

# A New Stygobitic Species of the Genus *Pseudocrangonyx* (Crustacea: Amphipoda: Pseudocrangonyctidae) from Central Honshu, Japan

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A new species of pseudocrangonyctid amphipod, *Pseudocrangonyx uenoi*, is described from cave streams in Taga town, central Honshu, Japan. Previous molecular phylogenetic studies reported the taxonomic status of the Taga populations of *Pseudocrangonyx* as an undescribed species. We present the diagnostic features to discriminate *P. uenoi* from the other congeners on the basis of morphological study. *Pseudocrangonyx uenoi* is closely related to *P. daejeonensis* Lee, Tomikawa, Nakano, and Min, 2018, but clearly distinguished from the latter by the presence of calceoli on female antenna 2, the 5-dentate lacinia mobilis of the left mandible, setation of the peduncular articles of pleopods, the number of articles of rami of the pleopods, a shorter terminal article of uropod 3, and a telson with a deeper cleft.

**Key Words:** Crangonyctoidea, new species, subterranean, limestone caves.

## Introduction

The *Pseudocrangonyx* Akatsuka and Komai, 1922 is the most diverse taxon among subterranean-amphipod genera known from East Asia (Holsinger 1994). So far, 25 species are known from Japan, the Korean Peninsula, China, and the Russian Far East (Tomikawa and Nakano 2018). In addition to the Russian Far East (see Sidorov and Gontcharov 2013), underground water habitats in the Japanese Archipelago have harbored a rich species diversity of this stygobitic group (Tomikawa 2017); and the Japanese *Pseudocrangonyx* amphipods are classified into eight species at present (Uéno 1971; Tomikawa and Nakano 2018). Moreover, several Japanese undescribed species have been recognized according to previous molecular phylogenetic studies (Tomikawa *et al.* 2016; Tomikawa and Nakano 2018), and thus their taxonomic status should be assessed by defining morphological characteristics.

*Pseudocrangonyx* amphipods inhabiting limestone caves in Taga town, central Honshu, Japan is a recognized unnamed species, and were discriminated from the other congeners as *Pseudocrangonyx* sp. 3 by Tomikawa *et al.* (2016). They are described herein as a new species of the genus with a complete morphological description of the specimens.

## Materials and Methods

Specimens were collected from limestone caves in Taga Town, Shiga Prefecture, Japan. When possible, the geographical coordinates for all cave entrances were obtained using a Garmin eTrex<sup>®</sup> GPS unit. 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 SZX7 stereomicroscope (Olympus, Tokyo, Japan). Specimens were examined using a 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).

### *Pseudocrangonyx uenoi* sp. nov.

(Figs 1–5)

*Pseudocrangonyx* sp. 3: Tomikawa *et al.* 2016: fig. 10; Zhao and Hou 2017: table 1, fig. 8; Lee *et al.* 2018: table 1, fig. 10; Tomikawa and Nakano 2018: fig. 11; Copilaş-Ciocianu *et al.* 2019: figs 2, 3.

