Two new hymenolepidid species (Cestoda, Hymenolepididae) from water shrews *Neomys fodiens* Pennant (Insectivora, Soricidae) in Bulgaria

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Abstract

Two new hymenolepidid species, *Coronacanthus magnihamatus* sp. nov. and *Triodontolepis boyanensis* sp. nov. are described from the European water shrew, *Neomys fodiens*, in Bulgaria. The most important differentiating features of *C. magnihamatus* are the length of the rostellar hooks (26–28 μ m, mean 27 μ m) and the thick-walled uterus, which does not form capsules in gravid proglottids. *T. boyanensis* is distinguished from other members in the genus by the number (16) and size (47–48 μ m, mean 48 μ m) of the rostellar hooks, the non-capsulate gravid uterus, containing relatively large number of eggs (35–70, mean 49) and the embryophore, possessing polar filaments. The types of uterine development in hymenolepidids of *Neomys* spp. are discussed.

Key words

Coronacanthus magnihamatus sp. nov., Triodontolepis boyanensis sp. nov., Cestoda, Neomys fodiens, Insectivora, Bulgaria

Introduction

Almost all adult cestodes occurring in water shrews (genus Neomys Kaup) belong to the family Hymenolepididae Ariola, 1899. The only non-hymenolepidid cestode of *Neomys* is Molluscotaenia estavarensis (Euzet et Jourdane, 1968). As has been previously revealed, the hymenolepidid fauna of Neomys, as well that of other shrew genera studied so far, such as Sorex Linnaeus, Crocidura Wagler, Suncus Ehrenberg, Blarina Gray and Diplomesodon Brandt, is characterised by a high level of host-specificity (Vaucher 1982, Mas-Coma et al. 1984, Tkach and Velikanov 1991, Velikanov and Tkach 1993). It should be mentioned that while some other shrew genera may at least share their hymenolepidid genera (e.g., Staphylocystis Villot, 1877, Hilmylepis Skryabin et Matevosyan, 1942, Pseudhymenolepis Joyeux et Baer, 1935), all hymenolepidids of *Neomys* are specific to this shrew genus. The hymenolepidid fauna of Neomys presently includes 15 valid species, the majority of them belonging to two genera, Coronacanthus Spassky, 1954 and Triodontolepis Yamaguti, 1959.

During faunistic studies on cestodes collected from the European water shrew, *Neomys fodiens* Pennant, in the vicinity of Sofia (Bulgaria) in 2001, we found specimens of several cestode species belonging to *Coronacanthus* and *Triodontolepis*. Examination of these specimens has revealed that one species of *Coronacanthus* and one of *Triodontolepis* are different in their rostellar hook size and number, as well as in strobilar characteristics, from the remaining species of these two genera. The descriptions of the new species are presented below.

Materials and methods

Specimens of the two new cestode species were collected from 2 specimens of the European water shrew, *Neomys fodiens*, trapped on the banks of the Boyana River, Mount Vitosha, in the vicinity of Sofia, Bulgaria, in July and October 2001. Tapeworms were isolated from the intestines, relaxed in tap water, fixed in 4% hot formalin solution and preserved in 70% ethanol. After the fixation, they were stained with iron acetocarmine (Georgiev *et al.* 1986), dehydrated in ethanol series, cleared in eugenol and mounted in Canada balsam. One scolex of *T. boyanensis* sp. nov. was mounted in Berlese's medium to facilitate the examination of the rostellar hooks. Details of the specimens studied, their localities and collection numbers are given below. Holotypes are deposited in The British Museum (Natural History) Collection, London (BMNH); the remaining specimens are at the Collection of the Parasite Biodiversity Group, Central Laboratory of General Ecology, Sofia, Bulgaria (PBG CLGE).

The metrical data are given as a range followed by mean values and the number of measurements taken (n) in parentheses. All measurements are in micrometres unless otherwise stated. The illustrations of the proglottids and genital ducts are presented in dorsal view.

