

First Record of *Gammarus koreanus* (Crustacea, Amphipoda, Gammaroidea) from Japan, Based on Morphology and 28S rRNA Gene Sequences

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(Received 8 June 2011; Accepted 25 November 2011)

Gammarus koreanus Uéno, 1940, which was originally described from the Korean Peninsula, is recorded from freshwaters of the Gotô Islands, Japan. This is the first record of its occurrence in Japan. Molecular analyses using partial sequences of the 28S rRNA gene strongly support the identity of Japanese populations with *G. koreanus*. The Japanese specimens differ from the original description of this species in the following features: antenna 2 lacking calceoli, inner margins of inner and outer rami of uropod 3 with plumose setae, and outer margin of outer ramus of uropod 3 without plumose setae. These differences are regarded as intraspecific variation.

Key Words: *Gammarus koreanus*, Amphipoda, first record, 28S rRNA, Japan, taxonomy, Gotô Islands, interspecific variation.

Introduction

The genus *Gammarus* Fabricius, 1775 is widely distributed in fresh, estuarine, and marine waters in the northern hemisphere, and more than 200 species have been described to date (Väinölä *et al.* 2007; Hou *et al.* 2009). Two species, *G. nipponensis* Uéno, 1940 and *G. sobaegensis* Uéno, 1966, have been reported from Japan. *Gammarus nipponensis* is endemic to Japan, and occurs in mountain streams or spring brooklets of Honshu, Shikoku, and Kyushu (including Tsushima and Iki islands) (Uéno 1940, 1941; Tomikawa, unpublished data). The other species, *G. sobaegensis*, recorded in Japan only from Akiyoshido Cave, Yamaguchi Prefecture (Karaman 1986), was originally described from the Korean Peninsula.

Gammarus koreanus Uéno, 1940 was first described as *G. (Rivulogammarus) pulex koreanus* by Uéno (1940) based on specimens collected from Kainei (Hoeryong) and Zenkyori (present-day name unknown) in the northeastern part of the Korean Peninsula. It was elevated to full specific rank by Karaman (1984, 1991). Hou *et al.* (2007) investigated phylogenetic relationships among species of *Gammarus* using molecular data and indicated that *G. koreanus* collected from China is closely related to *G. electrus* Hou and Li, 2003, *G. nekkensis* Uchida, 1935, *G. nipponensis*, and two undescribed species designated as *Gammarus* sp. 3 ZH-2007 and *Gammarus* sp. 4 ZH-2007.

During a field survey of freshwater amphipods in the Gotô Islands, Nagasaki Prefecture, Japan, made in 2010, *G. koreanus* was collected from two islands, Fukue Island and

Nakadôri Island. In this study, we report the occurrence of this species in Japan based on these newly collected specimens. In addition, molecular phylogenetic analyses were used to clarify phylogenetic relationships among populations of *G. koreanus*.

Materials and Methods

Samples. Specimens of *Gammarus koreanus* were collected from six localities of two islands, Nakadôri Island and Fukue Island, in the Gotô Islands, Nagasaki Prefecture (Fig. 1). They were collected by scooping with a fine-mesh handnet, and preserved in 99% ethanol at the site. Two anisogammarids, *Jesogammarus (Annanogammarus) debilis* Hou and Li, 2005 and *J. (Jesogammarus) hebeiensis* Hou and Li, 2004 were used as outgroup taxa (Table 1).

Morphological observation. All appendages of the specimens were dissected under a stereomicroscope (Olympus SZX7) in 99% ethanol and mounted in gum-chloral medium on glass slides. These slides and remaining bodies of the amphipods were examined using a compound microscope (Olympus BH2) and illustrated with the aid of a camera lucida. The body length from the tip of the rostrum along the dorsal curvature to the base of the telson was measured to the nearest 0.1 mm. The nomenclature of setal patterns on the mandibular palp follows Stock (1974). The specimens are deposited in the National Museum of Nature and Science, Tokyo (NSMT).

DNA extraction, PCR amplification, and DNA sequencing. Total genomic DNA was extracted from the

