

Three newly recorded free-living marine nematode species (Nematoda: Chromadorea) from Korea

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Abstract: Three free-living marine nematodes (*Desmoscolex* (*Desmoscolex*) *max* Timm, 1970, *Daptonema longiapophysis* Huang and Zhang, 2010, and *Pseudosteineria sinica* Huang and Li, 2010) were newly recorded in Korea. *Desmoscolex* (*D.*) *max* was found from subtidal coarse sediment around Wangdolcho in the East Sea. It was characterized by the presence of untypical setae arrangement, obviously elongated triangle-shaped head, long hairy cephalic setae, a long naked tail spinneret, and the absence of peduncle at the base of somatic setae. *Daptonema longiapophysis* was obtained from intertidal sandy sediments in the southern coast of Korea. It was characterized by the presence of setiform labial sensilla, spicules with a projection on both sides, and gubernaculum with dorso-caudal apophysis. *Pseudosteineria sinica* was discovered from the intertidal sediment in the Yellow Sea. It was characterized by unobservable amphideal fovea, different lengths of spicules, and gubernaculum with dorso-caudal apophysis. In this study, we provide detailed morphological features of three free-living marine nematodes by differential interference contrast microscopy.

Keywords: marine nematode, *Desmoscolex*, *Daptonema*, *Pseudosteineria*, taxonomy, Korea

INTRODUCTION

Marine nematodes are one of the most abundant animal groups, discovered in various marine habitats, from beach sediment to deep-sea environments (Nicholas 1984). In addition, many species have been reported from marine algal habitat as well as on the surface of other invertebrates (Porifera, Cnidaria, Bryozoa and Mollusca and so on) (Kito and Hope 1999). Free-living marine nematodes are used as environmental bio-indicator in marine benthic ecosystems because they show high biomass, species richness, and shorter generation period (Heip *et al.* 1985; Sandulli and De Nicola-Giudici 1991; Schratzberger *et al.* 2000; Balsamo *et al.* 2010). They also play an important role in the

marine food chain because they are predators that feed on other invertebrate such as unicellular algae, bacteria, other nematodes, and further prey on fish and other large invertebrates (Bouwman 1983; Heip *et al.* 1985; Giere 2008).

The first taxonomic study of free-living marine nematodes in Korea was began with the description of *Tenuidraconema koreensis* by Rho and Kim (2004). Since then, 70 species of free-living marine nematodes have been recorded by various taxonomists (Rho and Kim 2004, 2005; Lim and Chang 2006; Rho *et al.* 2010; Rho and Min 2011; Barnes *et al.* 2012; Hong *et al.* 2016; Jeong *et al.* 2019a, b, 2020; Lee and Rho 2019; Rho *et al.* 2020; Tchesunov *et al.* 2020, 2021, 2022; Lee *et al.* 2021a, b, 2022). Among them, 33 species were reported in the East Sea, 8 in the southern

coast, 5 in the Yellow Sea, and 24 in Jeju Island of Korea.

As a result of continuous biodiversity study of free-living marine nematodes around Korea, three nematode species were discovered from shallow intertidal and subtidal sediments in the benthic environment. Present paper deals with morphological study of three unrecorded free-living marine nematodes using a differential interference contrast (DIC) microscope.

MATERIALS AND METHODS

Specimen collecting. Specimens were collected from upper surface of the intertidal sandy beach of southern and western coasts of Korea using a hand scoop, and also obtained from shallow subtidal zone around the East Sea of Korea using Smith-McIntyre grab. Meiobenthic animals were roughly separated from the sediment the freshwater shock technique, and then quickly filtered through a 63 µm mesh-sieve and fixed in 5% buffered formalin in seawater (Kristensen and Higgins 1989). Afterwards, the meiobenthos was extracted by flotation in Ludox (DuPont, USA) HS 40 in three centrifugation cycles (Burgess 2001).

Morphological observation. Nematodes were picked out from the mixed meiobenthos under high magnification of dissecting microscope (LEICA 205C; Wetzlar, Germany). Target nematodes were transferred to 5% glycerin solution, and then dehydrated through a graded series of glycerine. Nematodes were mounted on between two coverslips using the HS slide (Shirayama *et al.* 1993). Mounted nematodes were measured and examined using an Olympus BX 53 microscope equipped with Nomarski differential interference contrast and photographed with an Olympus DP 26 digital camera. Measurements were carried out with an Olympus Cellsens corresponding imaging software (Olympus, Tokyo, Japan). Line drawings for the mounted specimens were developed by an Olympus BX 53 microscope with drawing tube, and were adjusted in Adobe illustrator CC program. All the measurements are given in µm.

SYSTEMATIC ACCOUNTS

Phylum Nematoda Potts, 1932
 Class Chromadorea Inglis, 1983
 Order Demoscolecida Filipjev, 1929
 Family Desmoscolecidae Shipley, 1896
 Genus *Desmoscolex* Claparède, 1863

Subgenus *Desmoscolex* Claparède, 1863

Desmoscolex (Desmoscolex) max Timm, 1970

(Figs. 1, 2)

Desmoscolex (Desmoscolex) max Timm, 1970, p. 26, figs. 26–28.

Desmoscolex max: Timm, 1978, p. 229, fig. 2E, F; Decraemer, 1984, p. 311, fig. 3C, D.

