

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

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Received: 12 November 2020

Revised: 17 November 2020

Revision accepted: 17 November 2020

Abstract: A new free-living marine nematode species of the genus *Belbolla* Andrassy, 1973 belonging to the family Enchelidiidae is described based on specimens collected from the sediment of a subtidal benthic environmental habitat in the East Sea, Korea. *Belbolla wonkimi* sp. nov. differs from its congeners by the combination of the following characteristics: a relatively long body (3263–3396 μm), the absence of ocelli, nine oesophageal bulbs in the posterior pharynx, well-developed two winged precloacal supplements, longer spicule length (115–130 μm , 1.6–1.8 anal body diameter long), and a triangle-shaped shorter gubernacular apophysis (17–18 μm). Comparative tables on the biogeographical and morphological characteristics of *Belbolla* species are presented. A DIC (differential interference contrast) photomicrograph of the new species is also provided. This is the first taxonomic report on the genus *Belbolla* from Korean waters.

Keywords: marine nematodes, morphology, free-living, DIC photomicrographs, taxonomy

INTRODUCTION

The genus *Belbolla* Andrassy, 1973 is characterized by the presence or absence of ocelli, presence of a large and narrow buccal cavity divided into two parts by a sclerotized transverse band, the presence of three teeth, of which one ventrosubdorsal is the largest, and the presence of a series of bulb-like muscular oesophageal swellings (4 to 10) in the posterior section of pharynx, which is the most important diagnostic characteristic of the genus within the family Enchelidiidae Filipjev, 1928 (Smol *et al.* 2014). So far, the genus *Belbolla* contains 15 valid species found from various marine habitats (Huang and Zhang 2005; Nasira *et al.* 2014; Gagarin and Thanh 2016; Gagarin and Tu 2016). Of these, four species of the genus *Belbolla* have been previously described around the Korean waters, i.e. *B. zhang*

Guo and Warwick, 2001 from the Bohai Sea, China and *B. huanghaiensis* Huang and Zhang, 2005, *B. stenocephalum* Huang and Zhang, 2005 and *B. warwicki* Huang and Zhang, 2005 from the Yellow Sea, China.

During a continuous ecological investigation on the biodiversity of the free-living marine nematofauna around the East Sea, Korea, one *Belbolla* nematode species was obtained from the washings of coarse sediments from a shallow subtidal benthic interstitial environment. Present paper deals with a new species of the genus *Belbolla* based on morphological study using a differential interference contrast (DIC) microscope. The new species is described and illustrated herein as *Belbolla wonkimi* sp. nov., and we discuss its taxonomic relationships with other valid species of the genus. This is the first taxonomic report of the genus *Belbolla* from the East Sea, Korea.

MATERIALS AND METHODS

Sampling of specimens

The marine nematodes were obtained from the washings of subtidal sediments, which were collected at a depth of 104 m using Smith McIntyre grab (OSIL, UK) at Gangneung on the eastern coast of Korea.

Sample processing and preparation of specimens

Meiobenthos were roughly separated from the sediments by decantation methods using a 67 µm mesh sieve in the survey field after freshwater rinsing for less than a few minutes to reduce the attachment from the sediments (Kristensen 1989), and then fixed in 4% neutralized formalin in sea water. After the initial sampling, meiobenthos were extracted once again from the rough samples using the Ludox flotation method (Burgess 2001) in the laboratory, and the concentrated samples were subsequently fixed in 4% neutralized formalin solution. The marine nematodes were picked up under a high magnification of dissecting microscope (Leica M205 C; Leica Microsystems, Germany). Specimens for morphological observation under a light microscope were dehydrated through a graded series of glycerin according to Seinhorst (1959) and mounted on a HS slide (Shirayama *et al.* 1993). The mounted nematodes were examined and photographed using an Olympus BX53 microscope equipped with an Olympus DP26 digital camera (Olympus, Japan). All drawings and measurements were made with the aid of a camera lucida.

