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A new species of *Sunamphitoe* Bate, 1857 (Crustacea: Amphipoda: Ampithoidae) from Hokkaido, Japan

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Abstract

A new species of ampithoid amphipod, *Sunamphitoe gigantea* sp. nov., is described from Katsurakoi, Hokkaido, Japan. The new species most closely resembles *S. eoa*, but can be distinguished from it by the shorter flagellum of antenna 1, the maxilla 1 inner plate that bears two slender setae, the short dactylus of male gnathopod 2, and the presence of a group of long setae on anterior margins of pereopods 3 and 4 bases. Nucleotide sequences of mitochondrial cytochrome c oxidase subunit I obtained from the type specimens also differentiated this new species from the congeners. Ontogenetic morphological changes of the male gnathopod 2 are briefly discussed.

Introduction

The ampithoid genus *Sunamphitoe* Bate, 1857 is known to be comprised of large-bodied herbivorous amphipods often associated with brown algae, specifically kelps (Poore *et al.*, 2008; Peart, 2017). The genus was recently verified to be a senior synonym of *Peramphithoe* Conlan & Bousfield, 1982, by both morphological and molecular phylogenetic analyses (Peart & Ahyong, 2016; Sotka *et al.*, 2016). With the species transferred from *Peramphithoe* (Peart & Ahyong, 2016) and also with subsequently described species (Peart, 2017; Griffiths, 2019), the genus *Sunamphitoe* is currently represented by 38 species. Among them, four species have been documented from Japan (Ishimaru, 1994): *S. orientalis* (Dana, 1853), *S. pelagica* (Milne-Edward, 1830), *S. plumosa* Stephensen, 1944 and *S. tea* (Barnard, 1965).

An extremely large-bodied *Sunamphitoe* species (maximum length, more than 40 mm) was found on the surface of a kelp *Saccharina longissima* which was collected by the second author from Katsurakoi, south-east coast of Hokkaido, Japan. In this paper, we herein describe and illustrate the species as *Sunamphitoe gigantea* sp. nov. Additionally, nucleotide sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) obtained from the type specimens were also provided for DNA barcoding. Phylogenetic analysis for COI sequences of *Sunamphitoe* species was also carried out.

Materials and methods

Sampling

Sampling was carried out at Katsurakoi, south-east coast of Hokkaido, Japan (Figure 1; 42°56′56.1″N 144°26′31.1″E) from April to November in 2017. The specimens were collected from the surface of *Saccharina longissima* fronds obtained from subtidal kelp beds (<3 m deep) by snorkelling.

Morphological observation

Body length was measured from the tip of rostrum along the dorsal margin to the posterior margin of telson (measurements were done on the curved body). Specimens were dissected under a binocular stereomicroscope, and appendages were fixed on slide mounts with Hoyer's medium. A part of pleopods were stored in 99% ethanol for subsequent DNA analysis. Observations and line drawings were made by using a light microscope and a binocular stereomicroscope with aid of drawing tube. All the specimens examined in this study were deposited in the National Museum of Nature and Science, Tokyo (NSMT).

DNA extraction and COI sequencing

The following DNA extraction and COI sequencing were carried out at the Bioengineering Lab. Co., Ltd (Kanagawa, Japan). Genomic DNA was extracted from a part of pleopods of seven specimens (including the holotype and the allotype) by using Lysis buffer for PCR (TaKaRa). The target sequences of COI were amplified using the method of two-step tailed

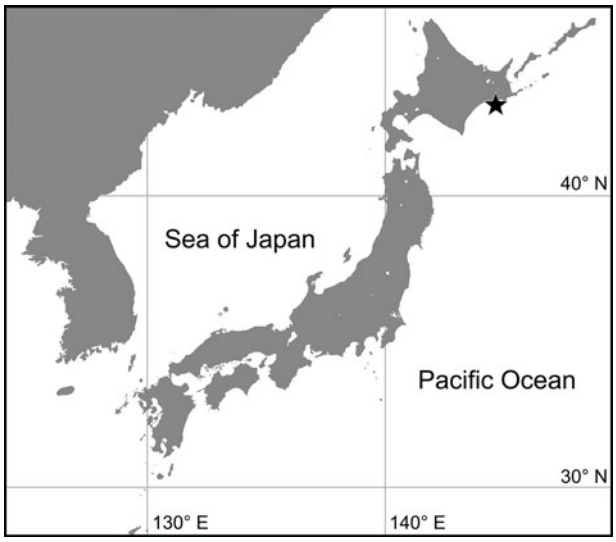

Fig. 1. Map of Japan showing the type locality (indicated by the black star) of *Sunamphitoe gigantea* sp. nov.

PCR procedure for library preparations. This two-step tailed PCR method consists of first-stage and second-stage PCRs. In the first-stage PCR, gene specific amplification is performed. In the second-stage PCR, index-sequences (for sample identification) and adapter-sequences (for subsequent sequencing) are added. The first-stage and second-stage PCR primers are shown in Table 1. The first-stage PCR was carried out with two kinds of primer sets (see Table 1): for each, a total volume of 20.0 µl containing 1.0 µl of template DNA (of which the concentration was not standardized), 0.5 µl of each primer (each 10.0 µM), 10.0 µl of 2× Gflex PCR Buffer, 0.4 µl of Tks Gflex (TaKaRa) and 7.6 µl of deuterium depleted water (DDW). The first-stage PCR conditions were as follows: the initial denaturing step was set at 94°C for 1 min; followed by 35 cycles of 10 s at 98°C, 15 s at 52°C and 30 s at 68°C; the final elongation was set for 5 min at 68°C. The second stage of PCR was carried out in a total volume of 10.0 µl containing 2.0 µl of mixture of first-stage PCR products performed with two kinds of primer set, 1.0 µl of 10 × Ex Buffer, 0.8 µl of dNTPs (each 2.5 mM), 0.5 µl of each primer (each 10.0 µM), 0.1 µl of Ex Taq (5 U μl^{-1}) (TaKaRa), and 5.1 µl of DDW. The programme of amplification for the second-stage PCR was 94°C for 2 min, 12 cycles (94°C for 30 s, 60°C for 30 s, 72°C for 30 s) and 72°C for 5 min. Concentration of the prepared amplicon libraries was evaluated using Synergy H1 (BioTek) and QuantiFluor dsDNA System (Promega). Quality of the library was evaluated using Fragment Analyzer and dsDNA 915 Reagent Kit (Advanced Analytical Technologies). The amplified products were then sequenced on an MiSeq (Illumina) 2 × 300 bp platform.

