

Three new species of subterranean amphipod (Pseudocrangonyctidae: *Pseudocrangonyx*) from limestone caves in South Korea (#54037)

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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 monophly with *P. joolaei* and *P. wonkimi*, which are indigenous to the Korean caves.

1 Three new species of subterranean amphipod 2 (Pseudocrangonyctidae: *Pseudocrangonyx*) from 3 limestone caves in South Korea

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14 Abstract

15 The genus *Pseudocrangonyx* is the most diverse subterranean amphipods of the groundwater
16 communities in Far East Asia. In Korea, the true species diversity of the group has been
17 underestimated owing to the records of morphological variants of *P. asiaticus*. To estimate their
18 true species diversity, we analyzed morphological characteristics and conducted molecular
19 analyses of specimens collected from Korean caves, which were treated as morphological
20 variants of *P. asiaticus*. Thus, three new subterranean species of pseudocrangonyctid amphipod,
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24 oxidase subunit I gene of the new species for molecular analyses. Molecular phylogenetic
25 analyses revealed that the three new species formed a monophyly with *P. joolaei* and *P.*
26 *wonkimi*, which are indigenous to the Korean caves.
27

28 Introduction

29 Amphipods are the most diverse group of organisms in groundwater communities (Holsinger
30 1994), and subterranean amphipods are of interest from a biogeographic perspective because of
31 their limited dispersal ability and restriction to groundwater aquifers (Holsinger 1993). Most of
32 the subterranean amphipods are troglobiont (stygobiont), generally characterized by
33 morphological features with loss of eyes and pigment and elongation of appendages (Holsinger
34 1994; Väinölä *et al.* 2007), and these characteristics result in the strikingly convergent
35 morphology of cave animals (Jones *et al.* 1992). Therefore, the classification of subterranean
36 organisms that are based solely on morphological characteristics exhibit several taxonomic
37 problems (Lefébure *et al.* 2006; Kornobis *et al.* 2011). Particularly, in the case of subterranean or
38 cave amphipod species that are difficult to distinguish morphologically, distinguishing species

39 through molecular analyses helps to resolve the species delimitation (Lefébure *et al.* 2006;
40 Trontelj *et al.* 2009; Hou & [redacted] 2010).

41 The stygobitic amphipod *Pseudocrangonyx* Akatsuka & Komai, 1922 is the most diverse
42 taxon among the subterranean amphipod genera found in Far East Asia, i.e., the Korean
43 Peninsula, the Japanese Archipelago, eastern China and Russian Far East (Sidorov & Holsinger
44 2007; Tomikawa & Nakano 2018). The first record [redacted] the Korean Peninsula was *P. asiaticus*
45 Uéno, 1934 from North Korea (Uéno 1940). But the type locality of *P. asiaticus* is located on the
46 Liaodong Peninsula, China (Uéno 1934). Since then, it has been reported that the species
47 inhabits several caves [redacted] South Korea through identification based on morphological
48 characters (Uéno 1966; Holsinger 1989). [redacted] no (1966) mentioned regional morphological
49 variants of the Korean populations [redacted] but did not regard it as a distinct species. At that time, as
50 mentioned above, there would have been obvious limitations in identifying subterranean
51 amphipods solely by morphological characteristics. Recent studies have shown that the species
52 diversity of the genus based on both molecular analyses and morphological identification may be
53 higher than previously known (Tomikawa *et al.* 2016; Tomikawa & Nakano 2018; Lee *et al.*
54 2020).

55 During cave surveys in the Korean Peninsula, we collected *Pseudocrangonyx* specimens
56 from two caves (Kwangcheonseon Cave and Hwanseon Cave), where Uéno (1966) reported one
57 of the morphological variants of *P. asiaticus* and specimens from the other cave (Deureune
58 Cave) was first found. Based on the results of morphological examination of the amphipods, we
59 described and illustrated them as three new *Pseudocrangonyx* species. Furthermore, we
60 determined the nuclear large subunit ribosomal RNA (28S rRNA) gene and mitochondrial
61 cytochrome *c* oxidase subunit I (COI) gene sequence data for molecular analyses of the three
62 new species. Additionally, a key to Korean *Pseudocrangonyx* species is provided in this study.
63

64 Materials & Methods

65 **Sample collection and morphological examination.** *Pseudocrangonyx* specimens were
66 collected from groundwater of three caves in Korea, namely Deureune Cave (Fig. 1A),
67 Kwangcheonseon Cave (Fig. 1B), and Hwanseon Cave (Fig. 1C). Specimens were fixed and
68 preserved in 99% ethanol. All appendages of the specimens were dissected in 80% ethanol and
69 mounted with gum-chloral medium on glass slides under an Olympus SZX7 stereomicroscope
70 (Tokyo, Japan). The specimens were examined using a Nikon Eclipse Ni light microscope
71 (Tokyo, Japan) and illustrated with the aid of a drawing tube. The body length from the tip of the
72 rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm.
73 The nomenclature of the setal patterns on the mandibular palp follows the method described by
74 Stock (1974). The specimens examined in this study have been deposited in the collection of the
75 Nakdonggang National Institute of Biological Resources, Korea (NNIBR).

76 **Molecular analyses.** Genomic DNA was extracted from the muscles of the appendages of the
77 specimens using the LaboPass Tissue Mini Kit (Cosmo GENETECH, Seoul, South Korea),
78 according to the manufacturer's instructions. The primer sets for the PCR reaction used in this

79 study were as follows: 28F and 28R for 28S rDNA (Hou *et al.* 2007); LCO1490 and HCO2198
80 for COI (Folmer *et al.* 1994). The sequences of 28S rDNA were aligned using MAFFT v. 7.388
81 L-INS-i (Katoh & Standley 2013), and COI was aligned using Geneious 8.1.9 (Biomatters,
82 Auckland, New Zealand), respectively. For genetic analysis, these two alignments were
83 combined. All data used in molecular analyses is provided, including the newly obtained
84 sequences in this study (Table 1). Pairwise comparisons of uncorrected *p*-distances for COI
85 sequence was calculated using MEGA X (Kumar *et al.* 2018). Phylogenetic trees were
86 constructed using maximum likelihood (ML) and Bayesian inference (BI). ML analysis was
87 performed using RAxML v. 8.2.10 (Stamatakis 2014) with the substitution model set as
88 GTRCAT immediately after nonparametric bootstrapping was conducted with 1,000 replicates.
89 The best fit-partitioning scheme for the ML analysis was identified with the Akaike information
90 criterion using PartitionFinder v. 2.1.1 (Lanfear *et al.* 2017) with the “greedy” algorithm. BI and
91 posterior probabilities were estimated using MrBayes v. 3.2.6 (Ronquist *et al.* 2012). Two
92 independent runs of four Markov chains were conducted for 10 million generations, and the tree
93 was sampled at every 100 generations. Parameter estimates and convergence were checked using
94 Tracer v. 1.7.1 (Rambaut *et al.* 2018), and the first 50001 trees were discarded based on results.
95 **Scanning electron microscopy.** The specimen for scanning electron microscope (SEM) imaging
96 was rinsed with TWEEN 20 (Model 036K00963; Sigma, USA) to remove residual debris, and
97 then the sample was dehydrated with a graded ethanol series (30%, 50%, 70%, 80%, 95%, and
98 100% ethanol; 10 min each) and Hexamethyldisilazane (Sigma, St. Louis, USA) for 1 h. Dried
99 sample was sputtered with platinum, and then observed with an SEM (Model Hitachi S-4300;
100 Japan).

101 The electronic version of this article in Portable Document Format (PDF) will represent a
102 published work according to the International Commission on Zoological Nomenclature (ICZN),
103 and hence the new names contained in the electronic version are effectively published under that
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107 through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The
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109 6D8E3D8539CE]. The online version of this work is archived and available from the following
110 digital repositories: PeerJ, PubMed Central and CLOCKSS.

111

112 **Results**

113

114 Order Amphipoda Latreille, 1816
115 Family Pseudocrangonyctidae Holsinger, 1989
116 Genus *Pseudocrangonyx* Akatsuka & Komai, 1922

117

118 *Pseudocrangonyx deureunensis* sp. nov.

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

122

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

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

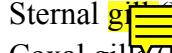
139 **Description.** Female holotype (NNIBRIV39838). Head (Fig. 3A) with short dorsal seta; rostrum
140 short; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes absent. Pereonites
141 1–6 with short dorsal setae; dorsal margin of pereonite 7 with long setae. Dorsal margins of
142 pleonites 1–3 with long setae (Fig. 3B). Posterior margin of epimeral plate 1 with 6 setae,
143 posteroventral corner with seta; ventral and posterior margins of plate 2 with 3 and 4 setae,
144 respectively, posteroventral corner with seta; ventral and posterior margins of plate 3 with 3
145 setae, respectively, posteroventral corner subquadrate with seta (Fig. 3B). Dorsal margin of
146 urosomites 1–3 with setae. Anteroventral corner of urosomite 1 with seta, posteroventral corner
147 of urosomite 3 with setae (Fig. 3B).

148 Antenna 1 (Fig. 3C) 0.51 times as long as body length, peduncular articles 1–3 in length
149 ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 3D) 2-articulate, more than shorter primary
150 flagellar article 1, terminal article with 3 setae and 1 aesthetascs; primary flagellum 18-articulate,
151 1 aesthetasc on some articles. Antenna 2 (Fig. 3E, F) 0.59 times as long as antenna 1; peduncular
152 article 5 with 2 calceoli; flagellum 0.65 times as long as peduncular articles 4 and 5 combined,
153 consisting of 9 articles, first 5 each with calceolus.

154 Upper lip (Fig. 3G) with rounded anterior margin, with fine setae. Mandibles (Fig. 3H–J)
155 with left and right incisors with 5-
156 dentate, respectively; left lacinia mobilis 5-dentate, right
157 lacinia bifid, with many teeth; molar process triturative; accessory setal rows of left and right
158 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

159 outer lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 3L) with inner and outer
160 plates, and palp; inner plate subovate with 5 plumose setae; outer plate subrectangular with 7
161 serrate teeth apically; palp 2-articulate, longer than outer plate, article 2 with weakly plumose
162 robust seta apically, and 3 apical and 4 subapical robust setae. Maxilla 2 (Fig. 4A) with oblique
163 inner row of 6 setae on inner plate. Maxilliped (Fig. 4B, C) with inner and outer plates, and palp;
164 inner plate subrectangular with 4 apical robust setae; outer plate suboval with apical and
165 subapical 4 robust setae, and some medial setae; palp 4-articulate, medial margin of article 2
166 lined with setae, article 4 with nail.

167 Gnathopod 1 (Fig. 4D, E) with subquadrate coxa, bearing seta on anterior dorsal margin and
168 anterodistal corner, width 1.8 times as long as depth; basis thick and short, anterior margin bare,
169 submargin with setae, posterior margin with 16 long setae; posterodistal corner of carpus with 2
170 serrate robust setae; propodus stout, subtriangular, palmar margin with 15 robust setae in 2 rows,
171 some distally notched; posterior margin of dactylus dentate (Fig. 4F). Gnathopod 2 (Fig. 4G, H)
172 with rounded coxa, with setae on its anterior to ventral margins, width 1.4 times as long as depth;
173 basis slender with anterior margin bare, posterior margin with 8 long setae; posterodistal corner
174 of carpus with 3 serrate robust setae; propodus  slender than that of gnathopod 1, palmar margin
175 with 18 robust setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig.
176 4I). Pereopod 3 (Fig. 5A) with subquadrate coxa bearing setae on anterodistal corner to ventral
177 margins, width 1.5 times as long as depth; anterior and posterior margins of basis with setae;
178 merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 0.9; posterior margin of dactylus with 2
179 setae (Fig. 5B). Pereopod 4 (Fig. 5C) with subquadrate coxa bearing setae on anterior margin,
180 anterodistal corner and ventral margin, width 1.6 times as long as depth; anterior and posterior
181 margins of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 1.0;
182 posterior margin of dactylus with 2 setae (Fig. 5D). Pereopod 5 (Fig. 5E) with bilobed coxa
183 bearing setae on anterior and posterior lobes; anterior and posterior margins of basis with setae;
184 merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 1.1; anterior margin of dactylus with 2
185 setae (Fig. 5F). Pereopod 6 (Fig. 5G) anterior coxa broken; bearing setae on posterior lobe;
186 anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of
187 1.0 : 1.0 : 1.1; anterior margin of dactylus with 2 setae (Fig. 5H). Pereopod 7 (Fig. 5I) with
188 subtriangular coxa, ventral margin weakly concave, with seta on ventral margin and posterodistal
189 corner; anterior and posterior margins of basis with setae; merus, carpus, and propodus in length
190 ratio of 1.0 : 1.2 : 1.2; anterior margin of dactylus with 2 setae (Fig. 5J).

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

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

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

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

198 10-, 9-, and 9-articulate, respectively; outer ramus of pleopods 1–3 11-, 10-, and 9-articulate,
199 respectively.