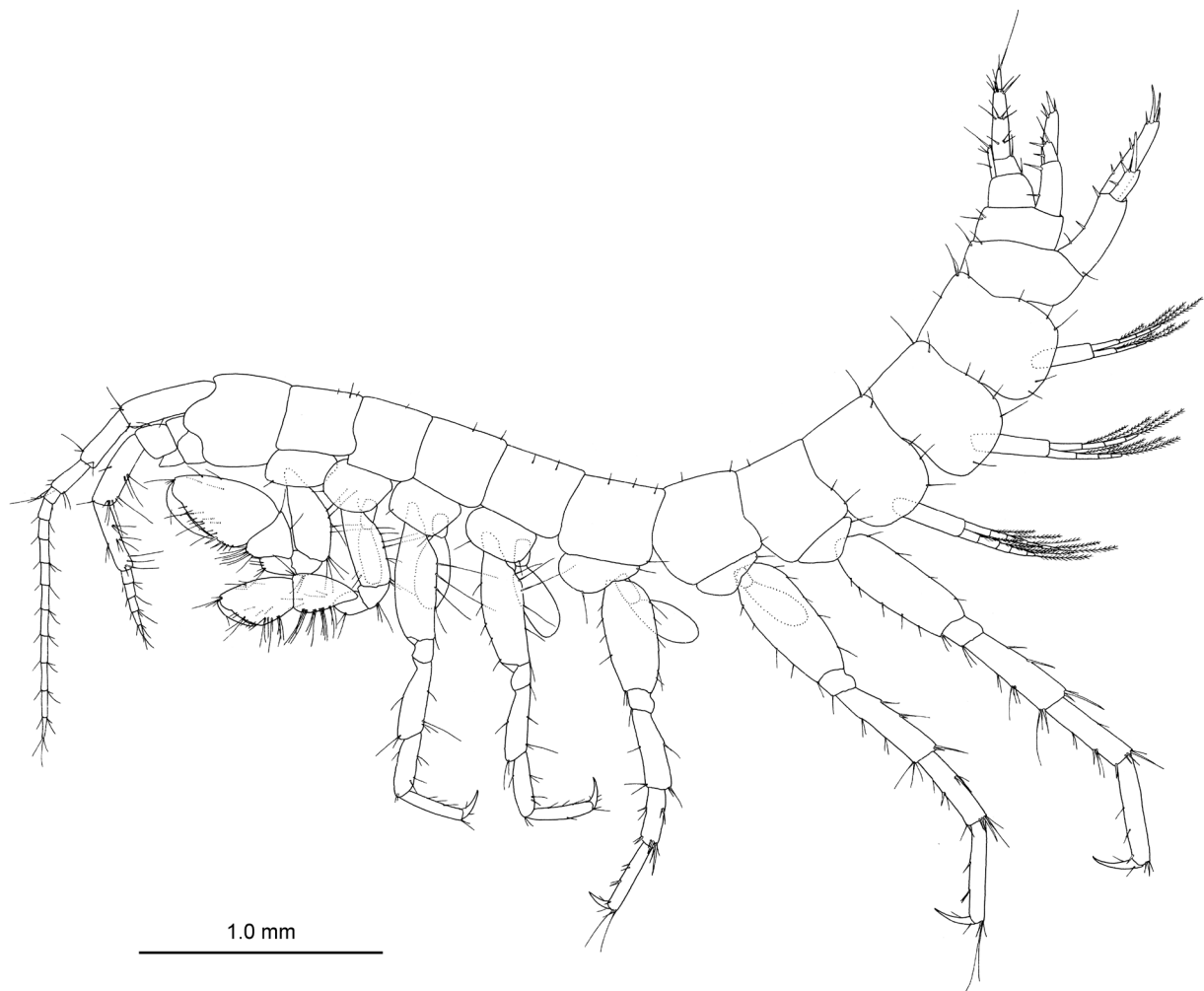


Fig. 1. *Pseudocrangonyx uenoi* sp. nov., holotype female (KUZ Z2514), 4.0 mm, Samenokōmōriana Cave, Taga, Shiga Prefecture, Japan. Habitus, lateral view.

**Diagnosis.** Antennal sinus with rounded angle; eyes completely absent; pereonites with short dorsal setae; urosomite 1 without ventral robust seta; dorsal margin of urosomite 3 lacking setae; sternal gill absent; antenna 1 0.4–0.5 times as long as body length; flagellar articles of antenna 2 with calceoli; mandible, palp article 3 slightly longer than article 2; maxilla 1, inner plate with 2–3 setae; maxilla 2, inner plate with oblique inner row of 2 setae; gnathopods 1 and 2, carpi without serrate robust setae on posterodistal corners; palmar margins of propodi of gnathopods 1 and 2 with 8–9 and 10–11 robust setae, respectively; pleopods, peduncles lacking marginal setae and inner margin of inner rami without bifid setae; uropod 1, inner ramus 1.6–2.0 times as long as outer ramus, inner margin of former with 3 robust setae, basal part with 1–2 slender setae, outer ramus with 1 marginal robust seta; uropod 2, inner ramus 1.2–1.5 times as long as outer ramus, its inner and outer margins with 0–2 and 1 robust setae, respectively, outer ramus with 0–1 inner and 1 outer marginal robust setae; uropod 3, terminal article 0.2–0.3 times as long as proximal article; and telson 1.3 times as long as wide, cleft for 17.1–20.4%.

**Material examined** Holotype: female 4.0 mm, KUZ Z2514, from Samenokōmōriana Cave (35.20445°N,

136.34383°E), Taga town, collected by T. Nakano and K. Tomikawa on 28 May 2017. Paratypes (5 specimens): male 4.3 mm, KUZ Z2515, female 4.6 mm, KUZ Z2516, data same as for holotype; male 2.4 mm, KUZ Z1963, from Kawachi Wind Cave (Kawachi Fuketsu Cave), Taga town, on 9 October 2009; female 2.9 mm, KUZ Z1964, from Samenokōmōriana Cave, Taga town, on 8 September 2008; female 2.1 mm, KUZ Z1965 from Gongen Cave, Taga town, on 30 September 2009.

