Finding of a new freshwater gammarid (*Gammarus gageoensis*) from South Korea

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*Gammarus gageoensis* n. sp., a new species of freshwater gammarid, was collected from Gageodo Island, South Korea. This new species is characterized in terms of its morphology by the flagellum of antenna 2 with calceoli, the pereopods 3 and 4 with numerous long curled setae on the posterior margins, the terminal article of the outer ramus in uropod 3, which is longer than the adjacent spines, and the presence of both rami with long simple setae. The morphological characters of the new species that are different to related species are presented in the text. This paper also compares the new species to related species by the partial sequence of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene. The sequence difference, consisting of a 39–44% difference relative to closely related species, is also large enough to designate this gammarid as a different species from other freshwater gammarid species. This is the first finding of a new species of Korean freshwater gammarid since 1992. Including this new species, a total of 10 species are known to Korea.

**Keywords:** Amphipoda; *Gammarus*; new species; Korea; CO1

Introduction

*Gammarus* Fabricius, 1775 (Amphipoda, Crustacea) is one among the 15 genera belonging to the family Gammaridae, represented by nearly 200 species worldwide at present (Vainolä et al. 2008). *Gammarus* is widely distributed throughout the Northern Hemisphere in various marine, brackish, freshwater and subterranean aquatic habitats up to 4500 m in altitude (Hou et al. 2007). This genus has some taxonomic problems because of high morphological diversity. These include morphological convergence of unrelated lineages in similar environments, and the evolutionary plasticity of many characteristics (Macdonald et al. 2005). Many faunistic studies have been done on this genus in Asia. Fifty-five species have thus far been reported in China (Hou 2002; Hou and Li 2002a, b, 2003, 2004; Hou et al. 2002; Wang et al. 2009), while only one species has been reported in Japan (Karaman 1984; Uéno 1940b).


The capability of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene to provide species-level identification has been demonstrated for most animal phyla (Hajibabaei et al. 2006; Clare et al. 2007; Elsasser et al. 2009; Zemlak et al. 2009). In previous studies, this gene has been suitably applied to freshwater amphipods (Meyran et al. 1997; Cristescu and Hebert 2005; Gomanenko et al. 2005; Lefebure et al. 2006; Witt et al. 2006; Hou et al. 2007; Venarsky et al. 2009). Therefore, genetic variation of the CO1 gene can be applicable to delineate species boundaries within the freshwater amphipods of Korea.

This paper describes a new species collected from Gageodo Island in South Korea based on combined analyses of its morphology and CO1 gene sequence.

Materials and methods

**Sample collection**

The specimens of freshwater *Gammarus* were collected with a fine-mesh hand net from three sites (Dae-ri, Hang-ri, and Daepung-ri) on Gageodo Island, Heuksan-myeon, Jeollanam-do, South Korea, on 30 April and 7 October 2009 (Figure 1). This island (34° 03' N, 125° 6' E), located in the south-westernmost part of the Korean peninsula, is 145 km away from the
mainland and 65 km southwest of Heuksando Island. Immediately after collecting the specimens, they were fixed in 95% ethyl alcohol for genetic analyses. Specimens were deposited in the National Institute of Biological Resources, South Korea.

**Morphological taxonomy**

The specimens were observed and dissected under a stereomicroscope (Olympus, SZX 12). All dissected appendages were mounted on glass slides with glycerin and figures were drawn using the drawing tube of a light microscope (LEICA, DM 2500). Pictures which were consecutive digital images on different focal planes (usually five or more shots per slide) were taken using a microscope digital camera (Moticam 2000) and the images were Z-stacked using Helicon Focus® software (Helicon Soft, Ltd). The body length was measured from the base of the first antennae to the base of the telson. Specimens were identified based on the nine morphological characteristics suggested by Karaman and Pinkster (1987).

