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RESEARCH ARTICLE

## Two new species of the family Tobrilidae (Nematoda: Triplonchida) from West Bengal, India

## Два новых видах семейства Tobrilidae (Nematoda: Triplonchida) из Западной Бенгалии, Индия

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**Abstract.** The article presents the first records of the genera *Epitobrilus* Tsalolikhin, 1981 and *Eutobrilus* Tsalolikhin, 1981 (Triplonchida: Tobrilidae), along with the description of two new species, *Epitobrilus brachystomus* **sp. nov.** and *Eutobrilus minutus* **sp. nov.**, from paddy fields in West Bengal, India. *Epitobrilus brachystomus* **sp. nov.** differs from the congeners in having the relatively shorter stoma, distinct crustaformeria cells at the oviduct-uterus junction, five to six setae including a subterminal one in the tail region, and the longer tail with a distinct cuticular protrusion at the tip. *Eutobrilus minutus* **sp. nov.** can be distinguished from *Eu. husmanni* (Altherr, 1958) by the differences in the body size, the distance between the stomatal pockets, the tail length, and the presence of a subterminal setae. From another closely related species, *Eu. nothus* Gagarin, 1989, it differs in the ratio of the body length to the tail length (c), the tail length relative to its diametre (c'), the distance from the vulva to the anterior body end (V %), and in the distance between the stomatal pockets.

**Резюме.** Впервые в Западной Бенгалии, Индия, в почве рисовых полей обнаружены нематоды родов *Epitobrilus* Tsalolikhin, 1981 и *Eutobrilus* Tsalolikhin, 1981 (Triplonchida: Tobrilidae). В статье приводятся описания двух новых видов *Epitobrilus brachystomus* **sp. nov.** и *Eutobrilus minutus* **sp. nov.** Первый вид отличается от других видов рода относительно более короткой стомой, отчетливой клеточной скорлуповой железой (крустаформерией) в месте соединения яйцевода и матки, 5-6 щетинками, включая субтерминальную в зоне хвоста, и более длинным хвостом с отчетливым кутикулярным выступом на его кончике. *Eutobrilus minutus* **sp. nov.** отличается от *Eu. husmanni* (Altherr, 1958) длиной тела, расстоянием между карманами стомы, длиной хвоста и наличием субтерминальной щетинки. От другого близкого вида *Eu. nothus* Gagarin, 1989 он отличается значениями индексов с и с', V %, а также расстоянием между карманами стомы.

**Keywords:** taxonomy, free-living nematodes, stomatal pockets, subterminal setae, *Epitobrilus, Eutobrilus*, new records, new species

**Ключевые слова:** таксономия, свободноживущие нематоды, карманы стомы, субтерминальные щетинки, *Epitobrilus, Eutobrilus*, новые находки, новые виды

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## Introduction

The soil-inhabiting nematode genera *Epitobri*lus Tsalolikhin, 1981 and Eutobrilus Tsalolikhin, 1981 are classified within the family Tobrilidae, under the order Triplonchida. Tsalolikhin distinguished Epitobrilus from Tobrilus Andrassy, 1959, based on a single stomatal pocket with an anterior tooth, spicules lacking proximal dilation, and echinate, submerged male supplements (Zulllini & Villa, 2005). Subsequent revisions by Zullini (2006) characterised the genus as having two stomatal pockets; the anterior pocket is connected to the base of the buccal cavity and contains a tooth, while the posterior pocket also has a distinct tooth. The distance between the teeth in the anterior and posterior pockets ranges from eight to 20 µm (Abebe et al., 2006). To date, Epitobrilus comprises 13 recognised species (Naumova & Gagarin, 2017; Nemys..., 2023), with records from Europe, Russia, Japan, East Africa, southern Argentina, and Canada (Andrassy, 1963; Tsalolikhin, 2001a; Zullini, 2006; Naumova & Gagarin, 2017).

