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# Systematic relationships of hymenolepidid cestodes of rodents and shrews inferred from sequences of 28S ribosomal RNA

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This study attempts to elucidate systematic relationships of hymenolepidid cestodes of rodents (18 species), shrews (13 species) and bats (one species) using sequences of partial 28S ribosomal RNA, with special reference to the genus *Rodentolepis*. The main finding is the presence of four multispecies clades of hymenolepidid cestodes showing pronounced morphological variation and frequent colonizations between unrelated hosts. Neither the hymenolepidid cestodes of shrews nor rodents were monophyletic. Also, the genus *Rodentolepis sensu* Vaucher in Czaplinski & Vaucher (1994, *Keys to the Cestode Parasites of Vertebrates*. Commonwealth Agricultural Bureaux International, Cambridge) is clearly non-monophyletic. Although rostellar morphology is obviously a key feature on specific and generic levels, on higher systematic levels it seems to be a rather poor indicator of phylogenetic affinity in hymenolepidid cestodes. The presence of clades with more than one rostellar type (armed rostellum present, rudimentary unarmed rostellum present and rostellum absent) also conflicts with the proposed subfamilial and tribal classifications of hymenolepidid cestodes. The overall evidence suggests that the recent trend of splitting hymenolepidid cestodes into multiple genera will produce a more stable and practical classification than the earlier practice of favouring a few, morphologically variable genera. New classifications of hymenolepidid cestodes should, however, consider both morphological and molecular evidence.

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## Introduction

Cestodes of the family Hymenolepididae (Cyclophyllidea) are ubiquitous and diverse parasites of birds, rodents, insectivores, bats and some other mammals. According to Czaplinski & Vaucher (1994), there are *c.* 230 and 620 species of hymenolepidids parasitizing mammals and birds, respectively. The monophyly of hymenolepidid cestodes was demonstrated by Mariaux (1998) on the basis of 18S rRNA sequences representing eight genera (four from mammals and four from birds). The same analysis also strongly suggested a sister group relationship between Hymenolepididae and Anoplocephalinae.

Despite high diversity, the phylogenetic relationships within Hymenolepididae are largely unknown, although the analysis of Mariaux (1998) revealed some supported structure among nine species from birds, rodents, shrews and bats. The other existing phylogenetic studies have dealt with relationships among a few well-known species, particularly the human-associated *Rodentolepis nana* (Siebold, 1852) and *Hymenolepis diminuta* (Rudolphi, 1819) and other rodent-associated species used in experimental research (Okamoto *et al.* 1997; Macnish *et al.* 2002, 2003). For the diverse hymenolepidids of shrews (Soricomorpha: Soricidae), there is even less phylogenetic information (two 18S rRNA sequences in Mariaux 1998).

Following the description and recognition *Hymenolepis* Weinland, 1858, the majority of hymenolepidids were long classified within this now unwieldy genus. The reviews of Skryabin & Matevosyan (1948) and Spasskii (1954) were the first major attempts to reorganize the mammalian hymenolepidids, the latter one recognizing 20 genera. The latest authorative review (Vaucher in Czaplinski & Vaucher 1994) listed 34 valid genera of mammalian hymenolepidids, eight of them primarily or exclusively from rodents, 20 from shrews, four from bats and two from other mammals.

*Hymenolepis* (*sensu stricto*) and *Rodentolepis* Spasskii 1954 are undoubtedly the most species-rich hymenolepidid genera of rodents. The type species *H. diminuta* and other species representing *Hymenolepis* (*sensu stricto*) are characterized by a rudimentary, unarmed rostellum/rostellar sac, and appear to form a morphologically well-defined assemblage. However, the systematic relationships among species variously assigned to *Rodentolepis* (with armed rostellum) are obscure, primarily reflecting the absence of comprehensive phylogenetic studies.

The latest, prevailing diagnosis of *Rodentolepis* (Vaucher in Czaplinski & Vaucher 1994) is rather wide, encompassing almost all armed hymenolepidids from rodents, but also from marsupials, bats and primates. Well-known taxa such as *R. nana* (from man) and *Rodentolepis fraterna* (Stiles, 1906) (from rodents) have been included within *Rodentolepis* by some authors, but there is still no consensus about their generic position. The same also concerns the armed *Rodentolepis microstoma* (Dujardin, 1845) from rodents, a potentially zoonotic species (Macnish *et al.* 2003) used as an experimental model.

The purpose of this study is to present the first wide-scale phylogenetic hypothesis of hymenolepidid cestodes of rodents and shrews, and to compare certain morphological features of cestodes (particularly the rostellum and hooks) to their inferred phylogeny. The results are used to reconsider the systematic relationships of these cestodes with special reference to species variously assigned to *Rodentolepis sensu* Vaucher in Czaplinski & Vaucher (1994).

## Materials and methods

This study deals with cestodes referable to Hymenolepididae *sensu* Khalil *et al.* (1994) from myomorph (Cricetidae, Muridae, Nesomyidae), sciuromorph (Gliridae) and castorimorph (Geomyidae) rodents, shrews (Soricomorpha: Soricidae) and bats (Chiroptera: Vespertilionidae) (Appendix).