After sequencing, the sequences with start regions which completely matched with the primer sequences were extracted, and then the primer sequences were removed by using the fastq_barcode_splitter in the Fastx Toolkit. Low-quality sequences (values <20) were removed. Short sequences (length <40 bp) and their paired sequences were discarded by using the Sickle Tools.

Target sequence of COI was divided into the first and the second half. For both first and second halves of target COI sequences, the processed sequences were merged by using the Paired-end merge script FLASH. The merged sequences having a high frequency were selected for both first and second half of COI sequences, and then these two sequences were combined into one total target sequence of COI by using CAP 3. All the obtained COI sequences were deposited into the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan.

Phylogenetic analysis

A phylogenetic analysis was conducted by using MEGA 7.0 software (Kumar *et al.*, 2016). In addition to the COI sequence of our materials, COI sequences of other *Sunamphitoe* spp. retrieved from INSDC were also used for the analysis (Table 2). All sequences were aligned using Clustal W (Thompson *et al.*, 1994). Nucleotide sequence divergences within and between species were calculated using Kimura 2-parameter distances. The maximum likelihood method was used to construct a tree. The strength of clade support was assessed with bootstrap resampling with 1000 replicates (Felsenstein, 1985). *Ampithoe valida* COI sequence (INSDC accession number: GU048489) was chosen as an outgroup.

Results

SYSTEMATICS

Order Amphipoda Latreille, 1816

Family Ampithoidae Boeck, 1871

Genus *Sunamphitoe* Bate, 1857

[Japanese name: Nise-hige-naga-yokoebi-zoku]

Species *Sunamphitoe gigantea* sp. nov.

[New Japanese name: Oni-hige-naga]

TYPE MATERIAL

All the specimens were collected from the surface of fronds of *Saccharina longissima*, which grew naturally in the rocky shore at Katsurakoi, Kushiro, Hokkaido, Japan (42°56′56.1″N 144° 26′31.1″E), by T. Onitsuka and A. Ito.

Holotype: 1 male 39.3 mm (NSMT-Cr 26733, INSDC LC472973), 19 October 2017.

Allotype: 1 female, 33.6 mm, NSMT-Cr 26734, INSDC LC472974, same date as holotype (note: 13 juveniles were also collected from the same nest).

Paratypes: 1 male, 25.5 mm, NSMT-Cr 26735, INSDC LC472976; 1 ovigerous female, 38.6 mm, NSMT-Cr 26736, INSDC LC472975 (note: NSMT-Cr 26735 and 26736 were collected from a single nest); 1 ovigerous female, 36.3 mm, NSMT-Cr 26737, 24 April 2017. – 1 male, 34.6 mm, NSMT-Cr 26738, INSDC LC472978; 1 ovigerous female, 42.6 mm, NSMT-Cr 26739, INSDC LC472979 (note: NSMT-Cr 26738 and 26739 were collected from a single nest); 1 female, 38.9 mm, NSMT-Cr 26740 (note: 82 juveniles were also collected from the same nest); 1 ovigerous female, 40.6 mm, NSMT-Cr 26741, 29 May 2017. – 1 male, 25.3 mm, NSMT-Cr 26742, INSDC LC472977, 17 November 2017.

DIAGNOSIS

Body very large, maximum length of more than 40 mm. Antenna 1 flagellum less than 2 times length of peduncle. Antenna 2 slender; flagellum without dense plumose setae on ventral margin. Mandibular palp present, with 3 articles. Maxilla 1 inner plate with 2 slender setae. Maxilla 2 inner plate narrow, outer plate broader than inner plate. Male gnathopod 2 enlarged; propodus, palm straight, not clearly defined, without distinct protrusion, with dense setae on posteroproximal corner; dactylus reaching to posterodistal angle of propodus, not beyond carpus. Female gnathopod 2 much smaller than that of male; propodus palm well defined, with spine. Pereopods 3 and 4 bases, anterior margin

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Table 1. Primer sets used for the first-stage and second-stage PCR. The first-stage PCR was carried out with two kinds of primer sets (LCO1490/HCO2049 and IntF/HCOmR)

Primer	Sequences
First-stage primer	
1st_LCO1490	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNGGTCAACAAATCATAAAGATATTGG-3′
1st_HCO2042	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTGATTAGGACGGATCA-3′
1st_IntF	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGWACWGGWTGAACWGT WTAYCCYCC-3′
1st_HCOmR	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAHACTTCNGGGTGKCCRAARAATCA-3′
Second-stage primer	
2ndF	5′-AATGATACGGCGACCACCGAGATCTACAC-Index-ACACTCTTTCCCTACACGACGC-3′
2ndR	5′-CAAGCAGAAGACGGCATACGAGAT-Index-GTGACTGGAGTTCAGACGTGTG-3′

Table 2. List of the species, their localities and INSDC accession numbers used for the genetic analysis

Species (N)	Locality	Accession number	Reference
Sunamphitoe aorangi (1)	Victoria, Australia	KP316317	Sotka *et al.* (2016)
S. baegryeongensis (3)	Korean Peninsula	JN575611–JN575613	Kim *et al.* (2012)
S. chujaensis (2)	Korean Peninsula	JN575621, JN575622	Kim *et al.* (2012)
S. gigantea sp. nov. (7)	**Hokkaido, Japan**	**LC472973–LC472979**	**This study**
S. eoa (2)	Korean Peninsula	JN575623, JN575624	Kim *et al.* (2012)
S. graxon (1)	Sydney, Australia	KP316323	Sotka *et al.* (2016)
S. namhaensis (7)	Korean Peninsula	JN575614–JN575620	Kim *et al.* (2012)
S. orientalis (1)	Hawaii Islands	KP316319	Sotka *et al.* (2016)
S. parmerong (1)	Port Jackson, Australia	KP316320	Sotka *et al.* (2016)
S. tea (5)	Korean Peninsula	JN575606–JN575610	Kim *et al.* (2012)
Ampithoe valida (1)	San Francisco, California	GU048489	Pilgrim & Darling (2010)

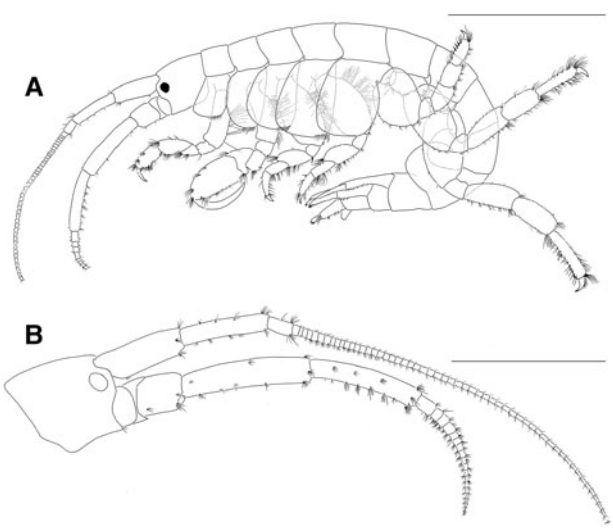

Fig. 2. *Sunamphitoe gigantea* sp. nov., holotype male, 39.3 mm (NSMT-Cr 26733). (A) habitus (setae partly omitted; coxal gills and pleopods omitted), lateral view; (B) head, lateral view. Scale bar: A, 10.0 mm; B, 5.0 mm.

with group of long setae subproximally. Pereopods 5–7 not enlarged; basis, posterodistal lobe absent or indistinct, not reaching ischium; merus, carpus not expanded. Uropod 3 peduncle about 3 times as long as rami. Telson, posterior margin rounded, not acute.

