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A new species of *Bestiolina* (Crustacea: Copepoda: Calanoida) from the Yellow Sea, with notes on the zoogeography of the genus

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Abstract.—A new species allocated to the genus *Bestiolina* Andronov, 1991 is described from the Yellow Sea. *Bestiolina coreana*, new species is closely related to *Bestiolina sinica* (Shen & Lee, 1966) but can be distinguished from its congeners by the number and presence of posterior surface spinules on the second and third exopodal segments of P2 to P4, and the number of spinules on the anterior surface of the second endopodal segment of P2 to P4 in both sexes. This species occurs primarily in brackish and/or coastal waters of Korea and is the first species to be recorded from the Yellow Sea. The zoogeography of the seven known species of *Bestiolina* is discussed.

Species of *Bestiolina* differ from other members of the family Paracalanidae such as *Paracalanus*, *Acrocalanus*, and *Parvocalanus* based on two morphological characteristics: outer margins of second and third exopodal segments of P2 to P4 lacking serrations, and the third endopodal segments of P2 to P4 have the setal formula 1, 2, 3. These characteristics were used by Andronov (1972) to remove three species *Acrocalanus inermis* (Sewell, 1912), *A. similis* (Sewell, 1914), and *A. sinicus* (Shen & Lee, 1966) from the genus *Acrocalanus* (Giesbrecht, 1888) and create a new genus *Bestiola*. Because the name *Bestiola* was preoccupied by a genus of insects, Andronov (1991) replaced it with *Bestiolina*. The genus *Bestiolina*, which is distributed worldwide in tropical and subtropical waters, now comprises seven species, including the one described here (Sewell 1912, 1914; Shen & Lee 1966, Andronov 1972, 1991; Li & Huang 1984, Ali et al. 2007). Most species of *Bestiolina* inhabit tropical/subtropical estuarine and/or coastal waters, where they are often the predominant group (Kimmerer 1984, McKinnon & Ayukai 1996).

In this study, we describe the new species of *Bestiolina* collected from the Yellow Sea, during a marine biodiversity survey aimed at populating a distributional database on copepods in Korean estuarine and coastal waters. We also discuss its morphological and zoogeographical relationship with congeners.

Materials and Methods

Copepods were collected from the southwestern coastal waters of Korea on 31 Aug 2007 and 12 Oct 2007 with a Norpac net (mesh size 200 μm; mouth diameter 45 cm) towed vertically from near bottom to the surface (35°22′24″N,

* Corresponding author.

126°20′30″E). The volume of water filtered was estimated from a flowmeter (General Oceanic Co.) attached on the net mouth. The samples were preserved immediately in 5% buffered formalin/seawater solution. Individuals of the new species were sorted out from the samples. Water temperature and salinity were measured in situ with a CTD (Alec Electronics Co. Model ACL1150-DK). Drawings were made with a camera lucida on a Nikon Eclipse 80i® microscope equipped with differential interference contrast optics, after the appendages were dissected and mounted on a slide with CMC-10® medium (Masters Chemical Company, Wood Dale, Illinois). The body size of individuals was measured from the head to the tip of the caudal rami, using an image analysis system (Image Pro Plus®, version 5.1 for Windows, Media Cybernetics, Silver Spring, Maryland). The rostrum, posterior parts of the last pedigerous somite, genital double-somite, and antennules of females were examined with a scanning electron microscope (Hitachi S-4700) to show some morphological details more clearly. Zoogeographical distributions of species of *Bestiolina* were determined from the web site of Razouls et al. (http://copepodes.obs-banyuls.fr). The descriptive terminology proposed by Huys & Boxshall (1991) was adopted. Abbreviations used in the text and figures: A1, antennule; A2, antenna; ae, aesthetasc; P1–P5, first to fifth legs. All type specimens are deposited in the National Institute of Biological Resources (NIBR), Seoul. Scale bars in figures are indicated in μm.

Systematics

Family Paracalanidae Giesbrecht, 1892
Genus *Bestiolina* Andronov, 1991
Bestiolina coreana, new species
Figs. 1–8

Type locality.—Yellow Sea (35°22′24″N, 126°20′30″E).

Material examined.—Holotype 1 ♀ (NIBRIV0000170172) from the type locality, collected by S. Y. Moon on 31 Aug 2007. Paratypes: 20 ♀♀ (NIBRIV0000170173), 20 ♂♂ (NIBRIV0000170174) in 70% ethanol. All from the type locality, collected by S. Y. Moon on 31 Aug 2007: 1 ♀ dissected on 10 glass slides and 1 ♂ on 10 slides, respectively, and 20 ♀♀, 18 ♂♂ in 70% ethanol from the type locality, and 10 ♀♀, 10 ♂♂ in 70% ethanol from Gamak Bay (34°41′19″N, 127°40′28″E), Yeosu, the southern waters of Korea, collected by H. Y. Soh on 27 Aug 2008, will be deposited in the third author's collection (HYS) in the Laboratory of Species Diversity and Ecology, Division of Marine Technology, Chonnam National University, Yeosu, Korea.

Description of female.—Body plump. Body length 0.90–0.95 mm ($\overline{X} \pm SD$ = 0.94 ± 0.09, n = 10). Rostral filaments (Fig. 7A) thick and short. Prosome-urosome ratio 3.12:1. Cephalosome and first pedigerous somite completely fused; fourth and fifth pedigerous somites completely separated; posterior corners of prosome rounded and with row of spinules posterodorsally (Figs. 1A, B, 7B). Urosome of 4 free somites: genital double-somite symmetrical, as long as wide; genital operculum (Fig. 7G, H) located ventroposteriorly. Caudal rami nearly symmetrical, each with 5 setae lacking setae I and II.

