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3 Running head: TOMIKAWA AND NAKANO: TWO NEW SPECIES OF

4 *PSEUDOCRANGONYX* FROM JAPAN

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6 **Two new subterranean species of *Pseudocrangonyx* Akatsuka**

7 **& Komai, 1922 (Amphipoda: Crangonyctoidea:**

8 ***Pseudocrangonyctidae*), with an insight into groundwater**

9 **faunal relationships in western Japan**

10

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20

## ABSTRACT

21

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

40

41 **Key Words:** molecular phylogeny, systematics, stygobitic fauna

## INTRODUCTION

42

43 Crangonyctoid amphipods constitute an important component of Holarctic subterranean  
44 habitats (Holsinger, 1993, 1994), with western Eurasian *Niphargus* Schiödte, 1849 and  
45 North American *Crangonyx* Bate, 1859 being highly diversified. In eastern Asia,  
46 amphipods that belong to *Pseudocrangonyx* Akatsuka & Komai, 1922 are one of the  
47 stygobitic groups indigenous to groundwater environments in this region (Holsinger,  
48 1994). In contrast to *Niphargus* and *Crangonyx*, which comprise approximately 300 and  
49 50 species, respectively (Zhang & Holsinger, 2003; Hekmatara *et al.*, 2013),  
50 *Pseudocrangonyx* so far contains only 23 species, six of them recorded from the  
51 Japanese Archipelago (Uéno, 1966; Narahara *et al.*, 2009; Tomikawa *et al.*, 2016).

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

59 Our understanding of the taxonomy of *Pseudocrangonyx* has been hampered by a  
60 lack of the molecular data of the true *P. shikokunis* Akatsuka & Komai, 1922 and *P.*  
61 *kyotonis* Akatsuka & Komai, 1922, which were originally described along with the  
62 genus. Topotypic specimens of *P. shikokunis* and *P. kyotonis* have not yet been  
63 collected. Although Tomikawa *et al.* (2016) speculated that the unidentified  
64 *Pseudocrangonyx* spp. 4 and 5 might comprise *P. shikokunis* and/or *P. kyotonis*, the  
65 phylogroup consisted of deeply diverged clades, which were discordant with the

66 morphological characters defined by the type specimens of *P. shikokunis* and *P.*  
67 *kyotonis*. Moreover, the group identified as *Pseudocrangonyx* sp. 5 contained a  
68 specimen of *P. coreanus* Uéno, 1966 (Narahara *et al.*, 2009).

69 The taxonomies of *P. shikokunis* and *P. kyotonis* have also complicated by  
70 misidentified records. Tomikawa *et al.* (2016) revealed that the population inhabiting  
71 Akiyoshi limestone caves identified as *P. shikokunis* (Uéno, 1927) and from Gifu  
72 reported as *P. kyotonis* (Nunomura, 1975) clearly represent *Pseudocrangonyx* spp. 2 and  
73 6, respectively. We therefore establish the taxonomic status of these two unidentified  
74 lineages.

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

84

## 85 MATERIALS AND METHODS

### 86 *Sampling and morphological observation*

87 Specimens of species of *Pseudocrangonyx* were collected from a cave each in Gifu,  
88 Okayama, and Kumamoto prefectures and two caves in Yamaguchi Prefecture, Japan.

89 The geographical coordinates for all cave entrances were obtained using a Garmin

90 eTrex<sup>®</sup> GPS unit (Garmin, Olathe, KS, USA). Specimens for molecular analyses were  
91 also newly collected from two locations, a well in Takarazuka, Hyogo Prefecture  
92 (~34.8861°N, ~135.3067°E) and Hakiiai-syonyudo Cave in Kumamoto Prefecture  
93 (32.41456°N, 130.86549°E). Amphipods inhabiting caves were collected by scooping  
94 groundwater environments with a fine-mesh hand net and fixed in 99% ethanol on-site.  
95 All appendages of the specimens of the undescribed species were dissected in 70%  
96 ethanol and mounted in gum-chloral medium on glass slides under an Olympus SZX7  
97 stereomicroscope (Olympus, Tokyo, Japan). Specimens were examined using a Nikon  
98 Eclipse Ni light microscope (Nikon, Tokyo, Japan) and illustrated with the aid of a  
99 camera lucida. The body length from the tip of the rostrum to the base of the telson was  
100 measured along the dorsal curvature to the nearest 0.1 mm. The nomenclature of the  
101 setal patterns on the mandibular palp follows Stock (1974). The specimens examined  
102 are deposited in the Zoological Collection of Kyoto University (KUZ).