The genus *Eutobrilus* is the most speciesdiverse genus within the family Tobrilidae (Naumova & Gagarin, 2023; Nemys..., 2023; Zullini & Villa, 2005). Tsalolikhin's separation of *Eutobri*lus from Tobrilus Andrassy, 1959 was based on several distinguishing characters, including the well-developed vaginal musculature, the cardiac glands, and six to nine echinate male supplements (Zulllini & Villa, 2005). Further revisions by Zullini (2006) characterised this genus by the presence of long anterior setae (40-60%) of the head diametre), short spicules (approximately 1/40th to 1/50th of the body length), and nearly overlapping stomatal pockets with two teeth, spaced 2 to 9 µm apart. Currently, Eutobrilus comprises 38 recognised species (Naumova & Gagarin, 2023; Nemys..., 2023). These species are recorded from lakes and rivers of Europe and Siberia. Iran, Mongolia, China, Japan, North and South Africa, Tanzania, New Guinea, Antarctic, North America, and Argentina (Swart & Heyns, 1988; Zullini, 2006; Gagarin, 2009; Gagarin & Naumova, 2011a, 2011b, 2012, 2018; Shahabi et al., 2017; Yunliang & Coomans, 2000; Naumova & Gagarin, 2023).

There have been no previous records of these two genera from India. In the Indian Tobrilidae fauna, *Brevitobrilus delhiensis* Sultana et Devi, 2015, the only species previously recorded (Sultana & Devi, 2015), has been complemented by another genus and species, *Tetratobrilus dentatus* Das, Das, Ghosh Jana et Ghosh, 2024, recently described from this region (Das et al., 2024).

In this study, we present the first records of the genera *Epitobrilus* and *Eutobrilus* from India, along with the descriptions of two new species, *E. brachystomus* **sp. nov.** and *Eu. minutus* **sp. nov.** 

## Materials and methods

Soil samples were collected from paddy fields in Lalgarh, Jhargram District in the southwestern part of West Bengal, India, to survey the diversity of soil nematodes. The samples were collected through sieving using the Baermann funnel technique (Viglierchio & Schmitt, 1983) with some modifications following Jana et al. (2010). Subsequently, extracted nematodes were fixed in a hot diluted formalin acetic acid (FAA) fixative (10 parts 40% formalin, one part acetic acid, and 89 parts distilled water). The specimens were then placed in a desiccator chamber to dehydrate and processed to prepare permanent glycerol preparations, which were used for species identification.

For morpho-taxonomic studies, light microscopy was employed. Line drawings were created either using a Dewinter microscope fitted with a drawing tube or digitally drawn from captured images using Adobe Photoshop 2021 software. Imaging was performed using a Zeiss Axio Vert. A1 optical microscope equipped with Zeiss Zen 2 Pro software and a Zeiss Axiocam 305 Color camera.

When measuring curved structures, the measurements were taken along the arc.

The holotypes and some paratypes of the new species are deposited in the Nemathelminthes Section of the National Zoological Collections of the Zoological Survey of India, Kolkata, West Bengal, India (NZC). Other paratypes are deposited in the Zoology Museum of Midnapore College, Midnapore, India (ZMMC).

#### Results

Order Triplonchida Cobb, 1919

Family Tobrilidae Filipjev, 1918

Subfamily Tobrilinae Filipjev, 1918

Tribe Epitobrilini Tsalolikhin, 2001

Genus Epitobrilus Tsalolikhin, 1981

#### *Epitobrilus brachystomus* sp. nov. (Figs 1, 2)

*Holotype.* Female, **India**, "West Bengal State, Lalgarh Vill., Jhargram Distr., 5–6 cm beneath surface, paddy fields, 22°35′28.5″N, 87°3′15.5″E, 4th March, 2022, coll. T. [Tandrani] Das" (NZC, slide No. WN4405/2).

*Paratypes.* Same data as for holotype: 1 female, slide No. WN4405/1 (NZC), 2 females, slide No. WN4405/2 (NZC), 3 females (ZMMC).

