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New species of *Kontrimavichusia* Makarikov & Binkienė, 2022 (Eucestoda: Hymenolepididae) from arvicoline rodents (Rodentia: Cricetidae) from the North Caucasus

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Abstract. Two previously unrecognized species attributable to the genus *Kontrimavichusia* Makarikov & Binkienė, 2022 in arvicoline rodents from the North Caucasus are described. *Kontrimavichusia testiculata* sp. nov. is described from *Microtus majori* (Thomas, 1906) from the northwestern Caucasus (Republic of Adygeya and Karachay-Cherkess Republic, Russia) and *Kontrimavichusia hobergi* sp. nov. is described from *Microtus daghestanicus* (Shidlovsky, 1919) from the central Caucasus (Republic of North Ossetia, Russia). *Kontrimavichusia testiculata* is readily distinguishable from *K. asymmetrica* (Janicki, 1904) and *K. hobergi* in having a larger number of testes (4–6 per proglottis), larger suckers and a longer cirrus and cirrus-sac. In addition, the new species differs from its congeners by the position of the cirrus-sac with regard to the poral osmoregulatory canals and position of distal end of the rostellar pouch relative to the posterior margins of the suckers. *Kontrimavichusia hobergi* can be readily distinguished from its congeners by the arrangement of the testes in a triangle and the position of the cirrus-sac with regard to the poral osmoregulatory canals. In addition, this previously unrecognized species differs from *K. asymmetrica* and *K. testiculata* by the smaller dimensions of the fully developed strobila and a narrower ovary. The cirrus-sac of *K. hobergi* is larger than that in *K. asymmetrica* but smaller than that in *K. testiculata*. We also used partial sequences of the nuclear ribosomal 28S rRNA gene and mitochondrial nad1 gen to justify the generic arrangement and independent status of these two new species which are characterized in the current manuscript.

Keywords. Cestoda, Hymenolepididae, *Kontrimavichusia asymmetrica*, *Kontrimavichusia* sp. nov., arvicoline rodents, North Caucasus.

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Introduction

The hymenolepidid cestode species *Kontrimavichusia asymmetrica* (Janicki, 1904) from arvicoline rodents from Europe has had a confusing taxonomic history; generic allocation has been revised several times since the original description. Initially, it was placed in *Hymenolepis* Weinland, 1858 (Janicki 1904, 1096), then transferred to *Rodentolepis* Spassky, 1954 (Spassky 1954), then returned again to *Hymenolepis* (e.g., Erhardová 1955; Vaucher 1967; Baer & Tenora 1970; Murai 1974; Genov 1984) or attributed to *Vampirolepis* Spassky, 1954 (e.g., Schmidt 1986; Murai 1989). Although there was no agreement on the generic affinities of this species, most recently it is usually considered as a member of the genus *Rodentolepis* based on morphological criteria (e.g., Ryzhikov *et al.* 1998; Santalla *et al.* 2002).

Subsequent phylogenetic studies showed that *K. asymmetrica* could not be referred to any known genus among the hymenolepidids from mammals and thus had uncertain taxonomic affinities (Haukisalmi *et al.* 2010; Greiman & Tkach 2012; Makarikov *et al.* 2015; Neov *et al.* 2019). Recently, the generic allocation of this species was clarified based on the integration of a morphological criteria and molecular phylogenetic analysis. A monotypic genus, *Kontrimavichusia* Makarikov & Binkienė, 2022, was proposed for this cestode.

Concurrently, it was suggested that the genus may include additional uncharacterized species since interspecific lineages within the *Kontrimavichusia* clade were detected in molecular-based comparisons (Makarikov & Binkienė 2022). Following this suggestion, we continued to study the species diversity within this complex. Two previously unrecognized species attributable to the genus *Kontrimavichusia*, based on morphological criteria, among arvicoline rodents from the North Caucasus were discovered. One of them, *Kontrimavichusia testiculata* sp. nov., is described from *Microtus majori* (Thomas, 1906) from the Republic of Adygeya and the Karachay-Cherkess Republic of Russia. Originally reported as *Rodentolepis* sp.1 from the northwestern Caucasus, it was suggested this cestode may represent an undescribed species (Makarikov *et al.* 2017). Subsequently, a detailed redescription of *K. asymmetrica* (sensu stricto) provided a basis to differentiate this putative taxon (Makarikov & Binkienė 2022). The other species *Kontrimavichusia hobergi* sp. nov. is described from *Microtus daghestanicus* (Shidlovsky, 1919) from the Republic of North Ossetia, Russia. The descriptions of these two new species and their morphological differentiation from the type species of *Kontrimavichusia* are provided herein. We also used partial sequences of the nuclear ribosomal 28S rRNA gene and mitochondrial nad1 gen to analyze relationships among species of hymenolepidids and to justify the generic arrangement and independent status of the new species.

Material and methods

Specimens of *Kontrimavichusia testiculata* sp. nov. were found in 7 out of 25 *Microtus majori*, prevalence 28%, collected in July 2014 from the suburbs of Nikel (44º10′31″ N, 40º09′24″ E), a village located in the Maykopsky District, Republic of Adygeya, Russia. Also, specimens attributable to this species were found in one out of 11 *Microtus majori* collected in September 2016 from the Djamagat River, suburbs of Teberda (43º27′21″ N, 41º47′44″ E), a town located in the Khabezsky District, Karachay-Cherkess Republic, Russia.

Specimens of *Kontrimavichusia hobergi* sp. nov. were found in 15 out of 26 *Microtus daghestanicus*, prevalence 57.7%, collected in July 2017 from the suburbs of Verkhniy Tsey (42º48′02″ N, 43º56′03″ E), a village located in the Alagirsky District, Republic of North Ossetia-Alania, Russia.

Host specimens were dissected fresh. Cestodes were isolated, rinsed and relaxed in water, and preserved in 70% ethanol. Specimens were stained with Ehrlich's haematoxylin, dehydrated in an ethanol series, cleared in clove oil and mounted in Canada balsam. Some scoleces and fragments of strobilae were

mounted in Berlese's medium to facilitate detailed examination of the rostellar hooks, suckers, cirrus armature and structure of the eggs. Additional tissue was subsampled from some strobila and stored in 96% ethanol for molecular analyses. Specimens were studied using standard light and differential interference contrast microscopy. In the descriptions, measurements are given in micrometers except where otherwise stated; they are presented as the range followed by the mean and the number of the measurements (n) in parentheses.

The type material and voucher specimens of the new species have been deposited in the collection of the Institute of Systematics and Ecology of Animals, Novosibirsk, Russia (ISEA). Mammalian taxonomy follows Musser & Carelton (2005).

Voucher specimens of *K. asymmetrica* deposited in the helminthological collections of the Geneva Museum of Natural History, Switzerland (MHNG) and ISEA were studied for comparison purposes. A list of examined collection material is outlined in Makarikov & Binkienė (2022).

Genomic DNA for the molecular phylogenetic analysis was extracted from fragments (1.5–2 mm long) of holotype, paratype and voucher specimens of *Kontrimavichusia testiculata* sp. nov. and *Kontrimavichusia hobergi* sp. nov. from the type locality and from voucher specimens of *K. asymmetrica* from Lithuania and the Republic of Bashkortostan, Russia, following the protocol of Tkach & Pawlowski (1999). Scoleces and the remaining strobila have been mounted on slides. DNA fragments approximately 1090 base pairs long at the 5' end of the nuclear large ribosomal subunit (28S) gene and approximately 755 base pairs long fragment of the mitochondrial NAD(P)H dehydrogenase 1 gene (nad1) were amplified by PCR and sequenced for inter- and intraspecific molecular comparisons. PCRs were run on an Eppendorf Mastercycler ep Gradient thermal cycler using OneTaq Quick-load Mastermix from New England Biolabs (Ipswich, MA) according to the manufacturer's instructions. All PCR protocols included 40 cycles. Forward primer 28S-5' (5'-TAC CCG CTG AAC TTA AGC ATA T-3') and reverse primer 28S-3' (5'-CTC CTT GGT CCG TGT TTC AAG AC-3') designed by Zehnder & Mariaux (1999) were used for amplification; annealing temperature 53ºC. Degenerate forward primer nad1f (5'-GGNTATTSTCARTNTCGTAAGGG-3') and degenerate reverse primer trnNR (5'-TTCYTGAAGTTAACAGCATCA-3') from Littlewood *et al.* (2008) were used for nad1 amplification; annealing temperature for these reactions was set at 45ºC. The same primers were used for sequencing both genes. Sequences were aligned using BioEdit software ver. 7.0.1 (Hall 1999). Pairwise comparisons of sequences of *Kontrimavichusia* spp. were calculated using MEGA X (Kumar *et al.* 2018). To build phylogenetic tree and reconstruct relationships between *Kontrimavichusia testiculata* sp. nov., *Kontrimavichusia hobergi* and *K. asymmetrica*, we used maximum likelihood (ML) with a general time reversible model as distance substitution. For phylogenetic analyses, we used newly obtained nucleotide sequences of 28S and nad1 genes of the two new species which were submitted to GenBank; *Kontrimavichusia testiculata* (5 and 7 respectively), accession numbers OR992632–OR992636 and PP133258–PP133264 and *Kontrimavichusia hobergi* (7 and 6 respectively), accession numbers OR992638–OR992642 and PP133265–PP133270. We also sequenced nad1 gene of *K. asymmetrica* (sensu stricto) from two relatively remote localities (Republic of Bashkortostan, Russia, and Lithuania), with GenBank accession numbers PP133271–PP133272 and PP133273. Nucleotide sequences of 28S of *K. asymmetrica* (sensu lato) and species of *Hymenolepis* were downloaded from the GenBank for comparison purposes (Lockyer *et al.* 2003; Haukisalmi *et al.* 2010; Nkouawa *et al.* 2016; Binkienė *et al.* 2019; Makarikov & Binkienė 2022). *Rodentolepis microstoma* (Dujardin, 1845) was used as an outgroup. Bootstrap values were calculated using MEGA as the percentage of 1000 replicates.

