Zoosyst. Evol. 93 (2) 2017, 189–210 | DOI 10.3897/zse.93.12125

PENSOFT

museum für naturkunde

# Two new species of *Jesogammarus* from Japan (Crustacea, Amphipoda, Anisogammaridae), with comments on the validity of the subgenera *Jesogammarus* and *Annanogammarus*

Ko Tomikawa[1], Takafumi Nakano[1], Naoto Hanzawa[2]

1 *Department of Science Education, Graduate School of Education, Hiroshima University, Higashi-Hiroshima 739-8524, Japan*

2 *Graduate School of Science and Engineering, Yamagata University, Yamagata 990-8560, Japan*

*http://zoobank.org/95D58718-953F-44B2-AA39-C5D1EF4B36BC*

Corresponding author: *Ko Tomikawa* (tomikawa@hiroshima-u.ac.jp)

Received 6 February 2017
Accepted 5 March 2017
Published 17 March 2017

Academic editor:
*Michael Ohl*

## Key Words

Gammaroidea
*Jesogammarus*
*Annanogammarus*
molecular phylogeny
cryptic species

## Abstract

Two new species of anisogammaris amphipod, *Jesogammarus* (*Jesogammarus*) *bousfieldi* and *J.* (*J.*) *uchiyamaryui*, are described from mountain streams in Yamagata Prefecture and from brackish waters on Iki and Fukue Islands, Nagasaki Prefecture, Japan. *Jesogammarus bousfieldi* is morphologically almost similar to *J. paucisetulosus* Morino, 1984. However, *J. bousfieldi* is distinguished from *J. paucisetulosus* by the more number of marginal setae on the pleonites. *Jesogammarus uchiyamaryui* resembles *J. ikiensis* Tomikawa, 2015, but the former differs from the latter by two setae on the posterior margin of peduncular article 2 of antenna 1, short and straight accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5, densely setose ventral margins of coxae of female gnathopods 1 and 2 and pereopod 3, and a shorter inner ramus of uropod 3. Phylogenetic analyses using nuclear 28S rRNA, mitochondrial cytochrome *c* oxidase subunit I, and the 16S rRNA markers showed the sister relationship between *J. bousfieldi* and *J. paucisetulosus*. However, the phylogenetic position of *J. uchiyamaryui* remains uncertain. Both new species were genetically highly diverged comparable to intraspecific divergence among other *Jesogammarus* species. The species diversity related to habitat and the subgeneric classification of *Jesogammarus* are briefly discussed.

## Introduction

The amphipod genus *Jesogammarus* Bousfield, 1979 inhabits fresh and brackish waters of the Japanese archipelago, the Korean Peninsula, and the Chinese continent (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). *Jesogammarus* was erected by Bousfield (1979) as a monotypic genus with the type species *Anisogammarus jesoensis* Schellenberg, 1937. In the same article, Bousfield established monotypic *Annanogammarus* Bousfield, 1979, of which the type species was designated *Gammarus annandalei* Tattersall, 1922.

Morino (1984) described a second species in *Jesogammarus*, *J. paucisetulosus* Morino, 1984 from Japan. Subsequently, Morino (1985) relegated *Annanogammarus* as a subgenus under *Jesogammarus* because the character combination of *J. paucisetulosus* and additional new species bridged the morphological gap between the two genera *Jesogammarus* and *Annanogammarus*. Morino concluded that *Annanogammarus* could be a distinct subgenus due to unequal coxal gill accessory lobes on gnathopod 2–pereopod 5 in length and simple robust setae on the palmar margin of female gnathopod 2. Molecular phylogenetic analyses of *Jesogammarus* recovered the monophyly of the subgenera *Jesogammarus* and *Annanogammarus*, respectively (Tomikawa et al. 2007, Tomikawa 2015).

The latest taxonomic study of the genus shows that *Jesogammarus* contains 18 species (Tomikawa 2015). The subgenus *Annanogammarus* consists of six freshwater species, and the subgenus *Jesogammarus* comprises 11 freshwater and one brackish-water species.

Two additional *Jesogammarus* species were collected during field surveys of fresh and brackish waters in Japan: one from mountain streams in Yamagata Prefecture and the other from brackish waters of Iki and Fukue Islands,

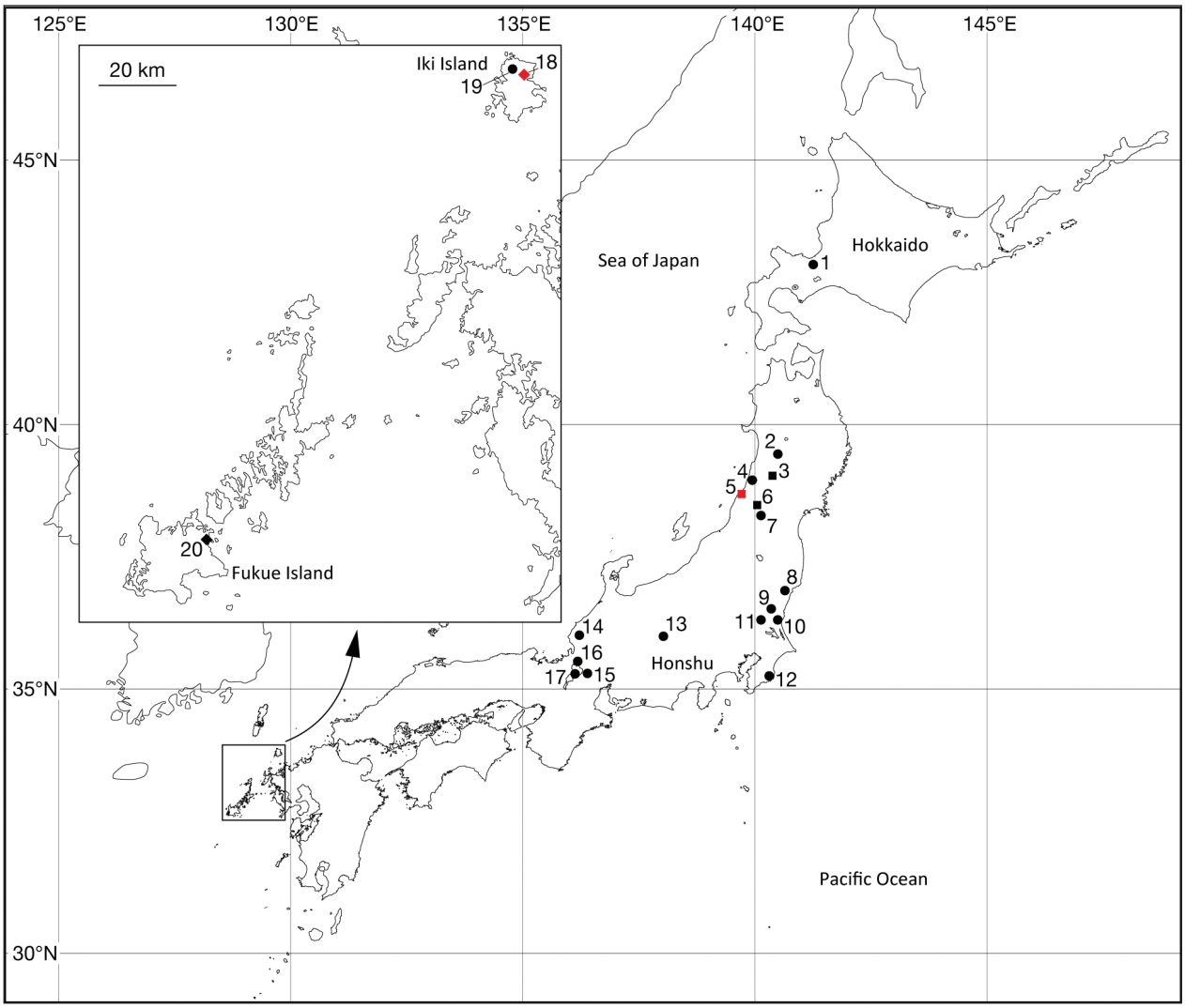


**Figure 1.** Map showing the collection localities of the specimens examined in this study. The closed squares indicate the localities of *J. bousfieldi* sp. n. The closed diamonds denote the localities of *J. uchiyamaryui* sp. n. The closed circles indicate the localities of the referred materials used for the phylogenetic analyses. The symbols in red denote the type locality of each of the new species. Names of localities are shown in Table 1.

Nagasaki Prefecture, Japan. A close examination of the external morphology revealed that these two species are distinct from their congeners and are described here as new species. In addition, the phylogenetic positions of these new species within *Jesogammarus* were estimated using nuclear 28S rRNA, the mitochondrial cytochrome *c* oxidase subunit I, and 16S rRNA sequences. A key to *Jesogammarus* species is provided and modified from that given in Tomikawa (2015).

## Material and methods

### Samples

Specimens of *Jesogammarus* were collected from three localities in Yamagata Prefecture and from two localities in Nagasaki Prefecture, Japan (Fig. 1). The specimens were collected by scooping with a fine-mesh hand-net and fixed in 99% ethanol on-site.

### Morphological observation

All appendages of the examined specimens of the undescribed species were dissected in 70% ethanol and mounted in gum-chloral medium on glass slides under a stereomicroscope (Olympus SZX7). Specimens were examined using a light microscope (Nikon Eclipse Ni) and illustrated with the aid of a camera lucida. The body length from the tip of the rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm. The nomenclature of the setal patterns on the mandibular palp follows Stock (1974). The specimens are deposited in the Tsukuba Collection Center of the National Museum of Nature and Science, Tokyo (NSMT) and the Zoological Collection of Kyoto University (KUZ).

### PCR and DNA sequencing

The extraction of genomic DNA from appendage muscles of the *Jesogammarus* materials preserved in 99% ethanol followed Tomikawa et al. (2014). Primer sets

**Table 1.** Samples used for the phylogenetic analyses. The information on the vouchers or isolate numbers is accompanied by the collection localities and the INSDC accession numbers. Sequences marked with an asterisk were obtained for the first time in the present study.

| # | Species | Voucher or isolate # | Locality | INSDC # | | |
|---|---|---|---|---|---|---|
| | | | | 28S | COI | 16S |
| Newly described *Jesogammarus* species in this study | | | | | | |
| 6 | *J. bousfieldi* sp. n. | KUZ Z1797 | Gassan, Tsuruoka, Yamagata Prefecture, Japan | LC214776* | LC214536* | LC214793* |
| 6 | *J. bousfieldi* sp. n. | KUZ Z1798 | Gassan, Tsuruoka, Yamagata Prefecture, Japan | LC214777* | LC214537* | LC214794* |
| 5 | *J. bousfieldi* sp. n. | KUZ Z1799 | Aburato, Tsuruoka, Yamagata Prefecture, Japan | LC214778* | LC214538* | LC214795* |
| 5 | *J. bousfieldi* sp. n. | KUZ Z1800 | Aburato, Tsuruoka, Yamagata Prefecture, Japan | LC214779* | LC214539* | LC214796* |
| 3 | *J. bousfieldi* sp. n. | KUZ Z1801 | Mamurogawa, Yamagata Prefecture, Japan | LC214782* | LC214541* | LC214798* |
| 3 | *J. bousfieldi* sp. n. | KUZ Z1802 | Mamurogawa, Yamagata Prefecture, Japan | LC214783* | LC214542* | LC214799* |
| 18 | *J. uchiyamaryui* sp. n. | KUZ Z1803 | Tanie River, Iki, Nagasaki Prefecture, Japan | LC214773* | LC214533* | LC214790* |
| 18 | *J. uchiyamaryui* sp. n. | KUZ Z1804 | Tanie River, Iki, Nagasaki Prefecture, Japan | LC214774* | LC214534* | LC214791* |
| 20 | *J. uchiyamaryui* sp. n. | KUZ Z1805 | Mukata, Goto, Nagasaki Prefecture, Japan | LC214788* | LC214545* | LC214802* |
| 20 | *J. uchiyamaryui* sp. n. | KUZ Z1806 | Mukata, Goto, Nagasaki Prefecture, Japan | LC214789* | LC214546* | LC214803* |
| *Jesogammarus* (*Annanogammarus*) | | | | | | |
| 17 | *J. annandalei* | G1162 | Lake Biwa, Shiga Prefecture, Japan | LC214786* | LC052248 | LC052269 |
| | *J. debilis* | IZCAS-I-A0325 | Fangshan, Beijing, China | EF582997 | | EF582846 |
| 15 | *J. fluvialis* | G83 | Samegai, Shiga Prefecture, Japan | LC214766* | LC052236 | LC052257 |
| 16 | *J. naritai* | G1167 | Lake Biwa, Shiga Prefecture, Japan | LC214787* | LC052249 | LC052270 |
| 13 | *J. suwaensis* | G88 | Lake Suwa, Nagano Prefecture, Japan | LC214767* | LC052237 | LC052258 |
| 13 | *J. suwaensis* | G89 | Lake Suwa, Nagano Prefecture, Japan | LC214768* | LC052238 | LC052259 |
| *Jesogammarus* (*Jesogammarus*) | | | | | | |
| 7 | *J. fujinoi* | G17 | Gobanmiki, Yamagata, Yamagata Prefecture, Japan | LC214762* | LC052232 | LC052253 |
| | *J. hebeiensis* | IZCAS-I-A0294 | Yanqing, Beijing, China | EF582998 | | EF582847 |
| 10 | *J. hinumensis* | G52 | Lake Hinuma, Ibaraki Prefecture, Japan | LC214765* | LC052235 | LC052256 |
| 14 | *J. hokurikuensis* | G383 | Takinami, Fukui, Fukui Prefecture, Japan | LC214771* | LC052241 | LC052262 |
| 19 | *J. ikiensis* | G515 | Katsumoto, Iki, Nagasaki Prefecture, Japan | LC214772* | LC052242 | LC052263 |
| 1 | *J. jesoensis* | G164 | Sapporo, Hokkaido, Japan | LC214769* | LC052239 | LC052260 |
| 2 | *J. mikadoi* | G13 | Rokugo, Akita Prefecture, Japan | LC214761* | LC052231 | LC052252 |
| 9 | *J. paucistulosus* | G1037 | Mito, Ibaraki Prefecture, Japan | LC214780* | LC052247 | LC052268 |
| 9 | *J. paucistulosus* | G1038 | Mito, Ibaraki Prefecture, Japan | LC214781* | LC214540* | LC214797* |
| 11 | *J. paucistulosus* | G1017 | Sakuragawa, Ibaraki Prefecture, Japan | LC214775* | LC214535* | LC214792* |
| 8 | *J. paucistulosus* | G1159 | Kitaibaraki, Ibaraki Prefecture, Japan | LC214784* | LC214543* | LC214800* |
| 8 | *J. paucistulosus* | G1160 | Kitaibaraki, Ibaraki Prefecture, Japan | LC214785* | LC214544* | LC214801* |
| 4 | *J. shonaiensis* | G192 | Sakata, Yamagata Prefecture, Japan | LC214770* | LC052240 | LC052261 |
| 12 | *J. spinopalpus* | G32 | Onjuku, Chiba Prefecture, Japan | LC214763* | LC052233 | LC052254 |
| Outgroup | | | | | | |
| | *Eogammarus kygi* | G1 | Naibetsu River, Eniwa, Hokkaido, Japan | LC214759* | LC052229 | LC052250 |
| | *Eogammarus possjeticus* | G3 | Akkeshi, Hokkaido, Japan | LC214760* | LC052230 | LC052251 |
| | *Spasskogammarus spasskii* | G35 | Akkeshi, Hokkaido, Japan | LC214764* | LC052234 | LC052255 |