Terminology and abbreviations

The terminology used for the description and measurements was followed by Huang and Zhang (2005). Abbreviations used in the text are as follows: a = body length divided by maximum body diameter; a.b.d. = anal body diameter; b = body length divided by oesophagus length; c = body length divided by tail length; c.d. = corresponding body diameter.

SYSTEMATIC ACCOUNTS

Phylum Nematoda Potts, 1932
Class Enoplea Inglis, 1983
Order Enoplida Filipjev, 1929

Family Enchelidiidae Filipjev, 1918

Genus *Belbolla* Andrassy, 1973

Type species

Belbolla tenuidens (Cobb, 1920) Andrassy, 1973

Valid species of the genus *Belbolla*

B. asupplementata Juario, 1974

B. californica (Allgén, 1951) Andrassy, 1973

B. gallanachmorae (Inglis, 1961) Andrassy, 1973

B. gracilis Gagarin and Thanh, 2016

B. heptabulba (Timm, 1961) Andrassy, 1973

B. huanghaiensis Huang and Zhang, 2005

B. intarma Belogurov and Belogurova, 1980

B. longispiculata Nasira, Shahina and Shamin, 2014

B. stenocephalum Huang and Zhang, 2005

B. sundaensis (Micoletzky, 1930) Andrassy, 1973

B. teissieri (Luc and Coninck, 1959) Andrassy, 1973

B. tenuidens (Cobb, 1920) Andrassy, 1973

B. vietnamica Gagarin and Tu, 2016

B. warwicki Huang and Zhang, 2005

B. zhangii Guo and Warwick, 2001

Belbolla wonkimi sp. nov. (Figs. 1, 2; Table 1)

Type material. Holotype male (NA00156498), in glycerin on HS slide, from Sacheon-myeon, 17 April 2018 (*leg.* H Lee, HG Kim and HJ Lee) was deposited in the nematode collection at the specimen conservation room of the Marine Biodiversity Institute of Korea (MABIK), Seochun, Korea. Two paratype males (KIOST NEM-1-2341, KIOST NEM-1-2342), mounted on HS slides, was deposited in the nematode collection at the specimen conservation room of the Bio-Resources Bank of Marine Nematodes (BRBNM), East Sea Research Institute, Korea Institute of Ocean Science & Technology (KIOST), Korea.

Etymology. The proposed specific name *wonkimi* is named after Prof. Won Kim of the School of Biological Sciences at Seoul National University, in recognition of his excellent mentoring during the first author's PhD research course (H. S. Rho).

Type locality. Subtidal zone of Sacheon-myeon (37° 44'16"N, 128° 59'05"E), Gangneung-si, Gangwon-do, Republic of Korea.

Habitat. Subtidal sediments with tiny shell gravels and detritus collected at a depth of 104 m.

Measurements. See Table 1 for measurements and morphometrics.

Table 1. Morphometrics of *Belbolla wonkimi* sp. nov. All measurements are in μm (a = body length divided by maximum body diameter; b = body length divided by oesophagus length; c = body length divided by tail length)

Characters	Holotype male	Paratype males	
		1	2
Total body length	3,328	3,263	3,396
Head diameter	15	15	15
Buccal cavity length	17	17	16
Buccal cavity width	10	11	11
Cephalic setae	11	10	10
Length of cervical setae	25	24	24
Nerve ring from the anterior end of body	374	383	365
Nerve ring c.d.	72	72	70
Oesophagus length	796	832	751
Oesophagus c.d.	91	89	88
Length of bulbed part of oesophagus	395	409	343
Maximum body diameter	101	97	97
Spicule length as arc	115	122	130
Length of gubernacular apophysis	18	18	17
Anal body diameter	70	75	72
Tail length	299	275	265
Tail length/a.b.d.	4.3	3.7	3.7
Length of the wing of the posterior precloacal supplement	19	19	24
Length of middle part of the posterior precloacal supplement	14	15	15
Total length of the posterior precloacal supplement	47	48	59
Length of the wing of the anterior precloacal supplement	21	18	24
Length of middle part of the anterior precloacal supplement	15	15	15
Total length of the anterior precloacal supplement	52	49	57
Distance of the posterior precloacal supplement from cloacal opening	350	351	385
Distance between precloacal supplements	193	193	189
a	33	34	35
b	4	4	5
c	11	12	13

Diagnosis. Body relatively long (3,263–3,396 μm in males); ocelli absent; nine well-developed oesophageal bulbs in the posterior section of pharynx; well developed two winged precloacal supplements; triangle-shaped gubernacular apophysis short and thick.

