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ORIGINAL ARTICLE

Diversity and abundance of naked lobose amoebae belonging to the classes Tubulinea and Discosea in pond sediments in Moscow urban parks

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Summary

Naked lobose amoebae (also known as gymnamoebae) nowadays are split into the Tubulinea and Discosea lineages within the Amoebozoa supergroup; few genera belong to the Variosea lineage. Morphological identification of amoeba species is difficult because it requires study of clonal cultures. Most of species nowadays require molecular data for precise identification. The deficiency of these data results in the lack of reliable information on the local amoebae faunas, their abundance and distribution in natural and artificial habitats. In this paper we provide faunistic and quantitative data on naked lobose amoebae from bottom sediments of ponds located in urban parks of Moscow, Russia. We applied enrichment cultivation, followed by light-microscopic study, and in some cases – electron-microscopic and molecular studies. In total, 29 amoebae species were found. Most of recovered isolates might be new to science. The number of amoebae cells in the sediments, estimated using the most probable number method, varied from 75 to 288 cells ml^{-1}. Overall, the diversity and abundance of naked amoebae in urban park ponds is comparable with that in intact biotopes, and they are not affected by the urbanization-driven pollution. These results suggest that urban parks play an essential role in maintaining and preserving eukaryotic microbial diversity in the anthropogenic environment.

Key words: amoebae, Amoebozoa, diversity, abundance, ecology, urban environment

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Introduction

Naked lobose amoebae, formerly known as gymnamoebae or Gymnamoebia sensu Page (1987), nowadays are split into the Tubulinea and Discosea lineages of the supergroup Amoebozoa; few genera belong to the Variosea lineage (Adl et al., 2019). In the system by Smirnov et al. (2011), these lineages are ranked as classes.

Amoeboid protists are among the most difficult objects for species identification. Morphological identification of naked amoebae is an expert task, it usually requires clonal cultures (Page, 1988; 1991; Smirnov and Goodkov, 1999; Rogerson and Patterson, 2002), and anyway often remains problematic (Smirnov and Brown, 2004; Smirnov, 2008; Kudryavtsev and Hausmann, 2009; Mesentsev and Smirnov, 2019; Mesentsev et al., 2021). Nowadays, molecular data are necessary for correct identification of most naked amoebae species (Mesentsev et al., 2020, 2022). As a result, faunistic and ecological studies of naked amoebae are still limited in number.

Observation of naked amoebae in fresh samples is almost unfeasible because they generally become detectable only when their abundance increases after several cycles of cell division, which usually takes a few days and depends on the cell size and growth conditions. Therefore, microscopical identification and enumeration of amoebae in natural samples requires application of an enrichment cultivation approach (Cutler, 1920; Severtsoff, 1922; Page, 1988; Darbshire et al., 1996). This approach is fundamentally handicapped because only the species that can grow and multiply in the used enrichment medium can be detected during observations (Smirnov, 2003, 2007). Thus, any faunistic study reveals just a subset of species inhabiting a particular habitat. Moreover, this approach does not allow researchers to differentiate cysts and trophozoites, because amoebae can excyst and multiply under appropriate conditions. However, it remains the only possible way to observe and study the diversity of amoebae at the morphological level.

Application of molecular methods, including environmental DNA studies, did not solve the problem of unveiling the amoeba diversity, mostly because of the absence of group-specific primers (Adl et al., 2014; Geisen et al., 2015). As a result, molecular ecological studies of naked lobose amoebae are limited and remain focused on pathogenic groups, like *Acanthamoeba* (Garcia et al., 2013; Abd El Wahab et al., 2018; Ren et al., 2018; Samba-Louaka et al., 2019). So far, few molecular studies have provided datasets showing representative environmental diversity of naked amoeba genotypes belonging to different lineages (Tsao et al., 2019).

For the same reasons, the quantification of naked amoebae in environmental samples is a challenging task. Amoebae cells cannot be directly counted in a sample. There were several attempts to apply fluorescent dyes for that purpose (Rogerson, 1988, 1991; Rogerson and Laybourn-Parry, 1992). However, this approach was not widely accepted due to the low selectivity of the method. At the morphological level, the dilution method remains the only practically available (Cutler, 1920; Singh, 1946; Darbyshire et al., 1974; Rønn et al., 1995; Smirnov et al., 1998). It is culture-based and quite laborious. However, it allows simultaneous observation of locomotive amoebae together with the enumeration of cells and provides cultures for further identification and study. In recent decades, the method has been improved and simplified by using the Poisson-based estimates of the most probable number (MPN) of amoebae cells in a single dilution set (Anderson 1998; Smirnov et al., 1998; Garstecki and Arndt, 2000). Like in other methods based on the enrichment cultivation, biases related to the selective recovery of species and the absence of the possibility to differentiate active and resting stages of amoebae remain valid. However, currently there are no other methods of amoeba enumeration.

In this paper, we estimated the abundance and species diversity of naked lobose amoebae belonging to the classes Tubulinea and Discosea (Amoebozoa) in the top layer of the bottom sediment of ponds located in three Moscow urban parks. The study was performed using an enrichment cultivation approach, followed by light-microscopic and molecular identification of isolated organisms.

Material and methods

Sampling

Samples of the upper layer of bottom sediment were collected in three ponds of Moscow urban parks: Apothecaries' Pond in "The Apothecaries' Garden" (55°46'44.4"N 37°38'10.4"E), Sobachiy pond in the park Izmailovsky (55°46'46.0"N 37°46'08.3"E), and Oleniy pond in the park Sokolniki (55°48'05.6"N 37°41'36.2"E) (Fig. 1) on August 20, 2020 (two sets of samples from each pond) and June 6, 2021 (one set of samples from each pond). Photographs of

Table 1. Temperature and hydrochemical characteristics of the studied ponds.