for the PCR and cycle sequencing reactions used in this study were as follows: for 28S rRNA (28S), 28SF and 28SR (Tomikawa et al. 2012); for cytochrome *c* oxidase subunit I (COI), Am-COI-H and Am-COI-T (Tomikawa 2015); for 16S rRNA (16S), 16STf (Macdonald III et al. 2005) and 16Sbr (Palumbi 1996; modified to correspond with "Fruit Fly"). The PCR reactions and DNA sequencing were performed using the identical method mentioned in Tomikawa (2015). In total, 59 sequences from the unidentified *Jesogammarus* specimens and *J. paucisetulosus* were newly obtained in this study, and deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (Table 1).

## Molecular phylogenetic analyses

Thirty-two published sequences were obtained from the INSDC for use in molecular phylogenetic analyses (Table 1). Along with the two Chinese *Jesogammarus* species, *J.* (*A.*) *debilis* Hou & Li, 2005 and *J.* (*J.*) *hebeiensis* Hou & Li, 2004, used in the previous phylogenetic study (Hou et al. 2007), three anisogammarid species, *Eogammarus kygi* (Derzhavin, 1923), *E. possjeticus* (Tzvetkova, 1967), and *Spasskogammarus spasskii* (Bulycheva, 1952), were included in the analyses as outgroup taxa.

The phylogenetic positions of the unidentified species among *Jesogammarus* were estimated based on the gene fragments of 28S, COI, and 16S sequences. The align-

ment of COI was trivial, as no indels were observed. The 28S and 16S sequences were aligned using MAFFT v. 7.305b L-INS-i (Katoh and Standley 2013). The lengths of 28S, COI and 16S sequences were 717, 362, and 420 bp, respectively. The concatenated sequences thus yielded 1,499 bp of alignment positions. One of each four completely identical sequence pairs (KUZ Z1803 and KUZ Z1804; KUZ Z1799 and KUZ Z1800; G1037 and G1038; and KUZ Z1805 and KUZ Z1806) was removed from the dataset using the "pgelimdupseq" command implemented in Phylogears v. 2.0.2014.03.08 (Tanabe 2008).

Phylogenetic relationships were estimated using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were conducted using RAxML v. 8.2.8 (Stamatakis 2014) with GTRCAT, immediately after nonparametric bootstrapping (Felsenstein 1985) (BS) conducted with 1,000 replicates. The best-fit partitioning scheme for the ML analysis was identified with the Akaike information criterion (Akaike 1974) using PartitionFinder v. 1.1.1 (Lanfear et al. 2012) with the "all" algorithm: each of 28S, 1st to 3rd positions of COI and 16S were, respectively, treated as a separate partition.

BI and Bayesian posterior probabilities (PPs) were estimated using MrBayes v. 3.2.6 (Ronquist et al. 2012). The best-fit models for each partition were identified with the Bayesian information criterion (Schwarz 1978) using PartitionFinder with the "all" algorithm: for 28S and the 2nd position of COI, K80+G; for 1st position of COI, SYM+I; for the 3rd position of COI, GTR+G; and for 16S, HKY+G. Two independent runs of four Markov chains were conducted for 5 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond 2013) and the first 20,001 trees were discarded based on the results.

## Taxonomy

### *Jesogammarus* (*Jesogammarus*) *bousfieldi* sp. n.

http://zoobank.org/EC1F1C3D-259C-4D7A-B88D-51AA229605A2
New Japanese name: Nagare-yokoebi
Figs 2–8

**Type materials.** Holotype: Male (9.8 mm), NSMT-Cr 25470, Aburato (38°45′23.9″N, 139°43′14.7″E), Tsuruoka, Yamagata Prefecture, Japan, 26 November 2013, collected by K. Tomikawa. Paratypes: 1 ovigerous female (6.6 mm), NSMT-Cr 25471, 1 male (8.2 mm), KUZ Z1799, 1 male (8.4 mm), KUZ Z1800, data same as for holotype; 1 female (8.9 mm), KUZ Z1797, 1 male (9.0 mm), KUZ Z1798, Gassan (38°33′53.2″N, 139°56′24.3″E), Tsuruoka, Yamagata Prefecture, Japan, 26 November 2013, collected by K. Tomikawa; 1 Male (10.4 mm), NSMT-Cr 25472, 1 ovigerous female (9.2 mm), NSMT-Cr 25473, 1 ovigerous female (7.8 mm), KUZ Z1801, 1 male (10.3 mm), KUZ Z1802, Otaki, Mamurogawa, Yamagata Prefecture, Japan, 15 April 2001, collected by K. Tomikawa.

**Type locality.** Japan, Yamagata Prefecture: Tsuruoka, Aburato.

**Description.** *Male* [NSMT-Cr 25470, 9.8 mm]. Head (Fig. 2) with short rostrum; ventral margin of lateral cephalic lobe weakly concave; antennal sinus rounded; eyes oval, major axis 0.2 × height of head. Dorsal surfaces of pereonites smooth (Fig. 2). Dorsal margins of pleonites 1–3 (Fig. 3A–C) with 4, 4, and 9 setae, respectively. Posterior margin of epimeral plate 1 rounded with seta, posteroventral corner with seta, anteroventral corner with 8 setae (Fig. 3D); posterior margin of plate 2 almost straight with 4 setae, posteroventral corner quadrate with seta, anteroventral corner with 4 setae, ventral submargin with 2 robust setae and 2 small setae (Fig. 3E); posterior margin of plate 3 almost straight with 5 setae, posteroventral corner quadrate with seta, anteroventral corner with 2 setae, ventral submargin with 2 robust setae (Fig. 3F). Urosomites 1–3 (Fig. 3G–I) with 8, 6, and 2 robust setae associated with slender setae on dorsal margins.

Antenna 1 (Fig. 3J): length 0.5 × body length; peduncular articles 1–3 in length ratio of 1.0 : 0.8 : 0.5; posterodistal corner of peduncular article 1 without robust seta, posterior margin of peduncular article 1 with 2 clusters and 1 pair of setae and 1 single seta, posterior margin of peduncular article 2 with 5 clusters and 1 pairs of setae, posterior margin of peduncular article 3 with 2 clusters and 1 pair of setae; accessory flagellum 6-articulate; primary flagellum 26-articulate, each article with 1 aesthetasc.

Antenna 2 (Fig. 3K): length 0.6 × antenna 1; posterior margin of peduncular article 4 with 5 clusters of setae, posterior margin of peduncular article 5 with 3 clusters and 1 pair of setae and 1 single seta; flagellum 13-articulate, calceoli present (Fig. 3L).

Mouthparts. Upper lip (= labrum) (Fig. 3M) with rounded distal margin, bearing fine setae. Mandibles (Fig. 3N–P) with left and right incisors 5- and 4-dentate, respectively, left lacinia mobilis 4-dentate, right one bifid, bearing many teeth; molar process triturative, with plumose seta; accessory setal rows of left and right mandibles with 7 and 6 blade-like setae, respectively; palp 3-articulate with length ratio of 1.0 : 4.4 : 4.0, palp article 1 bare, article 2 with 22 setae, article 3 with 2 clusters and 1 pair of A-setae, 1 pair of B-setae, and many C-, D-, and E-setae. Lower lip (= labium) (Fig. 3Q) with broad outer lobes, inner lobes indistinct. Maxilla 1 (Fig. 4A) with inner and outer plates and palp; medial margin and apical submargin of inner plate with 20 and 2 plumose setae, respectively; outer plate subrectangular, with 11 serrate teeth apically (Fig. 4B); palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 7 robust setae and 1 slender seta on its apical margin and 3 slender setae on its submargin, outer margin without setae. Maxilla 2 (Fig. 4C) with oblique inner row of 19 plumose setae on inner plate; outer plate almost as long as inner plate. Maxilliped (Fig. 4D) with inner and outer plates and palp; inner plate with 3 and 2 robust setae on

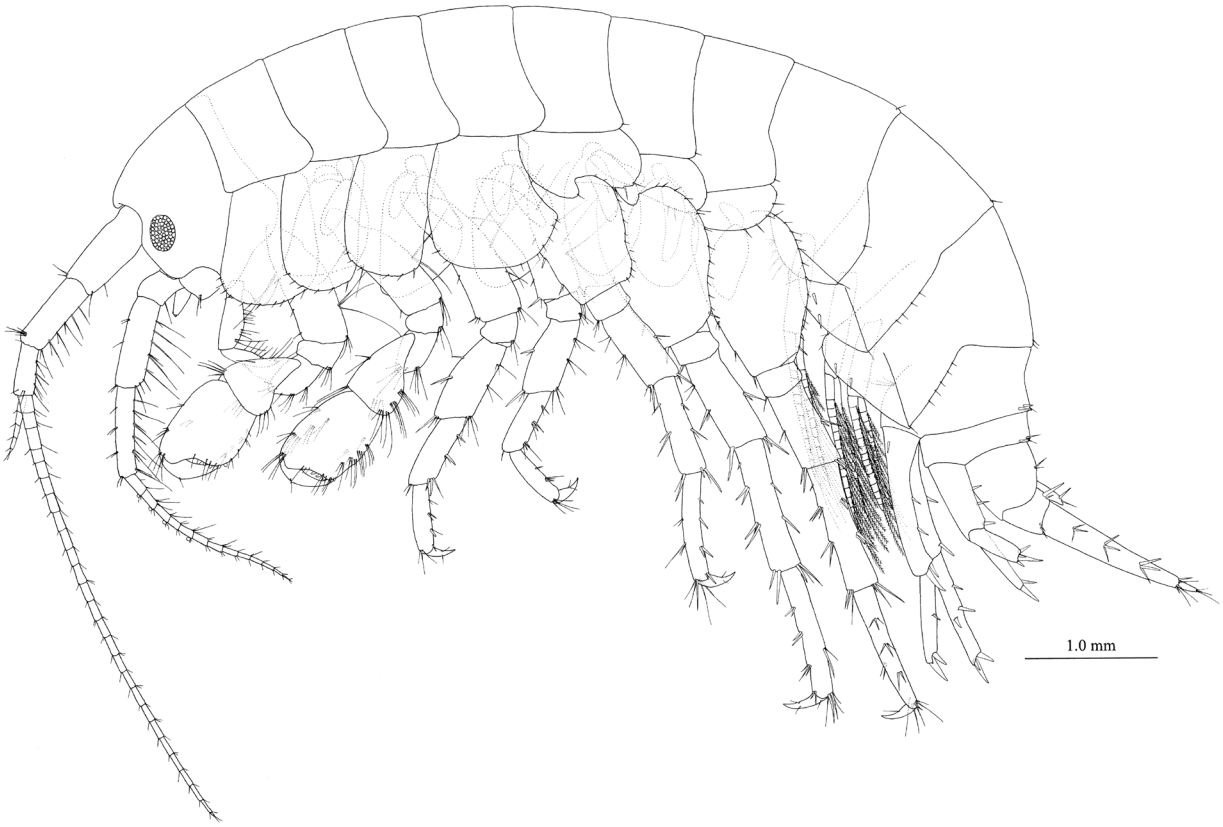


**Figure 2.** *Jesogammarus bousfieldi* sp. n., holotype, male (9.8 mm), NSMT-Cr 25470. Habitus, lateral view.

apical and inner margins, respectively; outer plate with plumose setae on apical margin and robust setae on inner margin; palp 4-articulate, article 2 with inner marginal and submarginal rows of setae, article 3 with facial setae, article 4 slightly curved inward, with slender nail.

Gnathopod 1 (= pereopod 1) (Fig. 4E): coxa (= article 1) with 13 setae on anterodistal to ventral margin; anterior and posterior margins of basis (= article 2) with long setae; carpus (= article 5) length 1.5 × width, anterior margin with 1 pair of setae; propodus (= article 6) length 1.4 × carpus and 1.4 × width, anterior margin with 1 pair and 1 cluster of setae, palmar margin oblique, weakly convex, with 15 peg-shaped robust setae (Fig. 4F); dactylus (= article 7) as long as palmar margin.

