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First records of *Hemicyclops tanakai* Itoh and Nishida, 2002 and *Tisbe ensifer* Fischer, 1860 (Crustacea, Copepoda) in Korea

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Extensive survey of invertebrates in Korea, led by the National Institute of Biological Resources in Incheon, continues to uncover many new and endemic species, but also elements of neighbouring faunas that were previously unknown here. *Hemicyclops tanakai* Itoh and Nishida, 2002 was originally described from estuarine mud-flats in Tokyo Bay, Japan. We report one male and two females from a sandy beach on Jeju Island, which represent its first record in Korea and second record ever. No morphological differences were observed between these two disjunct populations, so we expect more records of this species in Korea and Japan. *Tisbe ensifer* Fischer, 1860 was originally described from Madeira and later on reported from numerous other parts of the Northern Atlantic, as well as from the Indian Ocean. We report two females from a shallow littoral in Sokcho, which represents its first record in Korea and the first record in the entire Pacific Ocean. Because of morphological discrepancies reported in previous records we recognize that this species might be in reality a species complex, and we only tentatively identify our Korean specimens as *T.* cf. *ensifer*. We provide numerous light photographs of both species in addition to short descriptions, in hope that they might elucidate global problems of their zoogeography and dispersal of small marine invertebrates in general. Further samplings from Korea and other parts of the world will be necessary to test our identifications and phylogenetic relationships of Korean populations with molecular and other tools.

Keywords: Harpacticoida, littoral, marine interstitial, Poecilostomatoida, zoogeography

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Introduction

South Korea has become one of the most actively researched areas for invertebrate diversity in the last decade or so, mostly thanks to the efforts of the National Institute of Biological Resources in Incheon and supporting funding from the Korean Ministry of Environment (Lee and Karanovic, 2012). According to the most current review (Cho *et al*., 2011), the national inventory of Korea totals 36,921 species, consisting of 5,230 vascular plants and bryophytes, 4,587 algae, 4,085 fungi and lichens, 1,374 protists, 647 prokaryotes, 1,841 vertebrates, 13,384 insects, and 5,773 invertebrates other than insects. Copepods are relatively well studied here, both as free-living forms in marine (Soh, 2010; Lee *et al*., 2012) and freshwater environments (Chang, 2009; 2010), as well as parasites of other organisms (Kim, 2008). However, surveys of marginal and previously understudied habitats (Karanovic *et al*., 2012a; 2012b; Karanovic, 2014) or utilization of novel taxonomic methods, such as studies of microstructures (Karanovic and Cho, 2012; Karanovic and Lee, 2012; Karanovic *et al*., 2013) and DNA (Karanovic and Kim, 2014a; Kim *et al*., 2014; Karanovic *et al*., 2014; 2015), have resulted in numerous recent additions to the Korean copepod fauna. While most of the recent additions are endemic elements (see also Chang and Lee, 2012; Kim *et al*., 2014) some are actually elements of neighbouring faunas that were previously unknown here (Kim *et al*., 2011; Park and Lee, 2011; Nam and Lee, 2012; Park *et al*., 2012). Two copepod species that we report in this paper belong to the latter group. They were both collected in very small numbers and in a single location each, so their optimal ecological niche and/or season in Korea remain to be determined. To provide a wider perspective for our records we discuss systematic positions and taxonomic problems of the two species reported.

Materials and Methods

All specimens of *Hemicyclops tanakai* were collected by the senior author from sandy bottom in the intertidal zone, using the Karaman-Chappuis method. It consisted of digging a whole down to the water level and then decanting the inflowing interstitial water and filtering it through a plankton hand net (mesh size 30 μm). Specimens of *Tisbe ensifer* were collected with the same plankton net in shallow littoral (between 30 and 130 cm), by vigorous sweeping through an abundant macro-algal growth and scraping through a diverse growth on top of larger submerged rocks. All samples were fixed in 99% ethanol, sorted in the laboratory also in 99% ethanol using an Olympus SZX12 dissecting microscope with PLAPO objectives and magnification of up to 200x. Locality data and number of specimens are listed for each species separately and all specimens are deposited in NIBR.

Some specimens were dissected and mounted on microscope slides in Faure's medium (see Stock and von Vaupel Klein, 1996), and dissected appendages were then covered by a coverslip. For the urosome or the entire animal, two human hairs were mounted between the slide and coverslip, so the parts would not be compressed. Photographs of habitus were taken with a camera attached to our dissecting microscope, while photographs of dissected appendages were taken with a camera attached to a Leica MB2500 phase-interference compound microscope, equipped with N-PLAN (5×, 10×, 20×, 40× and 63× dry) or PL FLUOTAR (100× oil) objectives.

The terminology follows Huys and Boxshall (1991), except for the spelling of some appendages (antennula, mandibula, maxillula instead of antennule, mandible, maxillule), as an attempt to standardise the terminology for homologous appendages in different crustacean groups.