Results

Taxonomy

Order Cyclophyllidea van Beneden in Braun, 1900
Family Hymenolepididae Perrier, 1987
Genus *Kontrimavichusia* Makarikov & Binkienė, 2022

Kontrimavichusia testiculata sp. nov.
urn:lsid:zoobank.org:act:3CC2D714-6E24-4D8C-8B75-D8AC8D6178AA
Figs 1–2, 5–6, Tables 1–2

Diagnosis

Kontrimavichusia testiculata sp. nov. has morphological characters typical of the genus *Kontrimavichusia*, namely rhynchus armed with cricetoid-like hooks, apex of rostellum invaginable and blades of retracted hooks directed anteriorly, suckers armed with minute spines, ventral canals connected by irregularly spaced transverse anastomoses, copulatory part of vagina surrounded by circular musculature and covered externally by dense layer of intensely-stained cells, labyrinthine uterus extending beyond osmoregulatory canals into both lateral fields, situated dorsally to osmoregulatory canals and genital ducts and embryophore without polar filaments (Makarikov & Binkienė 2022).

Etymology

This specific epithet refers to the very distinctive morphological feature of the species, namely its relatively great number of testes.

Type material

Holotype

RUSSIA • Republic of Adygeya, Maykopsky District, suburbs of Nikel; 44º10′31″ N, 40º09′24″ E; 7 Jul. 2014; A.A. Makarikov leg.; ISEA AM14-134#1 (ex 253).

Paratypes

RUSSIA • 1 spec.; same data as for holotype; ISEA AM14-134#2 (ex 254) • 1 spec.; same data as for holotype; ISEA AM14-142#1 (ex 293) • 1 spec.; same data as for holotype; ISEA AM14-142#2 (ex 294) • 1 spec.; same data as for holotype; ISEA AM14-147#1 (ex 250) • 1 spec.; same data as for holotype; ISEA AM14-147#2 (ex 251) • 1 spec.; same data as for holotype; 4 Jul. 2014; ISEA AM14-72#1 • 1 spec.; same data as for holotype; ISEA AM14-72#2 (ex 325) • 1 spec.; same data as for holotype; ISEA AM14-72#3 (ex 326) • 1 spec.; same data as for holotype; 6 Jul. 2014; ISEA AM14-110#1 (ex 328) • 1 spec.; same data as for holotype; ISEA AM14-127#1 • 1 spec.; same data as for holotype; 10 Jul. 2014; ISEA AM14-178#3 (ex 261) • 1 spec.; same data as for holotype; 13 Jul. 2014; ISEA AM14-217#1.

Other material examined

RUSSIA • 1 spec.; Karachay-Cherkess Republic, suburbs of Teberda; 43º27′21″ N, 41º47′44″ E; 11 Sep. 2016; A.A. Makarikov leg.; ISEA AM16-403#1 (ex 341) • 1 spec.; same data as for preceding; ISEA AM16-403#2 (ex 342) • 1 spec.; same data as for preceding; ISEA AM16-403#3 (ex 343).

Type host

Microtus majori (Thomas, 1906) (Rodentia: Cricetidae: Arvicoline).

Description (Figs 1–2)

[Based on 8 stained and mounted specimens and 9 scolices mounted in Berlese's medium.] Fully developed strobila 72–128 (101; n = 6) mm long, with maximum width 2.6–3.4 (3.0; n = 6) mm at level gravid proglottides. Strobila flat, consisting of 410–530 craspedote proglottides. Scolex slightly compressed dorso-ventrally, 324–412 wide (374; n = 3), not clearly distinct from neck. Suckers small, thick-walled, rounded or oval, cup-shaped, 160–181 × 120–170 (171 × 144; n = 12), usually reaching lateral margins of scolex, armed with minute (less than 1 long) spines; spines covering entire sucker surface (Fig. 1A–B). Rostellar pouch 160–195 × 115–142 (175 × 128; n = 3), with muscular walls, its bottom not reaching level of posterior margin of suckers. Rostellum 115–153 × 50–88 (134 × 67; n = 3), sac-like, muscular, apex invaginable; when rostellar apparatus retracted, rostellar hooks with blades directed anteriorly (Fig. 1B). Rhynchus 80–102 long and 55–78 wide, with well-developed circular musculature, armed with single crown of 20–28 (n = 8) rostellar hooks of cricetoid-like type (Fig. 1C). Rostellar hooks with relatively short handle and straight blade; axis of blade situated to axis of guard at acute angle; guard narrow in anterior surface; handle and blade slightly shorter or equal in length with guard. Hook measurements: total length 17.5–22 (19.7; n = 28), handle 6.3–9 (7.6; n = 28), blade 6.3–9 (7.6; n = 28) and guard 8–10.5 (9.4; n = 28). Neck 300–360 wide (n = 3), approximately equal in width with scolex (Fig. 1A–B).

Ventral osmoregulatory canals 75–155 (110; n = 35) wide, connected by irregularly spaced transverse anastomoses (present in up to 54% proglottides) (Fig. 1D–E). Dorsal osmoregulatory canals very thin, 6–13 (9; n = 35) wide at level of hermaphroditic proglottides, usually situated directly dorsal (not shifted left or right) to ventral canals. Genital pores unilateral, dextral (Fig. 1D–E). Genital ducts pass dorsally to both ventral and dorsal longitudinal osmoregulatory canals. Development of proglottides gradual, protandrous.

Mature proglottides 210–280 × 1400–1780 (247 × 1612; n = 24), transversely elongate, trapezoid (Fig. 1D–E). Testes relatively large, almost equal in size, 145–210 × 125–175 (175 × 149; n = 40), round or oval, 4-6 in number (usually 5, 68.8%; n = 207), poral testes 1–3 (usually 2, 54.6% or 1, 44.9%; n = 207) separated from 2–5 (usually 3, 52.2% or 4, 34.3%; n = 207) antiporal testes by female gonads. Poral testes situated posteriorly, 2–3 antiporal testes testes most often situated posteriorly and 1–2 anteriorly. Cirrus-sac relatively short, with thick muscular walls, club-shaped, 370–445 × 55–77 (401 × 67; n = 31). Antiporal part of cirrus-sac substantially crossing poral ventral longitudinal canal (Fig. 1E–F). Genital atrium simple, cup-shaped, opens laterally, approximately in middle of lateral proglottis margin. Cirrus large, 152–200 × 30–42 (175 × 36; n = 28), cylindrical, armed with very small (up to 1.0–1.5 long), needle-shaped spines (Fig. 2A). Internal seminal vesicle with circular musculature, ovoid, 175–240 × 50–72 (212 × 61; n = 28), occupying half of cirrus-sac length (Fig. 1E–F). External seminal vesicle, 120–280 × 70–135 (185 × 101; n = 25), round or oval, clearly distinguishable from vas deferens, normally smaller than seminal receptacle.

Ovary 380–530 (455; n = 30) wide, median, transversely elongate, fan-shaped, irregularly lobed, ventral to male genital organs, occupying less than half of median field, overlapping testes (Fig. 1E). Vitellarium 85–115 × 180–275 (100 × 215; n = 30), postovarian, slightly shifted to lateral side of proglottis, slightly lobed. Vagina tubular, clearly distinct from seminal receptacle; ventral to cirrus-sac. Copulatory part of vagina 120–165 × 10–35 (145 × 18; n = 25), shorter than cirrus-sac, thick-walled, surrounded by circular musculature and covered externally by dense layer of intensely stained cells; proximal part of vagina infundibular (Figs 1F, 2A). Conductive part of vagina indistinct. Seminal receptacle relatively large, transversely elongate, 375–550 × 80–110 (488 × 92; n = 20).