Antennule: 25-segmented, reaching to distal part of third urosomite (Fig. 1B): ancestral segments II to IV incompletely separated; segments XXVII–XXVIII completely fused (Fig. 1C). Segmentation and setation patterns as follows (ancestral segment number – setae+ae): I-2+as, II–IV-4+ae, V-2+ae, VI-1+ae, VII-2+ae, VIII-1, IX-2+ae, X-I+1, XI-2+ae, XII-1, XIII-1, XIV-I+ae, XV-1, XVI-1+ae, XVII-1, XVIII-1+ae, XIX-1, XX-1, XXI-1+ae, XXII-1, XXIII-1, XXIV-1+1, XXV-1+1, XXVI-1+1, XXVII–XXVIII-5+ae. Proximal 9 articulated segments

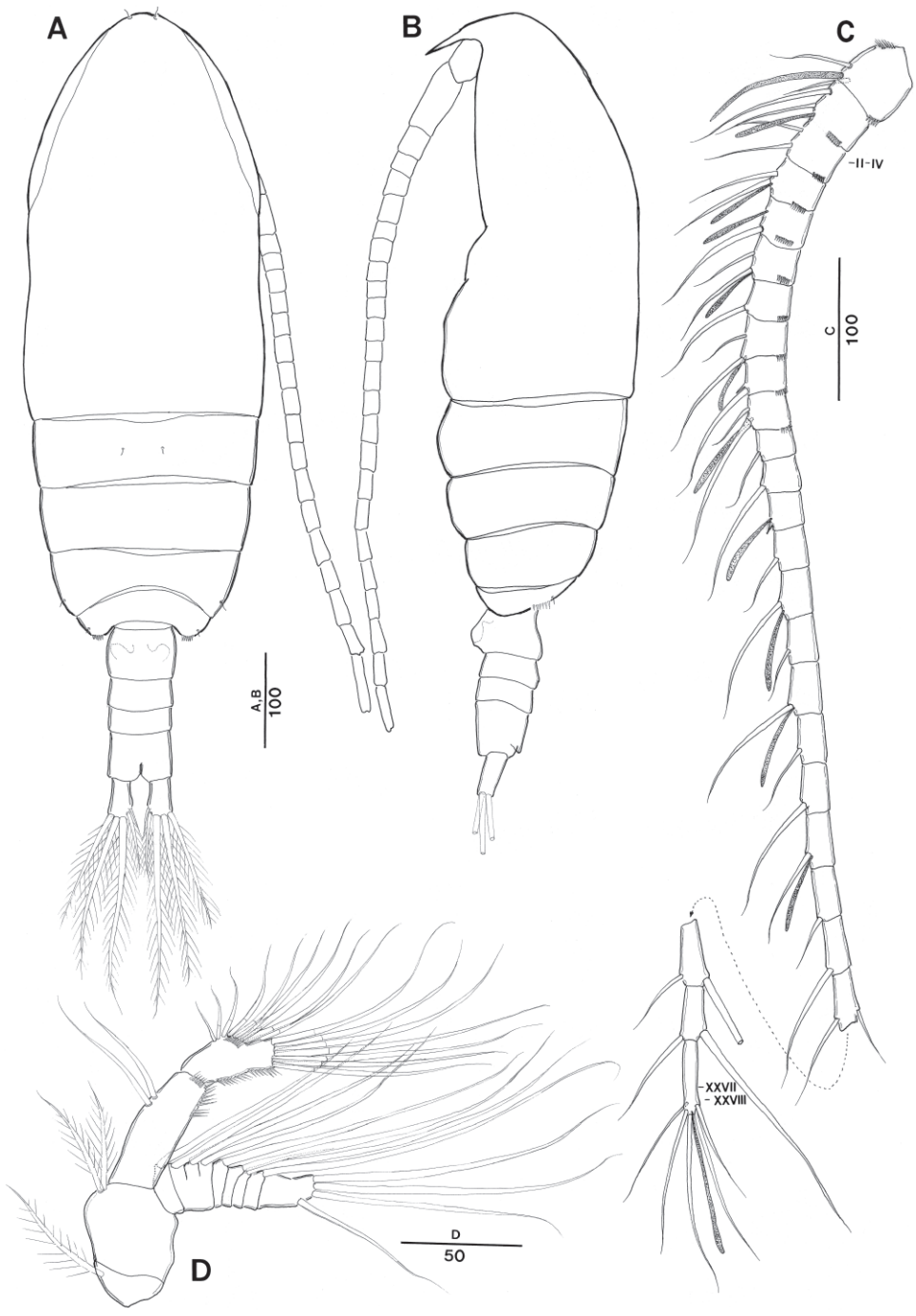

Fig. 1. *Bestiolina coreana*, female, holotype. A, habitus, dorsal view. B, habitus, left lateral view. C, antennule. D, antenna. Scale bars in μm.

with row of spinules on distal half of dorsal surface.

Antenna (Fig. 1D): Coxa and basis with 1 and 2 setae, respectively; endopod 2-segmented, proximal segment with 2 setae and row of spinules subterminally, distal segment with 9 setae subterminally, 7 setae terminally and oblique row of tiny spinules on anterior surface; exopod 7-segmented, with setal formula of 1, 3, 1, 1, 1, 1, 1+3.

Mandible (Fig. 2A): Gnathobase well developed, cutting edge with short teeth and dorsal seta; basis of palp with 4 setae; exopod 5-segmented, with setal formula of 1, 1, 1, 1, 2; endopod 2-segmented, proximal and distal segments with 4 and 11 setae, respectively.

Maxillule (Fig. 2B): Praecoxa and coxa incompletely fused; praecoxal arthrite with 14 setae, rows of spinules on posterior surface; coxa with 3 setae on proximal and distal endites, respectively, and 9 setae on epipodite; basis with 4 setae on endite; endopod and exopod with 14 and 11 setae, respectively.

Maxilla (Fig. 2C): Praecoxa and coxa incompletely fused, proximal praecoxal endite with 5 setae, distal and coxal endites with 3 setae each; basis with 4 setae; endopod 3-segmented, with 1, 3 and 4 setae on segments 1 to 3, respectively.

Maxilliped (Fig. 2D): Syncoxa robust, with setal formula of 1, 2, 2, 4 and oblique rows of tiny spinules on posterior surface; basis with 3 setae and row of spinules on medial surface; endopod 6-segmented, with setal formula of 2, 3, 4, 3, 3+1, 4.

Spine and seta formula of P1 to P4 as follows:

P1 (Fig. 3A): Coxa, basis and first endopodal segments with tiny spinules on posterior surface, respectively; first and second exopodal segments with tiny spinules on posterodistal surface; third exopodal segment with hairs and 2 seta-like spines subterminally. P2 (Fig. 3B): second endopodal segments with rows of spinules on posterior surface; second and third exopodal segments with tiny spinules on posterodistal surface, respectively. P3 (Fig. 3C) and P4 (Fig. 3D) with setules on posterodistal surface of second endopodal segment, respectively. P5 (Fig. 3E) strongly reduced to pair of rounded lobes.