Gnathopod 2 (= pereopod 2) (Fig. 4G): coxa with 6 marginal setae on ventral margin, posterodistal corner with 1 seta; anterior and posterior margins of basis with long setae; carpus length 1.5 × width, anterior margin with 1 pair of setae; propodus length 1.2 × carpus and 1.5 × width, respectively, anterior margin with 2 clusters of setae, palmar margin oblique, weakly convex, with 10 peg-shaped robust setae (Fig. 4H); dactylus as long as palmar margin.

Pereopod 3 (Fig. 4I): coxa with 7 marginal setae on ventral part, posteroproximal part with 2 setae; anterior and posterior margins of basis with long setae, anterodistal corner of basis with robust seta.

Pereopod 4 (Fig. 4J): coxa expanded with posterior concavity, bearing 2 setae on anterodistal corner and 6 setae on ventral margin; anterior and posterior margins of basis with long setae, anterodistal corner with robust seta.

Pereopod 5 (Fig. 5A): coxa bilobed, anterior lobe with apical seta, ventral margin of posterior lobe with 3 setae, posterodistal corner rounded with seta; posterior margin of basis weakly expanded, with 13 setae; anterior and posterior margins of merus to propodus with robust and slender setae.

Pereopod 6 (Fig. 5B): coxa bilobed, anterior lobe with anteroproximal setae, ventral margin of posterior lobe with 3 setae, posterodistal corner rounded with seta; posterior margin of basis weakly expanded with 12 setae, posterodistal corner with robust seta associated with small seta; anterior and posterior margins of merus to propodus with robust and slender setae.

Pereopod 7 (Fig. 5C): ventral margin of coxa weakly concave, bearing 2 setae on anterior part and 4 setae on posteroventral part; posterior margin of basis weakly expanded with 9 setae, posterodistal corner with robust seta associated with 2 small setae; anterior and posterior margins of merus to propodus with robust and slender setae.

Coxal gills on gnathopod 2 and pereopods 3–5 (Fig. 5D–G) with 2 accessory lobes, posterior lobes longer than anterior ones, gills on pereopods 6 and 7 (Fig. 5H, I) each with 1 accessory lobe.

Pleopods 1–3 (Fig. 5J) each with paired retinacula (Fig. 5L) on inner margin of peduncle, and bifid plumose setae (= clothes-pin setae) (Fig. 5K) on inner basal margin of inner ramus.

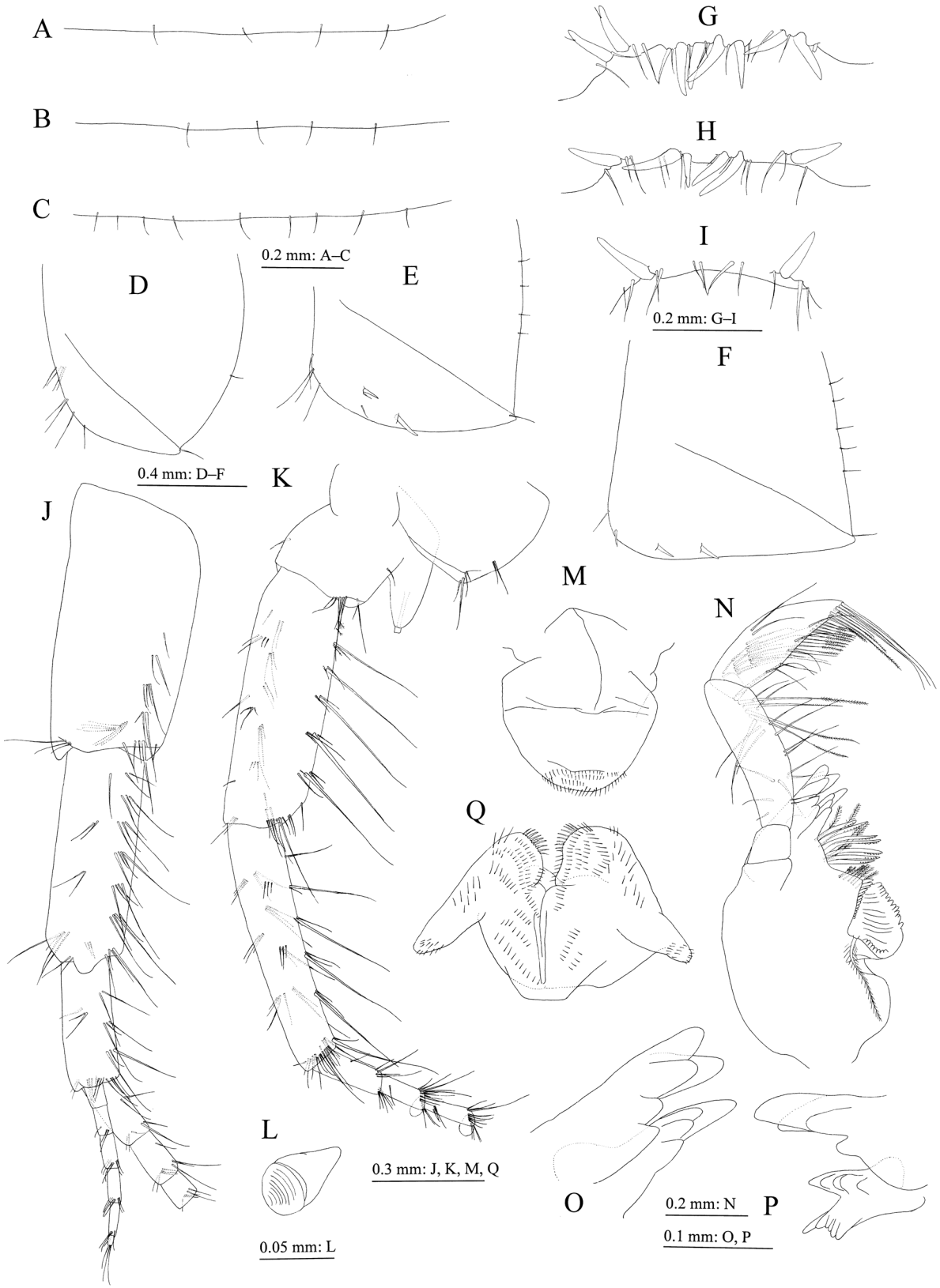


**Figure 3.** *Jesogammarus bousfieldi* sp. n., holotype, male (9.8 mm), NSMT-Cr 25470. **A–C** dorsal margins of pleonites 1–3, respectively, dorsal views; **D–F** epimeral plates 1–3, respectively, lateral views; **G–I** dorsal margins of urosomites 1–3, respectively, dorsal views; **J** peduncular articles 1–3, accessory flagellum, and flagellar articles 1–3 of antenna 1, medial view; **K** peduncular articles 1–5 and flagellar articles 1–3 of antenna 2, medial view; **L** calceolus of antenna 2, medial view; **M** upper lip, anterior view; **N** left mandible, medial view; **O** incisor and lacinia mobilis of left mandible, lateral view; **P** incisor and lacinia mobilis of right mandible, lateral view; **Q** lower lip, ventral view.

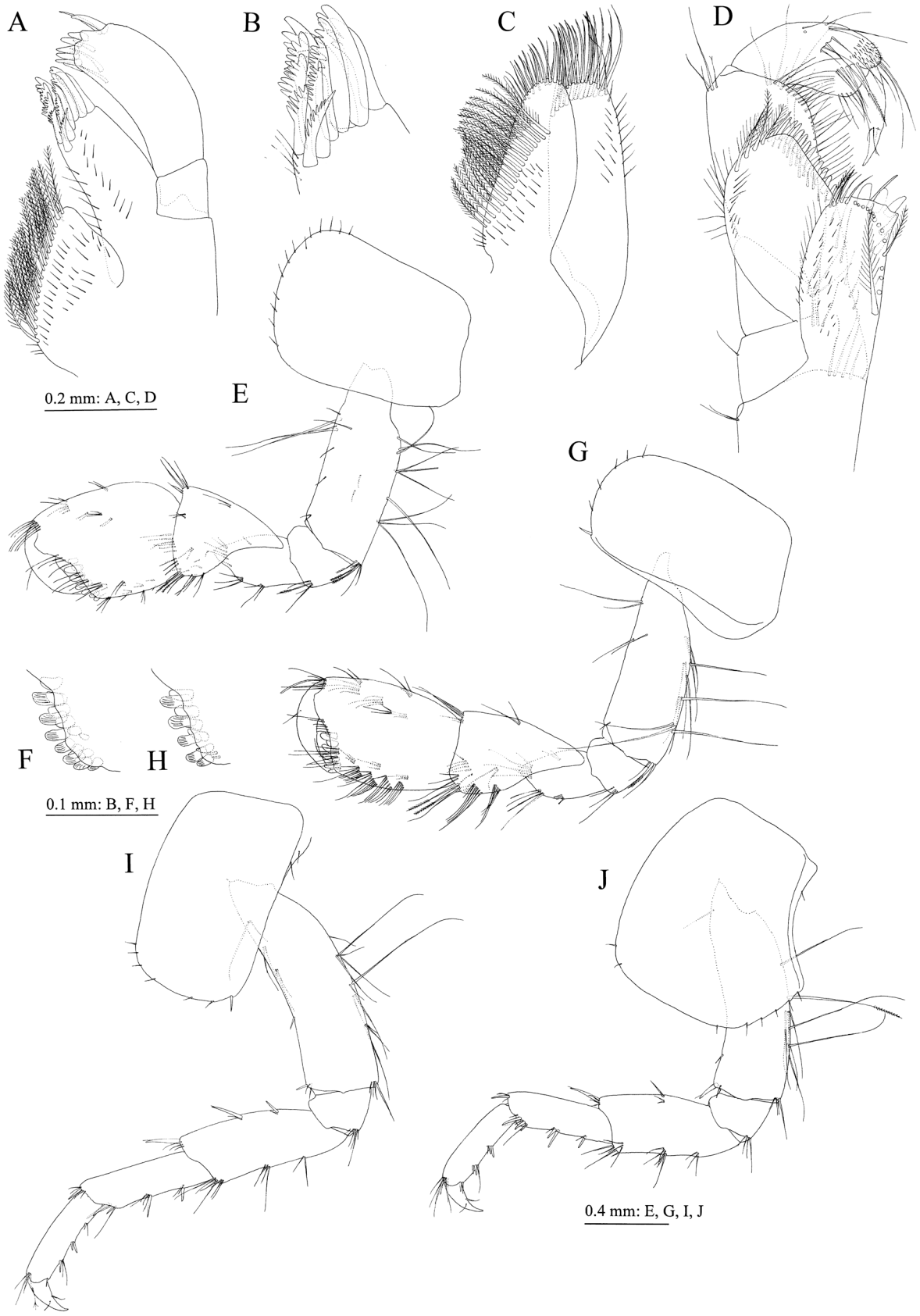


**Figure 4.** *Jesogammarus bousfieldi* sp. n., holotype, male (9.8 mm), NSMT-Cr 25470. **A** maxilla 1, dorsal view; **B** outer plate of maxilla 1, dorsal view; **C** maxilla 2, dorsal view; **D** maxilliped, dorsal view; **E** gnathopod 1, lateral view; **F** palmar margin of propodus of gnathopod 1, lateral view; **G** gnathopod 2, lateral view; **H** palmar margin of propodus of gnathopod 2, lateral view; **I** pereopod 3, lateral view; **J** pereopod 4, lateral view.