Uterus appears as perforated, transversely-elongate band, situated dorsally to testes, genital ducts and osmoregulatory canals and extending laterally beyond longitudinal osmoregulatory canals. With

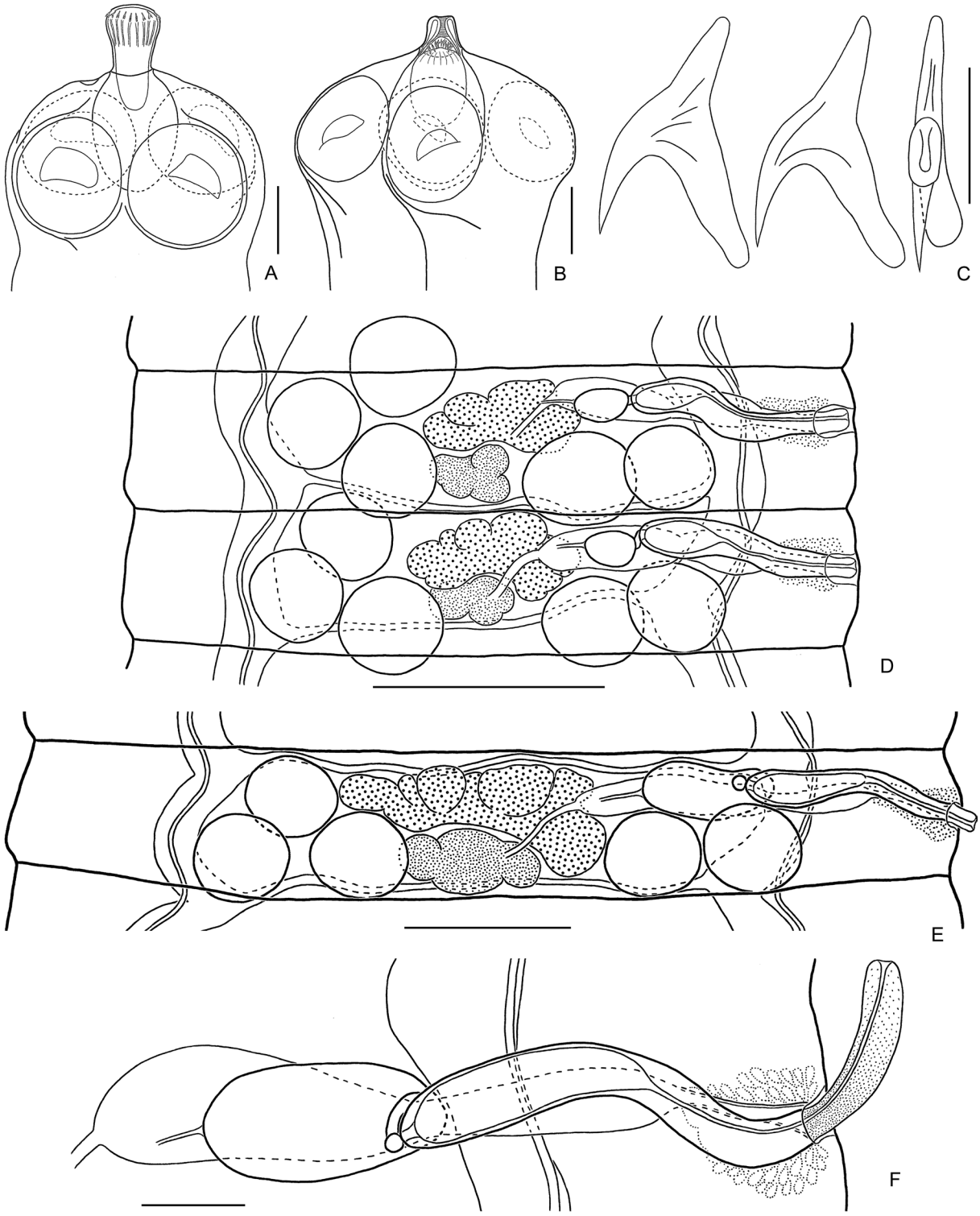

Fig. 1. *Kontrimavichusia testiculata* sp. nov. **A**. Holotype (ISEA AM14-134#1), scolex, dorso-ventral view. **B**. Paratype (ISEA AM14-147#2), scolex, sub-lateral view. **C**. Paratype (ISEA AM14-142#2), rostellar hooks in profile and frontal view (note narrow hook guard). **D**. Holotype, male mature proglottides, dorsal view. **E**. Holotype, hermaphroditic mature proglottis, dorsal view. **F**. Paratype (ISEA AM14-147#2), genital ducts, dorsal view. Scale bars: A–B, F = 100 μm; C = 10 μm; D–E = 300 μm.

proglottis development, uterus forms numerous diverticula on ventral side and becomes labyrinthine in terminal postmature proglottides. Testes persist in postmature proglottides; cirrus-sac and vagina persist in gravid proglottides (Fig. 2B). Gravid proglottides transversely elongate, 410–550 × 2650–3450 (504 × 2042; n = 20). Fully developed uterus labyrinthine, occupying entire median field, extending bilaterally, dorsally, beyond longitudinal osmoregulatory canals (Fig. 2C). Uterus contains numerous (up to 1800–2500) small eggs. Eggs 55–61 × 63–70, subspherical, with very thin outer coat (up to 0.5–0.8 thick); oncospheres 22–26 × 30–33 (Fig. 2D). Embryophores very thin, 27–32 × 35–38, without polar

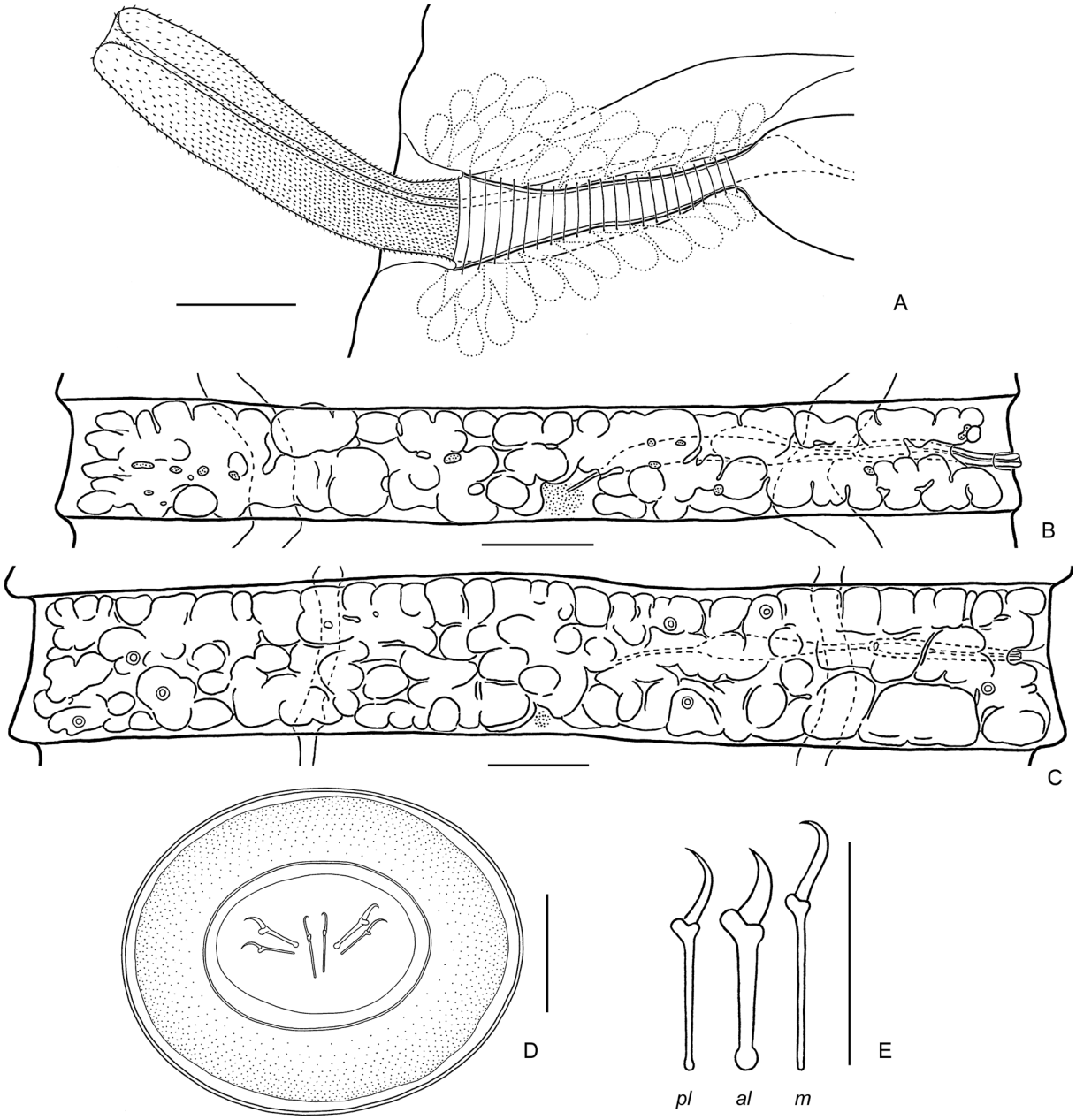

Fig. 2. *Kontrimavichusia testiculata* sp. nov. **A**. Paratype (ISEAAM14-147#2), cirrus and vagina, ventral view. **B**. Holotype (ISEAAM14-134#1), pregravid proglottis, showing appearance of uterine diverticula, dorsal view. **C**. Holotype, gravid proglottis, showing labyrinthine uterus, dorsal view. **D**. Holotype, egg. **E**. Holotype, embryonic hooks. Abbreviations: al = anterolateral; m = median; pl = postero-lateral. Scale bars: A = 50 μm; B–C = 300 μm; D = 20 μm; E = 10 μm.

filaments. Embryonic hooks small, antero-lateral hooks (10.3–10.6), much more robust than slender postero-lateral (10.0–10.5) and median (11.0–11.5) hooks (Fig. 2E).