	Apothecaries' Pond	Sobachiy Pond	Oleniy Pond
August 20, 2020			
Temperature, °C	15.1	18.1	18.1
pH	7.21	7.55	7.71
ORP, mV	–160	83	153
TDS, µS/cm	326	178	869
June 6, 2021			
Temperature, °C	16.0	18.8	19.5
pH	7.34	7.71	7.92
ORP, mV	Not measured	94	120
TDS, µS/cm	415	261	1203

Notes: ORP – Oxidation-reduction potential; TDS – total dissolved solids

these ponds and closer views of sampled biotopes are shown in Figure 1. Hydrochemical parameters were measured onsite using portable HANNA (USA) devices during the sampling (Table 1). The top layer of bottom sediments (ca. 20 cm^3) was collected in the littoral zone at a depth of approximately 50 cm in plastic bottles. In 2020, two samples were collected at a distance of ca. 50 cm; in 2021, one sample was collected in each pond. Samples were transported to the laboratory and inoculated on the same day.

Inoculation, observation and quantification

For species identification and enumeration of amoebae, samples were carefully shaken to mix the sampled material, placed in the graded tube and left for several minutes to settle down. The volume of the sediment was adjusted to 1 ml using sterile plastic pipette to remove the extra amount of sediments. The remaining 1 ml of the sediment was diluted in 1000 ml of 0.025% WG infusion (see Geisen et al., 2014 for the protocol) made on PJ medium (Prescott and James, 1955) and inoculated into fifty Petri dishes (60 mm in diameter) under constant shaking to ensure homogeneous distribution of the materials among dishes. In addition, twenty Petri dishes (90 mm in diameter) were filled with the same medium and inoculated with 0.2-0.5 ml of the sediment to better estimate the diversity of amoebae.

Cultures were observed for the presence of amoebae after 7 and 14 days of cultivation using an inverted Nikon TS100-F microscope. Amoebae-positive dishes were marked and a cumulative list of species found during the first and the second examinations was created for each dish. Preliminary identification of amoebae was performed following Page (1988, 1991) keys and other relevant literature. For detailed light-microscopic investigation, cells were transferred to glass slides and observed with an upright microscope Leica DM2500 equipped with phase contrast and differential interference contrast (DIC). Amoebae were photographed and video-recorded using a Nikon DS-Fi3 camera. For measurement, we used NisElements AR software (Nikon). For every found isolate, we attempted to establish clonal cultures using the same liquid WG medium and wMY agar medium (Spiegel et al., 1995), overlayed with liquid WG medium.

The treatment of MPN series was performed as suggested by Garstecki and Arndt (2000) and further modified by Smirnov (2002). In the 50-dish series, every amoeba species was counted individually that resulted in a table showing the occurrence of every taxon. The total number of findings was statistically treated (op. cit.) to get the MPN number for every individual species. To get the total number of amoebae, obtained MPN numbers were summarized.

DNA extraction from single cells

For the DNA extraction, single cells were collected, washed twice in millipore-filtered (0.22 µm) PJ solution and transferred with 1-2 µl of PJ in a 200 µm PCR tube. DNA was extracted using the Arcturus PicoPure DNA extraction Kit (thermo Fischer Scientific, USA) following the manufacturer's instructions. The 18S rRNA gene was amplified by PCR using eukaryotic primers RibA (forward) and RibB (reverse) (Medlin et al., 1988; Pawlowski, 2000). Thermal cycle parameters were: initial denaturation (10 min at 95 °C) followed

Fig. 1. Images of sampling sites and closer view of the sampled habitats: A–B – Sobachiy Pond; C–D – Oleniy Pond; E–F – Apothecaries' Pond. Not to scale.

by 39 cycles of 30 s at 94 °C, 60 s at 58 °C and 120 s at 72 °C, followed by 10 min at 72 °C for the final extension. Amplicons were purified in 1.5% agarose gel using Cleanup mini Purification Kit (Eurogene, Moscow, Russia) and sequenced using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit with the RibA, s6F, s12.2, s12.2R, s14 and s20R primers for 18S rRNA gene (Medlin et al., 1988; Pawlowski, 2000; Adl et al., 2014). Search in the GenBank database (Benson et al., 2013) was performed using BLASTN (Zhang et al., 2000) on the NCBI web-site (https://www.ncbi.nlm.nih.gov/).

Results

A total of 29 morphospecies of naked lobose amoebae were discovered during the study. Twenty-three species were found during the quantitative analysis and six additional species were observed in 90 mm Petri dishes used for the recovery of amoeba diversity (Table 2, Figs 2-3). The observed morphospecies belong to three classes: Tubulinea (6 morphospecies), Discosea (22 morphospecies) and Variosea (one morphospecies, *Flamella* sp.). Six isolates were assigned to known species based on morphological and molecular data, while the rest were identified to the genus or higher taxonomical levels generally based on light microscopy (electron-microscopy for several species). Most of the revealed isolates evidently represent new species that should be described during the future studies. Complete or partial SSU rRNA gene sequences were obtained from nine isolates (Table 2) and used to confirm their identification.

The species diversity in the ponds was analyzed based on the samples collected in 2021, because by that time we had better knowledge of the diversity of amoebae and identified more species within the genera *Cochliopodium* and *Korotnevella* than in 2020 (Table 2). The species number of naked lobose amoebae was the greatest in the Sobachiy (the maximum number of species was 18) and Oleniy ponds (12), whereas the lowest value (10) was observed in the Apothecaries' Pond.

One of the most interesting isolates was a strain of *Paradermamoeba valamo* Smirnov et Goodkov 1993 (Fig. 2, D), identified by light and electron microscopy. We obtained a SSU sequence of this strain and included this species in the phylogenetic tree of Amoebozoa (Smirnov et al., 2020). In addition, we obtained an SSU sequence for a strain of *Thecamoeba striata* (Figs 2, I; 3, F). This sequence was identical to the partial sequence of the currently lost CCAP 1583/4 strain of the species in all its 1083 bp length (Mesentsev et al., 2022). This means that the studied species represents *Thecamoeba striata* (Penard 1890) Schaeffer 1926. The result can be considered as a case of reliable re-isolation of a species from a distant location.