Description. Male (Holotype). Body comparatively long (3,328 μm), slender; anterior body region outstretched anteriorly and strongly tapered anteriorly from mid oesophagus to cephalic region; posterior body region often slightly curved ventrally in fixed specimen (Figs. 1C, 2A). Head diameter 15 μm distinctly narrowed. Body at level of end of posterior pharyngeal region 6.1 times as wide as head region width. Body cuticle, smooth, no lateral differentiation. Anterior parts of head around cephalic setae,

separated with slight constriction (Figs. 1A, 2B). Lip region not extruded in fixed specimen. Six short, thin labial setae on lip region arranged in one circle. Inner labial setae not observed. Ten cephalic setae (11 μm long) inserted at level of amphideal fovea and arranged in one circle. Maximum body width 101 μm . Buccal cavity wide (10 μm) with thick wall 1.7 times as long (17 μm) as wide. Buccal cavity with one large right dorsolateral tooth and two less prominent teeth, which are composed of dorsal and left ventrolateral in position (Fig. 1A). Buccal cavity large and wide and separated into two buccal chambers by a cuticular ring, which is smooth with no denticles. Amphideal fovea located at level of stoma. Ocelli absent. Several cervical setae, 25 μm in length. Oesophagus muscular and expands gradually

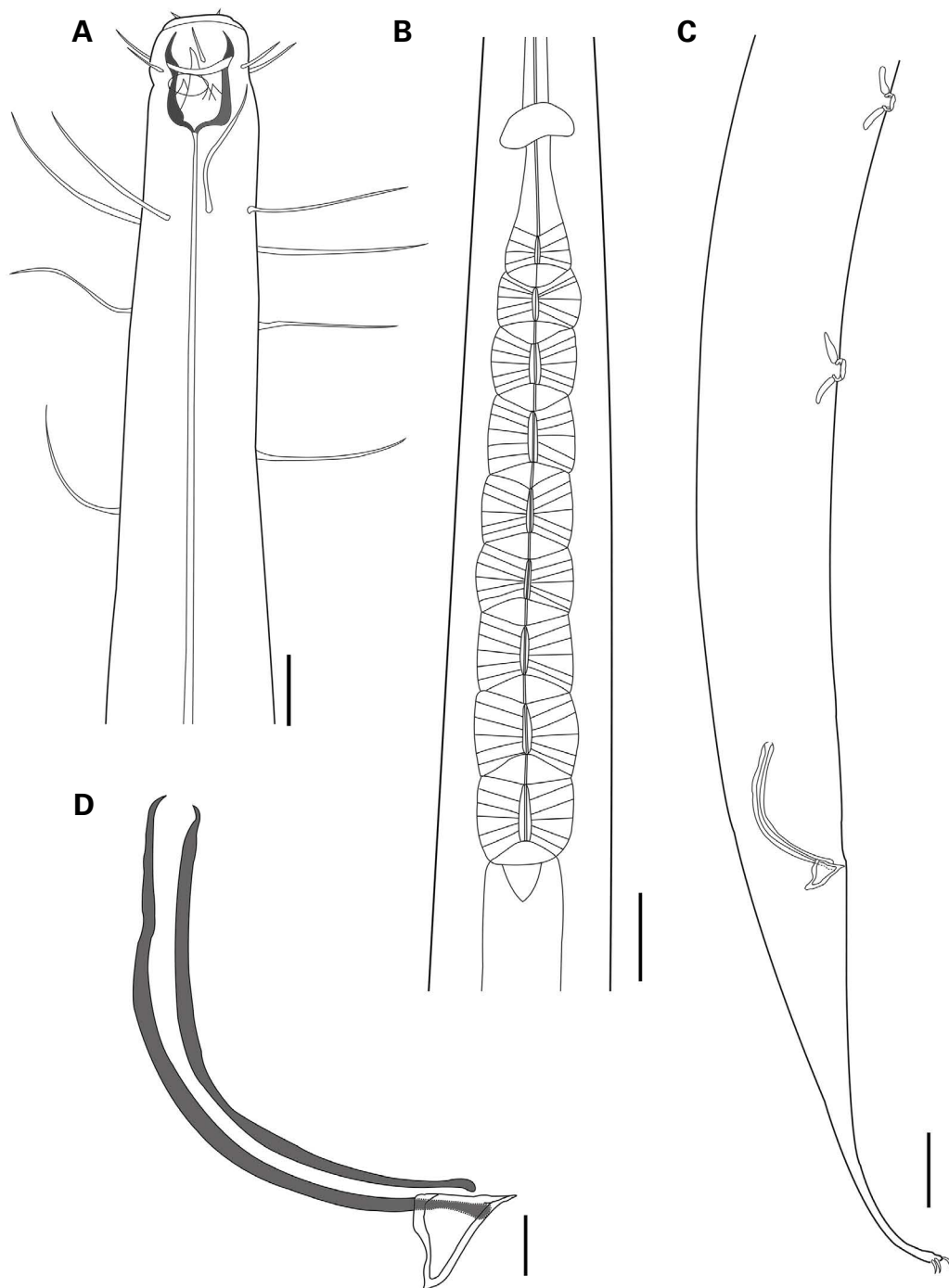


Fig. 1. *Belbolla wonkimi* sp. nov., holotype male. A, lateral view of male head end and cervical region; B, lateral view of male oesophageal region; C, lateral view of male posterior body region; and D, spicule and gubernaculum. Scale bars: A, D = 10 µm; B, C = 50 µm.

