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Article

Two New Species of Free-Living Nematode (Desmodoridae, Namatoda) from Mangrove Wetlands of China

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Abstract: Two new species of free-living nematode, *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 extended ventrally to support spicule, 6-8 precloacal tubular supplements, and short, conical tail with two protuberances. *Molgolaimus longicaudatus* sp. nov. is characterized by short cephalic setae, relatively small amphidial fovea, ventrally bent spicules with pronounced hamose curved proximal terminus, two undulate precloacal supplements, and relatively long conico-cylindrical tail. Nearly full-length SSU sequences of the two species were provided, and phylogenetic tree based on maximum likelihood analyses supported the taxonomic position of the two new species. Combined use of traditional morphology-based taxonomy and molecular technology would be a good choice for identification of free-living nematodes.

Keywords: *Metachromadora parobscura* sp. nov.; *Molgolaimus longicaudatus* sp. nov.; taxonomy; Beibu Gulf of the South China Sea; Small subunit (SSU) 18S rDNA gene

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 play a key role in nutrient cycling and affect carbon fixation in mangrove wetlands [1]. Furthermore, nematodes are sensitive to physicochemical conditions of sediment, thus being potential bioindicators for conservation purposes [2]. Hence, the identification of nematode species would improve our understanding of ecosystem's functional roles [3]. In the past years, community structure of nematodes was investigated, and also some nematode species were found and described from 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 subfamily Spiriniinae Gerlach & Murphy, 1965. Now, it is comprised of 32 valid species which are divided into five subgenera: *Bradulaimus* Stekhoven, 1951, *Metachromadora* Filipjev, 1918, *Metachromadoroides* Timm, 1961, *Metonyx* Chitwood, 1936, and *Neonyx* Cobb, 1933. And Maria et al. provided a revised dichotomous key to the genus [8]. The genus *Molgolaimus* Ditlevsen, 1921 belongs to subfamily Molgolaiminae Jensen, 1978. It was divided into four species groups by Fonseca et al. based on spicule length, and scatter-plot graphs of morphometric data, and illustrations of the different 33 *Molgolaimus* species were provided in the study [9]. Furthermore, *Molgolaimus haakonmosbiensis* sp. n. [10], *Molgolaimus kaikouraensis* sp. nov. [11] and *Molgolaimus euryformis*

sp. nov. [6] were described. Additionally, two *Microloaimus* species were transferred to the genus by Shi and Xu [12]. *Molgolaimus* now contains 38 valid species.

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 [13,14]. And also some scientists proposed the combined use of different genes would get 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 nematode. Desmodoridae is complicated due to lack of synapomorphy and it is also highly polyphyletic based upon small subunit of ribosomal DNA [15]. 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 techniques. Nearly full-length SSU sequences of this two species were obtained for phylogenetic analysis. This study will help to improve our 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. Samples were collected by pushing syringe (2.6 cm internal diameter) into the sediment to a depth of 5 cm. The sediment was then fixed in 5% formalin. Samples used for molecular analysis was stored in 95% ethanol and frozen at -20 °C. The nematodes were sorted using differential interference contrast microscope (Leica DM 2500) (CMS GmbH, Wetzlar, Germany and assembled in Shanghai, China). 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.

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 PCR tubes and DNA was extracted from nematodes following the protocol of the DNeasy Blood & Tissue kit (Qiagen, Germany). PCR was performed using an Ex taq polymerase kit (TaKaRa). Nearly full-length of the 18S rDNA gene was amplified using 18S-G18S4 (5'-GCTTGCTCTCAAAGATTAAGCC-3') and 18S-18P (5'-TGATCCWKCYGCAGGTTAC-3') [16]. Each PCR mixture (50 µl) contained 5 µl DNA template, 200 µmol/L of each dNTP, 1.5 µmol/L MgCl₂, 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 30s, annealing at 56°C for 1 min 30s, and extension at 72°C for 30s; and a final extension at 72°C for 7 min. PCR products were gel purified, cloned and sequenced. All sequences were deposited in GenBank. *Diplopeltis cirrhatus* was selected as the outgroup. A total of 23 18S rDNA sequences, belonging to the family Desmodoridae and Microloaimidae, 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 [17].

2.3. Abbreviations

De Man (1884) ratios 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 [18].

3. Results

3.1. Systematics 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
Diagnosis of the genus *Metachromadora* Filipjev, 1918 (Modified from Maria et al. 2013)

Cuticle finely or coarsely striated, extreme anterior end of the head unstriated, longitudinal lateral ridges present or absent. Somatic setae present. Cryptospiral amphidial fovea partly or not surrounded by striations. Inner labial sensilla papilliform, outer labial and cephalic sensilla papilliform or setiform, buccal cavity with large dorsal tooth, and subventral teeth present or absent. Pharynx with well developed posterior bulb with a thick cuticular lining and partitioned into two or three sections; male with one testis at left of the intestine; spicules with well developed velum and capitulum; precloacal supplements of various forms or absent; tail conical [8].

