TWO NEW NEMATODE SPECIES OF THE GENUS *EUTOBRILUS* (NEMATODA, TRIPLONCHIDA) FROM LAKE BAIKAL (RUSSIA)

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This paper describes and illustrates two new nematode species of the genus *Eutobrilus* found in Lake Baikal. *Eutobrilus tsalolikhini* sp. nov. is most similar to *E. mirandus* and *E. obesus* in body size and spicules length. It differs from *E. mirandus* in its thicker body, longer outer labial setae, larger stoma, and more supplements. It differs from *E. obesus* in a shorter tail, farther location of vulva from the anterior body end, longer outer labial setae, and more supplements. *Eutobrilus olkhonensis* sp. nov. is most similar to *E. fortis* and *E. godlewskii* in body size and supplements location but differs from both species in a thicker body, longer tail in males, shorter spicules, and fewer supplements.

Key words: diversity, free-living nematodes, morphology, taxonomy, Tobrilidae

Introduction

Lake Baikal is a fascinating freshwater body that is known throughout the world. Its shores and pure crystal clean water attract many tourists as well as experts and researchers. The ancient age of Lake Baikal (25-30 million years), the enormous depths of the water body (1637-1642 m) 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. The unique environments of Lake Baikal inevitably provided favourable conditions for the formation of an abundant and diverse fauna, including over 2600 species with a high level of endemism (over 56%) (Timoshkin, 2001). According to the latest records, the fauna of free-living nematodes comprises 116 species, belonging to 32 genera, 16 families and eight orders (Naumova & Gagarin, 2019). These species are found on rocky, sandy, and silty substrates, occupying all depths from the splash zone (above the water edge) to the deep zones. In terms of abundance, nematodes often dominate other benthic groups. Eutobrilus Tsalolikhin, 1981 is one of the most well-known and diverse genera of the family Tobrilidae. At present, it contains 36 valid species (Andrássy, 2007; Gagarin & Naumova, 2011a,b, 2012, 2018; Naumova & Gagarin, 2021). In Lake Baikal, 17 valid species (including the new species described below) were identified, namely E. affectiosus Shoshin, 1988, E. anguiculus (Tsalolikhin, 1977), E. assimulatus Shoshin, 1988, E. ayaensis Gagarin & Naumova, 2018, E. brzeskii Gagarin & Naumova, 2012, E. differtus Shoshin, 1988, E. fortis (Tsalolikhin, 1972), E. grandipapillatus (Brakenhoff, 1914), E. godlewskii Naumova & Gagarin, 2021, E. longicaudatoides Gagarin & Naumova, 2011, E. mirabilis (Shoshina, 2003) Andrássy, 2007, E. mirandus Gagarin & Naumova, 2011, E. obesus Gagarin & Naumova, 2012, E. peregrinator Tsalolikhin, 1983, E. prodigious Shoshin, 1988, E. selengaensis (Tsalolikhin, 1977), E. stefanskii Gagarin & Naumova, 2018. The aim of this paper is to describe two new nematode species inhabiting Lake Baikal.

Material and Methods

The nematodes were collected in the littoral zone of Lake Baikal from the following sites: central basin of Lake Baikal (Maloye More Strait), Elginsky Bay (53.154010° N, 107.174021° E), 30-cm depth, sand, collected on 16.07.2018; central basin of Lake Baikal (Maloye More Strait), Olkhon Island, near Saraysky Beach (52.12865°-53.12670° N, 107.21023°-107.20789° E), 15-20-m depth, sand with mica, collected on 17.06.2021. The samples contained numerous free-living nematodes, including both species described herein. Nematodes were preserved in 4% formaldehyde, tinted with Rose Bengal dye and mounted in glycerin on permanent slides (Williams & Williams, 1974; Ryss, 2003). All observations were made with the 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 programme for analysis and documentation of images from the preparations.

