

BIOLOGY, MORPHOLOGY,  
AND SYSTEMATICS OF HYDROBIONTS

Description of Two New Species of Free-Living  
Nematodes (Nematoda) from Vietnam

Nguyen Dinh Tu<sup>a</sup> and V. G. Gagarin<sup>b, \*</sup>

<sup>a</sup>Institute of Ecology and Biological Resources, Vietnamese Academy of Sciences and Technology, Hanoi, Vietnam

<sup>b</sup>Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences,  
Borok, Nekouzskii raion, Yaroslavl oblast, Russia

\*e-mail: gagarin@ibiw.ru

Received October 5, 2018; revised April 9, 2019; accepted September 23, 2019

**Abstract**—An illustrated description of two free-living nematode species—*Leptolaimoides brevicaudatus* sp. n. and *Paramonhystera affinis* sp. n.—from the water bodies of Vietnam is presented. *L. brevicaudatus* is similar to *L. thermastris* (Lorenzen, 1966); *L. gangioensis* Hoang Lai-Phu et al., 2009; and *L. asiaticus* Gagarin, Nguyen Vu Thanh, 2005 in regards to body size, but differs from these species by a shorter and less slender body, longer spicules, and the absence of precloacal supplements in males. *Paramonhystera affinis* sp. n. is morphologically similar to *P. parabutschlii* (Timm, 1961) and *P. levicula* (Lorenzen, 1972), but it has shorter and sparse somatic setae, six outer labial setae (versus four outer labial setae in both compared species), and shorter spicules.

**Keywords:** Vietnam, water bodies, free-living nematodes, new species

**DOI:** 10.1134/S1995082920030141

INTRODUCTION

The study of the fauna of free-living nematodes in the water bodies and watercourses in Vietnam began about 15 years ago in order to develop a database on the aquatic fauna of Vietnam. In recent years, large-scale studies on the fauna of this taxonomic group have been performed. The data on their fauna in fresh water bodies and mangroves are summarized in the works of Gagarin and Gusakov (Gusakov and Gagarin, 2017; Gagarin, 2018).

This study aims to give an illustrated description of two species of free-living nematodes new to science.

MATERIALS AND METHODS

Twenty-four samples of nematodes were collected in March 2017 by colleagues from the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam) in the bottom sediments of an artificial pond for shrimp aquaculture. This water body is located in the Quảng Ninh Province on an island in the South China Sea off the coast of Vietnam. Its area is 1000 m<sup>2</sup>. The bottom is overgrown by dense thickets of grass *Haloph-*

