

Research Article

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Description of two new species, *Microlaimus paraaffinis* sp. nov. and *Pseudomicrolaimus major* sp. nov. (Nematoda: Microlaimidae), from Yangma Island, the Yellow Sea, China

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Abstract

Two new species of Microlaimidae: *Microlaimus paraaffinis* sp. nov. and *Pseudomicrolaimus major* sp. nov. are described from Yangma Island, the Yellow Sea. *M. paraaffinis* sp. nov. is characterized by body length 662–785 µm, six inner and six outer labial sensilla papilliform, four short cephalic setae 3–4 µm in length, amphidial fovea cryptocircular at level of buccal cavity, spicules L-shaped with equal thickness, gubernaculum boat shaped, tail conico-cylindrical with short cylindrical portion. *P. major* sp. nov. is characterized by large body size, six inner labial sensilla papilliform, six outer labial sensilla and four cephalic sensilla setiform, eight subcephalic setae present, amphidial fovea cryptospiral, anterior pharynx region at the buccal cavity widened, posterior pharynx region with oval-shaped bulb, spicules curved with proximal portion enlarged, gubernaculum boat shaped, tail short and conical. Pictorial key to genus *Pseudomicrolaimus* is given.

Introduction

To study the diversity and taxonomy of free-living marine nematodes in Yangma Island, undisturbed sediments were acquired along the coast of the Yellow Sea in July, 2019. Marine nematodes are the dominant meiofauna group.

The genus *Microlaimus* was erected by de Man in 1880 with the type species of *M. globiceps* de Man, 1880 and was the most diverse group in the family Microlaimidae Micoletzky, 1922. Gerlach (1950) first reviewed the genus, described three new species, re-described 16 reported species, provided two new names, and subdivided the valid species into six groups mainly based on buccal cavity structure, cuticle, and tail shape. Wieser (1954) described five new species of *Microlaimus* and erected the genus *Paramicrolaimus* Wieser, 1954 in Microlaimidae. Jensen (1978) reviewed Microlaimidae with more differentiating characters, erected Molgolaimidae Jensen, 1978 to accommodate species with reflexed ovaries and one testis from *Microlaimus* and transferred eight *Microlaimus* species to *Molgolaimus* Ditlevsen, 1921 and regarded *M. labradorensis* Allgén, 1957, *M. tenuicaudatus* Allgén, 1959 and *M. tenuilaimus* Allgén, 1932 as species inquirendae for poor description, erected the genus *Aponema* Jensen, 1978 with type species of *A. torosum* (Lorenzen, 1973) Jensen, 1978 transferred from *Microlaimus*, four species to *Bolbolaimus* Cobb, 1920 based on buccal cavity and pharynx structure, three species from *Paramicrolaimus*. Taxonomic relationships of *Microlaimus* with the genus *Molgolaimus* (ovaries reflexed vs ovaries outstretched in *Microlaimus*) and other genera in Microlaimidae have been deeply discussed. Lorenzen (1981, 1994) discussed the phylogenetic position of Microlaimidae and considered Jensen's taxonomic characters variable, downgraded Molgolaimidae as Molgolaiminae Jensen, 1978 in Desmodoridae Filipjev, 1922 and erected the family Paramicrolaimidae Lorenzen, 1981 to accommodate *Paramicrolaimus*. Kovalyev and Tchesunov (2005) discussed taxonomic problems of *Microlaimus* with *Molgolaimus*, *Aponema*, and *Pseudomicrolaimus* Sergeeva, 1976 based on characters of ovary structure, tail shape, stoma and copulatory apparatus, described one new species of *M. paraconothelis* Kovalyev and Tchesunov, 2005, and placed *Microlaimus citrus* as *incertae sedis*. Tchesunov (2014) transferred *M. bathyalis* (Kovalyev and Miljutina, 2009) Tchesunov, 2014, *M. martinezi* (Miljutin and Miljutina, 2009) Tchesunov, 2014, *M. minutissimus* (Kovalyev and Miljutina, 2009) Tchesunov, 2014, *M. nanus* Blome, 1982, *M. nymphea* (Bussau and Vopel, 1999) Tchesunov, 2014, *M. westindicus* (Kovalyev and Miljutina, 2009) Tchesunov, 2014 from *Aponema* with absence of gubernaculum apophysis. Leduc (2016) discussed the differentiation between *Microlaimus* and *Bolbolaimus* with the character of a constriction setting off the head from the rest of the body, resurrected *M. wieseri* (Hopper, 1961) from *Bolbolaimus* by the presence of set off head, described one new species of *M. korari* Leduc, 2016, and listed 83 valid species. After Leduc (2016), *M. pecticauda* Murphy, 1966 and *M. spirifer* Warwick, 1970 were transferred to *Molgolaimus* (Shi and Xu, 2017), by now 87 valid species have been listed in Nemys (Bezerra *et al.*, 2023).

