Two new subterranean species of Pseudocrangonyx Akatsuka & Komai, 1922 (Amphipoda: Crangonyctoidea: Pseudocrangonyctidae), with an insight into groundwater faunal relationships in western Japan

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Two new subterranean species of *Pseudocrangonyx* Akatsuka & Komai, 1922 (Amphipoda: Crangonyctoidea: Pseudocrangonyctidae), with an insight into groundwater faunal relationships in western Japan

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ABSTRACT

Amphipods belonging to the crangonyctoid genus *Pseudocrangonyx* Akatsuka & Komai, 1922 constitute a major component of the subterranean environments in east Asia. The true species diversity of this group has been unsettled due to the lack of molecular data for *P. shikokunis* Akatsuka & Komai, 1922 and *P. kyotonis* Akatsuka & Komai, 1922 and the taxonomic status of the misidentified populations of these two species. The status of the misidentified populations is herein clarified. Morphological comparisons among the specimens of these populations and the name-bearing types of *P. shikokunis* and *P. kyotonis* demonstrate the two are distinctive species. Phylogenetic analyses using partial sequences of nuclear 28S rRNA and histone H3, mitochondrial cytochrome *c* oxidase subunit I, and 16S rRNA genes also confirm that each of the two populations represents a unique clade within the species of *Pseudocrangonyx*. Accordingly, the population indigenous to the limestone caves in western Japan, which was previously identified as *P. shikokunis*, is described as *P. akatsukai* **n. sp.**, and that reported as *P. kyotonis* from central Japan is described as *P. komaii* **n. sp.** The phylogenetic relationships within *P. akatsukai* **n. sp.** and an unidentified *Pseudocrangonyx* species elucidate the complex stygofaunal relationships in western Japan (western Honshu, Shikoku, and Kyushu). A key to *Pseudocrangonyx* species is also provided.

Key Words: molecular phylogeny, systematics, stygobitic fauna

INTRODUCTION

Crangonyctoid amphipods constitute an important component of Holarctic subterranean habitats (Holsinger, 1993, 1994), with western Eurasian *Niphargus* Schiödte, 1849 and North American *Crangonyx* Bate, 1859 being highly diversified. In eastern Asia, amphipods that belong to *Pseudocrangonyx* Akatsuka & Komai, 1922 are one of the stygobitic groups indigenous to groundwater environments in this region (Holsinger, 1994). In contrast to *Niphargus* and *Crangonyx*, which comprise approximately 300 and 50 species, respectively (Zhang & Holsinger, 2003; Hekmatara *et al.*, 2013), *Pseudocrangonyx* so far contains only 23 species, six of them recorded from the Japanese Archipelago (Uéno, 1966; Narahara *et al.*, 2009; Tomikawa *et al.*, 2016).

A molecular phylogenetic study by Tomikawa *et al.* (2016) revealed that the true species diversity of *Pseudocrangonyx* from Japan remains elusive, recognizing at least six unidentified species. Tomikawa *et al.* (2016) also showed that the several records of species of *Pseudocrangonyx* from non-type localities in Japan (e.g., Uéno, 1927; Nunomura, 1975) were based on misidentified specimens and highlighted that the systematic status of the unidentified species of *Pseudocrangonyx* should be clarified by using both morphological and molecular data.

Our understanding of the taxonomy of *Pseudocrangonyx* has been hampered by a lack of the molecular data of the true *P. shikokunis* Akatsuka & Komai, 1922 and *P. kyotonis* Akatsuka & Komai, 1922, which were originally described along with the genus. Topotypic specimens of *P. shikokunis* and *P. kyotonis* have not yet been collected. Although Tomikawa *et al.* (2016) speculated that the unidentified *Pseudocrangonyx* spp. 4 and 5 might comprise *P. shikokunis* and/or *P. kyotonis*, the phylogroup consisted of deeply diverged clades, which were discordant with the morphological characters defined by the type specimens of *P. shikokunis* and *P. kyotonis*. Moreover, the group identified as *Pseudocrangonyx* sp. 5 contained a specimen of *P. coreanus* Uéno, 1966 (Narahara *et al.*, 2009).

The taxonomies of *P. shikokunis* and *P. kyotonis* have also complicated by misidentified records. Tomikawa *et al.* (2016) revealed that the population inhabiting Akiyoshi limestone caves identified as *P. shikokunis* (Uéno, 1927) and from Gifu reported as *P. kyotonis* (Nunomura, 1975) clearly represent *Pseudocrangonyx* spp. 2 and 6, respectively. We therefore establish the taxonomic status of these two unidentified lineages.

The molecular phylogenies in Tomikawa *et al.* (2016) also revealed that the species of *Pseudocrangonyx* that inhabit the Japanese Archipelago do not form a monophyletic group. *Pseudocrangonyx elegantulus* Hou in Zhao & Hou (2017) from Henan, China, *P. daejeoensis* Lee, Tomikawa, Nakano & Min, 2018 from the Korean Peninsula support a complex biogeographical history of *Pseudocrangonyx* in continental Asia and the Japanese Archipelago. The present molecular phylogenetic trees based on an updated dataset, which includes newly collected specimens, elucidates the biogeographical relationships of the species of *Pseudocrangonyx* from western Japan.

MATERIALS AND METHODS

Sampling and morphological observation

Specimens of species of *Pseudocrangonyx* were collected from a cave each in Gifu, Okayama, and Kumamoto prefectures and two caves in Yamaguchi Prefecture, Japan. The geographical coordinates for all cave entrances were obtained using a Garmin eTrex® GPS unit (Garmin, Olathe, KS, USA). Specimens for molecular analyses were also newly collected from two locations, a well in Takarazuka, Hyogo Prefecture (~34.8861°N, ~135.3067°E) and Hakiai-syonyudo Cave in Kumamoto Prefecture (32.41456°N, 130.86549°E). Amphipods inhabiting caves were collected by scooping groundwater environments with a fine-mesh hand net and fixed in 99% ethanol on-site. All appendages of the specimens of the undescribed species were dissected in 70% ethanol and mounted in gum-chloral medium on glass slides under an Olympus SZX7 stereomicroscope (Olympus, Tokyo, Japan). Specimens were examined using a Nikon Eclipse Ni light microscope (Nikon, Tokyo, Japan) 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 examined are deposited in the Zoological Collection of Kyoto University (KUZ).

