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An approach to assembling MAGs of intracellular parasites with a reduced genomes from MDA data

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Motivation and Aim: Representatives of the group Opisthophagea are of interest from the evolutionary point of view. This group comprises two large lineages, Rozellida (also known as Rozellosporidia) and Microsporidia. In phylogenetic trees, they form a separate branch of Holomycota, closely related to the "true" fungi. Microsporidia comprise of a great diversity of species, whereas few representatives have been described for Rozellida. However, recent metagenomic studies show that both these lineages are abundant and inhabit diverse environments. They are intracellular parasites (in many cases even hyperparasites), and they show genome reduction, when part of their functions is performed at the expense of the host genome [1].
Their study is complicated by the fact that they are very difficult to isolate in sufficient numbers for sequencing. In most cases, we deal with metagenomes, and the MDA method is used to obtain material. This method gives uneven coverage, which further complicates sequencing and data analysis. As a result, metagenome-assembled genomes are incomplete, and we need a way to distinguish between genome elements lost during parasitic reduction, or simply not assembled due to various reasons – small amounts of DNA, low coverage, or complex community composition [2].
Methods and Algorithms: We assembled a pipeline to analyse MDA data from genome sequencing of different Opisthophagea specimens. The metagenome is assembled using SPAdes in a single-cell mode (adapted for uneven coverage), then binning with MaxBin2, the resulting bins are checked for completeness and contamination using BUSCO and BlobTools. Finally, we identify the target bin (MAG), and build a phylogenomic tree based on the BUSCO Fungi dataset using a set of publicly available microsporidian genomes. We use both a supermatrix-based approach (general concatenated alignment and phylogenetic reconstruction using the maximum likelihood method) and a supertree-based method – building a separate tree for each of the orthologs and obtaining a tree using Astral.
The resulting tree allows us to determine the taxonomic position of the studied specimen. We then distinguish the clade which the specimen belongs to, using branch length threshold, and then build a loss pattern for each of the genes of interest. We assume that the loss of a gene during reduction must be a synapomorphy, and that the absence of a gene for technical reasons may occur randomly and not be consistent with the topology of the tree. Thus for a range of genes we can reliably detect parasitic loss events.
Results: We analyzed several dozen samples of MDA metagenomes containing microsporidia genomes using our pipeline. For each sample, a target bin was selected, followed by further decontamination and quality assessment of the resulting MAG. The completeness assessment values by BUSCO for microsporidia were in range 20-45 %, and these values were consistent across all samples and were similar within clades. It is

also important to note that for publicly available genomes we obtained similar values. Thus, these values reflect parasitic genome reduction. By using phylogenomic analysis results, we compared the tree topologies for each ortholog with a consensus final tree and identified genes with shared patterns of losses.

Conclusion: The application of the developed approach and accumulation of information allows for the generation of a sufficiently accurate phylogenetic tree for various branches of Opisthophagea, as well as tracking gene loss patterns during parasitic genome reduction. With further increase in the number of specimens, the picture of the evolution of the entire Holomycota branch will become much clearer.

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