

## Abstract

Order Diplomonadida includes parasitic and free-living species that adapted to the oxygen-poor environment. They possess reduced form of mitochondria (hydrogenosome or mitosome). These organelles lack Krebs cycle and membrane electron-transport chain. ATP synthesis by oxidative phosphorylation and other mitochondrial metabolic pathways are modified or entirely absent. Main difference between hydrogenosome and mitosome is synthesis of hydrogen using the enzyme hydrogenase and ATP synthesis by substrate level phosphorylation in hydrogenosomes that are absent in mitosomes. The most studied diplomonads are a human pathogen *Giardia intestinalis* possessing the mitosomes and a salmon parasite *Spironucleus salmonicida* with hydrogenosomes. This thesis was focused on determining the type of mitochondrial organelles in angelfish parasite *Spironucleus vortens* and free living *Hexamita sp.* It has not been described whether they possess the hydrogenosomes or the mitosomes so far.

In both protists transmission electron microscopy revealed presence of double membrane vesicles, possibly their mitochondrial organelles. Homologous *S. vortens* anti-hydrogenase and anti-HydE antibodies were produced and tested in order to determine their cellular localization. Using the western blot analysis and immunofluorescence microscopy, hydrogenase was detected in the cytosol whereas HydE in mitochondrial organelles of *S. vortens*. Moreover, expression of HydE, HydG and IscU recombinant proteins was performed. All three proteins were detected in the organellar fraction. These results suggest that *S. vortens* possesses mitochondrial organelles with characteristics corresponding to the mitosomes.

*Hexamita sp.* genomic DNA and total RNA was sequenced and the genome was partially assembled. Sequences of proteins involved in ATP synthesis, production of hydrogen, conversion of serine into glycine, H-cluster synthesis and Fe-S cluster synthesis were predicted to reside in the mitochondrial organelle of *Hexamita*. Based on this genome, mitochondrial organelle of *Hexamita sp.* was described as the hydrogenosome.