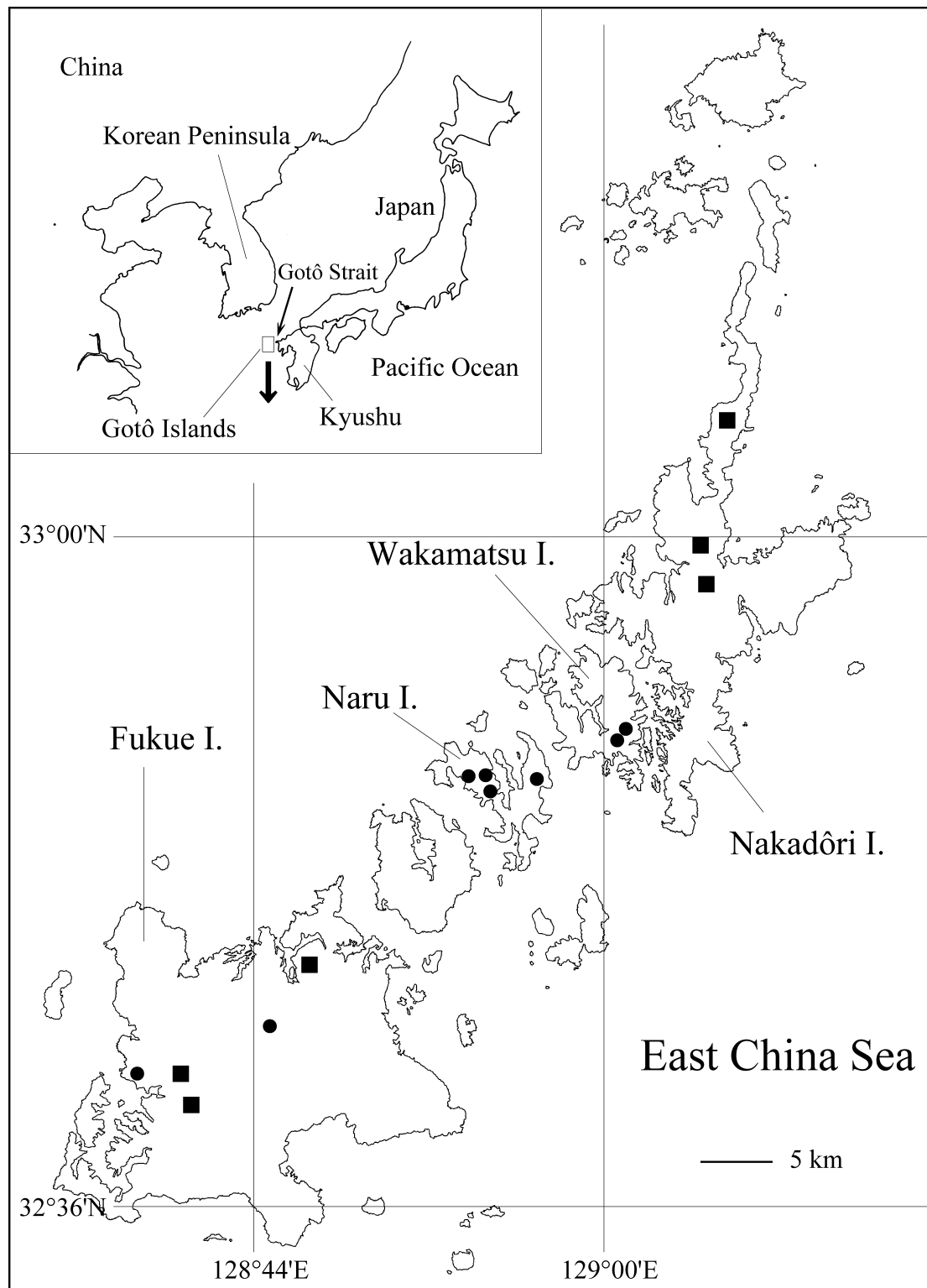


Fig. 1. Study area. Filled squares indicate the localities at which *Gammarus koreanus* Uéno, 1940 was collected. Filled circles indicate the sampling sites at which *G. koreanus* was not found.

pereopod musculature of each individual by means of the DNeasy blood and tissue Kit (Qiagen, Hilden, Germany); the final volume of the unquantitated DNA solution following extraction was 200 μ l. Partial regions of the 28S rRNA genes (about 700bp) were amplified by polymerase chain reaction (PCR) using the following primer pairs: 28SF [5'-AGG TCG TAA ACT CGA TCT AAG-3'] and 28SR [5'-

CAC ACA TGC TAT ACT CCT TGG-3'] (modified from Hou *et al.* 2007). PCR reactions containing 0.5 μ l template solution, 2 mM $MgCl_2$, 2.5 mM each dNTP, 10 pmol each primer, and 5 U/ μ l Taq polymerase (TaKaRa Ex Taq[®]) in 1 \times buffer provided by the manufacture were performed in 10- μ l volumes in an PC-320 thermal cycler (ASTEC). Amplification conditions were as follows: an initial denaturation

Table 1. Species, sampling localities, accession numbers, and number of specimens of taxa included in the phylogenetic analysis.

Species	Locality	No. of specimens	Haplotype nos (no. of individuals)	GenBank Accession No.
<i>Gammarus koreanus</i> Uéno, 1940	Arakawa R. (Fukue I.), Tamaouracho, Gotô city, Nagasaki Pref., Japan	2	GK1 (2)	AB631038
	Dondonbuchiaki waterfall (Fukue I.), Gotô city, Nagasaki Pref., Japan	2	GK2 (2)	AB631039
	Tsuridô R. (Nakadôri I.), Shin-kamigotôcho, Naga- saki Pref., Japan	2	GK3 (1), GK4 (1)	AB631040, AB631041
	Aiko R. (Nakadôri I.), Shin-kamigotôcho, Nagasaki Pref., Japan	2	GK5 (2)	AB631042
	Ômizu (Nakadôri I.), Shin-kamigotôcho, Nagasaki Pref., Japan	2	GK6 (2)	AB631043
	Ji'an, Jilin, China	1		EF582963
<i>Gammarus electrus</i> Hou and Li, 2003	Haidian, Beijing, China	1		EF582951
<i>Gammarus nekkensis</i> Uchida, 1935	Chengde, Hebei, China	1		EF582972
<i>Gammarus nekkensis</i> Uchida, 1935	Mentougou, Beijing, China	1		EF582973
<i>Gammarus</i> sp. 3 ZH-2007	Laiyuan, Hebei, China	1		EF582987
<i>Gammarus</i> sp. 4 ZH-2007	Fangshan, Beijing, China	1		EF582988
<i>Jesogammarus debilis</i> Hou and Li, 2005	Fangshan, Beijing, China	1		EF582997
<i>Jesogammarus hebeiensis</i> Hou and Li, 2004	Yanqing, Beijing, China	1		EF582998

for 7 min at 94°C; 35 cycles of denaturation for 45 s at 94°C, annealing for 1 min at 30–42°C depending on sample, and extension for 1 min at 72°C; and final extension for 7 min at 72°C. Amplification products were purified by the silica method (Boom *et al.* 1990). All sequencing reactions were performed according to the manufacturer's instructions using the BigDye Terminator v3.1 Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA). Cycle sequencing conditions were 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Sequencing reaction products were purified by ethanol precipitation. Labeled fragments were analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystem). Sequences were obtained from both strands of the gene segments for verification using the same primers. The nucleotide sequences have been registered in the DNA Databank of Japan (DDBJ) nucleotide-sequence database (linked to the EMBL and GenBank databases) under accession numbers AB631038–AB631043.

