Parasitology Research (2019) 118:73–88
https://doi.org/10.1007/s00436-018-6117-y

ORIGINAL PAPER

Phylogeny of hymenolepidid cestodes (Cestoda: Cyclophyllidea) from mammalian hosts based on partial 28S rDNA, with focus on parasites from shrews

Boyko Neov[1] · Gergana P. Vasileva[1] · Georgi Radoslavov[1] · Peter Hristov[1] · D. Timothy J. Littlewood[2] · Boyko B. Georgiev[1]

Received: 2 August 2018 / Accepted: 7 October 2018 / Published online: 18 October 2018

Abstract

The aims of the study are to enrich the partial 28S rDNA dataset for hymenolepidids by adding new sequences for species parasitic in the genera *Sorex*, *Neomys* and *Crocidura* (Soricidae) and to propose a new hypothesis for the relationships among mammalian hymenolepidids. New sequences were obtained for *Coronacanthus integrus*, *C. magnihamatus*, *C. omissus*, *C. vassilevi*, *Ditestolepis diaphana*, *Lineolepis scutigera*, *Spasskylepis ovaluteri*, *Staphylocystis tiara*, *S. furcata*, *S. uncinata*, *Vaucherilepis trichophorus* and *Neoskrjabinolepis* sp. The phylogenetic analysis (based on 56 taxa) confirmed the major clades identified by Haukisalmi et al. (Zool Scr 39:631–641, 2010) based on analysis of 31 species: *Ditestolepis* clade, *Hymenolepis* clade, *Rodentolepis* clade and *Arostrilepis* clade; however, the support was weak for the early divergent lineages of the tree and for the *Arostrilepis* clade. Novelties revealed include the molecular evidence for the monophyly of *Coronacanthus*, the non-monophyletic status of *Staphylocystis* and the polyphyly of *Staphylocystoides*. The analysis has confirmed the monophyly of *Hymenolepis*, the monophyly of hymenolepidids from glirids, the position of *Pararodentolepis* and *Nomadolepis* as sister taxa, the polyphyly of *Rodentolepis*, the position of *Neoskrjabinolepis* and *Lineolepis* as sister taxa, and the close relationship among the genera with the entire reduction of rostellar apparatus. Resolved monophyletic groups are supported by the structure of the rostellar apparatus. The diversification of the *Ditestolepis* clade is associated with soricids. The composition of the other major clades suggests multiple evolutionary events of host switching, including between different host orders. The life cycles of *Coronacanthus* and *Vaucherilepis* are recognised as secondarily aquatic as these taxa are nested in terrestrial groups.

Keywords Phylogeny · Taxonomy · Hymenolepididae · 28S rDNA gene

Introduction

The Hymenolepididae Perrier, 1897 is the most speciose cestode family, with more than 920 valid species (Mariaux et al. 2017). They are parasitic as adults in birds (mostly aquatic) and mammals. The diversity of hymenolepidids from mammals is estimated at 358 species (Mariaux et al. 2017). Hosts of mammalian hymenolepidids are several groups, with the majority of genera and species occurring in insectivores (Eulipotyphla), rodents (Rodentia) and bats (Chiroptera) (Vaucher 1971; Czaplinski and Vaucher 1994; Georgiev et al. 2006; Binkienė et al. 2011; Mariaux et al. 2017). Earlier views on phylogenetic relationships among hymenolepidids from mammals were expressed by proposals for their classification, especially by the erection of numerous genera based on morphology, host associations and life-cycle patterns, and believed to represent monophyletic groups (Spasskiy 1954; Vaucher 1992; Czaplinski and Vaucher 1994; Gulyaev and Kornienko 1999; Gulyaev et al. 2004; Gulyaev and Mel'nikova 2005).

An entire concept of the phylogenetic relationships among the hymenolepidid cestodes is lacking. Haukisalmi et al. (2010) postulated that hymenolepidids from "rodents and

Handling Editor: Julia Walochnik

✉ Boyko B. Georgiev
bbg@ecolab.bas.bg

[1] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

[2] Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

shrews” represented a monophyletic group, though this monophyly had not been tested by inclusion of taxa parasitic in birds. Their assumption is congruent with the results of a preliminary phylogenetic analysis based on 28S rRNA genes (V. V. Tkach, V. V. Kornyushin, B. B. Georgiev and D. T. J. Littlewood, unpublished) revealing hymenolepidids from mammals as forming a monophyletic group together with those from terrestrial (non-aquatic) birds.

Earlier molecular phylogenetic studies were focussed on the examination of the relationships between the major phyletic lineages or orders of cestodes (Mariaux 1998; Mariaux and Olson 2001; Olson and Tkach 2005; Waeschenbach et al. 2007, 2012). No molecular phylogenetic hypotheses have been proposed for the cestode order Cyclophyllidea as a whole or for the family Hymenolepididae. However, special attention was paid to the phylogeny of mammalian hymenolepidids based on sequencing partial (D1-D3) 28S rDNA and a hypothesis for their relationships, though based on a limited number of taxa, was proposed (Haukisalmi et al. 2010). Since the latter pioneer study on molecular phylogeny of mammalian hymenolepidids, it has become a standard practice to include molecular data when describing or redescribing species of this group (e.g. Greiman and Tkach 2012; Greiman et al. 2013; Tkach et al. 2013, 2018; Makarikov et al. 2015, 2018; Binkienė et al. 2015, 2018). This has contributed to a growing set of published gene sequence data available on GenBank, paving the way to more comprehensive elucidation of mammalian hymenolepidid phylogeny.

The aim of the present study is to expand the knowledge on the phylogenetic relationships among mammalian hymenolepidids by analysing a bigger, more comprehensive dataset of species than in previous studies. We used new 28S rDNA sequences of hymenolepidids from shrews obtained by us as well as recently published data by other researchers.

Materials and methods

Cestode sampling and identification

Shrews were collected by trapping from Boyana River, Vitosha Mts. (42.6369°, 23.2598°) and Kalimok Field Station (44.0116° 26.4397°) near Nova Cherna, Bulgaria. Adult cestodes were isolated from intestines. Specimens were preserved in 70% ethanol permitting both morphological and molecular study. Each cestode included in the analysis was divided into two parts. The anterior part (containing the scolex) was stained with iron acetocarmine (Georgiev et al. 1986) and dehydrated in alcohol series, cleared in dimethyl phthalate and mounted in Canada balsam for morphological identification of the species. Some specimens were mounted in Berlese’s medium (Swan 1936) when additional examination of the rostellar armament was deemed necessary. When more specimens were available in a host individual, some of them were mounted as entire specimens in order to be used as comparative material during the identification process. Specimens used for DNA extraction were deposited as voucher slides in the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER–BAS), Sofia (for acquisition numbers, see Table 1). The posterior parts of the specimens included in the molecular analysis were used as tissue samples for DNA extraction.

DNA extraction, PCR amplification and sequencing

Total DNA was isolated using Single Worm PCR Protocol (Williams et al. 1992). Samples were incubated in lysis buffer (containing 1× PCR buffer and 0.8 mg/ml Proteinase K) at 56 °C for 12 h followed by inactivation of the enzyme at 95 °C for 15 min. The amplification of a region of 28S rRNA gene that covers the variable regions D2-D3 was accomplished using the following primers D_2A 5′-ACAAGTACCGTGAGGGAAAGTTG-3′ and D_3B 5′-TCGGAAGGAACCAGCTACTA-3′ as suggested by De Ley et al. (1999). The PCR mixtures contained 25 μL of NZYTaq 2× Colourless Master Mix (Cat. No. MB04002; Nzytech, Lisbon, Portugal), 1 μM of each primer (FOR/REV) and 10 ng of template DNA in a total volume of 50 μL. All PCR reactions were carried out under the following conditions: initial denaturation at 94 °C for 5 min, 30 cycles (denaturation at 94 °C for 30 s; primer annealing at 50 °C for 30 s; extension at 72 °C for 90 s) and final extension at 72 °C for 10 min. PCR products were visualised on 1% agarose gel with GreenSafe staining (NZYTech, Lisbon, Portugal) under UV light. Fragment size was determined using GeneRulerTM 100 bp Ladder Plus (Fermentas, Thermo Scientific, Waltham, USA). All amplicons were purified by GeneJET™ PCR Purification Kit (Fermentas, Thermo Scientific, Waltham, USA) and sequenced in both directions by a PlateSeq kit (Eurofins Genomics, Ebersberg, Germany).

Phylogenetic analysis

The newly obtained 13 nucleotide sequences from D2-D3 28S rDNA were manually edited and aligned with MEGA software version 7.0 (Kumar et al. 2016). An analysis using Basic Local Alignment Search Tool (BLAST analysis, see www.ncbi.nlm.nih.gov/BLAST) was applied for comparison and possible identification with sequences available in GenBank for the family Hymenolepididae (Table 2).

For phylogenetic analyses, we used published sequences from several previous studies (Olson et al. 2001; Haukisalmi et al. 2010; Greiman and Tkach 2012; Greiman et al. 2013; Tkach et al. 2013, 2018; Binkienė et al. 2015, 2018; Makarikov et al. 2015, 2018; Nkouawa et al. 2016) and

Table 1 Cestode species sequenced and used in the course of present analysis

Cestode species	Host species	Host family	Locality (Bulgaria)	GenBank accession no.	Vouchers[1]
Ditestolepis diaphana (Cholodkowski, 1906)	*Sorex araneus*	Soricidae (Soricini)	Kalimok	MH587182	C0128.1.1
Coronacanthus integrus (Hamann, 1891)	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	KJ710324	C0128.1.5
Coronacanthus magnihamatus Vasileva, Tkach and Genov, 2005	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	KJ710326	C0128.1.7
Coronacanthus omissus Baer and Joyeux, 1943	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	KJ710323	C0128.1.6
Coronacanthus vassilevi Genov, 1980	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	KJ710325	C0128.1.8
Lineolepis scutigera (Dujardin, 1845)	*Sorex araneus*	Soricidae (Soricini)	Kalimok	MH587179	C0128.1.2
Neoskjrabinolepis sp.	*Sorex araneus*	Soricidae (Soricini)	Kalimok	MH587178	C0128.1.3
Spasskylepis ovaluteri Schaldybin, 1964	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	MH587183	C0128.1.9
Staphylocystis tiara (Dujardin, 1845)	*Crocidura suaveolens*	Soricidae (Crocidurinae)	Kalimok	MH587181	C0128.1.11
Staphylocystis furcata (Stieda, 1862)	*Sorex araneus*	Soricidae (Soricini)	Kalimok	MH587175	C0128.1.4
Staphylocystis uncinata (Stieda, 1862)	*Crocidura suaveolens*	Soricidae (Crocidurinae)	Kalimok	MH587177	C0128.1.12
Staphylocystis uncinata (Stieda, 1862)	*Crocidura suaveolens*	Soricidae (Crocidurinae)	Kalimok	MH587176	C0128.1.13
Vaucherilepis trichophorus Tkach, Vasileva and Genov, 2003	*Neomys fodiens*	Soricidae (Nectogalini)	Boyana River	MH587180	C0128.1.10

