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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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The basal Holomycota include three groups of obligate intracellular parasites — **Rozellida, Microsporidia** and **Aphelida**. These organisms seriously influence on a function of food webs in different types of aquatic ecosystems (Lafferty et al. 2008) and population dynamics of various microeukaryotes (Murareanu et al. 2021).

Since NGS provides a new opportunity for extensive study of phylogenetic diversity of unculturable species we obtained metabarcoding data on 9 samples from 2 freshwater habitats in Northwestern Russia and analyzed them with a special focus on basal Holomycota diversity and distribution (Bass et al. 2018; Chauvet et al. 2023).

RSF Russian Science Foundation

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Material and methods

PIPELINE STEPS	REAGENTS AND SORTWARE USED
extraction	Dneasy PowerLyzer PowerSoil DNA isolation Kit (Qiagen)
amplification	Q5 High Fidelity DNA Polymerase Kit (New England Biolabs)
	nested PCR 18sEUK581F/18sEUK1134R E572/1009R; single-step PCR EUK565F_NGS/UnonMet_R; single-step PCR 18F/530R or V1F/530R
NGS	Illumina MiSeq
data proceeding	Mothur quality control - contigs assembling - correcting sequencing errors - target reads filtering - merging duplicates - unique reads in reference alignment (SILVA) - preliminary clustering - reads classification - exclusion of chimeric and prokaryotic sequences - target taxa selection
	Cryptomycota and Microsporidia reads array; unclassified reads array
taxonomical attribution	BLAST
alignment	MAFFT v. 7.490, SeaView v. 4.6.1
phylogenetic analysis	IQ-TREE v1.6.12, GTR+I+G+F iTOL v.3 (tree visualization)