Distribution

Russia (Republic of Adygeya, Karachay-Cherkess Republic).

Remarks

Until the present study, *K. asymmetrica* was the only known representative of the genus *Kontrimavichusia*. *Kontrimavichusia testiculata* sp. nov. is readily distinguishable from the type species by the number of testes; the former species has 4–6 testes per proglottis while in *K. asymmetrica* those are usually 3. The cirrus-sac in *K. testiculata* substantially crosses the poral osmoregulatory canals, whereas the cirrus-sac of *K. asymmetrica* overlaps or rarely crosses the ventral longitudinal canal. Further, the distal end of the rostellar pouch does not attain the level of the posterior margins of suckers; in *K. asymmetrica* the rostellar pouch reaches to or slightly extends beyond the level of the posterior margins of the suckers. In addition, specimens of *K. testiculata* are characterized by larger suckers and a longer cirrus and cirrus-sac relative to *K. asymmetrica* (Table 1).

Kontrimavichusia hobergi sp. nov.
urn:lsid:zoobank.org:act:8EBE9219-C6BA-4B85-8740-3A3E05F51581
Figs 3–6, Tables 1–2

Diagnosis

Kontrimavichusia hobergi sp. nov. has morphological characters typical of the genus *Kontrimavichusia*, namely rhynchus armed with cricetoid-like hooks, apex of rostellum invaginable and blades of retracted hooks directed anteriorly, suckers armed with minute spines, ventral canals connected by irregularly spaced transverse anastomoses, copulatory part of vagina surrounded by circular musculature and covered externally by dense layer of intensely-stained cells, labyrinthine uterus extending beyond osmoregulatory canals into both lateral fields, situated dorsally to osmoregulatory canals and genital ducts and embryophore without polar filaments (Makarikov & Binkienė 2022).

Etymology

This species has been named in honour of the outstanding parasitologist Dr Eric P. Hoberg in recognition of his seminal and critical studies of parasites of vertebrates, helminth systematics, biogeography, ecology, phylogeny and evolution. It is also recognition of his significant contribution to the advancement of parasitology and the preservation of important archival materials on helminths during his tenure as chief curator of the U.S. National Parasite Collection from 1990 to 2014.

Type material

Holotype

RUSSIA • Republic of North Ossetia-Alania, Alagirsky District, suburbs of Verkhniy Tsey; 42°48′02″ N, 43°56′03″ E; 27 Jul. 2017; A.A. Makarikov leg.; ISEA AM17-236#3 (ex 379).

Paratypes

RUSSIA • 1 spec; same data as for holotype; ISEA AM17-236#1 • 1 spec; same data as for holotype; ISEA AM17-236#2 (ex 378) • 1 spec; same data as for holotype; ISEA AM17-240#2 • 1 spec; same data as for holotype; ISEA AM17-242 (ex 382) • 1 spec; same data as for holotype; ISEA AM17-243#1 (ex 377) • 1 spec; same data as for holotype; ISEA AM17-243#2 • 1 spec; same data as for holotype; SEA AM17-243#3 (ex 376) • 1 spec.; same data as for preceding; 25 Jul. 2017; ISEA AM17-206 (ex 477)

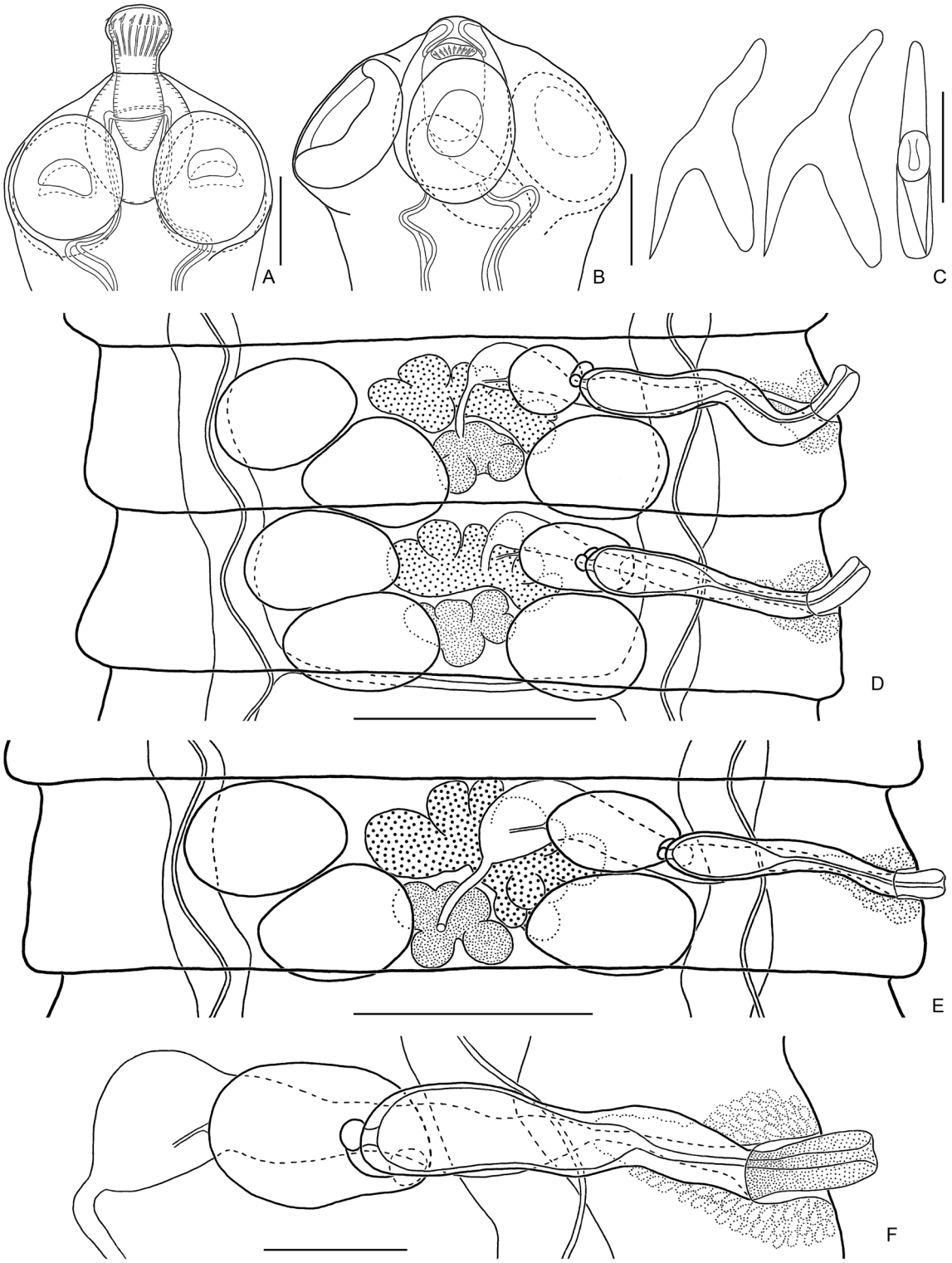

Fig. 3. *Kontrimavichusia hobergi* sp. nov. **A**. Paratype (ISEA AM17-242), scolex, dorso-ventral view. **B**. Paratype (ISEA AM1 17-243#3), scolex, sub-lateral view. **C**. Holotype (ISEA AM17-236#3) (left) and paratype (ISEA AM 17-236#2) (centre, right), rostellar hooks in profile and frontal view (note narrow hook guard). **D**. Holotype, male mature proglottides, dorsal view. **E**. Holotype, hermaphroditic mature proglottis, dorsal view. **F**. Holotype, genital ducts, dorsal view. Scale bars: A–B, F = 100 µm; C = 10 µm; D–E = 300 µm.

• 1 spec.; same data as for preceding; ISEA AM17-211 (ex 474) • 1 spec.; same data as for preceding; ISEA AM17-213 • 1 spec.; same data as for preceding; 26 Jul. 2017; ISEA AM17-222#1 (ex 368) • 1 spec.; same data as for preceding; ISEA AM17-222#2 (ex 369) • 1 spec.; same data as for preceding; ISEA AM17-226#1 (ex 482).

