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Two new nematode species of the genus *Paratrilobus* Micoletzky, 1922 (Nematoda, Triplonchida) from the water area of Lake Baikal (Russia)

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Abstract. This paper describes and illustrates two new nematode species of the genus *Paratrilobus* Micoletzky, 1922. The species *Paratrilobus tankhoyensis* sp. nov. was found at the estuary of the Pereyomnaya River (water area of Lake Baikal, near the Tankhoy railway station). *Paratrilobus tankhoyensis* sp. nov. is most similar to *P. expugnator* (Tsalolichin, 1976) in the body size, but differs in the comparatively thin body, shorter and thicker tail, shorter stoma and spicules. Another new species, *Paratrilobus aquaticus* sp. nov., was found in Posolsk Bank (natural underwater elevation of the bottom between the southern and central basins of Lake Baikal). The species is similar to *P. granulatus* Gagarin & Naumova, 2011 and *P. ultimatus* (Tsalolichin, 1977) in the structure of the preloacal supplements. It differs from the former in the absence of crystalloids, a comparatively longer pharynx, longer stoma and outer labial setae as well as the absence of subterminal seta. It differs from the latter in a longer pharynx, stoma and longer outer labial setae as well as a longer and more slender tail. We also discuss diagnostic features of the males of the genus *Paratrilobus*.

Keywords. Free-living nematodes, diversity, morphology, taxonomy.

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Introduction

The genus *Paratrilobus* belongs to the family Tobrilidae Filipjev, 1918 (De Coninck, 1965). According to Hodda (2011), the world's tobrilid fauna includes 162 species in 10 genera. More than 45 species of the Tobrilidae inhabit Lake Baikal (Naumova & Gagarin 2019a, Naumova & Gagarin 2019b).

The Hungarian nematologist Istvan Andrásy (2007) included eight valid species in the genus *Paratrilobus*: *P. brevis* (Tsalolichin, 1976), *P. delicatus* (Shoshin, 1988), *P. expugnator* (Tsalolichin, 1976), *P. grandipapilloides* Micoletzky, 1922, *P. ponticus* Tsalolichin, 1981, *P. rapis* Gagarin, 1991, *P. strenuus* (Gagarin, 1991) and *P. ultimus* (Tsalolichin, 1977). Later, one more species, *P. granulosis* Gagarin & Naumova, 2011, was described from Lake Baikal (Gagarin & Naumova 2011).

This paper aims to describe two new nematode species from the water area of Lake Baikal (*P. tankhoyensis* sp. nov. and *P. aquaticus* sp. nov.) and discuss some features of the genus *Paratrilobus*.

Material and methods

Nematodes were collected from the following two sites: water area of Lake Baikal, estuary of the Pereyomnaya River, near the Tankhoy railway station, (51.568452 N, 105.166531 E), 10 cm depth, collected on 19 July 2018; southern basin of Lake Baikal, Posolsk Bank (underwater elevation of the bottom between the southern and central basins, (52.07994 N, 105.90368 E), 51 m depth, sand and silt, collected on 5 July 2012.

The samples contained numerous free-living nematodes, including the species described herein. Nematodes were fixed by standard methods and mounted in glycerin on permanent slides. All observations were made using Olympus CX-21 and Nikon Eclipse 80i light microscopes with Nomarski DIC accessories. Images were taken using a Nikon DS-Fil digital camera and Intel Pentium Dual CPU E 2200 Processor Series for Desktop with the NIS-Elements D 3.2 program for analysis and documentation of images from the preparations.

Institutional abbreviations

- HM RAS = Helminthological Museum, Center for Parasitology, Institute of Ecology and Evolution, Russian Academy of Science, Moscow, Russia
LIN-SB = Limnological Institute, Siberian Branch, Russian Academy of Sciences, Irkutsk, Russia

Abbreviations

- a* = body length / greatest body diameter
b = body length / distance from anterior end to pharyngo-intestinal junction
c = body length / tail length
c' = tail length / tail diameter at anus or cloaca
diam.c.s. = body diameter at the level of cephalic setae, in μm
gub. = gubernaculum length, in μm
L = body length, in μm
o.l.s. = length of the outer labial setae, in μm
o.l.s.% = length of the outer labial setae as percentage (%) of body diameter at the level of cephalic setae
spic. = spicules length, in μm
stoma = stoma length, in μm
suppl. = number of supplements
V = distance from body anterior end to vulva expressed as percentage (%) of the body length

Results

Phylum Nematoda Cobb, 1932
Class Enoplea Inglis, 1983
Order Triplonchida Cobb, 1920
Family Tobrilidae Filipjev, 1918 (De Coninck, 1965)
Genus *Paratrilobus* Micoletzky, 1922

Type species

Paratrilobus grandipapilloides Micoletzky, 1922.