Results

Subphylum Crustacea Brünnich, 1772
Class Maxillipoda Dahl, 1956
Subclass Copepoda H. Milne Edwards, 1840
Order Poecilostomatoida Thorell, 1859
Family Clausidiidae Embleton, 1901
Genus *Hemicyclops* Boeck, 1873

Hemicyclops tanakai **Itoh and Nishida, 2002 (Figs. 1-3)**

Synonymy. *Hemicyclops tanakai* n. sp. - Itoh and Nishida (2002), p. 139, figs. 103; *Hemicyclops* sp. - Itoh (2001), p. 185, fig. 3C.

Type locality. Japan, Tokyo Bay, estuarine mud-flat on the mouth of the Tama River, mud-shrimp burrows in the intertidal zone, 35°32.0′N 139°46.2′E.

Specimens examined. Two females (NIBR IV 0000 287266 and NIBR IV 0000287267) dissected on one slide each; allotype male (NIBR IV 0000287268) dissected on one slide; all collected from Korea, Jeju Island, Sunshine Hotel beach, interstitial, 26 April 2014, leg. T. Karanovic. 33°32.755′N 126°39.753′E.

Diagnosis. Body length, excluding caudal setae, from 1220 to 1435 μm, male slightly longer than female. Colour of preserved specimen yellowish (Fig. 1A). Habitus robust, dorsoventrally compressed, with prosome/urosome ratio about 1.2 and greatest width at posterior end of cephalothorax. Prosome ovoid, about 1.4 times as long as wide, twice as wide as genital somite in male and 2.6 times as wide as genital double-somite in female. Female urosome (Fig. 1B) gradually tapering posteriorly, genital double-somite about twice as wide as anal somite; genital double-somite (Fig. 1C) 1.1 times as long as greatest width, expanded in anterior third. Male genital somite (Fig. 1A) expanded in posterior part, hearth-shaped, 0.8 times as long as wide, 2.7 times as wide as anal somite. Caudal rami (Figs. 1D, 3A) cylindrical, 2.1 times as long as wide. Antennula (Figs. 1E, 3B) seven-segmented, with fourth segment longest, armature formula in female 4.15.6.3.4+ae.2+ae.7+ae, and with one extra seta on third and fourth segments each in male. Antenna (Fig. 1E) four-segmented, armature formula 1.1.4.7, outer distal corner of third segment projected, fourth segment minute. Labrum (Fig. 3C) trapezoidal, with wide and centrally convex cutting edge and concave sides. Mandibular gnathobase (Fig. 1F) with one smooth spine and three pinnate setae. Maxillula (Fig. 1F) simple cuticular plate with eight setae. Maxilla (Fig. 1F) two-segmented, with two setae on large first segment, and four elements (two setae and two spines) on small second segment. Female maxilliped (Fig. 2A) four-segmented, first and second segment robust, second and third minute, armature formula 2.2.0.5. Male maxilliped (Fig. 3E) prehensile, with narrow and cylindrical first segment, expanded and triangular second segment, and minute third segment armed with large apical claw. All swimming legs (Fig. 2B-E) with three-segmented exopods and endopods, endopods about 1.4 times as long as exopods, only first leg with inner basal spine, first exopodal segment with just outer spine, first endopodal segment with just inner seta, second exopodal segment with outer spine and inner seta, second endopodal segment with one (first leg) or two (other legs) inner setae, spine/seta formula of third exopodal segments 1.2.2.1/7.7.7.7, spine/seta formula of third endopodal segments 1.3.3.3/