DESCRIPTION OF MALE

Based on holotype male, 39.3 mm (NSMT-Cr26733). *Body* (Figure 2A) very large, laterally compressed, smooth on surface.

Head. Head (Figure 2B) about 1.7 times as long as pereonite 1; rostrum indistinct; lateral cephalic lobes weakly angular, truncated, not rounded distally; eyes small, rounded or oval. *Antenna 1* slender, weakly setose; length ratio of peduncular articles 1–3 about 34:33:10, article 1 with 2 small spine distoventrally; flagellum longer than peduncle, but less than 2 times length of peduncle; accessory flagellum absent. *Antenna 2* slender, setose, shorter than antenna 1; length ratio of peduncular articles 3–5 about 7:20:17; flagellum subequal to peduncular article 5 in length, with 8 articles in left antenna 2 (broken distally), with 19 articles in right antenna 2 (intact), without dense plumose setae on ventral margin.

Mouth parts. Upper lip (Figure 3A) normal, setulose ventrally, posteriorly. *Lower lip* (Figure 3B) normal, setulose; outer plate deeply notched, lateral lobe longer than medial lobe, mandibular process developed, curved. *Mandible* (Figures 2 & 3C1): palp 3-articulated, article 1 longer than wide, unarmed, article 2 slightly longer than article 3, with several slender setae distomedially, article 3 tapering on distal half, with dense setae on distomedial margin; left, right incisors 8-, 9-dentate, respectively; left laciniae mobiles 8-dentate, right laciniae mobiles many dentate, dentation on right laciniae mobiles smaller than that of left laciniae mobiles; left, right accessory setal rows including 17, 16 setae, respectively; molar well developed. *Maxilla 1* (Figure 3D): palp 2-articulated, article 1 short, unarmed, article 2 incurved, beyond outer plate, with 13 spines apically, ventral surface with

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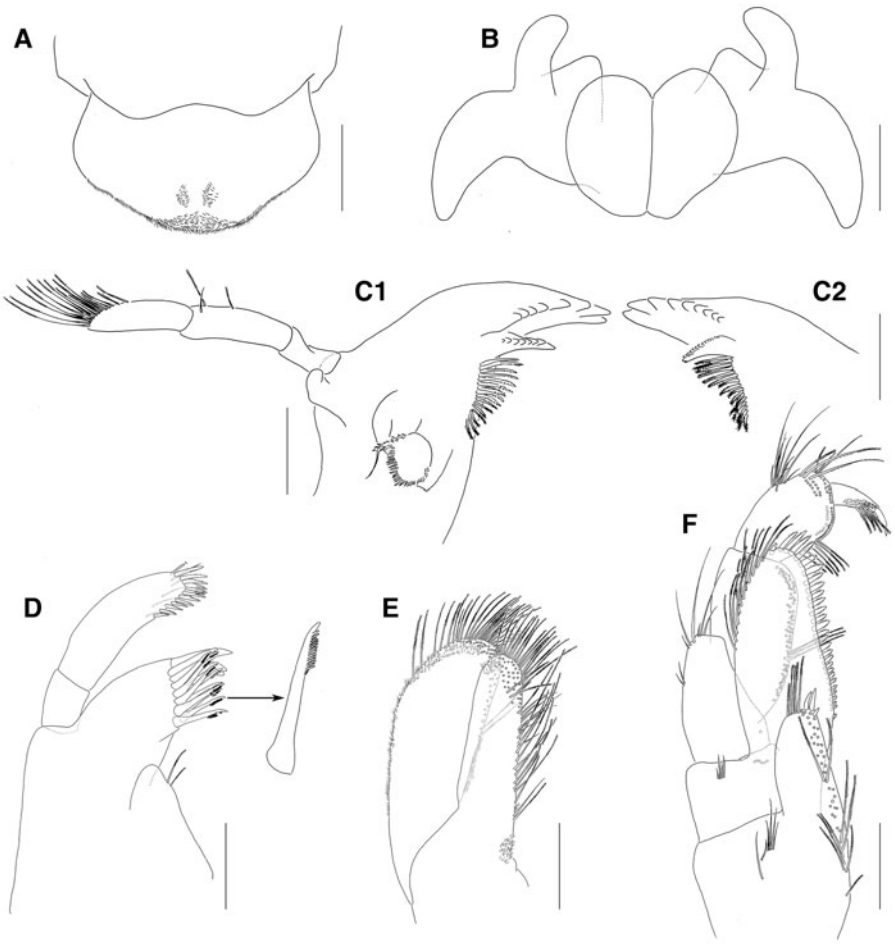

Fig. 3. *Sunamphitoe gigantea* sp. nov., holotype male, 39.3 mm (NSMT-Cr 26733). (A) upper lip, posterior view; (B) lower lip (setules omitted), ventral view; (C1) left mandible, medial view; (C2) incisor, laciniae mobilis and accessory setal row of right mandible, medial view; (D) left maxilla 1 and pectinate spine on the outer plate, dorsal view; (E) left maxilla 2 (setae and setules partly omitted), dorsal view; (F) maxilliped (setae partly omitted), dorsal view. Scale bars: 0.5 mm.