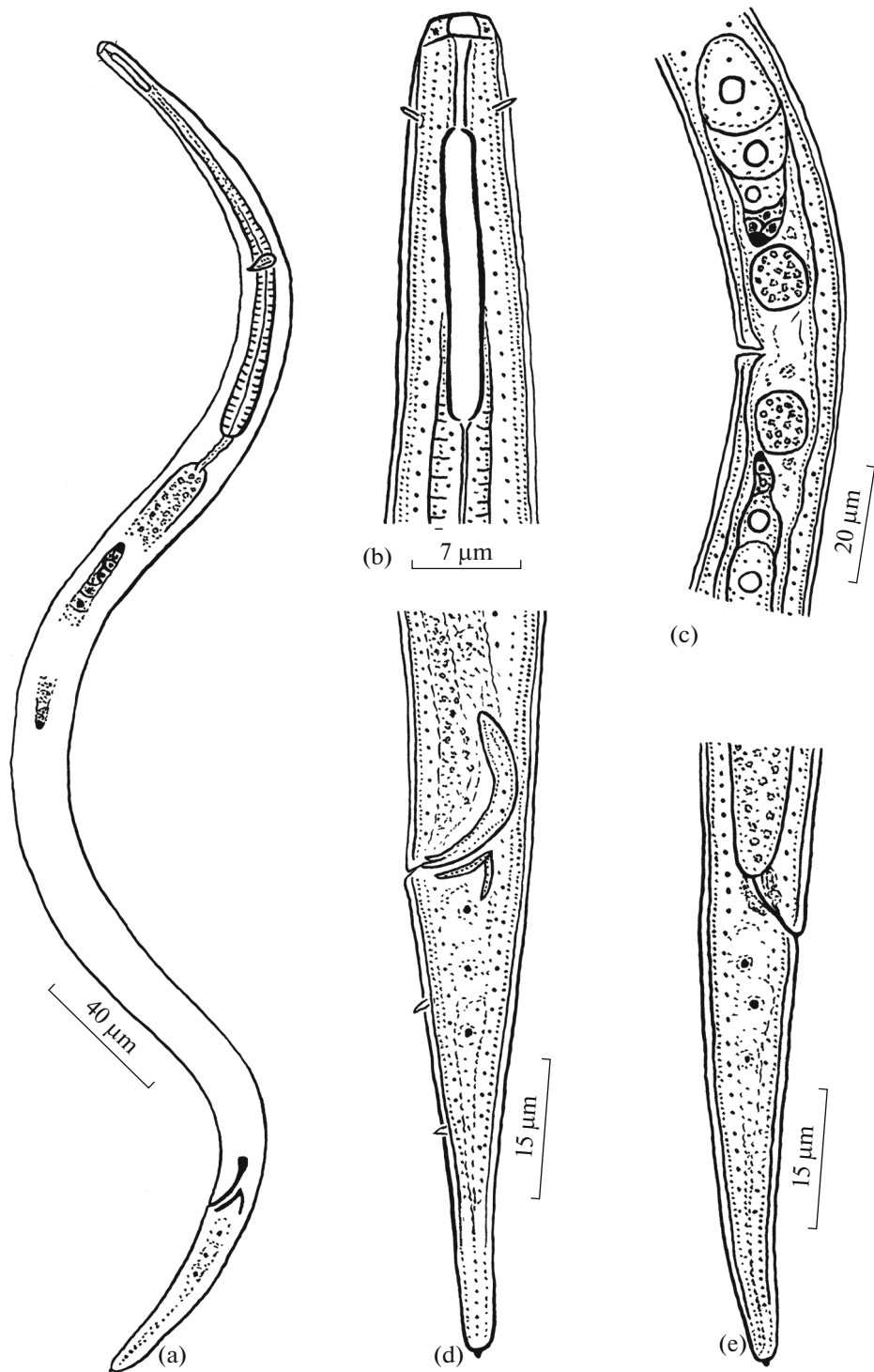
*ila beccarii* Ascherso, 871 and *Ruppia maritima* L., 1953. Material was also collected in the estuaries of the Van Uc River and Ien River in the mangroves. Samples were taken at a depth of 0.3–0.7 m using a cylinder with a diameter of 3.5 cm, a length of 10 cm, and washed through a sieve with a mesh diameter of 0.08 mm. Samples were fixed with hot (60–70°C) 4% formalin solution. The sample was then placed in 200-mL containers, a Ludox TM50 solution (1 : 1) was added, and the samples were centrifuged five times for 40 min. The nematodes were transferred to pure glycerol according to the Seinhorst method (Seinhorst, 1959) and then mounted in a drop of glycerol on a glass slide and sealed with a paraffin (wax) ring. A Nikon Eclipse 80i light microscope equipped with DIC contrast, a Nikon DS-Fil digital camera, and a PC equipped with NIS-Elements D 3.2 software for analysis and documentation were used for measuring, species definition, photographing, and drawings the nematodes.

In the samples, nematode species new to science were discovered. The description and illustrations of the two species are presented below.

RESULTS

**Description of new species.** Order Plectida Malakhov, 1982. Family Leptolaimidae Örley, 1880. Genus *Leptolaimoides* Vitiello, 1971.

**Abbreviations:** (*a*) ratio of body length to its width; (*b*) ratio of body length to pharynx length; (*c*) ratio of body length to tail length; (*c'*) ratio of tail length to body width in the anus or cloaca area; (*L*) body length, mm; and (*V*) the ratio of the distance from vulva to the anterior end of the body to the body length, %.