The type specimens of *P. coreanus*, *P. kyotonis*, and *P. shikokunis* deposited at the National Museum of Nature and Science, Tsukuba (NSMT), were examined: paratypes of *P. coreanus*, female 3.3 mm, NSMT-Cr 13521, and female 3.0 mm, NSMT-Cr 13522, Seongnam-dong, Chungju, South Korea; holotype of *P. kyotonis*, female 11.0 mm, NSMT-Cr 13500, Kyoto, Kyoto Prefecture, Honshu, Japan; and syntypes of *P shikokunis*, male 7.0 mm, NSMT-Cr 13501, and female 8.2 mm, NSMT-Cr 13502, both from Tomioka, Tokushima Prefecture, Shikoku, Japan.

PCR, DNA sequencing, and molecular phylogenetic analyses

Genomic DNA was extracted from appendage muscles following Tomikawa *et al.* (2014). Primer sets for the polymerase chain reaction (PCR) and cycle sequencing reaction (CS) for the nuclear 28S rDNA (28S), histone H3 (H3), and the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rDNA (16S) follow Tomikawa *et al.* (2016). The PCR and CS reactions and DNA sequencing were performed using a modified version of a method described by Tomikawa *et al.* (2016) using a T-100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The obtained sequences were assembled using DNA BASER (Heracle Biosoft, Piteşti, Romania). In total, 12 sequences from the three *Pseudocrangonyx* specimens were obtained and deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through DNA Data Bank of Japan (Supplementary material Table S1).

The phylogenetic relationships of the species studied were estimated based on 28S, H3, COI, and 16S sequences. The dataset was identical to that used by Tomikawa *et al.* (2016) with the addition of the two sequences obtained from the type material of *P. elegantulus* (Zhao & Hou, 2017), four sequences from the holotype of *P. daejeoensis* (Lee *et al.*, 2018), and the newly obtained 12 sequences (Supplementary material Table S1). The alignments of H3 and COI were trivial, as no indels were observed. The sequences of 28S and 16S were aligned using MAFFT v7.312 (Katoh & Standley, 2013). The lengths of the 28S, H3, COI, and 16S sequences were 1360, 328, 658, and 432 bp, respectively. The concatenated sequences yielded 2778 bp of aligned positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The ML phylogeny was reconstructed using RAxML v8.2.8 (Stamatakis, 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (BS) was conducted with 1000 replicates. The best-fit partition scheme was identified with the Akaike information criterion using PartitionFinder v2.1.2 (Lanfear *et al.*, 2017) with the "greedy" algorithm (Lanfear *et al.*, 2012): 28S/ H3 1st and 2nd positions/H3 3rd position/COI 1st position/COI 2nd position/COI 3rd position/16S.

BI and Bayesian posterior probabilities (PPs) were estimated using MrBayes v3.2.6 (Ronquist *et al.*, 2012). The best-fit partition scheme and models for each partition were selected with the Bayesian information criterion using PartitionFinder with the "greedy" algorithm: for 28S GTR+I+G; for H3 1st and 2nd positions and COI 2nd position, K80+I; for H3 3rd position, SYM+G; for COI 1st position, SYM+I+G; for COI 3rd position, GTR+I+G; and for 16S, GTR+I+G. Two independent runs for four Markov chains were conducted for 20 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v1.6.0 (http://tree.bio.ed.ac.uk/software/tracer/), and the first 50001 trees were discarded based on the results.

SYSTEMATICS

Family Pseudocrangonyctidae Holsinger, 1989

Genus *Pseudocrangonyx* Akatsuka & Komai, 1922

***Pseudocrangonyx akatsukai* n. sp.**

(Figs. 1A, 2–5)

Pseudocrangonyx shikokunis – Uéno, 1927: 361, fig. 4. — Torii, 1955: 423.

Pseudocrangonyx sp. 2 – Tomikawa *et al.*, 2016: fig. 10. — Lee *et al.*, 2018: fig. 10.

Type material: Holotype female (10.2 mm), KUZ Z1980, Taishodo Cave (34.27694°N, 131.32056°E), Mine, Yamaguchi Prefecture, Japan, 6 June 2015, collected by K.

Tomikawa, T. Nakano, and S. Tashiro. Paratypes: 1 female (9.6 mm), KUZ Z1968, 1 male (7.7 mm), KUZ Z1981, 1 female (8.7 mm), KUZ Z1982, 1 male (8.3 mm), KUZ Z1983, data same as for holotype; 1 female (7.7 mm), KUZ Z1967, 1 male (6.3 mm), KUZ Z1984, 1 female (6.8 mm), KUZ Z1985, Akiyoshido Cave (34.23333°N, 131.30528°E), date and collectors same as for holotype; 1 female (9.0 mm), KUZ Z1972, 1 male (7.1 mm), KUZ Z1986, 1 female (8.5 mm), KUZ Z1987, Uyamado Cave (34.94250°N, 133.57583°E), Niimi, Okayama Prefecture, Japan, 30 July 2015, collected by K. Tomikawa and S. Tashiro; 1 female (6.5 mm), KUZ Z1953, Gongen-shonyudo Cave (32.41402°N, 130.40839°E), Kamiamakusa, Kumamoto Prefecture, 22 October 2017, collected by K. Tomikawa and T. Nakano.

Diagnosis: Antennal sinus with rounded angle; eyes absent; pereonites 1–7 with short dorsal setae; urosomite 1 with ventral robust seta; dorsal margin of urosomite 3 lacking setae; sternal gill absent; antenna 1 reaching 0.55–0.73× body length; antenna 2 with calceoli in both sexes; mandible palp article 3 longer than article 2; maxilla 1 inner plate with 4–6 setae; maxilla 2 inner plate with oblique inner row of 4–6 setae; gnathopods 1, 2, carpi with serrate setae on posterodistal corners in both sexes; palmar margins of propodi of gnathopods 1, 2 with 9–11, 8–9 robust setae, respectively; pleopod peduncles with marginal setae, inner margin of inner rami with bifid setae; uropod 1 inner ramus 1.7× outer ramus length; inner, outer margins of inner ramus with 2 or 3, 1 or 2 robust setae, respectively; basal part with 1 or 2 slender setae, outer ramus with 1 or 2 marginal robust setae; uropod 2 inner ramus 1.4–1.5× outer ramus length; inner, outer margins with 3, 2 robust setae, respectively; outer ramus with 2 marginal robust setae; uropod 3 terminal article 0.1–0.2× length of proximal article; telson 1.1–1.3× long as wide, cleft for 6.6–12.3%.