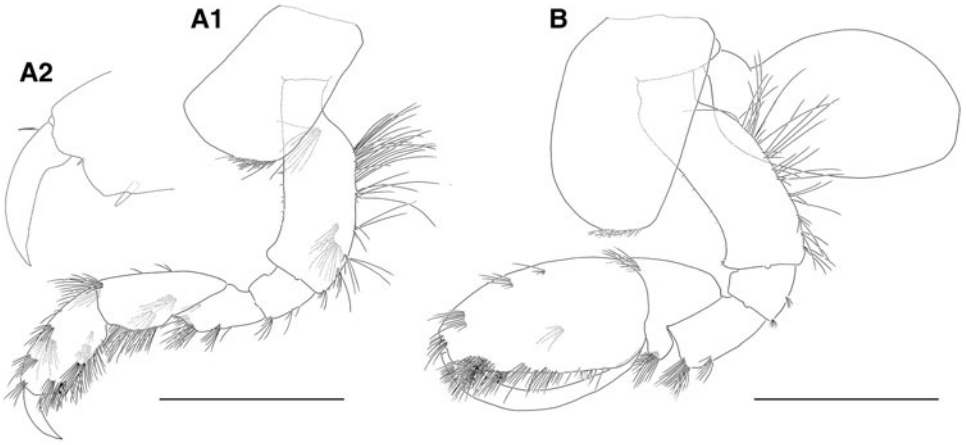

Fig. 4. *Sunamphitoe gigantea* sp. nov., holotype male, 39.3 mm (NSMT-Cr 26733). (A1) left gnathopod 1, lateral view; (A2) propodus palm and dactylus of left gnathopod 1 (setae omitted), lateral view; (B) left gnathopod 2, lateral view. Scale bars: A1, B, 3.0 mm; A2, 1.0 mm.

several slender setae subdistally; outer plate truncated, with several simple spines, pectinate spines on distal margin; inner plate small, with 2 serrulate spines on subapically medial margin. *Maxilla 2* (Figure 3E): outer plate broader than inner plate, slightly longer than inner plate, with dense setae distolaterally, distally, distomedially; inner plate with dense setae medially to distally. *Maxilliped* (Figure 3F): palp 4-articulate, article 4 medially covered with spinules, unguis acute, well developed; outer plate subovate, extending beyond distal end of palp article 2, with row of teethed spines medially to distally, serrulate setae on distal half of lateral margin; inner plate developed, with serrulate setae medially to distally, 3 spines mediodistally.

Pereon. Gnathopod 1 (Figure 4A1) subchelate, smaller than gnathopod 2, length ratio of basis to dactylus about 17:4:5:9:9:5; coxa subquadrate, deeper than broad, slightly broadened ventrally, with tuft of short setae posteroventrally; basis longer than coxa, posterior margin with dense long setae, medial surface with several groups of long setae, anterior margin with several short setae, anterodistal lobe very small; carpus with dense setae posteriorly to medially; propodus with dense setae posteriorly to medially, palm (Figure 4A2) transverse, defined by spine, posterodistal corner with small spine; dactylus longer than palm, with anteroproximal seta. *Gnathopod 2* (Figure 4B) subchelate, large, length ratio of basis to dactylus about 9:2:3:3:9:10; coxa

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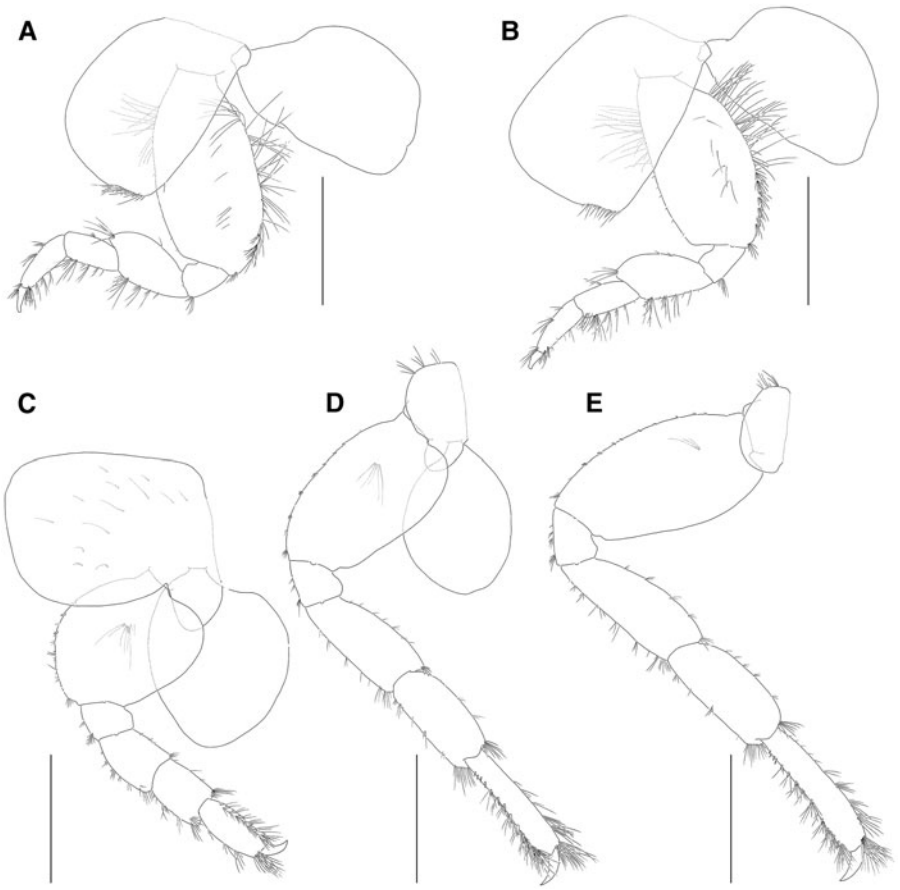

Fig. 5. *Sunamphitoe gigantea* sp. nov., holotype male, 39.3 mm (NSMT-Cr 26733). (A–E) left pereopods 3–7, lateral views. Scale bars: 3.0 mm.

subquadrate, rounded anteriorly to anteroventorally, deeper than broad, with tuft of short setae posteroventrally; basis subequal to coxa in length, posterior margin with dense long setae, anterior margin with several short setae, anterodistal lobe very small; carpus subtriangular, with dense setae posteriorly; propodus enlarged, palm straight without distinct protrusion, not clearly defined, with dense setae posteriorly, setae on posterodistal corner denser, lacking palm defining spines; dactylus curved, long, reaching to posteroproximal angle of propodus, not beyond carpus, lacking anteroproximal seta.