Another interesting finding was a representative of the genus *Endostelium* Olive, Bennett et Deasey 1984 (order Pellitida) (Fig. 2, K) as shown by the results of SSU gene sequencing. The species *Vannella simplex* Wohlfarth-Bottermann 1960, which is widely distributed worldwide (Smir-nov et al., 2002), was observed in the present stu-dy that adds another geographic location to its distribution range.

Data on the abundance of amoebae species and the total number of amoebae are presented in Table 2. The highest numbers of amoebae were found in Sobachiy and Oleniy ponds, while Apothecaries' Pond showed the lowest abundance of amoebae both in 2020 and 2021. The maximal difference in the total number of amoebae in two samples collected from the same pond in August 2020 did not exceed 20%. This was the case of Sobachiy pond, while in Oleniy pond the number of cells was almost the same in two independent samples. However, the abundance of amoebae in 2020 and 2021 considerably differed in all ponds. In Oleniy pond, the difference exceeded two times (up to 272 ml^{-1} in 2020 and 134 ml^{-1} in 2021). In two other ponds the difference was lower.

The total number of detected amoebae cells in one milliliter of the bottom sediment varied from 75 ml^{-1} in Apothecaries' Pond in August, 2020 to 288 ml^{-1} in Sobachiy pond in June, 2021.

Discussion

Species diversity

During this study, we discovered 29 morpho-species of naked lobose amoebae. This number is comparable to similar studies made on various freshwater and marine habitats. Environmental conditions in different habitats, the methods of sampling and study significantly vary from one study to another, so it might be difficult to compare the data directly (Kyle and Noblet, 1987; Smirnov and Goodkov, 1996; Smirnov et al., 1998; Smirnov, 2003; Rodriguez-Zaragoza et al., 2005; Kiss et al.,

Table 2. Species list and abundance of species in the bottom sediments of Moscow park ponds. In bold are species, identified or classified basing on molecular data (SSU gene sequencing).

	Species	Apothecaries' Pond			Sobachiy Pond			Oleniy Pond		
		20.08.2020 Sample #1	20.08.2020 Sample #2	06.06.2021 Sample #3	20.08.2020 Sample #1	20.08.2020 Sample #2	06.06.2021 Sample #3	20.08.2020 Sample #1	20.08.2020 Sample #2	06.06.2021 Sample #3
1	*Cochliopodium* sp. (1)	6	6	9	5	33	42	8	22	16
2	*Cochliopodium* sp. (2)			15			57			8
3	*Cochliopodium* sp. (3)			2			4			10
4	*Cochliopodium* sp. (4)			_			1			_
5	*Korotnevella* sp. (1)	3	4	4	46	54	29	33	54	12
6	*Korotnevella* sp. (2)			_			18			11
7	*Korotnevella* sp. (3)			_			5			_
8	***Mayorella* sp. (1)**	12	4	14	14	27	6	48	60	15
9	***Mayorella* sp. (2)**			19			1			4
10	*Vannella* simplex	60	30	0	39	10	37	37	35	29
11	*Vannella* sp.			16	51	27	71	43	48	19
12	***Saccamoeba* sp.**	1	1	3	11	9	0	8	4	0
13	*Stenamoeba* sp.	1	6	0	4	1	2	1	2	0
14	*Vexillifera* sp.	4	15	0	3	0	1	0	14	0
15	***Paradermamoeba levis***	2	0	3	22	6	6	57	16	2
16	***Thecamoeba striata***	0	0	0	1	0	1	0	0	0
17	*Flamella* sp.	0	9	0	2	0	0	5	8	0
18	«*Gocevia* sp.»	0	0	12	0	0	0	0	0	8
19	Hartmannellidae gen. sp.	0	0	0	3	0	0	14	1	0
20	*Dermamoeba* sp.	0	0	0	2	0	0	0	0	0
21	***Leptomyxa regia***	0	0	0	8	3	2	18	5	0
22	*Amoeba* sp.	0	0	0	1	0	1	0	2	0
23	Vannellidae gen. sp.	0	0	0	0	0	4	0	0	0
	Amoeba cells total, ml^{-1}, MPN	**89**	**75**	**97**	**212**	**170**	**288**	**272**	**271**	**134**
	Species total	**8**	**8**	**10**	**15**	**9**	**18**	**11**	**13**	**11**
	Found without counting of individuals in 90 mm dishes									
24	***Endostelium*. sp.**				+					
25	Pellitida gen. sp.	+								
26	***Paradermamoeba valamo***				+					
27	*Polychaos* sp.				+			+		
28	*Thecamoeba* cf. *quadrilineata*				+					
29	*Echinamoeba* sp.	+								