The genus *Pseudomicrolaimus* was first erected by Sergeeva in 1976 with the description of *P. murinae* Sergeeva, 1976 with the character of wide and funnel-shaped buccal cavity with



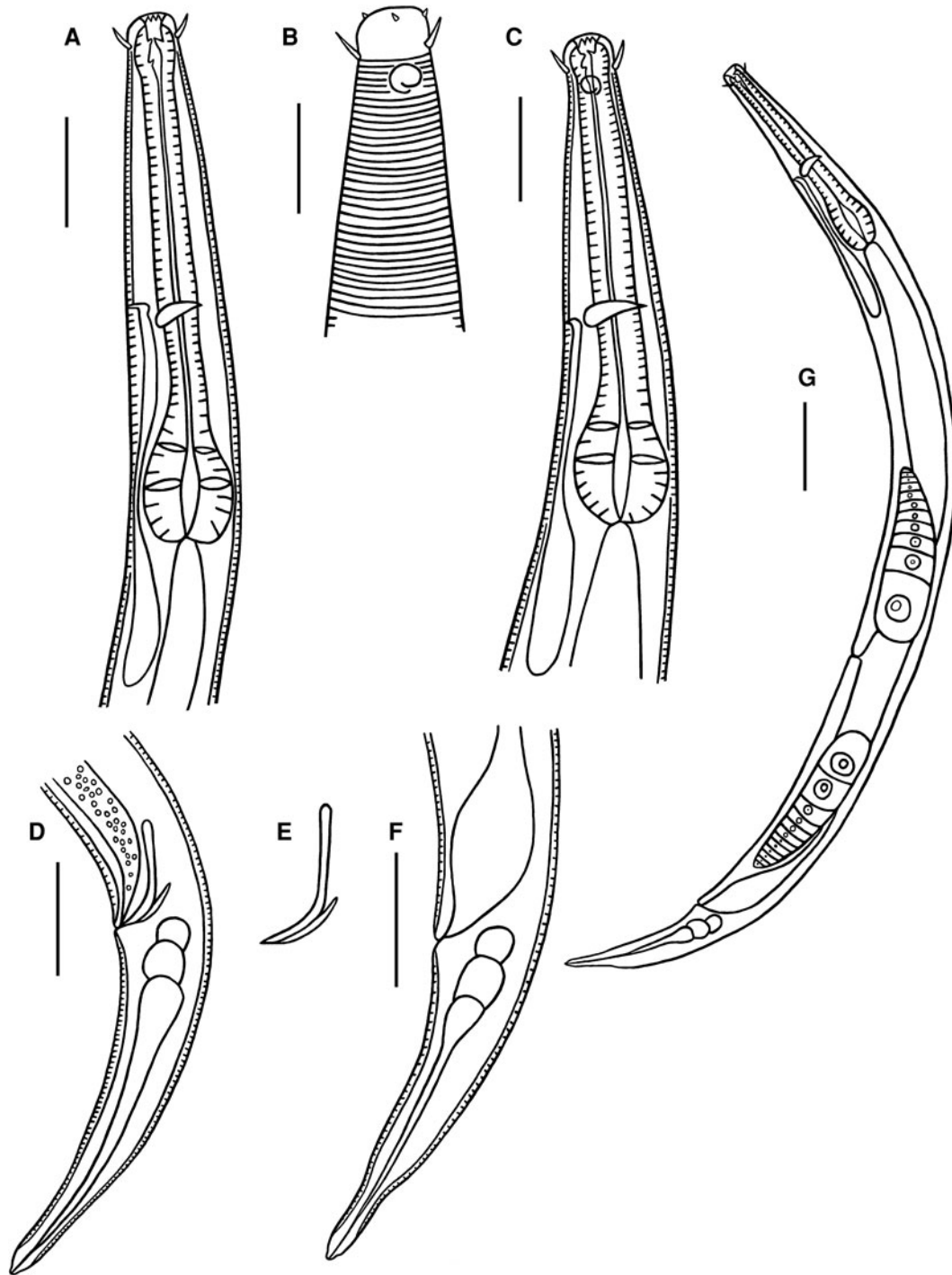


Figure 1. *Microlaimus paraaffinis* sp. nov. (A) Lateral view of male anterior portion, showing buccal cavity and pharynx region (holotype); (B) Lateral view of male anterior end, showing amphids (holotype); (C) Lateral view of female anterior end (19YMD2-2-46); (D) Lateral view of male posterior portion (holotype); (E) Lateral view of spicules and gubernaculum (holotype); (F) Lateral view of female posterior portion (19YMD2-2-46); (G) Lateral view of female body, showing female reproductive structure (19YMD2-2-46). Scale bars: A, B, C = 20 µm; D, F = 30 µm; G = 50 µm.

numerous denticles on the subventral side, dorsal tooth much larger than others. Jensen (1978) regarded *Pseudomicrolaimus* as a junior synonym of *Bolbolaimus* for most characters of these two genera agreed well. Genus *Pseudomicrolaimus* was reconsidered as a valid genus by Kovalyev and Tchesunov (2005) with presence of numerous denticles on the left subventral side of stoma. By now, three species, *P. murinae*, *P. dentatus* (Allgén, 1935) Sergeeva, 1976 and *P. denticulatus* (Gerlach, 1953) Kovalyev and Tchesunov, 2005 are considered valid.