[1] Accession numbers of the specimens used for DNA extraction ("hologenophores" according to Pleijel et al. 2008) in the IBER–BAS Helminthological Collection are presented

available in GenBank (Table 2). Phylogenetic analysis was performed using Bayesian inference (BI) with MrBayes (Ronquist et al. 2012). Prior to analysis, the best model of nucleotide substitution was selected using MrModeltest2 (Nylander et al. 2004); this was the general time reversible model, with estimates of invariant sites and gamma distributed among-site rate variation (GTR+G+I). The analysis was run for 1.5×10^7 generations, two separate runs, each with four chains, discarding 33% of resulting trees as burn-in. As outgroup, we used a sequence of *Dilepis undula* (Schrank, 1788) (see Table 2), a species of the family Dilepididae, believed to represent the most closely related family-group taxon, from which matching molecular data were available (Mariaux et al. 2017). For some clades revealed by the present analysis, we used the names proposed by Haukisalmi et al. (2010), though in the majority of cases, additional taxa were added based either on our new data or published sequences by other authors. Average standard deviation of split frequencies below 0.01 was observed and served as a proof of chains reaching convergence. Branches persisting in less than 50% of post burn-in samples were treated as polytomies. Nodal support was expressed as posterior probabilities.

The number of base differences per site from averaging over all sequence pairs between the main clades revealed was calculated in MEGA7 (Kumar et al. 2016). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). The analysis involved 63 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e. fewer than 5% alignment gaps, missing data and ambiguous bases were allowed at any position. There were a total of 930 positions in the final dataset.

Results

Genetic characterisation of newly sequenced hymenolepidid cestodes

Thirteen individuals of 12 cestode species isolated from shrews in Bulgaria were sequenced in the present study (Table 1). The sequences were deposited in GenBank (for accession numbers, see Table 1). New sequences spanned the D2-D3 fragment of 28S rRNA gene from position 376 to position 1522 according to a reference sequence, Acc. No. AY157181 *Hymenolepis diminuta* (Rudolphi, 1809) (Lockyer et al. 2003).

For seven species, i.e. *Coronacanthus integrus* (Hamann, 1891), *C. magnihamatus* Vasileva, Tkach and Genov 2005, *C. omissus* (Baer and Joyeux, 1943), *C. vassilevi* Genov, 1980, *Vaucherilepis trichophorus* Tkach, Vasileva and Genov, 2003, *Staphylocystis tiara* (Dujardin, 1845) and *S. uncinata* (Stieda, 1862), the obtained sequences represent the first available genetic data. Sequences were also obtained for an unidentified species of the genus *Neoskrjabinolepis* Spassky, 1947, probably representing an undescribed species; it differs by a single nucleotide substitution from *N. schaldybini* Spassky, 1947 (0.1% genetic difference) sequenced by Haukisalmi et al. (2010). For four species that were previously sequenced on the basis of specimens from elsewhere, i.e. *Staphylocystis furcata* (Stieda, 1862), *Lineolepis scutigera* (Dujardin, 1845), *Ditestolepis diaphana* (Cholodkowsky, 1906) and *Spasskylepis ovaluteri* Schaldybin, 1964, we obtained additional 28S rDNA sequences based on specimens from Bulgaria. The sequence of

Table 2 Published sequences of 28S rDNA of dilepidid (*Dilepis undula*, outgroup) and hymenolepidid cestodes deposited in GenBank used in the present phylogenetic analysis

Cestode species	GenBank accession no.	Host species	Host family	Geographic origin	Source
Hymenolepididae					
Armadolepis dryomi Makarikov et al., 2018	MG025955	*Dryomys nitedula*	Gliridae	Russia (Rostov Oblast')	Makarikov et al. (2018)
Armadolepis longisoma Makarikov et al., 2018	MG025954	*Glis glis*	Gliridae	Russia (Adygeya)	Makarikov et al. (2018)
Armadolepis tenorai Makarikov, 2017	MG025953	*Dryomys nitedula*	Gliridae	Kazakhstan	Makarikov et al. (2018)
Arostrilepis sp.	GU166223	*Lemmus sibiricus*	Cricetidae	Russia (Wrangel I.)	Haukisalmi et al. (2010)
Ditestolepis diaphana (Cholodkowsky, 1906)	GU166253	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Ditestolepis sp.	GU166261	*Sorex isodon*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Gulyaevilepis tripartita (Zarnowski, 1955)	GU166255	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
"Hymenolepididae sp. A"[1]	GU166277	*Glis glis*	Gliridae	France	Haukisalmi et al. (2010)
"Hymenolepididae sp. B"[1]	GU166239	*Glis glis*	Gliridae	Croatia	Haukisalmi et al. (2010)
"Hymenolepididae sp. C"	GU166273	*Neomys fodiens*	Soricidae (Nectogalini)	Finland	Haukisalmi et al. (2010)
Hymenolepis diminuta (Rudolphi, 1819)	GU166229	*Rattus rattus*	Muridae	Madagascar	Haukisalmi et al. (2010)
Hymenolepis erinacei (Gmelin, 1790)	KX928758	*Erinaceus roumanicus*	Erinaceidae	Lithuania	Binkienė et al. (2018)
Hymenolepis hibernia Montgomery, Montgomery and Dunn, 1987	HM138527	*Apodemus sylvaticus*	Muridae	Croatia	Haukisalmi et al. (unpublished, used by Binkienė et al. 2018)
Hymenolepis weldensis Gardner and Schmidt, 1988	GU166230	*Geomys bursarius*	Geomyidae	USA (Illinois)	Haukisalmi et al. (2010)
Hymenolepis sp.	GU166227	*Apodemus sylvaticus*	Muridae	Turkey	Haukisalmi et al. (2010)
Hymenolepis sp.	LC064142	*Homo sapiens*	Hominidae	China	Nkouawa et al. (2016)
Lineolepis scutigera (Dujardin, 1845)	GU166250	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Neoskrjabinolepis schaldybini Spasskii, 1947	GU166248	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Nomadolepis fareasta Makarikov, Mel'nikova and Tkach, 2015	KT161962	*Cricetulus barabensis*	Cricetidae	Russia (Amursk Oblast')	Makarikov et al. (2015)
Nomadolepis shiloi Makarikov Mel'nikova and Tkach, 2015	KT161963	*Micromys minutus*	Muridae	Russia (Novosibirsk Oblast')	Makarikov et al. (2015)
Pararodentolepis gnoskei (Greiman and Tkach, 2012)	JQ260806	*Suncus varilla minor*	Soricidae (Crocidurinae)	Malawi	Greiman and Tkach (2012)
Pararodentolepis sp.	KT161964	*Sylvisorex johnstoni*	Soricidae (Crocidurinae)	Uganda	Makarikov et al. (2015)
Pseudobothrialepis globosoides (Soltys, 1954)	GU166269	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (unpublished, used by Binkienė et al. 2015)
Rodentolepis asymmetrica (Janicki, 1904)	GU166232	*Microtus agrestis*	Cricetidae	Sweden	Haukisalmi et al. (2010)
Rodentolepis fraterna (Stiles, 1906)	GU166241	*Micromys minutus*	Muridae	Finland	Haukisalmi et al. (2010)
Rodentolepis fraterna	GU166268	*Mus domesticus*	Muridae	Spain (Canary Islands)	Haukisalmi et al. (2010)
Rodentolepis microstoma (Dujardin, 1845)	GU166266	*Mus domesticus*	Muridae	Spain (Canary Islands)	Haukisalmi et al. (2010)
Rodentolepis straminea (Goeze, 1782)	GU166264	*Apodemus sylvaticus*	Muridae	France	

Table 2 (continued)

Cestode species	GenBank accession no.	Host species	Host family	Geographic origin	Source
					Haukisalmi et al. (2010)
Rodentolepis sp.	GU166243	*Cricetulus barabensis*	Cricetidae	Russia (Buryatia)	Haukisalmi et al. (2010)
Soricinia bargusinica (Eltyschev, 1975)	KM516211	*Sorex* sp.	Soricidae (Soricini)	Russia (Irkutsk Oblast')	Binkienė et al. (2015)
Soricinia genovi Binkiene, Kornienko and Tkach, 2015	KM516213	*Neomys fodiens*	Soricidae (Nectogalini)	Bulgaria	Binkienė et al. (2015)
Soricinia infirma (Zarnowski, 1955)	GU166259	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Soricinia infirma	KM516215	*Sorex araneus*	Soricidae (Soricini)	Ukraine	Binkienė et al. (2015)
Soricinia quarta (Karpenko, 1983)	KM516212	*Sorex tundrensis*	Soricidae (Soricini)	Russia (Yakutia)	Binkienė et al. (2015)
Spasskylepis ovaluteri Schaldybin, 1964	GU166262	*Sorex caecutiens*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Staphylocystis brusatae (Vaucher, 1971)	JQ260805	*Crocidura suaveolens*	Soricidae (Crocidurinae)	Ukraine	Greiman and Tkach (2012)
Staphylocystis clydesengeri Tkach et al., 2013	KF257901	*Sorex vagrans*	Soricidae (Soricini)	USA (Montana)	Tkach et al. (2013)
Staphylocystis furcata (Stieda, 1862)	GU166274	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Staphylocystis schilleri (Rausch and Kuns, 1950)	KF257896	*Sorex palustris*	Soricidae (Soricini)	USA (Montana)	Tkach et al. (2013)
Staphylocystoides gulyaevi Greiman, Tkach and Cook, 2013	KC789834	*Sorex monticolus*	Soricidae (Soricini)	USA (Alaska)	Greiman et al. (2013)
Staphylocystoides oligospinosus Tkach, Kinsella and Greiman, 2018	MG273725	*Sorex cinereus*	Soricidae (Soricini)	USA (Montana)	Tkach et al. (2018)
Staphylocystoides parasphenomorphus Tkach, Kinsella and Greiman, 2018	MG273726	*Sorex cinereus*	Soricidae (Soricini)	Canada (Yukon)	Tkach et al. (2018)
Staphylocystoides parvissima (Voge, 1953)	KC789836	"*Sorex* sp."	Soricidae (Soricini)	USA (Oregon)	Greiman et al. (2013)
Staphylocystoides sphenomorphus (Locker and Rausch, 1952)	MG273724	*Sorex vagrans*	Soricidae (Soricini)	USA (Montana)	Tkach et al. (2018)
Staphylocystoides stefanskii (Zarnowski, 1954)	GU166258	*Sorex minutus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Urocystis prolifer Villot, 1880	GU166252	*Sorex araneus*	Soricidae (Soricini)	Finland	Haukisalmi et al. (2010)
Vampirolepis sp.	GU969051	*Eptesicus nilssoni*	Vespertilionidae	Finland	Haukisalmi et al. (2010)
Vampirolepis sp.	JQ260802	*Scotomanes ornatus*	Vespertilionidae	China	Greiman and Tkach (2012)
Vigisolepis spinulosa (Cholodkowsky, 1906)	JQ260803	*Sorex araneus*	Soricidae (Soricini)	Ukraine	Greiman and Tkach (2012)
Dilepididae					
Dilepis undula (Schrank, 1788)	AF286915	*Turdus merula*	Turdidae	UK	Olson et al. (2001)

