



Article Three New Species of Free-Living Marine Nematodes of the Order Enoplida Filipjev, 1929 (Nematoda) from the Yellow Sea, China

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Abstract: Three new nematode species belonging to the order Enoplida were discovered in the muddy and sandy sediment along the Shandong peninsula coast of the Yellow Sea during a biodiversity investigation of marine nematodes in the Yellow Sea. They are named *Belbolla octobulba* sp. nov., *Ironella gracilis* sp. nov. and *Oxystomina longiseta* sp. nov. *Belbolla octobulba* sp. nov. is characterized by having eight pharyngeal bulbs; spicules arcuate, broad and even; gubernaculum with a pair of dorsocaudal apophyses, about a third of the length of spicule; and two winged precloacal supplements. *Ironella gracilis* sp. nov. differs from known species by body slender, buccal cavity divided into conical vestibulum and cylindrical posterior portion, three sclerotized teeth located in the vestibulum; both circles of outer labial setae and cephalic setae 10 µm apart; elongated spicules weakly arcuate with central strip and cephalate proximal end; gubernaculum conical without apophysis; and a tubular precloacal supplement with a long seta at its anterior end. *Oxystomina longiseta* sp. nov. can be distinguished from other known species by the relatively long outer labial setae and cephalic setae; arcuate spicules expanded proximally with a median rib; gubernaculum small and oblong; and two unequal precloacal supplementary setae, anterior one short, posterior one very long. Updated keys to eighteen species of *Belbolla* and keys to four species of *Ironella* are given.

Keywords: *Belbolla octobulba* sp. nov.; *Ironella gracilis* sp. nov.; *Oxystomina longiseta* sp. nov.; taxonomy; biodiversity; Shandong peninsula

1. Introduction

Free-living marine nematodes are the most dominant and diverse meiofauna in marine benthic habitats. They interact in many ways with different organisms in many food webs, and they play a very important role in the recycling of organic matter and energy flow to the higher trophic levels and through the benthic ecosystem [1,2]. They can also be used for biomonitoring. Identifying nematode species is therefore very important. Globally, more than 9860 species of marine nematodes have been recorded to date [2–4], but only about 500 species have been identified in China, and there are still many unknown species.

The Yellow Sea is located on the edge of the western Pacific Ocean, between the Chinese mainland and the Korean Peninsula. It is a semi-enclosed inland shallow sea basin. The area is $38 \times 104 \text{ km}^2$, with an average depth of 44 m and a maximum depth of 140 m. Biodiversity surveys and taxonomical studies on nematodes in the Yellow Sea have been carried out in recent years. The abundance is $505 \pm 238-2538 \pm 676$ individuals per 10 cm^{-2} and the Shannon–Wiener diversity index is 1.859-3.79. More than 340 species were identified, and they belong to 2 classes, 13 orders, 45 families and 141 genera [2,3]. In abundance and occurrence, *Daptonema* [5] is the most dominant genus from the intertidal to subtidal zone up to 89 m deep. However, the study on nematodes in the intertidal zone of the Shandong peninsula is still not comprehensive. The total number of nematodes is unknown, and new species are always found in each sampling.

Currently, the classification of nematodes is usually based on morphological, molecular and developmental evidence. Molecular phylogenetic analyses have proven to be a



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). valuable tool to clarify relationships among marine nematode taxa, which are impossible to determine based on morphology alone [6,7]. In the present paper, only morphological descriptions are carried out for three new species; no molecular phylogenetic analyses are conducted on them, due to their clear phylogenetic status.

The genus *Belbolla* [8] belonging to the family Enchelidiidae [9] was first proposed as *Bolbella* by Cobb in 1920 with the description of *Bolbella tenuidens* [5]. Currently, eighteen valid species have been recognized within the genus globally (electronic database: http://nemys.ugent.be (accessed on 1 October 2023)). Five of them, namely *B. huanghaiensis* [10]; *B. sinica* [4]; *B. stenocephalum* [10]; *B. warwicki* [10]; and *B. zhangi* [11], were originally described from the Chinese sea area. The *Belbolla* species have a high occurrence rate in the subtidal zone of the Yellow Sea, but they are rare in the intertidal zone, and only *B. sinica* and this new species have been found in this survey area.

The genus *Ironella* belonging to the family Ironidae [12] was erected by Cobb in 1920 with *I. prismatolaima* as the type species [5]. To date, only three species have been recorded around the world (electronic database: http://nemys.ugent.be (accessed on 1 October 2023)). Unfortunately, *I. cobbi* [13] was described only from females found at the Atlantic Shore of the USA by Timm. *I. prismatolaima* [5] was described only from males found at the Atlantic Shore of the USA by Cobb. *I. riemanni* [14] was found by Riemann in 1966 [15] in the Elbe estuary and in Kandalaksha Bay (the White Sea) by Platonova and Mokievsky in 1994 [14]. The new species is the first recorded species within *Ironella* in the Chinese sea area.

The genus *Oxystomina* [16] belonging to the family Oxystominidae [17] was established by Filipjev in 1921, and assigned *O. elongata* [16,18] as the type species. At present, there have been 56 nominal species of the genus worldwide (electronic database: http://nemys. ugent.be (accessed on 1 October 2023)). However, only 41 species were confirmed as valid after several revisions by nematologists [19–22]. Shimada et al. provided two dichotomous keys to 36 species of *Oxystomina* in 2020 [22]. The specimens of *O. elegans* [23] and *O. elongata* [16,18] were often found in intertidal to subtidal sediments of the Yellow Sea. *O. longiseta* sp. nov. is the first new species of the genus found in the survey area.