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 slides C3-1-15, ♀1 on slide C3-1-7, and ♀2 on slide C3-1-8.

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 bscura*, referring to the new species is similar to *Metachromadora bscura* in most morphological characters.

3.1.4. Measurements

All measurement data are given in Table 1.

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

Morphological Characters	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)

Male: Body cylindrical 896–1020 µm long, finely striated, with striation starting immediately after the amphidial foveae. Lateral ridges, 2.0–3.0 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 middle of the tail. Blunt conical head. Six internal and six external labial sensillae prominent papilliform. Four cephalic setae, 5.0–6.0 µm long. No cervical setae. Somatic setae, 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 sudorsal). Amphideal fovea loop-shaped, with an open top and have double contours, positioned anterior to the first head cuticular annulus. Width of amphidial foveae is 13.0 - 15.0µm, about 50.0–62.5% of corresponding body diameter. Buccal cavity 33.0 - 38.0µm deep, with three teeth, a large dorsal tooth and two minute subventral teeth, dorsal tooth bigger. Bulb length is 39–43% of the total pharynx length, with bipartite cuticularized internal cavity. Cardia muscular, small. Nerve ring anterior to the pharynx bulb. Secretory–excretory pore not observed. Reproductive system monorchic with outstretched testis. Vas deferens right of intestine. Two equal spicules (63–68 µm), slender, ventrally curved with well-cephalated capitulum. Gubernaculum (30-31 µm) boat-shaped, parallel to spicule, with pointed end extended ventrally to support spicule, without apophyses. Six tubular precloacal supplements, about 11-17µm apart, first supplement 5-6 µm distant from 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 ventral side of the tail, the first one 38-44 µm distant from the cloaca. Caudal glands present, but poorly visible.