Results

Coronacanthus magnihamatus sp. nov. (Figs 1-10)

Description

Strobila ribbon-like, slender, $9-11 \text{ mm} (n=2) \log$; maximum width 0.46-0.47 mm (n = 2), at level of gravid proglottids. Scolex cup-shaped, large, $386-437 \times 327-354$ (n = 2); anterior part of scolex invaginates and forms deep anterior cavity with thick, muscular walls. Suckers small, oval, with weaklydeveloped musculature, situated on inner surface of apical cavity which can be erected so that suckers may appear almost at apical surface of scolex (Fig. 1); diameter of suckers 95-134 (118, n=4). Rostellum small, thick-walled, muscular, situated on bottom of apical cavity; measurements $103-106 \times$ 152-155 (n = 2); rostellum filled with intensely staining glandular cells. Rostellar sheath $142-168 \times 232-250$ (n = 2), almost oval, thick-walled, entirely filled with compact intensely staining glandular masses. Rostellum armed by a single crown of 37-38 (n = 2) hooks of coronacanthoid type (Fig. 2). Hooks (Fig. 3) with thin, short handle, sickle-shaped blade and well-developed guard, slightly shorter than blade. Hook measurements: total length 26-28 (27, n = 12), handle 5-6 (6, n = 12), blade 21–24 (23, n = 12) and guard 16–18 (17, n =12). Neck thin, well-differentiated from scolex, minimum width 147 (n = 1); internal segmentation begins 386 (n = 1) from posterior margin of scolex. Proglottids (Figs 4-7) almost acraspedote, with weakly-developed velum, always much wider than long. Longitudinal muscle bundles numerous. Genital pores unilateral, equatorial. Genital atrium (Figs 8 and 9) cylindrical, surrounded by intensely staining cells. Dorsal and ventral osmoregulatory canals without transverse anastomoses; diameter of dorsal osmoregulatory canals 3 (n = 10); diameter of ventral osmoregulatory canals 8-15 (11, n = 10). Genital ducts dorsal to osmoregulatory canals.

Testes (Figs 4 and 5) three, compact, oval; arranged in one row, one poral and two antiporal to female glands, overlapping them dorsally; maximum diameter of testes 39-49 (45, n = 20). External seminal vesicle (Figs 4–6) $57-77 \times 21-28$ 57

 $(64 \times 24, n = 10)$, elongate, dorsal to female glands, usually reaching mid-line of proglottid. Cirrus-sac (Figs 4–8) $101-111 \times 21-26$ (106×24 , n = 10), thin-walled, elongate; crosses poral osmoregulatory canals dorsally to them, does not reach mid-line of proglottid. Internal seminal vesicle (Figs 4–8) elliptical, situated in antiporal 1/4-1/3 of cirrus-sac. Evaginated cirrus (Fig. 9) cylindrical, covered by tiny, needleshaped spines, more densely distributed in basal portion of cirrus; length of evaginated cirrus 31-57 (42, n = 10), maximum width 10 (n = 10).

Ovary (Figs 4 and 5) 77–93 × 41–59 (82 × 50, n = 10), compact, elliptical, usually slightly poral to proglottid midline. Vitellarium (Figs 4 and 5) 28–36 × 18–34 (34 × 26, n = 10), compact, oval, situated antiporally and dorsally to ovary, close to posterior proglottid margin. Mehlis' gland (Figs 4 and 5) round, median, dorsal to vitellarium. Seminal receptacle (Figs 4–6) elongate, 41–64 (52, n = 10) × 18–26 (21, n = 10), dorsal to ovary and uterus and posterior to cirrus-sac and external seminal vesicle. Vagina (Fig. 8) with short, funnelshaped, thick-walled copulatory part, gradually tapering and passing into cylindrical, thick-walled conductive part; vagina opens and passes ventral and posterior to cirrus-sac.

Young uterus (Figs 4 and 5) appears as elliptical, transparent thick-walled sac, containing large, intensely staining cells; median and dorsal to ovary and vitellarium. Pregravid uterus (Fig. 6) sac-like, transversely elongate, thick-walled, occupies entire median field, sometimes crosses osmoregulatory canals. Fully-developed uterus (Fig. 7) fills entire proglottid; number of ripe eggs in gravid uterus 23-43 (35, n = 5). Eggs (Fig. 10) elliptical or irregular in shape; measurements of eggs $39-46 \times 31-41$ (42×37 , n = 10). Embryophore thick, elliptical, wrinkled in stained specimens; measurements $28-31 \times 23-26$ (30×25 , n = 10). Oncosphere elliptical $18-21 \times 15-18$ (21×17 , n = 10). Oncospheral hooks 10-13 (11, n = 10) long.

