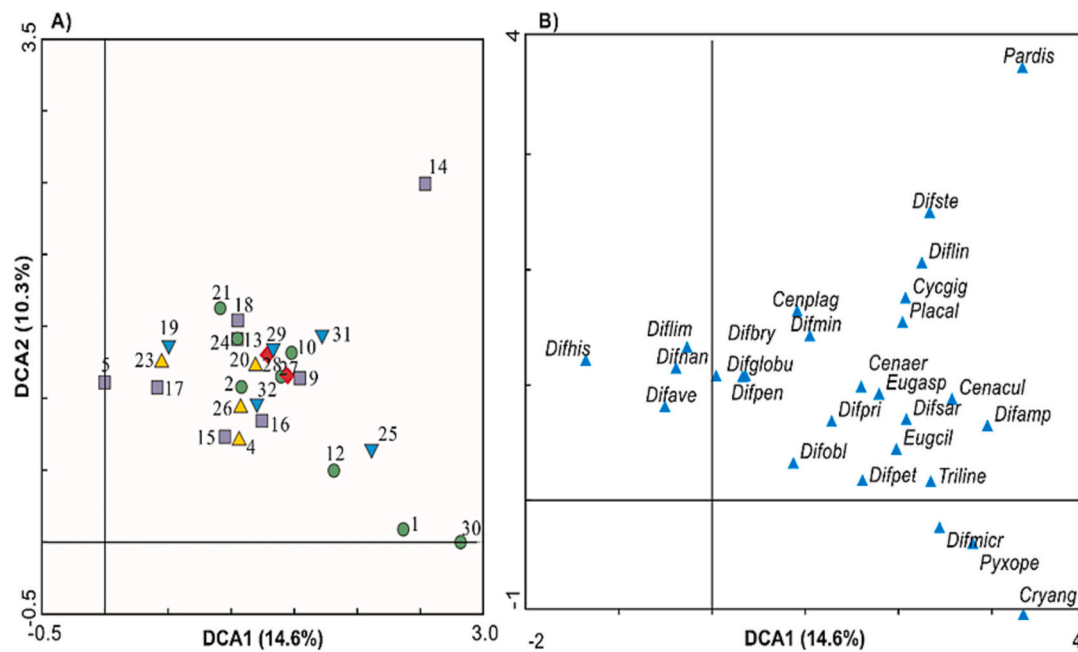


Fig. 5. DCA ordination of sampling sites (A) and corresponding TA taxa (B). The colors of the sampling sites in (A) correspond to the ones used in Fig. 4. TA taxon abbreviations are given in the Supplementary Table 1.

interpretations regarding the factors controlling TA diversity and community structures in the lakes of the QTP.

4.4. *Testate amoeba shell size in relation to lake elevation and implications for paleoenvironmental reconstructions*

The investigated lakes in our study were characterized by a 3035 m elevation gradient (1967–5002 m a.s.l.), which strongly affected the distribution and diversity of TA. TA taxon richness, TA diversity, shell mean size, and protozoic biosilicification were found to be negatively correlated with elevation. This is different from studies on elevational diversity gradients of TA in soil samples, which showed a bell-shaped pattern of TA biodiversity peaking at intermediate elevations following the distribution of vegetation (Krashevskaya et al., 2007). For example, Tsyganov et al. (2022) found 95 TA taxa, which mostly belonged to ubiquitous taxa (e.g., *Trinema lineare*, *Euglypha laevis*, *Cryptodiffugia oviformis*, and *Trinema complanatum*) besides TA taxa with limited geographic distribution (e.g., *Centropyxis latideflandriana*, *C. stenodeflandriana*, *Plagiopyxis cf. barroisi*, *Heleopera rectangularis*, and *Distomatopyxis couillardii*), in samples from Mount Fuji (904–2377 m a.s.l., Japan). Their statistical analyses revealed that TA assemblages were strongly mediated by vegetation. This is supported by the results of Wanner et al. (2022), who analyzed TA in soil samples from different heights (1730–4000 m a.s.l.) of Mt. Kinabalu, Borneo. These authors found 78 morphotypes of TA, which were mostly <100 μm and cosmopolitan besides *Certesella certesi* with limited geographical distribution, some rare taxa (e.g., *Padaungiella lageniformis cordiformis*, *Placocista jurassica*, *Trinema chardezi*), and two undescribed morphospecies. TA community assemblages were linked to elevation with a pronounced change at the tree line. In contrast, we found TA biodiversity in lake sediments to be mainly influenced by water temperature, pH, conductivity, and depth. Water temperature and pH in turn were strongly correlated to elevation. However, a vegetation effect on TA communities in lake sediments of the QTP driven by TA as well as nutrient inputs cannot be excluded. Future analyses of the surrounding catchments in the QTP will enlighten this aspect. In general, catchment properties have been found to be of great significance for TA assemblages in freshwater sediments (e.g., Roe et al., 2010; Oris et al., 2013).