Description: Female (KUZ Z1980, 10.2 mm). Head (Fig. 1A) with short dorsal setae; rostrum reduced; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes absent. Pereonites 1–7 with short dorsal setae (Fig. 1A); posterolateral margin of pereonites 5–7 with 1, 1, 4 setae, respectively (Fig. 1A). Dorsal margin of pleonites 1–3 with 14, 14, 19 setae, respectively (Fig. 2A–C). Posterior margin of epimeral plate 1 with 7 setae, posteroventral corner not produced with seta (Fig. 2D); ventral, posterior margins of plate 2 with 4 robust setae, 6 setae, respectively, posteroventral corner not produced, with 2 setae (Fig. 2E); ventral, posterior margins of plate 3 with 4 robust setae, 3 setae, respectively, posteroventral corner rounded, with seta (Fig. 2F). Ventral margin of urosomites 1 with robust seta (Fig. 1); dorsal margin of urosomites 1, 2 with 9, 8 setae, respectively (Fig. 2G, H), dorsal margin of urosomite 3 lacking setae (Fig. 2I).

Antenna 1 (Fig. 2J) 0.66× body length, length ratio of peduncular articles 1–3 1.0:0.9:0.5; accessory flagellum (Fig. 2K) 2-articulate, terminal article with 3 setae, 1 aesthetasc; primary flagellum 21-articulate, aesthetasc on some articles (Fig. 2L). Antenna 2 (Fig. 2M) 0.55× antenna 1 length; peduncular article 5 with 3 calceoli (Fig. 2N); flagellum 0.50× length peduncular articles 4, 5 combined, consisting of 7 articles, first 5 with calceolus.

Upper lip (labrum) (Fig. 2O) with rounded anterior margin, with fine setae. Mandibles (Fig. 2P–R) with left, right incisors 5-dentate; left lacinia mobilis 5-dentate, right lacinia bifid, with many teeth; molar process triturative, molar of right mandible with accessory seta; accessory setal rows of left, right mandibles with 8, 4 weakly pectinate setae, respectively; palp 3-articulate, article 3 longer than article 2 with 3 A-setae, about 17 D-setae, about 8 E-setae. Lower lip (Fig. 2S) with broad outer lobes, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 3A, B) with inner, outer plates, palp; inner plate subquadrate, medial margin with 6 plumose setae; outer plate subrectangular with 7 serrate teeth apically (Fig. 3B); palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 5 apical robust setae, 6 subapical slender setae. Maxilla 2 (Fig. 3C) with oblique inner row of 5 plumose setae plus simple seta on inner plate. Maxilliped (Fig. 3D) with inner, outer plates, palp; inner plate (Fig. 3E) with 5 apical, 2 subapical robust setae; outer plate with 4 apical plumose setae, 8 robust, some slender setae on medial margin; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 3F, G) with subquadrate coxa bearing setae on anterior to ventral margins of coxa, width 1.6× long as depth; anterior margin of basis bare, posterior margin of basis with many setae; posterodistal corner of carpus with 5 serrate setae (Fig. 3H); propodus stout, subtriangular, palmar margin with 11 robust setae in 2 rows, some distally notched (Fig. 3G); posterior margin of dactylus dentate (Fig. 3G). Gnathopod 2 (Fig. 3I, J) with rounded coxa bearing setae on its anterior margin, posterodistal corner, width 1.3× depth; basis with setae on anterodistal submargin, posterior margin; posterodistal corner of carpus with 4 serrate setae (Fig. 3K); propodus slender than that of gnathopod 1, with 9 robust setae along palmar margin in 2 rows, some distally notched (Fig. 3J); posterior margin of dactylus dentate (Fig. 3J). Pereopod 3 (Fig. 4A, B) with subquadrate coxa bearing setae on anterodistal, posteroventral corners, width 1.2× depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:0.9:0.9; posterior margin of dactylus with 2 setae (Fig. 4B). Pereopod 4 (Fig. 4C, D) with coxa bearing setae on anterodistal, posteroventral corners, width 1.5× depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:0.9:0.9; posterior margin of dactylus with 2 setae (Fig. 4D). Pereopod 5 (Fig. 4E–G) with weakly bilobed coxa bearing setae on anterior, posterior lobes; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:0.9:0.9; anterior margin of propodus with long setae (Fig. 4F); anterior margin of dactylus with 2 setae (Fig. 4G). Pereopod 6 (Fig. 4H, I) with coxa bearing concave lower margin, anterodistal, posteroproximal corners with setae; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:1.0:0.9; anterior margin of dactylus with 3 setae (Fig. 4I). Pereopod 7 (Fig. 4J, K) with coxa bearing shallowly concave lower margin, posteroproximal corner of coxa with seta; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:1.1:1.1; posterior margin of dactylus with 3 setae (Fig. 4K).

Coxal gills (Figs. 2I, 3A, C, E, H) on gnathopod 2, pereopods 3–6; sternal gills absent. Brood plates (Figs. 3I, 4A, C, E) slender on gnathopod 2, pereopods 3–5.

Peduncle of pleopod 1 (Fig. 5A) with seta on outer margins; peduncles of pleopods 2, 3 (Fig. 5D, E) lacking marginal setae. Pleopods 1–3 each with paired retinacula (Fig. 5B), bifid seta (clothes-pin seta; Fig. 5C) on inner basal margin of inner ramus.

Uropod 1 (Fig. 5F) with basofacial robust seta on peduncle; peduncle 1.3× longer than inner ramus; inner ramus 1.7× outer ramus length, inner, outer margins of inner ramus with 3, robust setae, respectively, basal part with 2 slender setae; outer ramus with marginal robust seta. Uropod 2 (Fig. 5G) with peduncle 0.9× longer than inner ramus; inner ramus 1.5× longer than outer ramus, inner, outer margins with 3, 2 robust setae, respectively; outer ramus with 2 marginal robust setae. Uropod 3 (Fig. 5H, I) with peduncle 0.3× outer ramus length; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.1× proximal article length, with 3 distal setae (Fig. 5I).

Telson (Fig. 5J) length 1.1× width, cleft for 9.2% of length, each telson lobe with 2 lateral, long penicillate setae, apical robust seta, subapical slender seta, apical short penicillate seta.

Male (KUZ Z1981, 7.7 mm). Antenna 1 (Fig. 5K, L) 0.62× body length, primary flagellum 19-articulate. Antenna 2 (Fig. 5M, N) 0.63× antenna 1 length, peduncular article 5 with calceoli; flagellum 0.53× length of peduncular articles 4, 5 combined, 8-articulate, articles 2–5 each with calceolus.

