
**BIOLOGY, MORPHOLOGY,
AND SYSTEMATICS OF HYDROBIONTS**

Species of Free-Living Nematodes (Nematoda) New to Science from Lake Baikal and Freshwater Bodies of the Russian Far East

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Abstract—Two free-living nematode species found the interstitial zone of the splash zone of Lake Baikal have been described. *Eumonhystera arenosa* sp. n. is similar to *E. filiformis* (Bastian, 1865) Andrassy, 1981, but it has longer body and spicules, a bulb-shaped expansion of pharynx, and shorter inner labial sensillae; it is also characterized by a different ratio between the tail length and the distance from vulva to anus. *Tripyla affinis* de Man, 1880 found in the water bodies of the Far East has been assigned to the new species *T. alekseevi* sp. n., since there are differences between these two closely related species. *Tripyla alekseevi* sp. n. is close morphologically to *T. affinis* de Man, 1880, but differs by longer inner labial sensillae, a narrower labial region, a smaller number of supplements in males, and a longer gubernaculum. A key for the five valid species of the genus *Tripyla* inhabiting Lake Baikal is given.

Keywords: splash zone, Lake Baikal, new species, free-living nematodes, *Eumonhystera arenosa* sp. n., *Tripyla alekseevi* sp. n.

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INTRODUCTION

Fauna of free-living nematodes of Lake Baikal comprises more than 100 species belonging to 7 orders, 11 families, and 33 genera; about 65% of these species are endemic to the lake [4, 6, our data].

Studies of the fauna of nematodes living in the interstitial zone of the splash zone of Lake Baikal have started recently, and the first results of these researches were published in 2012 [13, 14]. In total, 20 nematode species belonging to 15 genera, 11 families, and 6 orders have been found [5, 13–15]. Most of the findings are known and widespread species and five are new to science.

The aim of the work is to study the fauna of nematodes of Lake Baikal, describe the species new to science, and compare those with the nematodes from other freshwater bodies.

MATERIALS AND METHODS

The meiobenthos samples were collected in the interstitial zone of Listvenichnyi Bay in vicinity of the settlement of Listvyanka, Lake Baikal, Russia. One part of the samples was collected on May 18, 2012, by a UWITEC sampling tube at sandy sediments at a distance of ~50 cm above the water edge (samples nos. 496, 497, and 500); the sediment height in the tube was

~10 cm. The other part of the samples was collected on September 4, 2012, at a distance of 1 m above the water edge: a 40-cm-deep hole was dug in the sand, and the groundwater accumulated there was filtered through a hydrobiological net (sample no. 614).

The samples were fixed with 4% formaldehyde solution. The worms were identified and measured under an Olympus CX-21 microscope on Trypan Blue glycerin smears framed with a colorless varnish. Photographs were taken with a Nikon Eclipse 80i microscope equipped with a DIC phase contrast mode, a Nikon DS-Fil digital camera, and a PC with NIS-Elements D 3.2 software for analyzing and documenting images from the smears.

RESULTS

Order Monhysterida Filipjev, 1929. Family Monhysteridae de Man, 1876. Genus *Eumonhystera* (Bastian, 1865) Andrassy, 1981. *Eumonhystera arenosa* sp. n. (Figs. 1, 2).

Morphological characteristics of the holotype and paratypes are given in Table 1.

Material. Holotype ♂, smear no. 102/54, paratypes: 4♂♂, 5♀♀. The smears of holotype and three paratypes (2♂♂ and 1♀) are stored in the Helminthological Museum of the Russian Academy of Sciences,