2. Materials and Methods

Sediment samples were collected at twenty sampling points using a 2.9 cm diameter sawn-off syringe from the top sediment layer (0–8 cm deep) in the intertidal zone along the coast of Shandong Peninsula in February and August 2022 (Figure 1). The samples were fixed with an equal amount of 10% formalin solution. At the sorting, the samples were poured through two sieves (500 and 42 µm mesh sizes), and they were washed with tap water to remove silt and separate macrofauna from meiofauna [24,25]. Meiofauna was sorted under a stereoscopic microscope. Nematodes were processed in a solution of glycerol and ethanol [26]. Finally, they were mounted in glycerin on permanent slides. Observation and measurement were carried out using a differential interference contrast microscope (Leica DM 2500) (CMS Gmbh, Wetzlar, Germany and assembled in Shanghai, China) and Leica software of LAS X version 3.3.3. Type specimens were deposited in the Marine Biological Museum of the Chinese Academy of Sciences, Qingdao.

Abbreviations used in the table and keys are as follows: a = body length/max. body diameter; abd = cloacal or anal body diameter; b = body length / pharynx length; c = body length/tail length; c' = tail length/body diameter at cloaca or anus; V% = position of vulva as % of body length from anterior end.



Figure 1. Map of the sampling stations (red triangles refer to sampling stations in February, blue circles refer to sampling stations in August).

3. Results

The mean abundance of nematodes along the coast of Shandong peninsula is 1663 ± 502 individuals 10 cm^{-2} . More than 200 species have been discovered. Among these species, three species belonging to the order Enoplida have been identified as new species and described here as *Belbolla octobulba* sp. nov., *Ironella gracilis* sp. nov. and *Oxystomina longiseta* sp. nov.

3.1. Description of Belbolla octobulba sp. nov. (Figures 2-4)

3.1.1. Type Material

One male and two females were obtained and measured. Holotype male, paratype 1 (female) and paratype 2 (female) are all on slide 22YMDNQ3-2-1.

3.1.2. Etymology

The species epithet comes from the Latin words *octo* (eight) and *bulba* (bulbous), referring to the new species possessing eight pharyngeal bulbs.

3.1.3. Type Locality and Habitat

The holotype and paratypes were all collected from intertidal muddy sediments at the coast of Yangma Island, Yantai $(37^{\circ}26'1'' \text{ N}, 121^{\circ}36'37'' \text{ E})$ (Figure 1 Red 5).

3.1.4. Measurements

All measurement data are given in Table 1.

Channe share	Holotype	Paratype			
Characters	Holotype	្1	୍ୱ 2		
Body length	3185	4076	3853		
Maximum body diameter	96	139	134		
Head diameter	14	18	11		
Length of cephalic setae	10	10	9		
Length of buccal cavity	21	21	17		
Width of buccal cavity	10	11	11		
Nerve ring from anterior end	335	368	341		
Length of pharynx	717	760	740		
Body diameter at pharyngeal base	80	100	91		
Number of pharyngeal bulbs	8	8	8		
Tail length	274	273	248		
Body diameter at cloaca or anus	64	62	62		
Spicule length along arc	124	-	-		
Length of gubernacular apophysis	38	-	-		
Length of anterior supplement	53	-	-		
Length of posterior supplement	42	-	-		
Posterior supplement from cloaca	251	-	-		
Distance between both supplements	185	-	-		
Vulva from anterior end	-	1986	1853		
Body diameter at vulva	-	138	120		
V%	-	49	48		
a	33.0	29.3	28.8		
b	4.4	5.4	5.2		
с	11.6	14.9	15.5		
c′	4.3	4.4	4.0		

Table 1. Individual measurements of *Belbolla octobulba* sp. nov. (in μ m).

3.1.5. Description

Male. Body slender with thin and elongated neck. The front end of the body tapers to a very small diameter. Cuticle smooth with some somatic setae and mainly distributing in cervical and caudal region. Inner labial sensilla papilliform. Six outer labial setae and four cephalic setae situated in the same circle, located approximately one head diameter from the front of the body, each seta 10 μ m long. Amphidial fovea not observed. Buccal cavity barrel-shaped, divided into two parts by a sclerotized transverse ring accompanying some denticles (Figures 2C and 3A). A large right ventro-lateral tooth and two fewer prominent teeth (dorsal and left ventro-lateral in position) present. The anterior ten cervical setae (20–25 μ m long) arranged in one circle (about 40 μ m from head end), while the remaining setae arranged irregularly. The pharynx expanded gradually and modified into eight bulbs (Figures 3B and 4C). The nerve ring situated at 47% pharyngeal length from front end of body. Excretory system not seen. Tail 4.3 cloacal body diameters long, conico-cylindrical with the distal one-fourth cylindrical and some short caudal setae (Figure 3D). Tail tip slightly swollen with a terminal spinneret. Three caudal glands within the tail.

Reproductive system containing two opposed outstretched testes. Paired spicules equal in length, arcuate, broad and even, 124 μ m (1.9 cloacal body diameters) long as arc, 118 μ m long as chord. Gubernaculum with a pair of dorso-caudal apophyses, approximately one third of spicule in length. Two well-developed winged precloacal supplements situated close together relatively far from the cloaca (Figure 3C). The previous one slightly larger than the latter one. The latter supplement situated 251 μ m in front of the cloaca and distance between both supplements is 185 μ m. A total of 1–2 paired short setae just anterior to cloaca.

