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Ph.D. thesis

EVOLUTION OF INTESTINAL FLAGELLATES

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EDUCATION:

1996 – 2002: Master degree in Biology, Faculty of Science Charles University, thesis: *Phylogenetic analysis of the genus Tetratrichomonas*.

2002 – Postgraduate study of Parasitology, Faculty of Science, Charles University, thesis: *Evolution of intestinal flagellates*.

2003: Visiting student at laboratory of Andrew Roger, PhD. Dalhousie University, Halifax, NS, Canada

Introduction

Anaerobic flagellates of groups Metamonada and Slopalinida constitute important components of the complex intestinal ecosystems of both vertebrates and invertebrates. Evolution of the both groups is still poorly understood. Metamonada, which was once thought to be primarily amitochondrial and to belong to the separate kingdom Archezoa, are now classified as a part of the controversial eukaryotic “megagroup” Excavata. Metamonada consist of mostly parasitic Parabasala, Eopharyngia and Oxymonada, as well as of free-living *Trimastix* and *Carpediemonas*.

The view of the parabasalid evolution significantly changed with the application of molecular-phylogenetic methods. Now it has been widely accepted that Trichonymphida with rather complex morphology form the most basal branch of Parabasala and that the complex hypermastigote morphology, as well as the simple monocercomonad morphology, have arisen several times independently. Little is known about both intra- and interspecies molecular polymorphism, as well as about the host specificity of parabasalid species.

Classes Retortamonadea and Trepomonadea constitute together the group Eopharyngia. Two cell organizations which are believed to form monophyletic taxa occur in Trepomonadea: monozoic Enteromonadina and diplozoic Diplomonadina which have all cell structures mirror-like doubled. Diplomonads are thought to have arisen once from enteromonads.

The group Slopalinida comprises rather diverse families Proteromonadidae and Opalinidae which resemble each other by the structure of flagellar transitional zone and by presence of cortical microtubuli. No molecular data verifying the monophyly of Slopalinida are available so far. The enigmatic fungus-like genus *Blastocystis* probably forms a branch sister to Slopalinida. Slopalinida, *Blastocystis*, Oomycetes and some other stramenopiles are on the basis of similarities in the structure of the transitional flagellar zone thought to constitute a monophyletic taxon Bigyra.

Aims of the thesis

1. To obtain isolates of intestinal flagellates, to determine them and to characterize them by methods of molecular phylogenetics.
2. To add new taxa into the SSU rRNA tree of Parabasala and analyze its topology with an emphasis on the presence of the long-branch attraction artifact.
3. To analyze molecular polymorphism and host specificity of lineages within the genus *Tetratrichomonas* on the basis of sequential and RAPD analysis.
4. To determine phylogenetic position of monozoic representatives of the group Eopharyngia, to infer the relationship between classes Retortamonadea and Trepomonadea and confirm the possibility of the origin of diplozoic Diplomonadina from monozoic Enteromonadina.
5. To determine phylogenetic position of the family Opalinidae and confirm the close relationship of families Opalinidae, Proteromonadidae, and the genus *Blastocystis*.

Results

1. Isolates of intestinal flagellates

We obtained and cultured 130 isolates of intestinal flagellates belonging to eighteen genera of trichomonads, diplomonads, enteromonads, retortamonads and oxymonads, four of the genera being new, and to the genus *Blastocystis*. We also obtained uncultured isolates belonging to genera *Karotomorpha* and *Blastocystis*.

2. Evolution of Parabasala

We performed a thorough analysis of the topology of the parabasalid SSU rRNA tree; one branch (*Hexamastix*) was included. Using the slow-fast and taxa-exclusion methods, we showed that the position of the root on the branch of Trichonympida could be, in fact, caused by the long-branch attraction artifact. We confirmed the non-monophyly of Trichomonadidae, Monocercomonadidae and Hypermastigotea.

3. Molecular polymorphism and host specificity within the genus

Tetratrichomonas

We analyzed molecular polymorphism among 90 *Tetratrichomonas* isolates obtained from various vertebrate and invertebrate hosts using the sequences of the ITS region, SSU rRNA gene, α -tubulin gene and their concatenate. We demonstrated monophyly of the genus *Tetratrichomonas* in

respect to the genus *Trichomonas*. We showed that genera *Trichomonoides* and *Pentatrichomonoides* may, in fact, belong to the genus *Tetratrachomonas*. We divided the isolates into 16 clades. We infer from great genetic distances, large degree of host specificity and morphological characteristics that most or all of them represent independent species. This considerably increases the known diversity of this genus because only five species were described from the studied host taxa. At least one of the clades (*Tetratrachomonas gallinarum*) probably represents a complex comprised of several species, some of them being cryptic.

4. Phylogenetic position of retortamonads and enteromonads

We determined the phylogenetic position of the monozoic genera *Retortamonas* (Retortamonadea) and *Trimitus* (Enteromonadina) on the basis of the phylogeny of the SSU rRNA gene. The genus *Retortamonas* is sister to the diplozoic genus *Giardia* making Trepomonadea polyphyletic, but this position is not well-supported and is probably artificial. The enteromonad genus *Trimitus* branches within Hexamitinae diplomonads. Our unpublished data show that three enteromonad genera (*Trimitus*, *Enteromonas* and undescribed enteromonad) form two or three independent branches within Hexamitinae. This would mean that either diplozoic diplomonads must have arisen several times from monozoic enteromonads, or *vice versa*.

5. Phylogenetic position of opalinids

We determined the phylogenetic position of *Protoopalina intestinalis* based on analysis of the SSU rRNA gene. We demonstrated that the order Slopalinida is monophyletic, the genus *Blastocystis* forming its sister taxon. The group Bigyra is not monophyletic.

List of publications

Silberman JD, Simpson AGB, Kulda J, Cepicka I, Hampl V, Johnson PJ, Roger AJ (2002). Retortamonad flagellates are closely related to diplomonads – implications for the history of mitochondrial function in eukaryote evolution. *Mol Biol Evol*, 19: 777-786

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List of selected abstracts

Hampl V, **Cepicka I**, Tachezy J, Kulda J, Flegr J (2001). Phylogenetic position and relationship of flagellates of the genus *Monocercomonas* from different host species. *7th Meeting of PhD Students in Evolutionary Biology*. Bernried, Germany.

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Cepicka I, Hampl V, Kulda J, Flegr J (2003). Molecular polymorphism and host specificity in the genus *Tetratrichomonas*. *CIAR (Evolutionary Biology Program) Annual Meeting*. White Point Beach, Canada.

Cepicka I, Hampl V, Kulda J, Flegr J (2005). New evolutionary lineages, unexpected diversity and host specificity in the parabasalid genus *Tetratrichomonas*. *12th International Congress of Protozoology*. Guangzhou, China.

Kostka M, Hampl V, **Cepicka I**, Flegr J (2005). Phylogenetic position of *Protoopalina intestinalis* based on SSU rRNA sequence. *12th International Congress of Protozoology*. Guangzhou, China.

Kolisko M, **Cepicka I**, Hampl V, Kulda J, Flegr J (2005). The phylogenetic position of enteromonads: a challenge for the present models of diplomonad evolution. *12th International Congress of Protozoology*. Guangzhou, China.