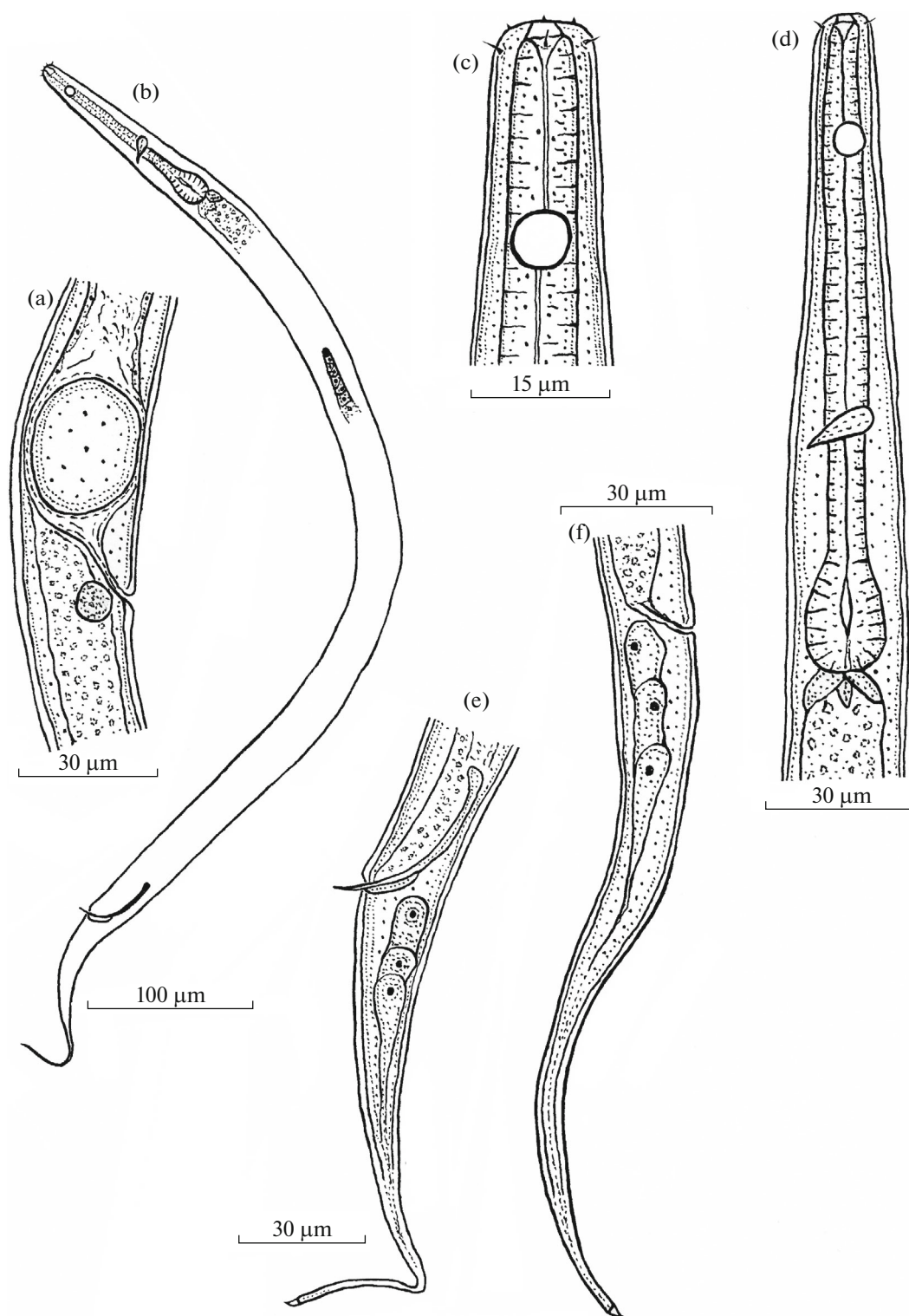


Fig. 1. *Eumonhystera arenosa* sp. n.: (a) body in the vulva area; (b) general view of the male; (c) head of the male; (d) anterior end of the male; and (e, f) tails of the male and female, respectively.



Fig. 2. Male (a, c, e, h, i) and female (b, d, f, g, j, k) *Eumonhystera arenosa* sp. n.: (a, b) general view, (c, d) head, (e) anterior end of the body, (f) body in the area of the cardium, (g) body in the vulva area, (h) the body in the area of cloaca, (i, j) tail, and (k) the tip of the tail.

Table 1. Morphometric characteristics of *Eumonhystera arenosa* sp. n.

Parameter	Holotype	Paratypes			
	♂	4♂♂		5♀♀	
		min–max	average	min–max	average
<i>L</i> , μm	807	785–930	853	807–910	839
<i>a</i>	34	26–38	33	28–37	31
<i>b</i>	6.4	6.4–7.2	6.8	5.8–7.0	6.5
<i>c</i>	6.2	6.0–6.9	6.4	5.3–5.5	5.4
<i>c'</i>	6.5	5.3–6.6	5.8	8.2–10.3	9.3
<i>V</i> , %	–	–	–	61–63	62
Width, μm:					
labial area	11	10–11	10	10–11	11
body (middle part)	24	23–36	30	27–33	29
body in the cloaca or anus areas	20	20–23	21	15–18	17
Length, μm:					
external labial papillae	2.0	2.0	2.0	2.0	2.0
pharynx	127	115–142	127	120–147	130
Distance, μm:					
from the anterior margin of the amphidial fovea to anterior end of the body	19	18–22	20	20–23	21
from the end of the pharynx to the vulva	–	–	–	348–425	390
from the end of the pharynx to the cloaca	550	545–665	594	–	–
from the vulva to the anus	–	–	–	150–175	164
Length, μm:					
tail	130	120–140	132	147–163	155
spicules (along the arch)	53	53–56	54	–	–
gubernaculum	12	–	12	–	–

Here and in Tables 2 and 3: (*L*) length of a body, μm; (*a*) ratio of the body length to width of body in its middle part; (*b*) ratio of the body length to the length of pharynx; (*c*) ratio of the body length to the tail length; (*c'*) ratio of the tail length to the body width at the anus or cloaca area; (*V*) ratio of the distance from the anterior end of the body to the vulva to the total body length, %.