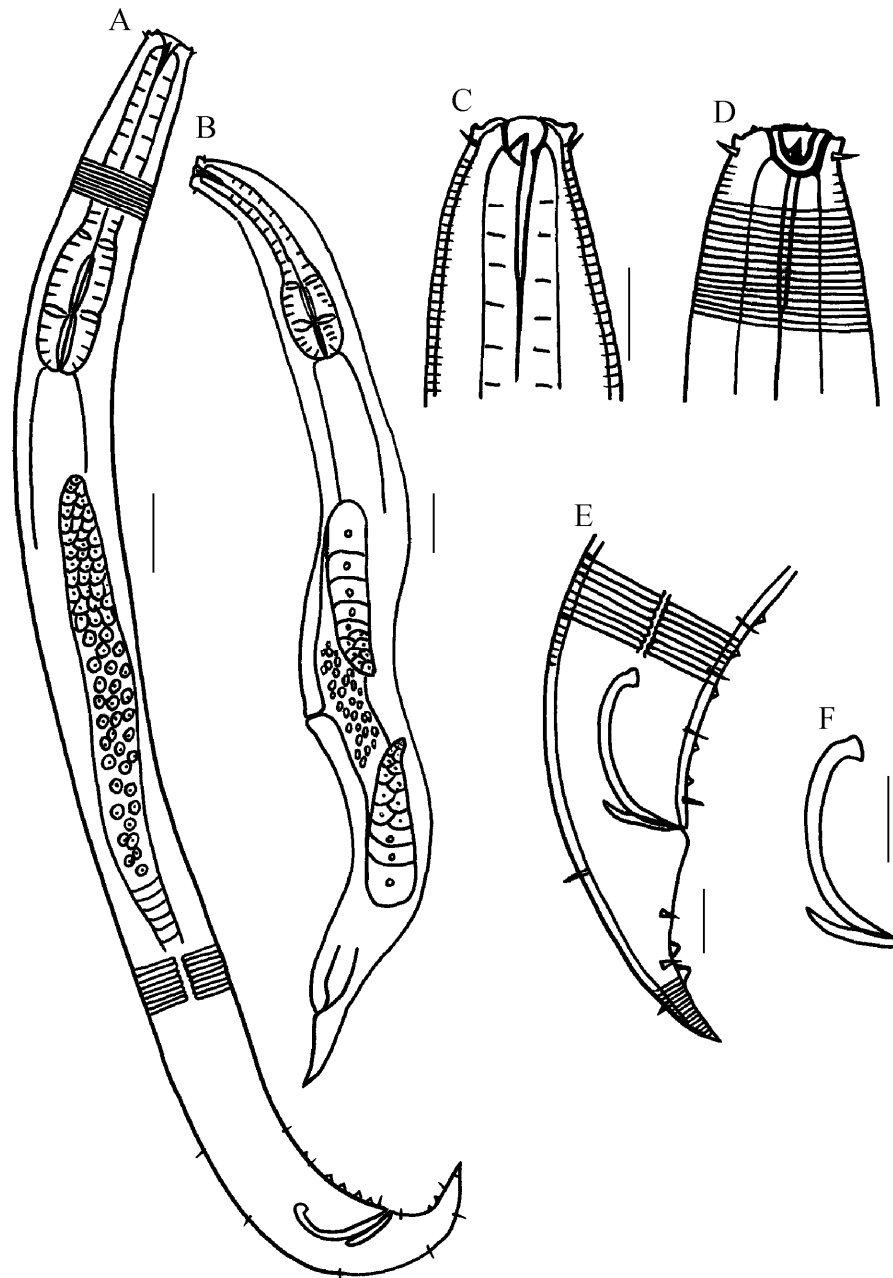


Figure 1. Drawing of *Metachromadora parobscura* sp. nov. (A) Entire view of male Anterior end of male, showing outer labial setae, cephalic setae, amphid (arrow 1) and epidermal gland cells (arrow 2); (B) entire view of female; (C) anterior end of holotype; (D) anterior end of holotype, showing amphid and tooth; (E) posterior end of male, showing spicule, gubernaculum, precloacal supplements and tail papillae; (F) magnifying spicule and gubernaculum. Scale bar: (A,B) = 50 µm; (C,D,E,F) = 20 µm.

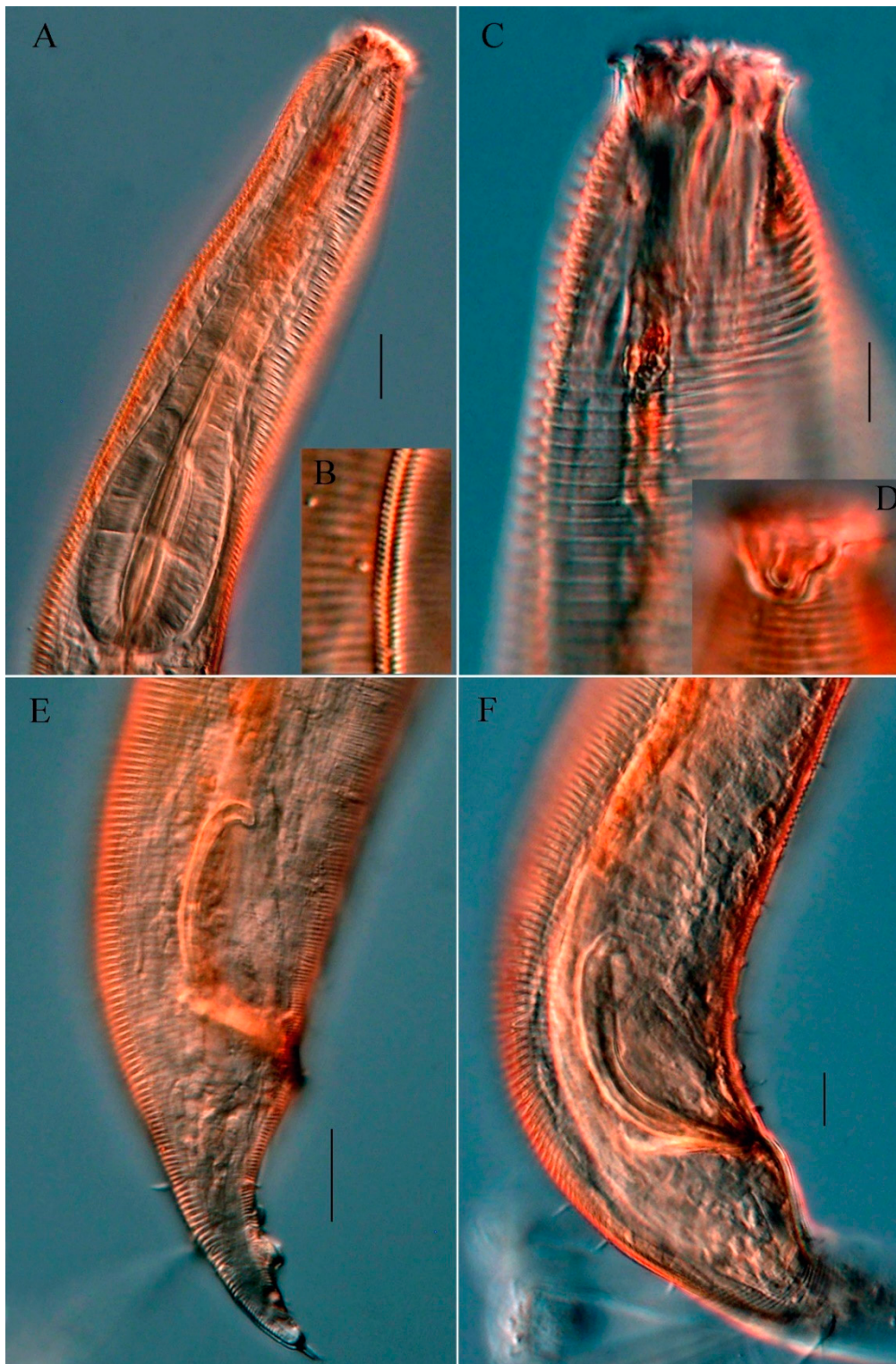


Figure 2. Micrograph of *Metachromadora parobscura* sp. nov. (A) Pharyngeal region of holotype, showing pharyngeal bulbs; (B) lateral ridge (C) lateral view of holotype anterior end, showing anterior sensilla and dorsal tooth; (D) amphid of holotype; (E) posterior end of male, showing spicule and tail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

