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Metachromadora parobscura sp. nov. and *Molgolaimus longicaudatus* sp. nov. (Nematoda, Desmodoridae) from Mangrove Wetlands of China

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Abstract: Two new species of free-living marine nematodes, *Metachromadora parobscura* sp. nov. and *Molgolaimus longicaudatus* sp. nov., from mangrove wetlands of Beihai, Guangxi province in China, are described. *Metachromadora parobscura* sp. nov. is characterized by eight longitudinal rows of somatic setae arranged from the posterior part of the body, loop-shaped amphidial foveae with an open top and double contours, pharynx with bipartite cuticularized internal cavity, spicules with well-developed capitulum, gubernaculum canoe-shaped, without apophysis, 6–8 precloacal tubular supplements, and a short, conical tail with two ventral protuberances. It could be easily distinguished from the known species by spicule length and numbers of precloacal supplements. *Molgolaimus longicaudatus* sp. nov. is characterized by short cephalic setae, relatively small amphidial fovea, slender spicules ventrally bent with pronounced hooked capitulum and tapered distal end, two poriform precloacal supplements, and a relatively long conico-cylindrical tail. It differs from other species by the shape of spicules and long tail. Nearly full-length SSU sequences (1542–1592 bp) of the two species were provided, and phylogenetic trees based on maximum likelihood analyses supported the taxonomic position of the two new species. The combined use of traditional morphology-based taxonomy and molecular approaches has been proven to be a good choice for identification of free-living nematodes.

Keywords: free-living marine nematodes; taxonomy; Beibu Gulf of the South China Sea; small subunit (SSU) 18S rDNA

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1. Introduction

The mangrove is one of the most productive ecosystems in the world and plays an important role in the maintenance of coastal biodiversity. Mangrove sediments harbor numerous marine nematodes, which participate in nutrient cycling and affect carbon fixation in mangrove wetlands [1]. Furthermore, nematodes are sensitive to the physicochemical conditions of the sediment, being potential bioindicators for conservation purposes [2]. The identification of nematode species would improve our understanding of ecosystems' functional roles [3]. In the past few years, the community structure of nematodes was investigated, and some nematode species were also found and described in the mangrove wetlands of China [4–6].

The family Desmodoridae belongs to the superfamily Desmodoroidea Filipjev, 1922, and it is composed of six subfamilies: Desmodorinae Micoletzky, 1924, Molgolaiminae Jensen, 1978, Prodesmodorinae Lorenzen, 1981, Pseudonchinae Gerlach & Riemann, 1973, Spiriniinae Gerlach & Murphy, 1965, and Stilbonematinae Chitwood, 1936 [7].

The genus *Metachromadora* Filipjev, 1918, belongs to the subfamily Spiriniinae Gerlach & Murphy, 1965. *Metachromadora* is a heterogeneous genus and not well defined [8]. It has been reviewed by Gerlach (1951), Wieser & Hopper (1967), Gerlach & Riemann (1972), Lorenzen (1981), and Maria (2013) [9,10]. It was first divided into five subgenera: *Bradylaimus*, *Chromadoropsis*, *Metonyx*, *Metachromadora*, and *Neonyx* by Gerlach (1951) [11].

Subsequently, Timm (1961) erected the sixth subgenus, *Metachromadoroides*, with species *M. vulgaris* and *M. complexa* [12]. Later, *Chromadoropsis* was reinstated to the genus level by Furstenberg & Vincx in 1988 [13]. Until now, *Metachromadora* consists of five subgenera and is still a large genus, containing 32 valid species. *Metachromadora* is a very widely distributed genus. It is found in marine habitats such as mangrove wetlands, sublittoral areas, coral reefs, estuaries, and the continental shelf, from muddy to sandy sediments.

The genus *Molgolaimus* Ditlevsen, 1921, belongs to the subfamily Molgolaiminae Jensen, 1978. The taxonomic status of *Molgolaimus* is complicated and has been amended several times. *Molgolaimus* was first placed in the family Microlaimidae by Gerlach and Riemann in 1973 [14]. The family Microlaimidae is characterized by two outstretched ovaries and two opposed testes or only anterior testis. Subsequently, Jensen (1978) erected the family Molgolaimidae based on female and male reproductive systems (a single anterior testis in males and two reflexed ovaries in females) [15]. Then, Lorenzen (1994) downgraded the family Molgolaimidae as a subfamily Molgolaiminae in Desmodoridae Filipjev, 1922 [16]. It was divided into four species groups by Fonseca et al. [17] based on spicule length, and scatter-plot graphs of morphometric data and illustrations of the different 33 *Molgolaimus* species were provided in the study. Furthermore, *Molgolaimus haakonmosbiensis* Portnova, 2009 [18], *Molgolaimus kaikouraensis* Leduc, Fu & Zhao, 2019 [19], and *Molgolaimus euryformis* Zhou, Zeng, Cai, Fu & Tan, 2020 [6] were described. Additionally, two *Microlaimus* species were transferred to the genus by Shi and Xu [20]. *Molgolaimus* now contains 38 valid species. *Molgolaimus* is a common free-living marine nematode genus from shallow waters to the deep sea, and it is also found in the mangrove forests. The genus often occurs in organically enriched and recently colonized sediments.

The choice of molecular markers in nematode barcoding studies was controversial. COI, 18S, and 28S nuclear ribosomal DNA (rDNA) have been suggested as ideal gene markers to discriminate marine nematodes [21,22]. Some scientists proposed the comprehensive use of different genes would obtain better results. 18S rRNA sequences contain both conserved and variable regions, suitable for primer design and taxonomic distinction. Therefore, the 18S rRNA sequence was a potential useful marker for the rapid molecular identification of nematodes. Desmodoridae is complicated due to a lack of synapomorphy, and it is also paraphyletic based upon small subunit of ribosomal DNA [23]. So in this study, we described two new species, one belonging to the genus *Metachromadora* and another to *Molgolaimus*, based on combined morphological and molecular approaches. Nearly full-length SSU sequences of the two species were obtained for phylogenetic analysis. This study will help to improve the assessment of marine nematode diversity and their evolutionary relationships.

2. Materials and Methods

2.1. Sampling and Morphological Analysis

Sediments were obtained from mangrove wetland located in Tieshan Harbor of Beihai, Guangxi province (109°36′ E, 21°36′ N) in October 2023. During the sampling process, four transects (A, B, C, and D) were established, and both of the new species were discovered at transect C. Mangrove plants Avicennia marina were distributed across transects A, B, and C, and Rhizophora stylosa was the dominant mangrove species at transect D. Samples were collected by pushing a syringe (2.6 cm internal diameter) into the sediment to a depth of 5 cm. Samples used for molecular analysis were stored in 95% ethanol and frozen at −20 °C. The sediments for morphological analysis were then fixed in 5% formalin and washed through two sieves with mesh sizes of 500 and 42 μm to separate meiofauna from macrofauna. Ludox™ with a gravity of 1.15 was used for separating fauna from sediment. Nematodes were observed using a stereoscopic microscope and transferred into a mixture of 5% glycerol, 5% pure ethanol, and 90% freshwater. After evaporation of ethanol, specimens were mounted on slides. The nematodes were identified using a differential interference contrast microscope (Leica DM 2500) (CMS GmbH, Wetzlar, Germany, assembled in Shanghai, China). The microphotographs were taken with a camera,

a Leica DMC 5400. The nematodes' line drawings were made with the aid of a camera lucida (Leica, 10×). All measurements were taken using Leica LAS X version 3.3.3, and all curved structures were measured along the arc or median line.

Abbreviations used in the table are as follows: a = body length divided by body maximum width, b = body length divided by the pharynx length, c = body length divided by the tail length, c′ = tail length divided by the anal body diameter, c.b.d. = corresponding body diameter, V% = distance from the vulva from the anterior end divided by the total body length [24].