Center for Parasitology of the Severtsov Institute for Ecology and Evolution of the Russian Academy of Sciences (RAS) (Moscow, Russia). The rest of the smears of the paratypes are stored in the collection of the Laboratory of Biology of Aquatic Invertebrates of the Limnological Institute of the Siberian Branch (SB), RAS (Irkutsk, Russia).

Location. Russia, Eastern Siberia, Lake Baikal, the interstitial zone of the splash zone of the Listvenichnyi Bay (settlement of Listvyanka, opposite the post office, 51°30'21" N, 104°31'15" E), ~50 cm above the water edge.

Male. Fine thin worm. Both ends of the body are narrowed. Cuticle thin, smooth, without somatic setae and cuticular pores. No crystalline bodies are found in the body cavity. The anterior margin of the head is slightly flattened. Lips are poorly expressed. The labial area is not separated from the rest of the body. Around the osculum, there are six internal labial sensillae in

the form of small papillae. At a distance of ~3 μm from the anterior end of the body, six external labial sensillae are localized in the form of small, fine setae; their length is 1/5–1/6 of the diameter of labial area. No head or neck sensillae are found. The amphidial foveae form a circle with a diameter of 41–46% of the body diameter at this section; they are located at a distance from the anterior end of the body that exceeds the width of the labial area 1.8–2.0 times. Eyes are absent. Stoma in the form of a short funnel, its walls thin, not cuticular. Pharynx is slender, muscular. The basal end of the pharynx forms a bulbous extension, the walls of the inner cavity of which are cuticularized. Cardium is small, surrounded by three petal-like glands. Renette, its duct and excretory pore were not found. One straight testis. Spicules thin, curved, with poorly expressed heads. The length of the spicules is 2.4–2.6 times larger than the diameter of the body in the area of the cloaca. The gubernaculum is a plate-shaped, sharpened from both edges. The gubernacu-

Table 2. Morphometric characteristics of *Eumonhystera filiformis* (Bastian, 1865) Andr ssy, 1981

Location, reference	Sex	<i>L</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>c'</i>	<i>V</i> , %	Lels	amph/Dla	tail/V-A	Ls	Ls/Dc
Different habitats [8]	♀♀	500–750	25–33	4.8–7.5	3.8–5.5	9–12	58–66	2.5–3.0	1.7–2.1	1.3–1.5	–	–
	♂♂	530–700	25–30	5.5–6.5	6.0–6.6	8–10	–	2.5–3.0	1.7–2.1	–	25–32	1.5–1.7
Lake Sevan, Armenia [2]	15♀♀	627–715	23–35	5.3–6.0	4.3–5.6	6.2–7.1	62–68	3.0	1.8–2.0	1.3–1.5	–	–
	3♂♂	590–730	17–28	4.9–5.9	4.8–5.9	5.0–6.0	–	3.0–3.5	1.9–2.0	–	27–35	1.6–1.7
Mineral spring on Sakhalin Island, Russia [2]	4♀♀	660–790	24–27	5.3–5.7	4.6–5.2	6.3–7.7	63–66	2.5–3.0	1.7–2.0	1.3–1.5	–	–
	1♀	660	25	5.4	5.5	5.1	–	3.0	1.7	–	35	1.7

Notes: (Lels) length of the external labial setae, μm ; (amph/Dla) ratio of the distance from the amphidial fovea to the anterior end of the body to the diameter of the labial area; (tail/V-A) ratio of the tail length to the distance from the vulva to the anus; (Ls) the length of the spicules, μm ; (Ls/Dc) ratio of the length of the spicules to the diameter of the body in the cloaca area.

lum length is 11–13 μm . Precloaca supplementary organs and precloaca setae are absent. The tail is relatively long, gradually narrowing. Caudal glands are present. Spinneret short, “beak-shaped.”