Diagnosis (after Andrásy 2007)

Body 1.9–5.5 mm long. Amphidial fovea at the stoma level. Buccal cavity spacious, funnel- or barrel-shaped with well-sclerotized walls. Pockets merged with buccal cavity, practically reduced. Teeth small, at base of stoma or just posterior to it. Vagina normal. Supplements six, rarely seven to eight, echinate, large, protractible; first and last supplements usually smaller than others.

Paratrilobus tankhoyensis sp. nov.

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Figs 1–2, Table 1

Diagnosis

Paratrilobus tankhoyensis sp. nov. is characterized by a 3687–5463 µm long body; cuticle smooth under light microscope; crystalloids absent; inner labial sensillae in shape of short and thick setae 6–10 µm long; six outer labial sensillae in shape of smooth non-articulated setae 27–33 µm long, 54–67% of labial region width for males and 44–56% for females; four cephalic sensillae in shape of thin and smooth setae 12–15 µm long; buccal cavity spacious, barrel-shaped. One pocket merged with buccal cavity; two small teeth located in stoma base. Spicules comparatively thin and long (70–76 µm in length), 1.2–1.6 times as long as cloacal body diameter; gubernaculum in shape of ‘gutter’; precloacal supplements 6–7 in number, comparatively large, echinate; ‘cap’ and ‘shoulder’ well developed; supplemental ampulla comparatively large; its contents located in top part of ampulla; first and last supplements smaller than others; tail elongate-conical, comparatively thick, with subterminal seta.

Etymology

The species epithet means ‘from Tankhoy’, the type locality name.

Material examined

Holotype

RUSSIA • ♂; Lake Baikal water area, Pereyomnaya River estuary, near Tankhoy railway station; 51.568452° N, 105.166531° E; 10 cm depth; 19 Jul. 2018; T.V. Naumova leg.; sand; HM RAS, slide 102/71 (1538-2).

Paratypes

RUSSIA • 1 ♂, 2 ♀♀; same collection data as for the holotype; T.V. Naumova leg.; HM RAS, slide 102/71 (1538-1, 1538-3, 1538-4) • 8 ♂♂, 8 ♀♀ same collection data as for the holotype; T.V. Naumova leg.; LIN–SB.