Pereopod 3 (Figure 5A) simple, length ratio of basis to dactylus about 17:3:6:5:5:2; coxa deeper than broad, with tuft of short setae posteroventrally, anterior margin roundly convex; basis expanded, anterior margin with group of long setae subproximally, posterior margin with dense long setae; merus expanded distoanteriorly; propodus slightly tapering distally. *Pereopod 4* (Figure 5B) similar to pereopod 3, but coxa slightly larger, broadened ventrally, anterior margin rather straight. *Pereopod 5* (Figure 5C) slightly shorter than pereopod 4, length ratio of basis to dactylus about 6:2:3:3:4:2; coxa bilobate, anterior lobe strongly enlarged, roundly quadrate, with scattered setae on medial surface, posterior lobe small, rounded, unarmed; basis expanded, with several small spines on anterior margin, group of long setae on medial surface, unarmed on posterior margin, without distinct posterodistal lobe; carpus broader than propodus; propodus with row of spines on flexor margin; dactylus falcate. *Pereopod 6* (Figure 5D) longer than pereopod 5, length ratio of basis to dactylus about 11:3:7:7:8:2; coxa bilobate, each lobe rounded, anterior lobe larger than posterior lobe with several setae anteriorly, posterior lobe small, unarmed; basis with row of small spines on anterior margin, group of setae on medial surface, unarmed posteriorly, posteroproximal corner rounded; propodus slightly expanded distally, with row of spines on flexor margin; dactylus falcate. *Pleopod 7* (Figure 5E) similar to pereopod 6 but slightly longer, coxa semicircular. Coxal gills present on coxae 2–6.

Pleon. Epimeral plates (Figure 6A) normally rounded, without distinct teeth or setae. *Pleopods* normal, similar to each other.

Urosome. Uropod 1 (Figure 6B) peduncle with dorsolateral row of spines on distal 0.6, dorsomedial row of spines on distal 0.7, ventrolateral row of slender setae on proximal 0.8, distal end with long ventral spur; outer ramus about 0.7 times as long as peduncle (distoventral spur excluded), inner ramus longer and more slender than outer ramus, both outer and inner rami bearing rows of spines on both lateral, mesial margins, lacking slender setae. *Uropod 2* (Figure 6C) peduncle with dorsolateral and dorsomedial rows of spines on distal half, without slender setae, distal end with ventral spur; outer ramus about 0.8 times as long as peduncle (distoventral spur excluded), inner ramus longer and more slender than outer ramus, both outer and inner rami bearing rows of spines on both lateral and mesial margins, lacking slender setae. *Uropod 3* (Figure 6D1) peduncle cylindrical, reaching beyond posterior margin on telson, with several tufts of slender setae laterally, medially, several spines dorsodistally, distolaterally, lateral half of distoventral margin slightly extended, concealing base of outer ramus, fringed with slender setae; outer ramus slightly tapering, about 0.4 times as long as peduncle, finely spinulate dorsolaterally to ventrolaterally (Figure 6D2), with several short setae laterally, 2 recurved spines distally; inner ramus subquadrate, slightly shorter than outer ramus, with several small spines dorsodistally, dense slender setae ventrodistally. *Telson* (Figure 6E), roundly trapezoid, wider than long, posterior margin roundly convex, both lateral margins with 3–5 slender setae, short pappose seta, small telsonic cusp, dorsal surface with 1–2 short simple seta(e), long simple seta on both left, right side.

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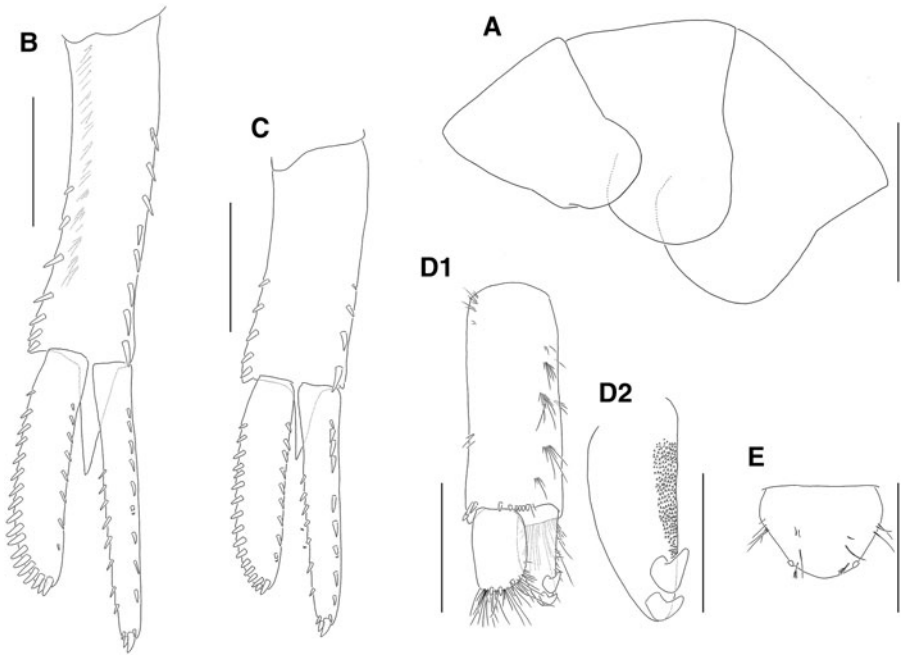

Fig. 6. *Sunamphitoe gigantea* sp. nov., holotype male, 39.3 mm (NSMT-Cr 26733). (A) epimeral plates 1–3, lateral view; (B) left uropod 1, dorsal view; (C) left uropod 2, dorsal view; (D1) right uropod 3 (spinulation on outer ramus omitted), dorsal view; (D2) spinulation on right uropod 3 outer ramus, dorsal view; (E) telson, dorsal view. Scale bars: A, 3.0 mm; B–D1, E, 1.0 mm; D2, 0.5 mm.

