First records of testate amoebae from the town of Ugolnye Kopi, Chukotka (Russian Arctic)

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Abstract

Testate amoebae are a useful group of species for biogeographic research. Recent knowledge of the distribution of testate amoebae in the Arctic is incomplete because of large geographic gaps in species distribution data. In this study, we present the first report of testate amoebae from the eastern part of Chukotka in the Russian Arctic, which may at least partially bridge the gap between Alaska and the studied regions of the Russian Arctic. Testate amoebae were collected from 11 waterbodies in the vicinity of the town of Ugolnye Kopi, which is located on the coast of Anadyr Bay in the Bering Sea. Testate amoebae were abundant and active in the studied water bodies, even in the extreme physical environment of Chukotka. The genus and species structure of testate amoebae have been described. We found clear differences in the species structure of the assemblages inhabiting the studied water bodies. Our results showed that most of the testate amoeba assemblages in this part of Chukotka were dominated by *Centropyxis pontigulasiformis*, which is a typical Arctic species, assemblages found in small water bodies show more affinity to those from Spitzbergen, and the assemblage dominated by *Cucurbitella mespiliformis* was not previously reported in the Arctic. These results highlight the limited knowledge of the abundance and diversity of testate amoebae over large areas of the globe.

Introduction

Testate amoebae are a polyphyletic group of protists characterized by cosmopolitan distribution (Smith et al. 2009) and are adapted to survive in a wide range of habitats from terrestrial to lacustrine and saltmarshes (Balik and Song 2000; Mitchell et al. 2008a; Marcisz et al. 2016). Testate amoebae are a good model for biogeographical research (Heger et al. 2011) due to a good preservation of shells in the sediments (Mitchell et al. 2008b) and high indicator potential

of testate amoebae (Freitas et al. 2022). This allows us to study the factors determining their distribution on a long-term scale across the globe.

The controversial discussion on the endemism and cosmopolitism of testate amoebae is still ongoing (Finlay 2004; Foissner 2007; Yang et al. 2010). The Arctic region seems to be an interesting case in this debate, as many of its ecosystems are young and simple. Although testate amoebae have a long history of study, the number of researches dedicated to their distribution in the Arctic remains very low. The distribution of testate amoebae in terrestrial habitats in the Arctic has been better studied than that in lakes. Beyens and Bobrov (2016) in their study counted 378 species in terrestrial habitats, compared to only 40 species in lakes (Beyens et al. 1986).

The geography of the study also has large gaps. There are reports from Canada, North America, Spitzbergen, the coast of the Laptev Sea and from the Novaya Zemlia (Schönborn 1966; Beyens and Chardez 1987, 1997; Bobrov et al. 1999; Trappeniers et al. 1999; Beyens et al. 2009; Anatoly and Wetterich 2012; Mazei et al. 2018) while the territory between Alaska and Novaya Zemlya remains a “white spot.” To fill this gap, we present the first data on testate amoebae from eastern Chukotka.

Study site

Samples were collected from small water bodies in the vicinity of the town and airport of Ugolnye Kopi in Chukotka (64.44.12° N, 177.40.28° E). Because of the extreme inaccessibility of different locations in this region, the choice of sampling sites was logistically constrained.

The town of Ugolye Kopi is located on the shores of the Anadyr Bay of the Bering Sea in the permafrost zone. The climate of the city is subarctic, maritime and harsh. The average temperature in January is -22°C; in July, it varies greatly from year to year, but on average, it is + 12°C. The warm period is very short. The topography of the sampling area is low-lying, with numerous small lakes, some of which freeze completely during winter. The map of the study area, the locations of the water bodies and photos of studied waterbodies are shown on Fig. 1 and on Fig. 2.

Material and methods

Eleven samples were collected on 3rd and 4th of August 2023 in eleven waterbodies. The depth of waterbodies did not exceed 2 m and the length did not exceed 10 m. At each waterbody, one sample was taken from a depth of 0.2–0.5 m in approximately 3 m from the shore. Top 3 cm of a total volume 3–5 cm^3 of bottom sediments were collected for testate amoeba analysis and fixed with 96% ethanol. The geographical location of waterbodies and sampling dates are presented in Table 1.

Table 1
The list of the samples with their location and date of sampling.
Samples ID correspond the number of waterbody on Fig. 1.