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

110

#### 111 *PCR, DNA sequencing, and molecular phylogenetic analyses*

112 Genomic DNA was extracted from appendage muscles following Tomikawa *et al.*  
113 (2014). Primer sets for the polymerase chain reaction (PCR) and cycle sequencing

114 reaction (CS) for the nuclear 28S rDNA (28S), histone H3 (H3), and the mitochondrial  
115 cytochrome *c* oxidase subunit I (COI) and 16S rDNA (16S) follow Tomikawa *et al.*  
116 (2016). The PCR and CS reactions and DNA sequencing were performed using a  
117 modified version of a method described by Tomikawa *et al.* (2016) using a T-100  
118 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The obtained sequences were  
119 assembled using DNA BASER (Heracle Biosoft, Pitești, Romania). In total, 12  
120 sequences from the three *Pseudocrangonyx* specimens were obtained and deposited  
121 with the International Nucleotide Sequence Database Collaboration (INSDC) through  
122 DNA Data Bank of Japan (Supplementary material Table S1).

123 The phylogenetic relationships of the species studied were estimated based on 28S,  
124 H3, COI, and 16S sequences. The dataset was identical to that used by Tomikawa *et al.*  
125 (2016) with the addition of the two sequences obtained from the type material of *P.*  
126 *elegantulus* (Zhao & Hou, 2017), four sequences from the holotype of *P. daejeoensis*  
127 (Lee *et al.*, 2018), and the newly obtained 12 sequences (Supplementary material Table  
128 S1). The alignments of H3 and COI were trivial, as no indels were observed. The  
129 sequences of 28S and 16S were aligned using MAFFT v7.312 (Kato & Standley,  
130 2013). The lengths of the 28S, H3, COI, and 16S sequences were 1360, 328, 658, and  
131 432 bp, respectively. The concatenated sequences yielded 2778 bp of aligned positions.

132 Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian  
133 inference (BI). The ML phylogeny was reconstructed using RAxML v8.2.8 (Stamatakis,  
134 2014) with the substitution model set as GTRCAT, immediately after nonparametric  
135 bootstrapping (BS) was conducted with 1000 replicates. The best-fit partition scheme  
136 was identified with the Akaike information criterion using PartitionFinder v2.1.2  
137 (Lanfear *et al.*, 2017) with the “greedy” algorithm (Lanfear *et al.*, 2012): 28S/ H3 1st

138 and 2nd positions/H3 3rd position/COI 1st position/COI 2nd position/COI 3rd  
 139 position/16S.

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

150

## 151 SYSTEMATICS

152 **Family Pseudocrangonyctidae** Holsinger, 1989

153 **Genus *Pseudocrangonyx*** Akatsuka & Komai, 1922

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

155 (Figs. 1A, 2–5)

156

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

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

159

160 *Type material*: Holotype female (10.2 mm), KUZ Z1980, Taishodo Cave (34.27694°N,  
 161 131.32056°E), Mine, Yamaguchi Prefecture, Japan, 6 June 2015, collected by K.



162 Tomikawa, T. Nakano, and S. Tashiro. Paratypes: 1 female (9.6 mm), KUZ Z1968, 1  
163 male (7.7 mm), KUZ Z1981, 1 female (8.7 mm), KUZ Z1982, 1 male (8.3 mm), KUZ  
164 Z1983, data same as for holotype; 1 female (7.7 mm), KUZ Z1967, 1 male (6.3 mm),  
165 KUZ Z1984, 1 female (6.8 mm), KUZ Z1985, Akiyoshido Cave (34.23333°N,  
166 131.30528°E), date and collectors same as for holotype; 1 female (9.0 mm), KUZ  
167 Z1972, 1 male (7.1 mm), KUZ Z1986, 1 female (8.5 mm), KUZ Z1987, Uyamado Cave  
168 (34.94250°N, 133.57583°E), Niimi, Okayama Prefecture, Japan, 30 July 2015, collected  
169 by K. Tomikawa and S. Tashiro; 1 female (6.5 mm), KUZ Z1953, Gongen-shonyudo  
170 Cave (32.41402°N, 130.40839°E), Kamiamakusa, Kumamoto Prefecture, 22 October  
171 2017, collected by K. Tomikawa and T. Nakano.