Females. General morphology is similar to that in male. Structure of cuticle and anterior end of body is similar to that in males. Cuticle smooth, without somatic setae. Lips are poorly developed. There are no crystalloid bodies. Six internal labial sensillae in the form of papillae and six external labial sensillae in the form of short setae. Head and neck sensillae are not found. The amphidial fovea form of a circle with a diameter of 40–44% of the corresponding body diameter; they are located at a distance exceeding the width of the labial area 2.0–2.1 times. Eyes are absent. Stoma has the shape of a short funnel. Pharynx muscular; there are bulbous-shaped thickenings at its basal end. Around the cardiac, there are three petal-shaped glands. Renette, its duct and excretory pore were not found. The length of the rectum is equal to or slightly larger than the diameter of the body at the anus.

One anterior straight ovary located to the right of the mid-gut. Vulva is postequatorial. The vulva lips are not sclerotized and do not protrude beyond the contours of the body. The vagina is short, inclined to the anterior end of the body; its walls are thin. In the uterus, numerous spermatozoa and one egg size of (34–40) \times (38–41) μm are found often. Postvulvar cell is present. The tail is relatively long, gradually narrowing. Tail length is equal to 0.9–1.0 of the distance from the vulva to the anus. The caudal glands are well-developed. Spinneret short, beak-shaped.

Diagnosis. Relatively small and slender worms ($L = 785\text{--}910 \mu\text{m}$, $a = 26\text{--}38$). Cuticle smooth. Somatic setae and crystalloid bodies are absent. The labial region is not separated from the rest of the body. Six internal sensillae look like small papillae and six external labial sensillae in the shape of 2- μm short setae. Head and neck sensillae not found. The amphidial fovea form a circle and are located at a distance of 1.8–2.1 of the labial area width from the anterior end of the body. Eyes absent. Pharynx muscular, forms a basal

bulbous-shape extension. Three cardiac glands. Vulva is postequatorial ($V = 61\text{--}63\%$). The vagina is short; the walls are thin. Postvulvar cell is present. The tail length in the females is 0.9–1.0 of the distance from the vulva to the anus. Spicules thin, with poorly expressed heads; their length is 53–56 μm , exceeding the diameter of the body in the cardiac area in 2.4–2.6 times. Gubernaculum has a shape of a short plate; the dorsal spur is absent. The tail is relatively long, gradually narrowing. Spinneret short, beak-shaped.

Differential diagnosis. The species is morphologically close to the widely distributed species *Eumonhystera filiformis* (Bastian, 1865) Andr ssy, 1981 (Table 2). *E. arenosa* sp. n. differs from *E. filiformis* by longer body ($L = 785\text{--}910 \mu\text{m}$ against $L = 500\text{--}790 \mu\text{m}$ in *E. filiformis*), by the presence of basal bulbous-shape expansion of pharynx (absent in *E. filiformis*), by shorter external labial setae (2.0 μm versus 2.5–3.0 μm in *E. filiformis*), by a different ratio of the tail length to the distance from the vulva to the anus in females (0.9–1.0 vs. 1.3–1.5 in *E. filiformis*), and by longer spicules (53–56 μm , 2.4–2.6 times greater of the body diameter in the cloaca area, versus 25–35 μm and 1.5–1.7 times in *E. filiformis*, respectively) [7, 8].

Morphological and taxonomic notes. The genus *Eumonhystera* (Bastian, 1865) Andr ssy, 1981 has been isolated from the genus *Monhystera* Bastian, 1865, because the latter was clearly a composite genus that included a rather large number of morphologically diverse Monhysteridae worms inhabiting the freshwater bodies and soil [7]. The genus *Eumonhystera* combined relatively small nematodes with a body length of 0.5 mm to 1.5 mm characterized by the absence of crystalloid bodies. The amphidial fovea are located from the anterior end of the body at a distance of 1.0–3.5 of the diameter of the labial area. Males are very rare, and the females, apparently, reproduce by parthenogenesis. The tail length in females is approximately equal to or greater than the distance from the vulva to the anus. Spicules in males are relatively short; their length, as a rule, does not exceed the double body diameter in the area of the cloaca. Currently, the genus

Table 3. Morphometric characteristics of *Tripyla alekseevi* sp. n.