Description. Female (measurements in Table 1). Following fixation, body slightly curved ventrally (Figs 1A, 2A); maximum body diametre at vulva or at pharyngo-intestinal junction. Cuticle thick and appearing smooth under light microscope. Labial sensilla in two concentric circles. Six lips with six papilliform inner labial sensilla. Six outer labial sensilla, each 4 µm long (27% of head diametre), and two pairs of slightly smaller cephalic sensilla. Amphid short, funnel-shaped (Figs 1F, 2F), 4 µm in diametre, located at the level of second stomatal pocket, 17 µm distant from anterior end. Body shorter than 1.1 mm. Lip region flat, continuous to end of body. Cheilostome distinct (Figs 1B', 2B), with sclerotised lining. Funnel-shaped stoma, 22 µm in length (including pockets), 5– 8 µm in diametre. Two subventral pockets located posterior to stoma (Figs 1B, 1B', 2B). Anterior pocket confluently attached to base of stoma, containing a single tooth. Posterior pocket connected to anterior pocket through narrow canal, containing a single tooth,  $5-8 \mu m$  distant from anterior pocket. Teeth apices  $5-8 \mu m$  apart. Nerve ring prominent, muscular band-like, at 73-83 µm from anterior end of body. Pharynx cylindrical, muscular, 155–165 µm long. Cardia simple, with cardiac gland cells (Figs 1C, 2C). Amphidelphic-didelphic gonad with epithelial cell lining (Figs 1D, 2D). Anterior and posterior gonads similar in size. Ovaries with lateral reflexion, ranging in length from 200 to 240 µm; thin-walled sac with distal germination region and prominent oocytes at basal part. Oviducts on either side similar in size, measuring 67–71 µm. Sphincter-like columella of irregularly arranged cells (crustaformeria) at oviduct-uterus junction. Vagina strongly muscular. Vaginal zonations distinct (Fig 1D', 2D'), short pars distalis vaginae opening through a narrow orifice (vulval pore), pars refringens vaginae guarded with thick cells, pars proximalis vaginae muscular, wider than long. Vulva distinct, with transverse slit-like opening, located at 40–44% of body length. Anus slit-like. Tail long, not filiform (Figs 1E, 2E), 115-128 µm long, with bluntly rounded tip bearing distinct cuticular bulb-like protrusion (Figs 1E', 2E'). Tail regions with five to six setae, including a subterminal one. Subterminal setae on dorsal side, 9 µm distant from tail terminus.

#### Male not found.

**Comparison.** Among the 13 known species of Epitobrilus, E. brachystomus sp. nov. is most closely related to E. flagellatus (Andrássy, 1963) in the body length (L) (0.93-1.01 mm vs. 0.96-1.04 mm), the ratio of the body length to the body diametre ('a') (23.2–28.9 vs. 27–34), the ratio of the body length to the distance from the pharyngo-intestinal valve to the anterior body end ('b') (4.8-5.1 vs. 4.2-5.0), and the distance from the vulva to the anterior body end ('V') (40-44 % vs. 43.0-45.2%) (Naumova & Gagarin, 2017), but notably differs in the length and structure of the stoma, as well as in the tail length. In E. brachystomus sp. nov., the tail is long, measuring  $115-128 \mu m$ , which is 1/8 of the body length ('c': 7.5-8.2), while in E. flagellatus, it is only

**Fig. 1.** Photos of *Epitobrilus brachystomus* **sp. nov.**, holotype, female (A–E') and paratype, female (F). **A**, body; **B**, anterior body end showing buccal cavity; **B'**, magnified view showing buccal cavity and pockets with teeth; **C**, pharyngo-intestinal junction; **D**, amphidelphic gonads with vulva; **D'**, magnified view showing zonations of vagina: pars distalis (*a*), pars refringens (*b*), pars proximalis vaginae (*c*); **E**, tail region with anus; **E'**, magnified view of tail region showing terminal features; **F**, anterior end showing amphid. Scale bars: 100  $\mu$ m (A), 20  $\mu$ m (B, C, D, D', E), and 10  $\mu$ m (B', E', F).





