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Metabarcoding study of the phylogenetic diversity of basal Holomycota (Opisthokonta) in the bottom sediments of Turovskoe Lake (Northwestern Russia)

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Motivation and Aim: Basal Holomycota include three groups of obligate intracellular parasites – Rozellida, Microsporidia and Aphelida [1]. Their representatives are suspected to influence on a function of food webs in different types of aquatic ecosystems [2] and population dynamics of various microeukaryotes [3]. Since NGS provides a new opportunity for extensive study of phylogenetic diversity of unculturable species we obtained metabarcoding data on 9 samples of freshwater bottom sediments from two closely located sampling sites (Turovskoye Lake and the stream between Turovskoye and Zaklinskoe lakes, Leningrad region, Russia) and analyzed them with a special focus on basal Holomycota [4, 5].
Methods and Algorithms: Amplification of the V4 region of 18S rRNA gene of rozellids and aphelids from the total environmental DNA extractions was performed through (1) nested PCR with eukaryotic primers 18sEUK581F/18sEUK1134R [6] and Holomycota-specific primers E572/1009R [7] subsequently or (2) single-step PCR with the primers biased against metazoans EUK565F_NGS/UnonMet_R [8]. Amplification of V1-V3 regions of 18S rRNA gene of Microsporidia was performed through single-step PCR with specific primers 18F/530R or V1F/530R [9]. Q5 High Fidelity DNA Polymerase Kit (New England Biolabs) was used for each amplification. Amplicon library construction and sequencing using Illumina MiSeq platform were provided by the Core Facility Center "BioBank" of the St. Petersburg State University Research Park (https://researchpark.spbu.ru/en/biobank-eng).
Quality-controlled reads were assembled into contigs using Mothur (https://mothur.org/wiki/MiSeq_SOP) [10], with the same software 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 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 alignments of basal Holomycota (2420 sequences from Genbank). 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: Each amplification approach resulted in sequencing a significant amount of SAR representatives (Ciliophora, Chrysophyta, Bacillariophyta), some Archaeplastida (Chlorophyta) and non-targeted Opisthokonta (Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota) from all freshwater samples. Both PCR protocols

used to obtain rozellids and aphelids (nested PCR with 18sEUK581f/18sEUK134r and E572/1009R primers subsequently and one-step PCR with anti-metazoan EUK565F_NGS/UnonMet_R primers) appeared comparable in terms of target sequences amplification efficiency, while the second protocol is faster and implies less loss of material. PCR protocols with microsporidia-specific primer systems 18F/530R and V1F/530R appeared equally suitable for amplification of a wide range Microsporidia corresponding to different phylogenetic lineages and also contributed to rozellids and aphelids dataset.

After data processing we obtained 56 OTUs (366 reads) of Microsporidia, 112 OTUs (954 reads) of Aphelida and 450 OTUs (3818 reads) of Rozellida. The discovered sequences contributed to molecular clades within the entire basal Holomycota tree, including both described species (*Rozella*, *Paraphelidium*, *Aphelidium*, *Paramicrosporidium*, *Mitosporidium*, *Morellospora* and numerous microsporidian species) and consistently reproduced in previous phylogenetic studies environmental clades (NAMAKO37, LKM46, NAMAKO35, hww6, LKM15, AZeuk2, LKM11, WIM27, dpeuk6, LKM46). Numerous sequences clustering with microsporidian sequences from aquatic habitats from clades 4(IV), 1(I), 3(V) [15, 16] were detected. A potentially new group of early microsporidia basal to Core Microsporidia + Metchnikovellida + RL107-1 (FN546176) was identified.

All analyzed samples showed rather similar diversity profiles, except the one taken from an especially silted area of Turovskoye Lake (T.07.21). Sequences contributed to most microsporidian clades, *Mitosporidium* clade, *Rozella* clade and Aphelida did not show any visible correspondence to sampling site or sampling time, probably following a continuous distribution pattern of potential multicellular hosts in closely located sampling sites. Sequences contributed to *Morellospora*, *Paramicrosporidium* and *Nucleophaga* clades shared patchy and sporadic distribution pattern of potential unicellular hosts sensitive to unstable microhabitat conditions.