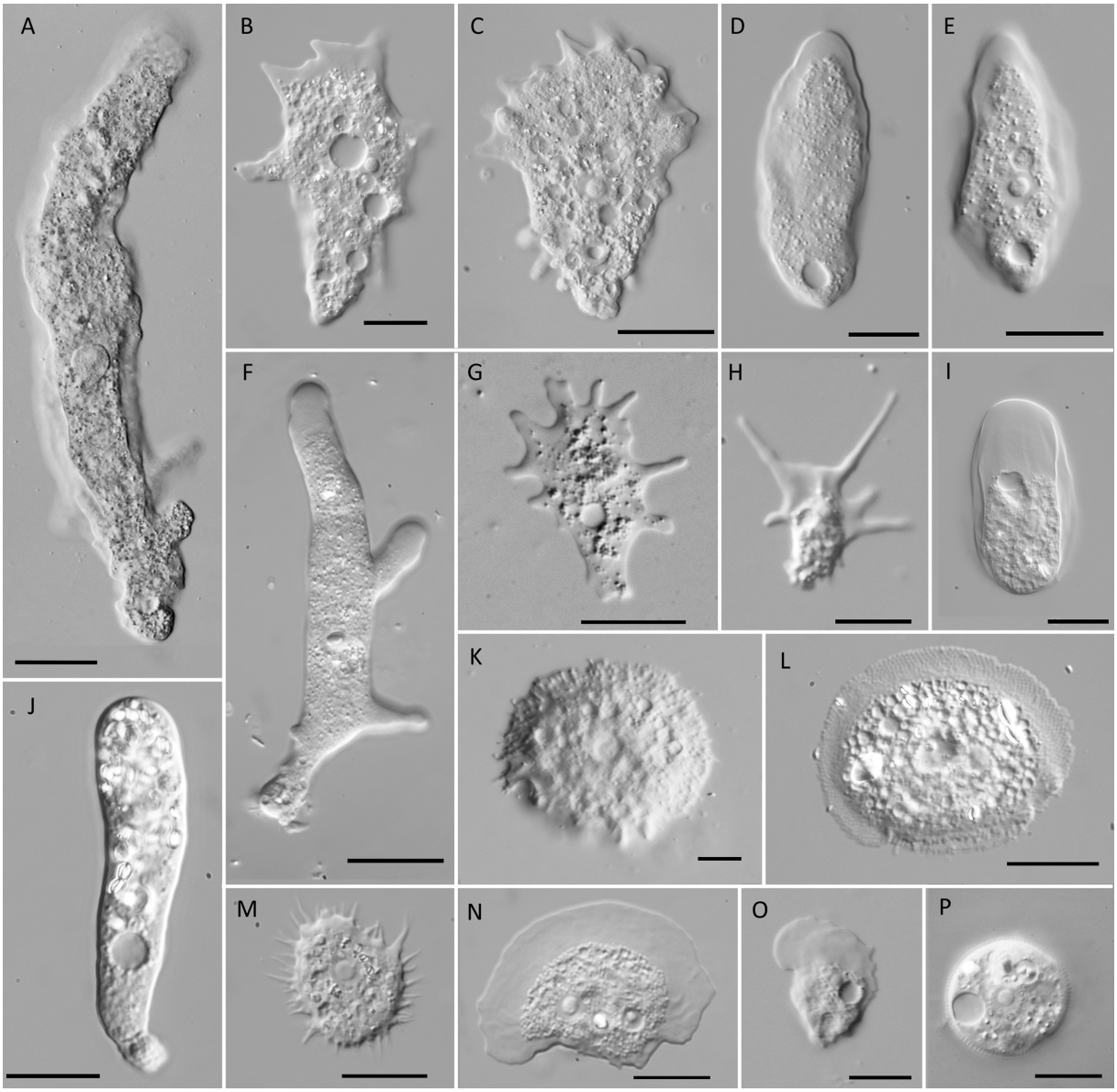

Fig. 2. DIC images of amoebae species recovered from the bottom sediment of the ponds, located in Moscow urban parks. Cells are moving on the surface of the object slide, under upright microscope. A – *Amoeba* sp.; B – *Mayorella* sp. (1); C – *Mayorella* sp. (2); D – *Paradermamoeba valamo*; E – *Paradermamoeba levis*; F – *Leptomyxa regia*; G – *Korotnevella* sp. (1); H – *Vexillifera* sp.; I – *Thecamoeba striata*; J – *Saccamoeba* sp.; K – *Endostelium* sp.; L – *Cochliopodium s*p. (4); M – *Echinamoeba* sp.; N – *Vannella simplex*; O – *Vannella* sp.; P – *Cochliopodium* sp. (3). Scale bars: A - 40 μm, B-G and I-N - 20; H, O-P – 10 μm.

2009). Overall, the number of species found in the studied habitats is comparable with that recovered by enrichment cultivation of different kinds from other freshwater lakes, or even exceeds these amounts. For instance, O'Dell (1979) discovered 15 species of amoeba in 12 samples of the upper layer of bottom sediments from the freshwater Papio Lake in 12 samples of the upper layer of bottom sediments. Smirnov and Goodkov (1996) reported 39 species of naked lobose amoebae in the bottom sediments of the Leshchevo Lake on Valamo Island as a result of observations for several years. Patsyuk and Dovgal (2012) noted up to 30 species of naked amoebae in various freshwater reservoirs. Probably the maximal recorded number of naked amoebae morphospecies was 64 species recovered during the long-term and

