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The significance of genomic studies of Aphelida (Aphelida, Hooycota, Opisthokonta) for solving questions of evolution in the Opisthokonta group

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Motivation and Aim: Aphelids [1, 2] are the significant object for evolutionary research because:
1) They are the closest evolutionary relatives of Fungi, but do not have morpho-physiological characteristics specific to fungi (osmotrophy, mycelial structure with apical growth, etc.);
2) They have a complex life cycle with alternating immobile plasmodial and motile amoebo-flagellate stages. This cycle, on the one hand, coincides with the life cycles of zoosporic fungi and unicellular Holozoa; on the other hand, there is primary data on the differences of the genetic bases of these life cycles [3, 4].
Thus, genomic and proteomic studies of aphelids allow:
1) Explore the prerequisites and initial stages of the fungal evolution.
2) Raise and solve questions on the evolution of cellular polymorphism in unicellular Opisthokonta and its connection with the origin of multicellularity in filamentous fungi and Metazoa.
We have performed:
1. Assessment of the possibility of enhancing the osmotrophic abilities in aphelids by analyzing the quantitative and qualitative composition of their transmembrane transporter proteins of the MFS (Major facilitator superfamily) superfamily [5, 6].
2. Study of gene expression in *Aphelidium insulamus* at the plasmodium stage.
Methods and Algorithms:
For analysis of MFS proteins:
Major facilitator superfamilyThe MFS-domain proteins initially were selected based on annotations among the hits of the BLASTP searching in the predicted proteomes of selected organisms with annotated genomes. The *Saccharomyces cerevisiae* proteins were taken as the initial queries. The hidden Markov models were built by the hmmbuild program of the hmmer, v.3.3.2 batch [7] for necessary protein families. The final search was performed using hmmsearch among predicted proteoms of representatives of various subgroups of Opisthokonta including four aphelid species (*Amoeboaphelidium protococcarum*, *Amoeboaphelidium occidentale*, *Paraphelidiun tribonematis* and *Aphelidium insulamus*). The multiple sequence alignment (MSA) was prepared in the M-Coffee aligner using the web server interface (https://tcoffee.org). The MSA was treated in TrimAl, v.1.4.rev15 with a gap threshold of 0.5. For the tree construction was

used IQ-Tree 2, v.2.0.3 [8] with determined LG+F+G4 substitution model and 100000 replicates of ultrafast bootstrap.
For gene expression analysis:
Library preparation for RNA-seq was performed using the NEBNext® Single Cell/Low Input RNA Library Prep Kit from New England Biolab (NEB) using a protocol for low input. Sequencing was performed on an Illumina HiSeq sequencer. For each sample, 15 million short paired reads 2x100 were obtained. Short reads were aligned to the already known A. insulamus transcriptome and quantified using the utitlites included in the BBtools package [9]. Further quantified reads were analysed in DEseq2 [10].
Results and Conclusions:
1. It has been shown that studied aphelids lack MFS proteins specific for fungi (both a specific fungal protein family Drug:H+ antiporters-2 (DAH-2) and specific fungal orthologs of the common sugar porters (SP) family) (Fig. 1). The repertoire of SP orthologs in aphelids turned out to be less diverse than in free-living opisthokonts, and one of the most limited among opisthokonts.
We argue that aphelids do not show signs of similarity with fungi in terms of their osmotrophic abilities. Moreover, the osmotrophic abilities of aphelids appear to be reduced in comparison with free-living unicellular opisthokonts.
Thus, it was concluded that the specific evolution of fungi began after the separation of the fungal and aphelid lineages.
2. Groups of genes with the similar expression level, genes having an expression level an order of magnitude greater than the median and genes with the maximum expression level in *A. insulamus* plasmodia were identified. Assumptions are made about the most important metabolic processes for plasmodia and genes whose products are potentially involved in interaction with the host and in the regulation of switching stages of the life cycle are noted.
It is planned to obtain gene expression profiles at other stages of the life cycle and build a general picture of changes in gene expression, metabolic and regulatory processes. Further inclusion of the obtained data in comparative studies.
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