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

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

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

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

219 Uropod 1 (Fig. 8A) with 2 basofacial setae on peduncle; inner ramus 0.76 times as long as
220 peduncle; outer margin and marginal with 3 and 2 robust setae, respectively, basal part with 2
221 slender setae; outer ramus with 2 outer margin robust setae. Uropod 2 (Fig. 8B, C) with peduncle
222 0.82 times as long as inner ramus; inner ramus 1.4 times as long as outer ramus, distal part with 2
223 serrate, 4 simple robust setae. Uropod 3 (Fig. 8D, E) with outer ramus terminal article 0.2 times
224 as long as proximal article.

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

226 **Distribution.** Known only from the type locality.

227 **Etymology.** The specific name is an adjective derived from the name of the cave where the new
228 species inhabits.

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

238

239 *Pseudocrangonyx kwangcheonseonensis* sp. nov.

240 urn:lsid:

241 [New Korean name: kwang-cheon-seon-dong-gul-yeop-sae-u]

242 (Figs. 2B, 9–14)

243

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

245 **Material examined.** Holotype female (10.6 mm), NNIBRIV35120, collected from
246 Kwangcheonseon Cave ($37^{\circ}31.11'N$, $128^{\circ}27.05'E$), Pyeongchang-gun, Gangwon-do, Korea, on
247 28 February 2017, by Y. G. Choi. Paratypes: 1 male (7.8 mm), NNIBRIV39840; 1 male (7.1
248 mm), NNIBRIV39841, collection data same as that for the holotype.

249 **Diagnosis.** Female larger than male; antennal sinus with rounded angle; eyes absent; pereonites
250 1–7 with dorsal setae; dorsal margin of urosomite 3 lacking setae; pereonites 2–4 each with
251 sternal gill; antenna 1 longer than half body length; antenna 2 with calceoli in both sexes;
252 mandible palp article 3 longer than article 2; maxilla 1 inner plate with 8 plumose setae;
253 gnathopods 1 and 2, carpi with serrate setae on posterodistal corners in both sexes; palmar
254 margins of propodi of gnathopods 1 and 2 with 24–26 and 20–21 robust setae, respectively;
255 pleopod peduncles lacking marginal setae, inner margin of inner rami with bifid setae; uropod 1,
256 inner and outer margins of inner ramus with 4 and 3 robust setae, basal part of inner ramus with
257 3 slender setae, outer ramus with 3 outer marginal robust setae; uropod 3 terminal article shorter
258 than adjacent robust setae; telson laterally concave and shallowly at the top.

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

268 Antenna 1 (Fig. 9A) 0.56 times as long as body length, peduncular articles 1–3 in length
269 ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 9B) 2-articulate, more than longer primary
270 flagellar article 1, terminal article with 3 setae and 1 aesthetascs; primary flagellum 21-articulate,
271 1 aesthetasc on some articles. Antenna 2 (Fig. 9C, D) 0.64 times as long as antenna 1;
272 peduncular article 5 with 4 calceoli; flagellum 0.52 times as long as peduncular articles 4 and 5
273 combined, consisting of 8 articles, first 6 each with calceolus.

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



278 E-setae. Lower lip (Fig. 9I) with broad outer lobes with fine setae, mandibular process of outer
279 lobe rounded apically; inner lobes indistinct. Maxilla 1 (Fig. 9J, K) with inner and outer plates,
280 and palp; inner plate subovate with 8 plumose setae; outer plate subrectangular with 7 serrate
281 teeth apically; palp 2-articulate, longer than outer plate, article 2 with plumose robust seta
282 apically. Maxilla 2 (Fig. 9L) with oblique inner row of 10 setae on inner plate. Maxilliped (Fig.
283 10A) with inner and outer plates, and palp; inner plate subrectangular with 4 apical robust setae;
284 outer plate suboval with apical and subapical 6 robust setae, and some medial setae; palp 4-
285 articulate, medial margin of article 2 lined with setae, article 4 with nail.

286 Gnathopod 1 (Fig. 10B, C) with subquadrate coxa, bearing seta on its anterior to ventral
287 margins, width 1.7 times as long as depth; basis thick and short, anterior margin bare, posterior
288 margin with 15 long setae; posterodistal corner of carpus with 2 serrate robust setae; propodus
289 stout, subtriangular, palmar margin with 24 robust setae in 2 rows, some distally notched;
290 posterior margin of dactylus dentate (Fig. 10D). Gnathopod 2 (Fig. 10E, F) with rounded coxa,
291 with setae on its anterior to ventral margins, width 1.3 times as long as depth; basis slender with
292 anterior margin bare, posterior margin with 16 long setae; posterodistal corner of carpus with 3
293 serrate robust setae; propodus  than that of gnathopod 1, palmar margin with 21 robust
294 setae in 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 10G). Pereopod
295 3 (Fig. 11A) with subquadrate coxa bearing setae on anterior margin to posteroventral corner,
296 width 1.4 times as long as depth; basis posterior margin with 17 long setae; merus, carpus, and
297 propodus in length ratio of 1.0 : 0.7 : 0.7; posterior margin of dactylus with 2 setae (Fig. 11B).
298 Pereopod 4 (Fig. 11C) with subquadrate coxa bearing setae on anterior margin to posteroventral
299 corner, width 1.6 times as long as depth; basis posterior margin with 12 long setae; merus,
300 carpus, and propodus in length ratio of 1.0 : 0.8 : 0.7; posterior margin of dactylus with seta (Fig.
301 11D). Pereopod 5 (Fig. 11E) with bilobed coxa bearing setae on anterior and posterior lobes;
302 anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of
303 1.0 : 1.0 : 0.9; anterior margin of dactylus with 1 seta (Fig. 11F). Pereopod 6 (Fig. 11G) with
304 weakly bilobed coxa bearing setae on anterior and posterior lobes; anterior and posterior margins
305 of basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 0.9. Pereopod 7
306 (Fig. 11H) anterior coxa broken, ventral margin weakly concave, with setae on ventral margin
307 and posterodistal corner; anterior and posterior margins of basis with setae; merus, carpus, and
308 propodus in length ratio of 1.0 : 1.1 : 1.0; anterior margin of dactylus with 2 setae (Fig. 11I).

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

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

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

313 Peduncles of pleopods 1–3 (Fig. 12B, D, F) lacking marginal setae, outer distal corners with
314 2, 4, and 1 seta, respectively. Pleopods 1–3 with paired retinacula (Fig. 12C, E, G), inner ramus
315 inner basal margin with 3, 2, and 2 bifid seta (clothes-pin seta), respectively; inner ramus of
316 pleopods 1–3 11-, 9-, and 9-articulate, respectively; outer ramus of pleopods 1–3 13-, 13-, and
317 10-articulate, respectively.

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

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

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

334 Gnathopod 1 (Fig. 13D, E) carpus with 2 serrate setae on posterodistal corner; palmar
335 margin of propodus with 26 robust setae in 2 rows, some distally notched (Fig. 13F). Gnathopod
336 2 (Fig. 13G, H) carpus with 2 serrate setae on posterodistal corner; palmar margin of propodus
337 with 20 robust setae in 2 rows, some  distally notched (Fig. 13I).

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

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

345 **Distribution.** Known only from the type locality.

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

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

357

358 *Pseudocrangonyx hwanseonensis* sp. nov.

359 urn:lsid:

360 [New Korean name: Hwan-seon-dong-gul-yeop-sae-u]

361 (Figs. 2C, 15–20)

362

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

364 **Material examined.** Holotype female (7.5 mm), NNIBRIV35118, collected from Hwanseon

365 Cave (37°19.52'N, 129°1.02'E), Samcheok-si, Gangwon-do, Korea, on 20 October 2018, by C. -

366 W. Lee. Paratypes: 1 female (7.7 mm), NNIBRIV39836; 1 male (6.3 mm), NNIBRIV39837,

367 collection data same as that for the holotype.

368 **Diagnosis.** Female larger than male; antennal sinus with rounded angle; eyes absent; pereonites

369 1–6 without short dorsal setae; dorsal margin of urosomite 3 lacking setae; pereonites 2–4 each

370 with 1 pair ~~ste~~^{ventr}al gill; antenna 1 0.53 times as long as body length; antenna 2 with calceoli in

371 both sexes; mandible palp article 3 longer than article 2; maxilla 1 inner plate with 4 plumose

372 setae; maxilla 2 inner plate with oblique inner row of 6 setae; gnathopods 1 and 2, carpi with

373 serrate setae on posterodistal corners in both sexes; palmar margins of propodi of gnathopods 1

374 and 2 with 13 and 13–15 robust setae, respectively; pleopod peduncles lacking marginal setae,

375 inner margin of inner rami with bifid setae; uropod 1 inner ramus 0.86 times as long as peduncle,

376 inner and outer margins of inner ramus with 3 and 1 robust setae, respectively, basal part of inner

377 ramus with 3 slender setae, outer ramus with 2 marginal robust setae; uropod 2 inner ramus 1.2

378 times as long as peduncle; inner and outer margins of inner ramus with 2 robust setae,

379 respectively, outer ramus with 2 outer marginal robust setae; uropod 3 terminal article 0.15 time

380 as long as length of proximal article; telson length 1.31 time as long as width, cleft for 36.8%.

381 **Description.** Female holotype (NNIBRIV35118). Head (Fig. 2C) without dorsal setae; rostrum

382 short; lateral cephalic lobe rounded; antennal sinus shallow with rounded angle; eyes absent.

383 Pereonites 1–6 without short dorsal setae; dorsal margin of pereonite 7 with long setae. Dorsal

384 margins of pleonites 1–3 with long setae (Fig. 2C). Ventral and posterior margins of epimeral

385 plate 1 with 1 and 5 setae, respectively, posteroventral corner with 1 seta; ventral and posterior

386 margins of plate 2 with 4 and 5 setae, respectively, posteroventral corner with 1 seta; ventral and

387 posterior margins of plate 3 with 4 setae, respectively, posteroventral corner with 1 seta (Fig.

388 2C). Dorsal margin of urosomites 1–2 with setae, urosomite 3 lacking dorsal setae. Anteroventral

389 corner of urosomite 1 with 1 seta, posteroventral corner of urosomite 3 with setae (Fig. 2C).

390 Antenna 1 (Fig. 15A) 0.53 times as long as body length, peduncular articles 1–3 in length

391 ratio of 1.0 : 0.7 : 0.4; accessory flagellum (Fig. 15B) ~~l~~iculate, more than shorter primary

392 flagellar article 1, terminal article with 3 setae and 1 ~~aesthetasc~~; primary flagellum 16-articulate,

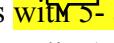
393 1 aesthetasc on some articles. Antenna 2 (Fig. 15C, D) 0.58 times as long as antenna 1;

394 peduncular article 5 with 2 calceoli; flagellum 0.53 times as long as peduncular articles 4 and 5

395 combined, consisting of 7 articles, first 3 each with calceolus.

396 Upper lip (Fig. 15E) with rounded anterior margin, with fine setae. Mandibles (Fig. 15F, G)

397 with left and right incisors ~~w~~ith 5-dentate, respectively; left lacinia mobilis 5-dentate, right

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

408 Gnathopod 1 (Fig. 16B, C) with subquadrate coxa, bearing setae on anterodistal corner to
409 ventral margin, width 1.6 times as long as depth; basis thick and short, anterior margin with 1
410 seta and some medial setae, posterior margin with 10 long setae; posterodistal corner of carpus
411 with 2 serrate robust setae; propodus stout, subtriangular, palmar margin with 13 robust setae in
412 2 rows, some distally notched; posterior margin of dactylus dentate (Fig. 16D). Gnathopod 2
413 (Fig. 16E, F) with subrounded coxa, with setae on its anterior to ventral corners, width 1.3 times
414 as long as depth; basis slender with anterior and posterior margin with 1 seta and 11 long setae,
415 respectively; posterodistal corner of carpus with 3 serrate robust setae; propodus slender than
416 that of gnathopod 1, palmar margin with 15 robust setae in 2 rows, some distally notched;
417 posterior margin of dactylus dentate (Fig. 16G). Pereopod 3 (Fig. 17A) with subquadrate coxa
418 bearing setae on anterior margin to posteroventral corner, width 1.4 times as long as depth;
419 anterior and posterior margins of basis with setae; merus, carpus, and propodus in length ratio of
420 1.0 : 0.8 : 0.8; posterior margin of dactylus with 2 setae (Fig. 17B). Pereopod 4 (Fig. 17C) with
421 subquadrate coxa bearing setae on anterodistal to posteroventral corners, width 1.4 times as long
422 as depth; basis posterior margin with 9 long setae; merus, carpus, and propodus in length ratio of
423 1.0 : 0.9 : 0.8; posterior margin of dactylus with 2 setae (Fig. 17D). Pereopod 5 (Fig. 17E) with
424 bilobed coxa bearing setae on anterior and posterior lobes; anterior and posterior margins of
425 basis with setae; merus, carpus, and propodus in length ratio of 1.0 : 0.9 : 1.0; anterior margin of
426 dactylus with 2 setae (Fig. 17F). Pereopod 6 (Fig. 17G) with weakly bilobed coxa bearing setae
427 on anterior and posterior lobes; anterior and posterior margins of basis with setae; merus, carpus,
428 and propodus in length ratio of 1.0 : 0.9 : 1.0; anterior margin of dactylus with 2 setae (Fig.
429 17H). Pereopod 7 (Fig. 17I) with subtriangular coxa, ventral margin weakly concave, with setae
430 on ventral margin and posterodistal corner; anterior and posterior margins of basis with setae;
431 merus, carpus, and propodus in length ratio of 1.0 : 1.0 : 1.1; anterior margin of dactylus with 2
432 setae (Fig. ).