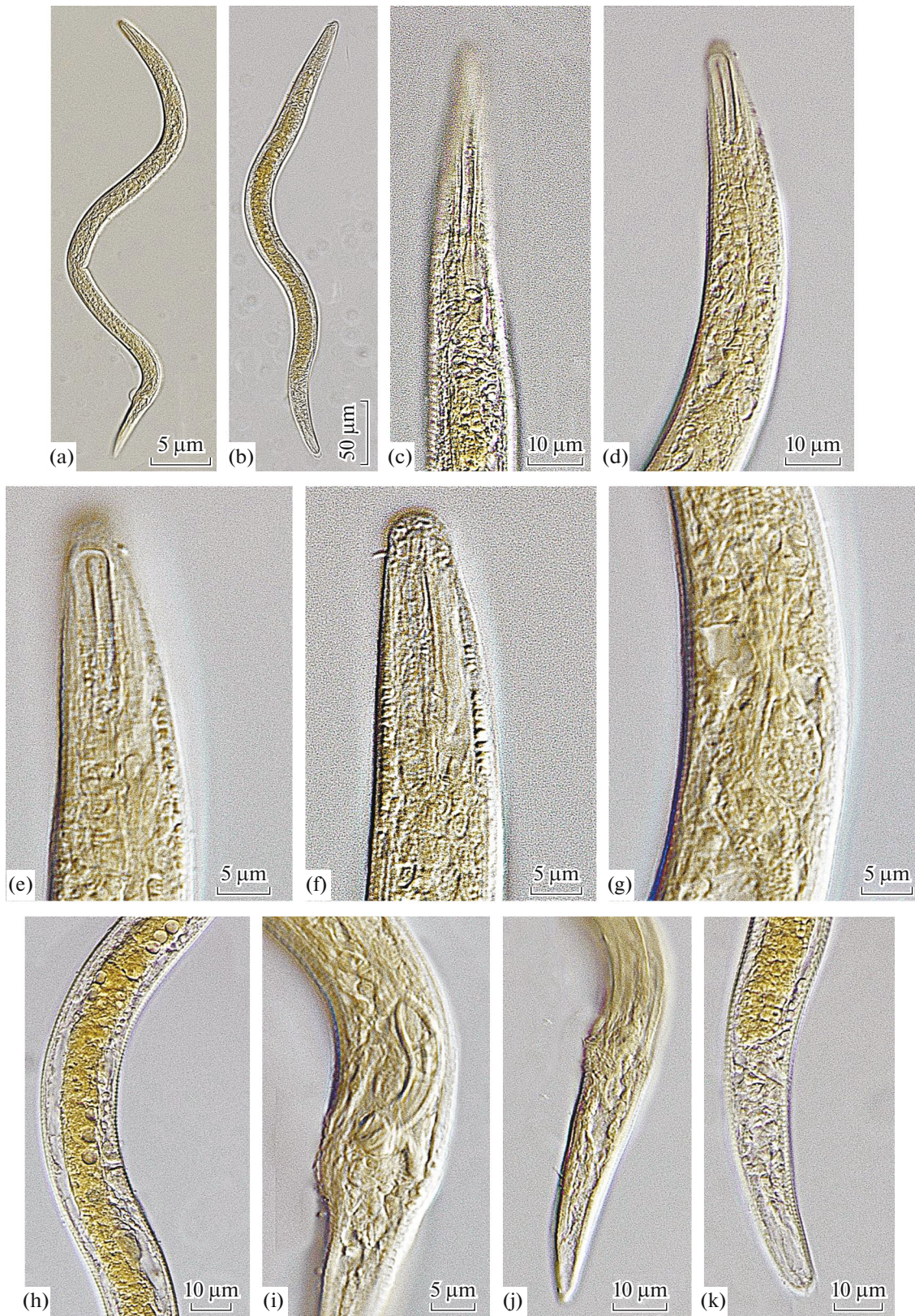


**Fig. 1.** Male (a, b, d) and female (c, e) *Leptolaimoides brevicaudatus* sp. n.: (a) general view, (b) head, (c) body in the vulva area, and (d, e) tail.

*Leptolaimoides brevicaudatus* Gagarin sp. n. (Figs. 1, 2).

**Material.** Holotype ♂, slide no. ND 4.2.19; paratypes: 4♂♂, 1♀. The holotype preparation is stored in the collection of the Nature Museum of the

Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam). Paratype preparations are stored in the collection of nematodes at the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).



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

**Table 1.** Morphometric characteristics of *Leptolaimoides brevicaudatus* sp. n.

Parameter	Holotype ♂	Paratypes		
		4♂		1♀
		min–max	mean	
<i>L</i> , μm	409	399–488	437	362
<i>a</i>	28	26–33	29	19
<i>b</i>	3.4	3.1–4.0	3.7	3.0
<i>c</i>	7.7	7.8–9.0	8.1	7.1
<i>c'</i>	5.3	5.0–6.3	5.8	3.8
<i>V</i> , %	–	–	–	52.5
Width of labial area, μm	6	6–7	6.5	7
Length of amphid fovea, μm	17	16–18	17	17
Distance from amphid fovea to the anterior end of the body, μm	7	6–8	7	7
Pharynx length, μm	77	69–81	75	121
Distance, μm:				
from pharynx end to anus	–	–	–	68
from pharynx end to cloaca	278	260–353	309	–
from vulva to anus	–	–	–	122
Tail length, μm	54	51–54	53	51
Body width, μm:				
in the middle part	15	14–16	15	19
in the area of anus or cloaca	12	12–14	13	9
Length of spicules (along the curve), μm	23	22–25	23	–
Gubernaculum length, μm	9	8–10	9	–

**Location.** The nematodes were found in two locations. Three ♂♂ were found in Northern Vietnam, Quảng Ninh Province, Hà Đông, artificial pond for shrimp aquaculture (21°16'77" N, 107°23'92" E). Depth 0.5 m, silt with turf, water salinity 20‰. One ♂ and one ♀ were also found in Northern Vietnam, Hai Phong Province, estuary of the Van Uc River, (20°40'28" N, 106°41'44" E). Mangroves, depth 1.1 m, silt, salinity 6‰.

