Three new species of subterranean amphipod (Pseudocrangonyctidae: *Pseudocrangonyx*) from limestone caves in South Korea

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The genus *Pseudocrangonyx* is the most diverse subterranean amphipods of the groundwater communities in Far East Asia. In Korea, the true species diversity of the group has been underestimated owing to the records of morphological variants of *P*. *asiaticus*. To estimate their true species diversity, we analyzed morphological characteristics and conducted molecular analyses of specimens collected from Korean caves, which were treated as morphological variants of *P*. *asiaticus*. Thus, three new subterranean species of pseudocrangonyctid amphipod, namely *P*. *deureunensis* **sp. nov.,** *P*. *kwangcheonseonensis* **sp. nov.,** and *P*. *hwanseonensis* **sp. nov.** were described from the groundwater of limestone caves in South Korea. Additionally, we determined sequences of nuclear large subunit ribosomal RNA and mitochondrial cytochrome *c* oxidase subunit I gene of the new species for molecular analyses. Molecular phylogenetic analyses revealed that the three new species formed a monophyly with *P*. *joolaei* and *P*. *wonkimi*, which are indigenous to the Korean caves.

Three new species of subterranean amphipod (Pseudocrangonyctidae: *Pseudocrangonyx*) from limestone caves in South Korea

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Abstract

The genus *Pseudocrangonyx* is the most diverse subterranean amphipods of the groundwater communities in Far East Asia. In Korea, the true species diversity of the group has been underestimated owing to the records of morphological variants of *P*. *asiaticus*. To estimate their true species diversity, we analyzed morphological characteristics and conducted molecular analyses of specimens collected from Korean caves, which were treated as morphological variants of *P*. *asiaticus*. Thus, three new subterranean species of pseudocrangonyctid amphipod, namely *P*. *deureunensis* **sp. nov.,** *P*. *kwangcheonseonensis* **sp. nov.,** and *P*. *hwanseonensis* **sp. nov.** were described from the groundwater of limestone caves in South Korea. Additionally, we determined sequences of nuclear large subunit ribosomal RNA and mitochondrial cytochrome *c* oxidase subunit I gene of the new species for molecular analyses. Molecular phylogenetic analyses revealed that the three new species formed a monophyly with *P. joolaei* and *P*. *wonkimi*, which are indigenous to the Korean caves.

Introduction

Amphipods are the most diverse group of organisms in groundwater communities (Holsinger 1994), and subterranean amphipods are of interest from a biogeographic perspective because of their limited dispersal ability and restriction to groundwater aquifers (Holsinger 1993). Most ~~of the~~ subterranean amphipods are troglobiont (stygobiont), generally characterized by morphological features ~~with~~ loss of eyes and pigment and elongation of appendages (Holsinger 1994; Väinölä *et al*. 2007), and these characteristics result in the strikingly convergent morphology of cave animals (Jones *et al*. 1992). Therefore, the classification of subterranean organisms that are based solely on morphological characteristics exhibit several taxonomic problems (Lefébure *et al*. 2006; Kornobis *et al*. 2011). Particularly, in the case of subterranean or cave amphipod species that are difficult to distinguish morphologically, distinguishing species through molecular analyses helps to resolve ~~the~~ species delimitation (Lefébure *et al*. 2006; Trontelj *et al*. 2009; Hou & Li 2010).

The stygobitic amphipod *Pseudocrangonyx* Akatsuka & Komai, 1922 is the most diverse taxon among the subterranean amphipod genera found in Far East Asia, i.e., the Korean Peninsula, the Japanese Archipelago, eastern China, and Russian Far East (Sidorov & Holsinger 2007; Tomikawa & Nakano 2018). The first record in the Korean Peninsula was *P. asiaticus* Uéno, 1934 from North Korea (Uéno 1940). But the type locality of *P. asiaticus* is located on the Liaodong Peninsula, China (Uéno 1934). Since then, it has been reported that the species inhabits several caves in the South Korea through identification based on morphological characters (Uéno 1966; Holsinger 1989). Uéno (1966) mentioned regional morphological variants of the Korean populations but did not regard it as a distinct species. At that time, as mentioned above, there would have been obvious limitations in identifying subterranean amphipods solely by morphological characteristics. Recent studies have shown that the species diversity of the genus based on both molecular analyses and morphological identification may be higher than previously known (Tomikawa *et al.* 2016; Tomikawa & Nakano 2018; Lee *et al.* 2020).

During cave surveys in the Korean Peninsula, we collected *Pseudocrangonyx* specimens from two caves (Kwangcheonseon Cave and Hwanseon Cave), where Uéno (1966) reported one of the morphological variants of *P. asiaticus* and specimens from ~~the other~~ cave (Deureune Cave) was first found. Based on the results of morphological examination of the amphipods, we described and illustrated them as three new *Pseudocrangonyx* species. Furthermore, we determined the nuclear large subunit ribosomal RNA (28S rRNA) gene and mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence data for molecular analyses of the three new species. Additionally, a key to Korean *Pseudocrangonyx* species is provided in this study.

Materials & Methods

Sample collection and morphological examination. *Pseudocrangonyx* specimens were collected from groundwater of three caves in Korea, namely Deureune Cave (Fig. 1A), Kwangcheonseon Cave (Fig. 1B), and Hwanseon Cave (Fig. 1C). Specimens were fixed and preserved in 99% ethanol. All appendages of the specimens were dissected in 80% ethanol and mounted with gum-chloral medium on glass slides under an Olympus SZX7 stereomicroscope (Tokyo, Japan). The specimens were examined using a Nikon Eclipse Ni light microscope (Tokyo, Japan) and illustrated with the aid of a drawing tube. 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 the method described by Stock (1974). The specimens examined in this study have been deposited in the collection of the Nakdonggang National Institute of Biological Resources, Korea (NNIBR).

Molecular analyses. Genomic DNA was extracted from the muscles of the appendages of the specimens using the LaboPass Tissue Mini Kit (Cosmo GENETECH, Seoul, South Korea), according to the manufacturer's instructions. The primer sets for the PCR reaction used in this study were as follows: 28F and 28R for 28S rDNA (Hou *et al*. 2007); LCO1490 and HCO2198 for COI (Folmer *et al*. 1994). The sequences of 28S rDNA were aligned using MAFFT v. 7.388 L-INS-i (Katoh & Standley 2013), and COI was aligned using Geneious 8.1.9 (Biomatters, Auckland, New Zealand), respectively. For phylogenetic analysis, these two alignments were combined. All data used in molecular analyses is provided, including the newly obtained sequences in this study (Table 1). Pairwise comparisons of uncorrected *p*-distances for COI sequence was calculated using MEGA X (Kumar *et al*. 2018). Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML analysis was performed using RAxML v. 8.2.10 (Stamatakis 2014) with the substitution model set as GTRCAT immediately after nonparametric bootstrapping was conducted with 1,000 replicates. The best fit-partitioning scheme for the ML analysis was identified with the Akaike information criterion using PartitionFinder v. 2.1.1 (Lanfear *et al*. 2017) with the "greedy" algorithm. BI and posterior probabilities were estimated using MrBayes v. 3.2.6 (Ronquist *et al*. 2012). Two independent runs of four Markov chains were conducted for 10 million generations, and the tree was sampled at every 100 generations. Parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut *et al*. 2018), and the first 50001 trees were discarded based on results.

Scanning electron microscopy. The specimen for scanning electron microscope (SEM) imaging was rinsed with TWEEN 20 (Model 036K00963; Sigma, USA) to remove residual debris, and then the sample was dehydrated with a graded ethanol series (30%, 50%, 70%, 80%, 95%, and 100% ethanol; 10 min each) and Hexamethyldisilazane (Sigma, St. Louis, USA) for 1 h. Dried sample was sputtered with platinum, and then observed with an SEM (Model Hitachi S-4300; Japan).

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Results

Order Amphipoda Latreille, 1816

Family Pseudocrangonyctidae Holsinger, 1989

Genus *Pseudocrangonyx* Akatsuka & Komai, 1922

***Pseudocrangonyx deureunensis* sp. nov.**

urn:lsid:
[New Korean name: deu-reu-ne-dong-gul-yeop-sae-u]
(Figs. 2A, 3–8)

Material examined. Holotype female (9.8 mm), NNIBRIV39838, collected from Deureune Cave (37°4.75′N, 128°59.36′E), Bonghwa-gun, Gyeongsangbuk-do, Korea, on 25 May 2018, by C.-W. Lee. Paratypes: 1 female (8.2 mm), NNIBRIV39835 (fig. 2A); 1 male (7.1 mm), NNIBRIV39839, collection data same as that for the holotype.

Diagnosis. Antennal sinus with rounded angle; eyes absent; pereonites 1–6 with short dorsal setae; dorsal margin of urosomite 1–3 with setae; pereonites 2–4 each with sternal gill; antenna 1 being 0.51 times as long as body length; antenna 2 with calceoli in both sexes; mandible palp article 3 longer than article 2; maxilla 1 outer plate with 7 serrate teeth; maxilla 2 inner plate with oblique inner row of 6 setae; gnathopods 1 and 2, carpi with serrate setae on posterodistal corners in both sexes; palmar margins of propodi of gnathopods 1 and 2 with 11–15 and 14–18 robust setae, respectively; pleopod peduncles lacking marginal setae, inner margin of inner rami with bifid setae; uropod 1 inner ramus 0.9 times as long as peduncle, inner and outer margins of inner ramus with 3 and 2 robust setae, respectively, basal part of inner ramus with 3 slender setae, outer ramus with 2 marginal robust setae; uropod 2 inner ramus 1.2 times as long as peduncle, outer ramus with 2 marginal robust setae; uropod 3 terminal article longer than adjacent robust setae; telson each lobe with 2 apical robust setae and 1 penicillate seta.

Description. Female holotype (NNIBRIV39838). Head (Fig. 3A) with short dorsal seta; rostrum short; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes absent. Pereonites 1–6 with short dorsal setae; dorsal margin of pereonite 7 with long setae. Dorsal margins of pleonites 1–3 with long setae (Fig. 3B). Posterior margin of epimeral plate 1 with 6 setae, posteroventral corner with seta; ventral and posterior margins of plate 2 with 3 and 4 setae, respectively, posteroventral corner with seta; ventral and posterior margins of plate 3 with 3 setae, respectively, posteroventral corner subquadrate with seta (Fig. 3B). Dorsal margin of urosomites 1–3 with setae. Anteroventral corner of urosomite 1 with seta, posteroventral corner of urosomite 3 with setae (Fig. 3B).

Antenna 1 (Fig. 3C) 0.51 times as long as body length, peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 3D) 2-articulate, more than shorter primary flagellar article 1, terminal article with 3 setae and 1 aesthetascs; primary flagellum 18-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 3E, F) 0.59 times as long as antenna 1; peduncular article 5 with 2 calceoli; flagellum 0.65 times as long as peduncular articles 4 and 5 combined, consisting of 9 articles, first 5 each with calceolus.

Upper lip (Fig. 3G) with rounded anterior margin, with fine setae. Mandibles (Fig. 3H–J) with left and right incisors with 5- dentate, respectively; left lacinia mobilis 5-dentate, right lacinia bifid, with many teeth; molar process triturative; accessory setal rows of left and right mandibles each with 6- pectinate setae, respectively; palp 3-articulate, article 3 with 5 A-, 15 D-, and 2 E-setae. Lower lip (Fig. 3K) with broad outer lobes with fine setae, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 3L) with inner and outer plates, and palp; inner plate subovate with 5 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 2 with weakly plumose robust seta apically, and 3 apical and 4 subapical robust setae. Maxilla 2 (Fig. 4A) with oblique inner row of 6 setae on inner plate. Maxilliped (Fig. 4B, C) with inner and outer plates, and palp; inner plate subrectangular with 4 apical robust setae; outer plate suboval with apical and subapical 4 robust setae, and some medial setae; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 4D, E) with subquadrate coxa, bearing seta on anterior dorsal margin and anterodistal corner, width 1.8 times as long as depth; basis thick and short, anterior margin bare, submargin with setae, posterior margin with 16 long setae; posterodistal corner of carpus with 2 serrate robust setae; propodus stout, subtriangular, palmar margin with 15 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 4F). Gnathopod 2 (Fig. 4G, H) with rounded coxa, with setae on its anterior to ventral margins, width 1.4 times as long as depth; basis slender with anterior margin bare, posterior margin with 8 long setae; posterodistal corner of carpus with 3 serrate robust setae; propodus slender than that of gnathopod 1, palmar margin with 18 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 4I). Pereopod 3 (Fig. 5A) with subquadrate coxa bearing setae on anterodistal corner to ventral margins, width 1.5 times as long as depth; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 0.9; posterior margin of dactylus with 2 setae (Fig. 5B). Pereopod 4 (Fig. 5C) with subquadrate coxa bearing setae on anterior margin, anterodistal corner and ventral margin, width 1.6 times as long as depth; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 1.0; posterior margin of dactylus with 2 setae (Fig. 5D). Pereopod 5 (Fig. 5E) with 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 : 1.0 : 1.1; anterior margin of dactylus with 2 setae (Fig. 5F). Pereopod 6 (Fig. 5G) anterior coxa broken; bearing setae on posterior lobe; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 1.1; anterior margin of dactylus with 2 setae (Fig. 5H). Pereopod 7 (Fig. 5I) with subtriangular coxa, ventral margin weakly concave, with seta on ventral margin and posterodistal corner; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 1.2 : 1.2; anterior margin of dactylus with 2 setae (Fig. 5J).

Sternal gill (Fig. 6A) on ventral surfaces of pereonites 2–4, respectively.

Coxal gills (Figs. 4G, 5A, C, E, G) on gnathopod 2 and pereopods 3–6.

Brood plates (Figs. 4G, 5A, C, E) slender with numerous setae, on gnathopod 2 and pereopods 3–5.

Peduncles of pleopods 1–3 (Fig. 6B, D, F) lacking marginal setae, outerdistal corners with 2 setae, respectively. Pleopods 1–3 with paired retinacula (Fig. 6C, E, G), inner ramus inner basal margin with 2, 2, and 1 bifid seta (clothes-pin seta), respectively; inner ramus of pleopods 1–3

10-, 9-, and 9-articulate, respectively; outer ramus of pleopods 1–3 11-, 10-, and 9-articulate, respectively.

Uropod 1 (Fig. 6H) with basofacial seta on peduncle; inner ramus 0.9 times as long as peduncle, inner and outer margins with 3 and 2 robust setae, respectively, basal part with 3 slender setae; outer ramus 0.7 times as long as inner, with 2 outer margin robust setae, inner margin bare. Uropod 2 (Fig. 6I) with inner ramus 1.2 times as long as peduncle, outer margin and marginal with 2 robust setae, respectively; outer ramus 0.7 times as long as inner ramus, inner margin bare and outer margin with 2 robust setae. Uropod 3 (Fig. 6J) with peduncle 0.24 times as long as outer ramus; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.2 times as long as proximal article, with 3 distal setae.

Telson (Fig. 6K) laterally straight, length 1.26 times as long as width, cleft for 36.5% of length, each telson lobe with 2 lateral penicillate setae, apical with 2 robust setae and penicillate seta.

Male paratype (NNIBRIV39839). Antenna 1 (Fig. 7A, B) 0.64 times as long as body length, primary flagellum 18-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 7C, D) 0.6 times as long as antenna 1; flagellum 0.61 times as long as peduncular articles 4 and 5 combined, consisting of 8 articles, articles 1–2 with calceolus.

Gnathopod 1 (Fig. 7E) carpus with serrate seta on posterodistal corner; palmar margin of propodus with 11 robust setae in 2 rows, some distally notched (Fig. 7F). Gnathopod 2 (Fig. 7G) carpus with 2 serrate setae on posterodistal corner; palmar margin of propodus with 14 robust setae in 2 rows, some distally notched (Fig. 7H).

Uropod 1 (Fig. 8A) with 2 basofacial setae on peduncle; inner ramus 0.76 times as long as peduncle; outer margin and marginal with 3 and 2 robust setae, respectively, basal part with 2 slender setae; outer ramus with 2 outer margin robust setae. Uropod 2 (Fig. 8B, C) with peduncle 0.82 times as long as inner ramus; inner ramus 1.4 times as long as outer ramus, distal part with 2 serrate, 4 simple robust setae. Uropod 3 (Fig. 8D, E) with outer ramus terminal article 0.2 times as long as proximal article.