Females. Body size slightly larger than male, lacking cervical and caudal setae. Tail equipped with two subterminal setae. Reproductive system didelphic, two ovaries opposed, reflexed. Vulva slightly raised (Figure 4D), located at 48–49% of body length from front end of body. Vagina sphincter well developed.

3.1.6. Differential Diagnosis and Discussion

Belbolla octobulba sp. nov. is characterized by having eight pharyngeal bulbs, spicules arcuate, broad and even, gubernaculum with a pair of dorso-caudal apophyses, about a third of the length of spicule, two typical winged precloacal supplements lying close together comparatively far from the cloacal opening, the previous supplement slightly larger than the latter one. At present, seven species in the genus, *Belbolla gallanachmorae* [8,27]; *B. longispiculata* [28]; *B. teissieri* [8,29]; *B. tenuidens* [8,9]; *B. stenocephalum* [10]; *B. sundoensis* [8,30]; and *B. zhangi* [11], have been found to have eight pharyngeal bulbs.

The new species is different from *B. gallanachmorae* by the distance from the posterior supplement to the proximal end of spicule almost equal to the spicule length vs. the distance longer than spicule length and gubernacular apophysis one third of spicule length vs. one fifth to one sixth of spicule length. The new species differs from *B. longispiculata* in having shorter spicules (1.9 vs. 10.8 cloacal body diameters) and different precloacal supplements (two winged vs. five papilliform). The new species differs from *B. teissieri* by longer body length (3.2 mm vs. 1.9 mm), longer spicules (115 µm vs. 39 µm) and shorter tail (4.3 cloacal body diameters with one-fourth posterior cylindrical part vs. 5.7 cloacal body diameters with half posterior cylindrical part). The new species differs from B. tenuidens by shorter length of supplements (0.7–0.8 vs. 1.4–1.5 cloacal body diameters long). The new species differs from B. stenocephalum in larger body size (3.1-4.1 mm in length and 96–139 μ m in maximum width vs. 2.2–2.7 mm and 62–72 μ m, respectively) and different spicules (stout with tapered distal tip vs. slender with swollen distal tip). The new species differs from *B. sundoensis* by distance between posterior supplement and the proximal end of spicule almost equal to the spicule length vs. the distance longer than the spicule length and gubernacular apophysis one third of spicule length vs. one fifth to one sixth of spicule length. The new species differs from *B. zhangi* by longer body length (3.1–4.1 mm vs. 2.2–2.8 mm) and length of gubernacular apophysis shorter than half spicule length vs. length of gubernacular apophysis longer than half spicule length. In addition, the new species differs from *B. sinica* [4] by a different number of pharyngeal bulbs (eight vs. seven), spicules arcuate and uniform vs. straight with proximal and distal ends tapered and middle part thickened, and gubernaculum with apophysis vs. without apophysis.

The distinction between *B. octobulba* sp. nov. and other known species is specified in the key below.



Figure 2. Drawing of *Belbolla octobulba* sp. nov. (**A**) Pharyngeal region of male, showing nerve ring and eight pharyngeal bulbs; (**B**) lateral view of male posterior end, showing two precloacal supplements, spicule, gubernaculum, gland cells and tail; (**C**) anterior end of male, showing buccal cavity with teeth, outer labial and cephalic setae, and cervical setae; (**D**) lateral view of entire female; (**E**) spicule and gubernaculum. Scale bar: (**A**,**B**) = 100 μ m; (**C**) = 20 μ m; (**D**) = 200 μ m; (**E**) = 50 μ m.



Figure 3. Micrograph of *Belbolla octobulba* sp. nov. (**A**) Anterior end of male, showing buccal cavity with teeth, outer labial and cephalic setae, and cervical setae; (**B**) pharyngeal region, showing nerve ring (arrow 1), bulbs and cardia (arrow 2); (**C**) lateral view of male cloacal region, showing two precloacal supplements, spicules and gubernaculum; (**D**) lateral view of male posterior end, showing spicule, gubernaculum and tail. Scale bar: (**A**) = $20 \ \mu$ m; (**B**–**D**) = $50 \ \mu$ m.



Figure 4. Micrograph of *Belbolla octobulba* sp. nov. (**A**,**B**) Anterior end of female, showing buccal cavity with teeth and cephalic setae; (**C**) pharyngeal region of female, showing bulbs; (**D**) lateral view of female middle region, showing vulva (arrow 1) and ovary (arrows); (**E**) lateral view of female posterior end, showing tail and anus. Scale bar: (**A**,**B**) = 20 μ m; (**C**–**E**) = 50 μ m.

- 3.1.7. Updated Key to Species of the Genus Belbolla (Based on Huang and Zhang, 2005) [10]
- 1. Absence of precloacal supplements...B. asupplementata (Juario, 1974) Andrássy, 1973 [8,31]
 - Presence of precloacal supplements . . .2
- 2. Pharynx with four or seven bulbs...3
 - Pharynx with eight, nine or ten bulbs ...7
- 3. Four pharyngeal bulbs; male with two papilliform and two winged precloacal supple ments. . . *B. vietnamica* Gagarin & Nguyen Dinh Tu, 2016 [32]
 - Seven pharyngeal bulbs; male with two precloacal supplements...4
 - Precloacal supplements winged; gubernacular apophysis absent ... 5
 - Precloacal supplements not winged; gubernacular apophysis present...6
- 5. Spicules slender with handle-shaped proximal end, gubernaculum absent. . .*B. heptab-ulba* (Timm, 1961) Andrássy, 1973 [8,33]
 - Spicules broad with tapered proximal end, gubernaculum present...*B. sinica* Wang, Guo & Wang, 2022 [4]
- 6. Tail conico-cylindrical, spicules broad, 1.3–1.5 abd...*B. warwicki* Huang & Zhang, 2005 [10]
 - Tail long, filiform, spicules slender, 2.5 abd. . . B. gracilis Gagarin & Thanh, 2016 [34]
- 7. Pharynx with eight bulbs...8

4.