The size of TA shells (expressed as bio-volume in some studies) is

considered as one of the important functional traits responding to many environmental variables including warming climate (Jassey et al., 2013; Marcisz et al., 2020). In our study the mean size of TA shells was negatively correlated with conductivity ($r_s = -0.627$, $p < 0.01$), pH ($r_s = -0.478$, $p < 0.05$), and elevation ($r_s = -0.421$, $p < 0.05$), but positively correlated with water depth and warm-wet climate conditions (Table 4). In general, abundances of smaller TA species were higher in shallow lakes or higher regions with harsh environmental conditions (e.g., higher values of water conductivity and pH, colder climate) compared to lakes in the SW of the QTP. This is consistent to some results of studies on peatlands, where a decrease in abundances of larger TA species has also been related to extreme weather conditions (Jassey et al., 2016; McKeown et al., 2019), suggesting that smaller species are generally more resistant to extreme climatic conditions. Furthermore, shell size shifts also reflected changes in functional groups of TA. For example, it was shown that a decrease in protist host biomass and diversity towards mountain tops impacts the functional composition of protists in soils of the Alps (Mazel et al., 2022). In the lakes of the QTP, TA shell size shifts occurred around 4400 m, where small size (<30 μm) TA species were found to be dominant (cf. Fig. 4D). Interestingly, a similar shift was also observed in phytoplankton communities of the QTP in a previous study (Li et al., 2021). In addition to temperature and conductivity TA shell size is also affected by other factors like nutrient availability, pollution, and environmental disturbances (Marcisz et al., 2020). Altogether, these results and our observations clearly emphasize the sensitivity of TA functional traits to environmental gradients, characterizing TA communities as well-suited proxies for paleoenvironmental reconstructions by fossil TA remains in lake sediments (cf. Qin et al., 2021b).

4.5. *Siliceous testate amoeba shells in lake sediments and implications for protozoic biosilicification under warming climate*

While protozoic biosilicification has been found to be significant in specific forest ecosystems (Puppe, 2020), there was no information on the potential of TA for Si cycling in lake sediments until now. However, previous studies indicated that freshwater lake sediments represent important reservoirs of recent and fossil protozoic biosilica (Fig. 6). In fact, TA idiosomes, i.e., the single building blocks of siliceous TA shells,