---

[1] Names used by Haukisalmi et al. (2010) are presented. According to Makarikov et al. (2018), these two forms belong to the genus *Armadolepis* but their species identification requires further studies

S. furcata was identical with one published by Haukisalmi et al. (2010). For the remaining three species, they were very similar to previously published examples for the same species: *L. scutigera* differed by three substitutions (0.26% difference), *D. diaphana* differed by 22 substitutions (1.96% difference) and *S. ovaluteri* differed by three deletions of totally 18 nucleotides and 14 substitutions (2.79% difference) from those sequenced by Haukisalmi et al. (2010).

Phylogenetic analysis

The phylogenetic tree of hymenolepidids from mammals (Fig. 1) supported early, deep divergence between the group of taxa with serial maturation of strobila (“*Ditestolepis* clade” of Haukisalmi et al. 2010) consisting of cestodes from soricids (the genera *Ditestolepis* Soltys, 1952, *Spasskylepis* Schaldybin, 1964 and *Gulyaevilepis* Kornienko and Binkiene, 2014) and the remaining mammalian hymenolepidids characterised (with a few exceptions) by gradual strobilar maturation and comprising parasites of shrews, rodents, chiropterans, humans and erinaceomorph mammals. Among the latter group, the deeper (“backbone”) branching was relatively weakly supported. However, several strongly supported clades were outlined, each of them characterised by a constant composition of taxa included. Since they confirmed the main clades revealed by Haukisalmi et al. (2010), we used for names proposed by these authors for consistency. These clades were as follows:

- “*Rodentolepis* clade”. This strongly supported clade consisted of parasites of rodents, chiropterans and soricids. Several well-defined subgroups were outlined. One of them was the subclade consisting of the genera *Nomadolepis* Makarikov, Gulyaev and Krivopalov, 2010, *Pararodentolepis* Makarikov and Gulyaev, 2009 and species of the genera *Rodentolepis* Spassky, 1954 and *Staphylocystis* Villot, 1877 representing parasites of either shrews or rodents. Other well-supported monophyletic groups in this clade were as follows: (i) hymenolepidids of glirid rodents (*Armadolepis* spp. and unidentified species from the same host group); (ii) species of *Vampirolepis* Spassky, 1954, a genus parasitic in bats; and (iii) a group of taxa parasitizing soricids plus the rodent cestode *Rodentolepis straminea* (Goeze, 1782); in the latter subclade, there was a clear separation of cestodes from *Sorex* spp. from those isolated from other hosts. The first sequence for *Staphylocystis tiara*, a parasite of crocidurine shrews, which had been obtained in the course of the present study, placed this species separately and distantly from other species considered its congeners.
- “*Hymenolepis* clade”. It was strongly supported by our analysis and consisted of species of the genus *Hymenolepis* Weinland, 1858 (parasitic in murid and geomyid rodents, erinaceomorphs and humans) plus *Rodentolepis asymmetrica* (Janicki, 1904), a parasite of cricetid rodents.
- “*Arostrilepis* clade”. It consisted of cestodes of soricine shrews (of the tribes Soricini and Nectogalini), with a notable exception representing a species of the genus *Arostrilepis* Mas-Coma and Tenora, 1997 parasitic in cricetid rodents. The backbone of this clade is weakly supported by our analysis. However, several monophyletic groups had strong support. These were the following: (i) *Neoskrjabinolepis* plus *Lineolepis* Spassky, 1959; (ii) the species of the genus *Coronacanthus* Spassky, 1954; (iii) and (iv) two strongly supported groups consisting of three and two Nearctic species of the genus *Staphylocystoides* Yamaguti, 1952, however being distant from one another; (v) *Soricinia quarta* (Karpenko, 1983) plus *S. genovi* Binkiene, Kornienko and Tkach, 2015. Though being weakly supported, a clade consisting of hymenolepidids of the genera *Arostrilepis*, *Soricinia* Spassky and Spasskaya, 1954 and *Pseudobothrialepis* Schaldybin, 1957, all characterised by the lack of rostellar apparatus or its rudiment, was outlined (Fig. 1, see also Fig. 3, bar E).

Each of the two species, *Urocystis prolifer* Villot, 1880 and *Vigisolepis spinulosa* (Cholodkowsky, 1906), cannot be attributed to any of the aforementioned clades.

The analysis of the number of base differences per site from averaging over all sequence pairs between the main clades of hymenolepidids from mammals resulted in values between 0.060 and 0.086 (Table 3), indicating similar levels of divergence between them.

Discussion

Comparison with the previous phylogenetic hypotheses for mammalian hymenolepidids

The general configuration of our phylogenetic tree (Fig. 1) is similar to the previous phylogenetic hypothesis for relationships among hymenolepidids from mammals (Haukisalmi et al. 2010). However, our study includes 56 taxa of the family Hymenolepididae, including 12 taxa sequenced by us (Table 1) and 25 species sequenced by other authors after 2010 (Table 2) (versus 31 taxa in the analysis of Haukisalmi et al. 2010). Our analysis revealed the same main phyletic lineages (clades): “*Hymenolepis* clade”, “*Rodentolepis* clade”, “*Ditestolepis* clade” and “*Arostrilepis* clade” (Haukisalmi et al. 2010), exhibiting a similar level of divergence between them (Table 3). The former two clades are well supported in our study. However, in contrast to the hypothesis of Haukisalmi et al. (2010), the *Arostrilepis* clade is weakly supported, a feature suggesting that the inclusion of additional

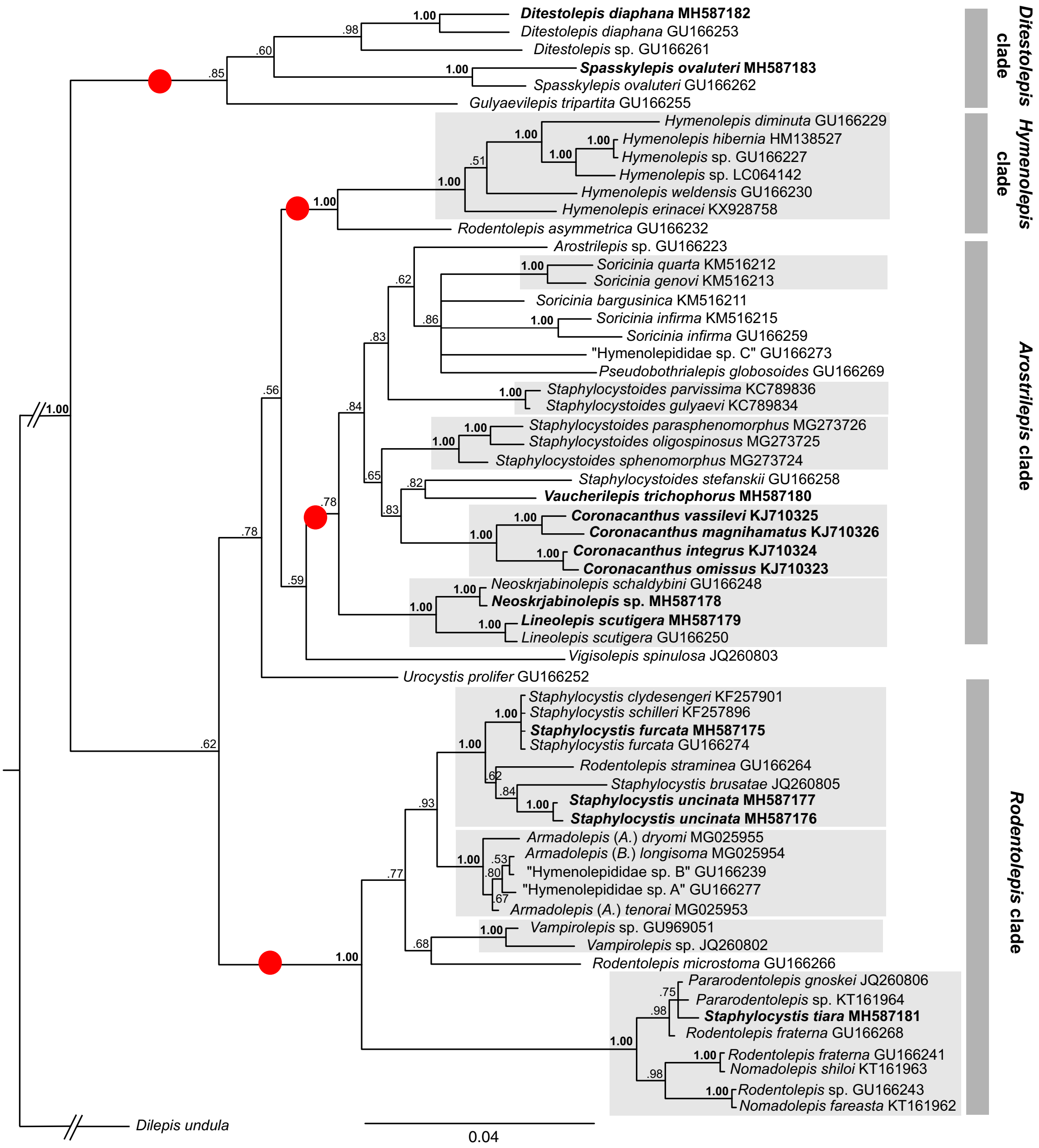


Fig. 1 Bayesian inference tree of phylogenetic relationships among hymenolepidid cestodes from mammals based on analysis of 28S rRNA gene of 56 ingroup taxa. *Dilepis undula* (family Dilepididae) is used as outgroup. The GenBank numbers are added after the binomial name of each species. Newly sequenced taxa are in bold. The major clades in this group recognised by Haukisalmi et al. (2010) and also outlined and complemented by the present study are marked by circles. Nodal support is given by posterior probabilities. Strongly supported subclades (posterior probability more than 0.95) are marked by rectangles