and evenly, 796 µm long and posteriorly modified into nine oesophageal bulbs (length of oesophageal bulb 395 µm) (Figs. 1B, 2E). Oesophagus corresponding diameter 91 µm. Nerve ring 374 µm from anterior end with 72 µm

corresponding body diameter and at 46.9% of oesophagus length. Cardia short, conoid. Male diorchic, testis opposed and outstretched, located left or right of intestine. Spicules long (115 µm long or 1.6 times as long as a.b.d.) and cylin-



Fig. 2. *Belbolla wonkimi* sp. nov., holotype male, DIC photomicrographs. A, lateral view of male habitus; B, lateral view of male head end and cervical region; C, lateral view of precloacal supplements; D, lateral view of spicule and gubernaculum E, lateral view of male oesophageal region; and F, lateral view of male posterior body region. Scale bars: A = 200 μ m; B, D = 20 μ m; C, E, F = 50 μ m.

dricul, strongly arcuated ventrally with rounded open head and blunt distal end; proximal end slightly cephalated (Figs. 1D, 2D). Gubernaculum small and wide, 18 μm long with triangle-shaped apophysis. Two winged precloacal supplements strongly developed; anterior precloacal supplement 543 μm lies from cloacal opening and posterior precloacal supplement lies 350 μm from cloacal opening (Figs. 1C, 2C). Tail long and slender, conico-cylindrical, 299 μm long, 4.3 times anal body diameter, tapering with distal third cylindrical and with slightly swollen tip with three minute terminal setae (Figs. 1C, 2F).

Female. Unknown.