Material examined. The specimen was collected from the subtidal zone of Wangdolcho, Hupo-myeon, Uljin-gun, Gyeongsangbuk-do, Korea, 26 Sep. 2019 (leg. H Lee). One female (MABIK NA00157826), in glycerin on a HS slide, was deposited in the nematode collection at the specimen conservation room of the Marine Biodiversity Institute of Korea (MABIK), Seocheon, Korea.

Habitat. Subtidal coarse sediment, 68 m depth (36°42'26.52"N, 129°43'07.22"E).

Measurements. Female ($n=1$): L=419; hd=20; cs=34; sd1=28; sd3=30; sd5=29; sd7=27; sd9=25; sd11=26; sd13=36; sd16=40; sv1=19; sv2=18; sv4=19; sv6=17; sv8=20; sv12=16; sv14=14; sv15=18; sv17=20; ph=82; t=89; tmr=46; t/tmr=1.9; tmrw=20; tmr/tmrw=2.3; mbd=67; mbde=42; a=6.3; b=5.1; c=4.7.

Description. Female. Body small, ventrally curved, slightly tapered anteriorly and posteriorly; tail end ring strongly curved dorsally. Body cuticle with 17 main rings of slightly different widths and lengths; interzone very wide with uncovered annules. Each main ring covered with layer of secretion and different sized foreign material (Figs. 1A, 2A).

Somatic setae arranged according to untypical desmoscolecoid setal pattern: eight pairs of subdorsal somatic setae on main rings 1, 3, 5, 7, 9, 11, 13, 16; nine pairs of subventral somatic setae on main rings 1, 2, 4, 6, 8, 12, 14, 15, 17. Subdorsal somatic setae gradually becoming finer to tip, without mid-central canal, longer than subventral somatic setae, and not inserted on peduncle. Pairs of subdorsal somatic setae on main rings 13 and 16 longer than rest of subdorsal somatic setae. Subventral somatic setae smaller than subdorsal somatic setae and relatively slender, not inserted on peduncle; cylindrical basal part broad, without mid-central canal, tapered to tip. Subventral somatic setae all about same length, except for those on main rings 15 and 17, which are slightly longer (Fig. 1A–C).

Head triangle-shaped, obviously elongated, longer than wide, anteriorly very tapered (Figs. 1A, 2A). Cephalic cuticle slightly covered with thin layer of fine granular desmos, except in amphideal zone. Long hairy cephalic setae situ-

