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New Record of Three Nematode Species of Genus *Enoplus* (Nematoda: Enoplidae) from Korea

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Abstract - Three unrecorded species of free-living marine nematodes, belonging to genus *Enoplus* Dujardin, 1845 collected from rocky intertidal seagrass on the eastern coast of Korea, are described and illustrated. *Enoplus taipingensis* Zhang and Zhou, 2012 is characterized by longer body size, a series of lateral setae throughout the tail in male, the presence of trumpet-shaped preloacal supplement with well-dilated proximal end, and the presence of spicules with five to eight semi-circular plates. *Enoplus meridionalis* Steiner, 1921 is characterized by the presence of trumpet-shaped preloacal supplement with slightly dilated proximal end, paired massive spicules, and tail with two pairs of stout terminal setae. *Enoplus mammillatus* Timm, 1959 is easily distinguished from the congeners by narrow tubular-shaped preloacal supplement. In this study, we provide detailed morphological features of three *Enoplus* species by differential interference contrast microscopy and scanning electron microscopy. This is the first report on the species of the genus *Enoplus* from the Korean waters.

Key words: taxonomy, marine nematodes, *Enoplus*, East Sea, Korea

INTRODUCTION

The genus *Enoplus* Dujardin, 1845 is currently composed of 36 valid species, one of the largest dominant species in the intertidal zone, and having a cosmopolitan distribution. The genus *Enoplus* Dujardin, 1845 is easily distinguished by its three solid mandibles without teeth, low lips with inner labial papilliform, and the presence of specific preloacal supplement (Platt and Warwick 1983). In this genus, most species known can be separated among species according to the shape of the male genital armature, spicules and gubernaculum (Wieser 1953).

Most taxonomic studies of free-living marine nematodes

have been conducted in Europe. Recently several papers of free-living marine nematodes have been published on China and Japan (Shimada and Kajihara 2014; Chunming *et al.* 2015). Until now, only seven *Enoplus* species, *E. anisospiculus* Hopper, 1968, *E. kurilensis* Fadeeva and Yushin, 1998, *E. michaelsoni* Linstow, 1896, *E. paralittoralis* Wieser, 1953, *E. paralpha* Fadeeva and Yushin, 1998, *E. taipingensis* Zhang and Zhou, 2012, and *E. velatus* Wieser, 1959, have been recorded in the Northwest Pacific Ocean (Kito 1976; Yoshimura 1980; Fadeeva and Yushin 1998; Zhang and Zhou 2012). Up to now, 40 free-living marine nematodes of the family Draconematidae, Comesomatidae, and Enchelidiidae have been recorded from Korea (Rho and Min 2011; Barnes *et al.* 2012; Hong and Lee 2014). However, the taxonomic report on the genus *Enoplus* of Korea in the intertidal algae is entirely unknown. The purpose of this study is to report taxonomic description of three unre-

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corded species belonging to genus *Enoplus* collected from rocky intertidal seagrass on the eastern coast of Korea.

MATERIALS AND METHODS

1. Sampling

Marine nematodes were obtained from washing of intertidal sediment. Samples were collected from rocky intertidal seagrass on the eastern coast of Korea. Samples were filtered through a 67 μm mesh sieve in the field after freshwater rinsing for less than a minute for osmotic shock (Kristensen and Higgins 1989), and then fixed in 5% formalin in sea water. Coarse detritus and tiny shell gravels were removed from the sample by decantation and the meiobenthos was roughly extracted by flotation in Ludox (Dupont) HS 40 (Burgess 2001).

2. Observation and drawing

The specimens were sorted from the mixed meiobenthos under LEICA 205C stereomicroscope. The specimens for morphological observation were transferred to anhydrous

glycerin between two cover slips on a H-S slide (Shirayama *et al.* 1993). Specimens were examined, photographed and drawn using Nomarski differential interference contrast (DIC) with an Olympus BX53 microscope equipped with a drawing tube and a DIXI 3000 camera, and quality enhanced portable photoshop software. Specimens selected for scanning electron microscopy (SEM) were fixed in 5% formalin, washed in distilled water, and critical point dried. Specimens were coated with gold/palladium, and examined with mini 4500 SEM.