**Description.** *Female* (KUZ Z2514, 4.0 mm). Head (Fig. 1) with short rostrum; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes absent. Pereonites 1–7 with short dorsal setae (Fig. 1); posterolateral margin of pereonites 6 and 7 each with 1 seta (Fig. 1). Dorsal margin of pleonites 1–3 with 4, 4, and 5 setae, respectively (Fig. 2A–C). Posterior margin of epimeral plate 1 with 2 setae, posteroventral corner not produced with 1 seta (Fig. 2D); ventral and posterior margins of plate 2 with 1 and 3 setae, respectively, posteroventral corner not produced, with 1 seta (Fig. 2E); ventral and posterior margins of plate 3 with 1 and 2 setae, respectively, posteroventral corner rounded, with 1 seta (Fig. 2F). Ventral margin of urosomite 1 without setae (Fig. 1); dorsal margin of urosomites 1 and 2 with 2 and 4

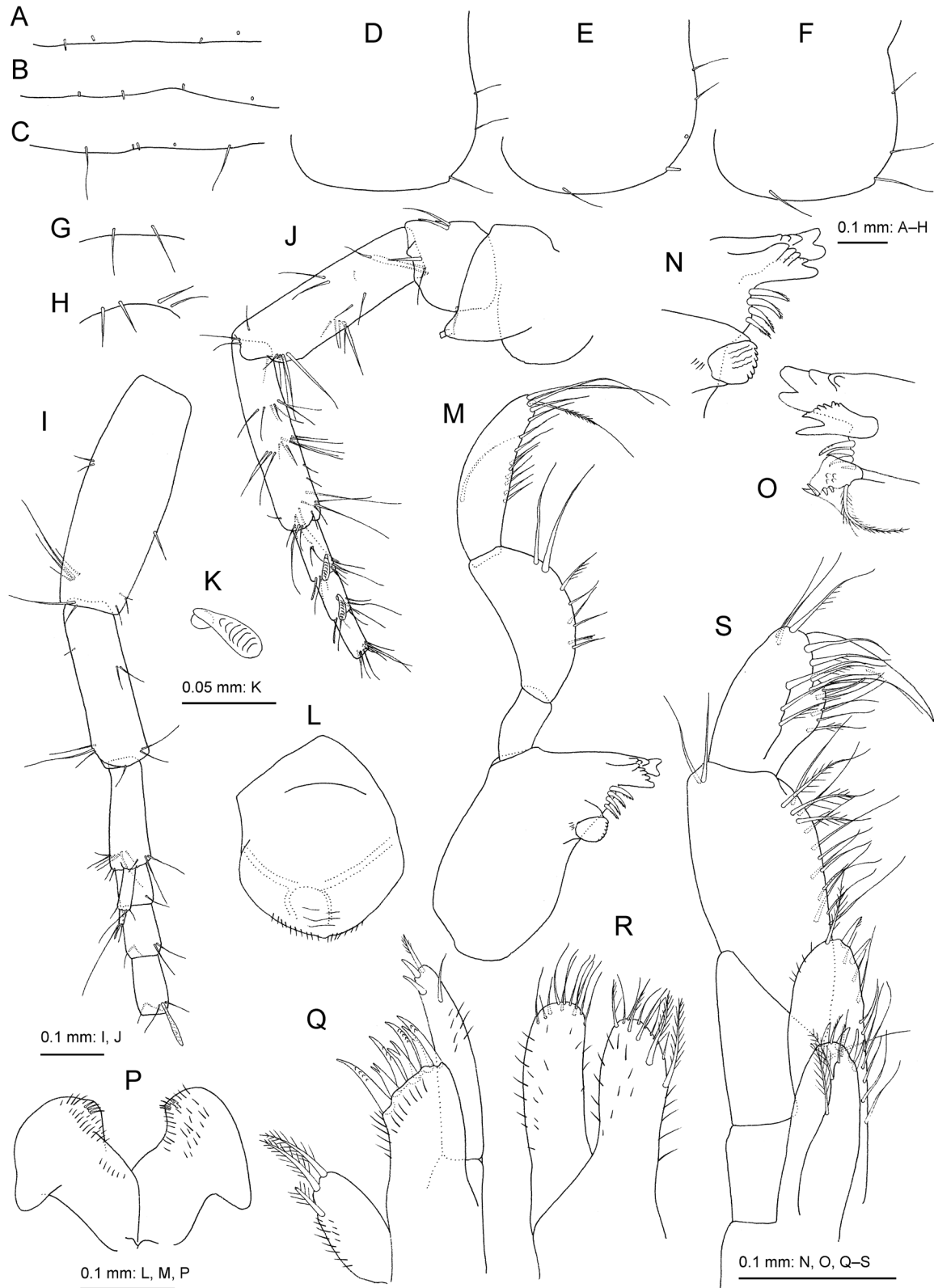


Fig. 2. *Pseudocrangonyx uenoi* sp. nov., holotype female (KUZ Z2514), 4.0 mm, Samenokōmoriana Cave, Taga, Shiga Prefecture, Japan. A–C, dorsal margins of pleonites 1–3, dorsal views; D–F, epimeral plates 1–3, lateral views; G, H, dorsal margins of urosomites 1 and 2, dorsal views; I, antenna 1, medial view, some distal articles of main flagellum omitted; J, antenna 2, medial view, some distal articles of flagellum omitted; K, calceolus on flagellum of antenna 2; L, upper lip, posterior view; M, left mandible, medial view; N, incisor, lacinia mobilis, and molar process of left mandible, medial view; O, incisor, lacinia mobilis, and molar process of right mandible, medial view; P, lower lip, ventral view; Q, maxilla 1, dorsal view; R, maxilla 2, dorsal view; S, maxilliped, dorsal view.