172 *Diagnosis:* Antennal sinus with rounded angle; eyes absent; pereonites 1–7 with short  
173 dorsal setae; urosomite 1 with ventral robust seta; dorsal margin of urosomite 3 lacking  
174 setae; sternal gill absent; antenna 1 reaching 0.55–0.73× body length; antenna 2 with  
175 calceoli in both sexes; mandible palp article 3 longer than article 2; maxilla 1 inner plate  
176 with 4–6 setae; maxilla 2 inner plate with oblique inner row of 4–6 setae; gnathopods 1,  
177 2, carpi with serrate setae on posterodistal corners in both sexes; palmar margins of  
178 propodi of gnathopods 1, 2 with 9–11, 8–9 robust setae, respectively; pleopod peduncles  
179 with marginal setae, inner margin of inner rami with bifid setae; uropod 1 inner ramus  
180 1.7× outer ramus length; inner, outer margins of inner ramus with 2 or 3, 1 or 2 robust  
181 setae, respectively; basal part with 1 or 2 slender setae, outer ramus with 1 or 2 marginal  
182 robust setae; uropod 2 inner ramus 1.4–1.5× outer ramus length; inner, outer margins  
183 with 3, 2 robust setae, respectively; outer ramus with 2 marginal robust setae; uropod 3  
184 terminal article 0.1–0.2× length of proximal article; telson 1.1–1.3× long as wide, cleft  
185 for 6.6–12.3%.

186 *Description:* Female (KUZ Z1980, 10.2 mm). Head (Fig. 1A) with short dorsal setae;  
187 rostrum reduced; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes  
188 absent. Pereonites 1–7 with short dorsal setae (Fig. 1A); posterolateral margin of  
189 pereonites 5–7 with 1, 1, 4 setae, respectively (Fig. 1A). Dorsal margin of pleonites 1–3  
190 with 14, 14, 19 setae, respectively (Fig. 2A–C). Posterior margin of epimeral plate 1  
191 with 7 setae, posteroventral corner not produced with seta (Fig. 2D); ventral, posterior  
192 margins of plate 2 with 4 robust setae, 6 setae, respectively, posteroventral corner not  
193 produced, with 2 setae (Fig. 2E); ventral, posterior margins of plate 3 with 4 robust  
194 setae, 3 setae, respectively, posteroventral corner rounded, with seta (Fig. 2F). Ventral  
195 margin of urosomites 1 with robust seta (Fig. 1); dorsal margin of urosomites 1, 2 with  
196 9, 8 setae, respectively (Fig. 2G, H), dorsal margin of urosomite 3 lacking setae (Fig.  
197 2I).

198 Antenna 1 (Fig. 2J)  $0.66 \times$  body length, length ratio of peduncular articles 1–3  
199 1.0:0.9:0.5; accessory flagellum (Fig. 2K) 2-articulate, terminal article with 3 setae, 1  
200 aesthetasc; primary flagellum 21-articulate, aesthetasc on some articles (Fig. 2L).

201 Antenna 2 (Fig. 2M)  $0.55 \times$  antenna 1 length; peduncular article 5 with 3 calceoli (Fig.  
202 2N); flagellum  $0.50 \times$  length peduncular articles 4, 5 combined, consisting of 7 articles,  
203 first 5 with calceolus.

204 Upper lip (labrum) (Fig. 2O) with rounded anterior margin, with fine setae.

205 Mandibles (Fig. 2P–R) with left, right incisors 5-dentate; left lacinia mobilis 5-dentate,  
206 right lacinia bifid, with many teeth; molar process triturative, molar of right mandible  
207 with accessory seta; accessory setal rows of left, right mandibles with 8, 4 weakly  
208 pectinate setae, respectively; palp 3-articulate, article 3 longer than article 2 with 3 A-  
209 setae, about 17 D-setae, about 8 E-setae. Lower lip (Fig. 2S) with broad outer lobes,