Materials and methods

In July 2019, undisturbed sediment samples were collected using a syringe (2.6 cm internal diameter) to a depth of 8 cm at the intertidal mud flats of Yangma Island and were vertically subdivided into 0–2 and 2–8 cm depth and fixed with 10% formalin in seawater. In laboratory, samples were stained with rose Bengal for more than 24 h and washed with tap water. Meiofauna was separated from heavier sediment particles by centrifugation in

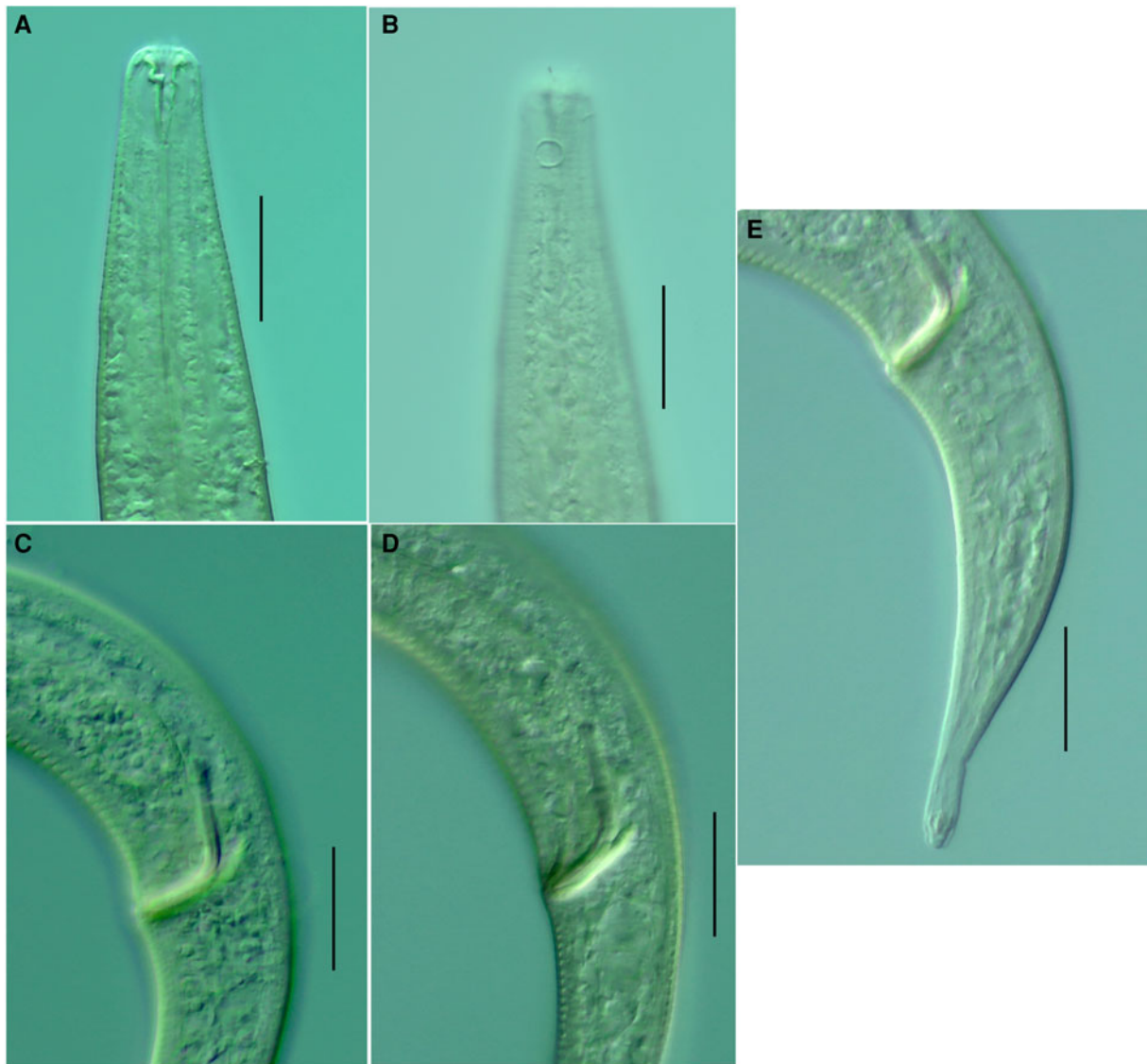


Figure 2. *Microlaimus paraaffinis* sp. nov. (A) Lateral view of male body, showing buccal cavity (holotype); (B) Lateral view of male anterior end, showing amphidial fovea (holotype); (C) Lateral view of male posterior region, showing spicules (holotype); (D) Lateral view of male posterior body, showing gubernaculum (holotype); (E) Lateral view of male posterior end, showing male tail (holotype). Scale bars: 20 μ m.

Ludox-TM (Sigma-Aldrich Co., USA) (de Jonge and Bouwman, 1977), transferred into grid-lined Petri dish and sorted under stereoscopic microscope. Nematodes were transferred into mixture of ethanol (50%) and glycerine (1:9 in volume ratio) with ethanol slowly evaporated, and mounted in glycerine on permanent slides. Descriptions were made using a differential interference contrast microscope (Axiscope-5, Zeiss, Germany). Line drawings were made through an iPad (Apple, USA) and photos were taken with the aid of ZEN software (provided by Zeiss corporation). Type specimens were deposited in the Institute of Oceanology, Chinese Academy of Sciences, Qingdao.

