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Redescription of the Free-living Marine Nematode Species, *Draconema japonicum* Kito, 1976 (Nematoda: Draconematidae), by Scanning Electron Microscopy

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Key Words:

Free-living
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SEM
Korea

Numerous specimens of adults and juveniles of *Draconema japonicum* Kito, 1976 have been found in intertidal and subtidal sediments and various algae around coast of South Korea. *Draconema japonicum* is recognized by the following characteristics: having an elongated loop-shaped amphideal fovea in male and horseshoe-shaped amphideal fovea in female, two pairs of uniformly tapered sublateral anal setae and two pairs of unevenly tapered subventral anal setae in male, eight to ten pairs in male and 13 to 15 pairs in female of posterior sublateral adhesion tubes, and five to six pairs of somatic setae on non-striated tail region. Scanning electron photomicrographs of the species are presented with a detailed morphological description and a key to the species of the genus *Draconema* Cobb, 1913. This is the first discovery of *D. japonicum* outside the Japanese waters.

Draconematidae is a small family of marine nematodes, including 14 genera, and occurs from the various habitats, from the pole to the equator and from the exposed intertidal sands to deep-sea hydrothermal vents (Decraemer et al., 1997). They are also quite common in algal habitats. Since the discovery of the draconematid species by the Claparéde in 1863, about 70 draconematid species belonging to 14 genera of 2 subfamilies have been recognized. Genus *Draconema* Cobb, 1913 is the most representative and type genus of draconematid nematodes. Presently seven species of the genus are known as valid in the world: *D. antarcticum* Allen and Noffsinger, 1978, *D. cephalatum* Cobb, 1913, *D. chilense* Allen and Noffsinger, 1978, *D. claparedii* (Mechnikov, 1876), *D. haswelli* (Irwin-Smith, 1918), *D. japonicum* Kito, 1976, and *D. ophicephalum* (Claparéde, 1863) (Allen and Noffsinger, 1978; Decraemer et al., 1997). Of these, *D. japonicum* was firstly described on the basis of the specimens collected from washing seaweeds of a *Sargassum* community from Oshoro on the Japan Sea coast in Hokkaido, Japan by Kito (1976). This species has the closest resemblance with an American species, *D. cephalatum*. When studying draconematid nematofauna in South Korea, we found out that *D. japonicum* was widely distributed in South Korea. However, any taxonomic study on *D. japonicum* has not yet been performed in South Korea. The present report contains redescription of the species with illustrations and scanning electron microscope (SEM) photomicrographs, in addition to a key to the species of the genus *Draconema*. This is the first discovery of *D. japonicum* outside the Japanses waters.

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Materials and Methods

The nematodes were obtained from the washings of intertidal and subtidal sediments and various algae, which were collected from three to 35 m deep by SCUBA diving on the eastern, southern, and western coast of South Korea. Samples were filtered in the field through a plankton net (67 μm in pore diameter) after freshwater rinsing for less than a minute for osmotic shock (Kristensen, 1989), and then fixed in 5% formalin. Specimens were mounted in anhydrous glycerin between two coverslips on H-S slides (Shirayama et al., 1993), and measured and photographed using a differential interference contrast (DIC) microscope (Olympus BX-60) equipped with Nomarski optics. All drawings were made with the aid of a camera lucida.

Several specimens were prepared for SEM examination. Five males and four females selected for SEM were prefixed overnight at 4°C in a 2.5% glutaraldehyde, followed by post fixation with 1% osmium tetroxide. After dehydration through a graded series of ethanol (50%-100%, 10% intervals) for 30 min each, the materials were critical point dried, coated with gold-palladium, and