210 mandibular process of outer lobe rounded apically; inner lobes indistinct. Maxilla 1  
211 (Fig. 3A, B) with inner, outer plates, palp; inner plate subquadrate, medial margin with  
212 6 plumose setae; outer plate subrectangular with 7 serrate teeth apically (Fig. 3B); palp  
213 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 5  
214 apical robust setae, 6 subapical slender setae. Maxilla 2 (Fig. 3C) with oblique inner  
215 row of 5 plumose setae plus simple seta on inner plate. Maxilliped (Fig. 3D) with inner,  
216 outer plates, palp; inner plate (Fig. 3E) with 5 apical, 2 subapical robust setae; outer  
217 plate with 4 apical plumose setae, 8 robust, some slender setae on medial margin; palp  
218 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

219 Gnathopod 1 (Fig. 3F, G) with subquadrate coxa bearing setae on anterior to ventral  
220 margins of coxa, width  $1.6\times$  long as depth; anterior margin of basis bare, posterior  
221 margin of basis with many setae; posterodistal corner of carpus with 5 serrate setae (Fig.  
222 3H); propodus stout, subtriangular, palmar margin with 11 robust setae in 2 rows, some  
223 distally notched (Fig. 3G); posterior margin of dactylus dentate (Fig. 3G). Gnathopod 2  
224 (Fig. 3I, J) with rounded coxa bearing setae on its anterior margin, posterodistal corner,  
225 width  $1.3\times$  depth; basis with setae on anterodistal submargin, posterior margin;  
226 posterodistal corner of carpus with 4 serrate setae (Fig. 3K); propodus slender than that  
227 of gnathopod 1, with 9 robust setae along palmar margin in 2 rows, some distally  
228 notched (Fig. 3J); posterior margin of dactylus dentate (Fig. 3J). Pereopod 3 (Fig. 4A,  
229 B) with subquadrate coxa bearing setae on anterodistal, posteroventral corners, width  
230  $1.2\times$  depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus,  
231 propodus 1.0:0.9:0.9; posterior margin of dactylus with 2 setae (Fig. 4B). Pereopod 4  
232 (Fig. 4C, D) with coxa bearing setae on anterodistal, posteroventral corners, width  $1.5\times$   
233 depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus,

234 propodus 1.0:0.9:0.9; posterior margin of dactylus with 2 setae (Fig. 4D). Pereopod 5  
235 (Fig. 4E–G) with weakly bilobed coxa bearing setae on anterior, posterior lobes;  
236 anterior, posterior margins of basis with setae; length ratio of merus, carpus, propodus  
237 1.0:0.9:0.9; anterior margin of propodus with long setae (Fig. 4F); anterior margin of  
238 dactylus with 2 setae (Fig. 4G). Pereopod 6 (Fig. 4H, I) with coxa bearing concave  
239 lower margin, anterodistal, posteroproximal corners with setae; anterior, posterior  
240 margins of basis with setae; length ratio of merus, carpus, propodus 1.0:1.0:0.9; anterior  
241 margin of dactylus with 3 setae (Fig. 4I). Pereopod 7 (Fig. 4J, K) with coxa bearing  
242 shallowly concave lower margin, posteroproximal corner of coxa with seta; anterior,  
243 posterior margins of basis with setae; length ratio of merus, carpus, propodus  
244 1.0:1.1:1.1; posterior margin of dactylus with 3 setae (Fig. 4K).

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

247 Peduncle of pleopod 1 (Fig. 5A) with seta on outer margins; peduncles of pleopods  
248 2, 3 (Fig. 5D, E) lacking marginal setae. Pleopods 1–3 each with paired retinacula (Fig.  
249 5B), bifid seta (clothes-pin seta; Fig. 5C) on inner basal margin of inner ramus.

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

258 distal setae (Fig. 5I).

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

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

266 Gnathopod 1 carpus with 3–5 serrate setae on posterodistal corner; palmar margin  
267 of propodus with 9 robust setae in 2 rows, some distally notched (Fig. 5O). Gnathopod  
268 2 carpus bearing 3 or 4 serrate setae on posterodistal corner; palmar margin of propodus  
269 with 8 robust setae in 2 rows, some distally notched (Fig. 5P).