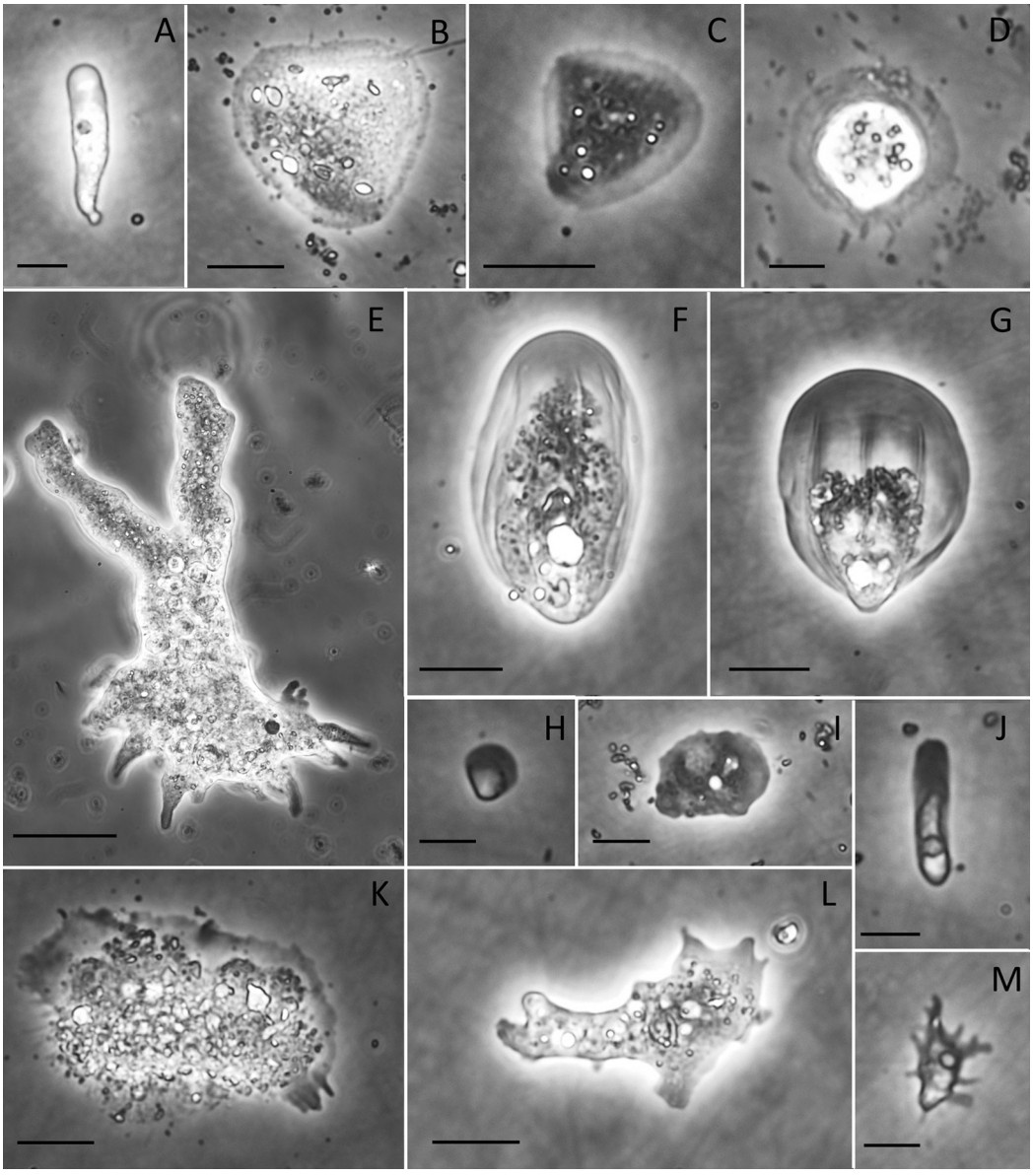

Fig. 3. Phase-contrast images of amoebae species recovered from the bottom sediment of the ponds, located in Moscow urban parks. Cells are moving on the surface of plastic Petri dish, in culture, under inverted microscope. A – Hartmannellida gen. sp.; B – *Cochliopodium* sp. (2); C – *Cochliopodium* sp. (1); D – *Cochliopodium* sp. (3); E – *Polychaos* sp.; F – *Thecamoeba striata*; G – *Thecamoeba* cf *quadrilineata*; H – Vannellida gen. sp.; I – *Vannella* sp.; J – *Stenamoeba* sp.; K – "*Gocevia* sp".; L – *Mayorella* sp. (1); M – *Korotnevella* sp. (2). Scale bars: A, D, H, J, M – 5 μm; C, F-G, L – 10 μm; I – 15 μm; E – 60 μm.

very detailed study of a productive pond Priest Pot in the English Lake District (Finlay et al., 2000; Finlay and Fenchel, 2004).

The data available for marine and brackish waters are also comparable. For example, 16 species were isolated from Chincoteague Bay (Sawyer, 1975a, 1975b) and can be considered as a moderate diversity. Sawyer (1980) listed up to 19 species from a single site in the Atlantic Ocean, and up to 20 species were recorded in the New York Bight Apex (Sawyer and Bodammer, 1983). Rogerson (1991) found 27 species in samples collected from the surface of seaweed near Isle of Cumbrae (the Firth of Clyde, Scotland). Butler and Rogerson (1995) found about 70 amoeba morphotypes in the bottom sediments of the Firth of Clyde. It is logical that long-term studies

and sampling of different biotopes at various seasons allow recovering more species rather than studies of a single type of biotopes (e.g., bottom sediments). So, the results obtained in the present study are comparable with data reported from other habitats using morphological methods (e.g., Smirnov, 2003; Patsyuk and Dovgal, 2012; Patsyuk, 2014).

In modern studies, the massive parallel sequencing method was applied to estimate the diversity of protists, including naked amoebae. These studies were mostly focused on pathogenic species (e.g., Garcia et al., 2013; Delafont et al., 2014, 2019; Scheikl et al., 2016). Geisen and coauthors (2015) carried out a metatranscriptomic analysis of the soil samples; however, they did not select individuals but counted the total number of amoebozoan genotypes. Other researchers performed a general metatranscriptomic analysis of 18S rRNA genes, providing only the percentage ratio of Amoebozoa to other eukaryotes (e.g., Stoeck et al., 2010). Few authors have attempted to estimate the overall diversity of Amoebozoa, but in most cases, they were facing serious technical problems, mostly related to the primer specificity. Tsao and coauthors (2019) found 79 phylotypes belonging to Tubulinea, Discosea and Variosea in the DNA isolated from the samples of cooling tower water. This amount exceeds the maximal numbers of species recovered in the morphological studies, and the taxonomic coverage in the cited study looks rather representative, showing that the molecular approach, as applied in this paper, may be a promising way for unveiling the lobose amoeba diversity.

Abundance of amoebae in pond sediments

The recovered abundance of amoebae in our study varied from 75 to 288 cells ml^{-1}, which is a modest value, comparing with the abundance of amoebae in many other habitats. Smirnov and coauthors (1998) recorded from 100 to 500 cells ml^{-1} of amoebae in the top layer of the bottom sediment of a freshwater Lake Leshevoe (Valamo Island, Ladoga Lake, Russia). Anderson (2007), in an annual study, found from 81 cells ml^{-1} of amoebae in January to 1568 cells ml^{-1} in June (early summer peak) and 1813 cells ml^{-1} in October (autumn peak). O'Dell (1979) counted up to 2100 cells of *Acanthamoeba polyphaga* in one gram of the bottom sediment of the Papio Lake. The wide range of abundance reported in those studies probably reflect both the biases of culture-based methods of amoeba count and the true variation in amoeba abundance in various substrates and biotopes at different seasons. Another reason is probably the pronounced heterogeneity of amoeba distribution in natural biotopes and the patchy distribution of many species (Anderson, 2002; Smirnov and Thar, 2003).