Table 3 The number of base differences per site from averaging over all sequence pairs between the main clades of hymenolepidids from mammals as revealed by the present study

		1	2	3
1	*Rodentolepis* clade			
2	*Arostrilepis* clade	0.070		
3	*Ditestolepis* clade	0.086	0.076	
4	*Hymenolepis* clade	0.071	0.060	0.083

species may lead to its breaking down into smaller monophyletic groups. In addition, the backbone of the phylogenetic tree is characterised by weak resolution, in contrast to that presented in the previous hypothesis (see Fig. 1 of Haukisalmi et al. 2010). This weak support is not surprising, since mostly cestodes from shrews from temperate latitudes have been used in the present analysis. The vast majority of species of the subfamily Crocidurinae (where, inter alia, the genera *Crocidura* Wagler, 1832 and *Suncus* Ehrenberg, 1832 belong, both occurring in Europe) and the soricine tribe Nectogalini (harbouring 7 genera, including *Neomys* Kaup, 1829 from temperate latitudes of Eurasia) are distributed in tropical areas (Wilson and Reeder 2005). It could be expected that the unexplored diversity of their hymenolepidids in tropics is much higher than in temperate latitudes. Denser sampling of these host groups, for both morphological and molecular studies, may contribute for developing more reliable phylogenetic hypotheses for this parasite group. The current diversity of soricine shrews in Europe consists of two genera only while, for the same territory, the number of fossil genera of this subfamily is 24 (Rzebik-Kowalska 2003). The present fauna of European Soricidae (and, apparently, the fauna of their cestodes) has been formed after the last glaciation when the species of this family (mostly those preserved in the South-European refugia) recolonised the continent (Hewitt 1999). Therefore, episodes of mass extinction of pre-glaciation shrews (and their cestodes) as well as the lack of parasite specimens originating from tropical shrews included in the phylogenetic analysis may partly explain the low resolution of the backbone of our tree. That more recently derived nodes were more robustly supported than deeper nodes in the phylogeny suggesting additional gene loci, and particularly those evolving more slowly than this section of 28S rDNA, may help in providing stability to the phylogeny. However, for the purposes of this study, where we are building on an extensive taxon set for which only one gene locus is available, our results provide some useful and robust insights.

Our analysis confirms the following: the monophyly of the genus *Hymenolepis* (sensu stricto) parasitic in hosts from various mammalian orders as revealed by Binkienė et al. (2018); the monophyly of hymenolepidids from glirid rodents (including armed and unarmed subgenera) and the paraphyly of the nominotypical subgenus of the genus *Armadolepis* Spassky, 1954 (Makarikov et al. 2018); the position of the genera *Pararodentolepis* and *Nomadolepis* as sister groups (Makarikov et al. 2015); the position of *Rodentolepis asymmetrica* as the sister group of *Hymenolepis* (sensu stricto) and distantly from other *Rodentolepis* spp. (Haukisalmi et al. 2010; Greiman and Tkach 2012; Binkienė et al. 2018); the position of *Neoskrjabinolepis* and *Lineolepis* as sister taxa (Haukisalmi et al. 2010; Greiman and Tkach 2012); the close phylogenetic relationships among the unarmed genera of the *Arostrilepis* clade, i.e. *Arostrilepis*, *Soricinia* and *Pseudobothrialepis* (Haukisalmi et al. 2010; Binkienė et al. 2015).

Novelties revealed by our analysis are mostly associated with the position of taxa, which have not been sequenced or have not been involved in similar analyses previously. For example, we provide the first evidence based on molecular data about the monophyly of the genus *Coronacanthus* erected or recognised on the basis of morphological data (Spasskiy 1954; Genov 1980; Czaplinski and Vaucher 1994; Vasileva et al. 2005). We have also revealed the non-monophyletic status of the genus *Staphylocystis* (harbouring parasites of shrews). Its polyphyly is due to the distant position of *S. tiara* and its relationship with the subclade harbouring the genera *Nomadolepis* and *Pararodentolepis*. In addition, the remaining congeners form a paraphyletic group, with *Rodentolepis straminea*, a parasite of rodents, belonging to the same clade. Our results also suggest the polyphyly of *Staphylocystoides* in its current taxonomic concept (Czaplinski and Vaucher 1994; Greiman et al. 2013; Tkach et al. 2018), since its species, though all belonging to the *Arostrilepis* clade, are distributed among three subclades (Fig. 1).

The position of the newly sequenced genus *Vaucherilepis* Tkach, Vasileva and Genov, 2003 remains unclear as its type and only species forms a weakly supported clade with a species of *Staphylocystoides*.

Morphological support of the revealed relationships

Hoberg et al. (1999) considered the rostellar apparatus of hymenolepidids as consisting of a sac-like rostellum, typically in combination with a rostellar sheath; they believed that the loss of rostellum is a secondary evolutionary event that happened independently in various phyletic lineages of cyclophyllideans. Therefore, we accept that the presence of a sac-like rostellar apparatus is the plesiomorphic state for this family. This type of rostellar apparatus consists of a rostellum, a protrusible apical part of the scolex, termed rhynchus (often bearing a crown of rostellar hooks) and a rostellar sheath (Fig. 2a, b; for further details of the structure of the sac-like rostellar apparatus, see Stoitsova et al. (1997)). Almost half of the species included in our analysis (23 out of 56) are characterised by this general (plesiomorphic) pattern of rostellar apparatus.

Notably, the major clades and subclades outlined by our analysis reveal various degrees of reduction or modification of the general rostellar pattern. The species of the *Ditestolepis* clade are characterised by the entire reduction of the rostellar sheath and partial reduction of the rostellum; the latter is transformed into a small "glandular apical organ" (termed by Gulyaev and Karpenko 1998), with almost entirely reduced musculature (Fig. 2c). In the *Hymenolepis* clade, where there are both cestodes with armed rostellum (*Rodentolepis asymmetrica*) and unarmed species (*Hymenolepis* s. str.), the rostellum of the latter is entirely reduced and the rhynchus (termed "anterior canal", see, e.g. Specian and Lumsden 1980) is invaginated into the rostellar sheath (Fig. 2d). Similarly, an entire reduction of the rostellum can also be observed in the *Rodentolepis* clade, among the subclade consisting of cestodes from glirid rodents, where the subgenus *Bremserilepis* Makarikov, 2017 is characterised by the presence of unarmed rhynchus invaginated into the rostellar sheath (Makarikov 2017; Makarikov et al. 2018). In the *Arostrilepis*

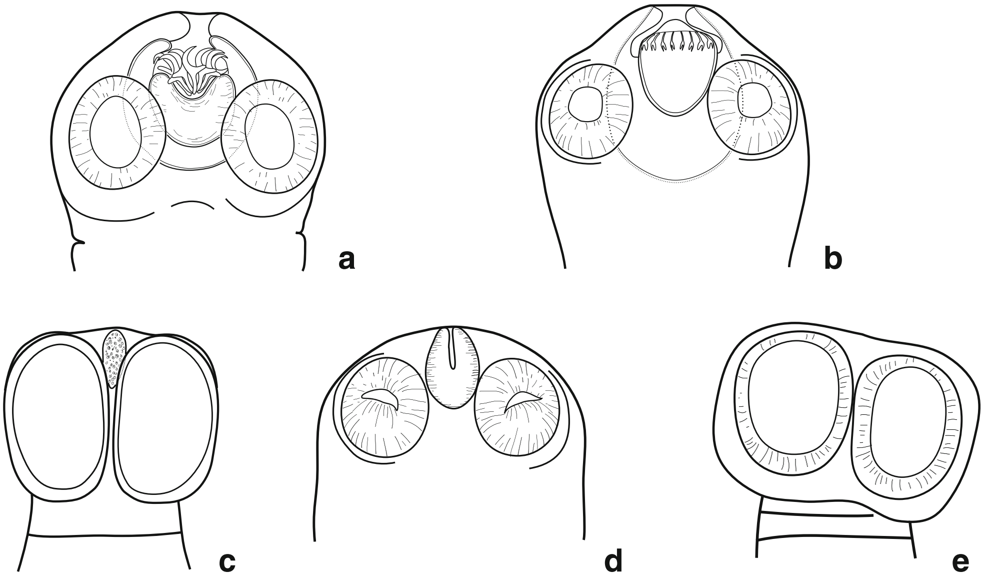


Fig. 2 Major types of rostellar apparatus of hymenolepidid cestodes from mammals (schematic presentation). **a**, **b** Scoleces with fully developed (not rudimentary) rostellar apparatus with invaginable rostellum (**a**) and retractile rostellum (**b**). **c**–**e** Scoleces with rudimentary rostellar apparatus. **c** Rostellar apparatus characterised by the entire reduction of the rostellar sheath and partial reduction of the rostellum, the latter being transformed into a glandular apical organ. **d** Rostellum entirely reduced and the rhynchus ("anterior canal") invaginated into the rostellar sheath. **e** The entire reduction of the rostellar apparatus (no vestigial structures of rostellum or rostellar sheath)

clade, the subclade consisting of the genera *Arostrilepis*, *Soricinia* and *Pseudobothrialepis* exhibits entire reduction of the rostellar apparatus, i.e. no vestigial structures of rostellum or rostellar sheath (Fig. 2e). Therefore, we confirm that the partial or entire reduction of the rostellar apparatus is an apomorphic condition (Hoberg et al. 1999) that appeared independently in all major phyletic lineages (clades) of hymenolepidids.