Female: Similar to male in general morphology. Few and scattered somatic setae, without a pattern of organization. Reproductive system amphidelphic with two ovaries opposed and reflexed, anterior ovary positioned to the left of the intestine, posterior ovary to right. Vagina 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.

3.1.6. Diagnosis

Metachromadora parobscura sp. nov. is characterized by eight longitudinal rows 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 extended ventrally to support spicule, 6-8 precloacal tubular supplements, and short, conical tail with two protuberances.

3.1.7. Differential Diagnosis

Due to the morphological characters, the new species is placed in *Metachromadoroides* Timm, 1961. There are nine valid species within the subgenus *Metachromadoroides* Timm, 1961: *M. (M.) complexa*, *M. (M.) pulvinata*, *M. (M.) remanei*, *M. (M.) vulgaris*, *M. (M.) zaixsi*, *M. (M.) minor*, and *M. (M.) orientalis*, *M. (M.) obscura*, and *M. (M.) xiamenensis*. Description 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 division. Precloacal supplemental male organs in the shape of short faintly cuticularized tubules or absent [19].

Metachromadoroides could be subdivided into two groups based on the precloacal supplements. Supplements absent: *M. (M.) zaixsi*, *M. (M.) orientalis* and *M. (M.) vulgaris*. These three species could be easily differentiated by internal division of pharyngeal bulb (bipartite in *M. (M.) orientalis* vs. tripartite in *M. (M.) zaixsi* and *M. (M.) vulgaris*) and length of anterior non-striated part (one amphidial fovea lengths in *M. (M.) zaixsi* vs. two in *M. (M.) vulgaris*). Supplements present: *M. (M.) complexa*, *M. (M.) xiamenensis* and *M. (M.) minor* have unique characteristics separately (setae absent, three small protuberances on ventral side of the tail and short slim body); *M. (M.) pulvinata*, *M. (M.) remanei* and *M. (M.) obscura* differed from each other by body length, and numbers of precloacal supplements (1720mm and 23 vs. 1100-1300mm and 5 vs. 786mm and 13-14).

Metachromadora parobscura sp. nov. is closely resembles *M. (M.) remanei* in De Man ratios. New species could be distinguished from it by body length (896-1020mm vs.1100-1300mm in *M. (M.) remanei*), spicules length (63-68 μm vs. 49-55 μm in *M. (M.) remanei*) and numbers of precloacal supplements (6-8 vs. 5 in *M. (M.) remanei*). Otherwise, the new species is morphologically similar to *M. (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. (M.) obscura*), amphid diameter as a percentage of the corresponding body diameter (50-62.5% vs. 65-75% in *M. (M.) obscura*), shorter spicules (63-68 μm vs. 75-78 μm in *M. (M.) obscura*), shorter gubernaculum (30-31 μm vs. 38-40 μm in *M. (M.) obscura*), smaller number of precloacal supplements (6-8 vs. 13-14 tubular supplements in *M. (M.) obscura*). Morphological differentiating characters of the species of *Metachromadoroides* are proposed (Table 2).

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

species	Body Length (μm)	Spicule Length (μm)	numbers of supplements	internal division of pharyngeal bulb	protuberances on the tail	a	b	c	c'	References
<i>M.(M.) complexa</i>	758-940	74	17	tripartite	2	8.4-11.3	3.4-4.4	7.3-12.1	1.3	[19]
<i>M.(M.) pulvinata</i>	1720	55	23	tripartite	2	18.1	5.4	15.6	2.0	[20]
<i>M. (M.) remanei</i>	1100-1300	49-55	5	bipartite	*	14	4.9	14	1.9-2.2	[21]
<i>M.(M.) vulgaris</i>	1100-1200	72	0	tripartite	0	14.1-20.0	4.4-4.9	12.1-14.5	1.8-2.8	[19]