3. Terminology and abbreviations

Abbreviations are as follows: L, total body length; esol, esophagus length; a, body length/max. body diameter; hd, head diameter on cephalic setae; b, total body length/esophagus length; c, total body length/tail length; bd, body diameter at the base of esophagus; M, maximum body diameter; cs, cephalic setae; mandl, mandibles length; nr, anterior end to nerve ring; nr (%), nr/esol; supl, supplement; spil, spicules length; abd, anal body diameter; s', spi/abd; gubL, gubernaculum length; t, tail length; t/abd, tail length/abd; V, anterior end to vulva; V (%), V/L.

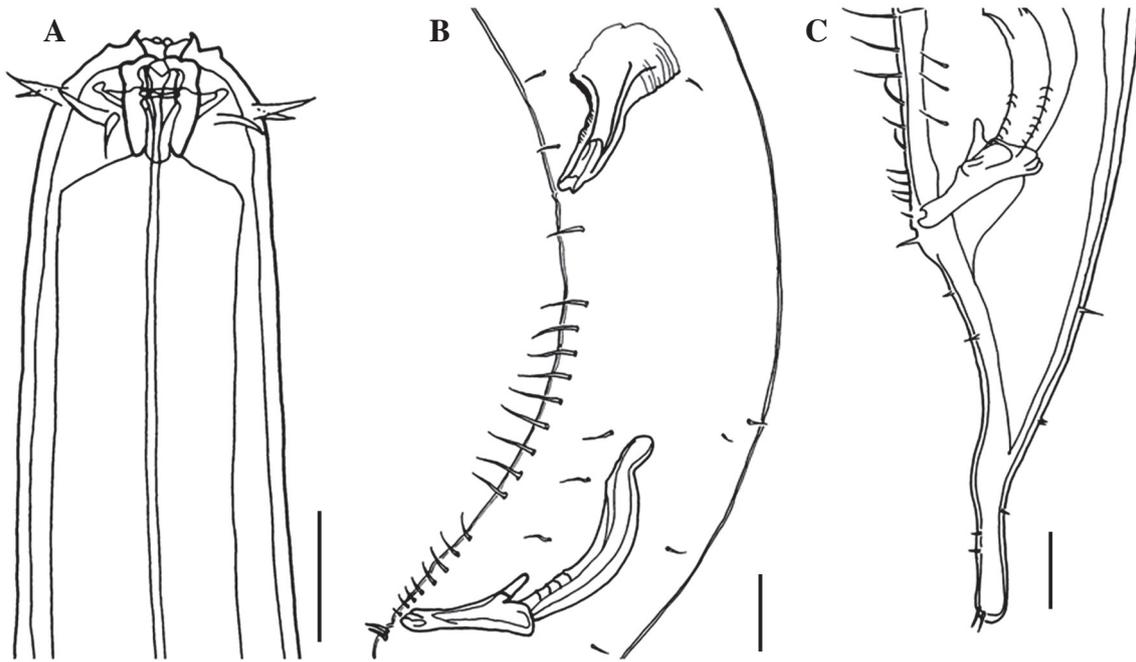


Fig. 1. *Enoplus taipingensis*, male, lateral view: A, anterior body region; B, posterior region showing precloacal supplement; C, spicules and gubernaculum. Scale bars: A-C = 50 μm .

SYSTEMATIC ACCOUNTS

Class Enoplea Inglis, 1983

Order Enoplida Filipjev, 1929

Family Enoplidae Dujardin, 1845

Genus *Enoplus* Dujardin, 1845 갑옷선충속 (신칭)

1. *Enoplus taipingensis* Zhang and Zhou, 2012

타이핑갑옷선충 (신칭)

Synonym: *Enoplus taipingensis* Zhang and Zhou, 2012, p. 102, Figs. 2-4.

Material examined: 5♂♂ and 3♀♀, Bugu-ri, Uljin-gun, Gyeongsangbuk-do, Korea (37°06'21.39"N, 129°22'37.42"E), collected on 14 July 2014. The specimens are deposited in the nematode collection of the Korea Institute of Ocean Science and Technology, Uljin, Korea. All are mounted in anhydrous glycerin between two coverslips on H-S slides, sealed with nail polish.