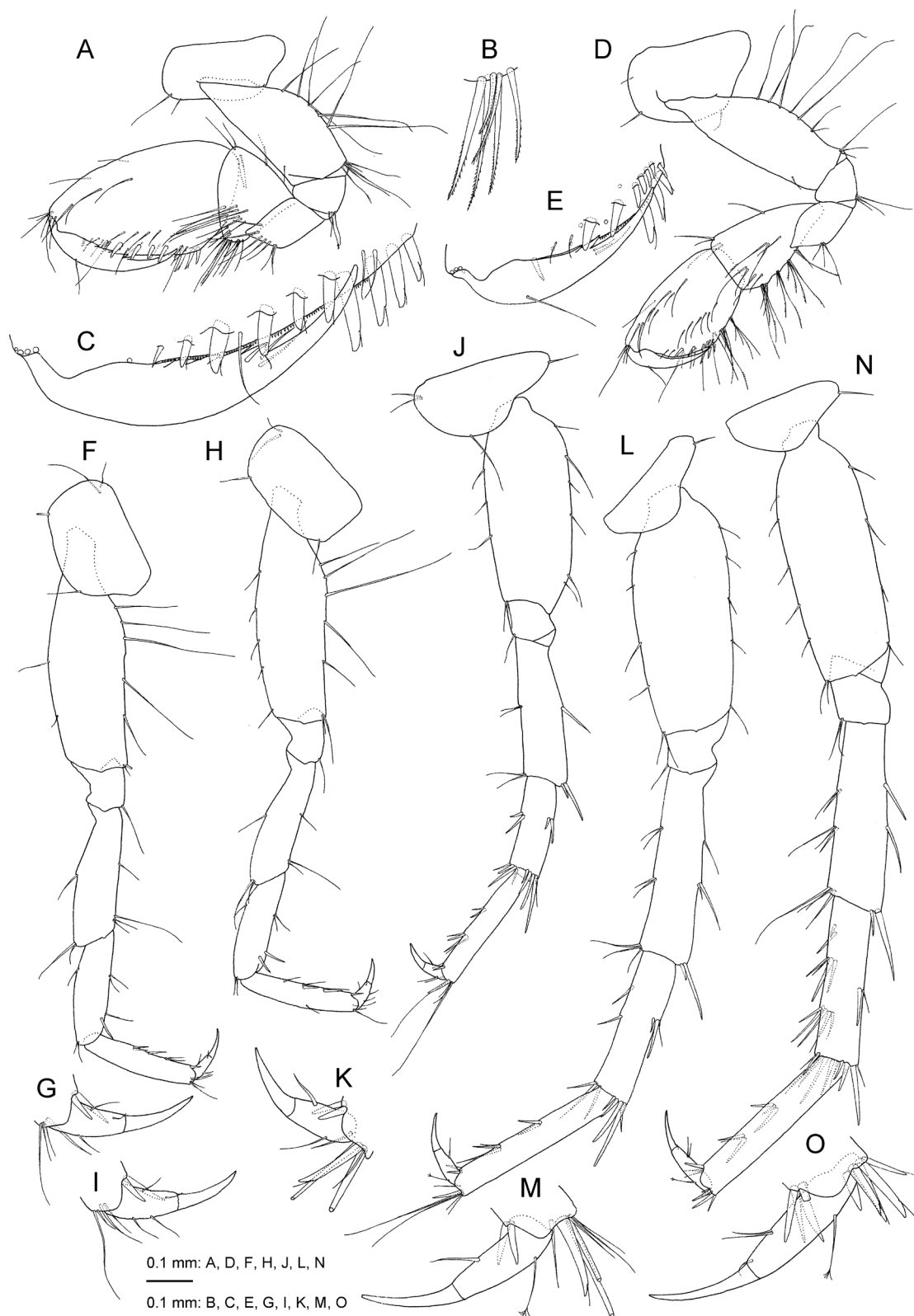


Fig. 3. *Pseudocrangonyx uenoi* sp. nov., holotype female (KUZ Z2514), 4.0 mm, Samenokōmoria Cave, Taga, Shiga Prefecture, Japan. A, gnathopod 1, medial view; B, serrate slender setae on posterodistal corner of carpus of gnathopod 1; C, palmar margin of propodus and dactylus of gnathopod 1, medial view; D, gnathopod 2, medial view; E, palmar margin of propodus and dactylus of gnathopod 2, medial view; F, pereopod 3, lateral view; G, dactylus of pereopod 3, lateral view; H, pereopod 4, lateral view; I, dactylus of pereopod 4, lateral view; J, pereopod 5, lateral view; K, dactylus of pereopod 5, lateral view; L, pereopod 6, lateral view; M, dactylus of pereopod 6, lateral view; N, pereopod 7, lateral view; O, dactylus of pereopod 7, lateral view.

