

ORIGINAL ARTICLE

Diversity and abundance of naked lobose amoebae (Amoebozoa: Tubulinea, Discosea, Variosea) in highly productive soil of Chernevaya taiga (Western Siberia, Russia)

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Summary

Amoebozoa is a poorly studied component of the soil microbiota. Data on amoebozoan diversity in different types of soil remains drastically limited. In this paper, we present results of our study of species composition and abundance of naked amoebae in tall-herbaceous rainforest ecosystem unique to Siberia - the Chernevaya taiga, located in the low mountains of Western Siberia (Russia). The soils of this ecosystem have high fertility, which causes plant gigantism. The abundance of naked amoebae in sampled soil, recovered by culture-based MPN method, varied from 194 to 265 g⁻¹ and was higher than in usual oligotrophic taiga soil from the nearby sampling site (130 g⁻¹). Fifteen amoebae species were recovered from the samples. Amoebae of the order Leptomyxida were highly abundant in all samples. Since we worked with soil transported for a long distance, we believe that the list of recovered species is a “minimal” one and could be expanded in the future. Relatively high abundance of naked amoebae suggests their significant influence on the microbiota of the soil of Chernevaya taiga.

Key words: Amoebozoa, soil, diversity, systematics, ecology

Introduction

Though basic studies of protist diversity in marine and estuarine benthic sediments proved the high abundance of naked lobose amoebae in different types of natural ecosystems (Arndt, 1993; Butler and Rogerson, 1995; Smirnov, 2002; Smirnov and Thar, 2003), data on their diversity are still limited (Grossmann et al., 2016; Mahe et al., 2017; Geisen et al., 2018).

Morphological identification of naked amoeboid protists, especially the species distinction, is a difficult task (Bovee, 1985; Page, 1988; Smirnov, 2008, 2011). It requires expert knowledge of amoebae diversity, establishing clonal cultures, application of electron microscopy, and anyway in many cases remains low-reliable. Traditional ecological studies of naked amoebae use morphospecies, identified at light-microscopic level (Butler and Rogerson, 2000), morphotypes (Garstecki and Arndt, 2000; Rogerson and Gwaltney, 2000; Rodriguez-Zaragoza et al., 2005) or size clusters (Bischoff, 2002). Even if molecular methods are applied, one still needs an expert approach to amoebae identification to link them with morphological species diversity and abundance (Perez-Juarez et al., 2018).

Long-term observations tend to reveal unstable species composition of local amoebae microcommunities (Smirnov and Goodkov, 1995; Smirnov, 2003), which is partially explained by changes in physical conditions (Whitford, 2002; Geisen et al., 2014; Lanzen et al., 2016), seasonal dynamics (Rodriguez-Zaragoza et al., 2005), effects of living macroorganisms on the habitat (Perez-Juarez et al., 2018) and fluctuations in the abundance associated with emergence and vanishing of suitable microhabitat conditions (Baldock and Sleigh, 1988; Taylor and Berger, 1980). Because of the instability of amoebae communities in space and time, studying their diversity in natural habitats is challenging. As a result, number of reliable studies dedicated to amoebae diversity and differential species abundance remains limited and this issue requires more attention.

The significant role of naked lobose amoebae in soil microcosm functioning is widely recognized (Foster and Dormaar, 1991; Ekelund and Rønn, 1994; Bonkowski, 2004; Singer et al., 2021). They are considered important for biomineralization processes in rhizosphere, regulation of bacterial and fungal biomass, predation of nematodes (Rodriguez-Zaragoza et al., 2005; Geisen et al., 2015a; Xiong

et al., 2018). However, data on amoebae species diversity in different types of soils are drastically limited. In this paper, we present data on the diversity and abundance of amoebae in soils of a unique type of ecosystem — the Chernevaya taiga — Siberian hemiboreal tall-herbaceous forests dominated by aspen and fir, limited in their distribution to hyperhumid areas of the Altai-Sayan Mountain region (Western Siberia, Russia) at elevations from 200 m to 900 m above sea level. There is almost no plant litter in these forests, since grass litter is almost completely mineralized within one year. The soils in these ecosystems are known for their extremely high productivity, causing gigantism of the perennial herbaceous plants and bushes (see Abakumov et al., 2020a, 2020b; 2023). Earlier we isolated a rare amoeba species *Thecochaos fibrillosum* from this habitat (Mesentsev et al., 2023). However, there is no data on overall amoebae diversity from this type of soil. The present paper is intended to partly fill in this gap.