Abbreviations are as follows: a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal body diameter; V%, position of vulva from anterior body end expressed as a percentage of total body length.

Description of new species

Order MICROLAIMIDA Leduc, Verdon and Zhao, 2017

Superfamily MICROLAIMOIDEA Micoletzky, 1922

Family MICROLAIMIDAE Micoletzky, 1922

Genus *Microlaimus* de Man, 1880

Diagnosis

Cuticle annulated, in some species also showing punctations or longitudinal bars. Head slightly set off. Spicules usually short and arcuate, seldom long and slender; gubernaculum present, often bent distally but without dorso-caudal apophyses. Papilloid preloacal supplements may be present (based on Leduc, 2016).

Type species

Microlaimus globiceps de Man, 1880

Microlaimus paraaffinis sp. nov.

(Figures 1–2, Table 1)

Type material

Four males and three females were measured and studied. Holotype: m#1 on slide 19YMD2-2-65; paratypes: m#2, m#3, m#4 on slides 19YMD2-2-65, 19YMD2-2-26, 19YMD2-4-5; f#1 f#2, f#3 on slides 19YMD2-2-46.

Type locality and habitat

Specimens were collected from intertidal muddy sediment at Yangma Island, Shandong Province. 37°26'N, 121°36'E.

Table 1. Individual measurements of *Microlaimus paraaffinis* sp. nov. (in μm except for ratios)

Characters	Holotype			Paratype			
	m#1	m#2	m#3	m#4	f#1	f#2	f#3
Total body length	736	728	714	785	662	718	745
Maximum body diameter	35	32	35	36	42	42	37
Head diameter	13	13	12	13	10	13	12
Length of cephalic setae	4	4	4	4	3	4	4
Buccal cavity depth	15	14	16	13	15	16	15
Diameter of amphidial fovea	5	5	4	6	5	5	5
Body diameter at amphidial fovea	14	12	12	15	13	15	14
Amphidial fovea from anterior end	10	9	9	11	10	11	11
Nerve ring from anterior end	74	73	79	80	80	75	74
Body diameter at nerve ring	27	26	27	31	26	27	27
Pharynx length	129	126	132	140	126	127	133
Body diameter at the base of pharynx	28	29	27	34	29	30	29
Spicules length along arc	32	35	32	31	–	–	–
Gubernaculum length	20	21	20	19	–	–	–
Anterior ovary length	–	–	–	–	112	122	120
Posterior ovary length	–	–	–	–	120	124	121
Vulva from anterior end	–	–	–	–	393	411	431
V%	–	–	–	–	59.4	57.2	57.9
Body diameter at vulva	–	–	–	–	41	39	37
Anal/cloacal body diameter	28	28	26	28	24	26	25
Tail length	105	103	95	108	92	98	111
a	21.0	22.8	20.4	21.8	15.8	17.1	20.1
b	5.7	5.8	5.4	5.6	5.3	5.7	5.6
c	7.0	7.1	7.5	7.3	7.2	7.3	6.7
c'	3.8	3.7	3.7	3.9	3.8	3.8	4.4

Etymology

The species name refers to similarity with species *M. affinis*.

Measurements

All measurement data are given in Table 1.

Description

Males. Body spindle shaped, 714–785 μm in length. Anterior body end slightly truncated, posterior body end elongated conical. Cuticle striated without dots or punctuations. Cuticle striation from posterior to cephalic setae to tail tip. Head smooth and slightly set off at the cephalic setae region. Six inner labial sensilla and six outer labial sensilla papilliform. Four cephalic setae short, 4 μm in length. Somatic setae absent. Amphidial fovea cryptocircular, 4–6 μm in diameter (33–42% corresponding body diameter), 9–11 μm from anterior body end. Buccal cavity wide, 3 μm in width and 13–16 μm in depth with a large dorsal tooth and two small ventrosublateral teeth, right ventrosublateral tooth situated approximately 3–4 μm anteriorly relative to left one, small longitudinal cuticularized folds present in cheilostoma. Pharynx muscular, 126–140 μm in length, anterior part slightly widened at the buccal cavity region, posterior part widened into bulb (24–32 μm in length). Nerve ring situated 73–80 μm from anterior end. Ventral gland immediately posterior to pharyngeal

bulb and excretory pore situated just anterior to nerve ring. Cardia not observed. Tail conico-cylindrical, 95–108 μm (3.7–3.9 cloacal body diameter) in length, with cylindrical portion short (18–19% of tail length). Three caudal glands present and spinneret absent.

Testes outstretched and opposed, anterior and posterior testis to the right of intestine. Spicules paired and L-shaped, slender with equal thickness and distal end tapered, 31–35 μm in length (1.1–1.3 cloacal body diameter). Gubernaculum simple, boat-shaped, 19–21 μm in length and parallel to distal end of spicules. Precloacal supplements absent.