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

276 *Variation:* Antenna 1 length 0.55 (female 6.5 mm, KUZ Z1953) to  $0.73\times$  (male 6.3 mm,  
277 KUZ Z1984, male 7.1 mm, KUZ Z1986) body length; primary flagellar articles of male  
278 7.1 mm (KUZ Z1986), each with 1 or 2 aesthetascs. Antenna 2 length up to  $0.66\times$   
279 antenna 1 length (female 6.5 mm, KUZ Z1953). Maxilla 1 medial margin of inner plate  
280 with 4 (female 6.5 mm, KUZ Z1953), 5 (male 7.1 mm, KUZ Z1986, female 8.5 mm,  
281 KUZ Z1987) setae. Maxilla 2 inner plate with oblique inner row of 4 (female 6.5 mm,

282 KUZ Z1953), 5 (males 7.7, 7.1 mm, KUZ Z1981, Z1986) setae. Peduncles of pleopods  
283 2, 3 of specimen from Kumamoto (KUZ Z1953) with marginal setae. Telson length 1.3×  
284 width (male 7.1 mm, KUZ Z1986, female 6.8 mm, KUZ Z1985), cleft for 7.3 (female  
285 6.8 mm, KUZ Z1985) to 12.3% (male 7.1 mm, KUZ Z1986).

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

288 *New Japanese name*: Akatsuka-mekurayokoebi.

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

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

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

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

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

314

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

316 (Figs. 1B, 6–10)

317

318 *Pseudocrangonyx kyotonis* – Nunomura, 1975: 11.

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

320

321 *Type material*: Holotype male (5.8 mm), KUZ Z1988, Miyama-shonyudo Cave  
322 (35.74889°N, 137.02472°E), Miyama, Gujohachiman, Gifu Prefecture, Japan, 18  
323 October 2015, collected by K. Tomikawa and S. Tashiro. Paratypes: 5 females (5.5 mm,  
324 4.2 mm, 5.1 mm, 4.6 mm, 4.0 mm), KUZ Z1976, Z1977, Z1989, Z1990, Z1991, data  
325 same as for holotype.

326 *Diagnosis*: Antennal sinus with rounded angle; eyes absent; pereonites 1–7 with short  
327 dorsal setae; urosomite 1 without ventral robust seta; dorsal margin of urosomite 3  
328 lacking setae; sternal gill absent; antenna 1 0.45–0.51× body length; female antenna 2  
329 with calceoli; mandible palp article 3 almost as long as article 2; maxilla 1 inner plate

330 with 4 setae; maxilla 2 inner plate with oblique inner row of 5 setae; gnathopods 1, 2  
331 carpi without serrate setae on posterodistal corners; palmar margins of propodi of  
332 gnathopods 1,2 with 13–21,14–18 robust setae, respectively; pleopods, peduncles  
333 lacking marginal setae, inner margin of inner rami without bifid setae; uropod 1 inner  
334 ramus 1.4× outer ramus length; inner, outer margins of uropod 1 inner ramus with 2 or  
335 3, 0 or 1 robust setae, respectively, basal part with 1 or 2 slender setae, outer ramus with  
336 marginal robust seta; uropod 2 inner ramus 1.4–1.6× outer ramus length, inner, outer  
337 margins with 3, 2 robust setae, respectively; outer ramus with 1 or 2 marginal robust  
338 setae; uropod 3 terminal article 0.1× proximal article length; telson length 1.3× width,  
339 cleft for 6.8–10.2%.

340 *Description:* Male (KUZ Z1988, 5.8 mm). Head (Fig. 1B) with short dorsal setae;  
341 rostrum reduced; lateral cephalic lobe rounded; antennal sinus with rounded angle; eyes  
342 absent. Pereonites 1–7 with short dorsal setae (Fig. 1B); posterolateral margin of  
343 pereonites 5–7 with 1, 1, 3 setae, respectively (Fig. 1B). Dorsal margin of pleonites 1–3  
344 with 10, 12, 11 setae, respectively (Fig. 6A–C). Posterior margin of epimeral plate 1  
345 with 4 setae, posteroventral corner not produced, with seta (Fig. 6D); ventral, posterior  
346 margins of plate 2 with 2 robust setae, 4 setae, respectively, posteroventral corner not  
347 produced, with seta (Fig. 6E); ventral, posterior margins of plate 3 with 2 robust setae, 5  
348 setae, respectively, posteroventral corner rounded, with seta (Fig. 6F). Ventral margin of  
349 urosomites 1 without setae (Fig. 1B); dorsal margin of urosomites 1, 2 with 4 slender, 6  
350 robust setae, respectively (Fig. 7G, H), dorsal margin of urosomite 3 lacking setae (Fig.  
351 6I).

