

The transcriptome of *Paraphelidium tribonemae* illuminates the ancestry of Fungi

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Aphelids are fairly unknown parasitoids of algae that have raised considerable interest due to their pivotal phylogenetic position in the holomycotan branch of the opisthokonts. Based on 18S rRNA genes, they form a monophyletic group with Rozellosporidia (Cryptomycota) and Microsporidia, albeit with modest support. This clade has been named as Opisthosporidia, which constitute the sister group to the fungi. Despite their high diversity, as revealed by molecular environmental studies, only four genera have been described. Here we present the first transcriptome for one aphelid, *Paraphelidium tribonemae*. After strict decontamination (aphelids cannot be cultured axenically), the predicted proteome was highly complete as compared to other eukaryotes. We carried out phylogenomic analyses with the widest possible taxon sampling using a concatenated supermatrix approach. Our analyses show that aphelids have a deep-branching position within fungi. We looked for functional markers of interest for the clade and detected genes involved in the chitin cell-wall synthesis pathway and several putative cellulolytic proteins, which suggest that they digest the algal cell-wall during infection. Comparative genomic analyses of functional genes with fungi, *Rozella allomycis* (Cryptomycota), microsporidians (including short and long-branching species) and other opisthokonts (including parasites and free-living species) confirmed that “opisthosporidian” parasites have reduced metabolism while aphelids group with fungi and other free-living species. Since aphelids and *Rozella* are phagotrophs, we specifically looked for phagotrophy-related proteins, which showed that aphelids have more affinities with *Rozella*. Overall, our results support the intermediate position of aphelids between parasitic microsporidians and *Rozella*, on the one hand, and osmotrophic fungi on the other, filling an important gap in opisthokont evolution.