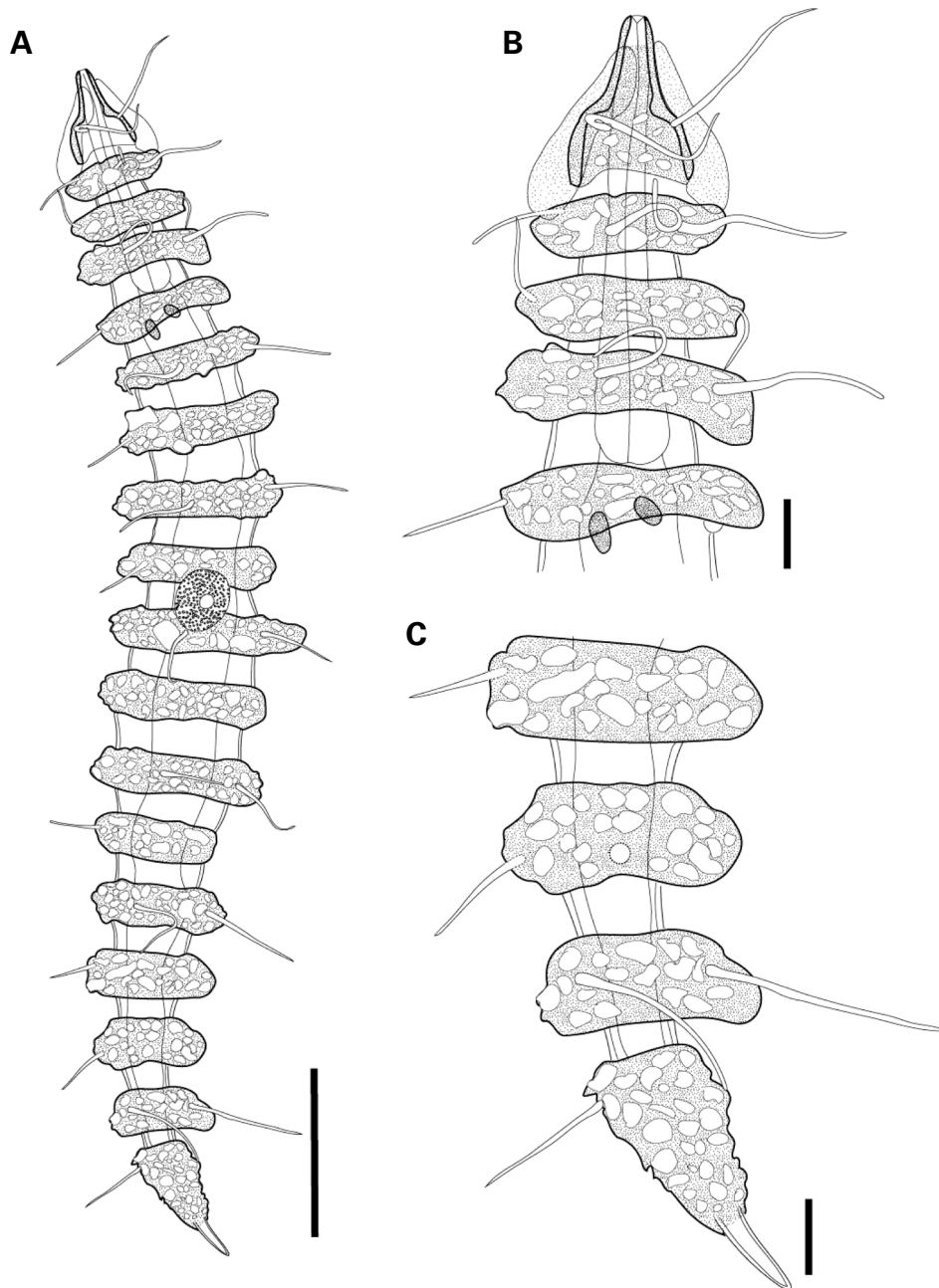


Fig. 1. *Desmoscolex (Desmoscolex) max* Timm, 1970, female in lateral view: A, Habitus; B, Anterior region; C, Tail region. Scale bars: 50 μ m in A and 10 μ m in B and C.

ated at almost middle of head, 34 μ m long; not jointed and not inserted on peduncles. Amphideal fovea very long (34 μ m) and elongated, largely covering head laterally, reached at anterior margin of main ring 1.

Digestive system typical of *Desmoscolex*. Buccal opening with minute buccal cavity. Oesophagus 82 μ m long, poster-

iorly extending to level of anterior margin of main ring 4 (Fig. 1B). A pair of ocelli situated at level of posterior margin of main ring 4. Vulva not observed. Annus situated at between main rings 15 and 16.

Tail with two main rings (Figs. 1C, 2C). End ring 46 μ m long, about 2.3 times longer than wide, consisting of con-

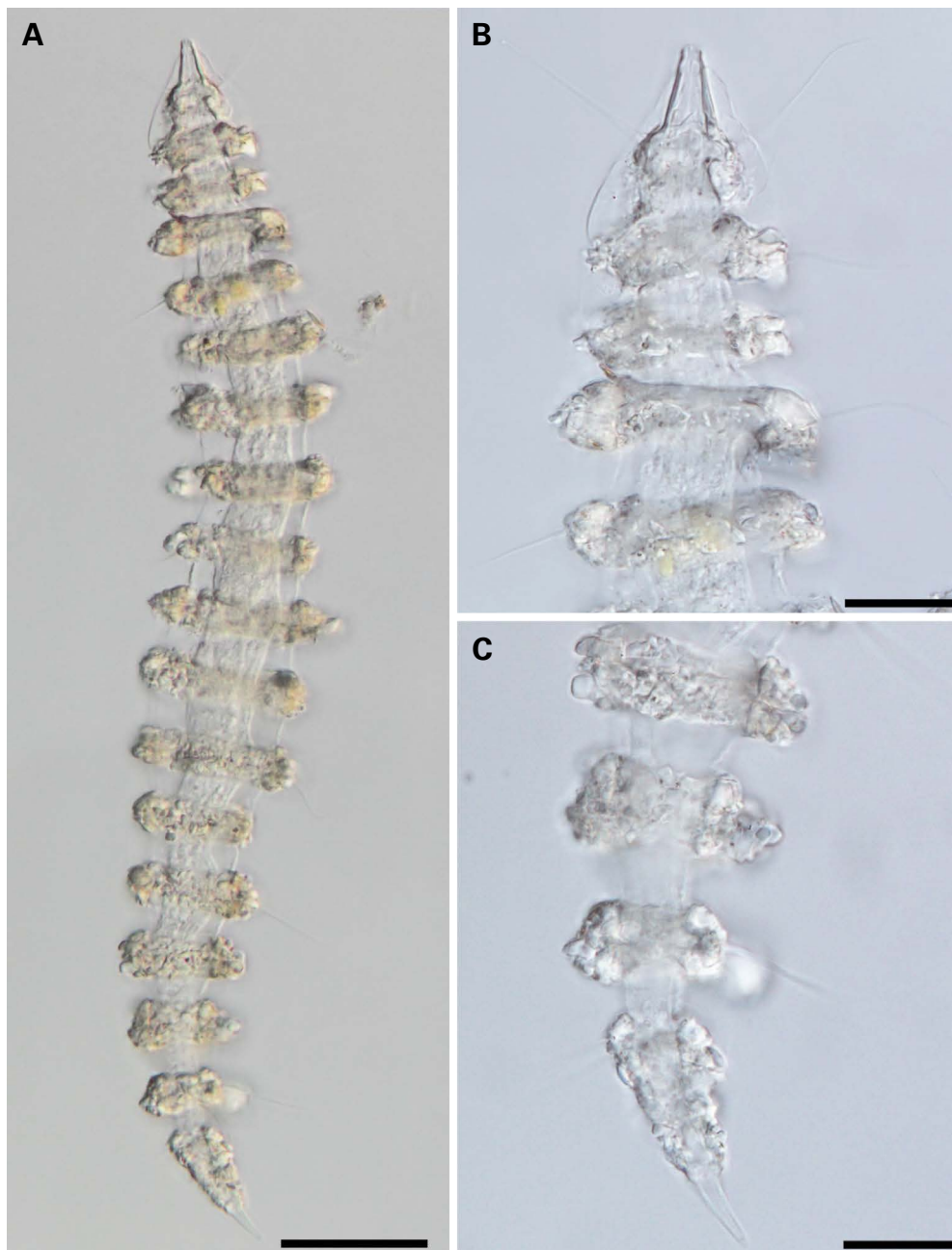


Fig. 2. *Desmoscolex (Desmoscolex) max* Timm, 1970, DIC photomicrographs, female in lateral view: A, Habitus; B, Anterior region; C, Tail region. Scale bars: 50 μm in A and 20 μm in B and C.

