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Short communication

First Record of the Brackish Water Amphipod *Jesogammarus* (*Jesogammarus*) *hinumensis* (Amphipoda: Anisogammaridae) from Korea with DNA Barcode Analysis among *Jesogammarus* Species

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ABSTRACT

Jesogammarus (*Jesogammarus*) *hinumensis* Morino, 1993 was discovered firstly from a brackish water region in Jeju Island, Korea. To identification of the specimens we conducted both of morphological and molecular analyses. This species is characterized by having large eyes and a robust seta on the mandibular palp article 1. The morphology of this Korean specimens was well matched with the original description without variation. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences of the present specimens were also completely identical to the sequences of *J.* (*J.*) *hinumensis* collected from the type locality of the species. Thus, we concluded that the *Jesogammarus* species from Jeju Island is *J.* (*J.*) *hinumensis*, based on both morphological and molecular data.

Keywords: COI, DNA barcode, *Jesogammarus hinumensis*, Jeju Island, Korea

INTRODUCTION

The anisogammarid genus *Jesogammarus* Bousfield, 1979 has been recorded from freshwater or brackish water in the Korean Peninsula, the Japanese Archipelago and the Chinese Continent, and currently comprises 20 species in two subgenera (Bousfield, 1979; Morino, 1984, 1985, 1986, 1993; Lee and Seo, 1990, 1992; Tomikawa and Morino, 2003; Tomikawa et al., 2003; Hou and Li, 2004, 2005; Tomikawa, 2015; Tomikawa et al., 2017). Two freshwater species, *Jesogammarus* (*Jesogammarus*) *ilhoii* Lee and Seo, 1992 and *J.* (*Annanogammarus*) *koreaensis* Lee and Seo, 1990 have been known from the Korean Peninsula (Lee and Seo, 1990, 1992). However, the species diversity of the genus in Korea is underestimated and many regions remain to be investigated.

Jesogammarus (*J.*) *hinumensis* Morino, 1993 was originally described from a brackish lake in Hinuma, Japan. Subsequently, this species has been recorded in various brackish water areas in Japan (Tomikawa, 2007). *Jesogammarus* (*J.*) *hinumensis* has been designated as an endangered species in Japan (Ariyama, 2012).

During field surveys of brackish water habitats in Jeju Island, the first author collected *Jesogammarus* specimens. Close examination of the external morphology and molecular analyses based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences revealed that these specimens are indubitably identical to *J.* (*J.*) *hinumensis*. In additional, for the future study using the DNA barcode of *Jesogammarus* in Korea, we provide revealed sequences by using two pairs of primers in the *COI* region. In this paper, we provide diagnosis and DNA barcode of this species collected from Jeju Island, Korea.

RESULTS AND DISCUSSION

Specimens were collected from a small brackish pond in Jeju Island (33°30.19′N, 126°53.51′E), Hado-ri, Jeju-si, Jeju-do, Korea using a fine-mesh hand-net on 11 Jun 2018 and 26 Apr 2019. Specimens were fixed and preserved in 99% ethanol. The Korean specimens completely agreed with the original description of *J.* (*J.*) *hinumensis* Morino, 1993. In addition to

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Table 1. Pairwise genetic distances among *Jesogammarus* species calculated using K2P model from *COI* gene sequences

Specific name	Locality	GenBank No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Genus *Jesogammarus*																		
Jesogammarus (*Jesogammarus*)																		
1. *J. hinumensis*	**Jeju Island**	**MN068361–MN068363**	–															
2. *J. hinumensis*	Lake Hinuma	LC052235	0.000	–														
3. *J. jesoensis*	Sapporo	LC052239	0.205	0.205	–													
4. *J. shonaiensis*	Sakata	LC052240	0.216	0.216	0.117	–												
5. *J. paucistulosus*	Mito	LC214540	0.219	0.219	0.236	0.232	–											
6. *J. spinopalpus*	Onjuku	LC052233	0.223	0.223	0.254	0.243	0.255	–										
7. *J. ikiensis*	Katsumoto	LC052242	0.232	0.232	0.331	0.277	0.260	0.217	–									
8. *J. fujinoi*	Gobanmiki	LC052232	0.235	0.235	0.118	0.103	0.240	0.259	0.309	–								
9. *J. hokurikuensis*	Takinami	LC052241	0.244	0.244	0.203	0.143	0.226	0.233	0.291	0.178	–							
10. *J. bousfieldi*	Mamurogawa	LC214541	0.246	0.246	0.240	0.255	0.269	0.222	0.222	0.287	0.282	–						
11. *J. mikadoi*	Rokugo	LC052231	0.269	0.269	0.218	0.224	0.294	0.265	0.330	0.225	0.259	0.276	–					
12. *J. uchiyamaryui*	Tanie River	LC214533	0.302	0.302	0.277	0.272	0.293	0.245	0.288	0.283	0.294	0.304	0.319	–				
Jesogammarus (*Annanogammarus*)																		
13. *J. suwaensis*	Lake Suwa	LC052237	0.223	0.223	0.249	0.268	0.234	0.225	0.241	0.283	0.271	0.260	0.285	0.237	–			
14. *J. naritai*	Lake Biwa	LC052249	0.223	0.223	0.258	0.268	0.234	0.233	0.246	0.273	0.271	0.255	0.290	0.246	0.006	–		
15. *J. fluvialis*	Samegai	LC052236	0.223	0.223	0.263	0.277	0.242	0.237	0.260	0.297	0.270	0.250	0.309	0.250	0.022	0.022	–	
16. *J. annandalei*	Lake Biwa	LC052248	0.241	0.241	0.271	0.300	0.242	0.242	0.259	0.296	0.279	0.273	0.319	0.249	0.034	0.034	0.031	–