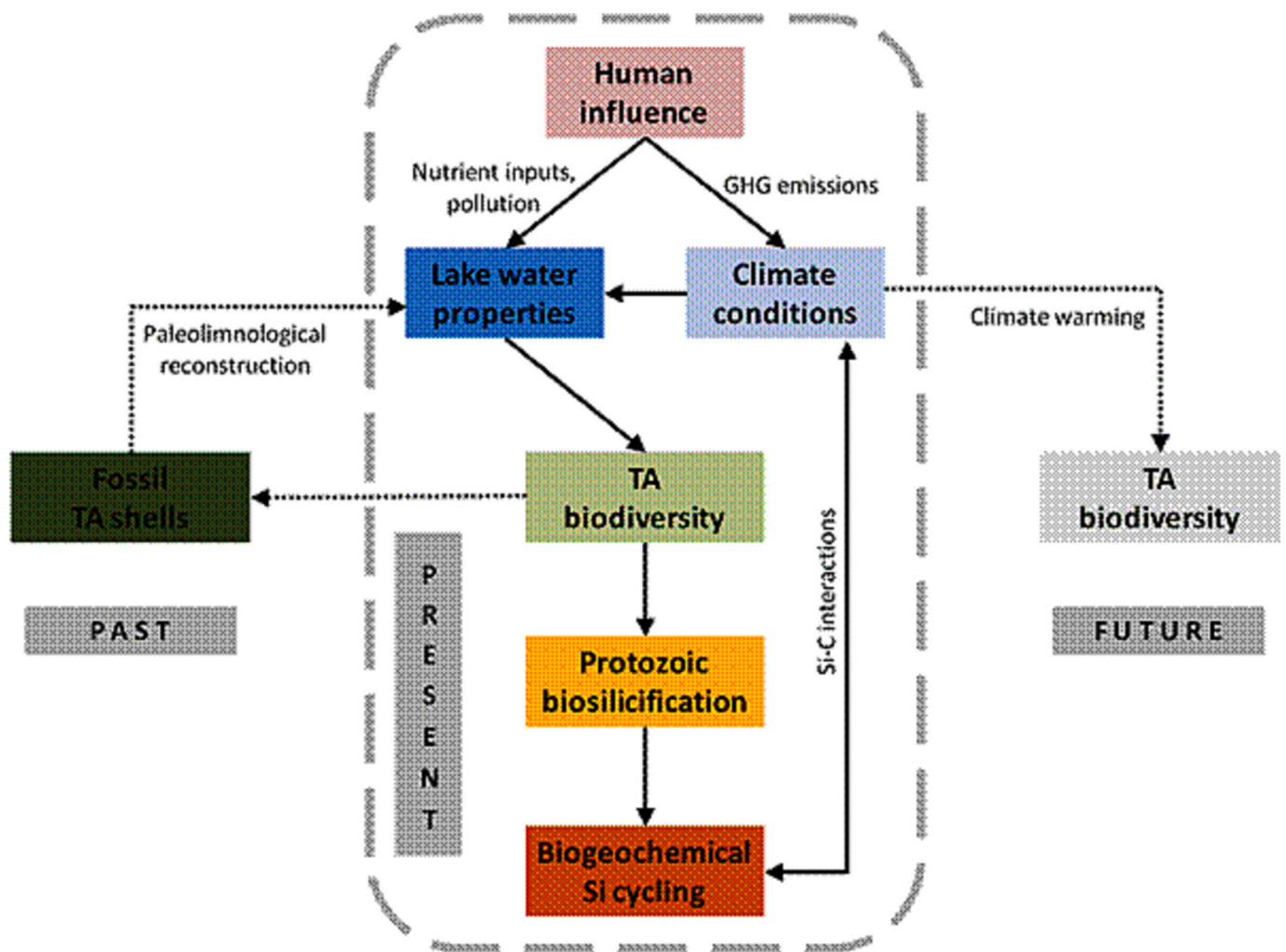


Fig. 6. Overview of the relations of TA biodiversity and protozoic silica cycling in lakes to environmental change in the past, present and future, with reference particularly to human activities and climate warming.

were found to be very abundant in freshwater lakes in the USA (Douglas and Smol, 1987; Siver et al., 2020), and Fennoscandia (Pienitz et al., 1995). These authors clearly ascribed the accumulation of protozoic silica plates in lake sediments mainly to inputs of TA shells/idiosomes by surface runoff originating from the surrounding lake catchments. In our study only intact idiosomic TA shells were considered for the quantification of protozoic biosilicification, and thus we have no information on the protozoic biosilica pool represented by single idiosomes, which can be assumed as the bigger one (see Puppe, 2020).

In general, we found the biosilicification potential of intact TA shells in lake sediments of the QTP to be quite low (max. 12 ng per 150 TA shells) compared to Asian peatlands, for example (see Qin et al., 2022). This can be directly ascribed to the fact that idiosomic TA taxa were only found in 25 % of the samples, which were thus clearly dominated by TA with xenosomic or organic shells. This is confirmed by previous studies that showed freshwater sediment samples to be dominated by TA taxa of the order Arcellinida (e.g., Escobar et al., 2008; Ju et al., 2014). However, we urgently need quantitative data of TA idiosomes in lake sediments of the QTP (and other lakes worldwide) to be able to finally evaluate their role as protozoic biosilica reservoirs. In this context, results of DNA analyses (18S rRNA gene sequencing) showed that lake sediments of the QTP are characterized by abundant micro-eukaryotic communities including diatoms and TA (Ren et al., 2022), indirectly indicating their potential for protistic biosilica accumulation.

Global warming has been found to increase silicate weathering

intensity (Deng et al., 2022). This might enhance Si bio-cycling, as more Si will be released, and thus become bio-available for organisms that are able to form bio-silica like plants, sponges, and protists including TA (Puppe et al., 2022). Moreover, shifts of the treeline due to climate warming (cf. Wang et al., 2022), and thus changes in the vegetation can be assumed to additionally affect Si cycling (e.g., by enhanced bio-weathering and accumulation of silica in the vegetation) in the QTP in the next decades. In fact, climate warming in the QTP is assumed to be two to three times faster compared to the global average (Li et al., 2014). This basically means that the rates of silicate weathering and soil erosion might be accelerated in this region leading to increased Si inputs in the lakes of the QTP now and in the near future (Fig. 6). Indeed, our analyses revealed that protozoic biosilicification was positively correlated with climatic conditions (temperature, precipitation) in the QTP (Table 4). Furthermore, warming in the QTP might enhance the probability and frequency of fires, which have been found to affect Si availability in mineral soils and TA communities with potential consequences for protozoic biosilicification (Qin et al., 2017; Schaller and Puppe, 2021).

We consider the QTP as a model region for the observation of global change effects on TA community compositions. From our results and their discussion above we conclude that analyses of TA assemblages in lake sediments are not only suitable for the reconstruction of environmental properties and protozoic biosilicification in the past, but also for future predictions (Fig. 6). Based on our results we assume protozoic biosilicification in the lakes of QTP to increase in the near future as a

direct response to global warming. This is underlined by the results of Qin et al. (2022), who found protozoic biosilicification to be positively correlated with annual mean temperatures in Asian peatlands. Furthermore, their results showed a time-dependent increase in protozoic biosilicification in the Dajihu peatland, China, within the last 2000 years. The climate-related increase of protozoic biosilicification might be accelerated by environmental disturbances (Qin et al., 2022). However, it remains unclear which consequences this acceleration of protozoic biosilicification has for the other parts (e.g., Si uptake by vegetation, soil weathering) of biogeochemical Si cycling.