Sample ID	Location	Sampling Date
1	64.726056° N, 177.748563° E	03.08.2023
2	64.726171° N, 177.748866° E	03.08.2023
3	64.7177624° N, 177.7406995° E	03.08.2023
4	64.7126889° N, 177.7486030° E	03.08.2023
5	64.7099809° N, 177.7439165° E	03.08.2023
6	64.7104145° N, 177.7341266° E	03.08.2023
7	64.7110931° N, 177.7296152° E	03.08.2023
8	64.7107491° N, 177.7292815° E	03.08.2023
9	64.7105547° N, 177.7285683° E	03.08.2023
10	64.7080061° N, 177.7241132° E	03.08.2023
11	64.7276276° N, 177.7499104° E	04.08.2023

For testate amoeba analysis, 3 ml of the sediments were taken from each sample, mixed with 1–3 ml of glycerin and investigated on the slide using a light microscope (Olympus CX41, Japan) at a magnification of ×200X. All the individuals were counted and identified. The following identification guides were used Todorov & Bankov (2019) and Mazei & Tsyganov (2006).

We used two diversity indices: total taxon richness and Shannon's diversity index. Cluster analysis based on Ward's method was used to quantify the relationships between individual samples. Sampling rarefaction of the entire dataset was used to estimate how taxon richness varied with the number of samples (Colwell et al. 2004). All data analyses were performed using PAST ver. 4.15 (Hammer and Harper 2001).

Results

We identified 44 species belonging to 15 genera, the number of shells per sample varied from 10 to 224 individuals (mean = 81) (Table 2). The number of genera per sample varied from 3 to 11 (mean = 6.4). The maximal occurrence was observed for genera *Difflugia* (11, herein and after number of samples where particular genera or species was found), *Centropyxis* (10), *Arcella* (8) and *Netzelia* (8). Species richness in samples ranged from 4 to 20 (mean = 11.9). Maximal occurrence was observed for species *Difflugia lobostoma* (9), *Arcella hemisphaerica* (8), *Netzelia gramen* (8), *Difflugia minuta* (7) and *Centropyxis pontigulasiformis* (6) (Fig. 2). A total of 26 species, *Arcella rotundata*, *A. gibbosa Centropyxys sylvatica, C. cassis, C. platistoma armata, Cyclopyxis kahli, Difflugia glans*, *D. pulex, D. geosphaerica, D. lithophila, Difflugia sp., D. oranensis, D. elegans, D. lucida, D. pristis, D. levandery, D. viscidula, D. claviformis, D. acuminata, D. mammilaris*, *Lagenodifflugia bryophila, Pseudodifflugia fulva, Trigonopyxis arcula* and *Trinema enchelys* were found in only one sample.

The most abundant genera were *Difflugia* (hereinafter 35% of the total number of identified shells), *Centropyxis* (24%), *Arcella* (18%) and *Cucurbitella* (7%), the most abundant species were *Arcella hemisphaerica* (15%), *Difflugia lobostoma* (13%) and *Centropyxis pontigulasiformis* (13%).

Table 2
The list of testate amoeba species and the number of counted tests in eleven samples from Ugolnye Kopi. Samples ID correspond number of waterbody on Fig. 1.

Species/Sample ID	1	2	3	4	5	6	7	8	9	10	11
Arcella gibbosa Penard, 1890	10									1	
Arcella hemisphaerica Perty, 1852	73	3	9		3		21	9		12	6
Arcella rotundata Playfair, 1918		1									
Arcella vulgaris Ehrenberg, 1830	4	3	7		1			1			
Centropyxis aculeata (Ehrenberg, 1838)			1			1		2			
Centropyxis aerophila Deflandre, 1929	3		14					2		1	
Centropyxis cassis (Wallich, 1864)		2		2							
Centropyxis constricta (Ehrenberg, 1841)		3			1				3		5
Centropyxis platystoma (Penard, 1890)		22	18		3	6		2			
Centropyxis platystoma armata Deflandre, 1929						1					
Centropyxis sp.		1									
Centropyxis sylvatica (Deflandre, 1929)		3				4					
Centropyxis pontigulasiformis (Beyens et al., 1986)	14	28	64		1	7	3				
Cucurbitella mespiliformis Penard, 1902	2							17		43	

Species/Sample ID	1	2	3	4	5	6	7	8	9	10	11
Cyclopyxis eurystoma Deflandre, 1929	1	1								1	
Cyclopyxis kahli Deflandre, 1929			1						1		
Cylindrifflugia acuminata (Ehrenberg, 1838)										1	
Cylindrifflugia elegans (Penard, 1890)						1					
Difflugia angulostoma Gauthier-Lièvre, Thomas, 1958	3		10			1					
Difflugia claviformis Penard, 1899	1										
Difflugia geosphaerica Ogden, 1991		9					1				
Difflugia glans Penard, 1902	1		18								
Difflugia globulosa Dujardin, 1837	1		4		1						
Difflugia levanderi Playfair, 1918			1								
Difflugia lithophila (Penard, 1902)	3										
Difflugia lobostoma Leidy, 1879	8	1	2		100	3	1	2	1		1
Difflugia lucida Penard, 1890						1					
Difflugia mammillaris Penard, 1893				19							