Type host: European water shrew, *Neomys fodiens* Pennant (Insectivora, Soricidae).

Site of infection: Small intestine.

Type locality: Boyana River, Mount Vitosha, vicinity of Sofia, Bulgaria, 30°37'N, 88°41'W.

Type specimens: BMNH 2004.8.17.1, holotype, a slide containing one mature specimen, stained whole-mount, small intestine, Mount Vitosha, Boyana River, 11.10.2001; para-type, PBG CLGE no. 1, small intestine, Mount Vitosha, Boyana River, 11.10.2001, one gravid specimen, stained whole-mount (1 slide).

Etymology: The name of the new species refers to its unusually large rostellar hooks.

Differential diagnosis

The peculiar structure of the scolex, the shape of the rostellar hooks and the strobilar morphology of *C. magnihamatus* correspond with the principal generic characters of *Coronacan-thus* (see Vaucher in Czaplinski and Vaucher 1994). Currently, this genus includes three species, *C. integrus* (Hamann, 1891), *C. omissus* (Baer et Joyeux, 1943) and *C. vassilevi* Genov,



Figs 1–6. *Coronacanthus magnihamatus* sp. nov.: **1** – scolex; **2** – entire crown of rostellar hooks; **3** – rostellar hooks; **4** – mature hermaphroditic proglottid; **5** – proglottid with young uterus; **6** – pregravid proglottid. Scale bars: 1 and 4–6 = 200 μ m, 2 = 100 μ m, 3 = 30 μ m



Figs 7–10. *Coronacanthus magnihamatus* sp. nov.: **7** – gravid proglottid; **8** – distal part of genital ducts; **9** – evaginated cirrus; **10** – egg. Scale bars: $7 = 200 \mu m$, 8 and $9 = 50 \mu m$, $10 = 30 \mu m$

1980. *C. magnihamatus* is easily distinguishable from all of them by the unusually long rostellar hooks (26–28 µm). The hook length in the remaining three species does not exceed 14 µm, i.e., 5–6 µm in *C. omissus*, 8–11 µm in *C. vassilevi* and 12–14 µm in *C. integrus* (see Andrejko and Spassky 1971, Genov 1980). *C. magnihamatus* differs from *C. omissus* and *C. vassilevi* by the longer strobila and larger diameter of the scolex. The rostellum of *C. magnihamatus* (103–106 × 152– 155) is also much larger compared with those of *C. omissus* (17–24 × 24–32) and *C. vassilevi* (47–60 × 55–74) (Genov 1980).

Judging by the morphology of the strobila, *C. magnihamatus* is most similar to the type-species of *Coronacanthus*, *C. integrus*. In addition to the number of the hooks (38 in *C. magnihamatus* vs. 62–68 in *C. integrus*), the new species differs from *C. integrus* by the presence of a longer cirrus-sac (101– 111 μ m vs. 66–74 μ m in *C. integrus*) and the structure of the gravid uterus. In *C. magnihamatus* it is thick-walled, fills entire proglottid but does not form uterine capsules (or cocoons as described by some authors), whilst the gravid uterus of *C. integrus* forms a well-developed thick-walled capsule. On the basis of these differences, we consider our specimens from *N. fodiens* to represent a new species of *Coronacanthus*.

Triodontolepis boyanensis sp. nov. (Figs 11-20)

Description

Strobila ribbon-shaped, with strong longitudinal musculature; maximum length 12–17 mm (15, n = 4); maximum width 0.57–0.71 mm (0.65, n = 4) at level of gravid proglottids; pos-