- Pharynx with nine or ten bulbs...15
- 8. Spicules 10.8 abd long with knob-like proximal end; five papilliform supplements...*B. longispiculata* Nasira, Shahina & Shamim, 2014 [28]
 - Spicules shorter than 2 abd; two precloacal supplements...9
- 9. Gubernacular apophysis longer than half spicule length ... 10
 - Gubernacular apophysis shorter than half spicule length ...11
- 10. Precloacal supplements 1.4–1.5 abd long...B. tenuidens (Cobb, 1920) Andrássy, 1973 [5,8]
 - Precloacal supplements 0.5–0.9 abd long, eight or nine pharyngeal bulbs...*B. zhangi* Guo & Warwick, 2001 [11]
- 11. Spicule with swollen distal tip ... B. stenocephalum Huang & Zhang, 2005 [10]
 - Spicule with tapered distal tip ...12
- 12. Distance between posterior supplement and proximal end of spicule equal or shorter than spicule length; gubernacular apophysis about one third of spicule length...13
 - Distance between posterior supplement and proximal end of spicule longer than spicule length; gubernacular apophysis one fifth to one sixth of spicule length...14
- 13. Body length 1.9 mm, spicules 39 μm, tail 5.7 abd with half posterior cylindrical part. . .*B. teissieri* (Luc. et de Conink, 1959) Andrássy, 1973 [8,29]
 - Body length 3.2 mm, spicules 115 μm, tail 4.3 abd with one-fourth posterior cylindrical part...*B. octobulba* sp. nov.
- 14. The wings of supplement almost equal to the diameter of its central part. . . *B. sundoensis* (Micoletzky, 1930) Andrássy, 1973 [8,30]
 - The wings of supplement twice the diameter of its central part . . . *B. gallanachmorae* (Inglis, 1961) Andrássy, 1973 [8,27]
- 15. Spicule with a hook at the distal end...B. intarma Belogurov & Belogurova, 1980 [35]
 - Spicule without hook at the distal end...16
- 16. Gubernacular apophysis shorter than $25 \ \mu m...17$
 - Gubernacular apophysis longer than 40 μm...18

- Female having ten pharyngeal bulbs. . .B. californica (Allgén, 1951) Andrássy, 1973 [8,36]
 Female having nine pharyngeal bulbs. . .B. wonkimi Rho, Lee, Lee & Min, 2020 [37]
- 18. Male having ten pharyngeal bulbs, a = 19.8...*B. insula* Belogurov, Fadeeva & Belogurova, 1983 [38]
 - Male having nine pharyngeal bulbs, a = 34.5–41.5...*B. huanghaiensis* Huang & Zhang, 2005 [10]

3.2. Description of Ironella gracilis sp. nov. (Figures 5–7)

3.2.1. Type Material

Four males and two females were acquired. Holotype male is on slide JM24(4-8). Paratypes male 2, male 3, female 1 and female 2 are successively on slides JM252(4-8), JM149(4-8), JM6(4-8) and JM665(4-8).

3.2.2. Etymology

The species is named after its relatively thin and slender body.

3.2.3. Type Locality and Habitat

Holotype and paratypes were all collected from intertidal fine sandy sediments at the Jimo coast of Qingdao along the Yellow Sea (120°41′31″ E, 36°22′32″ N) (Figure 1 Red 10).

3.2.4. Measurements

All measurement data are given in Table 2.

Table 2. Individual measurements of *Ironella gracilis* sp. nov. (in μ m).

Chausataus	Holotype –	Paratypes				
Characters		1്	്2	Q1	₽2	
Total body length	1469	1357	1244	1623	1513	
Maximum body diameter	19	18	19	27	26	
Head diameter	14	14	14	18	18	
Length of inner labial setae	3	3	3	2.5	2.5	
Length of outer labial setae	9	9	9	7	8	
Length of cephalic setae	9	9	10	9	9	
Distance between outer labial setae and cephalic seta	7	8	6	6	6	
Depth of buccal cavity	26	29	26	34	33	
Width of buccal cavity	4	5	4	7	7	
Nerve ring from anterior end	106	103	93	113	117	
Length of pharynx	316	304	284	326	329	
Body diameter at pharyngeal base	17	17	19	25	25	
Length of cardia	7	7	7	10	10	
Spicule length along arc	61	57	57	-	-	
Length of gubernaculum	7	7	7	-	-	
Vulva from anterior end	-	-	-	825	797	
V%	-	-	-	50.8	52.7	
Body diameter at cloaca or anus	16	15	15	17	16	
Tail length	99	96	85	110	122	
a	77.3	75.4	65.5	60.1	58.2	
b	4.6	4.5	4.4	5	4.6	
С	14.8	14.1	14.6	14.8	12.4	
c′	6.2	6.4	5.7	6.5	7.6	

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3.2.5. Description

Males. Body slender. Cuticle smooth. No somatic setae. Anterior end slightly widened. Buccal cavity large, divided into conical vestibulum and cylindrical posterior portion, 26–29 μ m in depth and 4–5 μ m in width (Figures 5A and 6A). Three sclerotized teeth situated at the vestibulum. Anterior sensilla in three circles. Six inner labial setae 3 μ m long; all the outer labial setae and cephalic setae 9 μ m long (i.e., 64% head diameter). Both circles of outer labial setae and cephalic setae 10 μ m apart. Amphid not seen. Pharynx expanded around buccal cavity and forming an anterior bulb, then narrow after the buccal cavity, cylindrical and slightly swelling at its base, not forming posterior bulb. Cardia small, conical. Excretory pore situated at the level of outer labial setae. Tail clavate, 5.7–6.4 cloacal body diameters in length, with half proximal conical and half distal cylindrical. Tail tip slightly swelling without terminal seta. Caudal glands precloacal.