Species/Sample ID	1	2	3	4	5	6	7	8	9	10	11
Difflugia manicata Penard, 1902			20		1	33		2			
Difflugia minuta Rampi, 1950		2	11		2	10			1	2	3
Difflugia mulanensis Yang, Meisterfeld, Zhang et Shen, 2005	8		3								
Difflugia oblonga Ehrenberg, 1838	2	1								6	1
Difflugia oranensis (Gauthier-Lièvre, Thomas, 1958)			2								
Difflugia penardi Hopkinson, 1909			1	9				1			
Difflugia pristis Penard, 1902		1									
Difflugia pulex Penard, 1902	2		8								
Difflugia sp.											2
Galeripora discoides (Ehrenberg, 1843)		1	1								3
Golemanskia viscidula (Penard, 1902)	1										
Lagenodifflugia bryophila (Penard, 1902)						1				1	
Netzelia gramen (Penard, 1902)	7	6	7			1	7	1	2	26	
Pontigulasia rhumbleri Hopkinson, 1919	1		4		1				2		
Pseudodifflugia fulva Archer, 1870			14	3							

Species/Sample ID	1	2	3	4	5	6	7	8	9	10	11
Trigonopyxis arcula (Leidy, 1879)		1									
Trinema enchelys (Ehrenberg, 1838)		4	3								
Zivkovicia spectabilis (Penard, 1902)	8	6	1					5			2
Total	153	99	224	33	114	70	33	44	10	94	23
Taxa richness (S)	20	20	24	4	10	13	5	11	6	10	8
Shannon Index (H)	2,15	2,42	2,67	1,07	0,68	1,85	1,07	1,95	1,72	1,53	1,95

Ward Cluster analysis (Fig. 4) distinguished 4 groups of samples. Group 1 included the sample 5 and dominated by *D. lobostoma* (Fig. 3, c1, c2), Group 2 included the sample 4 and dominated by *Difflugia mammilaris* (Fig. 3, h), Group 3 included two samples: 1 and 7 and was dominated by *A. hemisphaerica* (Fig. 3, l) and Group 4 included samples 2, 3, 6, 8–11 and was dominated by *C. pontigulasiformis* (Fig. 3, j) and subdominated by *D. manicata, C. mespiliformis* (Fig. 3, d1, d2) and *C. constricta (*Fig. 3, f).

The overall sample rarefaction curve (Fig. 5.) did not reach an asymptote, suggesting that the amoeba counts were insufficient to identify all taxa. Thus, our results should not be considered representative of a comprehensive description of the testate amoebae complex in this region.

The values of Shannon diversity indices ranged from 0.68 in sample 5 to 2.67 in sample 3, species riches in samples varied from 4 to 24 (mean = 11.9) (Table 2).

Discussion

Our results show that testate amoebae are present even under the harsh conditions of small water bodies in Chukotka, and the presence of tests at the reproduction stage (Fig. 2, g) suggests that testate amoebae are alive.

The species composition of the assemblages includes species previously reported as typical for soils, lakes, and wetlands in the Arctic and subarctic zones (Beyens et al. 1986, 1995; Azovsky and Mazei 2018; Mazei et al. 2018). We did not identify any tests that could not be assigned to the known species of testate amoebae.

The most common genera for the Arctic region are known to be *Centropyxis*, *Difflugia*, *Euglyph*a, *Nebela*, *Arcella* and *Trinema* (Beyens et al. 1995; Trappeniers et al. 1999; Mattheeussen et al. 2005; Beyens and Bobrov 2016), in our case the most common genera were *Centropyxis*, *Difflugia*, *Arcella* and *Cucurbitella* while we did not found any specimen belonging to genera *Euglypha* or *Nebela*, the genus *Trinema* was represented by only a few specimen. This phenomenon may be explained either by the absence of species of these genera in a given region or by local environmental conditions.

Among the most abundant species found in the area – *Arcella hemisphaerica* and *Centropyxis pontigulasiformis* – were previously reported as dominant (Schönborn 1966) or "flagship" (Beyens and Bobrov 2016) species in different habitats of the Arctic region; however, a high abundance of *Cucurbitella mespiliformis* was registered for the first time. We also found some taxa that have rarely been reported from the Arctic, including *Cylindrifflugia acuminata*, *Centropyxis constricta*, *D. claviformis*, *D. viscidula*, *Pseudodifflugia fulva*, and *Arcella vulgaris*. Some of these species were found in a single specimen in a single waterbody, for example, *D. viscidula* and *D. claviformis*, but others were found in multiple waterbodies and were relatively abundant, such as *C. constricta* and *A. vulgaris*. It is possible that the environmental conditions in the studied waterbodies were favorable for these taxa, but taxonomic uncertainty and a very limited number of samples make it impossible to draw a clear conclusion.