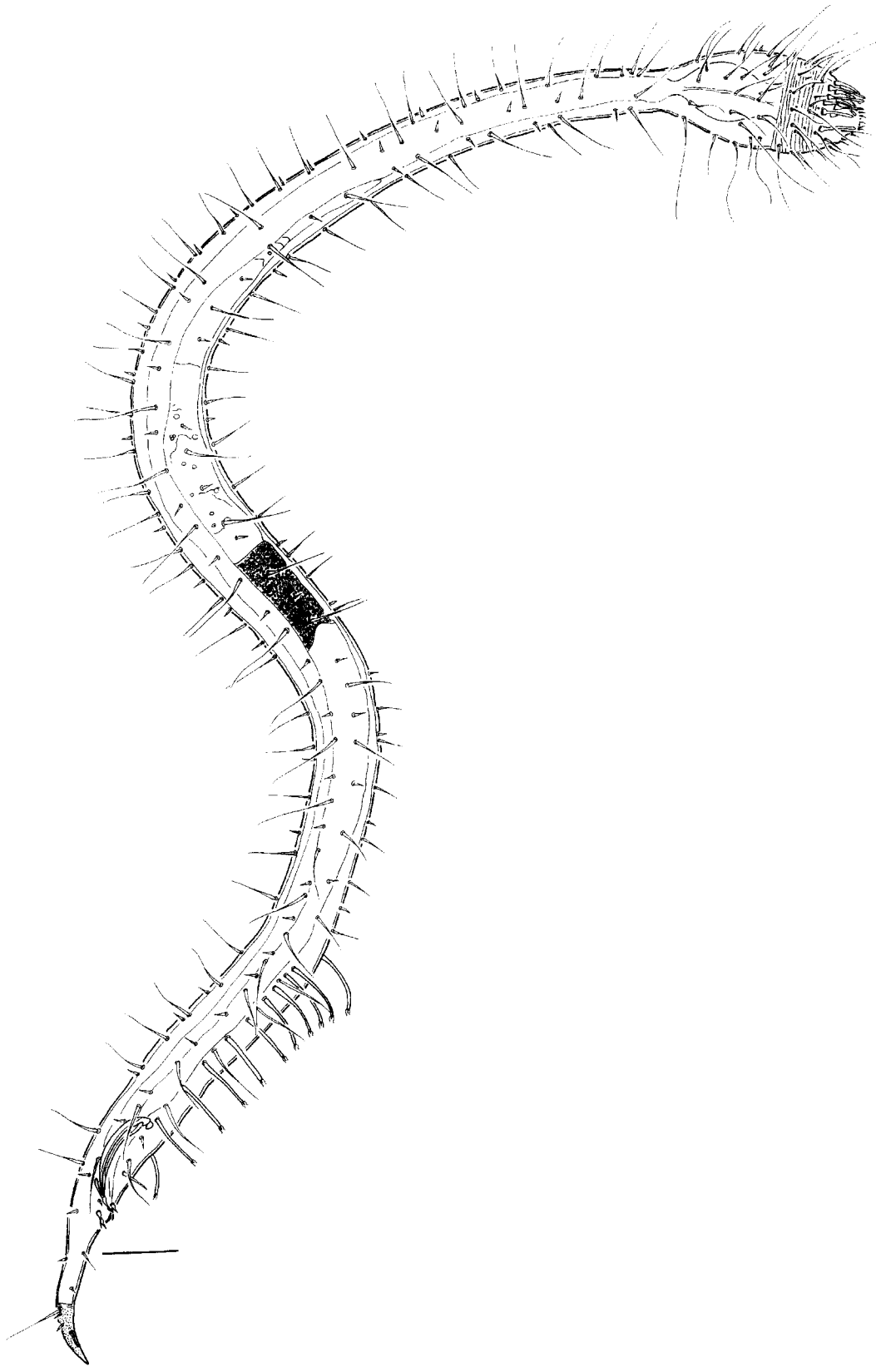

Fig. 1. *Draconema japonicum* Kito, 1976, male habitus, lateral vew. Scale bar=50 µm.

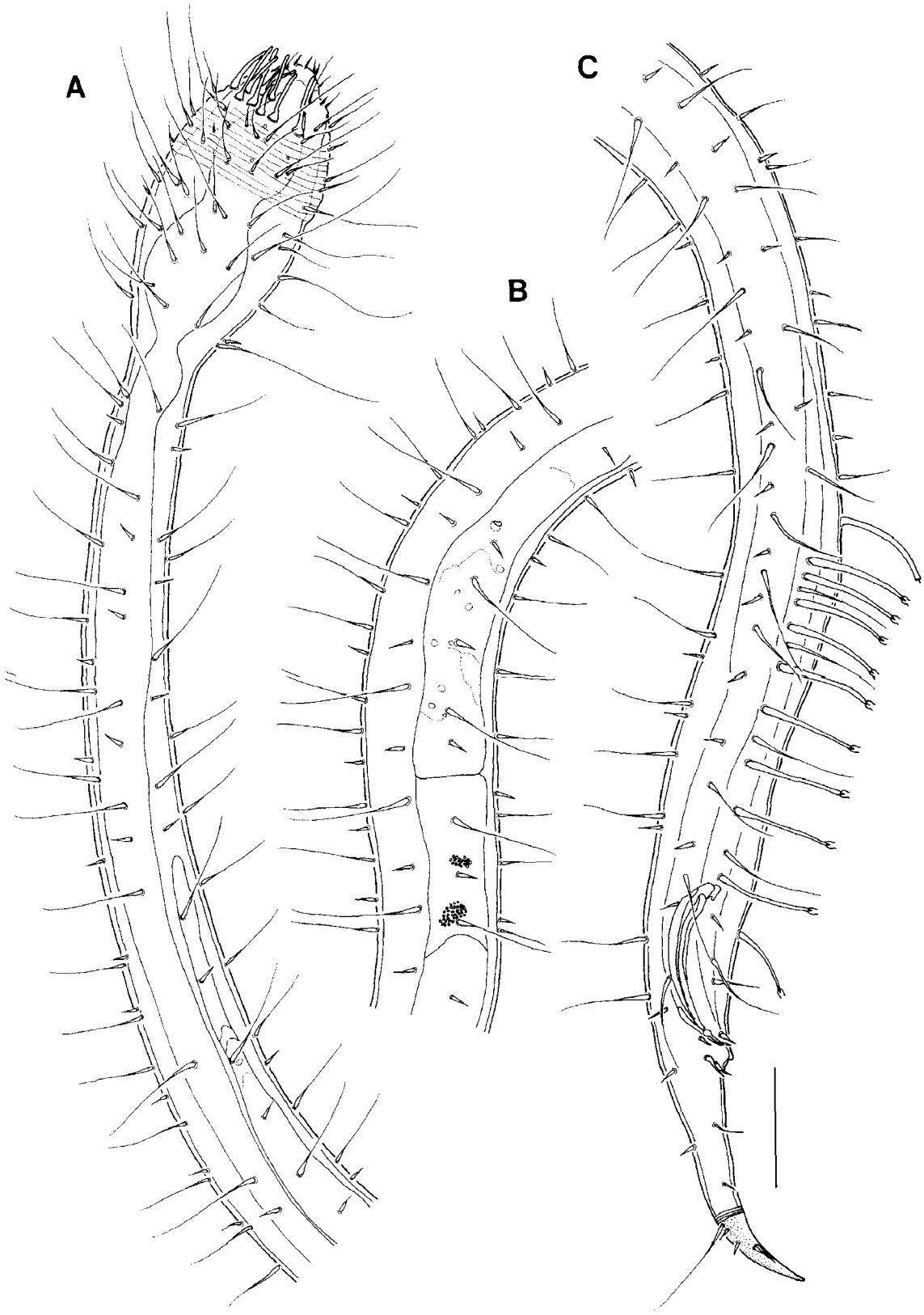

Fig. 2. *Draconema japonicum* Kito, 1976, male. A, Anterior region, lateral vew. B, Middle region, lateral view. C, Posterior region, lateral view. Scale bar=50 μm.