Telson (Fig. 8F) length 1.2 times as long as width, cleft for 39.1% of length.

Distribution. Known only from the type locality.

Etymology. The specific name is an adjective derived from the name of the cave where the new species inhabit.

Remarks. *Pseudocrangonyx deureunensis* **sp. nov.** is morphologically most similar to *P. joolaei* Lee *et al*., 2020 in having 1) eyes completely absent, 2) pereonites 1–6 with short dorsal setae, 3) ventral surface of pereonites 2–4 present sternal gill, 4) antenna 2 with calceoli in both sexes, 5) maxilla 1 outer plate with 7 serrate teeth, and 6) inner rami of pleopods with bifid setae on inner margin. The new species can be clearly distinguished from *P. joolaei* by the following features (features of *P. joolaei* in parentheses): 1) urosomite 3 with (without) dorsal setae, 2) antenna 1 longer (shorter) than as long as body length half, 3) carpi of male gnathopods 1 and 2 with 1–2 (with 3) serrate robust setae on posterodistal corner, 4) uropod 3 terminal article longer (shorter) than adjacent robust setae, and 5) telson with 2 (with 4) apical robust setae.

***Pseudocrangonyx kwangcheonseonensis* sp. nov.**
urn:lsid:
[New Korean name: kwang-cheon-seon-dong-gul-yeop-sae-u]
(Figs. 2B, 9–14)

Pseudocrangonyx asiaticus. —Uéno, 1966: 506–518 (in part), figs. 2–4, 5A–K.

Material examined. Holotype female (10.6 mm), NNIBRIV35120, collected from Kwangcheonseon Cave (37°31.11′N, 128°27.05′E), Pyeongchang-gun, Gangwon-do, Korea, on 28 February 2017, by Y. G. Choi. Paratypes: 1 male (7.8 mm), NNIBRIV39840; 1 male (7.1 mm), NNIBRIV39841, collection data same as that for the holotype.

Diagnosis. Female larger than male; antennal sinus with rounded angle; eyes absent; pereonites 1–7 with dorsal setae; dorsal margin of urosomite 3 lacking setae; pereonites 2–4 each with sternal gill; antenna 1 longer than half body length; antenna 2 with calceoli in both sexes; mandible palp article 3 longer than article 2; maxilla 1 inner plate with 8 plumose setae; gnathopods 1 and 2, carpi with serrate setae on posterodistal corners in both sexes; palmar margins of propodi of gnathopods 1 and 2 with 24–26 and 20–21 robust setae, respectively; pleopod peduncles lacking marginal setae, inner margin of inner rami with bifid setae; uropod 1, inner and outer margins of inner ramus with 4 and 3 robust setae, basal part of inner ramus with 3 slender setae, outer ramus with 3 outer marginal robust setae; uropod 3 terminal article shorter than adjacent robust setae; telson laterally concave and shallowly at the top.

Description. Female holotype (NNIBRIV35120). Head (Fig. 2B) with short dorsal setae; rostrum short; lateral cephalic lobe rounded; antennal sinus shallow with rounded angle; eyes absent. Pereonites 1–6 with short dorsal setae; dorsal margin of pereonite 7 with long setae. Dorsal margins of pleonites 1–3 with long setae (Fig. 2B). Posterior margin of epimeral plate 1 with 5 setae; ventral and posterior margins of plate 2 with 2 and 4 setae, respectively, posteroventral corner with seta; ventral and posterior margins of plate 3 with 3 setae, respectively, posteroventral corner with seta (Fig. 2B). Dorsal margin of urosomites 1–2 with setae, urosomite 3 lacking dorsal setae. Anteroventral corner of urosomite 1 with seta, posteroventral corner of urosomite 3 with setae (Fig. 2B).

Antenna 1 (Fig. 9A) 0.56 times as long as body length, peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 9B) 2-articulate, more than longer primary flagellar article 1, terminal article with 3 setae and 1 aesthetascs; primary flagellum 21-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 9C, D) 0.64 times as long as antenna 1; peduncular article 5 with 4 calceoli; flagellum 0.52 times as long as peduncular articles 4 and 5 combined, consisting of 8 articles, first 6 each with calceolus.

Upper lip (Fig. 9E) with rounded anterior margin, with fine setae. Mandibles (Fig. 9F–H) with left and right incisors with 6- and 5- dentate, respectively; left lacinia mobilis 5-dentate, right lacinia bifid, with many teeth; molar process triturative; accessory setal rows of left and right mandibles with 7- and 6- pectinate setae; palp 3-articulate, article 3 with 8 A-, 20 D-, and 5

E-setae. Lower lip (Fig. 9I) with broad outer lobes with fine setae, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 9J, K) with inner and outer plates, and palp; inner plate subovate with 8 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 2 with plumose robust seta apically. Maxilla 2 (Fig. 9L) with oblique inner row of 10 setae on inner plate. Maxilliped (Fig. 10A) with inner and outer plates, and palp; inner plate subrectangular with 4 apical robust setae; outer plate suboval with apical and subapical 6 robust setae, and some medial setae; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 10B, C) with subquadrate coxa, bearing seta on its anterior to ventral margins, width 1.7 times as long as depth; basis thick and short, anterior margin bare, posterior margin with 15 long setae; posterodistal corner of carpus with 2 serrate robust setae; propodus stout, subtriangular, palmar margin with 24 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 10D). Gnathopod 2 (Fig. 10E, F) with rounded coxa, with setae on its anterior to ventral margins, width 1.3 times as long as depth; basis slender with anterior margin bare, posterior margin with 16 long setae; posterodistal corner of carpus with 3 serrate robust setae; propodus slender than that of gnathopod 1, palmar margin with 21 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 10G). Pereopod 3 (Fig. 11A) with subquadrate coxa bearing setae on anterior margin to posteroventral corner, width 1.4 times as long as depth; basis posterior margin with 17 long setae; merus, carpus, and propodus in length ratio of 1.0 : 0.7 : 0.7; posterior margin of dactylus with 2 setae (Fig. 11B). Pereopod 4 (Fig. 11C) with subquadrate coxa bearing setae on anterior margin to posteroventral corner, width 1.6 times as long as depth; basis posterior margin with 12 long setae; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.7; posterior margin of dactylus with seta (Fig. 11D). Pereopod 5 (Fig. 11E) with 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 : 1.0 : 0.9; anterior margin of dactylus with 1 seta (Fig. 11F). Pereopod 6 (Fig. 11G) 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 : 1.0 : 0.9. Pereopod 7 (Fig. 11H) anterior coxa broken, ventral margin weakly concave, with setae on ventral margin and posterodistal corner; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 1.1 : 1.0; anterior margin of dactylus with 2 setae (Fig. 11I).

Sternal gill (Fig. 12A) on ventral surfaces of pereonites 2–4, respectively.

Coxal gills (Figs. 10E, 11A, C, E, G) on gnathopod 2 and pereopods 3–6.

Brood plates (Figs. 10E, 11A, C, E) slender with numerous setae, on gnathopod 2 and pereopods 3–5.

Peduncles of pleopods 1–3 (Fig. 12B, D, F) lacking marginal setae, outerdistal corners with 2, 4, and 1 seta, respectively. Pleopods 1–3 with paired retinacula (Fig. 12C, E, G), inner ramus inner basal margin with 3, 2, and 2 bifid seta (clothes-pin seta), respectively; inner ramus of pleopods 1–3 11-, 9-, and 9-articulate, respectively; outer ramus of pleopods 1–3 13-, 13-, and 10-articulate, respectively.

Uropod 1 (Fig. 12H) with basofacial seta on peduncle; inner ramus 0.76 times as long as peduncle, inner and outer margins with 4 and 3 robust setae, respectively, basal part with 3 slender setae; outer ramus 0.6 times as long as inner, with 3 outer marginal robust setae, inner margin bare. Uropod 2 (Fig. 12I) with inner ramus 1.1 times as long as peduncle, outer margin and marginal with 3 and 2 robust setae, respectively; outer ramus 0.7 times as long as inner ramus, inner margin bare and outer margin with 2 robust setae. Uropod 3 (Fig. 12J, K) with peduncle 0.29 times as long as outer ramus; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.07 times as long as proximal article, with 3 distal setae.

Telson (Fig. 12L) base laterally concave and shallowly at the top, length 1.33 times as long as width, cleft for 40.2% of length, each telson lobe with lateral penicillate setae, apical with 3 robust setae and 1 seta.

Male paratype (NNIBRIV39840). Antenna 1 (Fig. 13A, B) 0.54 times as long as body length, primary flagellum 18-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 13C) 0.6 times as long as antenna 1; flagellum 0.46 times as long as peduncular articles 4 and 5 combined, consisting of 7 articles, peduncular article 5 without calceoli and some flagellum with calceolus.

Gnathopod 1 (Fig. 13D, E) carpus with 2 serrate setae on posterodistal corner; palmar margin of propodus with 26 robust setae in 2 rows, some distally notched (Fig. 13F). Gnathopod 2 (Fig. 13G, H) carpus with 2 serrate setae on posterodistal corner; palmar margin of propodus with 20 robust setae in 2 rows, some distally notched (Fig. 13I).

Uropod 1 (Fig. 14A) with basofacial 1 seta on peduncle; inner ramus 0.81 times as long as peduncle; inner margin bare and outer margin with 3 robust setae, basal part with 2 slender setae; outer ramus with 1 marginal robust seta. Uropod 2 (Fig. 14B) with peduncle and inner ramus ratio 1.0 : 1.0; inner ramus 1.2 times as long as outer ramus, distal part with 5 serrate, 3 simple robust setae, 1 simple seta. Uropod 3 (Fig. 14C, D) with outer ramus terminal article 0.1 times as long as proximal article.

Telson (Fig. 14E) length 1.43 times as long as width, cleft for 45.7% of length.

Distribution. Known only from the type locality.

Etymology. The specific name is an adjective derived from the name of the cave where the new species inhabit.

Remarks. *Pseudocrangonyx kwangcheonseonensis* **sp. nov.** is morphologically most similar to *P. asiaticus* Uéno, 1934 in having 1) eyes completely absent, 2) sternal gill present 3) accessory flagellum of antenna 1 being as long as first article of primary flagellum, 4) antenna 2 longer than half of antenna 1 length, and 5) carpi of gnathopods 1 and 2 with serrate robust setae on posterodistal corner. The new species can be clearly distinguished from *P. asiaticus* by the following features (features of *P. asiaticus* in parentheses): 1) pereonites 1–7 with (without) short dorsal setae, 2) sternal gills present on pereonites 2–4 (pereonites 2–5), 3) maxilla 1 inner plate with 8 (with 4) plumose setae, 4) antenna 1 longer (shorter) than as long as body length half, and 5) inner ramus of uropod 1 with 3 (without) outer marginal robust setae.

***Pseudocrangonyx hwanseonensis* sp. nov.**
urn:lsid:
[New Korean name: Hwan-seon-dong-gul-yeop-sae-u]
(Figs. 2C, 15–20)

Pseudocrangonyx asiaticus. —Uéno, 1966: 506–518 (in part), figs. 5O, 7E.

Material examined. Holotype female (7.5 mm), NNIBRIV35118, collected from Hwanseon Cave (37°19.52′N, 129°1.02′E), Samcheok-si, Gangwon-do, Korea, on 20 October 2018, by C. -W. Lee. Paratypes: 1 female (7.7 mm), NNIBRIV39836; 1 male (6.3 mm), NNIBRIV39837, collection data same as that for the holotype.

Diagnosis. Female larger than male; antennal sinus with rounded angle; eyes absent; pereonites 1–6 without short dorsal setae; dorsal margin of urosomite 3 lacking setae; pereonites 2–4 each with 1 pair sternal gill; antenna 1 0.53 times as long as body length; antenna 2 with calceoli in both sexes; mandible palp article 3 longer than article 2; maxilla 1 inner plate with 4 plumose setae; maxilla 2 inner plate with oblique inner row of 6 setae; gnathopods 1 and 2, carpi with serrate setae on posterodistal corners in both sexes; palmar margins of propodi of gnathopods 1 and 2 with 13 and 13–15 robust setae, respectively; pleopod peduncles lacking marginal setae, inner margin of inner rami with bifid setae; uropod 1 inner ramus 0.86 times as long as peduncle, inner and outer margins of inner ramus with 3 and 1 robust setae, respectively, basal part of inner ramus with 3 slender setae, outer ramus with 2 marginal robust setae; uropod 2 inner ramus 1.2 times as long as peduncle; inner and outer margins of inner ramus with 2 robust setae, respectively, outer ramus with 2 outer marginal robust setae; uropod 3 terminal article 0.15 time as long as length of proximal article; telson length 1.31 time as long as width, cleft for 36.8%.

Description. Female holotype (NNIBRIV35118). Head (Fig. 2C) without dorsal setae; rostrum short; lateral cephalic lobe rounded; antennal sinus shallow with rounded angle; eyes absent. Pereonites 1–6 without short dorsal setae; dorsal margin of pereonite 7 with long setae. Dorsal margins of pleonites 1–3 with long setae (Fig. 2C). Ventral and posterior margins of epimeral plate 1 with 1 and 5 setae, respectively, posteroventral corner with 1 seta; ventral and posterior margins of plate 2 with 4 and 5 setae, respectively, posteroventral corner with 1 seta; ventral and posterior margins of plate 3 with 4 setae, respectively, posteroventral corner with 1 seta (Fig. 2C). Dorsal margin of urosomites 1–2 with setae, urosomite 3 lacking dorsal setae. Anteroventral corner of urosomite 1 with 1 seta, posteroventral corner of urosomite 3 with setae (Fig. 2C).

Antenna 1 (Fig. 15A) 0.53 times as long as body length, peduncular articles 1–3 in length ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 15B) 2-articulate, more than shorter primary flagellar article 1, terminal article with 3 setae and 1 aesthetascs; primary flagellum 16-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 15C, D) 0.58 times as long as antenna 1; peduncular article 5 with 2 calceoli; flagellum 0.53 times as long as peduncular articles 4 and 5 combined, consisting of 7 articles, first 3 each with calceolus.

Upper lip (Fig. 15E) with rounded anterior margin, with fine setae. Mandibles (Fig. 15F, G) with left and right incisors with 5-dentate, respectively; left lacinia mobilis 5-dentate, right lacinia bifid, with many teeth; molar process triturative; accessory setal rows of left and right mandibles with 5- and 4- pectinate setae; palp 3-articulate, article 3 with 5 A-, 12 D-, and 4 E-setae. Lower lip (Fig. 15H) with broad outer lobes with fine setae, mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 15I) with inner and outer plates, and palp; inner plate subovate with 4 plumose setae; outer plate subrectangular with 7 serrate teeth apically; palp 2-articulate, longer than outer plate, article 2 with plumose robust seta apically. Maxilla 2 (Fig. 15J) with slender outer plate; oblique inner row of 6 setae on inner plate. Maxilliped (Fig. 16A) with inner and outer plates, and palp; inner plate subrectangular with 6 apical robust setae; outer plate suboval with apical and subapical 3 robust setae, and some medial setae; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

Gnathopod 1 (Fig. 16B, C) with subquadrate coxa, bearing setae on anterodistal corner to ventral margin, width 1.6 times as long as depth; basis thick and short, anterior margin with 1 seta and some medial setae, posterior margin with 10 long setae; posterodistal corner of carpus with 2 serrate robust setae; propodus stout, subtriangular, palmar margin with 13 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 16D). Gnathopod 2 (Fig. 16E, F) with subrounded coxa, with setae on its anterior to ventral corners, width 1.3 times as long as depth; basis slender with anterior and posterior margin with 1 seta and 11 long setae, respectively; posterodistal corner of carpus with 3 serrate robust setae; propodus slender than that of gnathopod 1, palmar margin with 15 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 16G). Pereopod 3 (Fig. 17A) with subquadrate coxa bearing setae on anterior margin to posteroventral corner, width 1.4 times as long as depth; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.8; posterior margin of dactylus with 2 setae (Fig. 17B). Pereopod 4 (Fig. 17C) with subquadrate coxa bearing setae on anterodistal to posteroventral corners, width 1.4 times as long as depth; basis posterior margin with 9 long setae; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 0.8; posterior margin of dactylus with 2 setae (Fig. 17D). Pereopod 5 (Fig. 17E) with 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 : 0.9 : 1.0; anterior margin of dactylus with 2 setae (Fig. 17F). Pereopod 6 (Fig. 17G) 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 : 0.9 : 1.0; anterior margin of dactylus with 2 setae (Fig. 17H). Pereopod 7 (Fig. 17I) with subtriangular coxa, ventral margin weakly concave, with setae on ventral margin and posterodistal corner; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 1.1; anterior margin of dactylus with 2 setae (Fig. 17J).