**Fig. 2.** Drawings of *Epitobrilus brachystomus* **sp. nov.**, holotype, female (A–E') and paratype, female (F). **A**, body; **B**, anterior body end showing buccal cavity and pockets with teeth; **C**, pharyngo-intestinal junction; **D**, gonads with vulva; **D'**, magnified view showing the zonations of vagina: pars distalis (*a*), pars refringens (*b*), pars proximalis vaginae (*c*); **E**, tail region with anus; **E'**, magnified view of the tail region showing terminal features; **F**, anterior end showing amphid. Scale bars: 100 μm (A), 20 μm (B, C, D, D', E), 5 μm (E'), and 10 μm (F).

1/4 of the body length ('c': 3.4–3.9). The stoma of *E. brachystomus* **sp. nov.** is significantly shorter (8–10 µm in length) than that of *E. flagellatus* (27–30 µm) (Naumova & Gagarin, 2017). The

stoma of the new species is also remarkably shorter compared to other species of *Epitobrilus*, namely *E. allophysis* (Steiner, 1919) (36.0–38.5 μm; Yunliang & Coomans, 2000), *E. interstitialis* Naumova **Table 1.** Measurements of *Epitobrilus brachystomus* **sp. nov.** All measurements are in  $\mu$ m and in the form: mean  $\pm$  s.d. (range).

	Holotype female	Paratypes, females (n=6)	
Characters		Mean±SD	Range
L (total body length)	960	968±41	930-1010
<b>a</b> (body length / maximum body diametre)	25.6	$25.90{\pm}2.86$	23.2 - 28.9
${\bf b}$ (body length / distance from anterior body end to pharyngo-intestinal junction)	5.1	4.93±0.15	4.8–5.1
c	7.5	$7.90 \pm 0.36$	7.5 - 8.2
c'	5.6	$5.37 {\pm} 0.25$	5.1 - 5.6
V (%)	40	42.30±1.83	40.0 - 44.0
Head diametre	15	$15.00 {\pm} 0.00$	15
Maximum body diametre	38	$37.50 \pm 2.50$	35 - 40
Distance from nerve ring to anterior body end	78	$77.50 {\pm} 5.00$	73-83
Distance from anterior body end to pharyngo-intestinal junction	185	193.33±10.41	185-205
Length of buccal cavity	8	$8.33 \pm 1.44$	8-10
Diametre of buccal cavity	5	$5.83 \pm 1.44$	5-8
Distance between stomatal pockets (between margins)	5	$5.83 \pm 1.44$	5-8
Distance from first (anterior) stomatal pocket to anterior body end	10	$10.00 \pm 0.00$	10
Distance from second (posterior) stomatal pocket to anterior body end	15	15.83± 1.44	15–18
Distance from first (anterior) stomatal pocket teeth to anterior body end	10	$10.00 \pm 0.00$	10
Distance between teeth (apices) of two pockets	5	$5.83 \pm 1.44$	5-8
Pharynx length	155	$160.83 \pm 5.20$	155-165
Cardia length	13	$15.00 \pm 2.50$	13-18
Cardia diametre	18	$19.17 \pm 1.44$	18-20
Distance from vulva to anterior body end	390	$410.00 \pm 30.72$	390-440
Distance from vulva to anus	440	435.83±15.88	420-440
Length of female gonad	220	$220.83 \pm 16.65$	200 - 240
Length of anterior gonad	108	$106.67 \pm 3.82$	103-110
Length of posterior gonad	118	114.17±12.83	100-125
G1(%)	11	$11.00 \pm 0.85$	10-12
G2(%)	12	$11.80 \pm 1.83$	10-13
Total length of vagina	12	$12.36 \pm 0.56$	12-13
Pars proximalis vaginae length	8	$8.02 {\pm} 0.03$	8
Pars refringens vaginae length	2	$2.80 {\pm} 0.34$	2-3
Pars distalis vaginae length	1.6	$1.54{\pm}0.50$	1-2
Tail length	128	121.67±6.29	115-128
Tail diametre at anus	23	$22.50 \pm 0.00$	23