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

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

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

437 Peduncles of pleopods 1–3 (Fig. 18B, D, F) lacking marginal setae, outerdistal corners with
438 2, 2, and 1 setae, respectively. Pleopods 1–3 with paired retinacula (Fig. 18C, E, G), inner ramus
439 inner basal margin with 2, 2, and 1 bifid seta (clothes-pin seta), respectively; inner ramus of
440 pleopods 1–3 9-, 8-, and 7-articulate, respectively; outer ramus of pleopods 1–3 10-, 9-, and 8-
441 articulate, respectively.

442 Uropod 1 (Fig. 18H) with basofacial seta on peduncle; inner ramus 0.86 times as long as
443 peduncle, inner and outer margins with 3 and 1 robust setae, respectively, basal part with 3
444 slender setae; outer ramus 0.76 times as long as inner, with 2 outer marginal robust setae, inner
445 margin bare. Uropod 2 (Fig. 18I) with inner ramus 1.2 times as long as peduncle, outer margin
446 and marginal with 2 robust setae, respectively; outer ramus 0.73 times as long as inner ramus,
447 inner margin bare and outer margin with 2 robust setae. Uropod 3 (Fig. 18J, K) with peduncle
448 0.29 times as long as outer ramus; inner ramus absent; outer ramus 2-articulate, proximal article
449 with robust setae, terminal article 0.15 times as long as proximal article, with 4 distal setae.

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

452 Male paratype (NNIBRIV39837). Antenna 1 (Fig. 19A, B) 0.53 times as long as body
453 length, primary flagellum 14-articulate, 1 aesthetasc on some articles. Antenna 2 (Fig. 19C, D)
454 0.63 times as long as antenna 1; flagellum 0.58 times as long as peduncular articles 4 and 5
455 combined, consisting of 7 articles, first 2 each with calceolus.

456 Gnathopod 1 (Fig. 19E, F) carpus with 2 serrate setae on posterodistal corner; palmar margin
457 of propodus with 13 robust setae in 2 rows, some distally notched (Fig. 19G). Gnathopod 2 (Fig.
458 19H, I) carpus with 3 serrate setae on posterodistal corner; palmar margin of propodus with 13
459 robust setae in 2 rows, some distally notched (Fig. 19J).

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

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

467 **Distribution.** Known only from the type locality.

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

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

477 teeth, 3) antenna 1 longer (shorter) than as long as body length half, and 4) uropod 3 terminal
478 article shorter (longer) than adjacent robust setae.

479

480 **Key to the species of Korean *Pseudocrangonyx*.**

481 1	Sternal gills absent	2
482 –	Sternal gills present	4
483 2	Female body size larger than 6.0 mm	3
484 –	Female body size smaller than 6.0 mm	<i>P. daejeonensis</i> Lee <i>et al.</i> , 2018
485 3	Uropod 3 terminal article longer than adjacent robust setae	<i>P. minutus</i> Jung <i>et al.</i> , 2020
486 –	Uropod 3 terminal article shorter than adjacent robust setae	<i>P. wonkimi</i> Lee <i>et al.</i> , 2020
487 4	Carpus of gnathopod with serrate robust setae on posterodistal corner	5
488 –	Carpus of gnathopod without serrate robust setae on posterodistal corner	<i>P. coreanus</i> Uéno, 1966
489	
490 5	Sternal gills on pereonites 2 to 4	6
491 –	Sternal gills on pereonites 2 to 5	9
492 6	Sternal gills total number 3	7
493 –	Sternal gills total number 6	<i>P. hwanseonensis</i> sp. nov.
494 7	Urosomite 3 without dorsal setae	8
495 –	Urosomite 3 with dorsal setae	<i>P. deureunensis</i> sp. nov.
496 8	Accessory flagellum of antenna 1 exceeding first article of primary flagellum	<i>P. kwangcheonseonensis</i> sp. nov.
497	
498	
499	
500 –	Accessory flagellum of antenna 1 not exceeding first article of primary flagellum	<i>P. joolaei</i> Lee <i>et al.</i> , 2020
502	
503 9	Maxilla 1 inner plate with less than 7 plumose setae	10
504 –	Maxilla 1 inner plate with 7 plumose setae	<i>P. villosus</i> Jung <i>et al.</i> , 2020
505 10	Telson cleft less than 40% for length	11
506 –	Telson cleft more than 40% for length	12
507 11	Sternal gills on pereonites 2 to 5 (1+1+1+1)	<i>P. asiaticus</i> Uéno, 1934
508 –	Sternal gills on pereonites 2 to 5 (1+1+0+1)	<i>P. crassus</i> Jung <i>et al.</i> , 2020
509 12	Uropod 2 outer ramus with 2 inner marginal robust setae	<i>P. gracilipes</i> Jung <i>et al.</i> , 2020
510 –	Uropod 2 outer ramus without inner marginal robust setae	<i>P. concavus</i> Jung <i>et al.</i> , 2020
511	
512	Molecular Analyses. The uncorrected COI <i>p</i> -distance among the species of the genus	
513	<i>Pseudocrangonyx</i> in Korean caves is shown in Table 2; this divergence was calculated based on	
514	the 657 aligned positions from the data set. The range of interspecific variation was 11.7–17.0%.	
515	However, the maximum intraspecific variation was 0.2% within each species. In the	
516	phylogenetic analyses (Fig. 21), the topologies of the BI and ML trees were almost identical.	

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

519

520 Discussion

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

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

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

554

555 Conclusions

556 In summary, this is a study on three new species described from caves in Korea. Two new
557 species of them were found from caves treated as the morphological variants of *P. asiaticus*
558 Uéno, 1934, the other new species was found from a new cave. All new species may receive a
559 unique species status within the genus *Pseudocrangonyx* based on morphological examination
560 and molecular analyses in this study. These results suggest that the species diversity of *Pseudocrangonyx*
561 may be higher than what has been known in the Korean Peninsula so far.
562 Although we have failed to obtain the molecular data of true *P. asiaticus*, if we obtain the data
563 through future study, it is possible that the true species diversity may be elucidated of the
564 subterranean amphipod *Pseudocrangonyx* in the Far East Asia including the Korean Peninsula.
565

566 **Acknowledgement**

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568 continued support, especially the support of Mr. Yong Gun Choi.
569

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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.

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

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

Table 2(on next page)

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

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

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

2

Figure 1

The collection locality of the specimens examined in this study.

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



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 Hanseonensis* sp. nov., lateral view.

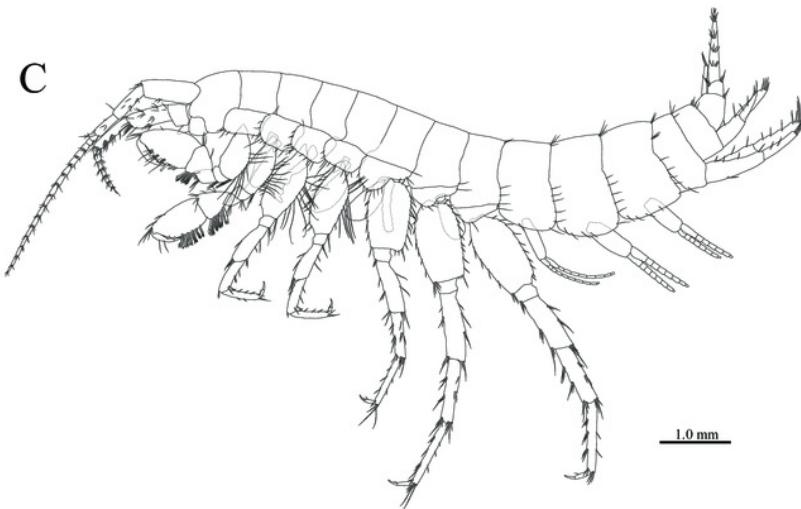
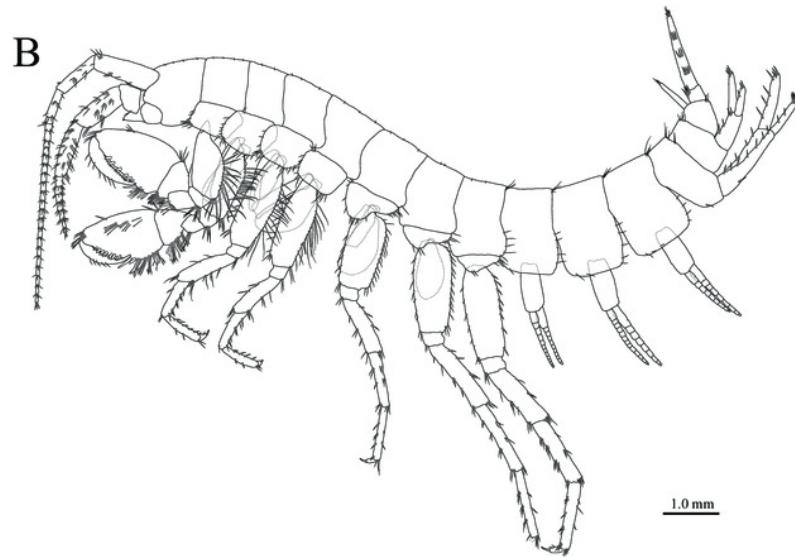


Figure 3

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.

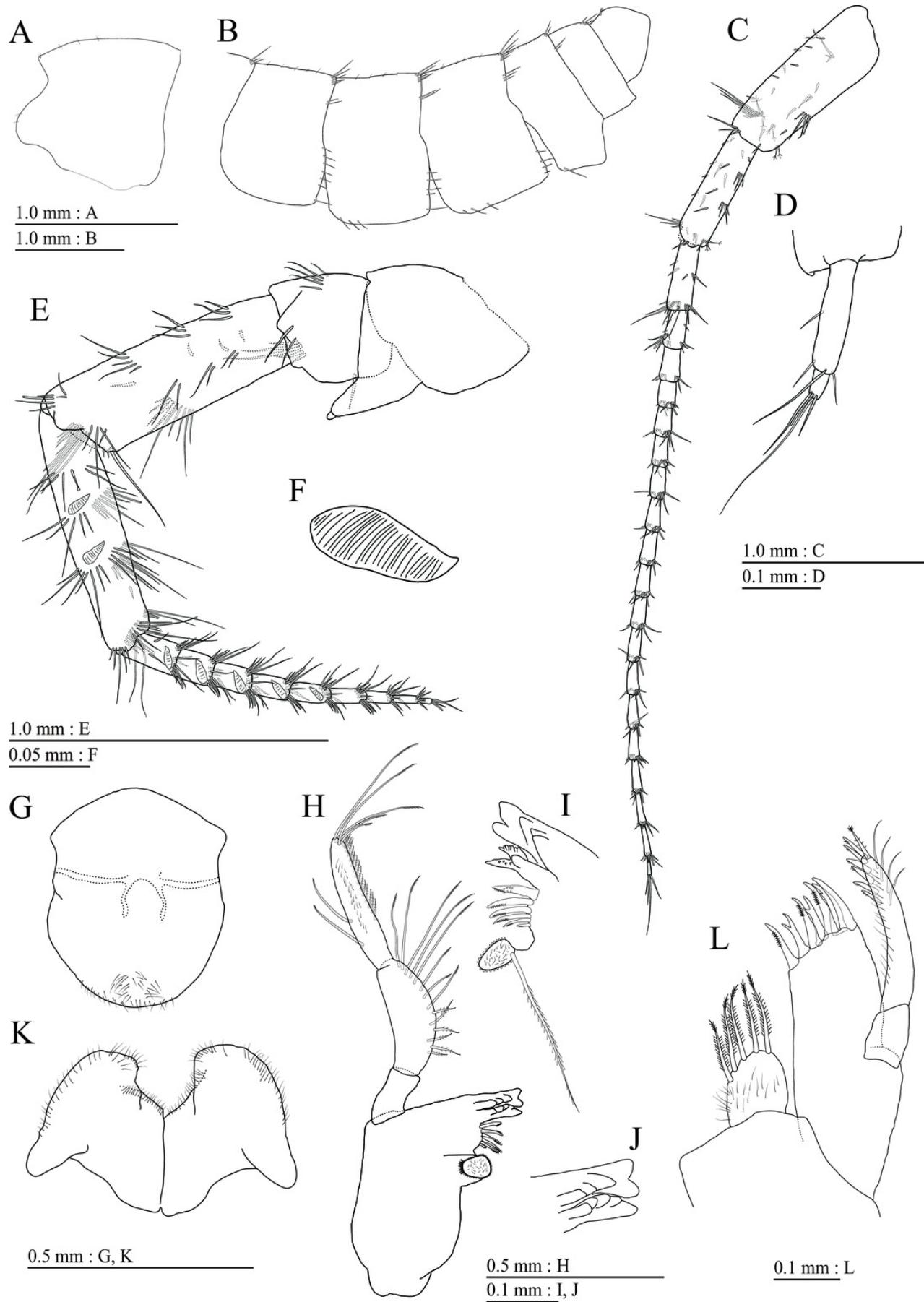


Figure 4

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.