Sternal gill (Fig. 18A) on ventral surfaces of pereonites 2–4, paired, respectively.

Coxal gills (Figs. 16E, 17A, C, E, G) on gnathopod 2 and pereopods 3–6.

Brood plates (Figs. 16E, 17A, C, E) slender with numerous setae, on gnathopod 2 and pereopods 3–5.

Peduncles of pleopods 1–3 (Fig. 18B, D, F) lacking marginal setae, outerdistal corners with 2, 2, and 1 setae, respectively. Pleopods 1–3 with paired retinacula (Fig. 18C, E, G), inner ramus inner basal margin with 2, 2, and 1 bifid seta (clothes-pin seta), respectively; inner ramus of pleopods 1–3 9-, 8-, and 7-articulate, respectively; outer ramus of pleopods 1–3 10-, 9-, and 8-articulate, respectively.

Uropod 1 (Fig. 18H) with basofacial seta on peduncle; inner ramus 0.86 times as long as peduncle, inner and outer margins with 3 and 1 robust setae, respectively, basal part with 3 slender setae; outer ramus 0.76 times as long as inner, with 2 outer marginal robust setae, inner margin bare. Uropod 2 (Fig. 18I) with inner ramus 1.2 times as long as peduncle, outer margin and marginal with 2 robust setae, respectively; outer ramus 0.73 times as long as inner ramus, inner margin bare and outer margin with 2 robust setae. Uropod 3 (Fig. 18J, K) with peduncle 0.29 times as long as outer ramus; inner ramus absent; outer ramus 2-articulate, proximal article with robust setae, terminal article 0.15 times as long as proximal article, with 4 distal setae.

Telson (Fig. 18L) length 1.31 times as long as width, cleft for 36.8% of length, each telson lobe with lateral penicillate 2 setae, apical robust setae and 1 short penicillate seta.

Male paratype (NNIBRIV39837). Antenna 1 (Fig. 19A, B) 0.53 times as long as body length, primary flagellum 14-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 19C, D) 0.63 times as long as antenna 1; flagellum 0.58 times as long as peduncular articles 4 and 5 combined, consisting of 7 articles, first 2 each with calceolus.

Gnathopod 1 (Fig. 19E, F) carpus with 2 serrate setae on posterodistal corner; palmar margin of propodus with 13 robust setae in 2 rows, some distally notched (Fig. 19G). Gnathopod 2 (Fig. 19H, I) carpus with 3 serrate setae on posterodistal corner; palmar margin of propodus with 13 robust setae in 2 rows, some distally notched (Fig. 19J).

Uropod 1 (Fig. 20A) with basofacial seta on peduncle; inner ramus 0.79 times as long as peduncle; inner and outer margins with 3 and 1 robust setae, respectively, basal part with 3 slender setae; outer ramus with 2 margin robust setae. Uropod 2 (Fig. 20B) with peduncle 0.90 times as long as inner ramus; inner ramus 1.3 times as long as outer ramus, distal part with 6 serrate, 2 simple robust setae. Uropod 3 (Fig. 20C, D) with outer ramus terminal article 0.22 times as long as proximal article.

Telson (Fig. 20E) length 1.25 times as long as width, cleft for 40.0% of length.

Distribution. Known only from the type locality.

Etymology. The specific name is an adjective derived from the name of the cave where the new species ~~inhabit.~~

Remarks. *Pseudocrangonyx hwanseonensis* **sp. nov.** is morphologically similar to *P. asiaticus* Uéno, 1934 in having 1) eyes completely absent, 2) pereonites 1–6 without short dorsal setae, 3) urosomite 1 with ventral robust seta, 4) maxilla 1 inner plate with 4 plumose setae, 5) antenna 2 longer than half of antenna 1 length, and 6) carpi of gnathopods 1 and 2 with serrate robust setae on posterodistal corner. The new species can be clearly distinguished from *P. asiaticus* by the following features (features of *P. asiaticus* in parentheses): 1) sternal gills of 1 pair (single) present on each pereonites 2–4 (pereonites 2–5), 2) maxilla 1 outer plate with 7 (with 5) serrate teeth, 3) antenna 1 longer (shorter) than as long as body length half, and 4) uropod 3 terminal article shorter (longer) than adjacent robust setae.

Key to the species of Korean *Pseudocrangonyx*.

1 Sternal gills absent 2
– Sternal gills present 4
2 Female body size larger than 6.0 mm 3
– Female body size smaller than 6.0 mm .... *P. daejeonensis* Lee *et al*., 2018
3 Uropod 3 terminal article longer than adjacent robust setae ...... *P. minutus* Jung *et al*., 2020
– Uropod 3 terminal article shorter than adjacent robust setae ...... *P. wonkimi* Lee *et al*., 2020
4 Carpus of gnathopod with serrate robust setae on posterodistal corner 5
– Carpus of gnathopod without serrate robust setae on posterodistal corner .... *P. coreanus* Uéno, 1966
5 Sternal gills on pereonites 2 to 4 6
– Sternal gills on pereonites 2 to 5 9
6 Sternal gills total number 3 7
– Sternal gills total number 6 .... *P. hwanseonensis* **sp. nov.**
7 Urosomite 3 without dorsal setae 8
– Urosomite 3 with dorsal setae .... *P. deureunensis* **sp. nov.**
8 Accessory flagellum of antenna 1 exceeding first article of primary flagellum .... *P. kwangcheonseonensis* **sp. nov.**
– Accessory flagellum of antenna 1 not exceeding first article of primary flagellum .... *P. joolaei* Lee *et al*., 2020
9 Maxilla 1 inner plate with less than 7 plumose setae 10
– Maxilla 1 inner plate with 7 plumose setae .... *P. villosus* Jung *et al*., 2020
10 Telson cleft less than 40% for length 11
– Telson cleft more than 40% for length 12
11 Sternal gills on pereonites 2 to 5 (1+1+1+1) .... *P. asiaticus* Uéno, 1934
– Sternal gills on pereonites 2 to 5 (1+1+0+1) .... *P. crassus* Jung *et al*., 2020
12 Uropod 2 outer ramus with 2 inner marginal robust setae ...... *P. gracilipes* Jung *et al*., 2020
– Uropod 2 outer ramus without inner marginal robust setae ..... *P. concavus* Jung *et al*., 2020

Molecular Analyses. The uncorrected COI *p*-distance among the species of the genus *Pseudocrangonyx* in Korean caves is shown in Table 2; this divergence was calculated based on the 657 aligned positions from the data set. The range of interspecific variation was 11.7–17.0%. However, the maximum intraspecific variation was 0.2% within each species. In the phylogenetic analyses (Fig. 21), the topologies of the BI and ML trees were almost identical.

Results of the present analyses showed that the species of the genus *Pseudocrangonyx*, inhabiting individual caves, were distinct new species.

Discussion

Three new species described in this paper are similar to *P. asiaticus* Uéno, 1934 in morphology, and they share following characteristics: relatively large body size (about 8.0–10.0 mm), eyes completely absent, present of basal setae on urosomite 1, sternal gills present, carpi of gnathopods 1 and 2 with serrate robust setae on posterodistal corner. However, the three new species have following characteristics that distinguished them as distinct new species: 1) *P. deureunensis* **sp. nov.,** urosomite 3 with dorsal setae; 2) *P. kwangcheonseonensis* **sp. nov.,** maxilla 1 inner plate with 8 plumose setae, telson base laterally concave, and shallow at the top; and 3) *P. hwanseonensis* **sp. nov.,** sternal gills of 1 pair present on each pereonites 2–4. Furthermore, the genetic distance among the three species for the COI gene showed significant differentiation (12.5–13.4%) sufficient to designate the species as distinct new species, as revealed in a previous study (12–20%) of the genus *Pseudocrangonyx* (Zhao & Hou 2017).

Most species of geographically separated subterranean communities are likely to be of independent origin owing to their poor dispersal and small range (Trontelj *et al*. 2009; Trontelj *et al*. 2012). Likewise, our molecular phylogenetic analyses revealed that the species within the genus *Pseudocrangonyx*, indigenous to the Korean Peninsula caves formed monophyletic clade (Fig. 21: in gray box) and this result implies that the genus *Pseudocrangonyx* was inhabiting the groundwater environment, where dispersal is ~~not free relatively, such as that~~ in a cave, and might have independent origin in each habitat. A previous study (Lee *et al*. 2020) has shown that *P. joolaei* Lee *et al*., 2020 and *P. akatsukai* Tomikawa & Nakano, 2018 form a clade; however, the result of the present study showed clade of Korean cave *Pseudocrangonyx* and Japanese *P. akatsukai*. This means that the Korean cave *Pseudocrangonyx* species may form a single lineage with the true *P. asiaticus*, which is geographically adjacent and morphologically similar, rather than forming a clade with the Japanese species ~~through future phylogenetics study~~. Unfortunately, we could not obtain ~~the~~ molecular data ~~of~~ *P. asiaticus* Uéno, 1934, and it is not clear whether the true *P. asiaticus* inhabit the Korean Peninsula. Therefore, additional molecular data of *P. asiaticus* should be examined to confirm the ~~inhibition~~ of true *P. asiaticus* in the Korean Peninsula. It is also possible that the true species diversity of *Pseudocrangonyx* amphipods inhabiting the Korean Peninsula may be elucidated by additional molecular data.

Finally, the ~~existence of~~ pseudocrangonyctids is ~~related to biogeographic interest and~~ the origin and evolution of subterranean amphipod fauna in the Far East region (Sidorov & Holsinger 2007). Therefore, further studies on molecular phylogenetic analyses of *Pseudocrangonyx* are essential to enhance the understanding of species diversity and evolutionary history of the Far Eastern subterranean Crangonyctoidea species.

Conclusions

In summary, this is a study on three new species described from caves in Korea. Two new species ~~of them~~ were found from caves ~~treated as~~ ~~the~~ morphological variants of *P. asiaticus* Uéno, 1934~~,~~ the other new species was found from a new cave. All new species may receive a unique species status within the genus *Pseudocrangonyx* based on morphological examination and molecular analyses in this study. These results suggest that the species diversity of genus *Pseudocrangonyx* may be higher than what has been known in the Korean Peninsula so far. Although we have failed to obtain the molecular data of true *P. asiaticus*, if we obtain the data through future study, it is possible that the true species diversity may be elucidated of the subterranean amphipod *Pseudocrangonyx* in the Far East Asia including the Korean Peninsula.

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Table 1 (on next page)

Data used for molecular analyses. Sequences marked with an asterisk were obtained for the first time in the present study.

Table 1. Data used for molecular analyses. Sequences marked with an asterisk were obtained for the first time in the present study.

Species	Voucher or isolate	Locality or Country	GenBank No.	
			28S	COI
Genus *Pseudocrangonyx*				
P. deureunensis **sp. nov.**	NNIBRIV39838	Bonghwa, Korea	MW026427*	MW026424*
	NNIBRIV39835			MW026425*
	NNIBRIV39839			MW026426*
P. kwangcheonseonensis **sp. nov.**	NNIBRIV35120	Pyeongchang, Korea	MW026433*	MW026430*
	NNIBRIV39840			MW026431*
	NNIBRIV39841			MW026432*
P. hwanseonensis **sp. nov.**	NNIBRIV35118	Samcheok, Korea	MW026439*	MW026436*
	NNIBRIV39836			MW026437*
	NNIBRIV39837			MW026438*
P. wonkimi	NNIBRIV35119	Hampyeong, Korea	MT316536	MT316534
	NNIBRIV36158	Hampyeong, Korea		MT316535
P. joolaei	NNIBRIV21629	Goesan, Korea	LC467007	LC467001
	NNIBRIV21630	Goesan, Korea		LC467002
P. daejeonensis	NNIBRIV1	Daejeon, Korea	LC322136	LC322137
P. akatsukai	KUZ Z1967 (G1277)	Yamaguchi, Japan	LC171506	LC171507
P. komaii	KUZ Z1976 (G1297)	Yamaguchi, Japan	LC171541	LC171542
P. gudariensis	NSMT-Cr 24605	Aomori, Japan	LC171498	LC171499
P. yezonis	KUZ Z1970 (G1280)	Hokkaido, Japan	LC171518	LC171519
P. uenoi	KUZ Z1964 (G405)	Shiga, Japan	LC171491	LC171492
P. elegantulus	IZCAS I-A1602-2	China	KY436646	KY436647
P. holsingeri		Russian Far East	KJ871679	KF153111
P. korkishkoorum	B1	Russian Far East	KJ871678	KF153107
P. korkishkoorum	N1	Russian Far East	KJ871676	KF153105
P. tiunovi		Russian Far East	KJ871674	KF153110
P. febras		Russian Far East		KF153114
P. susanaensis		Russian Far East		KF153113
P. sympatricus		Russian Far East		KF153112
Outgroup Genus *Crangonyx*				
C. floridanus	G1322	Chiba, Japan	LC171549	LC171550

Table 2(on next page)

Intra and interspecific variation calculated from COI of Korean cave *Pseudocrangonyx*.

Table 2. Intra and interspecific variation calculated from COI of Korean cave *Pseudocrangonyx*.

Specific name	Intraspecific (%)	Interspecific (%)				
		1	2	3	4	5
P. deureunensis sp. nov.	0.2	-				
P. kwangcheonseonensis sp. nov.	0.2	13.2–13.4	-			
P. hwanseonensis sp. nov.	-	13.2–13.4	12.5–12.6	-		
P. wonkimi Lee *et al*., 2020	-	16.9–17.0	13.9–14.0	14.6	-	
P. joolaei Lee *et al*., 2020	0.2	15.2–15.4	14.8–15.1	11.7–11.9	14.9–15.1	-

Figure 1

The collection locality of the specimens examined in this study.

(A) Deureune cave; (B) Kwangcheonseon cave; (C) Hwanseon cave.


124°
126°
128°
130°
132°
42°
40°
38°
36°
34°
C H I N A
SEOUL
B
C
A
K O R E A
Daejeon
Daegu
Kwangju
Busan
Honshu
Hiroshima
J A P A N
Kyushu
Jejudo
0
100
200km

Figure 2

Habitus of three new species.

(A) Paratype of *Pseudocrangonyx deureunensis* sp. nov., lateral view; (B) Holotype of *Pseudocrangonyx Kwangcheonseonensis* sp. nov., lateral view; (C) Holotype of *Pseudocrangonyx Hwanseonensis* sp. nov., lateral view.

