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Research article

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Two new nematode species of the genus *Tobrilus* Andrassy, 1959 (Nematoda, Triplonchida) from Lake Baikal, Russia

Tatyana V. NAUMOVA^{1,*} & Vladimir G. GAGARIN²

¹Limnological Institute, Siberian Branch of the Russian Academy of Sciences,
Irkutsk, 664033, Russia.

²Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, 152742,
Yaroslavl Province, Russia.

*Corresponding author: tvnaum@lin.irk.ru

²Email: gagarin@ibiw.yaroslavl.ru

¹[urn:lsid:zoobank.org:author:835C52CB-795C-48BF-B5A0-6DA2C303E060](https://zoobank.org/author:835C52CB-795C-48BF-B5A0-6DA2C303E060)

²[urn:lsid:zoobank.org:author:E28EA8D1-CBFE-4518-AA60-7F4388FBAD64](https://zoobank.org/author:E28EA8D1-CBFE-4518-AA60-7F4388FBAD64)

Abstract. Two new nematode species of the genus *Tobrilus* Andrassy, 1959 from Lake Baikal are described and illustrated. The first species *Tobrilus elginus* sp. nov. was found in the littoral zone of Maloye More Bays. The second species *Tobrilus juliae* sp. nov. dwells on bodies of dead sponges *Lubomirskia baicalensis* (Pallas, 1776). *Tobrilus elginus* sp. nov. is most similar to *T. amabilis* Tsalolikhin, 1974 and *T. bekmanae* Tsalolikhin, 1975. In contrast to the first species it has a shorter body and spicules, longer gubernaculum and a shorter supplements row. Its body is shorter and thinner, tail and supplement row are shorter and the vulva is more posterior as compared to the second species. The body size of *Tobrilus juliae* sp. nov. is most similar to *T. securus* Gagarin & Naumova, 2011 and *T. saprophagus* Naumova & Gagarin, 2017. From the first of these species it differs by the thinner body, shorter tail, comparatively shorter outer labial setae and shorter spicules. It differs from the second species by a thinner body, shorter male tail and shorter labial setae.

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

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Introduction

Lake Baikal is a fascinating freshwater reservoir known all over the Earth. Its shores and pure, crystal clean water attract numerous tourists as well as experts and researchers. The ancient age of the Lake (25–30 million years), the enormous depths of the water body (1637–1642 m) and its huge 7–8 km depression including sediment layers containing one fifth of the world's surface fresh water and its high oxygen saturation (up to 9.5 mg/l at the 1600 m depth point) make it a wonder of our planet. In 1996, UNESCO announced Lake Baikal a World's Heritage Object.

Unique environments of the Lake inevitably provided favourable conditions for the formation of an abundant and diverse fauna including over 2600 species with a high level of endemism (more than 56%) (Timoshkin 2001). The fauna of free-living nematodes comprises 114 species in 32 genera, 16 families and 8 orders according to the latest records (Naumova & Gagarin 2019). These worms are encountered in rocky, sandy and silty substrates occupying all depths from the splash zone (above the water edge) to the abyssal zones. In terms of abundance, nematodes often dominate among other benthos groups.

The current world fauna records of *Tobrilus* Andr ssy, 1959 include 22–24 valid species (Zullini 2006; Andr ssy 2007), ten of which are present in Lake Baikal: *T. amabilis* Tsalolikhin, 1974, *T. bekmanae* Tsalolikhin, 1975, *T. incognitus* Tsalolikhin, 1972, *T. latens* Tsalolikhin, 1974, *T. longisetosus* Gagarin & Naumova, 2011, *T. macramphis* Tsalolikhin, 1977, *T. methanus* Gagarin & Naumova, 2016, *T. saprophagus* Naumova & Gagarin, 2017, *T. securus* Gagarin & Naumova, 2011, *T. undophylus* Shoshin, 1988 (Tsalolikhin 1974, 1975; Gagarin & Naumova 2011, 2016; Naumova & Gagarin 2017). A key for the identification of *Tobrilus* species from Lake Baikal based on male characters was published by Gagarin & Naumova (2016).

The aim of this paper is to describe two new nematode species inhabiting Lake Baikal: *T. elginus* sp. nov. and *T. juliae* sp. nov.