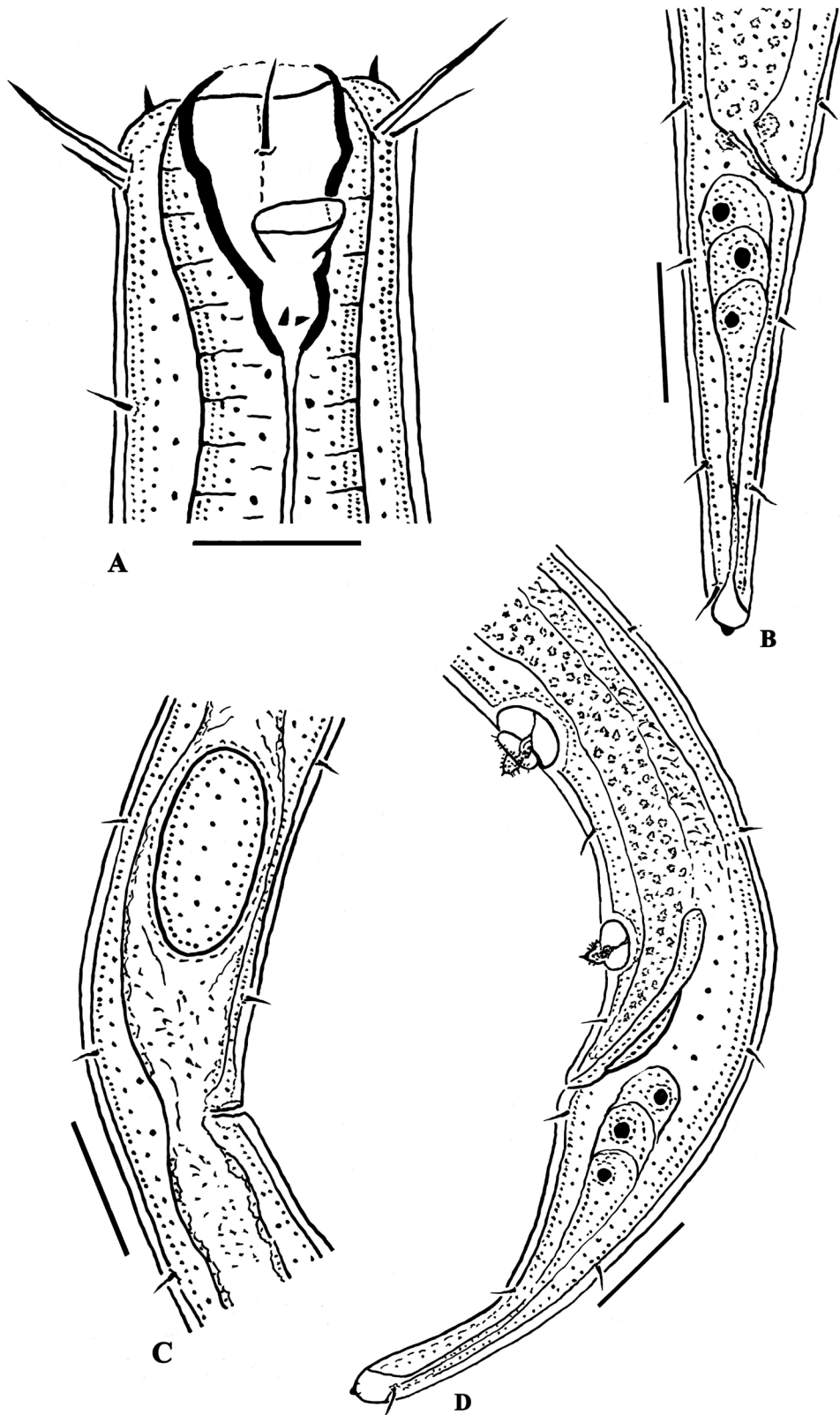


Fig. 1. *Paratrilobus tankhoyensis* sp. nov. **A.** Male anterior end. **B.** Female posterior end. **C.** Female vulva region. **D.** Male posterior body end. A, D: holotype (HM RAS 102/71 1538-2); B–C: paratype (HM RAS 102/71 1538-1). Scale bars: A = 30 μm ; B = 60 μm ; C = 80 μm ; D = 40 μm .



Fig. 2. *Paratrilobus tankhoyensis* sp. nov., light micrographs. **A.** Entire male. **B.** Entire female. **C.** Male pharyngeal region. **D.** Male anterior end. **E.** Female anterior end. **F.** Female vulva region. **G.** Male cloacal region. **H.** Male precloacal supplement. **I.** Male posterior body end. **J.** Female posterior body end. A, H–I: holotype (HM RAS 102/71 1538-2); B, E, J: paratype (HM RAS 102/71 1538-3); C–D, G: paratype (HM RAS 102/71 1538-4), F: paratype (HM RAS 102/71 1538-1). Scale bars: A–B = 500 μ m; C–E = 20 μ m; F, I–J = 50 μ m; G–H = 10 μ m.

Table 1. Measurements (in μm) of *Paratrilobus tankhoyensis* sp. nov., presented as mean and range.

Character	Holotype ♂	♂♂	♀♀
Number of specimens	1	10	10
Body length (L)	4265	4323 (3687–4793)	4923 (4130–5463)
<i>a</i>	64	65 (59–71)	63 (57–67)
<i>b</i>	5.9	5.7 (5.1–5.9)	5.8 (5.4–6.3)
<i>c</i>	33	32 (25–38)	25 (23–28)
<i>c'</i>	2.5	2.6 (2.3–3.0)	3.4 (2.9–3.7)
V, %	–	–	48 (47–52)
Labial region diameter	50	50 (45–55)	60 (53–67)
Body diameter	67	67 (60–77)	78 (70–90)
Anal or cloacal diameter	52	52 (48–60)	59 (52–70)
Stoma length	45	46 (45–48)	51 (50–55)
Outer labial setae length	27	30 (28–31)	30 (28–33)
Cephalic setae length	12	14 (13–15)	13 (12–15)
Pharynx length	725	765 (725–838)	854 (762–960)
Posterior pharynx end to vulva	–	–	1524 (1300–1688)
Posterior pharynx end to cloaca	3410	3424 (2812–3830)	–
Vulva to anus	–	–	2350 (1888–2638)
Tail length	130	136 (125–150)	196 (180–220)
Spicula length	75	74 (70–76)	–
Gubernaculum length	38	35 (35–38)	–
Number of supplements	6	6–7	–