Cestode tissue samples were fixed and preserved in 70–100% ethanol and DNA extracted using E.Z.N.A.$^{\text{TM}}$ Tissue Kit (OMEGA Bio-Tek, Inc., Norcross, Georgia, USA). Forty-four specimens of hymenolepidid cestodes were successfully amplified and sequenced for partial 28S ribosomal RNA (D1–D3) using three alternative pairs of primers: (i) LSU5 (forward, 5′-TAGGTCGACCCGCTGAAYTT**Y**AGCA-3′) of Littlewood *et al.* (2000), except that one 'A' was replaced with 'Y', and 1200R (reverse, 5′-GCATAGTTCACCATCTTTCGG-3′) of Lockyer *et al.* (2003) (*c.* 1400 bp); (ii) XZ-1 (forward, 5′-ACCCGCTGAA**T**TTAAGCATAT-3′) of Waeschenbach *et al.* (2007), which differs from the original XZ-1 of van der Auwera *et al.* (1994) by having one 'Y' was replaced with 'T', and 1500R (reverse, 5′-GCTATCCTGAGGGAAACTTCG-3′) of Littlewood *et al.* (2008) (*c.* 1660 bp); and (iii) U178 (forward, 5′-GCACCCGCTGAAYTTAAG-3′) and L1642 (reverse, 5′-CCAGCGCCATCCATTTTCA-3′) (*c.* 1500 bp), both from Lockyer *et al.* (2003). Standard 50 μL PCR was performed using hot start, cycling conditions following those of Lockyer *et al.* (2003), Waeschenbach *et al.* (2007) and Littlewood *et al.* (2008). Successfully amplified DNA was purified using E.Z.N.A.$^{\text{TM}}$ Cycle Pure Kit (OMEGA Bio-Tek, Inc., Norcross, Georgia, USA). Purified PCR products were direct sequenced using dye terminators and visualized with an Applied Biosystems, Inc. (Foster City, California, USA) 3730xl DNA analyser at Macrogen Inc. (Korea).

Sequences were assembled and edited in Geneious Pro v. 4.8 (Biomatters Ltd, Auckland, New Zealand) (Drummond *et al.* 2009) and aligned with ClustalW (Thompson *et al.* 1997). Unambiguously aligned and gapped sites were deleted. The best model for the phylogenetic analysis was GTR+I+γ, as suggested by the Akaike information criterion implemented in jModelTest (Posada 2008). Phylogenetic analyses were based on the Bayesian approach (Huelsenbeck *et al.* 2001), performed using MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003). Various combinations of three cestode species representing different cyclophyllidean families, i.e. *Raillietina sonini*

Spasskaya & Spasskii, 1971 (Davaineidae) (EU665462), *Dilepis undula* (Schrank, 1788) (Dilepididae) (AF286915) and *Anoplocephala perfoliata* (Goeze, 1782) (Anoplocephalidae) (AY569769), were tested as outgroups. A separate analysis was performed for the '*Rodentolepis* clade' using two hymenolepidid species (*Vigisolepis spinulosa* Cholodkowsky, 1906 and *H. diminuta*) as outgroups. For both data sets, MrBayes was run for 5 million generations, sampled every 1000 generations, 500 000 generations were discarded as 'burnin' and a 50% majority-rule consensus tree was computed from the post-burnin samples. Node support was expressed as posterior probabilities, >95% probabilities being considered significant. Two independent runs performed for both data sets converged in identical topologies and bootstrap values, suggesting that likelihood plateaus represented global optima rather than local ones.

Classification of hymenolepidid cestodes and their mammalian hosts follow Czaplinski & Vaucher (1994) and Wilson & Reeder (2005), respectively. Cestode material and GenBank accession numbers for 28S sequences are listed in Appendix.

## Results

*Rodentolepis straminea* (7 sequences), *R. microstoma* (2), *R. fraterna* (2), *R.* cf. *fraterna* (2) and *Hymenolepis* sp. (2) included identical sequences (Appendix), which were discarded from phylogenetic analyses. Also, two of the three sequences of *Staphylocystis furcata* were identical and differed from the third one by two nucleotides; this species was also represented by a single sequence in phylogeny reconstruction. In addition, the present sequences of *H. diminuta* and *R. microstoma* were identical with conspecific sequences from GenBank (AF286917 and AF286918, respectively).

The deep phylogenetic structure of hymenolepidid cestodes of rodents and shrews remained unresolved. The basal polytomy comprised four supported multispecies clades (A–D in Fig. 1) and four species without clear associations with other species [i.e. *Urocystis prolifer* Villot, 1880, '*Hymenolepis*' *muris-sylvatici* (Rudolphi, 1819), *Rodentolepis evaginata* (Barker & Andrews, 1915) and *Paraoligorchis* sp.]. The apparent dichotomy of clade D vs. clades A–C had consistently low, non-significant support values (<70%). The main phylogenetic structure was not sensitive to the use of different (combinations of) outgroups, except that the support of '*Arostrilepis* clade' was lower (81–94%) when *A. perfoliata* (Anoplocephalidae) was included in the outgroup. The phylogram presented in Fig. 1 is based on an analysis using *R. sonini* (Davaineidae) and *D. undula* (Dilepididae) as outgroups.

One of the four multispecies clades (D) included species from rodents, shrews and bats and another (C) from rodents and shrews. In addition, the four species without clear associations with other species parasitize both rodents (*H. muris-sylvatici*, *R. evaginata* and *Paraoligorchis* sp.) and shrews (*U. prolifer*). Thus, neither the hymenolepidid cestodes of shrews nor rodents are monophyletic, implying that parasite colonizations have occurred among these host groups. The results also suggest that murine (Muridae) and arvicoline (Cricetidae) rodents have been colonized by hymenolepidids on at least two independent occasions each.