Legs	Coxa	Basis	Exopod	Endopod
P1	0-0	0-1	0-1; 0-1; II,I,4	0-1; 1,2,2
P2	0-1	0-0	I-1; I-1; III,I,5	0-1; 0-2; 1,2,3
P3	0-1	0-0	I-1; II-1; III,I,5	0-1; 0-2; 1,2,3
P4	0-1	0-0	I-1; II-1; III,I,5	0-1; 0-2; 1,2,3

Description of male.—Body more slender than female (Fig. 4A, B). Total body length 0.85–0.96 mm ($\overline{X} \pm SD = 0.94 \pm 0.08$, $n = 10$). Prosome-urosome ratio 2.4:1. Cephalosome bearing dorsal hump and completely fused with first pedigerous somite; fourth and fifth pedigerous somite completely separated; posterior corners of prosome rounded (Fig. 4A). Rostral filaments thick, but more slender than in female. Urosome of 5 free somites, second urosomal somite longest. Caudal rami nearly symmetrical, about 2 times longer than wide, and each with 5 setae, lacking setae I and II.

Antennule: 20-segmented, extending to distal part of second urosomite (Fig. 4C): ancestral segments I to IV, V to VIII, and IX to X completely fused. Segmentation and setation patterns as follows: I–IV-6+6ae, V–VIII-5+4ae, IX–X-2+I+2ae, XI-2+ae, XII-2, XIII-1, XIV-0, XV-1, XVI-2, XVII-1, XVIII-2, XIX-0, XX-1, XXI-1, XXII-1, XXIII-1, XXIV-1+1, XXV-1+1, XXVI-1+1, XXVII–XXVIII-4+ae.

Antenna (Fig. 4D): Biramous, but atrophied: coxa and basis completely fused, with single seta; endopod 2-segmented, proximal endopod segment without seta, distal one furnished with rows of spinules, subterminally with 5 setae and terminally 6 setae; exopod incompletely fused, with 5 setae.

Mandible (Fig. 5A): Without coxal gnathobase; basis bearing single seta medially; exopod 4-segmented with setal formula of

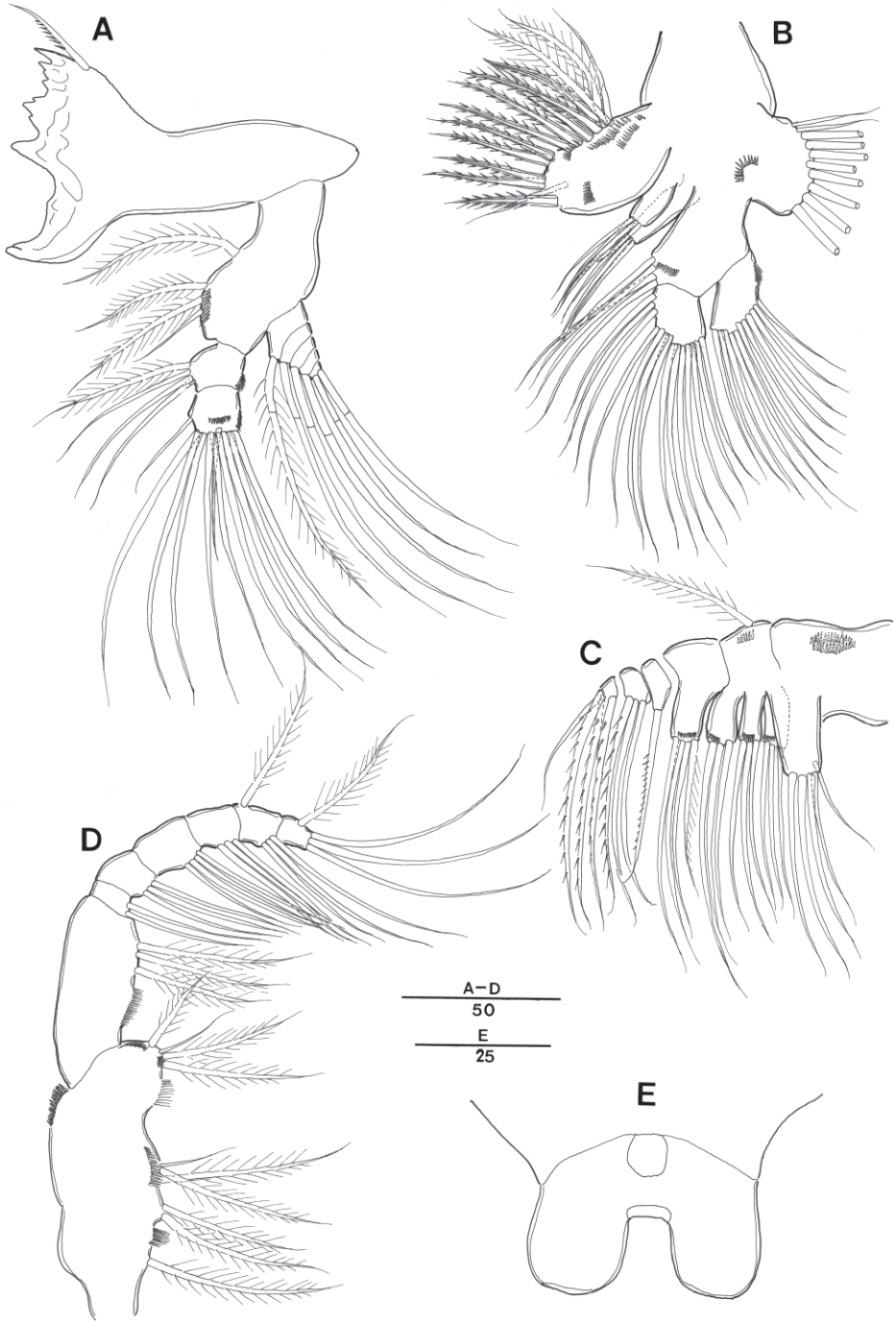

Fig. 2. *Bestiolina coreana*, female, holotype. A, mandible. B, maxillule. C, maxilla. D, maxilliped. E, P5. Scale bars in μm.