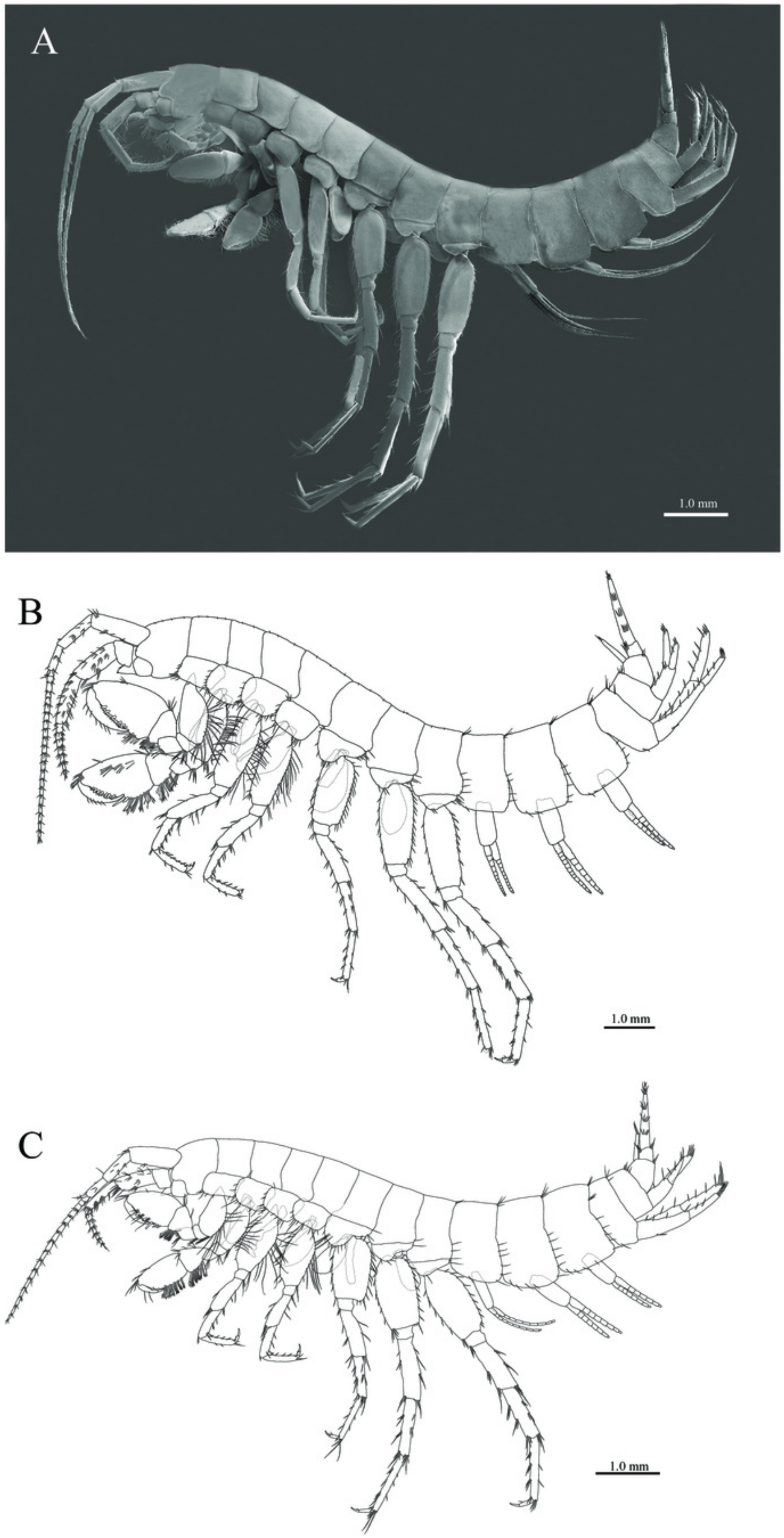
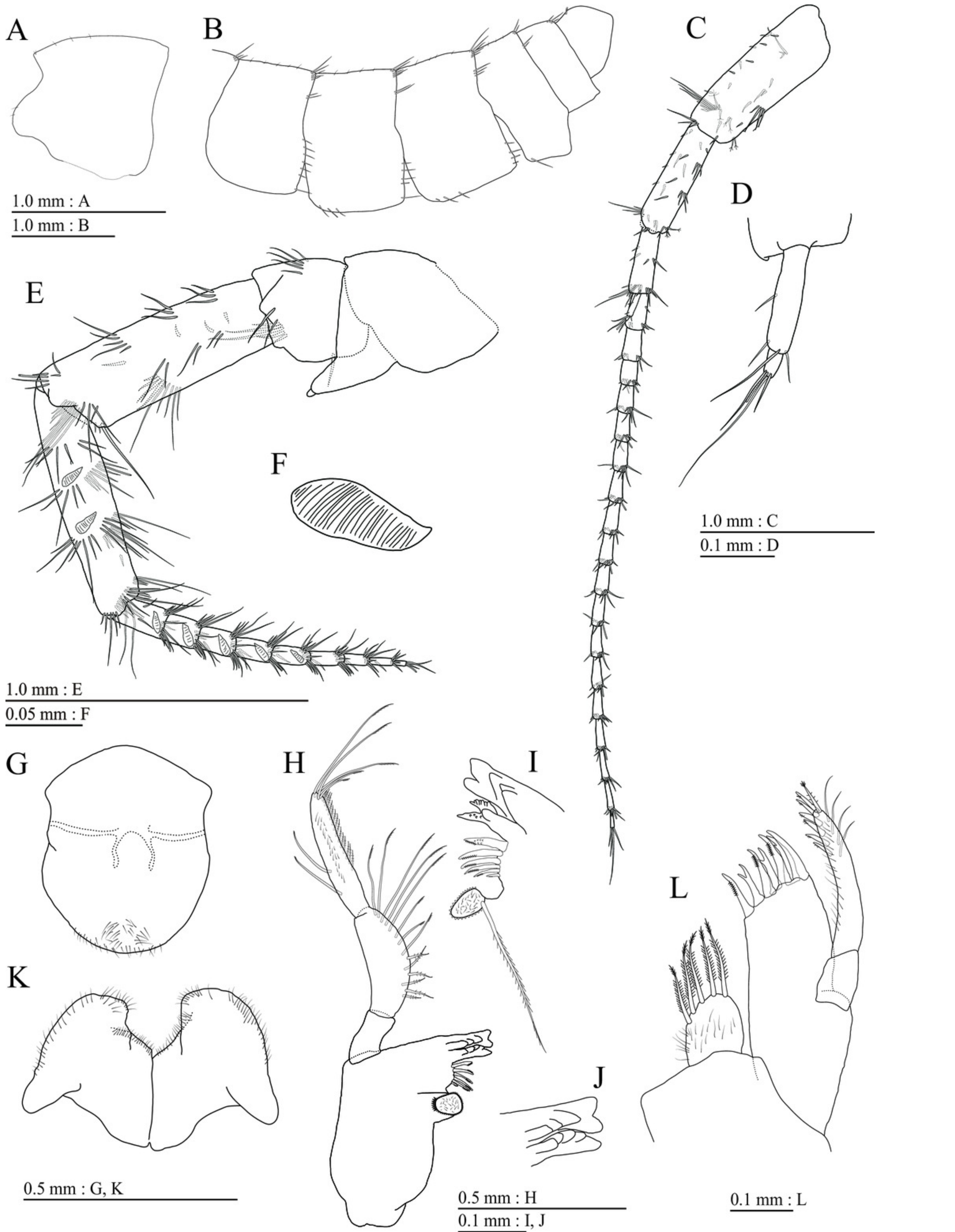

A
1.0 mm
B
1.0 mm
C
1.0 mm

Figure 3

Holotype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39838).

(A) Head, lateral view; (B) Epimeral plates 1–3 and urosomites 1–3, lateral view; (C) Antenna 1, medial view; (D) Accessory flagellum of antenna 1, medial view; (E) Antenna 2, medial view; (F) Calceolus of antenna 2, medial view; (G) Upper lip, posterior view; (H) Left mandible, medial view; (I) Incisor, lacinia mobilis, and molar process of right mandible, medial view; (J) Incisor and lacinia mobilis of left mandible, medial view; (K) Lower lip, ventral view; (L) Maxilla 1, dorsal view.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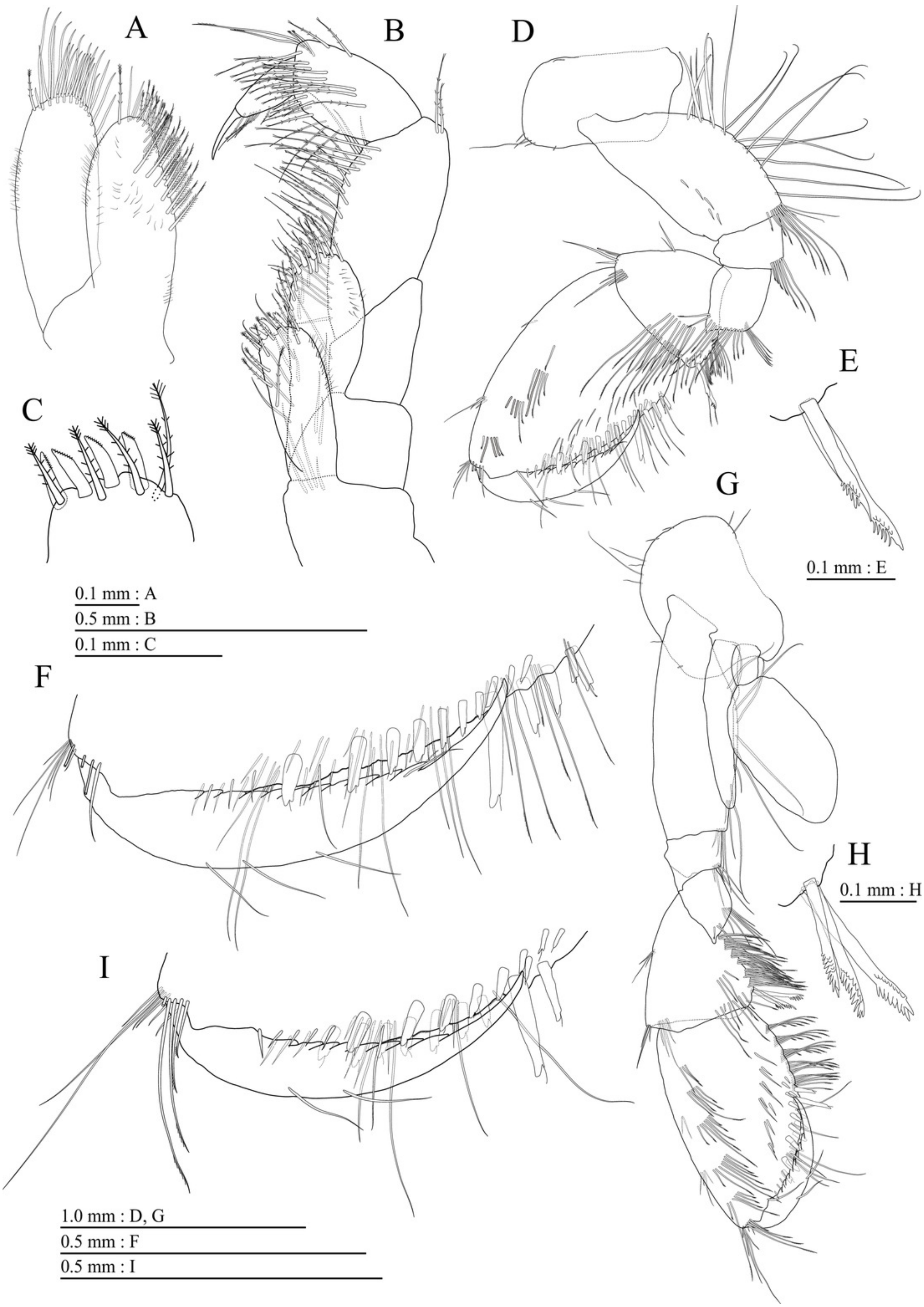
1.0 mm : A
1.0 mm : B
1.0 mm : C
0.1 mm : D
1.0 mm : E
0.05 mm : F
0.5 mm : G, K
0.5 mm : H
0.1 mm : I, J
0.1 mm : L

Figure 4

Holotype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39838).

(A) Maxilla 2, dorsal view; (B) Maxilliped, dorsal view; (C) Apical setae on inner plate of maxilliped, dorsal view; (D) Gnathopod 1, medial view; (E) Serrate setae on posterodistal corner of carpus of gnathopod 1, lateral view; (F) Palmar margin of propodus and dactylus of gnathopod 1, medial view; (G) Gnathopod 2, medial view; (H) Serrate setae on posterodistal corner of carpus of gnathopod 2, lateral view; (I) Palmar margin of propodus and dactylus of gnathopod 2, medial view.


A
B
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E
0.1 mm : E
G
0.1 mm : A
0.5 mm : B
0.1 mm : C
F
H
0.1 mm : H
I
1.0 mm : D, G
0.5 mm : F
0.5 mm : I

Figure 5

Holotype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39838).

(A) Pereopod 3, medial view; (B) Dactylus of pereopod 3, medial view; (C) Pereopod 4, medial view; (D) Dactylus of pereopod 4, medial view; (E) Pereopod 5, medial view; (F) Dactylus of pereopod 5, medial view; (G) Pereopod 6, medial view; (H) Dactylus of pereopod 6, medial view; (I) Pereopod 7, medial view; (J) Dactylus of pereopod 7, medial view.

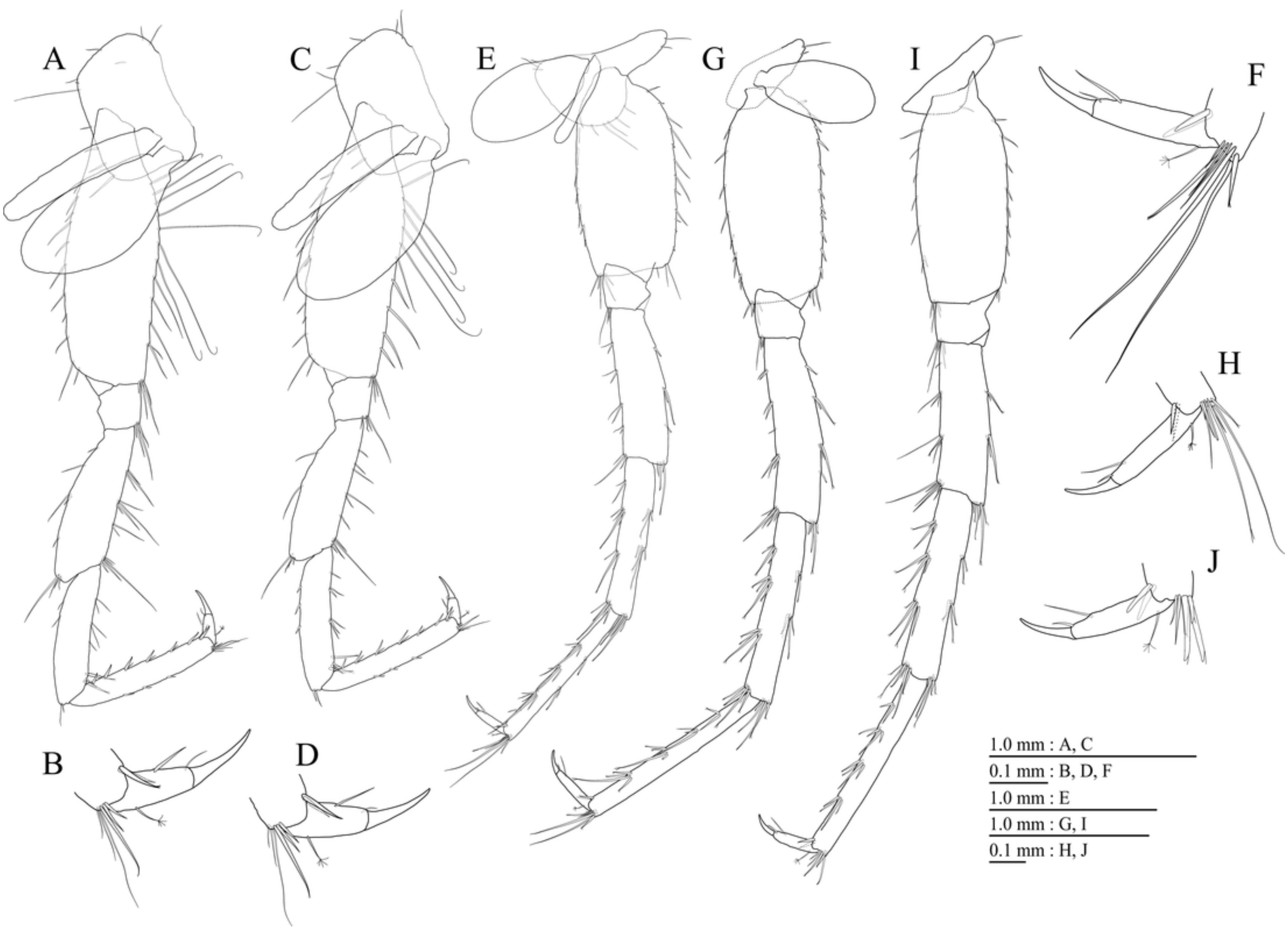
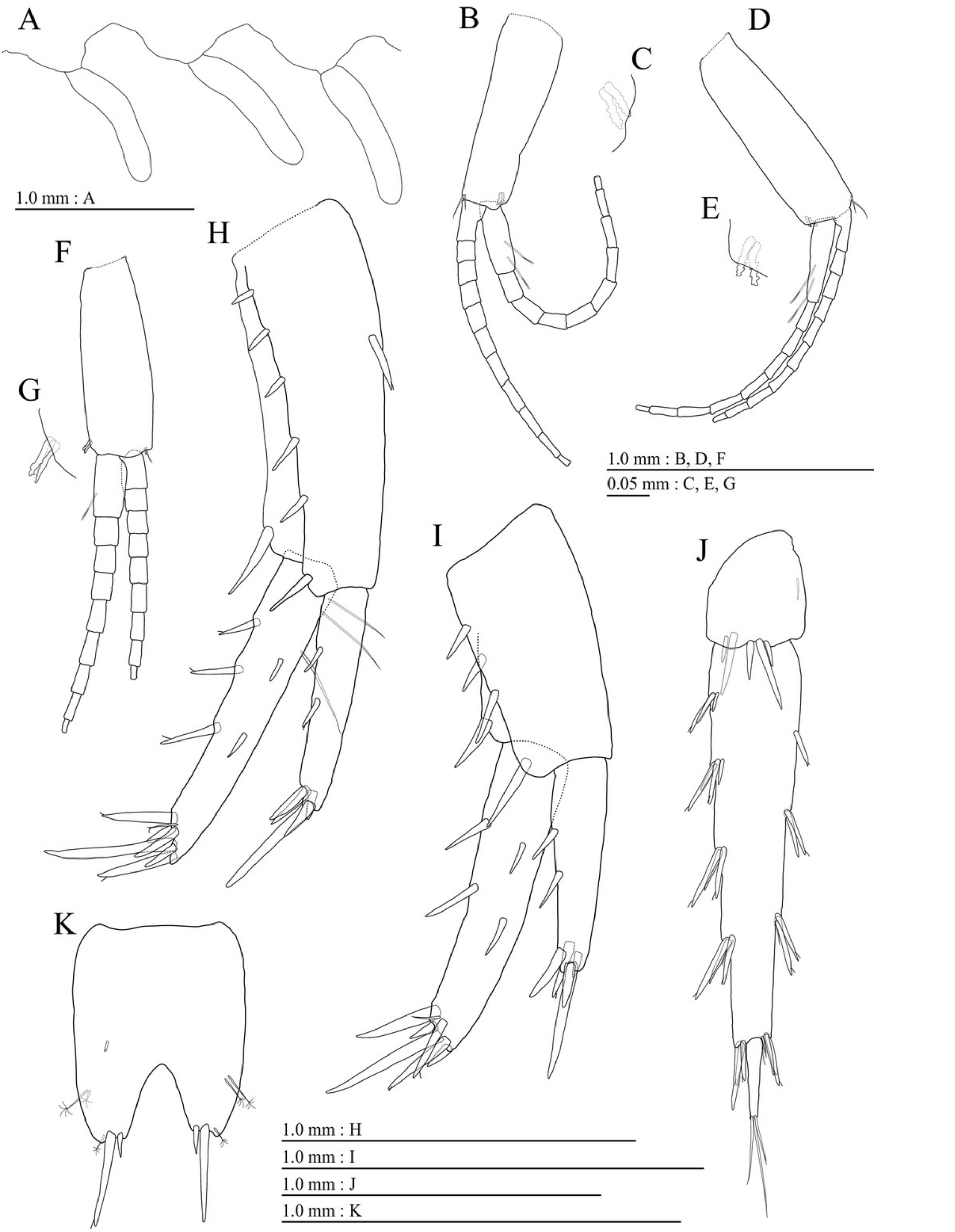