Testis difficult to identify. Spicules 57–61 μ m long (about 3.8 cloacal diameters), weakly arched with central strip, proximal end cephalate and distal end tapered (Figure 6C). Gubernaculum conical, 7 μ m long, surrounding spicules distally, without apophyses. A tubular precloacal supplement 8 μ m long, 9 μ m in front of cloaca, with a bended seta at its anterior end, 8 μ m in length (Figure 6D).

Females closely resemble males in most characteristics, except the body and the buccal cavity slightly large. Reproductive system didelphic, two ovaries reflexed and opposed; the anterior ovary somewhat shorter than the posterior one. Both anterior and posterior ovaries located to the left side of the intestine. Vulva raised, located in the mid-body (Figure 7C).

3.2.6. Differential Diagnosis and Discussion

Ironella gracilis sp. nov. is characterized by body slender, buccal cavity divided into conical vestibulum and cylindrical posterior portion with three sclerotized teeth in the vestibulum, both the circles of outer labial setae and cephalic setae 10 μ m apart, outer labial setae and cephalic setae 9 μ m long, spicules elongated, weakly arched, with central strip and cephalate proximally, gubernaculum conical without apophysis, a tubular precloacal supplement just in front of cloaca and with a long thick seta at its front end.

I. gracilis sp. nov. is most similar to *I. riemanni* [14] in body size. However, it differs from the latter species by body slender (demanian value a = 65.5-77.3 vs. 34.7–50.7 in males and 58.2–60.1 vs. 32.5–35 in females), vestibulum separated and with sclerotized walls (vs. vestibulum not separated and not sclerotized), tubular precloacal supplement large and close to cloaca (vs. small and far from cloaca, 20 µm in front of cloaca). The new species is similar to *I. cobbi* [13] in having vestibulum separated and with sclerotized walls, but differs from *I. cobbi* by slender body (a = 58.2-60.1 vs. 34 in females), both circles of outer labial setae and cephalic setae far apart (distance between the two circles of setae equal to 1/3 of the corresponding diameter vs. 1/6 of the corresponding diameter in the latter species). From the identification key below, the distinctions between *I. gracilis* sp. nov. and its congeners can be deduced.

3.2.7. Identification Key to Species of Ironella (Updated from Platonova & Mokievsky [14])

- 1. Lips twisted at anterior ends into horizontal tubes, microodontia present. . .*I. prismato laima* Cobb, 1920 [5]
 - Lips not twisted, microodontia absent...2
- 2. Distance between the circles of outer labial setae and cephalic setae equal to 1/6 cbd... *I. cobbi* Timm, 1954 [13]
 - Distance between the circles of cephalic setae equal to 1/3 cbd...3
- 3. Vestibulum separated and with sclerotized walls...*I. gracilis* sp. nov.
 - Vestibulum not separated and not sclerotized.... I. riemanni Platonova & Mokievsky, 1994 [14]



Figure 5. Drawing of *Ironella gracilis* sp. nov. (**A**) Anterior end of male, showing inner labial setae (1), outer labial setae (2), cephalic setae (3), buccal cavity, teeth and anterior bulb (arrow); (**B**) entire female; (**C**) entire male; (**D**) cloacal region of male, showing spicule, gubernaculum (arrow) and precloacal supplement; (**E**) posterior end of male, showing tail. Scale bar: (**A**,**D**,**E**) = 20 μ m; (**B**,**C**) = 50 μ m.



Figure 6. Microscopic images of *Ironella gracilis* sp. nov. (**A**) Anterior end of male, showing outer labial setae (arrow 1), cephalic setae (arrow 2) and buccal cavity; (**B**) anterior end of male, showing buccal cavity teeth; (**C**) lateral view of male posterior end, showing spicules and tail (broken); (**D**) lateral view of male posterior end, showing spicules, gubernaculum (arrow 1) and precloacal supplement with seta (arrow 2). Scale bar: (**A**–**D**) = 10 μ m.



Figure 7. Microscopic images of *Ironella gracilis* sp. nov. (**A**) Anterior end of female, showing buccal cavity and anterior setae; (**B**) anterior end of female, showing buccal cavity, tooth and anterior bulb; (**C**) lateral view of female middle region, showing vulva (arrow), ovary and eggs; (**D**) posterior end of female, showing anus (arrow) and tail. Scale bars: (**A**,**B**) = 10 µm; (**C**) = 30 µm; (**D**) = 20 µm.

3.3. Description of Oxystomina longiseta sp. nov. (Figures 8–10)3.3.1. Type Material

Three males and three females were acquired. Holotype male, paratype 1 (male) and paratype 3 (female) are on slide 22WHRLW2-8-12; paratype 2 (male) and paratype 4 (female)

are on slide 22WHRLW2-8-13; and paratype 5 (female) is on slide 22WHRLW2-8-11.