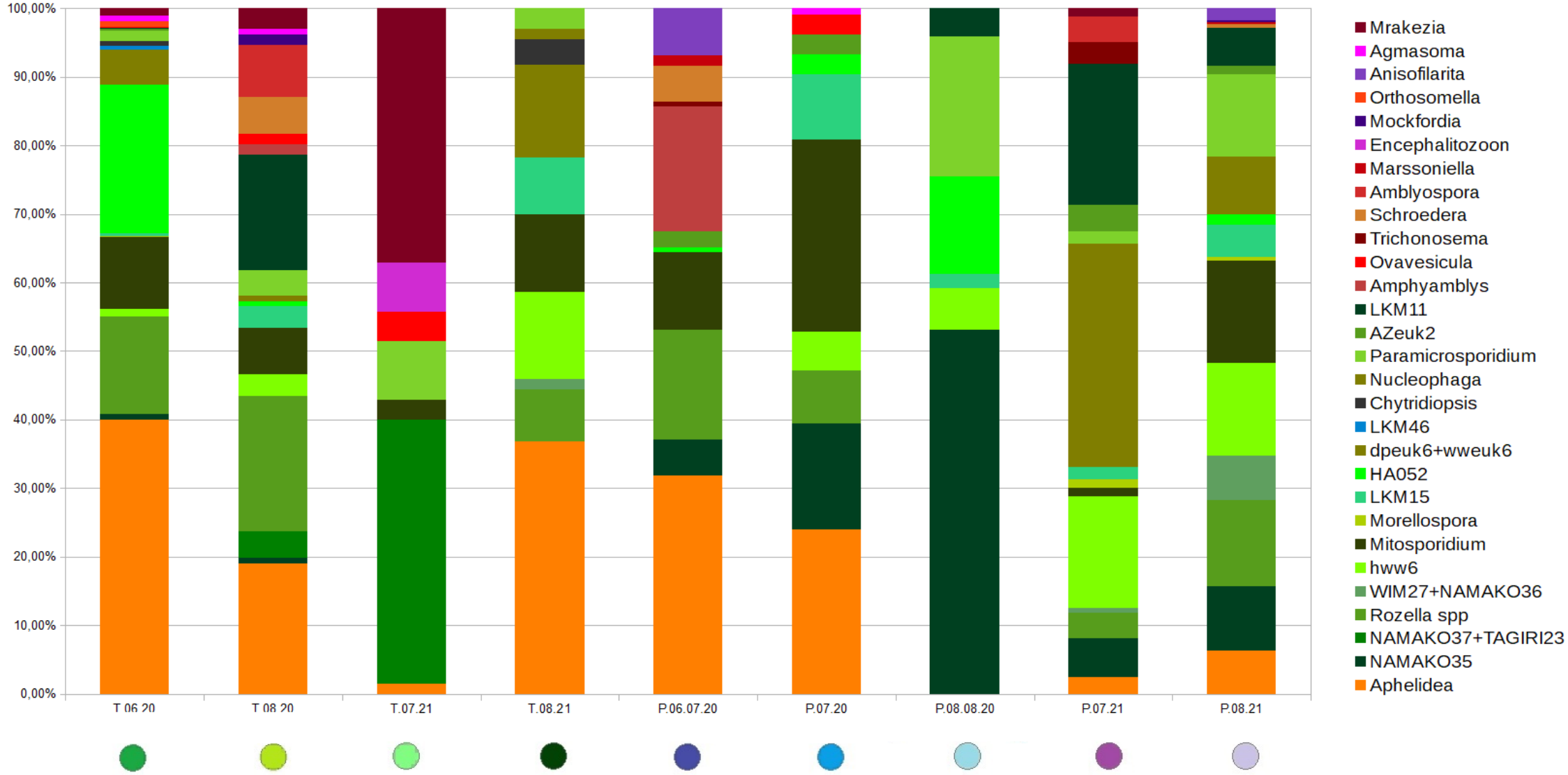
SAMPLING DETAILS

Nine samples of freshwater bottom sediments were taken from two closely located sampling sites – Turovskoye lake and the stream (Protoka) between Turovskoye and Zaklinskoe lakes (Leningrad region, Russia).