Description

Male

Body comparatively long, thin. Cuticle smooth under light microscope, 1.5–2.0 μm thick. Body diameter at posterior pharynx end 1.3–1.4 times as large as width of labial region. Crystalloids absent. Somatic setae sparse, short, 8–11 μm long. Labial region slightly offset from adjacent body; lips well developed. Six inner labial sensillae in shape of thick, short setae, 6–9 μm long. Six outer labial sensillae in shape of smooth non-articulated setae, long 54–67% of labial region width. Four cephalic sensillae in shape of thin, smooth setae. Cheilostom of average size. Buccal cavity spacious, barrel-shaped, with thick walls. One pocket offset from buccal cavity, on its base two small teeth. Stoma 0.9 times as long as labial region width. Amphidial fovea cup-shaped, opening at level of buccal cavity. Pharynx muscular, comparatively long, expanding gradually along entire length. Cardiac glands large, rounded, 22–25 μm in diameter. Ventral gland, its canal, ampulla, excretory pore not seen.

Testes paired, situated to left of intestine; anterior testis outstretched, posterior testis reflexed. Vas deferens well developed. Spicules comparatively thin, slightly curved, long, 1.2–1.6 times as long as cloacal body diameter. Gubernaculum in shape of ‘gutter’, 46–50% of spicule length. Precloacal supplements 6–7 in number, first, last supplements smaller than others. Supplement ampulla comparatively large, its contents located in top part of ampulla. Supplements comparatively large, echinate, ‘cap’, ‘shoulder’

well developed. Supplement row 475–588 μm long. Preloacal supplement located at spicules level. Tail elongate-conical, comparatively thick, with subterminal seta. Caudal glands well developed; spinneret in shape of short conical tube.

Female

General morphology similar to males in structure of cuticle, anterior body end. Six inner labial sensillae in shape of thick and short setae, 8–10 μm long. Outer labial sensillae in shape of smooth non-articulated setae, long 44–56% of labial region width. Cardia small, surrounded by three round glands. Prerectum not observed. Rectum length equal to or slightly less than anal body diameter. Reproductive system didelphic, amphidelphic. Ovaries situated to left of intestine, reflexed, comparatively short. Oocytes numerous. Vulva transverse slit, situated to mid-body or slightly anterior to mid-body. Vulval lips not sclerotized, not protruding outside body contour. Cuticular wrinkles around vulva, vulva glands not seen. Vagina short, with thick walls. Uterus containing numerous spermatozoa, 1–2 eggs, measuring 112–132 \times 52–80 μm . Tail elongate-conical, comparatively thick, with subterminal seta. Caudal glands well developed.

Remarks

Paratrilobus tankhoyensis sp. nov. is most similar to *P. expugnator* (Tsalolichin, 1976) in body size (Tsalolichin 1976). The new species differs from it in the comparatively thin body ($a = 57\text{--}71$ vs $a = 32\text{--}44$ in *P. expugnator*), shorter and thicker tail ($c = 23\text{--}38$, $c' = 2.3\text{--}3.7$ vs $c = 8.2\text{--}10.4$, $c' = 5.0\text{--}7.5$ in *P. expugnator*), shorter stoma (stoma length 45–55 μm vs 70–80 μm long in *P. expugnator*), and shorter spicules (spicules length 70–76 μm vs 90 μm long in *P. expugnator*) (Tsalolichin 1976).

Paratrilobus aquaticus sp.nov.

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Figs 3–4, Table 2

Diagnosis

Paratrilobus aquaticus sp. nov. is characterized by a 2015–2203 μm body length; cuticle finely annulated; crystalloids absent; six inner labial sensillae papilliform; six outer labial sensillae in the shape of smooth non-articulated setae 18–20 μm long (53–58% of labial region width); four cephalic sensillae in shape of thin and smooth setae 10–13 μm long; buccal cavity spacious, barrel-shaped; one pocket connected with buccal cavity by wide gleam and contains two small teeth. Spicules comparatively thin, 51 μm long, 1.2 times as long as cloacal body diameter; gubernaculum in shape of ‘gutter’. Preloacal supplements 6 in number, about same size, located approximately at identical distance from each other; supplements echinate, not very protruded over body surface; contents of ampulla located at base; cap armed, numerous small thorns and one large central thorn. Tail slender, comparatively long; three caudal glands and spinneret well developed; subterminal seta not observed.

Etymology

The species epithet comes from the habitat ('water').

Material examined

Holotype

RUSSIA • ♂; Lake Baikal, Posolsk Bank (underwater elevation of the bottom between the southern and central basins of Lake Baikal); 52.07994° N, 105.90368° E; 51 m depth; 5 Jul. 2012; T.V. Naumova leg.; sand and silt; HM RAS, slide 102/64 (768-2).