Figure 8. Drawing of *Oxystomina longiseta* sp. nov. (**A**) Anterior end of male, showing outer labial setae, cephalic setae, amphid (arrow 1) and epidermal gland cells (arrow 2); (**B**) anterior end of female, (**C**) entire male; (**D**) posterior end of male, showing short and long precloacal seta, spicule and epidermal gland cells (arrow); (**E**) magnifying spicule and gubernaculum; (**F**) entire female. Scale bar: (**A**,**B**,**E**) = 10 μ m; (**C**,**F**) = 50 μ m; (**D**) = 20 μ m.



Figure 9. Micrograph of *Oxystomina longiseta* sp. nov. (A–C) Lateral view of male anterior end, showing outer labial setae (1), cephalic setae (2) and amphid; (D) lateral view of male posterior end, showing long precloacal seta, spicule, gubernaculum (arrow 1) and tail; (E) lateral view of male cloacal region, showing spicules, longer (arrow 1) and shorter (arrow 2) precloacal setae; (F) magnifying spicule. Scale bar: (A–F) = $10 \mu m$.



Figure 10. Micrograph of *Oxystomina longiseta* sp. nov. (**A**) Lateral view of female anterior end, showing outer labial setae, cephalic setae and amphid (arrow); (**B**) lateral view of female middle region, showing vulva and vagina (arrow), ovary (arrow 1) and degraded anterior uterus (arrow 2). Scale bar: (**A**) = 10 μ m; (**B**) = 20 μ m.

3.3.2. Etymology

The species epithet is derived from the Latin words *longus* (long) and *seta* (seta), referring to the new species possessing very long precloacal seta.

3.3.3. Type Locality and Habitat

Holotype and paratypes were all collected from intertidal fine sandy sediments at the Riluowan Bay of Weihai (37°31′11″ N, 121°1′8″ E) (Figure 1 Blue 7).

3.3.4. Measurements

All measurement data are given in Table 3.

Table 3. Individual measurements of *Oxystomina longiseta* sp. nov. (in μ m).

Characters	Holotype	Paratypes					
		്1	₫1	♀1	₽2	♀3	
Total body length	2387	2221	2205	2019	2349	2540	
Maximum body diameter	20	21	21	19	23	23	
Head diameter	4	4	4	4	4	4	
Length of outer labial setae	13	16	17	13	14	14	
Length of cephalic setae	14	15	14	14	13	13	
Distance between two circles of setae	11	11	11	10	11	11	
Length of amphidial fovea	6	6	8	8	8	9	
Width of amphidial fovea	5	4	4	4.5	4	4	
Amphid from anterior end	30	29	32	38	32	31	
Body diameter at amphid level	9	8	8	9	9	8	
Length of pharynx	486	532	460	476	503	562	
Body diameter at pharyngeal base	19	19	20	18	20	19	
Spicule length along arc	26	27	26	-	-	-	
Length of precloacal setae	35/8	41/11	38/8	-	-	-	

Characters	Holotype	Paratypes				
		₫1	്1	♀1	♀ 2	₽3
From precloacal setae to cloaca	19	18	18	-	-	-
Body diameter at cloaca or anus	19	19	18	18	18	17
Tail length	90	86	85	80	85	82
Vulva from anterior end	-	-	-	1019	1200	1305
Body diameter at vulva	-	-	-	20	24	24
y %	-	-	-	50.5	51.1	51.4
a	119	106	105	106	102	111
b	4.9	4.2	4.8	4.2	4.7	4.5
С	26.5	25.8	25.9	25.2	27.6	31.0
c′	4.7	4.5	4.7	4.4	4.7	4.8

Table 3. Cont.

3.3.5. Description

Males. Body slender, elongated and very thin at the anterior end. Cuticle smooth. Epidermal gland cells scattering throughout the body. Inner labial sensilla papilliform and difficult to observe. Six outer labial setae 13–17 μ m long. Four cephalic setae 14–15 μ m long, located at 11 μ m (about three times of head diameter) from front end of body. Amphidial fovea longitudinal oval (Figure 8A,B and Figure 9A–C), 5–6 μ m long and 4–5 μ m wide, located at 29–31 μ m from front end of body. Buccal cavity minute. Pharynx slender with an expanded base. Nerve ring difficult to distinguish. No excretory pore and ventral cell observed. Cardia conical. Tail conico-cylindrical with a clavate distal part, 4.5–4.7 cloacal body diameters long, without terminal setae. Caudal glands extend into the precloacal region.

Reproductive system monorchic with an anterior outstretched testis. Spicules arcuate, 26–27 μ m long, enlarged proximal end with a median rib, distal end tapered. Gubernaculum small, oblong, 4 μ m long. One short and one long precloacal supplementary setae in front of cloaca; the anterior one thin and short, 8–11 μ m long; the posterior one thick and very long, 35–41 μ m long; located at the level of proximal end of spicule, or 18–19 μ m in front of cloaca opening.

Females. Similar to males in most respects, but the body slightly thick. Reproductive system monodelphic, single posterior reflexed ovary. Vulva raised, situated at mid-body. Vagina straight, trumpeted, encircled by sphincter muscle. Uterus a wide and short tube. The degraded anterior uterus present (Figure 10B).

3.3.6. Differential Diagnosis and Discussion

Oxystomina longiseta sp. nov. can be easily identified by longer outer labial and cephalic setae within the genus; spicules with enlarged proximal end and a median rib; gubernaculum small and oblong; two unequal precloacal supplementary setae, anterior one thin and short, posterior one thick and very long.