In the separate phylogenetic analysis for the '*Rodentolepis* clade' (Fig. 2), the main phylogenetic division was between the divergent subclade of *R. fraterna*-like cestodes and the rest of the species. The crown clade of the latter assemblage was formed by *R. straminea* + *S. furcata* and two unidentified species from glirids.

The main rostellar types have been illustrated in Fig. 3. One of the three main types (rostellum absent) formed a monophyletic crown lineage within the '*Arostrilepis* clade' (C), but the other two types (unarmed or armed rostellum present) did not form inclusive clades. The species of the '*Ditestolepis* clade' (A) were all characterized by an unarmed, rudimentary rostellum, but the three other multispecies clades showed combinations of armed and unarmed scoleces (either with or without rostellum). In addition, the four basal species without clear connections with other species or clades included both armed and unarmed scoleces. The results indicate three unequivocal losses of rostellum [Hymenolepididae sp. A, *Arostrilepis* spp. and *Hymenolepis* (*sensu stricto*) spp.], but probably no change/reversal from unarmed to armed condition.

Within the *Rodentolepis* clade (D), the unidentified Hymenolepididae sp. A from *Glis* is unique among the present assemblage, because its rostellum and hooks are rudimentary and probably non-functional and the hook shape does not correspond to that of the other species in the *Rodentolepis* clade. In addition, its sister species (Hymenolepididae sp. B), also from *Glis*, has lost the whole rostellum and rostellar sac. This pattern is further complicated by the fact that the two known hymenolepidid species of European glirids do not correspond morphologically to the two forms of this study (see Discussion).

The number of hooks also shows some correspondence with the inferred phylogeny. In the *Rodentolepis* clade, the number of hooks is high (19–36) if (normal) hooks are present, compared with the armed species in the *Arostrilepis* clade (10–20 hooks). *Urocystis prolifer*, one of the basal species, is very deviant in this respect (*c.* 190 tiny hooks). The clade formed by *Lineolepis* Spasskii, 1959 and *Neoskrjabinolepis* Spasskii, 1947 is characterized by a constant number (10) of hooks, but the same (10), constant number of hooks has been independently acquired in *R. evaginata*.

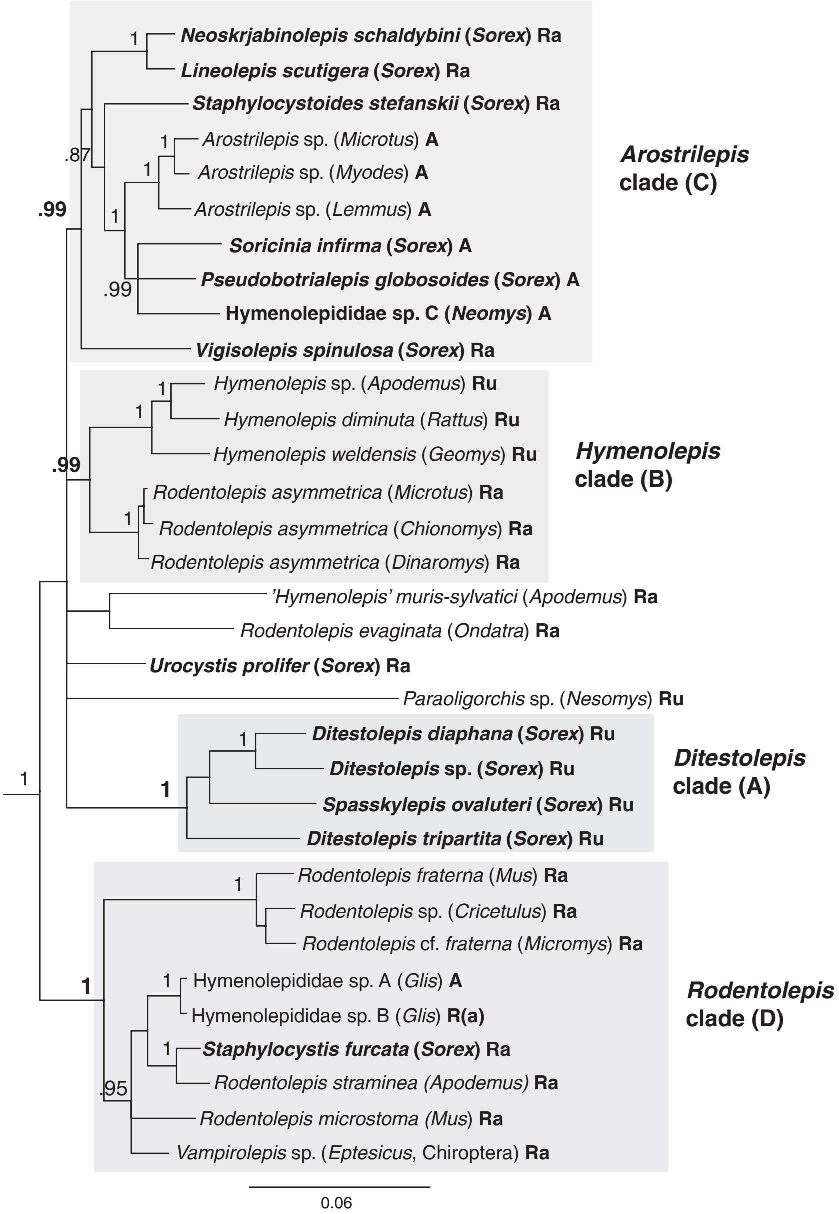

**Fig. 1** Bayesian inference tree of phylogenetic relationships among hymenolepidid cestodes of rodents, shrews and bats. Parasites of shrews in bold. A–D denote the four multispecies clades of cestodes. Host genera in parentheses after the cestode names. Posterior support (when >80%) depicted at nodes. Letters in bold after the host genera indicate the four main types of rostellum: Ra, armed rostellum present; R(a), armed rostellum with rudimentary hooks present; Ru, unarmed rostellum present; A, rostellum and hooks absent.