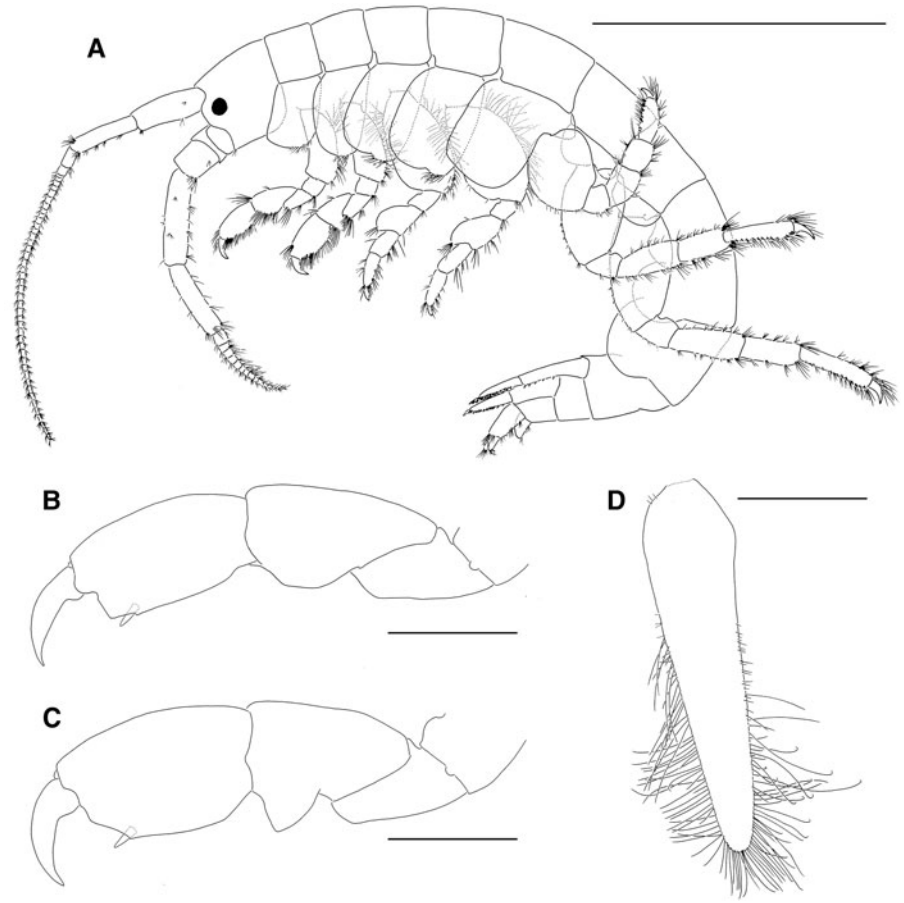

Fig. 7. *Sunamphitoe gigantea* sp. nov., allotype female 33.6 mm (NSMT-Cr 26733). (A) habitus (setae partly omitted; coxal gills, oostegites and pleopods omitted), lateral view; (B) merus to dactylus of left gnathopod 1 (setae omitted), lateral view; (C) merus to dactylus of left gnathopod 2 (setae omitted), lateral view; (D) oostegite of left pereopod 3, lateral view. Scale bar: A, 10.0 mm; B–D, 1.0 mm.

DESCRIPTION OF FEMALE

Generally similar to males (Figure 7A); gnathopod 1 (Figure 7B) similar to that of males, but carpus slightly stouter; gnathopod 2 (Figure 7C) smaller than that of males, propodus much smaller than that of males, palm well defined, oblique, bearing spine; coxae 2–5 with oostegites (Figure 7D), each oostegite similar, tapering distally, marginally setose on about distal half, some of setae curled distally.

VARIATIONS

Peduncular article 1 of antenna 1 bears 1 or 2 small spines distoventrally. Flagellar articles of antennae 1 and 2 increase numbers in large individuals. Coxae are sometimes notched on the ventral margin. Spines and setae on pereopods and uropods vary in numbers individually.

Male gnathopod 2 shows ontogenetic morphological change: in small males, palm well defined bearing a spine, and dactylus

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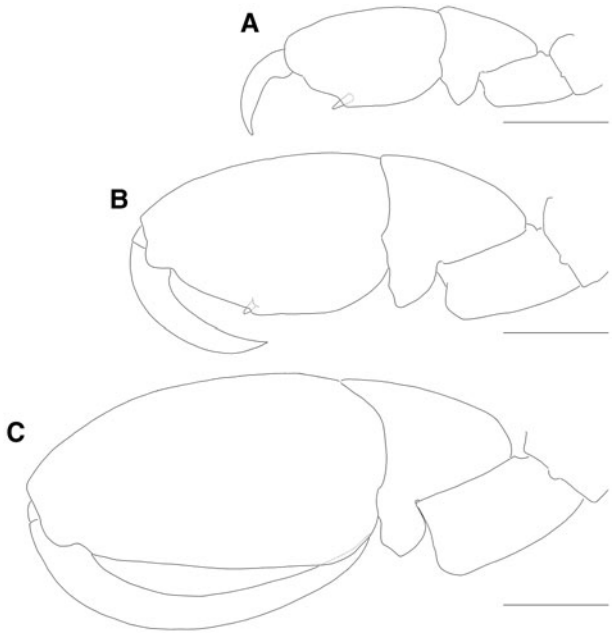

Fig. 8. Ontogenetic morphological change in male gnathopod 2. (A–C) merus to dactylus of left gnathopod 2 (setae omitted), lateral view: (A) paratype male, 25.3 mm (NSMT-Cr 26742); (B) paratype male, 34.6 mm (NSMT-Cr 26737); (C) holotype male, 39.3 mm (NSMT-Cr 26733). Scale bars: 1.0 mm.

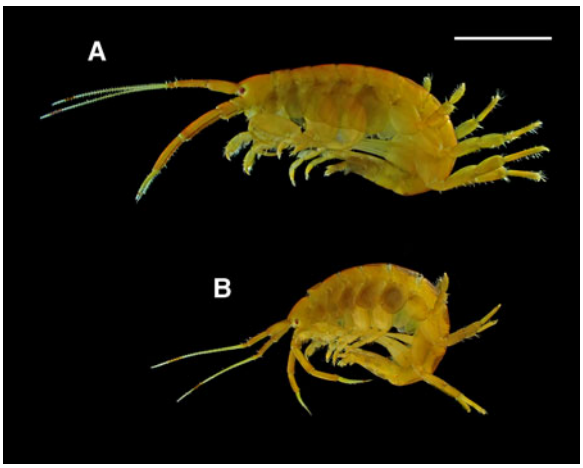

Fig. 9. *Sunamphitoe gigantea* sp. nov. (A) holotype male, 39.3 mm (NSMT-Cr 26733), lateral view; (B) allotype female 33.6 mm (NSMT-Cr 26734), lateral view. Scale bar: 10.0 mm.

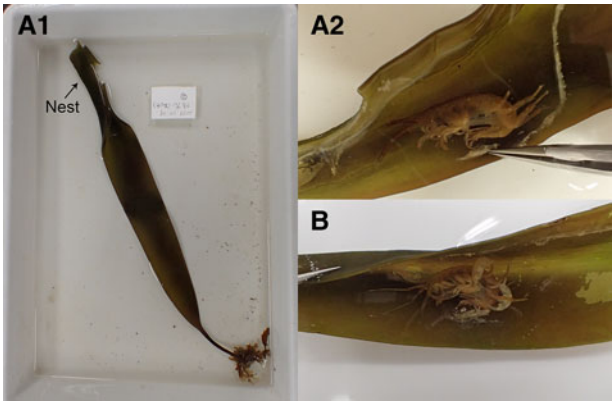

Fig. 10. Nests of *Sunamphitoe gigantea* sp. nov. and their inhabitants. (A1) nest occupied by the holotype male; (A2) holotype male (NSMT-Cr 26733) in the nest; (B) allotype female (NSMT-Cr 26734) and its juveniles in a nest.