Females. Similar to males in most characters. Reproductive system didelphic-amphidelphic with two ovaries outstretched. Anterior ovary to the left of the intestine (112–122 μm in length), posterior ovary to the right (120–124 μm in length). Vulva located slightly posterior to the mid-body, 393–431 μm from the anterior end. Vagina short and sclerotized.

Differentiation diagnosis and discussion

The new species presents as main characteristics: cuticle striated, four short cephalic setae, buccal cavity with one large dorsal tooth and two small ventrosublateral teeth, amphidial fovea cryptocircular at level of posterior buccal cavity, somatic setae absent, spicules L-shaped and 31–35 μm in length, gubernaculum simple and

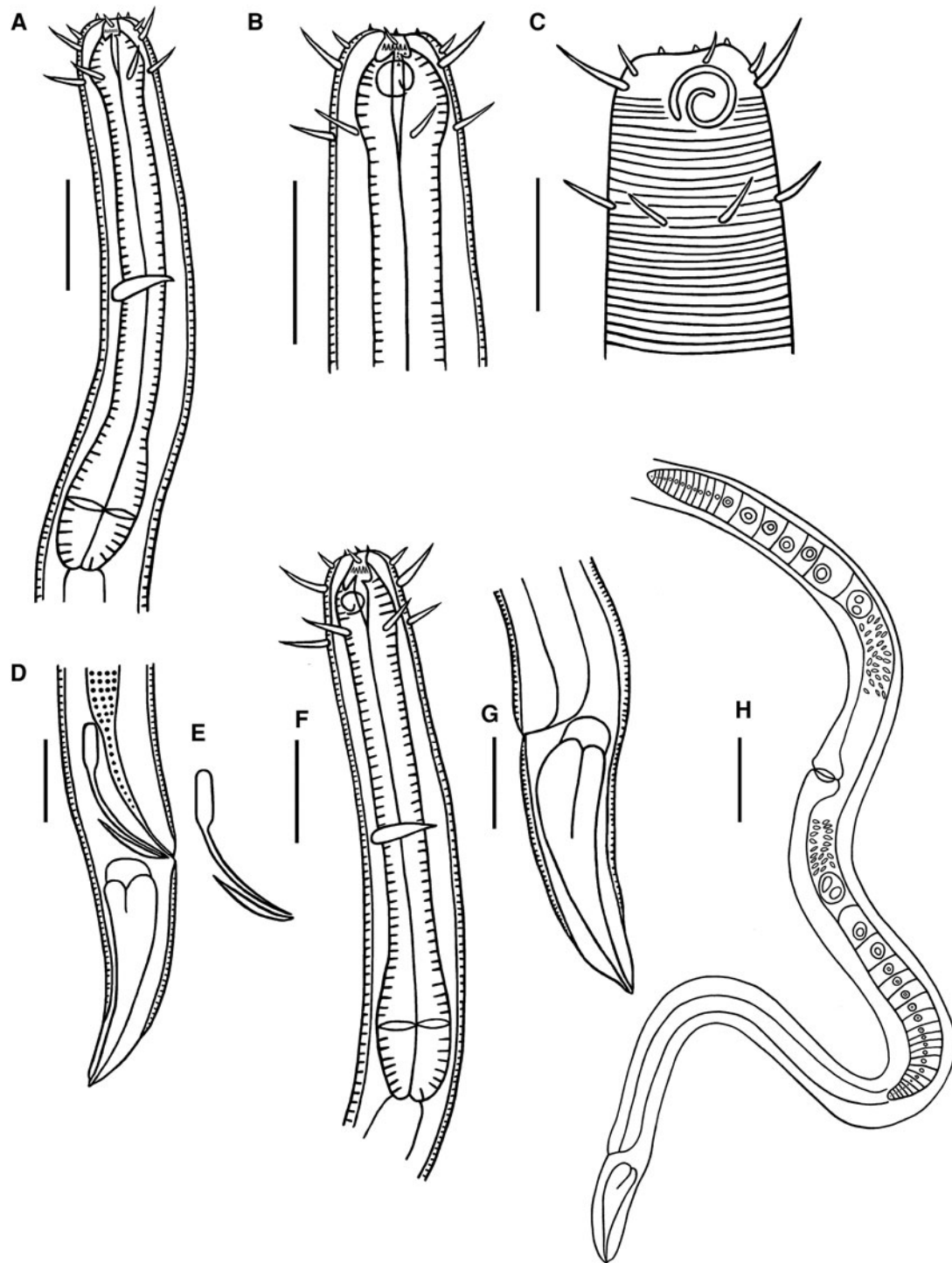


Figure 3. *Pseudomicrolaimus major* sp. nov. (A) Lateral view of male anterior portion, showing buccal cavity and pharynx region (holotype); (B) Lateral view of male anterior portion, showing amphids and buccal cavity (holotype); (C) Lateral view of male anterior portion, showing cuticle and amphids (holotype); (D) Lateral view of male posterior portion, showing tail (holotype); (E) Spicules and gubernaculum (holotype); (F) Lateral view of female anterior end (19YMD1-1-9); (G) Lateral view of female posterior portion (19YMD1-1-9); (H) Lateral view of female middle portion, showing female reproductive structure (19YMD1-1-9). Scale bars: A, B, C, D, F = 20 μm ; G = 50 μm .

boat-shaped, precloacal supplements absent, tail conico-cylindrical with cylindrical portion short.