Among the hymenolepidid genera with the general (plesiomorphic) pattern of rostellar apparatus, two types of rostella were recognised (Czaplinski and Vaucher 1994). "Invaginable rostellum" is that with an anterior part able to invaginate and, as a result, hooks are with anteriorly directed blades when rostellum is withdrawn (Fig. 2a); in contrast, "rectractile rostellum" is with hook blades directed posteriorly when the rostellum is withdrawn (Fig. 2b). These two types of armed rostellar apparatus correspond to the "Type A" and "Type C" recognised by Vaucher (1971) who has explained their morphological and functional differences on the basis of examination of histological sections of intestinal walls with scoleces attached in situ. In our phylogenetic tree (Fig. 1), excluding forms with rudimentary rostella, the retractile type of rostellum is characteristic for the taxa belonging to the *Rodentolepis* clade and the *Hymenolepis* clade while the invaginable type of rostellum occurs only in the *Arostrilepis* clade (Fig. 3).

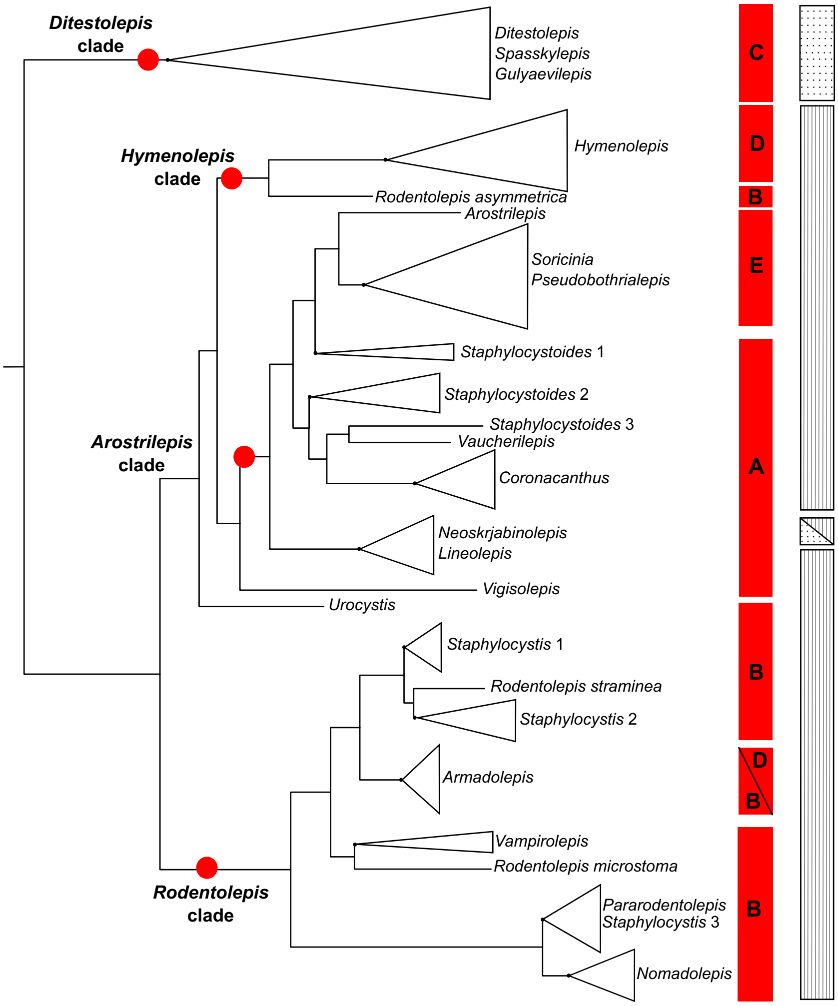
In addition to the abovementioned peculiarities of the rostellar apparatus, other morphological characters may support the clades revealed by the present analysis. In the *Ditestolepis* clade, all the members are characterised by serial maturation of strobila; however, this character is homoplasious, since serial maturation of strobila occurs also in the genus *Neoskrjabinolepis* of the *Arostrilepis* clade (Kornienko et al. 2006, 2008). The majority of mammalian hymenolepidids are characterised by gradual maturation of the strobila (Fig. 3).

The subclade consisting of the genera *Nomadolepis* Makarikov, Gulyaev and Krivopalov, 2010 and *Pararodentolepis* Makarikov and Gulyaev, 2009 (within the *Rodentolepis* clade) is characterised by fraternoid shape of rostellar hooks, median position of vitellarium, bi-lobed uterus not extending beyond the osmoregulatory canals and embryophores with polar filaments (Makarikov and Gulyaev 2009; Makarikov et al. 2010, 2015; Greiman and Tkach 2012). Some of these characters fit well to the preliminary data on the morphology of *Staphylocystis tiara* (Dujardin, 1845), a species placed by our analysis in this clade and distantly from its congeners. This demonstrates the need for redescription of the latter species and its possible affiliation to a genus belonging to this clade, probably to *Pararodentolepis*.

The remaining species of *Staphylocystis* are grouped into two clades, one consisting of species parasitic in soricine and the other parasitic in crocidurine shrews (Fig. 1). These two clades are also supported by specific morphological characters of their rostellar hooks, being fraternoid in the former and cricetoid in the latter clade. Further morphological studies are needed in order to justify the possible erection of a new genus for one of these groups.

The subclade consisting of the genera *Neoskrjabinolepis* and *Lineolepis* (within the *Arostrilepis* clade) is morphologically supported by the number of rostellar hooks (10) and the transformation of gravid proglottides into oophores

Fig. 3 Distribution of the various types of rostellar apparatus and strobilar development of hymenolepidid cestodes from mammals across the phylogenetic tree produced by the present analysis. The labels A–E of rostellar apparatus correspond to those in Fig. 2. For the strobilar development, dotted rectangles correspond to taxa (or clades) characterised by serial maturation; striped rectangles correspond to taxa (or clades) with gradual maturation



responsible for the group dispersion of eggs (Kornienko et al. 2008; Korneva and Kornienko 2013).

The subclade consisting of the genera *Arostrilepis*, *Soricinia* and *Pseudobothrialepis* (belonging also to the *Arostrilepis* clade), in addition to the entire reduction of the rostellar apparatus, is also supported by the elongate fusiform embryophores with polar processes of its members as described by Karpenko and Gulyaev (1990), Makarikov et al. (2011) and Binkienė et al. (2015).

The revealed polyphyly of the genus *Staphylocystoides* with molecular data, with species belonging to three subclades, is morphologically supported by several characters. The type species *S. sphenomorphus* (Locker and Rausch, 1952) together with *S. oligospinosus* Tkach, Kinsella and Greiman, 2018 and *S. parasphenomorphus* Tkach, Kinsella and Greiman, 2018 form a well-supported monophyletic group; these species are characterised by slender, more elongate strobila (Tkach et al. 2018). The type species has rostellar hooks forming sigmoid curvature in dorso-ventral plane (Gulyaev et al. 2007); however, this character is not mentioned in the description of the two recently described species (Tkach et al. 2018). In contrast, the remaining two Nearctic species included in our analysis, *S. parvissima* (Voge, 1953) and *S. gulyaevi* Greiman, Tkach and Cook, 2013 are characterised by very short strobila of a few proglottides and the shape of hooks as presented by Greiman et al. (2013) is rather cricetoid than fraternoid, thus differing substantially from the shape of the hooks in the other Nearctic clade. The Palaearctic *S. stefanskii* (Zarnowski, 1954) is also characterised by rostellar hooks with sigmoid configuration in dorso-ventral plane (Gulyaev et al. 2007); in addition, it has medial vitellarium in contrast to the Nearctic species having vitellarium situated antiporally, at the level between the antiporal testis and medial testis. Therefore, our results suggest the heterogeneity of the genus *Staphylocystoides* in its current taxonomic concepts (Czaplinski and Vaucher 1994; Gulyaev et al. 2007; Greiman et al. 2013; Tkach et al. 2018); a re-evaluation of the genus with additional morphological and molecular data is warranted.

Our analysis reveals that two characters that have been considered important from taxonomic point of view are homoplasious. The embryophore is provided with polar filaments in two subclades of the *Rodentolepis* clade, i.e. in *Pararodentolepis–Nomadolepis* subclade (Makarikov and Gulyaev 2009; Greiman and Tkach 2012; Makarikov et al. 2015) as well as in *Rodentolepis microstoma* (see Cunningham and Olson 2010). The gravid uterus is restricted entirely in the median field of proglottis in all the members of the *Ditestolepis* clade (Gulyaev 1991; Gulyaev et al. 2004; Kornienko and Binkiene 2014), *Pararodentolepis–Nomadolepis* subclade (Makarikov and Gulyaev 2009; Makarikov et al. 2015) and *Armadolepis* subclade (Makarikov 2017; Makarikov et al. 2018) of the *Rodentolepis* clade as well as *Lineolepis–Neoskrjabinolepis* subclade (Kornienko et al. 2008; Korneva and Kornienko 2014) of the *Arostrilepis* clade. Since these characters have been widely ignored in previous taxonomic descriptions, we need more detailed morphological studies in order to understand the possible independent origin of these structures in various groups.

The distribution of the species currently recognised as members of *Rodentolepis* in four distinct monophyletic groups (clades and subclades) suggests the artificial character of this genus and requires deeper morphological and taxonomic examination in order to justify the erection of new genera.