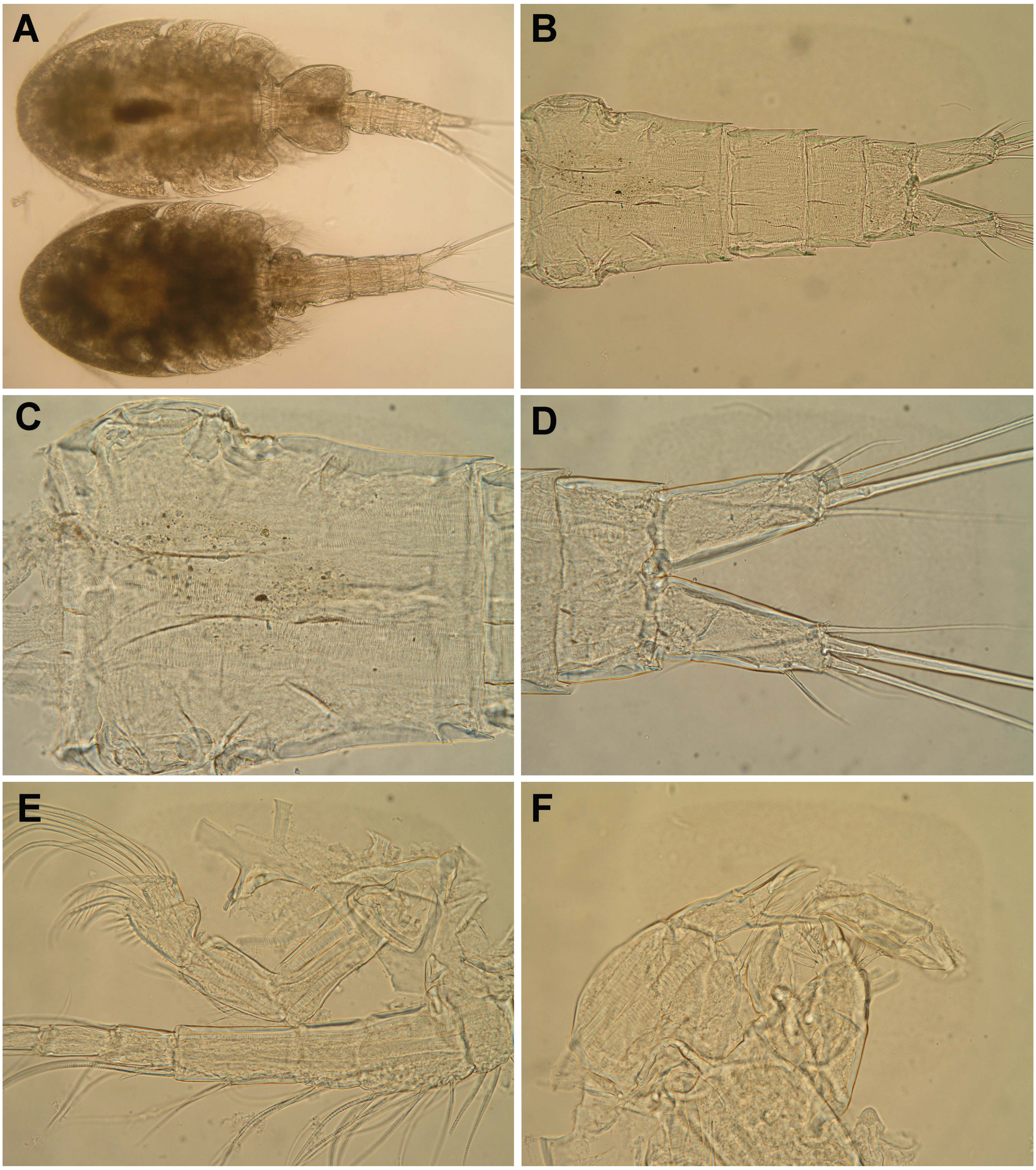

Fig. 1. *Hemicyclops tanakai* Itoh and Nishida, 2002 from Korea. A. male (top) and female (bottom) habitus. B. female urosome. C. female genital double-somite. D. female caudal rami. E. female anntennula and antenna. F. female mandibula, maxillula and maxilla.

5.3.3.2. Fifth leg (Figs. 2F, 3F) two-segmented, first segment short with single outer seta, second segment ovoid, about 1.7 times as long as wide, with three spines and one seta.

Order Harpacticoida G.O. Sars, 1903

Family Tisbidae Stebbing, 1910

Genus *Tisbe* Lilljeborg, 1853

Tisbe cf. _ensifer_ Fisher, 1860 (Figs. 4, 5)

Synonymy. *Tisbe ensifer* Fisch. - Fisher (1860), p. 668, figs. 67-70; *Idya furcata* n. sp. - Brady (1880), p. 172, plate 67, fig. 9; *Idya ensifera* (Fischer) - Grandori (1912),