The shape of normal rostellar hooks in *Rodentolepis* clade is partially connected with the inferred phylogeny, because the representatives of the divergent *fraterna* clade all have 'fraternoid' hooks, i.e. with a long, curved handle and a short blade and guard. The other subclade shows, however, considerable variation in hook shape and development. *Rodentolepis microstoma* and *R. straminea* have very similar 'cricetoid' hooks, i.e. with a short handle and a pronounced guard (cf. Baer & Tenora 1970), although they are not closely related. The hooks of *Vampirolepis* sp. and *S. furcata* resemble somewhat those of the *fraterna* clade.

## Discussion

### *General patterns*

Of the several proposed classifications for (mammalian) hymenolepidids, the two most recent ones (Schmidt 1986;

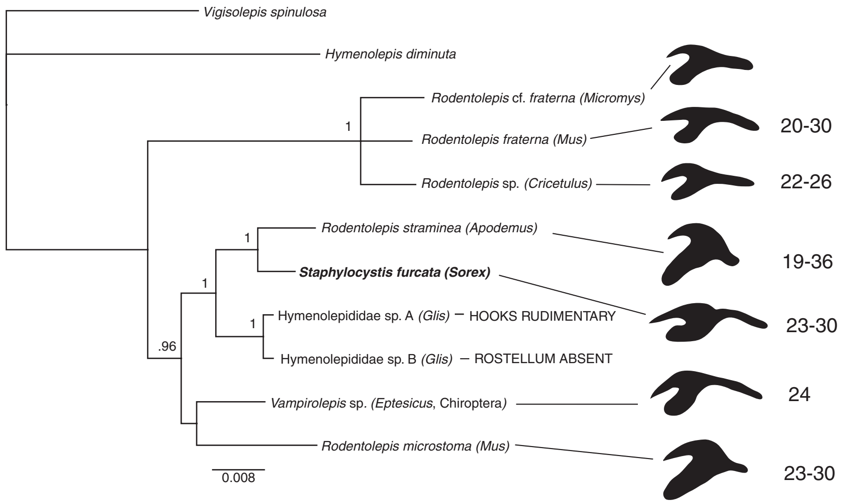

**Fig. 2** Bayesian inference tree of phylogenetic relationships within the '*Rodentolepis* clade' of hymenolepidid cestodes. Typical shape of rostellar hooks and their number (when known) indicated for each armed species with normal (functional) hooks. The hook of *Rodentolepis fraterna* has been redrawn from Baer & Tenora (1970); other illustrations are original. Labels as in Fig. 1.

Czaplinski & Vaucher 1994) are considered here. According to Schmidt (1986), mammalian hymenolepidids are distributed among three subfamilies, i.e. Hymenolepidinae Perrier, 1897, Pseudhymenolepidinae Joyuex & Baer, 1935 and Ditestolepidinae Yamaguti, 1959. However, Pseudhymenolepidinae and Ditestolepidinae were not considered valid in the classification of Czaplinski & Vaucher (1994). According to the latter authors, all mammalian hymenolepidids represent the subfamily Hymenolepidinae, with one exception [*Monogynolepis taglei* (Olsen, 1966) from chinchillid rodents] representing Diploposthinae Poche, 1926.

Due to their monophyly and lack of deep phylogenetic structure, the hymenolepidid cestodes of rodents and shrews are here suggested to represent the subfamily Hymenolepidinae, although the monophyly of this group among all hymenolepidids could not be tested. No species from birds could be included and representatives of *Pseudhymenolepis* Joyuex & Baer, 1935 and Pseudhymenolepidinae *sensu* Schmidt (1986) were also lacking. However, the present material includes the type genus *Hymenolepis* and its type species *H. diminuta* (from *Rattus*).

It may be argued that the four multispecies clades recognized in this study should each form an independent subfamily or a tribe. Such a classification attempt would, however, be severely hampered by problems of finding apomorphies that uniquely characterize each group. For example, in three of the four multispecies clades there are species with and without rostellum and hooks, characters that have traditionally played a central role in classification of hymenolepidids (and other cestodes). In addition, the *Rodentolepis* clade actually consists of three different rostellar types. Assuming that armed rostellum has been the original condition in Hymenolepididae, the loss of armature has occurred as least on four independent occasions.

Among species considered here, only *Paraoligorchis* sp. from an endemic Madagascan rodent has regularly more than three testes per proglottid. The unresolved, basal phylogenetic position of *Paraoligorchis* sp. suggests that the number of testes is a phylogenetically important feature among hymenolepidid cestodes. The high genetic divergence of *Paraoligorchis* sp. is evidently due to the fact that the endemic Madagascan rodents have been isolated from their African ancestors for 24–20 Myr (Poux *et al.* 2005). This scenario gives a minimum age of 20 Myr for the Madagascan *Paraoligorchis* sp. and probably a more ancient origin for the hymenolepidid cestodes of rodents and shrews at large.