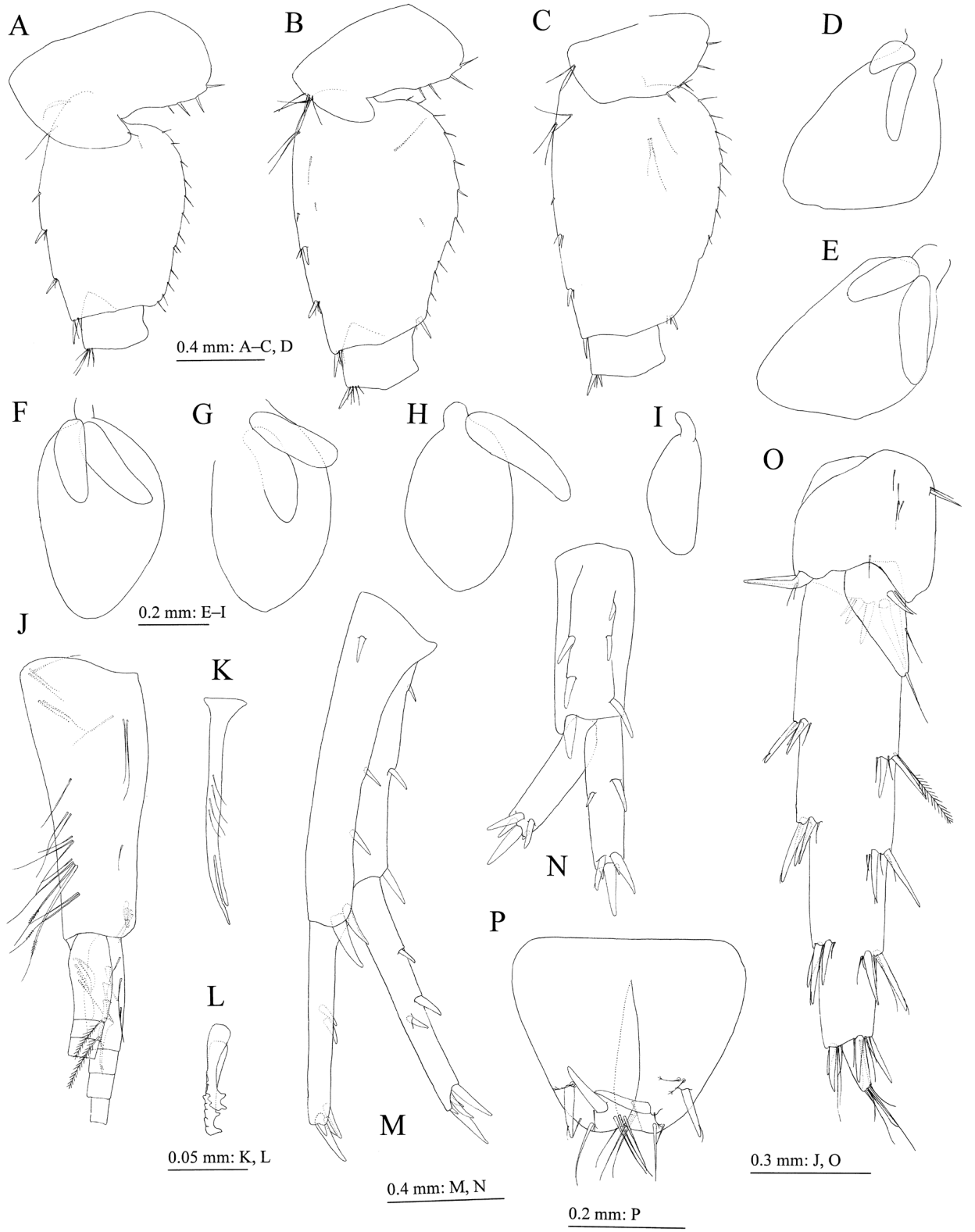


**Figure 5.** *Jesogammarus bousfieldi* sp. n., holotype, male (9.8 mm), NSMT-Cr 25470 (A–C and E–P) and paratype, female (6.6 mm), NSMT-Cr 25471 (D). **A** coxa–ischium of pereopod 5, lateral view; **B** coxa–ischium of pereopod 6, lateral view; **C** coxa–ischium of pereopod 7, lateral view; **D–I** coxal gills on gnathopod 2 and pereopods 3–7, lateral views; **J** pleopod 1, lateral view, distal parts of rami omitted; **K** bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of pleopod 1, lateral view; **L** retinacula on peduncle of pleopod 1, lateral view; **M** uropod 1, dorsal view; **N** uropod 2, dorsal view; **O** uropod 3, dorsal view; **P** telson, dorsal view.

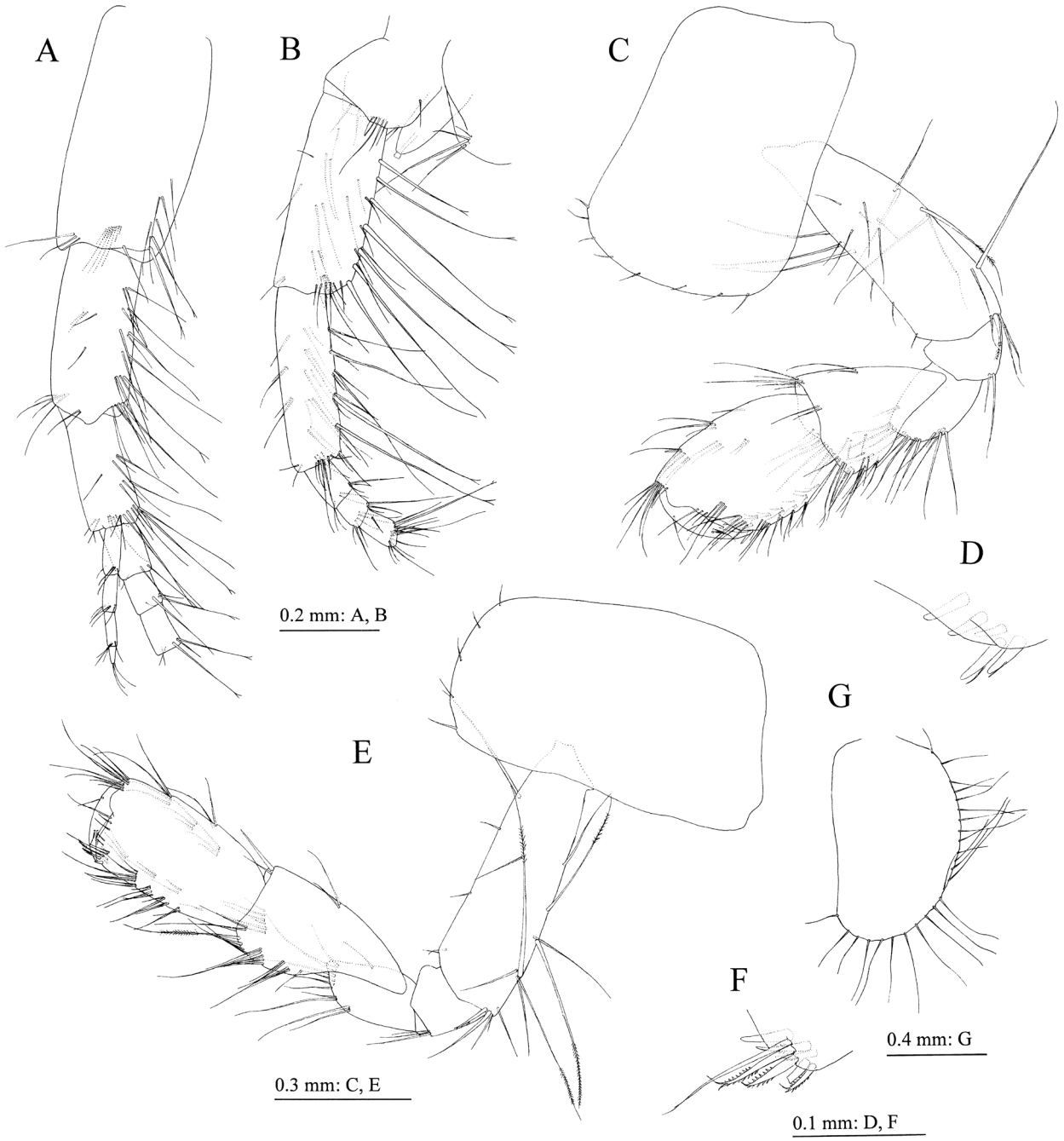


**Figure 6.** *Jesogammarus bousfieldi* sp. n., paratype, female (6.6 mm), NSMT-Cr 25471. **A** peduncular articles 1–3, accessory flagellum, and flagellar articles 1–3 of antenna 1, medial view; **B** peduncular articles 1–5 and flagellar articles 1–3 of antenna 2, medial view; **C** gnathopod 1, lateral view; **D** palmar margin of propodus of gnathopod 1, lateral view; **E** gnathopod 2, lateral view; **F** palmar margin of propodus of gnathopod 2, lateral view; **G** brood plate of gnathopod 2, lateral view.

Uropods. Uropod 1 (Fig. 5M): peduncle with robust seta on basofacial part, inner and outer margins each with 2 robust setae, inner and outer distal corners with 1 and 2 robust setae, respectively; inner ramus length 0.7 × peduncle, inner margin with 2 robust setae and outer margin with robust seta; outer ramus length 0.8 × inner ramus, inner and outer margins each with robust seta. Uropod 2 (Fig. 5N): peduncle with 2 robust setae on inner and outer margins, respectively, inner and outer distal corners each with robust seta; inner ramus length 0.8 × peduncle, its inner and outer margins each with robust seta; outer ramus length 0.9 × inner ramus, marginally bare. Uropod 3 (Fig. 5O): peduncle length 0.3 × outer ramus; inner ramus length 0.2 × outer ramus, with 2 slender setae and robust seta on inner margin, and apical seta; outer ramus 2-articulate, inner and outer margins

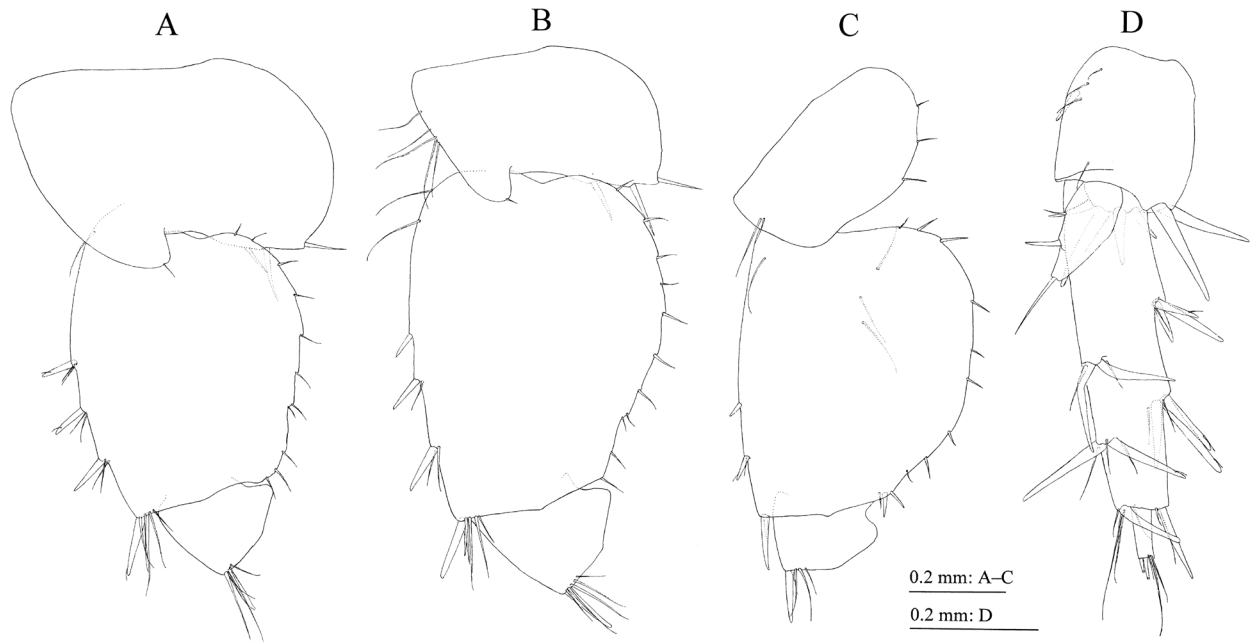


**Figure 7.** *Jesogammarus bousfieldi* sp. n., paratype, female (6.6 mm), NSMT-Cr 25471. **A** coxa–ischium of pereopod 5, lateral view; **B** coxa–ischium of pereopod 6, lateral view; **C** coxa–ischium of pereopod 7, lateral view; **D** uropod 3, dorsal view.

**Figure 8.** *Jesogammarus bousfieldi* sp. n., not preserved, precopula pair (male: upper, female: lower). Photographed by Ryu Uchiyama.

of article 1 each with 3 clusters of setae, some of which robust, inner margin with plumose seta, article 2 length 0.1 × article 1, with simple setae apically.

Telson (Fig. 5P) length 0.8 times × width, cleft for 79% of length; each lobe with 2 robust setae and slender setae.

*Female* [NSMT-Cr 25471, 6.6 mm]. Antenna 1 (Fig. 6A): peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.5; posterior margin of peduncular article 1 with 3 pairs of setae and single seta, posterior margin of peduncular article 2 with 5 clusters of setae, posterior margin of peduncular article 3 with 2 clusters of setae; accessory flagellum 4-articulate; primary flagellum 19-articulate.

Antenna 2 (Fig. 6B): posterior margin of peduncular article 4 with 1 cluster and 4 pairs of setae and single seta, posterior margin of peduncular article 5 with 3 clusters and 2 pairs of setae; flagellum 12-articulate, calceoli absent.

Gnathopod 1 (Fig. 6C): posteroproximal part of coxa without setae; carpus length 1.4 × width; propodus slightly longer than merus and 1.5 × width; palmar margin (Fig. 6D) with 5 robust setae.

Gnathopod 2 (Fig. 6E): posteroproximal part of coxa without setae; carpus length 1.9 × width; propodus almost as long as merus, lenght 1.8 × width; palmar margin (Fig. 6F) with 3 robust and 4 pectinate robust setae.

Posterior margin of bases of pereopods 5–7 more expanded than in male (Fig. 7A–C).

Brood plates (= oostegites) (Fig. 6G): broad, with numerous marginal setae.

Uropod 3 (Fig. 7D): peduncle length 0.4 × outer ramus; inner ramus length 0.3 × outer ramus; inner and outer margins of article 1 of outer ramus each with 2 clusters of setae, plumose setae absent, article 2 length 0.2 × article 1.

Egg number 6 (16 in female from Mamurogawa [NSMT-Cr 25473]).

**Etymology.** *Jesogammarus bousfieldi* was named in remembrance of the late Dr Edward Lloyd Bousfield, who enthusiastically guided and encouraged many Japanese amphipodologists, and sadly passed away on 7 September 2016.

**Distribution and habitat.** This species is known only from Yamagata Prefecture. The specimens were collected from small mountain streams. Ovigerous females were collected from November to April.

**Remarks.** *Jesogammarus bousfieldi* resembles *J. paucisetulosus* closely in having 1) small eyes, 2) posterodistal corner of peduncular article 1 of antenna 1 without robust seta, 3) posterior margins of peduncular articles antennae 1 and 2 with many long setae, 4) outer margin of palp article 2 of maxilla 1 without setae, and 5) ventral margins of coxae of female gnathopods 1 and 2 with few setae. However, *J. bousfieldi* differs from *J. paucisetulosus* by the number of setae on the dorsal margins of pleonites 1–3: each pleonite with more than 4 setae in *J. bousfieldi* vs. 0–3 in *J. paucisetulosus*.