M. paraaffinis sp. nov. similar to *M. arenicola* Schulz, 1938, *M. affinis* Gerlach, 1958, *M. borealis* Steiner, 1916, *M. campiensis* Lima, Neres and Esteves, 2022, *M. papillatus* Gerlach, 1956, *M. pinguis* Wieser, 1954, *M. undulatus* Gerlach, 1953 in body length and amphidial fovea position. *M. paraaffinis* sp. nov. differs from *M. arenicola* in the testis number (two testes vs one testis), spicules shape (*L*-shaped, slender with equal thickness vs crescentic shaped, with proximal end knobbed), and tail shape (conico-

cylindrical vs conical). *M. paraaffinis* sp. nov. differs from *M. affinis* in de Man *c* value (6.7–7.5 vs 9.5–10), spicules length and shape (31–35 μm , *L*-shaped vs 24 μm , slightly curved), gubernaculum length (19–21 μm vs 11 μm), absence of precloacal supplements (6–7 supplements present, redescribed by Kovalev and Tchesunov, 2005) and tail length and shape (conico-cylindrical, 92–111 μm vs conical, 77 μm). *M. paraaffinis* sp. nov. differs from *M. borealis* in the smaller amphidial fovea (33–42% vs 50%, measured according to Steiner, 1916), shorter cephalic setae (0.3 head diameter vs 0.75 head diameter, measured