Table 3. Kimura 2-parameter distances of COI sequences (%) among *Sunamphitoe* species

	S. aorangi	*S. baegryeongensis*	*S. chujaensis*	*S. eoa*	*S. gigantea* sp. nov.	*S. graxon*	*S. namhaensis*	*S. orientalis*	*S. parmerong*	*S. tea*	*A. valida*
Sunamphitoe aorangi	–										
S. baegryeongensis	17.8–18.3	0.2–0.4									
S. chujaensis	17.4–17.7	15.5–16.3	0.6								
S. eoa	18.5	19.5–20.1	16.9–17.4	0.0							
S. gigantea sp. nov.	**16.4–17.2**	**16.9–17.9**	**14.7–16.0**	**17.2–18.0**	**0.0–1.6**						
S. graxon	19.0	16.7–17.2	16.9–17.1	21.0	**18.1–18.4**	–					
S. namhaensis	17.4–18.4	15.5–17.3	14.2–16.2	18.8–19.6	**14.9–17.0**	14.6–15.9	0.4–2.4				
S. orientalis	16.2	13.8–14.0	15.2–15.7	19.7	**15.1–16.2**	16.2	13.1–14.3	–			
S. parmerong	17.7	17.5–18.0	14.7	20.4	**16.9–17.7**	19.9	17.1–18.7	17.7	–		
S. tea	22.0–22.3	15.7–17.0	17.5–18.1	20.8–22.2	**15.7–17.8**	17.7–18.5	15.3–16.3	15.3–16.0	17.0–17.5	0.2–1.6	
Ampithoe valida	20.1	17.6–18.1	20.0–20.5	20.3	**16.9–17.9**	19.7	15.2–17.2	19.0	19.2	19.4–20.2	–

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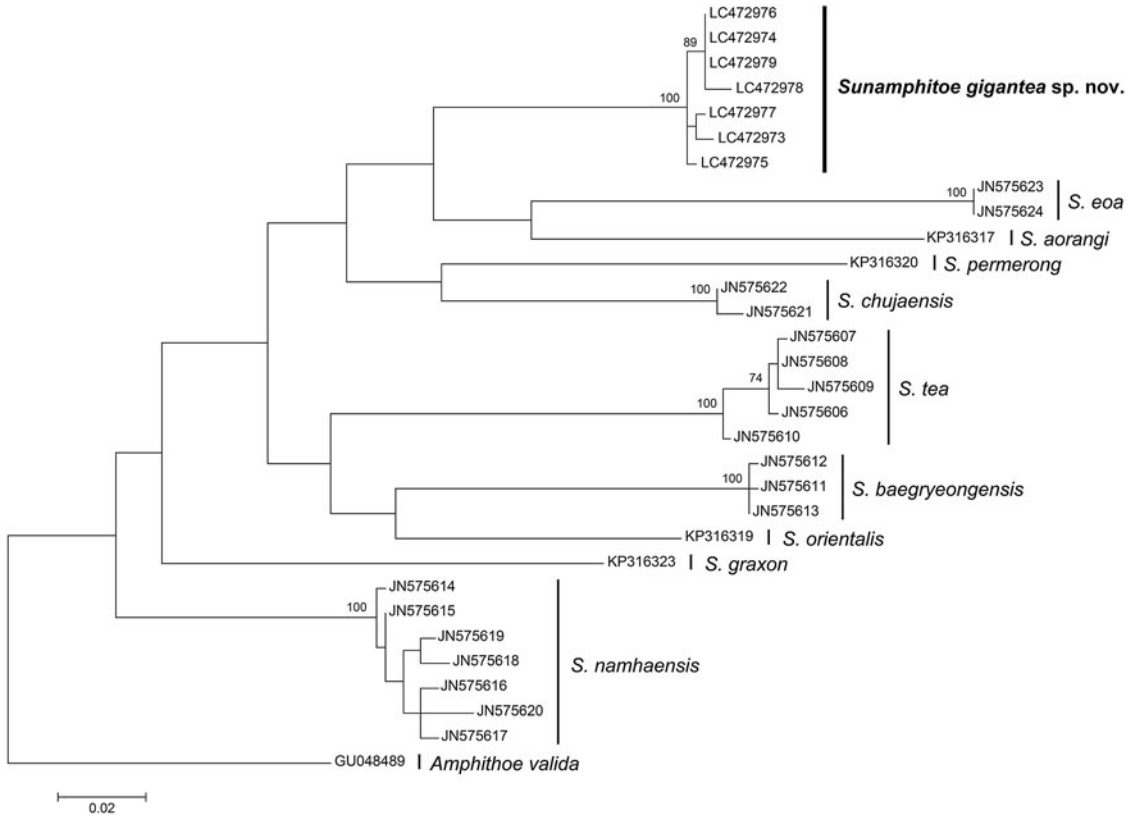

Fig. 11. Maximum likelihood tree of *Sunamphitoe* species and an outgroup *Ampithoe valida*, based on 506 base pairs of COI sequences. Values at nodes indicate bootstrap values above 70%.

is much shorter than propodus (Figure 8A, B), while in large males, posterior margin of propodus is straight and the palm is obscure without spine, and dactylus is elongate reaching proximal end of propodus (Figure 8C).

COLOURATION IN LIFE

Body (Figure 9) generally yellowish (or sometimes greenish) light brown without mottling, without sexual dimorphism; eyes red; antennae 1, 2 flagellae white or whitish yellow, with dark brown band subdistally.

DISTRIBUTION

Known only from type locality, Katsurakoi, Kushiro, Hokkaido, Japan (Figure 1).

HABITAT

Found on the surface of fronds of a kelp *Saccharina longissima* inhabiting subtidal rocky shore.

ECOLOGICAL NOTE

Specimens of the new species were found occupying a nest which was constructed by the apical part of kelp blade being rolled up (Figure 10) as reported in some other congeners (e.g. Cerda *et al.*, 2010). The following four patterns were observed as inhabitant(s) of single nest: (1) single male only, (2) single female only, (3) single female with many juveniles and (4) a pair of male and ovigerous female.

Male gnathopod 2 shows ontogenetic morphological changes (see *VARIATIONS*). Furthermore, it was found that a male and an ovigerous female coexisted in a nest, though the male gnathopod 2 was not fully developed (NSMT-Cr 26735 and 26736; NSMT-Cr 26738 and 26739). This suggests that male sexual maturity may occur prior to morphological maturity of their gnathopod 2. Males were smaller than coexisting ovigerous females in these two cases.