Remarks. The genus *Belbolla* Andr ssy, 1973 was first erected as *Bolbella* by Cobb (1920) based on *Bobella tenuidens* Cobb, 1920 as a type species of the genus (Andr ssy 1973), and classified within the family Enchelidiidae Filipjev, 1928 belongs to the order Enoplida Filipjev, 1929 based on the following combination of characteristics: the presence or absence of ocelli, the presence of a large and narrow buccal cavity divided into two parts by a sclerotized transverse band, the presence of three teeth, of which one ventrosubdorsal is the largest, and the presence of a series

Table 2. Comparison of the characteristics of *Belbolla wonkimi* sp. nov. with its congeners

Taxa	Characters		
	Body length (μm)	Number of oesophageal bulb of male	Number of precloacal supplement
<i>B. asupplementata</i> Juario, 1974	M: 2053–2060 F: 1700–2229	7	0
<i>B. californica</i> (Allg�n, 1951)	M: 2390 F: 2350	7–9	2
<i>B. gallanachmorae</i> (Inglis, 1961)	M: 2900 F: –	8	2
<i>B. gracilis</i> Gagarin and Thabh, 2016	M: 911 F: 1262–1317	7	2
<i>B. heptabulba</i> (Timm, 1961)	M: 1130–1160 F: 1270–1400	7	2
<i>B. huanghaiensis</i> Huang and Zhang, 2005	M: 3037–3700 F: 2458–2808	9	2
<i>B. intarma</i> Belogurov and Belogurova, 1980	M: 3700 F: –	9	2
<i>B. longispiculata</i> Nasira, Shahina and Shamin, 2014	M: 2638 F: 3490	8	5
<i>B. stenocephalum</i> Huang and Zhang, 2005	M: 2226–2394 F: 2554–2669	8	2
<i>B. sundaensis</i> (Micoletzky, 1930)	M: – F: 2640	8	2
<i>B. teissieri</i> (Luc and Coninck, 1959)	M: 1899 F: –	8	3
<i>B. tenuidens</i> (Cobb, 1920)	M: 2700 F: 2900	8	2
<i>B. vietnamica</i> Gagarin and Tu, 2016	M: 1057–1091 F: –	4	4
<i>B. warwicki</i> Huang and Zhang, 2005	M: 1470–1772 F: 1230–1390	7	2
<i>B. wonkimi</i> sp. nov.	M: 3263–3396 F: –	9	2
<i>B. zhangii</i> Guo and Warwick, 2001	M: 2230–2750 F: 2450–2780	8	2

M: male; F: female; -: unknown

of bulb-like muscular oesophageal swellings (4 to 10) in the posterior section of pharynx, which is the most remarkable taxonomic key character of the genus within the family Enchelidiidae (Smol *et al.* 2014). So far, 15 species within the genus *Belbolla* have been described previously from various localities of the intertidal and shallow subtidal habitats in oceans all over the world (Huang and Zhang 2005; Nasira *et al.* 2014; Gagarin and Thanh 2016; Gagarin and Tu 2016) (Table 3). *Belbolla* can be artificially subdivided into two groups on the basis of the number of oesophageal bulb, as adopted in the keys by Belogurov and Belogurova (1980) and Huang and Zhang (2005) (Table 2). The first group is characterized by having four, seven or eight oesophageal bulbs in male, and comprises 13 recorded species: *B. asupplementata*, *B. californica*, *B. gallanachmorae*, *B. gracilis*, *B. heptabulba*, *B. longispiculata*, *B. stenocephalum*, *B. sundaensis*, *B. teissieri*, *B. tenuidens*, *B. vietnamica*, *B. warwicki*, and *B. zhangii*. On the other hand, the second group is characterized by having nine oesophageal bulbs, and in-