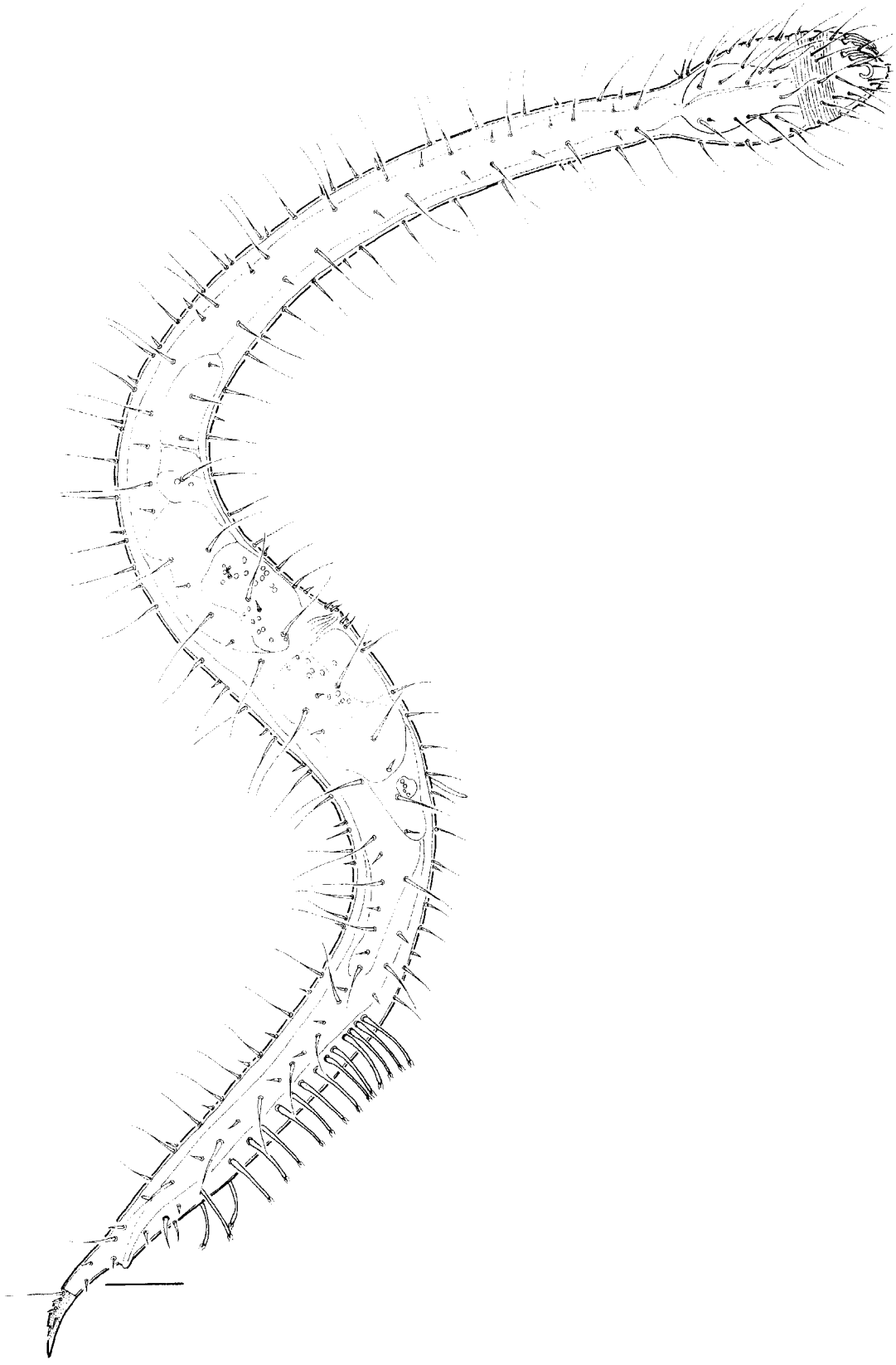

Fig. 3. *Draconema japonicum* Kito, 1976, female habitus, lateral vew. Scale bar = 50 μm.