Molecular phylogenetic analyses. Since Hou *et al.* (2007) indicated that *Gammarus koreanus* is closely related to *G. electrus*, *G. nekkensis*, *G. nipponensis*, *Gammarus* sp. 3 ZH-2007, and *Gammarus* sp. 4 ZH-2007, previously reported sequences of these six species were included in the phylogenetic analyses. The nucleotide sequences were aligned with the multiple alignment algorithm in Clustal W (Thompson *et al.* 1994) with default setting (gap opening penalty = 15, gap extension penalty = 6.66, transition weight = 0.5). Phylogenetic relationships were reconstructed by the neighbor-joining (NJ) (Saitou and Nei 1987), equally weighted maximum parsimony (MP), and maximum likelihood (ML) methods with MEGA 5.03 software (Tamura *et al.* 2011). Indels were treated as missing data in all analyses. In the NJ analysis, the Kimura 2-parameter (K2P) model (Kimura 1980) of nucleotide substitution was used to estimate genetic distances. In the MP analysis, a tree was obtained using the Close-Neighbor-Interchange algorithm, in which the initial trees were obtained with the random addition of sequences (10 replicates). In the ML analysis, the Kimura 2-parameter with gamma (K2P+G) model (gamma distribution shape parameter = 1.713) was selected as the best-fit model using the Bayesian information criterion (BIC) in MEGA 5.03. To estimate statistical support for branching patterns, 10,000 bootstrap replications (Felsenstein 1985) were performed for the NJ, and 1,000 for the MP and ML analyses, respectively.

Taxonomic Account

Gammarus koreanus Uéno, 1940

[New Japanese name: Chôsen-yokoebi]

(Figs 2–4)

Gammarus (*Rivulogammarus*) *pulex koreanus* Uéno, 1940: 78–81, figs 74–90; Lee and Kim 1980: 44; Barnard and Barnard 1983: 468.

Gammarus koreanus: Karaman 1984: 142; Karaman 1991: 48–54, figs VI 6, 7, VII–XI; Hou *et al.* 2007: 598.

Material examined. Fukue Island: NSMT-Cr 21670–21673, 3 males (10.4–11.6 mm) and 1 female (8.0 mm), Arakawa River, 32°39'50"N, 128°42'3"E, Tamanoura-cho, Gotô city, Nagasaki Prefecture, 25 January 2010, coll. K. Tomikawa and S. Tashiro; NSMT-Cr 21975, 1 male (8.9 mm), Nanatakejinja, 32°40'58"N, 128°41'23"E, Tamanoura-cho, Gotô city, Nagasaki Prefecture, 25 January 2010, coll. K. Tomikawa and S. Tashiro; NSMT-Cr 21974, 1 male (9.5 mm), Dondonbuchidaki waterfall, 32°44'37"N, 128°47'7"E, Gotô city, Nagasaki Prefecture, 25 January 2010, coll. K. Tomikawa and S. Tashiro. Nakadôri Island: NSMT-Cr 21674–21677, 4 males (9.5–11.9 mm), Tsuridô River, 32°59'44"N, 129°4'12"E, Shin-kamigotôcho, Nagasaki Prefecture, 23 January 2010, coll. K. Tomikawa and S. Tashiro; NSMT-Cr 21678–21679, 2 males (7.8–10.0 mm), Aiko River, 32°57'40"N, 129°4'44"E, Shin-kamigotôcho, Nagasaki Prefecture, 23 January 2010, coll. K. Tomikawa and S. Tashiro; NSMT-Cr 21973, 4 males (8.3–11.7 mm), Ômizu, 33°4'24"N, 129°5'58"E, Shin-kamigotôcho, Nagasaki Prefecture, 23 January 2010, coll. K. Tomikawa and S. Tashiro.

Description. *Male.* Head (Fig. 2A) slightly shorter than pereonites 1 and 2 combined, rostrum short, ventral margin of lateral cephalic lobe straight, antennal sinus rounded. Dorsal margins of pleonites 1–3 each with 4 setae. Epimeral plates 1–3 with 1, 1, and 2 robust setae on each ventral margin, and 3 short setae on each posterior margin; posteroventral corner of plates 2 and 3 pointed (Fig. 2A). Urosomites 1–3 with 4, 4, and 2 robust setae on dorsal margin, respectively.

Antenna 1 (Fig. 2A) longer than half of body length; each article of primary flagellum with aesthetasc, calceoli absent. Antenna 2 (Fig. 2B,C) shorter than antenna 1; posterior margins of peduncular articles 4 and 5 with 2 and 3 clusters of short setae, respectively, except in specimens from Tsuridô River, Nakadôri Island, these with 3 and 4 clusters of long setae, respectively (Fig. 2C); flagellum longer than half of peduncle length, calceoli absent.

