

BIOLOGY, MORPHOLOGY,
AND SYSTEMATICS OF HYDROBIONTS

Two New Species of Free-Living Nematodes
of the Family Linhomoeidae (Nematoda, Monhysterida)
from Mangrove Forests in Vietnam

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Abstract—An illustrated description of two species of free-living nematodes of the family Linhomoeidae Filipjev, 1922, new to science, found in the sediments of a thick mangrove forest in the Yên River, Vietnam, is presented. *Desmolaimus minor* sp. n. is morphologically similar to *D. calvus* Gerlach, 1956, but has a shorter body, two-separate internal cavity of pharynx, and setiform labial and cephalic sensilla. A review of species of the genus *Desmolaimus* de Man, 1888 is made, and a pictorial key to determinate valid species of this genus is provided. *Terschellingia yenkinsis* sp. n. is similar to *T. lissa* Timm, 1962 and *T. longissimicaudata* Timm, 1962. It differs from *T. lissa* by the presence of precloacal supplements in males, the presence of cervical setae, a longer tail, and shorter spicules; it differs from *T. longissimicaudata* by a comparatively longer tail, shorter spicules, and a longer dorsal apophysis of gubernaculum.

Keywords: Vietnam, mangrove forests, Yên River, free-living nematodes, *Desmolaimus minor* sp. n., *Terschellingia yenkinsis* sp. n.

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INTRODUCTION

The fauna of free-living nematodes from mangrove habitats in the coastal zone of the sea and river estuaries in Vietnam has been studied since 2012. A total of 67 nematode species have been found in this cenosis inhabiting the mouth of the Yên River, of which 23 are new to science (12 species have already been described and illustrated [1, 2, 8, 9]).

The aim of this work is an illustrated description of two free-living species of nematodes which are new to science.

MATERIALS AND METHODS

The material for the work was 23 samples of nematodes collected from the mouth of the Yên River (Dongh Rui, Sông Yên, Quảng Ninh Province), northern Vietnam, in March 2014. Nematodes were sampled using a plastic cylinder 3.5 cm in diameter and 10 cm in length at eight stations. Samples were fixed with hot (60–70°C) 4% formalin solution. After decanting, a sample was placed in a container with a volume of 200 mL, supplemented with a Ludox-TM50 solution, and centrifuged 5 times for 40 min each time. After that the nematodes were transferred to pure glycerin and then mounted in a small drop of glycerin on a

slide and sealed with a ring of paraffin wax. Measurements, identification of worms, photographing, and drawings were made using a Nikon Eclipse80i light microscope with equipment for observing a DNA contrast, a Nikon DS-Fil digital camera, and a personal computer with the installed NIS-Elements D3.2 software for analyzing and documenting images from mounts.

The abbreviations used in the text and tables are as follows: *L*, body length; *a*, body length to pharynx length ratio; *c*, body length to tail length ratio; *c'*, ratio of tail length to body width at anus or cloaca; and *V*, ratio of length from anterior body end to vulva to total body length, %.

RESULTS AND DISCUSSION

Description of new species. Order Monhysterida Filipjev, 1929. Family Linhomoeidae Filipjev, 1922. Genus *Desmolaimus* de Man, 1880. *Desmolaimus minor* Gagarin sp. n. (Figs. 1, 2).

Material. Holotype ♂; inventory no. of mount, TY 3.1.13; paratypes: 4 ♀♀, 4 ♂♂. The mounts of the holotype and paratypes are deposited at the Vietnam National Museum of Nature (Hanoi, Vietnam).