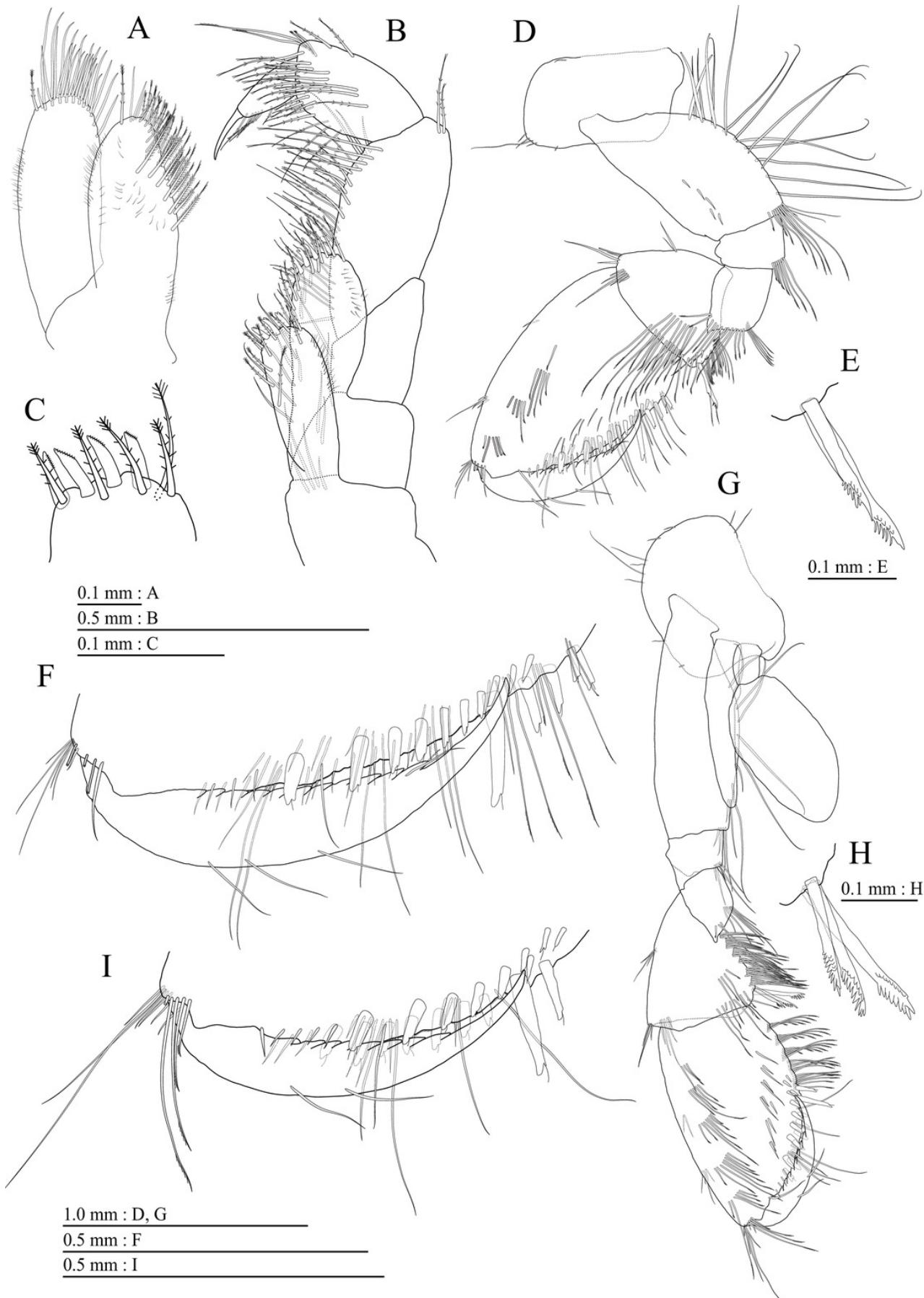


Figure 5

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.

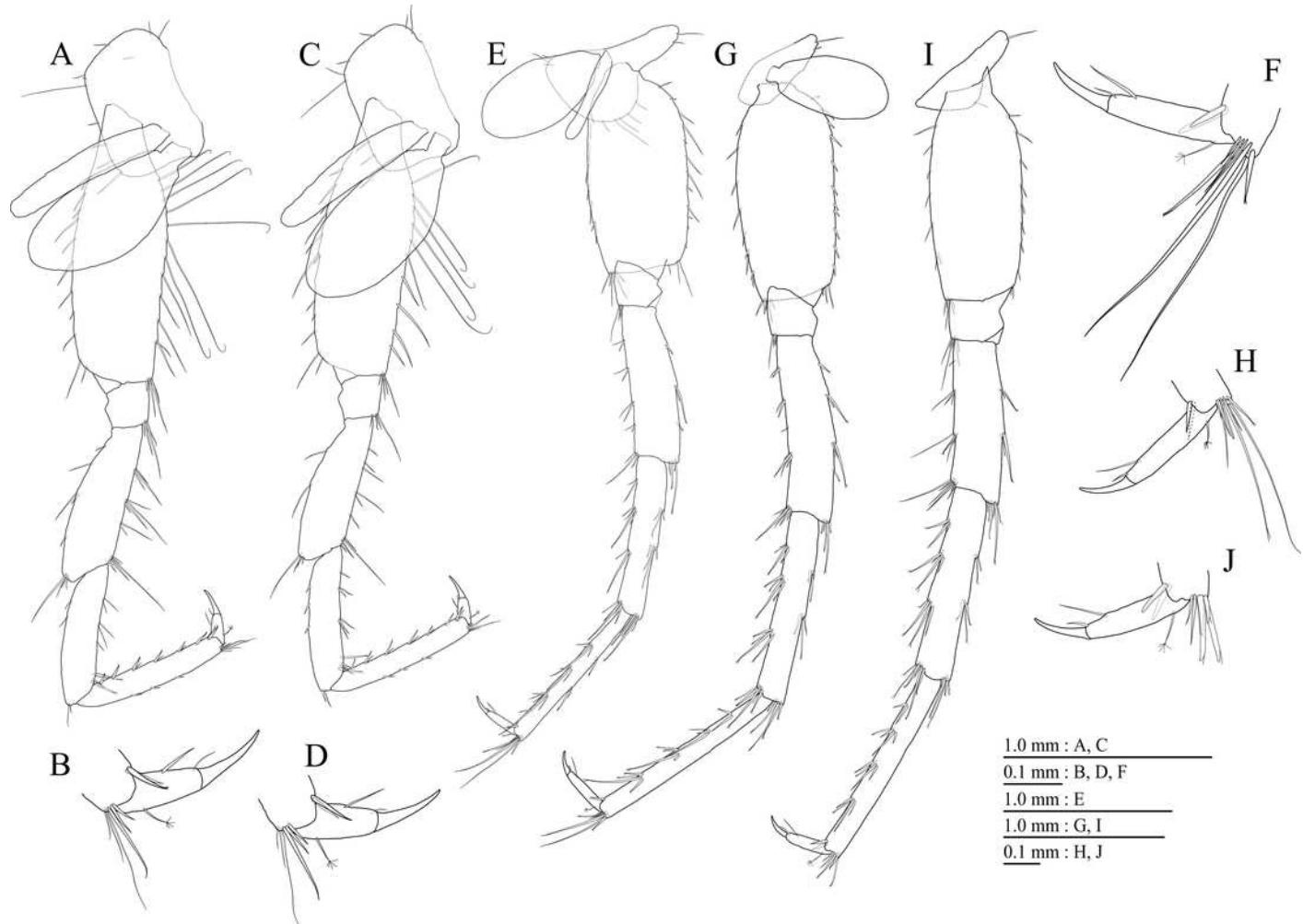


Figure 6

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.

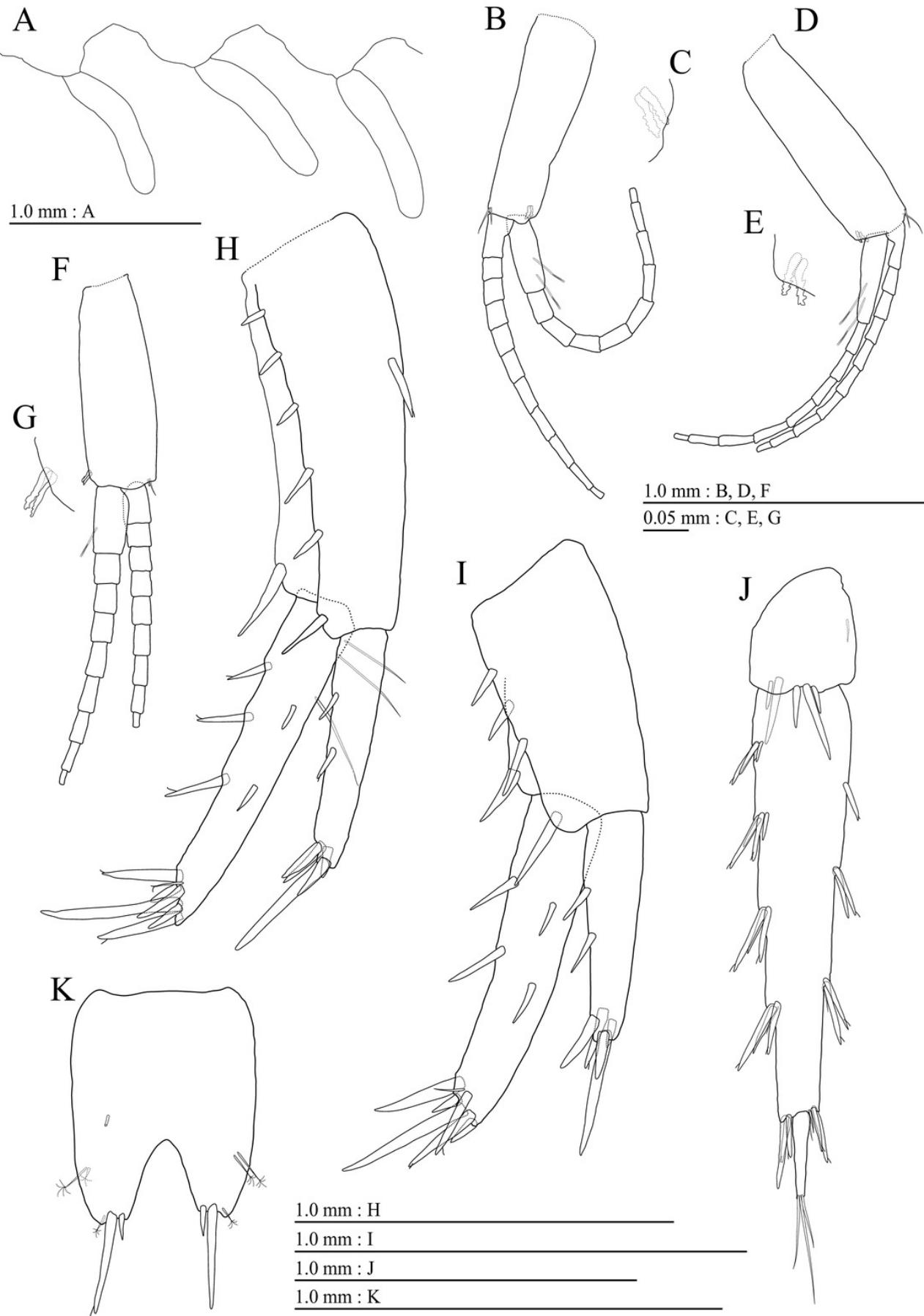


Figure 7

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.