Upper lip (Fig. 2E) with fine setae on rounded ventral margin. Lower lip (Fig. 2F) with broad outer lobes, inner lobes indistinct. Mandibles (Fig. 2G,H) with left and right incisors 5- and 4-dentate, respectively; left lacinia mobilis 4-dentate, right one bifid, bearing many teeth; each accessory setal row consisting of weakly pectinate setae; palp 3-articulate, article 3 with A, B, D, and E-setae. Maxilla 1 (Fig. 3A) with many plumose setae on medial margin of inner plate, inner surface setulose; outer plate subrectangular, with 11 serrate apical teeth, inner face setulose; palp 2-articulate, exceeding tip of outer plate, article 1 short, without marginal setae, article 2 with 8 robust and 3 slender apical setae. Maxilla 2 (Fig. 3B) with row of setae on medial margin of inner plate, oblique setal row of inner plate consisting of plumose setae. Maxilliped (Fig. 3C) with both apical and medial setae, including some robust and some plumose setae on outer plate; palp 4-articulate, article 2 with marginal and submarginal rows of setae, article 3 with facial setae on inner surface.

Gnathopod 1 (Fig. 3D,E) with setae on anterior and posterior margins of basis; palmar margin of propodus (Fig. 3E)

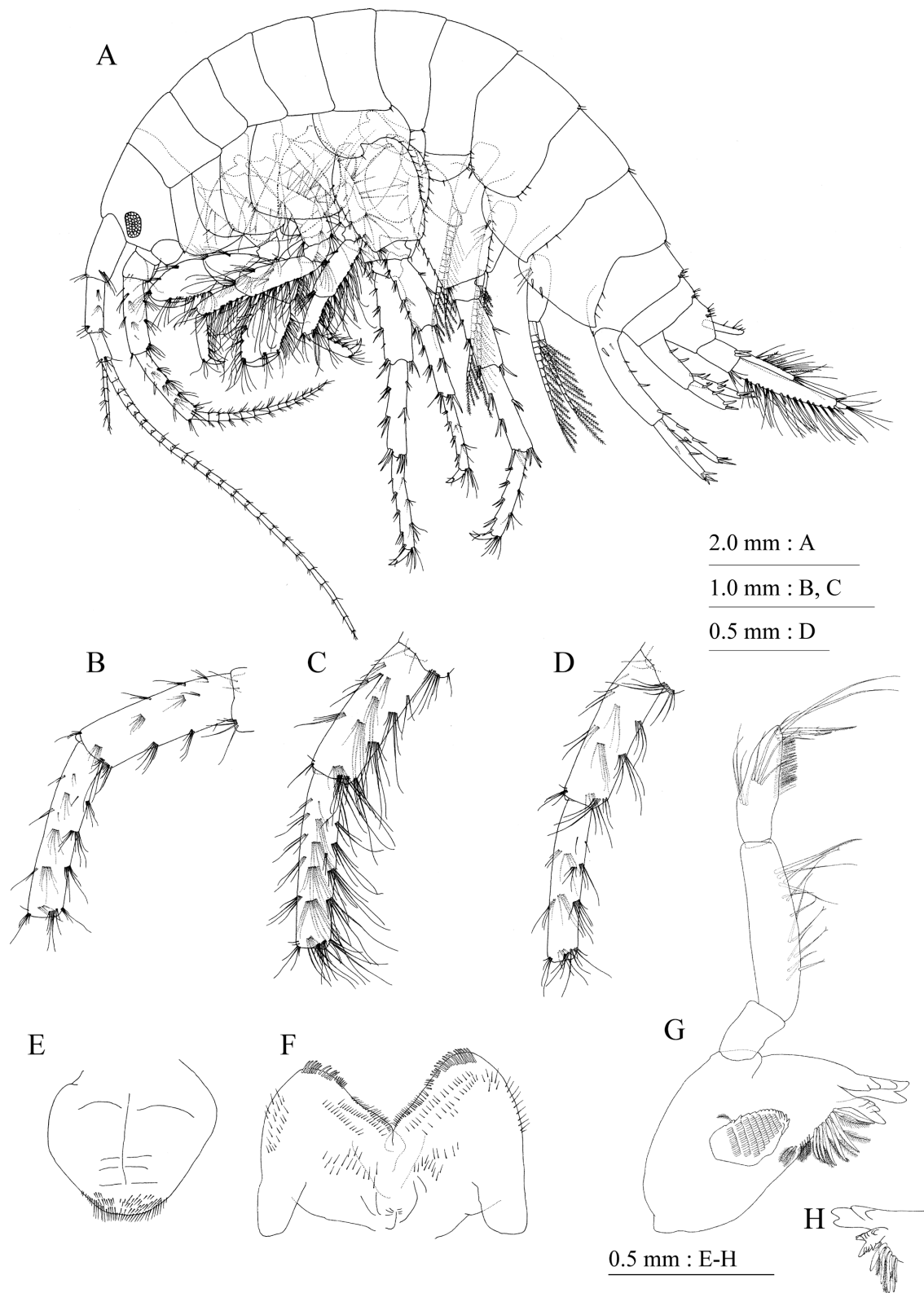


Fig. 2. *Gammarus koreanus* Uéno, 1940. A, B, E-H, Male (10.4 mm), NSMT-Cr 21670, Arakawa River, Fukue Island, Gotô Islands (Tamanouracho, Gotô city, Nagasaki Prefecture); C, male (9.5 mm), NSMT-Cr 21674, Tsuridou River, Nakadôri Island (Shin-kamigotôcho, Nagasaki Prefecture); D, female (8.0 mm), NSMT-Cr 21673, same locality. A, Habitus, lateral view; B-D, peduncular articles 4 and 5 of antenna 2, medial view; E, upper lip, anterior view; F, lower lip, ventral view; G, left mandible, medial view; H, incisor, lacinia mobilis, and accessory setal row of right mandible, medial view.