Diagnosis: Cuticle smooth; trumpet-shaped precloacal supplement present; spicules with five to eight semi-circular plates; tail with a series of lateral setae.

Measurements: See Table 1.

Description: *Male:* Body length 5,987~7,059 µm long, large, rather stout, slightly tapers to anterior region (Fig. 2A). Maximum body diameter 177~218 µm. Cuticle smooth and thick. Head bluntly rounded, typically with three low lips. Head with inner circle of six prominent labial papillae and outer circle of 10 (6+4) cephalic setae, six longer setae 26~33 µm, 32~48% of the head diameter. Mandibles solid, 26~33 µm wide, measured across anterior extremity, about 34~43% of the head diameter on cephalic setae (Figs. 1A, 2B, 3A). Amphids openings small rounded pocket-like, situated at between anterior to posterior edge of cephalic capsule (Fig. 3B). Three short lateral cervical setae situated at posterior to cephalic capsule, arranged in triangle. Esophagus cylindrical, 846~1,095 µm. Nerve ring encircling esophagus, 388~440 µm from anterior end, situated at 35~45% of esophagus length from anterior end. No definite eyespots. Dark brown pigment diffuse present anteriorly and variable in form (Fig. 2B). Cervical and somatic setae stout, sparsely distributed throughout body along dorsal and ventral margins of lateral hypodermal chords. Spicules symmetrical, curved, 272~311 µm long, inflated proximally and

Table 1. Measurement of *Enoplus taipingensis*

(in µm)

	♂1	♂2	♂3	♂4	♂5	♀1	♀2	♀3
L	7058.4	6514.9	6757.1	6434.9	5986.6	7232.7	5447.6	6544.5
esol	1014.5	1146.6	1046	1102	897.7	1143.8	943.3	1134.2
a	32.3	33.5	34.8	34.5	33.8	33.9	23.8	34.7
b	7.0	5.7	6.5	5.8	6.7	6.3	5.8	5.8
c	19.8	20.5	18.5	19.5	18.7	22.0	14.6	18.2
hd	69	69.7	77.4	80.2	71.7	82.9	76.5	76.7
bd	164.2	161.8	169	159.6	157.7	173.9	190.8	163.3
M	218.5	194.7	194.3	186.4	177.2	213.5	229.2	188.7
cs	33.2	31.6	30.6	28.4	30.9	26.2	31.1	31.6
cs/hd	0.48	0.45	0.40	0.35	0.43	0.32	0.41	0.41
Mandl	26.2	27	30	30.3	30.7	32.6	25.8	29.1
(mandl/hd)	(0.38)	(0.39)	(0.39)	(0.38)	(0.43)	(0.39)	(0.34)	(0.38)
nr	440.5	435.5	401.7	387.8	402.6	468.5	358.8	432
nr/eso(%)	43.42	37.98	38.40	35.19	44.85	40.96	38.04	38.09
supl	115.8	127	120.4	120.1	115.3			
spil	232.2	212.7	205.9	212.9	221.8			
spil/sup	2.01	1.67	1.71	1.77	1.92			
s'	1.70	1.63	1.45	1.51	1.77			
gubL	77.4	95.8	88.9	88.3	94			
abd	136.8	130.6	142.3	140.8	125.2	128.3	130.6	126.6
t	357	317.7	365.5	330.2	320.5	328.2	373.5	358.9
t/abd	2.61	2.43	2.57	2.35	2.56	2.56	2.86	2.83
V						4113.9	2660.6	3893.0
V(%)						56.9	48.8	59.5



Fig. 2. *Enoplus taipingensis*, DIC photomicrographs of male (A-F) and female (G-H), lateral view: A, habitus; B, head region; C, precloacal setae; D, precloacal supplement; E, spicules region; F, tail region; G, vulva region; H, tail region. Scale bars: A = 200 μ m, B, D, E = 20 μ m, C, F-H = 40 μ m.