Our study is another example for the effects of global change on TA assemblages and Si cycling on the microbial level. In this context, TA are not only affected by warming climate, but also land-use change, peatland degradation, and salinization caused by rising sea-levels (Qin et al., 2020, 2022; Wanner et al., 2020). Since TA represent key players in biological Si cycling in some ecosystems (Puppe, 2020), changes in TA community compositions might have severe consequences for Si cycling at the landscape scale. The protection and restoration of endangered ecosystems can help to mitigate the effects of global change on TA communities (cf. Qin et al., 2020).

5. Conclusions

Our analyses of TA communities in the model region QTP, the so-called “third pole” of the Earth, revealed new insights into the relationships between TA biodiversity and protozoic biosilicification in lakes under climate warming. As TA biodiversity is relatively high in these lakes, we consider the QTP to be still comparably undisturbed. However, climate warming is a big challenge for this region and climate-related changes in TA communities will be directly reflected in changes of protozoic biosilicification. Our study thus represents another example for anthropogenic impacts on biogeochemical Si cycling, even in outlying, high-altitude regions like the QTP. What we need now is (i) further research to enlighten the interactions of TA biodiversity, protozoic biosilicification, and environmental properties in more detail (e.g., on a micro-habitat scale), (ii) the establishment of a (long-term) monitoring of TA communities in the QTP to detect climate-related changes in these bio-indicator communities, and (iii) a limitation and regulation of human disturbances in the QTP to preserve the “Asian Water Tower” as effectively as possible.

CRedit authorship contribution statement

Yangmin Qin: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Anatoly Bobrov:** Data curation, Investigation. **Daniel Puppe:** Data curation, Formal analysis, Software, Writing – original draft. **Hui Li:** Data curation, Investigation. **Baiying Man:** Data curation, Investigation. **Jing Gong:** Data curation, Investigation. **Jie Wang:** Data curation, Investigation. **Yongde Cui:** Data curation, Investigation. **Yansheng Gu:** Data curation, Investigation. **Ulrike Herzsuh:** Data curation, Investigation. **Shucheng Xie:** Conceptualization, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All relevant data are presented within the paper. Underlying data can be obtained on request from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.169661>.

References

- Bobrov, A., Wetterich, S., 2012. Testate amoebae of arctic tundra landscapes. *Protistol* 7, 51–58.
- Booth, R.K., 2002. Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *J. Paleolimnol.* 28, 329–348.
- Deng, K., Yang, S., Guo, Y., 2022. A global temperature control of silicate weathering intensity. *Nat. Commun.* 13, 1781. <https://doi.org/10.1038/s41467-022-29415-0>.
- Douglas, M.S., Smol, J.P., 1987. Siliceous protozoan plates in lake sediments. *Hydrobiologia* 154, 13–23. <https://doi.org/10.1007/BF00026827>.
- Ehrmann, O., Puppe, D., Wanner, M., Kaczorek, D., Sommer, M., 2012. Testate amoebae in 31 mature forest ecosystems – densities and micro-distribution in soils. *Eur. J. Protistol.* 48 (3), 161–168.
- Escobar, J., Brenner, M., Whitmore, T.J., Kenney, W.F., Curtis, J.H., 2008. Ecology of testate amoebae (thecamoebians) in subtropical Florida lakes. *J. Paleolimnol.* 40, 715–731. <https://doi.org/10.1007/s10933-008-9195-5>.
- Han, C., Li, X., Fan, Q., Wei, H., Cheng, Y., 2022. Distribution characteristics and responding to ecological environment of terrestrial ostracod on the Qinghai-Tibetan Plateau. *J. Lake Sci.* 34 (3), 868–880. <https://doi.org/10.18307/2022.0313> (2022, In Chinese).
- Herzsuh, U., 2006. Palaeo-moisture evolution at the margins of the Asian monsoon during the last 50 ka. *Quat. Sci. Rev.* 25, 163–178. <https://doi.org/10.1016/j.quascirev.2005.02.006>.
- Herzsuh, U., Birks, H.J.B., Mischke, S., Zhang, C., Böhner, J., 2010. A modern pollen-climate calibration set based on lake sediments from the Tibetan Plateau and its application to a Late-Quaternary pollen record from the Qilian Mountains. *J. Biogeogr.* 37, 752–766. <https://doi.org/10.1111/j.1365-2699.2009.02245>.
- Jassey, V.E.J., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Défarge, F., Delarue, F., Bernard, N., Mitchell, E.A.D., Toussaint, M.L., Francez, A.J., Gilbert, D., 2013. Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. *Glob. Chang. Biol.* 19, 811–823. <https://doi.org/10.1111/gcb.12075>.
- Jassey, V.E.J., Lamentowicz, M., Bragazza, L., Hofsommer, M.L., Mills, R.T.E., Buttler, A., Signarbieux, C., Robroek, B.J.M., 2016. Loss of testate amoeba functional diversity with increasing frost intensity across a continental gradient reduces microbial activity in peatlands. *Eur. J. Protistol.* 55, 190–202. <https://doi.org/10.1016/j.ejop.2016.04.007>.
- Ju, L.H., Yang, J., Liu, L.M., Wilkinson, D.M., 2014. Diversity and distribution of freshwater testate amoebae (Protozoa) along latitudinal and trophic gradients in China. *Microb. Ecol.* 68, 657e670. <https://doi.org/10.1007/s00248-014-0442-1>.
- Kaupilla, T., Kihlman, S., Mäkinen, J., 2006. Distribution of Arcellaceans (testate amoebae) in the sediments of a mine water impacted bay of lake retunen, Finland. *Water Air Soil Pollut.* 172, 337–358. <https://doi.org/10.1007/s11270-006-9099-9>.
- Kihlman, S., Kaupilla, T., 2009. Mine water-induced gradients in sediment metals and arcellacean assemblages in a boreal freshwater bay (Petkellahti, Finland). *J. Paleolimnol.* 42, 533–550. <https://doi.org/10.1007/s10933-008-9303-6>.
- Kihlman, S., Kaupilla, T., 2012. Effects of mining on testate amoebae in a Finnish lake. *J. Paleolimnol.* 47, 1–15. <https://doi.org/10.1007/s10933-011-9541-x>.
- Krashevskaya, V., Bonkowski, M., Maraun, M., Scheu, S., 2007. Testate amoebae (protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* 51, 319–331.
- Langen, A., Simachew, A., Gessesse, A., Chmolewska, D., Jonassen, I., Øvreås, L., 2013. Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *PLoS One* 8 (8), e72577.
- Leps, J., Smilauer, P., 2003. *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press, Cambridge, p. 284. Li, H.K.
- Li, B., Yu, Z., Liang, Z., Acharya, K., 2014. Hydrologic response of a high altitude glacierized basin in the central Tibetan Plateau. *Global Planet. Change* 118, 69–84. <https://doi.org/10.1016/j.gloplacha.2014.04.006>.
- Li, Z., Gao, Y., Wang, S., Lu, Y., Sun, K., Jia, J., Wang, Y., 2021. Phytoplankton community response to nutrients along lake salinity and altitude gradients on the