2.2. Molecular Analysis

Specimens were rinsed with phosphate buffer three times to remove ethanol. Subsequently, nematodes were cut into several pieces with a sterile scalpel, transferred into Eppendorf tubes, and DNA was extracted from nematodes following the protocol of the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). PCR was performed using an Ex taq polymerase kit (TaKaRa, Tokyo, Japan). Nearly full-length of the 18S rDNA was amplified using 18S-G18S4 (5′-GCTTGTCTCAAAGATTAAGCC-3′) and 18S-18P (5′-TGATCCWKCYGCAGGTTCAC-3′) [25]. Each PCR mixture (50 µL) contained 5 µL DNA template, 200 µmol/L of each dNTP, 1.5 µmol/L $MgCl_2$, 0.3 µmol/L of each primer, 2.5 U of Ex Taq DNA polymerase, and 5 µL of 10× reaction buffer. The PCR program was as follows: denaturation at 94 °C for 5 min; 38 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1min and 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 7 min. After verification in a 1% agarose gel stained with ethidium bromide, PCR products were purified. Sequencing was carried out in both directions with the ABI 3730XL DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA) by Sangon Biotech Co., Ltd. (Shanghai, China). All sequences were deposited in GenBank. A total of 23 18S rDNA sequences, belonging to the family Desmodoridae and Microlaimidae, were downloaded from the GenBank database. The sequences were subsequently edited and aligned using the program Geneious v. 9.0.2 (Biomatters Ltd., Auckland, New Zealand), Maximum likelihood bootstrapping analysis was performed with RAxML HPC v. 8, using the locus-specific model partitions with the default parameters and the GTRGAMMA model as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1000 bootstrap replicates [26]. *Diplopeltis cirrhatus* was selected as the outgroup.

3. Results and Discussion

3.1. Description of *Metachromadora parobscura sp. nov.*

Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Desmodorida De Coninck, 1965
Superfamily Desmodoroidea Filipjev, 1922
Family Desmodoridae Filipjev, 1922
Subfamily Spiriniinae Gerlach & Murpy, 1965
Genus *Metachromadora* Filipjev, 1918
Metachromadora parobscura sp. nov.

3.1.1. Type Material

Four males and two females were measured and studied. Holotype: ♂1 on slide C3-1-5. Paratypes: ♂2 on slide C3-1-5, ♂3 and ♂4 on slide C3-1-15, ♀1 on slide C3-1-7, and ♀2 on slide C3-1-8. The type material is deposited in the Institute of Oceanology, Chinese Academy of Sciences.

3.1.2. Type Locality and Habitat

Muddy surface sediment (0–5 cm) in mangrove wetland, located in Tieshan Harbor in Beihai, Guangxi province (109°36′ E, 21°36′ N).

3.1.3. Etymology

The species epithet comes from the species name *Metachromadora obscura* Nguyen Dinh Tu, Gagarin, Phan Ke Long, Nguyen Thi Xuan Phuong & Nguyen Vu Thanh, 2016, referring to the new species is similar to *Metachromadora obscura* in most morphological characteristics.

3.1.4. Measurements

All measurement data are provided in Table 1.

Table 1. Measurements of individuals of *Metachromadora parobscura* sp. nov. (in μm except ratios).

Morphological Characteristics	Holotype	Paratype				
	♂**1**	♂**2**	♂**3**	♂**4**	♀**1**	♀**2**
a	14.0	14.0	17.3	15.3	11.7	12.2
b	4.1	4.2	4.4	4.4	4.3	4.2
c	11.5	13.2	14.8	12.5	15.6	17.7
c′	2.0	1.9	2.0	1.7	2.0	2.2
Total body length	896	977	1020	915	935	976
Maximum body diameter	64	70	59	60	80	80
Head diameter	22	24	24	23	26	28
Amphideal fovea width	15	13	13	15	17	16
Amphideal fovea c.b.d.	25	24	26	24	27	24
Amphideal fovea as % of c.b.d.	60	54	50	63	63	67
Pharynx length	216	233	234	210	220	232
Pharynx c.b.d.	59	67	57	60	71	75
Length of double bulb	77	84	91	90	95	92
Bulb as % of pharynx length	35.6	36.1	38.9	42.9	43.0	40.0
Spicule length as arch	68	63	68	64	-	-
Gubernacular length	31	30	31	31		
Cloacal/anal body diameter	39	39	39	43	30	25
Distance from first precloacal supplement to anus	5	5	5	6	-	-
Distance from last precloacal supplement to anus	85	101	114	106	-	-
Precloacal supplement number	6	8	6	8	-	-
Distance from the first protuberance to cloaca	38	43	44	38	-	-
Distance from the second protuberance to cloaca	50	52	52	48	-	-
Tail length	78	74	69	73	60	55
Vulva from anterior end	-	-	-	-	520	575
Vulva c.b.d.	-	-	-	-	77	80
V%	-	-	-	-	55.6	58.9

3.1.5. Description of *Metachromadora parobscura* sp. nov. (Figures 1 and 2)

Males: Body cylindrical, 896–1020 μm long. Cuticle with fine striation starting immediately after the amphidial foveae. Lateral ridges, 2–3 μm wide (~1/30 of the body diameter at mid-body), beginning at the anterior end of the pharynx bulb and extending as far as the middle of the tail (Figure 2B). Blunt conical head. Six internal and six external labial sensilla prominent papilliform. Four cephalic setae 5–6 μm long (Figure 1A,C). No cervical setae. Somatic setae are short and stout, arranged in eight longitudinal rows from the posterior part of the body (two rows at each lateral side, two rows subventral, and two rows subdorsal). Amphideal fovea are loop-shaped with an open top and have double contours, positioned anterior to the first head cuticular annulus. Width of *amphidial foveae* 13–15 μm, about 50.0–62.5% of corresponding body diameter (Figures 1D and 2D). Buccal cavity 33–38 μm deep, with three teeth, a large dorsal tooth, and two minute subventral

teeth, the dorsal tooth is bigger (Figure 2C). Bulb length 39–43% of the total pharynx length, with bipartite cuticularized internal cavity (Figure 2A). Cardia muscular, small. Nerve ring anterior to the pharynx bulb. Secretory–excretory pore not observed.

Reproductive system monorchic with outstretched testis. Vas deferens at the right side of the intestine. Paired spicules (63–68 μm), equal, slender, ventrally curved, with an enlarged capitulum. Gubernaculum (30–31 μm) canoe-shaped, parallel to spicule, without apophyses (Figures 1F and 2F). Six tubular precloacal supplements, about 11–17 μm apart, first supplement 5–6 μm anterior to the cloaca. Tail conical (1.7–2.0 times cloacal body diameter) with spinneret (7–8 μm). Terminal part of tail smooth, 8–10 μm long, 10–14% of total tail length. Two protuberances positioned on the ventral side of the tail, the first one 38–44 μm posterior to the cloaca. Caudal glands are present but poorly visible (Figures 1E and 2E).

Females: Similar to males in general morphology. Few and scattered somatic setae. Reproductive system amphidelphic with two ovaries opposed and reflexed, anterior ovary positioned to the left of the intestine, posterior ovary to the right of the intestine. Vagina is very sclerotized. Two glands present. Vulva in the shape of transversal slit, located posterior to mid-body (56–59% of total length), occupying about five annules. Sperm cells are always found within the uterus. Tail without protuberance and setae (Figure 1B).

3.1.6. Differential Diagnosis and Discussion

Metachromadora parobscura sp. nov. is characterized by eight longitudinal rows of somatic setae arranged from the posterior part of the body, loop-shaped amphidial fovea with an open top and double contours, pharynx with bipartite cuticularized internal cavity, spicules with well-developed capitulum, gubernaculum canoe-shaped, without apophysis, 6–8 precloacal tubular supplements, and a short, conical tail with two ventral protuberances.