**Description.** The morphometric characteristics of the holotype and paratypes are given in Table 1.

**Male.** The body is thin, short. The cuticle is finely annulated. Somatic bristles and pores not found. A lateral area in the form of two small ridges stretches along the entire body. The width of the lateral area is 4.0–4.5 μm. The anterior end of the body is narrowed. The width of the labial area is 2.3–2.7 times smaller than the body diameter at the cardium area. The labial area is not isolated from the rest of the body. External labial sensillae barely visible, in the form of small papillae. Four head sensillae in the form of larger papillae. Cheilostoma is relatively large. Pharyngostoma in the form of a narrow tube, 21–23 μm in length. The pharynx is muscular, slightly expanding to its base. Cardium in the form of a long muscular tube,

20–24 μm in length. Renette, its pair and ducts not detected. Amphid fovea in the form of a narrow long cylinder, 16–18-μm length, separated from the anterior end of the body at a distance of 6–9 μm.

Seminal glands are paired, opposed. The anterior testis is straight, located on the right side from the middle intestine; posterior testis short, bent. The spicules are thin, curved, with a head. The spicules are 1.8–2.0 times longer than the body width in the cloaca area. The gubernaculum has the form of a simple plate, 8–10 μm in length, with a curved caudal process of about the same length. Supplementary organs not found. Caudal setae not found. Caudal glands are present, but poorly visible. The spinneret is small, triangular.

**Females.** Similar to males in general morphology. The structure of the anterior end of the body and the cuticle structure are similar to that in males. The cuticle is finely annulated. Lateral areas stretch along the entire body as two ridges. The anterior end of the body is narrowed. Labial sensillae in the form of small, barely noticeable papillae. Four head sensillae in the form of larger papillae. Narrow tubelike pharyngostoma of 20-μm length. The pharynx is muscular, expanding to its base. The cardium is long, muscular,

and does not extend into the lumen of the middle intestine. The reproductive system is didelphic, amphidelphic; ovaries are antidiromic, short. Bends of the ovaries reach the vulva. The anterior ovary is located on the right from the intestine, the posterior one, on the left from the intestine. The vulva is equatorial, in the form of a transverse gap. The vagina is short, straight. The uterus is extensive. Each uterus has one rounded spermatheca. The tail is elongated-conical. The caudal glands are barely visible. The spinneret is small, triangular.

**Differential diagnosis.** Recently, the genus *Leptolaimoides* Vitiello, 1971 comprises 12 valid species (Holovachov, 2015; Hoang Lai-Phu et al., 2009). In regards to the body size, *Leptolaimoides brevicaudatus* sp. n. is close to *L. thermastris* (Lorenzen, 1966), *L. cangioensis* Hoang Lai-Phu et al., 2009, and *L. asiaticus* Gagarin, Nguyen Vu Thanh, 2005. It differs from the first species by a shorter and less slender tail ( $c = 7.1-9.0$ ,  $c' = 3.8-6.3$  versus  $c = 4.0-4.7$ ,  $c' = 7.5$  in *L. thermastris*), longer amphid fovea (16–20  $\mu\text{m}$  in length versus 8–12  $\mu\text{m}$  in *L. thermastris*), and longer spicule (22–25- $\mu\text{m}$  length versus 13  $\mu\text{m}$  in *L. thermastris*) (Lorenzen, 1972); it differs from *L. cangioensis* by a shorter and less slender tail ( $c = 7.1-9.0$ ,  $c' = 3.8-6.3$  versus  $c = 4.0-6.5$ ,  $c' = 7.8-10.0$  in *L. cangioensis*), longer amphid fovea (16–20  $\mu\text{m}$  length versus 5–7  $\mu\text{m}$  in *L. cangioensis*), simply structured lateral areas (in *L. cangioensis* they are areolated), a longer spicule (22–25  $\mu\text{m}$  in length versus 14–15  $\mu\text{m}$  in *L. cangioensis*), and by the absence of supplements in males (there are two supplements ahead cloaca in *L. cangioensis* males); it differs from *L. asiaticus* by a shorter and less slender tail ( $c = 7.1-9.0$ ,  $c' = 3.8-6.3$  versus  $c = 3.7-6.2$ ,  $c' = 7.5-11.4$  in *L. asiaticus*), longer spicule (22–25  $\mu\text{m}$  in length versus 15–17  $\mu\text{m}$  in *L. asiaticus*), and the absence of supplements in males (there are three supplements ahead cloaca in *L. asiaticus*) (Gagarin, Nguyen Vu Thanh, 2005).