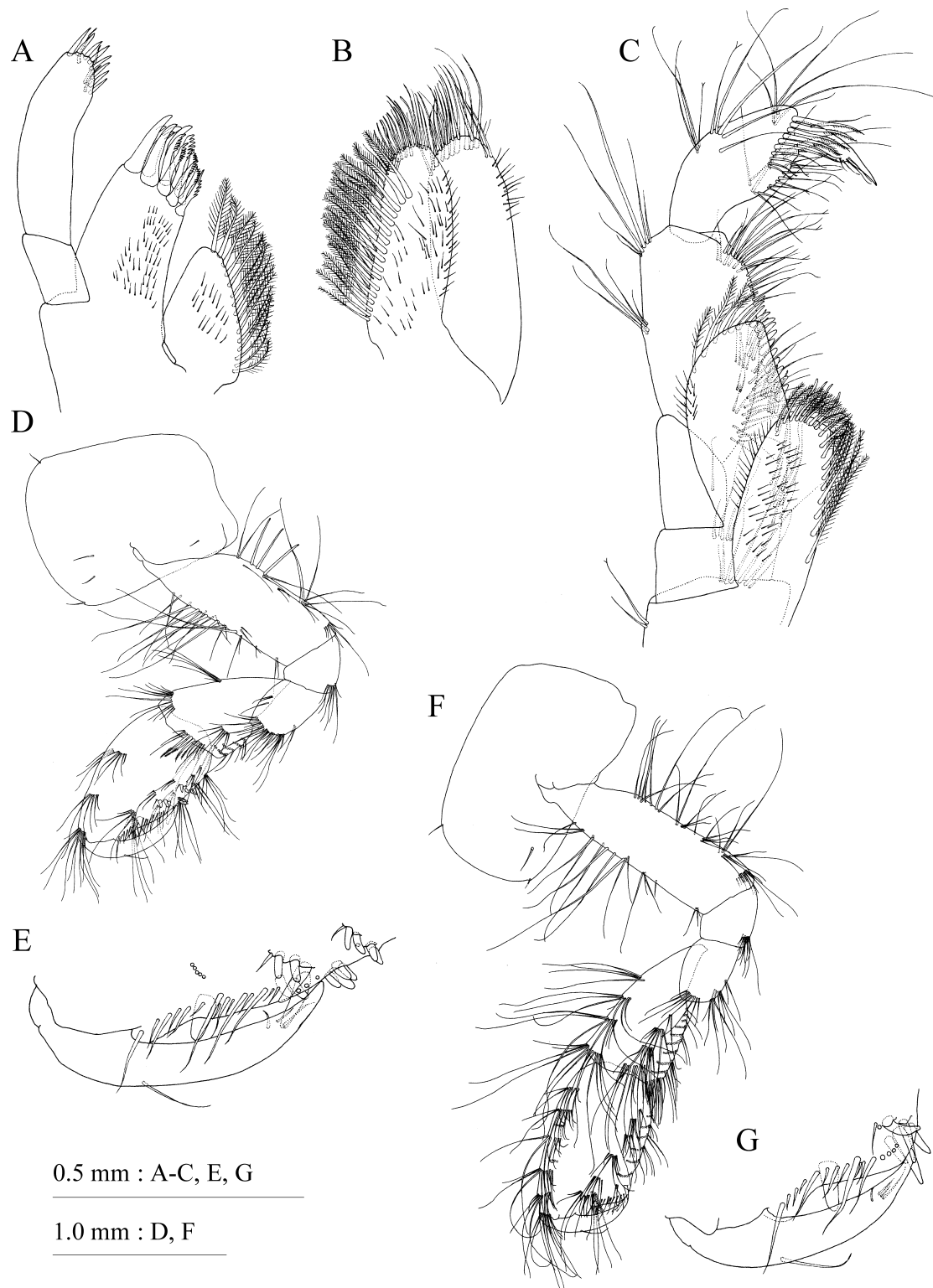


Fig. 3. *Gammarus koreanus* Uéno, 1940, male (10.4 mm), NSMT-Cr 21670, Arakawa River, Fukue Island (Tamanouracho, Gotô city, Nagasaki Prefecture). A, Maxilla 1, dorsal view; B, maxilla 2, dorsal view; C, maxilliped, dorsal view; D, gnathopod 1, medial view; E, palmar margin of propodus and dactylus of gnathopod 1, medial view; F, gnathopod 2, medial view; G, palmar margin of propodus and dactylus of gnathopod 2, medial view.

weakly concave, with 15 robust setae; dactylus curved inward. Gnathopod 2 (Fig. 3F,G) also with setae on anterior and posterior margins of basis; anterior margin of propodus with apically curled long setae; palmar margin of propodus (Fig. 3G) weakly concave, with 6 robust setae; dactylus

curved inward. Posterior margin of meri of pereopods 3 and 4 with apically curled long setae (Fig. 4C,D). Anterior margins of meri and carpi of pereopods 5–7 with robust and short setae. Lower margin of coxa 4 (Fig. 4D) straight, with posterior concavity; coxae 5 and 6 bilobed. Coxal gills pres-