All the morphological features of the new species are consistent with the description of the subgenus *Metachromadoroides*. There are nine valid species within *Metachromadoroides* Timm, 1961: *M. complexa*, *M. pulvinata*, *M. remanei*, *M. vulgaris*, *M. zaixsi*, *M. minor*, *and M. orientalis*, *M. obscura*, *and M. xiamenensis*. Diagnosis of Subgenus *Metachromadoroides* Timm, 1961 (modified from Timm 1961) is as follows: cuticle heavily annulated, without longitudinal striation on head; lateral ridges present; stoma cylindrical, with dorsal tooth; smaller subventral teeth present or absent; amphidial foveae circular or loopshaped, having double contour; esophageal bulb with two or three internal divisions; precloacal supplemental in the shape of short, faintly cuticularized tubules or absent [12]. *Metachromadoroides* could be subdivided into two groups based on the precloacal supplements. Group 1, supplements absent: *M. zaixsi*, *M. orientalis,* and *M. vulgaris.* These three species could be easily differentiated by internal division of pharyngeal bulb (bipartite in *M. orientalis* vs. tripartite in *M. zaixsi* and *M. vulgaris*) and length of anterior non-striated part (one amphidial fovea length in *M. zaixsi* vs. two in *M. vulgaris*), respectively. Group 2, supplements present: *M. complexa*, *M. xiamenensis,* and *M. minor* have unique characteristics separately (setae absent, three small protuberances on the ventral side of the tail, and a short slim body); *M. pulvinata*, *M. remanei,* and *M. obscura* differed from each other by body length and numbers of precloacal supplements (1720 μm and 23 vs. 1100–1300 μm and 5 vs. 786 μm and 13–14).

Metachromadora parobscura sp. nov. closely resembles *M. remanei* in De Man ratios. New species could be distinguished from it by body length (896–1020 μm vs. 1100–1300 μm in *M. remanei*), spicule length (63–68 μm vs. 49–55 μm in *M. remanei*), and number of precloacal supplements (6–8 vs. 5 in *M. remanei*) [11]. Otherwise, the new species is morphologically similar to *M. obscura*. But differs from it by relatively shorter and thinner tail (c = 11.5–14.8, c′ = 1.7–2.0 vs. c = 10.7–12.1, c′ = 1.4–1.6 in *M. obscura*), amphid diameter as a percentage of the corresponding body diameter (50–62.5% vs. 65–75% in *M. obscura*), shorter spicules (63–68 μm vs. 75–78 μm in *M. obscura*), shorter gubernaculum (30–31 μm vs. 38–40 μm in *M. obscura*), smaller number of precloacal supplements (6–8 vs. 13–14 tubular supplements in *M. obscura*) [27].

Only one species of the genus, *Metachromadora xiamenensis*, has been previously described in China. It is also from the mangrove habitats. 18 tubular precloacal supplements and a tail with three small protuberances make it different from the new species [6]. Morphological differentiating characteristics of the species of *Metachromadoroides* are proposed (Table 2).

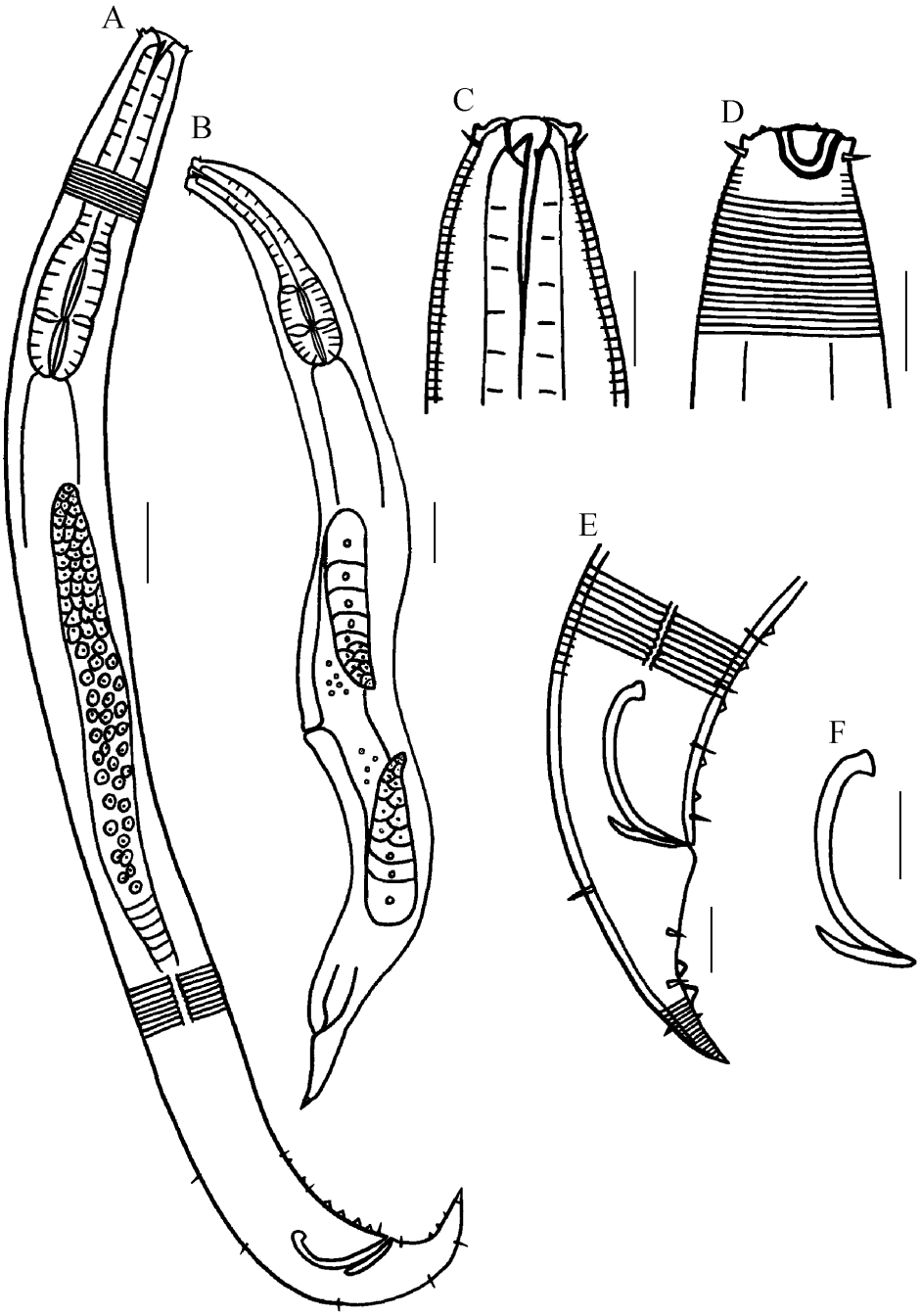

Figure 1. Line drawing of *Metachromadora parobscura* sp. nov. (**A**) Entire view of male, showing outer labial setae, cephalic setae, and epidermal gland cells; (**B**) entire view of female; (**C**) anterior end of holotype; (**D**) anterior end of holotype, showing amphid; (**E**) posterior end of male, showing spicule, gubernaculum, precloacal supplements, and tail protuberances; (**F**) magnifying spicule and gubernaculum. Scale bar: (**A**,**B**) = 50 μm; (**C**–**F**) = 20 μm.

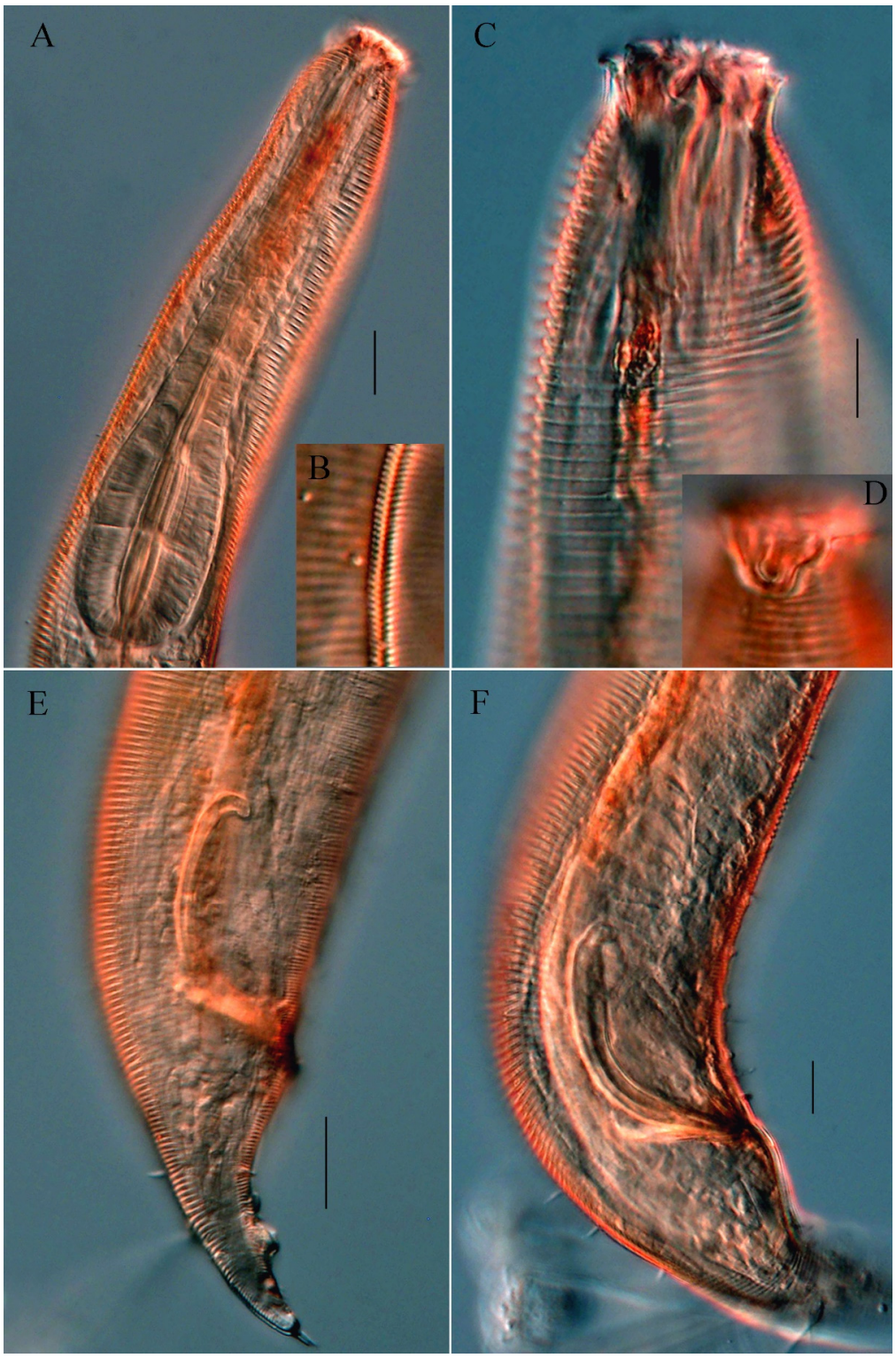

Figure 2. Microphotograph of *Metachromadora parobscura* sp. nov. (**A**) Pharyngeal region of holotype, showing pharyngeal bulbs; (**B**) lateral ridge; (**C**) lateral view of holotype anterior end, showing anterior sensilla and dorsal tooth; (**D**) amphid of holotype; (**E**) posterior end of male, showing spicule and tail protuberances; (**F**) lateral view of male cloacal region, showing spicule, gubennaculum, and precloacal supplements. Scale bar: (**A**,**E**) = 20 μm; (**C**,**F**) = 10 μm.

Table 2. Morphological differentiating data of male *Metachromadora* (*Metachromadoroides*).

Species	Body Length (μm)	Spicule Length (μm)	Numbers of Supplements	Internal Division of Pharyngeal Bulb	Protuberances on the Tail	a	b	c	c′	References
M. complexa	758–940	74	17	tripartite	2	8.4–11.3	3.4–4.4	7.3–12.1	1.3	[12]
M. pulvinata	1720	55	23	tripartite	2	18.1	5.4	15.6	2.0	[28]
M. remanei	1100–1300	49–55	5	bipartite	*	14	4.9	14	1.9–2.2	[11]
M. vulgaris	1100–1200	72	0	tripartite	0	14.1–20.0	4.4–4.9	12.1–14.5	1.8–2.8	[12]
M. zaixsi	890–1160	40–52	0	tripartite	0	10.5–19.3	4.7–7.2	6.8–10.1	1.7–2.8	[10]
M. minor	594–641	35–36	12–16	tripartite	*	12–15	4.2–4.7	13.5–14.9	1.4–1.5	[29]
M. orientalis	859–1133	59–63	0	bipartite	**	13–15	4.8–5.3	15.3–19.2	1.0–1.3	[30]
M. obscura	786–1008	75–78	13–14	bipartite	2	11–15	4.2–4.5	10.7–12.1	1.4–1.6	[27]
M. xiamenensis	741–888	46–56	18	bipartite	3	11.0–14.3	4.4–5.2	10.1–12.2	1.5–1.7	[6]
M. parobscura sp. nov.	896–1020	63–68	6–8	bipartite	2	14.0–17.3	4.1–4.4	11.5–14.8	1.7–2.0	this study

* not described, ** described as: small protuberance with three or four setae.

3.2. Description of *Molgolaimus longicaudatus sp. nov.*

Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Desmodorida De Coninck, 1965
Superfamily Desmodoroidea Filipjev, 1922
Family Desmodoridae Filipjev, 1922
Subfamily Molgolaiminae Jensen, 1978
Genus *Molgolaimus* Ditlevsen, 1921
Molgolaimus longicaudatus sp. nov.

3.2.1. Type Material

Four males and three females were measured and studied. Holotype: ♂1 on slide C3-1-49. Paratypes: ♂2 on slide C3-1-48, ♂3 on slide C3-1-50, ♂4 on slide C3-1-51, ♀1 on slide C3-1-47, ♀2 on slide C3-1-49, and ♀3 on slide C3-1-50. The type material is deposited in the Institute of Oceanology, Chinese Academy of Sciences.

3.2.2. Type Locality and Habitat

Muddy surface sediment (0–5 cm) in mangrove wetland, located in Tieshan Harbor in Beihai, Guangxi province (109°36′ E, 21°36′ N).

3.2.3. Etymology

The species name is composed of the Latin adjectives longus and caudatus, referring to the new species with a relatively long tail.

3.2.4. Measurements

All measurement data are given in Table 3.

Table 3. Measurements of individuals of *Molgolaimus longicaudatus* sp. nov. (in μm except ratios).

Morphological Characteristics	Holotype	Paratype					
	♂**1**	♂**2**	♂**3**	♂**4**	♀**1**	♀**2**	♀**3**
a	31.0	30.9	28.3	30.1	28.2	24.8	28.3
b	8.0	7.4	7.7	8.2	7.9	7.9	8.2
c	5.7	5.4	5.7	5.6	6.7	6.9	6.1
c′	7.6	7.7	7.2	7.4	6.9	6.4	7.2
Total body length	775	742	735	752	789	793	791
Maximum body diameter	25	24	26	25	28	32	28
Head diameter	6	6	6	6	6	7	5
Cephalic setae length	2	2	2	2	2	2	2
Amphideal fovea width	3	3	4	4	4	3	3

Table 3. *Cont.*

Morphological Characteristics	Holotype	Paratype					
	♂**1**	♂**2**	♂**3**	♂**4**	♀**1**	♀**2**	♀**3**
Amphideal fovea c.b.d.	9	8	9	9	9	8	8
Amphidial fovea from anterior end	6	6	6	7	8	5	6
Amphideal fovea as % of c.b.d.	33.3	37.5	44.4	44.4	44.4	37.5	37.5
Nerve ring width	14	15	15	14	15	16	15
Nerve ring c.b.d.	20	20	21	20	20	21	20
Pharynx length	97	100	96	92	100	101	97
Pharynx c.b.d.	22	23	22	23	23	25	22
Diameter of esophageal bulb	17	19	18	18	17	19	17
Spicule length as arch	33	35	33	32	-	-	-
Gubernacular length	11	11	11	12	-	-	-
Cloacal or anal body diameter	18	18	18	18	17	18	18
Cloacal/anal body diameter	1.8	1.9	1.8	1.8	-	-	-
Distance from first precloacal supplement to anus	34	31	41	25	-	-	-
Distance from second precloacal supplement to anus	62	68	72	43	-	-	-
Tail length	136	138	130	134	117	115	129
Vulva from anterior end	-	-	-	-	348	339	343
Vulva c.b.d.					28	30	26
V%					44.1	42.7	43.3

3.2.5. Description of *Molgolaimus longicaudatus* sp. nov. (Figures 3–5)

Males: Body slender and cylindrical, tapering gradually towards both ends (Figure 3E). Cuticle annules indistinct. Head separated from body by a faint constriction. Inner labial and outer labial sensilla not observed. Four cephalic setae 2 µm long, located posterior to cephalic constriction (Figures 3C and 4B). Amphideal fovea round, posterior to the cephalic constriction, 3–4 µm in diameter, corresponding to 33.3–44.4% of corresponding diameter, about 6–7 µm from the anterior end (Figures 3C and 4A). Buccal cavity small, weakly sclerotized, narrow, and with small teeth (Figure 3C). Pharynx corpus narrow cylindrical, ending in a pronounced spherical muscular bulb (17–19 µm diameter), and the bulb heavily cuticularized (Figures 3A and 4A). Nerve ring located slightly anterior to middle of pharynx length. Excretory pore not observed. Cardia cylindrical, 6–9 µm long.

Reproductive system monorchic, with a single anterior outstretched testis, left to the intestine. Vas deferens long and thin. Spicules slender, 32–35 µm long (1.8–1.9 abd), ventrally bent with hooked capitulum and tapered distal end (Figure 4D). Gubernaculum arcuate and parallel to the spicules, without apophyses, its length corresponding to one third of the spicule length (Figure 3F). Two small precloacal supplements, poriform, 25–41 µm and 43–68 µm distant from the cloaca, respectively (Figures 3B and 4C). Tail conico-cylindrical, conical in the proximal half, and distally characterized by a cylindrical prolongation (Figure 5A). Caudal glands not observed.

Females: Similar to males, but with a larger maximum body diameter and a shorter tail. Reproductive system didelphic with reflexed ovaries, the anterior branch right to the intestine and posterior branch to the left (Figure 5B). Vulva located anterior to mid-body (42.7–44.1% of total length) (Figure 3D).

3.2.6. Differential Diagnosis and Discussion

Molgolaimus longicaudatus sp. nov. is characterized by short cephalic setae (2 µm), relative small amphidial fovea (3–4 µm in diameter), ventrally bent spicules with pronounced hooked capitulum, two poriform precloacal supplements, and a long conico-cylindrical tail (115–138 µm). Female reproductive system didelphic with two reflexed ovaries.

The genus *Molgolaimus* is characterized by round amphidial fovea, pharynx with a pronounced spherical bulb, and spicules of variable length and shape. Fonseca et al. (2006)

provided an illustrated polytomous identification key to 33 species of the genus, which were classified into four groups according to absolute spicule length. This new species is placed in group 1b1, which is characterized by short spicules (<35 μm) and ratio spicules divided by the anal body diameter ranging from 1 to 3 [17]. There are seven species in the group: *M. drakus*, *M. exceptionregulum*, *M. gazii*, *M. pecticauda*, *M. mareprofundus*, *M. sapiens*, and *M. spirifer*. *Molgolaimus* in this group could be subdivided into three subgroups based on the number of precloacal supplements. Subgroup 1, supplements absent: *M. pecticauda*, *M. spirifer* and *M. sapiens*. *M. pecticauda* and *M. spirifer* could be easily differentiated clearly from other species of the group by their amphids (amphids with a tubiform structure in *M. pecticauda* and multispiral amphid fovea in *M. spirifer*, vs. unispiral or circular in other species of the group). *M. sapiens* differs in spicule with S-shaped blade. Subgroup 2, one supplement: *M. drakusthe*, *M. mareprofundus, and M. exceptionregulum. M. drakusthe* is characterized by a slim body, the shortest spicules, and amphidial fovea at 3 hd from the anterior end. *M. mareprofundus* resembles *M. exceptionregulum* but differs from it by the smaller body and slender spicules. Subgroup 3, two supplements: *M. gazii* is shorter and characterized by sexual dimorphism amphids. *Molgolaimus longicaudatus* sp. nov. can be distinguished by the longest tail (130–138 μm) and the spicules shape (pronounced hamose curved proximal terminus). *Molgolaimus longicaudatus* sp. nov. is morphologically close to *M. exceptionregulum* and *M. mareprofundus* but differs from them by the larger body length (735–793 μm vs. 500–605 μm and 610–630 μm), longer tail (c = 5.4–6.9 vs. c = 7.0–8.9, c = 7.2–7.6), and the presence of two precloacal supplements (one in *M. exceptionregulum* and *M. mareprofundus*) [17].

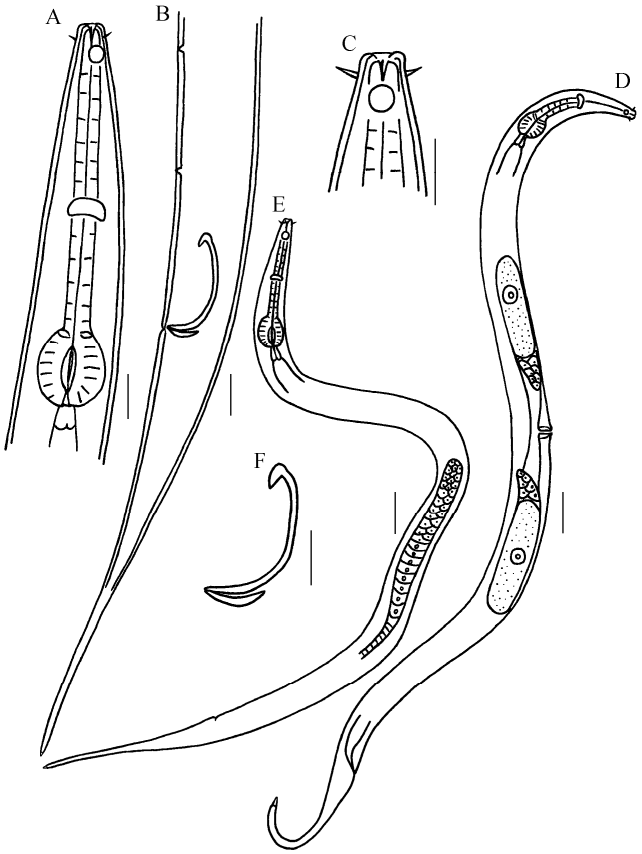

Figure 3. Line drawing of *Molgolaimus longicaudatus* sp. nov. (**A**) Pharyngeal region of holotype, showing amphid, nerve ring, and pharyngeal bulb; (**B**) lateral view of holotype posterior end, showing spicule, gubernaculum, and precloacal supplements; (**C**) lateral view of holotype anterior end, showing cephalic setae, buccal cavity with a dorsal tooth, and amphidial fovea; (**D**) entire view of female; (**E**) entire view of male; (**F**) magnifying spicule and gubernaculum. Scale bar: (**A**–**C**,**F**) = 10 μm; (**D**,**E**) = 30 μm.

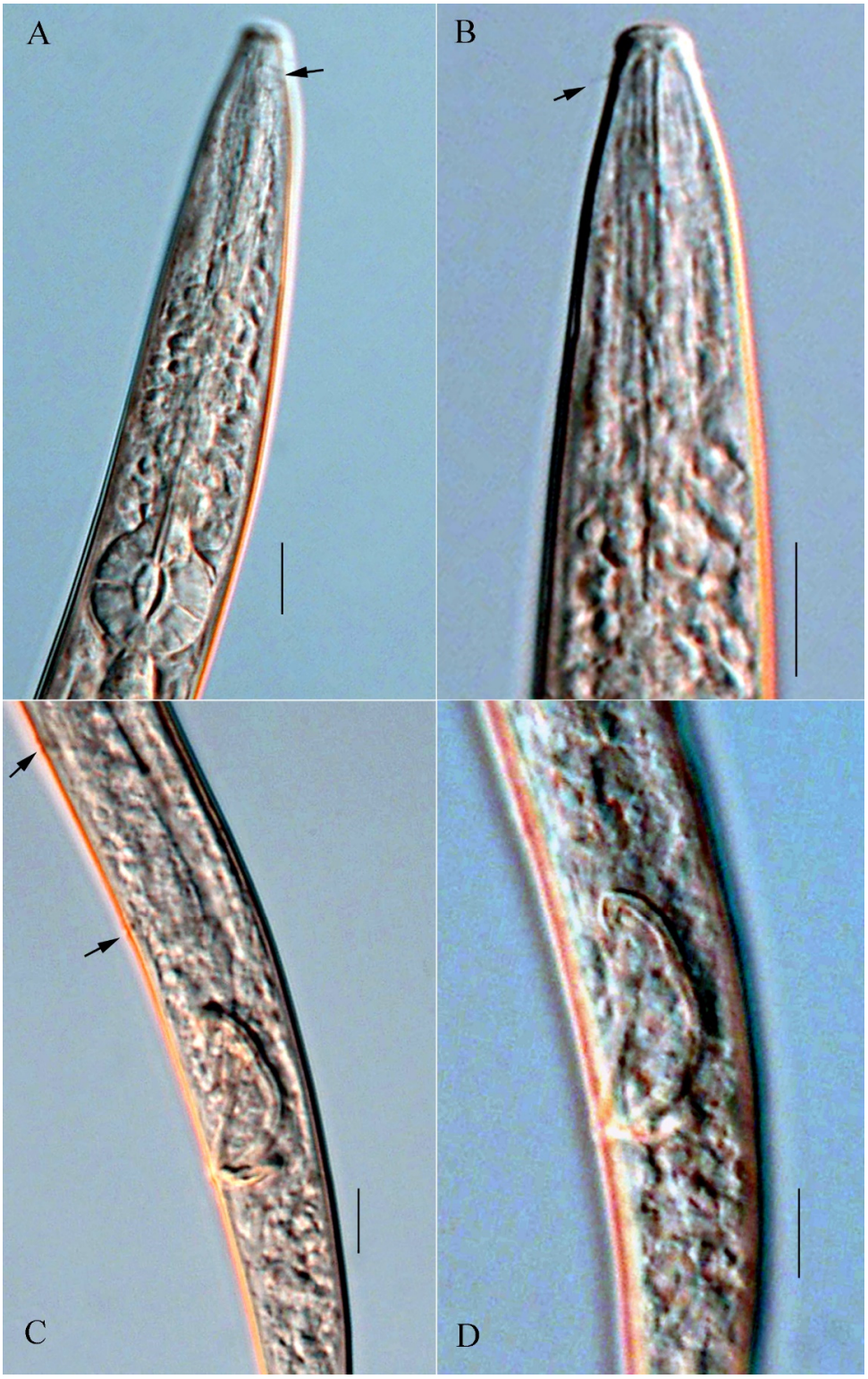

Figure 4. Microphotograph of *Molgolaimus longicaudatus* sp. nov. (**A**) Pharyngeal region of holotype, showing amphid (arrow) and pharyngeal bulb; (**B**) lateral view of holotype anterior end, showing cephalic setae (arrow) and buccal cavity; (**C**) lateral view of holotype cloacal region, showing spicule, gubernaculum, and precloacal supplements (arrows); (**D**) lateral view of holotype cloacal region, showing spicule. Scale bar: (**A**–**D**) = 10 μm.

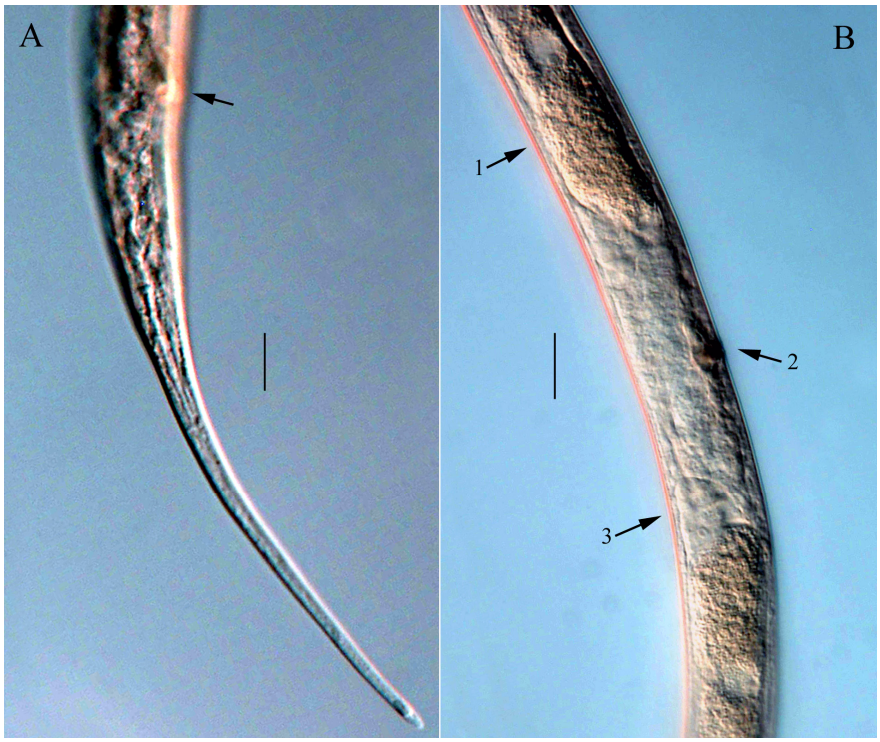

Figure 5. Microphotograph of *Molgolaimus longicaudatus* sp. nov. (**A**) Tail of male and arrow showing cloacal; (**B**) Middle of female, showing egg (arrow 1), vulva (arrow 2), and ovary (arrow 3). Scale bar: (**A**) = 10 μm; (**B**) = 20 μm.

So far, *Molgolaimus euryformis* is the only species of this genus that has been discovered in mangrove wetlands in China. It belongs to group 1a in the identification key and is characterized by morphological characteristics of spicules (short with ventral apophysis) and gubernaculum (with a block-shaped hook on the terminus) [6]. It could be easily distinguished from the new species. The morphological differentiating characteristics of the *Molgolaimus* species of group1b1 (based on the classification of Fonseca et al. [17]) were provided (Table 4).

Table 4. Morphological differentiating data of male *Molgolaimus* (species belonging to group1b1 were listed in this Table).

Species	Body Length (μm)	Spicule Length (μm)	Numbers of Supplements	a	b	c	c′	Spic/abd	References
M. pecticauda	750–850	-	absent	23.6–30.6	6.6–7.2	7.7–10.2	-	-	[31]
M. sapiens	435–620	35	absent	19.5–24.5	5.4–6.8	8.8–10.5	2.6–3.5	1.9	[17]
M. spirifer	880–1060	22–25	absent	36.6–38.0	6.2–7.3	8.5–11.2	5.4–5.7	1.2–1.5	[32]
M. drakus	495–575	19–20	1	35.5–42.7	6.1–7.0	7.0–7.9	5.3–6.2	1.4–1.6	[17]
M. exceptionregulum	585–600	28–31	1	28.4–31.5	5.7–6.1	7.5–8.9	3.6–4.6	1.6–1.8	[17]
M. mareprofundus	610–630	29–32	1	24.1–30.7	5.8–6.7	7.2–7.6	4.6–5.1	1.6–1.9	[17]
M. gazii	382–430	29	2	27.5	5.6	6.0–6.1	-	2.2–2.9	[33]
M. longicaudatus sp. nov.	735–775	32–35	2	28.3–31.0	7.4–8.2	5.4–5.7	7.2–7.7	1.8–1.9	This study

3.3. Molecular Phylogenetic Analysis

In this study, nearly full-length SSU sequences (1542–1592 bp) of the two new species were provided. All sequences were deposited in GenBank, and the accession numbers were PP923590 and PP923591 (*Metachromadora parobscura* sp. nov.), PP923592 and PP923593 (*Molgolaimus longicaudatus* sp. nov.). Sequence alignment indicated that the amplified 18S rRNA fragments contained some variable regions, and it was suitable for DNA barcoding studies of Desmodoridae. Then, a taxonomic phylogenetic tree was constructed based on maximum likelihood analyses. The new species, *Molgolaimus longicaudatus* sp. nov., formed a separate clade and was represented by a bootstrap support of 100%. The other

species of Desmodoridae comprised another well-supported clade (bootstrap support of 100%). *Metachromadora parobscura* sp. nov. in this clade formed high-support clade clustering with other *Metachromadora* species (bootstrap = 98%). Five sequences of the genus *Metachromadora* were included in the phylogenetic tree, and only *M. remanei* was identified to the species level. Blasting results showed that the sequence of *M. remanei* (Accession number AM234620.1) had the highest similarity to the new species in the NCBI genebank. The percentage of base differences was 5.2% (82/1574). The phylogenetic tree showed the taxonomic relationships between *M. parobscura* sp. nov. and *M. remanei* (Figure 6). Thus, the phylogenetic positions of these two new species were confirmed.

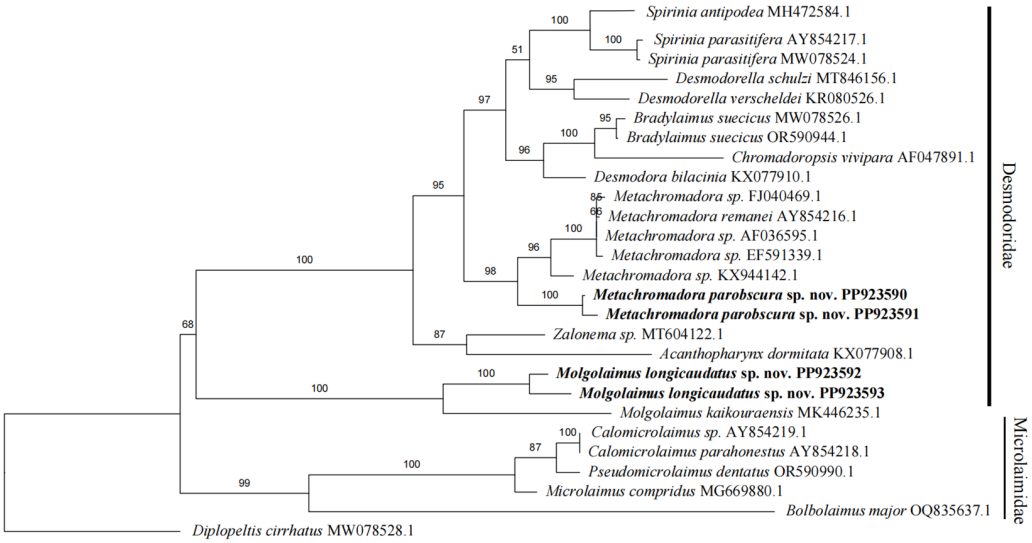

Figure 6. Phylogenetic tree generated from maximum likelihood (ML) analysis based on 18S rRNA sequences. ML bootstrap values are displayed above each branch. New species are shown in bold.

Species of *Molgolaimus* are very similar to those of Microlaimidae, but with the reflexed ovaries. Shi et al. (2016) transferred *Microlaimus pecticauda* and *Microlaimus spirifer* from the family Microlaimidae to *Molgolaimus* of the family Desmodoridae [20]. Recently, Leduc et al. (2018) placed *Molgolaimus demani* Jensen, 1978, in the Microlaimidae [18]. In order to confirm the taxonomic position of *Molgolaimus longicaudatus* sp. nov., the phylogenetic tree included members belonging to the family Desmodoridae and Microlaimidae (Figure 6). As shown in the phylogenetic tree, the family Microlaimidae and Desmodoridae exhibits two monophyletic lineages, with the larger Desmodoridae clade encompassing three subfamilies (Desmodorinae, Molgolaiminae, and Spiriniinae). *Molgolaimus longicaudatus* sp. nov. showed a close relationship with *Molgolaimus kaikouraensis* (MK446235.1) and exhibited good differentiation from species of other subfamilies. Results showed that the new species *Molgolaimus longicaudatus* sp. nov. clustered within the Desmodoridae clade, supporting the opinion of Lorenzen [16].

4. Conclusions

Two new species of free-living nematodes, *Metachromadora parobscura* sp. nov. and *Molgolaimus longicaudatus* sp. nov., from mangrove wetlands in Beihai were discovered. Differentiating morphometric data were given to facilitate species identification. Nearly full-length SSU sequences of the two species were provided. ML topology performed with SSU sequences supported the taxonomic position of the two new species, and they were both assigned a firm place within the phylogeny. Sequences in this study provided a foundational basis and feasibility for future monitoring of the two nematode species by using molecular approaches such as qPCR, multiplex PCR, LF-RPA assay, and so on. It is essential for the conservation of mangrove ecosystems. But variable sequences in databases are seldom, and most of the sequences are too short or not assigned to species levels. It limits the resolving abilities of molecular tools for nematode identification. With the

enrichment of the nematode DNA database, it would be particularly valuable in identifying those closely related species and contributing to a more robust nematode taxonomy.