### *Jesogammarus* (*Jesogammarus*) *uchiyamaryui* sp. n.

http://zoobank.org/7E6DE7E7-4892-4526-B42B-A2414895D665
New Japanese name: Uchiyama-yokoebi
Figs 9–14

**Type materials.** Holotype: Male (10.3 mm), NSMT-Cr 25474, Tanie River (33°49′16.9″N, 129°44′0.4″E), Ashibe, Iki, Nagasaki Prefecture, Japan, 8 March 2012, collected by K. Tomikawa and S. Tashiro. Paratypes: 1 ovigerous female (9.2 mm), NSMT-Cr 25475, 1 male (12.1 mm), KUZ Z1803, 1 male (12.0 mm), KUZ Z1804, data same as for holotype; 1 male (8.6 mm), KUZ Z1805, 1 male (9.1 mm), KUZ Z1806, Mukata (32°42′54.7″N, 128°50′19.3″E), Goto, Nagasaki Prefecture, Japan, 15 December 2015, collected by K. Tomikawa and S. Tashiro.

**Type locality.** Japan, Nagasaki Prefecture: Iki, Ashibe, Tanie River.

**Description.** *Male* [NSMT-Cr 25474, 10.3 mm]. Head (Fig. 9) with short rostrum; ventral margin of lateral cephalic lobe weakly concave; antennal sinus rounded; eyes reniform, major axis 0.4 × height of head. Dorsal surfaces of pereonites smooth (Fig. 9). Dorsal margins of pleonites 1–3 (Fig. 10A–C) each with 2 setae. Posterior margin of epimeral plate 1 rounded with seta, posteroventral corner with seta, anteroventral to ventral margin with 7 setae (Fig. 10D); posterior margin of plate 2 weakly sinusoid with seta, posteroventral corner quadrate with seta, ventral margin and submargin with 3 and 2 robust setae, respectively (Fig. 10E); posterior margin of plate 3 slightly waved with seta, posteroventral corner weakly pointed with seta, anteroventral to ventral margin with 4 robust and 1 small setae (Fig. 10F). Urosomites 1–3 (Fig. 10G–I) with 10, 6, and 6 robust setae on dorsal margins.

Antenna 1 (Fig. 10J): length 0.6 × body length; peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.5; posterodistal corner of peduncular article 1 with robust seta, posterior margin of peduncular article 1 with single seta, posterior margin of peduncular article 2 with 1 cluster and 1 pair of setae, posterior margin of peduncular article 3 with pair of setae; accessory flagellum 5-articulate; primary flagellum 28-articulate, each article with 1 aesthetasc.

Antenna 2 (Fig. 10K): length 0.7 × antenna 1; posterior margin of peduncular article 4 with 2 clusters and 1 pair of setae, posterior margin of peduncular article 5 with 2 clusters of setae; flagellum 14-articulate, calceoli present (Fig. 10L).

Mouthparts. Upper lip (= labrum) (Fig. 10M) with rounded distal margin, bearing fine setae. Mandibles (Fig. 10N–P) with left and right incisors 5- and 4-dentate, respectively, left lacinia mobilis 5-dentate, right one bifid, bearing many teeth; molar process triturative, with plumose seta; accessory setal rows of left and right mandibles each with 6 blade-like setae; palp 3-articulate with length ratio of 1.0 : 2.3 : 2.0, palp article 1 bare, article 2 with 16 setae, article 3 with 1 cluster and 2 pairs of A-se-

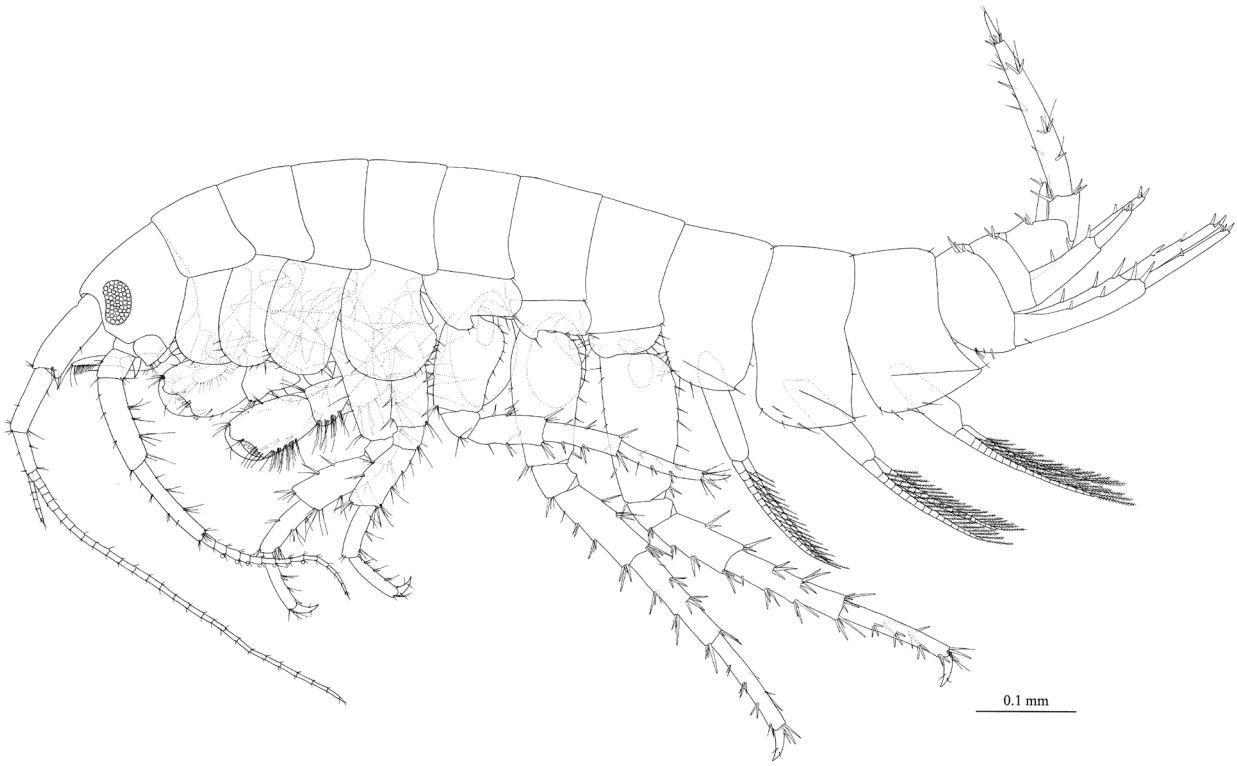


**Figure 9.** *Jesogammarus uchiyamaryui* sp. n., holotype, male (10.3 mm), NSMT-Cr 25474. Habitus, lateral view.

tae, single B-seta, and many C-, D-, and E-setae. Lower lip (= labium) (Fig. 10Q) with broad outer lobes, inner lobes indistinct. Maxilla 1 (Fig. 11A) with inner and outer plates and palp; medial margin of inner plate with 16 plumose setae 2 plumose setae, apical submargin with 4 setae; outer plate subrectangular, with 11 serrate teeth apically (Fig. 11B); palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 5 robust setae and 1 slender seta on its apical margin and 4 slender setae on its submargin, outer margin with 2 setae. Maxilla 2 (Fig. 11C) with oblique inner row of 16 plumose setae on inner plate; outer plate slightly longer than inner plate. Maxilliped (Fig. 11D) with inner and outer plates and palp; inner plate with 3 and 2 robust setae on apical and inner margins, respectively; outer plate with plumose setae on apical margin and robust setae on inner margin; palp 4-articulate, article 2 with inner marginal and submarginal rows of setae, article 3 with facial setae, article 4 slightly curved inward, with slender nail.

Gnathopod 1 (Fig. 11E): coxa with 12 setae on anterodistal to to posterodistal margin; anterior and posterior margins of basis with long setae; carpus length 1.5 × width, anterior margin with single seta; propodus length 1.3 × carpus and 1.4 × width, anterior margin with 2 clusters of setae and single seta, palmar margin oblique, weakly convex, with 15 peg-shaped robust setae (Fig. 11F); dactylus as long as palmar margin.

Gnathopod 2 (Fig. 11G): coxa with 8 marginal setae on anterodistal to posterodistal margin, medial surface with 2 setae; anterior and posterior margins of basis with long setae; carpus length 1.7 × width, anterior margin with 1 pair of setae; propodus length 1.1 × carpus and 1.5 × width, respectively, anterior margin with 1 cluster and 1 pair of setae, palmar margin oblique, weakly convex, with 14 peg-shaped robust setae (Fig. 11H); dactylus as long as palmar margin.

Pereopod 3 (Fig. 11I): coxa with 4 and 2 marginal setae on anterodistal and posterodistal parts, respectively; anterior and posterior margins of basis with long setae, anterodistal corner of basis without robust seta.

Pereopod 4 (Fig. 11J): coxa expanded with posterior concavity, bearing 2 setae on anterodistal corner and 4 setae on ventral margin; anterior and posterior margins of basis with long setae, anterodistal corner with robust seta.

Pereopod 5 (Fig. 12A): coxa bilobed, anterior lobe with 2 apical setae, ventral margin of posterior lobe with 2 setae, posterodistal corner not pointed with seta; posterior margin of basis weakly expanded, with 9 setae; anterior and posterior margins of merus to propodus with robust and slender setae.

Pereopod 6 (Fig. 12B): coxa bilobed, anterior lobe with apical seta, anterior margin with long setae, ventral margin of posterior lobe with 2 setae, posterodistal corner weakly pointed with seta; posterior margin of basis weakly expanded with 9 setae, posterodistal corner with robust seta; anterior and posterior margins of merus to propodus with robust and slender setae.

Pereopod 7 (Fig. 12C): ventral margin of coxa weakly concave, bearing 3 setae on anterior margin and 4 setae on posteroventral margin; posterior margin of basis

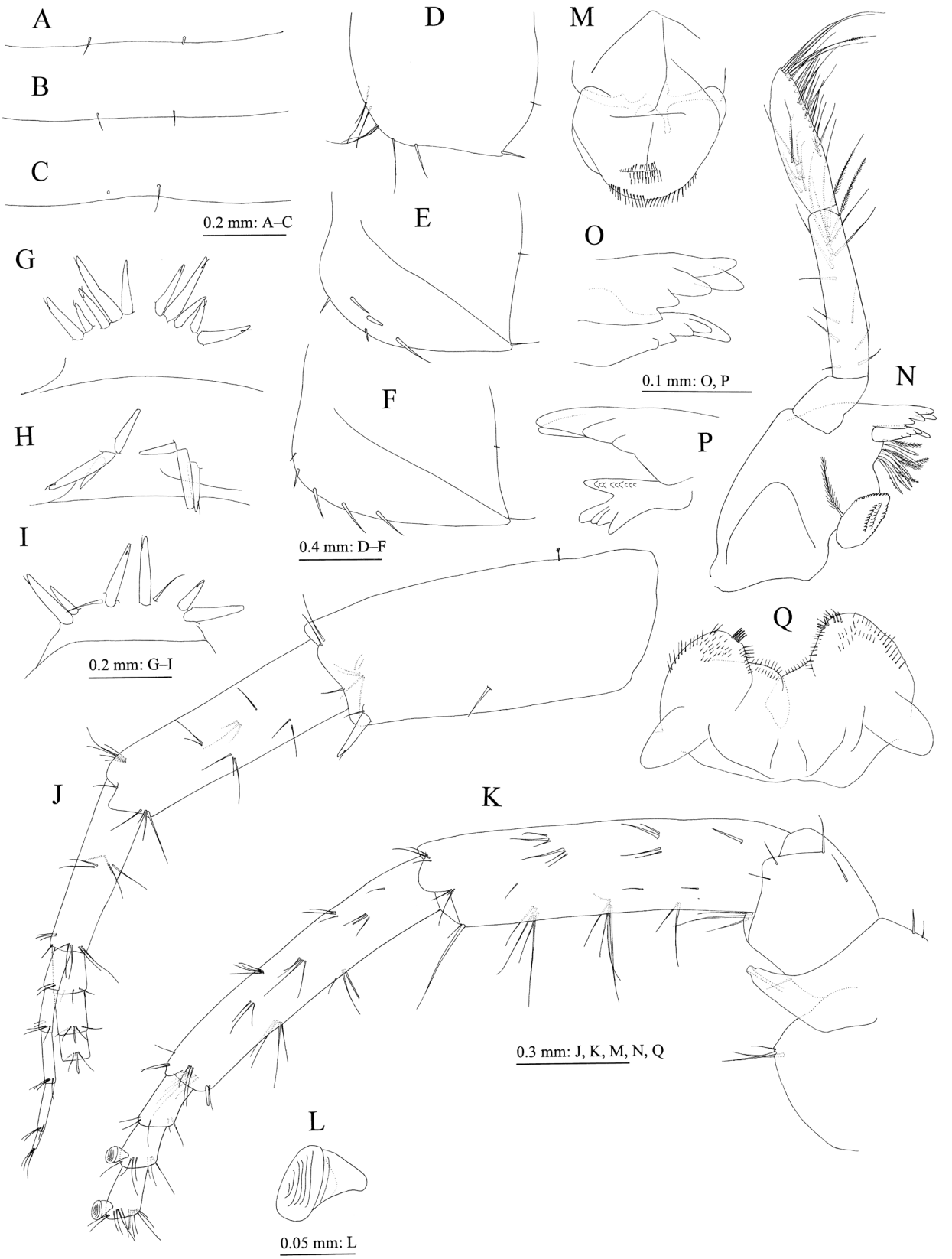


**Figure 10.** *Jesogammarus uchiyamaryui* sp. n., holotype, male (10.3 mm), NSMT-Cr 25474. **A–C** dorsal margins of pleonites 1–3, respectively, dorsal views; **D–F** epimeral plates 1–3, respectively, lateral views; **G–I** dorsal margins of urosomites 1–3, respectively, dorsal views; **J** peduncular articles 1–3, accessory flagellum, and flagellar articles 1–3 of antenna 1, medial view; **K** peduncular articles 1–5 and flagellar articles 1–3 of antenna 2, medial view; **L** calceolus of antenna 2, medial view; **M** upper lip, anterior view; **N** left mandible, medial view; **O** incisor and lacinia mobilis of left mandible, lateral view; **P** incisor and lacinia mobilis of right mandible, lateral view; **Q** lower lip, ventral view.