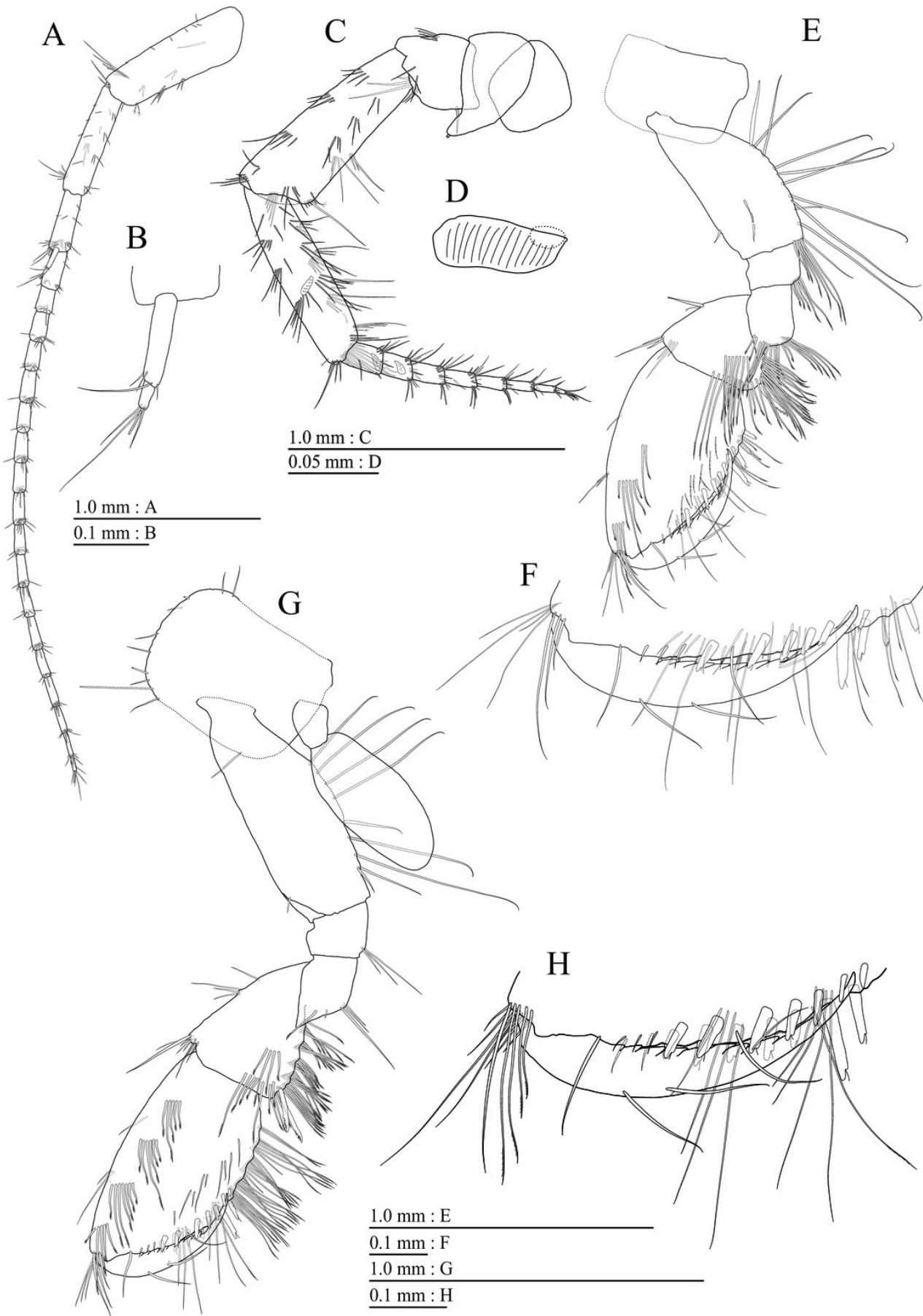


Figure 8

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.

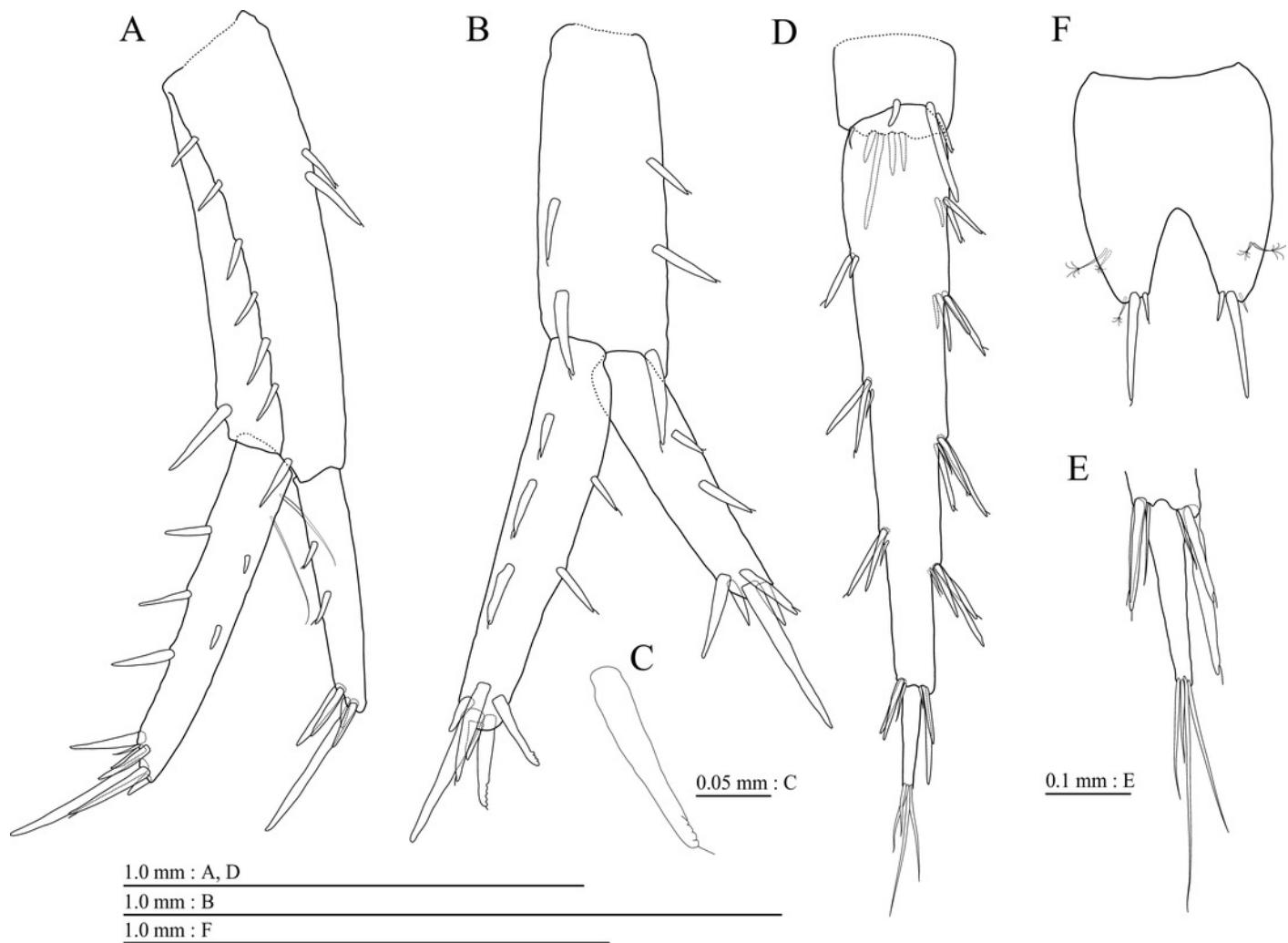


Figure 9

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.

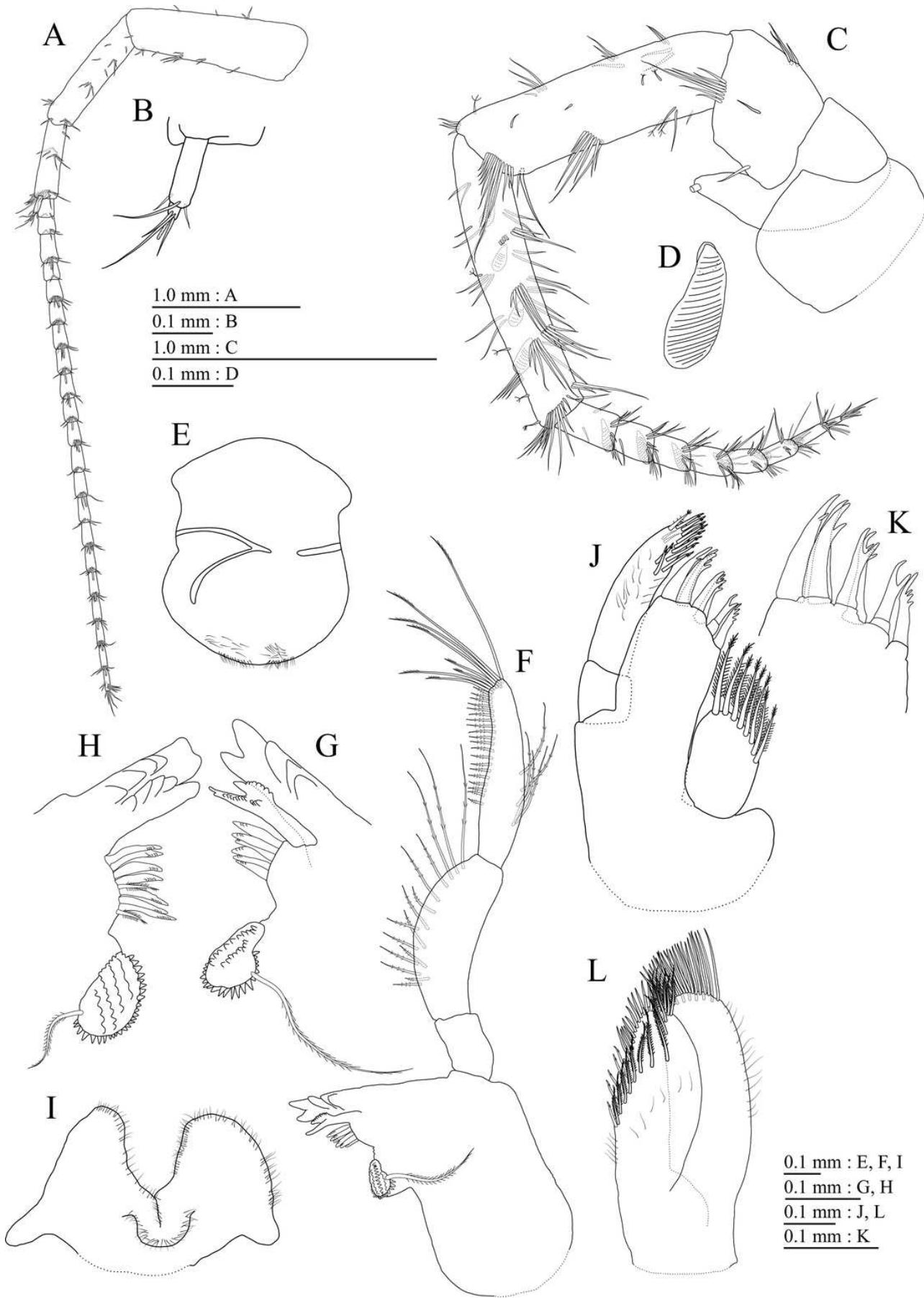


Figure 10

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.

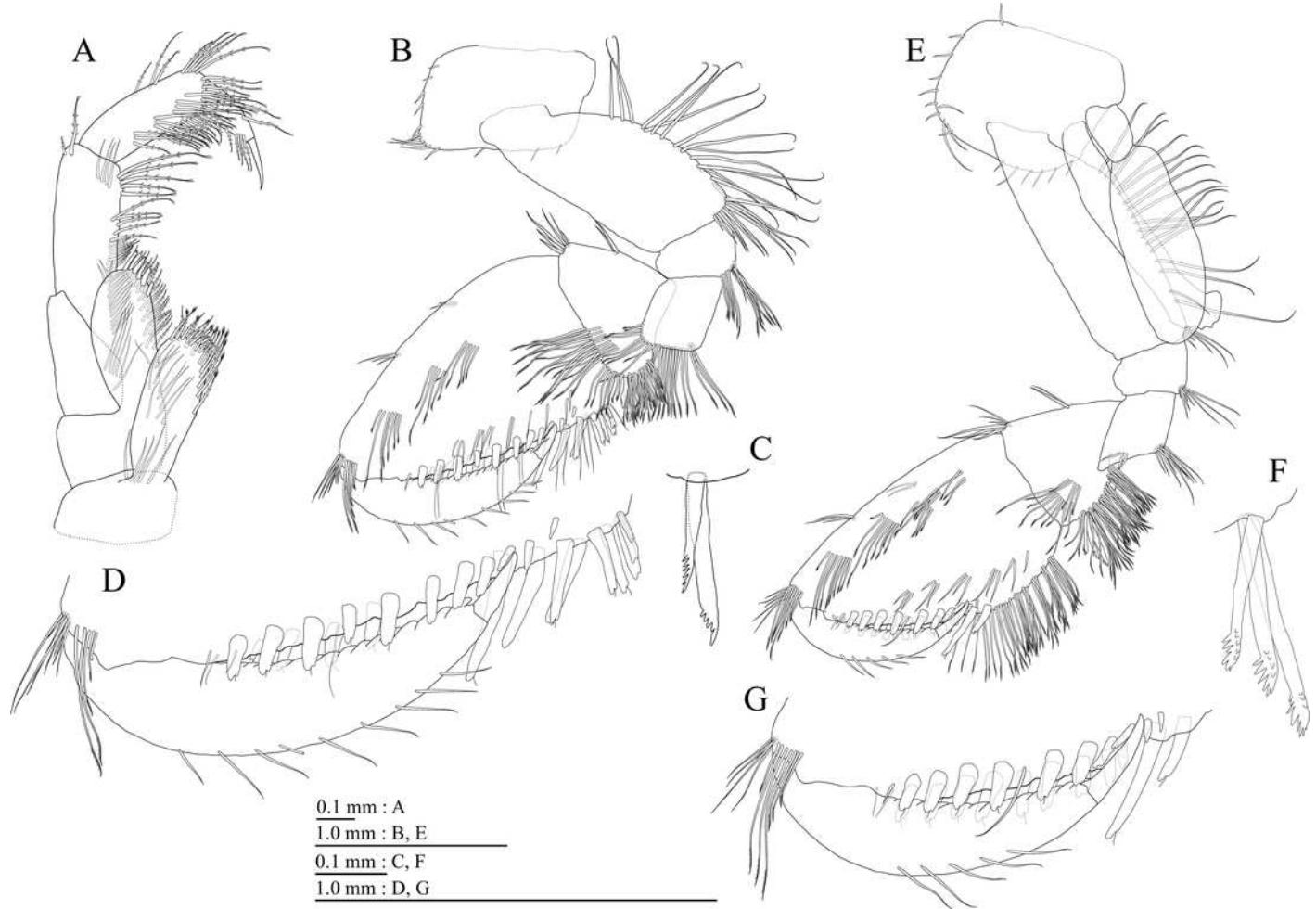


Figure 11

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.