Figure 6

Holotype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39838).

(A) Sternal gills on pereonites 2–4, lateral view; (B) Pleopod 1, lateral view; (C) Retinacula on peduncle of pleopod 1, lateral view; (D) Pleopod 2, lateral view; (E) Retinacula on peduncle of pleopod 2, lateral view; (F) Pleopod 3, lateral view; (G) Retinacula on peduncle of pleopod 3, lateral view; (H) Uropod 1, dorsal view; (I) Uropod 2, dorsal view; (J) Uropod 3, dorsal view; (K) Telson, dorsal view.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1.0 mm : B, D, F
0.05 mm : C, E, G
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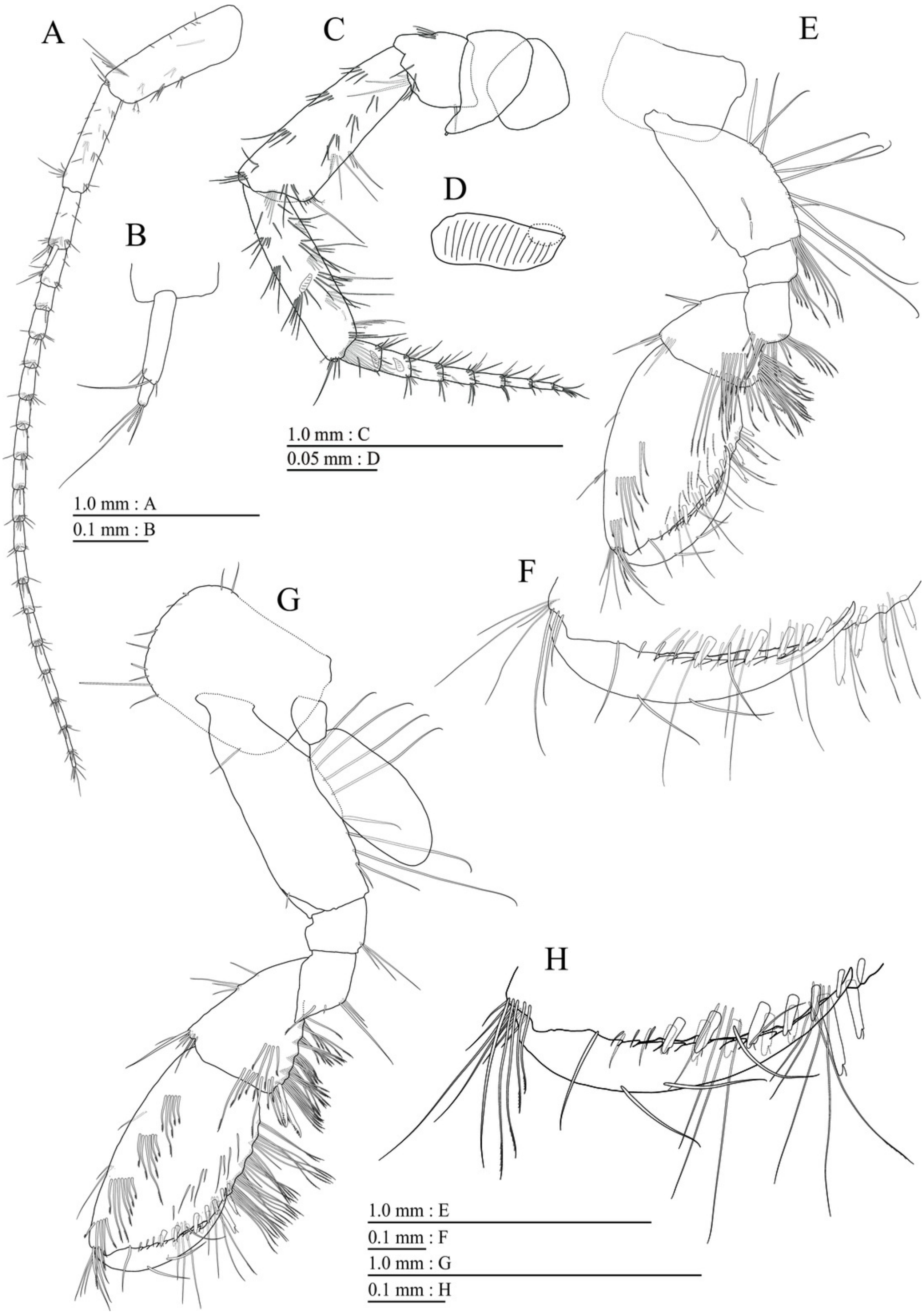
I
J
K
1.0 mm : H
1.0 mm : I
1.0 mm : J
1.0 mm : K

Figure 7

Paratype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39839).

(A) Antenna 1, medial view; (B) Accessory flagellum of antenna 1, medial view; (C) Antenna 2, medial view; (D) Calceolus of antenna 2, medial view; (E) Gnathopod 1, medial view; (F) Palmar margin of propodus and dactylus of gnathopod 1, medial view; (G) Gnathopod 2, medial view; (H) Palmar margin of propodus and dactylus of gnathopod 2, medial view.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1.0 mm : A
0.1 mm : B
1.0 mm : C
0.05 mm : D
1.0 mm : E
0.1 mm : F
1.0 mm : G
0.1 mm : H

Figure 8

Paratype of *Pseudocrangonyx deureunensis* sp. nov. (NNIBRIV39839).

(A) Uropod 1, dorsal view; (B) Uropod 2, ventral view; (C) Distal robust seta on inner ramus of uropod 2, ventral view; (D) Uropod 3, dorsal view; (E) Terminal article of uropod 3, dorsal view; (F) Telson, dorsal view.

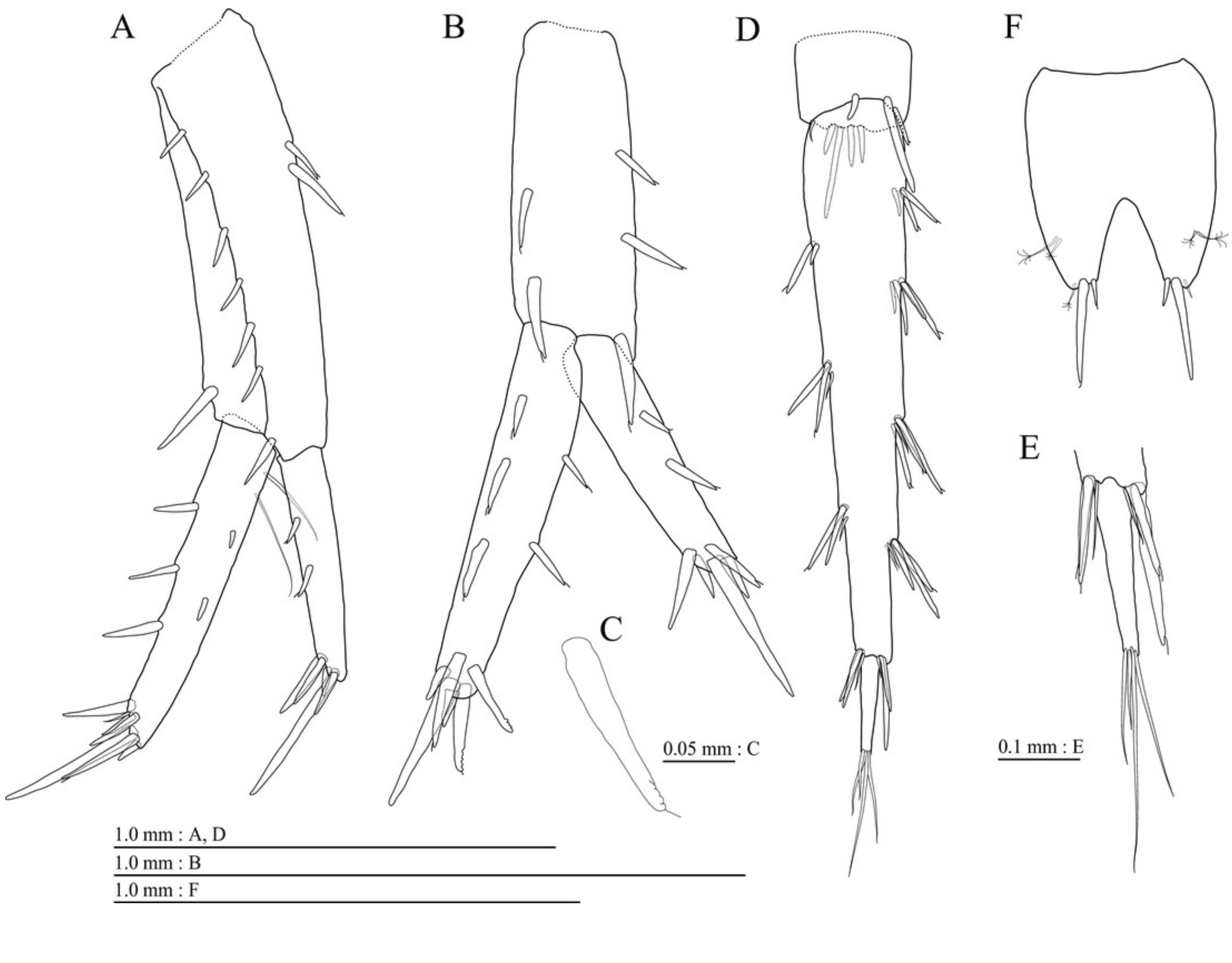
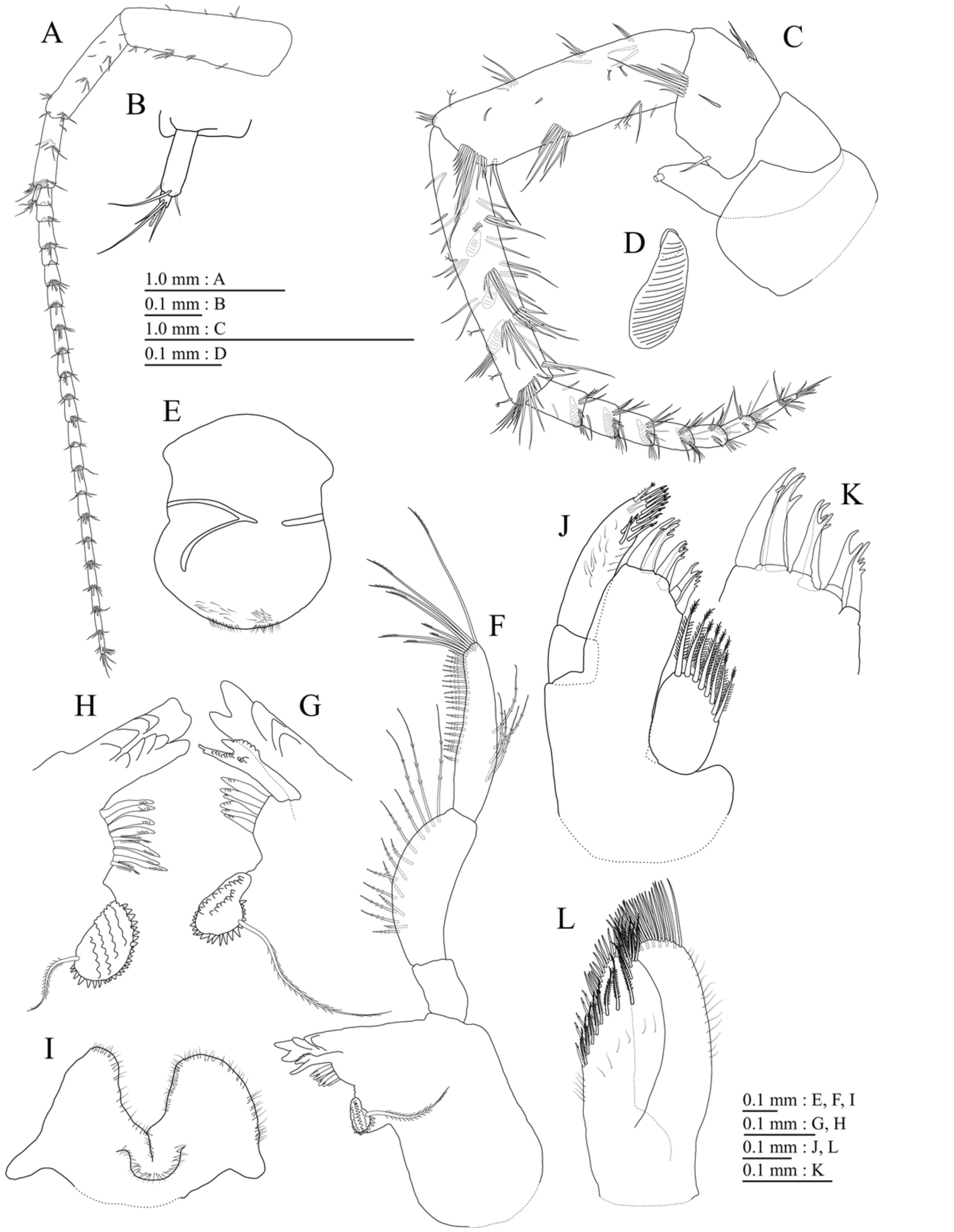


Figure 9

Holotype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV35120).