Other material examined

RUSSIA • 1 spec.; same data as for holotype; 26 Jul. 2017; ISEA AM17-221#3 (ex455) • 1 spec.; same data as for preceding; ISEA AM17-221#4 (ex456).

Type host

Microtus daghestanicus (Shidlovsky, 1919) (Rodentia: Cricetidae: Arvicoline).

Description (Figs 3–4)

[Based on 11 stained and mounted specimens and 7 scoleces mounted in Berlese's medium.] Fully developed strobila 65–78 (74; n = 7) mm long, with maximum width 1.7–2.1 (1.8; n = 5) mm at level gravid proglottides. Strobila flat, consisting of 400–480 craspedote proglottides. Scolex slightly compressed dorso-ventrally, 245–360 wide (301; n = 3), not clearly distinct from neck. Suckers small, thick-walled, rounded or oval, cup-shaped, 120–155 × 100–120 (138 × 109; n = 12), usually reaching lateral margins of scolex, armed with minute (less than 1 long) spines; spines covering entire sucker surface (Fig. 3A–B). Rostellar pouch 130–164 × 95–125 (148 × 112; n = 3), with muscular walls, its bottom not reaching level of posterior margin of suckers. Rostellum 105–161 × 50–70 (130 × 61; n = 3), sac-like, muscular, apex invaginable; when rostellar apparatus retracted, rostellar hooks with blades directed anteriorly (Fig. 3B). Rhynchus 60–73 long and 50–75 wide, with well-developed circular musculature, armed with single crown of 18–22 (n = 7) rostellar hooks of cricetoid-like type (Fig. 3C). Rostellar hooks with relatively short handle and straight blade; axis of blade situated to axis of guard at acute angle; guard narrow in anterior surface; handle and blade slightly shorter or equal in length with guard. Hook measurements: total length 20–24 (21.8; n = 39), handle 8–9.5 (8.7; n = 39), blade 7.8–9.4 (8.6; n = 39) and guard 8–10.6 (9; n = 39). Neck 200–240 wide (n = 3), approximately equal in width with scolex (Fig. 3A–B).

Ventral osmoregulatory canals 40–110 (79; n = 36) wide, connected by irregularly spaced transverse anastomoses (present in up to 23% proglottides) (Fig. 3D). Dorsal osmoregulatory canals very thin, 3–8 (5; n = 36) wide at level of hermaphroditic proglottides, usually situated directly dorsal (not shifted left or right) to ventral canals. Genital pores unilateral, dextral (Fig. 3D–E). Genital ducts pass dorsally to both ventral and dorsal longitudinal osmoregulatory canals. Development of proglottides gradual, protandrous.

Mature proglottides 180–240 × 970–1140 (210 × 1085; n = 29), transversely elongate, trapezoid (Fig. 3D–E). Testes 3, relatively large, almost equal in size, 135–210 × 95–150 (169 × 119; n = 36), round or oval, most often situated in triangle with flat angle (anterior antiporal testis shifted to lateral side of proglottis in relation to posterior antiporal testis) or, rarely, in triangle with right angle or in one row, poral testis separated from two antiporal testes by female gonads. Number and distribuition of testes constant, no variation in testes number observed, proglottis with three antiporal testes or two poral testes appear infrequent. Cirrus-sac relatively short, with thick muscular walls, club-shaped, 270–320 × 56–75 (296 × 66; n = 28). Antiporal part of cirrus-sac substantially crossing poral ventral longitudinal canal (Fig. 3E–F). Genital atrium simple, cup-shaped, opens laterally, approximately in middle of lateral proglottis margin. Cirrus large, 100–128 × 24–39 (112 × 31; n = 25), cylindrical, armed with very small (up to 1.0–1.5 long), needle-shaped spines (Fig. 4A). Internal seminal vesicle with circular musculature, ovoid, 130–180 × 52–70 (155 × 58; n = 28), occupying half of cirrus-sac length (Fig. 3E–F). External

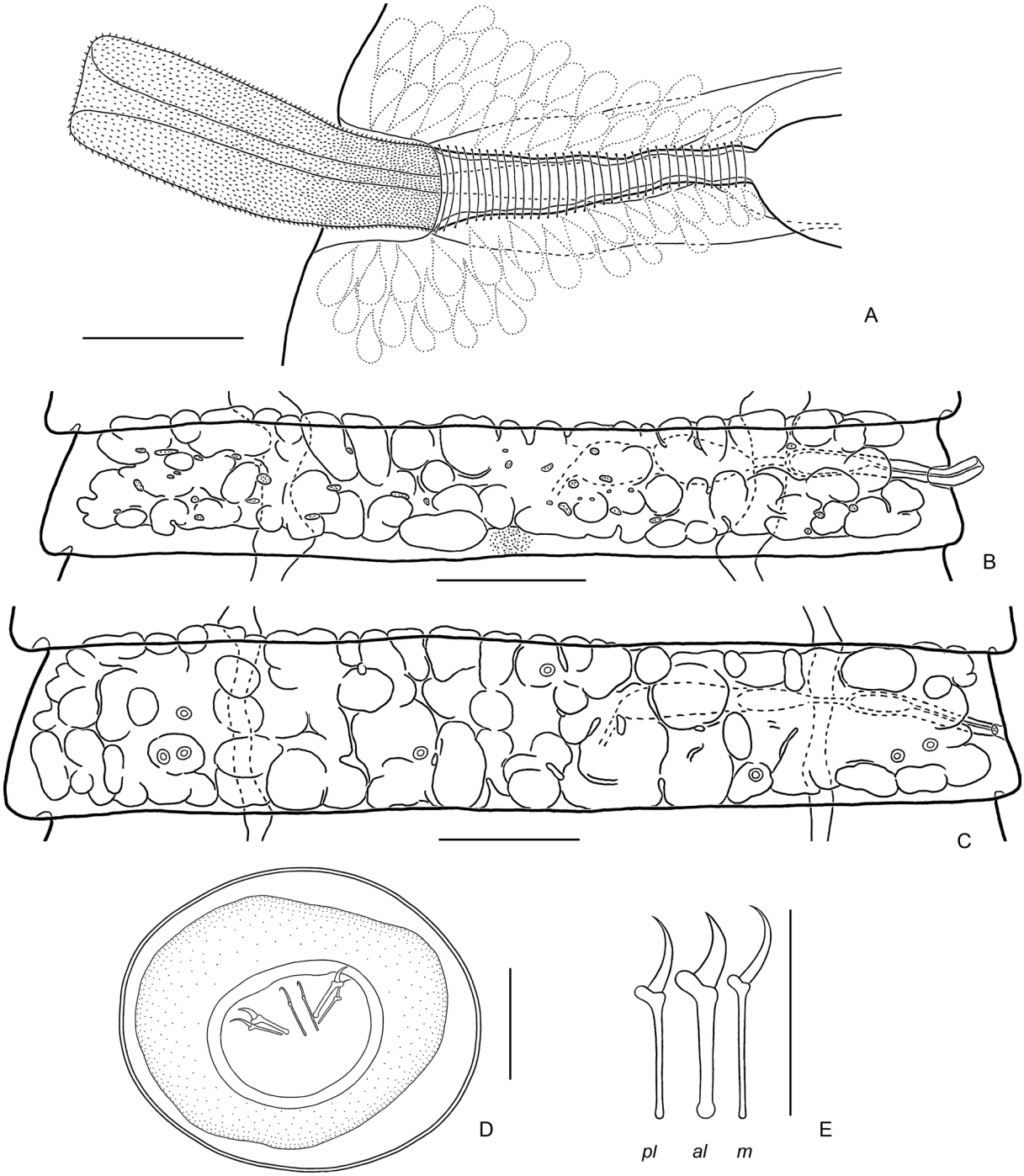

Fig. 4. *Kontrimavichusia hobergi* sp. nov. **A**. Paratype (ISEA AM17-236#1), cirrus and vagina, ventral view. **B**. Holotype (ISEA AM17-236#3), pregravid proglottis, showing appearance of uterine diverticula, dorsal view. **C**. Holotype, gravid proglottis, showing labyrinthine uterus, dorsal view. **D**. Holotype, egg. **E**. Holotype, embryonic hooks. Abbreviations: al = anterolateral; m = median; pl = postero-lateral. Scale bars: A = 50 µm; B–C = 300 µm; D = 20 µm; E = 10 µm.

seminal vesicle, 105–190 × 82–120 (127 × 93; n = 28), round or oval, clearly distinguishable from vas deferens, normally smaller than seminal receptacle.