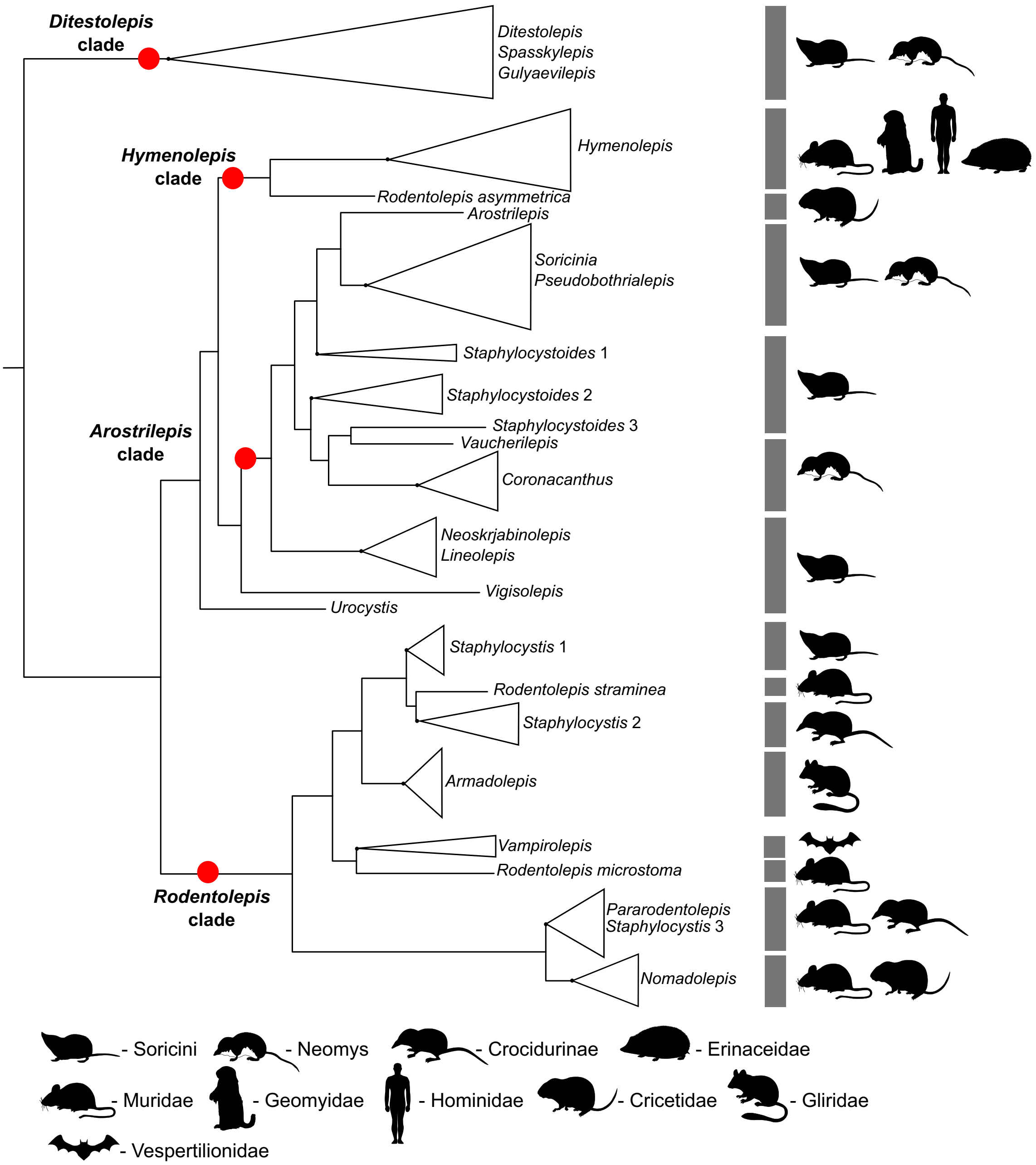


Fig. 4 Distribution of definitive host associations of hymenolepidid cestodes from mammals across the phylogenetic tree produced by the present analysis

Definitive-host and life-cycle associations

Among the four major clades outlined in hymenolepidids from mammals, only the *Ditestolepis* clade is strongly associated with soricid shrews (Fig. 4). Each of the remaining three major clades contains parasites of two or more orders of mammals: *Arostrilepis* clade—parasites of cricetid rodents and soricids; *Hymenolepis* clade—parasites of rodents, primates and erinaceomorphs; and *Rodentolepis* clade—parasites of rodents, chiropterans and soricid shrews. Such distribution of parasite–host associations across the phylogenetic tree resolved by our study suggests the presence of multiple events of host switching in the course of the formation of the current diversity of mammalian hymenolepidids, including host switching between members of different mammalian orders.

However, we revealed several lineages as closely associated with certain mammalian groups, with their present diversity probably reflecting the host–parasite coevolution or multiple colonisations of hosts of the same group. These are the following: (i) the *Ditestolepis* clade, probably corresponding to the tribe Ditestolepidini (in the concept of Gulyaev 1991), strongly associated with hosts of the family Soricidae; (ii) the subclade containing the genera with entirely reduced rostellar apparatus (*Soricinia* and *Pseudobothrialepis*), also parasitizing soricids; (iii) the genus *Coronacanthus* hosted by *Neomys* shrews and (iv) the genus *Armadolepis* diversified within glirid rodents.

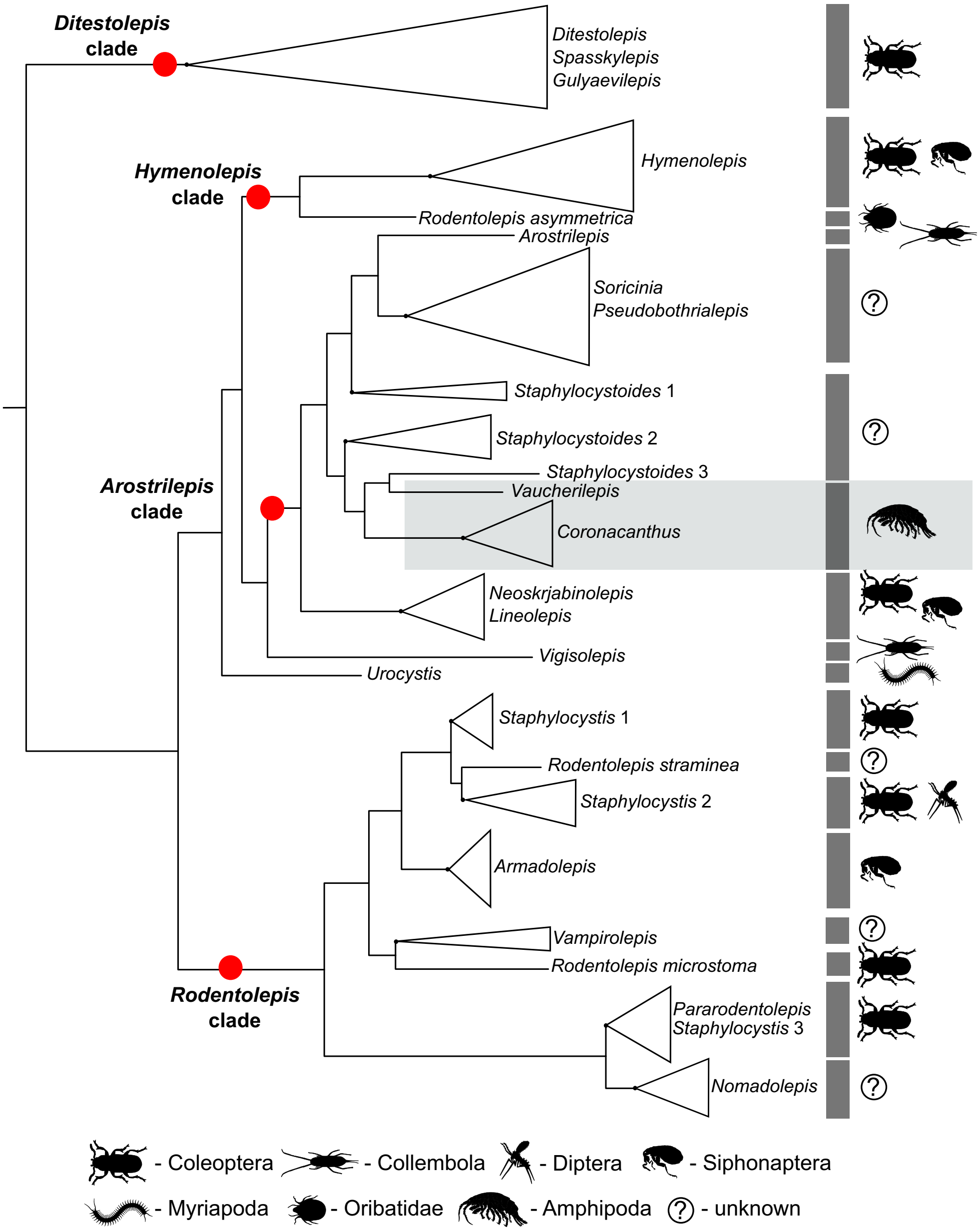


Fig. 5 Distribution of intermediate host associations of hymenolepidid cestodes from mammals across the phylogenetic tree produced by the present analysis

Only few of the species included in the present analysis are with known intermediate hosts (Fig. 5). Coleopterans are intermediate hosts of *Ditestolepis diaphana*, *Hymenolepis diminuta*, *H. weldensis* Gardner and Schmidt, 1988, *H. hibernia* Montgomery, Montgomery and Dunn, 1987, *H. erinacei* (Gmelin, 1789), *Neoskrjabinolepis schaldybini*, *Staphylocystis furcata*, *S. uncinata*, *Rodentolepis microstoma* (Dujardin, 1845) and *R. fraterna* (Stiles, 1906) (Prokopic 1971, Genov 1984, Sulgostowska et al. 2015). Siphonapterans (fleas) are hosts of *Hymenolepis diminuta* and *Lineolepis scutigera* (see Genov 1984) as well as of a species of *Armadolepis* (see Vaucher and Quentin 1975). Metacestodes of *Urocystis prolifer* have been recorded in myriapods (Baer and Della Santa 1960; Kisielewska 1960). Mites are known as intermediate hosts of *Rodentolepis asymmetrica* (see Prokopic and Mauer, 1969), collembolans of *Arostrilepis* spp. (Ishigenova et al. 2018) and *Vigisolepis spinulosa* (see Prokopic 1968), while dipterans have been recorded as hosts of *Staphylocystis brusatae* Vaucher, 1971 (see Quentin et al. 1971). The taxonomic affiliations of intermediate hosts are distributed across the phylogenetic tree generated by the present study, and no clear pattern of association can be inferred. The formation of the suprageneric monophyletic groups revealed by the present study does not seem to be associated with adaptation to type of invertebrate group as intermediate hosts.