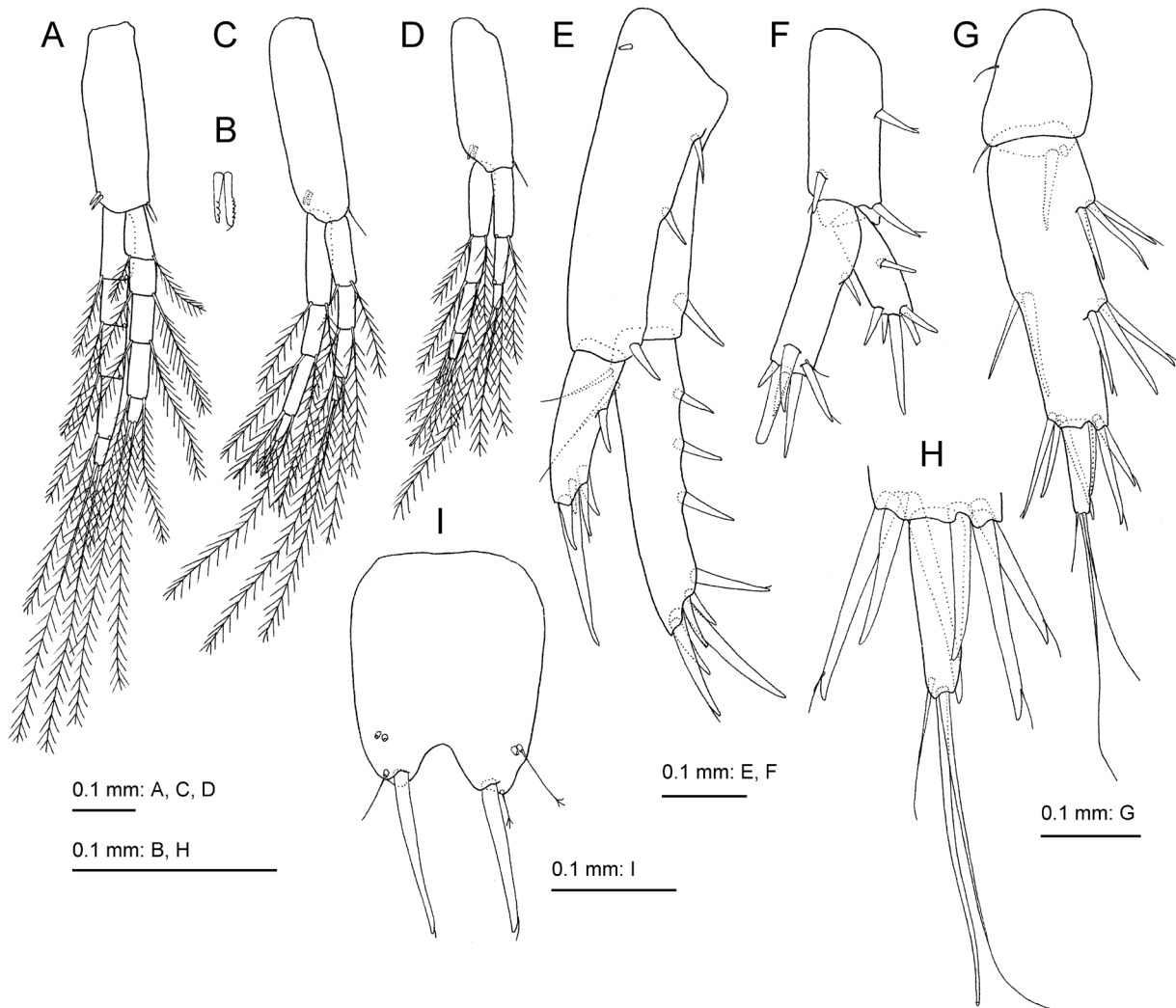


Fig. 4. *Pseudocrangonyx uenoi* sp. nov., holotype female (KUZ Z2514), 4.0 mm, Samenokōmoria Cave, Taga, Shiga Prefecture, Japan. A, pleopod 1, medial view; B, retinacula on peduncle of pleopod 1, medial view; C, D, pleopods 2 and 3, medial views; E–G, uropods 1–3, dorsal views; H, terminal article of uropod 3, dorsal view; I, telson, dorsal view.

slender setae, respectively (Fig. 2G, H), dorsal margin of urosomite 3 lacking setae (Fig. 1).

Antenna 1 (Fig. 2I) 0.5 times as long as body length, peduncular articles 1 to 3 in length ratio of 1.0:0.7:0.5; accessory flagellum 2-articulate; primary flagellum 1.3 times as long as peduncular articles 1–3 combined, 11-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 2J) 0.7 times as long as antenna 1; peduncular articles without calceoli; flagellum 0.4 times as long as peduncular articles 4 and 5 combined, consisting of 5 articles, first 2 of which with calceolus (Fig. 2K).