**Etymology.** Species name means “short-tailed” or “with short tail.”

Order Monhysterida Filipjev, 1929. Family Xyalidae Chitwood, 1951. Genus *Paramonhystera* Steiner, 1916.

*Paramonhystera affinis* sp. n. (Figs. 3, 4).

**Material.** Holotype ♂, slide no. PL 3.2.12; paratypes: One ♂, four ♀♀. The holotype and paratype preparation of the male is stored in the collection of the Nature Museum of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam). Paratype preparations of females are stored in the collection of nematodes at the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).

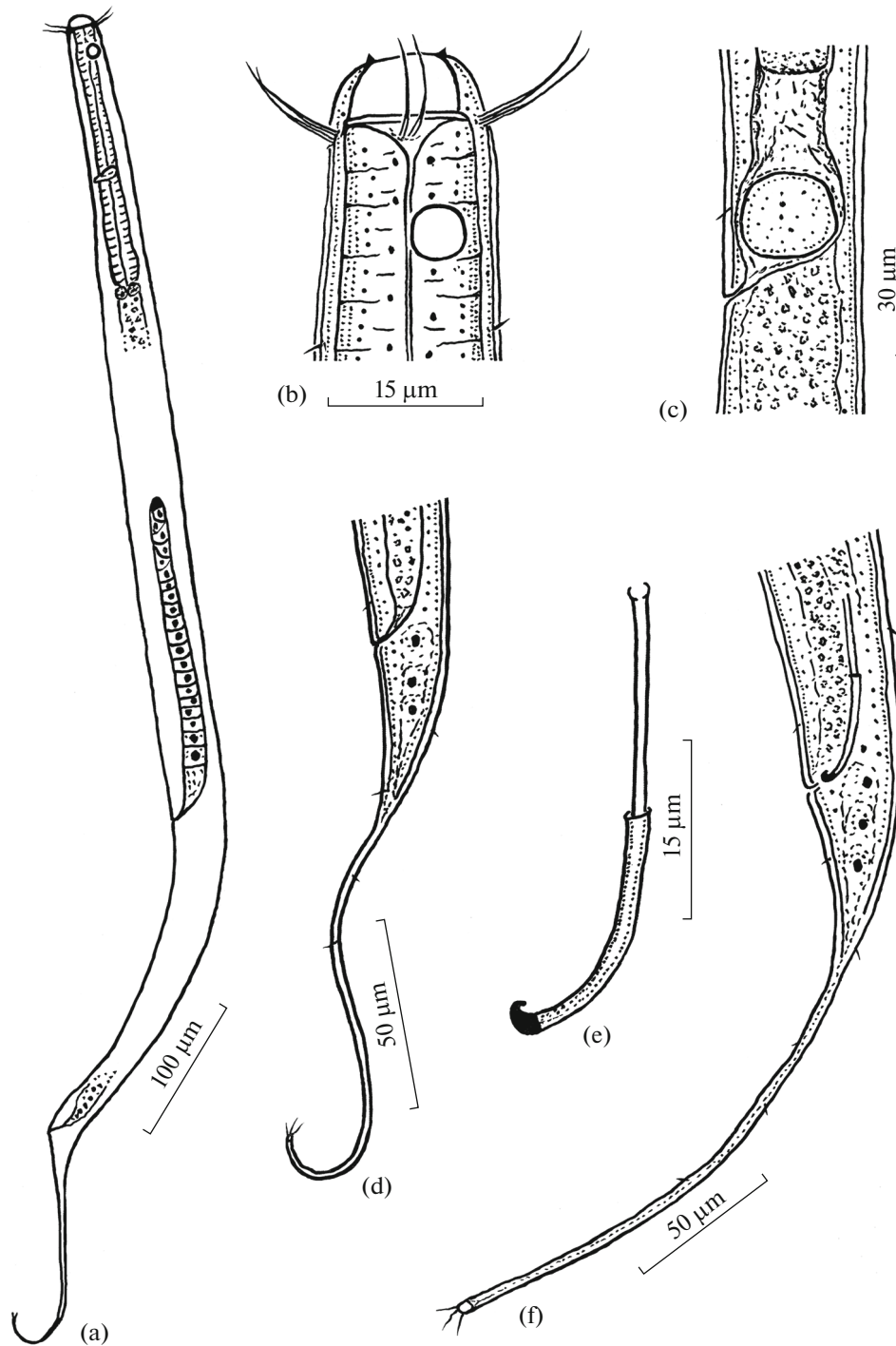
**Location.** Northern Vietnam, Quảng Ninh Province, artificial pond for shrimp aquaculture (20°48'84" N, 106°56'02" E). Depth 0.3 m, silty sand,

