

Phylogenomics reveals early evolution of microsporidia

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Motivation and Aim: Microsporidia are a widespread and species-rich group of obligate intracellular parasites of animals and some protists. They combine highly specialized and primitive features in their organization. The biosynthetic pathways and molecular machineries are seriously reduced in these parasites, and the genomes are small and compactly organized. Recent metagenomic studies demonstrated a huge “hidden” diversity of early microsporidia and related groups of organisms, while only a limited number of described representatives is known. The isolation and thorough study of new organisms from this group is of special interest. One of the main challenges is to reconstruct the early steps of evolutionary history of this remarkable group of parasites, which was strongly influenced by reductive evolution.

Methods and Algorithms: To get a sufficient amount of DNA for genome sequencing of miniature intracellular organisms, we applied a single-cell genomics approach. Whole genome amplifications of DNA from individually isolated infected host cells were performed using multiple displacement amplification (MDA). The MDA products were checked for the presence of target DNA using PCR with microsporidia-specific primers for amplification of the SSU rRNA gene. Verified MDA products were used for library preparations and sequencing on the Illumina HiSeq2500 platform. The genomes were assembled using SPAdes in a single-cell mode and binned with MaxBin2, to identify the target bin within the primary metagenomic assembly (parasite + host + bacteria). The resulting target bins were checked for completeness and contamination using BUSCO and BlobTools. The phylogenomic trees based on the BUSCO Fungi dataset were built using a set of publicly available genomes of microsporidia and related organisms. Augustus program was used for *ab initio* determination of functional sequences. Repetitive elements in the genomes were searched with RepeatModeler and the Dfam database. Functional annotation was carried out using the eggNOG-mapper package. Prediction of functional domains was based at the PFAM and eggNOG databases. Functional orthologs of the predicted proteins were determined using the KEGG database.

Results: Early microsporidia were isolated from a broad range of hosts: from protists (regarines from marine annelids, free-living amoebae) to Metazoa (tardigrades, insects). Several new species were identified. The sequenced genomes were rather small and compact. They varied in size from 3.5 to 9.1 Mbp; from 2000 to 3750 ORFs were identified; gene density varied in the range from 0.45 to 0.65 gene/kb. The diversity of mobile elements was represented mainly by simple repeats, rolling circles and low

complexity elements, while in some lineages LINEs, LTR elements and DNA transposons were rather abundant. In some lineages we observed the complete or partial reduction of the mitochondrial genome, while in others, it was not affected by a tendency to degeneration. Phylogenomic analysis suggested the possible branching pattern of early microsporidian lineages. It also demonstrated the widespread occurrence of co-infections with two or more hyperparasitic microsporidian species in one superhost. For example, in the case of metchnikovellid parasites of gregarines from marine annelids, the co-occurring species (apparently represented only by the early proliferative stages) often remained hidden until genomic data became available. The comparative genomic analysis supposed that early evolution of microsporidia involved a series of independent losses and acquisitions of genes and molecular machineries in different lineages, probably as a result of adaptations to the various hosts.

Conclusion: Phylogenomic and comparative genomic analyses of known and recently isolated microsporidia allowed to propose a hypothetical scenario of the early evolution in this group.

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