Material and methods

Three samples containing the material from top 10 cm of the soil were collected from a location near Tomsk city (Russia, Western Siberia, Tomskaya oblast; 56.30693° N, 85.47063° E) (see Abakumov et al., 2023 for details on this site). Samples 1, 2, and 3 were collected from the top 10 cm layer of Chernevaya taiga in July 2020. The other three samples (marked as samples T1-T3) were collected in July 2021. Sample T1 was taken from the top 10 cm of Luvic Stagnic Phaeozem of Chernevaya taiga (56.306629° N, 85.470616° E), while sample T2 was taken from the intermediate area between soil of Chernevaya taiga and oligotrophic soil (56.307012° N, 85.470421° E). The soil was diagnosed as Albic Luvisol. The sample T3 contained oligotrophic soil from the closest sampling site (56.481131° N, 84.798967° E). This soil was diagnosed as Albic Luvisol (Epidystric). The classification of soils is given according to IUSS Working Group WRB (2022). The temperature of soil in the sampled top 10 cm layer during both years of sampling did not exceed +16 °C (Abakumov et al., 2020a).

Samples were gently mixed to homogenize soil; 0.01 g of soil (natural moisture) was placed in 60 mm sterile Petri dishes filled with culturing medium (0.025% cerophyll on PJ with addition of soil extract made on the same soil) (Prescott and James, 1955; Page, 1988). For quantification of amoebae, 50

dishes were established for each of the six analyzed samples. Enrichment cultures were incubated for 14 days at +16 °C. Quantitative and qualitative assessment of the diversity of naked lobose amoebae was performed using an inverted Nikon TMF100 microscope equipped with phase contrast.

Abundance of protists was calculated using MPN (most probable number) method as described by Garstecki and Arndt (2000) with modifications described by Smirnov (2002) and Surkova et al. (2022). In the 50-dish series, every amoeba species was counted individually; this 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. Abundance of flagellates, ciliates, heterolobosean amoebae, testate amoebae and other microeukaryotes (nematodes, rotifers) was accessed quantitatively without identification. Numbers of organisms other than naked amoebae cannot be properly counted by MPN method. Ciliates and flagellates to the certain extent may be an exception, but results are usually biased with selective species growth (Bamforth, 1992; Berthold and Palzenberger, 1992). So, here the recovered abundance of other groups is provided for comparison between habitats only.

To improve data on amoebae diversity, 15 enrichment cultures in 90 mm Petri dishes with PJ medium and rice grains were established for each of the 6 analyzed soil samples. They were incubated at room temperature for 7 days, then maintained at +16 °C. Cultures were checked on the 7th, 14th and 30th days. Observations on these cultures were used to accumulate data on species diversity. These data were applied for identification of amoebae strains in dilution series and documentation of isolated strains.

Results

In 300 observed Petri dishes, 15 species of naked lobose amoebae were found. If we add the species *Thecochaos fibrillosum*, described from these samples earlier (Mesentsev et al., 2023), the total number of species recovered from these samples is 16. Members of all three lineages containing naked lobose amoebae, namely Tubulinea (Fig. 1, A-H), Discosea (Fig. 1 I-Y) and Variosea (Fig. 1, Z) were represented. They were identified up to genus (or higher taxon, when appropriate) at the light-

microscopic level, so provided names should be accepted with caution. However, light-microscopic data were sufficient to reliably differentiate observed amoebae and repetitively recognize them during the screening of dishes.

Four species, namely – *Cochliopodium* sp. 1 (Fig. 1, W-X), *Cochliopodium* sp. 2 (Fig. 1, Y), *Leptomyxa sylvatica* (Fig. 1, A, see Glotova et al., 2021) and *Leptomyxa* sp. 2 (Fig. 1, B) occurred most frequently in Chernevaya taiga soil samples (samples 1-3, and T1). *Saccamoeba* sp. (Fig. 1, E-F), *Vannella simplex* (Fig. 1, Q-R), *Korotnevella* sp. (Fig. 1, M-N), *Flamella* sp. (Fig. 1, Z) were less common; while representatives of *Vexillifera* sp. (Fig. 1, O-P), Thecamoebidae gen. sp. (Fig. 1, I-J), *Mayorella* sp. (Fig. 1, K-L), Hartmannellidae gen. sp. (Fig. 1, G-H), «unidentified amoebozoan» (Fig. 1, C-D), *Vannella* sp. (Fig. 1, S-T) and Vannellidae gen. sp. (Fig. 1, U-V) occurred rarely.