Parameters	Lake Baikal (original data)					Freshwater bodies of the Far East [1]	
	Holotype	Paratypes				3♂♂	28♀♀
	♂	10♂♂		10♀♀			
		min–max	average	min–max	average		
<i>L</i> , μm	1232	1060–1400	1212	1050–1238	1150	1100–1300	1200–1800
<i>a</i>	32	26–35	32	31–39	34	28–36	25–28
<i>b</i>	5.8	5.6–6.3	5.9	5.5–6.2	5.8	4.3–5.7	4.8–5.7
<i>c</i>	5.6	5.6–6.4	6.1	5.4–6.4	5.9	6.0–7.9	5.3–6.7
<i>c'</i>	6.3	5.8–7.3	6.6	6.4–7.8	7.4	5.0–6.0	6.0–7.0
<i>V</i> , %	–	–	–	48–53	50	–	52–57
Width, μm:							
labial area	21	18–21	19	17–20	19	18–20	18–22
body (middle part)	39	35–42	38	31–37	34	34–40	48–58
body in the cloaca or anus areas	32	27–32	30	25–30	27	29–37	33–41
Length, μm:							
external labial papillae	4.0	4.0–5.0	4.5	4.0	4.0	4.0	5.0
head setae	2.0	1.5–2.0	2.0	2.0	2.0	2.0–3.0	2.0
pharynx	211	190–245	206	185–225	200	235–290	222–312
Distance, μm:							
from the anterior end of the body to the tooth	18	17–20	18	17–20	19	14	13–15
from the end of the pharynx to the vulva	–	–	–	340–435	381	–	–
from the end of the pharynx to the cloaca	801	700–935	807	–	–	–	–
from the vulva to the anus	–	–	–	345–415	373	–	–
Length, μm:							
tail	220	165–220	199	170–230	196	168–190	287–320
spicules (along the arch)	45	40–45	42	–	–	45	–
gubernaculum	15	14–16	15	–	–	14–18	–
Total number of supplemental organs	9	8–11	10	–	–	7–10	–
Number of supplemental organs in the area of pharynx	1	1–2	1	–	–	–	–

Eumonhystera comprises 38 valid species [8, 12]. *E. filiformis* is a cosmopolitan species; it belongs to the most common species of the genus. It inhabits the inland waters, as well as the damp moist soils and moss. Morphologically, the species is characterized by the presence of a postvulvar glandular cell in females; the amphidial fovea are located at a distance of 1.7–2.1 of the diameter of labial area from the anterior end of the body; the tail length in females is 1.3–1.5 times longer than the distance from the vulva to the anus; the length of the spicules is 25–35 μm, which exceeds the body diameter at the cloaca area 1.5–1.7 times. Males are very rare and are found only in the freshwater bodies of the Netherlands [17], lakes of Poland [18], Lake Sevan (Armenia), and in a mineral spring at Sakhalin Island (Russia) [2].

Etymology. Species name means “sandy,” “sand.”

Order Triplonchida Cobb, 1920. Family Tripylidae de Man, 1876. Genus *Tripyla* Bastian, 1965. *Tripyla alekseevi* sp. n. Morphological characteristics of the holotype and paratypes are given in Table 3.

Material. Holotype ♂, smear no. 102/55, paratypes: 10♂♂, 10♀♀. Smears of the holotype and three paratypes (1♂ and 2♀♀) are stored in the Helminthological Museum of the Russian Academy of Sciences, Center for Parasitology of the Severtsov Institute of Ecology and Evolution of the RAS (Moscow, Russia). The rest of the smears of the paratypes are stored in the collection of the Laboratory of Biology of Aquatic Invertebrates of the Limnological Institute of the SB RAS (Irkutsk, Russia).

Location. Russia, Eastern Siberia, Lake Baikal, interstitial zone of the splash zone in Listvenichnyi Bay (settlement of Listvyanka, opposite the sealarium,

51°30'16" N, 104°31'23" E), ~1 m above the water edge.

Male. A relatively small fine worm. Cuticle annulate, somatic setae are absent. The thickness of the cuticle in the middle part of the body is 2.0–2.5 μm . The anterior end of the body is slightly narrowed. Three low lips. The labial area is not separated from the rest of the body. Six internal labial sensillae have the shape of small papillae, ~1- μm -long. Six external labial sensillae have the shape of large papillae, 4.0- to 5.0- μm long. At a distance of 5 μm from the external labial sensillae, there are four head sensillae shaped like thin bristles, 1.5- to 2.0- μm -long. The amphidial fovea are pocket-shaped, localized slightly below the level of the head setae. Stoma is not expressed. Pharynx muscular, only slightly widens to its base. In the pharynx lumen, a small dorsal tooth is located at a distance of 17–20 μm from the anterior end of the body. Subventral teeth are not found. The cardium is small, surrounded by the three round-shape cardiac glands. Renette, its duct and excretory pore are not found.

Paired testes are opposite each other. Spicules are relatively thick, with no heads. The length of the spicules exceeds the width of the body in the area of the cloaca 1.4–1.5 times. Gubernaculum has the shape of a simple plate, 14- to 18- μm -long. Supplementary organs has the shape of small, barely noticeable papillae. The number of supplements has been counted only in three males from Lake Baikal. One male had nine supplements (one in the pharynx region), the other had eight supplements (also one in the pharynx region), and the third had eleven supplements (two in the pharynx region). The tail is relatively long, gradually narrowing. The caudal glands and the spinneret are well-developed.