Materials and methods

Nematodes were collected in the littoral zone of Lake Baikal from the following sites:

- Southern basin of Lake Baikal, near Cape Berezovy (51°30'11" N, 104°32'33" E), 5 m depth, bodies of dead sponges *Lubomirskia baicalensis* (Pallas, 1776) collected by divers on 6 September 2016.
- Middle basin of Lake Baikal (Maloye More), Yelginsky Bay (53°09'14" N, 107°10'26" E), 30 cm depth, sand, collected on 16 July 2018.

The samples contained numerous free-living nematodes, including both 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 ver. 3.2 program for analysis and documentation of images from the preparations.

Repositories

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

L = body length

V = distance from body anterior end to vulva expressed as percentage (%) of the body length

Results

Phylum Nematoda Potts, 1932
Class Enoplea Inglis, 1983
Order Triplonchida Cobb, 1920
Family Tobrilidae de Coninck, 1965
Genus *Tobrilus* Andr ssy, 1959

Type species

Tobrilus gracilis (Bastian, 1865) = *Trilobus gracilis* Bastian, 1865.

Diagnosis (after Andr ssy 2007; Holovachov & Shoshin 2014)

Body 1.0–4.5 μm long. Cuticle smooth or very finely annulated. Buccal cavity cup-or funnel-shaped, with overlapping subventral pocket and teeth close to each other. Vagina moderately strong. Spicules comparatively short. Supplements 3 to 13, mostly six, small, non-echinate, but with a small central thorn, submerged, not protruding. Spacing between supplements nearly equal.

Tobrilus elginus sp. nov.

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Figs 1–2, Table 1 (morphometric)

Diagnosis

Tobrilus elginus sp. nov. is characterized by a 2200–2995 μm long body; cuticle smooth under light microscope; crystalloids absent; inner labial sensillae papilliform; six outer labial sensillae in the shape of smooth non-articulated setae 14–17 μm long, 45–57% for males and 44–59% for females of labial region width; four cephalic sensillae on the shape of thin and smooth setae 6–8 μm long; buccal cavity spacious, funnel-shaped; dorsal pocket and its tooth absent; both subventral pockets overlapping adjacent to each other. Spicules comparatively thick, slightly curved, short, 50–53 μm long, 1.1–1.3 long as the cloacal body diameter; gubernaculum in the shape of ‘gutter’; precloacal supplements 6 in number, small, submerged; tail slender, long, elongate-conical, with terminal seta.

Type material

Holotype

RUSSIA • ♂; Lake Baikal (Maloye More Strait), Elginsky Bay; 53°09'14" N, 107°10'26" E; 30 cm depth; 16 Jul. 2018; sand; HM RAS slide reference number 102/69.

Paratypes

RUSSIA • 2 ♂♂, 1 ♀; same collection data as for holotype; HM RAS slide reference number 102/69 (1507-1, 1507-3, 1507-5) • 7 ♂♂, 13 ♀♀; same collection data as for holotype; LIN-SB.

Etymology

The specific epithet means ‘from Elga’, the type locality name.

Description

Male

Body comparatively long and thin. Cuticle smooth under light microscope, 1.5–2.0 μm thick. Body diameter at the posterior pharynx end 1.7–2.0 times the width of the labial region. Crystalloids absent. Somatic setae rare and short, 5–7 μm long. Labial region slightly offset from the adjacent body; lips