Sequences obtained in the present study are in bold.

K2P, Kimura two-parameter; *COI*, cytochrome *c* oxidase subunit I.

Fig. 1. *Jesogammarus* (*Jesogammarus*) *hinumensis* Morino, 1993. Male 14.7 mm (NNIBR22377), Jeju Island, Korea. Habitus. Scale bar = 5.0 mm.

J. (*J.*) *hinumensis*, two species of *Jesogammarus* have robust setae on the mandibular palp article 1: *J.* (*J.*) *fontanus* Hou and Li, 2004 from a well in China and *J.* (*J.*) *spinopalpus* Morino, 1985 from freshwaters in Japan. *Jesogammarus* (*J.*) *hinumensis* differs from these two species by large eyes (vs. medium), the mandibular palp article 1 with one (vs. three) robust seta, and a few dorsal setae on pleonites 1−3 that are up to two (vs. more than six setae). The specimens examined in this study have been deposited in the collection of the Nakdonggang National Institute of Biological Resources, Korea (NNIBR22377−NNIBR22382).

The phylogenetic position of the newly identified *Jesogammrus* amphipod within the genus was estimated based on the sequences of the mitochondrial *COI* gene. DNA extraction and PCR reactions with *COI* primers Am-COI-H [CG(AG)GC(CGT)TA(CT)TT(CT)AC(CT)TC(ATC)GC(AC)ACTAT] and Am-COI-T [CGTCG(AGT)GG(CT)AT(ACG)CC(ACGT)CT(AGT)A(AG)(ATC)CCTA] were performed according to the methods described by Tomikawa (2015). Three sequences from the three *Jesogammarus* specimens were newly obtained in this study (NCBI Nos. MN068361, MN068362 and MN068363). Also, we provide sequences performed with universal primers LCO1490 and HCO2198 (Folmer et al., 1994) (NCBI Nos. MN068364, MN068365 and MN068366). Sequences of three specimens are completely identical from two *COI* regions. The sequences determined from primers Am-COI-H and Am-COI-T were used for the distance analysis (Tomikawa et al., 2007).

The sequences were aligned using Geneious 8.1.9 (Biomatters Ltd, Auckland, New Zealand). The *COI* gene sequences of other *Jesogammarus* species were obtained from GenBank. Sequence analyses were conducted using MEGA 7.0 (Kumar et al., 2016). Nucleotide sequence divergences were calculated using the Kimura two-parameter distance (Kimura, 1980). In our analysis, the sequences of the present specimens were completely identical to the sequence from *J.* (*J.*) *hinumensis* from the type locality of the species (Lake Hinuma, Japan), while the genetic distance between *J.* (*J.*) *hinumensis* and other *Jesogammarus* species are ranged from 20.5% to 30.2% (Table 1). Thus, we concluded that *Jesogammarus* species from Jeju Island is *J.* (*J.*) *hinumensis* based on both morphological and molecular data.