<i>M.(M.) zaixsi</i>	890-1160	40-52	0	tripartite	0	10.5-19.3	4.7-7.2	6.8-10.1	1.7-2.8	[22]
<i>M.(M.) minor</i>	594-641	35-36	12-16	tripartite	*	12-15	4.2-4.7	13.5-14.9	1.4-1.5	[23]
<i>M.(M.) orientalis</i>	859-1133	59-63	0	bipartite	**	13-15	4.8-5.3	15.3-19.2	1.0-1.3	[24]
<i>M.(M.) obscura</i>	786-1008	75-78	13-14	bipartite	2	11-15	4.2-4.5	10.7-12.1	1.4-1.6	[25]
<i>M.(M.) xiamenensis</i>	741-888	46-56	18	bipartite	3	11.0-14.3	4.4-5.2	10.1-12.2	1.5-1.7	[26]
<i>M. (M.) parobscura sp. nov.</i>	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. Systematics 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
Description of the Genus *Molgolaimus* Ditlevsen, 1921 (Modified from Fonseca et al., 2006)
Cuticle finely striated or apparently smooth. Amphidial fovea round and posterior to the cephalic constriction. Cephalic setae close to the cephalic constriction. Buccal cavity small, narrow and with small teeth. Pharynx with a pronounced spherical bulb. Male reproductive system monorchic. Spicules of variable length and shape from short and bent to long and sinusoidal or straight. Gubernaculum with or without apophysis. Precloacal supplements often present. Female reproductive system didelphic-amphidelphic, with ovaries reflexed. Tail of varying shape and length [9].

3.2.1. Type Material

Four males and four 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.

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).

Morphological Characters	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
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 and 4)

Male: Body slender and cylindrical, tapering gradually towards both ends. Cuticle annules not obvious. Head separated from body by a faint constriction. Inner labial and outer labial sensilla indistinct. Four cephalic setae, 2 µm long, located posterior to cephalic constriction. Amphideal fovea round, posterior to the cephalic constriction, 3-4 µm in diameter, corresponding to 33.3-44.4 % of c.b.d., about 6-7 µm from the anterior end. Buccal cavity small and narrow, without teeth. Buccal cavity small, weakly sclerotized, narrow and with small teeth. Pharynx corpus narrow cylindrical, ending in apronounced spherical muscular bulb (17-19µm diameter), and the bulb heavily cuticularised. 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 of the intestine. Vas deferens long and thin. Spicules 32-35 µm long (1.8-1.9 abd), ventrally bent and pronounced hamose curved proximal terminus. Gubernaculum simple and parallel to the spicules, without apophyses, its length corresponding to one third of the spicule length. Two precloacal supplements, 25-41 µm and 43-68 µm distant from the cloaca. Tail conico-cylindrical, conical in the proximal half and distally characterized by a cylindrical prolongation. Caudal glands not observed.