**Molecular data**

Total genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer’s instructions. The target DNA segment of the CO1 gene was amplified by the polymerase chain reaction (PCR) method with the primers of LCO1490 5'-GGTCAACAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). PCR amplification involved initial denaturation at 94°C for 2 min, followed by 10 cycles of 10s at 94°C, 30 s at 37°C, and 60 s at 72°C. This was followed by 25 cycles of 10 s at 94°C, 30 s at 48°C, 60 s at 72°C, and finally a 7 min extension at 72°C.

PCR products were purified with the QIAquick® Gel Extraction Kit (Qiagen), and both strands were sequenced in an automated sequencer ABI 3100
The sequences were initially aligned using the Clustal X multiple sequence alignment program (Thompson et al. 1997). The CO1 gene sequence of related species on *G. pulex* (EF570334), *G. electrus* (EF570035) and *G. sinuolatus* (EF570339) were obtained from GenBank. Sequence analyses were conducted using MEGA 4.0 (Tamura et al. 2007). Nucleotide sequence divergence was calculated using the Kimura two-parameter distances. A neighbor-joining method was used to construct a phylogenetic tree. Confidence in the resulting relationships was assessed using a bootstrap procedure with 1000 replications.

**Systematic account**

**Order Amphipoda Latreille, 1816**  
**Suborder Gammaridea Latreille, 1803**  
**Family Gammaridae Leach, 1813**  
**Genus Gammarus Fabricius, 1775**  

1*Gammarus gageoensis*, new species

**Material examined.** Holotype (NIBRIV0000170243): male (Figure 2); Daeg-ri (34° 03’ N, 125° 07’ E), Gageodo Island, Heuksan-myeon, Jeollanam-do, South Korea, 30 Apr. 2009, collected by H.J. Jang and M.S. Kim. Paratypes: four males (NIBRIV0000170245) and three females (NIBRIV0000170244, NIBRIV0000170246); other data same as the holotype. Sixteen males (NIBRIV0000170247) and twelve females (NIBRIV0000170248); other data same as the holotype. Ten males (NIBRIV0000170249); other data same as the holotype, Dae-ri (34° 05’ N, 125° 06’ E) of the same island, 7 Oct. 2009, collected by M.S. Kim and D.H. Ahn.

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**Diagnosis.** *Gammarus gageoensis* n. sp. is identified by the following diagnostic characters. The peduncle articles 4 and 5 of antenna 2 with a few tufts of short setae on anterior margins; flagellum with calceoli. The carpus and propodus of gnathopod 2 with long curved setae on dorsal margins. The merus and carpus of pereopods 3 and 4 densely setose, with long curved setae on posterior margins. Inner ramus of uropod 3 about half as long as outer ramus, terminal article of outer ramus longer than adjacent spines, both rami with long simple setae.

Description of male (NIBRIV0000170243). Body length 11.6 mm.

Head (Figure 3A): cephalic lateral lobe truncate; inferior antennal sinus deep; eyes subreniform, medium in size.

Antenna 1 (Figure 3B–D): 44% of body length; peduncle articles 1–3 in length ratio 1: 0.64: 0.43, with distal setae; peduncle articles 2 and 3 with two and one tufts of slightly long setae, respectively; primary flagellum with 25 articles, each article with short distal setae; accessory flagellum with four articles, article 4 very short.

Antenna 2 (Figure 3E–G): 68% as long as antenna 1; peduncle articles 3–5 in length ratio 1: 2.58: 2.61; article 3 with distal setae; articles 4 and 5 with three longitudinal rows composed of tufts of setae; article 4 with two, two and three tufts of setae on interior, anterior and posterior margins, respectively; length of setae on anterior margin slightly shorter than width of article 4; article 5 with three, four and four tufts of setae on interior, anterior and posterior margins, respectively; setae on anterior margin and width of article 5 subequal in length; flagellum with 12 articles, articles 1–5 with calceoli.