Figs 11–16. *Triodontolepis boyanensis* sp. nov.: **11** – scolex; **12** – entire crown of rostellar hooks; **13** – rostellar hooks; **14** – mature hermaphroditic proglottid; **15** – proglottid with young uterus, **16** – proglottid with developing uterus. Scale bars: $11 = 200 \mu m$, $12 \text{ and } 14-16 = 100 \mu m$, $13 = 50 \mu m$

terior terminal part of strobila gradually tapering. Scolex (Fig. 11) very large, $437-482 \times 309-399$ (463×339 , n = 3), anterior part conically protruded, maximum width at level of suckers. Rhynchus well-developed, 129–142 \times 134–147 (133 \times 139, n = 3), with muscular walls. Suckers large, 134–154 $(143, n = 16) \times 108 - 134$ (118, n = 14), elliptical, muscular. Rostellum small, $64-95 \times 119-126$ (74 × 124, n = 3), thickwalled, muscular, with slightly conically tapering posterior part; large intensely staining glandular cells situated in its anterior part. Rostellar sheath highly elongate, 321–366 (338, n = 3 × 148–167 (159, n = 3), thick-walled, reaching beyond posterior margins of suckers; intensely staining glandular cells fill entire rostellar sheath. Rostellum armed by a single crown of 16 hooks (Fig. 12). Each hook (Fig. 13) of bifurcoid type, with thin, slightly curved handle, curved blade and welldeveloped bifurcate guard. Measurements of hooks: total length 47–48 (48, n = 5), handle 20–21 (21, n = 5), blade 29-31 (30, n = 5) and guard 23-24 (24, n = 5). Neck well-differentiated from scolex, minimum width 186–231 (207, n = 4); internal segmentation begins 386-534 (431, n = 4) from posterior margin of scolex. Proglottids (Figs 14-17) craspedote, always much wider than long. Longitudinal muscle bundles numerous. Genital pores unilateral, apparently pre-equatorial. Genital atrium (Figs 18 and 19) thick-walled, surrounded by intensely staining cells. Dorsal and ventral osmoregulatory canals without transverse anastomoses; diameter of dorsal osmoregulatory canals 5-10 (8, n = 10); diameter of ventral osmoregulatory canals 21-31 (26, n = 10). Genital ducts dorsal to osmoregulatory canals.

Testes (Figs 14–16) three, compact, oval; usually in one row, one poral and two antiporal to female glands, overlapping them dorsally; antiporal testes frequently reach antiporal osmoregulatory canals; maximum diameter of testes 52-67 (59, n = 20). External seminal vesicle (Figs 14–18) $80-101 \times$ 23–36 (90 \times 27, n = 10), large, elongate, dorsal to female glands, usually reaching mid-line of proglottid. Cirrus-sac (Figs 14–18) 124–157 \times 21–26 (136 \times 22, n = 15), thin-walled, highly elongate, crosses poral osmoregulatory canals dorsally, does not reach mid-line of proglottid. Internal seminal vesicle (Figs 14-18) elliptical, situated in antiporal 1/5-1/4 of cirrus-sac. Evaginated cirrus (Fig. 19) short, with slightly conically tapering tip; basal part armed by tiny triangular spines, distal part covered with larger needle-shaped spines; measurements of partly evaginated cirrus $15-54 \times 15-23$ (25×22 , n = 10).

Ovary (Figs 14 and 15) $90-103 \times 36-44$ (96×41 , n = 10), compact, elliptical, usually slightly poral to mid-line of proglottid, ventral to vitellarium. Vitellarium (Figs 14 and 15) $36-46 \times 28-34$ (42×31 , n = 10), compact, elliptical, almost median, posterior and dorsal to ovary and uterus. Seminal receptacle (Figs 14–16 and 18) sac-like, 31-52 (42, n = 10) × 18-26 (23, n = 10), elliptical, dorsal to ovary and uterus and posterior to cirrus-sac and external seminal vesicle. Vagina (Figs 18 and 19) with short, funnel-shaped copulatory part, gradually tapering and passing into thin conductive part; vagina opens and passes ventrally to cirrus-sac.

Uterus (Fig. 15) initially appears as elliptical, transparent, empty sac, with thick walls, containing large intensely staining cells; median and ventral to ovary and vitellarium. Developing uterus (Fig. 16) sac-like, transversely elongate, thickwalled. With further maturation, uterus occupies median field and sometimes crosses osmoregulatory canals; anterior and posterior sacculations appear in uterine walls. Fully-developed uterus (Fig. 17) fills entire proglottid; number of eggs in gravid uterus 35–70 (49, n = 10). Eggs of elliptical or irregular shape, 41–52 × 28–39 (48 × 32, n = 15) in size; egg sur-

face wrinkled in stained, whole-mounted specimens (Fig. 20). Embryophore thin, elliptical, forms two long polar filaments. Oncosphere elliptical, $21-28 \times 15-18$ (24×17 , n = 15). Oncospheral hooks 8–10 (8, n = 10) long.