The fact that Ward's cluster analysis distinguished four clusters within 11 samples collected in fairly close waterbodies may indicate high heterogeneity in their environmental conditions.

Group 1 was dominated by *D. lobostoma* and was characterized by minimal values of Shannon diversity indices and low species richness. This may indicate a high concentration of suspended inorganic material at the sampling site or waterbody (Schwind et al. 2018). The very low number of individuals and species found in the sample assigned to Group 2 may indicate extremely unfavorable conditions for most of the species in the given waterbody or sampling site. Samples assigned to Group 3 were found in small waterbodies, and the dominance of *A. hemisphaerica* was consistent with that previously described in Spitzbergen (Schönborn 1966). The most abundant species in Group 4 was *Centropyxis pontigulasiformis*, which is mentioned as a potential "flagship" species for the Arctic (Beyens and Bobrov 2016). The samples assigned to this group were characterized by the maximum values of the Shannon indices and species richness. Interestingly, this group was dominated by *C. mespiliformis*, which is a typical benthic algivorous species (Balik and Song 2000), and *D. manicata*, which also uses Cyanophyta and diatoms (Burbidge and Schröder-Adams 1998) and organisms associated with "zoochlorellae" (Christopher and Patterson 1983) as a food source. *C. constricta* is a ubiquitous species previously reported on the Eastern North American Coast (Collins et al. 1990).

Conclusion

Testate amoebae play a significant role in lake ecosystems and can be used as model organisms to study global microbial biogeography. However, our knowledge of the species diversity and distribution of testate amoebae in several regions is still limited. Our study of testate amoebae in Chukotka fills a gap in the Arctic and shows that testate amoebae can survive even in cold polar environments. This is the next step towards understanding the distribution and biogeography of protists in the Arctic. Future studies are required to explain the differences in the species structure of testate amoebae assemblages in Chukotka and other Arctic regions demonstrated in our study.