ical anterior part (32 μm long) and well-developed naked spinneret (14 μm long). Terminal somatic setae situated at level of anterior margin of end ring subventrally. End ring, with exception of terminal spinneret, totally covered with desmos. Caudal glands not observed.

Remarks. *Desmoscolex (D.) max* Timm, 1970 belongs to

the species group with 17 main rings and the untypical setal pattern. *Desmoscolex (D.) max* is mainly distinguished from its congeners in having the following characteristics: (1) the presence of untypical setae arrangement (eight subdorsal somatic setae and nine subventral somatic setae), (2) the presence of obviously elongated triangle-shaped

head, (3) the presence of long hairy cephalic setae, (4) the absence of peduncle at the base of somatic setae, and (5) end ring has a long naked spinneret. The present Korean specimen of *D. (D.) max* agrees well with Timm's (1970) original description, especially in having the elongated, triangle shaped head and a long naked spinneret in the end ring. However, the Korean specimens of *D. (D.) max* are not slightly accorded with Timm's (1970) original description in the number of subventral somatic setae as follows: compared to 10 of the original description, there are 9 of Korean specimen, and main ring 10 do not have subventral somatic setae in the Korean specimen.

Distribution. Galapagos Islands (Timm 1970, 1978), Moçambique Channel (Decraemer, 1984) and Korea (Present study).

Order Monhysterida Filipjev, 1929

Family Xyalidae Chitwood, 1951

Genus *Daptonema* Cobb, 1920

***Daptonema longiapophysis* Huang and Zhang, 2010**

(Figs. 3, 4; Table 1)

Daptonema longiapophysis Huang and Zhang, 2010, p. 391–394, figs. 1, 2.

Material examined. Specimens were collected from Ttangkkeutsongho Beach, Songji-myeon, Haenam-gun, Jeollanam-do, Korea, 27 May 2020 (leg. HS Rho and H Lee). One male (MABIK NA00157825), in glycerin on a HS slide, was deposited in the nematode collection at the specimen conservation room of the Marine Biodiversity Institute of Korea (MABIK), Seocheon, Korea. Three males (KIOST NEM-1-2673, KIOST NEM-1-2674, KIOST NEM-1-2675) in glycerin on HS slide, were deposited in the nematode collection at the specimen conservation room of the Bio-Resources Bank of Marine Nematodes (BRBMN), East Sea Research Institute, Korea Institute of Ocean Science & Technology (KIOST), Korea.

Habitat. Intertidal fine sand beach (34°18'54.00"N, 126°31'06.00"E).

Measurements. See Table 1 for detailed measurements and morphometric ratios.

Description. Males. Body cylindrical and slender (1,421–1,565 µm long), gradually tapering towards both extremities (Fig. 4A). Cuticle with coarse annulations, which begins in buccal cavity and extends to tail tip (Fig. 4C). Maximum body diameter at mid body level 39–44 µm wide. Head end spherical (18–21 µm wide), with six large and raised lips, each with six setiform labial sensilla. Six

Table 1. Morphometrics of Korean *Daptonema longiapophysis* specimens

	Male 1	Male 2	Male 3	Male 4	Mean ± sd
Total body length	1,428	1,465	1,421	1,565	1,470 ± 57.3
a	36	38	34	35	36 ± 1.5
b	4	4	4	4	4 ± 0.1
c	8	8	8	8	8 ± 0.2
Head diameter at cephalic setae level	18	19	21	19	19 ± 1.1
Body diameter at pharynx level	33	36	35	38	36 ± 1.8
Maximum body diameter	39	39	42	44	41 ± 2.2
Length of labial setae	4	5	5	4	5 ± 0.5
Length of the longer cephalic setae	16	18	18	18	17 ± 0.7
Length of the shorter cephalic setae	11	14	15	14	14 ± 1.5
Buccal cavity width	11	12	14	13	13 ± 1.3
Distance from anterior end to nerve ring	114	121	119	120	119 ± 2.7
Body diameter at nerve ring level	29	32	32	34	32 ± 2.0
Pharynx length	361	357	363	391	368 ± 13.4
Spicules length along the arc	23	27	28	25	26 ± 2.0
Spicule length along the chord	21	23	25	23	23 ± 1.7
Gubernaculum apophysis	25	26	25	25	25 ± 0.3
Anal body diameter	31	33	32	36	33 ± 1.8
Tail length	181	195	187	194	189 ± 5.4

All measurements are in µm (a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length).