The new species resembles *O. alpatovi* [19,39], *O. affinis* [40] and *O. elongate* [16,18] in having two unequal precloacal setae, but it differs from *O. alpatovi* by longer anterior sensory setae (longer than three head diameters vs. shorter than one head diameter) and much longer precloacal setae (the longer one 35–41 μ m vs. 18 μ m long), proximal end of spicule without median rib in the latter species. The new species differs from *O. affinis* in having very slender body with shorter tail (a = 102–111 vs. 56 in the latter species; c = 25.2–31 vs. 14); the latter species possessing very short anterior sensory setae and precloacal setae and proximal end of spicules not enlarged. The new species distinguishes from *O. elongate* by very slender body (a = 102.1–119.4 vs. 47–56), longer outer labial and cephalic setae (vs. 2.5 μ m long), and much longer precloacal setae (the longer one 35–41 μ m vs. about 10 μ m long); spicule enlarged proximally with a median rib and tapered distally (vs. spicule with a slight bump ventrally at the proximal tip without median rib and rounded distally), gubernaculum oblong (vs. curved enclosing spicule tips). Relatively long anterior sensory setae and very long precloacal seta can be used to distinguish the new species from its similar congeners.

4. Conclusions and Discussion

Three new species, *Belbolla octobulba* sp. nov., *Ironella gracilis* sp. nov. and *Oxystomina longiseta* sp. nov., were discovered in the intertidal zone along the Shandong peninsula coast of the Yellow Sea. The genus *Belbolla* is common in the Yellow Sea, and five species within the genus were found, namely, *B. huanghaiensis* [10]; *B. sinica* [4]; *B. stenocephalum* [11]; *B. warwicki* [10]; and *B. zhangi* [11]. *B. octobulba* sp. nov. is the second new species after *B. sinica* found in the intertidal zone of the Yellow Sea. The new species can be easily identified from its congeners by eight pharyngeal bulbs, arcuate spicules broad and even, gubernacular apophyses one third of the length of spicule and two winged precloacal supplements. Even though only one male specimen was found for the identification, it was still considered as a new species due to its distinctive characteristic. *Ironella* is a rare genus, containing only three known species worldwide. *Ironella gracilis* sp. nov. is the first species found in the Chinese sea area. *Oxystomina* is a common genus in the Yellow Sea. The specimens of *O. elegans* [23] and *O. elongata* [16,18] were often found. *O. longiseta* sp. nov. is the first new species of the genus found in the Yellow Sea.

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References

- 1. Heip, C.; Vincx, M.; Vranken, G. The ecology of marine nematodes. *Oceanogr. Mar. Biol.* 1985, 23, 399–489.
- Hodda, M. Phylum Nematoda: A classification, catalogue and index of valid genera, with a census of valid species. Zootaxa 2022, 5114, 1–289. [CrossRef] [PubMed]
- Chu, M.D.; Hao, Y.D.; Huang, Y. Descriptions of *Leptolaimus sinensis* sp. nov. and *Perspiria brevicaudata* sp. nov. (Nematoda) from the Yellow Sea, China. *Zootaxa* 2023, 5258, 524–536. [CrossRef] [PubMed]
- 4. Wang, M.N.; Guo, W.; Wang, C.M. Two new species, *Parabathylaimus gracilis* sp. nov. and *Belbolla sinica* sp. nov. (Nematoda: Enoplida), from Yangma Island of the Yellow Sea, China. *Zootaxa* **2022**, *5200*, 344–354. [CrossRef] [PubMed]
- 5. Cobb, N.A. One hundred new nemas (type species of 100 new genera). Contrib. A Sci. Nematol. 1920, 9, 217–343.
- Leduc, D.; Zhao, Z.Q.; Verdon, V.; Xu, Y. Phylogenetic position of the enigmatic deep-sea nematode order Rhaptothyreida: A molecular analysis. *Mol. Phylogenetics Evol.* 2018, 122, 29–36.
- Leduc, D.; Fu, S.J.; 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]
- Andrássy, I. Über vier homonyme Nematodengattungen. Nematologica 1973, 19, 403–404.
- 9. Filipjev, I.N. Free-living marine Nematodes of the Sevastopol Area. Pt. I. *Trudy Osoboi Zoologicheskoi Laboratorii Sebastopolskoi Stantsii Rossiikoi* 1918, 4, 1–614.
- 10. Huang, Y.; Zhang, Z.N. Three new species of the genus *Belbolla* (Nematoda: Enoplida) from the Yellow Sea, China. *J. Nat. Hist.* **2005**, *39*, 1689–1703. [CrossRef]
- 11. Guo, Y.Q.; Warwick, R.M. Three new species of free-living nematodes from the Bohai Sea, China. J. Nat. Hist. 2001, 35, 1575–1586. [CrossRef]
- 12. De Man, J.G. Onderzoekingen over vrij in de aarde levende Nematoden. Tijdschr. Ned. Dierk. Vereen. 1876, 2, 78–196.
- 13. Timm, R.W. A survey of the marine nematodes of Chesapeake Bay, Maryland. Cathol. Univ. Am. Biol. Stud. 1954, 23, 70.
- 14. Platonova, T.A.; Mokievsky, V.O. Revision of the marine nematodes of the family Ironidae (Nematoda: Enoplida). Zool. Inst. St. Petersburg Zoosyst. Ross. **1994**, *3*, 5–17.