et Gagarin, 2017 (50–63 µm; Naumova & Gagarin, 2017), and *E. setosus* (Altherr, 1963) (18–20 µm; Abebe & Coomans, 1997; Zullini & Villa, 2005). In *E. flagellatus*, there are no caudal setae (setae are located anterior to the anus level), while in E. brachystomus sp. nov., these setae are limited to the caudal region only. Additionally, distinct crustaformeria cells are present at the oviductuterus junction in the new species. The lip-like structures of pars distalis and pars refringens vaginae differentiate new species from the congeners. The new species can be distinguished from another species, E. parvipapillatus (Kreis, 1932)\*, which has similarities in the anterior part of the body, by the absence of the distinct anterior buccal pocket separated from the stoma by a notch (Tsalolikhin, 2001a). The posterior pocket is also well demarcated from the anterior one by a distinct constriction in E. parvipapillatus. Additionally, both E. flagellatus and E. parvipapillatus lack any special cuticular bulge at the tail tip.

**Etymology.** The specific name is derived from the Ancient Greek words  $\beta \rho \alpha \chi \dot{\upsilon} \varsigma$  (short) and  $\sigma \tau \dot{\upsilon} \mu \alpha$  (stoma, mouth).

#### Tribe Tobrilini Filipjev, 1918

Genus Eutobrilus Tsalolikhin, 1981

## *Eutobrilus minutus* sp. nov. (Figs 3, 4)

*Holotype.* Female, **India**, "West Bengal State, Lalgarh Vill., Jhargram Distr., 5–6 cm beneath surface, paddy fields, 22°35′28.5″N, 87°3′15.5″ E, 4th March, 2022, coll. T. [Tandrani] Das" (NZC, slide No. WN4406/1).

*Paratypes*. Same data as for holotype: 1 female (NZC, slide No. WN4406/2), 4 females (ZMMC).

**Description.** *Female* (measurements in Table 2). Cuticle thick, with smooth surface under light microscope. Following fixation, specimens ventrally curved (Figs 3A, 4A). Body shorter than

1.6 mm. Vestibulum low, not much protruding beyond rest of body level. Six inner labial sensilla papilliform, while six outer labial sensilla and four cephalic sensilla thin and smooth. Outer labial sensilla 6 µm long (28% of head diametre), slightly protruding beyond adjacent body curvature. Buccal cavity wide, funnel-shaped, walls moderately sclerotised (Figs 3B, 4B). Stoma with two closely and longitudinally arranged distinct and voluminous subventral pockets. Anterior pocket  $6 \times 5 \,\mu m$ . while, posterior pocket slightly longer ( $8 \times 5 \mu m$ ). Anterior and posterior pockets not overlapping, connected to base of stoma one behind another. Distance from anterior pocket to cephalic end 10-18 µm. Each pocket containing a small tooth, tooth apices 5–10 µm apart. Amphid funnel-shaped (Figs 3B', 4B'), 3 µm in diametre, located at level and lateral to first stomatal pocket, 11 µm distant from anterior end of body. Pharynx muscular. Cardia simple, pharyngo-intestinal junction 15-20 µm long, with prominent cardiac gland cells (Figs 3C, 4C). Reproductive system amphidelphic-didelphic, with epithelial cell lining (Figs 3D, 4D). Ovaries reflexed, 230-500 µm long. Vulva prominent, with small slit-like opening (Figs 3D', 4D'), at 40-46% of body length. Rounded vulval lips lightly sclerotised, not protruding beyond body contour. Pars distalis vaginae short, pars refringens vaginae slightly sclerotised, pars proximalis vaginae joined to voluminous uteri. Uterus on either side connected to oviducts through deep constriction. Anus slit-like. Tail long, 115-163  $\mu$ m, not filiform, with rounded tip (Figs 3E, 4E). Post-anal region with four or six setae, including a subterminal one. Subterminal setae located on dorsal side, 7 µm anterior to tail tip.

Male not found.