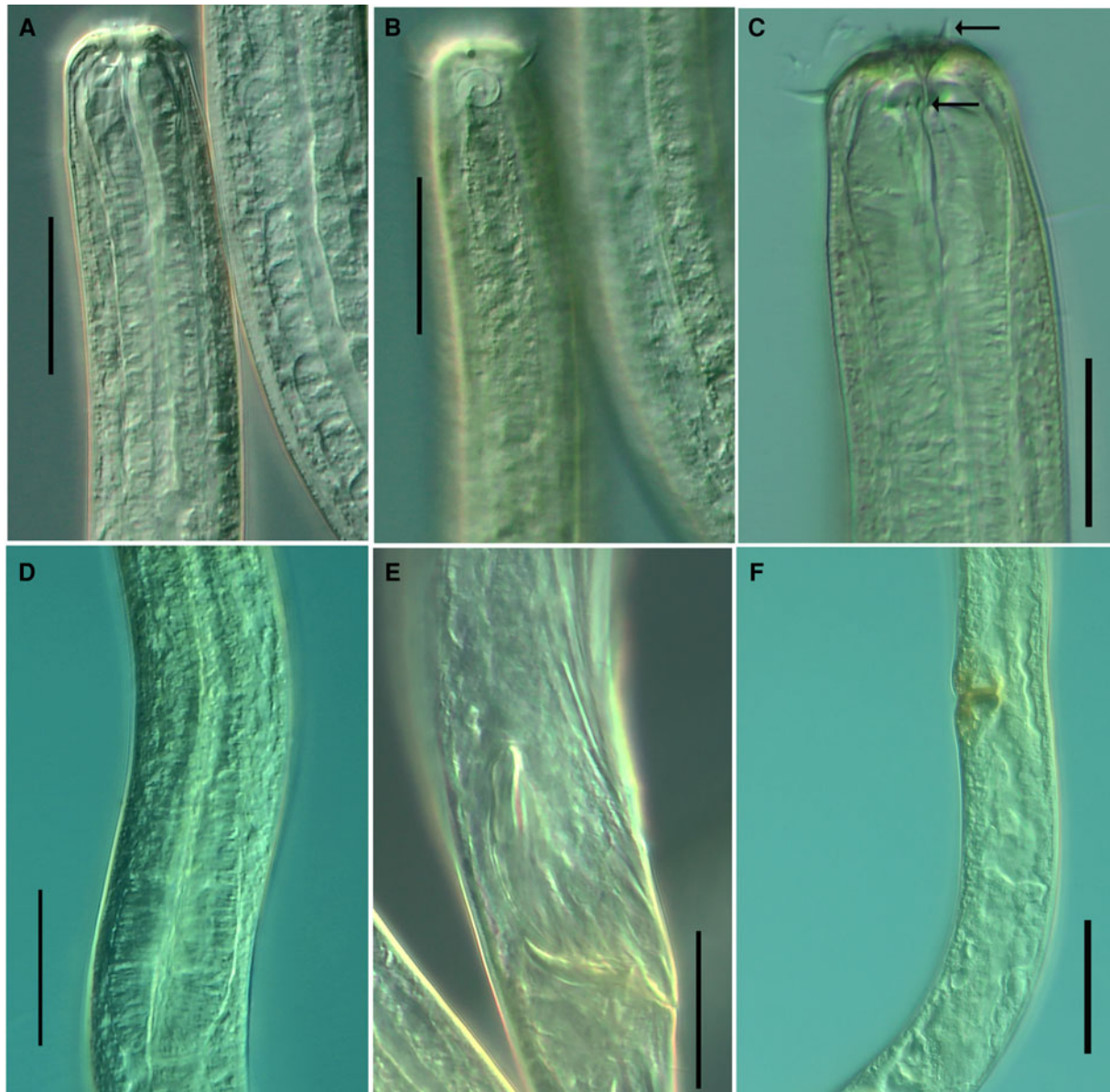


Figure 4. *Pseudomicrolaimus major* sp. nov. (A) Lateral view of male anterior end, showing buccal cavity (holotype); (B) Lateral view of male anterior end, showing amphidial fovea (holotype); (C) Lateral view of male anterior region, showing denticles and inner labial setae (arrows) (19YMD1-1-9); (D) Lateral view of male pharynx region (19YMD1-1-9); (E) Lateral view of male posterior body, showing spicules and gubernaculum (holotype); (F) Lateral view of female showing vulva (19YMD1-1-9). Scale bars: A, B, C, D, E = 20 μ m, F = 50 μ m.

according to Steiner, 1916), and tail length (92–111 μ m vs 66–68 μ m). *M. paraaffinis* sp. nov. differs from *M. campiensis* in the shorter cephalic setae (3–4 μ m vs 5–12 μ m), spicules shape and length (slender with equal thickness, 31–35 μ m vs slender with proximal end enlarged, 38–43 μ m), absence of precloacal supplements (7 papilliform supplements in *M. campiensis*) and tail length (3.7–4.4 anal body diameter vs 2.6–3.2 anal body diameter). *M. paraaffinis* sp. nov. differs from *M. papillatus* in outer labial sensilla and cephalic setae length (papilliform, 3–4 μ m vs 5 μ m, 8.5 μ m), amphidial fovea diameter (4–5 μ m vs 8 μ m), spicules length and shape (31–35 μ m, equal thickness vs 26 μ m, proximal end offset), and absence of precloacal supplements (six precloacal supplements present in *M. papillatus*). *M. paraaffinis* sp. nov. differs from *M. pinguis* in amphidial fovea diameter (4–5 μ m vs 10–10.5 μ m), spicules shape (*L*-shaped vs slightly curved), and tail length (3.7–4.4 anal body diameter vs 2.25–2.6 anal body diameter). *M. paraaffinis* sp. nov. differs from *M. undulatus* in shorter cephalic setae length (3–4 μ m vs 7 μ m), amphidial

fovea diameter (33–42% vs 53%), spicules length and shape (31–35 μ m, *L*-shaped vs 27 μ m, slightly knee-shaped), gubernaculum shape (boat-shaped vs twisted), and shorter tail length (92–111 μ m vs 72–83 μ m).

Order MICROLAIMIDA Leduc, Verdon and Zhao, 2017
 Superfamily MICROLAIMOIDEA Micoletzky, 1922
 Family MICROLAIMIDAE Micoletzky, 1922
 Genus *Pseudomicrolaimus* Sergeeva, 1976

Diagnosis

Cuticle strongly annulated, head truncated and not set off, cephalic setae close to the front end, amphidial fovea unispiral or circular, buccal cavity rather wide and strongly sclerotized, with a large dorsal tooth, and transversal rows of denticles, pharyngeal tissue inflated around the buccal cavity, posterior oval bulb present, copulatory apparatus strongly sclerotized, no dorso-caudal gubernaculum apophyses (based on Tchesunov, 2014).

Table 2. Individual measurements of *Pseudomicrolaimus major* sp. nov. (in μm except for ratios)

Characters	Holotype		Paratype			
	m#1	m#2	m#3	f#1	f#2	f#3
Total body length	3120	2586	3258	3403	2777	3267
Maximum body diameter	37	37	36	40	40	37
Head diameter	22	24	20	25	26	26
Length of outer labial setae	6	5	6	5	5	6
Length of cephalic setae	10	10	7	8	8	10
Distance of subcephalic setae from anterior end	13	13	11	11	11	14
Buccal cavity depth	20	18	16	18	13	17
Diameter of amphidial fovea	10	9	9	8	7	11
Body diameter at amphidial fovea	26	25	24	26	27	28
Amphidial fovea from anterior	12	9	9	9	12	8
Nerve ring from anterior end	100	115	109	100	95	99
Body diameter at nerve ring	30	31	31	30	31	31
Pharynx length	188	196	195	191	193	204
Body diameter at the base of pharynx	33	31	31	32	33	33
Spicules length along arc	65	62	63	–	–	–
Gubernaculum length	30	31	27	–	–	–
Anterior ovary length	–	–	–	299	342	279
Posterior ovary length	–	–	–	336	324	313
Vulva from anterior end	–	–	–	2236	1779	2091
V%	–	–	–	65.7	64.1	64.0
Body diameter at vulva	–	–	–	38	37	37
Anal/cloacal body diameter	34	33	33	34	32	30
Tail length	81	80	95	90	85	94
a	84.3	69.9	90.5	85.1	69.4	88.3
b	16.6	13.2	16.7	17.8	14.4	16.0
c	38.5	32.3	34.3	37.8	32.7	34.8
c'	2.4	2.4	2.9	2.6	2.7	3.1

Type species

Pseudomicrolaimus murinae Sergeeva, 1976

Pseudomicrolaimus major sp. nov.