Results and Discussion

Phylum Nematoda Potts, 1931 Class Enoplea Inglis, 1983 Order Triplonchida Cobb, 1920 Family Tobrilidae de Coninck, 1965 Genus *Eutobrilus* Tsalolikhin, 1981

Type species. Eutobrilus grandipapillatus (Brakenhoff, 1914) Tsalolikhin, 1981 – Trilobus grandipapillatus Brakenhoff, 1914.

Diagnosis. It is made according to Andrássy (2007) and Holovachov & Shoshin (2014). The body length varies from 1.5 mm to 4.7 mm. Cuticle is smooth or very finely annulated. Buccal cavity is cup- or funnel-shaped, with an overlapping subventral pocket and teeth close to each other. Vagina is moderately strong. Spicules are comparatively short. Supplements are of 5 to 9 (rarely 13), echinate, protrusible; the first and last supplements are generally smaller than the others; two posterior supplements are often more remote from the others.

Taxonomy

Eutobrilus tsalolikhini Naumova & Gagarin sp. nov.

Type material. Holotype is male (slide reference number: 1506-3); two paratypes are males and two paratypes are females (slides reference number: 1506-2, 1506-5, 1509-2, 1509-3); they are deposited in the Helminthological Museum collection of the Centre for Parasitology, A.N. Severtsov Institute of Ecology and Evolution of the RAS (Moscow, Russia). Six paratypes are males and eight paratypes are females (slides reference number: 1504-1, 1504-2, 1504-3, 1504-4, 1504-5, 1505-4, 1505-5, 1508-1, 1508-2, 1508-3, 1508-4, 1508-5, 1516-4, 1516-5); they are deposited in the Limnological Institute, Siberian Branch of the RAS (Irkutsk, Russia).

Type locality and habitats. Russia, central basin of Lake Baikal (Maloye More Strait), Elginsky Bay. Samples were collected on 16.07.2018 (leg. T.V. Naumova). Individuals of the new species were found at 30-cm depth.

Etymology. The species is named in honour of the Russian scientist, nematologist, Professor, Dr. Sc. Semyon Ya. Tsalolikhin.

Description of males. Body is comparatively long and thin (Table 1, Fig. 1, Fig. 2). Cuticle is finely annulated; 2.0-µm thick. Body diameter at the pharynx base is 2.3-2.6 times wider than the labial region. Crystalloids are numerous. Somatic setae are rare and short, 4-5 µm long. Labial region is slightly offset from the adjacent body; lips are well developed. Six inner labial sensillae are papilliform. Six outer labial sensillae are in the shape of smooth, nonarticulated setae, 18-21 µm long, 51-62% of the labial region width. Four cephalic sensillae are in the shape of thin and smooth setae are 10–12 µm long. Both circles of setae are drawn close together. Cheilostom is of average size. Buccal cavity is spacious, funnel-shaped, with thick walls. Dorsal pocket and its tooth are absent. Subventral pockets are located close to each other. Stoma is 1.0–1.1 times longer than labial region width. Amphidial fovea is cup-shaped, opening at the level of the buccal cavity. Pharynx is muscular, comparatively long, gradually expanding along the entire length. Cardial glands are large, rounded, $20-22 \ \mu m$ in diameter. Ventral gland, its canal, ampulla, and excretory pore are not seen. Testes are paired, opposed, located to the left of the intestine; anterior testis is outstretched, posterior testis is reflexed. Vas deferens is well developed. Spicules are slender, slightly curved, short, 1.2–1.4 times longer than the cloacal body diameter, apically bifurcate. Gubernaculum is in the shape of a straight «gutter», 27–30 µm long. Precloacal supplements are 8-11 in number, echinate, protrusible. Supplementary pads are armed with small thorns and one longer thorn. Ampullae contents are at the top of ampullae. Supplements are approximately identical in size and located almost equidistant from each other. The supplement closest to cloaca is at 50-82 µm away from it; supplement row is 450-650 µm long. Tail is slender, elongate-conical. Caudal glands are well developed; spinneret is present. Subterminal seta is absent.