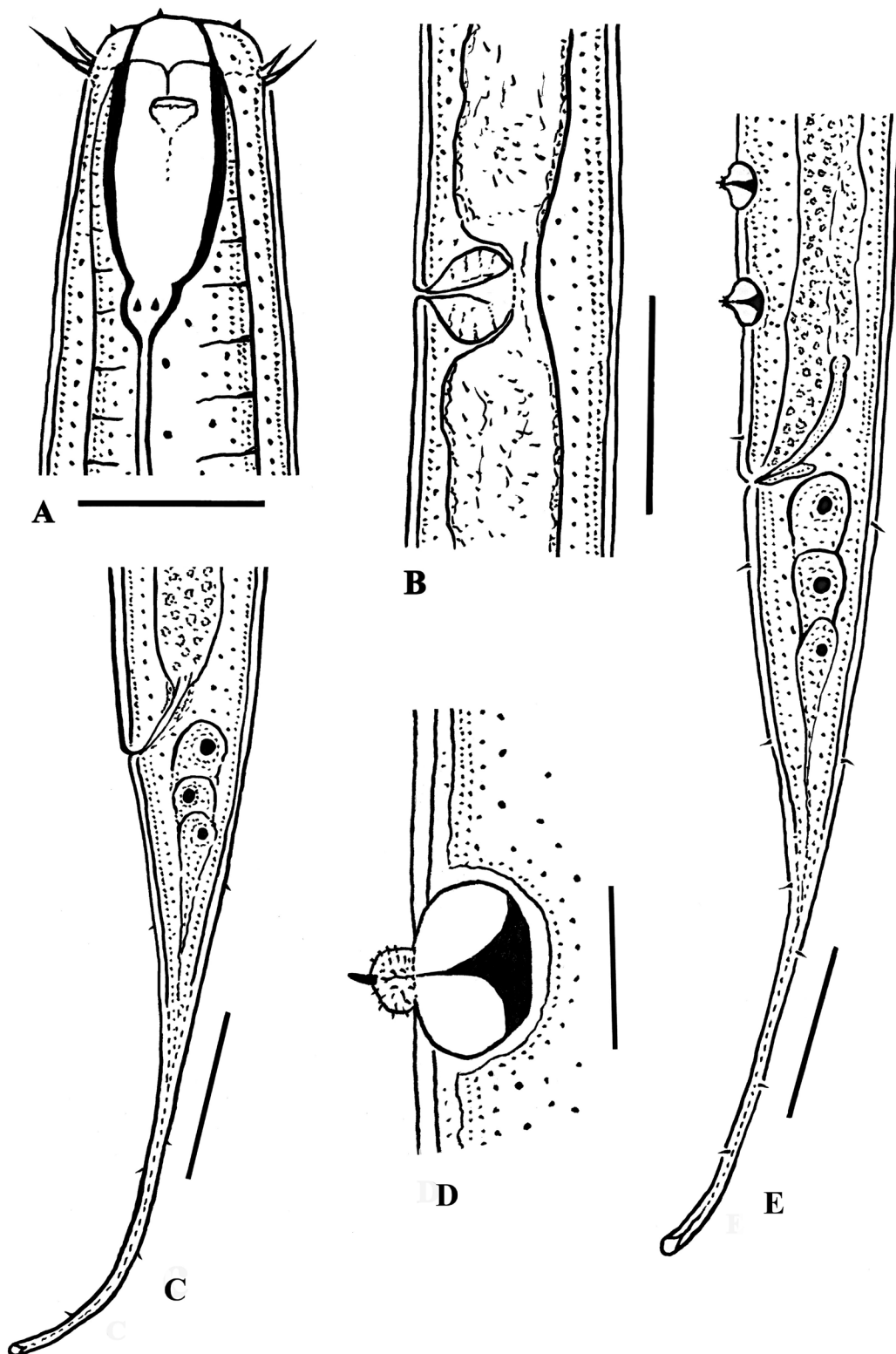


Fig. 3. *Paratrilobus aquaticus* sp. nov. **A.** Male anterior end. **B.** Female vulva region. **C.** Female posterior body end. **D.** Male precloacal supplement. **E.** Male posterior body end. **A, D–E:** holotype (HM RAS 102/64 768-2); **B–C:** paratype (HM RAS 102/64 768-4). Scale bars: **A** = 30 µm; **B** = 70 µm; **C** = 60 µm; **D** = 20 µm; **E** = 50 µm.