**Comparison.** Among the species of *Eutobrilus, Eu. minutus* **sp. nov.** is closely related to *Eu. husmanni* (Altherr, 1958)\*\*, *Eu. nothus* Gagarin, 1989, *Eu. brzeskii* Gagarin et Naumova, 2012,

#### Executive Editor's comments.

<sup>\*</sup> Tsalolikhin (2001a), based on material from Biwa Lake (Japan), considered *Tobrilus parvipapillatus* Kreis, 1932, described from the basin of the Paraguay and Parana rivers, a distinct species in the genus *Epitobrilus* and provided its distinguishing characters in a key; however, other authors (Zullini, 2006; Nemys..., 2024) list it as a species inquirenda.

<sup>\*\*</sup>Tsalolikhin (2001b) considered this species within the genus *Raritobrilus* Tsalolikhin, 1981, but Zullini (2006) placed this generic name in synonymy with *Eutobrilus*, a position supported by Gagarin (2009).



**Fig. 3.** Photos of *Eutobrilus minutus* **sp. nov.**, holotype, female. **A**, body; **B**, anterior body end showing buccal cavity and pockets with teeth; **B'**, anterior body end showing amphid; **C**, pharyngo-intestinal junction; **D**, composite image showing gonads with vulva; **D'**, magnified view showing the zonations of vagina: pars distalis (*a*), pars refringens (*b*), pars proximalis vaginae (*c*); **E**, tail region with anus. Scale bars: 100  $\mu$ m (A), 10  $\mu$ m (B, B'), 20  $\mu$ m (C, E), and 30  $\mu$ m (D, D').

and *Eu. olkhonensis* Naumova et Gagarin, 2023. All these species have either nearly contiguous or non-overlapping, yet extremely proximate, stomatal pockets located one behind another (Zullini & Villa, 2005; Gagarin & Naumova, 2012; Shahabi et al., 2017; Naumova & Gagarin, 2023). Other species of *Eutobrilus* have overlapping stomatal pockets.

*Eutobrilus minutus* **sp. nov.** exhibits similarity to *Eu. husmanni* in the presence of closely located

stomatal pockets and a similar ratio of 'b' (the body length to the distance from the pharyngo-intestinal valve to the anterior body end) (Zullini & Villa, 2005). However, it differs in the shorter stoma (8–15  $\mu$ m vs. 15–18  $\mu$ m) and body (0.94–1.5 mm vs. 1.70–2.48 mm), significantly different ratio of the body length to the body diametre ('a') (20.8– 31.3 vs. 43–55), much shorter tail (115–163  $\mu$ m vs. 193–232  $\mu$ m), smaller ratios of the body length to the tail length (c) (7.1–10.0 vs. 9.2–11.0) and



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**Fig. 4.** Drawings of *Eutobrilus minutus* **sp. nov.**, holotype female. **A**, body; **B**, anterior body end showing buccal cavity and pockets with teeth; **B'**, anterior body end showing amphid; **C**, pharyngo-intestinal junction; **D**, composite image showing gonads with vulva; **D'**, magnified view showing the zonations of vagina: pars distalis (*a*), pars refringens (*b*), pars proximalis vaginae (*c*); **E**, tail region with anus. Scale bars: 100  $\mu$ m (A), 20  $\mu$ m (B, C, E), 10  $\mu$ m (B'), and 30  $\mu$ m (D, D').

**Table 2.** Measurements of *Eutobrilus minutus* **sp. nov.** All measurements are in  $\mu$ m and in the form: mean  $\pm$  s.d. (range).