Declarations

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Figures

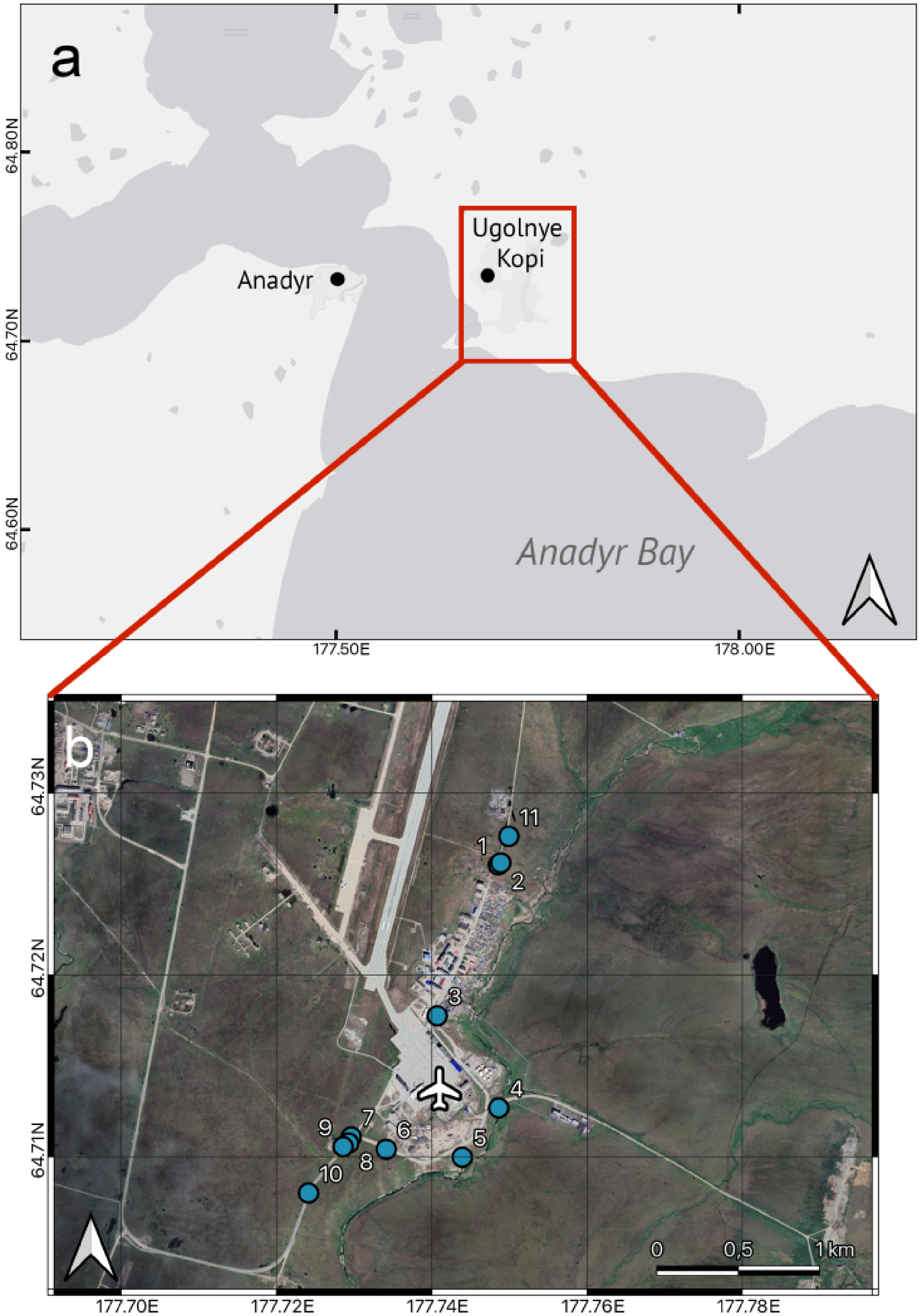

Figure 1

The map of the study area (a) and the locations of the water bodies (b). The aircraft icon marks the airport Anadyr.