Type host: European water shrew, *Neomys fodiens* (Insectivora, Soricidae).

Site of infection: Small intestine.

Type locality: Boyana River, Mount Vitosha, vicinity of Sofia, Bulgaria, 30°37'N, 88°41'W.

Type specimens: Holotype, BMNH 2004.8.17.2, a slide with a gravid specimen, stained whole-mount, small intestine, Mount Vitosha, Boyana River, 26.07.2001; paratypes, PBG CLGE Nos 4–1, 2, 54, small intestine, Mount Vitosha, Boyana River, 26.07.2001, 3 gravid specimens, stained whole-mounts (3 slides), one scolex, mounted in Berlese's medium (1 slide).

Etymology: The new species is named after the Boyana River, the type-locality of the species.

Differential diagnosis

Currently, the genus Triodontolepis includes 7 species possessing rostellar hooks with characteristic 'bifurcate' hook guards. Judging by this feature and the morphology of the scolex and strobila, the above described specimens from N. fodiens conform to the generic characters of Triodontolepis (see Vaucher in Czaplinski and Vaucher 1994). Depending on the number of rostellar hooks, Triodontolepis can be divided into two groups. The first group includes species with 10 rostellar hooks, i.e., T. bifurca (Hamann, 1891), T. torrentis Murai, 1987, T. skrjabini Spassky et Andrejko, 1968 and T. sumavensis (Prokopič, 1957); the latter two species may have 12 hooks and one of the authors (VVT) has observed a specimen of T. skrjabini with 11 hooks which should be considered a teratology. The second group comprises Triodontolepis spp. having more than 10 rostellar hooks, i.e., 16-21 hooks in T. hamanni (Mrázek, 1891) (see Mrázek 1891, Tkach 1991), 30 in T. rysavyi Prokopič, 1972 (see Prokopič 1972) and 34 rostellar hooks in T. kurashvilii Prokopič et Matsaberidze, 1971 (see Prokopič and Matsaberidze 1971). Concerning the number of the hooks, T. boyanensis belongs to the second group of Triodontolepis spp. In addition to the number of the rostellar hooks, the new species differs from T. rysavyi and T. kurashvilii by the length of the hooks (47–48 µm in T. boyanensis vs. 26 µm in T. rysavyi and 30-32 µm in T. kurashvilii), larger vitellarium and by the cirrus-sac, which does not cross the mid-line of the proglottid. The significant distinguishing feature of the new species is the lack of uterine capsules, whilst



Figs 17–20. *Triodontolepis boyanensis* sp. nov.: **17** – gravid proglottid; **18** – distal part of genital ducts; **19** – evaginated cirrus; **20** – eggs. Scale bars: $17 = 200 \mu m$, 18 and $20 = 50 \mu m$, $19 = 20 \mu m$

both *T. rysavyi* and *T. kurashvilii* possess one or two thickwalled capsules per gravid proglottid (Prokopič and Matsaberidze 1971, Prokopič 1972).

Judging by the number of the rostellar hooks *T. boyanensis* is most similar to *T. hamanni*. The two species differ by the length of the rostellar hooks, i.e., 47–48 μ m in *T. boyanensis* vs. 25–30 μ m in *T. hamanni* (see Vaucher 1971, Murai 1987) and the length of the cirrus-sac (124–157 μ m in *T. boyanensis* vs. 70–100 μ m in *T. hamanni*) (Baer 1931, Murai 1987). In addition, these two species also differ in the structure of the

gravid uterus since the gravid proglottids in *T. hamanni* have well-developed, thick-walled uterine capsule, containing up to 40 eggs (Murai 1987).

The presence of a thick-walled uterine capsule is a common feature of *Triodontolepis* spp., with two exceptions, *T. boyanensis* and *T. torrentis*. They are both characterised by the presence of a sac-like gravid uterus, which does not form a capsule (present study, Tkach 1991). Despite this similarity, *T. boyanensis* differs considerably from *T. torrentis* in the number of rostellar hooks (16 in *T. boyanensis* vs. 10 in *T. tor*- rentis), their size (47–48 μ m in *T. boyanensis* vs. 38–41 μ m in *T. torrentis*) and number of eggs in uterus (35–70, mean 49 in *T. boyanensis* vs. 16–22 in *T. torrentis*) (present study, Tkach 1991). In addition, we observed the presence of long polar filaments of the embryophores in *T. boyanensis*, which has not been reported in the previous descriptions of *Triodontolepis* spp. On the basis of these comparisons, we recognize the above described specimens from *N. fodiens* as a new species of *Triodontolepis*.