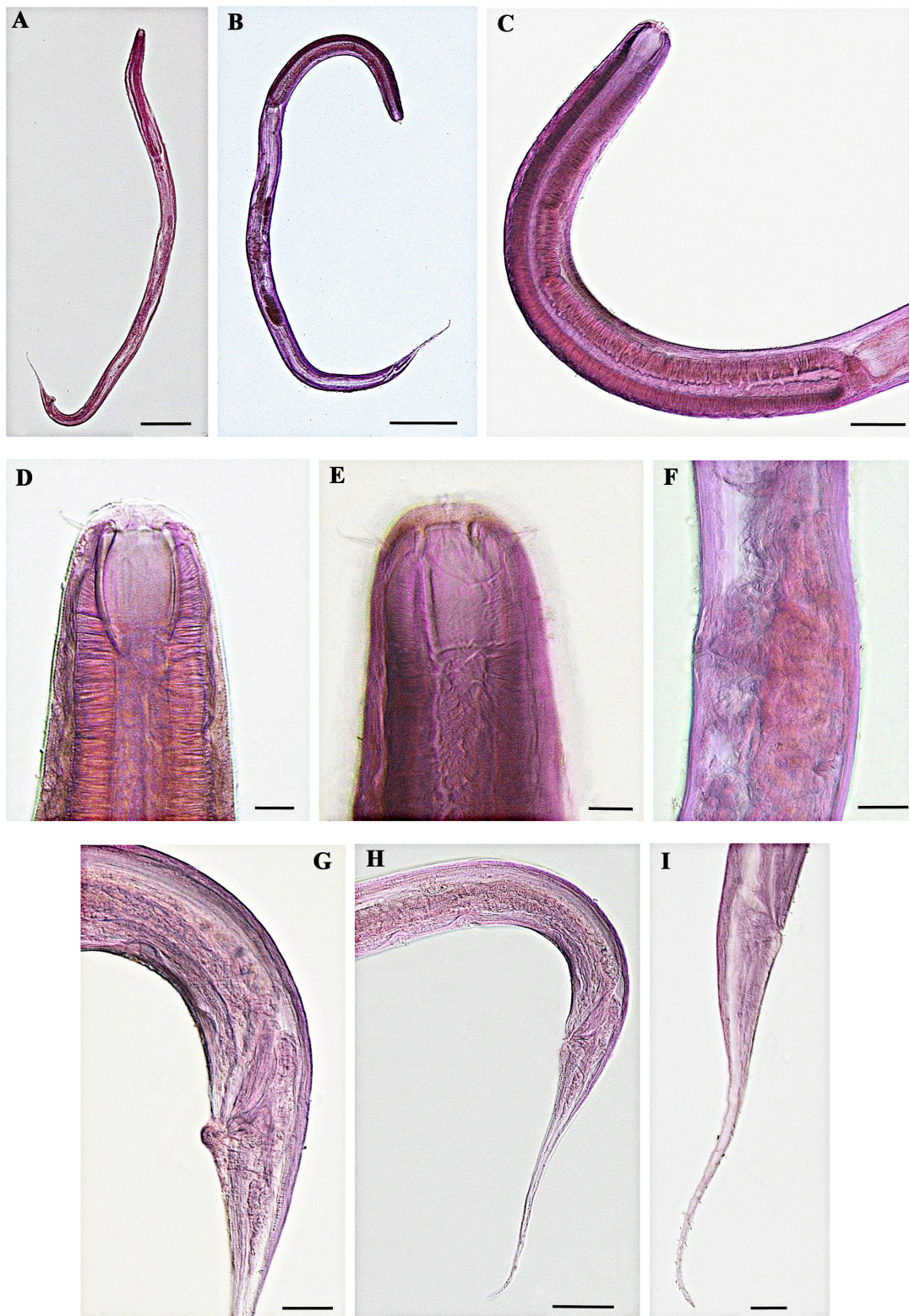


Fig. 4. *Paratrilobus aquaticus* sp. nov., light micrographs. **A.** Entire male. **B.** Entire female. **C.** Female pharyngeal region. **D.** Male anterior end. **E.** Female anterior end. **F.** Female vulva region. **G.** Male cloacal region. **H.** Male posterior body end. **I.** Female posterior body end. **A, D, G–H:** holotype (HM RAS 102/64 768-2); **B–C, I:** paratype (HM RAS 102/64 768-4); **E–F:** paratype (LIN-SB 765-3). Scale bars: **A–B** = 200 μ m; **C, H** = 50 μ m; **D–E** = 10 μ m; **F–G, I** = 20 μ m.

Table 2. Measurements (in μm) of *Paratrilobus aquaticus* sp. nov., presented as mean and range.