The MPN values showing the abundance of naked lobose amoebae in samples from the Chernevaya taiga soil (1-3, and T1) were comparable. Those values were higher than the MPN value of amoebae in oligotrophic soil sample (T3), collected from the same geographical area, obtained and transferred under the same conditions (Table 1). According to the MPN values, the total abundance of ciliates and testate amoebae was also higher in Chernevaya taiga soil samples, while heterolobose amoebae and flagellates were almost equally represented in high-productive and oligotrophic soil samples. Rare occurrence of micrometazoans (Nematoda, Rotifera) was documented in all samples. It should be stressed that conditions for taxa other than amoebae in our enrichment cultures probably were not optimal, so provided abundances are appropriate only for comparison between dishes in the same series of experiments.

Discussion

Physical and chemical conditions, as well as plants growth characteristics have a crucial importance for rhizosphere microbiota (Aslani et al., 2021; Ceja-Navarro et al., 2021). Protist communities are sensitive to microhabitat conditions, such as spatial distribution of preferable prey bacteria, even more than pH and soil moisture (Geisen et al., 2020; Oliverio et al., 2020; Xiong et al., 2020).

Naked lobose amoebae are considered to be a difficult group for faunistic and ecological studies, in

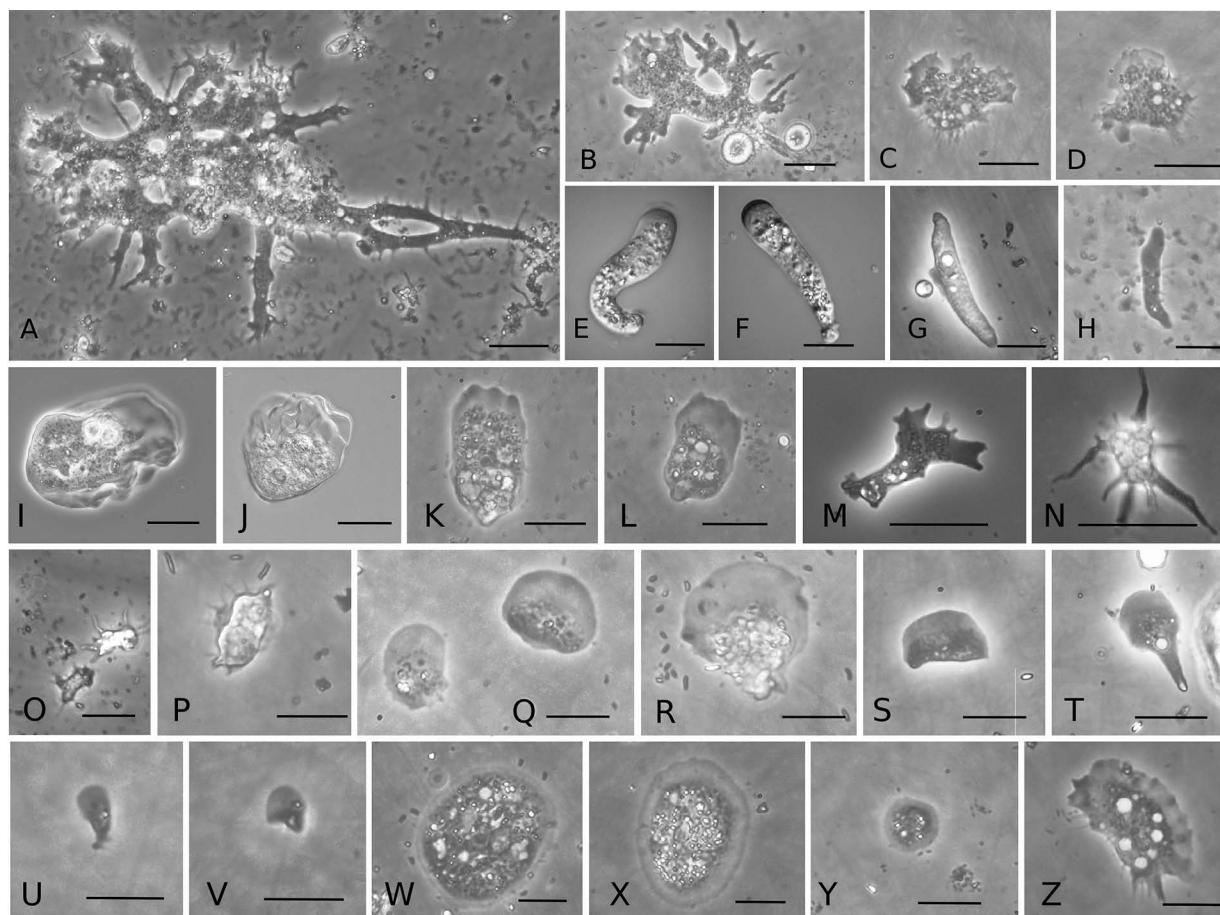


Fig. 1. Light microscopy of naked lobose amoebae strains found in the samples from Chernevaya taiga soil (phase contrast). A – *Leptomyxa sylvatica*; B – *Leptomyxa* sp.; C-D – an unidentified amoebozoan; E-F – *Saccamoeba* sp.; G-H – Hatmannellidae gen. sp.; I-J – Thecamoebidae gen. sp.; K-L – *Mayorella* sp.; M-N – *Korotnevella* sp.; O-P – *Vexillifera* sp.; Q-R – *Vannella simplex*; S-T – *Vannella* sp.; U-V – Vannellidae gen. sp.; W-X – *Cochliopodium* sp. 1; Y – *Cochliopodium* sp. 2; Z – *Flamella* sp. Scale bar is 20 μ m throughout.

particular – in soils (Smirnov and Brown, 2004), but experimental studies revealed higher MPN values and recovery potential of amoebae communities in planted soil microcosms than in bare samples (Rodriguez-Zaragoza et al., 2005; Rosenberg et al., 2009; Cortes-Perez et al., 2014). Predatory activity of free-living amoebae provides the release of nutrients back to the soil and influences the community composition and population dynamics of their prokaryotic and eukaryotic prey (Geisen et al., 2018).

Representatives of Hartmannellidae, *Flamella*, *Korotnevella*, *Echinamoeba*, *Acanthamoeba*, *Mayorella*, *Saccamoeba* and *Leptomyxa* are commonly described from various agricultural and natural soil habitats (Rodriguez-Zaragoza et al., 2005; Cortes-Perez et al., 2014; Perez-Jaurez et al., 2018; Patsyuk, 2020). Surprisingly, in the analyzed samples from