### *Ditestolepis clade (A)*

*Ditestolepis diaphana*, *Ditestolepis tripartita*, *Ditestolepis* sp. and *Spasskylepis ovaluteri* formed one of the basal clades in the present material, which appears to support the subfamily Ditestolepidinae. Interestingly, the distance-based phylogenetic methods place the *Ditestolepis* clade as a sister group of all other hymenolepidids of rodent and shrews (results not shown), suggesting that the position of this clade and the status of Ditestolepidinae should be evaluated using additional molecular markers. The species in this clade share a rudimentary, unarmed rostellum (or rostellar sac). The most distinctive morphological features of the type species *D. diaphana* are the serial development of proglottids and the fusion of gravid proglottids to form a 'syncapsule'. The

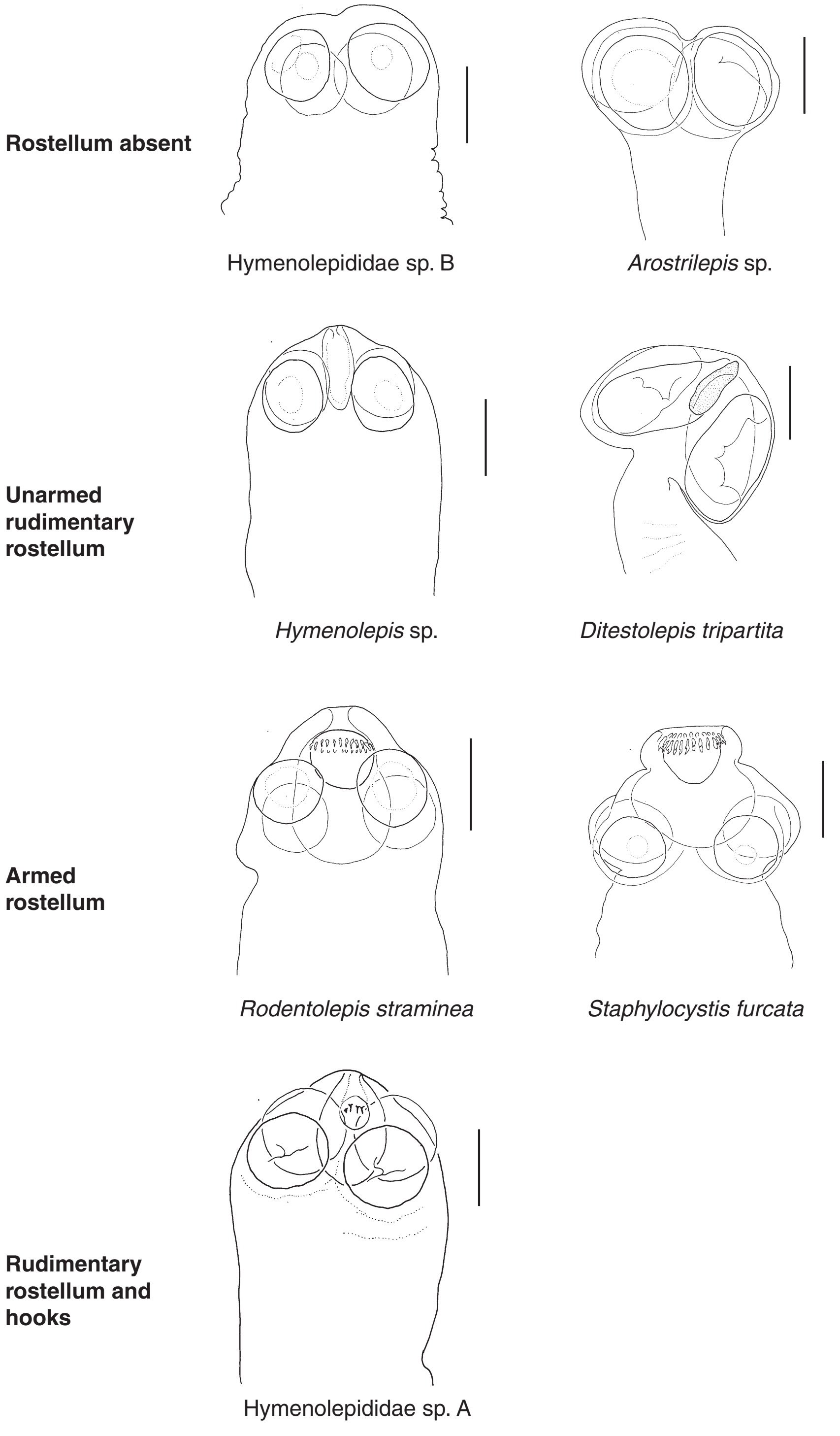

**Fig. 3** Examples of main types of scolex and rostellum in hymenolepidid cestodes of rodents and shrews. Scale-bars 0.20 mm.

gravid proglottids of other species in this clade do not fuse, although the terminal proglottids of *D. tripartita* are capsule-like. Also, serial development of proglottids occurs in *D. tripartita*, but not in *Ditestolepis* sp. and *S. ovaluteri*.