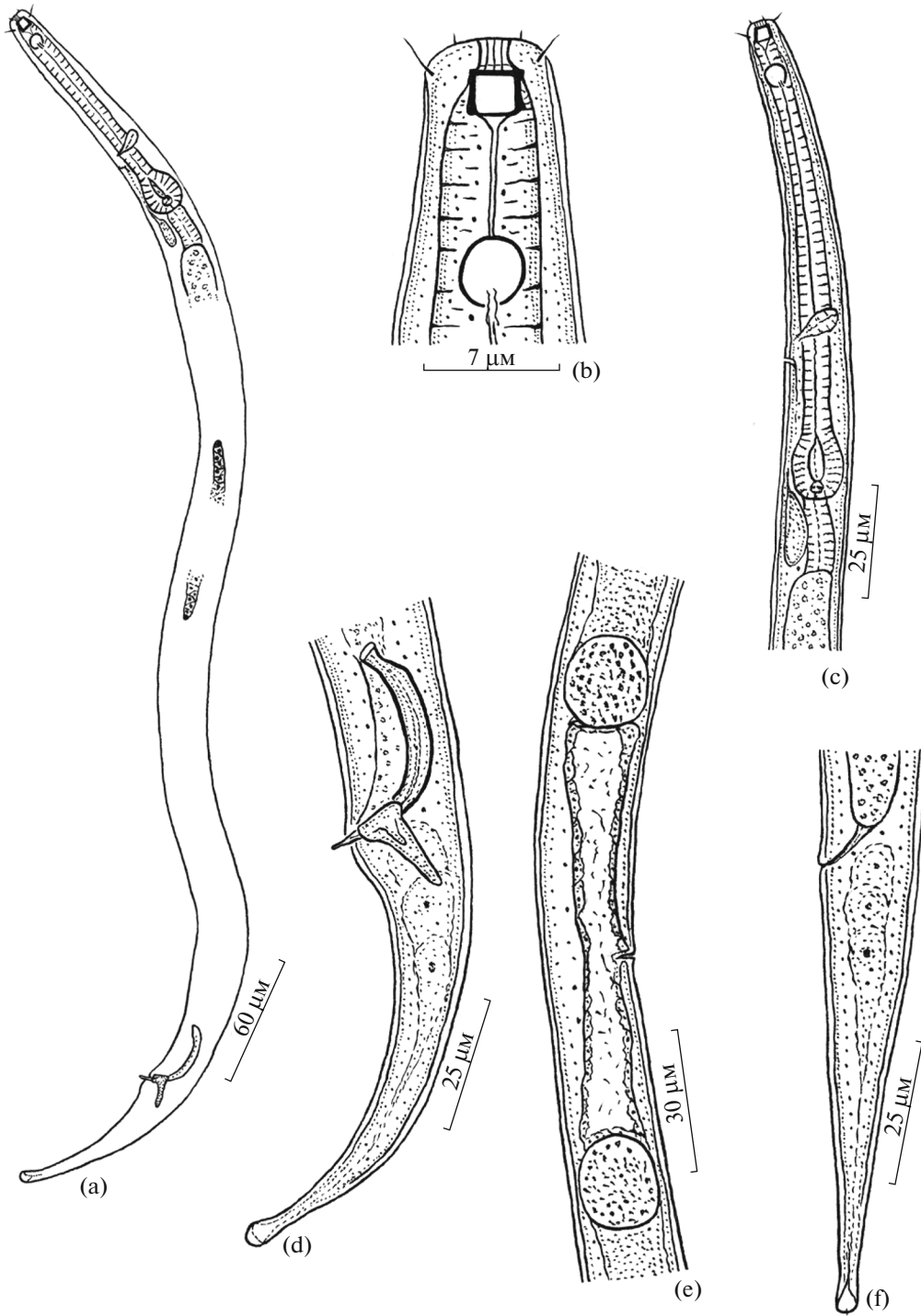


Fig. 1. Structure of a male (a, b, c, d) and female (e, f) *Desmolaimus minor* sp. n.: (a) general view, (b) head, (c) anterior end of body, (d, f) tail, and (e) body in the area of vulva.

Locality. Northern Vietnam, Quảng Ninh Province, the Yên River estuary. Coordinates: 21°13'01'' N, 107°22'36'' E. Mangrove forest; depth, 1.2 m; sediment, silty sand; water salinity, 4.2‰.

Description. Morphometric characteristics of the holotype and paratypes are listed in Table 1.

Male. Small, relatively thin worm. Both body ends narrowed. Cuticle smooth, without somatic setae and cuticular pores. Anterior head edge flattened. Lips poorly expressed. Mouth orifice surrounded by six thin labial setae ~1 µm long. Four longer cephalic setae, 2.5–3.0 µm long, constituting 40–45% of labial area width, located slightly posteriorly. Cervical setae

