

Evolutionary processes in the formation of the diversity of hymenolepidid cestodes parasitizing on insectivore mammals of the family Soricidae

Shrews were collected by trapping in several localities in Bulgaria. After parasitological autopsy adult hymenolepidid cestodes were isolated from the shrew intestines. Each cestode included in the analysis was divided into two parts. The anterior part was stained and mounted in Canada balsam. The posterior parts of the specimens included in the analysis were used as tissue samples for DNA extraction. PCR reactions were carried out resulting in the amplification of several fragments from the nuclear genes for 28S and 18S rRNA and the mitochondrial gene *cox1*. The obtained fragments were sequenced and later used for phylogenetic reconstruction using also data published by previous researchers. Phylogenetic analyses were performed for each of the three gene fragments and also a combined analyses with all the data was done.

The general configuration of 28S phylogenetic tree is similar to the previous phylogenetic hypothesis for relationships among hymenolepidids from mammals. However, our study includes 56 taxa of the family Hymenolepididae (versus 31 taxa in the only previously published major analysis of the group). Our analysis confirmed the existence of 4 major monophyletic clades of mammalian hymenolepid cestodes (“Ditestolepis clade”, “Hymenolepis clade”, “Arostrilepis clade” and Rodentolepis clade). The Ditestolepis clade position was revealed as basal to the other three clades, however the support for this topology was weak. The data confirmed the monophyly of several hymenolepidid genera, like Hymenolepis and Coronacanthus and also the monophyly of the hymenolepidids from glirid rodents. This study also revealed the non-monophyletic status of several other genera like Staphylocystis and Staphylocystoides. Close phylogenetic relationships among other groups was also demonstrated – e.g. between the hymenolepidids with unarmed rostellum – the genera Arostrilepis, Soricinia and Pseudobothrialepis; also the position as sister taxa of Pararodentolepis and Nomadolepis.

Mapping of morphological and anatomical characters to the phylogenetic analysis revealed that several characters, considered important from taxonomic point of view are homoplasious, since they occur in distant groups – the type of strobilar maturation, the general reduction of rostellar apparatus (although the cestodes with full reduction of the rostellum are closely related), presence of polar filaments in the embryophore.

From the four main phyletic groups of mammalian hymenolepidids, only the Ditestolepis clade is closely associated with insectivore mammals of the family Soricidae. All of the other three clades are associated with definitive hosts of two or more mammalian orders. There is no strong association between the studied cestode clades and their intermediate hosts.

The analyses of the 18S rRNA and *cox1* genes as well as the combined analysis confirm the validity of the four main mammalian hymenolepidid clades but the basal relationship among them remains unresolved.

Revealed data demonstrates that in the formation of the diversity of the mammalian hymenolepidid species both the processes of colonization of new hosts and coevolution have played an important role.