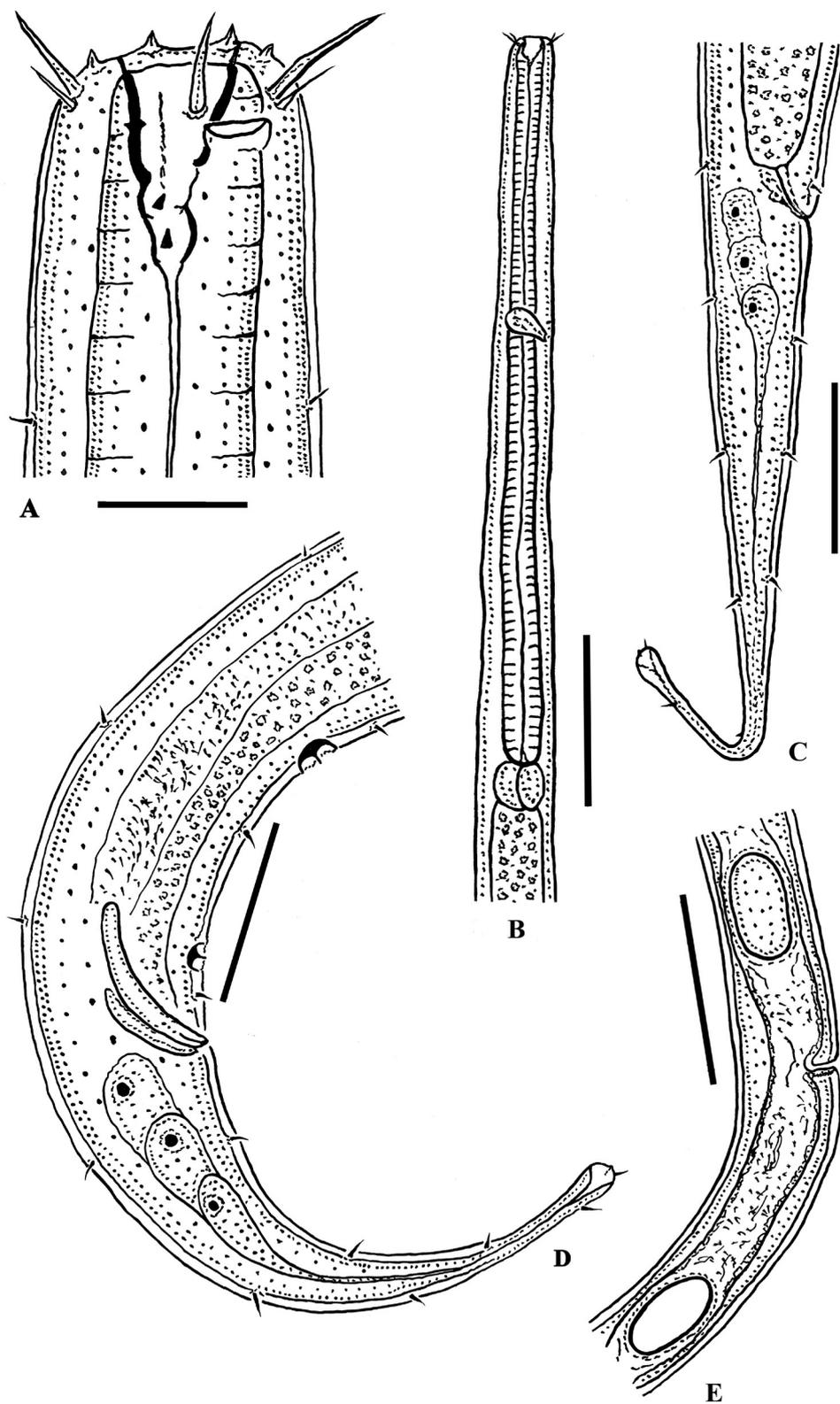


Fig. 1. *Tobrilus elginus* sp. nov. **A.** Male anterior end. **B.** Male pharyngeal region. **C.** Female posterior body end. **D.** Male posterior body end. **E.** Female vulva region. Scale bars: A = 20 μm ; B, E = 100 μm ; C–D = 50 μm .