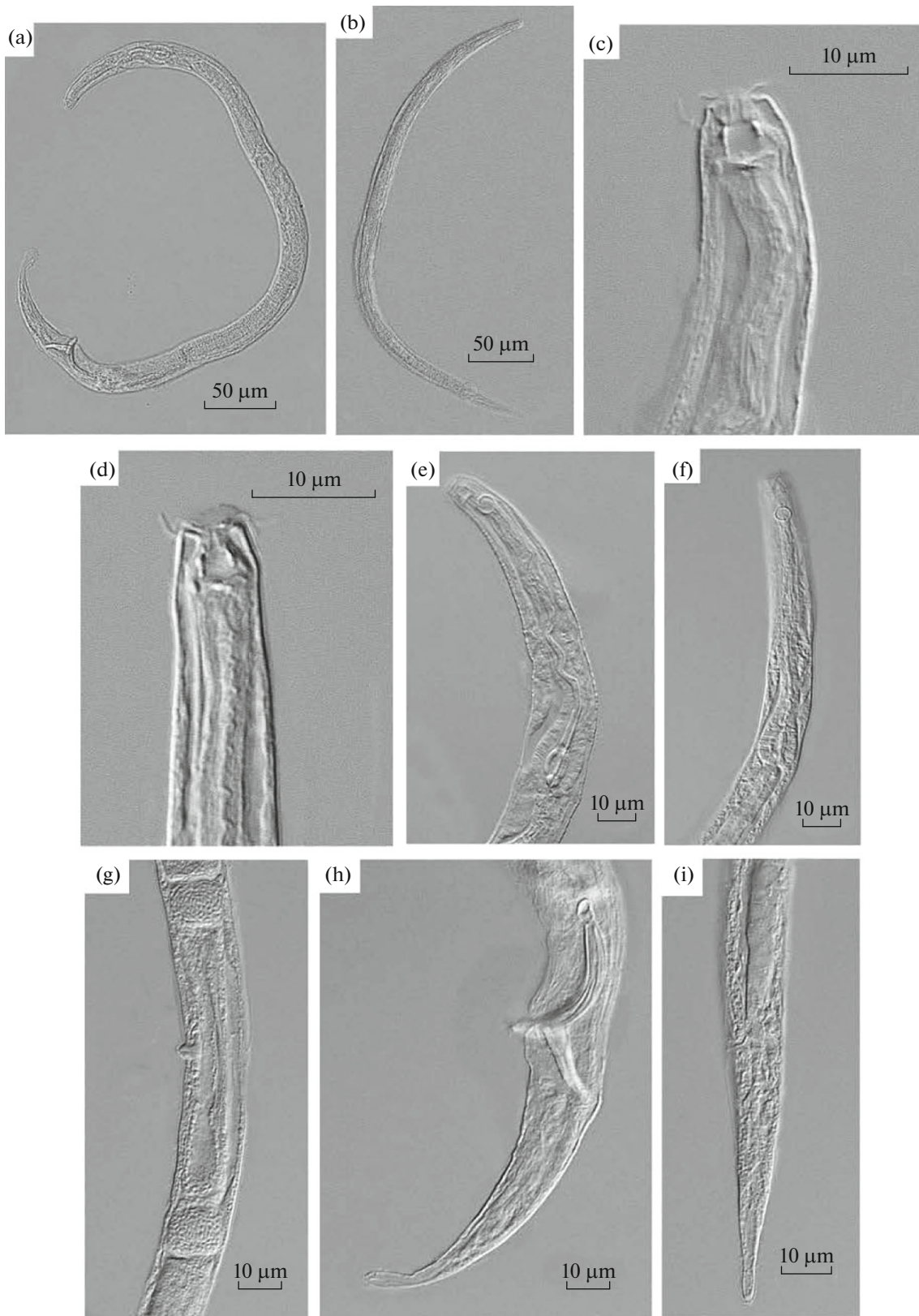


Fig. 2. Male (a, c, e, h) and female (b, d, f, g, i) *Desmolaimus minor* sp. n.: (a, b) general view, (c, d) head, (e, f) anterior end of body, (g) body in the area of vulva, and (h, i) tail.

Table 1. Morphometric characteristics of *Desmolaimus minor* sp. n.

Character	Holotype	Paratypes			
	♂	4 ♂♂		4 ♀♀	
		min–max	mean	min–max	mean
<i>L</i> , μm	548	515–588	560	440–517	492
<i>a</i>	27	26–29	27	26–33	27
<i>b</i>	5.7	5.5–5.9	5.7	5.6–6.9	6.2
<i>c</i>	6.8	6.8–7.5	7.3	6.0–6.6	6.3
<i>c'</i>	4.6	3.5–4.6	4.0	7.1–8.3	7.1
<i>V</i> , %	–	–	–	50.7–53.8	52.0
Width, μm:					
labial area	6.0	5.5–6.5	6.0	5.5–6.5	6.0
middle body	20	20–22	21	15–20	18
body at anus or cloaca	17	16–18	17	9–12	11
Length, μm:					
external labial setae	2.5	2.5–3.0	2.7	2.5–3.0	2.8
stoma	3.5	3.0–4.0	3.5	3.0–4.0	3.5
pharynx	96	93–102	98	75–86	80
Distance, μm:					
from anterior edge of amphidial fovea to anterior body end	9.0	8.5–9.5	9.0	8.3–9.8	9.2
from pharynx end to vulva	–	–	–	153–203	176
from pharynx end to cloaca	369	353–408	385	–	–
from vulva to anus	–	–	–	138–168	158
Length, μm:					
tail	83	69–78	77	71–86	78
spicules (by arch)	39	37–39	38	–	–
dorsal apophysis of gubernaculum	17	17–19	18	–	–

not found. Cheilostoma with cuticulated ribs. Pharyngostoma relatively extensive, with very cuticulated walls, separated from cheilostoma by easily discernible cuticulated ring. Total stoma length equal to or slightly larger than labial area width. Amphidial fovea in form of circle broken at bottom, its diameter reaching 35–40% of respective body diameter; fovea located at ~1.5 diameter of labial area from anterior body end. Pharynx slender, muscular, with well-pronounced basal bulb. Internal cavity of bulb extensive, with cuticulated walls, separated into two compartments: anterior (large) and posterior (small). A similar structure of the internal cavity of basal bulb was observed in *Desmolaimus zeelandicus* de Man, 1880 and *D. bulbulus* Lorenzen, 1969 [22, 23]. Cardia muscular, relatively long, its length equal to or slightly greater than the length of basal bulb of pharynx. Renette located in the area of cardia, with its excretory pore located posteriorly to nerve ring.

Testes paired, opposed. Spicules slender, slightly curved, with well-expressed heads. Length of spicules

1.2–2.3 times body diameter at cloaca. Gubernaculum small, with large dorsal process 17–19 μm long. Tail elongated, conical, gradually tapering. Precloacal supplements not found. Tail terminus slightly swollen. Caudal setae not found. Caudal glands present, but poorly developed. Spinneret present.

Females are similar to males in general morphology. Structure of cuticle and anterior body end similar to those in males. Cuticle smooth. Six labial setae ~1 μm long and four cervical setae 2.5–3.0 μm long. Cervical setae not found. Cheilostoma with cuticulated ribs. Pharyngostoma four-angled, with much cuticulated external wall, separated from cheilostoma by a well-expressed cuticular ring. Amphidial fovea in form of a circle broken in its lower part. Pharynx slender, muscular, with pronounced basal bulb. Internal cavity of bulb separated into two compartments: anterior (extensive) and posterior (smaller). Cardia muscular, long. Renette located in the area of cardia, with its excretory pore located slightly below

nerve ring. Rectum length equal to or slightly smaller than body diameter at anus.