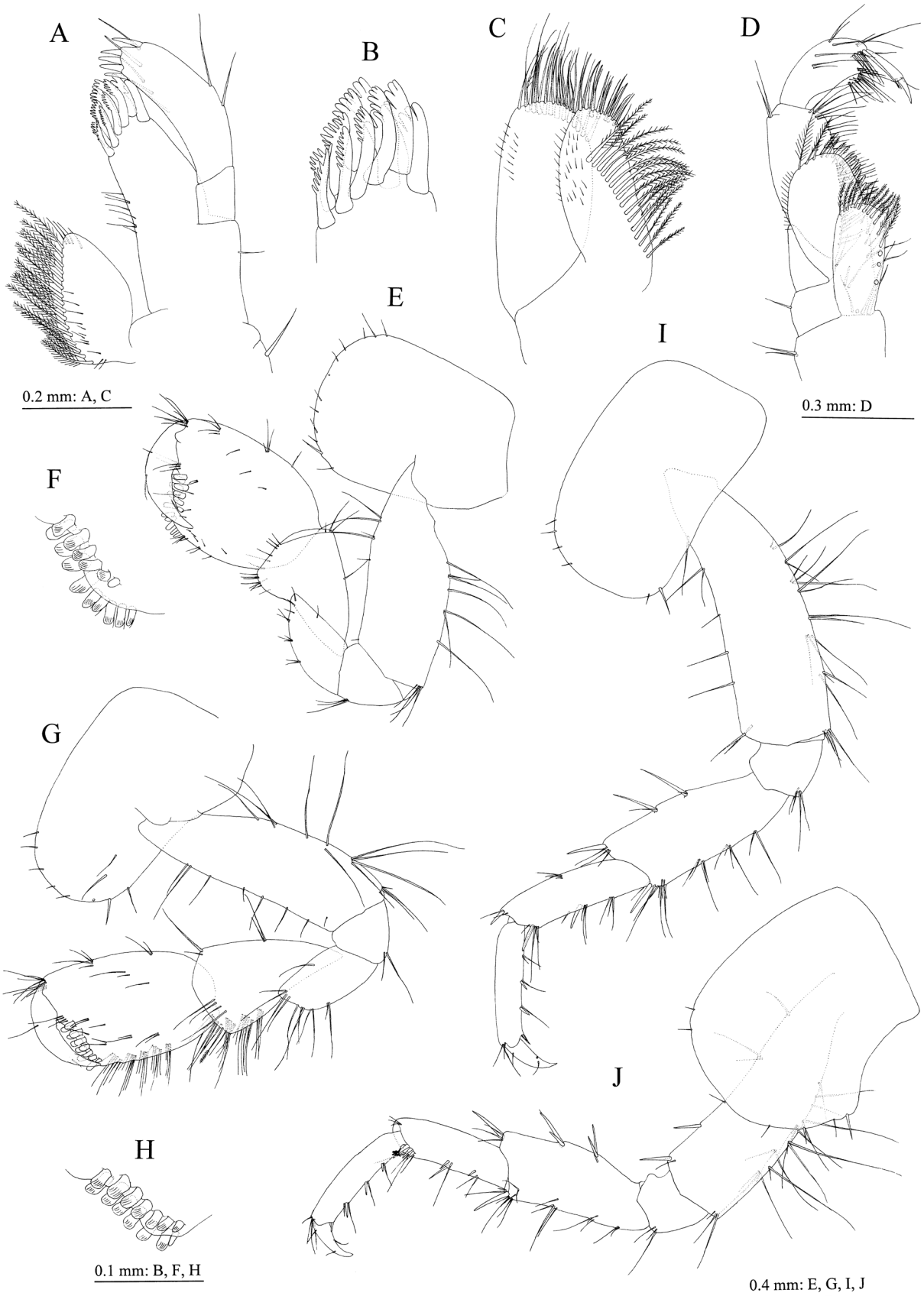


**Figure 11.** *Jesogammarus uchiyamaryui* sp. n., holotype, male (10.3 mm), NSMT-Cr 25474. **A** maxilla 1, dorsal view; **B** outer plate of maxilla 1, dorsal view; **C** maxilla 2, dorsal view; **D** maxilliped, dorsal view; **E** gnathopod 1, lateral view; **F** palmar margin of propodus of gnathopod 1, lateral view; **G** gnathopod 2, lateral view; **H** palmar margin of propodus of gnathopod 2, lateral view; **I** pereopod 3, lateral view; **J** pereopod 4, lateral view.

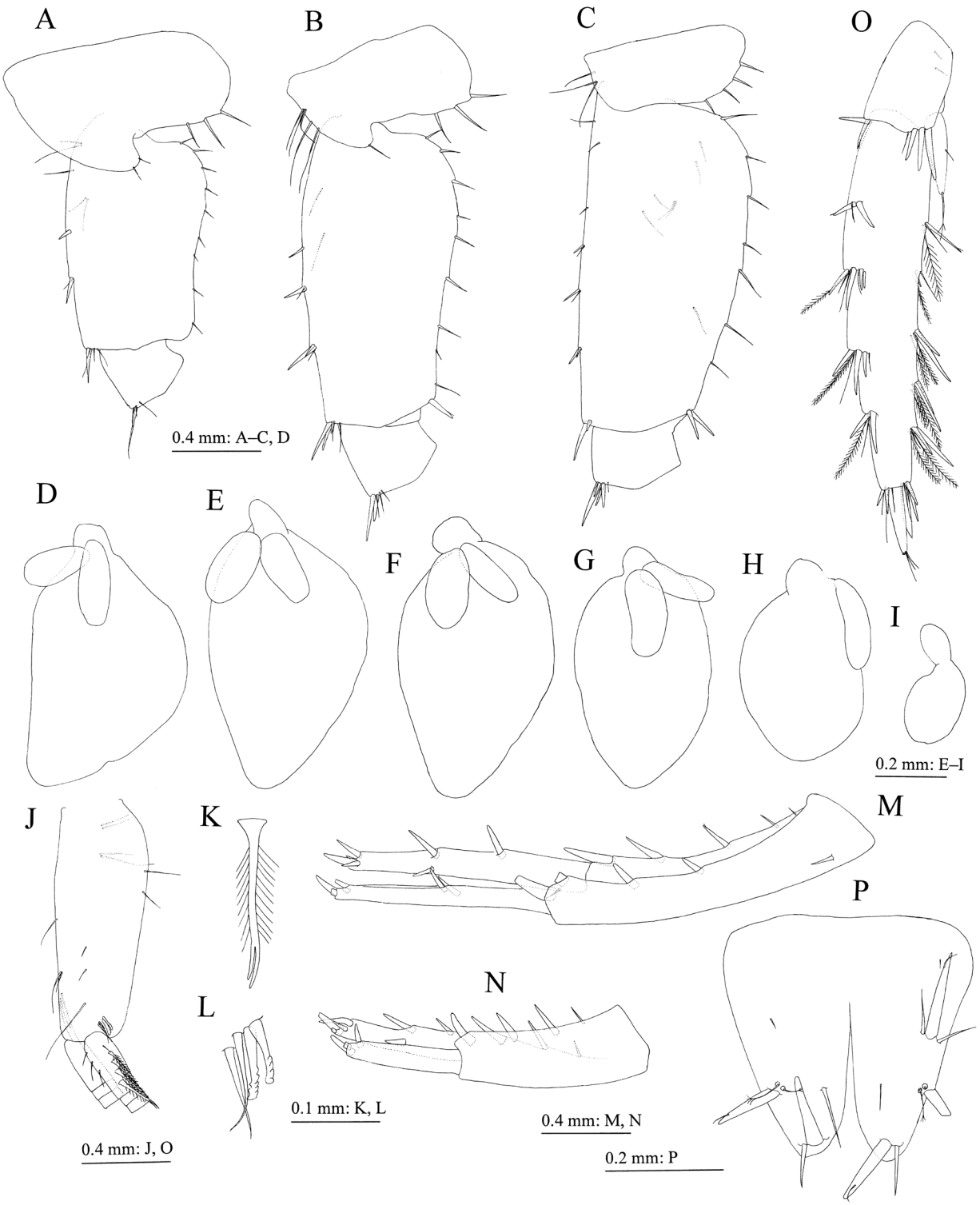


**Figure 12.** *Jesogammarus uchiyamaryui* sp. n., holotype, male (10.3 mm), NSMT-Cr 25474. **A** coxa–ischium of pereopod 5, lateral view; **B** coxa–ischium of pereopod 6, lateral view; **C** coxa–ischium of pereopod 7, lateral view; **D–I** coxal gills on gnathopod 2 and pereopods 3–7, lateral views; **J** pleopod 1, lateral view, distal parts of rami omitted; **K** bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of pleopod 1, lateral view; **L** retinacula on peduncle of pleopod 1, lateral view; **M** uropod 1, dorsal view; **N** uropod 2, dorsal view; **O** uropod 3, dorsal view; **P** telson, dorsal view.

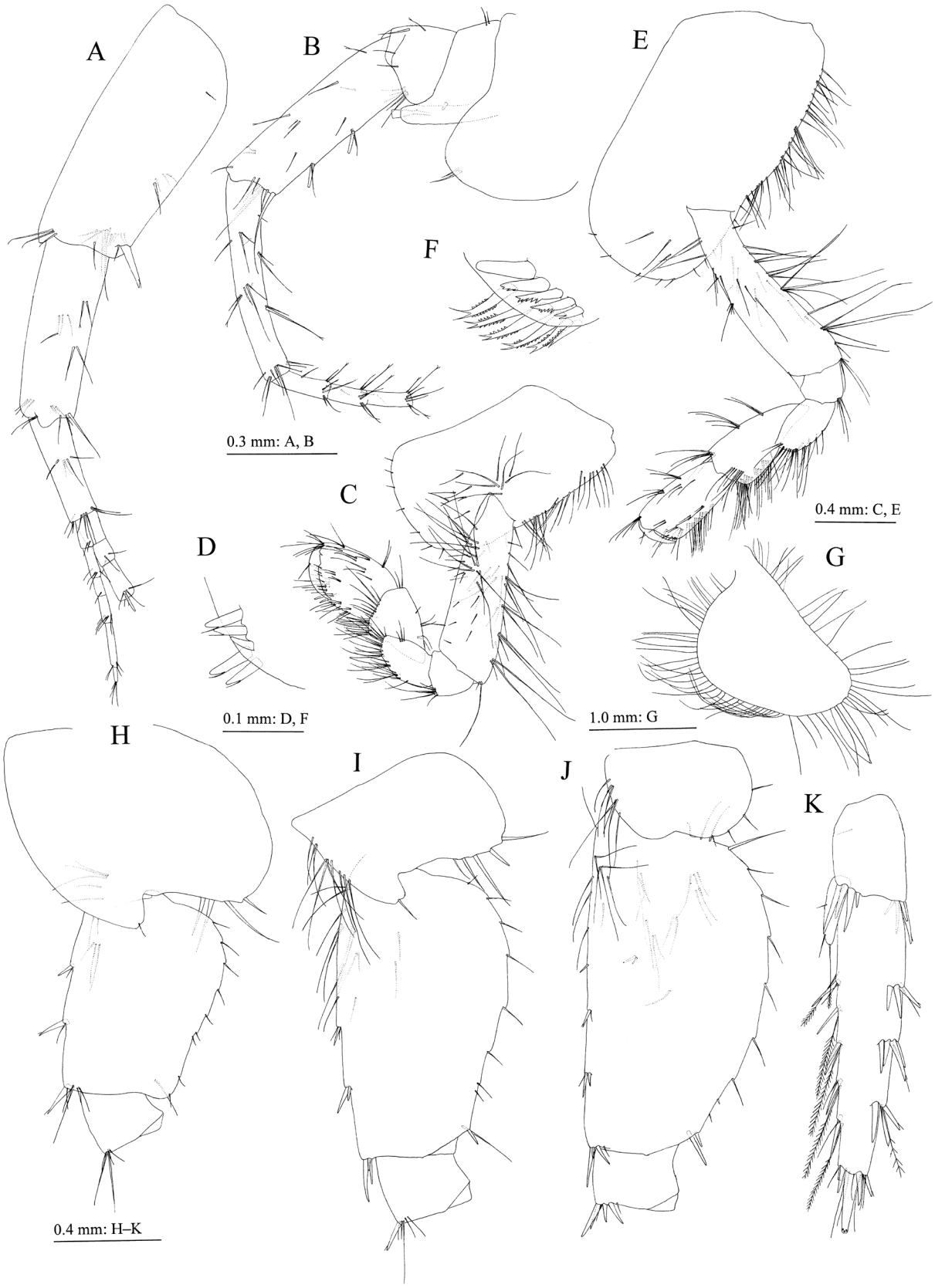


**Figure 13.** *Jesogammarus uchiyamaryui* sp. n., paratype, female (9.2 mm), NSMT-Cr 25475. **A** peduncular articles 1–3, accessory flagellum, and flagellar articles 1–3 of antenna 1, medial view; **B** peduncular articles 1–5 and flagellar articles 1–3 of antenna 2, medial view; **C** gnathopod 1, lateral view; **D** palmar margin of propodus of gnathopod 1, lateral view; **E** gnathopod 2, lateral view; **F** palmar margin of propodus of gnathopod 2, lateral view; **G** brood plate of gnathopod 2, lateral view; **H** coxa–ischium of pereopod 5, lateral view; **I** coxa–ischium of pereopod 6, lateral view; **J** coxa–ischium of pereopod 7, lateral view; **K** uropod 3, dorsal view.