352 Antenna 1 (Fig. 6J) 0.45× body length, length ratio of peduncular articles 1–3  
353 1.0:0.7:0.4; accessory flagellum (Fig. 6K) 2-articulate, terminal article with 3 setae, 1



354 aesthetasc; primary flagellum 13-articulate, 1 aesthetasc on some articles. Antenna 2  
355 (Fig. 6L)  $0.67\times$  antenna 1 length; peduncular article 5 with 1 calceolus (Fig. 6M);  
356 flagellum  $0.58\times$  length of peduncular articles 4, 5 combined, consisting of 7 articles,  
357 first 4 with calceolus.

358 Upper lip (Fig. 6N) with rounded anterior margin bearing fine setae. Mandibles  
359 (Fig. 6O–Q) with left, right incisors 5-dentate; left lacinia mobilis 5-dentate, right  
360 lacinia bifid, with many teeth; molar process triturative, molar of right mandible with  
361 accessory seta; accessory setal rows of left, right mandibles with 4, 3 weakly pectinate  
362 setae, respectively; palp 3-articulate, article 3 almost as long as article 2, with 3 A-setae,  
363 about 10 D-setae, about 5 E-setae. Lower lip (Fig. 6R) with broad outer lobes,  
364 mandibular process of outer lobe apically rounded; inner lobes indistinct. Maxilla 1  
365 (Fig. 7A, B) with inner, outer plates, palp; inner plate subquadrate, medial margin with  
366 4 plumose setae; outer plate subrectangular with 7 serrate teeth apically (Fig. 7B); palp  
367 2-articulate, longer than outer plate, article 1 lacking marginal setae, article 2 with 3  
368 robust setae, slender seta apically, robust seta plus slender seta subapically. Maxilla 2  
369 (Fig. 7C) with oblique inner row of 5 plumose setae on inner plate. Maxilliped (Fig. 7D,  
370 E) with inner, outer plates, palp; inner plate (Fig. 7E) with 3 apical, 2 subapical robust  
371 setae; outer plate with 4 apical plumose setae, 3 robust, some slender setae on medial  
372 margin; palp 4-articulate, medial margin of article 2 lined with setae, article 4 with nail.

373 Gnathopod 1 (Fig. 7F, G) with subquadrate coxa bearing setae on anterodistal  
374 corner of coxa, width  $1.8\times$  depth; anterior margin of basis bare, posterior margin of  
375 basis with 6 setae; posterodistal corner of carpus without serrate setae; propodus stout,  
376 ovate, palmar margin with 10 lateral, 11 medial robust setae, some distally notched (Fig.  
377 7G); posterior margin of dactylus dentate (Fig. 7G). Gnathopod 2 (Fig. 7H, I) with

378 subquadrate coxa bearing setae on anterodistal, posteroventral corners, width 1.5×  
379 depth; basis with setae on anterodistal submargin, posterior margin; posterodistal corner  
380 of carpus without serrate setae; propodus more slender than propodus of gnathopod 1,  
381 with 7 lateral, 11 medial robust setae along palmar margin, some distally notched (Fig.  
382 7I); posterior margin of dactylus dentate (Fig. 7I). Pereopod 3 (Fig. 7J, K) with  
383 subquadrate coxa bearing setae on anterodistal, posteroventral corners, width 1.6×  
384 depth; anterior, posterior margins of basis with setae; length ratio of merus, carpus,  
385 propodus 1.0:0.8:0.8; posterior margin of dactylus with 2 setae (Fig. 7K). Pereopod 4  
386 (Fig. 8A, B) with coxa bearing setae on anterodistal, posteroventral corners, ventral  
387 margin, width 1.8× depth; anterior, posterior margins of basis with setae; length ratio of  
388 merus, carpus, propodus 1.0:0.9:0.8; posterior margin of dactylus with 2 setae (Fig. 8B).  
389 Pereopod 5 (Fig. 8C, D) with weakly bilobed coxa, bearing setae on anterior, posterior  
390 lobes; anterior, posterior margins of basis with setae; ratio of merus, carpus, propodus  
391 1.0:0.7:0.9; anterior margin of dactylus with 2 setae (Fig. 8D). Pereopod 6 (Fig. 8E, F)  
392 with coxa bearing concave lower margin, posteroproximal corner with seta; anterior,  
393 posterior margins of basis with setae; ratio of merus, carpus, propodus 1.0:0.8:0.9;  
394 anterior margin of dactylus with 2 setae (Fig. 8F). Pereopod 7 (Fig. 8G, H) with coxa  
395 bearing shallowly concave lower margin, posteroproximal corner of coxa with seta;  
396 anterior, posterior margins of basis with setae; ratio of merus, carpus, propodus  
397 1.0:0.9:1.0; posterior margin of dactylus with seta (Fig. 8H).