The majority of life cycles of mammalian hymenolepidids are associated with terrestrial environments due to the habitat preferences of their definitive hosts. However, the genera *Vaucherilepis* and *Coronacanthus*, which use aquatic amphipods as intermediate hosts (Prokopic et al. 1970; Tkach 1994; Tkach et al. 2003), are nested among species from other mammalian hosts with obviously terrestrial life cycles (Fig. 5). This position suggests the secondary acquisition of aquatic life cycles of cestodes parasitic in *Neomys* shrews, which is congruent with the hypothesis for the secondary adaptation of the shrews of the tribe Nectogalini to an aquatic environment (He et al. 2010). In view of understanding the origin of the cestode parasites from *Neomys* spp., it could be worth comparing its cestode parasites with those from the other members of the tribe Nectogalini that includes mostly shrews living in terrestrial habitats and having great taxonomic diversity in East Asia. *Neomys* is the only genus of this tribe studied in detail for cestode parasites, with 24 hymenolepidid species recorded in its members (Binkienė et al. 2011, 2015). Other Nectogalini examined for cestode parasites are three species of the genus *Soriculus* Blyth, 1854 from Nepal, with eight cestode species recorded from them (Sawada et al. 1995); however, their descriptions are inadequate and do not allow detailed morphological comparisons.

Interestingly, we found *Spasskylepis ovaluteri* from *Neomys fodiens* in Bulgaria. This parasite is believed to be a specific cestode of *Sorex* spp. (Gulyaev et al. 2004). The only published sequence for this species was based on a specimen isolated from *Sorex caecutiens* Laxmann, 1788 from Finland (Haukisalmi et al. 2010). The specimens from Bulgaria used in the present study correspond very well to the description by Gulyaev et al. (2004) and a redescription based on our samples is to be published elsewhere. The record of *S. ovaluteri* in *Neomys* is congruent with the observation that Eurasian water shrews feed mainly on terrestrial prey; aquatic prey comprises only 11–27% of their diet (He et al. 2010). In support of the wide overlap of diets of *Sorex* and *Neomys* shrews, Binkienė et al. (2011) listed 11 cestode species believed to be specific for *Sorex* spp. and having also records in *Neomys*.

Concluding remarks

Except for the *Ditestolepis* clade diversified within the Soricidae, the composition of the remaining phyletic lineages of mammalian hymenolepidids suggests multiple events of host switching, including between hosts belonging to different mammalian orders. However, for the entire reconstruction of the evolutionary history of mammalian hymenolepidids, we need denser taxon sampling, mostly from tropical representatives of the major host groups that better reflect the huge diversity of this cestode group as revealed by morphological studies. Mariaux et al. (2017) summarised that mammalian hymenolepidids include 358 species belonging to 52 genera, suggesting adequate representative sampling remains a considerable task. Additionally, we recognise that further molecular data capable of resolving deeper nodes will be important to estimate more robust phylogenies and test further the hypotheses raised in this study.

Acknowledgements We are grateful to the staff of the Kalimok Field Station of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, for facilitating partly field studies. Sampling of host individuals was permitted by the Ministry of Environment and Waters of the Republic of Bulgaria, licences NSZP-153/11.05.2012 and NSZP-350/11.09.2014.

Funding The present study used facilities developed at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, in the frames of the projects WETLANET (EC FP7, Programme CAPACITIES, Grant 229802) and CEBDER (National Science Fund of the Republic of Bulgaria, Grant DOO2-15/17.02.2009). This work was partly funded by the National Science Foundation, PBI grants DEB 0818696 and DEB 0818823.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors carefully reviewed the ethical standards of the journal and hereby certify that the procedures used with the investigated species comply fully with those standards. The methods used in the current study were approved by the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

References

Baer J-G, Della Santa E (1960) Matériaux pour servir à une révision des espèces du genre *Hymenolepis* Weinland (Cestoda), parasites de Musaraignes. I. *Hymenolepis prolifer* (Villot, 1880) Stammer, 1955. Bulletin de la Société Neuchâteloise des Sciences Naturelles 83:71–77

Binkienė R, Kontrimavichus V, Hoberg EP (2011) Overview of the cestode fauna of European shrews of the genus *Sorex* with comments on the fauna in *Neomys* and *Crocidura* and an exploration of historical processes in post-glacial Europe. Helminthologia 48:207–228. https://doi.org/10.2478/s11687-011-0031-5

Binkienė R, Kornienko SA, Tkach VV (2015) *Soricinia genovi* n. sp. from *Neomys fodiens* in Bulgaria, with redescription of *Soricinia globosa* (Baer, 1931) (Cyclophyllidea: Hymenolepididae). Parasitol Res 114:209–218. https://doi.org/10.1007/s00436-014-4180-6

Binkienė R, Miliūtė A, Stunžėnas V (2018) Molecular data confirm the taxonomic position of *Hymenolepis erinacei* (Cyclophyllidea: Hymenolepididae) and host switching, with notes on cestodes of Palaearctic hedgehogs (Erinaceidae). J Helminthol. https://doi.org/10.1017/S0022149X18000056

Cunningham LJ, Olson PD (2010) Description of *Hymenolepis microstoma* (Nottingham strain): a classical tapeworm model for research in the genomic era. Parasit Vectors 3:123. https://doi.org/10.1186/1756-3305-3-123

Czaplinski B, Vaucher C (1994) Family Hymenolepididae Ariola, 1899. In: Khalil LF, Jones A, Bray RA (eds) Keys to the Cestode parasites of vertebrates. CAB International, Wallingford, pp 595–663

De Ley P, Felix MA, Frisse LM, Nadler SA, Sternberg PW, Thomas WK (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1:591–612

Genov T (1980) Morphology and taxonomy of the species of genus *Coronacanthus* Spassky, 1954 (Cestoda: Hymenolepididae) in Bulgaria. Helminthologia 17:245–255

Genov T (1984) Helminths of insectivorous mammals and rodents in Bulgaria. Izdatelstvo na Balgarskata Akademiya na Naukite, Sofia (in Bulgarian)

Georgiev B, Biserkov V, Genov T (1986) *In toto* staining method for cestodes with iron acetocarmine. Helminthologia 23:279–281

Georgiev BB, Bray RA, Littlewood DTJ (2006) Small mammals as definitive hosts of cestodes. In: Morand S, Krasnov BR, Poulin R (eds) Micromammals and macroparasites. From evolutionary ecology to management. Springer, Tokyo, pp 23–62

Greiman SE, Tkach VV (2012) Description and phylogenetic relationships of *Rodentolepis gnoskei* n. sp. (Cyclophyllidea: Hymenolepididae) from a shrew *Suncus varilla minor* in Malawi. Parasitol Int 61:343–350. https://doi.org/10.1016/j.parint.2012.01.003

Greiman SE, Tkach VV, Cook JA (2013) Description and molecular differentiation of a new *Staphylocystoides* (Cyclophyllidea: Hymenolepididae) from the Dusky Shrew *Sorex monticolus* in Southeast Alaska. J Parasitol 99:1045–1049. https://doi.org/10.1645/13-302.1

Gulyaev VD (1991) Morphology and taxonomy of Ditestolepidini – cestodes (Cyclophyllidea) of shrews with the serial-metameric structure of the strobila. Zoologicheskiy Zhurnal 70:44–53 (In Russian)

Gulyaev VD, Karpenko SV (1998) Cestodes of the genus *Mathevolepis* (Cestoda: Cyclophyllidea, Hymenolepididae) from the common shrews of the Holarctic region. Parazitologiya 32:507–518 (In Russian)

Gulyaev VD, Kornienko SA (1999) On the morphology of *Cryptocotylepis globosoides* (Cestoda: Hymenolepididae) – a cestode from water shrews from Palaearctic region. Parazitologiya 33:49–54 (In Russian)

Gulyaev VD, Mel'nikova YA (2005) A new cestode from moles, *Talpolepis* gen. n., and redescription of *T. peipingensis* (Hsü, 1935) comb. n. (Cyclophyllidea: Hymenolepididae). In: Problemy tsestodologii 3. Zoologicheskiy Institut RAN, Saint Petersburg, pp 130–139 (In Russian)

Gulyaev VD, Lykova KA, Mel'nikova YA, Bibik EV (2004) On independence of the species *Spasskylepis ovaluteri* (Cestoda, Cyclophyllidea, Hymenolepididae), a parasite of *Sorex* shrews in Palaearctic Region. Zoologicheskiy Zhurnal 83:387–394 (In Russian)

Gulyaev VD, Dokuchaev NE, Kornienko SA (2007) Cestodes of the genus *Staphylocystoides* Yamaguti, 1959 (Cestoda, Hymenolepididae) from Beringian soricine shrews. Vestnik Severo-Vostochnogo Nauchnogo Tsentra Dal'nevostochnogo Otdeleniya RAN 4:75–84 (In Russian)

Haukisalmi V, Hardman LM, Foronda P, Feliu C, Laakkonen J, Niemimaa J, Lehtonen JT, Henttonen H (2010) Systematic relationships of hymenolepidid cestodes of rodents and shrews inferred from sequences of 28S ribosomal RNA. Zool Scr 39:631–641. https://doi.org/10.1111/j.1463-6409.2010.00444.x

He K, Li YJ, Brandley MC, Lin LK, Wang YX, Zhang YP, Jiang XL (2010) A multi-locus phylogeny of Nectogalini shrews and influences of the paleoclimate on speciation and evolution. Mol Phylogenet Evol 56:734–746. https://doi.org/10.1016/j.ympev.2010.03.039

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biol J Linn Soc 68:87–112

Hoberg EP, Jones A, Bray RA (1999) Phylogenetic analysis among the families of the Cyclophyllidea (Eucestoda) based on comparative morphology, with new hypotheses for co-evolution in vertebrates. Syst Parasitol 42:51–73

Ishigenova LA, Berezina OG, Makarikov AA (2018) Postembryonic development of *Arostrilepis microtis* Gulyaev et Chechulin, 1997 (Eucestoda: Hymenolepididae) in springtails (Collembola: Entomobryidae). Parasitol Res 117:2411–2417. https://doi.org/10.1007/s00436-018-5929-0

Karpenko SV, Gulyaev VD (1990) Redescription of the shrew cestode *Pseudobothrialepis mathevossianae* Schaldybin, 1957 (Hymenolepididae). Izvestiya Sibirskogo Otdeleniya Akademii Nauk SSSR, Seriya Biologicheskikh Nauk 9:68–71 (In Russian)

Kisielewska K (1960) Life cycle of the tapeworm *Pseudodiorchis prolifer* (Villot, 1890) comb. nova (= *Pseudodiorchis multispinosa* Żarnowski, 1955). Acta Parasitol Pol 8:197–204

Korneva ZV, Kornienko SA (2013) Morphology and ultrastructure of the uterus of *Lineolepis scutigera* (Dujardin, 1845) Karpenko, 1985 (Cestoda, Cyclophyllidea, Hymenolepididae) in formation of uterine capsules. Inland Water. Biology 6:259–267