Ovary 280–396 (330; n = 25) wide, median, transversely elongate, fan-shaped, irregularly lobed, ventral to male genital organs, occupying less than half of median field, overlapping testes (Fig. 3E). Vitellarium 70–116 × 120–185 (85 × 145; n = 25), postovarian, slightly shifted to lateral side of proglottis, slightly

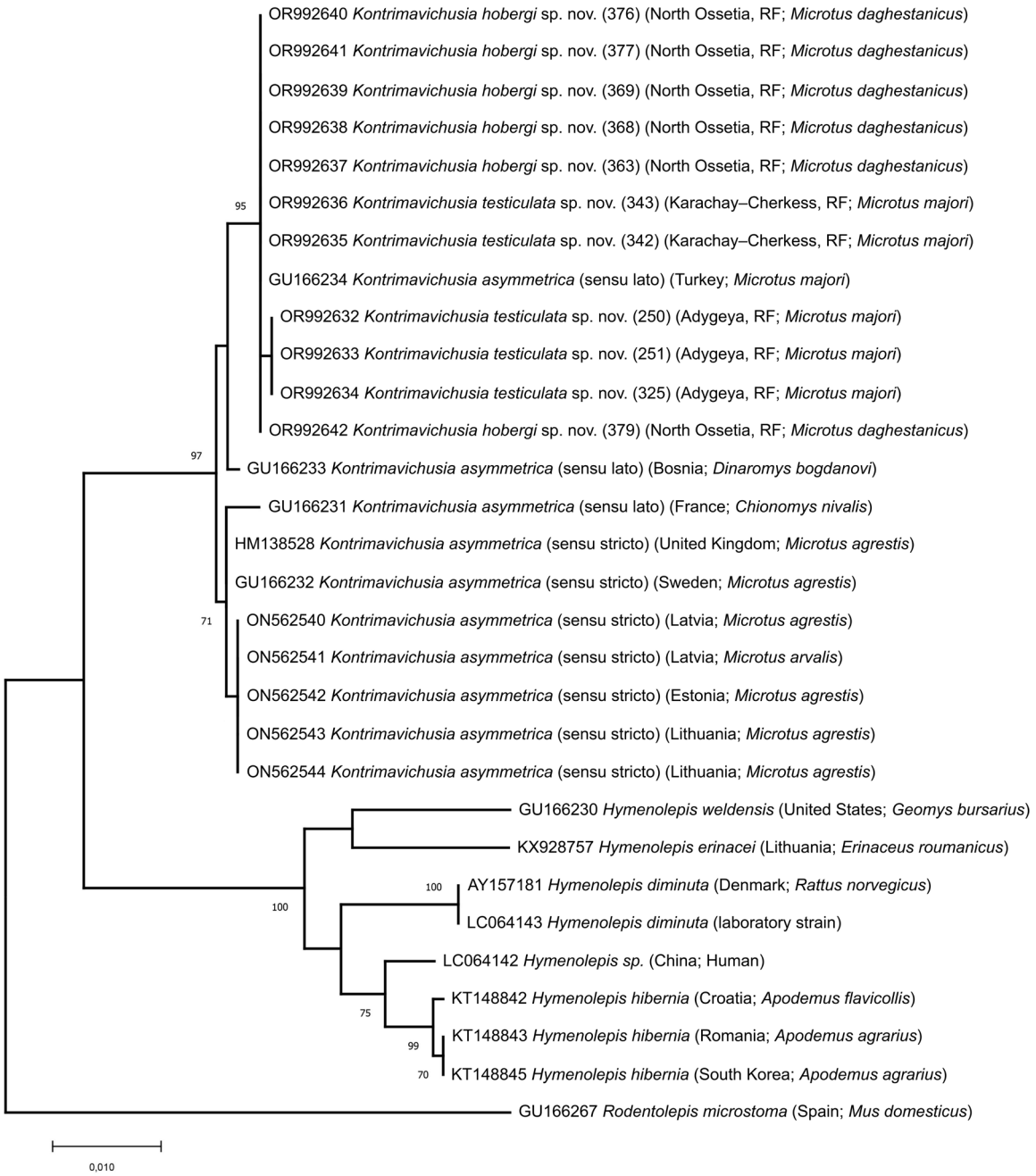

Fig. 5. Maximum likelihood phylogenetic tree of *Kontrimavichusia* Makarikov & Binkienė, 2022 and *Hymenolepis* Weinland, 1858 based on analysis of partial sequences of the 28S rRNA gene. Bootstrap support given for maximum likelihood analysis based on 1000 replicates. Bootstrap support values lower than 70% are not shown.

lobed. Vagina tubular, clearly distinct from seminal receptacle; ventral to cirrus-sac. Copulatory part of vagina 94–120 × 8–32 (105 × 16; n = 25), shorter than cirrus-sac, thick-walled, surrounded by circular musculature and covered externally by dense layer of intensely stained cells; proximal part of vagina infundibular (Figs 3F, 4A). Conductive part of vagina indistinct. Seminal receptacle relatively large, transversely elongate, 315–470 × 55–100 (387 × 75; n = 25).

Uterus appears as perforated, transversely-elongate band, situated dorsally to testes, genital ducts and osmoregulatory canals and extending laterally beyond longitudinal osmoregulatory canals. With proglottis development, uterus forms numerous diverticula on ventral side and becomes labyrinthine in terminal postmature proglottides. Testes persist in postmature proglottides; cirrus-sac and vagina persist in gravid proglottides (Fig. 4B). Gravid proglottides transversely elongate, 260–390 × 1690–2040 (325 × 1856; n = 20). Fully developed uterus labyrinthine, occupying entire median field, extending bilaterally, dorsally, beyond longitudinal osmoregulatory canals (Fig. 4C). Uterus contains numerous (up to 1500–1600) small eggs. Eggs 52–57 × 60–66, subspherical, with very thin outer coat (up to 0.7–1 thick); oncospheres 19–23 × 26–31 (Fig 4D). Embryophores very thin, 23–28 × 33–38, without

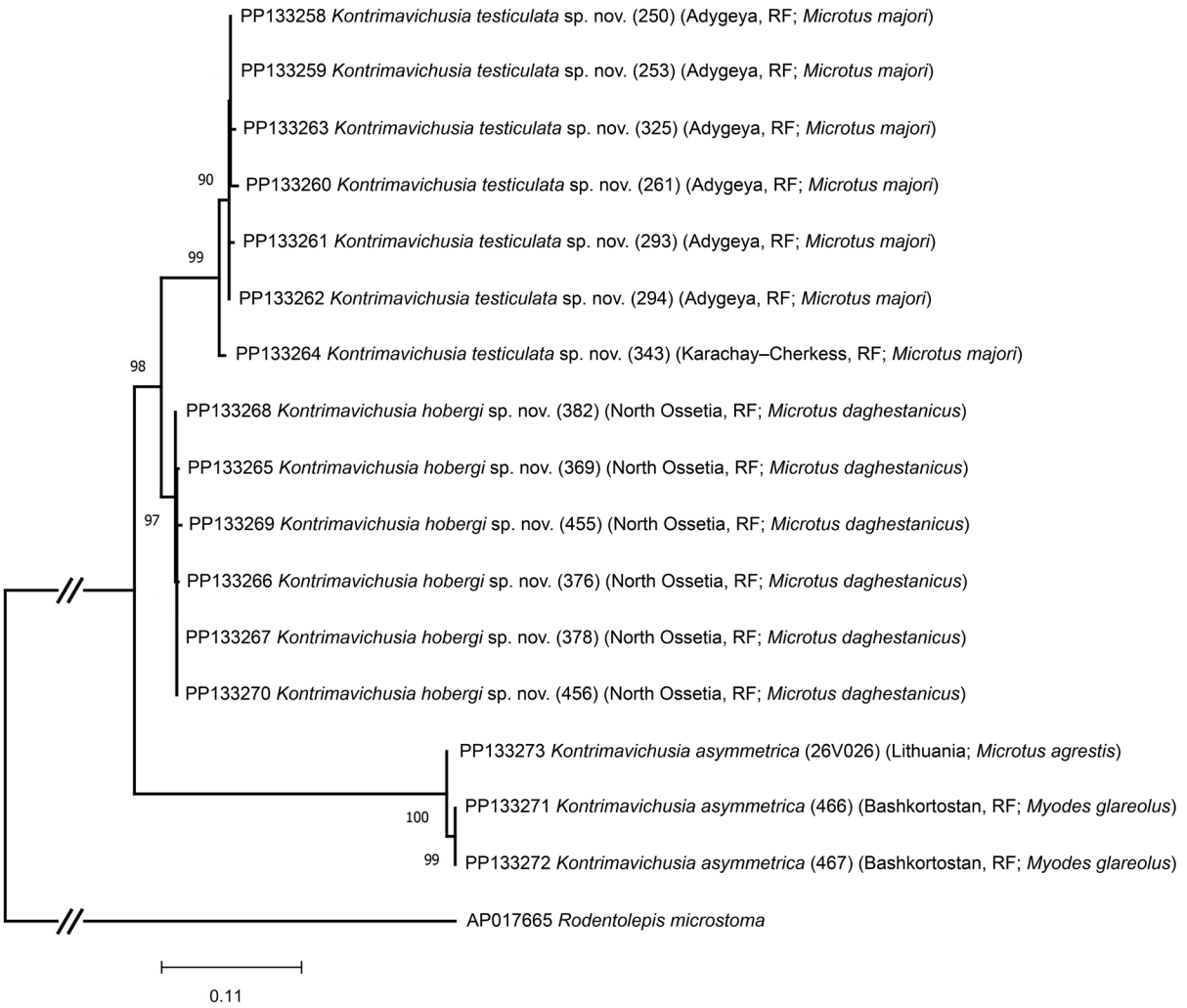

Fig. 6. Maximum likelihood phylogenetic tree of species of *Kontrimavichusia* Makarikov & Binkienė, 2022 based on analysis of partial sequences of the nad1 gen. Bootstrap support given for maximum likelihood analysis based on 1000 replicates. Bootstrap support values lower than 70% are not shown.

polar filaments. Embryonic hooks small, antero-lateral hooks (9.8–10.7), much more robust than slender postero-lateral (9.8–10.5) and median (10.3–11) hooks (Fig. 4E).