water salinity 24‰. Samples were taken in March 2016.

**Description.** The morphometric characteristics of the holotype and paratypes are given in Table 2.

**Male.** The body is thin and relatively short. The anterior end of the body is narrowed. The cuticle is finely annulated, thin. The cuticle thickness in the middle part of the body is ~1.0  $\mu\text{m}$ . Somatic setae rare and short, 1.0–1.5- $\mu\text{m}$  length. Six internal labial sensillae in the form of small papillae. Six external labial sensillae and six head sensillae in the form of thin bristles 11- $\mu\text{m}$ -length; they are arranged in one circle. Cheilostoma is relatively large. Faringostoma in the form of a funnel. Amphid fovea in the form of a circle 5  $\mu\text{m}$  in diameter (30% of the body width at this level) are located 10  $\mu\text{m}$  from the anterior end of the body. The pharynx is muscular, only slightly expanding to its base. The cardium is small, surrounded by three oval formations having a glandular structure. Renette, its ducts and pore not found. One testis, straight, located to the right of the middle intestine. The spicules are thin, slightly curved, with small heads. Their length exceeds the body width twofold in the area of the cloaca. Two gubernaculums in the form of a tube or “socket” cover the distal ends of the spicules. The apical ends of the gubernaculums are armed with clawlike formations. The gubernaculum is about half the length of the spicules. Precloacal complementary organs not found. The tail is long, consisting of two parts. It is conical at the base, then thin, flagellum-shaped. Flagellum length reaches 75% of the total tail length. Caudal setae short. The tail terminus is armed with three long setae. Caudal glands and spinneret are present.