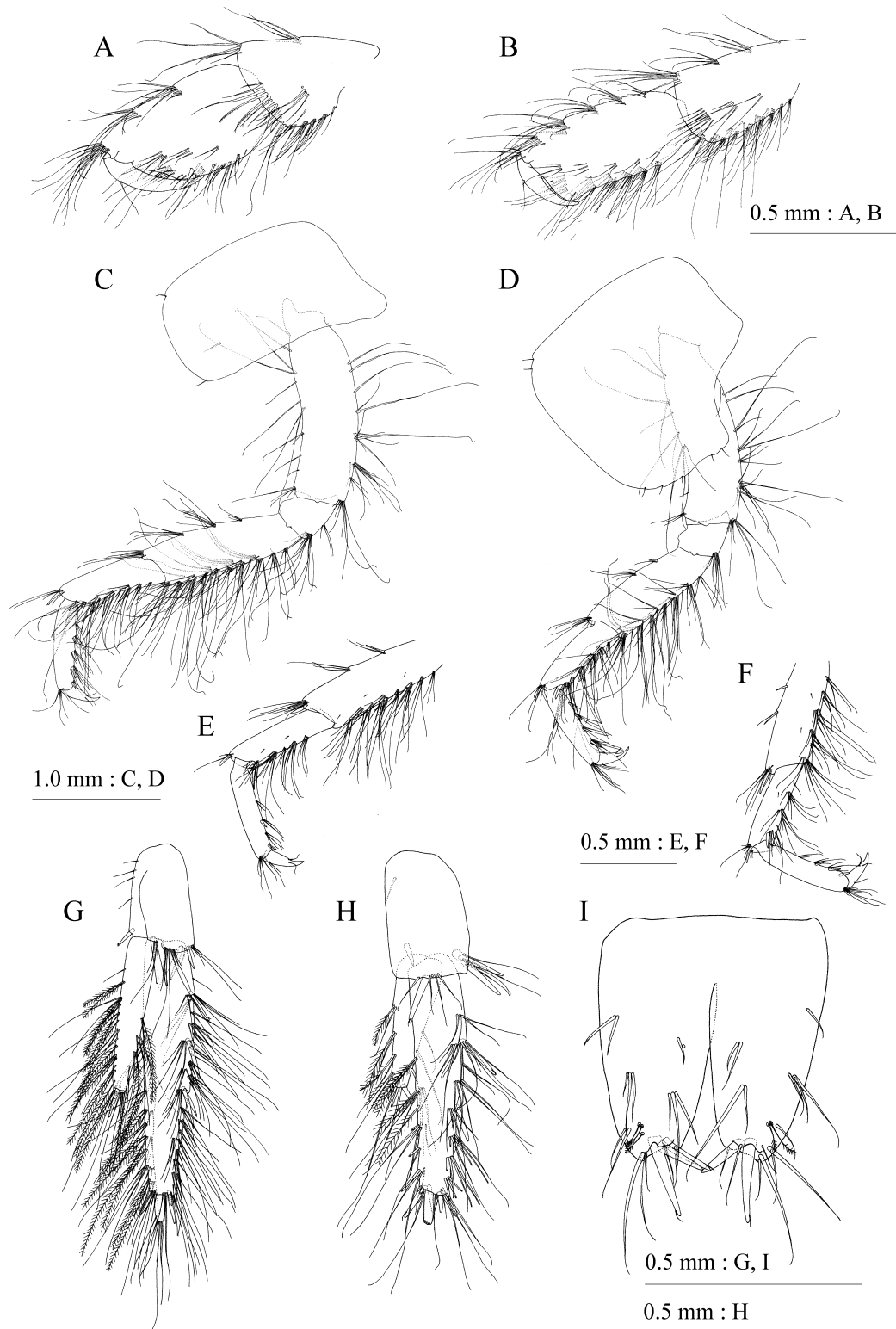


Fig. 4. *Gammarus koreanus* Uéno, 1940. A, B, E, F, H, female (8.0 mm), NSMT-Cr 21673, Arakawa River, Fukue Island (Tamanouracho, Gotô city, Nagasaki Prefecture); C, D, G, I, male (10.4 mm), NSMT-Cr 21670, same locality. A, Carpus, propodus, and dactylus of gnathopod 1, medial view; B, carpus, propodus, and dactylus of gnathopod 2, medial view; C, pereopod 3, lateral view; D, pereopod 4, lateral view; E, merus, carpus, propodus, and dactylus of pereopod 3, lateral view; F, merus, carpus, propodus, and dactylus of pereopod 4, lateral view; G and H, uropod 3, dorsal and ventral views, respectively; I, telson, dorsal view.

ent on gnathopod 2 and pereopods 3–7; accessory lobe absent.

Pleopods 1–3 each with retinacula, inner basal margin of inner ramus with bifid plumose setae. Uropod 1 with robust

basofacial seta on peduncle; outer ramus shorter than peduncle, with 2–3 and 0–2 robust setae on outer and inner margins, respectively; inner ramus as long as outer, with 0–1 outer and 2–3 inner marginal robust setae, respectively.