cludes the remaining two species (*B. huanghaiensis* and *B. intarma*), with the present new species. *Belbolla wonkimi* sp. nov. is characterized by the following combination of characteristics: (1) relatively long body (3,263–3,396 μm); (2) the absence of ocelli; (3) nine oesophageal bulbs in the posterior pharynx; (4) well developed two winged precloacal supplements; (5) longer spicule length (115–130 μm , 1.6–1.8 a.b.d. long); and (6) triangle-shaped shorter gubernacular apophysis length (17–18 μm). In having the nine muscular oesophageal bulbs in the pharynx, which is the most remarkable diagnostic characteristic of the genus, *Belbolla wonkimi* sp. nov. is very similar to *B. huanghaiensis* and *B. intarma* among the congeners. However, the new species differs from *B. huanghaiensis* by: (1) the shape of gubernacular apophysis (triangle-shaped vs. long dorso-caudally directed); (2) the very shorter gubernacular apophysis (17–18 μm vs. 45–60 μm); (3) the relatively longer tail in male (265–299 μm vs. 230–252 μm); (4) distance of the posterior precloacal supplement from cloacal opening in

Table 3. Ecological and biogeographical characteristics of the genus *Belbolla* including *Belbolla wonkimi* sp. nov.

Taxa	Geographic distribution	Ocean	Ecological habitat
<i>B. asupplementata</i> Juario, 1974	German Bight	Atlantic Ocean	Marine; sublittoral zone (39 m depth)
<i>B. californica</i> (Allgén, 1951)	California, San Diego, USA	Pacific Ocean	Marine
<i>B. gallanachmorae</i> (Inglis, 1961)	West coast of Scotland	Atlantic Ocean	Marine; holdfast of <i>Laminaria saccharina</i> (> 15 m depth)
<i>B. gracilis</i> Gagarin and Thab, 2016	Yen River Delta, Vietnam	Pacific Ocean	Estuary; mangrove forests (1 m depth)
<i>B. heptabulba</i> (Timm, 1961)	Bay of Bengal	Indian Ocean	Marine; bottom mud and Siphonocladus
<i>B. huanghaiensis</i> Huang and Zhang, 2005	Yellow Sea, China	Pacific Ocean	Marine; sublittoral sediment (> 77 m depth)
<i>B. intarma</i> Belogurov and Belogurova, 1980	Tatar Strait, Russia	Pacific Ocean	Marine; sediment (155 m depth)
<i>B. longispiculata</i> Nasira, Shahina and Shamin, 2014	River Indus, Pakistan	Indian Ocean	Mouth of River Indus; sandy mud
<i>B. stenocephalum</i> Huang and Zhang, 2005	Yellow Sea, China	Pacific Ocean	Marine; sublittoral sediment (> 41 m depth)
<i>B. sundaensis</i> (Micoletzky, 1930)	Sunda Island, Indonesia	Pacific Ocean	Marine
<i>B. teissieri</i> (Luc and Coninck, 1959)	Roscoff, France	Atlantic Ocean	Marine
<i>B. tenuidens</i> (Cobb, 1920)	Woods Hole, Mass., USA	Atlantic Ocean	Marine; intertidal sand
<i>B. vietnamica</i> Gagarin and Tu, 2016	Yen River Estuary, Vietnam	Pacific Ocean	Estuary; mangrove forest (1 m depth)
<i>B. warwicki</i> Huang and Zhang, 2005	Yellow Sea, China	Pacific Ocean	Marine; sublittoral sediment (> 41 m depth)
<i>B. wonkimi</i> sp. nov.	East Sea, Korea	Pacific Ocean	Marine; subtidal sediment (104 m depth)
<i>B. zhangii</i> Guo and Warwick, 2001	Bohai Sea, China	Pacific Ocean	Marine; sublittoral sediment (> 38.5 m depth)

male (350–385 μm vs. 210–255 μm); and (5) distance between precloacal supplements in male (189–193 μm vs. 115–140 μm). *Belbolla wonkimi* sp. nov. can be also easily distinguished from *B. intarma* by the relatively longer tail in male (265–299 μm vs. 215 μm), and the shape and relative length of spicules (smooth on the distal end of spicules, 115–130 μm vs. hook on the distal end of spicule, 110 μm).

ACKNOWLEDGEMENTS

The authors appreciate the anonymous reviewers for their helpful comments that greatly improved the manuscript. This research was supported by the Marine Biotechnology Program of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) (No. 20170431) (PM61960, PE99813).

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