pointed distally, and provided with hook shaped 5~8 semi-circular plates (Figs. 1B, 2E, 3D-G). Two pairs of stout setae situated at posterior lip of cloacal opening. Long stout setae present on subventral sides between openings of supplement and of precloacal region (Figs. 1B, 2C). A series of lateral setae situated throughout tail (Fig. 3I). A piece of gubernaculum 97~103 μ m long, with lateral projections. Pre-cloacal supplement trumpet-shaped with well-dilated proximal end, 113~127 μ m long (Figs. 1B, 2D, 3H). Tail

conico-cylindrical, 320~366 μ m long, about 2.4~2.6 times of anal body diameter (Figs. 1C, 2F, 3C).

Female: Similar to male in general appearance. Body length 5,448~7,273 μ m long; maximum body diameter 189~229 μ m. Reproductive system monodelphic, with two opposed, reflexed ovaries. Vulva 3,890~4,110 μ m from anterior end, situated at 57~ 60% of total body length (Fig. 2G). Tail length 328~374 μ m long, about 2.6~2.9 times of anal body diameter (Fig. 2H).

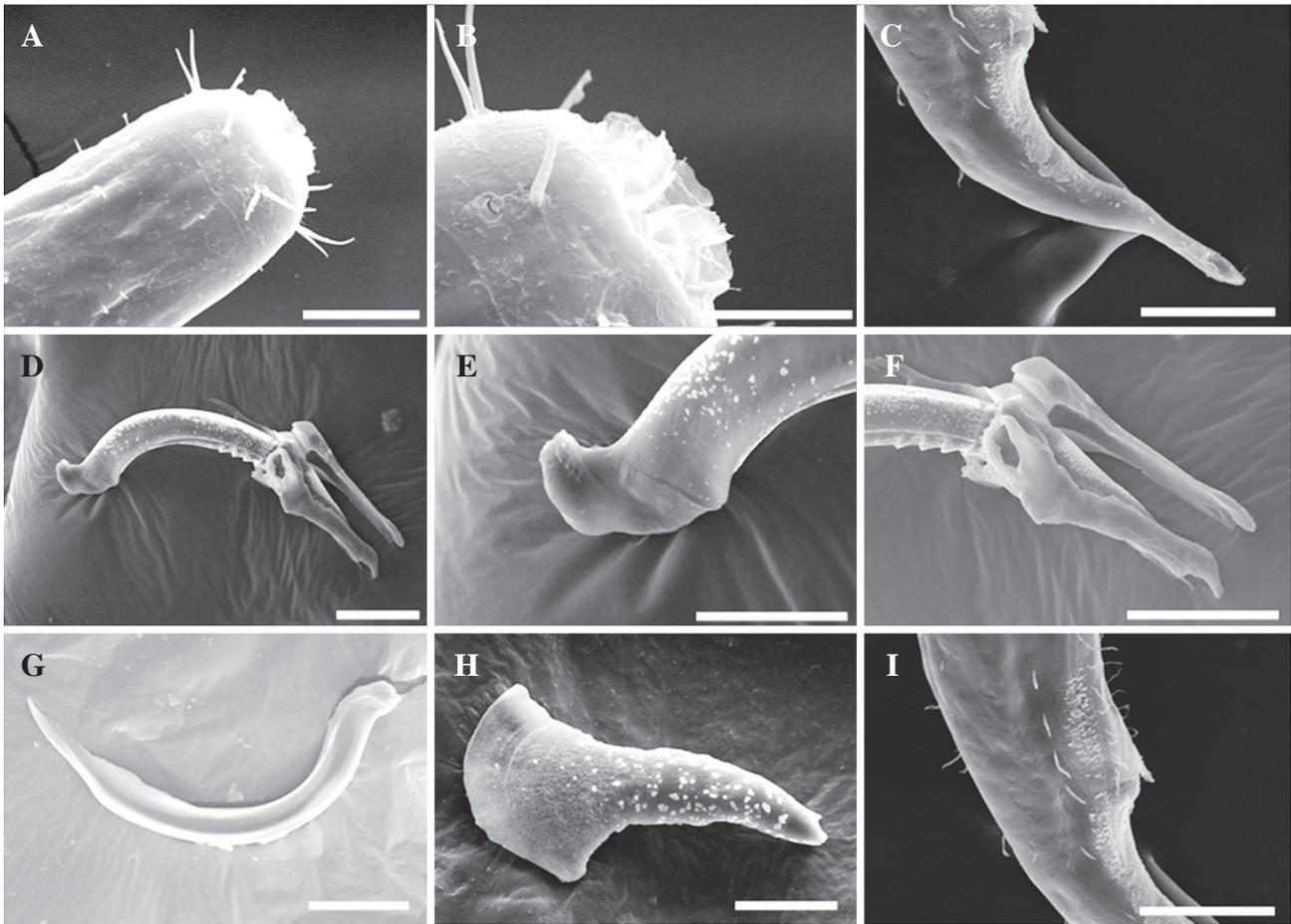


Fig. 3. *Enoplus taipingensis*, SEM photomicrographs of male, lateral view: A, head; B, anterior body region showing amphidial fovea and lips; C, tail region; D, spicules and gubernaculum; E, spicules region, S-shaped structure; F, gubernaculum and semi-circular plate; G, spicules; H, preloacal supplement; I, a series of lateral setae on the tail. Scale bars: A, D, G = 50 μm , B, E, F, H = 30 μm , C, I = 100 μm .