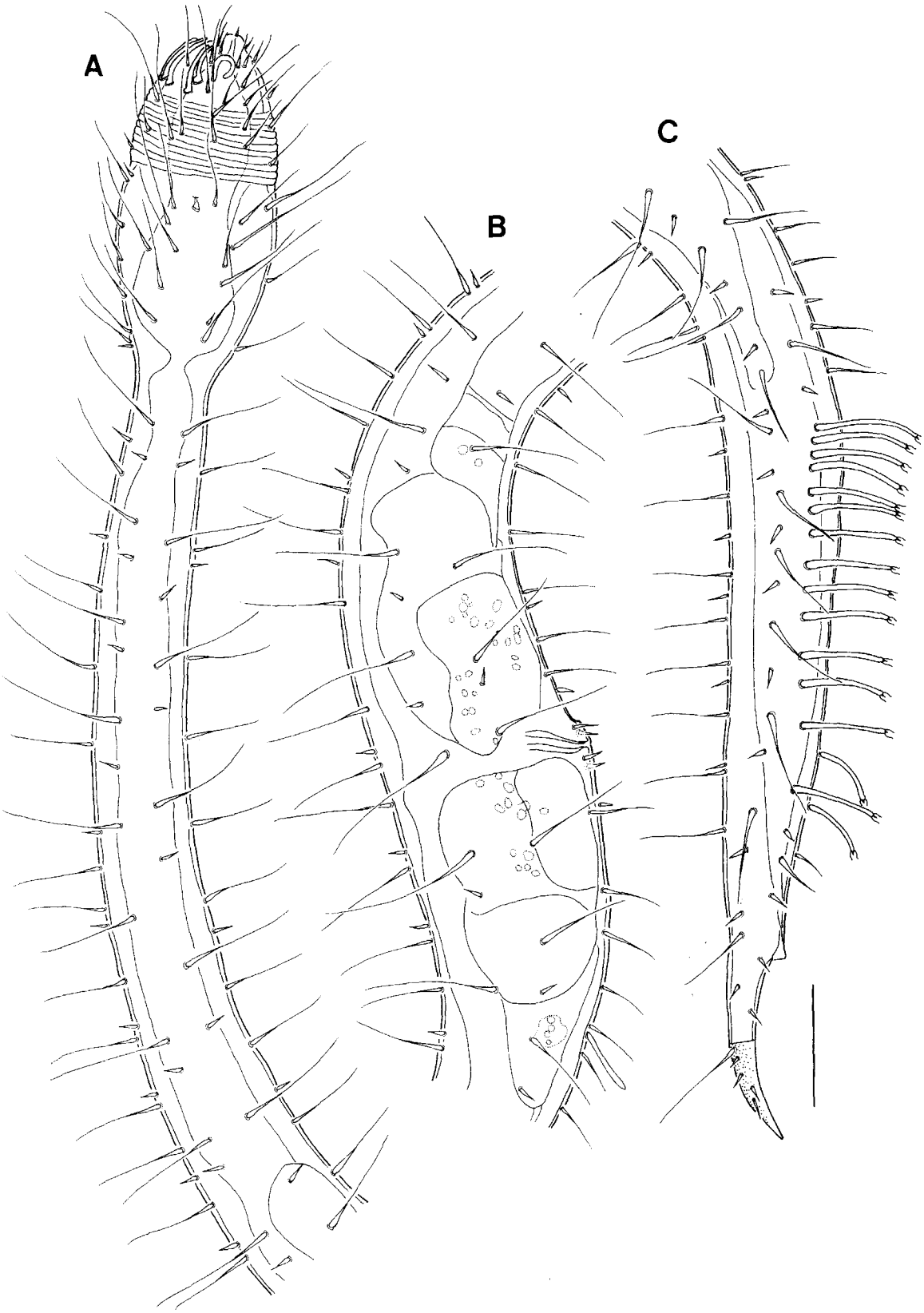

Fig. 4. *Draconema japonicum* Kito, 1976, female. A, Anterior region, lateral vew. B, Middle region, lateral view. C, Posterior region, lateral view. Scale bar=50 μm.

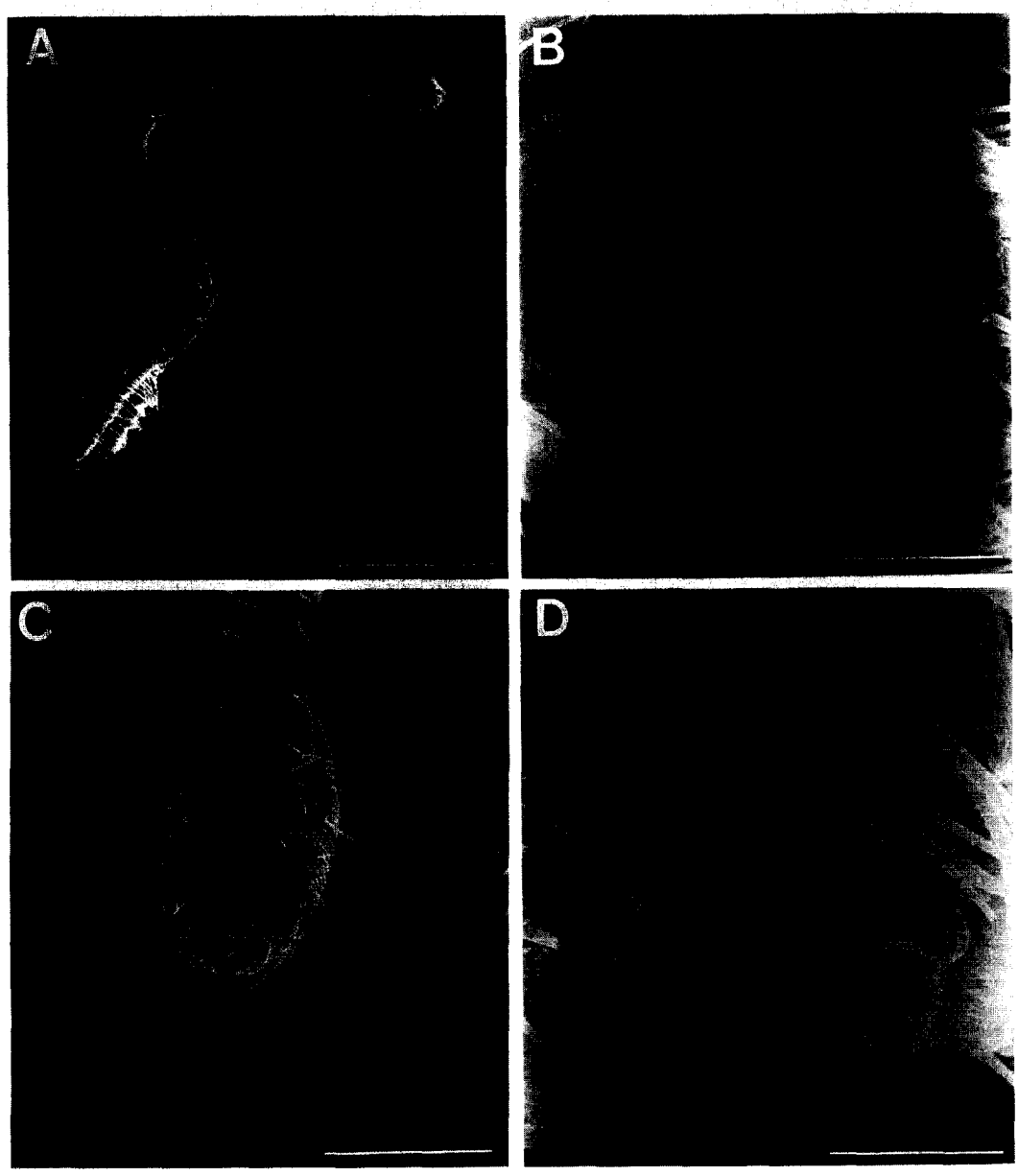

Fig. 5. *Draconema japonicum* Kito, 1976, male. A, Habitus, lateral view. B, Differentiated annules, lateral view. C, Head region, lateral view. D, Amphideal fovea, lateral view. Scanning electron micrographs. Scale bars=222 µm (A), 18.8 µm (B), 55 µm (C), and 8.6 µm (D).

examined in a Hitachi S-520 scanning electron microscope.