The abundance of naked lobose amoebae in other biotopes, like mosses and soil, may be even higher than in water habitats. Direct count of large amoebae in *Sphagnum*-dominated bog reported up to 15575 amoebae cells per one cubical decimeter of substrate, which corresponds to ca. 15 cells cm^{-3} (Rogerson, 1982). A much greater abundances of amoebae can be found in soil. The reported amounts vary from 3200 cells g^{-1} (Menapace, 1975) to 421000 - 1690000 cells g^{-1} (Brzezinska-Dudziak, 1953). Taken together, the data on water, soil and other studied biotopes suggest that amoeboid protists play an important role in mass and energy flows in the environment. Overall, the diversity and abundance of naked amoebae in urban park ponds, reported in the present study, is comparable with those in various intact biotopes, not affected by urbanization. This evidences the essential role of urban parks in maintaining and preservation of biodiversity in the anthropogenic environment.

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References

Abd El Wahab W.M., El-Badry A.A. and Hamdy D.A. 2018. Molecular characterization and phylogenetic analysis of *Acanthamoeba* isolates in tap water of Beni-Suef. Egypt. Acta Parasitol. 63: 826–834. https://doi.org/10.1515/ap-2018-0101

Adl S.M., Bass D., Lane C.E., Lukeš J. et al. 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. J. Eukaryot. Microbiol.

66: 4–119. https://doi.org/10.1111/jeu.12414

Adl S.M., Habura A. and Eglit Y. 2014. Amplification primers of SSU rDNA for soil protists. Soil Biology and Biochemistry. 69: 328–342. https://doi.org/10.1016/j.soilbio.2013.10.024

Anderson R. 1998. Densities and diversity of gymnamoebae in relation to some inshore aquatic habitats at Bermuda. J. Eukaryot. Microbiol. 45: 151–155. https://doi.org/10.1111/j.1550-7408.1998.tb05083.x

Anderson R. 2002. Laboratory and field-based studies of abundances, small-scale patchiness, and diversity of gymnamoebae in soils of varying porosity and organic content: evidence of microbiocoenoses. J. Eukaryot. Microbiol. 49: 17–23. https://doi.org/10.1111/j.1550-7408.2002.tb00334.x

Anderson O.R. 2007. A seasonal study of the carbon content of planktonic naked amoebae in the Hudson Estuary and in a productive freshwater pond with comparative data for ciliates. J. Eukaryot. Microbiol. 54: 388–391. https://doi.org/10.1111/j.1550-7408.2007.00276.x

Benson D.A., Cavanaugh M., Clark K., Karsch-Mizrachi I. et al. 2013. GenBank. Nucleic Acids Res. 41(D1): 36–42. https://doi.org/10.1093/nar/gks1195

Butler H. and Rogerson A. 1995. Temporal and spatial abundance of naked amoebae (Gymnamoebae) in marine benthic sediments of the Clyde Sea area, Scotland. J. Eukaryot. Microbiol. 42: 724–730. https://doi.org/10.1111/j.1550-7408.1995.tb01624.x

Bzezinska-Dudziak B. 1953. Oznaczanie ilosci ameb w glebie wg zmodytikowanej metody Singh'a. Acta Microbiol. Pol. 3: 121–124.

Cutler D.W. 1920. A method for estimating the number of active protozoa in the soil. The Journal of Agricultural Science. 10: 135–143. https://doi.org/10.1017/S0021859600006419

Darbshire J.F., Anderson O.R. and Rogerson A. 1996. Protozoa. In: Methods for the examination of organismal diversity in soils and sediments (Ed: Hall G.S.). CAB International ed. Wallingford, pp. 79–90.

Darbyshire J.F., Wheatley R.E., Greaves M.P. and Inkson R.H.E. 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. Revue d'Ecologie et de Biologie du Sol, France. 11: 465–475.

Delafont V., Mougari F., Cambau E., Joyeux M. et al. 2014. First evidence of amoebae-mycobacteria association in drinking water network. Environ. Sci. Technol. 48: 11872–11882. https://doi.org/10.1021/es5036255

Delafont V., Perrin Y., Bouchon D., Moulin L. et al. 2019. Targeted metagenomics of microbial diversity in free-living amoebae and water samples. Methods Mol. Biol. 1921: 421–428. https://doi.org/10.1007/978-1-4939-9048-1_26

Finlay B.J. and Fenchel T. 2004. Cosmo-politan metapopulations of free-living microbial eukaryotes. Supplementary Information. Protist. 155: 237–244. https://doi.org/10.1078/143446104774199619

Finlay B.J., Maberly S.C. and Berninger U.G. 2000. Microbial diversity in Priest Pot: a productive pond in the English Lake District. The Freshwater Biological Association, Ambleside, in collaboration with the NERC Centre for Ecology and Hydrology, Windermere.

Garcia A., Goci P., Cieloszyk J., Fernandez M.T. et al. 2013. Identification of free-living amoebae and amoeba-associated bacteria from reservoirs and water treatment plants by molecular techniques. Environ. Sci. Technol. 47: 3132–3140. https://doi.org/10.1021/es400160k

Garstecki T. and Arndt H. 2000. Seasonal abundances and community structure of benthic rhizopods in shallow lagoons of the southern Baltic Sea. Europ. J. Protistol. 36: 103–115. https://doi.org/10.1016/S0932-4739(00)80027-9

Geisen S., Tveit A.T., Clark I.M., Richter A. et al. 2015. Metatranscriptomic census of active protists in soils. ISME J. 9: 2178–2190. https://doi.org/10.1038/ismej.2015.30

Geisen S., Weinert J., Kudryavtsev A., Glotova A. et al. 2014. Two new species of the genus *Stenamoeba* (Discosea, Longamoebia): cytoplasmic MTOC is present in one more amoebae lineage. Europ. J. Protistol. 50: 153–165. https://doi.org/10.1016/j.ejop.2014.01.007

Kiss Á.K., Ács É., Kiss K.T. and Török J.K. 2009. Structure and seasonal dynamics of the protozoan community (heterotrophic flagellates, ciliates, amoeboid protozoa) in the plankton of a large river (River Danube, Hungary). Europ. J. Protis-tol. 45: 121–138. https://doi.org/10.1016/j.ejop.2008.08.002

Kudryavtsev A. and Hausmann K. 2009. *Thecamoeba aesculea* n. sp. (Amoebozoa, Thecamoebidae), a terrestrial amoeba with affinities to *Th. sphaeronucleolus* (Greeff, 1891). Acta Protozool. 48: 91–96.