Upper lip (Fig. 2L) with rounded anterior margin, bearing fine setae. Mandibles (Fig. 2M–O) with left and right incisors both 5-dentate; left lacinia mobilis 5-dentate, right lacinia bifid, bearing many teeth; molar process weakly tritriturate, molar of right mandible with accessory plumose seta; accessory setal rows of left and right mandibles both with 4 weakly pectinate setae; palp 3-articulate, article 3 length 1.1 times as long as article 2 with 1 A-, about 9 D-, and 4 E-setae. Lower lip (Fig. 2P) with broad outer lobes, man-

dibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 2Q) with inner and outer plates, and palp; inner plate subovate, its medial margin with 3 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 2 robust setae and 1 slender seta apically, and 1 slender seta subapically. Maxilla 2 (Fig. 2R) with oblique inner row of 2 plumose setae on inner plate. Maxilliped (Fig. 2S) with inner and outer plates, and palp; inner plate with 3 apical and 1 subapical robust setae; outer plate with 1 apical plumose seta, and 2 robust and some slender setae on medial margin; palp 4-articulate, medial margin of article 2 lined with setae.

Gnathopod 1 (Fig. 3A) with subquadrate coxa bearing 2 setae on anterodistal corner of coxa, width 2.2 times as long as depth; anterodistal submargin of basis with short seta, posterior margin of basis with long setae; posterodistal corner of carpus with weakly serrate slender setae (Fig. 3B), lacking serrate robust setae; propodus stout, ovate, palmar margin with 4 lateral and 5 medial robust setae, some