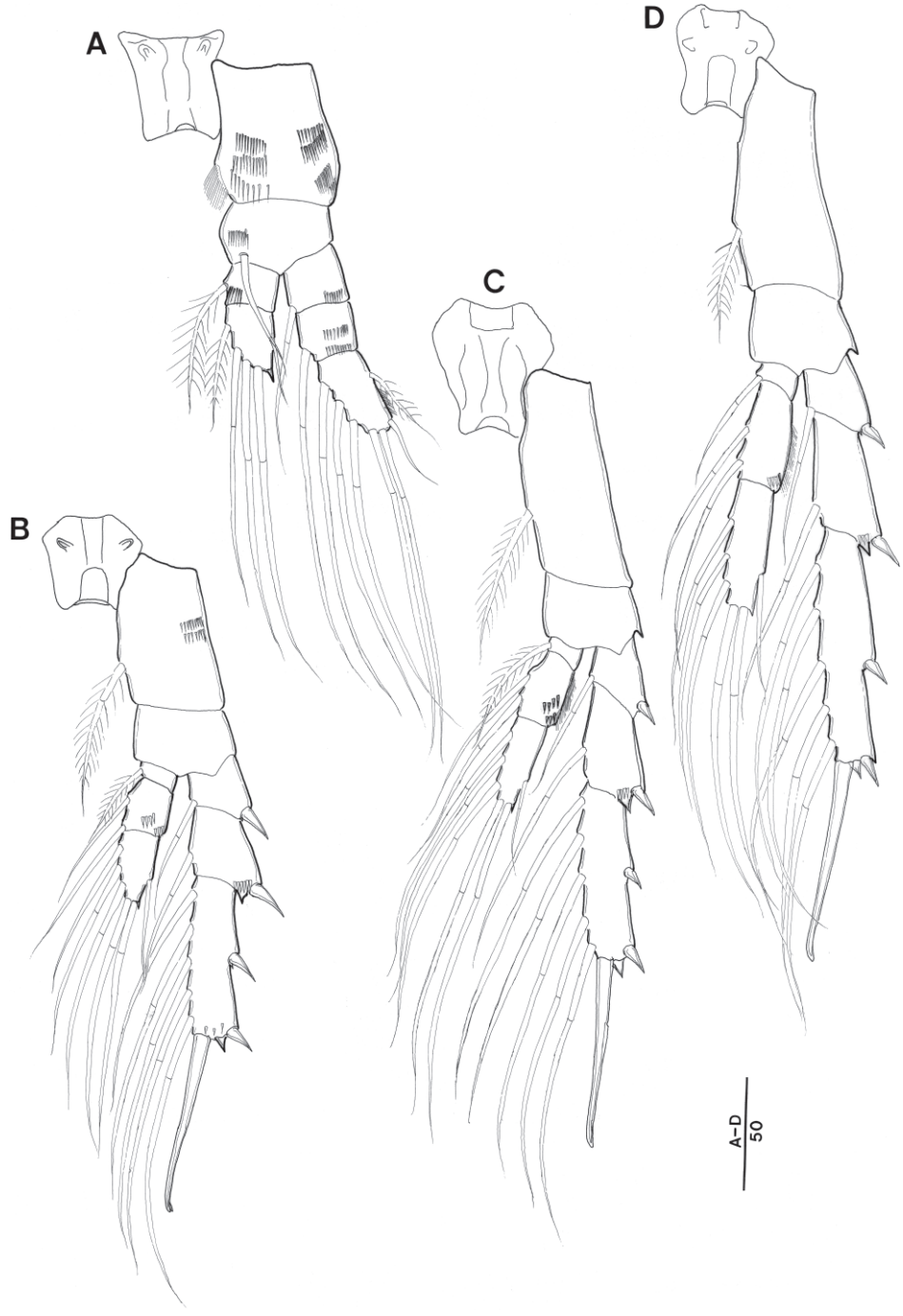

Fig. 3. *Bestiolina coreana*, female, holotype. A, P1. B, P2. C, P3. D, P4. Scale bars in μm.