Terminology mostly follows Decraemer (1989). Abbreviations used in the text are as follows: abd = anal body diameter; CAT = cephalic adhesion tubes; gub = length of gubernaculum; L = body length; mbd Ph = maximum body diameter in pharyngeal region; (mbd) = minimum body diameter; mbd = maximum body diameter at mid body level; PAT = posterior adhesion tubes; ph = length of pharynx; spic = length of spicule measured along the median line; SlATl = length of sublateral adhesion tubes; SlATn = number of sublateral adhesion tubes; SvATl = length of subventral adhesion tubes; SvATn = number of subventral adhesion tubes; t = tail length; tmr = length of non-striated tail end; V = position of the vulva as a percentage of the total body length from anterior; a, b, c = proportions of de Man. Scale bars in figures are indicated in µm.

Description

Family Draconematidae Filipjev, 1918
Genus *Draconema* Cobb, 1913
Draconema japonicum Kito, 1976
(Figs. 1-8)

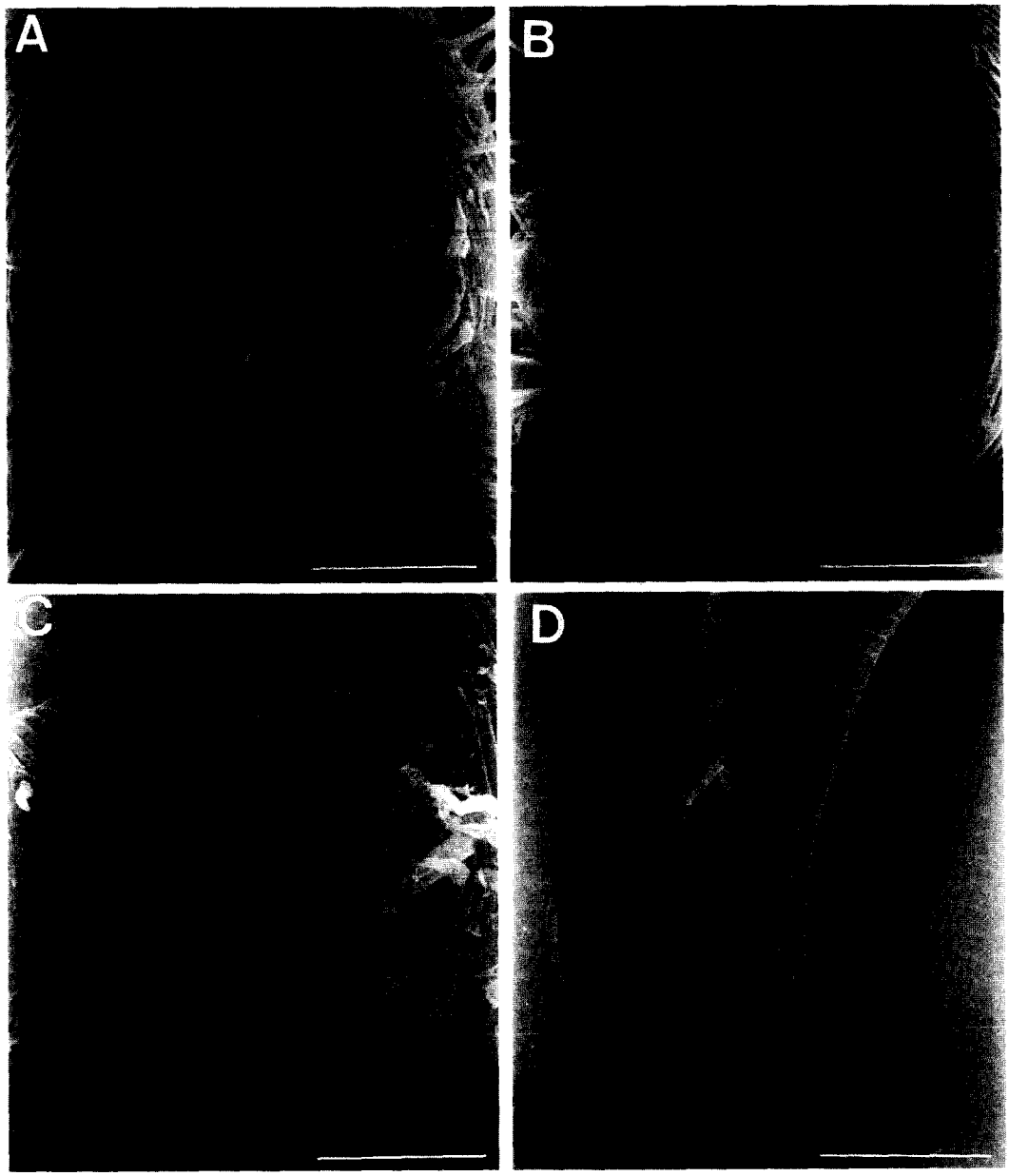

Fig. 6. *Draconema japonicum* Kito, 1976, male. A, Mouth region, enface view. B, Cephalic adhesion tubes, dorsal view. C, Cloacal opening and precloacal setae, ventral view. D, Non-striated tail end, lateral view. Scanning electron micrographs. Scale bars=17.6 μm (A), 18.8 μm (B), 12 μm (C), and 10 μm (D).

Draconema japonicum Kito, 1976, p. 573, Fig. 3; Kito, 1979, p. 88, Figs. 1-5.

Material examined: 8 inds., Gyoungju, Bonggil, (35°44′51″N, 129°29′07″E), 4 Sep. 1996, H. S. Rho and S. H. Kim; 15 inds., Jeju Is., Hyeobjae (33°23′42″N, 126°14′29″E), 23 Aug. 1998, H. S. Rho and S. H. Kim; 6 inds., Udo Is., Sanhosa beach (33°29′07″N, 126°56′69″E), 26 Aug. 1998, H. S. Rho and S. H. Kim; 35 inds., Jeju Is., Beomseom (33°13′21″N, 126°30′34″E), 3 Mar. 2000, H. S. Rho and S. H. Kim; 5 inds., Jeju Is., Seongsanpo (33°27′91″N, 126°56′25″E), 9 Jun. 2001, H. S. Rho and S. H. Kim; 30 inds., Ulleung Is., Gadubong (37°30′29″N, 130°55′65″E), 20 Oct. 2001, H. S. Rho and S. H. Kim; 10 inds, Taean, Chaesukpo (33°29′07″N, 126°56′69″E), 13 Feb. 2003, H. S. Rho and J. W. Choi. All are mounted in anhydrous glycerine between two coverslips on H-S slides, sealed with nail polish. Five males and four females are gold-coated on the aluminum stub for SEM preparation. Specimens are kept in the author's collection at the specimen room of the School of Biological Sciences,