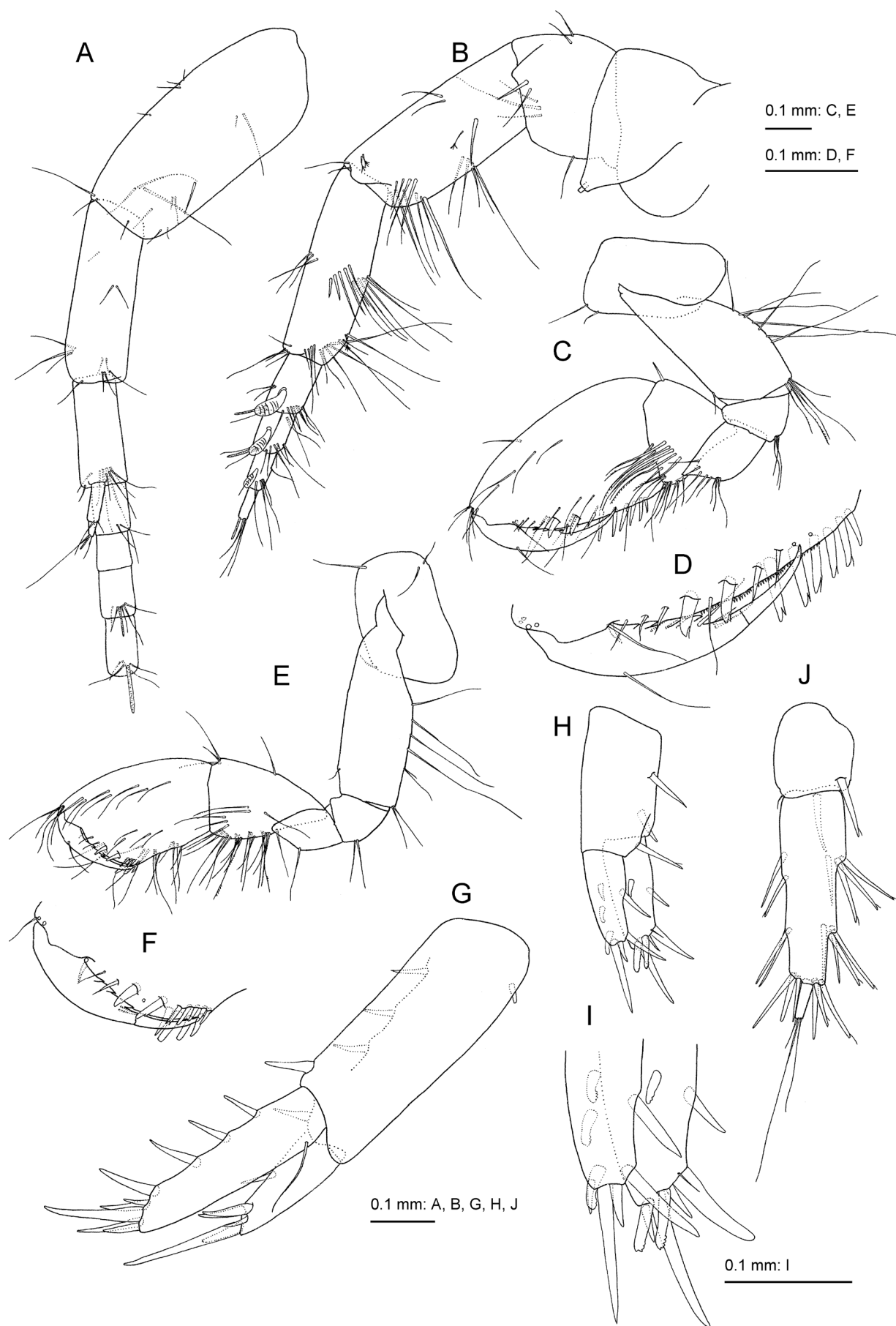


Fig. 5. *Pseudocrangonyx uenoi* sp. nov., paratype male (KUZ Z2515), 4.3 mm, Samenokōmoriana Cave, Taga, Shiga Prefecture, Japan. A, antenna 1, medial view, some distal articles of main flagellum omitted; B, antenna 2, medial view; C, gnathopod 1, medial view; D, palmar margin of propodus and dactylus of gnathopod 1, medial view; E, gnathopod 2, medial view; F, palmar margin of propodus and dactylus of gnathopod 2, medial view; G, uropod 1, ventral view; H, uropod 2, dorsal view; I, distal setae on inner and outer rami of uropod 2, dorsal view; J, uropod 3, dorsal view.

distally notched (Fig. 3C); posterior margin of dactylus dentate (Fig. 3C). Gnathopod 2 (Fig. 3D) with subquadrate coxa bearing setae on its anterodistal and posteroventral corners, width 1.5 times as long as depth; basis with short seta on anterodistal submargin and long setae on posterior margin; posterodistal corner of carpus without serrate robust setae; propodus weakly slender than that of gnathopod 1, with 3 lateral and 7 medial robust setae along palmar margin (Fig. 3E); posterior margin of dactylus dentate (Fig. 3E). Pereopod 3 (Fig. 3F) with subquadrate coxa bearing setae on its anterodistal and posteroventral corners, width 1.5 times as long as depth; anterior and posterior margins of basis with short and long setae, respectively; merus, carpus, and propodus in length ratio of 1.0:0.8:0.8; anterior and posterior margins of dactylus with 1 and 2 setae, respectively (Fig. 3G). Pereopod 4 (Fig. 3H) with coxa bearing setae on its anterodistal and posteroventral corners, width 1.7 times as long as depth; anterior and posterior margins of basis with short and long setae, respectively; merus, carpus, and propodus in length ratio of 1.0:0.8:0.9; anterior and posterior margins of dactylus each with 2 setae (Fig. 3I). Pereopod 5 (Fig. 3J) with weakly bilobed coxa bearing setae on anterior and posterior lobes; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0:0.7:0.8; anterior and posterior margins of dactylus each with 1 seta (Fig. 3K). Pereopod 6 (Fig. 3L) with coxa bearing concave lower margin, posteroproximal corner with 1 seta; anterior and posterior margins of basis with short setae; merus, carpus, and propodus in length ratio of 1.0:0.8:0.9; anterior and posterior margins of dactylus each with 1 seta (Fig. 3M). Pereopod 7 (Fig. 3N) with subtriangular coxa, posteroproximal corner of coxa with 1 seta; anterior and posterior margins of basis with short setae; merus, carpus, and propodus in length ratio of 1.0:0.9:1.0; anterior and posterior margins of dactylus with 3 and 1 setae (Fig. 3O).

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

Coxal gills (Fig. 1) on gnathopod 2 and pereopods 3–6; sternal gills absent.

Peduncles of pleopods 1–3 (Fig. 4A, C, D) marginally bare, each with paired retinacula (Fig. 4B), outerdistal corners of pleopods 1–3 with 2, 1, and 1 short setae, respectively; inner basal margin of inner ramus without bifid setae.

Uropod 1 (Fig. 4E) with 1 basofacial slender seta on peduncle; peduncle 1.1 times as long as inner ramus; inner ramus 2.0 times as long as outer ramus, inner margin of inner ramus with 3 robust setae, outer margin bare, basal part with 2 slender setae; outer ramus with 1 marginal robust seta. Uropod 2 (Fig. 4F) with peduncle 1.1 times as long as inner ramus; inner ramus 1.5 times as long as outer ramus, its outer margin with 1 seta; outer ramus with 1 outer marginal robust seta. Uropod 3 (Fig. 4G) with peduncle 0.4 times as long as outer ramus; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.3 times as long as proximal article, shorter than distal robust setae on proximal article, with 3 distal setae (Fig. 4H).

Telson (Fig. 4I) length 1.3 times as long as wide, cleft for 20.4% of length, each telson lobe with 2 lateral long penicil-

late setae, 1 apical robust seta, and 1 apical penicillate seta.

**Male** (KUZ Z2515, 4.3 mm). Antenna 1 (Fig. 5A) 0.5 times as long as body length, primary flagellum 13-articulate. Antenna 2 (Fig. 5B) 0.6 times as long as antenna 1, peduncular articles without calceolus; flagellum 0.5 times as long as peduncular articles 4 and 5 combined, 4-articulate, articles 1–3 each with calceolus.