Gnathopod 1 carpus with 3–5 serrate setae on posterodistal corner; palmar margin of propodus with 9 robust setae in 2 rows, some distally notched (Fig. 5O). Gnathopod 2 carpus bearing 3 or 4 serrate setae on posterodistal corner; palmar margin of propodus with 8 robust setae in 2 rows, some distally notched (Fig. 5P).

Uropod 1 (Fig. 5Q) with peduncle 1.4× inner ramus length; inner, outer margins of inner ramus each with 2 robust setae, basal part with slender seta; outer ramus with 2 marginal robust setae. Uropod 2 (Fig. 5R) with peduncle almost as long as inner ramus; inner ramus 1.4× outer ramus length, distal part with 6 serrate, 4 simple robust setae, penicillate seta (Fig. 5S). Uropod 3 (Fig. 5H, I) with outer ramus terminal article 0.2× proximal article length. Telson length 1.2× width, cleft for 6.6% of length.

Variation: Antenna 1 length 0.55 (female 6.5 mm, KUZ Z1953) to 0.73× (male 6.3 mm, KUZ Z1984, male 7.1 mm, KUZ Z1986) body length; primary flagellar articles of male 7.1 mm (KUZ Z1986), each with 1 or 2 aesthetascs. Antenna 2 length up to 0.66× antenna 1 length (female 6.5 mm, KUZ Z1953). Maxilla 1 medial margin of inner plate with 4 (female 6.5 mm, KUZ Z1953), 5 (male 7.1 mm, KUZ Z1986, female 8.5 mm, KUZ Z1987) setae. Maxilla 2 inner plate with oblique inner row of 4 (female 6.5 mm, KUZ Z1953), 5 (males 7.7, 7.1 mm, KUZ Z1981, Z1986) setae. Peduncles of pleopods 2, 3 of specimen from Kumamoto (KUZ Z1953) with marginal setae. Telson length 1.3× width (male 7.1 mm, KUZ Z1986, female 6.8 mm, KUZ Z1985), cleft for 7.3 (female 6.8 mm, KUZ Z1985) to 12.3% (male 7.1 mm, KUZ Z1986).

Etymology: The species name is a noun in the genitive case debased on the name of the late Dr. Kozo Akatsuka, who the first studied the taxonomy of *Pseudocrangonyx*.

New Japanese name: Akatsuka-mekurayokoebi.

Distribution and habitat: The species is indigenous to the montane caves of Chugoku Mountains in western Honshu, Japan. It also inhabits the limestone cave in Kamishima Island in the Amakusa Islands off western Kyushu, Japan. Individuals were collected from small streams in the caves.

Remarks: *Pseudocrangonyx akatsukai* **n. sp.** is most similar to *P. shikokunis* described from Shikoku Island, Japan. Both species have eyes that are absent; mandible and palp of article 3 is longer than article 2; inner plate of maxilla 1 with more than four setae; inner plate of maxilla 2 with an oblique inner row of more than four setae; carpi of gnathopods 1 and 2 with serrate setae on the posterodistal corners; peduncles of pleopods with marginal setae and the inner margin of the inner rami with bifid setae; and telson, distally concave. The new species can nevertheless be differentiated from *P. shikokunis* by the armature of the urosomite 1, presence of ventral robust seta, and a shorter telson, 1.1–1.3 (*versus* 1.5) times its width.

Pseudocrangonyx akatsukai **n. sp.** is similar to *P. kyotonis* and *P. elegantulus* in all lacking eyes, article 3 off the mandibular palp is longer than article 2, and presence of serrate setae on the posterodistal corners of the carpi of female gnathopods 1 and 2 (Akatsuka & Komai, 1922; Zhao & Hou, 2017). The new species differs from *P.*

kyotonis in having a longer antenna 1, which is 0.55–0.73 (*versus* 0.39) times as long as body length, and more setose inner plate of the maxilla 1, having 4–6 (*versus* 3) medial setae. The new species differs from *P. elegantulus* in having serrate setae on the posterodistal corner of the carpus of male gnathopod 2 (none in *P. elegantulus*), marginal setae on the pleopod 1 peduncle (none in *P. elegantulus*), and the telson cleft is up to 12.3% (*versus* 27%) of its length.

Nomenclatural statement: A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank.org:pub:

***Pseudocrangonyx komaii* n. sp.**

(Figs. 1B, 6–10)

Pseudocrangonyx kyotonis – Nunomura, 1975: 11.

Pseudocrangonyx sp. 6 – Tomikawa *et al*., 2016: fig. 10. — Lee *et al*., 2018: fig. 10.

Type material: Holotype male (5.8 mm), KUZ Z1988, Miyama-shonyudo Cave (35.74889°N, 137.02472°E), Miyama, Gujohachiman, Gifu Prefecture, Japan, 18 October 2015, collected by K. Tomikawa and S. Tashiro. Paratypes: 5 females (5.5 mm, 4.2 mm, 5.1 mm, 4.6 mm, 4.0 mm), KUZ Z1976, Z1977, Z1989, Z1990, Z1991, data same as for holotype.

Diagnosis: Antennal sinus with rounded angle; eyes absent; pereonites 1–7 with short dorsal setae; urosomite 1 without ventral robust seta; dorsal margin of urosomite 3 lacking setae; sternal gill absent; antenna 1 0.45–0.51× body length; female antenna 2 with calceoli; mandible palp article 3 almost as long as article 2; maxilla 1 inner plate with 4 setae; maxilla 2 inner plate with oblique inner row of 5 setae; gnathopods 1, 2 carpi without serrate setae on posterodistal corners; palmar margins of propodi of gnathopods 1,2 with 13–21,14–18 robust setae, respectively; pleopods, peduncles lacking marginal setae, inner margin of inner rami without bifid setae; uropod 1 inner ramus 1.4× outer ramus length; inner, outer margins of uropod 1 inner ramus with 2 or 3, 0 or 1 robust setae, respectively, basal part with 1 or 2 slender setae, outer ramus with marginal robust seta; uropod 2 inner ramus 1.4–1.6× outer ramus length, inner, outer margins with 3, 2 robust setae, respectively; outer ramus with 1 or 2 marginal robust setae; uropod 3 terminal article 0.1× proximal article length; telson length 1.3× width, cleft for 6.8–10.2%.