These major differences favour the idea that all four species in the *Ditestolepis* clade represent different genera; this view is supported by the high genetic divergence among them. An alternative view is to assign all these species into *Ditestolepis*, which would invalidate the genus *Spasskylepis* Schaldybin, 1964, and also the related *Cucurbilepis* Sadovskaya, 1965, *Sinuterilepis* Sadovskaya, 1965 and *Ecrinolepis* Spasskii & Karpenko, 1983 (as proposed by Vaucher in Czaplinski & Vaucher 1994), the latter with serial development of proglottids. However, the basal relationships of the *Ditestolepis* clade remained unresolved, and it cannot be totally excluded that *Ditestolepis* spp. comprise a monophyletic group that is sister to *S. ovaluteri*.

### *Hymenolepis clade (B)*

The phylogenetic affinity between *Hymenolepis* (*sensu stricto*) spp. and *Rodentolepis asymmetrica* (Janicki, 1904) is unexpected, as they represent different rostellar types (rudimentary, unarmed rostellum vs. armed, functional rostellum). The implication is that the ancestor of *Hymenolepis* (*sensu stricto*) spp. has lost rostellar hooks and functional rostellum following colonization from arvicoline rodents. A rudimentary rostellum characterizes all *Hymenolepis* (*sensu stricto*), but a very similar structure has developed independently at least in two unrelated clades of hymenolepidid cestodes.

This study provides strong evidence for the monophyly of *Hymenolepis* (*sensu stricto*) in rodents. The true *H. diminuta* from *Rattus*, a very widespread species, is genetically divergent from the similar cestode in *Apodemus* spp. in Europe. This gives support for the independent status of *H. hibernia*, described from *Apodemus sylvaticus* from Ireland (Montgomery *et al*. 1987). In fact, there is evidence to suggest that the genus *Hymenolepis* (*sensu stricto*), and *H. diminuta* in particular, actually includes a number of undescribed species. According to the host–parasite database of the Natural History Museum, London (Gibson *et al*. 2005), *H. diminuta* has been reported worldwide not only from *c*. 80 species of myomorph and sciuromorph rodents, but also from insectivores and humans. In addition, interspecific morphological differences in the *H. diminuta* clade are limited, and plausible species recognition without molecular tools is evidently very challenging. Our ongoing molecular phylogenetic studies have revealed several new, more or less cryptic species in the *H. diminuta* clade (Haukisalmi, V., Hardman, L. M., Lehtonen, J. T., Foronda, P., unpublished data); their hosts include endemic Madagascan nesomyid rodents (Myomorpha) and tenrecids (Tenrecomorpha), suggesting an ancient radiation of this cestode clade.

### *Arostrilepis clade (C)*

The revealed phylogenetic association between *Arostrilepis* Mas-Coma & Tenora, 1997, *Soricinia* Spasskii & Spasskaya, 1954, *Pseudobotrialepis* Schaldybin, 1957, *Staphylocystoides* Yamaguti, 1959, *Neoskrjabinolepis* Spasskii, 1947, *Lineolepis* Spasskii, 1959 and *Vigisolepis* Matevosyan, 1945 is also surprising and has not been suggested earlier. The structure of the clade is clearly indicative of the loss of rostellum and hooks in the ancestor of *Arostrilepis* + *Soricinia* + *Pseudobotrialepis* + Hymenolepididae sp. C and colonization of rodents by the ancestor of *Arostrilepis* spp. The presence of Hymenolepididae sp. C within this clade also indicates colonization into *Neomys* from *Sorex*. Hymenolepididae sp. C, which may represent *Hymenolepis fodientis* Vaucher, 1971, probably should be assigned to an independent, new genus, because of its divergent phylogenetic position. Despite the shared characteristic of the crown clade (absence of rostellum and hooks), the constituent species are very different with respect to the body size and size and morphology of the scolex, and we cannot presently suggest any characters that could be used to unite these species.

This study also provides the first molecular evidence for the independent status and monophyly of *Arostrilepis* spp. The representatives of this taxonomically challenging group have been usually referred to as *Hymenolepis horrida* (von Linstow, 1901) or *Arostrilepis horrida* (von Linstow, 1901); additionally, two species referable to *Arostrilepis* Mas-Coma & Tenora, 1997 have been described from arvicoline rodents in Siberia and eastern Beringia (Kontrimavichus & Smirnova 1991; Gulyaev & Chechulin 1997). However, the unpublished molecular data of K. Galbreath and E. P. Hoberg (see Cook *et al*. 2005) suggest a presence of at least 10 independent species in the Beringian arvicoline rodents alone, each being usually restricted to a certain host genus. In addition, *Taenia horrida* Linstow, 1901 was originally described from an unknown rodent host in Europe, and it is possibly not conspecific with any of the clades recognized by Galbreath and Hoberg or those identified in this study. Thus, despite limited interspecific divergence, the present *Arostrilepis* assemblage probably includes four different species. The morphological differences among *Arostrilepis* spp. are evidently minor, and this clade should be subjected to a comprehensive phylogenetic analysis and taxonomic revision. The present results do not support the family Arostrilepididae Mas-Coma & Tenora, 1981.

### *Rodentolepis clade (D)*

The phylogenetic structure of the *Rodentolepis* clade indicates frequent host switching among various rodents, shrews and bats. The topology of the phylogenetic tree and host distribution suggest that murid rodents, possibly *Mus*, have been the original hosts for the *Rodentolepis* clade. The frequent host colonizations in this clade seem to have been accompanied by multiple, pronounced changes in the morphology of the rostellum and hooks.