(A) Antenna 1, medial view; (B) Accessory flagellum of antenna 1, medial view; (C) Antenna 2, lateral view; (D) Calceolus of antenna 2, lateral view; (E) Upper lip, posterior view; (F) Right mandible, medial view; (G) Incisor, lacinia mobilis, and molar process of right mandible, medial view; (H) Incisor, lacinia mobilis, and molar process of left mandible, medial view; (I) Lower lip, dorsal view; (J) Maxilla 1, dorsal view; (K) Apical robust setae on outer plate of maxilla 1, dorsal view; (L) Maxilla 2, dorsal view.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1.0 mm : C
0.1 mm : D
0.1 mm : E, F, I
0.1 mm : G, H
0.1 mm : J, L
0.1 mm : K

Figure 10

Holotype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV35120).

(A) Maxilliped, dorsal view; (B) Gnathopod 1, medial view; (C) Serrate setae on posterodistal corner of carpus of gnathopod 1, lateral view; (D) Palmar margin of propodus and dactylus of gnathopod 1, medial view; (E) Gnathopod 2, medial view; (F) Serrate setae on posterodistal corner of carpus of gnathopod 2, lateral view; (G) Palmar margin of propodus and dactylus of gnathopod 2, medial view.

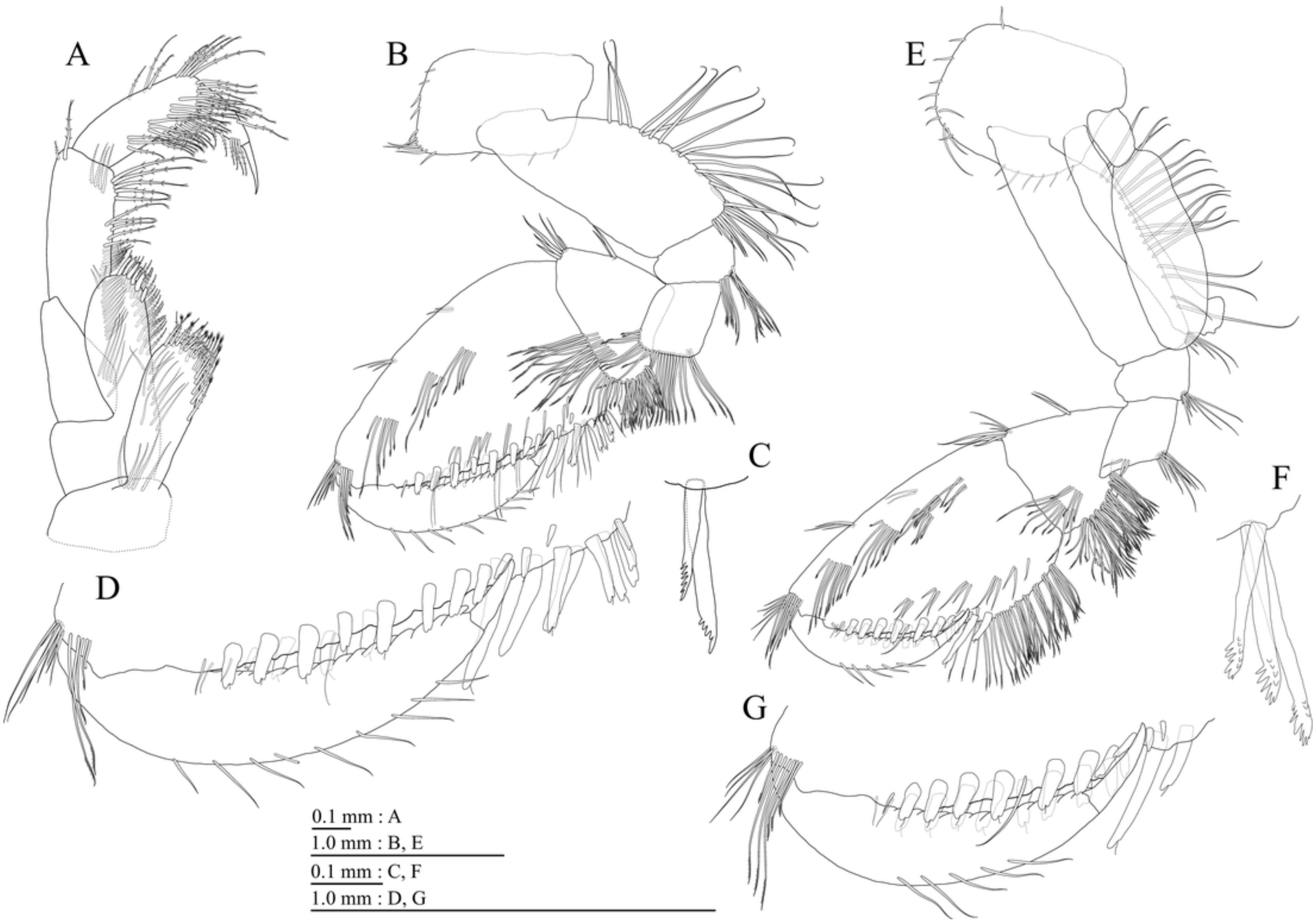


Figure 11

Holotype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV35120).

(A) Pereopod 3, medial view; (B) Dactylus of pereopod 3, medial view; (C) Pereopod 4, medial view; (D) Dactylus of pereopod 4, medial view; (E) Pereopod 5, medial view; (F) Dactylus of pereopod 5, medial view; (G) Pereopod 6, medial view; (H) Pereopod 7, medial view; (I) Dactylus of pereopod 7, medial view.

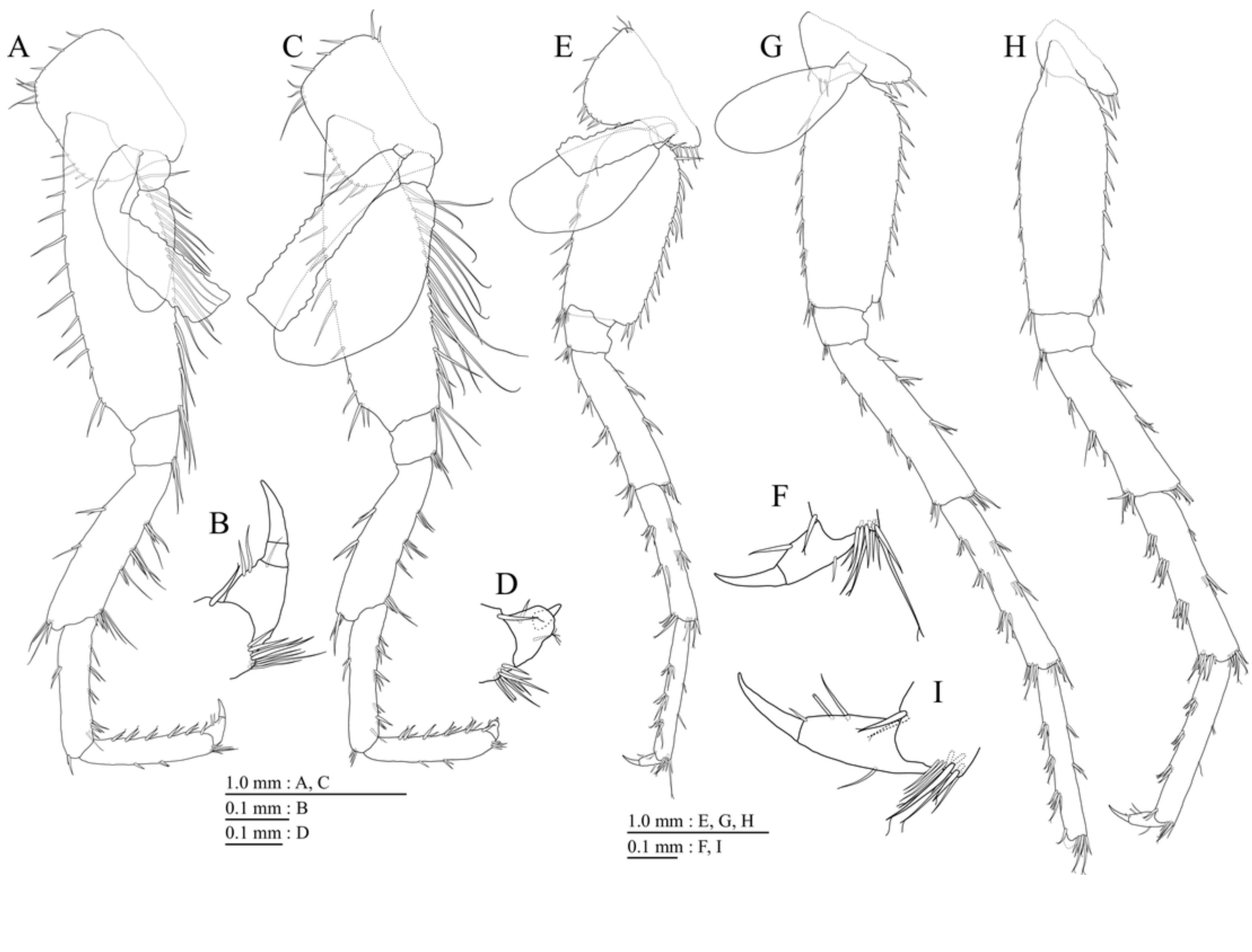
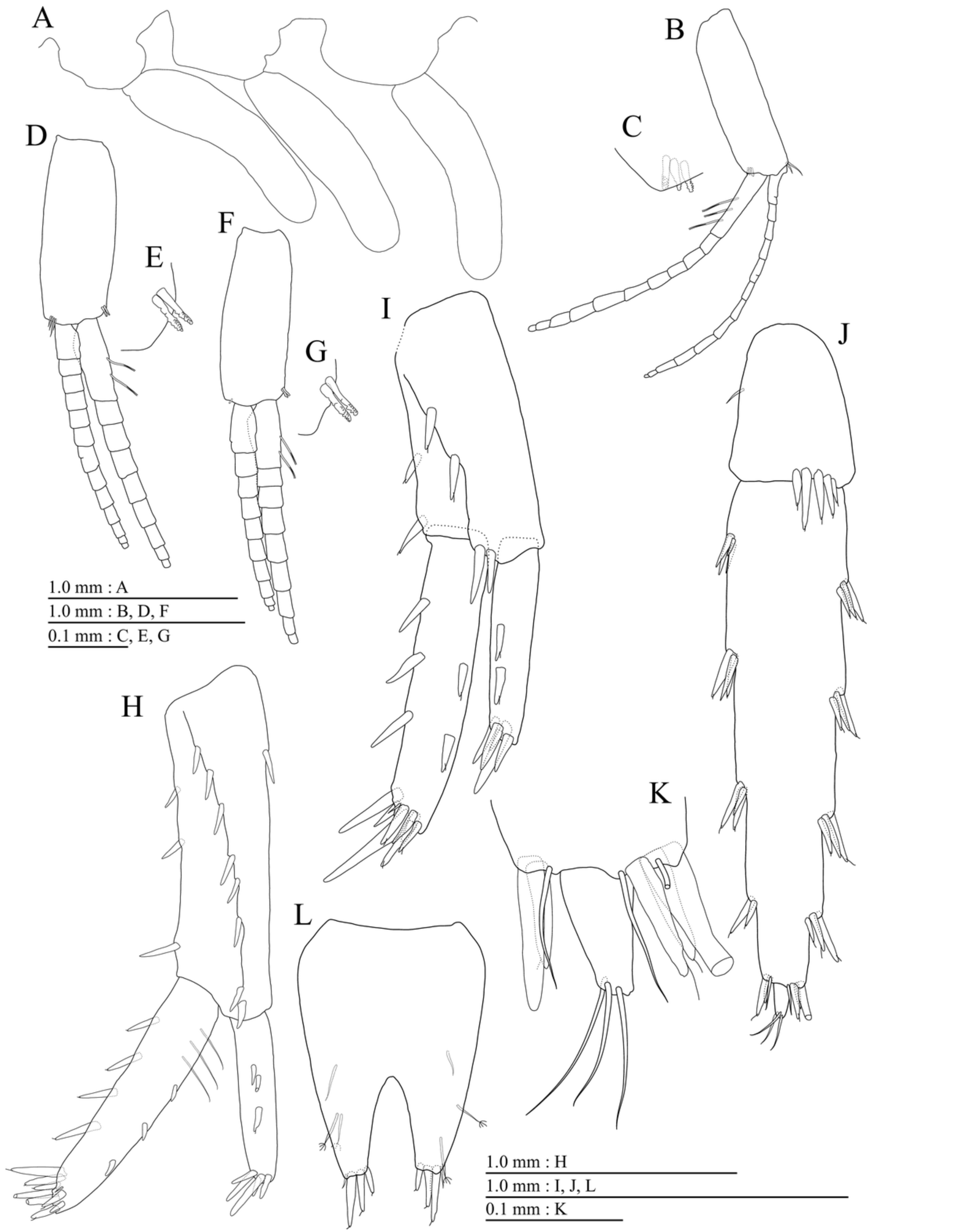


Figure 12

Holotype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV35120).

(A) Sternal gills on pereonites 2–4, lateral view; (B) Pleopod 1, lateral view; (C) Retinacula on peduncle of pleopod 1, lateral view; (D) Pleopod 2, lateral view; (E) Retinacula on peduncle of pleopod 2, lateral view; (F) Pleopod 3, lateral view; (G) Retinacula on peduncle of pleopod 3, lateral view; (H) Uropod 1, dorsal view; (I) Uropod 2, dorsal view; (J) Uropod 3, dorsal view; (K) Terminal article of uropod 3, dorsal view; (L) Telson, ventral view.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1.0 mm : A
1.0 mm : B, D, F
0.1 mm : C, E, G
1.0 mm : H
1.0 mm : I, J, L
0.1 mm : K

Figure 13

Paratype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV39840).

(A) Antenna 1, lateral view; (B) Accessory flagellum of antenna 1, lateral view; (C) Antenna 2, medial view; (D) Gnathopod 1, medial view; (E) Serrate setae on posterodistal corner of carpus of gnathopod 1, lateral view; (F) Palmar margin of propodus and dactylus of gnathopod 1, medial view; (G) Gnathopod 2, medial view; (H) Serrate setae on posterodistal corner of carpus of gnathopod 2, lateral view; (I) Palmar margin of propodus and dactylus of gnathopod 2, medial view.