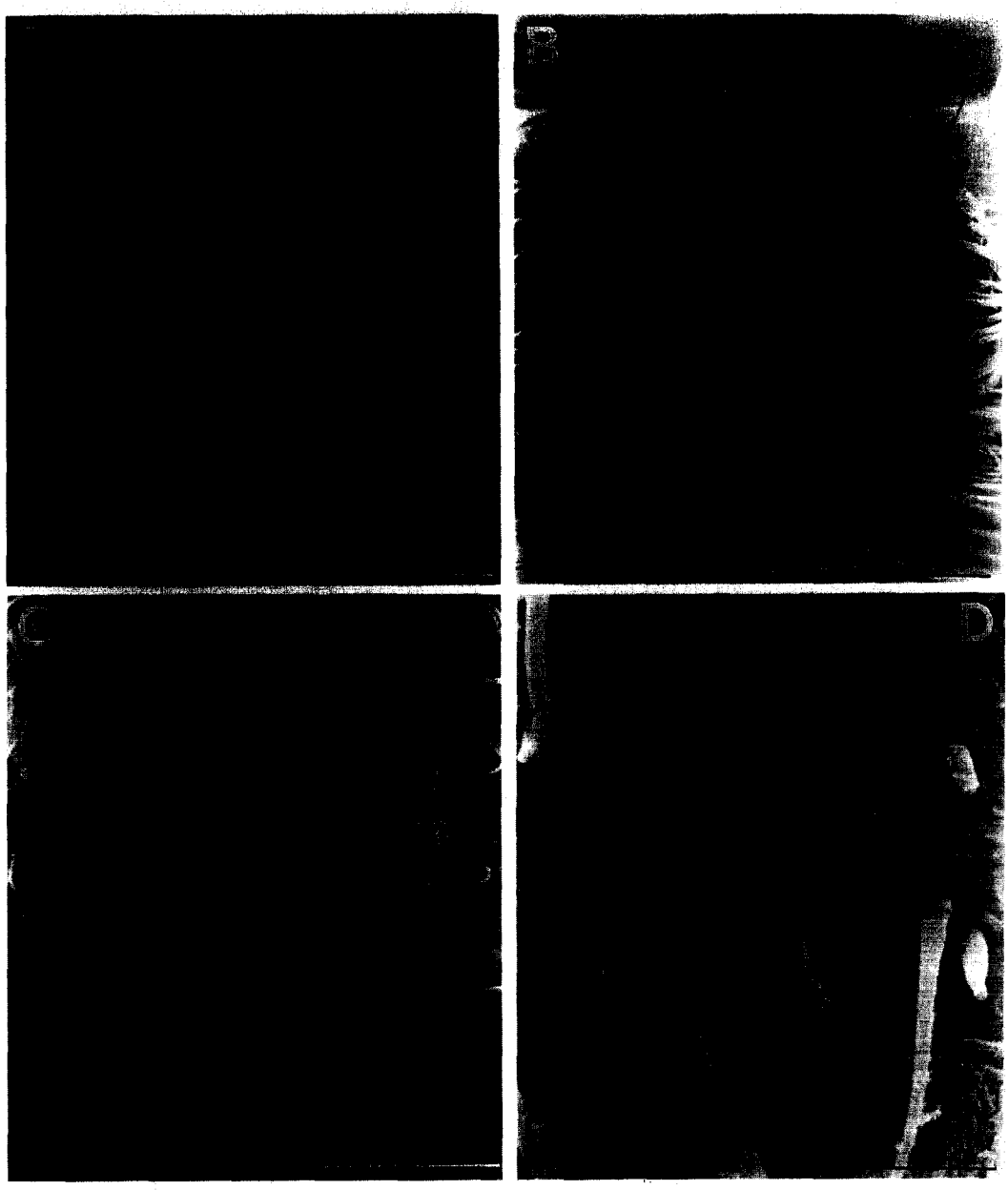

Fig. 7. *Draconema japonicum* Kito, 1976, female. A, Habitus, lateral view. B, Differentiated annules, dorsal view. C, Head region, oblique enface view. D, Amphideal fovea, lateral view. Scanning electron micrographs. Scale bars=200 μm (A), 28 μm (B), 25.2 μm (C), and 6 μm (D).

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Habitats: The nematodes were obtained from the washings of intertidal and subtidal sediments and various algae on the eastern, southern and western coast of South Korea.

Male: Body characteristic for *Draconema* in general, slender, with strongly swollen pharyngeal region, 4.5% of total body length; greatest body width at level of pharyngeal region in both sexes; cylindrical long trunk and tail ending in conical tip (Figs. 1A, 5A). Cuticle striated; annules widest in pharyngeal region; anterior 8-12 annules conspicuously differentiated (Figs. 2A, 5B), about 2 μm wide and those of following part about 1 μm wide; mid-body striae and tip of tail smooth.

Somatic setae densely distributed on swollen region (Fig. 5C). Somatic setae, hair-like with broadened base, varying in length from mainly long (63 μm) to very short (8 μm) setae, with marked insertions and more or less arranged in ten longitudinal rows in pharyngeal region and arranged in eight longitudinal rows on following