- Qinghai-Tibet Plateau. *Ecol. Indic.* 128 (107848–10784). <https://doi.org/article/f2caa93b79744f0ca36129190cdf0cccd>.
- Lutz, A.F., Immerzeel, W.W., Shrestha, A.B., Bierkens, M.F.P., 2014. Consistent increase in High Asia's runoff due to increasing glacier melt and precipitation. *Nat. Clim. Chang.* 4, 587–592. <https://doi.org/10.1038/nclimate2237>.
- Macumber, A.L., Roe, H.M., Prentice, S.V., Sayer, C.D., Bennion, H., Salgado, J., 2020. Freshwater testate amoebae (Arcellinida) response to eutrophication as revealed by test size and shape indices. *Front. Ecol. Evol.* 8, 568904 <https://doi.org/10.3389/fevo.2020.568904>.
- Mao, D., Wang, Z., Yang, H., et al., 2018. Impacts of climate change on Tibetan lakes: patterns and processes. *Remote Sens. (Basel)* 10 (3), 358.
- Marcisz, K., Jassey, V.E.J., Kosakyan, A., Krashevskaya, V., Lahr, D.J.G., Lara, E., Lamentowicz, L., Lamentowicz, M., Macumber, A., Mazei, Y., Mitchell, E.A.D., Nasser, N.A., Patterson, R.T., Roe, H.E., Singer, D., Tsyganov, A.N., Fournier, B., 2020. Testate amoeba functional traits and their use in paleoecology. *Front. Ecol. Evol.* 8, 575966 <https://doi.org/10.3389/fevo.2020.575966>.
- Mazei, Y., Tsyganov, A.N., 2006. Freshwater testate amoebae. *KMK, Moscow*.
- Mazel, F., Malard, L., Niculita-Hirzel, H., Yashiro, E., Mod, H.K., Mitchell, E.A.D., Singer, D., Buri, A., Pinto, E., Guex, N., Lara, E., Guisan, A., 2022. Soil protist function varies with elevation in the Swiss Alps. *Environ. Microbiol.* 24 (4), 1689–1702. <https://doi.org/10.1111/1462-2920.15686>.
- McKeown, M.M., Wilmshurst, J.M., Duckert, C., Wood, J.R., Mitchell, E.A.D., 2019. Assessing the ecological value of small testate amoebae (<45 µm) in New Zealand peatlands. *Eur. J. Protistol.* 68, 1–16. <https://doi.org/10.1016/j.ejop.2018.12.002>.
- Meisterfeld, R., 2002. Testate amoebae with filopodia. In: *The Illustrated Guide to the Protozoa*, 2, pp. 1054–1084.
- Mischke, S., Herzsich, U., Massmann, G., Zhang, C., 2007. An ostracod-conductivity transfer function for Tibetan lakes. *J. Paleolimnol.* 38, 509–524.
- Mitchell, E.A.D., Charman, D.J., Warner, B.G., 2008. Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodivers. Conserv.* 17, 2115–2137. <https://doi.org/10.1007/s10531-007-9221-3>.
- Nasser, N.A., Patterson, R.T., Roe, H.M., Galloway, J.M., Falck, H., Palmer, M.J., Spence, C., Sanei, H., Macumber, A.L., Neville, L.A., 2016. Lacustrine Arcellinina (testate amoebae) as bioindicators of arsenic contamination. *Microb. Ecol.* 72, 130–149. <https://doi.org/10.1007/s00248-016-0752-6>.
- Nasser, N.A., Patterson, P.T., Roe, R.M., Galloway, J.M., Falck, H., Sanei, H., 2020. Use of Arcellinina (testate lobe amoebae) arsenic tolerance limits as a novel tool for biomonitoring arsenic contamination in lakes. *Ecol. Indic.* 113, 106177 <https://doi.org/10.1016/j.ecolind.2020.106177>.
- Ogden, C.G., 1983. Observations on the systematics of the genus *Diffugia* in Britain (Rhizopoda, Protozoa). *Bull. Br. Mus. Nat. Hist. (Zool.)* 44, 1–73.
- Oris, F., Lamentowicz, M., Genries, A., et al., 2013. Holocene changes in climate and land use drove shifts in the diversity of testate amoebae in a subalpine pond. *J. Paleolimnol.* 49, 633–646. <https://doi.org/10.1007/s10933-013-9680-3>.
- Patterson, R.T., Kumar, A., 2000. Assessment of Arcellacea (thecamoebian) assemblages, species and strains as contaminant indicators in variably contaminated James Lake, north eastern Ontario. *J. Foraminiferal Res.* 30, 310–320. <https://doi.org/10.2113/0300310>.
- Patterson, R.T., Barker, T., Burbridge, S.M., 1996. Arcellaceans (thecamoebians) as proxies of arsenic and mercury contamination in northeastern Ontario lakes. *J. Foraminiferal Res.* 26, 172–183. <https://doi.org/10.2113/gsfjr.26.2.172>.
- Patterson, R.T., Dalby, A., Kumar, A., Henderson, L.A., Boudreau, R.E.A., 2002. Arcellaceans (thecamoebians) as indicators of land-use change: settlement history of the swan Lake area, Ontario as a case study. *J. Paleolimnol.* 28 (3), 297–316. <https://doi.org/10.1023/A:1021621622090>.