Chernevaya taiga and surrounding area *Acanthamoeba* strains were not observed. The reasons for the absence of this taxon, common in soil habitats, are not clear and may be a bias of the experiment. Other genera previously recovered from soil ecosystems were represented, as well as small-sized species of Vannellida, Himatistenida and Dactylopodia.

Only a few specific soil microcommunities have been previously observed with a detailed description of naked lobose amoebae diversity, and the accumulated data can hardly be directly compared. A case study of the unique ecosystem may be called one of the trends in soil naked lobose amoebae ecology, and most of these studies were devoted to arid soils (Robinson et al., 2002; Rodriguez-Zaragoza and Steinberger, 2004; Bamforth, 2008; Barness et al., 2009; Fernandez, 2015). However, heterotrophic protists generally demonstrate higher diversity in

Table 1. Abundance of microeukaryotes in the samples collected in 2020 and 2021, estimated by the MPN method (most probable numbers) (g^{-1} of soil of natural moisture). Numbers of organisms other than naked amoebae cannot be properly counted by MPN method used, so they are provided for comparison between samples only.

Group of organisms	2020			2021		
	1	2	3	T1	T2	T3
naked lobose amoebae	194	232	212	256	208	130
testate amoebae	10	–	–	16	16	2
heteroloboseans amoebae	16	16	12	44	24	30
flagellates	212	196	160	392	332	282
ciliates	142	96	134	96	102	48
rotifera	–	2	2	2	10	4
nematods	6	18	14	4	8	6

continuously moist soils (Krashevskaya et al., 2012; Tsyganov et al., 2013; Geisen et al., 2014). Studies focused on naked lobose amoebae suggested that diversity varies between dry and wet seasons, and variations generally correlate with water availability (Perez-Juarez et al., 2018).

Data obtained in culture-based experiments are hardly comparable between different studies, but are reliably comparable within a single study, performed under the uniform sampling, transfer and cultivation conditions. The present data show higher abundance of naked lobose amoebae in Chernevaya taiga soil rather than in oligotrophic soil originating from the same geographic area (Table 1). The MPN values of individual species abundance significantly varied between different samples of Chernevaya taiga soil, while the variability of MPN values of total amoebae abundance was much lower (Table 2). It may be a result of amoebae species commitment to microhabitat conditions (Smirnov and Thar, 2003) and size-specific prey peaks.