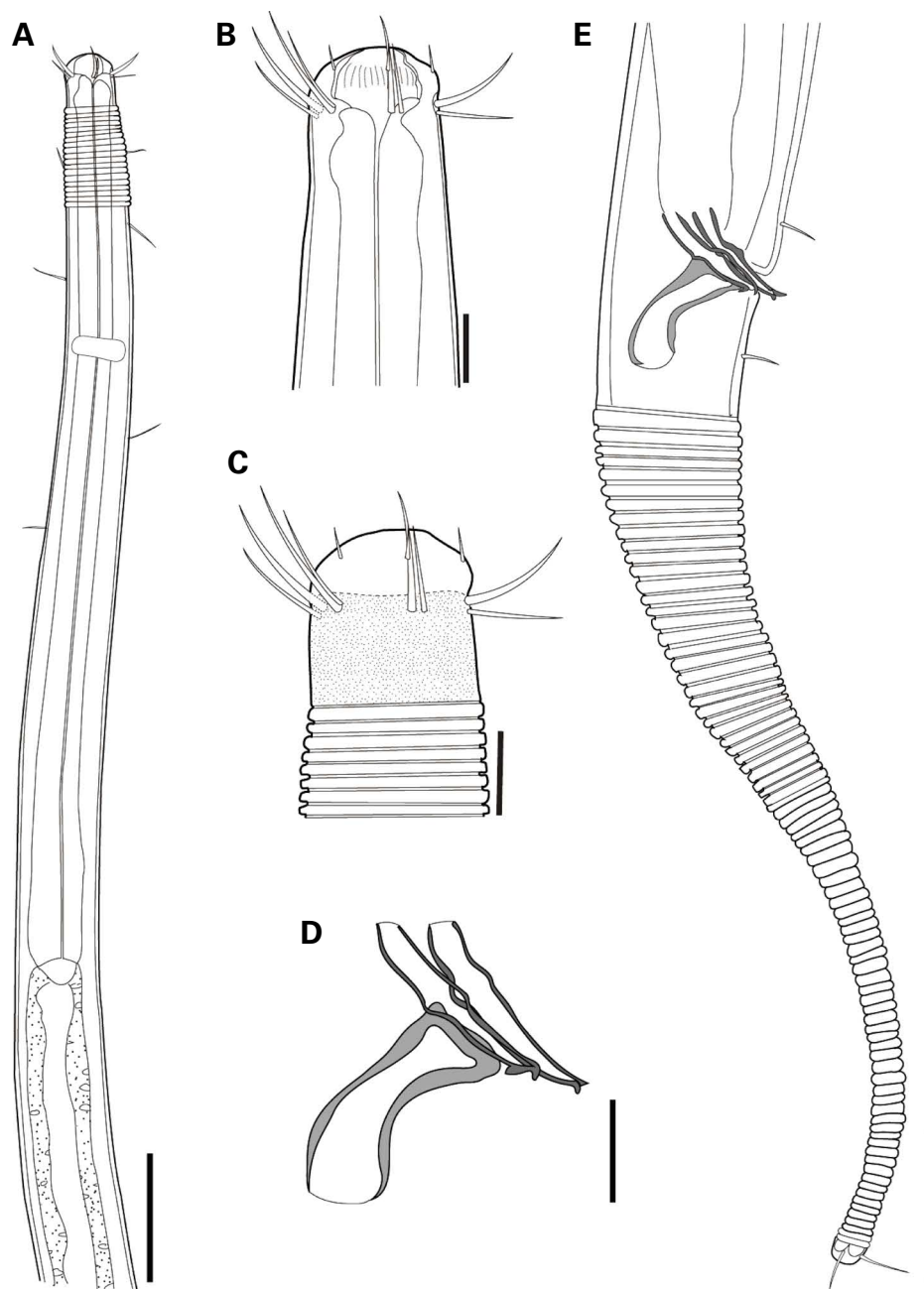


Fig. 3. *Daptonema longiapophysis* Huang and Zhang, 2010, male in lateral view: A, Anterior region; B, Head region; C, Head region (surface view); D, Spicules and gubernaculum; E, Spicules and tail region. Scale bars: 50 μ m in A and E and 10 μ m in B to D.

relatively short outer labial setae (11–15 μ m long) and six longer cephalic setae (16–18 μ m long) arranged in one circle (Fig. 3C). Buccal cavity spacious and conical (11–14 μ m wide) without teeth (Figs. 3B, 4B). Somatic setae thin filamentous, scattered throughout body. Amphideal fovea not visible. Pharynx cylindrical, 357–391 μ m long, about 24–26% of total body length. Pharyngo-intestinal junction

with cardia. Nerve ring encircling pharynx, situated at 31–34% of pharynx length from anterior end (Fig. 3A). Tail with anteriorly conical, tapering with distal third cylindrical, 181–195 μ m long. Tail tip bearing two terminal setae. Three caudal glands with common opening (Figs. 3E, 4F).