Ovaries two, both straight, without bends, located right of gut. Vulva in form of transverse slit, located approximately in mid-body. Labia of vulva not cuticulated, but slightly extend beyond the contours of body. Vagina relatively short, its walls thickened. Each ovary has rounded spermathecae 16–18 μm in diameter. Uteri extensive. One individual had one egg $35 \times 20 \mu\text{m}$ in its uterus. Tail elongated, conical. Caudal glands poorly developed.

Diagnosis. Body short, relatively slender ($\delta\delta$ $L = 515\text{--}588$, $a = 26\text{--}29$; ♀♀ $L = 440\text{--}517 \mu\text{m}$, $a = 26\text{--}33$). Cuticle smooth. Lips not expressed. Six labial setae $\sim 1 \mu\text{m}$ long and four cephalic setae $2.5\text{--}3.0 \mu\text{m}$ long. Cervical setae not found. Cheilostoma with ribs. Pharyngostoma four-angled, with much cuticulated walls, and separated from cheilostoma by pronounced cuticular ring. Amphidial fovea in the form of a circle broken at the bottom. Pharynx with well-pronounced bulbus. Internal cavity of bulbus separated into two compartments: anterior extensive and posterior small. Cardia long. Spicules large, with well expressed heads. Their length 2.1–2.3 times body diameter at cloaca. Gubernaculum with large dorsal process. Tail elongated, conical. Its terminus slightly swollen.

Differential diagnosis. The new species is the smallest one among the valid species of the genus (Table 2). Morphologically, it is closer to the species *Desmolaimus calvus* Gerlach, 1956, described from mangrove forests on the coast of Brazil [10]. It differs from the latter by a shorter body ($L = 440\text{--}588 \mu\text{m}$ vs. $L = 863 \mu\text{m}$ in *D. calvus*), the presence of labial and cephalic sensilla in the form of setae (in *D. calvus* they are in the form of papillae), and the presence of two-separate internal cavity of pharynx (in *D. calvus* it is single, not separated) [10].

Morphological and taxonomic remarks. According to different authors [7, 13], the genus *Desmolaimus* de Man, 1880 includes 12–14 species. Of them, eight were described by Allg n [3, 5], but four were attributed to the category of “species inquirenda” rather than valid species [9]. Three species (*D. conicaudatus* Allg n, 1959, *D. macrocirculus* Allg n, 1959, and *D. propinquus* Allg n, 1959) were described from a single individual, male or female. The description is brief; it lacks the morphological traits that are included in the diagnosis of the genus *Desmolaimus*. Thus, the structure of stoma is not provided. The illustrations are schematic; they also do not contain a depiction of stoma. Accordingly, these three species are attributed by the author to the category “species inquirenda”; the only species recognized as valid is *D. zosteriae*, described by Allg n [3]. Therefore, eight valid species were accepted in the composition of the genus: *D. brasiliensis* Gerlach, 1963, *D. bulbosus* Lorenzen, 1969, *D. calvus* Gerlach, 1956, *D. courti* Leduc, Gwyther, 2008, *D. longicaudatus* Kreis, 1929,

D. zeelandicus de Man, 1880, *D. zosteriae* Allgen, 1933, and *D. minor* sp. n.

Morphological characteristics of these species are provided in Table 2; a pictorial key to their identification, in Fig. 3.

Etymology. The species name means *small in size*, or *little*.

Genus *Terschellingia* de Man, 1888. *Terschellingia yensis* Gagarin sp. n. (Figs. 4, 5).

Material. Holotype δ ; inventory no. of mount TY 2.1.14; paratypes: 10 $\delta\delta$, 10 ♀♀ . The holotype mount is deposited at the Vietnam National Museum of Nature; the mounts of the paratypes are in the collection of the Department of Nematology, Institute of Ecology and Biological Resources (Hanoi, Vietnam).

Locality. Northern Vietnam, Quang Ninh Province, Yen River estuary. Coordinates: $21^{\circ}12'68''$ N, $107^{\circ}24'88''$ E. Mangrove forests of the Yen River; depth, 1.0 m; sediment, silty sand; water salinity, 7.7‰.

Description. Morphometric characteristics of the holotype and paratypes are listed in Table 3.

Males. Relatively thin and small worms with long tail. Tail in fixed mounts, usually bent dorsally. Cuticle thin-ringed, 0.5–0.7 μm thick mid-body. Somatic setae short and rare. Cephalic end narrowed. Lips not expressed. Internal labial sensilla in form of small papillae. Six external labial sensilla in form of thin setae 4.2–4.6 μm in length. Cephalic sensilla not found. Four cervical setae located behind amphidial fovea, 4.4–5.0 μm long. Amphidial fovea in the form of a circle 6.5–7.2 μm in diameter, which equals 55–60% body width at this level, located at 3.4–4.0 μm from anterior body end. Stoma small, funnel-shaped. No teeth found in stoma. Pharynx muscular, comparatively short. Basal bulbus of pharynx large, well developed. Its internal cavity relatively extensive, its walls sclerotized. Cardia small, protruding into lumen of midgut. Position of renette could not be detected. Excretory pore opens slightly behind nerve ring.