Remarks: *Enoplus taipingensis* Zhang and Zhou, 2012 was firstly described in the Taiping Bay, Chingdao, China. *Enoplus taipingensis* has been previously reported from only type locality. The Korean specimens were discovered from the seagrass bed of intertidal rocky shore of the East Sea, Korea. *Enoplus taipingensis* is characterized by longer body size, a series of lateral setae throughout the tail in male, and the presence of spicules with five to eight semi-circular plates and preloacal supplement with three projections in distal end. *Enoplus taipingensis* is most like *E. michaelsoni* Linstow, 1896 by possessing trumpet-shaped preloacal supplement and similar spicules structure with semi-circular plates. However, *E. taipingensis* can be significantly different from *E. michaelsoni* by longer body length (5,987~7,060 μm vs. 3,360~4,800 μm), slightly larger spicules length (206~232 μm vs. 174~192 μm in Yoshimura 1980), and

the presence of special series of lateral setae throughout the tail in male. The present Korean specimens agree well with Zhang and Zhou's (2012) original description, especially in the number of three lateral cervical setae arranged in triangle and the presence of characteristic lateral setae of throughout the tail in male. However, the Korean specimens of *E. taipingensis* are not well accorded with the original description by having a longer preloacal supplement length (115~127 μm vs. 62~83 μm).

Habitat: The nematodes were obtained from the sediments of rocky intertidal seagrass bed on the eastern coast of Korea collected at a depth of 1 m by hands with scoop. Sediments include tiny shell gravels and coarse detritus.

Distribution: China, Korea.