Spicules paired, slightly curved, and 23–28 μ m long arc, 21–25 μ m long chord. Both spicules' proximal end with

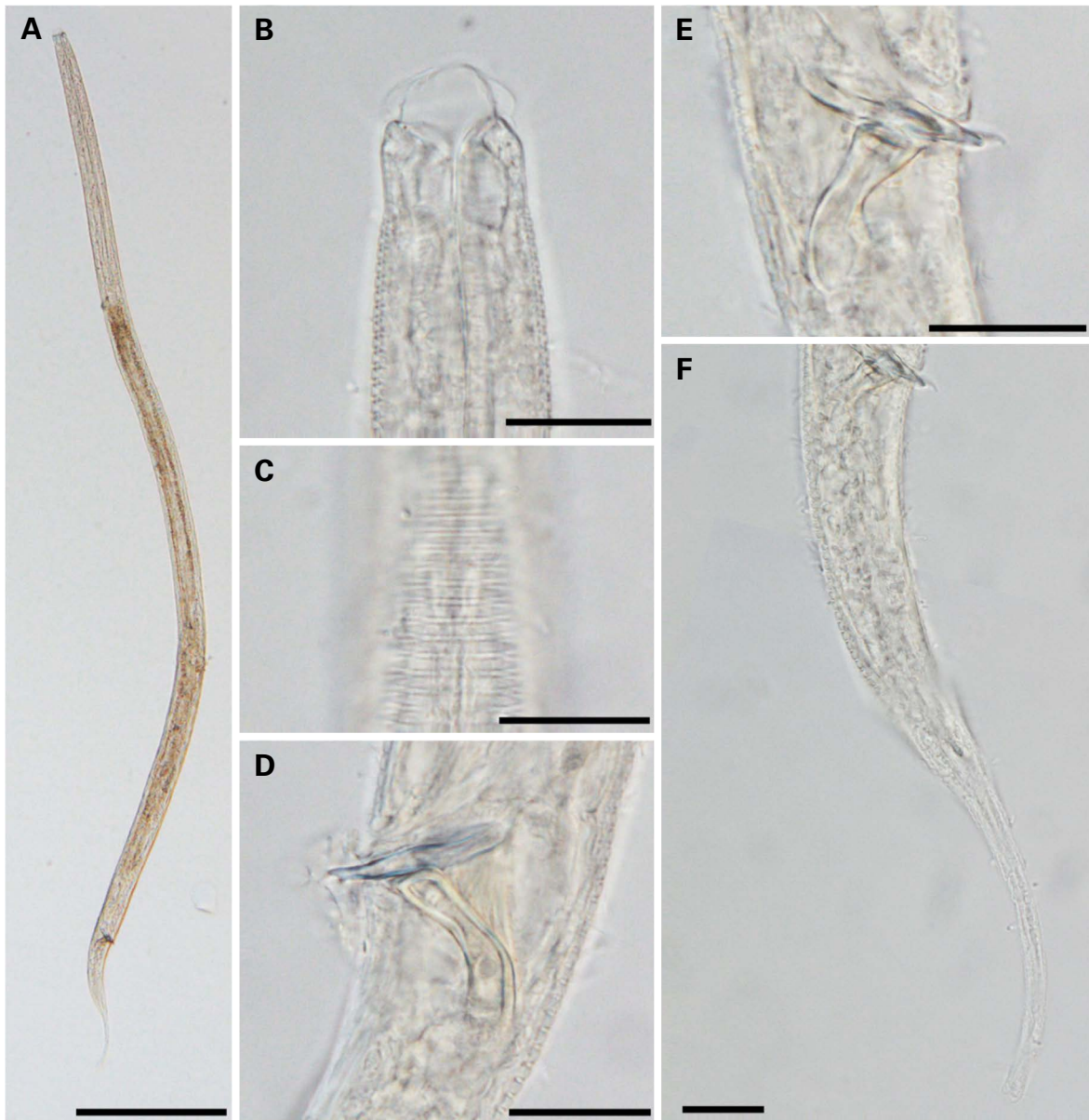


Fig. 4. *Daptonema longiapophysis* Huang and Zhang, 2010, DIC photomicrographs, males in latera view: A, Habitus; B, Head region; C, Cuticles (surface view); D, Spicules and gubernaculum (KIOST NEM-1-2675); E, Spicules and gubernaculum (MABIK NA00157825); F, Tail region. Scale bars: 200 μ m in A and 20 μ m in B to F.

capitulum, and distal end with bifid tip. Spicules with a projection in middle part each of dorsal and ventral side. Gubernaculum loop shaped (25–26 μ m long), parallel to spicule, with long dorso-caudal apophysis (Figs. 3D, 4D, E). One pre-cloacal and one post-cloacal setae present.

Remarks. *Datonema longiapophysis* was firstly described from intertidal sandy sediments on the Rizhao coast of Shandong province and the Huangdao coast of Qingdao

from the Yellow Sea, China. *Daptonema longiapophysis* is characterized by the presence of setiform labial sensilla, spicules with a projection on both sides, and gubernaculum with dorso-caudal apophysis longer than spicules length.

The present Korean specimens of *D. longiapophysis* are very similar to *D. deconincki* Sharma, 1985 in having the spicules with bifid distal tip and gubernaculum with long dorso-caudal apophysis (Sharma 1985). However, *D. longi-*

apophysis can be easily distinguished from *D. deconincki* by the relatively longer body length (1,421–1,565 μm vs. 942–1,157 μm in males), shorter spicules length (23–28 μm vs. 45–51 μm), and lower ratio of gubernaculum apophysis to spicules length (0.9–1.1 vs. 1.8). The present Korean specimens of *D. longiapophysis* are most like the original description by Huang and Zhang (2010), especially in the body size and structure, the shape of head and tail, the presence of spicules with a projection in the middle part, and the presence of long dorsal-caudal apophysis. However, the Korean specimens of *D. longiapophysis* are not accorded with the original description by having a bifid disal tip in the spicules.

Distribution. China (Huang and Zhang 2010) and Korea (Present study).