Testes paired, opposed. Spicules arcuate, their length 1.6–1.8 times body diameter at cloaca. Gubernaculum relatively small with pair of dorsal processes 12–15 μm long. Precloacal supplements in the form of small, barely noticeable papillae, four or five in number. Tail very long, always bent to dorsal side and clearly divided into two segments. Anterior segment conical, short; posterior thin, whiplike (flagellum). Length of anterior segment reaches 9–12% total tail length. Caudal setae and spinneret present.

Females. Similar to males in general morphology, but larger, with relatively longer tail. Cuticle slightly ringed. Somatic setae rare. Labial sensilla in form of small papillae. Six external labial sensilla in form of thin setae 4.2–4.6 μm long. Cephalic sensilla not found. Cervical setae 4.0–4.5 μm long and located immediately after amphidial fovea. Amphidial fovea in

Table 2. Morphometric characteristics of valid species of the genus *Desmolaimus*

Species	Sex	<i>L</i> , mm	<i>a</i>	<i>b</i>	<i>c</i>	<i>c'</i>	<i>V</i> , %	<i>l_{st}</i>	<i>l_{st}/l</i> , %	<i>cst</i>	<i>fv/l</i> , %	<i>sp</i>	<i>sp/cl</i>	Reference
<i>D. brasiliensis</i>	♀♀	3120	115	19.5	20.8	8.0	50	14	100	+	1.0	—	—	[12]
	♂♂	2056	103	14.3	19.4	5.5	—	14	100	+	1.0	19	1.0	
<i>D. bulbosus</i>	♀♀	910	38	8.8	6.5	8.0	46	2	25	+	1.1	—	—	[17]
	♂♂	840	34	7.6	8.0	5.2	—	2	22	+	1.3	21	1.0	
<i>D. calvus</i>	♀	863	29	7.2	8.0	5.0	52	pap	—	—	1.3	—	—	[10]
<i>D. courti</i>	♀♀	2181–2372	67–82	14.6–17.2	23.0–24.5	4.5–5.1	49–61	14–15	71–72	+	0.7–0.8	—	—	[16]
	♂♂	1743–2012	67–81	12.6–13.4	15.0–18.3	4.7–4.8	—	15	71–83	+	0.9	22–27	0.9–1.1	
<i>D. longicaudatus</i>	♀♀	1260	30	11.5	5.7	8.5	47.3	3.2	18	+	0.5	—	—	[15]
<i>D. minor</i> sp. n.	♀♀	440–517	27–33	5.6–6.9	6.0–6.6	7.1–8.3	50.7–53.8	2.5–3.0	40–45	—	1.5	—	—	Data by author
	♂♂	515–588	26–29	5.5–5.9	6.8–7.5	3.5–4.6	—	2.5–3.0	40–44	—	1.5	31–39	2.1–2.3	
<i>D. zeelandicus</i>	♀♀	1101–2100	24–57	9–12	8–13	4.2–6.3	49–53	3.0–6.5	20–35	+	0.9–1.2	—	—	[18, 20]
	♂♂	1400–2100	24–57	9–12	8–13	—	—	3.0–6.5	20–35	+	0.9–1.2	32–36	0.9–1.2	
<i>D. zosteræ</i>	♀♀	1200	30	7.7	?	?	?	7.0	50	—	0.9	—	—	[3]
	♂♂	1175	44	6.9	9.8	4.5	—	7.0	50	—	0.9	31	1.1	

l_{st}, length of external labial setae, µm; *l_{st}/l*, ratio of length of external labial setae to diameter of labial area; *cst*, presence of cervical setae; *fv/l*, ratio of distance from anterior edge of amphidial fovea to diameter of labial area; *sp*, spicule length, µm; *sp/cl*, ratio of spicule length to body diameter at cloaca; and *pap*, papillae. The plus sign means present; a dash means absent; and a questions mark means that data are not provided in work [3].