The divergent, basal '*fraterna* clade' clearly consists of three independent, genetically distinct species, which are recognizable by their characteristic (fraternoid) hook shape. Haukisalmi *et al*. (2009) provided preliminary morphological differences between *R. fraterna* from *Mus*, *Rodentolepis* sp. from *Cricetulus barabensis* (present material), and *Rodentolepis sinensis* (Oldham, 1929), also from *C. barabensis*. Thus, *Rodentolepis* sp. from *C. barabensis* and *R.* cf. *fraterna* from *Micromys minutus* represent undescribed species. The systematic position of *R. nana* from man cannot be solved by the present material, but the molecular phylogenetic analysis of Macnish *et al*. (2002) showed that the representatives of the *nana/fraterna* complex from man and rodents are closely related and they form a strongly supported clade with respect to *R. microstoma*. Thus, it can be predicted that *R. nana* from man also belongs to the *fraterna* clade; this prediction is supported by the hook shape of *R. nana* (see Baer & Tenora 1970). It should be noticed that the true *R. fraterna*, which occurs primarily in *Mus*, was also found from *Apodemus uralensis* in this study.

*Rodentolepis straminea* and *R. microstoma* have almost identical (cricetoid) rostellar hooks, for which reason they have sometimes been considered conspecific (Baer & Tenora 1970). However, Casanova *et al*. (2001) found fixed allelic differences between these species, suggesting independent status. Morphological differences were very limited, the most important one concerning the presence/absence of polar filaments in eggs (see below). The present results fully support the independent status of *R. straminea* and *R. microstoma* as host-specific parasites of *Apodemus* and *Mus*, respectively.

Within the *Rodentolepis* clade, there were two species (or forms) from glirid rodents with deviating rostellar morphology, i.e. Hymenolepididae sp. A with rudimentary hooks and rostellum and Hymenolepididae sp. B without a rostellum (or hooks). Despite this major morphological difference, the two cestodes in the present analysis formed a well-supported clade with minor genetic divergence. Neither of these species corresponds to the two known hymenolepidid species of European glirids, i.e. *Rodentolepis myoxi* (Rudolphi, 1819) with normal, functional hooks and *Hymenolepis sulcata* (von Linstow, 1879) with rudimentary, unarmed rostellum. These patterns suggest either a recent radiation of hymenolepidids of glirids accompanied by pronounced morphological divergence or, alternatively, a presence of one or more polymorphic hymenolepidid species in glirids in Europe. Further morphological and genetical studies are needed to solve this puzzle.

The morphology of eggs has been largely neglected in the classification of *Rodentolepis*-like cestodes. However, Casanova *et al*. (2001) showed that the morphologically similar *R. straminea* and *R. microstoma* can be best separated by the presence of polar filaments in the eggs of the latter species (absent in *R. straminea*). The present, unpublished observations show that all species within the *fraterna* subclade have polar filaments and they are also present in *S. furcata*, but absent in *Vampirolepis* sp. The present cestodes from *Glis* were not gravid, but other, evidently related species from glirids (*H. sulcata* and *R. myoxi*) lack polar filaments. This pattern suggests that polar filaments have been present in the common ancestor of the *Rodentolepis* clade, and that they have been secondarily lost in the cestodes of glirids, bats and in *R. straminea*. Although polar filaments obviously have limited phylogenetic significance, they are probably useful in future classification attempts of *Rodentolepis*-like cestodes on specific and generic level.

Based on the present results, the genus *Rodentolepis sensu* Vaucher in Czaplinski & Vaucher (1994) is clearly non-monophyletic. Five of the species in the *Rodentolepis* clade have traditionally been assigned to *Rodentolepis*, but the clade also includes a species of *Staphylocystis* and *Vampirolepis*. In addition, two species positioned outside the *Rodentolepis* clade, i.e. *R. asymmetrica* and *R. evaginata*, are morphologically referable to *Rodentolepis sensu* Vaucher in Czaplinski & Vaucher (1994). Merging all species in the *Rodentolepis* clade would be problematic because of the major morphological differences, particularly concerning the two deviating species from glirids. Thus, the *Rodentolepis* clade should be reorganized to encompass at least six genera, including new genera for the *R. fraterna* clade and *R. microstoma*. Two new genera are obviously needed also for *R. asymmetrica* and *R. evaginata*.

## Conclusions

The main finding of this study is the presence of four multispecies clades of hymenolepidid cestodes in rodents and shrews showing pronounced morphological variation and frequent colonizations between unrelated hosts. Although rostellar morphology is obviously a key feature on specific and generic levels, on higher systematic levels it seems to be a rather poor indicator of phylogenetic affinity in hymenolepidid cestodes. The presence of clades with more than one rostellar type also conflicts with the proposed subfamilial and tribal classifications of hymenolepidid cestodes. The overall evidence suggests that the recent trend of splitting hymenolepidid cestodes into multiple genera will produce a more stable and practical classification than the earlier practice of favouring a few, morphologically variable genera. However, it is obvious that new generic classifications of hymenolepidid cestodes should consider both morphological and molecular evidence.

