

## Abstract

Free-living diplomonads are an interesting, but underrated part of genus Diplomonadida. Belonging to Fornicata (Metamonada), diplomonads are anaerobic protists lacking classical mitochondrion. Most of them are endobiotic with *Giardia intestinalis* being the most well known as well as the main focus of many studies. Free-living diplomonads on the other hand, whilst claimed to be secondarily free-living and therefore are an interesting subject of phylogenetic studies, have been largely neglected.

We obtained and analyzed 65 new sequences of diplomonad SSU rRNA gene, of which 58 belonged to free-living species (specifically genera *Gyromonas*, *Hexamita*, *Trepomonas* and *Trimitus*). We also performed a light-microscopic morphological study of selected strains. The ultrastructure of *Trepomonas rotans* was studied for the first time.

Our phylogenetic analysis revealed genus *Hexamita* and also genus *Trepomonas* as polyphyletic. *Trepomonas rotans*, which forms a separate branch related to genus *Hexamita*, may actually be a novel genus. This is further supported by the fact that this species has the cell covered in scales, which is a trait unique in Diplomonadida. Our results show a complex evolutionary process in free-living diplomonads, therefore being genuinely worthwhile of further research.