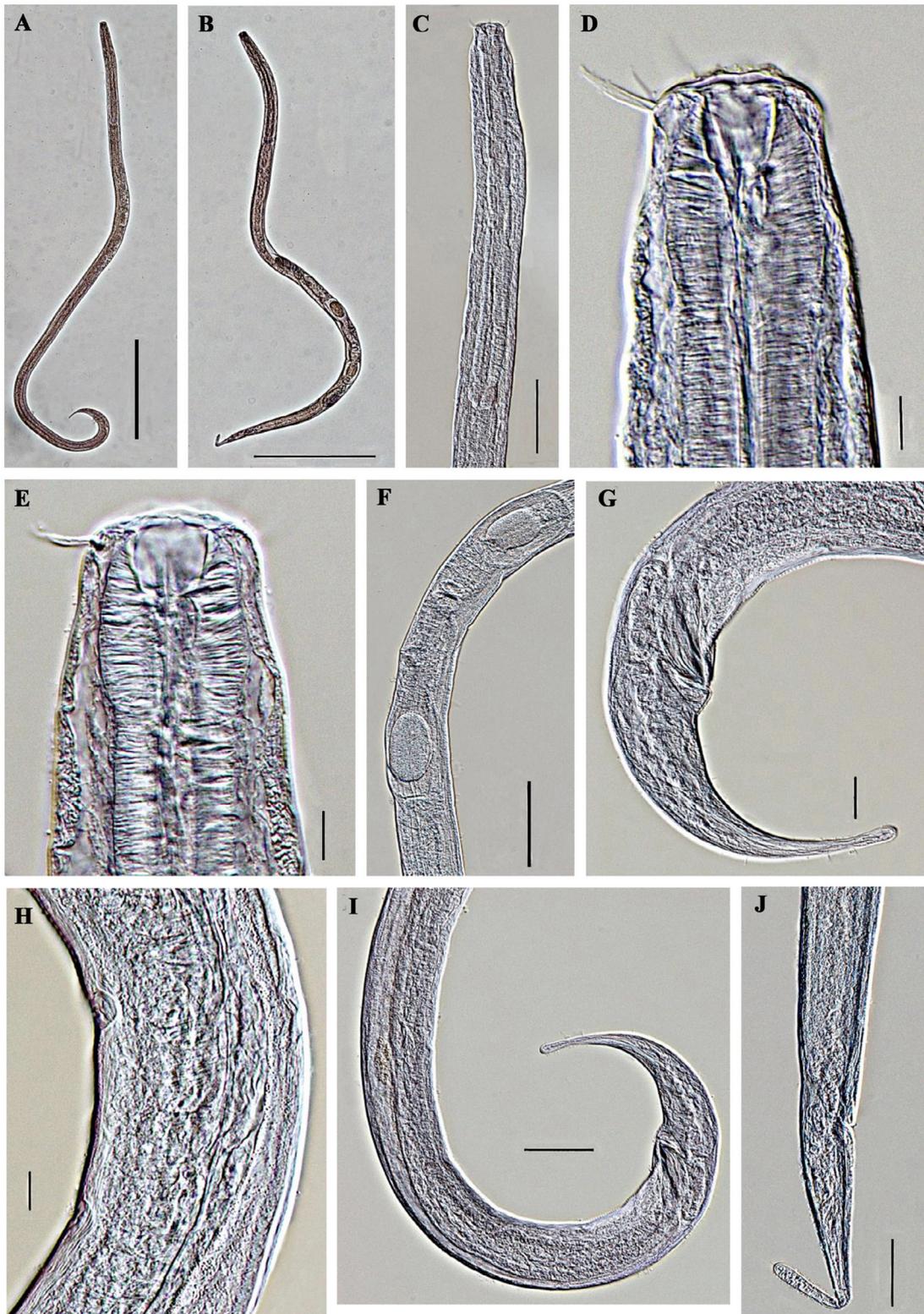


Fig. 2. *Tobrilus elginus* sp. nov., light micrographs. **A.** Entire male. **B.** Entire female. **C.** Male pharyngeal region. **D.** Male anterior end. **E.** Female anterior end. **F.** Female vulva region. **G, I.** Male posterior body end. **H.** Male supplement region. **J.** Female posterior body end. Scale bars: A–B = 500 μ m; C, F = 100 μ m; D–E, H = 10 μ m; G = 20 μ m; I–J = 50 μ m.

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

Character	Holotype ♂	♂♂	♀♀
Number of specimens	1	10	14
Body length (L)	2648	2551 (2235–2845)	2410 (2200–2995)
<i>a</i>	47	46 (39–56)	42 (37–46)
<i>b</i>	5.4	5.2 (4.8–5.6)	4.7 (4.5–4.9)
<i>c</i>	15.6	15.8 (14.2–18.7)	12.5 (11.0–13.4)
<i>c'</i>	3.9	3.8 (3.3–4.4)	6.0 (5.0–6.9)
V, %	–	–	61 (57–64)
Labial region diameter	28	28 (27–30)	30 (27–32)
Body diameter	57	56 (45–65)	58 (53–65)
Anal or cloacal diameter	44	43 (40–47)	32 (30–40)
Stoma (buccal cavity) length	33	31 (28–33)	32 (28–37)
Outer labial setae length	15	15 (14–17)	15 (14–17)
Cephalic setae length	8	7 (6–8)	7 (5–8)
Nerve ring from anterior body end	130	127 (110–135)	132 (120–150)
Pharynx length	490	487 (440–525)	505 (475–532)
Posterior pharynx end to vulva	–	–	956 (792–1180)
Posterior pharynx end to cloaca	1988	1903 (1638–2188)	–
Vulva to anus	–	–	757 (660–1090)
Tail length	170	162 (150–175)	193 (170–228)
Spicules length	52	51 (50–53)	–
Gubernaculum length	23	24 (20–26)	–
Number of supplements	6	6	–