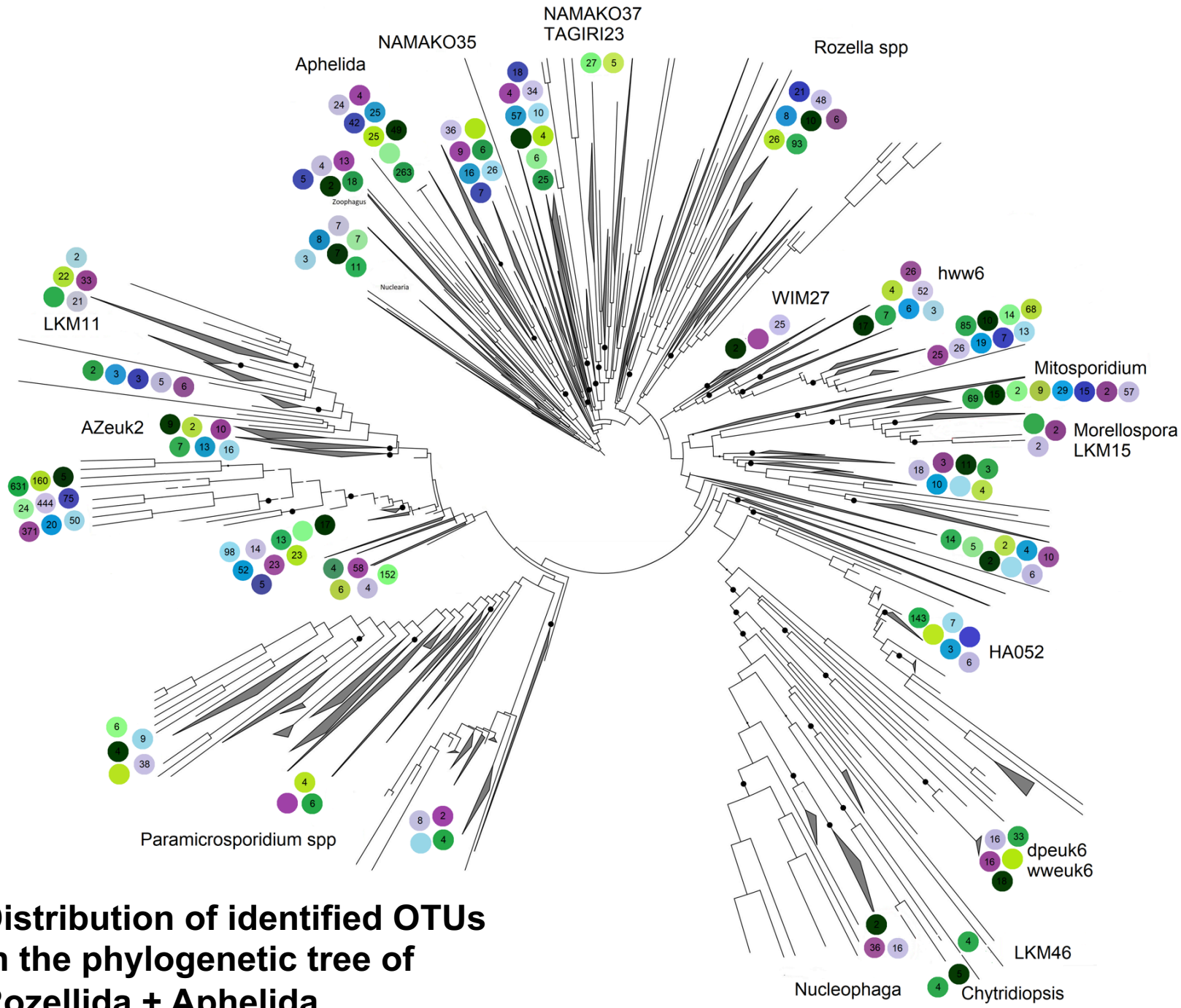
Both PCR protocols used to obtain rozellids and aphelids appeared comparable in terms of efficiency, while the one step protocol is faster and implies less loss of material.