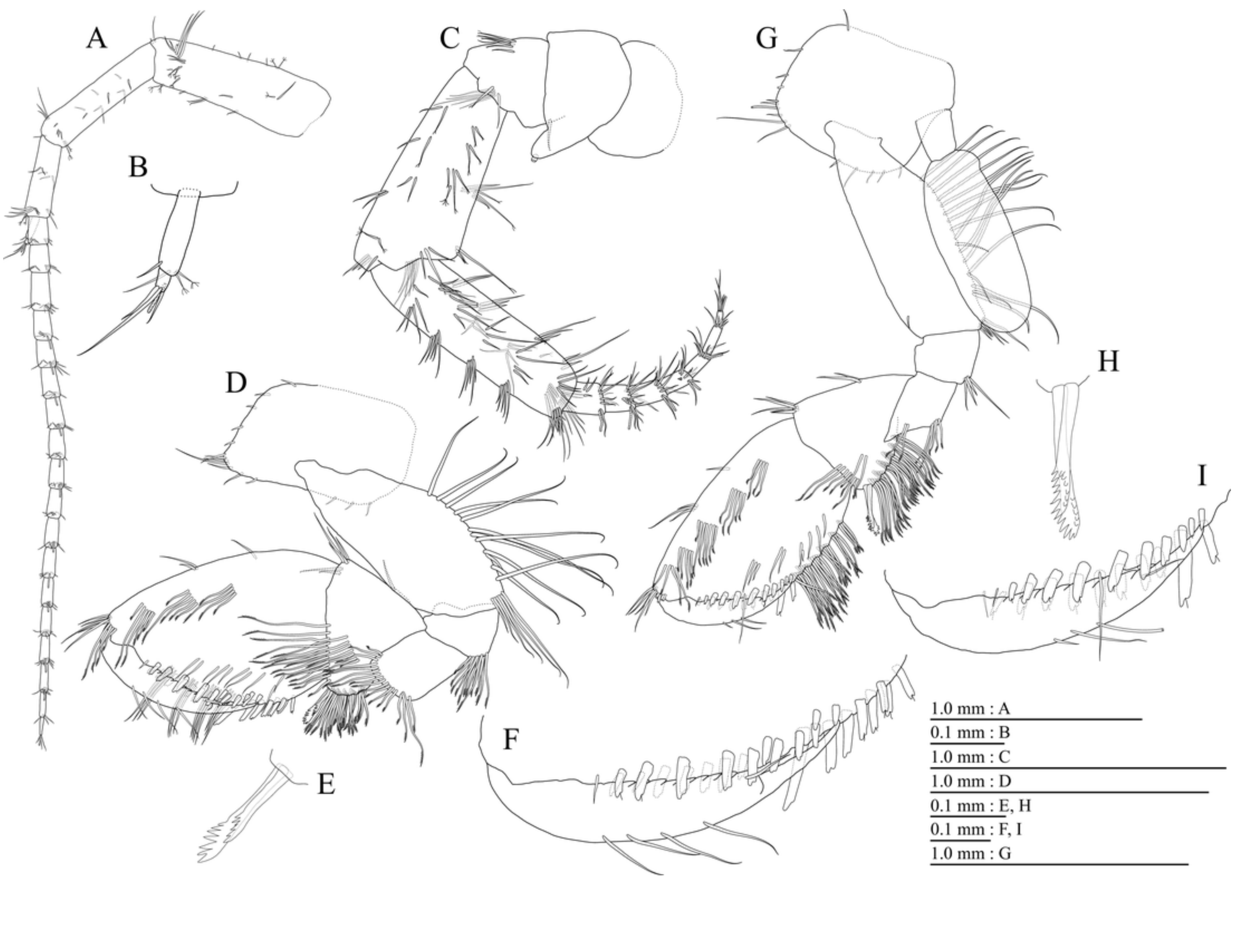


Figure 14

Paratype of *Pseudocrangonyx kwangcheonseonensis* sp. nov. (NNIBRIV39840).

(A) Uropod 1, dorsal view; (B) Uropod 2, dorsal view; (C) Uropod 3, dorsal view; (D) Terminal article of uropod 3, dorsal view; (E) Telson, dorsal view.

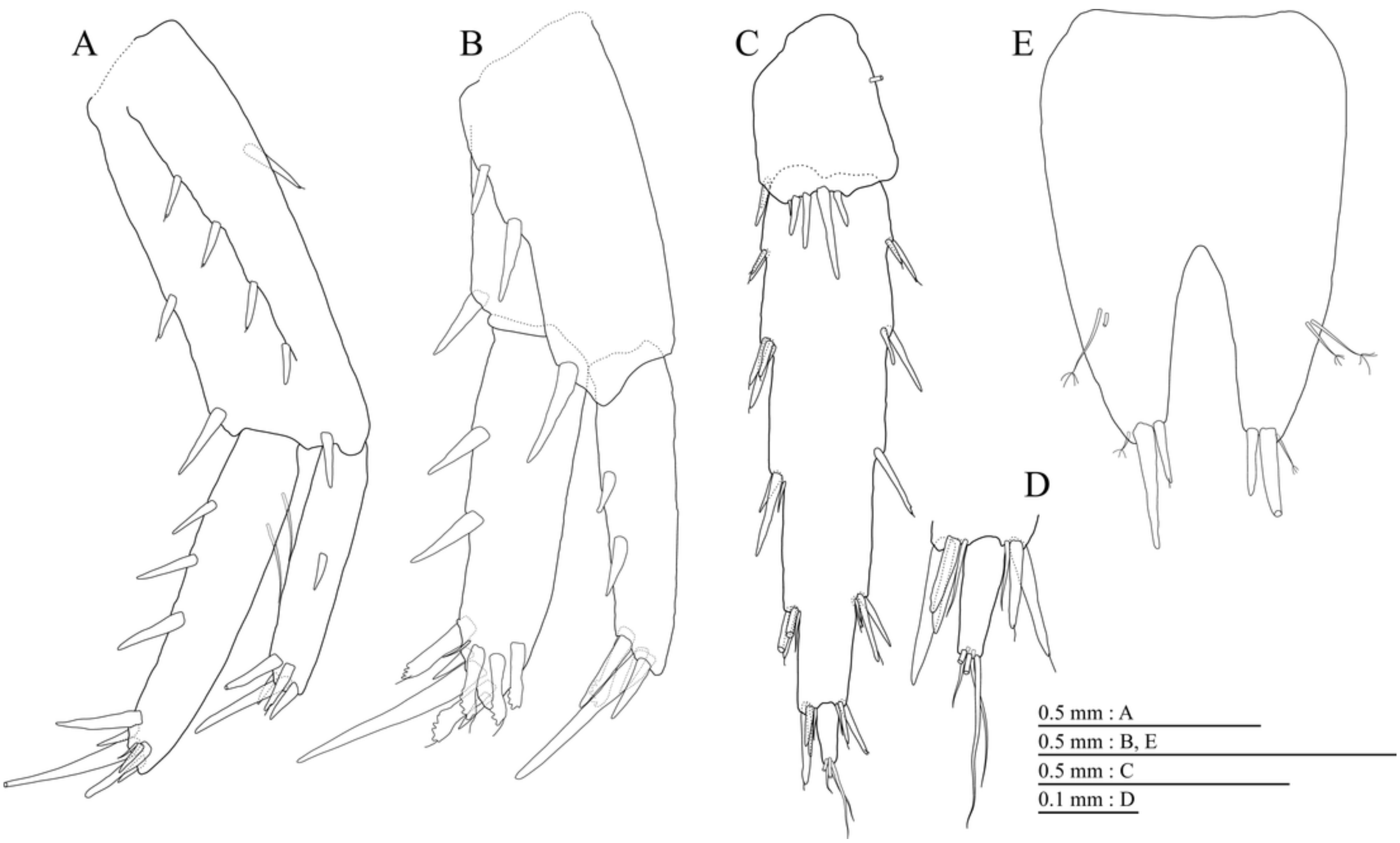
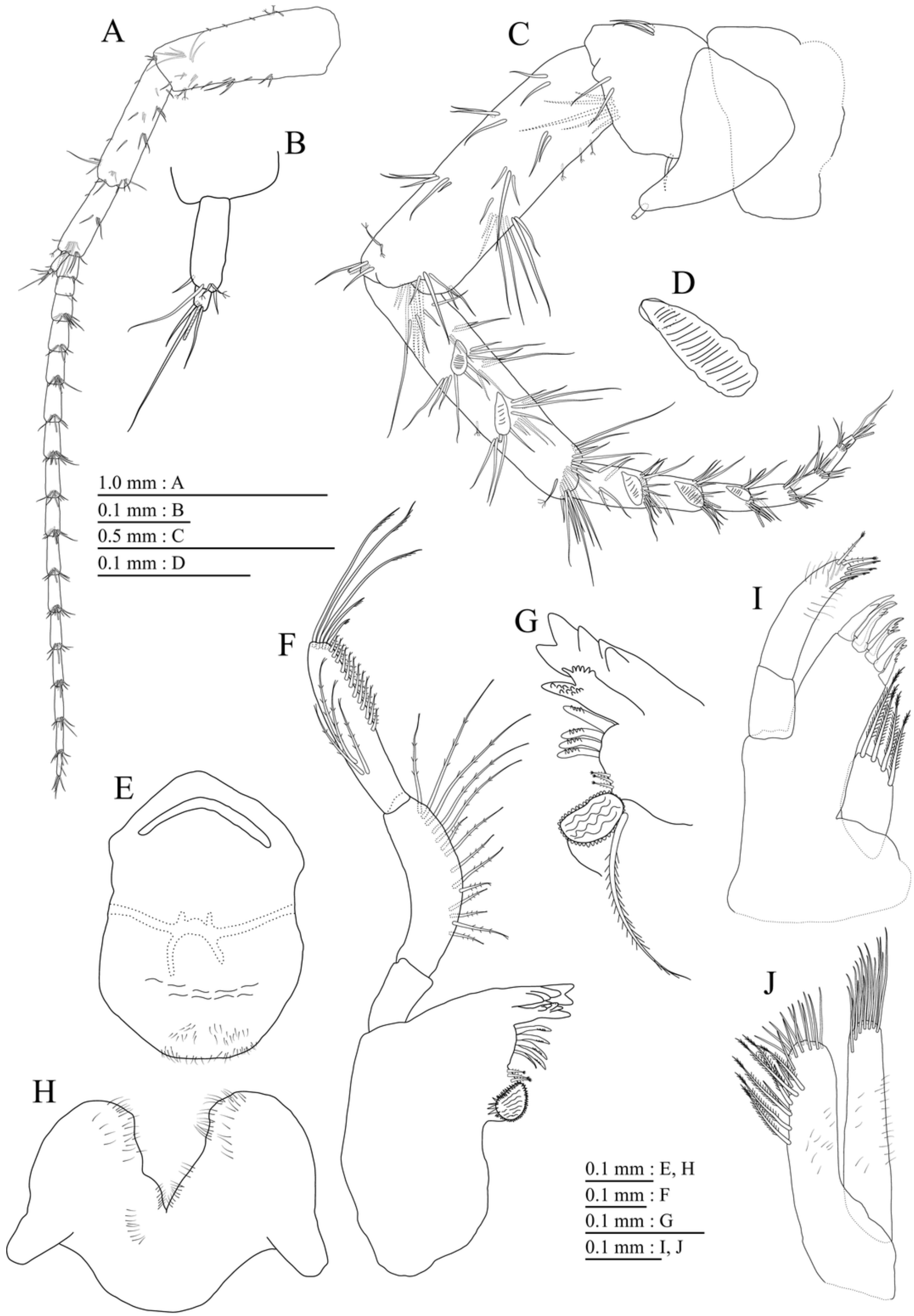


Figure 15

Holotype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV35118).

(A) Antenna 1, medial view; (B) Accessory flagellum of antenna 1, medial view; (C) Antenna 2, medial view; (D) Calceolus of antenna 2, medial view; (E) Upper lip, posterior view; (F) Left mandible, medial view; (G) Incisor, lacinia mobilis, and molar process of right mandible, medial view; (H) Lower lip, ventral view; (I) Maxilla 1, dorsal view; (J) Maxilla 2, dorsal view.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1.0 mm : A
0.1 mm : B
0.5 mm : C
0.1 mm : D
0.1 mm : E, H
0.1 mm : F
0.1 mm : G
0.1 mm : I, J

Figure 16

Holotype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV35118).

(A) Maxilliped, dorsal view; (B) Gnathopod 1, lateral view; (C) Serrate setae on posterodistal corner of carpus of gnathopod 1, lateral view; (D) Palmar margin of propodus and dactylus of gnathopod 1, lateral view; (E) Gnathopod 2, lateral view; (F) Serrate setae on posterodistal corner of carpus of gnathopod 2, lateral view; (G) Palmar margin of propodus and dactylus of gnathopod 2, lateral view.

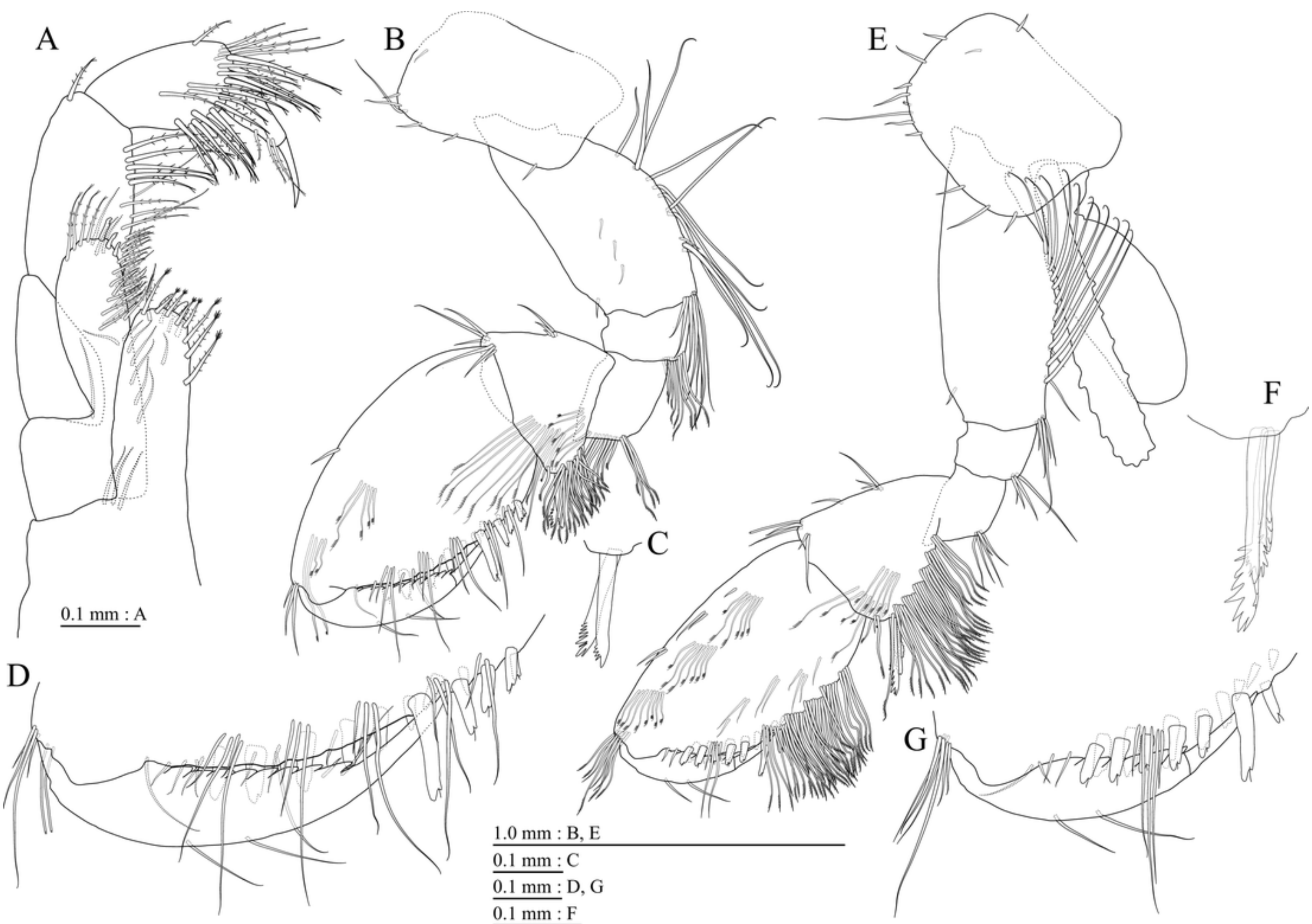


Figure 17

Holotype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV35118).

(A) Pereopod 3, lateral view; (B) Dactylus of pereopod 3, lateral view; (C) Pereopod 4, lateral view; (D) Dactylus of pereopod 4, lateral view; (E) Pereopod 5, lateral view; (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.