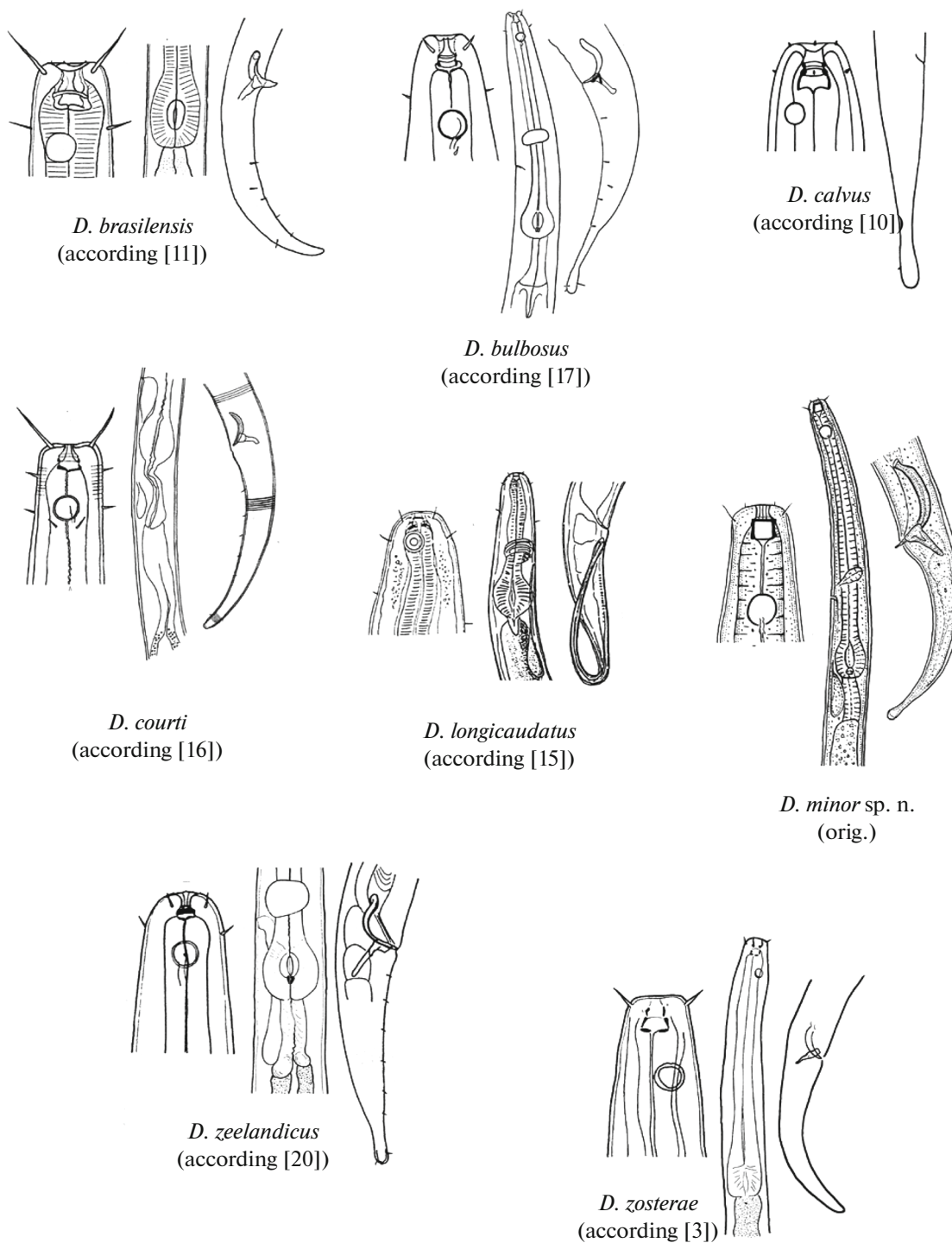


Fig. 3. Pictorial key for identifying the valid species of the genus *Desmolaimus*.

form of circle 6.0–6.6 μm in diameter, located at 4.5–5.0 μm from anterior body end. Stoma small, lacking teeth. Pharynx muscular, with pronounced basal bulbus. Cardia protrudes into lumen of midgut. Rectum length slightly smaller than body diameter at anus.

Gonads paired, straight; vulva preequatorial, in the form of a transverse slit. Labia of vulva not cuticulated

and not protruding beyond contours of body. Vagina short, length equal to $\sim 1/3$ body width at this level. Uteri extensive, containing numerous spermatozoa and 1–3 eggs (42–48) \times (18–20) μm . Tail long, consisting of anterior conical segment and long flagellum. Length of anterior segment equals to 8–10% total tail length. Caudal setae and spinneret present.