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

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

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

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

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

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

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

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

422 Uropod 1 (Fig. 10F) with basofacial slender seta on peduncle; inner ramus with 2  
423 marginal robust setae, basal part with 2 slender setae; outer ramus with marginal robust  
424 seta. Uropod 2 (Fig. 10G) with peduncle 0.9× inner ramus length; inner ramus 1.6×  
425 outer ramus length, inner, outer margins with 3, 2 robust setae, respectively, distal part

426 with 6 simple robust setae, short seta; outer ramus with marginal robust seta, distal part  
427 with 5 simple robust setae. Uropod 3 (Fig. 10H, I) with fewer robust setae on proximal  
428 article of outer ramus than in male.

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

431 *New Japanese name*: *Komai-mekurayokoebi*.

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

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

441 *Pseudocrangonyx komaii* **n. sp.** is similar to *P. coreanus* and *P. febras* Sidorov,  
442 2009 from Russia in lacking eyes, presence of ventral setae on urosomite 1, serrate setae  
443 on the posterodistal corner of gnathopod 1 carpus in females, bifid setae on the inner  
444 rami of pleopods, and in having a distally concaved telson (Uéno, 1966; Sidorov, 2009).  
445 The new is distinguished from *P. coreanus* by the number of robust setae on the palmar  
446 margin of the gnathopod propodus, more than 20 (*versus* less than 10) in male  
447 gnathopod 1, more than 10 (*versus* less than 10) in female gnathopod 1, more than 10  
448 (*versus* less than 10) in gnathopod 2, and absence (*present in P. coreanus*) of marginal  
449 setae on pleopod 1 peduncle. The new species differs from *P. febras* by distinct (*versus*

450 indistinct) antennal sinus, a shorter antenna 1 that is 0.6 times shorter than body length  
451 (*versus* 0.7 times longer), absence of serrate setae on the posterodistal corner of the  
452 gnathopod 2 carpus of females, and the outer margin of uropod 1 inner ramus with 0 or  
453 1 (*versus* three) robust setae.

#### 454 *Molecular phylogenies*

455 The obtained BI tree (mean ln-Likelihood [ $L$ ] = -15264.629; Fig. 11A) showed an  
456 almost identical topology to that of the ML tree ( $\ln L = -15778.578$ ; not shown). The  
457 results of the present analyses are generally concordant to those in Tomikawa *et al.*  
458 (2016), Zhao & Hou, (2017), and Lee *et al.* (2018). The trees failed to determine the  
459 precise phylogenetic position of *P. komaii* **n. sp.** within the genus *Pseudocrangonyx*.

460 The monophyly of *P. tiunovi* (Russia) + *P. korkishkoorum* (Russia) + *P. elegantulus*  
461 (China) + *P. yezonis* (Japan) + *P. akatsukai* **n. sp.** was well supported in both analyses  
462 (BS = 97%, PP = 0.99). This clade was split into three sub-clades, while their  
463 relationships remain uncertain. The monophyly of *P. elegantulus* and *P. yezonis* was  
464 recovered (BS = 95%, PP = 0.99). The Russian *P. tiunovi* and *P. korkishkoorum* formed  
465 a monophyletic group with high-support values (BS = 100, PP = 0.99). The specimens  
466 identified as *P. akatsukai* **n. sp.** formed a well-supported monophyletic lineage (BS =  
467 99%, PP = 1.0). The Russian clade and *P. akatsukai* **n. sp.** formed a clade in ML  
468 analyses, but this relationship was not fully supported (BS = 65%). The obtained  
469 phylogenies failed to reconstruct the robust relationships among *P. akatsukai* **n. sp.**  
470 specimens.