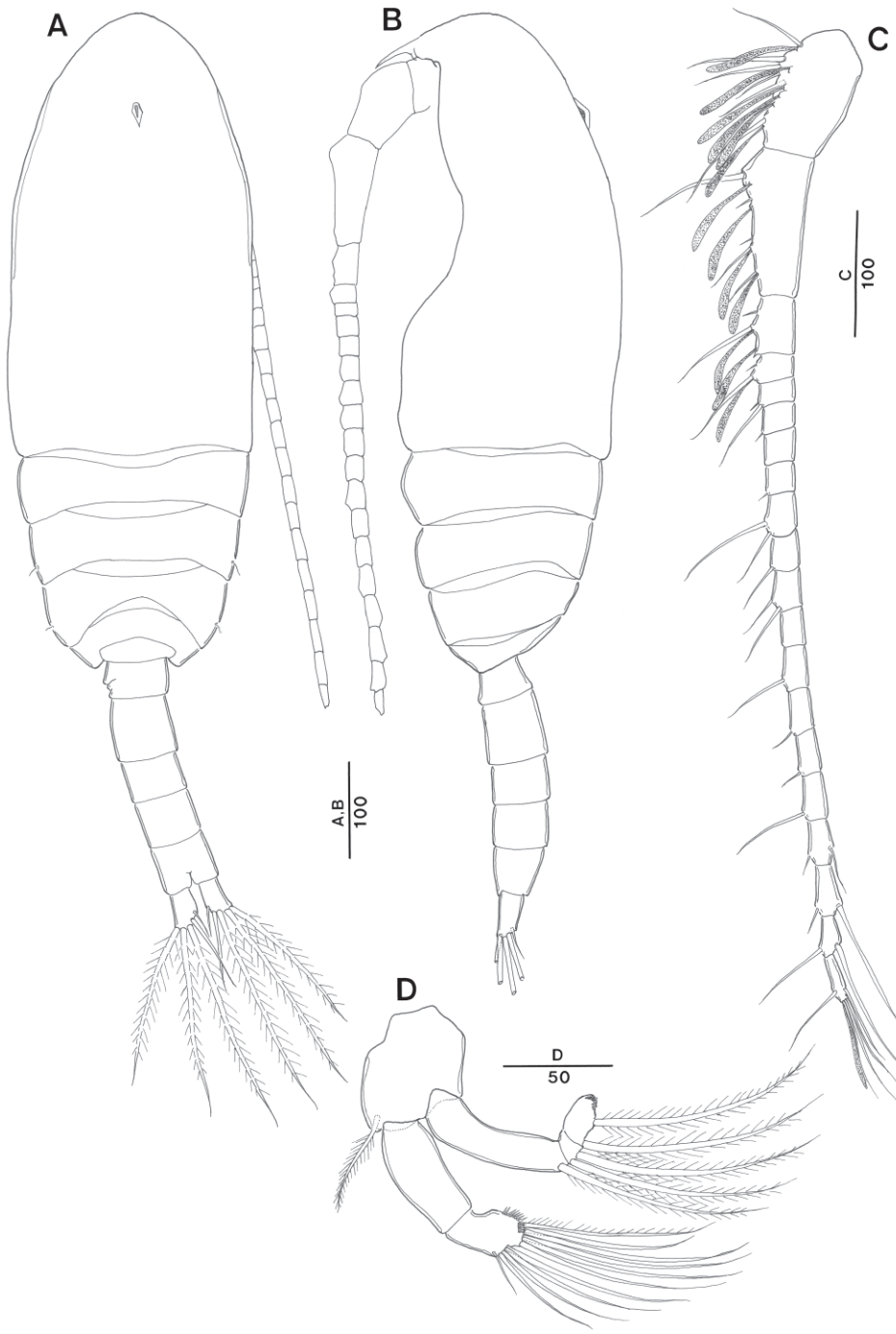

Fig. 4. *Bestiolina coreana*, male, paratype. A, habitus, dorsal view. B, habitus, left lateral view. C, antennule. D, antenna. Scale bars in μm.

1, 1, 1, 3; endopod 2-segmented, first endopodal segment with single seta, second endopodal segment with 8 setae.

Maxilliped (Fig. 5B): Comprising robust syncoxa, basis, and 2-segmented endopods: syncoxa without seta; basis with single seta and row of spinules medially; proximal endopodal segment with 5 setae, of which outer seta robust; distal segment with 4 setae, of which 1 outer and 1 distal setae robust. Maxillule and maxilla rudimentary.

Seta and spine formula of P1 to P4 (Fig. 5C–F) and ornamentation generally as in female, but with some differences: posterior spinules on coxa, basis and first endopodal segment of P1 absent, first exopodal and second exopodal segments of P1 with tiny spinules terminally; posterior spinules on second and third exopodal segments of P2 absent.

P5 strongly asymmetrical (Fig. 5G): Right leg rudimentary; left leg longer than urosome, uniramous, and 5-segmented; basis and first exopodal segment unarmed; second exopodal segment with pointed process on distal margin; terminal one with pointed process and inner long apical spine.

Variability.—There are variations in number of spinules on the posterior surface of coxal, endopodal and exopodal segments of P2 to P4 among individuals from the Yellow Sea (Fig. 6): in P2 (Fig. 6A–E) number of spinules on posterior surface of coxal segment variable, second endopodal segment with 3+3, 4+3, 4+2, 4+3 and 3+3 spinule patterns on posterior surface; second exopodal segment with 4 to 6 spinules on posterior surface; in P3 (Fig. 6F, G) second endopodal segment with 5+3 and 4+3 spinule patterns on posterior surface, second exopodal segment with 4 or 3 spinules on posterodistal surface; in P4 (Fig. 6H, I) second endopodal segment with 4 or 3 spinules on posterodistal surface.

Etymology.—The species name *coreana* refers to its type locality, Republic of Korea.

Remarks.—*Bestiolina coreana* closely resembles *B. sinica* (Shen & Lee, 1966), but it differs from the latter in the following characteristics (Table 1): 1) the second and third exopodal segments of P2 with small and large spinules on the posterodistal surface, and 2) the second exopodal segment of P3 and P4 with rows of spinules on the anterior surface. *Bestiolina coreana* is found predominantly in waters with a temperature greater than 20°C and in salinities ranging from 28 to 32 psu in the Younggwang coastal region in August to October, when a large volume of fresh water flows into the region from the Watan and Bulgap rivers. When the salinity increased above 32.0 psu, *B. coreana* was replaced by *Paracalanus parvus* s. l. and the occurrence of the former was restricted to less-saline coastal waters. *Bestiolina coreana* disappeared when the water temperature decreased below 20°C (Fig. 8).

Discussion

Systematic discussion.—The genus *Bestiolina* was separated from the other genera within the Paracalanidae based on the following characteristics: 1) the absence of serration on the outer margins of second and third exopodal segments of P2 to P4, 2) the female P5 reduced to two rounded lobes, and 3) the male P5 with reduced rounded lobe of right leg and 5-segmented left leg, with tiny non-articulated process and relatively long terminal spine.

Bestiolina coreana is distinguished from other members of its genus in the following characteristics: the presence and number of spinules on the posterior surfaces of exopodal and endopodal segments of P2 to P4. These differences are shown in Table 1. However, this species shows variations in the number of spinules on the posterior surface of the second endopodal and third exopodal segments of P2 to P4. The presence and number of spinules on the second exopo-