Females. General morphology is similar to that in males. Structure of cuticle and anterior end of body same as in males. Cuticle annulate, without somatic setae. Three low lips. Six internal labial sensillae have the shape of small papillae. Six external labial sensillae have the shape of large papillae, 4.0- μm -long. Four head sensillae have the shape of thin bristles, 2- μm -long. Amphidial fovea pocket-shaped and located just below the head setae. Stoma not expressed. Pharynx muscular, slightly dilated in basal area. In the lumen of the esophagus, there is a small dorsal tooth located 13–20 μm from the anterior end of the body. There are three round glands around the cardium. The length of the rectum is less than the diameter of the body in the anus area.

Ovaries are paired, with bends. Bends are short, not reaching the vulva. Vulva is equatorial, and it has the shape of a transverse slit. The vulva lips protrude slightly behind the contours of the body. The vagina is straight; its walls are thin, nonmuscular. In the uterus, numerous spermatozoa and 1–3 rounded eggs (30–60) \times (26–48) μm in size. The tail is relatively long,

gradually narrowing. The caudal glands and the spinneret are well developed.

Diagnosis. Slender, small worms ($L = 1050\text{--}1400 \mu\text{m}$, $a = 25\text{--}39$). Cuticle annulate, without somatic setae. Six internal labial sensillae have the shape of small papillae; six external labial sensillae have the shape of large papillae, 4.0- to 5.0- μm -long; four head sensillae in the shape of thin bristles, 1.5- to 3.0- μm -long. There is a small dorsal tooth in the lumen of the esophagus. Spicules are 40- to 45- μm -long, which is 1.4–1.5 times larger than the diameter of the body in the area of the cloaca. The gubernaculum is 14- to 18- μm -long. Seven to eleven supplementary organs have the shape of small, hardly noticeable papillae; one or two of them are located in the pharynx region. The tail is relatively long, gradually narrowing. The caudal glands and the spinneret are well developed.

Differential diagnosis. The species *Tripyla alekseevi* sp. n. is morphologically close to the species *T. affinis* de Man, 1880; the latter is fairly widespread in moist soils and freshwater bodies around the world [16, 19]. *T. alekseevi* sp. n. differs from *T. affinis* by larger external labial papillae (4.0–5.0 μm versus 1.0–2.0 μm in *T. affinis*), a narrower labial area (width of 17–22 μm versus 23–29 μm in *T. affinis*), a smaller number of supplementary organs (7–11, including 1–2 in the pharynx region versus 14–19 and 4–7 in *T. affinis*, respectively) and a longer gubernaculum (14–18 μm versus 9–14 μm in *T. affinis*) [11].

Morphological and taxonomic notes. The species *Tripyla affinis* de Man, 1880 was first found and described in soils in the Netherlands [17]. It was then repeatedly found in soils and freshwater bodies around the world [16]. The main morphological features of this species are a relatively small body size and very short external labial and head sensillae; their length does not exceed 1–2 μm . The species was described twice and illustrated during the revision of the genus [10, 11]. In 1991, Russian nematologist Valentin M. Alekseev described a fairly large population of nematodes from the genus *Tripyla* from several reservoirs of the Far East, he attributed these nematodes to *T. affinis* [1]. Morphologically, these nematodes differed in some aspects from the generally accepted description of *T. affinis*. The main distinguishing feature was the length of the external labial sensillae; they were more than twice as long as that of *T. affinis*. Morphologically similar specimens of the genus *Tripyla* were found within this study in the interstitial zone of Lake Baikal (Table 3). They are morphologically similar to the worms from the freshwater bodies of the Far East (Table 3) and differed from *T. affinis* in four morphological features. Therefore, the authors attribute these worms to an independent species of the genus *Tripyla*: *T. alekseevi* sp. n.

Etymology. The species is named in honor of the Russian nematologist V.M. Alexeev.