Korneva ZV, Kornienko SA (2014) Interaction of the uterus and developing eggs in cyclophyllidean cestodes with different fecundity. Biol Bull 41:139–148

Kornienko SA, Binkiene R (2014) Redescription and systematic position of *Soricinia tripartita* Zarnowski, 1955 (Cestoda: Cyclophyllidea), a cestode species parasitic in shrews of the genus *Sorex*, including erection of *Gulyaevilepis* gen. n. Folia Parasitol 61:141–147. https://doi.org/10.14411/fp.2014.015

Kornienko SA, Gulyaev VD, Mel'nikova YA (2006) On morphology and systematics of cestodes of the genus *Neoskrjabinolepis* (Cyclophyllidea, Hymenolepididae). Zoologicheskiy Zhurnal 85:134–145 (In Russian)

Kornienko SA, Gulyaev VD, Mel'nikova YA, Georgiev BB (2008) *Neoskrjabinolepis nuda* n. sp. from shrews on Sakhalin Island, Russia, with a taxonomic review of *Neoskrjabinolepis* Spasskii, 1947 (Cestoda: Cyclophyllidea: Hymenolepididae). Syst Parasitol 70:147–158. https://doi.org/10.1007/s11230-008-9140-z

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lockyer AE, Olson PD, Littlewood DTJ (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biol J Linn Soc 78:155–171. https://doi.org/10.1046/j.1095-8312.2003.00141.x

Makarikov AA (2017) A taxonomic review of hymenolepidids (Eucestoda, Hymenolepididae) from dormice (Rodentia, Gliridae), with descriptions of two new species. Acta Parasitol 62:1–21. https://doi.org/10.1515/ap-2017-0001

Makarikov AA, Gulyaev VD (2009) *Pararodentolepis* gen. n., a new genus of cestodes from rodents, with the description of *P. sinistra* sp. n. (Cyclophyllidea: Hymenolepididae). Parazitologiya 43:454–459 (In Russian)

Makarikov AA, Gulyaev VD, Krivopalov AV (2010) *Nomadolepis* (Cyclophyllidea, Hymenolepididae), a new genus of cestodes from rodents. Zoologicheskiy Zhurnal 89:948–955 (In Russian)

Makarikov AA, Gulyaev VD, Kontrimavichus VL (2011) A redescription of *Arostrilepis horrida* (Linstow, 1901) and descriptions of two new species from Palaearctic microtine rodents, *Arostrilepis macrocirrosa* sp. n. and *A. tenuicirrosa* sp. n. (Cestoda: Hymenolepididae). Folia Parasitol 58:108–120

Makarikov AA, Mel'nikova YA, Tkach VV (2015) Description and phylogenetic affinities of two new species of *Nomadolepis* (Eucestoda, Hymenolepididae) from Eastern Palearctic. Parasitol Int 64:453–463. https://doi.org/10.1016/j.parint.2015.06.009

Makarikov AA, Stakheev VV, Tkach VV (2018) Phylogenetic relationships of the genus *Armadolepis* Spassky, 1954 (Eucestoda, Hymenolepididae), with descriptions of two new species from Palaearctic dormice (Rodentia, Gliridae). Syst Parasitol 95:65–79. https://doi.org/10.1007/s11230-017-9765-x

Mariaux J (1998) A molecular phylogeny of the Eucestoda. J Parasitol 84: 114–124

Mariaux J, Olson PD (2001) Cestode systematics in the molecular era. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp 127–134

Mariaux J, Tkach VV, Vasileva GP, Waeschenbach A, Beveridge I, Dimitrova YD, Haukisalmi V, Greiman SE, Littlewood DTJ, Makarikov AA, Phillips AJ, Razafiarisolo T, Widmer V, Georgiev BB (2017) Cyclophyllidea van Beneden in Braun, 1900. In: Caira JN, Jensen K (eds) Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Lawrence, pp 77–148

Nkouawa A, Haukisalmi V, Li T, Nakao M, Lavikainen A, Chen X, Henttonen H, Ito A (2016) Cryptic diversity in hymenolepidid tapeworms infecting humans. Parasitol Int 65:83–86. https://doi.org/10.1016/j.parint.2015.10.009

Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey J, Buckley T (2004) Bayesian phylogenetic analysis of combined data. Syst Biol 53:47–67. https://doi.org/10.1080/10635150490264699

Olson PD, Tkach VV (2005) Advances and trends in the molecular systematics of the parasitic Platyhelminthes. Adv Parasitol 60:165–243. https://doi.org/10.1016/S0065-308X(05)60003-6

Olson PD, Littlewood DTJ, Bray RA, Mariaux J (2001) Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). Mol Phylogenet Evol 19:443–467. https://doi.org/10.1006/mpev.2001.0930

Pleijel F, Jondelius U, Norlinder E, Nygren A, Oxelman B, Schander C, Sundberg P, Thollesson M (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Mol Phylogenet Evol 48:369–371. https://doi.org/10.1016/j.ympev.2008.03.024

Prokopic J (1968) Parasite specificity as the result of ecological parasite-host relations. Helminthologia 9:491–196 (In Russian)

Prokopic J (1971) The life cycle of the cestode *Rodentolepis erinacei* (Gmelin, 1789). Folia Parasitol 18:27–32

Prokopic J, Mauer Z (1969) Některé poznatky o biologii tasemnic z drobnych savcù. Zpravy Československé Spolecnosti Parasitologické 9:22–30

Prokopic J, Dimitrova E, Genov T, Karapchanski I (1970) Cysticercoids of cestodes of insectivores in Bulgaria. Izvestiya na Tsentralnata Khelmintologichna Laboratoriya 14:173–184

Quentin JC, Jourdane J, Rioux JA, Leger N, Houin R, Croset H (1971) Présence du cysticercoïde d'*Hymenolepis brusatae* Vaucher, 1971 chez *Phlebotomus perniciosus* Newstead, 1911 et *Phlebotomus mascittii* Grassi, 1908. Ann Parasitol Hum Comp 46:589–593

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchar MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Rzebik-Kowalska B (2003) Distribution of shrews (Insectivora, Mammalia) in time and space. Deinsea 10:499–508

Sawada I, Harada M, Oda S (1995) Further studies on cestode parasites of some micromammalians (Insectivora) from Akademgorodok City and Altai Region, Russia. Proceedings of the Japanese Society of Systematic Zoology 54:19–27

Spasskiy AA (1954) Classification of mammalian hymenolepidids. Trudy Gel'mintologicheskoy Laboratorii Akademii Nauk SSSR 7: 120–167 (In Russian)

Specian RD, Lumsden RD (1980) The microanatomy and fine structure of the rostellum of *Hymenolepis diminuta*. Zeitschrift für Parasitenkunde 63:71–88

Stoitsova SR, Georgiev BB, Dacheva RB, Vinarova MI (1997) Scolex glands associated with the rostella in three species of the Dilepididae (Cestoda: Cyclophyllidea). Acta Zoologica (Stockholm) 78:187–193

Sulgostowska T, Solarz K, Madej G, Klimaszewski K (2015) Mites and internal parasites associated with the common dung beetle *Geotrupes* (*Anoplotrupes*) *stercorosus* (Hartmann in Scriba, 1791) in Poland. Acta Parasitol 60:622–630

Swan DC (1936) Berlese's fluid: remarks upon its preparation and use as a mounting medium. Bull Entomol Res 27:389–391. https://doi.org/10.1017/S0007485300058259

Tkach VV (1994) Description of cysticercoid of *Coronacanthus vassilevi* Genov, 1980 (Cestoda: Hymenolepididae). Parasite 1:161–165. https://doi.org/10.1051/parasite/1994012161

Tkach VV, Vasileva GP, Genov T (2003) Description of *Vaucherilepis trichophorus* sp. nov., gen. nov. (Cyclophyllidea, Hymenolepididae) from water shrews and gammarid crustaceans in Bulgaria and Ukraine. Acta Parasitol 48:87–97

Tkach VV, Makarikov AA, Kinsella JM (2013) Morphological and molecular differentiation of *Staphylocystis clydesengeri* n. sp. (Cestoda, Hymenolepididae) from the vagrant shrew, *Sorex vagrans* (Soricomorpha, Soricidae), in North America. Zootaxa 3691:389–400. https://doi.org/10.11646/zootaxa.3691.3.7

Tkach VV, Kinsella JM, Greiman SE (2018) Two new species of *Staphylocystoides* Yamaguti, 1959 (Cyclophyllidea: Hymenolepididae) from the masked shrew *Sorex cinereus* in North America. J Parasitol 104:157–167. https://doi.org/10.1645/17-151

Vasileva GP, Tkach VV, Genov T (2005) Two new hymenolepidid species (Cestoda, Hymenolepididae) from water shrews *Neomys fodiens* pennant (Insectivora, Soricidae) in Bulgaria. Acta Parasitol 50:56–64

Vaucher C (1971) Les Cestodes parasites des Soricidae d'Europe. Etude anatomique, révision taxonomique et biologie. Rev Suisse Zool 78: 1–113. https://doi.org/10.5962/bhl.part.97061

Vaucher C (1992) Revision of the genus *Vampirolepis* Spasskij, 1954 (Cestoda: Hymenolepididae). Mem Inst Oswaldo Cruz 87:299–304. https://doi.org/10.1590/S0074-02761992000500056

Vaucher C, Quentin JC (1975) Présence du cysticercoïde de *Hymenolepis myoxi* (Rud., 1819) chez la Puce du Lérot et redescription du Ver adulte. Bulletin de la Société Neuchâteloise des Sciences Naturelles 98:27–34

Waeschenbach A, Webster BL, Bray RA, Littlewood DTJ (2007) Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. Mol Phylogenet Evol 45:311–325. https://doi.org/10.1016/j.ympev.2007.03.019

Waeschenbach A, Webster BL, Littlewood DTJ (2012) Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. Mol Phylogenet Evol 63:834–847. https://doi.org/10.1016/j.ympev.2012.02.020

Williams BD, Schrank B, Huynh C, Shownkeen R, Waterston RH (1992) A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Genetics 131:609–624

Wilson DE, Reeder DM (eds) (2005) Mammal species of the world. A taxonomic and geographic reference, 3rd edn. Johns Hopkins University Press, Baltimore http://www.departments.bucknell.edu/biology/resources/msw3