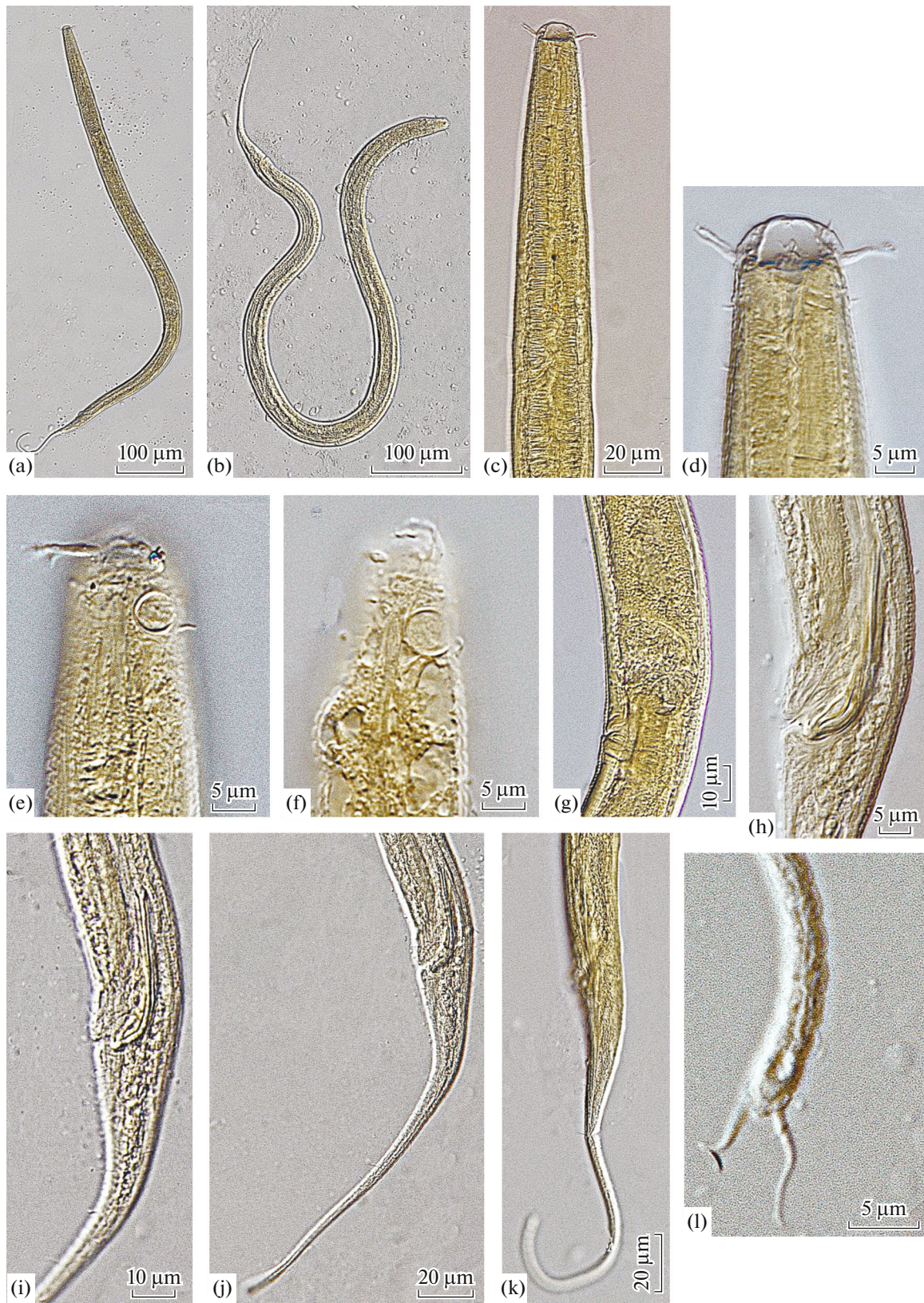
**Female s.** Similar to males in general morphology. The cuticle is finely annulated. The inner labial sensillae is in the form of papillae. Six external labial sensillae and six head sensillae in the form of setae of the same size; they are arranged in one circle. Cheilostoma large; pharyngostoma in the form of a funnel. Amphid fovea in the form of a circle with a diameter of ~4  $\mu\text{m}$  located 8–11  $\mu\text{m}$  from the anterior end of the body. The pharynx is muscular, slightly expanding to its base. There are three oval formations having a glandular structure around the cardium. The length of the rectum is smaller than the width of the body in the anus area. The ovary is single, anterior, and straight; it is located to the left of the intestine. The vulva is post-equatorial, in the form of a transverse fissure. The vagina is short, tilted to the anterior end of the body. The uterus is extensive. One female carries one egg (19 × 18  $\mu\text{m}$ ) in the uterus. Posterior uterus and post-vulvar cell not found. The tail is long and consists of two parts. Its anterior segment is conical; the posterior one is thin, in the shape of a flagellum. Flagellum reaches 68–72% of the total tail length. The tail terminus carries three long bristles. Caudal glands and spinneret are present.



**Fig. 3.** Female (a, b, c, d) and male (e, f) *Paramonhystra affinis* sp. n.: (a) general view, (b) head, (c) body in the vulva area, (d, e) tail, (e) spicule, and (f) gubernaculum.

**Differential diagnosis.** Recently, genus *Paramonhystra* Steiner, 1916 comprises 24 valid species (Gagarin, Nguyen Thi Thu, 2008; Bezerra, 2018). *Paramonhystra affinis* sp. n. is the closest morphologically to the two species: *P. parabütschlii* (Timm, 1961) Gagarin, Nguyen Thi Thu, 2008 and *P. levicula* (Lorenzen, 1972) Lorenzen, 1973. The first species is

described from the Bay of Bengal, one male (Timm, 1963). *P. affinis* sp. n. differs from this specimen by a slender body ( $a = 37$  versus  $a = 22$  in *P. parabütschlii*), larger number of the head setae (6 versus 4 in *P. parabütschlii*), longer external labial setae (10–12  $\mu\text{m}$  in length versus 7–8  $\mu\text{m}$  in *P. parabütschlii*), and shorter spicules (43  $\mu\text{m}$  in length versus 50  $\mu\text{m}$  in



**Fig. 4.** Male (a, c, d, f, h, i, j, l) and female (b, e, g, k) *Paramonhystra affinis* sp. n.: (a, b) general view, (c) anterior end of the body, (d, e, f) head, (g) body in the vulva area, (h, i) body in the cloaca area, (j, k) tail, and (l) tail terminus.