Kyle D.E. and Noblet G.P. 1987 Seasonal distribution of thermotolerant free-living amoebae. II. Lake Issaqueena. J. Protozool. 34: 10–15. https://doi.org/10.1111/j.1550-7408.1987.tb03122.x

Medlin L., Elwood H.J., Stickel S. and Sogin M.L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene. 71: 491–499. https://doi.org/10.1016/0378-1119(88)90066-2

Menapace D., Klein D.A., Mcclellan J.F. and Mayeux J.V. 1975. A simplified overlay plaque technic for evaluating responses of small free-living amebae in grassland soils. J. Protozool. 22: 405–410. https://doi.org/10.1111/j.1550-7408.1975.tb05192.x

Mesentsev Y., Bondarenko N., Nassonova E. and Smirnov A. 2021. *Vannella primoblina* n. sp. – an unusual species of the genus *Vannella* (Amoebozoa, Discosea, Vannellida) with pronounced dorsal ridges and folds. Europ. J. Protistol. 77: 125757. https://doi.org/10.1016/j.ejop.2020.125757

Mesentsev Y., Kamyshatskaya O., Nassonova E. and Smirnov A. 2022. T*hecamoeba vumurta* n. sp. (Amoebozoa, Discosea, Thecamoebida) from freshwater pond sediment – a sibling species of *T. striata* (Penard, 1890) Schaeffer, 1926. Europ. J. Protistol. 83: 125866. https://doi.org/10.1016/j.ejop.2022.125866

Mesentsev Y., Kamyshatskaya O. and Smirnov A. 2020. *Thecamoeba foliovenanda* n. sp. (Amoebozoa, Discosea, Thecamoebida) – one more case of sibling species among amoebae of the genus *Thecamoeba*. Europ. J. Protistol. 76: 125716. https://doi.org/10.1016/j.ejop.2020.125716

Mesentsev Y.S. and Smirnov A.V. 2019. *Thecamoeba cosmophorea* n. sp. (Amoebozoa, Discosea, Thecamoebida) — an example of sibling species within the genus *Thecamoeba*. Europ. J. Protistol. 67: 132–141. https://doi.org/10.1016/j.ejop.2018.12.003

O'Dell W.D. 1979. Isolation, enumeration and identification of amebae from a Nebraska lake. J. Protozool. 26: 265–269. https://doi.org/10.1111/j.1550-7408.1979.tb02777.x

Page F.C. 1987. The classification of "naked" amoebae (Phylum Rhizopoda). Arch. Protistenkd. 133: 199–217. https://doi.org/10.1016/S0003-9365(87)80053-2

Page F.C. 1988. A New Key to Freshwater and Soil Gymnamoebae. Freshwater Biological Association, the Ferry House, Ambleside, Cumbria

Page F.C. 1991. Nackte Rhizopoda, in: Nackte Rhizopoda und Heliozoea (Protozoenfauna Band 2) (Ed: Gustav Fischer). Verlag, Stuttgart, New York, pp. 3–170.

Patsyuk M.K. 2014. Morphotypes in naked amoebas (protista): distribution in water bodies of Zhytomyr and Volyn Polissia (Ukraine) and possible ecological significance. Vestnik Zoologii. 48: 547–552. doi:10.2478/vzoo-2014-0065

Patsyuk M.K. and Dovgal I.V. 2012. Biotopic distribution of naked amoebes (Protista) in Ukrainian Polissya area. Vestnik Zoologii. 46: 355–560

Pawlowski J. 2000. Introduction to the molecular systematics of foraminifera. Micropaleontology. 46: 1–12.

Prescott D.M. and James T.W. 1955. Culturing of *Amoeba proteus* on *Tetrahymena*. Expl. Cell Res. 8: 256–258. https://doi.org/10.1016/0014-4827(55)90067-7

Ren K., Xue Y., Rшnn R., Liu L. et al. 2018. Dynamics and determinants of amoeba community, occurrence and abundance in subtropical reservoirs and rivers. Water Res. 146: 177–186. https://doi.org/10.1016/j.watres.2018.09.011

Rodriguez Zaragoza S., Mayzlish E. and Steinberger Y. 2005. Seasonal changes in free-living amoeba species in the root canopy of *Zygophyllum dumosum* in the Negev Desert, Israel. Microb. Ecol. 49: 134–141. https://doi.org/10.1007/s00248-003-1056-1

Rogerson A. 1982 An estimate of the annual production and energy flow of the large naked amoebae population inhabiting a *Sphagnum* bog. Arch. Protistenkd. 126: 145–149. https://doi.org/10.1016/S0003-9365(82)80045-6

Rogerson A. 1988. DAPI-staining for the rapid examination of nuclei and parasomes in marine gymnamoebae. Arch. Protistenkd. 135: 289–298. https://doi.org/10.1016/S0003-9365(88)80076-9

Rogerson A. 1991. On the abundance of marine naked amoebae on the surface of five species of macroalgae. FEMS Microbiol. Lett. 85: 301–312.

Rogerson A. and Laybourn-Parry J. 1992. The abundance of marine naked amoebae in the water column of the Clyde estuary. Estuarine, Coastal and Shelf Science. 34: 187–196. https://doi.org/10.1016/S0272-7714(05)80104-0

Rogerson A. and Patterson D.J. 2002. The naked ramicristate amoebae (Gymnamoebae). In: An illustrated guide to the protozoa (Eds: Lee J.J, Leedale G.F. and Bradbury P.). Society of Protozoologists: Kansas, pp. 1023–1052.

Rønn R., Ekelund F. and Christensen S. 1995. Optimising soil extract and broth media for MPN-enumeration of naked amoebae and heterotrophic flagellates in soil. Pedobiologia. 39: 10–19.