Deposition: KIOST NEM-1-43, KIOST NEM-1-44, KIOST NEM-1-45, KIOST NEM-1-46, KIOST NEM-1-47, KIOST

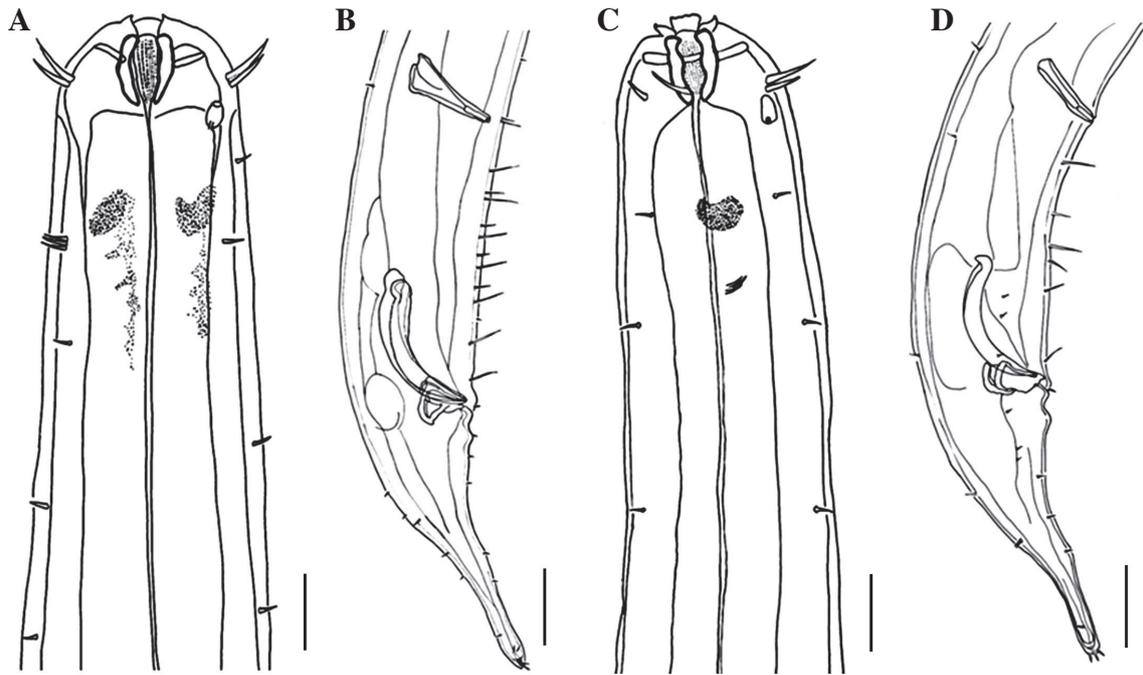


Fig. 4. *Enoplus meridionalis*, male (A, B) and *Enoplus mamillatus*, male (C, D), lateral view: A, anterior body region showing amphidial fovea and cephalic capsule; B, posterior region showing preloacal supplement, spicules and gubernaculum; C, anterior body region showing amphidial fovea and cephalic capsule; D, posterior region showing preloacal supplement, spicules and gubernaculum. Scale bars: A, C = 20 μ m, B, D = 50 μ m.

NEM-1-50, KIOST NEM-1-51, KIOST NEM-1-52.

Identifiers: Hyo Jin Lee.

2. *Enoplus meridionalis* Steiner, 1921

남방갯웃선충 (신칭)

Synonyms: *Enoplus communis* var. *meridionalis* Steiner, 1921, p. 30, Fig. 7.

Enoplus meridionalis: Inglis, 1971, p. 71, Figs. 20-26.

Material examined: 3♂♂, Bugu-ri, Uljin-gun, Gyeongsangbuk-do, Korea (37°06'21.39"N, 129°22'37.42"E), collected on 9 July 2014 by Hyo Jin Lee; Jangsa-dong, Sokcho-si, Gangwon-do, Korea (38°13'37.77"N, 128°35'16.92"E), collected on 2 Oct 2014. Two specimens are deposited in the nematode collection of the Korea Institute of Ocean Science and Technology, Uljin, Korea. One specimen is kept in the collection of the authors. All are mounted in anhydrous glycerin between two coverslips on H-S slides, sealed with nail polish.

Diagnosis: Cuticle smooth; trumpet-shaped preloacal supplement with slightly dilated proximal end; massive spic-

ules present; tail with two pairs of stout terminal setae.

Measurements: See Table 2.

Description: *Male:* Body length 3,339~4,194 μ m long, slender and cylindrical, slightly tapers to anterior region (Fig. 5A). Maximum body diameter 95~119 μ m. Cuticle smooth and thick. Head extended, typical with three low lips and inner circle of six prominent labial papillae. Head with outer circle of 10 (6+4) cephalic setae, six longer setae 15.3~16.6 μ m long, about 0.3~0.4 of head diameter. Mandibles solid, 12~13 μ m wide, measured across anterior extremity, about 27~32% of head diameter on cephalic setae (Figs. 4A, 5B). Amphids openings small rounded pocket-shaped; situated at between anterior to the posterior edge of the cephalic capsule. Esophagus cylindrical, 470~578 μ m. Eyespot present (Fig. 4A). Few setae 3~4 μ m long, scattered throughout anterior esophageal region, and three stout setae on each side in behind eyespot. Tail cylindrical; ends in slight swelling with four subterminal setae, 219~233 μ m long, about 2.6~2.9 times of anal body diameter (Figs. 4B, 5E). Spicules symmetrical, curved, 106~131 μ m long; gubernaculum, 34~40 μ m long, with lateral pro-