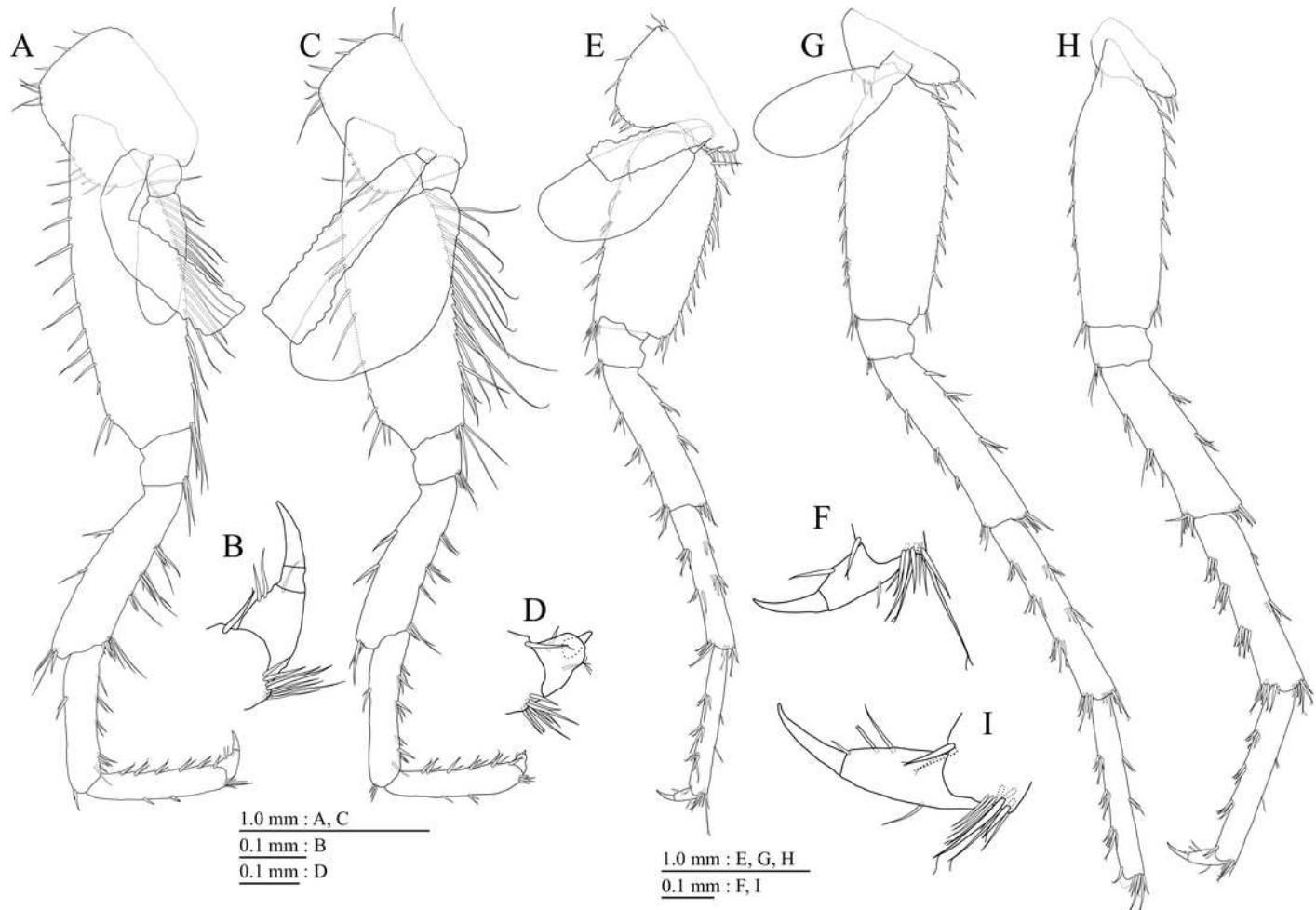


Figure 12

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.

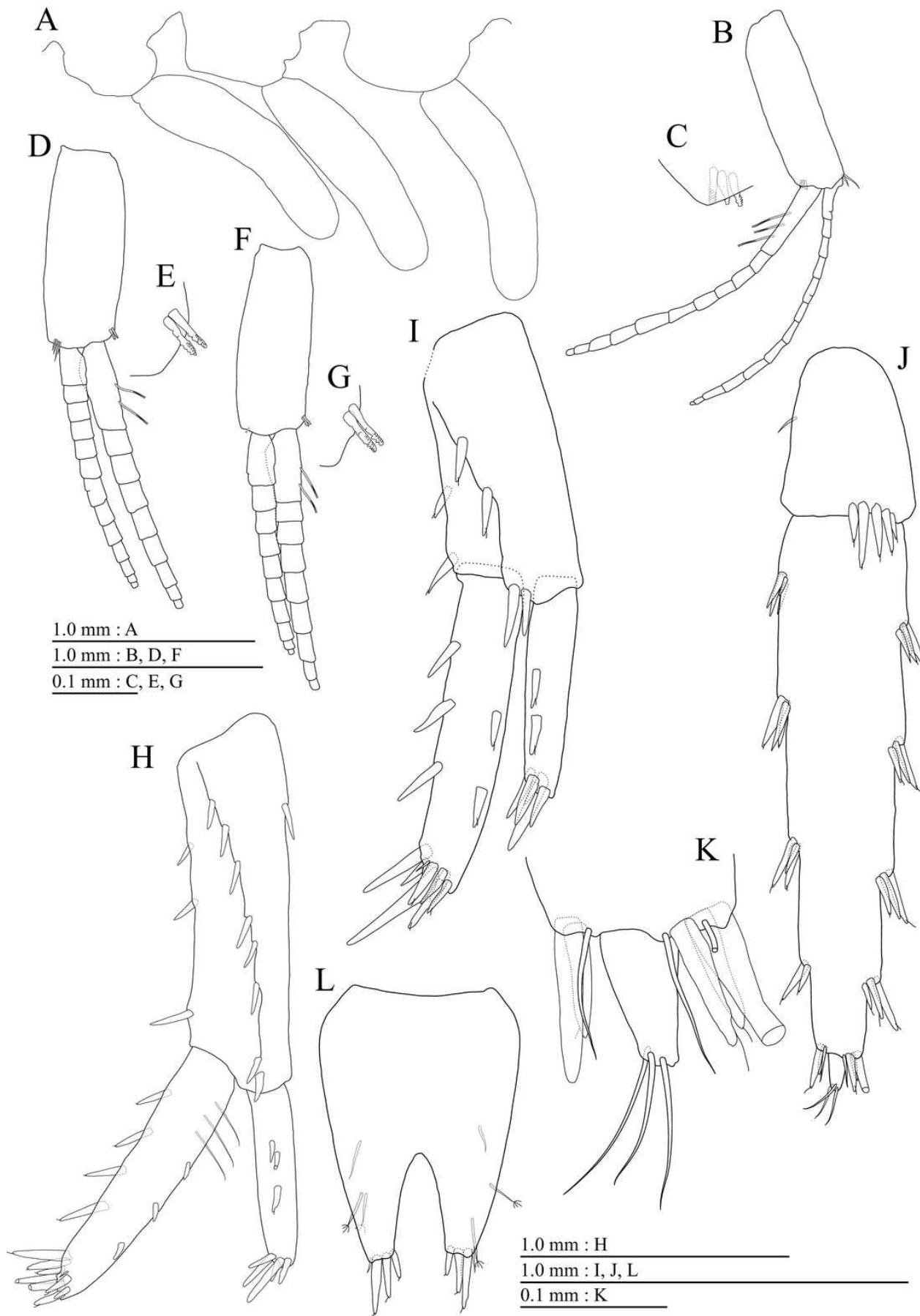


Figure 13

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.

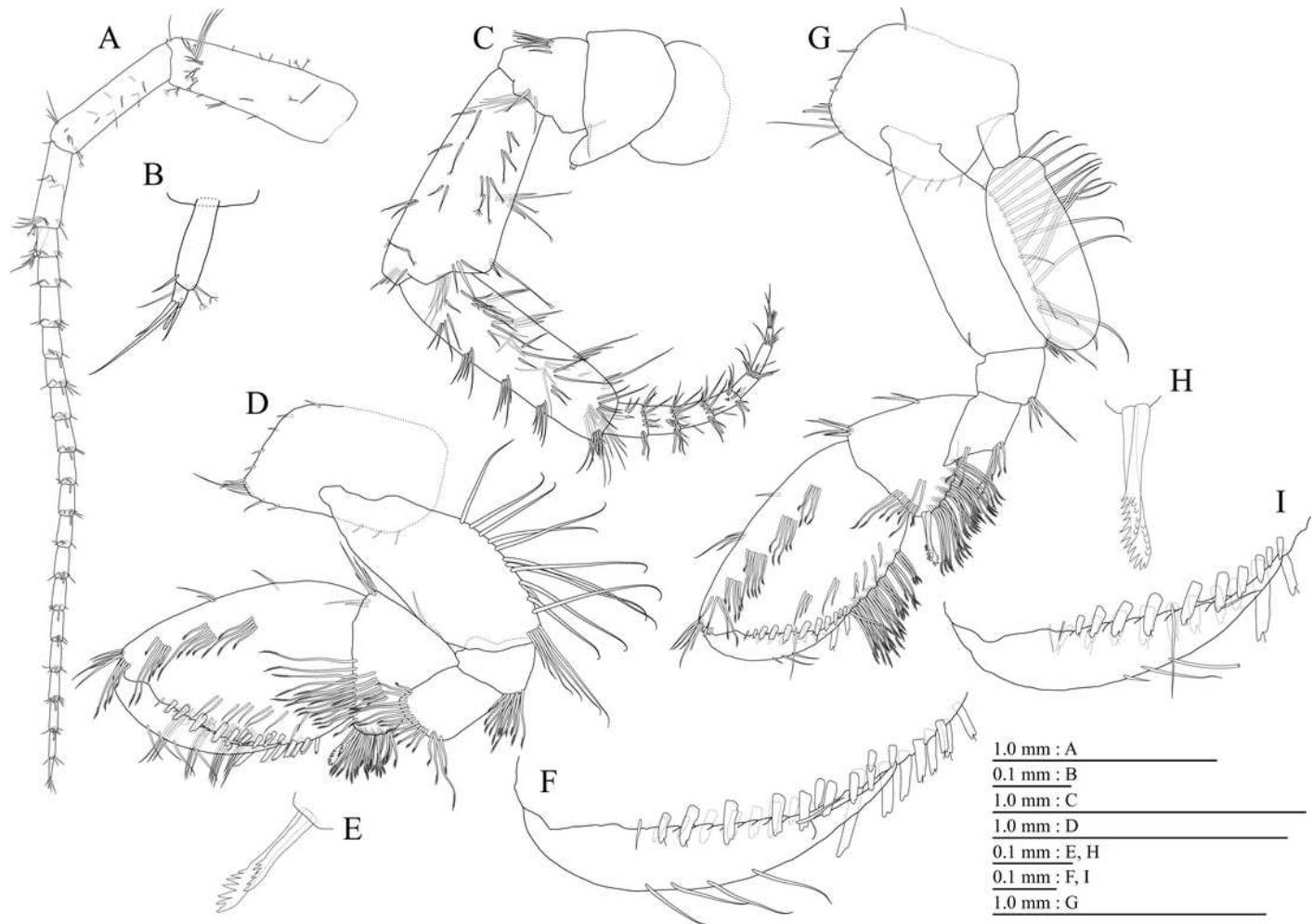


Figure 14

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.