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## Appendix

Cestode material, their background information and GenBank accession numbers for 28S rRNA sequences. The letters 'A–E' in the last column indicate identical sequences.

| Cestode species | Host species | Host group | Country | Locality | GenBank Acc. no. |
|---|---|---|---|---|---|
| (A) Rodents | | | | | |
| *Arostrilepis* sp. | *Microtus pennsylvanicus* | Cricetidae, Arvicolinae | USA | Alaska, Fairbanks | GU166226 |
| *Arostrilepis* sp. | *Myodes rutilus* | Cricetidae, Arvicolinae | Russia | Buryatia, Verhnaya Berezovka | GU166224 |
| *Arostrilepis* sp. | *Lemmus sibiricus* | Cricetidae, Arvicolinae | Russia | Wrangel Island | GU166223 |
| Hymenolepididae sp. A | *Glis glis* | Gliridae | France | Py | GU166277 |
| Hymenolepididae sp. B | *Glis glis* | Gliridae | Croatia | Delnice | GU166239 |
| *Hymenolepis diminuta* | *Rattus rattus* | Muridae, Murinae | Madagascar | Ranomafana | GU166229 |
| *Hymenolepis weldensis* | *Geomys bursarius* | Geomyidae | USA | Indiana, Bloomington | GU166230 |
| *Hymenolepis* sp. | *Apodemus sylvaticus* | Muridae, Murinae | Turkey | Elmabag | GU166227 (A) |
| *Hymenolepis* sp. | *Apodemus agrarius* | Muridae, Murinae | Romania | Pojejena | A |
| '*Hymenolepis*' *muris-sylvatici* | *Apodemus sylvaticus* | Muridae, Murinae | Croatia | Migalovci | GU166246 |
| *Paraoligorchis* sp. | *Nesomys rufus* | Nesomyidae | Madagascar | Ranomafana | GU166257 |
| *Rodentolepis asymmetrica* | *Microtus agrestis* | Cricetidae, Arvicolinae | Sweden | Gualöv | GU166232 |
| *Rodentolepis asymmetrica* | *Chionomys nivalis* | Cricetidae, Arvicolinae | France | Bourg-Saint-Maurice | GU166231 |
| *Rodentolepis asymmetrica* | *Dinaromys bogdanovi* | Cricetidae, Arvicolinae | Bosnia | Zelengora Mountains | GU166233 |
| *Rodentolepis evaginata* | *Ondatra zibethicus* | Cricetidae, Arvicolinae | USA | Alaska, BLB NP[1] | GU166245 |
| *Rodentolepis fraterna* | *Mus musculus domesticus* | Muridae, Murinae | Spain | Canary Islands, Tenerife | GU166268 (B) |
| *Rodentolepis fraterna* | *Apodemus uralensis* | Muridae, Murinae | Kazakhstan | Taldykorgan | B |
| *Rodentolepis* cf. *fraterna* | *Micromys minutus* | Muridae, Murinae | Finland | Vantaa and Lappeenranta | GU166241 (C), C |
| *Rodentolepis microstoma* | *Mus musculus domesticus* | Muridae, Murinae | Spain | Canary Islands, La Gomera | GU166266 |
| *Rodentolepis straminea* | *Apodemus sylvaticus* | Muridae, Murinae | France | Py | GU166264 (D) |
| *Rodentolepis straminea* | *Apodemus flavicollis* | Muridae, Murinae | Bosnia | Šator Mountain | D |
| *Rodentolepis straminea* | *Apodemus mystacinus* | Muridae, Murinae | Turkey | Ayder | D |
| *Rodentolepis straminea* | *Apodemus agrarius* | Muridae, Murinae | Croatia | Migalovci | D |
| *Rodentolepis straminea* | *Apodemus flavicollis* | Muridae, Murinae | Croatia | Loze | D |
| *Rodentolepis* sp. | *Cricetulus barabensis* | Cricetidae, Cricetinae | Russia | Buryatia, Ganzurinov | GU166243 |
| (B) Shrews | | | | | |
| *Ditestolepis diaphana* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU166253 |
| *Ditestolepis tripartita* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU166255 |
| *Ditestolepis* sp. | *Sorex isodon* | Soricidae | Finland | Konnevesi | GU166261 |
| Hymenolepididae sp. C | *Neomys fodiens* | Soricidae | Finland | Konnevesi | GU166273 |
| *Lineolepis scutigera* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU166250 |
| *Monocercus arionis* (Dilepididae) | *Sorex araneus* | Soricidae | Finland | Vantaa | GU166276 |
| *Neoskrjabinolepis schaldybini* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU166248 |
| *Pseudobotrialepis globosoides* | *Sorex minutus* | Soricidae | Finland | Konnevesi | GU166271 |
| *Soricinia infirma* | *Sorex araneus* | Soricidae | Finland | Pallasjärvi | GU166260 |
| *Spasskylepis ovaluteri* | *Sorex caecutiens* | Soricidae | Finland | Pallasjärvi | GU166262 |
| *Staphylocystis furcata* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU969050 |
| *Staphylocystis furcata* | *Sorex araneus* | Soricidae | Finland | Vantaa | GU166274 (E), E |
| *Staphylocystoides stefanskii* | *Sorex minutus* | Soricidae | Finland | Konnevesi | GU166258 |
| *Urocystis prolifer* | *Sorex araneus* | Soricidae | Finland | Vantaa | GU166251 |
| *Vigisolepis spinulosa* | *Sorex araneus* | Soricidae | Finland | Konnevesi | GU166254 |
| (C) Bats | | | | | |
| *Vampirolepis* sp. | *Eptesicus nilssoni* | Chiroptera, Vespertilionidae | Finland | Kuopio | GU969051 |

[1] Bering Land Bridge National Preserve.