Fig. 5. *Enoplus meridionalis*, DIC photomicrographs of male, lateral view: A, habitus; B, head region; C, precloacal setae; D, precloacal supplement and spicules; E, tail region. Scale bars: A = 100 μ m, B = 10 μ m; C-E = 20 μ m.

jections (Figs. 4B, 5D). Two pairs of stout setae situated on posterior lip of cloacal opening. Precloacal region with long and stout subventral setae; longest setae 18~20 μ m long (Fig. 5C). Precloacal supplement, 55~73 μ m long, trumpet-shaped with slightly dilated proximal end (Figs. 4B, 5D).

Remarks: *Enoplus meridionalis* Steiner, 1921 is mainly characterized by the following combination of characters: the presence of trumpet-shaped precloacal supplement with slightly dilated proximal end, different shaped massive pair-

ed spicules and tail with two pairs of stout terminal setae. *Enoplus meridionalis* is most related to *E. heardensis* Mawson, 1958 and *E. harlockae* Inglis, 1964 by having a trumpet-shaped precloacal supplement. However, *E. meridionalis* is easily discernible from the two morphologically related species by the shape of gubernaculum (the median and the lateral pieces of gubernaculum slightly curve posterior to the spicules in *E. meridionalis* vs. the median and the lateral pieces curve upwards to enfold the spicules in *E. harlockae*)



Fig. 6. *Enoplus mamillatus*, DIC photomicrographs of male, lateral view: A, habitus; B, head region; C, ampidual fovea region; D, precloacal supplement and spicules; E, tail region. Scale bars: A = 100 μ m, B, C = 10 μ m, D, E = 20 μ m.

and having a shorter gubernaculum length (34~40 μ m in *E. meridionalis* vs. 70~80 μ m in *E. heardensis*). The present Korean specimens agree well with Steiner's (1921) original description, especially in the shape of pre-cloacal supplement, spicules and gubernaculum. However, the Korean specimens of *E. meridionalis* are not well accorded with the original description by having a slightly longer body length (3,339~4,194 μ m vs. 2,531~2,545 μ m).

Habitat: The nematodes were obtained from the sediments of rocky intertidal seagrass bed on the eastern coast of Korea collected at a depth of 1 m by hands with scoop. Sediments include tiny shell gravels and coarse detritus.

Distribution: America, Australia, North Atlantic, Korea.

Deposition: KIOST NEM-1-369, KIOST NEM-1-372, NE-EN-11.

Identifiers: Hyo Jin Lee.

3. *Enoplus mammillatus* Timm, 1959

유두돌기갑옷선충 (신칭)

Synonym: *Enoplus mammillatus* Timm, 1959, p. 205, Pl. 1.

Material examined: 1♂, Namae-ri, Yangyang-gun, Gangwon-do, Korea (37°06'21.39"N, 129°22'37.42"E), collected on 15 July 2014. The specimen is deposited in the nematode collection of the Korea Institute of Ocean Science and Technology, Uljin, Korea. All are mounted in anhydrous glycerin between two coverslips on H-S slides, sealed with nail polish.

Diagnosis: Cuticle smooth; narrow tubular-shaped preloacal supplement present; spicules with cephalated posterior end.

Measurements: See Table 2.