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References

1. Naidoo, G.; Naidoo, K.; Swart, A. Oil Pollution Effects on Nematodes in Mangrove Sediment: A Microcosm Study. *Res. Square* **2021**. [CrossRef]
2. Spedicato, A.; Zeppilli, D.; Thouzeau, G.; Michaud, E. Nematode diversity patterns in mangroves: A review of environmental drivers at different spatial scales. *Biodivers. Conserv.* **2023**, *32*, 1451–1471. [CrossRef]
3. Sahraean, N.; Van Campenhout, J.; Rigaux, A.; Mosallanejad, H.; Leliaert, F.; Moens, T. Lack of population genetic structure in the marine nematodes *Ptycholaimellus pandispiculatus* and *Terschellingia longicaudata* in beaches of the Persian Gulf, Iran. *Mar. Ecol.* **2017**, *38*, e12426. [CrossRef]
4. Xiao, Y.P.; Guo, Y.Q. Three new free-living marine nematode species of *Subsphaerolaimus* Lorenzen, 1978, *Halichoanolaimus* de Man, 1886 and *Belbolla* Andrássy, 1973 from mangrove wetlands in Taiwan. *Zootaxa* **2023**, *5361*, 301–322. [CrossRef]
5. Wang, Y.; Pang, X.P.; Guo, Y.Q. Study on marine nematodes community in Jinhai bay mangrove wetland, Beihai, Guangxi. *Oceanol. Limnol. Sin.* **2020**, *51*, 583–590.
6. Zhou, X.P.; Zeng, J.L.; Cai, L.Z.; Fu, S.J.; Tan, W.J. Two New Species of Free-Living Marine Nematodes of the Desmodoridae from Mangrove Wetlands of Xiamen Bay, China. *J. Ocean Univ. China* **2020**, *19*, 143–150. [CrossRef]
7. Armenteros, M.; Ruiz-Abierno, A.; Decraemer, W. Revision of Desmodorinae and Spiriniinae (Nematoda: Desmodoridae) with redescription of eight known species. *Eur. J. Taxon.* **2014**, *96*, 1–32. [CrossRef]
8. Vincx, M. Free-Living Marine Nematodes from the Southern Bight of the North Sea. Ph.D. Thesis, State University of Ghent, Gent, Belgium, 1989; p. 678.
9. Maria, T.F.; Smol, N.; Esteves, A.M. Two new species of *Metachromadora* (Nematoda: Desmodoridae) from Guanabara Bay, Rio de Janeiro, Brazil, and a revised dichotomous key to the genus. *J. Mar. Biol. Assoc.* **2014**, *94*, 105–114. [CrossRef]
10. De Ward, C.T.P. New species of *Hopperia* (Nematoda, Comesomatidae) and *Metachromadora* (Nematoda, Desmodoridae) from Patagonia, Chubut, Argentina. *Zootaxa* **2004**, *542*, 1–15. [CrossRef]
11. Gerlach, S.A. Nematoden aus der familie der chromadoridae von den deutschen Küsten. *Kiel. Meeresforsch.* **1951**, *8*, 106–132.
12. Timm, R.W. The marine nematodes of the Bay of Bengal. *Proc. Pak. Acad. Sci. USA* **1961**, *1*, 25–88.
13. Furstenberg, J.P.; Vincx, M. Three new species of *Chromadoropsis* species (Nematoda, Desmodoridae) from Southern Africa and the North Sea. *S. Afr. J. Zool.* **1988**, *23*, 215–223.
14. Gerlach, A.; Riemann, F. *The Bremerhaven Checklist of Aquatic Nematodes. A Catalogue of Nematoda Adenophorea Excluding the Dorylaimida. Part 1*; Veröff Inst Meer Bremerhaven: Bremen, Germany, 1973; Volume 4, pp. 1–736.
15. Jensen, P. Revision of Microlaimidae, erection of Molgolaimidae fam. n., and remarks on the systematic position of *Paramicrolaimus* (Nematoda, Desmodorida). *Zool Scr.* **1978**, *7*, 159–173. [CrossRef]
16. Lorenzen, S. *The Phylogenetic Systematics of Free Living Nematodes*; The Ray Society: London, UK, 1994; p. 383.
17. Fonseca, G.; Vanreusel, A.; Decraemer, W. Taxonomy and biogeography of *Molgolaimus* Ditlevsen, 1921 (Nematoda: Chromadoria) with reference to the origins of deep sea nematodes. *Antarct. Sci.* **2006**, *18*, 23–50. [CrossRef]
18. Portnova, D. Free-living nematodes from the deep-sea Håkon Mosby Mud Volcano, including the description of two new and three known species. *Zootaxa* **2009**, *2096*, 197–213. [CrossRef]
19. Leduc, D.; Fu, S.; Zhao, Z.Q. New nematode species from the continental slope of New Zealand (Chromadorea, Microlaimida, and Chromadorida), and unexpected placement of the genus *Molgolaimus* Ditlevsen, 1921. *Mar. Biodiv.* **2019**, *49*, 2267–2280. [CrossRef]
20. Shi, B.; Xu, K. *Spirobolbolaimus undulatus* sp. nov. in intertidal sediment from the East China Sea, with transfer of two *Microlaimus* species to *Molgolaimus* (Nematoda, Desmodorida). *J. Mar. Biol. Assoc.* **2017**, *97*, 1335–1342. [CrossRef]

21. Macheriotou, L.G.K.B.; Nguyen, D.T.; Phuong Nguyen, T.X.; Noppe, F.; Armenteros, M.; Boufahja, F.; Rigaux, A.; Vanreusel, A.; Derycke, S. Metabarcoding free-living marine nematodes using curated 18S and CO1 reference sequence databases for species-level taxonomic assignments. *Ecol. Evol.* **2019**, *9*, 1211–1226. [CrossRef]
22. Avó, A.P.; Daniell, T.J.; Roy, N.; Solange, O.; Jordana, B.; Helena, A. DNA barcoding and morphological identification of benthic nematodes assemblages of estuarine intertidal sediments: Advances in molecular tools for biodiversity assessment. *Front. Mar. Sci.* **2017**, *4*, 66. [CrossRef]
23. van Megen, H.; van den Elsen, S.; Holterman, M.; Karssen, G.; Mooyman, P.; Bongers, T.; Holovachov, O.; Bakker, J.; Helder, J. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* **2009**, *11*, 927–950. [CrossRef]
24. Platt, H.M.; Warwick, R.M. Free-living Marine Nematodes, Part I, British Enoplids. In *Synopses of the British Fauna (New Series)*; The Linnean Society of London: London, UK, 1998; p. 248.
25. De Ley, I.T.; De Ley, P.; Vierstraete, A.; Karssen, G.; Moens, M.; Vanfleteren, J. Phylogenetic analysis of *Meloidogyne* small subunit rDNA. *J. Nematol.* **2002**, *34*, 319–327. [PubMed]
26. Stamatakis, A. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef] [PubMed]
27. Nguyen, D.T.; Gagarin, V.G.; Phan, K.L.; Nguyen TX, P.; Nguyen, V.T. Two new species of free-living marine nematodes (Nematoda) from an area near the mouth of the Yen River of Vietnam. *Biol. Morya* **2016**, *42*, 439–448.
28. Wieser, W.; Hopper, B. Marine nematodes of the east coast of North America. I. Florida. *Bull. Mus. Comp. Zool.* **1967**, *135*, 239–344.
29. Gagarin, V.G.; Nguyen, V.T. Three new species of free-living marine nematodes of the order Desmodorida (Nematoda) from Vietnam. *Zool. Zhurnal* **2010**, *89*, 398–406.
30. Gagarin, V.G.; Tu, N.D. Two new species of free living nematodes (Nematoda and Chromadorea) from mangrove thicket in Vietnam. *Inland Water Biol.* **2014**, *7*, 338–347. [CrossRef]
31. Murphy, D.G. An initial report on a collection of Chilean marine nematodes. *Mitt. Hamb. Zool. Mus. Inst.* **1966**, *63*, 29–50.
32. Warwick, R.M. Fourteen new species of free-living marine nematodes from the Exe estuary. *Bull. Br. Mus. Nat. Hist. (Zool.)* **1970**, *19*, 137–177. [CrossRef]
33. Muthumbi, A.W.; Vincx, M. Nematodes from the Indian Ocean: Description of six new species of the genus *Molgolaimus* Ditlevsen, 1921 (Nematoda: Desmodoridae). *Bull. De L'institut R. Dae Sci. Nat. Belgique. Biol.* **1996**, *66*, 17–28.

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