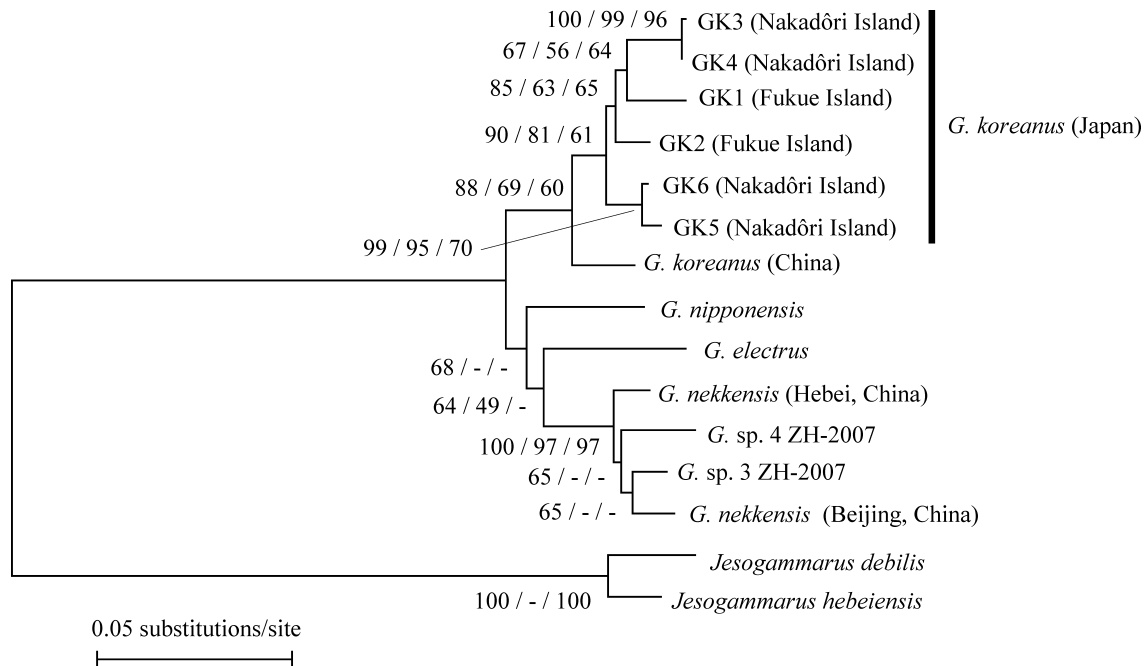


Fig. 5. Neighbor-joining tree based on genetic distances estimated from partial sequences of the 28S rRNA gene of *Gammarus koreanus* Uéno, 1940 and related species. Distances were calculated from Kimura's (1980) two-parameter model using a 563-bp data set. Numbers beside internal branches indicate bootstrap probabilities of NJ, MP, and ML trees based on 10,000, 1,000, and 1,000 replicates, respectively.

Uropod 2 with outer ramus shorter than peduncle, bearing 2–3 and 0–1 robust setae on outer and inner margins, respectively; inner ramus longer than outer, with 1–2 and 2–3 robust setae on outer and inner margins, respectively. Uropod 3 (Fig. 4G) with 2-articulate outer ramus, inner margin with plumose setae; inner ramus 49–63% as long as outer ramus, both outer and inner rami with plumose setae. Telson (Fig. 4I) slightly longer than wide, cleft for 72–85% of length.

Female. Antennae 2 (Fig. 2D) with 2 clusters of setae each on posterior margin of peduncular articles 4 and 5. Propodus of gnathopods 1 and 2 (Fig. 4A,B) more slender than in male. Posterior margins of meri of pereopods 3 and 4 each with long, straight setae (Fig. 4E,F). Inner ramus of uropod 3 about half as long as of outer ramus (Fig. 4H).

Distribution. Previously known from the northern part of the Korean Peninsula (Uéno 1940); Ji'an, Jilin, in China (Hou *et al.* 2007); and the Primorye region of Far-East Russia (Karaman 1991). In Japan, this species is known only from Fukue Island and Nakadôri Islands, the Gotô Islands, Nagasaki Prefecture (this study).

Habitat. Mountain streams.

Molecular phylogenetic analysis. A total of 621–709 bp of 28S partial nuclear gene sequences was obtained from 10 individuals representing five localities for *G. koreanus* from Japan. Excluding gap sites, the mutually alignable segments of the 28S rRNA sequences of 10 specimens representing *G. koreanus* from Japan, one representing *G. koreanus* from China, one representing *G. nipponensis* from Japan, and six specimens representing four species of *Gammarus* from China, *i.e.*, 18 sequences in all, was 563 bp in length, with 157 variable sites and 93 parsimony-informative sites.

Nucleotide frequencies among all samples were biased, with 43.2% A+T (mean: A = 22.4%, C = 26.4%, G = 30.4%, T = 20.8%). The average ratio of transitions (Ti) to transversions (Tv) was 1.14:1. Maximum parsimony analysis recovered eight most parsimonious trees. Tree length was 358 steps, the consistency index (CI) was 0.833, and the retention index (RI) was 0.894.

Since the NJ, MP, and ML trees showed similar topology (the placements of *G. nipponensis*, *G. electrus*, *G. nekkensis*, *Gammarus* sp. 3 ZH-2007, and *Gammarus* sp. 4 ZH-2007 differed slightly depending on the analysis), the NJ tree is shown here as a representative of all the trees (Fig. 5). The monophyly of *Gammarus koreanus* in China and Japan is supported by moderate bootstrap values in all trees (NJ = 88%, MP = 69%, and ML = 60%). Japanese *G. koreanus* forms a monophyletic group with moderate bootstrap values in all trees (NJ = 90%, MP = 81%, and ML = 61%). The genetic divergences between Japanese and Chinese *G. koreanus* are 3.4–4.9% of the uncorrected pairwise distances (*p*-distance) (Table 2). Japanese specimens of *G. koreanus* collected from Nakadôri Island (GK3 to GK6) and Fukue Island (GK1 and GK2) are not all reciprocally monophyletic. The sequence divergences among haplotypes of Japanese *G. koreanus* are 0.2–3.5% of the *p*-distances (Table 2).

Discussion

The present specimens from Japan were identified as *G. koreanus* on account of their having apically curled long setae on the anterior margin of the propodus of gnathopod 2 and on the posterior margin of the merus of male pereopods