Samba-Louaka A., Delafont V., Rodier M.H. Cateau, E. and Héchard Y. 2019. Free-living amoebae and squatters in the wild: ecological and

molecular features. FEMS Microbiol. Rev. 43: 415–434. https://doi.org/10.1093/femsre/fuz011

Sawyer T.K. 1975a. Marine amoebae from surface waters of Chincoteague Bay, Virginia: one new genus and eleven new species within the families Thecamoebidae and Hyalodiscidae. Transactions of the American Microscopical Society. 94: 305–323. https://doi.org/10.2307/3225496

Sawyer T.K. 1975b. Marine amoebae from surface waters of chincoteague bay, Virginia: two new genera and nine new species within the families Mayorellidae, Flabellulidae, and Stereomyxidae. Transactions of the American Microscopical Society. 94: 305–323. https://doi.org/10.2307/3225533

Sawyer T.K. 1980. Marine amoebae from clean and stressed bottom sediments of the Atlantic ocean and gulf of Mexico. J. Protozool. 27: 13–32. https://doi.org/10.1111/j.1550-7408.1980.tb04225.x

Sawyer T.K. and Bodammer S.M. 1983. Marine amoebae (Protozoa: Sarcodina) as indicators of healthy or impacted sediments in the New York Bight Apex. In: protozoa in hypoxic sediments–gulf of mexico (Eds: Ketchum H., Park P.K. and Kester D.R.) Wastes in the ocean, Vol. 1: Industrial and sewage wastes in the ocean. Wiley Interscience, New York, pp 337–352.

Scheikl U., Tsao H.F., Horn M., Indra A. et al. 2016. Free-living amoebae and their associated bacteria in Austrian cooling towers: a 1-year rou-tine screening. Parasitol. Res. 115: 3365–3374. https://doi.org/10.1007/s00436-016-5097-z

Severtzoff L.B. 1922. Method of counting, culture medium and pure cultures of soil amoebae. Centralbl. F. Bakt. Etc. I Abt. Originale. 92: 1–2.

Singh B.N. 1946 A method of estimating the numbers of soil Protozoa, especially amoebae, based on their differential feeding on bacteria. Ann. Applied Biology. 33: 112–119. https://doi.org/10.1111/j.1744-7348.1946.tb06282.x

Smirnov A.V. 2002. Vertical distribution and abundance of gymnamoebae (Rhizopoda) in bottom sediments of the brackish water Nivå Bay (Baltic Sea, The Sound). Protist. 153: 239–250. https://doi.org/10.1078/1434-4610-00101

Smirnov A.V 2003. Optimizing methods of the recovery of gymnamoebae from environmental samples: a test of ten popular enrichment media, with some observations on the development of cultures. Protistology. 3: 45–57.

Smirnov A.V. 2007. Cryptic freshwater amoeba species in the bottom sediments of Nive Bay (Øresund, Baltic Sea). Europ. J. Protistol. 43: 87–94. https://doi.org/10.1016/j.ejop.2006.11.002

Smirnov A.V. 2008. Amoebas, Lobose. Encyclopedia of Microbiology, Moselio Schaechter, Editor, Oxford: Elsevier, pp. 558–577. https://doi.org/10.1016/B978-012373944-5.00359-X

Smirnov A.V and Brown S. 2004. Guide to the methods of study and identification of soil gymnamoebae. Protistology. 3: 148–190.

Smirnov A.V., Chao E., Nassonova E.S. and Cavalier-Smith T. 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist. 162: 545–570. https://doi.org/10.1016/j.protis.2011.04.004

Smirnov A.V. and Goodkov A.V 1996. Systematic diversity of gymnamoebae in the bottom sediments of a freshwater lake in Karelia (Lobosea, Gymnamoebia). Zoosystematica Rossica. 4: 201–203.

Smirnov A.V. and Goodkov A.V. 1999. An Illustrated list of basic morphotypes of Gymnamoebia (Rhizopoda, Lobosea). Protistology. 1: 20–29.

Smirnov A. V., Kulishkin N. S., Surkova A. A., Mesentsev Y. S. et al. 2020. Molecular phylogeny of *Paradermamoeba valamo* (Amoebozoa, Discosea, Dermamoebida). Protistology, 14: 219–226. https://doi.org/10.21685/1680-0826-2020-14-4-1

Smirnov A.V., Lentsman N.V. and Nassonova E.S. 1998. Vertical distribution of naked and testate amoebae (Lobosea: Gymnamoebia, Testacealobosia) in the bottom sediments of a freshwater lake (in Russian), Proc. St. Petersburg Natl. Soc. 92: 74–81 (In Russian, English summary).

Smirnov A.V., Nassonova E., Holzmann M. and Pawlowski J. 2002. Morphological, ecological and molecular studies of *Vannella simplex* Wohlfarth-Bottermann 1960 (Lobosea, Gymnamoebia), with a new diagnosis of this species. Protist. 153: 367–377. https://doi.org/10.1078/14344610260450109

Smirnov A. and Thar R. 2003. Spatial distribution of gymnamoebae (Rhizopoda, Lobosea) in brackish-water sediments at the scale of centimeters and millimeters. Protist. 154: 359–369. https://doi.org/10.1078/143446103322454121

Spiegel F.W. Moore D.L. and Feldman J. 1995. *Tychosporium acutostipes*, a new protostelid which modifies the concept of the Protosteliidae. Mycologia. 87: 265–270. https://doi.org/10.2307/3760912

Stoeck T., Bass D., Nebel M., Christen R. et al. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19

(SUPPL. 1), pp. 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Tsao H.F., Scheikl U., Herbold C., Indra A. et al. 2019. The cooling tower water microbiota: seasonal dynamics and co-occurrence of bacterial and protist phylotypes. Water Res. 159: 464–479. https://doi.org/10.1016/j.watres.2019.04.028

Zhang Z., Schwartz S., Wagner L. and Miller W. 2000. A greedy algorithm for aligning DNA sequences. J. Computational Biology. 7: 203–214. https://doi.org/10.1089/10665270050081478