- Patterson, R.T., Roe, H.M., Swindles, G.T., 2012. Development of an Arcellacean (testate lobe amoebae) based transfer function for sedimentary phosphorus in lakes. *Palaeogeogr. Palaeoclimatol.* 348–349, 32–44. <https://doi.org/10.1016/j.palaeo.2012.05.028>.
- Payne, R.J., 2013. Seven reasons why protists make useful bioindicators. *Acta Protozool.* 52, 105–113. <https://doi.org/10.4467/16890027AP.13.0011.1108>.
- Pienitz, R., Douglas, M.S., Smol, J.P., Huttunen, P., Meriläinen, J., 1995. Diatom, chrysophyte and protozoan distributions along a latitudinal transect in Fennoscandia. *Ecography* 18, 429–439. <https://doi.org/10.1111/j.1600-0587.1995.tb00146.x>.
- Puppe, D., 2020. Review on protozoic silica and its role in silicon cycling. *Geoderma* 365, 114224. <https://doi.org/10.1016/j.geoderma.2020.114224>.
- Puppe, D., Kaczorek, D., Wanner, M., Sommer, M., 2014. Dynamics and drivers of the protozoic Si pool along a 10-year chronosequence of initial ecosystem states. *Ecol. Eng.* 70, 477–482. <https://doi.org/10.1016/j.ecoleng.2014.06.011>.
- Puppe, D., Ehrmann, O., Kaczorek, D., Wanner, M., Sommer, M., 2015. The protozoic Si pool in temperate forest ecosystems - quantification, abiotic controls and interactions with earthworms. *Geoderma* 243–244, 196–204.
- Puppe, D., Wanner, M., Sommer, M., 2018. Data on eglyphid testate amoeba densities, corresponding protozoic silicon pools, and selected soil parameters of initial and forested biogeosystems. *Data Brief* 21, 1697–1703.
- Puppe, D., Kaczorek, D., Schaller, J., 2022. Biological impacts on silicon availability and cycling in agricultural plant-soil systems. In: *Silicon and Nano-Silicon in Environmental Stress Management and Crop Quality Improvement*. Academic Press, pp. 309–324.
- Qin, Y., Booth, R.K., Gu, Y., Wang, Y., Xie, S., 2009. Testate amoebae as indicators of 20th century eutrophication in Lake Zhangdu, China. *Fund. Appl. Limnol.* 175 (1), 29–38. <https://doi.org/10.1127/1863-9135/2009/0175-0029>.
- Qin, Y., Xie, S., Smith, H.G., Swindles, G.T., Gu, Y., 2011. Diversity, distribution and biogeography of testate amoebae in China: implications for ecological studies in Asia. *Eur. J. Protistol.* 47, 1–9. <https://doi.org/10.1016/j.ejop.2010.09.004>.
- Qin, Y., Fournier, B., Lara, E., Gu, Y., Wang, H., Cui, Y., Zhang, X., Mitchell, E.A.D., 2013. Relationships between testate amoeba communities and water quality in Lake Donghu, a large alkaline lake in Wuhan, China. *Front. Earth Sci.* 7, 182–190. <https://doi.org/10.1007/s11707-013-0352-4>.
- Qin, Y., Payne, R., Yang, X., Yao, M., Xue, J., Gu, Y., Xie, S., 2016. Testate amoebae as indicators of water quality and contamination in shallow lakes of the Middle and Lower Yangtze Plain. *Environ. Earth Sci.* 75, 627. <https://doi.org/10.1007/s12665-016-5442-7>.
- Qin, Y., Payne, R., Gu, Y., Mazei, Y., Wang, Y., 2017. Short-term response of testate amoebae to wildfire. *Appl. Soil Ecol.* 116, 64–69. <https://doi.org/10.1016/j.apsoil.2017.03.018>.
- Qin, Y., Puppe, D., Payne, R., Li, L., Li, J., Zhang, Z., Xie, S., 2020. Land-use change effects on protozoic silicon pools in the Zaju National Wetland Park, China. *Geoderma* 368, 114305.
- Qin, Y., Zhang, L., Swindles, G.T., Gu, Y., Yang, H., Qi, S., 2021a. A nearby 40-year paleoenvironmental record from the swan oxbow, Yangtze River, China, inferred from testate amoebae and sedimentary pigments. *J. Paleolimnol.* 66, 29–40. <https://doi.org/10.1007/s10933-021-00183-z>.
- Qin, Y., Li, H., Mazei, Y., Kurina, I., Swindles, G.T., Bobrov, A., Tsyganov, A.N., Gu, Y., Huang, X., Xue, J., Lamentowicz, M., Marcisz, K., Roland, T., Payne, Richard J., R.J., Mitchell, E.A.D., Xie, S., 2021b. Developing a continental-scale testate amoeba hydrological transfer function for Asian peatlands. *Quat. Sci. Rev.* 258, 106868 <https://doi.org/10.1016/j.quascirev.2021.106868>.