Upper lip (Figure 3H): convex, with fine setae.  
*Mandible* (Figure 3I,J): left incisor 5 dentate, lacinia mobilis with four weak teeth, spine row with nine stout plumose setae, molar triturative with one plumose seta; article 2 of palp bearing three pectinate setae and 10 simple setae, article 3 about 0.66 times the length of article 2, with two A-setae on outer face, three B-setae on inner face, 30 D-setae, and four E-setae; right incisor 4 dentate, lacinia mobilis bifurcate with many weak dentitions apically.

Lower lip (Figure 3K): concave, inner plate absent.  
*Maxilla 1* (Figure 4A–C): inner plate with 17 plumose setae; outer plate having 10 serrated spines; article 2 of left palp with six slender spines, one stift seta and one seta on medial surface; article 2 of right palp with six stout spines and one stift seta.

*Maxilla 2* (Figure 4D): inner plate with 17 plumose setae on inner margin; outer plate a little longer than inner plate, bearing apical setae.  
*Maxilliped* (Figure 4E): inner plate with three blunt apical spines and one subapical spine; outer plate broad, having 12 blade spines on medial margin and eight pectinate apical setae.

*Gnathopod 1* (Figure 4F,G): coxal plate 1 weakly dilated in ventral margin, bearing 12 minute setae on ventral margin, one short setae and two minute setae on anterior corner, one short seta on posterior corner, one short stiff seta on inner margin; basis with long setae on anterior and posterior margins; carpus and propodus in length ratio 1: 1.22; carpus with subparallel anterior and posterior margins; propodus pyriform in shape, palm oblique, having one large medial spine and 10 spines on posterior margin; dactylus with one seta on outer margin.
Gnathopod 2 (Figure 5A,B, Figure 6A–D): coxal plate 2 relatively narrow distally, lower margin about 0.53 times as long as the lateral margin, bearing three short setae on anterior corner, one short seta on posterior corner; one long stiff seta and one short seta on inner margin; basis similar to that of gnathopod 1; carpus and propodus in length ratio 1: 1.13, both with long curled setae on dorsal margins; propodus quadrangular in shape, palm weakly oblique, having 1 large medial spine and 4 small spines on posterior corner.

Pereopod 3 (Figure 5C,D, Figure 6E–H): coxal plate 3 subrectangular, bearing three setae on anterior corner and one seta on posterior corner, one long stiff seta on inner margin; basis with long setae on proximal anterior and posterior margins; merus with many long curled setae on posterior margin, two tufts of setae on anterior margin, length of these setae 2 times as long as their width; carpus with long curled and straight setae on posterior margin, accompanied by a few spines; length of these setae longer than width; propodus having four pairs of one or two spines on posterior margin; dactylus with one plumose seta on posterior margin and two seta at the hinge of the nail.

Pereopod 4 (Figure 5E,F, Figure 6I,J): coxal plate 4 with posterior excavation, bearing two setae on
anterior corner, and five setae on posterior corner; basis to dactylus similar to that of pereopod 3; armature of merus to propodus weaker than those of pereopod 3; setae of merus to propodus shorter than pereopod 3; dactylus similar to that of pereopod 3.

Pereopods 5–7 (Figure 5G,D, Figure 7A–D): pereopods 6 and 7 longer than pereopod 5; subequal in length coxal plates 5–7 with a few setae only; posterior margin of basis nearly straight on pereopod 5, weakly concave distally on pereopods 6 and 7, bearing a row of

Figure 3. *Gammarus gageoensis* n. sp., male, holotype: A, head; B, antenna 1; C, anterior margin of antenna 1; D, articles of the primary flagellum of antenna 1; E, antenna 2; F, anterior margin of antenna 2; G, articles of the flagellum of antenna 2; H, upper lip; I, left mandible; J, incisor of right mandible; K, lower lip.
short setae, anterior margin with spines; merus to propodus with spines accompanied by a few short setae along anterior and posterior margins; dactylus with one plumose seta on posterior margin and two seta at the hinge of the nail.