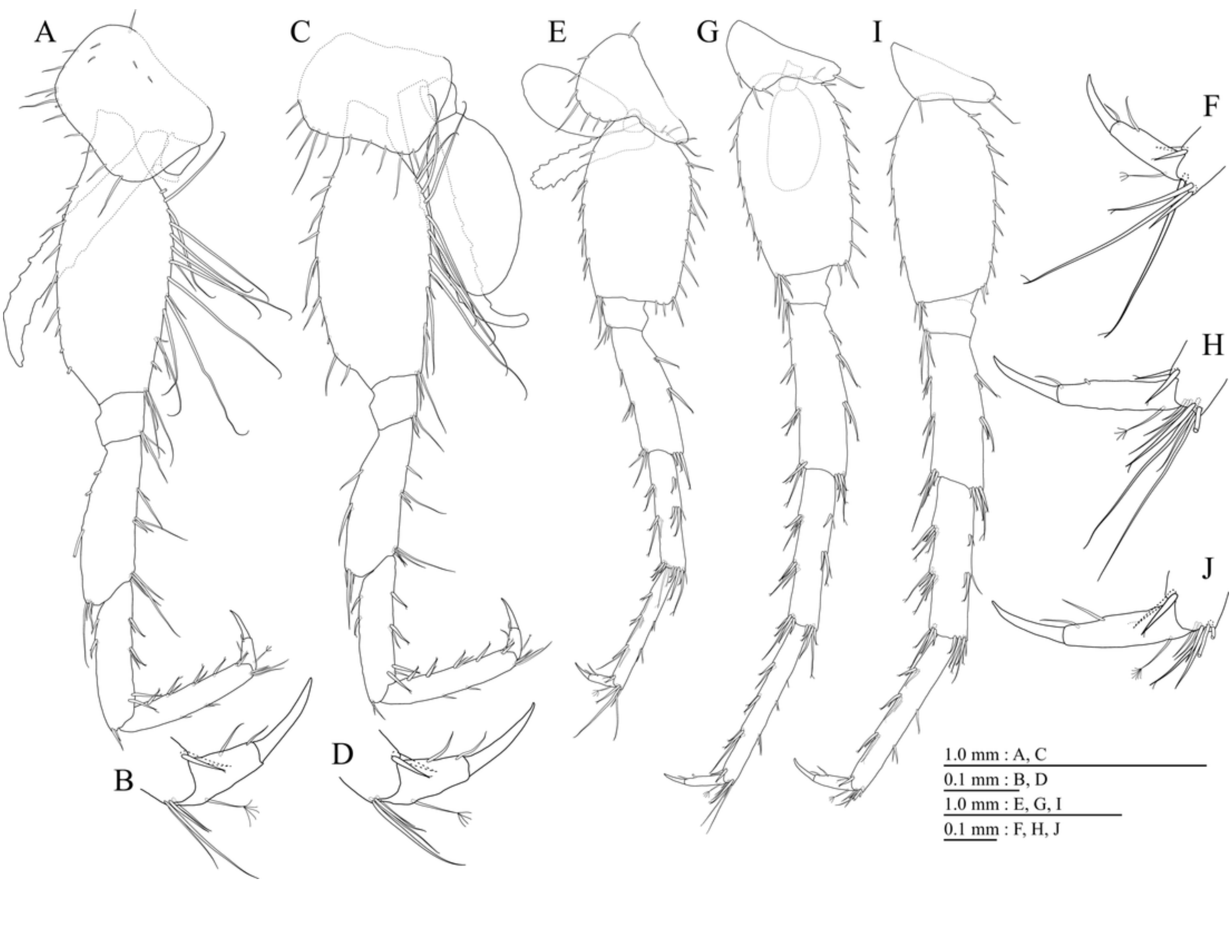
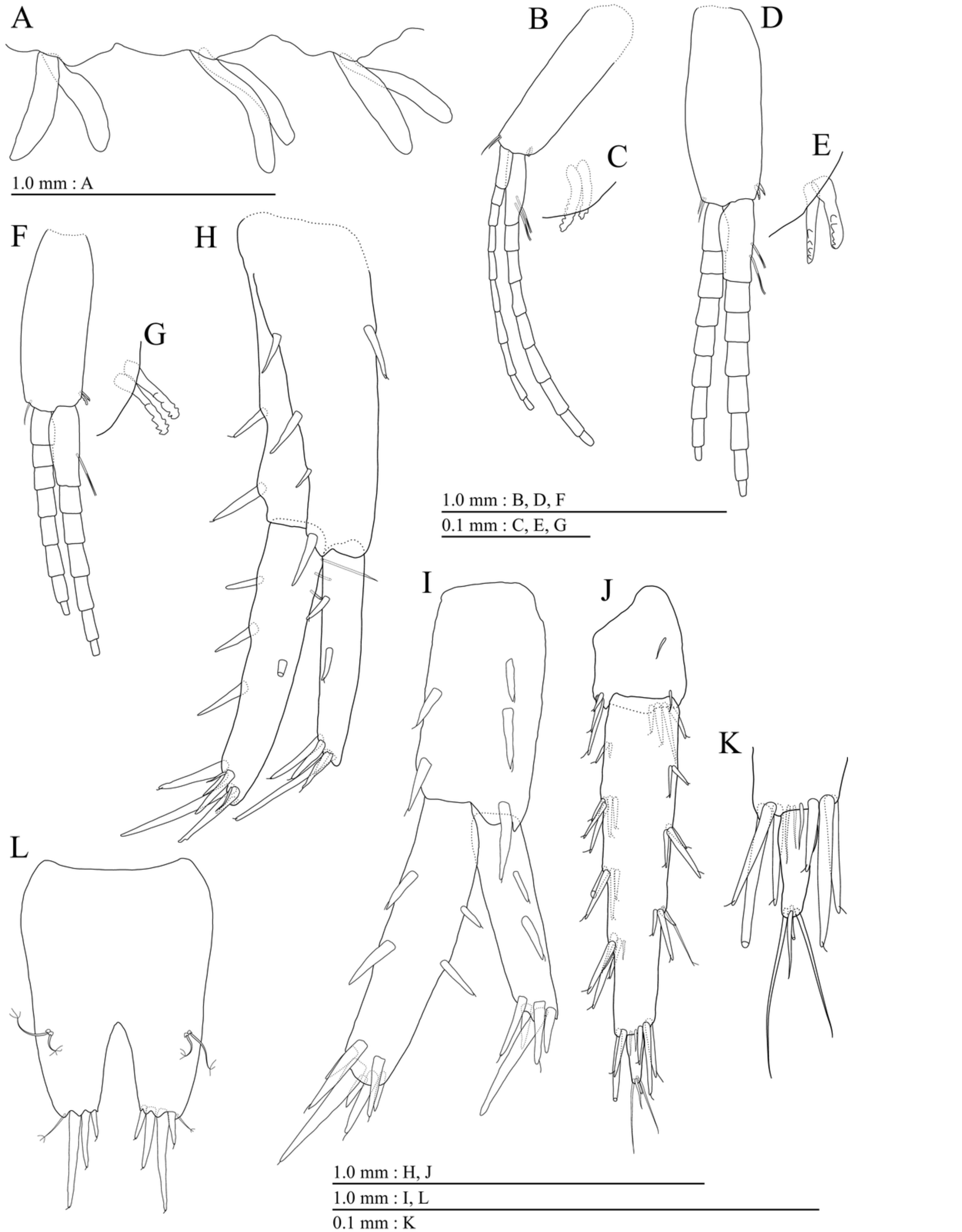


Figure 18

Holotype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV35118).

(A) Sternal gills on pereonites 2–4, lateral view; (B) Pleopod 1, lateral view; (C) Retinacula on peduncle of pleopod 1, lateral view; (D) Pleopod 2, lateral view; (E) Retinacula on peduncle of pleopod 2, lateral view; (F) Pleopod 3, lateral view; (G) Retinacula on peduncle of pleopod 3, lateral view; (H) Uropod 1, dorsal view; (I) Uropod 2, ventral view; (J) Uropod 3, dorsal view; (K) Terminal article of uropod 3, dorsal view; (L) Telson, dorsal view.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0.1 mm : C, E, G
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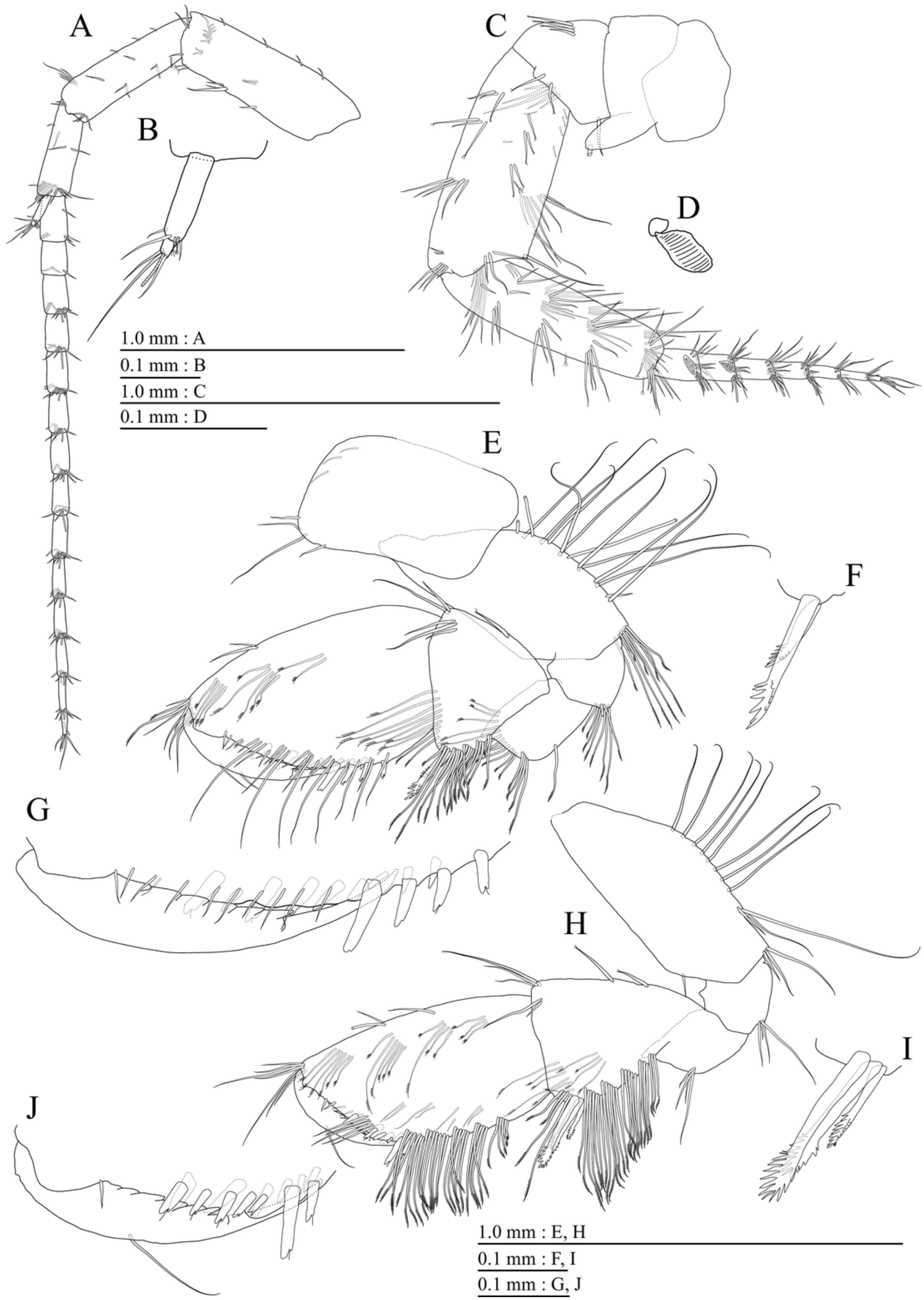
J
K
L
1.0 mm : H, J
1.0 mm : I, L
0.1 mm : K

Figure 19

Paratype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV39837).

(A) Antenna 1, medial view; (B) Accessory flagellum of antenna 1, medial view; (C) Antenna 2, medial view; (D) Calceolus of antenna 2, medial view; (E) Gnathopod 1, lateral view; (F) Serrate setae on posterodistal corner of carpus of gnathopod 1, lateral view; (G) Palmar margin of propodus and dactylus of gnathopod 1, lateral view; (H) Gnathopod 2, lateral view; (I) Serrate setae on posterodistal corner of carpus of gnathopod 2, lateral view; (J) Palmar margin of propodus and dactylus of gnathopod 2, lateral view.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0.1 mm : D
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I
J
1.0 mm : E, H
0.1 mm : F, I
0.1 mm : G, J

Figure 20

Paratype of *Pseudocrangonyx hwanseonensis* sp. nov. (NNIBRIV39837).

(A) Uropod 1, dorsal view; (B) Uropod 2, dorsal view; (C) Uropod 3, dorsal view; (D) Terminal article of uropod 3, dorsal view; (E) Telson, dorsal view.

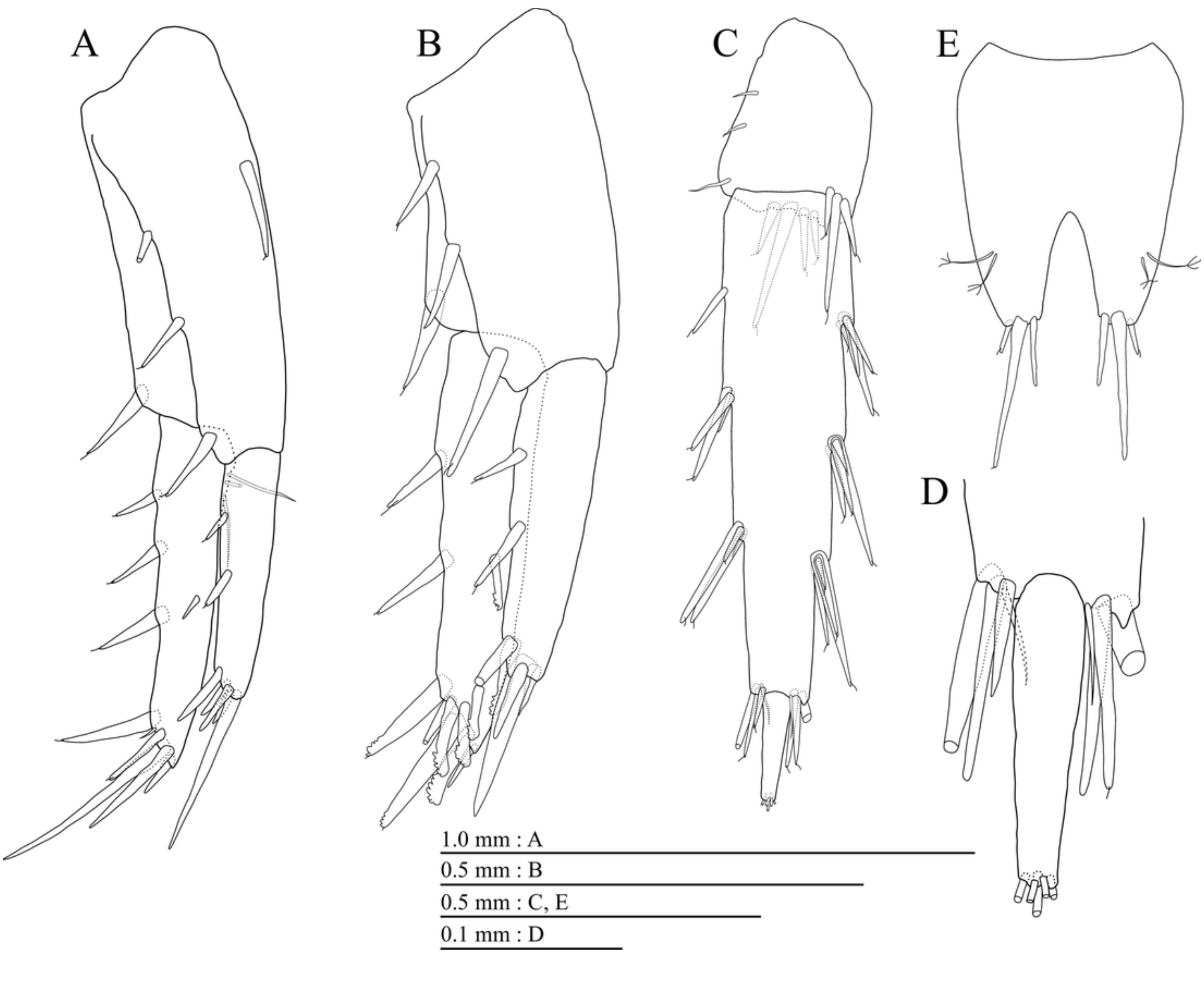

Figure 21

Maximum likelihood and Bayesian inference analyses based on nuclear 28S rRNA and mitochondrial COI sequences. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities.