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Culture independent barcoding of naked lobose amoebae (Amoebozoa: Tubulinea, Discosea, Variosea) from freshwater and soil habitats

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Motivation and Aim: Within the complex framework of the protist distribution patterns debate [1] naked lobose amoebae (NLA) are considered one of the most challenging phylogenetic group. Despite their abundance in many types of natural habitats [2], they are rarely accurately described in ecological studies due to the lack of easily assessed identification characters [3]. Since the conventional two-stepped molecular barcoding approach [4, 5] often requires a labor-consuming stage of amoebae cultivation [6], it has been still applied only for some culturable species and, in the recent decade – to single cells isolated from environment [7]. NGS metabarcoding provides a new tool to describe undiscovered amoebae diversity and could be applied for numerous minimally processed environmental samples.

Methods and Algorithms: Total environmental DNA was extracted from 9 freshwater bottom sediment samples (Turovskoe lake and stream between Turovskoe and Zaklynskoe lakes, Northwestern Russia) and 2 soil samples (Tomsk, Western Siberia, Russia and Etna, Italy) using Dneasy PowerLyzer PowerSoil DNA isolation Kit (Qiagen). Primers biased against metazoans EUK565F_NGS/UnonMet_R [8] and Q5 High Fidelity DNA Polymerase Kit (New England Biolabs) were used in order to amplify V4 marker region of 18S rRNA gene.

Amplicon library construction and sequencing using Illumina MiSeq platform were provided by the Core Facility Center “BioBank” of the Research Park of St. Petersburg State University (https://researchpark.spbu.ru/en/biobank-eng). After quality control reads were assembled into contigs using Mothur (https://mothur.org/wiki/MiSeq_SOP) [10], the same software was also used for data proceeding up to unique sequences insertion into the eukaryotic reference alignment from the SILVA database (https://mothur.org/wiki/silva_reference_files/) and their classification. Filtered target sequences and unclassified sequences were aligned using MAFFT v. 7.490 (https://mafft.cbrc.jp/alignment/server) [11], taxonomically attributed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and added into local alignments of main phylogenetic lineages of NLA. SeaView v. 4.6.1 [12] was used for alignments editing. ML analysis was performed by IQ-TREE v1.6.12 [13] with the model GTR+I+G+F, resulting phylogenetic trees were visualized using iTOL v.3 (http://itol.embl.de) [14].

Results: High-level taxonomic profiling showed a strong prevalence of Opisthokonta (Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota) in soil samples and SAR (Ciliophora, Chrysophyta, Bacillariophyta) in freshwater bottom sediment samples. Though automatic OTUs classification allowed to recover particular Variosea (Schizoplasmodiidae) from different samples, most of Amoebozoa sequences were obtained from unclassified reads array.

Prevalent amount of NLA sequences, obtained from almost every analyzed soil and freshwater sample, corresponded to Variosea (291 reads affiliated to *Filamoeba*, *Arboramoeba*, *Heliamoeba*, *Flamella*, *Telaepolella*, *Ischnamoeba*, *Dictyamoeba*, *Angulamoeba*) and Tubulinea (435 reads affiliated to *Flabellula*, *Leptomyxa*, *Copromyxa*, *Ptolemeba*, *Hartmannella*, *Echinamoeba*, *Vermamoeba*).

Less numerous target sequences appeared within Cutosea (70 reads affiliated to *Squamamoeba*, *Phreatamoeba*, *Mastigamoeba*) and Flabellinia (61 reads affiliated to *Mycamoeba*, *Vannella*, *Korotnevella*). Representatives of Longamoebia (15 reads affiliated to *Dermamoeba*) were obtained only from two freshwater samples (T.06.20, P.08.21).

Recovered diversity of several widespread genera commonly used for culture-based barcoding in laboratory conditions (*Vannella*, *Korotnevella*, *Mayorella*) appeared surprisingly limited. At the same time metabarcoding data visibly contributed into the diversity of *Filamoeba*, *Angulamoeba*, *Echinamoeba*, *Vermamoeba*, *Mycamoeba*, though these amoebae are rarely cultivated and assessed in traditional ecological studies. A noticeable proportion of newly obtained sequences clustered only with environmental Amoebozoa sequences deposited in Genbank with no morphological data affiliated. Patchy and sporadic patterns of sequences distribution within analyzed freshwater and soil samples might reflect population explosion events in certain conditions on a microhabitat level.

Conclusion: Metabarcoding data on NLA from freshwater bottom sediments and soil allowed to identify representatives of all major phylogenetic groups. Among them there are numerous unculturable species, unavailable for culture-based barcoding. Since a significant part of natural diversity of NLA still remains underdescribed and their communities are likely to be sensitive to local conditions favorability, metabarcoding appears particularly promising and complements traditional culture-based methods in amoebae ecological studies.

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