Description: *Male:* Body length 3,238 µm long, elongated cylindrical, tapered slightly to the anterior end (Fig. 6A). Maximum body diameter 90.6 µm. Cuticle smooth. Head rounded, 39.5 µm wide, typically with three low lips; each lips surrounded by inner circle of six small labial papillae. Head with 10 cephalic setae, six longer setae 13.8 µm, about 0.3 times of head diameter and four shorter setae 10.7 µm. Mandible well-developed, 11.7 µm wide, 16 µm long, about 30% of the head diameter at cephalic setae (Figs. 4C, 6B). Amphids opening small rounded pockets, 5 µm wide, 7 µm long, located anterior to base of cephalic capsule (Fig. 6C). Esophagus cylindrical, 486 µm long. Broadly spreading eye pigment located in anterior end of esophagus (Fig. 4C). Cervical-somatic setae about 4 µm long, scattered throughout body along subdorsally and subventrally. Tail cylindrical, with a pair of terminal setae posteriorly. Tail length 218 µm long, about 2.5 times of anal body diameter (Figs. 4D, 6E). Spicules, 103 µm long, massive, symmetrical, slightly curved toward cephalated posterior end. Gubernaculum 35.7 µm long, slightly triangular shaped with lateral projections. Preloacal supplement narrow strait-shaped, 53.6 µm long (Figs. 4D, 6D). Preloacal lip opening with a pair of stout cloacal setae. Long stout setae located on between openings of supplement and preloacal region, the longest setae 23.5 µm (Fig. 4D).

Remarks: *Enoplus mammillatus* Timm, 1959 was firstly described from the Arabian Sea. *Enoplus mammillatus* is easily distinguished from the other species of the genus by narrow tubular-shaped preloacal supplement. This species

Table 2. Measurement of *Enoplus meridionalis* and *E. mammillatus*. (in µm)

	<i>E. meridionalis</i>		<i>E. mammillatus</i>	
	♂1	♂2	♂3	♂1
L	3339.0	3641.2	4194.2	3238.1
esol	470.6	529.4	578.3	485.7
a	33.6	38.4	35.2	35.7
b	7.1	6.9	7.3	6.7
c	14.6	16.7	18.0	14.9
hd	44.2	37.3	47.1	39.5
bd	76.9	74.9	97.3	79.0
M	99.3	94.9	119.3	90.6
cs (shorter)	12.9	10.7	12.0	10.7
cs (longer)	16.4	15.3	16.6	13.8
cs/hd	0.37	0.41	0.35	0.35
Amphids (wide/long)	4.6/7.8	6.0/8.4	3.8/8.8	5.2/6.9
cervical-setae	3.4	3.7	4.6	4.7
mandl (mandl/hd)	13.6 (0.31)	12.1 (0.32)	12.8 (0.27)	11.7 (0.30)
mandl	17.8	16.3	18.0	16.0
supl	58.6	52.0	61.8	53.6
spil	131.1	105.9	111.9	103.0
spil/supl	2.24	2.04	1.81	1.92
s'	1.5	1.4	1.3	1.2
gubL	39.7	34.1	36.8	35.7
supl-anus length	173.6	211.0	208.4	200.0
subventral setae	17.9	18.7	20.0	23.5
abd	87.3	75.6	86.2	87.2
t	228.5	218.6	233.1	217.5
t/abd	2.6	2.9	2.7	2.5

is morphologically similar to *E. benhami* Ditlevsen 1930, *E. micrognathus* Allgen 1947, and *E. paralpha* Fadeeva and Yushin, 1998 by narrow tubular-shaped preloacal supplement. However, *E. mammillatus* can be significantly different from *E. benhami* by longer preloacal supplement length (54 µm vs. 33 µm). *Enoplus micrognathus* differs from *E. mammillatus* by the position of preloacal supplement, which is very closely located in front of the anus. Also, *E. mammillatus* has spicules of posterior end with hooks, but *E. paralpha* has only smooth spicules. The present Korean specimen collected from rocky intertidal seagrass on the eastern coast of Korea is also very similar with *E. mammillatus*. Most taxonomic characters of the present specimen are very similar with the original description of *E. mammillatus*, but the Korean specimen has no semicircular plate in spicules.

Habitat: The nematodes were obtained from the sediments of rocky intertidal seagrass bed on the eastern coast of Ko-

rea collected at a depth of 1 m by hands with scoop. Sediments include tiny shell gravels and coarse detritus.

Distribution: Arabian Sea, Korea.

Deposition: KIOST NEM-1-398.

Identifiers: Hyo Jin Lee.

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