- Qin, Y., Puppe, D., Li, H., Li, H.K., Mazei, Y., Tsyganov, A.N., Man, B.Y., Huang, X.Y., Gu, Y., Xie, S., 2022. Peatland degradation in Asia threatens the biodiversity of testate amoebae (Protozoa) with consequences for Protozoic silicon cycling. *Geoderma* 420, 115870. <https://doi.org/10.1016/j.geoderma.2022.115870>.
- Qiu, J., 2008. China: the third pole. *Nature* 454, 393–396. <https://doi.org/10.1038/454393a>.
- Ren, Z., Zhang, Y., Li, X., Zhang, C., 2022. Biogeography of micro-eukaryotic communities in sediment of thermokarst lakes are jointly controlled by spatial, climatic, and physicochemical factors across the Qinghai-Tibet plateau. *Front. Ecol. Evol.* 10, 901107 <https://doi.org/10.3389/fevo.2022.901107>.
- Roe, H.M., Patterson, R.T., 2006. Distribution of thecamoebians (testate amoebae) in small lakes and ponds, Barbados, West Indies. *J. Foraminiferal Res.* 36, 116–134. <https://doi.org/10.2113/36.2.116>.
- Roe, H.M., Patterson, R.T., 2014. Arcellacea (testate amoebae) as bio-indicators of road salt contamination in lakes. *Microb. Ecol.* 68, 299–313. <https://doi.org/10.1007/s00248-014-0408-3>.
- Roe, H.M., Patterson, R.T., Swindles, G.T., 2010. Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the Greater Toronto Area and their potential as water quality indicators. *J. Paleolimnol.* 43, 955–975. <https://doi.org/10.1007/s10933-009-9380-1>.
- Schaller, J., Puppe, D., 2021. Heat improves silicon availability in mineral soils. *Geoderma* 386, 114909. <https://doi.org/10.1016/j.geoderma.2020.114909>.
- Schröder, D., Wolters, V., De Ruyter, P.C., 2003. C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* 102 (2), 294–308. <https://doi.org/10.1034/j.1600-0579.2003.12064.x>.
- Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
- Shen, Y., 1983. Protozoa of the Tibetan Plateau. In: Jiang, X.Z., Shen, Y.F., Gong, X.J. (Eds.), *Aquatic Invertebrates of the Tibetan Plateau*. Science press, Beijing, pp. 48–100 (in Chinese).
- Siver, P.A., Lott, A.M., Torres, P., 2020. Abundance and distribution of testate amoebae bearing siliceous plates in freshwater lakes and ponds along the east coast of North America: importance of water depth and pH. *Freshw. Sci.* 39 (4), 791–803. <https://doi.org/10.1086/711691>.
- Tsyganov, A.N., Malysheva, E.A., Zharov, A.A., Sapelko, T.V., Mazei, Y.A., 2019. Distribution of benthic testate amoeba assemblages along a water depth gradient in freshwater lakes of the Meshchera lowlands, Russia, and utility of the micro fossils for inferring past lake water level. *J. Paleolimnol.* 62, 137–150. <https://doi.org/10.1007/s10933-019-00080-6>.
- Tsyganov, A.N., Bobrov, A., Shimano, S., Mitchell, E.A.D., Hagiwara, Y., Wall, A.A.J., Mazei, N.G., Chernyshov, V.A., Zhong, Y., Sogame, Y., Mazei, Y., 2022. Distribution of soil testate amoeba assemblages along an elevation gradient on Mount Fuji (Japan). *Eur. J. Protistol.* 125894 <https://doi.org/10.1016/j.ejop.2022.125894>.
- Wall, A.A.J., Gilbert, D., Magny, M., Mitchell, E.A.D., 2010. Testate amoeba analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure. *J. Paleolimnol.* 43, 683–704. <https://doi.org/10.1007/s10933-009-9360-5>.
- Wang, C.C., 1974. Protozoa from Qomolangma Feng (mount Jolmolungma) area. In: *The Scientific Reports of the Comprehensive Scientific Expedition of the Qomolangma Feng Area (1966–1968), Biology and Alpine Physiology*. Science Press, Beijing, pp. 145–172 (in Chinese).
- Wang, C.C., 1977. Protozoa from some districts of Tibetan Plateau. *Acta Zool. Sin.* 23, 131–160 (in Chinese).
- Wang, S., Dou, H., 1998. *Chinese Lakes Inventory*. Science Press, Beijing.
- Wang, X., Wang, T., Xu, J., Shen, Z., Yang, Y., Chen, A., Wang, S., Liang, E., Piao, S., 2022. Enhanced habitat loss of the Himalayan endemic flora driven by warming-forced upslope tree expansion. *Nat. Ecol. Evol.* 6, 890–899. <https://doi.org/10.1038/s41559-022-01774-3>.
- Wanner, M., Birkhofer, K., Puppe, D., Shimano, S.D., Shimizu, M., 2020. Tolerance of testate amoeba species to rising sea levels under laboratory conditions and in the South Pacific. *Pedobiologia* 79, 150610. <https://doi.org/10.1016/j.pedobi.2019.150610>.