Description: Male (KUZ Z1988, 5.8 mm). Head (Fig. 1B) with short dorsal setae; rostrum reduced; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes absent. Pereonites 1–7 with short dorsal setae (Fig. 1B); posterolateral margin of pereonites 5–7 with 1, 1, 3 setae, respectively (Fig. 1B). Dorsal margin of pleonites 1–3 with 10, 12, 11 setae, respectively (Fig. 6A–C). Posterior margin of epimeral plate 1 with 4 setae, posteroventral corner not produced, with seta (Fig. 6D); ventral, posterior margins of plate 2 with 2 robust setae, 4 setae, respectively, posteroventral corner not produced, with seta (Fig. 6E); ventral, posterior margins of plate 3 with 2 robust setae, 5 setae, respectively, posteroventral corner rounded, with seta (Fig. 6F). Ventral margin of urosomites 1 without setae (Fig. 1B); dorsal margin of urosomites 1, 2 with 4 slender, 6 robust setae, respectively (Fig. 7G, H), dorsal margin of urosomite 3 lacking setae (Fig. 6I).

Antenna 1 (Fig. 6J) 0.45× body length, length ratio of peduncular articles 1–3 1.0:0.7:0.4; accessory flagellum (Fig. 6K) 2-articulate, terminal article with 3 setae, 1 aesthetasc; primary flagellum 13-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 6L) 0.67× antenna 1 length; peduncular article 5 with 1 calceolus (Fig. 6M); flagellum 0.58× length of peduncular articles 4, 5 combined, consisting of 7 articles, first 4 with calceolus.

Upper lip (Fig. 6N) with rounded anterior margin bearing fine setae. Mandibles (Fig. 6O–Q) with left, right incisors 5-dentate; left lacinia mobilis 5-dentate, right lacinia bifid, with many teeth; molar process triturative, molar of right mandible with accessory seta; accessory setal rows of left, right mandibles with 4, 3 weakly pectinate setae, respectively; palp 3-articulate, article 3 almost as long as article 2, with 3 A-setae, about 10 D-setae, about 5 E-setae. Lower lip (Fig. 6R) with broad outer lobes, mandibular process of outer lobe apically rounded; inner lobes indistinct. Maxilla 1 (Fig. 7A, B) with inner, outer plates, palp; inner plate subquadrate, medial margin with 4 plumose setae; outer plate subrectangular with 7 serrate teeth apically (Fig. 7B); palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 3 robust setae, slender seta apically, robust seta plus slender seta subapically. Maxilla 2 (Fig. 7C) with oblique inner row of 5 plumose setae on inner plate. Maxilliped (Fig. 7D, E) with inner, outer plates, palp; inner plate (Fig. 7E) with 3 apical, 2 subapical robust setae; outer plate with 4 apical plumose setae, 3 robust, some slender setae on medial margin; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 7F, G) with subquadrate coxa bearing setae on anterodistal corner of coxa, width 1.8× depth; anterior margin of basis bare, posterior margin of basis with 6 setae; posterodistal corner of carpus without serrate setae; propodus stout, ovate, palmar margin with 10 lateral, 11 medial robust setae, some distally notched (Fig. 7G); posterior margin of dactylus dentate (Fig. 7G). Gnathopod 2 (Fig. 7H, I) with subquadrate coxa bearing setae on anterodistal, posteroventral corners, width 1.5× depth; basis with setae on anterodistal submargin, posterior margin; posterodistal corner of carpus without serrate setae; propodus more slender than propodus of gnathopod 1, with 7 lateral, 11 medial robust setae along palmar margin, some distally notched (Fig. 7I); posterior margin of dactylus dentate (Fig. 7I). Pereopod 3 (Fig. 7J, K) with subquadrate coxa bearing setae on anterodistal, posteroventral corners, width 1.6× depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:0.8:0.8; posterior margin of dactylus with 2 setae (Fig. 7K). Pereopod 4 (Fig. 8A, B) with coxa bearing setae on anterodistal, posteroventral corners, ventral margin, width 1.8× depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus 1.0:0.9:0.8; posterior margin of dactylus with 2 setae (Fig. 8B). Pereopod 5 (Fig. 8C, D) with weakly bilobed coxa, bearing setae on anterior, posterior lobes; anterior, posterior margins of basis with setae; ratio of merus, carpus, propodus 1.0:0.7:0.9; anterior margin of dactylus with 2 setae (Fig. 8D). Pereopod 6 (Fig. 8E, F) with coxa bearing concave lower margin, posteroproximal corner with seta; anterior, posterior margins of basis with setae; ratio of merus, carpus, propodus 1.0:0.8:0.9; anterior margin of dactylus with 2 setae (Fig. 8F). Pereopod 7 (Fig. 8G, H) with coxa bearing shallowly concave lower margin, posteroproximal corner of coxa with seta; anterior, posterior margins of basis with setae; ratio of merus, carpus, propodus 1.0:0.9:1.0; posterior margin of dactylus with seta (Fig. 8H).

Coxal gills (Figs. 7H, J, 8A, C, E) on gnathopod 2, pereopods 3–6; sternal gills absent.

Peduncles of pleopods 1–3 (Fig. 9A, C, D) lacking marginal setae, each with paired retinacula (Fig. 9B); inner basal margin of inner ramus without bifid setae.

Uropod 1 (Fig. 9E) with basofacial robust seta on peduncle; peduncle 1.3× inner ramus length; inner ramus 1.4× outer ramus length, inner, outer margins of inner ramus with 3 setae, robust seta, respectively, basal part with slender seta; outer ramus with marginal robust seta. Uropod 2 (Fig. 9F) with peduncle 0.8× inner ramus length; inner ramus 1.4× outer ramus length, inner, outer margins with 3, 2 weakly serrate robust setae, respectively, distal part with 4 serrate, 2 simple robust setae; outer ramus with 2 marginal robust setae, distal part with serrate seta plus 4 simple robust setae. Uropod 3 (Fig. 9G, H) with peduncle 0.3× outer ramus length; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.1× proximal article length, with 3 distal setae (Fig. 9H).

Telson (Fig. 9I) 1.3× longer than wide, cleft for 6.8% of length, each telson lobe with 2 lateral long penicillate setae, 2 apical robust setae, apical slender seta.

Female (KUZ Z1989, 5.1 mm). Antenna 1 (Fig. 10A, B) 0.51× body length, primary flagellum 14-articulate. Antenna 2 (Fig. 10C) 0.73× antenna 1 length, peduncular article 5 with 2 calceoli; flagellum 0.54× length of peduncular articles 4, 5 combined, 7-articulate, articles 1–4 each with calceolus. Mandibular article 3 1.1× article 2 length.