GENETIC ANALYSIS

In total, 643–658 bp of COI sequences were obtained from seven specimens including the holotype and the allotype (INSDC accession numbers, LC472973–LC472979 for finally obtained COI sequences, DRA008087 for raw fastq data). During the alignment procedure, all positions containing gaps or missing data were eliminated, and then, a total of 506 bp was used for the analysis. Intra-specific divergence of COI sequences within the new species was less than 2%, while inter-specific divergences between the new species and congeners were greater than 14% (Table 3).

In the phylogenetic tree of the COI sequences (Figure 11), all the sequences of *S. gigantea* sp. nov. formed a monophyletic clade that was supported by 100% bootstrap value. The sister clade of *S. gigantea* sp. nov. comprised of *S. eoa* and *S. aorangi*, though it was supported by a low bootstrap value. Species-level clade was supported by a high bootstrap value for each *Sunamphitoe* species, indicating the usefulness of COI sequences for DNA barcoding. However, all the higher-level clades (higher than species level) were supported by low bootstrap values, indicating that using only COI sequences may be insufficient for determining the phylogenetic relationship among *Sunamphitoe* species and other kinds of sequencing may be necessary for further validation.

ETYMOLOGY

The species name '*gigantea*' is derived from their large body size.

REMARKS

Among congeners, *Sunamphitoe gigantea* sp. nov. most resembles *S. eoa* (von der Brüggen, 1907) in the conspicuously large body (maximum body length is 42.6 mm in *S. gigantea* sp. nov. and

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Table 4. Morphological comparison among *Sunamphitoe gigantea* sp. nov., *S. baegryeongensis* and *S. eoa*

Morphological characters	*S. gigantea* sp. nov.	*S. baegryeongensis* (after Kim & Kim, 1988; Kim *et al*., 2012)	*S. eoa* from NW Pacific (after von der Brüggen, 1907; Gurjanova, 1938; Tzvetkova, 1967; Kim *et al*., 2012)	*S. eoa* from NE Pacific (after Barnard, 1954)
Maximum body length	42.6 mm	9 mm	38 mm in Russia 20.5 mm in Korea	11.5 mm
Length of antenna 1	Shorter than 1.5 times length of antenna 2	About 2 times as long as antenna 2	About 2 times as long as antenna 2	Unknown (at least, longer than antenna 2)
Number of slender setae on inner plate of maxilla 1	2	1	3	1
Propodus of male gnathopod 2	Without large processes on posterodistal corner, palm obscure without spines, palm margin straight, in large individuals Without a large process on posterodistal corner, palm well defined with a spine, palm margin straight to slightly convex, in small individuals	Without large processes on posterodistal corner, palm obscure without spines, palm margin strongly concave	Without large processes on posterodistal corner, palm obscure without spines, palm margin straight	With a large process on posterodistal corner, palm defined with spine, palm margin slightly concave
Dactylus of male gnathopod 2	Just reaching to posteroproximal end of propodus in large individuals Shorter than propodus in small individuals	Shorter than propodus	Very long, reaching to middle portion of merus	Shorter than propodus
Group of long setae on the sub-proximal area of anterior margin on bases of pereopods 3 and 4	Present	Absent (at least, not described or not shown in figures in the literatures)	Absent (at least, not described or not shown in figures in the literatures)	Absent (at least, not described or not shown in figures in the literature)
Posterodistal corner on basis of pereopod 7	Unarmed	Bearing a spine	Bearing slender setae	Bearing slender setae
Distoventral spur on peduncle of uropod 2	Present, acutely produced, reaching to about proximal 0.3 of rami	Absent to obsolete	Present, acutely produced, reaching to about proximal 0.1–0.2 of rami	Present, but not strongly or acutely produced
Length of peduncle of uropod 3	About 2.5–3.0 times longer than rami	About 2 times longer than rami	About 2.5–3.0 times longer than rami	About 2 times longer than rami

38 mm in *S. eoa* according to Tzvetkova, 1967), well developed mandibular palp, slender antenna 2, undefined male gnathopod 2 palm with the straight margin, unenlarged pereopods 5–7. However, the new species can be distinguished from *S. eoa* by the following points: (1) antenna 1 flagellum is much shorter than that of *S. eoa*; (2) maxilla 1 inner plate bears 2 slender setae in the new species, while that of *S. eoa* bears 3 setae; (3) gnathopod 2 dactylus of large male just reaches to posteroproximal end of propodus, whereas that of *S. eoa* reaches beyond carpus; (4) pereopods 3 and 4 bases bear a group of long setae on the sub-proximal area of anterior margin, while those of *S. eoa* lack long setae on its anterior margin. Moreover, *S. gigantea* sp. nov. and *S. eoa* also differ genetically in COI (17.2–18.0%) greater than the threshold distances (3.5–4%) proposed for amphipod species discrimination (Witt *et al.*, 2006; Rock *et al.*, 2007; Hou *et al.*, 2009). Therefore, we concluded that *S. gigantea* sp. nov. undoubtedly represents a novel species. The morphological comparisons among the new species and closely related species were summarized in Table 4.

Immature males of this new species are also close to Barnard's (1954) description of *S. eoa* from Oregon (note: Conlan & Bousfield (1982) suggested that Barnard's (1954) material might be another species rather than *S. eoa*). However, immature males of the new species differ from Barnard's material in the following characters: (1) gnathopod 2 palm is well defined with a small spine; (2) gnathopod 2 propodus lacks a large process on posterodistal corner; (3) pereopod 3 basis has a group of long slender setae on anterior margin; (4) distoventral spur on uropod 2 peduncle is longer; and (5) uropod 3 peduncle is longer.

Sunamphitoe gigantea sp. nov. also resembles *S. baegryeongensis* (Kim & Kim, 1988), however, is easily distinguished from it by the following points: (1) *S. gigantea* sp. nov. is much larger (maximum body length more than 40 mm) than *S. baegryeongensis* (maximum body length 9 mm); (2) in large males, propodus palm of gnathopod 2 is rather straight in *S. gigantea* sp. nov., while strongly concave in *S. baegryeongensis*; (3) pereopod 7 basis of *S. gigantea* sp. nov. is unarmed on posterior margin, while that of *S. baegryeongensis* bears a small spine on the posterodistal corner; (4) uropod 3 peduncle of *S. gigantea* sp. nov. is much longer than that of *S. baegryeongensis*; (5) they also differ genetically (COI, 16.9–17.9%).

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