Chernevaya taiga represents one of the richest soil biotopes, characterized by the high content of biogenic elements and diverse fungal and bacterial community (Abakumov et al., 2020a). These properties appear to be favorable for amoebae. Interestingly, the soil in Chernevaya taiga has no plant litter since grass remnants are getting almost completely mineralized within one year. However, a representative of the amoeba genus *Thecochaos*, previously described exclusively from mosses, was found in this soil (Mesentsev et al., 2023). It means that some species normally inhabiting the organic-rich moss of the forest floor still occur in this exceptionally nutrient-rich soil, but like real inhabitants of the soil. It may be a specific property of the unique ecosystem of Chernevaya taiga. An interesting observation is the finding of amoeba that we were not able to identify reliably (Fig. 1,

C-D). This organism may be some variosean or a mycetozoa trophozoite. Unfortunately, we were not able to establish a culture of this organism or get sequence data on it.

Studied samples did not show extraordinary abundance and diversity of naked amoebae. Since we worked with soil transported for a long distance, we believe that the list of recovered species is a “minimal” one and could be expanded in the future. Despite some early evidences of higher abundances of amoebae in soil habitats if compared to freshwater sites (Menapace, 1975), we obtained MPN values rather comparable to data on freshwater habitats from modern studies with a similar estimation approach (Surkova et al., 2022). Since enrichment cultures in the laboratory could never reconstruct entire microhabitat conditions, cultivable diversity, which we used to evaluate amoebae abundance, might not reflect actual diversity that participates in biomass and energy flows in the environment (Geisen et al., 2015b). For example, no mycetozoa were recovered under the culture conditions used. In addition to experimental data (Kreuzer et al., 2006; Ekelund et al., 2009; Bonkowski and Clarholm, 2012), a better understanding of the role of amoeboid protists in soil ecosystems requires new environmental data sources, more uniformly covering the diversity of taxa, such as metagenomic approaches or novel cultivation techniques (Sherpa et al., 2015; Salazar-Ardiles et al., 2022; Chauque et al., 2023).

The abundance of other groups of organisms cannot be reliably estimated with culture-based methods. It can only be noted that rotifers and nematodes were registered in almost all samples, while testate amoebae were predominantly found in the year 2021 (Table 1). This may be a result of slightly different transporting and culture conditions or a reflection of the local heterogeneity of protists

Table 2. Individual abundance of naked lobose amoebae strains recovered from the samples collected in 2020 and 2021, estimated by the MPN method (most probable numbers) (g^{-1} of soil of natural moisture).

Organism	2020			2021		
	1	2	3	T1	T2	T3
<i>Cochliopodium</i> sp.1	20	78	52	24	22	6
<i>Cochliopodium</i> sp.2	52	58	20	36	78	38
<i>Flamella</i> sp.	48	18	20	18	10	2
<i>Saccamoeba</i> sp.	10	8	6	16	8	10
Hatmannellidae gen. sp.	–	–	–	2	6	16
<i>Leptomyxa sylvatica</i>	16	10	62	102	28	44
<i>Leptomyxa</i> sp.	6	2	10	20	24	4
unidentified amoebozoan	–	–	6	4	–	–
<i>Vannella simplex</i>	4	4	10	8	2	2
<i>Vannella</i> sp.	4	2	4	–	–	–
Vannellidae gen. sp.	18	22	8	2	4	4
<i>Vexillifera</i> sp.	4	20	–	8	8	–
<i>Korotnevela</i> sp.	8	4	–	8	10	–
<i>Mayorella</i> sp.	4	6	8	6	6	4
Thecamoebidae gen. sp.	–	–	–	2	2	6

at the sampled soil site. Traditional culture-based MPN estimates of the number of ciliates and flagellates were criticized for the tendency to overestimate the numbers when compared to direct counts (Bamforth, 1992) and selective recovery of taxa (Foissner, 1987; Ekelund and Rønn, 1994; Rønn et al., 1995). Therefore, we only can compare the relative abundance between our samples, but no to extrapolate data to other studies. It is possible to note that detected abundances of ciliates and flagellates (Table 1) are generally in the same order of magnitude as of naked amoebae. These figures are much smaller than maximal detected numbers of flagellates and ciliates in soils that count to 10000–100000 per gram of dry weight of soil (Ekelund and Rønn, 1994 and references therein). So, as well as for naked amoebae, we cannot say that the abundance of studied protozoan groups recovered in these experiments is extraordinary in Chernevaya taiga soil.

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