Coxal gills of gnathopod 2 (Figure 5A, Figure 6C) and pereopods 3–7 (Figure 5C,E,G, Figure 6G,I, Figure 7A,C): all gills sac-like; gills 2–6 about as long as basis; gill of pereopod 7 relatively small, about one-third of length of basis.

Epimeral plates (Figure 7E): plate 1 subrounded, with five setae on anterior corner, three setae on posterior margin; plate 2 with two spines on ventral margin and five setae on posterior margin; plate 3 with
three spines on ventral margin and four setae on posterior margin.

Pleopods 1–3 (Figure 7F–I): pleopods well developed, peduncle with some long setae and two retina-cula accompanied by two setae; both rami having about 10–15 articles, fringed with plumose setae.

Urosomites 1–3 (Figure 7J): dorsally flat; urosomite 1 with 0–1–1–0 spines on dorsal margin from left to right, urosomite 2 with 1–1–1–1 spines, urosomite 3 with two spines and one spine on left and right sides and two tufts of three setae on medial dorsal margin.
Figure 6. *Gammarus gageoensis* n. sp., male, holotype: A, coxal plate 2; B, stiff setae on inner margin; C, gnathopod 2; D, carpus and propodus of gnathopod 2; E, coxal plate 3; F, stiff setae on inner margin; G, pereopod 3; H, merus to the propodus of pereopod 3; I, merus to the propodus of pereopod 4; J, pereopod 4; K, uropod 3. Scale bars = 0.5 mm (A, C–E, G–K), 0.25 mm (B, F).
Uropod 1 (Figure 7K): peduncle longer than rami, bearing one basofacial spine, two and two spines on outer and inner margins, two and one spine on outer and inner distal corners; inner ramus with one spine and one stiff seta on inner margin and five distal spines; outer ramus slightly shorter than inner ramus, with one spine on inner margin and five spines on distal margin.

Uropod 2 (Figure 7L): peduncle with two spines on each side; outer ramus longer than inner ramus, with 1 spine on each ramus and 5 distal spines.
Uropod 3 (Figure 6K, Figure 7M,N): peduncle with dorsal setae and distal spines accompanied by setae; inner ramus about 0.4 times the length of outer ramus; outer ramus two articles, proximal article with three marginal spines and three distal spines, terminal article longer than adjacent spines; both rami with numerous long simple setae.

Telson (Figure 7O): cleft nearly to the base, almost equal in length and width, each lobe with two distal spines accompanied by eight long setae.

Figure 8. *Gammarus gageoensis* n. sp., female, paratype: A, gnathopod 1; B, gnathopod 2; C, pereopod 3; D, dactylus of pereopod 3; E, pereopod 4; F, dactylus of pereopod 4; G, oostegite of pereopod 5; H, uropod 3; I, telson.
and three short penicillate setae, with groups of facial long setae.

Description of female (NIBRIV0000170244). Body length 9.2 mm. General appearance similar to male. Differences from male as follows:

Antenna 2: calceoli absent.

Gnathopod 1 (Figure 8A): propodus oval in shape; palm margin not as oblique as that of male, having two spines on posterior margin and two spines on posterior corner.

Gnathopod 2 (Figure 8B): carpus and propodus with straight setae on dorsal margin, propodus sub-rectangular in shape; palm transverse, having two spines on posterior corner.

Pereopods 3–4 (Figure 8C–F): merus to propodus with long straight setae on posterior margins.

Oostegites (Figure 8B,C,E,G): oostegites on pereopods 2–5, bearing many marginal setae.

Uropod 3 (Figure 8H): peduncle with three distal spines; inner ramus about 0.4 times the length of outer ramus; outer ramus two articles, proximal article with two marginal spines and two distal spines, terminal article longer than adjacent spines; both rami with numerous long, simple setae.