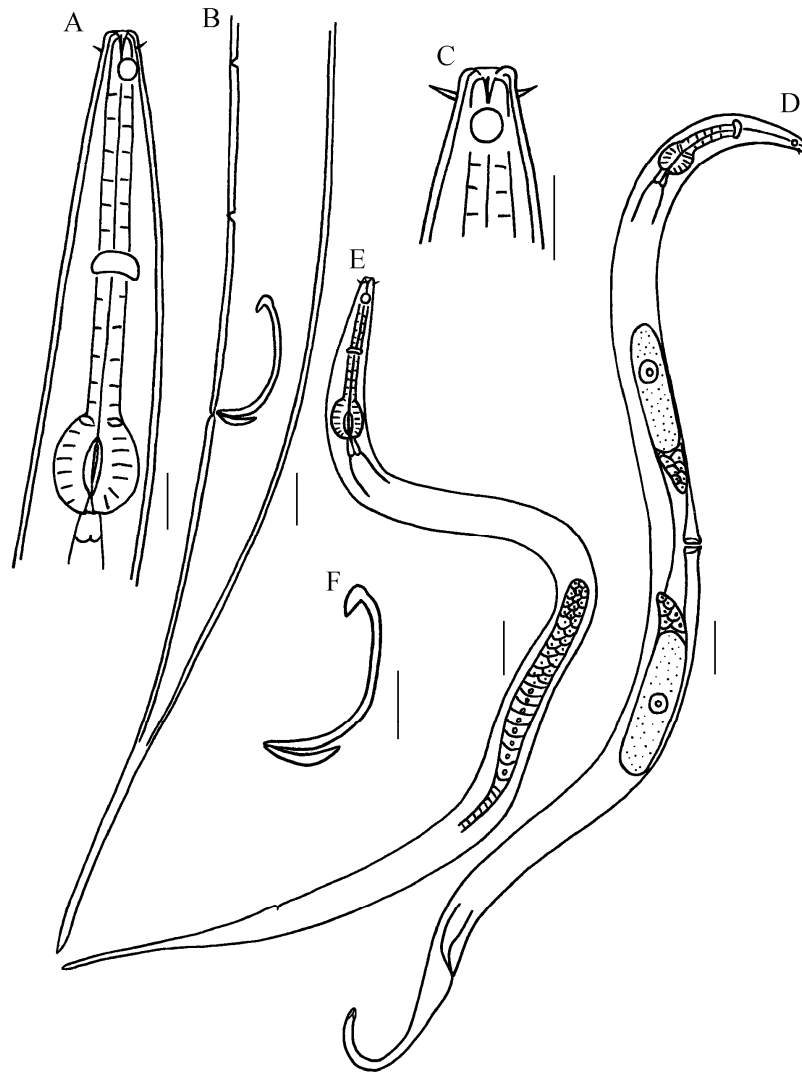


Figure 3. Micrograph of *Molgolaimus longicaudatus* sp. nov. (A) Pharyngeal region of holotype, showing amphid, nerve ring and pharyngeal bulb; (B) lateral view of holotype posterior end, showing spicule, gubernaculum and precloacal supplements; (C) lateral view of holotype anterior end, showing cephalic 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,B,C,F) = 10 µm; (D,E) = 30 µm.

Female: Similar to male, but with larger maximum body diameter and shorter tail. Reproductive system didelphic with reflexed ovaries, the anterior branch right of the intestine and posterior branch to the left. Vulva located anterior to mid-body (42.7-44.1% of total length).

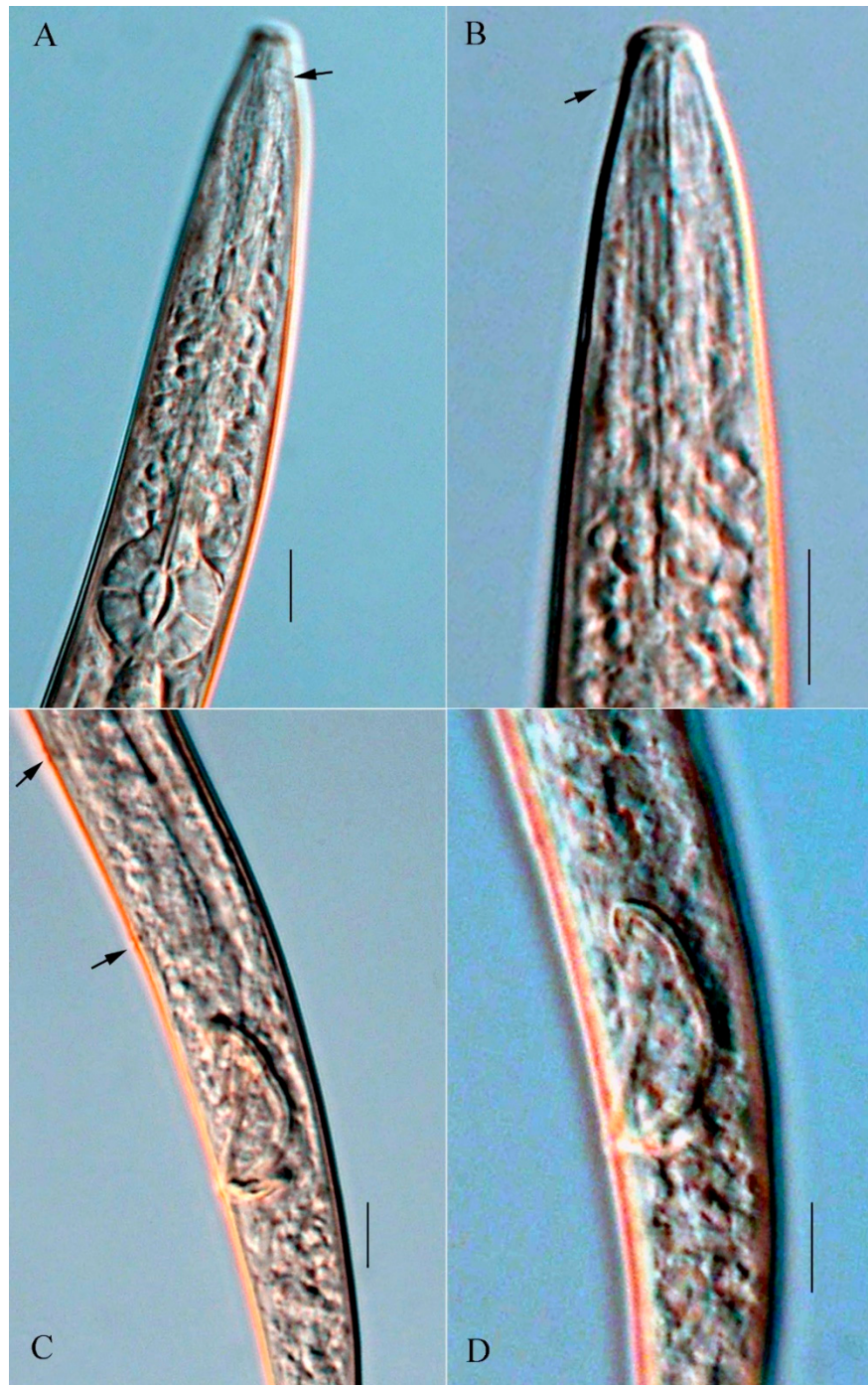


Figure 4. Micrograph of *Molgolaimus longicaudatus* sp. nov. (A) Pharyngeal region of holotype, showing amphid (arrow) and pharyngeal bulb; (B) lateral view of holotype anterior end, showing cephalic setae (arrow) and buccal cavity; (C) lateral view of holotype cloacal region, showing spicule, gubernaculum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

3.2.6. Diagnosis

Molgolaimus longicaudatus sp. nov. is characterized by short cephalic setae (2 μ m), relative small amphidial fovea (3-4 μ m in diameter), ventrally bent spicules with pronounced hamose curved proximal terminus, two undulate precloacal supplements, long conico-cylindrical tail (115-138 μ m). Female reproductive system didelphic with both ovaries reflexed.

3.2.7. Differential Diagnosis

Fonseca et al. provides an illustrated polytomous identification key to 33 species of the genus, which were classified into four groups according to absolute spicule length [9]. This new species is morphologically closely related to the species in group 1b1 of the identification key, characterized by short spicules (< 35 μm), ratio spicules divided by the anal body diameter ranging from 1 to 3. There are seven species in the group: *M. drakus*, *M. exceptionregulum*, *M. gazii*, *M. pecticauda*, *M. mareprofundus*, *M. sapiens*, *M. spirifer*. *Molgolaimus* could be subdivided into three groups based on the number of precloacal supplements. Supplements absent: *Molgolaimus pecticauda* and *Molgolaimus spirifer* could be easily differentiated clearly from other species of the group by its amphid (amphid with a tubiform structure and multispiral amphid fovea, vs unispiral or circular). *M. sapiens* differs in spicule with S-shaped blade. One supplements: *M. drakus* is characterized by 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. Two supplements: *M. gazii* is more 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). Regardless of precloacal supplements, *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 supplement (one in *M. exceptionregulum* and *M. mareprofundus*). Morphological differentiating characters of the *Molgolaimus* species of the group1b1(based on classification of Fonseca et al. [9])was provided (Table 4).

Table 4. Morphological differentiating data of male *Molgolaimus*. (belonging to the group1b1 based on classification of Fonseca et al. [9])

species	Body Length (μm)	Spicule Length (μm)	numbers of supplements	a	b	c	c'	spic/abd	References
<i>M. pecticauda</i>	750-850	-	absent	23.6-30.6	6.6-7.2	7.7-10.2	-	-	[27]
<i>M. sapiens</i>	435-620	35	absent	19.5-24.5	5.4-6.8	8.8-10.5	2.6-3.5	1.9	[9]
<i>M. spirifer</i>	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	[28]
<i>M. drakus</i>	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	[9]
<i>M. exceptionregulum</i>	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	[9]
<i>M. mareprofundus</i>	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	[9]
<i>M. gazii</i>	382-430	29	2	27.5	5.6	6.0-6.1	-	2.2-2.9	[29]
<i>M. longicaudatus</i> 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 specieswere were provided. All sequences were deposited in GenBank, and the accession numbers were shown in Figure 5. Sequences alignment indicated that the amplified 18S rRNA fragments contained some variable regions, and it is 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 represented by a bootstrap support of 100%. It showed a close relationship with *Molgolaimus kaikouraensis*(MK446235.1) and exhibited good differentiation from species of other subfamilies. The other species of Desmodoridae comprised another well-supported monophyletic clade (bootstrap support of 100%). *Metachromadora parobscura* sp. nov. in this clade formed a high-support clade clustering with other *Metachromadora* species (bootstrap = 98%).Thus the phylogenetic positions of these two species were confirmed.