Description of females. General morphology is similar to that of males in the structure of the cuticle and anterior body. Cardia is small, surrounded by three round glands. Prerectum is not observed. Rectum length is equal to or slightly greater than anal body diameter. Reproductive system is didelphic, amphidelphic. Ovaries are located to the left of the intestine, reflexed and comparatively short. Oocytes are numerous. Vulva is a transverse slit and equatorial. Vulval lips are not sclerotised and not protruding outside the body contour. Cuticular wrinkles near the vulva and vulva glands are not seen. Vagina is short, with thin walls. Uterus contains numerous spermatozoa and 2–7 eggs, measuring 70–75 \times 42–62 μm . Tail is slender, long, elongate-conical. Caudal glands are well developed. Subterminal seta is absent.



Fig. 1. Holotype male and paratype female of *Eutobrilus tsalolikhini* sp. nov. Designations: A – Male anterior end; B – Supplement; C – Vulva region; D – Male tail; E – Female tail; F – Female reproductive system. Scale bars: A = 25 μ m; B = 15 μ m; C = 120 μ m; D, E = 45 μ m; F = 200 μ m.

Table 1. Morphometrics of males and females of Eutobrilus tsalolikhini sp. nov.

		Paratypes				
Characters	Holotype male	Males $(n = 8)$		Females $(n = 10)$		
		range	mean	range	mean	
<i>L</i> , μm	2397	2158–2433	2313	2442-4120	2805	
a	31	25-31	28	20-40	26	
b	4.7	4.2–4.7	4.5	4.2–5.5	4.5	
с	15.0	15.0-18.3	16.8	12.7–15.9	14.2	
<i>c</i> ′	3.5	3.0-3.5	3.1	3.0-5.0	4.0	
V, %	-	_	-	46-51	48	
Labial region diameter, µm	33	33–36	34	39–45	42	
Mid-body diameter, µm	78	75–90	84	80-125	116	
Anal or cloacal body diameter, µm	45	40-47	44	44-65	50	
Stoma (buccal cavity) length, µm	35	33–36	35	40-45	42	
Outer labial setae length, µm	20	18-21	20	20-22	21	
Cephalic setae length, µm	12	10-12	11	10-12	11	
Distance from the anterior body end to nerve ring, µm	129	110–135	126	120-151	132	
Pharynx length, µm	512	488-550	519	558–745	621	
Distance from the pharynx base to the vulva, µm	-	_	-	538–1288	726	
Distance from the pharynx base to the cloaca, µm	1725	1535-1750	1655	_	_	
Distance from the vulva to anus, µm	-	-	-	1025-1825	1260	
Tail length, μm	160	130–161	138	168–262	198	
Spicules length, µm	58	53–58	56	_	_	
Gubernaculum length, µm	28	27-30	28	_	_	
Number of supplements	9	8-11	9	_	_	

Note: L – body length; a – ratio of body length to body width in its middle part; b – ratio of body length to pharynx length; c – ratio of body length to tail length; c' – ratio of tail length to body width at the anus or cloacal region; V – ratio of the distance from the anterior end of the body to the vulva to the total body length.



Fig. 2. *Eutobrilus tsalolikhini* sp. nov., light micrographs. Designations: A – Entire male; B – Entire female; C – Male anterior end; D – Male head; E – Female head; F – Female vulva region; G – Male supplement region; H – Male posterior end; I – Female posterior end. Scale bars: B = 200 μ m; A, C = 100 μ m; F = 50 μ m; E, H, I = 20 μ m; D, G = 10 μ m.

Diagnosis. Eutobrilus tsalolikhini sp. nov. is characterised by a large body (2158–4120 µm long). Cuticle is finely annulated. Crystalloids are numerous in the body cavity. Inner labial sensillae are papilliform. Six outer labial sensillae are in the shape of smooth, non-articulated setae, 18-22 µm long, 47-62% of labial region width. Four cephalic sensillae in the shape of thin and smooth setae are $10-12 \ \mu m$ long. Buccal cavity is spacious funnel-shaped. Dorsal pocket and its tooth are absent. Subventral pockets are located close to each other. Spicules are slender, slightly curved, 53–58 µm long, 1.2–1.4 long as the cloacal body diameter. Gubernaculum is in the shape of straight «gutter». Precloacal supplements are 8-11 in number, echinate, protrusible. Ampullae contents are at the top ampullae. Tail is slender, long, elongateconical. Subterminal seta is absent.