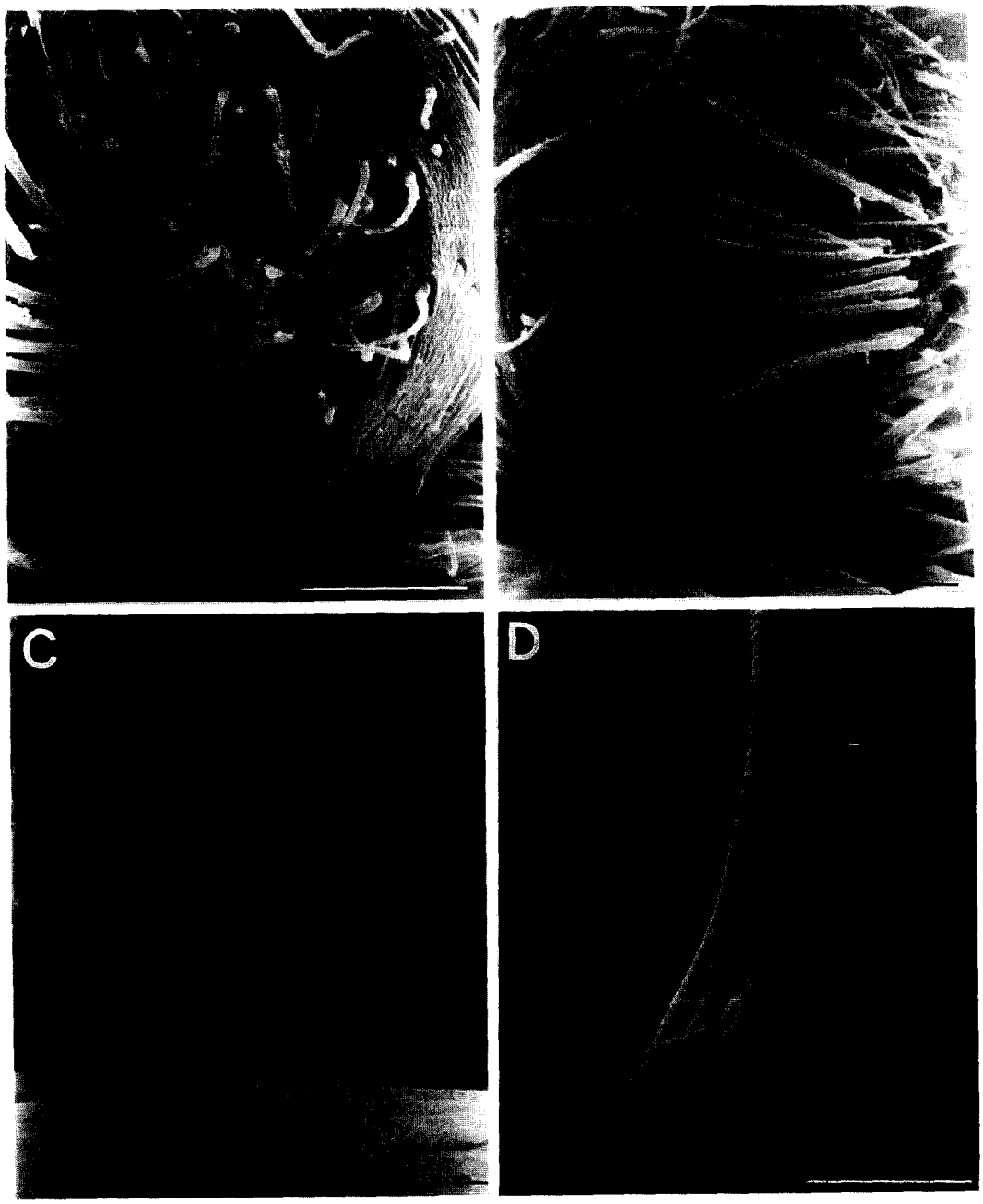

Fig. 8. *Draconema japonicum* Kito, 1976, female. A, Mouth region, enface view. B, Cephalic adhesion tubes, dorsal view. C, Vulva and paravulval setae, ventral view. D, Non-striated tail end, lateral view. Scanning electron micrographs. Scale bars=12 μm (A), 15 μm (B), 8.6 μm (C), and 18.8 μm (D).

middle part of body, 4 sublateral, 2 subdorsal and 2 subventral; ventral rows of setae shorter than others rows of setae at middle part of body (Fig. 2B). Longest somatic setae (63 μm) on swollen anterior part. Each kind of characteristic tubular setae (cephalic adhesion tubes and posterior adhesion tubes) arranged on dorsal side of head and ventral preanal part.

Rostrum with fine vacuolar ornamentation near posterior border. Lip region introverted in most fixed specimens, when extruded, surrounded by six low lips, each bearing short six external labial setae, 6 μm long (Fig. 6A). Amphideal fovea large, elongated loop-shaped, ventral arm, extending posteriorly, longer than dorsal arm (Fig. 4D). On dorsal surface between both amphideal fovea twelve stout cephalic adhesion tubes arranged in two transverse rows of six pairs of tubular setae; bending ventrally and enlarged at base, up to 27 μm long; setae of posterior rows longer than anterior setae and arranged

almost at boundary of coarse annuels (Fig. 6B). Subcephalic setae in two transverse rows, one shortly behind cephalic setae and second row near posterior head end. Four cephalic setae, 20 μm long with marked insertion near anterior end of helmet.

Stoma narrow, unarmed. Pharynx with enlarged corpus, separated by short isthmus from muscular posterior bulb; nerve ring at level of isthmus. Cardia well developed. Intestine narrow cylindrical, straightforward, gradually widening posteriorly, and lying dorsal of genital system. Excretory organ not observed.

Reproductive system typical of the Draconematidae, with single anterior testis. Testis outstretched; started at about 23% of body length from anterior end. Spicules 93 μm long, slightly arcuate; corpus very slender, slightly wider at both extremities and ventrally with weakly sclerotized velum; capitulum offset, with ventral apophysis (Fig. 2C). Gubernaculum 23 μm long, thin, parallel to spicules; distally dilated around spicules end from outside. Anal setae composed of two pairs of uniformly tapered sublateral anal setae and two pairs of unevenly tapered subventral anal setae; two pairs anterior and two pairs posterior to anus (Fig. 6C). Anal flap short, 2.5-3 μm long, not crenate.

All posterior adhesion tubes with well marked bell-shaped end and situated precloacally. Posterior adhesion tubes arranged on four longitudinal rows: two sublateral rows each consisting of nine (left side) and nine (right side) adhesion tubes, and four intermingled somatic setae; two subventral rows each consisting of 17 (left side) and 18 (right side) adhesion tubes, without intermingled somatic setae. Posterior adhesion tubes becoming slightly shorter caudally.