Gnathopod 1 with 6 lateral, 7 medial robust setae on palmar margin (Fig. 10D). Gnathopod 2 with 6 lateral, 8 medial robust setae on palmar margin (Fig. 10E).

Brood plates slender, on gnathopod 2, pereopods 3–5.

Uropod 1 (Fig. 10F) with basofacial slender seta on peduncle; inner ramus with 2 marginal robust setae, basal part with 2 slender setae; outer ramus with marginal robust seta. Uropod 2 (Fig. 10G) with peduncle 0.9× inner ramus length; inner ramus 1.6× outer ramus length, inner, outer margins with 3, 2 robust setae, respectively, distal part with 6 simple robust setae, short seta; outer ramus with marginal robust seta, distal part with 5 simple robust setae. Uropod 3 (Fig. 10H, I) with fewer robust setae on proximal article of outer ramus than in male.

Etymology: The specific name is a noun in the genitive case formed from the name of the late Professor Taku Komai, who established the genus *Pseudocrangonyx*.

New Japanese name: *Komai-mekurayokoebi*.

Distribution and habitat: Known only from its type locality in Gujohachiman, Gifu Prefecture. Specimens were collected from a small stream in the cave.

Remarks: *Pseudocrangonyx komaii* **n. sp.** resembles *P*. *kyotonis* in having a head without eyes, short antenna 1 that is less than half of body length, and bifid setae on the inner rami of pleopods. The new species can be clearly distinguished from *P*. *kyotonis* by the presence (absent *in P*. *kyotonis*) of calceoli on female antenna 2, the mandibular palp of article 3 is equal in length to article 2 (*versus* longer than article 2 in *P*. *kyotonis*), and posterodistal corners of female gnathopods carpi lacking serrate setae (present *in P*. *kyotonis*).

Pseudocrangonyx komaii **n. sp.** is similar to *P. coreanus* and *P. febras* Sidorov, 2009 from Russia in lacking eyes, presence of ventral setae on urosomite 1, serrate setae on the posterodistal corner of gnathopod 1 carpus in females, bifid setae on the inner rami of pleopods, and in having a distally concaved telson (Uéno, 1966; Sidorov, 2009). The new is distinguished from *P. coreanus* by the number of robust setae on the palmar margin of the gnathopod propodus, more than 20 (*versus* less than 10) in male gnathopod 1, more than 10 (*versus* less than 10) in female gnathopod 1, more than 10 (*versus* less than 10) in gnathopod 2, and absence (*present in P. coreanus*) of marginal setae on pleopod 1 peduncle. The new species differs from *P. febras* by distinct (*versus* indistinct) antennal sinus, a shorter antenna 1 that is 0.6 times shorter than body length (*versus* 0.7 times longer), absence of serrate setae on the posterodistal corner of the gnathopod 2 carpus of females, and the outer margin of uropod 1 inner ramus with 0 or 1 (*versus* three) robust setae.

Molecular phylogenies

The obtained BI tree (mean ln-Likelihood [L] = −15264.629; Fig. 11A) showed an almost identical topology to that of the ML tree (ln L = −15778.578; not shown). The results of the present analyses are generally concordant to those in Tomikawa *et al.* (2016), Zhao & Hou, (2017), and Lee *et al.* (2018). The trees failed to determine the precise phylogenetic position of *P. komaii* **n. sp.** within the genus *Pseudocrangonyx*.

The monophyly of *P. tiunovi* (Russia) + *P. korkishkoorum* (Russia) + *P. elegantulus* (China) + *P. yezonis* (Japan) + *P. akatsukai* **n. sp.** was well supported in both analyses (BS = 97%, PP = 0.99). This clade was split into three sub-clades, while their relationships remain uncertain. The monophyly of *P. elegantulus* and *P. yezonis* was recovered (BS = 95%, PP = 0.99). The Russian *P. tiunovi* and *P. korkishkoorum* formed a monophyletic group with high-support values (BS = 100, PP = 0.99). The specimens identified as *P. akatsukai* **n. sp.** formed a well-supported monophyletic lineage (BS = 99%, PP = 1.0). The Russian clade and *P. akatsukai* **n. sp.** formed a clade in ML analyses, but this relationship was not fully supported (BS = 65%). The obtained phylogenies failed to reconstruct the robust relationships among *P. akatsukai* **n. sp.** specimens.

Both of the newly added OTUs collected from Hyogo (KUZ Z1979; locality 19 in Fig. 11B) and Kumamoto (KUZ Z1952; locality 26) belonged to the clade comprising specimens tentatively identified as *Pseudocrangonyx* sp. 5 (BS = 97%. PP = 1.0). The

Kumamoto specimen was sister to the lineage consisting of the other individuals, which was supported only in BI tree (PP = 0.99).

Nomenclatural statement: A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank.org:pub:

DISCUSSION

The present molecular phylogenies highlight the phylogenetic relationships and distribution of the western Japan species of *Pseudocrangonyx*. Previous studies showed that two genetically highly diverged phylogroups (*Pseudocrangonyx* sp. 2 = *P. akatsukai* **n. sp.** and *Pseudocrangonyx* sp. 5) are distributed in the western tip of Honshu Island (Chugoku District), and their putative ranges may overlap in this region (Tomikawa *et al.*, 2016; Zhao & Hou, 2017). We found that *P. akatsukai* **n. sp.** and *Pseudocrangonyx* sp. 5 are also found in Kyushu Island (Supplementary material Fig. S2).

Previous (Tomikawa *et al.*, 2016; Zhao & Hou, 2017; Lee *et al.*, 2018) and present studies have reconstructed the phylogenetic position of *P. akatsukai* **n. sp.**, which is phylogenetically close to *P. yezonis* and found in northern Japan, and three continental species, *P. elegantulus*, *P. korkishkoorum*, and *P. tiunovi*. Although the obtained phylogenies could not resolve the precise relationships among the *P. akatsukai* **n. sp.** populations, our results clearly show that this new species is indigenous to underground water habitats in the montane region in Chugoku District and a small islet, Amakusa-Kamishima, Amakusa Islands, adjacent to Kyushu (Supplementary material Fig. S2).