Telson (Figure 8I): cleft nearly to the base, almost equal in length and width, each lobe with one distal spine accompanied by long setae, with groups of facial long setae.

Habitat. Specimens were collected from a brook on the slope of Doksil Mountain in the Gageodo Island.

Molecular data. CO1 gene sequences (GenBank accession numbers GU270650–GU270652) were obtained from three specimens of the new species. Sequence alignment was straightforward without any insertion and deletion. Intra-specific variation of the CO1 gene sequence of the new species ranges between 0 and 0.2%, while inter-specific variation ranges from a low of 39% (G. gageoensis n. sp. and G. pulex Linnaeus, 1758) to a high of 44% (G. gageoensis n. sp. and G. electrus Hou & Li, 2003) (Figure 9, Table 1).

Etymology. The species name ‘gageoensis’ is derived from the name of the type locality, ‘Gageodo Island’.

Type locality. Dae-ri (34° 03’ N, 125° 07’ E), Gageodo Island, Heuksan-myeon, Jeollanam-do, South Korea.

Remarks. Gammarus gageoensis n. sp. resembles G. zeongogensis in (1) the peduncle 4 and 5 of antenna 2 with a few tufts of short setae on anterior margins, flagellum with calceoli, (2) the pereopods 5–7 with a few short setae and spines on posterior margins. This new species differs from G. zeongogensis in (1) the carpus and propodus of gnathopod 2 with long curled setae on dorsal margins, (2) the pereopods 3 and 4 with long curled setae on posterior margins, (3) the inner ramus that is shorter than half of outer ramus of uropod 3, and both rami with long simple setae, (4) the telson with many long setae on dorsal margin.

This species is also comparable to G. hoonsooi, G. parucispinus Hou and Li, 2002, G. electrus and G. sinuolatus Hou and Li, 2004 in (1) the both rami of uropod 3 are densely set with long simple setae, (2) the telson with long setae on dorsal margin. Following are the characteristics that distinguish the news species from G. hoonsooi and G. parucispinus: (1) the antenna 2 with calceoli, (2) the dorsal margins of carpus and propodus of gnathopod 2 and posterior margins of pereopods 3, 4 with long curled setae. And the present species can be easily distinguished from G. electrus by (1) the gnathopod 1 with long straight setae on posterior margin of propodus, (2) the pereopod 4 with long curled setae on posterior margin, (3) the terminal article of outer ramus of uropod 3 longer than adjacent

Table 1. A matrix of the pair-wise genetic distance of the CO1 gene sequence of this study. Sequence divergence using the K2P distance parameter.

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spines. *Gammarus gageoensis* n. sp. is separated from *G. sinuolatus* by the pereopods 3, 4 with long curled setae on posterior margin. A comparison between *G. gageoensis* n. sp. and the related species is given in Table 2.

The inter-specific sequence variation within the CO1 gene region ranges from 39% to 44% (K2P distance) for *G. gageoensis* n. sp. and the related species (Figure 9, Table 1). The sequence divergence provides enough evidence for this gammarid to be a different species. Previous studies also mentioned a similar level of sequence variation among freshwater amphipods. Among these, Meyran et al. (1997) described 36.4% CO1 gene sequence variation between *G. pulex* and *G. marinus*. Similarly, Cristescu and Hebert (2005) reported ranges from 17% to 28% (Tamura–Nei’s distance) for Ponto Caspian amphipods, while Witt et al. (2006) reported 35.2% (K2P distance) for *Hyalalla* species. Hou et al. (2007) reported that divergences among freshwater *Gammarus* species were 36.9% for the CO1 gene (K2P distance). The sequence divergence, which is 39–44% among closely related species, provides enough evidence for a new species.

**Acknowledgements**

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**References**


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<th><em>G. zeongogensis</em></th>
<th><em>G. hoonsooi</em></th>
<th><em>G. parucispinus</em></th>
<th><em>G. electrus</em></th>
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