Order Monhysterida Filipjev, 1929

Family Xyalidae Chitwood, 1951

Genus *Pseudosteimeria* Wieser, 1956

***Pseudosteimeria sinica* Huang and Li, 2010**

(Figs. 5, 6; Table 2)

Pseudosteimeria sinica Huang and Li, 2010, p. 2454–2458, figs. 1, 2.

Material examined. Specimens were collected from Gamami Beach, Hongnong-eup, Yeonggwang-gun, Jeollanam-do, Korea, 19 Jun 2018 (leg. HG Kim and HJ Lee). One male (MABIK NA00157824), in glycerin on a HS slide, was deposited in the nematode collection at the specimen conservation room of the Marine Biodiversity Institute of Korea (MABIK), Seocheon, Korea. Two males (KIOST NEM-1-2676, KIOST NEM-1-2677) in glycerin on HS slide, were deposited in the nematode collection at the specimen conservation room of the Bio-Resources Bank of Marine Nematodes (BRBMN), East Sea Research Institute, Korea Institute of Ocean Science & Technology (KIOST), Korea.

Habitat. Intertidal mud-sand beach (35°23'55.90"N, 126°24'17.85"E).

Measurements. See Table 2 for detailed measurements and morphometric ratios.

Description. Males. Body slender, 1,297–1,580 μm long, gradually tapering towards both extremities (Fig. 6A). Cuticle transversely annulated, beginning at base of buccal cavity and extends to tail tip (Fig. 6C). Maximum body diameter at mid body level, 59–62 μm long. Head end slightly inflated with six lips. Labial region with six inner labial sensilla papilliform and 12 outer labial sensillae setiform arranged

Table 2. Morphometrics of Korean *Pseudosteimeria sinica* specimens

	Male 1	Male 2	Male 3	Mean \pm sd
Total body length	1,580	1,558	1,297	1,478 \pm 128.7
a	26	25	22	24 \pm 1.8
b	5	5	5	5 \pm 0.1
c	8	8	8	8 \pm 0.1
Head diameter at cephalic setae level	21	20	20	20 \pm 0.5
Body diameter at pharynx level	52	51	50	51 \pm 1.0
Maximum body diameter	61	62	59	61 \pm 1.1
Length of cephalic setae	9	9	7	8 \pm 0.7
Length of the longest subcephalic setae	51	51	43	48 \pm 3.9
Length of the shortest subcephalic setae	8	7	12	9 \pm 2.3
Buccal cavity width	10	12	12	11 \pm 0.9
Distance from anterior end to nerve ring	114	118	99	110 \pm 8.3
Body diameter at nerve ring level	42	40	36	39 \pm 2.7
Pharynx length	315	303	249	289 \pm 28.4
Right spicule length along the arc	52	47	48	49 \pm 2.3
left spicule length along the arc	60	62	57	60 \pm 2.3
Gubernaculum length	35	32	39	35 \pm 2.7
Anal body diameter	43	44	37	41 \pm 2.8
Tail length	194	191	163	183 \pm 13.9

All measurements are in μm (a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length).