- Wanner, M., Sogame, Y., Shimizu, M., 2022. An elevation transect study of testate amoeba communities up to 4000 m asl on Mount Kinabalu, Borneo. *Eur. J. Protistol.* 83, 125868 <https://doi.org/10.1016/j.ejop.2022.125868>.
- Wu, Q.L., Chatzinotas, A., Wang, J., Boenigk, J., 2009. Genetic diversity of eukaryotic plankton assemblages in eastern Tibetan lakes differing by their salinity and altitude. *Microb. Ecol.* 58, 569–581.
- Wu, Y., Wang, S., Ni, Z., Li, H., May, L., Pu, J., 2021. Emerging water pollution in the world's least disturbed lakes on Qinghai-Tibetan Plateau. *Environ. Pollut.* 272, 116032 <https://doi.org/10.1016/j.envpol.2020.116032>.
- Yang, X., Kamenik, C., Schmidt, R., Wang, S., 2003. Diatom-based conductivity and water-level inference models from eastern Tibetan (Qinghai-Xizang) Plateau lakes. *J. Paleolimnol.* 30, 1–19. <https://doi.org/10.1023/A:1024703012475>.
- Yang, J., Feng, W., Miao, W., 2004. A taxonomic catalogue of freshwater and soil testacea in China, with a discussion of their faunal similarity. *Acta Hydrobiol. Sin.* 28, 426–433 (in Chinese).
- Yang, J., Zhang, W., Feng, W., Shen, Y., 2005. Testate amoebae (Protozoa: Rhizopoda) from Northwest Yunnan, China. *J. Freshw. Ecol.* 20, 583–590. <https://doi.org/10.1080/02705060.2005.966477>.
- Zhang, G., Ran, Y., Wan, W., Luo, W., Chen, W., Xu, F., Li, X., 2021. 100 years of lake evolution over the Qinghai-Tibet Plateau. *Earth Syst. Sci. Data* 13, 3951–3966. <https://doi.org/10.5194/essd-13-3951-2021>.
- Zhu, M., Zhang, H., Chang, F., Li, H., Duan, L., Meng, H., Bi, R., Lu, Z., 2017. The seasonal variations of the water quality of Erhai during 2015-2016. *Adv. Environ. Prot.* 7, 297–308 (s). [10.12677/aep.2017.74042](https://doi.org/10.12677/aep.2017.74042).