well developed. Six inner labial sensillae papilliform. Six outer labial sensillae in the shape of smooth, non-articulated setae 14–17 μm long, 45–57% of labial region width. Four cephalic sensillae in the shape of thin and smooth setae, 6–8 μm long. Both circles of setae drawn close together. Cheilostom of the average size. Buccal cavity spacious, funnel-shaped, with thick walls. Dorsal pocket and its tooth absent. Both subventral pockets overlapping adjacent to each other. Its teeth small, close to each other (~ 5 μm away). Stoma 1.1–1.3 times as long as labial region width. Amphidial fovea cup-shaped, opening at the level of buccal cavity. Pharynx muscular, comparatively long, expanding gradually along entire length. Cardiac glands large, rounded, 20–23 μm in diameter. Ventral gland, its canal, ampulla and excretory pore not seen.

Testes paired, opposed, situated to the left of intestine; anterior testes outstretched, posterior testis reflexed. Vas deferens well developed. Spicules thick, slightly curved, short, 1.1–1.3 times as long as the cloacal body diameter, apically bifurcate. Gubernaculum in the shape of ‘gutter’, 42–53% of the spicule length. Precloacal supplements 6 in number, small, submerged. Supplement ampulla flattened, contents concentrated at ampulla base. Supplement cap absent. Central thorn protruding slightly above cuticle. Supplements approximately identical in size and situated about equidistant from each other. The closest to cloaca supplement at 21–30 μm away from it; supplement row 355–435 μm long. Tail slender, elongate-conical, with terminal seta 6–8 μm long. Caudal glands well developed; spinneret in the shape of a short, conical tube.

Female

General morphology similar to that of males in structure of cuticle and anterior body end. Cardia small, surrounded by three round glands. Prerectum not observed. Rectum length equal to or slightly greater than anal body diameter. Reproductive system didelphic, amphidelphic. Ovaries situated to the left of intestine, reflexed and comparatively short. Oocytes numerous. Vulva a transverse slit and situated slightly posterior to mid-body. Vulval lips not sclerotized and not protruding outside the body contour. Cuticular wrinkles around vulva and vulva glands not seen. Vagina short, with thick walls. Uterus containing numerous spermatozoa and 1–2 eggs, measuring 65–105 \times 26–50 μm . Tail slender, long, elongate-conical, with terminal seta. Caudal glands well developed.

Remarks

Tobrilus elginus sp. nov. is most similar to *T. amabilis* and *T. bekmanae*. The new species differs from the first species in the shorter body (σ L = 2.2–2.8 mm, ϕ L = 2.2–2.9 mm vs σ L = 2.8–3.0 mm, ϕ L = 3.0–3.1 mm in *T. amabilis*), shorter spicules (50–53 μm long vs 55–58 μm long in *T. amabilis*), longer gubernaculum (20–26 μm long vs 16–17 μm long in *T. amabilis*), shorter supplement row (355–435 μm long vs 487 μm long in *T. amabilis*) (Tsalolikhin 1974). *Tobrilus elginus* sp. nov. differs from *T. bekmanae* in the shorter body (σ L = 2.2–2.8 mm, ϕ L = 2.2–2.9 mm vs σ L = 2.6–3.1 mm, ϕ L = 2.9–3.8 mm in *T. bekmanae*), thinner body (σ a = 39–56, ϕ a = 37–46 vs σ a = 25–40, ϕ a = 28–35 in *T. bekmanae*), shorter tail (σ c = 14.2–18.7, c' = 3.3–4.4, ϕ c = 11.0–13.4, c' = 5.0–6.9 vs σ c = 8.5–13.7, c' = 6, ϕ c = 7.4–10.0, c' = 9 in *T. bekmanae*), a more posterior vulva (V = 57–64% vs V = 41–50% in *T. bekmanae*), shorter supplement row (355–435 μm long vs 334 μm long in *T. bekmanae*) (Tsalolikhin 1975).

Tobrilus juliae sp. nov.