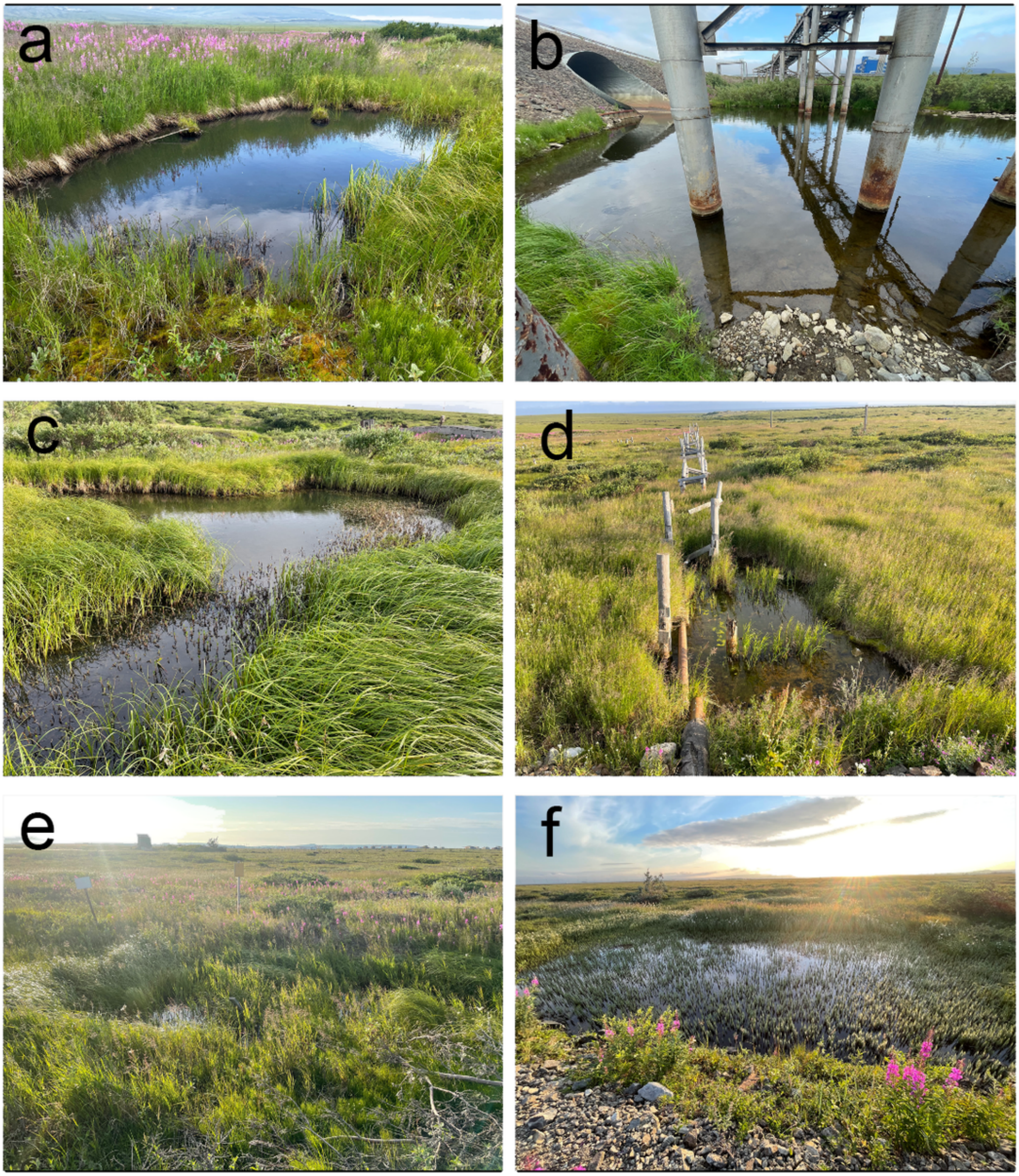

Figure 2

The photos of some of studied waterbodies, a (2), b (4), c (5), d (6), e (7), f (10). Numbers correspond the number of waterbody on Fig. 1.