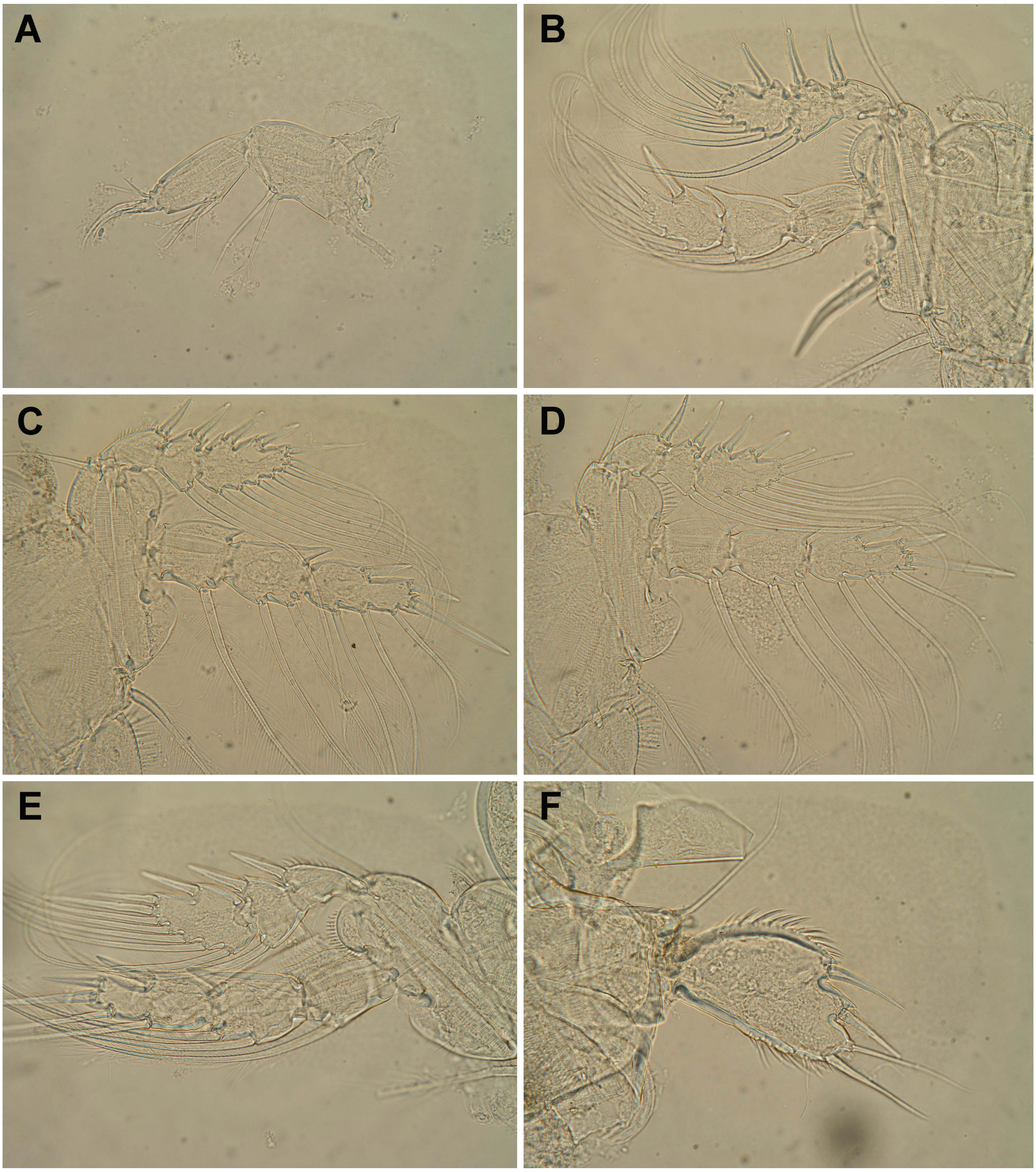

Fig. 2. *Hemicyclops tanakai* Itoh and Nishida, 2002 from Korea, female. A. maxilliped. B. first swimming leg. C. second swimming leg. D. third swimming leg. E. fourth swimming leg. F. fifth leg.

p. 21; Pesta (1920), p. 584, fig. K24; Sars (1905), p. 90, plate 53, fig. 1; *Idyaea ensifera* (Fischer) - Sars (1909), p. 21; Pesta (1927), p. 45; Lang (1934), p. 1, figs. 3-4; *Idyaea ensifera* var. *indica* n. var. - Sewell (1924), p. 817, plate 52, fig. 1; *Tisbe ensifera* var. *indica* (Sewell) - Sewell (1940), p. 160; *Tisbe ensifera* Fischer - Gestraecker (1878), p. 799; Gurney (1927), p. 497; Willey (1930), p. 83; *Tisbe ensifer* Fischer - Lang (1948), p. 372, fig. 165/1; Chislenko (1967), p. 525, fig. 27; Kornev and Chertoprud (2008), p. 106, fig. 5/38/A, B. [non] *Tisbe ensifera* (Fischer) - Chiba (1956), p. 65, fig. 63.

Type locality. Portugal, Madeira Island, ponds and wet washed seaweed on sandy beaches, approximately 32°39′