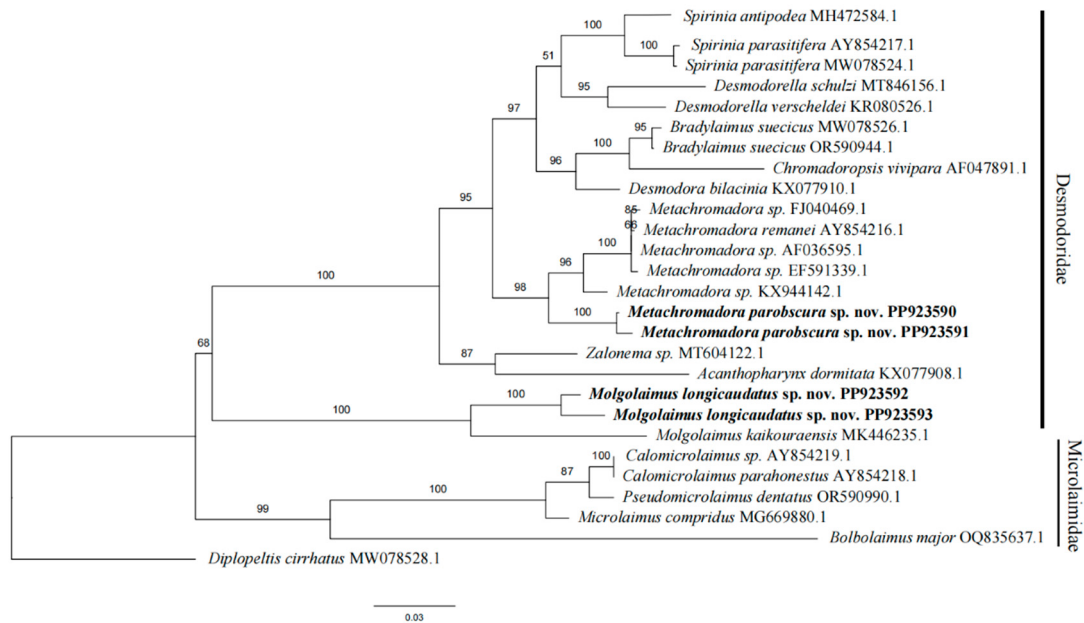


Figure 5. Phylogenetic tree generated from maximum likelihood (ML) analysis based on 18S rRNA sequences. ML bootstrap values are displayed above each branch. New species are shown in bold.

4. Discussion

Metachromadora is a heterogeneous genus and was not well defined [30]. It has been reviewed by Gerlach (1951), Wieser & Hopper (1967), Gerlach & Riemann (1972), Lorenzen (1981) and Maria (2013) [8,31]. It has been first divided into five subgenera: *Bradyaimus*, *Chromadoropsis*, *Metonyx*, *Metachromadora*, and *Neonyx* by Gerlach (1951)[21]. Subsequently, Timm (1961) erected the sixth subgenus, *Metachromadoroides*, with species *M. vulgaris* and *M. complexa* [19]. Later, *Chromadoropsis* was reinstated to the genus level by Furstenberg & Vincx, in 1988 [32]. Until now, *Metachromadora* consists of five subgenera and is still a large genus, containing 32 valid species. Although *Metachromadora serrata* Gerlach, 1963 and *Metachromadora spiralis* Gerlach, 1955 are considered valid species, they were regarded belonging to a doubtful genus or as incertae sedis [8,20]. The sequences of *M. (M.) parobscura* sp. nov. were blasted with sequences in the NCBI genebank. *M. (M.) remanei* (Accession number AM234620.1), which was identified to species level, was with the highest similarity to the new species, percentage of identity was 94.75% and percentage of base differences was 5.2%(82/1574). Because *M. (M.) parobscura* sp. nov. most closely resembles *M. (M.) remanei* in De Man ratios, different geographic speciation of *M. (M.) remanei*, from estuarine waters around southwest Britain and Mira estuary of Portugal separately (Accession number AM234620.1 and KX944141.1), were also aligned with MegAlign (DNASTar), and percentage of base differences between these two geographic speciation was only 2.4% (33/1389). So Possibility that the new species was the geographic existence of *M. (M.) remanei* was excluded. The phylogenetic tree based on 18S rRNA sequences also showed the taxonomic relationships between *M. (M.) parobscura* sp. nov. and *M. (M.) remanei*.

The taxonomic status of *Molgolaimus* was complicated and emended several times. *Molgolaimus* was firstly placed in family Microlaimidae by Gerlach and Riemann in 1973[33]. 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) [34]. Then, Lorenzen (1994) downgraded the family Molgolaimidae as a subfamily Molgolaiminae in Desmodoridae Filipjev, 1922 [35]. Shi et al. (2016) transferred *Microlaimus pecticauda* and *Microlaimus spirifer* from the family Microlaimidae to *Molgolaimus* of the family Desmodoridae [12]. But recently, Leduc et al. (2018) again placed *Molgolaimus demani* Jensen, 1978. in the Microlaimida [36]. Sometimes, it is hard to determine a new species belong to which of these two families. In order to confirm the

taxonomic position of *M.longicaudatus* sp. nov., the phylogenetic tree included members belonging to the the family Desmodoridae and Microlaimidae(Figure 5). As shown in the phylogenetic tree, the family Microlaimidae and Desmodoridae exhibits two monophyletic lineage, and the larger Desmodoridae clade encompassing three subfamilies (Desmodorinae, Molgolaiminae and Spiriniinae). Results showed that the new species *Molgolaimus longicaudatus* sp. nov. clustered within the Desmodoridae clade, supporting the opinion of Lorenzen.

5. Conclusions

Two new species of free-living nematode, *Metachromadora parobscura* sp. nov. and *Molgolaimus longicaudatus* sp. nov. , from mangrove wetlands in Beihai were discovered. And differentiating morphometric data were given. 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. Because available sequences in databases were seldom, most of sequences were too short or not assigned to species levels, it limited the resolving abilities of molecular tools for nematodes identification. With the enrichment of nematodes DNA database, it would be particularly valuable in identifying those closely related species, and contributes to a more robust nematodes taxonomy.

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