PCR protocols with the primer pairs 18F/530R and V1F/530R appeared equally suitable for amplification of a wide range Microsporidia and also contributed to rozellids and aphelids dataset.

Taxonomic profiling: basal Holomycota



Totally 56 OTUs (366 reads) of Microsporidia, 112 OTUs (954 reads) of Aphelida and 450 OTUs (3818 reads) of Rozellida were obtained. They contributed to the diversity of various clades within the **basal Holomycota tree**, including both the clades with the **described representatives** (*Rozella, Paraphelidium, Aphelidium, Paramicrosporidium, Mitosporidium, Morellospora* and numerous microsporidian species) and the known **environmental clades** (NAMAKO37, LKM46, NAMAKO35, hww6, LKM15, AZeuk2, LKM11, WIM27, dpeuk6, LKM46).



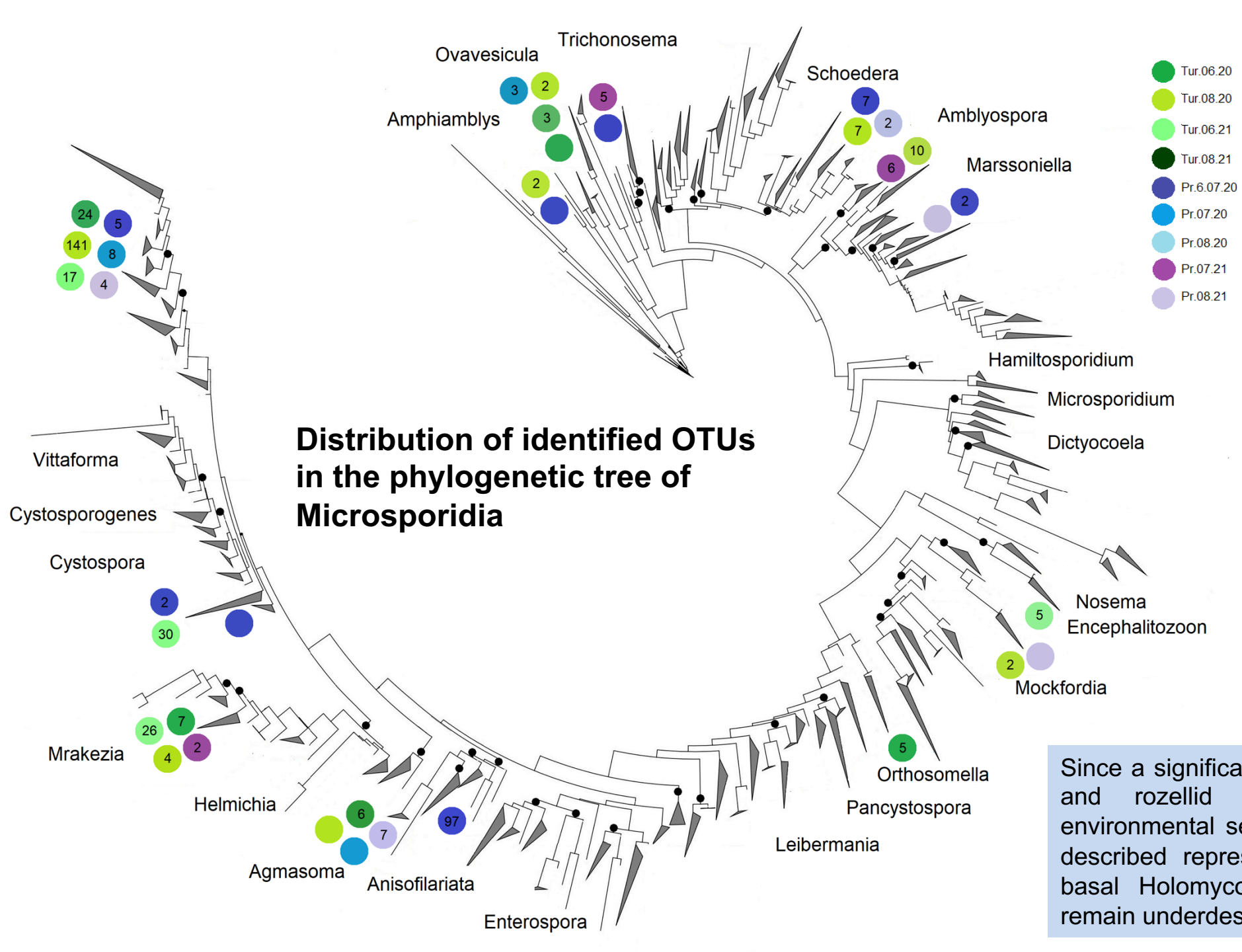
Distribution of identified OTUs in the phylogenetic tree of Rozellida + Aphelida

Phylogenetic reconstruction of Rozellida and Aphelida based on 18S rRNA gene. ML tree generated with IQ-TREE. Black blobs indicate the bootstrap support over 90%. Colored circles indicate the sampling sites, from which the sequences were originated.

- Tur.06.20
- Tur.08.20
- Tur.06.21
- Tur.08.21
- Pr.6.07.20
- Pr.07.20
- Pr.08.20
- Pr.07.21
- Pr.08.21

Sequences contributed to *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.



Phylogenetic reconstruction of Microsporidia based on 18S rRNA gene. ML tree generated with IQ-TREE. Black blobs indicate the bootstrap support over 90%. Colored circles indicate the sampling sites, from which the sequences were originated.

The identified OTUs clustered with the microsporidian sequences from aquatic habitats from clades 4(IV), 1(I), 3(V) (sensu Vossbrinck and Debrunner-Vossbrinck 2005; Vossbrinck et al. 2014).

A potentially new group of early microsporidia basal to Core Microsporidia + Metchnikovellida + RL107-1 (FN546176) was identified.

Since a significant fraction of obtained microsporidian and rozellid sequences formed clades with environmental sequences from Genbank without any described representatives, phylogenetic diversity of basal Holomycota in different ecosystems clearly remain underdescribed and require further study.