Differential diagnosis. *Eutobrilus tsalolikhini* sp. nov. is close to *E. mirandus* and *E. obesus* in body size and spicules length. The new species differs from *E. mirandus* by having a thicker body in males (a = 25-

31 vs. $\bigcirc a = 32-45$), longer outer labial setae (18–22 µm vs. 10–13 µm) and stoma (33–45 µm vs. 25–31 µm), more posterior vulva (V = 46–51% vs. V = 25–31%), number of supplements (8–11 vs. 6–7) and a different tail shape (Gagarin & Naumova, 2011a). *Eutobrilus tsalolikhini* sp. nov. differs from *E. obesus* in its shorter tail (c = 12.7-18.3 vs. c = 6.7-9.3), the more posterior location of the vulva from the anterior body end (V = 46-51% vs. V = 40-46%), longer outer labial setae (18–22 µm vs. 8–10 µm), number of supplements (8–11 vs. 7–8), the thicker tail (vs. very thin tail in *E. obesus*) (Gagarin & Naumova, 2012).

Eutobrilus olkhonensis Naumova & Gagarin sp. nov. **Type material.** Holotype is male (slide reference number: 1668-3); two paratypes are males and four paratypes are females (slides reference number: 1668-1, 1668-2, 1706-1, 1706-2, 1706-3, 1706-4); they are deposited in the Helminthological Museum collection of the Center for Parasitology, A.N. Severtsov Institute of Ecology and Evolution of the RAS (Moscow, Russia). Six paratypes are males and six paratypes are females (slides reference number: 1667-1, 1667-2, 1667-3, 1667-4, 1667-5, 1669-1, 1669-2, 1708-1, 1708-3, 1710-4, 1723-1, 1723-4); they are deposited in the Limnological Institute, Siberian Branch of the RAS (Irkutsk, Russia).

Type locality and habitats. Russia, central basin of Lake Baikal (Maloye More Strait), Olkhon Island, near Saraysky Beach. Samples were collected on 17.06.2021 (leg. T.V. Naumova). Individuals of the new species were found at 15–20-m depth.

Etymology. The specific epithet means from «Olkhon Island», the type locality name.

Description of males. Body is comparatively short and thick (Table 2, Fig. 3, Fig. 4). Cuticle is faintly annulated, 2-µm thick. Somatic setae are sparse, short, 4–7 µm long. Crystalloids are absent. Labial region is slightly offset from the adjacent body; lips are well developed. Six inner labial sensillae are papilliform. Six outer labial sensillae in the shape of smooth non-articulated setae are 7-8 µm long, 26-30% of labial region width. Four cephalic sensillae are in shape of smooth setae. Cheilostom is of average size. Buccal cavity is spacious, funnel-shaped, with thick walls. Dorsal pocket and its tooth are absent. Subventral pockets are located close to each other or less often overlap each other. Stoma is 1.1–1.2 times longer than labial region width. Amphidial fovea is cup-shaped, opening at the level of the buccal cavity. Pharynx is muscular, comparatively long, gradually expanding along the entire length. Cardial glands are large, rounded. Ventral gland, its canal ampulla and excretory pore are not seen. Testes are paired, opposed, situated to the left of the intestine; anterior testis is outstretched, posterior testis reflexed. Vas deferens is well developed. Spicules are slender, slightly curved, 55–58 μ m long, 1.1–1.3 times longer than the cloacal body diameter. Gubernaculum is in the shape of "gutter", 25–27 μ m long. Precloacal supplements are echinate, located in two groups (2 + 3), supplement closest to the cloaca smaller than others. Supplementary pads are armed with small thorns and one longer and thicker thorn. Ampulla contents are at the top of the ampullae. Tail is slender, long, elongate-conical, with subterminal seta. Caudal glands and spinneret are well developed.