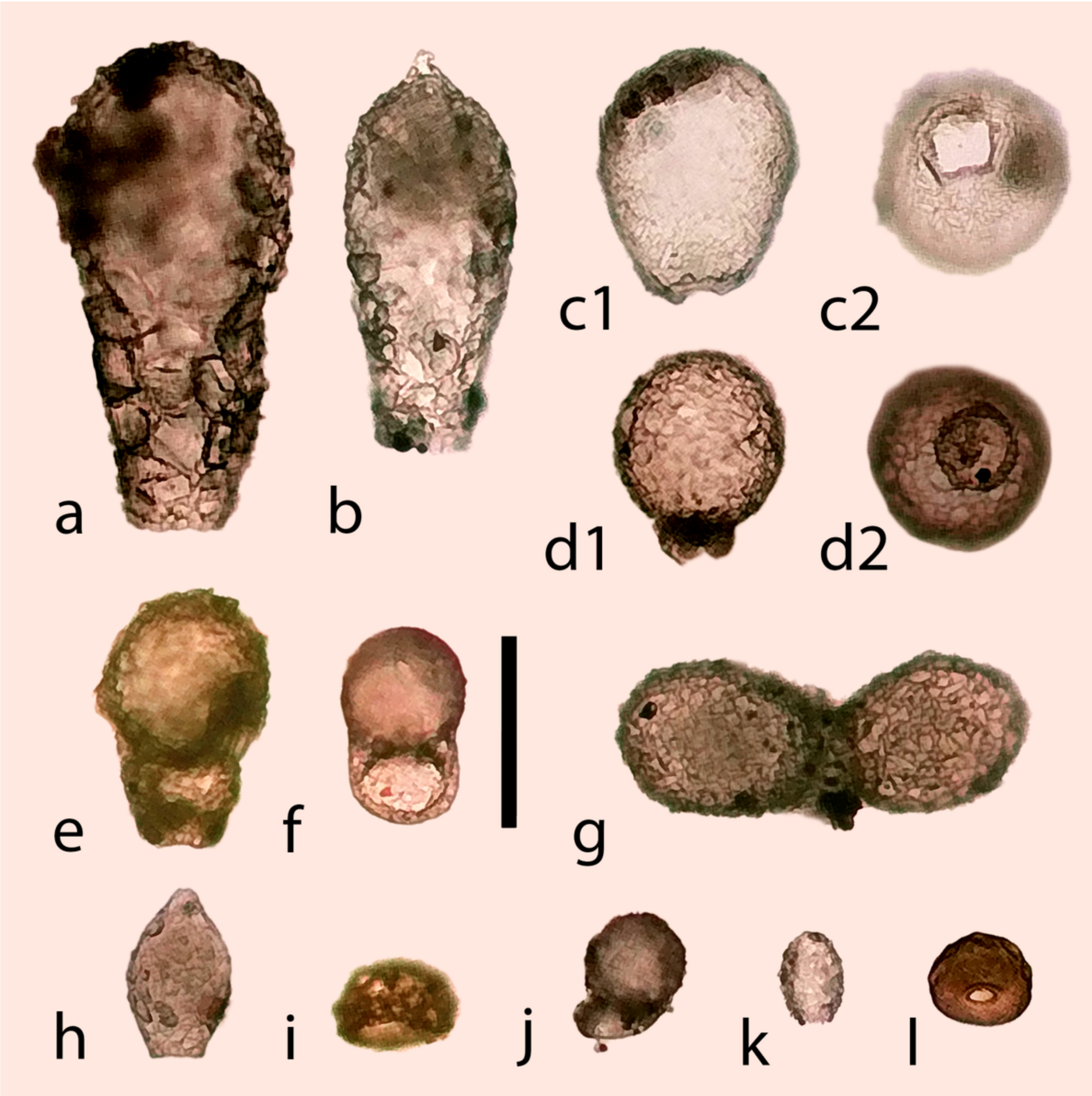

Figure 3

Amoeba tests, found in samples from small waterbodies near Ugolnye Kopi: **a** – *Difflugia oblonga*, **b** – *Cylindrifflugia acuminata*, **c1** – *Difflugia lobostoma*, **c2** – *Difflugia lobostoma*, aperture view, **d1** – *Cucurbitella mespiliformis*, **d2** – *Cucurbitella mespiliformis*, aperture view, **e** – *Zivkovicea spectabilis*, **f** – *Centropyxis constricta*, **g** – reproduction of *C.mespiliformis*, **h** – *Difflugia mammilaris*, **i** – *Cyclopyxix euristoma*, **j** – *Centropyxis pontigulasiformis*, **k** – *Difflugia penardi*, **l** – *Arcella hemisphaerica*. Scale bar – 100 μm.

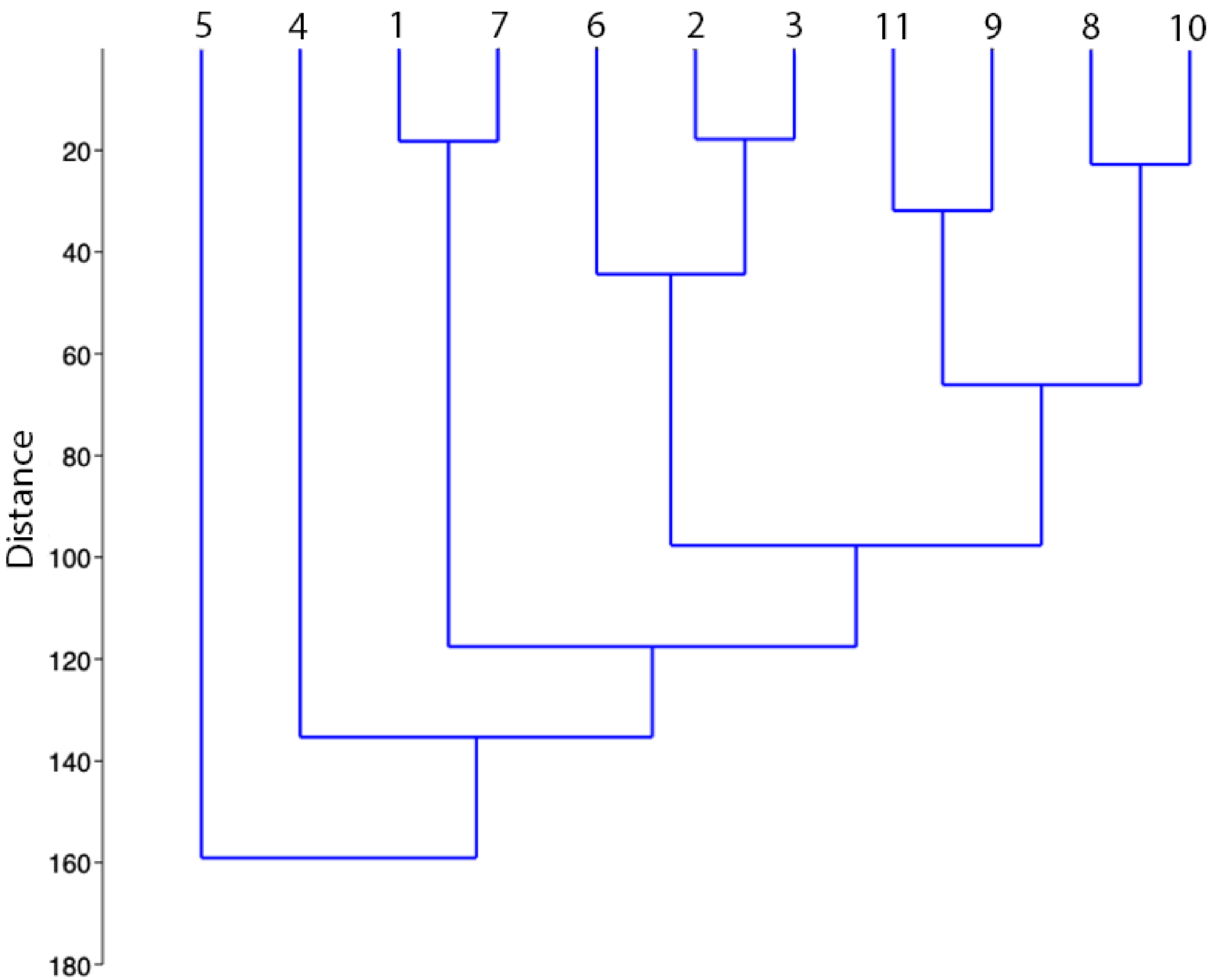

Figure 4

Cluster analysis dendrogram of testate amoeba relative abundance data for all samples. Analysis based on Ward’s method clustering**.** Numbers on the diagram correspond the number of the waterbody on Fig. 1.