Character	Holotype ♂	Paratypes ♀♀
Number of specimens	1	4
Body length (L)	2097	2079 (2015–2203)
<i>a</i>	28	29 (26–30)
<i>b</i>	3.9	3.4 (3.3–3.7)
<i>c</i>	8.9	8.3 (7.5–8.8)
<i>c'</i>	5.3	6.3 (6.0–6.8)
V, %	–	52.4 (51.8–53.0)
Labial region diameter	34	33 (31–35)
Body diameter	75	72 (68–80)
Anal or cloacal diameter	43	41 (39–43)
Stoma length	50	50 (48–52)
Outer labial setae length	18	19 (18–20)
Cephalic setae length	11	12 (10–13)
Pharynx length	536	609 (589–630)
Posterior pharynx end to vulva	–	479 (434–578)
Posterior pharynx end to cloaca	1326	–
Vulva to anus	–	738 (697–787)
Tail length	235	253 (240–267)
Spicula length	51	–
Gubernaculum length	20	–
Number of supplements	6	–

Paratypes

RUSSIA • 3 ♀♀; same collection data as for the holotype; T.V. Naumova leg.; LIN–SB (765-3, 765-5, 767-5) • 1 ♀ same collection data as for the holotype; T.V. Naumova leg.; HM RAS slide 102/64 (768-4).

Description

Male

Body comparatively short. Cuticle finely annulated, 1.5 μm thick. Crystalloids absent. Somatic setae sparse, short. Labial region comparatively high, slightly offset from adjacent body, lips well developed. Six inner labial sensillae papilliform. Six outer labial sensillae in shape of smooth setae long 53% of labial region width. Four cephalic sensillae in shape of thin setae. Cheilostom of average size. Buccal cavity spacious, barrel-shaped, with thick walls. One pocket connected with buccal cavity by wide gleam, containing two small teeth. Stoma 1.5 times as long as labial region width. Amphidial fovea cup-shaped, opening at level of buccal cavity. Pharynx muscular, comparatively long, expanding gradually along length. Cardiac glands large, rounded, 30 μm in diameter. Ventral gland, its canal, ampulla, excretory pore not observed.

Testes paired, situated to left of intestine; anterior testis outstretched, posterior testis short, reflexed. Vas deferens well developed. Spicules slender, ventral curved, 1.2 times as long as cloacal body diameter.

Gubernaculum in shape of gutter, 39% of spicule length. Precloacal supplement 6 in number, about one size, located approximately at equal distances from each other. Supplements echinate, not very protruded over body surface. Ampulla contents concentrated in ampullae base. Shoulder absent. Cap armed, numerous small thorns, one large central thorn. Precloacal supplement located anteriorly to spicules level. Tail slender, comparatively long. Three caudal glands, spinneret well developed. Subterminal setae not seen.

Female

General morphology similar to that of males in structure of cuticle, anterior body end. Six outer labial sensillae in shape of smooth non-articulated setae long 53–58% of labial region width. Cardia surrounded by three round glands. Rectum length equal to or slightly greater than anal body diameter. Reproductive system didelphic, amphidelphic. Ovaries situated to left of intestine, reflexed. Oocytes numerous. Vulva transverse slit, situated slightly posterior to mid-body. Vulval lips not sclerotized, not protruded outside body contour. Cuticular wrinkles around vulva, vulva glands not observed. Vagina straight line, with well-expressed spherical vaginal chamber, thick walls. Generated eggs in uterus not observed. Tail slender, comparatively long. Subterminal seta not seen.

Remarks

Paratrilobus aquaticus sp. nov. is similar to *P. granulosus* Gagarin & Naumova, 2011 and *P. ultimus* (Tsalolichin, 1977) in the structure of the precloacal supplements. From the former species, it differs in the absence of crystalloids, a comparatively longer pharynx ($b = 3.3–3.9$ vs $b = 4.5–5.9$ in *P. granulosus*), longer outer labial setae (18–20 μm long, 53–58% of labial region width vs 6.5–8.5 μm long, 30–35% of labial region width in *P. granulosus*), the vulva position ($V = 51.8–53.0\%$ vs 39.1–46.1% in *P. granulosus*), the longer stoma (48–52 μm long vs 28–33 μm long in *P. granulosus*), and the absence of a subterminal seta (Gagarin & Naumova 2011). From the latter species, it differs in the longer pharynx ($b = 3.3–3.9$ vs $b = 4.7–5.9$ in *P. ultimus*), a longer and more slender tail ($c = 7.5–8.9$, $c' = 5.5–6.8$ vs $c = 10.9–16.4$, $c' = 3.5–4.5$ in *P. ultimus*), the longer stoma (48–52 μm long vs 21 μm in *P. ultimus*), longer outer labial setae (18–20 μm long, 53–58% of labial region width vs 6–7 μm long, 25–30% of labial region width in *P. ultimus*), and the vulva position ($V = 51.8–53.0\%$ vs $V = 39–43\%$ in *P. ultimus*) (Tsalolichin 1977).