Description of females. General morphology is similar to that of males in the structure of cuticle and the anterior body end. Six inner labial sensillae are papilliform. Six outer labial sensillae are in the shape of smooth, non-articulated setae, $8-10 \mu m$ long; four cephalic sensillae are smooth, $4-5 \mu m$ long. Cardia is small, surrounded by three round glands. Prerectum is not observed. Rectum length is equal to or slightly less than anal body diameter. Reproductive system is didelphic. Ovaries are located to the left of intestine, reflexed, comparatively short. Oocytes are numerous. Vulva is transverse slit, located in mid-body. Vulval lips are not sclerotised, not protruding outside the

body contour. Cuticular wrinkles near vulva and vulva glands are not seen. Vagina is comparatively long, with thick walls. Uterus contains numerous spermatozoa and 1–2 eggs, measuring $58-75 \times 35-55 \mu m$. Tail is slender, elongate-conical, with subterminal seta. Caudal glands and spinneret are well developed.

Diagnosis. Eutobrilus olkhonensis sp. nov. is characterised by its small size (body 1251-1570 µm long). Cuticle is faintly annulated. Crystalloids are absent. Inner labial sensillae are papilliform. Six outer labial sensillae are in the shape of smooth, non-articulated setae, 7-10 µm long, 21-30% of labial region width. Four cephalic sensillae are in the shape of thin and smooth setae 4-5 µm long. Buccal cavity is spacious, funnel-shaped. Dorsal pocket and its tooth are absent. Subventral pockets are located close to each other or less often overlap each other. Spicules are slender, slightly curved, short (55-58 µm), 1.1-1.2 times longer than cloacal body diameter. Gubernaculum is in the shape of «gutter» with curved distal end. Precloacal supplements are echinate are in two groups (2+3) and the supplements closest to the cloaca smaller than the outers. Supplementary pads are armed with small thorns and one longer and thicker thorn. Ampulla contents are at its top. Tail is slender, elongate-conical, with subterminal seta.



Fig. 3. *Eutobrilus olkhonensis* sp. nov. Designations: A – Male anterior end; B – Supplement; C – Female tail; D – Vulva region; E – Male posterior end; F – Female reproductive system. Scale bars: A = 20 μ m; B = 15 μ m; C = 50 μ m; D, F = 100 μ m; E = 80 μ m.

	Holotype male	Paratypes				
Characters		Males $(n = 8)$		Females $(n = 10)$		
		range	mean	range	mean	
<i>L</i> , μm	1456	1373-1597	1461	1251-1570	1394	
a	19	17-21	18	12–19	15	
b	4.5	4.3-4.8	4.5	3.9–4.4	4.1	
С	10.2	8.7-10.4	9.8	6.7-8.0	7.3	
<i>c'</i>	2.9	2.6-3.4	3.0	3.1–4.3	3.8	
V, %	-	_	-	46-51	49	
labial region diameter, μm	27	26–29	28	30–38	33	
Mid-body diameter, µm	75	73–87	79	73–122	96	
Anal or cloacal body diameter, µm	50	45-54	49	45-65	51	
Stoma (buccal cavity) length, µm	30	28-31	29	30–37	33	
Outer labial setae length, µm	7	7–8	7	8-10	9	
Cephalic setae length, µm	4	4	4	4–5	4	
Distance from the anterior body end to nerve ring, µm	100	100-125	111	110–130	116	
Pharynx length, µm	325	288-388	321	310-380	337	
Distance from the pharynx base to the vulva, µm	-	_	—	300-425	349	
Distance from the pharynx base to the cloaca, µm	988	950-1105	990	-	_	
Distance from the vulva to anus, µm	-	_	-	458–580	518	
Tail length, μm	143	135-167	149	175-210	191	
Spicules length, µm	56	55-58	56	-	_	
Gubernaculum length, µm	25	25-27	26	-	_	
Number of supplements	5	5	5	-	_	

Table 2. Morphometr	rics of males	and females	of Eutobrilus	olkhonensis sp.	nov.
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Note: L – body length; a – ratio of body length to body width in its middle part; b – ratio of body length to pharynx length; c – ratio of body length to tail length; c' – ratio of tail length to body width at the anus or cloacal region; V – ratio of the distance from the anterior end of the body to the vulva to the total body length.