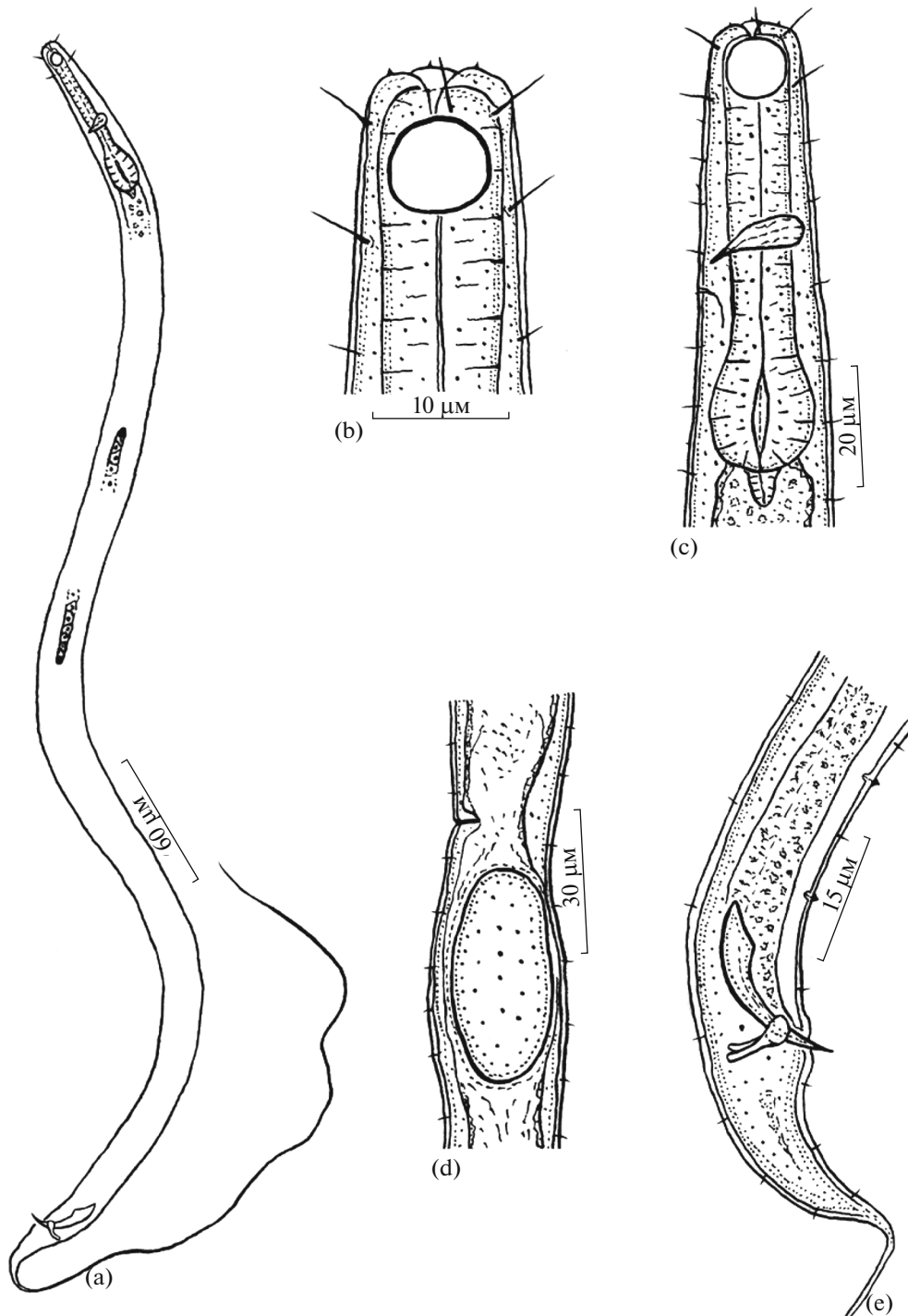


Fig. 4. Structure of a male (a, b, c, e) and female (d) *Terschellingia yenensis* sp. n.: (a) general view, (b) head, (c) anterior end of body, (d) body in the area of vulva, and (e) body in the area of cloaca.

Diagnosis. Relatively short and thin worms with a relatively long tail ($\sigma\sigma$ $L = 771\text{--}983\ \mu\text{m}$, $a = 39\text{--}53$, $c = 2.5\text{--}3.1$; ♀♀ $L = 908\text{--}1021\ \mu\text{m}$, $a = 44\text{--}57$, $c = 2.4\text{--}2.8$). Cuticle thin-ringed. Somatic setae rare. Internal labial sensilla in form of small papillae. External labial sensilla in form of thin setae. Cephalic sen-

silla not found. Cervical setae located immediately after amphidial fovea. Amphidial fovea circle-shaped, located at 0.3–0.5 width of lip area from anterior body end. Stoma small, without teeth. Esophagus muscular, with large, well-developed basal bulb. Spicules curved, 1.6–1.8 times body diameter at cloaca. Guber-