**Figure 14.** *Jesogammarus uchiyamaryui* sp. n., not preserved, precopula pair (male: upper, female: lower). Photographed by Ryu Uchiyama.

weakly expanded with 7 setae, posterodistal corner with 2 robust setae; anterior and posterior margins of merus to propodus with robust and slender setae.

Coxal gills on gnathopod 2 and pereopods 3–5 (Fig. 12D–G) with 2 accessory lobes, both anterior and posterior lobes short, subequal, gills on pereopods 6 and 7 (Fig. 12H, I) each with 1 accessory lobe.

Pleopods 1–3 (Fig. 12J) each with paired retinacula (Fig. 12L) on inner margin of peduncle, and bifid plumose setae (= clothes-pin setae) (Fig. 12K) on inner basal margin of inner ramus.

Uropods. Uropod 1 (Fig. 12M): peduncle with robust seta on basofacial part, inner and outer margins with 4 and 2 setae, respectively, inner and outer distal corners with 1 and 2 robust setae, respectively; inner ramus length 0.7 × peduncle, inner margin with 2 robust setae and outer margin with 1 robust and 1 small setae; outer ramus length 0.9 × inner ramus, inner and outer margins each with robust seta. Uropod 2 (Fig. 12N): inner and outer margins of peduncle each with 2 setae, outer distal corner with robust seta; inner ramus length 0.7 × peduncle, its inner and outer margins each with robust seta; outer ramus length 0.9 × inner ramus, marginally bare. Uropod 3 (Fig. 12O): peduncle length 0.2 × outer ramus; inner ramus length 0.2 × outer ramus, with inner marginal seta and apical seta; outer ramus 2-articulate, inner and outer margins of article 1 each with 4 clusters of setae, some of which robust, both inner and outer margins with plumose setae, article 2 length 0.2 × article 1, with simple setae apically.

Telson (Fig. 12P) almost as long as wide, cleft for 63% of length; each lobe with 2 or 3 robust setae and slender setae.

*Female* [NSMT-Cr 25475, 9.2 mm]. Antenna 1 (Fig. 13A): peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.4; posterior margin of peduncular article 1 with 2 single setae, posterior margin of peduncular article 2 with 2 pairs of setae, posterior margin of peduncular article 3 with cluster of setae; accessory flagellum 6-articulate; primary flagellum 31-articulate.

Antenna 2 (Fig. 13B): posterior margin of peduncular article 4 with 2 clusters of setae, posterior margin of peduncular article 5 with 2 pairs of setae and single seta; flagellum 13-articulate, calceoli absent.

Gnathopod 1 (Fig. 13C): posterior margin of coxa with many setae; carpus length 1.4 × width; propodus length 1.2 × merus and 1.6 × width, respectively; palmar margin (Fig. 13D) with 5 robust setae.

Gnathopod 2 (Fig. 13E): posteroproximal part of coxa with numerous setae; carpus length 2.2 × width;

propodus length 0.9 × merus and 2.1 × width, respectively; palmar margin (Fig. 13F) with 1 robust and 10 pectinate robust setae.

Posterior margin of bases of pereopods 5–7 more expanded than in male (Fig. 13H–J).

Brood plates (Fig. 13G): broad, with numerous marginal setae.

Uropod 3 (Fig. 13K): peduncle length 0.3 × outer ramus; inner ramus length 0.2 × outer ramus; inner and outer margins of article 1 of outer ramus with 5 and 3 clusters/pairs of setae, article 2 length 0.2 × article 1.

Egg number 154.

**Etymology.** The specific name honors Mr Ryu Uchiyama (nature photographer), who provided many photos of living amphipods throughout KT's amphipodological study.

**Distribution and habitat.** This species is known from Iki and Fukue Islands, Nagasaki Prefecture. The specimens were collected from river mouths subject to tidal action. An ovigerous female was collected in March.

**Remarks.** *Jesogammarus uchiyamaryui* is morphologically similar to *J. ikiensis* Tomikawa, 2015 in having 1) dorsal margin of pereonites 5–7 without setae, 2) a few (<4) setae on dorsal margin of pleonites 1–3, 3) large eyes, robust seta on posterodistal corner of peduncular article 1 of antenna 1, and 4) mandibular palp article 1 without robust setae. However, *J. uchiyamaryui* differs from *J. ikiensis* by the following features (features of *J. ikiensis* in parentheses): 1) posterior margin of peduncular article 2 of antenna 1 with two (three or four) setae, 2) accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 short and straight (long and curved), 3) ventral margins of coxae of female gnathopods 1 and 2 and pereopod 3 with numerous long setae (a few short setae), and 4) inner ramus of uropod 3 shorter than 0.2 (0.2–0.3) times as long as outer ramus. *Jesogammarus uchiyamaryui* is also similar to *J. spinopalpus* Morino, 1985 in having 1) short accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 and 2) densely setose ventral margins of coxae of female gnathopods 1 and 2. However, the former differs from the latter by the following features (features of *J. spinopalpus* in parentheses): 1) eyes large (small), 2) dorsal margins of pleonites 1–3 each with two setae (numerous), 3) the mandibular palp article 1 without robust setae (present), 4) inner ramus of uropod 3 shorter than 0.2 times as long as outer ramus (longer than 0.3), and 5) posterior margin of bases of female pereopods 5–7 with short (long) setae.

### Molecular phylogenies

The BI tree (mean ln $L = -8918.44$; Fig. 15) had an almost identical topology to that of the ML tree (ln $L = -9156.46$; not shown). The monophyly of the genus *Jesogammarus* was well-supported (BS = 100%, PP = 1.0). The genus *Jesogammarus* consisted of three monophyletic lineages: *J. uchiyamaryui* lineage (BS = 100%, PP = 1.0), the subgenus *Annanogammarus* lineage (BS = 100%, PP = 1.0), and the subgenus *Jesogammarus* lineage (BS = 99%, PP = 1.0). However, our phylogenetic analyses failed to resolve precise the phylogenetic relationships among these three lineages.

The monophyly of the four species within the *Annanogammarus* lineage inhabiting Japan was well supported (BS = 94%, PP = 1.0). The *Jesogammarus* lineage comprised four subclades; however, the detailed phylogenetic relationships among these four lineages remain unresolved. The first subclade (BS = 100%, PP = 1.0) contains the Chinese *J. hebeiensis* and *J. spinopalpus* from the Boso Peninsula on Honshu, Japan (locality #12 in Fig. 1). The second subclade (BS = 91%, PP = 0.99) comprised *J. hinumensis* Morino, 1993 inhabiting brackish habitats of Japan and *J. ikiensis* from Iki Island. The third lineage (BS = 100%, PP = 1.0) included Japanese *J. mikadoi* Tomikawa, Morino & Mawatari, 2003 and four species defined as the *J. jesoensis* complex by Tomikawa et al (2016): the monophyly of the *J. jesoensis* complex was well-supported (BS = 97%, PP = 1.0). *Jesogammarus paucistulosus* and *J. bousfieldi* formed the last subclade (BS = 98%, PP = 1.0). *J. paucistulosus* and *J. bousfieldi* formed a well-supported clade (BS = 100%, PP = 1.0; respectively). The *Jesogammarus paucistulosus* specimens were divided into two lineages: individuals collected from the southern part of Ibaraki Prefecture (#10, 11) formed a well-supported lineage (BS = 100%, PP = 1.0), and the remainder inhabiting the northern part of Ibaraki (#8) formed the other well-supported clade (BS = 100%, PP = 1.0). The *J. bousfieldi* specimens were also split into two lineages. Individuals from Mamurogawa (#3) formed a well-supported clade (BS = 100%, PP = 1.0), while the other amphipods inhabiting the southern part of Yamagata Prefecture (#5, 6) grouped with a monophyletic lineage (BS = 100%, PP = 0.99).

## Discussion

The species diversity of *Jesogammarus* inhabiting Japan has been extensively investigated (Tattersall 1922, Schellenberg 1937, Bousfield 1979, Morino 1984, 1985, 1986, 1993, Tomikawa and Morino 2003, Tomikawa et al. 2003, Tomikawa 2015). However, the present findings of the two new *Jesogammarus* species collected from Japan suggest that the true species diversity of the Japanese *Jesogammarus* remains unknown.

The freshwater *J. bousfieldi* described from Yamagata Prefecture has been treated as a population of *J. paucisetulosus*, which was thought to be widely distributed in Ibaraki, Yamagata and Niigata Prefectures (Tomikawa 2007, Tomikawa and Morino 2012). The latter species was originally described from a spring brooklet in Mito, Ibaraki Prefecture, Japan (Morino 1984). The *J. bousfieldi* specimens were clearly distinguished from those of *J. paucisetulosus* collected from Ibaraki Prefecture by the number of marginal setae on dorsal margins of pleonites 1–3. Additionally, our molecular phylogenetic analy-

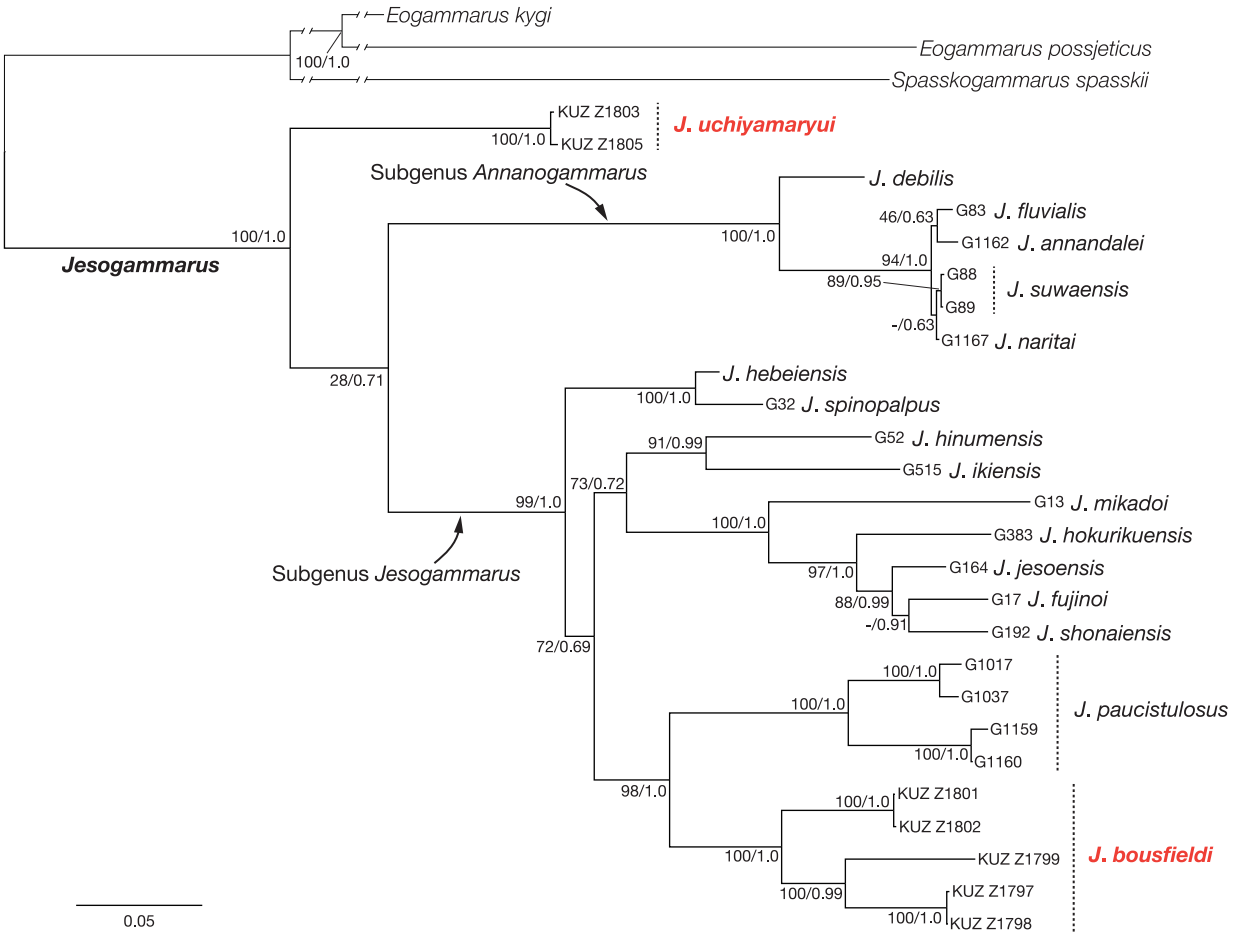


**Figure 15.** Bayesian inference tree for 1,499 bp of nuclear 28S rRNA and mitochondrial COI and 16S rRNA markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities.

ses showed that *J. bousfieldi* was highly diverged from the true *J. paucistulosus* although they possessed sister-species relationships. Their genetic divergence could be comparable to the detected intraspecific divergences among other *Jesogammarus* species. Thus, we concluded that *J. bousfieldi* could be regarded as a distinct new species. Unfortunately, we could not examine specimens of *J. paucisetulosus* from Niigata Prefecture. A further taxonomic study is necessary to determine the taxonomic position of the population living there.