**Table 2.** Morphometric characteristics of *Paramonhystera affinis* sp. n.

Parameter	Holotype ♀	Paratypes		
		1♂	4♀	
			min–max	mean
<i>L</i> , μm	848	961	855–904	873
<i>a</i>	26	37	25–28	27
<i>b</i>	5.1	5.2	5.4–5.8	5.6
<i>c</i>	5.5	6.9	5.4–6.2	5.9
<i>c'</i>	8.2	6.6	7.5–8.8	8.1
<i>V</i> , %	57.6	–	58.6–61.2	60.3
Width of labial area, μm	16	15	14–16	15
Length of head setae, μm	11	11	10–12	10
Distance, μm:				
from amphid fovea to the anterior end of the body, μm	11	10	8–11	9
from pharynx end to vulva	320	–	345–394	367
from pharynx end to cloaca	–	638	–	–
from vulva to anus	204	–	187–209	200
Width, μm:				
in the middle part of the body	32	26	31–34	33
in the area of anus or cloaca	19	21	17–20	19
Length, μm:				
pharynx	165	184	153–160	156
tail	153	139	145–153	150
spicules (along the curve)	–	43	–	–
gubernaculum	–	23	–	–

*P. parabütschlii* (Timm, 1963). It differs from *P. levicula* by a larger number of external labial setae (six versus four in *P. levicula*); shorter and sparse somatic setae, especially on the anterior end of the body and on the tail (their length is ~1 μm versus 3–5 μm in *P. levicula*); the vulva is located much far from the anterior end of the body (*V* = 58.6–60.3% versus 53% in *P. levicula*); and there are shorter spicules (43 μm in length versus 64 μm, and twofold versus fourfold body diameter at cloaca area) (Lorenzen, 1972).

**E t y m o l o g y.** Species name means “similar.”

#### ACKNOWLEDGMENTS

We thank V.A. Gusakov (Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences) for taking the microphotographs of the new species of nematodes.

#### FUNDING

This work was carried out as part of a State Task of the Federal Agency for Scientific Organizations of Russia (theme no. AAAA-A18-118012690105-0) with partial financial support from the Vietnam National Foundation

for Science and Technology Development (NAFOSTED), grant no. FWO 106-NN.2015.04.

#### 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.

#### REFERENCES

- Bezerra, T.N., Decraemer, W., Eisendle-Flockner, U., et al., *Paramonhystera* Steiner, 1916, in Nemys: World Database of Nematodes, 2018. <http://marinespecies.org/aphia.php?taxdetails&id=227185>. Accessed September 11, 2018.
- Gagarin, V.G., An annotated checklist of the free-living nematodes from mangrove thickets of Vietnam, *Zootaxa*, 2008, no. 4403 (2), p. 261.
- Gagarin, V.G. and Nguen Tkhi Tkhu, Two new species of monchisterides (Nematoda, Monhysterida) from the Red River mouth in Vietnam, *Zool. Zh.*, 2008, vol. 87, no. 4, p. 505.



- Gagarin, V.G. and Nguen Vu Tkhan, New species of free-living nematodes of the Leptolaimidae family from the Kem River mouth, Vietnam, *Zool. Zh.*, 2005, vol. 84, no. 7, p. 771.
- Gusakov, V. and Gagarin, V.G., An annotated checklist of the main representatives of meiobenthos from inland water bodies of Central and Southern Vietnam. I. Round worms (Nematoda), *Zootaxa*, 2017, vol. 4300, no. 1.
- Hoang, L.-Ph., Dietrich, B., Nguyen, V.Th., and Ulrich, S.-P., Five new species of the genus *Leptolaimoides* Vitiello, 1971 (Nematoda: Leptolaimidae) from Can Gio Mangrove Biosphere Reserve, Vietnam, *Russ. J. Nematol.*, 2009, vol. 17, no. 1, p. 17.
- Holovachov, O., Swedish *Plectida* (Nematoda). The genus *Leptolaimoides* Vitiello, 1971, *Zootaxa*, 2015, vol. 3955, no. 1, p. 83.
- Lorenzen, S., Diagnosen einiger freilebender Nematoden von der schleswig-holsteinischen Westküste, *Veroeff. Inst. Meeresforsch. Bremerhaven*, 1966, vol. 10, no. 2, p. 31.
- Lorenzen, S., Die nematodenfauna in verlappungsgebiet für industrieabwasser nordwestlich von helgoland, *Zool. Anz.*, 1972, vol. 187, nos. 3–4, p. 223.
- Seinhorst, J.V., A rapid method for the transfer of nematodes from fixative to anhydrous glycerin, *Nematologica*, 1959, vol. 4, p. 67.
- Timm, R.W., New marine nematodes of the Bay of Bengal, *Proc. Pak. Acad. Sci.*, 1963, vol. 1, no. 1, p. 1.

*Translated by D. Martynova*