The type locality of *P. akatsukai* **n. sp.** (locality 22 in Fig. 11B and Supplementary material Fig. S2) and a second locality, Uyamado Cave (locality 29), in Chugoku

District are located in the Akiyoshi accretionary complex, a geological unit that consists of a Carboniferous-Permian oceanic assemblage. The northernmost part of Kyushu is also composed of this accretionary unit (Isozaki *et al.*, 2010; Nakazawa *et al.*, 2011; Kojima *et al.*, 2016). The deep phylogenetic divergence between the populations of *P. akatsukai* **n. sp.** indigenous to Taishodo and Akiyoshido caves (locality 22) and Uyamado Cave (locality 29) could be associated with the geological disjunction between the two limestone regions of the Akiyoshi accretionary complex. The remaining locality, Gongen-shonyudo Cave in Amakusa-Kamishima Island (locality 27), belongs to a different geological unit characterized as the Cretaceous Higo metamorphic complex (Tashiro *et al.*, 1986; Miyazaki *et al.*, 2016). The presence of *P. akatsukai* **n. sp.** on this island thus indicates a past stygobitic connection during the formation of the limestone areas in Chugoku District and Amakusa-Kamishima Island.

The BI tree showed that the OTUs identified as *Pseudocrangonyx* sp. 5 can be split into two sub-clades: a lineage that consists of the individual from the cave in the central Kyushu Mountains, and a clade that contains individuals in Honshu and Shikoku (Supplementary material Fig. S2). The precise phylogenetic relationships within this unidentified species, however, remains unclear; only the monophyly of the amphipods collected from a small islet (locality 23 in Fig. 11B and Supplementary material Fig. S2) and Rakanana Cave in Shikoku (locality 25) was supported in both analyses. The *Pseudocrangonyx* sp. 5 individuals were only collected from subterranean habitats peripheral to the Chugoku Mountains, whereas individuals from Shikoku and Kyushu are found in caves located deep in the mountainous regions of these islands.

The results help elucidate the stygofaunal relationships in western Japan. The occurrence of *P. akatsukai* **n. sp.** indicates a close relationship between the underground water habitats from the central to the western Chugoku Mountains and those in the Amakusa Islands; both habitats could have been connected through northern Kyushu during a past geological event. *Pseudocrangonyx* sp. 5 are widely distributed in western Japan, so the stygofauna of the Chugoku Mountains in western Honshu, Shikoku, and central Kyushu might be closely related to each other. Additional specimens of this genus should be examined to elucidate the biogeographical history of *Pseudocrangonyx* in western Japan.

KEY TO SPECIES OF *PSEUDOCRANGONYX*

Pseudocrangonyx camtschaticus Birstein, 1955 is not included in this key because the original description does not provide appropriate morphological features to discriminate this species from the remaining 24 congeners, including *P. akatsukai* **n. sp.** and *P. komaii* **n. sp.**

1. Eyes absent ... 2
   – Trace of eyes present ... 20
2. Telson entire ... 3
   – Telson emarginated ... 4
3. Telson tapering, length 1.2× width ... *P. kseinae* Sidorov, 2012
   – Telson not tapering, length 1.7× width ... *P. levanidovi* Birstein, 1955
4. Inner plate of maxilla 1 with more than 4 setae ... 5
   – Inner plate of maxilla 1 with less than 4 setae ... 16
5. Posterodistal corner of carpus of female gnathopod 2 without serrate setae ... 6
   – Posterodistal corner of carpus of female gnathopod 2 with serrate setae ... 8
6. Female antenna 2 with calceoli ... *P. komaii* **n. sp.**

– Female antenna 2 without calceoli ... 7

7. Antenna 1 0.4× shorter than body length; posterodistal corner of carpus of female gnathopod 1 without serrate setae ... *P. cavernarius* Hou & Li, 2003

– Antenna 1 0.7× longer than body length; posterodistal corner of carpus of female gnathopod 1 with serrate setae ... *P. korkishkoorum* Sidorov, 2006

8. Telson laterally concave ... *P. manchuricus* Oguro, 1938

– Telson laterally straight, not concave ... 9

9. Sternal gills present ... *P. asiaticus* Uéno, 1934

– Sternal gills absent ... 10

10. Dorsal margins of pereopods 1–6 with long setae ... *P. yezonis* Akatsuka & Komai, 1922

– Dorsal margins of pereopods 1–6 without long setae ... 11

11. Posterodistal corner of carpus of female gnathopod 1 without serrate setae ... 12

– Posterodistal corner of carpus of female gnathopod 1 with serrate setae ... 13

12 Antenna 1 more than 0.5× longer than body length; terminal article of female uropod 3 0.05× proximal article length ... *P. elenae* Sidorov, 2011

– Antenna 1 0.3× shorter than body length; terminal article of female uropod 3 length 0.2× proximal article length ... *P. holsingeri* Sidorov & Gontcharov, 2013

13. Peduncle of pleopod 1 with marginal setae ... 14

– Peduncle of pleopod 1 without marginal setae ... 15

14. Urosomite 1 with ventral robust seta; telson 1.1–1.3× width ... *P. akatsukai* **n. sp.**

– Urosomite 1 without ventral robust seta; telson 1.5× width ... *P. shikokunis* Akatsuka & Komai, 1922

15. Female antenna 2 with calceoli; telson cleft along 24–27% of length ... *P.*

elegantulus Hou in Zhao & Hou, 2017

– Female antenna 2 without calceoli; telson cleft along 15% of length ... *P. tiunovi* Sidorov & Gontcharov, 2013

16. Posterodistal corner of carpus of female gnathopod 2 with serrate setae ... 17

– Posterodistal corner of carpus of female gnathopod 2 without serrate setae ...18

17. Posterodistal corner of carpus of female gnathopod 1 with serrate setae ... *P. kyotonis* Akatsuka & Komai, 1922

– Posterodistal corner of carpus of female gnathopod 1 without serrate setae ...18

18. Antenna 1 0.7× body length ... *P. febras* Sidorov, 2009

– Antenna 1 0.3× body length ... *P. sympatricus* Sidorov & Gontcharov, 2013

19. Female antenna 2 with calceoli ... *P. coreanus* Uéno, 1966

– Female antenna 2 without calceoli ... 19

19. Inner ramus of uropod 2 with marginal robust seta ... *P. daejeonensis* Lee, Tomikawa, Nakano & Min, 2018

– Inner ramus of uropod 2 with 4 marginal robust setae ... *P. gudariensis* Tomikawa & Sato in Tomikawa *et al.*, 2016

20. Outer plate of maxilla 1 with 5 serrate teeth ... *P. bohaensis* (Derzhavin, 1927)

– Outer plate of maxilla 1 with 7 serrate teeth ... 21

21. Telson cleft along 6.2% of length ... *P. birsteini* Labay, 1999

– Telson cleft along 16.3–20% of length ... 22

22. Outer ramus of uropod 2 with robust setae ... *P. relicta* Labay, 1999

– Outer ramus of uropod 2 without robust setae ... *P. susanaensis* Labay, 1999

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Samples used for the molecular phylogenetic analyses, with voucher or isolate numbers, collection locality, and INSDC accession numbers.

