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First record of *Ishizakiella supralittoralis* (Ostracoda, Cytheroidea, Leptocytheridae) from South Korea with a key to species of the genus

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Ishizakiella supralittoralis (Schornikov, 1974) was collected from rock pools near Busan, South Korea, and is redescribed herein. It is the first record of this species from Korea, and also the first finding of a living population of *Ishizakiella* McKenzie & Sudijono, 1981 from the continental margins. Here we also comment on the diversity of *Ishizakiella* in Japan, possible existence of cryptic species, and provide a key to all six currently known species of this genus, including the two fossil ones.

Keywords: biodiversity, Podocopida, taxonomy

INTRODUCTION

The superfamily Cytheroidea is poorly known from the Korean Peninsula. So far, only two papers have been published dealing with this group, among other ostracods. Lee et al. (2000) conducted a study on the ostracods as indicators of water pollution. On that occasion they collected 84 samples, mostly along the South Korean east coast and identified, rather astonishing, 500 ostracod species. Because this research was mainly conducted for the purpose of the environmental studies, only a small proportion of these species have been named, and the greatest majority was identified based on the shell only. Even if identified, the identity of most species was not certain, i.e labeled as cf. Of the 500 species, 441 belong to Cytheroidea. Later on, Annapurna et al. (2009) reported nine podocopid ostracods from the South Korean east coast, seven belonging to Cytheroidea, none of which being previously recorded by Lee et al. (2000), bringing the number of Korean Cytheroidea to 448 species. While Annapurna et al. (2009) provide a very brief taxonomic description of their species (but no illustrations or other remarks), Lee et al. (2000) merely provide a list. It is therefore hard to rely any new investigation on the Korean fauna on these two previous reports. Unfortunately, the materials collected by these authors are not available for study any more.

During a recent study of the Korean biodiversity several samples have been taken from the South Korean east coast, containing abundant ostracods diversity. In this paper we are dealing with *Ishizakiella supralittoralis* (Schornikov, 1974) collected from rock pools near Busan. This species was not previously recorded from Korea by Lee *et al.* (2000) or Annapurna *et al.* (2009). The former reports *I. cf. ryukyuensis* Tsukagoshi, 1994, while the latter reports no species belonging to this genus. *Ishizakiella* McKenzie & Sudijono (1981) belongs to the family Leptocytheridae (Cytheroidea). Lee *et al.* (2000) reported two other lepthocytherid species: *Cluthia cf. ishizaki* Zao, 1988 and *C. japonica* Tabuki, 1986. Annapurna *et al.* (2009) provided a very brief description of *Lepthocythere multipunctata* (Seguenza, 1883), but placed it erroneously in the family Cytheridae, and subfamily Cytherinae.

The genus Ishizakiella was described by McKenzie & Sudijono (1981) to include their new fossil species, I. foveoreticulata McKenzie & Sudijono, 1981 from Plio-Pleistocene deposits of Java. Later on, Tsukagoshi (1994) described Recent I. ryukyuensis Tsukagoshi, 1994 from Okinawa and Iriomonte, and also included the following species in the genus: I. miurensis (Hanai, 1957) living from Kanagawa, Japan; I. pacifica (Hanai, 1957), a new name for Cythere inflata Brady, 1890, known as fossil from Fiji and Samoa; I. novaezealandica (Hartmann, 1982), a Recent species described and known only from New Zealand; and I. supralittoralis (Schornikov, 1974) described as living species from Kuril Islands, and later found in Japan as a living population and as shells from around Vladivostok (see Hanai, 1957; Schornikov, 1974; Hartmann, 1982; and Tsukagoshi, 1994). With the exception of the type species, I. foveoreticulata and I. ryukyuensis, all other species were previously members of Tanella Kingma, 1948 which is still a valid genus. The difference between the two genera is in the ornamentation of the shell (ornamentation of *Ishizakiella* consisting of pits more densely distributed), more prominent sexual dimorphism of *Ishizakiella* and minor details of the soft parts. Titteron & Whatley (2007) doubted validity of the genus *Ishizakiella*, placing its type species in the genus *Leptocythere* Müller, 1927. The two genera are indeed very similar, but we have decided to retain the genus *Ishizakiella* for the purpose of this study, possible revision being out of the scope of the present paper.