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Figs 3–4, Table 2 (morphometric)

Diagnosis

Tobrilus juliae sp. nov. is characterized by a 1800–2386 μm long body; cuticle smooth under light microscope; crystalloids absent; inner labial sensillae papilliform; six outer labial sensillae in the shape of smooth, non-articulated setae 4–6 μm long for males (24–35% of labial region width) and 5–6 μm long for females (28–38% of labial region width); four cephalic sensillae on the shape of thin and smooth setae 3–4 μm long for males and 2–4 μm long for females; buccal cavity spacious, barrel-shaped; dorsal pocket and its tooth absent; both subventral pockets overlapping adjacent to each other. Spicules slightly curved, 45–50 μm long, 1.2–1.5 long as the cloacal body diameter; gubernaculum in the shape of ‘gutter’; precloacal supplements 6–7 in number, small, submerged; tail short, elongate-conical, with terminal seta.

Type material

Holotype

RUSSIA • ♂; Lake Baikal (Southern basin), near Cape Berezovy; 51°30'11" N, 104°32'33" E; 5 m depth; 6 Sep. 2016; bodies of dead sponges *Lubomirskia baicalensis* collected by divers; HM RAS slide reference number 102/70.

Paratypes

RUSSIA • 9 ♂♂, 10 ♀♀; same collection data as for holotype; LIN-SB; 1 ♀; same collection data as for holotype; HM RAS slide reference number 102/70 (1390-1).

Etymology

The species named in gratitude to Ms Julia Zvereva.

Description

Male

Body comparatively long and thin. Cuticle smooth under light microscope, 1.0–1.5 µm thick. Body diameter at the posterior pharynx end 2.1–2.5 times the width of the labial region. Crystalloids absent. Somatic setae rare and short, 3–4 µm long. Labial region only slightly offset from the adjacent body; lips well developed. Six inner labial sensillae papilliform. Six outer labial sensillae in the shape of smooth, non-articulated setae 4–6 µm long, 24–35% of labial region width. Four cephalic sensillae in the shape of thin and smooth setae 3–4 µm long. Both circles of setae drawn close together. Cheilostoma of the average size. Buccal cavity spacious, barrel-shaped, with thick walls. Dorsal pocket and its tooth absent. Both subventral pockets overlapping adjacent to each other. Its teeth small, close to each other (distance ~ 3 µm). Stoma 1.0–1.2 as long as labial region width. Amphidial fovea cup-shaped, opening at the level of buccal cavity. Pharynx muscular, comparatively long, expanding gradually along entire length. Cardiac glands large, rounded, 15–17 µm in diameter. Ventral gland, its canal, ampulla and excretory pore not seen.

Testes paired, opposed, situated to the left of intestine; anterior testis outstretched, posterior testis reflexed. Vas deferens well developed. Spicules slightly curved, comparatively short, 1.2–1.5 times as long as the cloacal body diameter, apically bifurcate. Gubernaculum in the shape of ‘gutter’, 43–47% of the spicule length. Preloacal supplements 6–7 in number, small, submerged. Supplement ampulla flattened, its contents concentrated at ampulla base. Supplement cap absent. Central thorn protruding slightly above cuticle. Supplements approximately identical in size and situated about equidistant from each other. The closest to cloaca supplement at 11–20 µm away from it; supplement row 175–290 µm long. Tail short, elongate-conical, with terminal seta 3–5 µm long. Caudal glands well developed; spinneret in the shape of a short, conical tube.

Female

General morphology similar to that of males in structure of cuticle and anterior body end. Six outer labial sensillae in the shape of smooth setae 5–6 µm long, 28–38% of labial region width; four cephalic sensillae in the shape of thin, smooth setae 2–4 µm long. Cardia small, surrounded by three round glands. Rectum length equal to or slightly greater than anal body diameter. Reproductive system didelphic, amphidelphic. Ovaries situated to the left of intestine, reflexed and comparatively short. Oocytes numerous. Vulva a transverse slit and situated slightly posterior to mid-body. Vulval lips not sclerotized and not protruding outside the body contour. Cuticular wrinkles around vulva and vulva glands not observed. Vagina short. Uterus containing numerous spermatozoa and 1–2 eggs, measuring 70–72 × 40–43 µm. Tail short, elongate-conical, with subterminal seta. Caudal glands well developed.