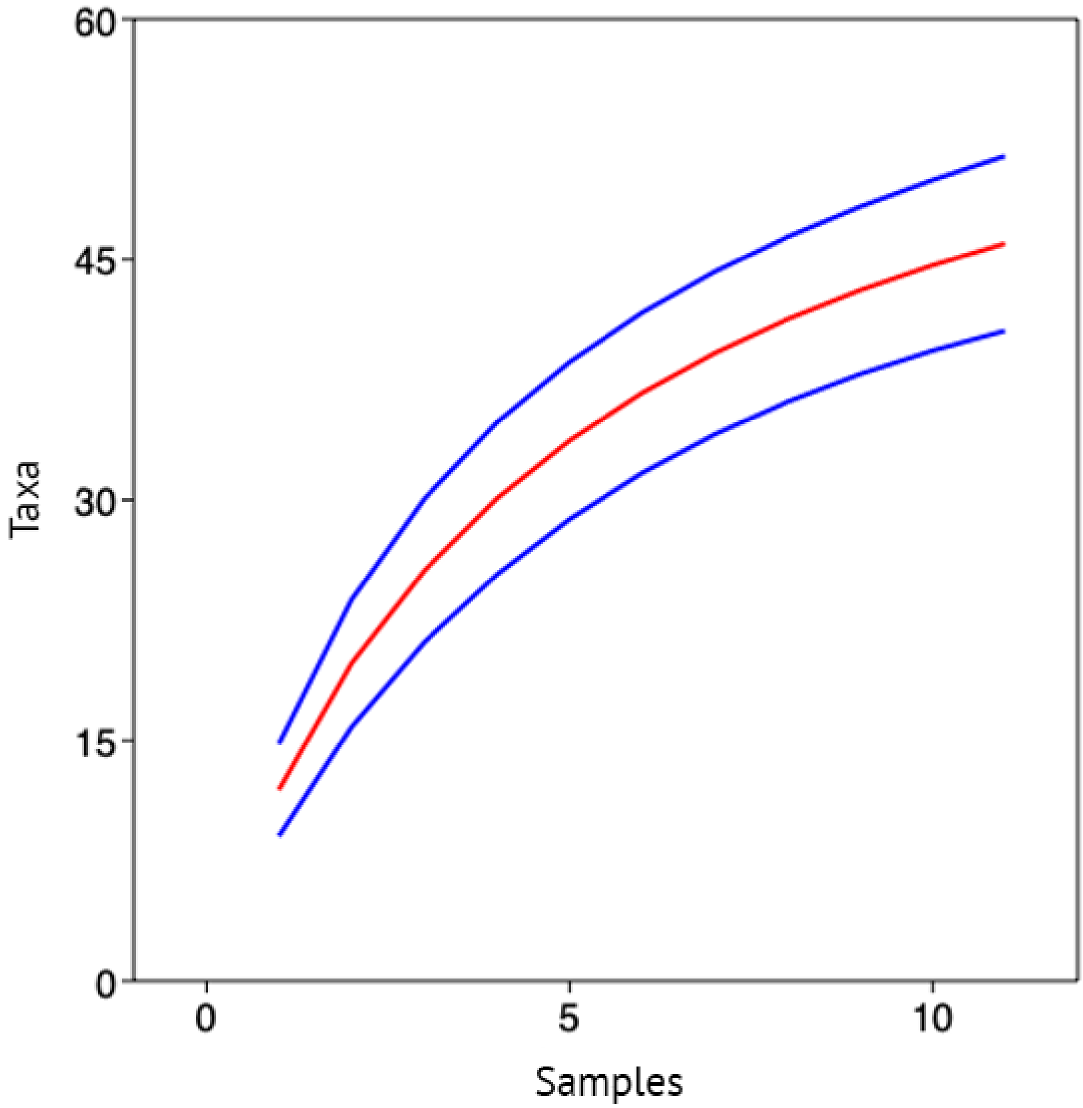

Figure 5

Overall sample rarefaction curve (red line) for entire dataset based on Mao's Tau showing standard errors (blue lines)