Distribution

Russia (Republic of North Ossetia–Alania).

Remarks

Kontrimavichusia hobergi sp. nov. is readily distinguishable from *K. asymmetrica* by the triangular arrangement of the testes; those in the type species most often are situated in one row. The number of testes distinguishes the new species from *K. testiculata* sp. nov.: the former has three 3 per proglottis, the latter has 4–6 per proglottis. The cirrus-sac in *K. hobergi* substantially crosses the poral osmoregulatory canals; in contrast, the cirrus-sac of *K. asymmetrica* overlaps or rarely crosses the ventral longitudinal canal. The distal end of the rostellar pouch does not attain the level of the posterior margins of the suckers in *K. hobergi*, directly contrasting with the condition in *K. asymmetrica*. In addition, specimens of *K. hobergi* differ from *K. asymmetrica* and *K. testiculata* by distinctly smaller dimensions of the fully developed strobila. Further, the cirrus-sac is larger than in *K. asymmetrica* but smaller than in *K. testiculata*; the cirrus of the new species is smaller than in *K. testiculata*. The ovary of *K. hobergi* is narrower than in both congeners (Table 1).

Molecular phylogenetic analysis

Phylogeny was based on the nuclear ribosomal 28S rRNA gene. The length of the alignment after trimming was 1042 nucleotides. Consistent with a previous study, the monophyly of each of the two clades corresponding to *Kontrimavichusia* and *Hymenolepis* is well supported (0.97 and 1.0 posterior probability, respectively) (Makarikov & Binkienė 2022). Further, we demonstrated at least four independent phylogenetic lineages within the *Kontrimavichusia* clade including the two species from the North Caucasus described herein, with differences between all available sequences up to 1–11 bp (Fig. 5). Current analyses strongly support the generic allocation of those species to the genus. One of those lineages is represented by specimens of *K. asymmetrica* (sensu stricto) from Europe from *Microtus agrestis* (Linnaeus, 1761) (GenBank: GU166232, HM138528, ON562540 and ON562542–ON562544) and *M. arvalis* (Pallas, 1778) (GenBank: ON562541). The second lineage consists of species from the North Caucasus, namely *K. testiculata* sp. nov. and *K. hobergi* sp. nov. including the sequence from *M. majori* from Turkey (GU166234) attributed to *K. asymmetrica* (sensu lato) by Haukisalmi *et al.* (2010). It distinctly differs from the *K. asymmetrica* lineage by 8–9 bp. However, interspecific differences based on 28S gene in the Caucasian cluster are not pronounced. The two remaining lineages represented by specimens from *Chionomys nivalis* (Martins, 1842) from France (GU166231) and *Dinaromys bogdanovi* (Martino, 1922) from Bosnia (GU166233). Those differ from Caucasian linage by up to 11 bp and 7 bp respectively.

Phylogeny was based on the mitochondrial nad1 gen. The length of the alignment after trimming was 731 nucleotides. Until present, sequences of nad1 gen of *K. asymmetrica* were missing in GenBank. We used for analysis the sequences of *K. asymmetrica* (sensu stricto) from Eastern Europe (Lithuania and the Republic of Bashkortostan, Russia) and two new species from the North Caucasus *K. testiculata* sp. nov. and *K. hobergi* sp. nov. that we obtained. Three well-supported lineages within *Kontrimavichusia* corresponded to morphologically recognized species (Fig. 6). Unlike the 28S rRNA gene sequences, mitochondrial gene nad1 provided strong evidence for the description of *K. testiculata* and *K. hobergi*. The interspecific pairwise distances between lineages of *K. testiculata* and *K. hobergi* vary within 4.76–5.81% (33–40 bp). Also, both species distinctly differ from *K. asymmetrica* (sensu stricto) by 13.36–14.35% (84–92 bp) and 14.51–15.32% (91–98 bp) respectively (Table 2).

Table 1. Comparative morphometric data of species of *Kontrimavichusia* Makarikov & Binkienė, 2022 (measurements in micrometres except where otherwise stated).

species	*Kontrimavichusia asymmetrica*	*Kontrimavichusia testiculata* sp. nov.	*Kontrimavichusia hobergi* sp. nov.
source	Makarikov & Binkienė (2022)	present study	present study
host species	*Microtus agrestis* (Linnaeus, 1761), *M. arvalis* (Pallas, 1778)	*Microtus majori* (Thomas, 1906)	*Microtus daghestanicus* (Shidlovsky, 1919)
strobila length (mm)	98–160 mm	72–128 mm	**65–78 mm**
strobila width (mm)	2.5–4.45 mm	2.6–3.4 mm	**1.7–2.1 mm**
scolex width	245–325	324–412	245–360
suckers size	120–144 × 105–125	**160–181 × 120–170**	120–155 × 100–120
rostellar pouch size	168–185 × 83–115	160–195 × 115–142	130–164 × 95–125
rostellum size	113–135 × 42–81	115–153 × 50–88	105–161 × 50–70
rostellar hooks number	18–23	20–28	18–22
rostellar hooks size	20–22.5	17.5–22	20–24
testes size	155–215 × 110–168	145–210 × 125–175	135–210 × 95–150
cirrus-sac size	225–270 × 48–66	**370–445 × 55–77**	**270–320 × 56–75**
cirrus size	110–166 × 25–36	**152–200 × 30–42**	100–128 × 24–39
cirrus spines, presence	armed	armed	armed
external seminal vesicle size	75–152 × 70–105	120–280 × 70–135	105–190 × 82–120
ovary width	410–690	380–530	**280–396**
vitellarium size	70–105 × 192–276	85–115 × 180–275	70–116 × 120–185
seminal receptacle size	240–445 × 80–110	375–550 × 80–110	315–470 × 55–100
egg size	42–50 × 47–54	55–61 × 63–70	52–57 × 60–66
oncosphere size	18–21 × 20–25	22–26 × 30–33	19–23 × 26–31
embryonic hooks size	9.0–10.5	10.0–11.5	9.8–11

Note: the measurements of *Kontrimavichusia testiculata* sp. nov. and *K. hobergi* sp. nov. highlighted in bold show the most remarkable differences between the described and new species.

Among these, the level of intraspecific differences of the lineages based on nad1 gen has the following values: in the lineage of *K. asymmetrica* (sensu stricto) the two specimens from the same locality (Republic of Bashkortostan, Russsia) have no intraspecific variability, while the sequence originated from the relatively remote population (more than 2000 km) in Lithuania differs from these by 0.69% (5 bp) (Table 2); in *K. testiculata* sp. nov. from the Republic of Adygeya the intraspecific differences reached up to 0.97% (7 bp), while the sequences from Karachay-Cherkess Republic showed slightly higher values of intraspecific variability 1.11%–1.81% (8–13 bp); all sequences of *K. hobergi* sp. nov. originating from the same locality showed up to 0.14%–0.41% (1–3 bp) intraspecific variability.

Discussion

The number of testes in the family Hymenolepididae is traditionally considered as a generic level character (Skrjabin & Matevosyan 1945; Mas-Coma & Galan-Puchades 1991; Czaplinski & Vaucher 1994). In the following genera of hymenolepidids from rodents the presence of numerous testes (more than three per proglottis) was used to discriminate among genera: *Chitinolepis* Baylis, 1926; *Hymenandrya* Smith, 1954; *Paraoligorchis* Wason & Johnson, 1977; *Pseudandrya* Fuhrmann, 1943; *Pseudanoplocephala* Baylis, 1927 and *Sudarikovina* Spassky, 1951 (Czaplinski & Vaucher 1994; Gulyaev & Chechulin 1996).

Table 2. Pairwise uncorrected genetic distances (below diagonal) and total nucleotide differences (above diagonal) in sequences of nad1 gen between specimens of *Kontrimavichusia* Makarikov & Binkienė, 2022.