S2 Figure. Distributions of two *Pseudocrangonyx* phylogroups in western Japan.

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FIGURE LEGENDS

Figure 1. *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980 (**A**); *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988 (**B**).

Figure 2. *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980. Dorsal margins of pleonites 1–3, dorsal views (**A–C**); epimeral plates 1–3, lateral views (**D–F**); dorsal margins of urosomites 1–3, dorsal views (**G–I**); antenna 1, medial view, some distal articles of main flagellum omitted (**J**); accessory flagellum of antenna 1, medial view (**K**); aesthetasc and associate setae on main flagellum of antenna 1, medial view (**L**); antenna 2, medial view (**M**); calceolus on flagellum of antenna 2 (**N**); upper lip, posterior view (**O**); left mandible, medial view (**P**); incisor, lacinia mobilis, and molar process of left mandible (**Q**); incisor, lacinia mobilis, and molar process of right mandible (**R**); lower lip, ventral view (**S**).

Figure 3. *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980. Maxilla 1, dorsal view (**A**); apical robust setae on outer plate of maxilla 1 (**B**); maxilla 2, dorsal view (**C**); maxilliped, dorsal view (**D**); apical setae on inner plate of maxilliped, dorsal view (**E**); gnathopod 1, lateral view (**F**); palmar margin of propodus and dactylus of gnathopod 1, medial view (**G**); serrate setae on posterodistal corner of carpus of gnathopod 1 (**H**); gnathopod 2, lateral view (**I**); palmar margin of propodus and dactylus of gnathopod 2, medial view (**J**); serrate setae on posterodistal corner of carpus of gnathopod 2 (**K**).

Figure 4. *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980. Pereopod 3, lateral view (A); dactylus of pereopod 3, lateral view (**B**); pereopod 4, lateral view (**C**); dactylus of pereopod 4, lateral view (**D**); pereopod 5, lateral view (**E**); propodus and dactylus of pereopod 5 (**F**); dactylus of pereopod 5, lateral view (**G**); pereopod 6, lateral view (**H**); dactylus of pereopod 6, lateral view (**I**); pereopod 7, lateral view (**J**); dactylus of pereopod 7, lateral view (**K**).

Figure 5. *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980 (**A–J**); paratype, male (7.7 mm), KUZ Z1981 (**K–U**). Pleopods 1–3, medial views, plumose setae on rami omitted (**A, D, E**); retinacula on peduncle of pleopod 1, medial view (**B**); bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of pleopod 1, medial view (**C**); uropods 1–3, dorsal views (**F–H**); terminal article of uropod 3, dorsal view (**I**); telson, dorsal view (**J**); antenna 1, medial view, some distal articles of main flagellum omitted (**K**); aesthetasc and associate setae on main flagellum of antenna 1, medial view (**L**); antenna 2, medial view (**M**); calceolus on flagellum of antenna 2, medial view (**N**); palmar margins of propodi and dactyli of gnathopods 1 and 2, medial views (**O–P**); uropod 1, dorsal view (**Q**); uropod 2, dorsal view (**R**); distal setae on inner ramus of uropod 2, dorsal view (**S**); uropod 3, ventral view (**T**); terminal article of uropod 3, ventral view (**U**).

Figure 6. *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988. Dorsal margins of pleonites 1–3, dorsal views (**A–C**); epimeral plates 1–3, lateral views (**D–F**); dorsal margins of urosomites 1–3, dorsal views (**G–I**); antenna 1, medial view, some distal articles of main flagellum omitted (**J**); accessory flagellum of antenna 1, medial view (**K**); antenna 2, medial view (**L**); calceolus on flagellum of antenna 2 (**M**); upper lip, posterior view (**N**); left mandible, medial view (**O**); incisor, lacinia mobilis, and molar process of left mandible (**P**); incisor, lacinia mobilis, and molar process of right mandible (**Q**); lower lip, ventral view (**R**).

Figure 7. *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988. Maxilla 1, dorsal view (**A**); apical robust setae on outer plate of maxilla 1 (**B**); maxilla 2, dorsal view (**C**); maxilliped, dorsal view (**D**); apical setae on inner plate of maxilliped, dorsal view (**E**); gnathopod 1, lateral view (**F**); palmar margin of propodus

and dactylus of gnathopod 1, lateral view (**G**); gnathopod 2, lateral view (**H**); palmar margin of propodus and dactylus of gnathopod 2, lateral view (**I**); pereopod 3, lateral view (**J**); dactylus of pereopod 3, lateral view (**K**).

Figure 8. *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988. Pereopod 4, lateral view (**A**); dactylus of pereopod 4, lateral view (**B**); pereopod 5, lateral view (**C**); dactylus of pereopod 5, lateral view (**D**); pereopod 6, lateral view (**E**); dactylus of pereopod 6 (**F**); pereopod 7, lateral view (**G**); dactylus of pereopod 7, lateral view (**H**).

Figure 9. *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988. Pleopods 1–3, medial views, plumose setae on rami omitted (**A, C, D**); retinacula on peduncle of pleopod 1, medial view (**B**); uropods 1–3, dorsal views (**E–G**); terminal article of uropod 3, dorsal view (**H**); telson, dorsal view (**I**).

Figure 10. *Pseudocrangonyx komaii* **n. sp.**, holotype female (5.1 mm), KUZ Z1989. Antenna 1, medial view, some distal articles of main flagellum omitted (**A**); accessory flagellum of antenna 1, medial view (**B**); antenna 2, medial view (**C**); palmar margins of propodi and dactyli of gnathopods 1 and 2, medial views (**D–E**); uropods 1–3, dorsal views (**F–H**); terminal article of uropod 3, dorsal view (**I**).

Figure 11. Phylogenetic tree and map for the specimens examined in this study. Bayesian inference tree for 2778 bp of nuclear 28S rRNA plus histone H3 and mitochondrial COI and 16S rRNA markers; numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities (**A**). Collection localities of the specimens used for the phylogenetic analysis (**B**).