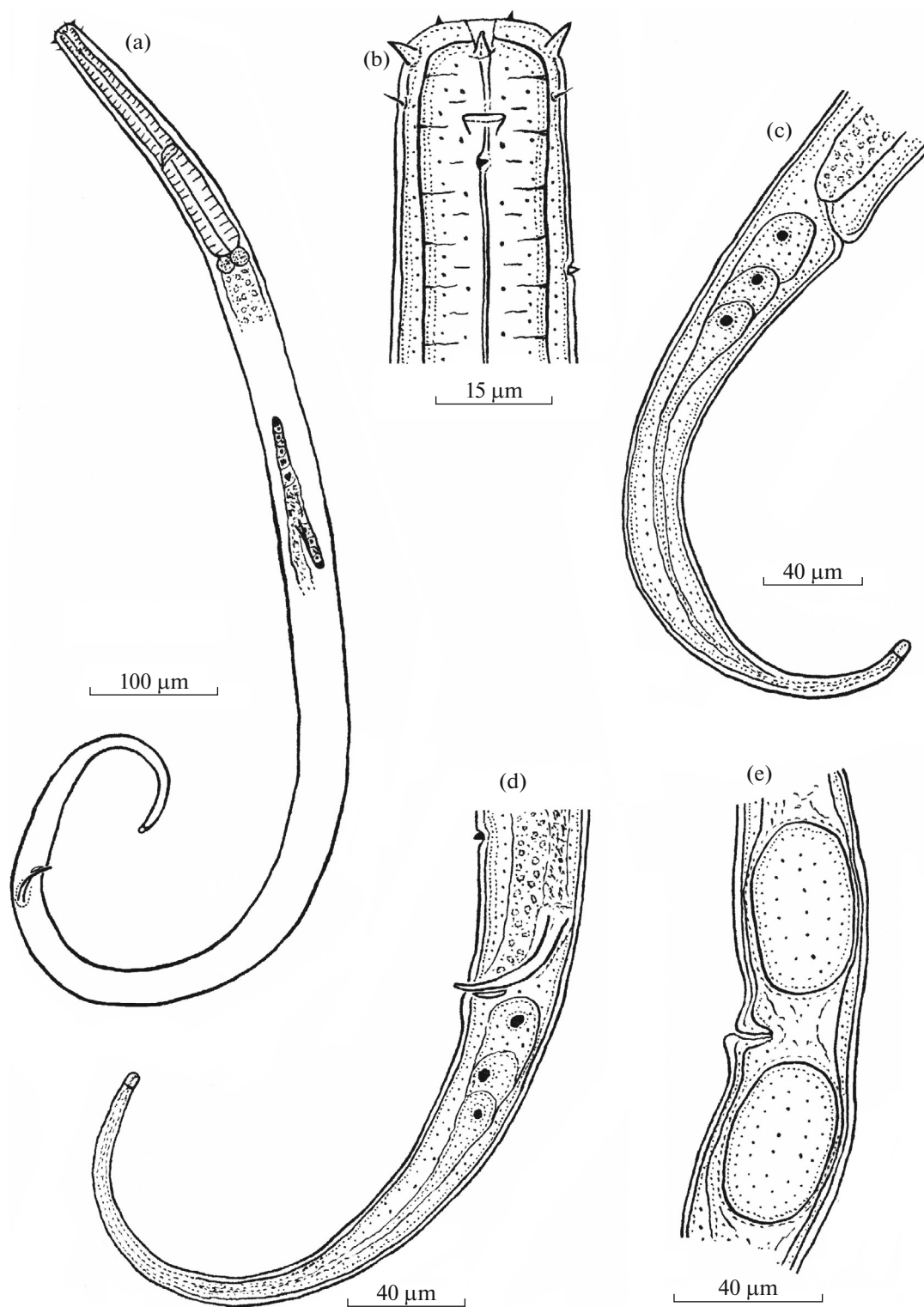


Fig. 3. *Tripyla alekseevi* sp. n.: (a) general view of a male, (b) head of a male, (c) tail of a female, (d) tail of a male, and (e) body in the vulva area with two eggs.