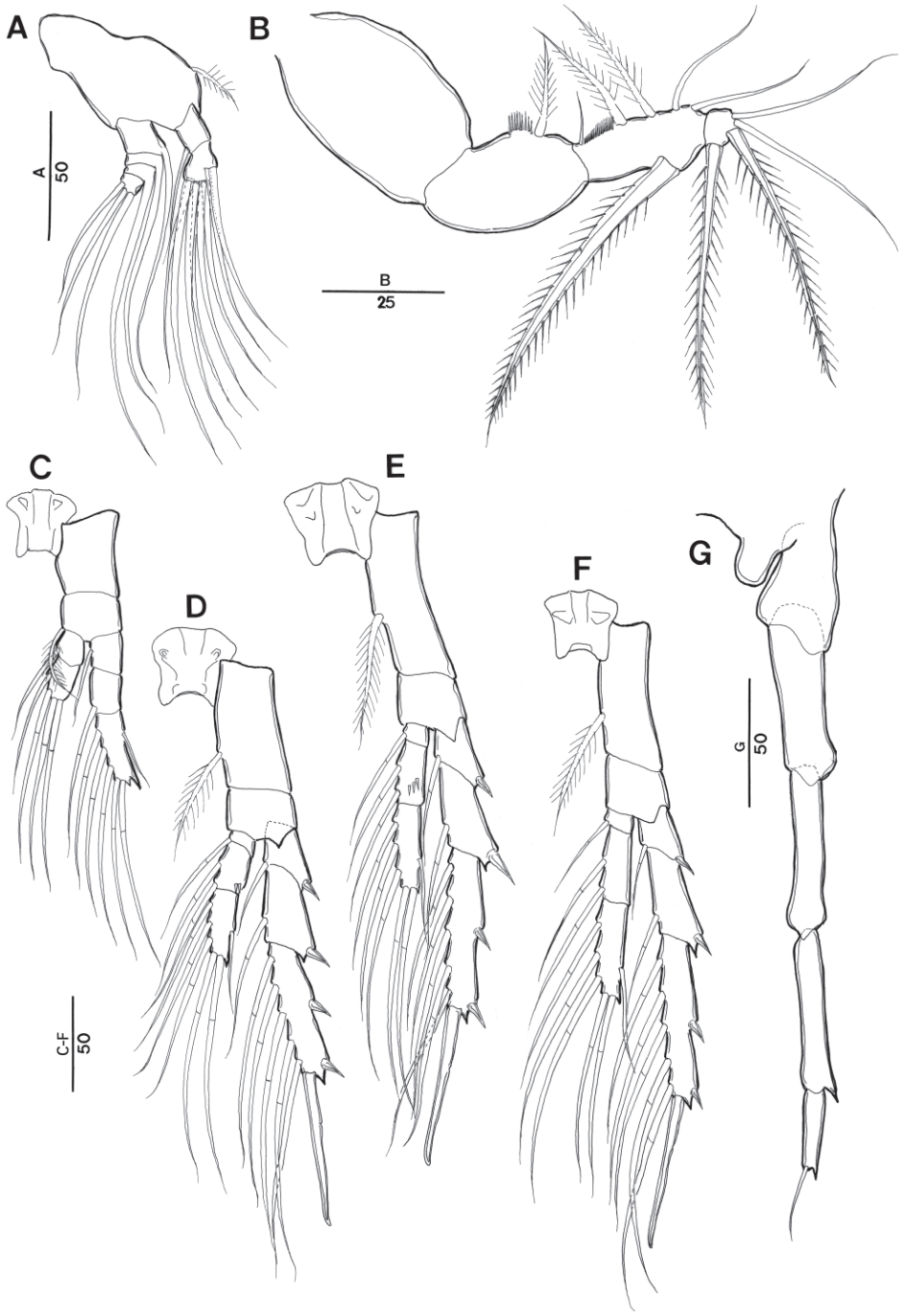

Fig. 5. *Bestiolina coreana*, male, paratype. A, mandible. B, maxilliped. C, P1. D, P2. E, P3. F, P4. G, P5. Scale bars in μm.

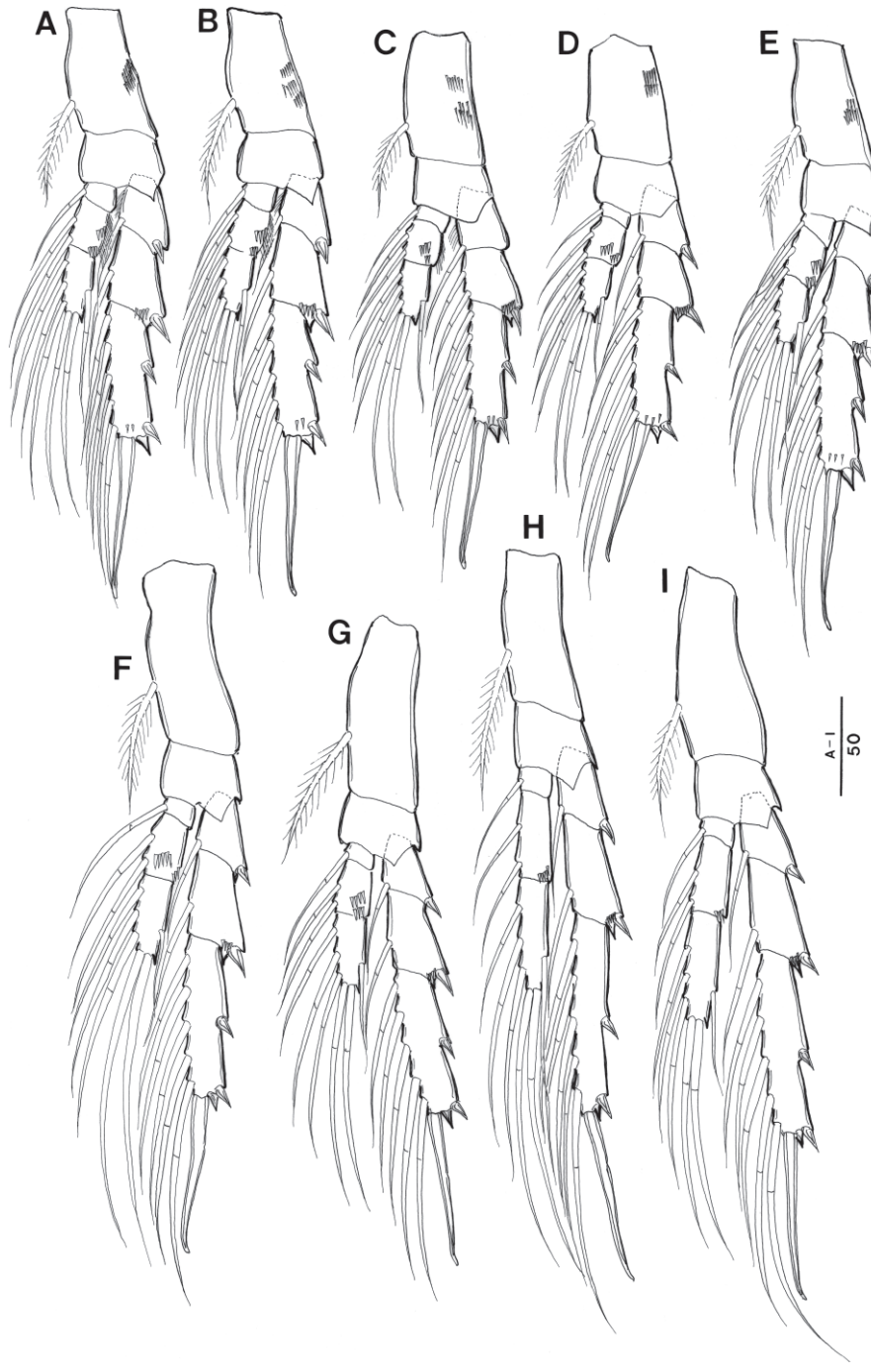

Fig. 6. Variant of number of spinules of *Bestiolina coreana*, female, paratype. A–E, P1. F, G, P3. H, I, P4. Scale bars in μm.