Table 2. Uncorrected pairwise sequence differences (%: p-distance) of partial 28S rRNA gene sequences among species and haplotypes of *G. koreanus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>G. electrus</i>	—												
2. <i>G. koreanus</i> (China)	6.9	—											
3. <i>G. koreanus</i> (GK1)	9.8	4.9	0.0										
4. <i>G. koreanus</i> (GK2)	8.3	3.4	2.7	0.0									
5. <i>G. koreanus</i> (GK3)	9.6	4.7	3.2	2.9	—								
6. <i>G. koreanus</i> (GK4)	9.5	4.6	3.0	2.7	0.2	—							
7. <i>G. koreanus</i> (GK5)	9.5	4.2	3.5	2.7	3.4	3.2	0.0						
8. <i>G. koreanus</i> (GK6)	8.8	3.5	3.0	2.4	3.2	3.0	0.7	0.0					
9. <i>G. nekkensis</i> (Hebei)	6.8	5.9	8.6	7.6	9.0	8.8	8.1	7.4	—				
10. <i>G. nekkensis</i> (Beijing)	6.6	6.8	9.0	7.6	9.8	9.6	8.4	8.1	2.7	—			
11. <i>G. nipponensis</i>	7.6	6.4	8.6	7.1	8.6	8.4	7.8	7.3	5.9	6.3	—		
12. <i>G. sp.</i> 3 ZH-2007	6.8	6.8	9.1	8.1	9.8	9.6	9.0	8.3	2.2	2.0	6.4	—	
13. <i>G. sp.</i> 4 ZH-2007	7.8	7.3	9.6	8.9	10.3	10.1	9.1	8.8	3.4	3.0	6.8	3.5	—

3 and 4; no long setae on the anterior margins of the merus and carpus of pereopods 5–7; and a short inner ramus of uropod 3 (less than 70% as long as the outer ramus). They differ from the original description of *G. koreanus* by Uéno (1940) in the following minor points (information from the original description is given in parentheses): antenna 2 lacking (bearing) calceoli, inner margins of inner and outer rami of uropod 3 with (without) plumose setae, and outer margin of outer ramus of uropod 3 without (with) plumose setae. In some species of *Gammarus*, the presence or absence of calceoli on antenna 2 is variable (e.g., *G. fossarum* Koch in Panzer, 1836 and *G. laborifer* Karaman and Pinkster, 1977: cf. Karaman and Pinkster 1977). Dr. Z. Hou (personal communication) kindly informed us that specimens of *G. koreanus* collected from Jian, Jilin, China (43.8°N, 126.5°E) also lack calceoli on antenna 2. Karaman (1991) exposed the considerable intraspecific variability of the occurrence of marginal plumose setae on both rami of uropod 3.

Although we could not examine specimens from the type locality of *G. koreanus*, the specimen collected from Jilin, China (located about 300 km northwest of the type locality of this species), and Japanese ones form a monophyletic group in our trees based on 28S rRNA partial sequences (Fig. 5). The sequence divergences among Japanese and Chinese haplotypes were 3.4–4.9% and smaller than interspecific divergences among related species within the genus: 6.6–6.8% between *G. electrus* and *G. nekkensis*, 7.6% between *G. electrus* and *G. nipponensis*, and 5.9–6.3% between *G. nipponensis* and *G. nekkensis*. Hence, the differences among Japanese and Chinese haplotypes could well be intraspecific. The close genetic relationship supports well the identification of Japanese *G. koreanus* based on morphological data.

Specimens collected from Tsuridô River in Nakadôri Island differ from the other Japanese and Chinese specimens in the setation of the peduncular articles 4 and 5 on antenna 2: the former have 3 and 4 clusters of long setae on peduncular articles 4 and 5, respectively, whereas the latter bear 2 and 3 clusters of short setae, respectively, on these segments (Z. Hou, personal communication). The present molecular

analysis suggests that numerous long setae were developed only once in the clade from the Tsuridô River (GK3 and GK4), although we examined only a few of specimens. The function of the posterior setae on the peduncular articles of antenna 2 of *Gammarus* is uncertain, and these morphological differences might reflect ecological traits, especially in feeding and/or mating. Further studies are needed to clarify the functions of these setae.

The specimens from Fukue Island and Nakadôri Island did not form mutually exclusive monophyletic groups in our gene tree (the Arakawa River clade [GK1] of Fukue Island clustered with the Tsuridô River clade [GK3 and GK4] of Nakadôri Island). This non-monophyletic pattern exhibited by six individuals of different islands may not only reflect ancestral polymorphism, but also recent gene flow. Dispersal of this species among the islands of the Gotô chain (Nakadôri, Naru, Wakamatsu, and Fukue islands) could have occurred at times of low sea level, such as during the last glacial period. *Gammarus koreanus* was not found on Naru Island and Wakamatsu Island, which are located between Fukue Island and Nakadôri Island, despite our intensive search (Fig. 1), but we suppose that *G. koreanus* could not survive on these small islands which have no rivers.

The Gotô Islands were separated from Kyushu about 8,500 years ago (Ohshima 1991), when the Gotô Strait was formed. Since the Gotô Islands were parts of Kyushu until recently, many species of the islands are in common with Kyushu. For this reason, the Gotô Strait has not been thought to be an important zoogeographical boundary (Kawai 1980; Abe 2005). However, we found that *G. koreanus* is distributed in the Gotô Islands but not in Kyushu (including Tsushima and Iki islands), and that *G. nipponensis* occurs in Kyushu instead. This indicates that the Gotô Strait is a distributional boundary at least between *G. koreanus* and *G. nipponensis*. In order to understand the formation of the present distribution pattern of both species, detailed information on distribution of *G. koreanus* (especially on islands around the Korean Peninsula), genetic relationships among populations of both species, and the geological his-

tory of the Tsushima land bridge is needed.

Acknowledgements

We thank Dr. Zhong-E Hou of Chinese Academy of Sciences for providing valuable information on Chinese *G. koreanus*. Thanks are also due to Dr. Hiroyuki Ariyama (Marine Fisheries Research Center, Research Institute of Environment, Agriculture and Fisheries, Osaka Prefectural Government) and an anonymous reviewer for their critical readings of the manuscript. This study was partly supported by a grant from the Nihon Kyoiku Koumuin Kousaikai.

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