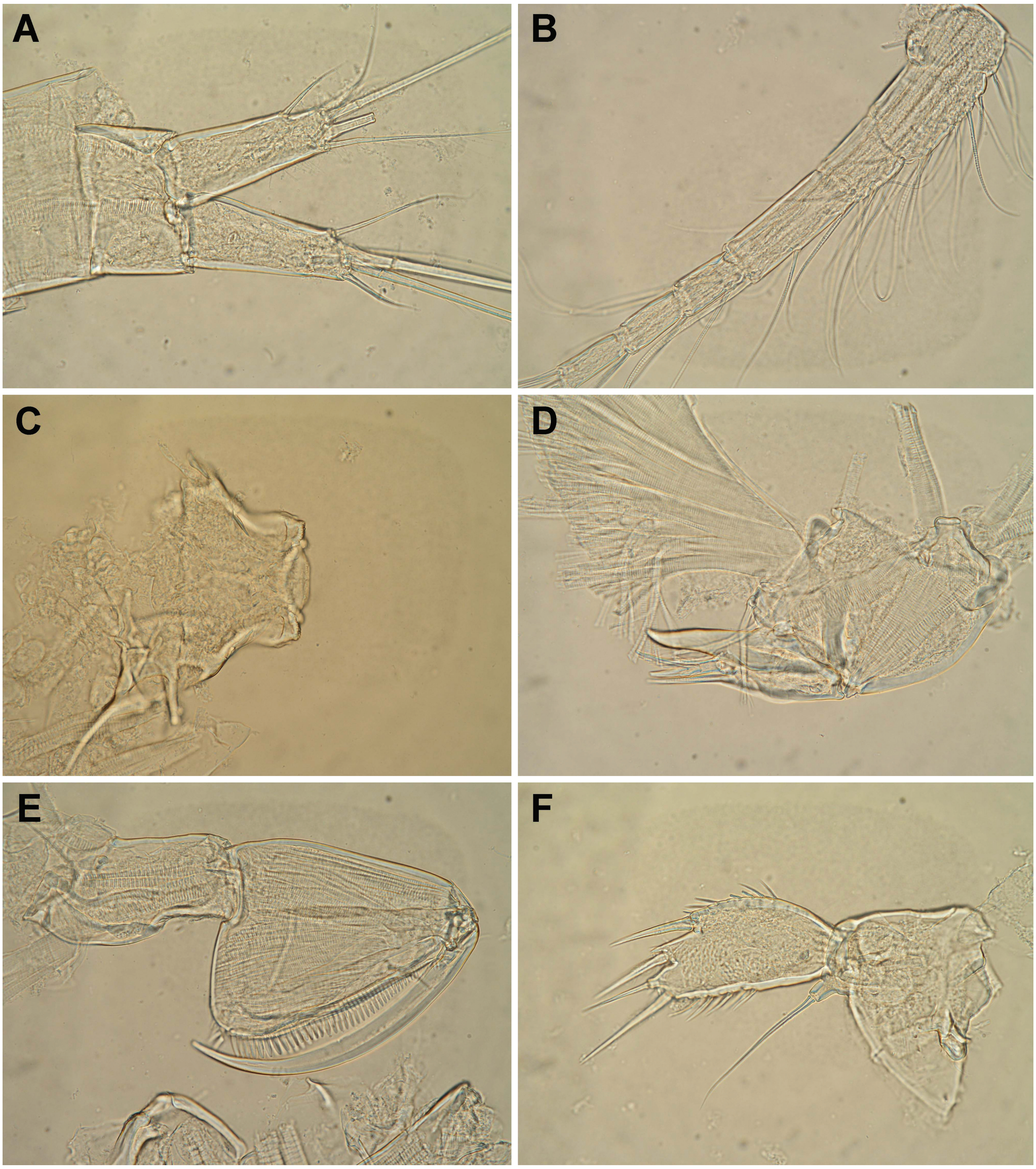

Fig. 3. *Hemicyclops tanakai* Itoh and Nishida, 2002 from Korea, male. A. caudal rami. B. antennule. C. labrum. D. maxilla. E. maxilliped. F. fifth leg.

N 16°54′W.

Specimens examined. Two females dissected on one slide each (ovigerous NIBR IV 0000287269, non-ovigerous NIBR IV 0000287270); both collected from Korea, Sokcho, new boat harbor, shallow littoral, 23 September 2011, leg. T. Karanovic. 38°10.134′N 128°36.512′E.

Diagnosis. Body length, excluding caudal setae, from 780 to 845 μm. Habitus (Fig. 4A) robust, dorsoventrally compressed, with prosome/urosome ratio about 1.8, and greatest width at posterior end of cephalothorax. Prosome ovoid, about 1.7 times as long as wide, 2.3 times as wide as genital double-somite. Urosome (Fig. 4B) gradually tapering posteriorly, genital double-somite 0.9 times as long as wide, 1.6 times as wide as anal so-

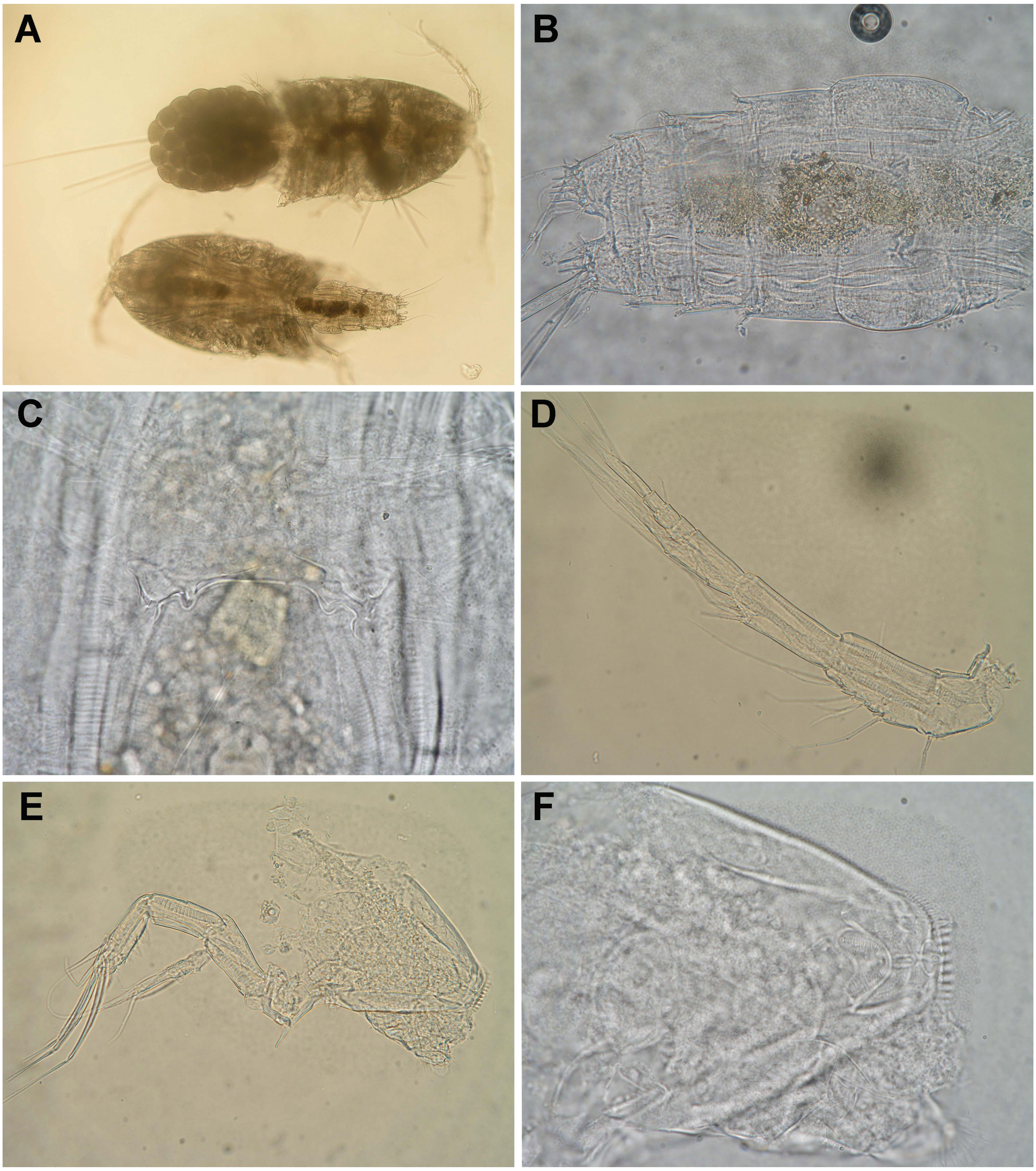

Fig. 4. *Tisbe* cf. *ensifer* Fischer, 1860 from Korea, female. A. ovigerous (top) and non-ovigerous (bottom) female habitus. B. urosome. C. genital field, with reduced sixth legs. D. antennule. E. antenna and labrum. F. labrum.

mite, only slightly expanded in anterior half, with central subdivision suture along dorsal and lateral surfaces. Genital operculum (Fig. 4C) covered with extremely reduced sixth leg. Caudal rami (Fig. 4B) half as long as wide. Atennula (Fig. 4D) eight-segmented, with the proportion of segmental lengths (proximal to distal) 1 : 2.9 : 3.1 : 1.8 : 0.6 : 0.6 : 0.5 : 1.6, and armature formula 1.14.9.4+ae.2.8.6+ae. Antenna (Fig. 4E) with minute unarmed coxa, large basis with single seta, two-segmented robust endopod with one seta on first segment and nine elements (including four geniculated), and four segmented slender exopod with armature formula 1.1.1.3. Labrum (Fig. 4F) trapezoidal, with narrow and straight cutting edge ornamented with continuous row of strong

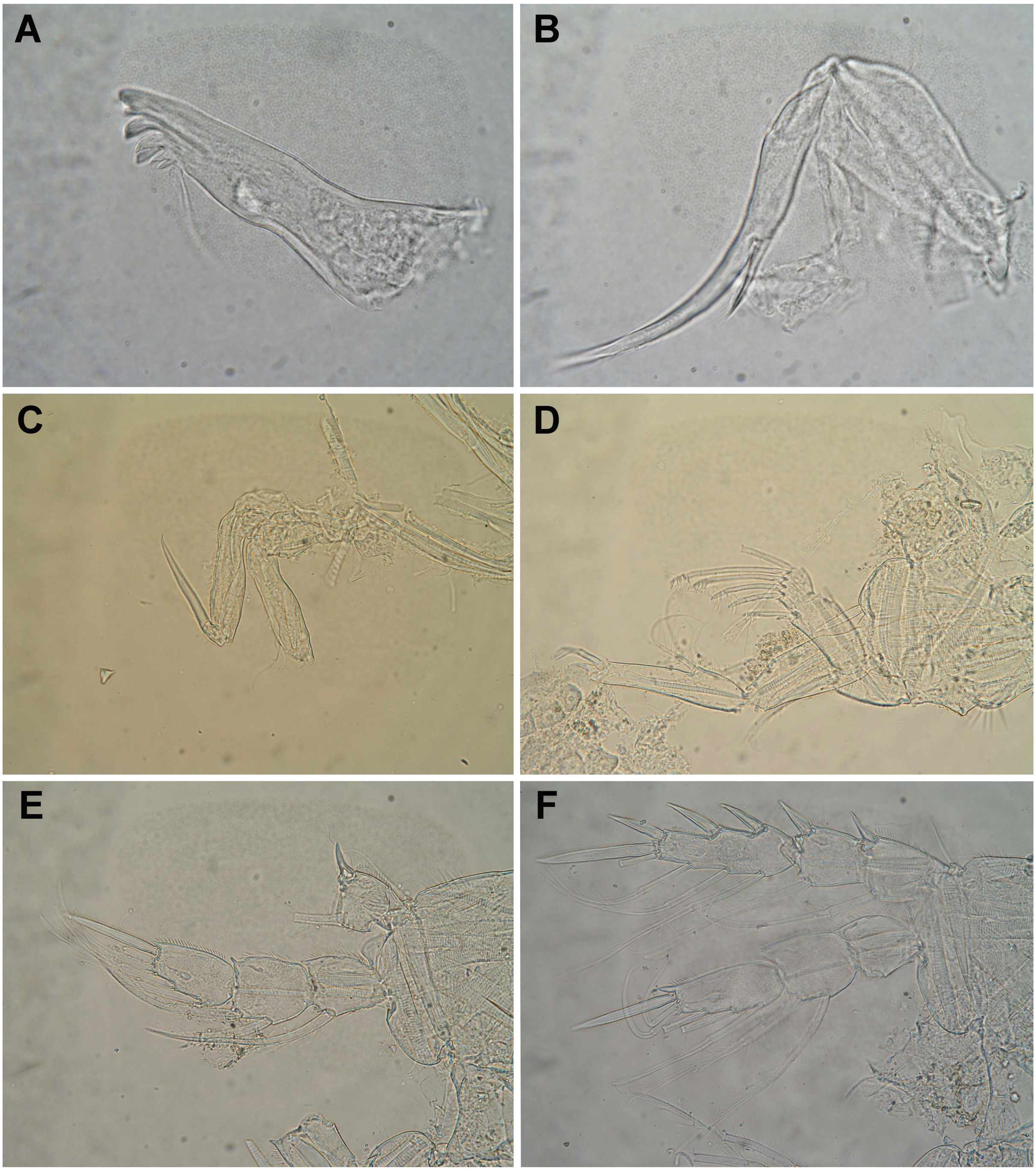

Fig. 5. *Tisbe* cf. *ensifer* Fischer, 1860 from Korea, female. A. coxal gnathobase of mandibula. B. maxilla. C. maxilliped. D. first swimming leg. E. second swimming leg with last two exopodal segments broken off. F. third swimming leg.

spinules. Mandibula (Fig. 5A) with narrow cutting edge on coxal gnathobase, armed with three complex teeth in ventral part, four simple teeth in dorsal part, and single dorsalmost unipinnate seta. Maxilla (Fig. 5B) with single minute endite on large syncoxa bearing two smooth setae, allobasis drawn out into strong arched claw with one short spiniform seta in proximal third. Maxilliped (Fig. 5C) prehensile and slender, basis 3.8 times as long as wide, 2.5 times as long as syncoxa, and 1.1 times as long as apical claw on minute endopod. All swimming legs (Fig. 5D-F) with three-segmented endopods and exopods. First swimming leg (Fig. 5D) with strong inner basal spine and all segments of different lengths, first endopodal segment 2.5 times as long as wide, slightly shorter than second endopodal but much wider, about as long as first two exopodal segments combined, with single long inner seta, second endopodal segment 3.8 times as long as wide, almost nine times as long as third endopodal segment, with single inner seta, third endopodal segment minute, as long as wide, with one minute slender seta and two comb-like short spines, first exopodal segment 1.8 times as long as wide, with single large outer spine, second exopodal segment 1.7 times as long as wide, twice as long as first exopodal segment and 2.6 times as long as third exopodal segment, with short outer spine and short inner seta, third exopodal segment 0.8 times as long as wide, with five comb-like spines and two long setae. Second to fourth legs (Fig. 5E, F) similar to each other, with first and second endopodal and exopodal segments of similar size and slightly larger third exopodal and exopodal segments, first and second exopodal segments with one outer spine and one inner seta, first endopodal segment with single inner seta, second endopodal segment with two inner setae, spine/seta formula of third exopodal segments 4.4.4/3.4.4, spine/seta formula of third endopodal segments 1.1.1/4.5.4. Fifth leg with three setae on short endopodal lobe, exopod about 7.5 times as long as wide, with five setae in distal part.

Discussion

Hemicyclops tanakai was originally described by Itoh and Nishida (2002) from burrows of the mud shrimp *Upogebia major* (de Haan, 1849) in estuarine mud-flats of the Tama River in Tokyo Bay, Japan. They also collected several specimens from the body surface of the ghost shrimp *Nihonotrypaea japonica* (Ortmann, 1892) and from burrows of the ocypodid crab *Macrophthalmus japonicus* (de Haan, 1835). We found it in the interstitial sediments of a sandy beach on Jeju Island, which represents its first record in Korea and second record ever. No morphological differences were observed between these two disjunct populations, so we expect more records of this species, both in Korea and Japan. It seems that the species exploits a variety of intertidal habitats with sandy or muddy sediments. Itoh and Nishida (2002) were able to keep some specimens alive for a month, in a petri dish containing filtered seawater and bottom sediment including sessile diatoms, which suggests that this species exploits shrimp burrows opportunistically and is not dependent on their hosts for survival.

Hemicyclops tanakai can be distinguished from all other congeners by an enlarged and hearth-shaped male genital somite (Fig. 1A). The genus *Hemicyclops* Boeck, 1873 contains today 46 valid species (see Karanovic, 2008; Moon and Kim, 2010; Walter and Boxshall, 2016), which is more than all other seven clausidiid genera combined. The generic name *Saphirella* T. Scott, 1894 was widely used for juvenile copepodid stages of *Hemicyclops*, a situation recognized by Nicholls (1944) and later confirmed through rearing experiments by Itoh and Nishida (1991) and Kim and Ho (1992). *Hemicyclops* is also the most diverse genus ecologically in the family Clausidiidae, with free-living representatives in marine interstitial and littoral, but also in association with a great variety of hosts (often inhabiting their burrows), including sponges, scleractinian corals, gastropods and bivalve mollusks, polychaetes, crustaceans and echiurans (Humes, 1984; Boxshall and Humes, 1987). All species appear to be intertidal forms or inhabitants of shallow waters, found predominantly in temperate and tropical regions of the world oceans (Mulyadi, 2005). Most recent revisionary work of *Hemicyclops* taxonomy was that of Vervoort and Ramirez (1966), who recognized 23 valid species and synonymized several previously described ones. Since then another 23 species have been described in this genus (see Karanovic, 2008; Moon and Kim, 2010; Ohtsuka *et al*., 2010). Also, Hamond (1968) reported and illustrated a *Hemicyclops* male that he could not identify to the species level, leaving it in the open nomenclature (as *Hemicyclops* sp.), and Yeatman (1983) described the male of *H. adhaerens* (Williams, 1907) for the first time. Unfortunately, some species descriptions were based on a very limited set of morphological characters, and today it would be impossible to construct a functional key to species without excluding several members, and this would make any future revision and/or subsequent descriptions of other new species more complicated. A full revision of *Hemicyclops* is needed, and it would have to involve redescriptions of most species described in the nineteenth and the first half of the twentieth century, a task that is well beyond the scope of this paper.

In Korea seven species of *Hemicyclops* have been reported to far, all described as new and presumably endemic (see Ho and Kim, 1990; 1991; Kim, 2000; Moon and Kim, 2010): *H. ctenidis* Ho and Kim, 1990; *H. gomsoensis* Ho and Kim, 1991; *H. membranatus* Moon and Kim, 2010; *H. nasutus* Moon and Kim, 2010; *H. parilis* Moon and Kim, 2010; *H. saxatilis* Ho and Kim, 1991; and *H. ventriplanus* Kim, 2000. *Hemicyclops tanakai* is the first non-endemic element here, and given the proximity of Korea and Japan and very close connections between their faunas (both freshwater and marine: see Karanovic and Lee, 2012; Karanovic *et al*., 2012b; 2013) it might be expected that some Korean species will be found in Japan as well. *Hemicyclops tanakai* differs from other Korean species, besides its unusual male genital somite (note that males of *H. nasutus* have not been described yet), in a number of obvious morphological characters, including the shape of the female genital double somite (all species), shape of the caudal rami (in *H. ctenidis*, *H. nasutus*, *H. saxatilis*), shape or armature of the maxilliped (in *H. ctenidis*, *H. parilis*, *H. ventriplanus*), shape or armature of the swimming legs (*H. membranatus*, *H. saxatilis*), shape of the female fifth leg (*H. ctenidis*, *H. membranatus*, *H. ventriplanus*), and armature of the male sixth leg (*H. gomsoensis*, *H. ventriplanus*). Additionally, each species can be distinguished from *H. tanakai* by the proportion of some armature elements on various appendages, ornamentation (spinule pattern) of some segments, and detailed proportions of the habitus. As all species can be distinguished easily from each other based on the female genital double-somite alone, we see no reason yet to provide a key to aid in their identification. Given the very conservative nature of appendage segmentation and armature in this genus, to understand phylogenetic relationships between its members we will have to rely either on molecular markers or some rarely explored microstructures, such as cuticular organs on somites (see Karanovic and Kim, 2014b; Karanovic *et al*., 2016).

Tisbe ensifer was originally described from Madeira by Fischer (1860), and was later on reported from numerous other parts of the Northern Atlantic (see Sars, 1905; Lang, 1948; Chislenko, 1967; Kornev and Chertoprud, 2008). Sewell (1924) described a separate subspecies from the Indian Ocean (also found in additional localities by Sewell, 1940), but this was considered a synonym by Lang (1948) and all subsequent workers. We report two females from a shallow littoral in Sokcho, which represents its first record in Korea and the first record in the entire Pacific Ocean. Because of morphological discrepancies reported in previous records we recognize that this species might be in reality a species complex, and we only tentatively identify our Korean specimens as *T.* cf. *ensifer*. Most frequently reported geographic variability was in the exact shape of the female fifth leg exopod (more or less elongated; see Lang, 1948; Chislenko, 1967), but this might be a consequence of different drawing skills and equipment used (or not used!) by different researchers, especially in the first half of the twentieth century. Specimens reported by Chiba (1956) (as *T. ensifera*) belong to a completely different (and probably not closely related species), as already noted by Chislenko (1967). In addition to some other differences from all other specimens reported previously, those reported by Chiba (1956) have a completely different proportion of antennular segments, with the second segment about as long as the first one (vs. more than twice as long). It is possible that some other reported populations also represent distinct species (as speculated by Chislenko, 1967), but given the scarcity of illustrations provided by most authors, it is impossible to establish this with any certainly. Thus, a redescription of the types (or topotypes) of this species is essential for establishing specific statuses of its disjunct populations from other parts of the world, including those from Korea. To assist this effort, we provide a selection of photographs of our examined specimens.

Acknowledgements

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