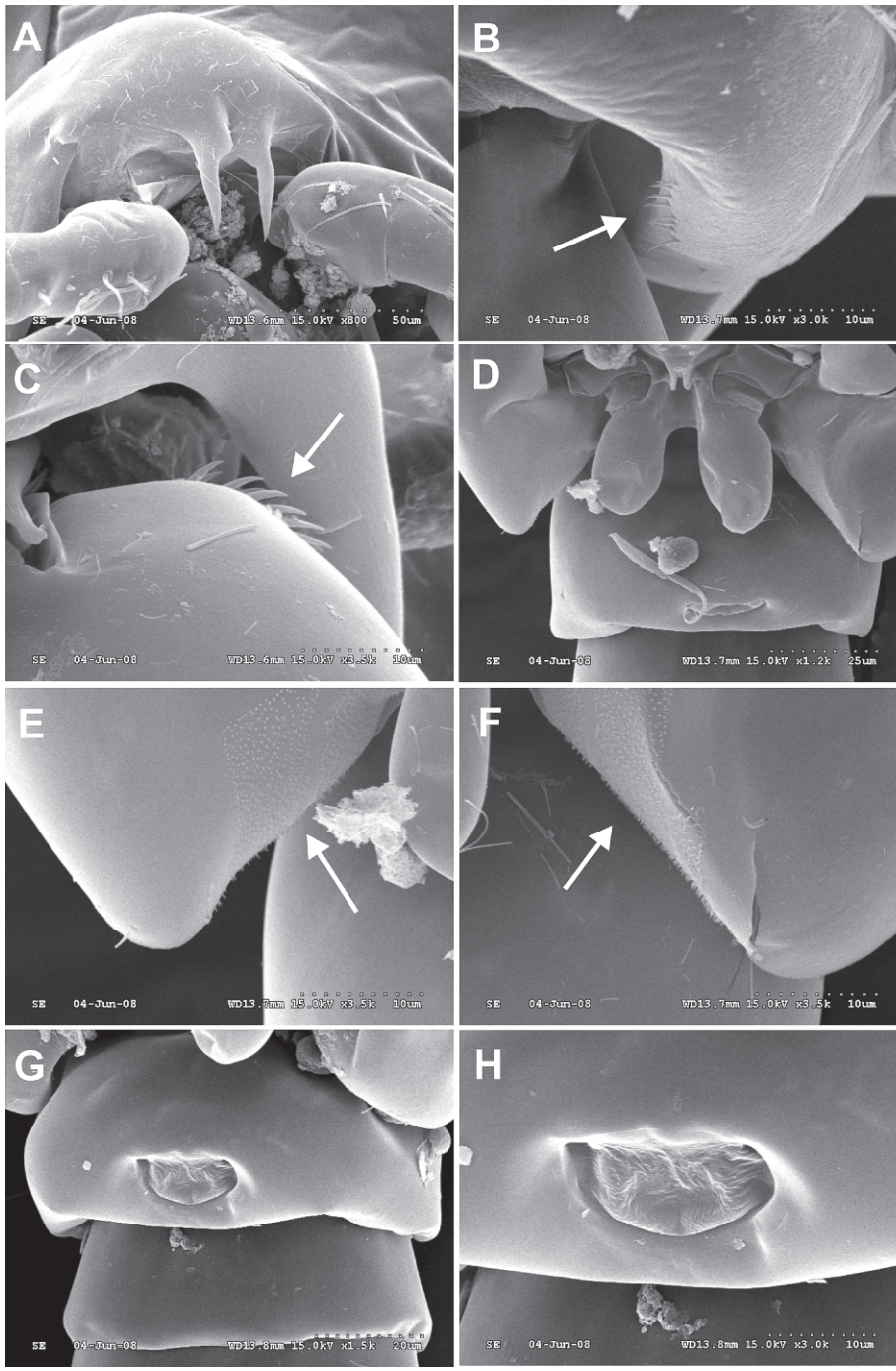

Fig. 7. SEM micrographs of *Bestiolina coreana*, female. A, rostral filaments. B, last pedigerous somite, dorsal view. C, first antennular segment, row of spinules. D, P5. E, F, last pedigerous somite, right and left ventral view, respectively. G, genital double-somite, ventral view. H, genital aperture, ventral view. Arrows indicate the spinules and setules.

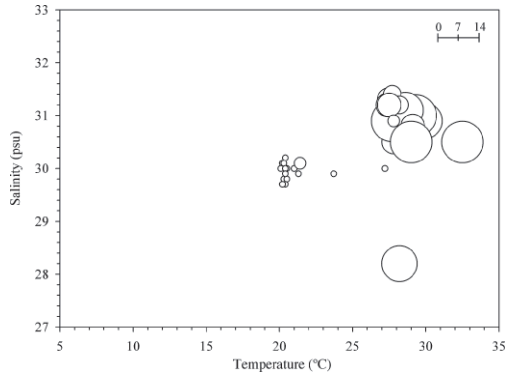

Fig. 8. Temperature-Salinity-Abundance diagram for *Bestiolina coreana*. Abundance (ind./m^3) of species is estimated by multiplying numbers on scale by 2×10^3 for *B. coreana*.

dal segment of P2 to P4 appear to be specific at the species level of *Bestiolina* (Andronov 1991), although all species are not described in great detail. These facts suggest that the ornamentation on the second exopodal segment of P2 to P4 may play an important role in separating *Bestiolina* species.