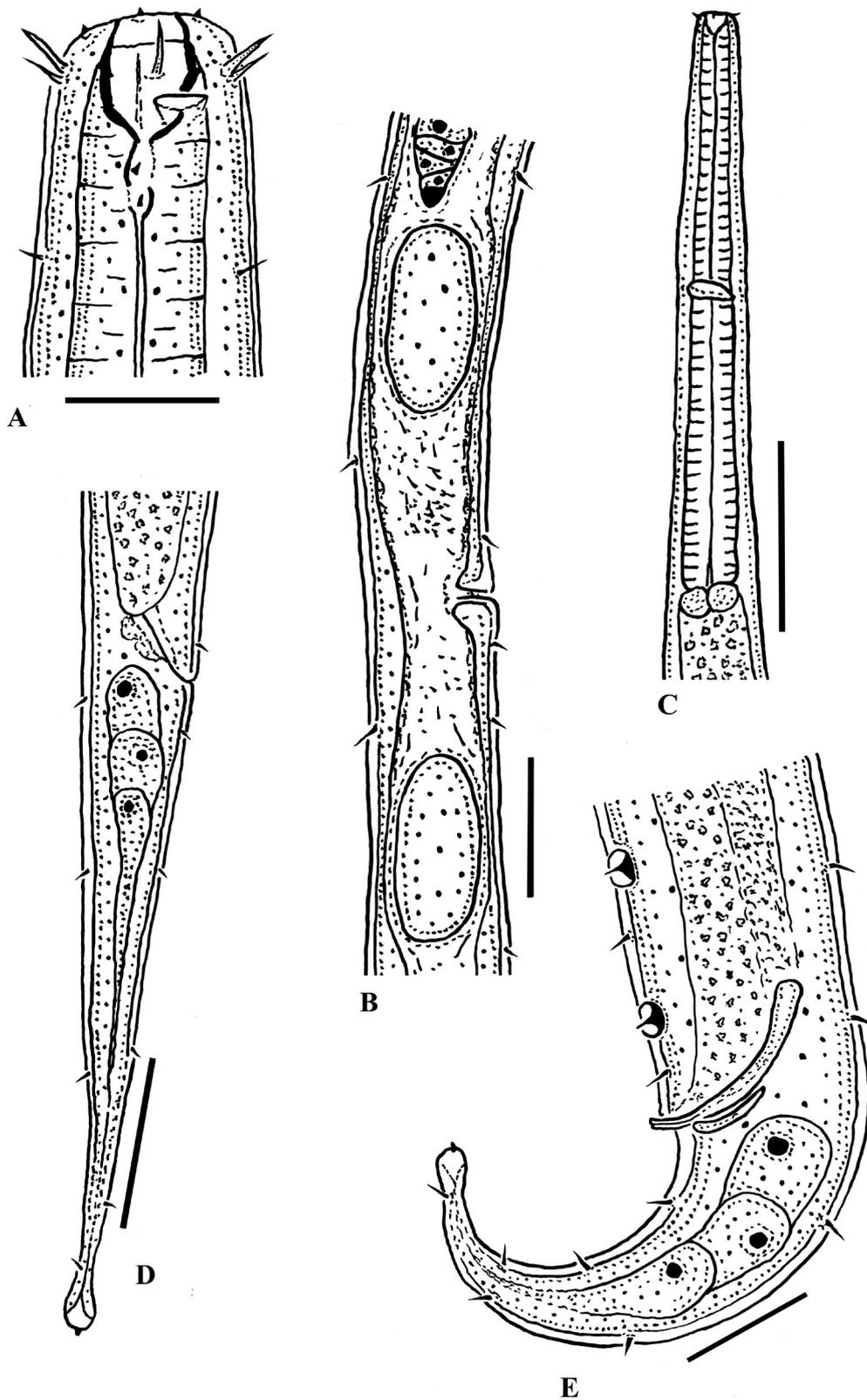


Fig. 3. *Tobrilus juliae* sp. nov. A. Male anterior end. B. Female vulva region. C. Male pharyngeal region. D. Female posterior body end. E. Male posterior body end. Scale bars: A = 15 μ m; B = 50 μ m; C = 100 μ m; D = 40 μ m; E = 30 μ m.