Fig. 4. *Eutobrilus olkhonensis* sp. nov., light micrographs. Designations: A – Entire male; B – Entire female; C – Male anterior end; D – Male head; E – Female head; F – Female vulva region; G – Male cloaca region; H – Male posterior end; I – Female posterior end. Scale bars: A, B = 100 μ m; F = 50 μ m; C, H, I = 20 μ m; D, E, G = 10 μ m.

Differential diagnosis. Eutobrilus olkhonensis sp. nov. is most similar to E. fortis (Tsalolikhin, 1972) and E. godlewskii Naumova & Gagarin, 2021 in body size and supplement arrangement. The new species differs from E. fortis in its thicker body (a = 12-21 vs. a = 22-35), longer tail in males (A) c = 8.7-10.4 vs. c = 10.4-14.0, longer outer labial setae (7-10 µm long vs. 2 µm long), shorter spicules (55-58 µm long vs. 62 µm long), and fewer supplements (5(2 + 3) vs. 6(2 + 4)) (Tsalolikhin, 1972). Eutobrilus olkhonensis sp. nov. differs from *E. godlewskii* by having a thicker body (a = 12-21) vs. a = 23-34), longer tail in males (c = 8.7-10.4vs. $\bigcirc c = 12.6-14.5$), shorter spicules (55–58 µm vs. $63-70 \mu m$), and fewer supplements (5(2+3) vs. 6-7(2+4-5)) (Naumova & Gagarin, 2021).

The combination of the shape and structure of stoma (pockets less separated from each other), the simple vagina and the protrusible supplements characterise the genus *Eutobrilus* within the family Tobrilidae. Regarding the number of species, it is the largest genus in this family (Andrássy, 2007). The genus occurs in Europe, Asia, Africa, North America, Australia, and Antarctica. In the genus *Eutobrilus*, more than half of the species (19 of 36) are inhabitants of Lake Baikal, where they occur in the depth range of 0–1610 m as well as in the splash zone (0.5 m above the water edge). Most of the species (15 of 19) are endemic (Naumova & Gagarin, 2019).

We believe that the knowledge on the species diversity of Baikal representatives of the genus *Eu*-

tobrilus is far from complete awaiting new exciting contributions. The use of molecular-genetic approaches would enable 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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ДВА НОВЫХ ВИДА НЕМАТОД РОДА *EUTOBRILUS* (NEMATODA, TRIPLONCHIDA) ИЗ ОЗЕРА БАЙКАЛ (РОССИЯ)

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В статье приведены иллюстрированные описания двух новых видов нематод рода *Eutobrilus*, найденные в оз. Байкал. *Eutobrilus tsalolikhini* sp. nov. наиболее близок к *E. mirandus* и *E. obesus* по длине тела и длине спикул. Он отличается от *E. mirandus* более толстым телом, более длинными наружными губными щетинками, более крупной стомой и большим количеством супплементов. От *E. obesus* отличается более коротким хвостом, дальним положением вульвы от переднего конца тела, более длинными наружными губными щетинками, большим количеством супплементов. *Eutobrilus olkhonensis* sp. nov. наиболее близок к *E. fortis* и *E. godlewskii* по длине тела и расположению супплементов, но отличается от обоих видов более толстым телом, более длинным хвостом у самцов, короткими спикулами и меньшим количеством супплементов.

Ключевые слова: Tobrilidae, морфология, разнообразие, свободноживущие нематоды, таксономия