Discussion

All hymenolepidids of Neomys spp. whose life histories are presently known (13 out of 15 species) have aquatic life cycles, involving gammarid crustaceans as intermediate hosts (Prokopič and Groschaft 1961; Prokopič et al. 1970; Matsaberidze et al. 1986; Tkach 1991, 1994, 1998; Tkach and Lisitsina 1996; Tkach et al. 2003). This fact obviously relates to the ecological preferences of their hosts. Cestodes of this group are characterised by a peculiar pattern of uterine development. A survey of the structure of the gravid uterus of Coronacanthus spp. (Andrejko and Spassky 1971, Vaucher 1971, Genov 1980), Triodontolepis spp. (Joyeux and Baer 1952, Andrejko and Spassky 1971, Prokopič and Matsaberidze 1971, Vaucher 1971, Prokopič 1972, Murai 1987, Tkach 1991), Hymenolepis alpestris (Baer, 1931) (Jourdane 1971), Cryptocotylepis globosoides (Soltys, 1954) (Gulyaev and Kornienko 1999), Neomylepis magnirostellata (Baer, 1931) (Tkach 1998) and Vaucherilepis trichophorus Tkach, Vasileva et Genov, 2003 (Tkach et al. 2003), shows two main types of uterine development.

In the first type, the gravid uterus forms one or two thickwalled, compact capsules per proglottid, enclosing approximately from 6 to 25 eggs. This type of the uterine structure is found in almost all *Triodontolepis* spp. (except for *T. torrentis* and *T. boyanensis*, see above), *V. trichophorus* and *Coronacanthus integrus*. The second type is characterised by the development of thick-walled, sac-like, non-capsulate gravid uterus, which occupies entire median field of proglottid, sometimes crosses the osmoregulatory canals, and contains from 10 to 70 eggs. This type is characteristic for *T. torrentis*, *T. boyanensis*, *Coronacanthus omissus*, *C. vassilevi*, *C. magnihamatus*, *H. alpestris*, *Cryptocotylepis globosoides* and *N. magnirostellata*.

These two types of gravid uterus demonstrate two different strategies in the dispersion of the eggs. Andrejko and Spassky (1971) supposed that the group dissemination of eggs (by uterine capsules) of *C. integrus* is a reason for the high intensity of gammarid infection by cysticercoids (7–12 cysticercoids per host). One of the authors (VVT) in the course of studies of gammarid infection by water shrew cestode larvae in the Ukrainian Carpathians has found that the intensity of infection of gammarids is at least in some accordance with the type of uterine development. For instance, only single cysticercoids of *T. torrentis* (not having uterine capsules) are usually found per infected gammarid, with two cysticercoids per host being very rare. In the same streams, an average of 8–9 cysticercoids of *T. skrjabini* (possessing uterine capsules) were found in each infected gammarid. It should be mentioned that the prevalence of infection of gammarids with larvae of *T. torrentis* was usually higher than with cysticercoids of *T. skrjabini*. Similarly, another hymenolepidid of water shrews, *V. trichophorus*, possesses uterine capsules in gravid proglottids and average number of the cysticercoids of that species per infected gammarid in the Ukrainian Carpathians varied from 6 to 10 (Tkach *et al.* 2003).

From another point of view, non-capsulate uterus usually contains more eggs, which probably possess different adaptations for successfully accomplishing their life cycle. Unfortunately, the details of the fine structure of the eggs of hymenolepidids of *Neomys* are practically absent. Our observations on *C. magnihamatus* and *T. boyanensis* show that their eggs possess peculiarities as different thickness of the outer egg envelope or presence of polar filaments of the embryophore (see Figs 10 and 20). We suppose that further studies of the egg and uterus morphology and ultrastructure will contribute substantially to our understanding of the life cycles and the adaptive mechanisms in hymenolepidid cestodes of water shrews.

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