- 15. Riemann, F. Die interstitielle Fauna im Elbe-Aestuar. Verbreitung und Systematik. Arch. Hydrobiol. 1966, 1, 1–279.
- 16. Filipjev, I.N. Free-living marine Nematodes of the Sevastopol area. Trudy Osob. Zool. Lab. Sebastop. Biol. Sta. 1921, 2, 351-614.
- 17. Chitwood, B.G. Nomenclatorial notes, l. Proc. Helminth. Soc. Wash. 1935, 2, 51-54.
- Bütschli, O. Zur Kenntnis der freilebenden Nematoden, insbesondere der des Kieler Hafens. Abh. Senck. Naturf Gesel. 1874, 9, 1–56.
- 19. Wieser, W. Reports of the Lund University Chile expedition 1948–1949: 1. Enoploidea. Acta Univ. Lund 1953, 49, 1–155.
- 20. Gerlach, S.A. Diagnosen neuer Nematoden aus der Kieler Bucht. Kieler Meeresforsch. 1956, 12, 85–109.
- Lorenzen, S. Entwurf eines phylogenetischen Systems der freilebenden Nematoden. Verött Inst. Meeresforsch. Bremerh. 1981, 7, 1–472.
- 22. Shimada, D.; Takeda, N.; Tsune, A.; Murakami, C. Three new species of free-living marine nematodes (Nematoda: Enoplida) from the Clarion-Clipperton Fracture Zone (CCFZ), North Pacific. *Zootaxa* **2020**, *4859*, 507–526. [CrossRef] [PubMed]
- 23. Platonova, T.A. Exploration of the fauna of the seas VIII (XVI). Fauna and flora of the Possjet Bay of the Sea of Japan. *Zool. Inst. Acad. Sci. USSR* **1971**, *8*, 72–108.
- 24. de Jonge, V.N.; Bouwman, L.A. A simple density separation technique for quantitative isolation of meiobenthos using the colloidal silica Ludox-TM. *Mar. Biol.* **1977**, *42*, 143–148. [CrossRef]
- 25. Chen, Y.Z.; Zhou, R.G.; Zhu, H.L.; Guo, Y.Q. Two new species of free-living marine nematode of the genus *Anticyathus* Cobb, 1920 (Linhomoeidae) from Mangroves Sediment of Shenzhen and Shantou, China. J. Mar. Sci. Eng. 2022, 10, 1107. [CrossRef]
- McIntyre, A.D.; Warwick, R.M. Meiofauna techniques. In *Methods for the Study of Marine Benthos*; Holme, N.A., McIntyre, A.D., Eds.; Blackwell Scientific Publications: Oxford, UK, 1984; pp. 217–244.
- 27. Inglis, W.G. Two new species of free-living marine nematodes from the west coast of Scotland. *Hydrobiologia* **1961**, *18*, 284–292. [CrossRef]
- Nasira, K.; Shahina, F.; Shamim, S. Descriptions of *Bathyeurystomina minima* sp. n. and *Belbolla longispiculata* sp. n. with observations on Pareurystomina vaughtae and Eurystomina indica (Enoplida: Enchelidiidae) from Pakistan. *Int. J. Nematol.* 2014, 24, 87–96.
- 29. Luc, M.; De Coninck, L.A. Nématodes libres marins de la région de Roscoff. Archs Zool. Exp. Gén. 1959, 98, 103–165.
- Micoletzky, H. Freilebende marine Nematoden von den Sunda-Inseln. I. Enoplidae. (Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16 53) (edited by H. A. KREIS). *Vidensk. Meddr Dansk Naturh. Foren.* 1930, 87, 243–339.
- 31. Juario, J.V. Neue freilebende Nematoden aus dem Sublitoral der Deutschen Bucht. *Veröff. Inst. Meeresforsch. Bremerh.* **1974**, *14*, 275–303.
- 32. Gagarin, V.G.; Nguyen, D.T. *Adoncholaimus minor* sp. n. and *Belbolla vietnamica* sp. n. (Nematoda, Enoplida) from mangrove forest of the Yen River Estuary in Vietnam. *Int. J. Nematol.* **2016**, *26*, 1–8.
- 33. Timm, R.W. The Marine Nematodes of the Bay of Bengal. Proc. Pak. Acad. Sci. 1961, 1, 25–88.
- 34. Gagarin, V.G.; Thanh, N.V. Two new nematode species (Nematoda) from the mangroves of the Yen River Delta, Vietnam. *Inland Water Biol.* **2016**, *9*, 39–47. [CrossRef]
- 35. Belogurov, O.I.; Belogurova, L.S. Morphology of *Belbolla intarma* sp. n., diagnosis and a table for the species of the genus *Belbolla*. *Biologiya Morya* **1980**, *4*, 74–77. (In Russian)
- Allgén, C.A. Pacific Freeliving Marine Nematodes. (Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16. LXXVI). Vidensk. Meddr. Fra Dansk Naturh. Foren. 1951, 113, 263–411.
- Rho, H.S.; Lee, H.; Lee, H.J.; Min, W. A new free-living marine nematode species of the genus *Belbolla* (Enoplida, Enchelidiidae) from a subtidal zone of the East Sea, Korea, with some ecological and biogeographical information. *Environ. Biol. Res.* 2020, 38, 578–585. [CrossRef]
- 38. Belogurov, O.I.; Fadeeva, L.S.; Belogurova, L.S. Studies of Nematodes of the Subfamily Eurystomininae (Enoplida, Enchelidiidae) from the Far East Seas of the USSR. *Zool. Zh.* **1983**, *62*, 15–24.
- Filipjev, I.N. Les Nématodes libres des mers septentrionales appartenant a la famille des Enoplidae. Arch. Naturgesch. 1927, 91, 1–216.
- 40. Gerlach, S.A. Brasilianische Meeres-Nematoden I. Bol. Inst. Ocean. São Paulo 1956, 5, 3–69.

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