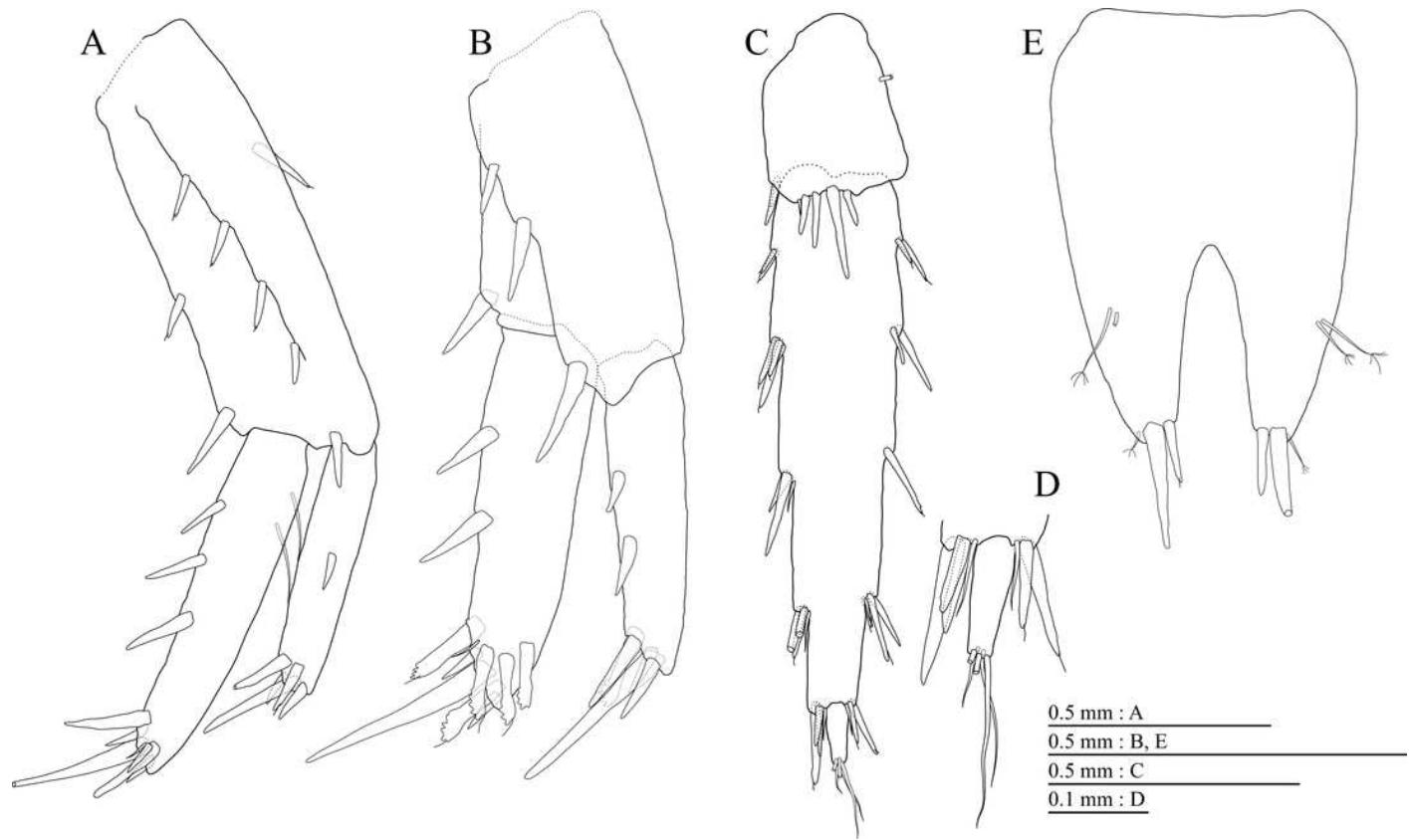


Figure 15

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.

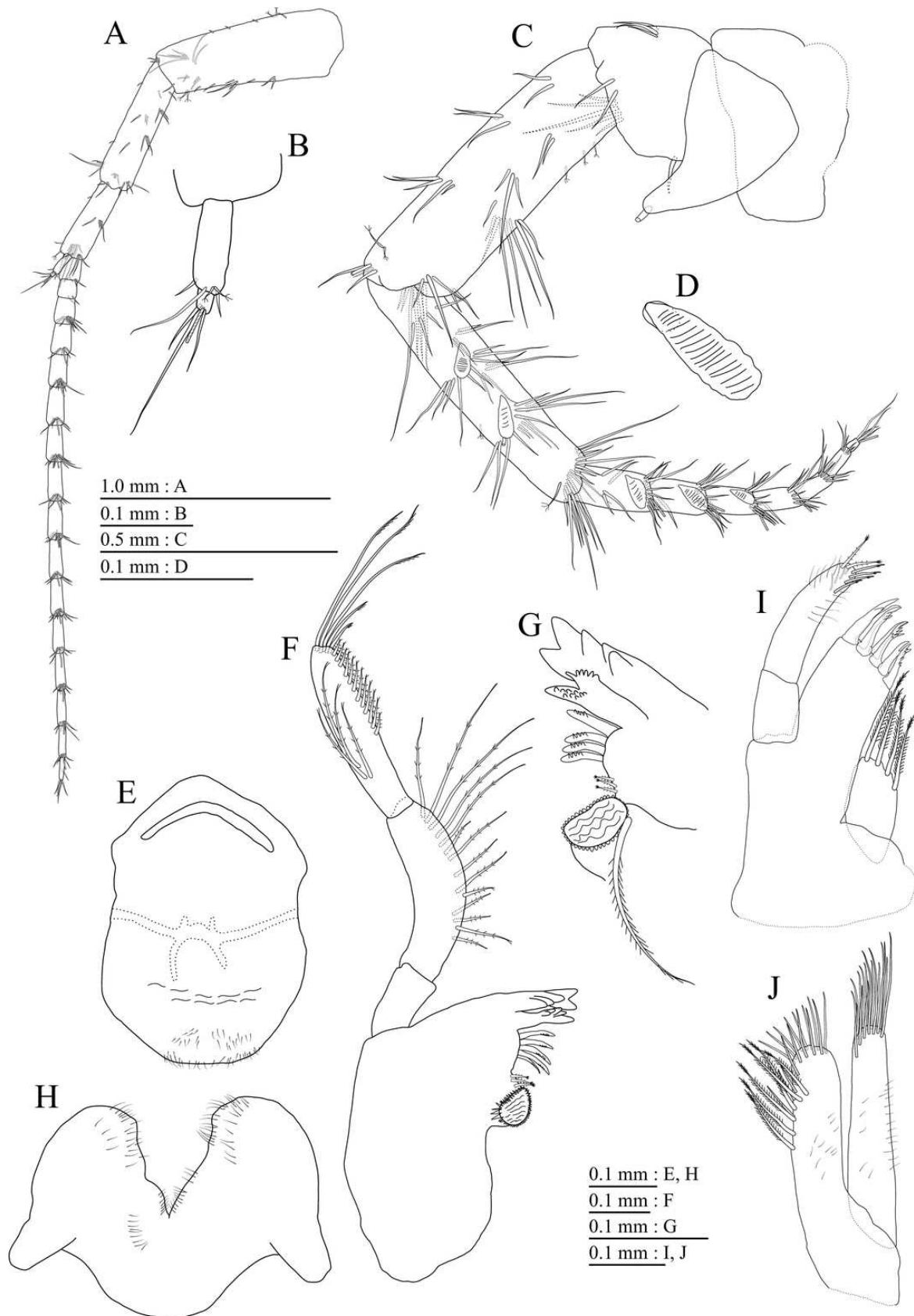


Figure 16

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.

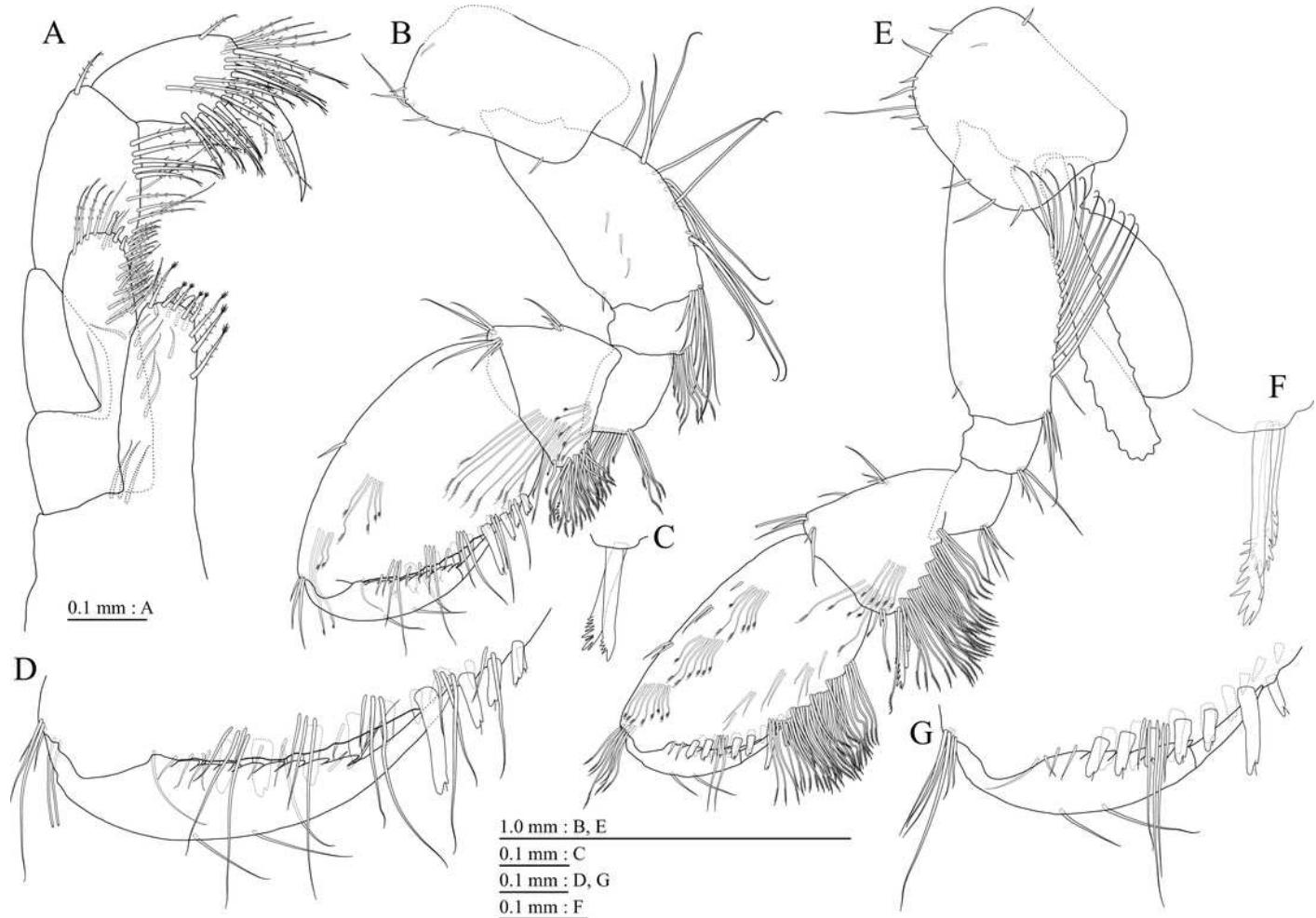


Figure 17

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.

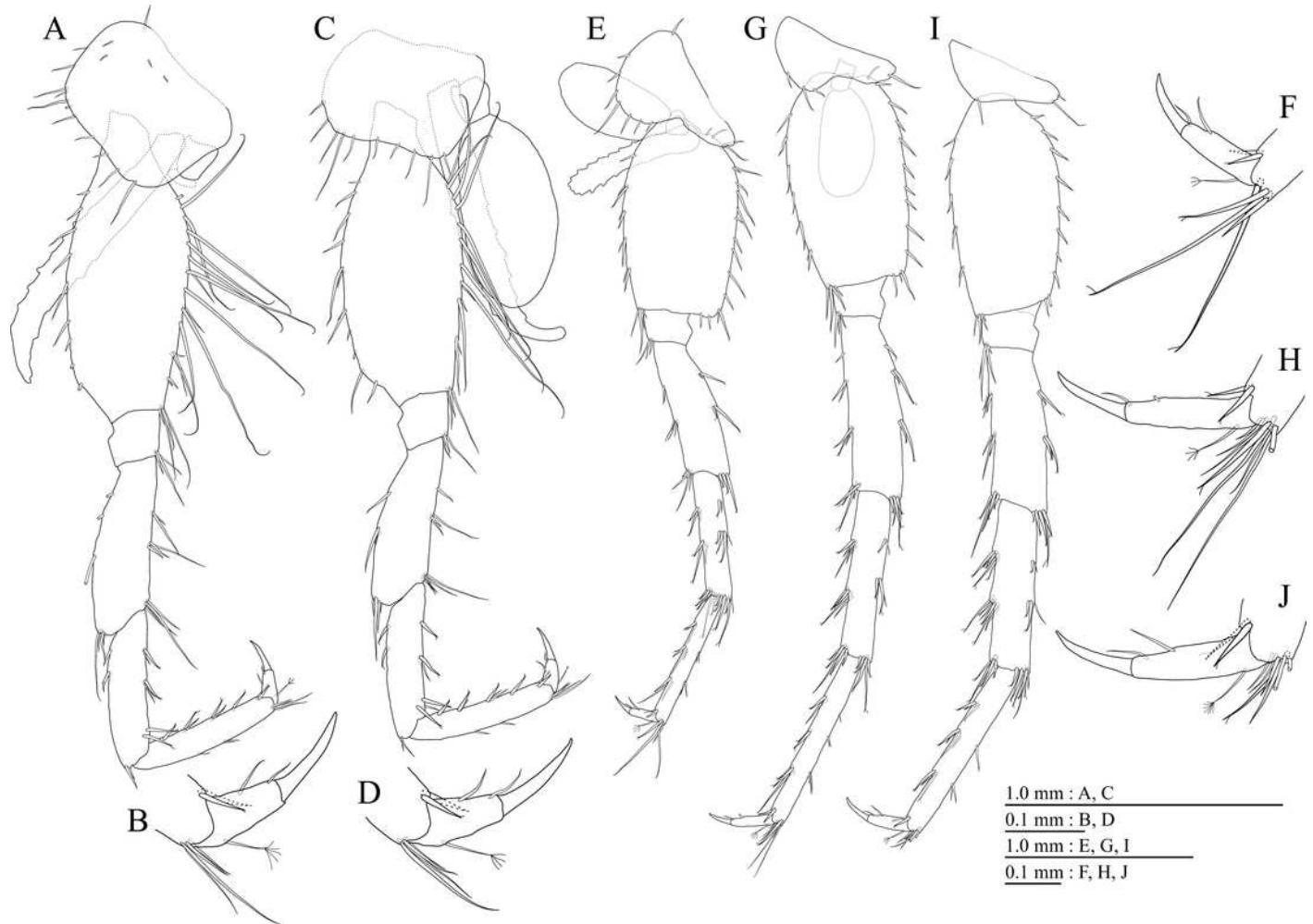


Figure 18

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.