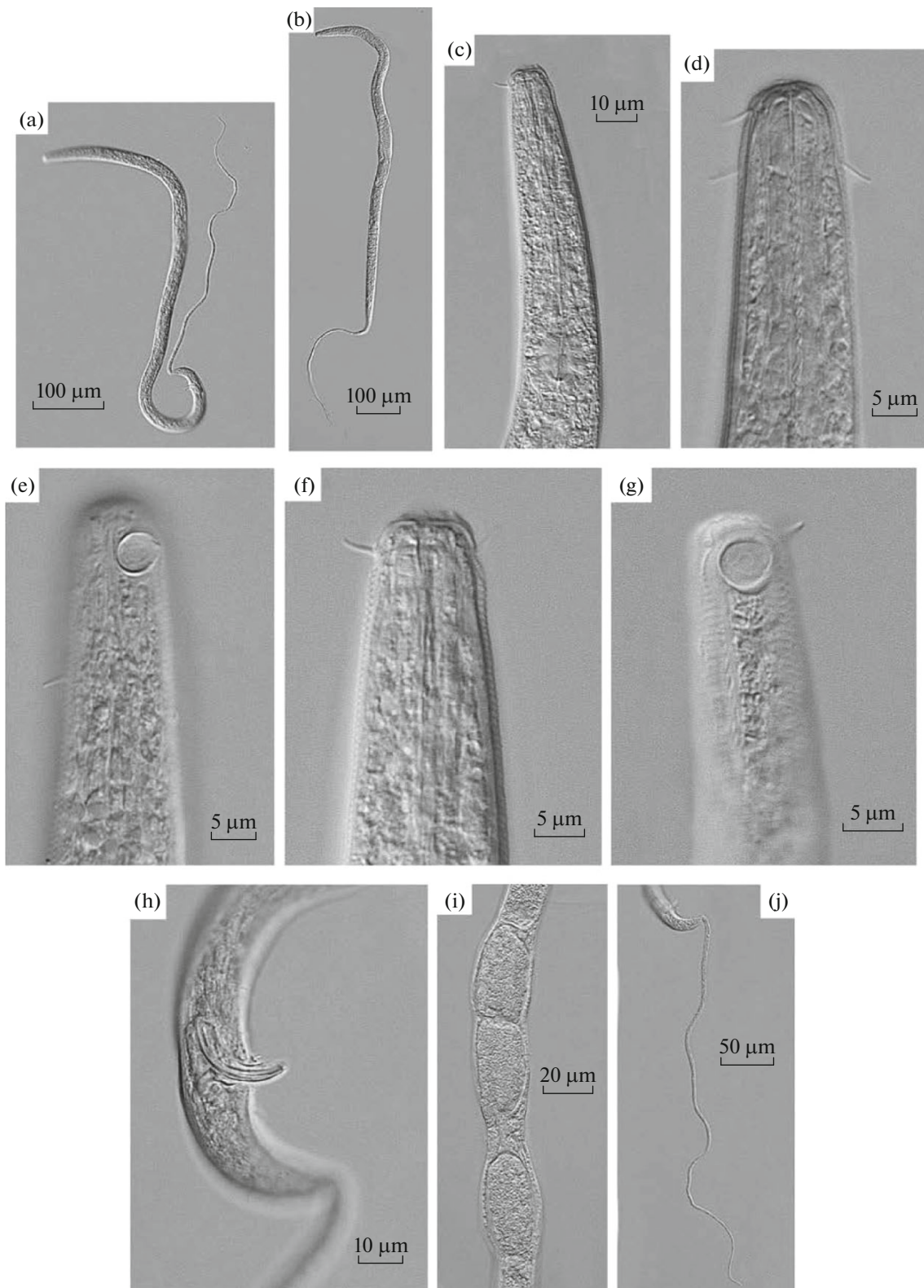


Fig. 5. Male (a, c, d, e, h, j) and female (b, f, g, i) *Terschellingia yenensis* sp. n.: (a, b) general view, (c) anterior end of body, (d, e, f, g) head, (h) body in the area of cloaca, (i) body in the area of vulva, and (j) tail.

Table 3. Morphometric characteristics of *Terschellingia yenensis* sp. n.

Character	Holotype	Paratypes	
	♂	10 ♂♂	10 ♀♀
<i>L</i> , μm	830	826 ± 58(771–983)	969 ± 44(908–1031)
<i>a</i>	52	46 ± 6(39–53)	48 ± 4(44–57)
<i>b</i>	11.5	10.6 ± 7.0(9.2–11.5)	12.4 ± 1.0(10.0–13.3)
<i>c</i>	2.8	2.7 ± 0.2(2.5–3.2)	2.6 ± 0.1(2.4–2.8)
<i>c'</i>	24.4	21.7 ± 3.9(16.6–28.6)	30.9 ± 1.4(29.8–33.1)
<i>V</i> , %	–	–	33.7 ± 1.4(32.6–36.6)
Body width, μm:			
at cephalic setae	9	10 ± 1(8–11)	9 ± 1(7–11)
in middle body	16	18 ± 2(15–21)	20 ± 1(18–21)
at anus or cloaca	12	14 ± 1(12–15)	11 ± 1(10–12)
Length, μm:			
external labial setae	4.5	4.3 ± 0.1(4.2–4.6)	4.2 ± 0.1(4.0–4.5)
pharynx	72	78 ± 8(69–91)	79 ± 5(74–90)
tail	293	303 ± 23(279–365)	379 ± 32(336–423)
spicules (by arch)	24	25 ± 1(23–26)	–
dorsal apophysis of gubernaculum	13	14 ± 1(13–16)	–
Distance, μm:			
from anterior body end to amphidial fovea	4.0	3.7 ± 0.1(3.4–4.0)	4.7 ± 0.1(4.5–5.0)
from pharynx end to vulva	–	–	250 ± 14(228–270)
from pharynx end to cloaca	465	445 ± 41(395–528)	–
from vulva to anus	–	–	261 ± 9(240–273)

Values before parentheses are the mean value of the character and its error; those in parentheses are the minimum and maximum values of the character.

naculum with paired dorsal processes. Precloacal supplements in form of small papillae, four or five in number. Tail very long, consisting of anterior conical segment and posterior thin whiplike segment. Length of anterior segment reaches 8–12% total tail length.

Differential diagnosis. By its morphological characteristics, *Terschellingia yenensis* sp. n. belongs to the species group *longicaudata* of the genus *Terschellingia* [6, 8]. In body length, it is close to *T. lissa* Timm, 1962 and *T. longissimicaudata* Timm, 1962. *T. yenensis* sp. differs from both species by the presence of precloacal supplements in males (male *T. lissa* and *T. longissimicaudata* lack precloacal supplements). Moreover, the new species is distinguished from *T. lissa* by the presence of cervical setae (*T. lissa* individuals lack these setae), a longer tail (♀♀ *c* = 2.4–2.8, *c'* = 29.8–33.1, ♂♂ *c* = 2.5–3.2, *c'* = 16.6–28.6 vs. ♀♀ *c* = 3.6–3.9, *c'* = 12.8–16.2, ♂♂ *c* = 3.8–4.4, *c'* =

9.0–10.8 in *T. lissa*), and shorter spicules (23–26 μm vs. 32–35 μm in *T. lissa*) [19]. It differs from *T. longissimicaudata*, which was described from only one male, by a relatively longer tail (♂♂ *c'* = 16.6–28.6 vs. *c'* = 16.3 in *T. longissimicaudata*), shorter spicules (24–26 μm vs. 27 μm in *T. longissimicaudata*), and a longer dorsal apophysis of gubernaculum (14–16 μm vs. 9 μm) [19].

Etymology. The species name means “from the Yên” (the name of the river where the species was found).

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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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