Mandibular palp article 3 length 1.3 times as long as article 2. Maxilla 1 with 2 plumose setae on its medial margin.

Gnathopod 1 with 6 lateral and 2 medial robust setae on palmar margin (Fig. 5C, D). Gnathopod 2 with 4 lateral and 7 medial robust setae on palmar margin (Fig. 5E, F).

Uropod 1 (Fig. 5G) with inner ramus 1.6 times as long as outer ramus, bearing 1 slender seta on basal part. Uropod 2 (Fig. 5H, I) with inner ramus 1.2 times as long as outer ramus, its inner and outer margins with 1 simple and 1 weakly serrate robust setae, respectively, distal part with 2 serrate and 4 simple robust setae and 1 slender seta; inner and outer margins of outer ramus with 1 simple and 2 serrate robust setae, respectively, distal part with 1 weakly serrate and 4 simple robust setae. Uropod 3 (Fig. 5J) with more robust setae on proximal article of outer ramus than that of female, terminal article 0.2 times as long as proximal article. Telson cleft for 17.2% of length.

**Variation.** Antenna 1 0.4 times as long as body length; antenna 2 0.6 times as long as antenna 1; maxilla 1 inner plate with 2 plumose setae on its medial margin; telson cleft for 17.1% (female 4.6 mm, KUZ Z2516).

**Distribution.** The species is known from limestone caves in Taga Town, Shiga Prefecture, Japan. Specimens were collected from small pools and streams in the caves.

**DNA sequences.** In total, 9 sequences of the present paratypes were determined by Tomikawa *et al.* (2016): KUZ Z1963, 2 sequences—28S (LC171488; 1297 bp), and H3 (LC171489; 328 bp); KUZ Z1964, 4 sequences—28S (LC171491; 1297 bp) H3 (LC171493; 328 bp), COI (LC171492; 658 bp), and 16S (LC171490; 416 bp); and KUZ Z1965, 3 sequences—28S (LC171495; 1297 bp), H3 (LC171496; 328 bp), and 16S (LC171494; 416 bp).

**Etymology.** The specific name is dedicated to the late Professor Masuzo Uéno, who was the past director of the now defunct Otsu Hydrobiological Station of Kyoto University in Shiga Prefecture, and greatly contributed to the *Pseudocrangonyx* systematics.

**Remarks.** A previous phylogenetic study (Lee *et al.* 2018) showed that *Pseudocrangonyx uenoi* sp. nov. formed a monophyletic lineage with *P. daejeonensis* Lee, Tomikawa, Nakano, and Min, 2018, which was described from the interstitial water in the Korean Peninsula. Both species have a small body size that is shorter than 5 mm; eyes that are completely absent; urosomite 1 without ventral setae; carpi of gnathopods 1 and 2 without serrate robust setae on the posterodistal corners; and inner rami of pleopods without bifid setae on its inner margins. However, these characteristics may not be synapomorphies between *P. uenoi* sp. nov. and *P. daejeonensis* because they are also shared with the other *Pseudocrangonyx* species (Tomikawa *et al.* 2016).

*Pseudocrangonyx uenoi* sp. nov. can be clearly differenti-

ated from *P. daejeonensis* by female antenna 2 with calceoli (*versus* without calceoli in *P. daejeonensis*); the lacinia mobilis of the left mandible being 5-dentate (*versus* 4-dentate); a peduncle of pleopod 1 without marginal setae (*versus* with a marginal seta); a peduncle of pleopod 3 with a seta on the outerdistal corner (without setae); pleopodal rami being up to 5-articulate (*versus* less than 5-articulate); the terminal article of uropod 3 being short, not reaching the tips of the robust setae on the distal part of the proximal article of uropod 3 (*versus* long, reaching past the tips of the robust setae); and the telson cleft being 17.1–20.4% (*versus* 8%) of its length.

*Pseudocrangonyx uenoi* sp. nov. also resembles *P. komaii* Tomikawa and Nakano, 2018 from central Japan in its absent eyes, its ventral setae being on urosomite 1, its postero-distal corners of gnathopods 1 and 2 carpi without serrate robust setae, its marginal setae being on the pleopodal peduncle, its bifid setae being on the inner rami of pleopods, its having calceoli on antenna 2 in both sexes, and its short terminal article of uropod 3 not reaching the tip of the robust setae on the distal part of the proximal article. The new species can be distinguished from *P. komaii* by the absence of calceoli on peduncular article 5 of antenna 2 (*versus* present in *P. komaii*), the pleopodal rami being up to 5-articulate (*versus* up to 8-articulate), the terminal article of uropod 3 being 0.2–0.3 (*versus* 0.1) times as long as the proximal article of uropod 3, and the telson cleft being 17.1–20.4% (*versus* 6.8%) of its length.

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