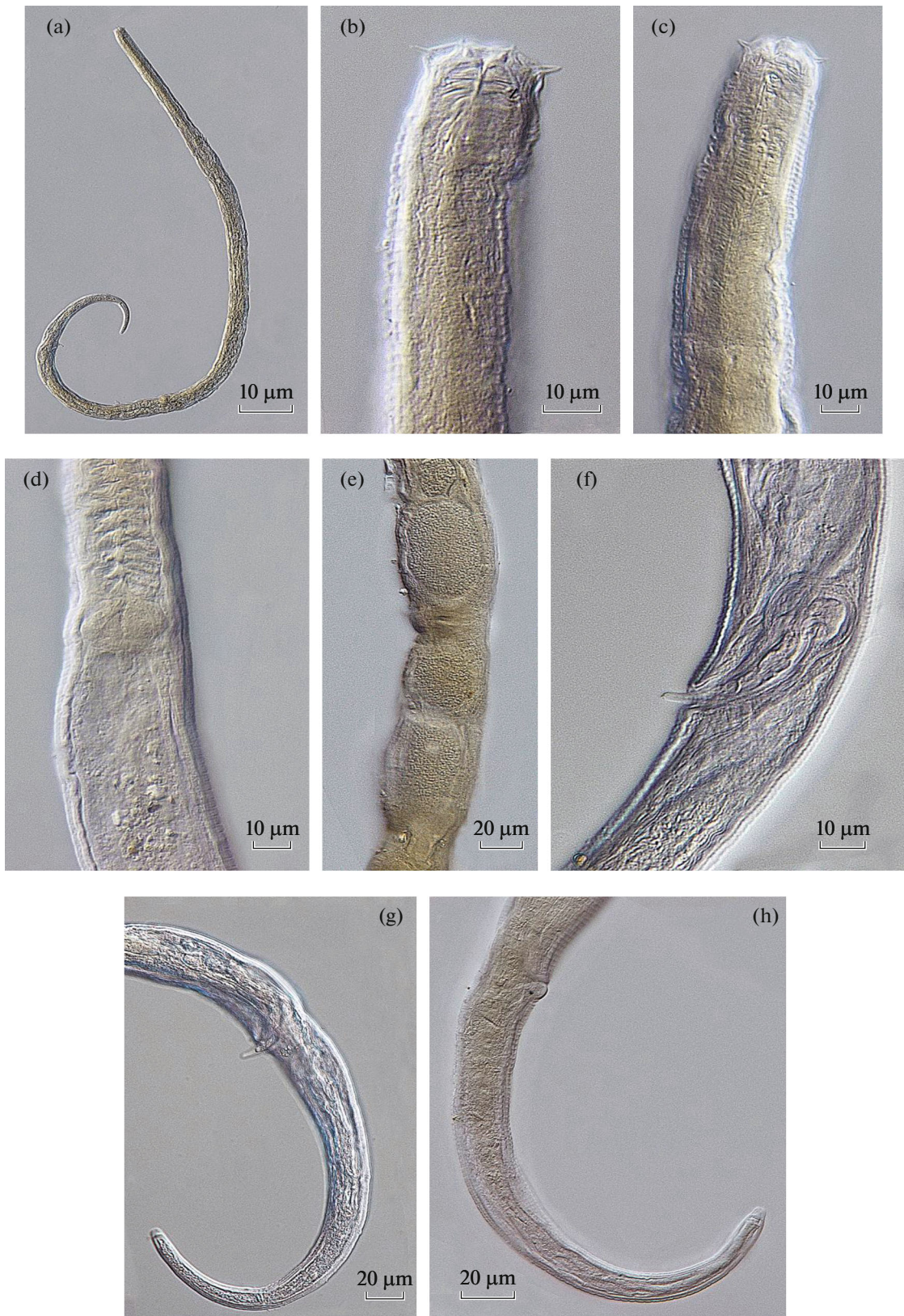


Fig. 4. Male (a, b, d, f, g) and female (c, e, h) *Tripyla alekseevi* sp. n.: (a) general view, (b, c) anterior end of the body, (d) body in the area of cardium, (e) body in the vulva area, (f) body in the cloaca area, and (g, h) tail.

Valid species of *Tripyla* genus inhabiting Lake Baikal. Five valid species of the genus *Tripyla* are currently found in Lake Baikal: *T. dybowskii* Tsalolikhin, 1976, *T. filicaudata* de Man, 1880, *T. setifera*

Bütschli, 1873, *T. infia* Brzeski, Winiszewska-Slipinska, 1993, and *T. alekseevi* sp. n. [3–6, 13]. The key for species identification is presented below.

Taxonomic key for identifying valid species of the genus *Tripyla* from Lake Baikal

- | | |
|---|----------------------------|
| 1. External labial sensillae are long, their length $>1/4$ of the labial area width | proceed to 2 |
| (or) external labial sensillae are shorter, their length $\leq 1/4$ of the labial area width | proceed to 3 |
| 2. Tail length exceeds the body diameter in the anus area 4–6 times | <i>T. setifera</i> |
| (or) tail length exceeds the body diameter in the anus area 9–13 times | <i>T. filicaudata</i> |
| 3. Body length 2.58–3.53 mm; length of the spicules 85–92 μm | <i>T. dybowskii</i> |
| (or) body length <2.5 mm; length of the spicules <60 μm | proceed to 4 |
| 4. Body length 1.28–2.20 mm; length of the external labial sensillae ~ 2 μm ; 17–20 supplemental organs | <i>T. infia</i> |
| (or) body length 1.05–1.24 mm; length of the external labial sensillae 4–5 μm ; 7–10 supplemental organs | <i>T. alekseevi</i> sp. n. |

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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