Contrary to the highly diverged freshwater *Jesogammarus* species, the number of known species indigenous to brackish habitats is limited: only *J. hinumensis* has been recorded from brackish lakes and estuaries in Japan (Morino 1993). Therefore, *Jesogammarus uchiyamaryui* is the second brackish-water species in this genus. This species is morphologically similar to the freshwater *J. ikiensis*. Although both species are distributed on Iki Island (Tomikawa 2015), they were not collected from the same localities or at the same time on the island (Tomikawa, personal observation). The habitat preferences of these two species seem to be completely different. Moreover, the phylogeny showed that *J. uchiyamaryui* was not a sister species of *J. ikiensis*, indicating that they did not share the last common ancestor. Because species diversity of the brackish *Jesogammarus* remains far from investigated, it is highly possible that additional undescribed species may be found in brackish-water environments within the distributional range of the genus *Jesogammarus*.

The morphological characteristics and the phylogenetic positions of the two new species raises a question about the validity of the two subgenera, *Jesogammarus* and *Annanogammarus*, under the genus *Jesogammarus*. These two subgenera are characterized only by two morphological characters: 1) accessory lobes of coxal gills of gnathopod 2–pereopod 5 subequal in length or posterior accessory lobe longer than anterior accessory lobe in the subgenus *Jesogammarus* vs. unequal in length, posterior accessory lobe often rudimentary in the subgenus *Annanogammarus*; and 2) palmar margin of propodus of female gnathopod 2 with pectinate robust setae in the subgenus *Jesogammarus* vs. without pectinate robust setae in the subgenus *Annanogammarus*. *Jesogammarus bousfieldi* and *J. uchiyamaryui* bear these subgeneric diagnostic characteristics that are identified with the subgenus *Jesogammarus*. Contrary to the monophyly of *Annanogammarus* implicated by previous phylogenetic studies (Tomikawa et al. 2007, To-

mikawa 2015), the phylogenies obtained here failed to recover the monophyly of the subgenus *Jesogammarus*. Although the precise phylogenetic position of *J. uchiyamaryui* remains uncertain, the findings highlight that the subgeneric classification under the genus *Jesogammarus* may be abrogated along with synonymizing *Annanogammarus* with *Jesogammarus*. Nevertheless, a future systematic study is essential to reveal whether *Annanogammarus* should be treated as a subjective junior synonym of *Jesogammarus*.

**Key to species of *Jesogammarus***

1 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; palmar margin of propodus of female gnathopod 2 with pectinate setae ..... 2 (subgenus *Jesogammarus*)
– Accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 weakly developed, anterior and posterior lobes unequal in length, often posterior lobe rudimentary; palmar margin of propodus of female gnathopod 2 without pectinate setae ..... 12 (subgenus *Annanogammarus*)
2 Article 1 of mandibular palp with setae ..... 3
– Article 1 of mandibular palp without setae ..... 6
3 Dorsal margin of pleonites 1–3 each with 1–2 setae; eye large; article 1 of mandibular palp with 1 robust seta; female pereopods densely setose ..... *J. hinumensis* Morino, 1993
– Dorsal margin of pleonites 1–3 each with more than 4 setae; eye small to medium; article 1 of mandibular palp with 2 or 3 robust setae; female pereopods not densely setose ..... 4
4 Peduncular article 1 of antenna 1 with robust seta on posterodistal corner ..... *J. spinopalpus* Morino, 1985
– Peduncular article 1 of antenna 1 with slender seta on posterodistal corner ..... 5
5 Inner ramus of uropod 3 length 1/4 × outer ramus; inner margin of outer ramus of uropod 3 with 4–6 plumose setae ..... *J. fontanus* Hou & Li, 2004
– Inner ramus of uropod 3 length 1/3 × outer ramus; inner margin of outer ramus of uropod 3 with about 10 plumose setae ..... *J. hebeiensis* Hou & Li, 2004
6 Dorsal margin of pereonites 1–3 each with 2 long setae ..... *J. mikadoi* Tomikawa, Morino & Mawatari, 2003
– Dorsal margin of pereonites 1–3 without setae ..... 7
7 Posterodistal corner of peduncular article 2 of antenna 1 without robust seta; posterior margin of peduncular article 2 of antenna 1 with more than 5 setae and/or setal bundles; outer margin of palp article 2 of maxilla 1 without setae ... 8
– Posterodistal corner of peduncular article 2 of antenna 1 with robust seta (occasionally lacking); posterior margin of peduncular article 2 of antenna 1 with less than 4 setae and/or setal bundles; outer margin of palp article 2 of maxilla 1 with setae ..... 9
8 Dorsal margins of pleonites 1–3 each with more than 4 setae ..... *J. bousfieldi* sp. n.
– Dorsal margins of pleonites 1–3 each with 0–3 setae ..... *J. paucisetulosus* Morino, 1984
9 Accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 short and straight ..... *J. uchiyamaryui* sp. n.
– Accessory lobes of coxal gills on gnathopod 2 and pereopods 3–5 long and curved ..... 10
10 Dorsal margins of pleonites 1–3 each with 2 or 3 setae; posterior margin of peduncular article 2 of antenna 1 with 3 or 4 setae and/or setal bundls ..... *J. ikiensis* Tomikawa, 2015
– Dorsal margins of pleonites 1–3 each with more than 7 setae; posterior margin of peduncular article 2 of antenna 1 with 2 setae and/or setal bundls ..... 11
11 Palmar margin of propodus of male gnathopod 2 without pectinate setae ..... *J. jesoensis* complex
– Palmar margin of propodus of male gnathopod 2 with pectinate setae ..... *J. ilhoii* Lee & Seo, 1992
12 Dorsal margin of pleonite 3 with robust setae; posterior margin of peduncular article 4 and 5 with more than 5 long-setal bundles ..... *J. naritai* Morino, 1985
– Dorsal margin of pleonite 3 without robust setae; posterior margin of peduncular article 4 and 5 with less than 3 short-setal bundles ..... 13
13 Posterodistal corner of bases of pereopods 5–7 with long setae ..... *J. annandalei* (Tattersal, 1922)
– Posterodistal corner of bases of pereopods 5–7 without long setae ..... 14
14 Dorsal margins of pleonites 1–3 each with 2–4 setae ..... *J. fluvialis* Morino, 1985
– Dorsal margins of pleonites 1–3 each with more than 10 setae ..... 15
15 Posterodistal corner of peduncular article 1 of antenna 1 with robust seta; palmar margin of propodus of female gnathopod 2 with simple setae only ..... *J. koreanus* Lee & Seo, 1990
– Posterodistal corner of peduncular article 1 of antenna 1 without robust seta; palmar margin of propodus of female gnathopod 2 with weakly pectinate setae ..... *J. debilis* Hou & Li, 2005

## Acknowledgements

The authors are grateful to Dr Ronald Vonk (Naturalis) and Dr Michael Ohl (Museum für Naturkunde) for their critical comments on this manuscript. We thank Dr Hiroshi Morino (NSMT) for providing *J. paucisetulosus* specimens, Noriyoshi Shimura for providing the *J. uchiyamaryui* specimens, and Tomoko Hirata (Hiroshima University) for supporting the molecular phylogenetic analyses. Thanks are also due to Ryu Uchiyama for providing photographs of live specimens. KT thanks Satoko Tashiro for supporting the field work. This study was partly supported by JSPS KAKENHI Grant Numbers JP25242015, JP25840140, and JP15J00720. The open access publication of this manuscript was supported by the Museum für Naturkunde, Berlin.

## References

Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723. https://doi.org/10.1109/TAC.1974.1100705

Bousfield EL (1979) The amphipod superfamily Gammaroidea in the northeastern Pacific region: systematics and distributional ecology. Bulletin of the Biological Society of Washington 3: 297–357.

Bulycheva AI (1952) Novye vidy bokoplavov (Amphipoda, Gammaridea) iz Japonskogo Morja. Akademija Nauk SSSR, Trudy Zoologicheskogo Instituta 12: 195–250.

Derzhavin AN (1923) Malacostraca der Susswasser-Gewasser von Kamtschatka. Russkii Gidrobiologicheskii Zhurnal 2: 180–194.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.2307/2408678

Hou Z, Fu J, Li S (2007) A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. Molecular Phylogenetics and Evolution 45: 596–611. https://doi.org/10.1016/j.ympev.2007.06.006

Hou Z, Li S (2004) Two new freshwater species of the genus *Jesogammarus* (Crustacea: Amphipoda: Anisogammaridae) from China. The Raffles Bulletin of Zoology 52: 455–466.

Hou Z, Li S (2005) Amphipod crustaceans (Gammaridea) from Beijing, P. R. China. Journal of Natural History 39: 3255–3274. https://doi.org/10.1080/00222930500289590

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Lee KS, Seo IS (1990) One new species of freshwater *Jesogammarus* (Crustacea, Amphiposa, Anisogammaridae) from South Korea. Korean Journal of Systematic Zoology 6: 251–260.

Lee KS, Seo IS (1992) One new species of freshwater *Jesogammarus* (Crustacea, Amphiposa, Anisogammaridae) from South Korea. Korean Journal of Zoology 35: 344–349.

Macdonald III KS, Yampolsky L, Duffy JE (2005) Molecular and morphological evolution of the amphipod radiation of Lake Baikal. Molecular Phylogenetics and Evolution 35: 323–343. https://doi.org/10.1016/j.ympev.2005.01.013

Morino H (1984) On a new freshwater species of Anisogammaridae (Gammaroidea: Amphipoda) from central Japan. Publications of the Itako Hydrobiological Station 1: 17–23.

Morino H (1985) Revisional studies on *Jesogammarus–Annanogammarus* group (Amphipoda: Gammaroidea) with descriptions of four new species from Japan. Publications of the Itako Hydrobiological Station 2: 9–55.

Morino H (1986) A new species of the subgenus *Annanogammarus* (Amphipoda: Anisogammaridae) from Lake Suwa, Japan. Publications of the Itako Hydrobiological Station 3: 1–11.

Morino H (1993) A new species of the genus *Jesogammarus* (Amphipoda: Anisogammaridae) from Brackshi waters of Japan. Publications of the Itako Hydrobiological Station 6: 9–16.

Palumbi SR (1996) Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (Eds) Molecular Systematics. Second Edition. Sinauer Associates, Inc., Sunderland, 205–247.

Rambaut A, Drummond AJ (2013) Tracer v. 1.6. http://tree.bio.ed.ac.uk/software/tracer/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Schellenberg A (1937) Schlüssel und diagnosen der dem süßwasser *Gammarus* nahestehenden einheiten ausschließlich der arten des Baikalsees und Australiens. Zoologischer Anzeiger 117: 267–280.

Schwarz G (1978) Estimating the dimension of a model. The Annals of Statistics 6: 461–464. https://doi.org/10.1214/aos/1176344136

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stock JH (1974) The systematics of certain Ponto-Caspian Gammaridae (Crustacea, Amphipoda). Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 70: 75–95.

Tanabe AS (2008) Phylogears v. 2.0.2014.03.08. http://www.fifthdimension.jp/

Tattersall WM (1922) Zoological results of a tour in the Far East. Part 8. Amphipoda with notes on an additional species of Isopoda. Memoirs of the Asiatic Society of Bengal 6: 435–459.

Tomikawa K (2007) Taxonomy and phylogeny of the genus *Jesogammarus* (Crustacea: Amphipoda: Anisogammaridae). Bulletion of the Graduate School of Education, Hiroshima University, Part 2, 56: 23–29.

Tomikawa K (2015) A new species of *Jesogammarus* from the Iki Island, Japan (Crustacea, Amphipoda, Anisogammaridae). ZooKeys 530: 15–36. https://doi.org/10.3897/zookeys.530.6063

Tomikawa K, Kobayashi N, Kyono M, Ishimaru S-i, Grygier MJ (2014) Description of a new species of *Sternomoera* (Crustacea: Amphipoda: Pontogeneiidae) from Japan, with an analysis of the phylogenetic relationships among the Japanese species based on the 28S rRNA gene. Zoological Science 31: 475–490. https://doi.org/10.2108/zs140026

Tomikawa K, Kobayashi N, Morino H, Hou Z, Mawatari SF (2007) Phylogenetic relationships within the genus *Jesogammarus* (Crustacea, Amphipoda, Anisogammaridae) deduced from mitochondrial

COI and 12S sequences. Zoological Science 24: 173–180. https://doi.org/10.2108/zsj.24.173

Tomikawa K, Morino H (2003) Two new freshwater species of the genus *Jesogammarus* (Crustacea: Amphipoda: Anisogammaridae) from northern Japan. Zoological Science 20: 229–241. https://doi.org/10.2108/zsj.20.229

Tomikawa, K, Morino H (2012) An annotatied inventory with a key of freshwater Amphipoda (Crustacea) from Japan. Taxa, Proceedings of the Japanese Society of Systematic Zoology 32: 39–51.

Tomikawa K, Morino H, Mawatari SF (2003) A new freshwater species of the genus *Jesogammarus* (Crustacea: Amphipoda: Anisogammaridae) from northern Japan. Zoological Science 20: 925–933. https://doi.org/10.2108/zsj.20.925

Tomikawa K, Tashiro S, Kobayashi N (2012) First record of *Gammarus koreanus* (Crustacea, Amphipoda, Gammaroidea) from Japan, based on morphology and 28S rRNA gene sequences. Species Diversity 17: 39–48. https://doi.org/10.12782/sd.17.1.039

Tzvetkova NL (1967) K faune ekologii bokoplavov (Amphipoda, Gammaridea) Zaliva Poc'et (Japonskoe More). Akademija Nauk SSSR, Zoologicheskii Institut, Issledovanija Fauny Morei 5: 160–195.