Tail gradually tapering to conical smooth tip end, 30 μm anal diameter long. Non-striated tail end 41% of total tail length; with five pairs of somatic setae: a pair of longest setae and two pairs of small setae inserted on subdorsally and two pair of small setae inserted on sublaterally (Fig. 6D). Its cuticle finely vacuolated.

Female: Similar to male in most respect (Figs. 3A, 7A, 8A, 8B). Strongly swollen pharyngeal region, 10% of total body length. Cuticle striated; annules widest in pharyngeal region; anterior 8-12 annules conspicuously differentiated (Figs. 4A, 7B). Body diameter at level of vulva wider than that on swollen pharyngeal region in well-grown individual.

Digestive system as in male. Ovaries paired, opposed and reflexed (Fig. 4B). Vagina short, bipartite with sclerotized distal part; vulva near mid-body, not encircled by any projections, and two pairs of paravulval setae present, 10 μm long (Fig. 8C).

Amphideal fovea usually large, horseshoe-shaped with distal ends of arms open (Fig. 7C, D).

All with well marked bell-shaped end and situated precloacally (Fig. 4C). Posterior adhesion tubes arranged on four longitudinal rows: two sublateral rows each consisting of 14 (left side) and 14 (right side) adhesion tubes, and two subventral rows each consisting of 16 (left side) and 16 (right side) adhesion tubes, without intermingled somatic setae. Posterior adhesion tubes becoming slightly shorter caudally.

Anal flap short, 2.5-3 μm long, not crenate. Non-striated tail end 22% of total tail length, with five pairs of somatic setae: a pair of longest setae and two pairs of small setae situated on subdorsally and two pair of small setae situated on sublaterally (Fig. 8D).

Male measurements: L=1433, mbd = 53, (mbd) = 27, mbd Ph = 65, ph = 122, abd = 30, t = 123, tmr = 50, spic = 93, gub = 23, SlATl = 50, SlATn = 9, SvATl = 50, SvATn = 17-18, a = 27, b = 12, c = 12.

Female measurements: L=1300, mbd = 90, (mbd) = 32, mbd Ph = 67, ph = 133, abd = 23, t = 97, tmr = 50, SlATl = 47, SlATn = 14, SvATl = 33, SvATn = 15, a = 14, b = 10, c = 13, V = 56.

Variations: Some differences are observed from the Korean specimens in the number of somatic setae on non-striated tail end in male (the Korean specimens have five or six pairs of somatic setae on non-striated tail end, while the type specimen has only six pairs of setae on non-striated tail end) and the number of posterior sublateral adhesion tubes in male (eight to ten pairs of posterior sublateral adhesion tubes in the Korean specimens vs only nine posterior sublateral adhesion tubes in the type specimen). No other significant variation is observed amongst our specimens examined.

Remarks: A total of seven species are currently recorded in the genus *Draconema*: *D. antarcticum* Allen and Noffsinger, 1978, *D. cephalatum* Cobb, 1913, *D. chilense* Allen and Noffsinger, 1978, *D. claparedii* (Mechnikov, 1876), *D. haswelli* (Irwin-Smith, 1918), *D. japonicum* Kito, 1976, and *D. ophicephalum* (Claparéde, 1863) (Allen and Noffsinger, 1978; Decraemer et al., 1997). The classification of the genus *Draconema* depends on the character combinations such as the shape of amphideal fovea in both sexes, the number and arrangement of posterior sublateral and subventral adhesion tubes in both sexes, the shape and number of anal setae in male, the number and position of setae on tail region in both sexes, and the shape of female gonophore (Allen and Noffisinger, 1978). *Draconema japonicum* is easily distinguished from its congeners in having the following character combination: (1) presence of eight to 12 differentiated anterior annules, (2) having a elongated loop-shaped amphideal fovea in male and horseshoe-shaped amphideal fovea in female, (3) presence of a pair of strongly dilated gubernaculum around distal part of spicules, which is most characteristic in this species, (4) the number and arrangement of the precloacal setae in

male (the precloacal setae is composed of two pairs of uniformly tapered sublateral anal setae and two pairs of unevenly tapered subventral anal setae), and (5) the presence of eight to ten pairs in male and 13 to 15 pairs in female of posterior sublateral adhesion tubes. Our specimens fit well with Kito's (1976) original description except some minor discrepancies as mentioned in the "Variations" section. The reported mouth opening of *Draconema japonicum* by Kito (1976) has six low lips, each bearing a short labial seta and two chitinous ribs. During the present investigation, however, we observed only six low lips and six labial setae in the Korean specimens of *D. japonicum* with the scanning electron microscope and recognized that the exact observation on mouth opening is so difficult to notice with the light microscope, due to the lateral body position in *D. japonicum*.

During an investigation of the free-living marine draconematid nematofauna in South Korea, we found out that *D. japonicum* was widely distributed in South Korea as the dominant nematode species. As indicated in the specific name, *D. japonicum* was originally described on the basis of the specimens collected from washing seaweeds of a *Sargassum* community from Oshoro on the Japan Sea coast in Hokkaido, Japan, and this taxonomic report based on morphological characters using scanning electron microscope is the first record of *D. japonicum* outside the Japanese waters.

Distribution: Japan (Kito, 1976, 1979) and South Korea.

A key to the species of the genus *Draconema* Cobb, 1913

1. Male with 14 to 19 SIAT.
   Female with 18 to 24 SIAT 2
   Male with 7 to 10 SIAT.
   Female with 9 to 16 SIAT 3
2. All anal setae unevenly tapered in male .. *D. chilense*
   All anal setae uniformly tapered in male
   ..... *D. ophicephalum*
3. Two pairs of sublateral anal setae uniformly tapered and two pairs of subventral anal setae unevenly tapered in male 4
   All anal setae uniformly tapered in male 5
4. Gubernaculum dilated around distal part of spicule
   ..... *D. japonicum*
   Gubernaculum not dilated around distal part of spicule ..... *D. cephalatum*
5. Anal flap not crenated in male 6
   Anal flap slightly crenated in male ..... *D. haswelli*
6. Males and females with long setae on non-striated tail end ..... *D. antarcticum*
   Males and females without long setae on non-striated tail end ..... *D. claparedii*

Acknowledgements

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