	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**	**15.**	**16.**
1. PP133258 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	–	0	4	3	1	2	9	37	37	36	35	38	35	90	90	87
2. PP133259 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	0.0000	–	4	3	1	2	9	37	37	36	35	38	35	90	90	87
3. PP133260 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	0.0055	0.0055	–	7	5	6	13	38	39	38	37	40	38	87	87	84
4. PP133261 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	0.0041	0.0041	0.0097	–	2	5	10	36	36	35	34	37	35	91	91	87
5. PP133262 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	0.0014	0.0014	0.0069	0.0027	–	3	8	36	36	35	34	37	35	89	89	86
6. PP133263 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Adygeya	0.0027	0.0027	0.0083	0.0069	0.0041	–	9	37	37	36	35	37	36	92	92	89
7. PP133264 *K. testiculata* sp. nov. ex *Microtus majori* (Thomas, 1906), Karachay-Cherkess	0.0124	0.0124	0.0181	0.0138	0.0111	0.0125	–	35	35	34	33	35	34	92	92	89
8. PP133265 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0536	0.0536	0.0550	0.0520	0.0520	0.0536	0.0506	–	2	1	2	3	1	96	96	91
9. PP133266 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0536	0.0536	0.0565	0.0520	0.0520	0.0536	0.0506	0.0028	–	1	2	3	1	97	97	92
10. PP133267 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0519	0.0519	0.0549	0.0504	0.0505	0.0520	0.0490	0.0014	0.0014	–	1	2	0	96	96	91
11. PP133268 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0505	0.0505	0.0535	0.0490	0.0490	0.0506	0.0476	0.0028	0.0028	0.0014	–	3	1	96	96	91
12. PP133269 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0551	0.0551	0.0581	0.0536	0.0536	0.0551	0.0506	0.0041	0.0041	0.0027	0.0041	–	2	98	98	93
13. PP133270 *K. hobergi* sp. nov. ex *Microtus daghestanicus* (Shidlovsky, 1919), North Ossetia	0.0519	0.0519	0.0549	0.0504	0.0505	0.0520	0.0490	0.0014	0.0014	0.0000	0.0014	0.0027	–	96	96	91
14. PP133271 *K.* asymmetrica ex *Microtus glareolus* (Shidlovsky, 1919), Bashkortostan	0.1397	0.1397	0.1340	0.1415	0.1379	0.1433	0.1435	0.1495	0.1513	0.1492	0.1491	0.1532	0.1492	–	0	5
15. PP133272 *K.* asymmetrica ex *Microtus glareolus* (Shidlovsky, 1919), Bashkortostan	0.1397	0.1397	0.1340	0.1415	0.1379	0.1433	0.1435	0.1495	0.1513	0.1492	0.1491	0.1532	0.1492	0.0000	–	5
16. PP133273 *K* .asymmetrica ex *Microtus agrestis* (Linnaeus, 1761), Lithuania	0.1392	0.1392	0.1336	0.1411	0.1374	0.1428	0.1430	0.1454	0.1473	0.1452	0.1451	0.1492	0.1452	0.0069	0.0069	–

Although specimens of *K. testiculata* sp. nov., unlike other representatives of the genus, have more than three testes per proglottis there is no evidence to separate this species in a distinct genus; all morphological characters are typical for *Kontrimavichusia*. Molecular analyses also clearly support the placement of *K. testiculata* in this genus. Similarly, it was discovered that the number of testes apparently does not have generic significance for other hymenolepidids of the genus *Arostrilepis* Mas-Coma & Tenora, 1997. Currently the genus includes 16 nominal species all having three testes per proglottis (e.g., Makarikov *et al.* 2013, 2020). However, this genus had been shown to be paraphyletic with respect to *Hymenandrya thomomyis* Smith, 1954 having 7–15 testes per proglottis (Haas *et al.* 2020; Galbreath *et al.* 2023). Also, recently it was shown that *Pseudanoplocephala crawfordi* Baylis, 1927, with numerous testes, is clustered in a subclade of *Hymenolepis* (all cestodes characterized by three testes), based on molecular phylogenetic data (Jia *et al.* 2014).

Among this assemblage *Arostrilepis*-*Hymenandrya*, *Hymenolepis*-*Pseudanoplocephala* and *Kontrimavichusia*, it was shown that the number of testes does not appear to be a generic feature. The present data do not support revision of supraspecific taxa based on the number of testes. In any case, a detailed study of the phylogenetic relationships of these hymenolepidids should be carried out, since it may be quite obvious that morphological characters, including the number of testes, may have different taxonomic significance in different groups of cestodes. It seems more likely that a secondary increase in the number of testes occurred in different groups of hymenolepidids of small mammals independently and at different times. This issue needs further study.

The shape of the spines or microtriches and their pattern of distribution on suckers are usually used as distinctive characters for differentiation among species and genera (Mas-Coma & Galan-Puchades 1991; Czaplinski & Vaucher 1994). However, no visible differences among *K. asymmetrica* and the two new species from the North Caucasus were detected by light microscopy.

Of interest are the two phylogenetic lineages based on the 28S gene published in GenBank and attributed to *K. asymmetrica* (Haukisalmi *et al.* 2010). Those sequences significantly differ both from the type species and from *K. testiculata* sp. nov. and *K. hobergi* sp. nov. These are the following: cestode specimens collected from *C. nivalis* from France (GU166231) and from *D. bogdanovi* from Bosnia (GU166233). Also, specimens collected from *M. majori* from Turkey (GU166234) distinctly differ from *K. asymmetrica* but do not show interspecific differences from *K. testiculata* or *K. hobergi* in this region of DNA. It is possible that these specimens of *K. asymmetrica* (sensu lato) may be conspecific to one of the described species from the Caucasus (i.e., GU166234), or represent yet undiscovered species (i.e., GU166231, GU166233 and GU166234). Unfortunately, there are no morphological vouchers for these sequences (Haukisalmi *et al.* 2010; Makarikov & Binkienė 2022); their status requires further study. The present phylogenetic analysis based on the 28S gene has shown that interspecific distances within the genus *Kontrimavichusia* can reach up to 11 base pairs. However, this gene has apparent limitations for differentiating among species, as the two new species from the North Caucasus, which clearly differ in morphological features, are indistinguishable in this region of DNA. For a reliable differentiation between species of these hymenolepidids it is necessary to use more variable genes. Further, the presence of a species complex among specimens attributed to *Hymenolepis hibernia* Montgomery, Montgomery & Dunn, 1986 cannot be excluded. As even based on the 28S gene the 3 sequences deposited in GenBank (KT148842, KT148843 and KT148845) differed up to 5 positions and that exceeds the limits of intraspecific variability in this relatively conserved region of DNA (Tkach *et al.* 2013).

The mitochondrial gene nad1 has been used in different groups of hymenolepidids from small mammals and apparently this marker is a reliable one for distinguishing among species. For instance, the proposed interspecific differences among species of *Staphylocystis* Villot, 1877 based on nad1 gen vary within

33–49 bases (Tkach *et al.* 2013; Greiman *et al.* 2013). Results of pairwise comparisons of the two new species from the North Caucasus showed the close values of interspecific differences in 33–40 bases. While specimens of *K. testiculata* sp. nov. and *K. hobergi* sp. nov. even more distanced from *K. asymmetrica* in 84–92 and 91–98 bases, respectively. At the same time, intraspecific variability in species of *Kontrimavichusia* can reach up to 13 bases and is found in specimens from remote or orographically isolated populations. Thus, molecular analysis of nad1 sequences together with morphological data provides compelling evidence for the description of the two new species from the North Caucasus belonging to the genus *Kontrimavichusia*.

The diversity of relief and climate in different parts of the Greater Caucasus leads to extraordinary diversity and heterogeneity of its nature in general and the animals in particular. These factors are closely related to the fact that the North Caucasus represents one of the important centers of speciation in the Western Palaearctic which includes a large number of endemics. For instance, among small mammals of the Caucasus a significant portion of the diversity is represented by endemics (Sokolov & Tembotov 1989). In this regard, it can be assumed that the fauna of their helminths also has features of uniqueness and includes species endemic to this region. Thus, it was noted that 5 out of 27 species of cestodes from shrews reported in the North Caucasus are endemic to this region (Kornienko *et al.* 2021). Although the fauna of cestodes from rodents from the Northern Caucasus has not yet been sufficiently studied, it has been suggested that at least four currently undescribed putative species of cestodes from the northwestern Caucasus may be endemic to this region (Makarikov *et al.* 2017). One of these species was later described from the fat dormouse *Glis glis* (Linnaeus, 1766) as *Armadolepis longisoma* Makarikov, Stakheev & Tkach, 2018, the second species is described herein as *K. testiculata* sp. nov. (Makarikov *et al.* 2018; present study). An additional potentially endemic species was found in the central Caucasus and described in this paper as *K. hobergi* sp. nov. Thus, the expanding knowledge on the diversity of helminths in small mammals of the Northern Caucasus confirms the presence of a unique cestode fauna in this region which remains to be evaluated in detail.

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