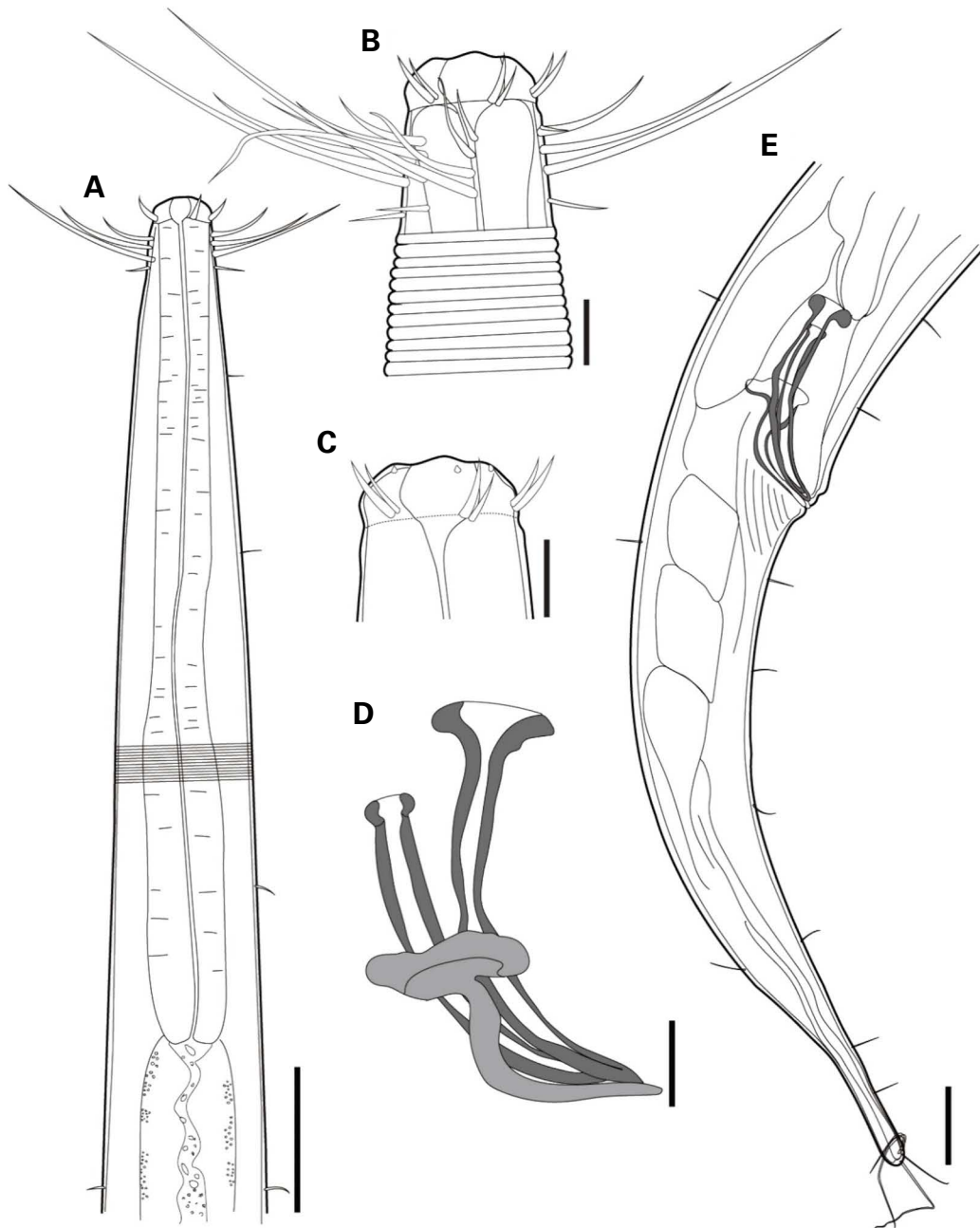


Fig. 5. *Pseudosteineria sinica* Huang and Li, 2010, males in lateral view: A, Anterior region; B, Head end including subcephalic setae; C, Lip region including labial and cephalic setae; D, Spicules and gubernaculum (KIOST NEM-1-2676); E, Spicules and tail region. Scale bars: 50 μm in A, 10 μm in B to D, and 20 μm in E.

in two circles. Six longer outer labial setae 7–9 μm long, six shorter cephalic setae 5–6 μm long. (Fig. 5C). Buccal cavity funnel-shaped, without teeth. Subcephalic setae arranged in eight longitudinal rows on both sides of body, and located at directly behind cephalic setae. Each group with three to five subcephalic setae, increasing gradually from anterior to

posterior setae in every row. Length of shortest subcephalic setae 7–12 μm long and longest one 43–51 μm long (Figs. 5B, 6B). Somatic setae scattered throughout body. Amphideal fovea not visible. Pharynx cylindrical, 249–315 μm long, about 19–20% of total body length. Pharyngo-intestinal junction with cardia. Nerve ring encircling pharynx,



Fig. 6. *Pseudosteineria sinica* Huang and Li, 2010, DIC photomicrographs, male in lateral view: A, Habitus; B, Head region; C, Cuticles (surface view); D, Spicule and gubernaculum (KIOST NEM-1-2676); E, Spicule and gubernaculum (MABIK NA00157824); F, Tail region. Scale bars: 200 μ m in A and 20 μ m in B to F.

situated at 36–40% of pharynx length from anterior end (Fig. 5A). Tail conico-cylindrical, 163–194 μ m long with distal fourth cylindrical part. Tail tip bearing three to four terminal setae; longest setae up to 32–42 μ m long (Figs. 5E, 6F). Three caudal glands observed.

Spicules paired, slightly curved, and unequal in length. Left spicule longer (57–62 μ m long as arc) than right spicule (47–52 μ m long as arc), and left spicule with a slight contraction in middle part. Both spicules' proximal end with large capitulum and distal taper tip. Gubernaculum arcuated, tapered distal part, with dorso-caudal apophysis (Figs. 5D, 6D, E). Precloacal supplements absent.

Remarks. The genus *Pseudosteineria* was first erected by Wieser (1956), and currently 16 valid species have been

reported from all over the world (Wieser 1956; Tchesunov 2000; Huang and Li 2010; Sun *et al.* 2019; Cidreira *et al.* 2020). *Pseudosteineria sinica* Huang and Li, 2010 was firstly described from intertidal sandy sediment at Rizhao coast of the Yellow Sea, China. *Pseudosteineria sinica* is characterized by unobservable amphideal fovea, different length of spicules: left spicule divided into two sections jointed in the middle and longer than right spicule, both spicules with proximal capitulum, tapered distally, and gubernaculum with dorso-caudal apophysis.

The present Korean specimens of *P. sinica* are very similar to *P. inaequispiculata* (Platonova 1971) by unequal spicules, but *P. inaequispiculata* are clearly distinguished by having round amphideal fovea and the absence of guber-

naeculum apophysis (Platonova 1971). The present Korean specimens are most like the original description by Huang and Li (2010) in the following features, such as the absence of amphideal fovea, the presence of different length of spicules, and gubernaculum with dorso-caudal apophysis. However, the Korean specimens of *P. sinica* are not slightly accorded with the original description by the number of subcephalic setae (three to five vs. three to four), and shorter length of the shortest subcephalic setae (8–12 μm vs. 15–18 μm).

Distribution. China (Huang and Li 2010) and Korea (Present study).

CRedit authorship contribution statement

Hyo Jin Lee: Data curation and Writing-Original draft preparation. Heegab Lee: Investigation. Hyun Soo Rho: Writing-Reviewing, Editing and Funding acquisition.

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