(Figures 3–4, Table 2)

Type material

Three males and three females were measured and studied. Holotype: m#1 on slide 19YMD1-1-4; paratypes: m#2, m#3 on slides 19YMD1-1-9, 19YMD1-1-4; f#1 on slides 19YMD1-1-9; f#2, f#3 on slides 19YMD1-1-4.

Type locality and habitat.

Specimens were collected from intertidal muddy sediment at Yangma Island, Shandong Province. 37°26'N, 121°36'E.

Etymology

The species name refers to large body size of the nematode.

Measurements

All measurement data are given in Table 2.

Description

Males. Body yellow brownish, long, stout and cylindrical, 2586–3258 μm in length. Anterior body end truncated, posterior body end conical. Cuticle annulated. Anterior sensilla in three crowns, six inner labial sensilla papilliform (2 μm in length), six outer labial setae 5–6 μm in length, and four cephalic setae 7–10 μm in length. Eight subcephalic setae present (8–9 μm in length, 11–13 μm from posterior amphidial fovea end). Somatic setae absent. Amphidial fovea cryptospiral at level of buccal cavity, 7–10 μm in diameter (36%–38% corresponding body diameter), 9–12 μm from anterior body end. Buccal cavity wide, 7 μm in width and 16–20 μm in depth with a large cuticularized dorsal tooth and two small ventro-sublateral teeth at the same level, small denticles present at left ventro-sublateral side of buccal cavity. Pharynx muscular, 188–196 μm in length, anterior part widened at the buccal cavity region, posterior part widened into long oval-shaped bulb. Nerve ring situated 100–115 μm from anterior end. Excretory-secretory system not observed. Cardia not observed. Tail short and conical, 80–95 μm (2.4–2.9 cloacal body diameter) in length. Three caudal glands present and spinneret absent.

Testes outstretched and opposed, the anterior testis to the right and the posterior one to the left of intestine. Spicules paired,

curved, 62–65 µm in length (1.9 cloacal body diameter). Proximal third of spicules widened (oval or rectangular-shaped) and the rest portion slender. Gubernaculum simple, boat-shaped, and parallel to distal end of spicules.

Females. Similar to males in most characters. Reproductive system didelphic-amphidelphic with two ovaries outstretched. Anterior ovary to the left of the intestine (279–342 µm in length), posterior ovary to the right (313–336 µm in length). Vulva located slightly posterior to the mid-body, 1779–2236 µm from the anterior end. Vagina short and sclerotized.

Differentiation diagnosis and discussion

The new species presents as main characteristics: body size relatively large, cuticle striated, buccal cavity with one large dorsal tooth and two small subventral teeth at the same level, small denticles present at left subventral side of buccal cavity, amphidial fovea cryptospiral, eight subcephalic setae present, somatic setae absent, spicules curved 62–65 µm in length with proximal portion widened and other portion slender, gubernaculum simple and boat-shaped, tail conical with three caudal glands.

P. major sp. nov. differs from *P. denticulatus* (Cobb, 1920; Gerlach, 1953) in the longer body (2586–3403 µm vs 2025–2139 µm), subcephalic setae position (11–14 µm from posterior end of amphidial fovea vs level with posterior end of amphidial fovea), and spicules length and shape (62–65 µm, proximal portion widened vs 33 µm, proximal end offset). *P. major* sp. nov. differs from *P. murinae* (Sergeeva, 1976) in the longer body (2586–3403 µm vs 841–1167 µm), buccal cavity tooth number (one dorsal tooth vs two dorsal teeth), presence of subcephalic setae (absence in *P. murinae*), spicules length and shape (62–65 µm, proximal portion widened vs 32.5–37.5 µm, proximal portion slightly cephalated). *P. major* sp. nov. differs from *P. dentatus* (Allgén, 1935) in the longer body (2586–3403 µm vs 950 µm), presence of subcephalic setae (absence in *P. dentatus*), spicules length and shape (62–65 µm, proximal portion widened vs 30 µm, proximal end cephalated).

Dichotomous key for species of genus *Pseudomicrolaimus* Sergeeva, 1976

1. subcephalic setae present 2
 - subcephalic setae absent 3
2. Spicules proximal portion widened, subcephalic setae posterior to posterior amphidial end. *P. major* sp. nov.
 - Spicules proximal end offset, subcephalic setae level with posterior amphidial end *P. denticulatus*
3. Two dorsal teeth present *P. murinae*
 - One dorsal tooth present *P. dentatus*

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Authors' contributions. Wen Guo: sample collection, preparation of *Microlaimus paraaffinis* sp. nov. and draft preparation; Mengna Wang: preparation of *pseudomicrolaimus major* sp. nov.; Chunming Wang: picture drawing, manuscript editing.

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Competing interest. None.

Data availability statements. The authors confirm that the data supporting the findings of this study are available within the article.

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