Remarks

Tobrilus juliae sp. nov. is most similar to *T. securus* and *T. saprophagus* in body size. From the first species it differs in the thinner body ($a = 41\text{--}63$ vs $a = 25\text{--}40$ in *T. securus*), shorter tail ($c = 11.3\text{--}23.4$, $c' = 2.4\text{--}6.1$ vs $c = 6.7\text{--}8.4$, $c' = 6.3\text{--}9.3$ in *T. securus*), comparatively shorter outer labial setae (24–35% of labial region width vs 40–45% of labial region width in *T. securus*) and shorter spicules (45–50 μm long vs 52–58 μm long in *T. securus*) (Gagarin & Naumova 2011). From the second species *T. juliae* sp. nov. differs in the thinner body ($a = 41\text{--}63$ vs $a = 30\text{--}43$ in *T. saprophagus*), shorter tail in males ($\text{♂ } c = 17.5\text{--}23.4$ vs $\text{♂ } c = 11.5\text{--}16.7$ in *T. saprophagus*) and shorter outer labial setae (4–7 μm long, 24–35% labial region width vs 10–12 μm long, 37–46% of labial region width in *T. saprophagus* (Naumova & Gagarin 2017).



Fig. 4. *Tobrilus juliae* sp. nov., light micrographs. **A.** Entire male. **B.** Entire female. **C.** Male pharyngeal region. **D.** Female anterior end. **E.** Male anterior end. **F.** Female vulva region. **G.** Male posterior body end. **H.** Female posterior body end. Scale bars: A–B = 250 μm ; C, F, H = 50 μm ; D–E, G = 10 μm .

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

Character	Holotype ♂	♂♂	♀♀
Number of specimens	1	10	11
Body length (L)	2067	2121 (1891–2386)	2038 (1800–2298)
<i>a</i>	52	53 (43–63)	51 (41–58)
<i>b</i>	6.8	6.8 (6.4–7.3)	6.7 (6.3–7.3)
<i>c</i>	20.7	20.8 (17.5–23.4)	12.9 (11.3–14.2)
<i>c'</i>	2.6	2.7 (2.4–3.6)	5.3 (4.6–6.1)
V, %	–	–	44 (43–47)
Labial region diameter	17	17 (15–19)	17 (15–18)
Body diameter	40	40 (33–45)	41 (35–48)
Anal or cloacal diameter	38	38 (30–40)	30 (28–35)
Stoma (buccal cavity) length	20	19 (18–20)	19 (18–22)
Outer labial setae length	6	5 (4–6)	6 (5–7)
Cephalic setae length	4	4 (3–4)	3 (2–4)
Nerve ring from anterior body end	105	103 (100–121)	117 (110–132)
Pharynx length	305	313 (290–342)	304 (275–338)
Posterior pharynx end to vulva	–	–	594 (530–645)
Posterior pharynx end to cloaca	1662	1705 (1488–1943)	–
Vulva to anus	–	–	981 (825–1180)
Tail length	100	102 (90–115)	158 (140–170)
Spicules length	47	48 (45–50)	–
Gubernaculum length	23	22 (20–23)	–
Number of supplements	7	6–7	–

Discussion

Tobrilus is considered the most primitive genus within the family Tobrilidae; the small and simple supplements in variable (non-constant) number seem to support this opinion (Andrássy 2007). The genus is distributed in Europe, Asia, Africa, both Americas and Australia (Andrássy 2007). Half of the species in the genus *Tobrilus* (12 of 22–24 valid species) are inhabitants of Lake Baikal where they are encountered in the depth range of 0 to 1520 m, as well as in the splash zone (0.5 m above the water edge). Most of the species are endemic and only two have been found elsewhere in water bodies of the Baikal region (Gagarin 1993; Naumova *et al.* 2010). Evidently, Lake Baikal is the epicenter of *Tobrilus* speciation.

The genus *Tobrilus* is considered a basic form for the whole Tobrilidae family. According to Hodda (2011), the world tobrilid fauna includes 162 species in 10 genera. At present, Baikal is the home of 43 species of the Tobrilidae family, i.e., ¼ of the world fauna (Naumova & Gagarin 2019).

The authors believe that the knowledge on the species diversity of Baikal representatives of the genus is far from complete and that new exciting contributions are to be expected. Use of molecular-genetic approaches would enable the researchers to highlight the phylogeny of this group and estimate the evolution rate within the genus. These are the objectives of future investigations.

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