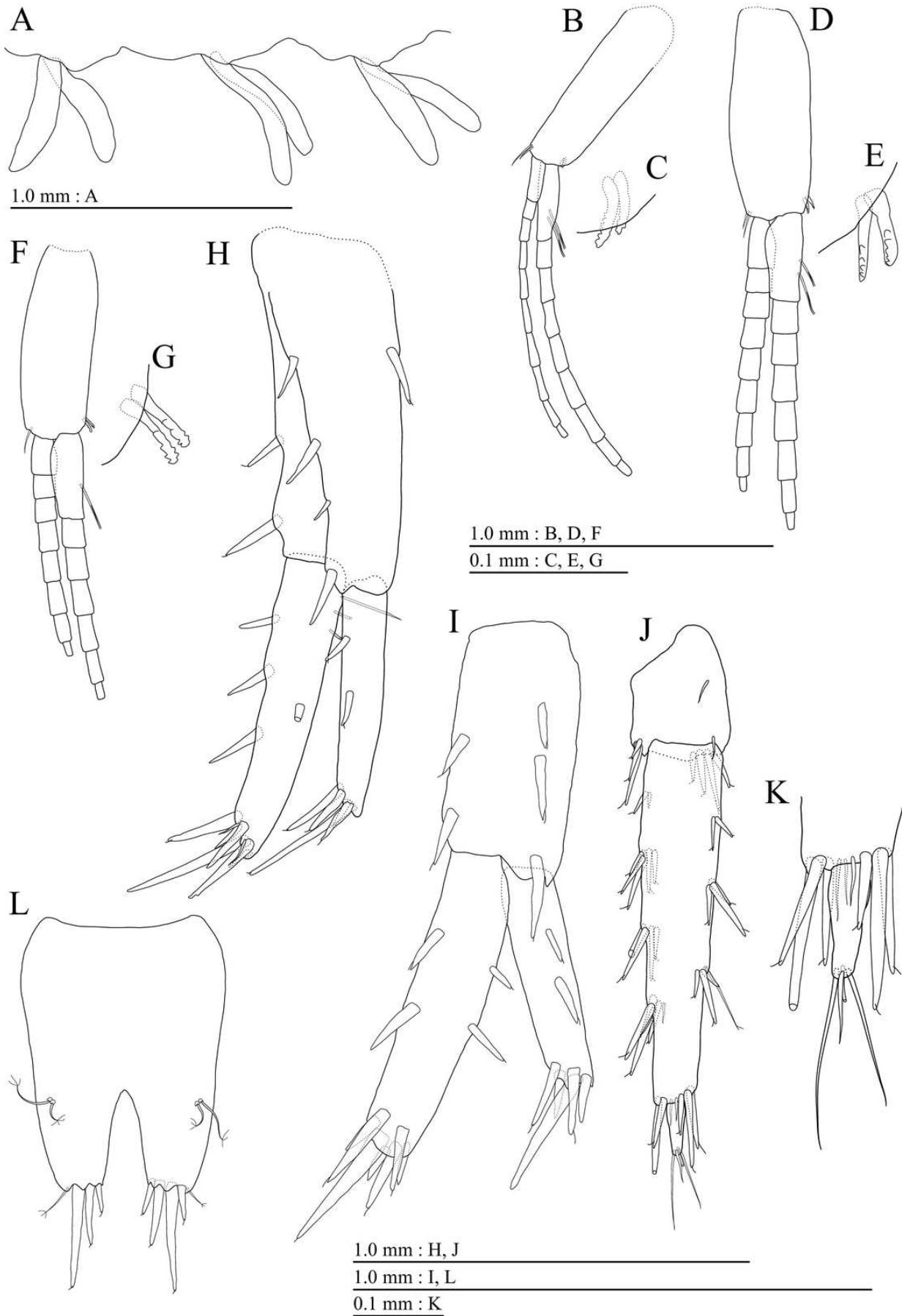


Figure 19

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.

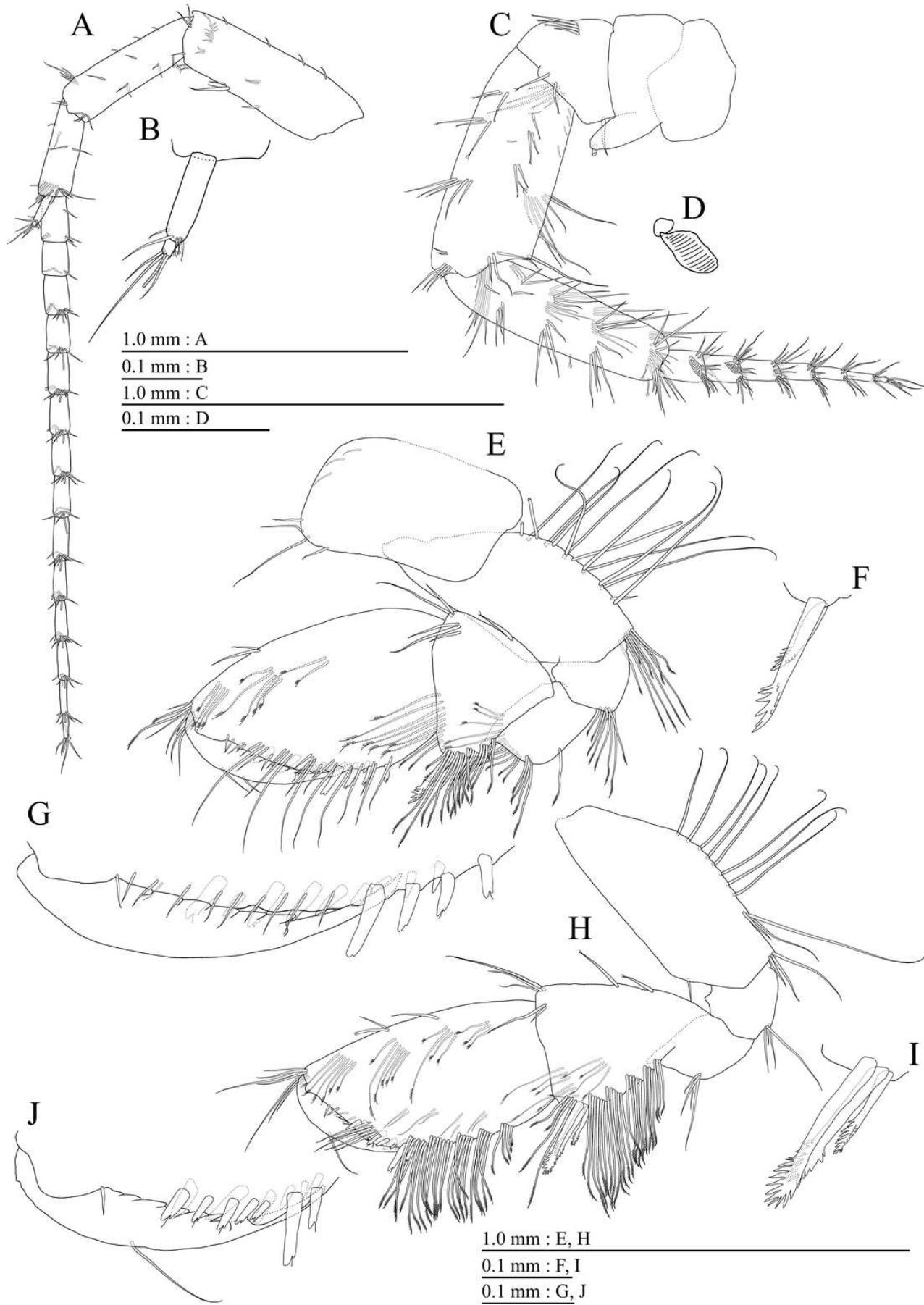


Figure 20

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.

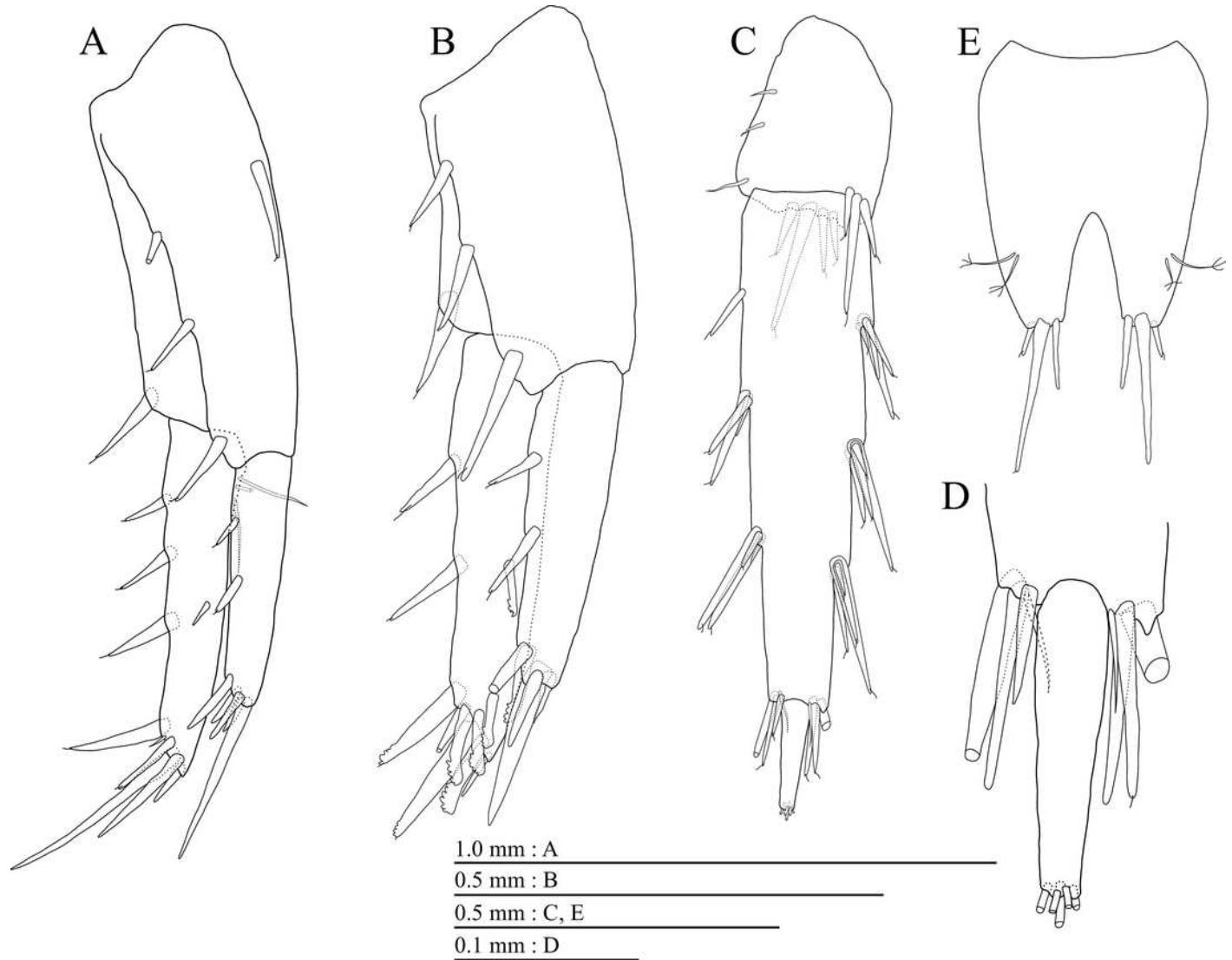


Figure 21

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.