Ostracods belonging to the genus *Ishizakiella* inhabit brackish habitats such as river mouths, lagoons, and brackish lakes (Schornikov, 1974; Tsukagoshi, 1994). They lack a planktonic larval stage and have a relatively restricted distribution. The present finding of a living population of *I. supralittoralis* in Korea is the first certain record of the genus along continental margins.

MATERIALS AND METHODS

Material was collected from rock pools in Igidae with hand-net (mesh size $63 \,\mu$ m) (Fig. 1). The salinity was 35.5‰, and temperature 24.2°C. The collected samples were fixed with 99% Et-OH, and sorted in the laboratory of the Hanyang University. All drawings were prepared using drawing tube attached to an Olympus BX51 interference microscope. Scanning electron photographs were taken with the microscope HITACHI S-4700 at Korea University. All the material is deposited at the National Institute of Biological Resources (NIBR), Incheon.

Abbreviation: A1-antennule; A2-antenna; H-height; Llength; L5-L7-limbs; LV-left valve; RV-right valve; Md -mandibula; Mxl-Maxillula; UR-uropodal ramus; W-width.

Systematic Part

Superfamily Cytheroidea Baird, 1850 Family Leptocytheridae Hanai, 1857 Genus *Ishizakiella* Mckenzie & Sudijono, 1981

Ishizakiella supralittoralis (Schornikov, 1974) (Figs. 2-5)

Synonymy. *Tanella supralitoralis* Schornikov, 1974: 158, fig. 11.

Ishizakiella supralittoralis (Schornikov)-Tsukagoshi (1994): 303, figs. 7A, 8A-J, 12A.

Material examined. 2 males (NIBRIV0000245044, NI BRIV0000245045) and 2 females (NIBRIV0000245046,

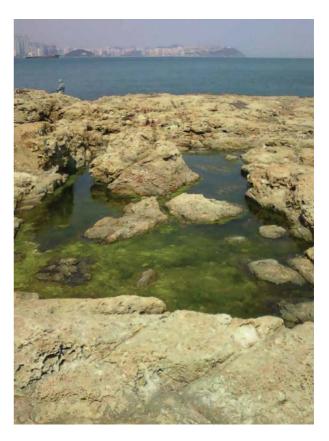


Fig. 1. Sampling locality, Rock Pools in Igidae (35° 07'20.2"N, 129° 07'30.1"E), Busan, South Korea.

NIBRIV0000245047) dissected, approximately 15 specimens kept undissected in 99% Et-OH, and 1 male and 1 female on one SEM-stub from Rock Pools in Igidae (35° 07'20.2"N, 129° 07'30.1"E), Busan, South Korea, 25th of April, 2011, collected by Hyunsu Yoo.

Redescription. *Male*: Shell subrectangular in lateral view (Figs. 2C, D, 5C, D), with greatest H situated on the first third of L equaling 50% of total L. Dorsal margin slopping gently down from anterior to the posterior end with a small bend around middle L. Both anterior and posterior margins widely rounded and equally wide. Ventral margin almost straight. From inside view, flange narrow and running around anterior, ventral and posterior margin. Calcified inner lamella well-developed on both ends. Line of concrescence narrow and marginal pore canals very short, but dense. Hinge merodont: LV anteriorly and posteriorly with sockets (Figs. 5D, F), medially with shallow continuous socket (Fig. 5E), while RV anteriorly and posteriorly with teeth (Figs. 5H, J), while medially with single continuous bar (Fig. 5I). Surface ornamentation consisting of relatively small pits. One anterior and one posterior more prominent ridge present, along with numerous smaller and discontinuous ones. Surface also relatively densely covered with hair-like setae, which spec-

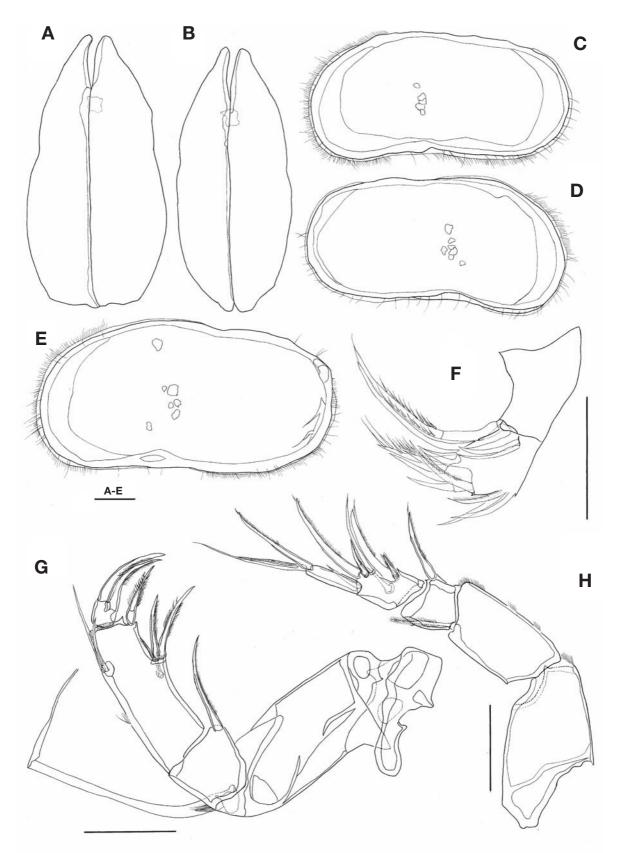


Fig. 2. *Ishizakiella supralittoralis* (Schornikov, 1974). A, E, F-H. female; B-D. male: A, B. shell, dorsal view. C, E. RV-lateral view from the inside. D. LV-lateral view from the inside. F. second, third and fourth segments on the Md palp. G. A2. H. A1. A-E Scales 0.1 F-H Scales 0.05 mm.

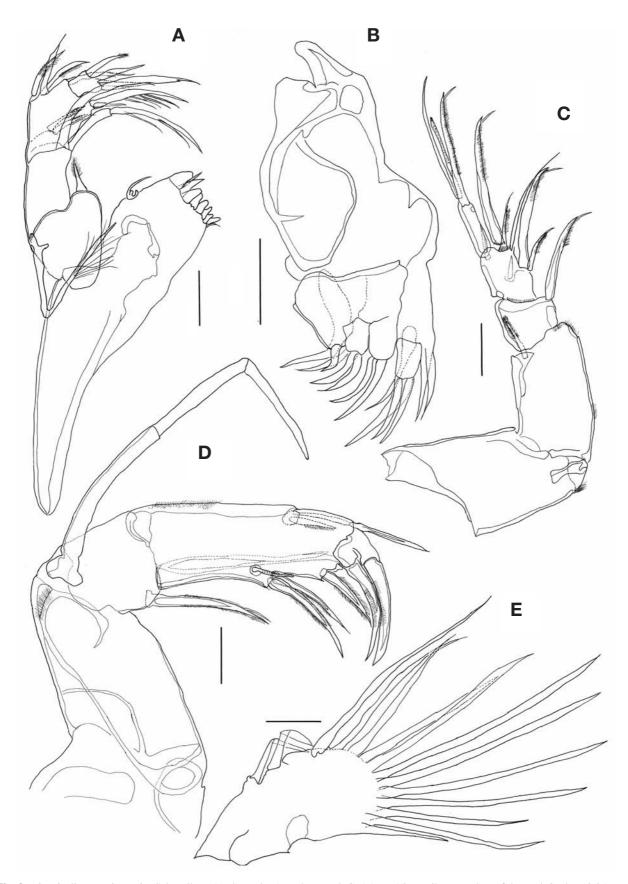


Fig. 3. Ishizakiella supralittoralis (Schornikov, 1974). Male: A. Md. B. Mxl. C. A1. D. A2. E. vibratory plate of the Mxl. Scales=0.05 mm.

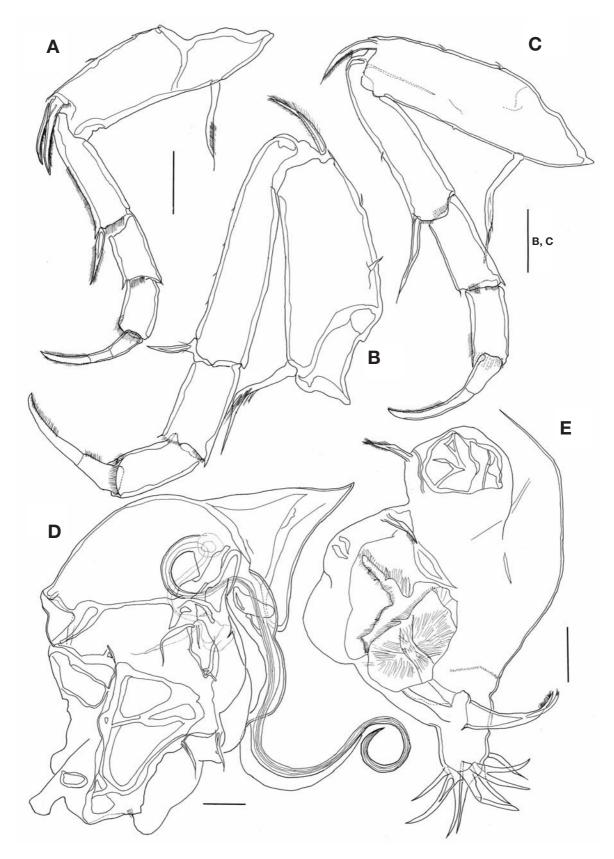


Fig. 4. Ishizakiella supralittoralis (Schornikov, 1974). A-D. male, E. female: A. L5. B. L7. C. L6. D. Hemipenis. E. UR and the posterior end of the body. Scales=0.05 mm.

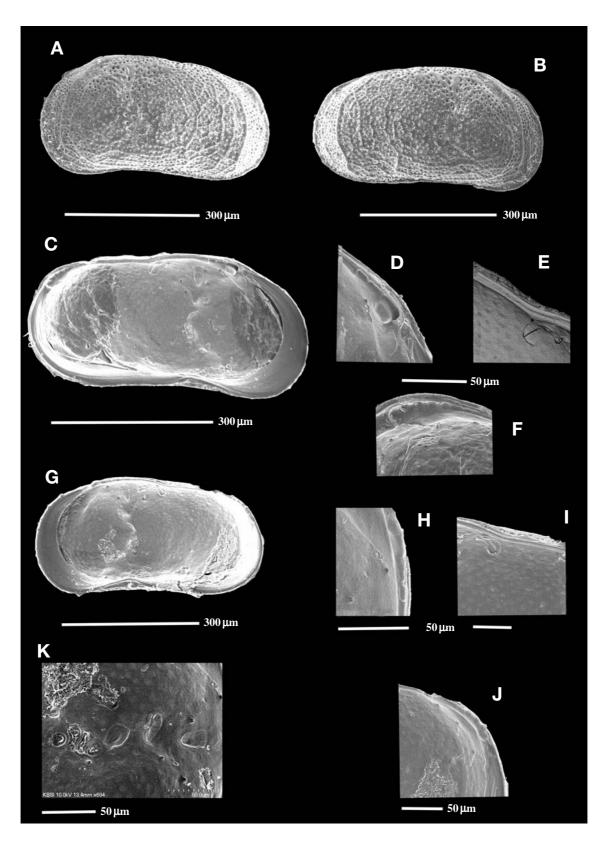


Fig. 5. SEM photos of *Ishizakiella supralittoralis* (Schornikov, 1974). A, B. female, C-K. male: A. LV, lateral view from the outside. B. RV, lateral view from the outside. C. LV, lateral view from the inside. D. anterior part of the hinge on the LV. E. medial part of the hinge on the LV. F. posterior part of the hinge on the LV. G. RV, lateral view from the inside. H. anterior part of the hinge on the RV. I. medial part of the hinge on the RV. J. posterior part of the hinge on the RV. K. muscular scars imprints.

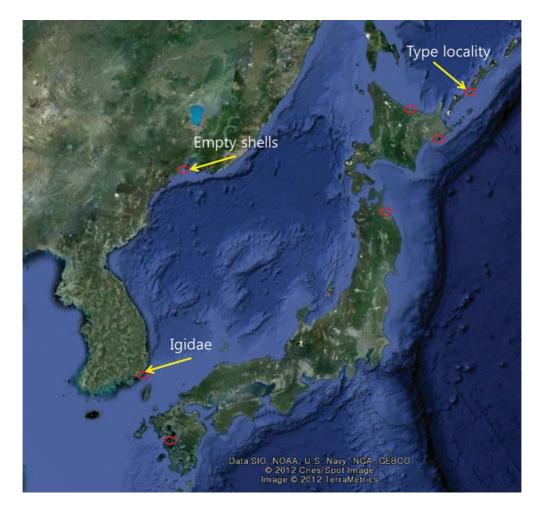


Fig. 6. Present distribution of Ishizakiella supralittoralis (Schornikov, 1974).

ially dense frontally and caudally. Muscular scar imprints consisting of a row of four vertical scars, dorsally to which another three larger scars present. In dorsal view (Fig. 2B) carapace narrow, slightly constrict anterior to middle L. Greatest W equaling 38% of L. One eye prominent and pigmented. L=0.48 mm.

A1 (Fig. 3C): 5-segmented. First segment bare, second segment postero-dorsally with one short (not reaching distal end of same segment) plumose seta. Third segment short, and anteriorly with strong, distally plumose seta, which reaching distal end of fourth segment. Fourth segment with total of seven setae: three situated more medially and four more distally. Two proximal setae short (one situated dorsally bare, one situated ventrally plumose); third medial seta three times longer, distally plumose and with terminal spinula. Two distal setae on fourth segment long (one bare, other plumose and with terminal spinula), and two three times shorter (one dorsal bare, ventral plumose). Terminal segment with one plumose seta terminating with spinula, and one smooth seta proximally fused with aesthetasc. Length ratio of four distal

segments equaling 2.8:1:1.15:1.15. Almost all setae on A1 very strong, all segments hirsute on their distal margins.

A2 (Fig. 3D): 4-segmented. Exopod transformed into spinneret seta which with two sutures. First endopodal segment with one strong seta postero-distally with overpassing middle of following segment. Second endopodal segment with two equally long setae postero medially (exceeding distal end of terminal segment), and accompanied with one short aesthetasc; postero-distally second endopodal segment with one plumose seta; anteriorly with two setae (one exceeding distal end of terminal segment, other two times shorter) situated more distally. Terminal segment with two strong claws. Length ratios of three endopodal segments equaling: 2.8:5.4:1.

Md (Fig. 3A): Exopod with one long vibratory seta. First segment of the palp with one posterior, plumose seta. Second segment with two long setae, each carrying distally spin-like setulae, and two short (situated mediodistally) plumose setae. Third segment with three subequal, plumose setae which only reaching distal end of terminal segment; two setae postero distally (one long and with long spine-like setulae, other short) and one posterodistal seta. Terminal segment square-shaped and with four strong setae.

Mxl (Fig. 2B, E): Exopod without reflexed setae. Palp 2-segmented. First segment with three setae: two lateral and one medial. Terminal segment with three setae, one completely fused with segment. Masticatory lobes small and each with two or three setae.

L5 (Fig. 4A): First segment dorsally with two tiny setae and two long setae distally; ventrally same segment with one long, plumose, proximal seta. Second segment with one distal plumose seta. Third and fourth segments bare. Terminal claw gently curved and short, slightly longer than two terminal segments combined. All segments hirsute especially along margins.

L6 (Fig. 3C): First segment dorsally with two short setae situated more proximally on the segment and one ventral, long seta; dorso-distally same segment with one plumose seta. Second segment with one distal seta, exceeding distal end of following segment. Third and fourth segments bare. All segments hirsute especially along margins

L7 (Fig. 4B): Similar to L6 except than seta on second segment much shorter.

Hemipenis (Fig. 4D): Distal lobe triangular and with antero-distal end sharply pointed. Postero-ventral end of distal lobe cylindrical. Ejaculatory tube smooth around margins. Clasping part cylindrical with rounded tip. Hemipenis capsule with couple of sclerified rods, but no musculous net present.

Female: Carapace: Strong sexual dimorphism present especially in dorsal view (Fig. 1A). Females with much broader posterior end, -transformed into broading pouch. In lateral view (Figs. 2E, 5A, B) dorsal margin with more prominent medial bend. Calcified inner lamella posterior-ly narrower than in males. Hinge same as in males. L= 0.51 mm.

A1 (Fig. 2H): Similar to male, except that posterior seta on second segment longer, and all setae appearing more slender than in males.

A2 (Fig. 2G): Exopod with only one suture observed.

Md palp (Fig. 2F): long setae on second and third segment of palp with less developed spine-like setae.

Genital filed and end of the body (Fig. 2E): Two setae representing UR present. Body ends in conspicuous process with two main branches each with numerous filaments.

Other appendages same as in male.

Remarks. The population collected in Korea slightly differs from the original description from Kuril Island (Schornikov, 1974) and subsequent finding in Japan (Tsukagoshi, 1994). Females of the Korean population seem to be much narrower in dorsal view. The other two findings

are also different from each other, and in the original description the females have the broadest posterior end, while the Japanese population is somewhere in-between these from Kuril Island and Korea. The hinge part in the Japanese population is slightly different, as the teeth and sockets on the posterior part are much better differentiated, i.e. one can easily observe up to six sockets/teeth in the population from Japan, while in the Korean specimens the teeth/sockets seem rather fused together. Tsukagoshi (1994) did not provide drawings or descriptions of the soft parts. In comparison to Schornikov's (1974) description of the soft parts, the Korean population differs in the following: 1. all the setae on the A1 are slightly shorter and stronger; 2. setae on A2, especially antero-distal ones on the second endopodal segment are also shorter, and the terminal claws are equally long, while in the Kuril Island population the more posterior (inner claw) is shorter; 3. spinulae on the long setae of the Md palp are better developed in the Korean population; 4. both tiny dorsal setae are present on the L7 in the Korean population, while on the drawings of Schornikov (1974) none is apparent, but because of their small size the setae may have been overlooked; 5. end of the female body looks different in two populations, namely the one from Korea has slightly stronger main process (in the Kuril Island specimens it is more slender), and the second branch coming ventrally has less filaments. The importance of the last character is not clear, and the appearance of it may be influenced by the slide preparation, since many of the filaments may become folded.

We have also noticed some variability within the Korean population: one or two setae internally on the first segment of the Md palp, and three or four setae on the first segment of the Mxl palp.

DISCUSSION

Ishizakiella supralittoralis (Schornikov, 1974) can be easily distinguished from other species of the genus, both fossil and Recent ones, based on the shell structure. Namely, only in this species the marginal zone is narrow and the marginal pore canals are not long and branched. Tsukagoshi (1994) considered this a pedomorphic character, since juveniles of other Ishizakiella McKenzie & Sudijono, 1981 species also have unbranched marginal canals. Ishizakiella supralittoralis is also less ornamented than its congeners and it is the only one where the central bar of the hinge is not crenulated. In fact, the ornamentation of *I. supralittoralis* resembles most closely the one found in the fossil species, I. pacifica (Hanai, 1957). When the soft parts are concerned, I. supralittoralis is the only species where the walking limbs carry very small (sometimes hardly visible) dorsal setae. These setae are much

more prominent in the other three Recent species. While the Japanese, I. ryukyuensis Tsukagoshi, 1994 and I. miurensis (Hanai, 1974) both have a serrated part on the ejaculatory process of the hemipenis, the New Zealand, I. novaezealandica (Hartmann, 1982) and I. supralittoralis have smooth ejaculatory process. Based on the description of Hartmann (1982), there are no other features that would more closely relate the New Zealand species to I. supralittoralis, than to the two Japanese species. Tsukagoshi (1994) considered that I. novaezealandica most closely resembles I. ryukyuensis. The molecular results are ambiguous in this regard (Yamaguchi, 2000). While on the phylogenetic tree of the mitochondrial COI gene I. supralittoralis groups as a sister taxon of I. novaezealandica, on the phylogenetic tree based on the inferred amino acid sequences this species clusters with the two Japanese species. The close relationship of I. miurensis and I. ryukyuensis is well supported on both trees of Yamaguchi (2000) and the one published latter on (Yamaguchi, 2003). Both papers deal with substantial samples, 29 and 21 populations respectively from all over Japan. In both papers the pairwise distances strongly reflect geographical distribution, close populations clustering together and showing less divergence than the populations from distant localities. Pairwise divergence value within I. supralittoralis collected from seven localities in Japan (2 from Hokkaido; 1 from northern Honshu, and 4 from Kyushu) are relatively small, ranging from 0 to 6.3%. On the other hand, the divergences are much higher among the two Japanese species: for *I. miurensis* they range from 0 to 16.1%, while for I. ryukyuensis from 0 to 19.6%. Ishizakiella ryukyuensis is distributed only on the Nansei Islands, while the distribution of I. miurensis ranges from Kyushu to northern part of Honshu. Neither has been reported from Hokkaido. In the second analysis, however, Yamaguchi (2003) reports a population of I. ryukyuensis from Kii Peninsula (Honshu). This population exhibits some remarkable differences in the hemipenis morphology compared to the Nansei Island populations, which Yamaguchi (2003) explains as character displacement, between species with parapatric distribution, supported with the well know variability in the hemipenis structure of some Cythere Müller, 1785 species (Tsukagoshi, 1994). The results are, however, accompanied with the molecular analysis of the mitochondrial COI, which shows a remarkable divergence of the Hokkaido population compared to the Nansei Islands one, and the pairwise distances within populations of I. ryukyuensis range from 1.3 to 21.2%, while the values between I. ryukyuensis and I. miurensis range from 20.6 to 28.9%, i.e. divergences between some populations of I. ryukyuensis are greater than between the two species. These values are well above those recorded in other crustaceans, as one of the advantages of COI gene is that it often shows low levels of genetic variation within species, but high level of divergence (usually >15% for crustaceans) between species (Lefébure *et al.*, 2006; Karanovic & Cooper, 2011). Therefore, we think that the Japanese populations of both species, but especially of *I. ryukyuensis* have several cryptic species. The hemipenis structure of the Honshu population along with the molecular results clearly point towards this. Japanese archipelago seems to have played an important role in diversification of the genus *Ishizakiella* and careful studies of the microcharaters will most probably support, in some cases, large COI sequence divergences among the populations, and lead to descriptions of new species.

We identified the Korean population as *I. supralittoralis* because of the following reasons: rather small morphological difference which we judge from the description of Schornikov (1974) and later brief report from Japan (Tsukagoshi, 1994); wide distribution (from Kuril Island to Kyushu, and one record of the shell close to Vladivostok) (Fig. 6); and finally, because the populations in Japan, even though separated by a wide distance, have small COI divergence (which suggests high gene flow). Further study of *Ishizakiella* species from Korea involving mitochondrial DNA will better clarify the position of the Korean populations.

Ishizakiella supralittoralis was found in mixoeuhaline waters in Korea (35.2%). In other localities (Kuril Island and Japan) the salinity ranges from 0.48 to 26‰, showing a rather wide tolerance to different salinity levels. Similar tolerance has been shown for other Japanese species of Ishizakiella as well (Tsukagoshi, 1994). Despite this high salinity tolerance, large open sea seems to act as a barrier to the wide distribution of Ishizakiella species. Yamaguchi (2000; 2003) postulated that the common ancestor of the East Asian Ishizakiella species migrated northward to the Japanese Archipelago along the continental margin before the Pleistocene, and that subsequent formation of land bridges between the Korean Peninsula and the Japanese Archipelago as well as the one between Nansei Islands and Honshu contributed towards further speciation (see Yamaguchi, 2000). If this hypothesis is correct, it would mean that I. supralittoralis in Korea is a remnant of the oldest population of this species.

Key to species of Ishizakiella

- Ishizakiella pacifica (Hanai, 1957)
- Ornamentation much more harsh, consisting of large fovea with secondary ornamentation inside them3

- RV of female in external view without a clear postero
 -ventral square-shaped process (sometimes this margin square-shaped, but no process present) ------4

- 5. Serration on the copulatory duct strongly pronounced, tassel-like Ishizakiella miurensis (Hanai, 1957)

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