Zoogeography of the genus Bestiolina.—*Bestiolina* as now defined contains the following species: *B. similis* (Sewell, 1914), *B. amoyensis* (Li & Huang, 1984), *B. inermis* (Sewell, 1912), *B. sinica* (Shen & Lee, 1966), *B. zeylonica* (Andronov, 1972), *B. arabica* Ali et al., 2007, and *B. coreana*. The zoogeographical distribution of *Bestiolina* is shown in Fig. 9 on the basis of Razouls et al. (http://copepodes.obs-banyuls.fr). *Bestiolina similis* has been distributed in tropical and subtropical waters of the Pacific and Indian oceans between 35°N and 35°S (Sewell 1914, Wellershaus 1969, Greenwood 1976, Andronov 1972, 1991). *Bestiolina sinica* and *B. amoyensis* occur mainly in coastal regions of the South China Sea (Shen & Lee 1966, Li & Huang 1984, Lo et al. 2004). *Bestiolina coreana* occurs in brackish waters of the coast of the Yellow Sea and the southern waters of Korea. *Bestiolina inermis* is distributed in the western Pacific Ocean and northern Indian Ocean (Andronov 1972, Kimmerer 1984, Calbet et al. 2000). *Bestiolina arabica* and *B. zeylonica* have confined distributions in the coastal regions of the Arabian Gulf and Sri Lanka, respectively (Andronov 1972, Ali et al. 2007). These distributional patterns show that the speciation of *Bestiolina* could have originated from the Indo-Malayan region, as was proposed for another brackish/coastal calanoid genus *Tortanus* by Ohtsuka & Reid (1998). In particular, the radiation of the Indo-Pacific species of *Bestiolina* could have been brought about by changes in the inflow of fresh water from East and Southeast Asia during the Pleistocene glacial periods, as hypothesized for the speciation mechanisms of the *Tortanus* subgenus, *Eutortanus* (Ohtsuka et al. 1992, Ohtsuka & Reid 1998). However, the testing of this hypothesis awaits a more detailed knowledge of *Bestiolina* taxonomy, biodiversity and molecular variability.

Key to Females of the Genus *Bestiolina*

1. Presence of row of spinules on the distal margin of fifth pedigerous somite 2
– Absence of row of spinules on the distal margin of fifth pedigerous somite 4
2. Presence of number of spinules on the posterodistal surface of second exopodal segment of P4 .... *Bestiolina coreana*
– Presence of number of spinules on the posterodistal surface of first exopodal segment of P4 ..... *B. amoyensis*
– Absence of spinules on the posterodistal surface of first and second exopodal segments of P4 3
3. Presence of number of spinules on the posterodistal surface of first to third exopodal segments of P2 .... *B. zeylonica*
– Absence of spinules on the posterodistal surface of first to third exopodal segments of P2 ... *B. sinica*

Table 1.—Presence and number of spinules on the anterior surface of the exopods and endopods of swimming legs 2–4 of the seven species of genus *Bestiolina*. These characteristics were taken from Ali et al. (2007), except for *B. coreana*.

Character	Swimming leg (P)	*B. coreana* (present study)	*B. similis* (Sewell 1914)	*B. amoyensis* (Li & Huang 1984)	*B. arabica* Ali et al., 2007	*B. inermis* (Sewell 1912)	*B. sinica* (Shen & Lee 1966)	*B. zeylonica* (Andronov 1972)
Number of spinules on the posterior surface of the first to third exopodal segments of swimming legs 2 to 4	P2	0, 6, 3	0, 0, 3	2, 1, 1	Absent	0, 3, 0	Absent	3, 3, 2
	P3	0, 4, 0	0, 0, 3	1, 1, 2	Absent	?	Absent	0, 3, 2
	P4	0, 3, 0	Absent	1, 2, 1	Absent	?	Absent	Absent
Number of spinules on the anterior and posterior surface of the second endopodal segment of swimming legs 2 to 4	P2	4+3	0+5	0+5	3	4	4+4	4
	P3	4+3	0+5	0+4	3	?	5+4	4+3
	P4	0+4	Absent	0+small spinules	Absent	?	0+4	0+small spinules
Posterior corners of prosome rounded and with row of spinules posterodorsally		Present	Absent	Present	Absent	Absent	Present	Present

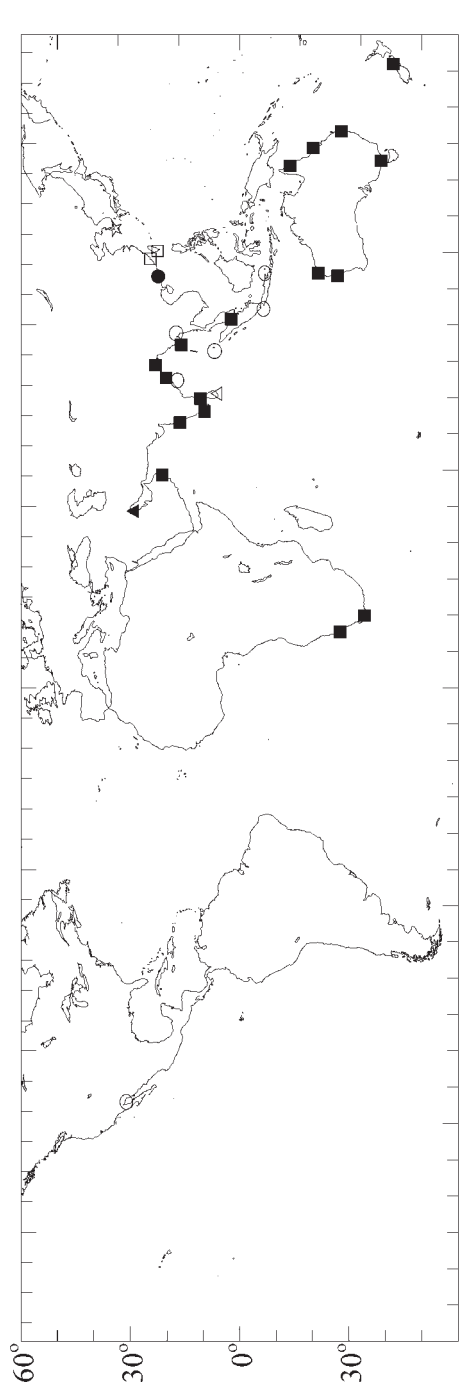

Fig. 9. Schematic illustration of zoogeographical distribution of genus *Bestiolina* in the world. *Bestiolina similis* (■), *B. amoyensis*, (□) *B. inermis* (O), *B. sinica* (●), *B. zeylonica* (△), *B. arabica* (▲), and *B. coreana* (☆).

4. Presence of number of spinules on the posterodistal surface of the second exopodal segment of P2 .... *B. inermis*
– Absence of spinules on the posterodistal surface of the second exopodal segment of P2 5
5. Presence of number of spinules on the posterodistal surface of the third exopodal segment of P2 and P3 .... *B. similis*
– Absence of spinules on the posterodistal surface of the third exopodal segment of P2 and P3 .... *B. arabica*

Acknowledgments

We express our sincere thanks to two anonymous reviewers and Dr. J. Reid for their critical comments. Also, we thank Mr. Min Ho Seo for collecting zooplankton samples. This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of the Environment, Korea and in part by the KORDI project "Development of port environmental risk assessment technology (PE98464)."

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