Conclusion: Since a significant fraction of obtained microsporidian and rozellids sequences formed clades with environmental sequences from Genbank, and these clades lack any described representatives, phylogenetic diversity of basal Holomycota in different ecosystems clearly remain underdescribed and require further study.

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References

1. Karpov S., Mamkaeva M., Aleoshin V., Nassonova E., Lilje O., Gleason F. Morphology, phylogeny, and ecology of the aphelids (Aphelidea, Opisthokonta) and proposal for the new superphylum Opisthosporidia. *Front Microbiol.* 2014;5:112. doi 0.3389/fmicb.2014.00112
2. Lafferty K., Allesina S., Arim M., Briggs C. et al. Parasites in food webs: the ultimate missing links. *Ecol Lett.* 2008;11(6):533-46. doi 10.1111/j.1461-0248.2008.01174.x
3. Murareanu B., Sukhdeo R., Qu R., Jiang J., Reinke A. Generation of a Microsporidia species attribute database and analysis of the extensive ecological and phenotypic diversity of Microsporidia. *mBio* 2021;12(3):e0149021. doi 10.1128/mbio.01490-21
4. Bass D., Chech L., Williams B., Berney C., Dunthon M., Mahe F., Torruella G., Stentiford G., Williams T. Clarifying the relationships between microsporidia and cryptomycota. *J Eukaryot Microbiol.* 2018;65(6):773-782. doi 10.1111/jeu.12519
5. Chauvet M., Monjot A., Lepere C. High diversity of microsporidian parasites and new planktonic hosts in freshwater and marine ecosystems. *Limnol Oceanogr.* 2023;68:928-941. doi 10.1002/lno.12321

6. Carnegie R., Meyer G., Blackbourn J., Cochennec N., Franck B., Bower S. Molecular detection of the oyster parasite *Mikrocytos mackini*, and a preliminary phylogenetic analysis. *Dis Aquat Org.* 2003;54:219-27. doi 10.3354/dao054219
7. Taya Y., Kinoshita G., Mohamed W., Moustafa M., Ogata S., Chatanga E., Ohari Y., Kusakisako K., Matsuno K., Nonaka N., Nakao R. Applications of blocker nucleic acids and non-metazoan PCR improves the discovery of the eukaryotic microbiome in ticks. *Microorganisms.* 2021;9(5):1051. doi 10.3390/microorganisms9051051
8. Bass D., Del Campo J. Microeukaryotes in animal and plant microbiomes: Ecologies of disease? *Eur J Protistol.* 2020;76:125719. doi 10.1016/j.ejop.2020.125719
9. Weiss L., Vossbrinck C. Molecular biology, molecular phylogeny and molecular diagnostic approaches to the Microsporidia. In: Wittner M., Weiss L.M. The Microsporidia and Microsporidiosis. Amer Society for Microbiology, 1999. doi 10.1128/9781555818227.CH4
10. Schloss P. Reintroducing mothur: 10 years later. *Appl Environ Microbiol.* 2020;86:e02343-19. doi 10.1128/AEM.02343-19
11. Katoh K., Rozewicki J., Yamada K. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinf.* 2019;20(4):1160-1166. doi 10.1093/bib/bbx108
12. Gouy M., Guindon S., Gascuel O. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol*. 2010;27(2):221-224. doi 10.1093/molbev/msp259
13. Nguyen L., Schmidt H., von Haeseler A., Minh B. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 2015;32(1):268-274. doi 10.1093/molbev/msu300
14. Letunic I., Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 2016;44(W1):W242-W245. doi 10.1093/nar/gkw290
15. Vossbrinck C., Debrunner-Vossbrinck B. Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitol (Praha)* 2005;52(1-2):131-142. doi 10.14411/fp.2005.017
16. Vossbrinck C., Debrunner-Vossbrinck B., Weiss L. Phylogeny of the Microsporidia. In: Weiss L.M, Becnel J.J. (Eds.). Microsporidia: Pathogens of Opportunity. John Wiley & Sons, 2014;203-220. doi 10.1002/9781118395264.ch6