Order Amphipoda Latreille, 1816
Family Anisogammaridae Bousfield, 1977
Genus *Jesogammarus* Bousfield, 1979

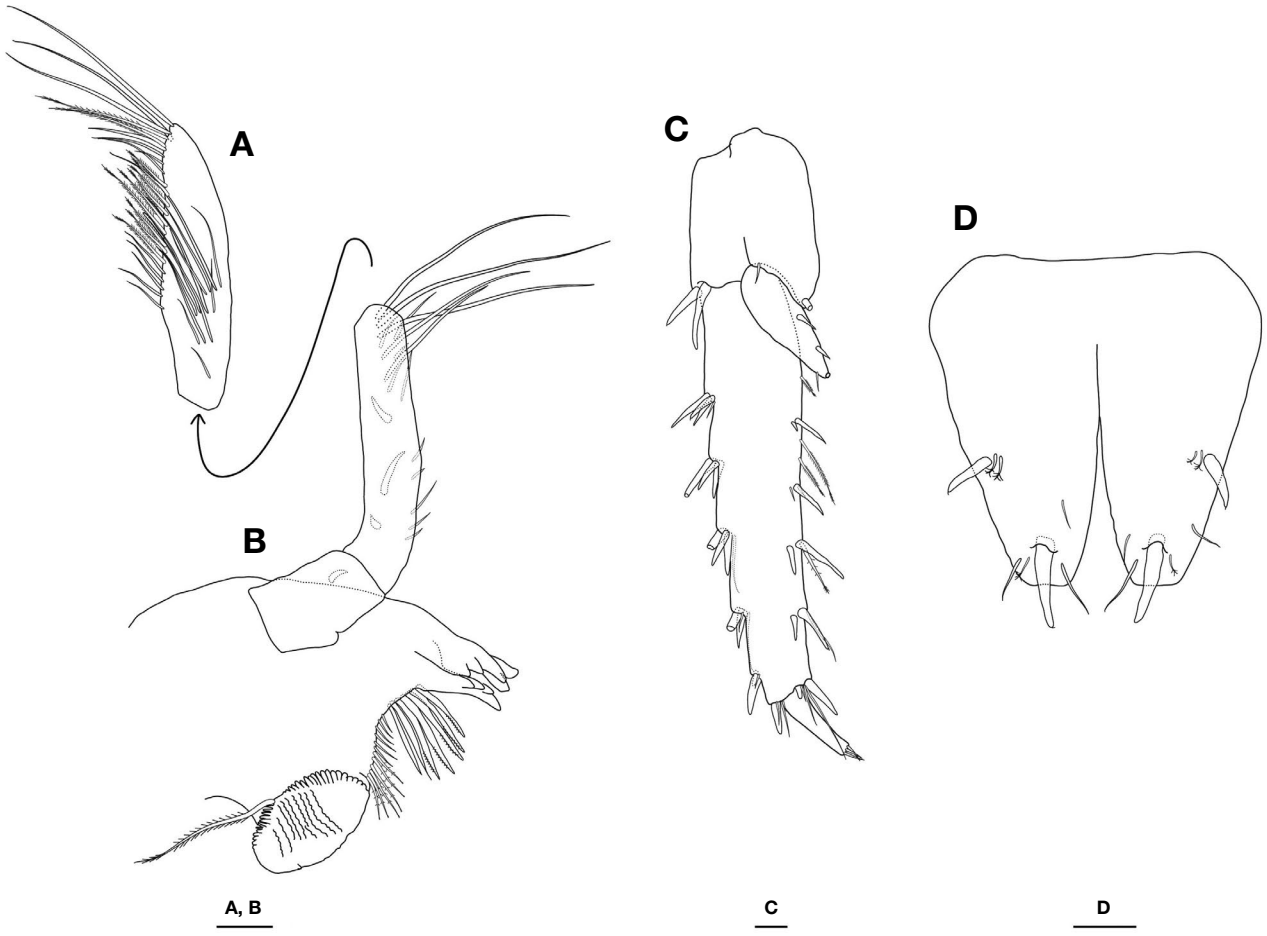

Fig. 2. *Jesogammarus* (*Jesogammarus*) *hinumensis* Morino, 1993. A, Mandible palp, lateral view; B, Mandible, medial view; C, Uropod 3, dorsal view; D, Telson, dorsal view. Scale bars: A–D = 0.1 mm.

[1]*_Jesogammarus_ (_Jesogammarus_) _hinumensis_ Morino, 1993 (Fig. 1)

Jesogammarus (*Jesogammarus*) *hinumensis* Morino, 1993: 9, figs. 1–4; Tomikawa, 2007: 25.

Diagnosis. Eyes large; pereonites without dorsal setae; dorsal margin of pleonites 1–3 each with 1–2 setae. Peduncles of antennae 1 and 2 with a few short setae on posterior margins. Mandible, palp article 1 with 1 robust seta (Fig. 2). Accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 well developed, both anterior and posterior lobes subequal in length or posterior lobe longer than anterior one. Female gnathopod 2, palmar margin of propodus with pectinate setae. Female pereopods densely setose. Uropod 3, inner ramus 0.15–0.27 times as long as outer ramus (Fig. 2); terminal article of outer ramus 0.1–0.2 times as long as proximal article. Telson length 1.0–1.3 times as long as wide (Fig. 2).

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