Discussion

We compared males of valid species of the genus *Paratrilobus* (except for *P. rapis* described only from females) according to morphological characters (Table 3). On grounds of the supplement structure, the genus can be divided into two species groups:

1) comparatively large supplements with ‘shoulder’, cap and ampulla contents located in the top part of ampulla (*P. grandipapilloides*, *P. brevis*, *P. delicatus*, *P. expugnator*, *P. ponticus* and *P. tankhoyensis* sp. nov., i.e., ‘*grandipapilloides*’ species group)

2) comparatively small supplements; ‘shoulder’ is absent, cap comparatively small, ampulla contents located in ampulla base (*P. ultimus*, *P. granulosus*, *P. aquaticus* sp. nov., i.e., ‘*ultimus*’ species group).

We cannot give the rank of subgenus to these groups, but we must take into account the heterogeneity of the genus.

The species *P. strenuus* was found in Lake Tajmyr (Siberia, Russia) and was originally described from 32 females as *Eutobrilus strenuus* Gagarin, 1991 (Gagarin 1991). Subsequently, the species was transferred to the genus *Quasibrilus* Tsalolichin, 1976, because the buccal cavity in specimens of this species is small, one pocket with two small teeth is hardly visible and well isolated from the buccal

Table 3. Diagnostic morphometric characters of males of valid species of the genus *Paratrilobus* Micoletzky, 1922.

Species	L	a	b	c	c'	diam.c.s.	stoma	o.l.s	o.l.s.%	spic.	gub.	suppl.
‘ <i>grandipapilloides</i> ’ species group												
<i>grandipapilloides</i>	2800–3030	28–34	3.6–3.7	10.8–11.2	4–5	56–67	55–69	10–11	10–12	76–77	30	6
<i>brevis</i>	3300	22	3.9	12.2	4.0	58–68	70–78	13–16	20–21	77	31	6
<i>delicatus</i>	2500–2800	40–50	4.8–6.3	17.0–20.2	2.0–3.5	30–33	30–33	15	45–50	58–63	18–20	6
<i>expugnator</i>	4800	44	5.0	8.8	7.5	51–60	70–80	25	50	90	40	6
<i>ponticus</i>	1900–2900	21–29	3.6–4.6	12.7–21.6	2.0	39	44–45	9.0	20–25	73–75	31–32	6
<i>tankhoyensis</i> sp. nov.	3687–4793	59–71	5.1–5.9	24.6–38.7	2.3–3.0	45–55	45–48	27–31	54–67	70–76	34–38	6–7
‘ <i>ultimus</i> ’ species group												
<i>ultimus</i>	1900–2500	21–35	5.0–5.7	11.7–16.4	3.5	23–25	21	6–7	25–30	50–54	?	6–8
<i>granulosus</i>	2128–2633	23–27	4.6–5.9	8.2–11.1	4.2–5.8	21–29	28–30	6.5–8.5	30–35	54–57	15–17	6–7
<i>aquaticus</i> sp. nov.	2097	28	3.9	8.9	5.5	34	50	18	53	51	20	6

cavity (Gagarin 1993). Andr ssy (2007) placed this species in the genus *Paratrilobus*, but we consider this an erroneous decision and return this species to the genus *Quasibrilus*, because a stoma structure in this species does not correspond to the morphological diagnosis of the genus *Paratrilobus* (Andr ssy 2007). The main feature of the genus *Paratrilobus* is the buccal cavity structure: it is spacious, funnel- or barrel-shaped with well-sclerotized walls; pockets are almost reduced.

The distribution area of the genus is within the borders of Eurasia. *Paratrilobus grandipapilloides* was described from freshwater bodies of Germany (Micoletzky 1922), but later, it was also found in Lake Onega, Lake Tajmyr and Lake Baikal (Gagarin 1990; Zullini 2006; Naumova & Gagarin 2019a). *Paratrilobus ponticus* was known from Dnepro-Bugs estuary of the Black Sea (Tsalolichin 1981). *Paratrilobus rapis* was described from Lake Tajmyr (Siberia, Russia) (Gagarin 1991). Seven species of the genus were found and described from Lake Baikal (Naumova & Gagarin 2019a). Therefore, Lake Baikal is the largest natural centre of speciation of this genus.

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