Character	Holotype female	Paratypes, females (n=5)	
Characters		Mean±SD	Range
L (total body length)	1430	1214±211	943-1590
<b>a</b> (body length / maximum body diametre)	23.8	23.91±2.83	20.8-31.3
${\bf b}$ (body length / distance from anterior body end to pharyngo-intestinal junction)	5.3	4.98±0.47	4.2–5.6
c	8.5	8.71±0.93	7.1 - 10.0
c′	5.5	$5.27 \pm 0.78$	4.3 - 6.6
V (%)	42	$42.69 \pm 2.10$	40-46
Head diametre	23	$21.25 \pm 3.61$	18-28
Maximum body diametre	60	48.83±13.73	40-70
Distance from nerve ring to anterior body end	73	77.71±12.59	60-105
Distance from anterior body end to pharyngo-intestinal junction	260	$241.88 \pm 39.89$	193–310
Length of buccal cavity	10	$10.42 \pm 2.09$	8-15
Diametre of buccal cavity	10	$10.83 \pm 2.68$	8-15
Distance between stomatal pockets (between margins)	8	7.71±1.98	5-13
Distance from first (anterior) stomatal pocket to anterior body end	13	11.25±1.99	10-18
Distance from second (posterior) stomatal pocket to anterior body end	20	18.13±2.85	13–23
Distance from first (anterior) stomatal pocket teeth to anterior body end	13	12.71±2.49	10–18
Distance between teeth (apices) of two pockets	8.0	7.29±1.29	5-10
Pharynx length	210	$171.88 \pm 52.38$	98 - 250
Cardia length	20	17.08±1.79	15.0 - 20.0
Cardia diametre	28	$25.63 \pm 5.75$	18-38
Distance from vulva to anterior body end	600	$519.79 \pm 99.22$	420-730
Distance from vulva to anus	670	$556.25 \pm 107.08$	410-740
Length of female gonad	380	$322.92{\pm}80.40$	230 - 500
Length of anterior gonad	193	$160.63 \pm 48.30$	108 - 270
Length of posterior gonad	185	$162.29 \pm 33.75$	125 - 230
G1(%)	13	$12.99 \pm 1.76$	10-17
G2(%)	13	$13.29 \pm 1.06$	12-16
Total length of vagina	18	$18.79 \pm 2.57$	16-19
Pars proximalis vaginae length	14	$13.5 \pm 2.30$	12 - 14
Pars refringens vaginae length	3	3.13±0.17	3
Pars distalis vaginae length	2	$1.23 \pm 0.30$	1-2
Tail length	168	$137.92{\pm}14.10$	115-163
Tail diametre at anus	30	26.67±5.57	23-38

the tail length to the diametre of the anus ('c") (4.3-6.6 vs. 5.7), and the presence of subterminal setae (Zullini & Villa, 2005).

Eutobrilus nothus exhibits morphological similarities to the new species, including very closely located stomatal pockets with a distance of  $6-8 \mu m$ , the body length (L), the ratio of the body length to the body diametre ('a'), the ratio of the body length to the distance from the pharyngo-intestinal valve to the anterior body end ('b'). the tail length, and the distance from the anterior and posterior tooth to the anterior body end (Shahabi et al., 2017). However, the two species differ in the ratio of the body length to the tail length ('c') (9.1–12.7 vs. 7.1–10.0), the tail length relative to the diametre of the anus ('c") (3.2-5.1 vs. 4.3-6.6), V (%) (46.0-51.0 vs. 39.7-46.0), the gonad length, and the sclerotisation of vulval lips (absent vs. present) (Shahabi et al., 2017).

*Eutobrilus brzeskii* can be distinguished from the new species by the sclerotisation of the vulval lip, the absence of subterminal caudal setae, the greater body length (3.70-4.40 mm vs. 0.94-1.50 mm), the tail length (488-637 µm vs. 115-163 µm), and other morphometric characters, except for having similarly distanced stomatal pockets (Gagarin & Naumova, 2012).

Though *Eu. minutus* **sp. nov.** and *Eu. olkhon*ensis share a similar body length (L), the new species differs from the latter in several morphometric measurements, including 'a' (20.8–31.3 vs. 12.0–19.0), 'b' (4.2–5.6 vs. 3.9–4.4), 'c' (6.7– 8.0 vs. 7.1–10.0), 'c" (4.3–6.6 vs. 3.1–4.3), 'V%' (39.7–46.0 vs. 46.0–51.0), the tail length (115– 163 µm vs. 175–210 µm), and the stoma length (8–15 µm vs. 30–37 µm), as well as the shape of the tail (long, not filiform vs. elongate-conoid) (Naumova & Gagarin, 2023).

Notably, *Eutobrilus minutus* **sp. nov.** exhibits the smallest body length among all known *Eutobrilus* species.

**Etymology.** The specific name is the Latin adjective *minutus* meaning "very small", "minute".

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