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

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

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

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479

## DISCUSSION

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

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

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

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

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

520 The results help elucidate the stygofaunal relationships in western Japan. The  
521 occurrence of *P. akatsukai* **n. sp.** indicates a close relationship between the underground

522 water habitats from the central to the western Chugoku Mountains and those in the  
 523 Amakusa Islands; both habitats could have been connected through northern Kyushu  
 524 during a past geological event. *Pseudocrangonyx* sp. 5 are widely distributed in western  
 525 Japan, so the stygofauna of the Chugoku Mountains in western Honshu, Shikoku, and  
 526 central Kyushu might be closely related to each other. Additional specimens of this  
 527 genus should be examined to elucidate the biogeographical history of *Pseudocrangonyx*  
 528 in western Japan.

529

#### 530 KEY TO SPECIES OF *PSEUDOCRANGONYX*

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

535 1. Eyes absent ... 2

536 – Trace of eyes present ... 20

537 2. Telson entire ... 3

538 – Telson emarginated ... 4

539 3. Telson tapering, length 1.2× width ... *P. kseinae* Sidorov, 2012

540 – Telson not tapering, length 1.7× width ... *P. levanidovi* Birstein, 1955

541 4. Inner plate of maxilla 1 with more than 4 setae ... 5

542 – Inner plate of maxilla 1 with less than 4 setae ... 16

543 5. Posterodistal corner of carpus of female gnathopod 2 without serrate setae ... 6

544 – Posterodistal corner of carpus of female gnathopod 2 with serrate setae ... 8

545 6. Female antenna 2 with calceoli ... *P. komaii* **n. sp.**



- 546 – Female antenna 2 without calceoli ... 7
- 547 7. Antenna 1 0.4× shorter than body length; posterodistal corner of carpus of female
- 548 gnathopod 1 without serrate setae ... *P. cavernarius* Hou & Li, 2003
- 549 – Antenna 1 0.7× longer than body length; posterodistal corner of carpus of female
- 550 gnathopod 1 with serrate setae ... *P. korkishkoorum* Sidorov, 2006
- 551 8. Telson laterally concave ... *P. manchuricus* Oguro, 1938
- 552 – Telson laterally straight, not concave ... 9
- 553 9. Sternal gills present ... *P. asiaticus* Uéno, 1934
- 554 – Sternal gills absent ... 10
- 555 10. Dorsal margins of pereopods 1–6 with long setae ... *P. yezonis* Akatsuka & Komai,
- 556 1922
- 557 – Dorsal margins of pereopods 1–6 without long setae ... 11
- 558 11. Posterodistal corner of carpus of female gnathopod 1 without serrate setae ... 12
- 559 – Posterodistal corner of carpus of female gnathopod 1 with serrate setae ... 13
- 560 12. Antenna 1 more than 0.5× longer than body length; terminal article of female
- 561 uropod 3 0.05× proximal article length ... *P. elenae* Sidorov, 2011
- 562 – Antenna 1 0.3× shorter than body length; terminal article of female uropod 3
- 563 length 0.2× proximal article length ... *P. holsingeri* Sidorov & Gontcharov, 2013
- 564 13. Peduncle of pleopod 1 with marginal setae ... 14
- 565 – Peduncle of pleopod 1 without marginal setae ... 15
- 566 14. Urosomite 1 with ventral robust seta; telson 1.1–1.3× width ... *P. akatsukai* **n. sp.**
- 567 – Urosomite 1 without ventral robust seta; telson 1.5× width ... *P. shikokunis*
- 568 Akatsuka & Komai, 1922
- 569 15. Female antenna 2 with calceoli; telson cleft along 24–27% of length ... *P.*

- 570 *elegantulus* Hou in Zhao & Hou, 2017
- 571 – Female antenna 2 without calceoli; telson cleft along 15% of length ... *P. tiunovi*
- 572 Sidorov & Gontcharov, 2013
- 573 16. Posterodistal corner of carpus of female gnathopod 2 with serrate setae ... 17
- 574 – Posterodistal corner of carpus of female gnathopod 2 without serrate setae ...18
- 575 17. Posterodistal corner of carpus of female gnathopod 1 with serrate setae ... *P.*
- 576 *kyotonis* Akatsuka & Komai, 1922
- 577 – Posterodistal corner of carpus of female gnathopod 1 without serrate setae ...18
- 578 18. Antenna 1 0.7× body length ... *P. febras* Sidorov, 2009
- 579 – Antenna 1 0.3× body length ... *P. sympatricus* Sidorov & Gontcharov, 2013
- 580 19. Female antenna 2 with calceoli ... *P. coreanus* Uéno, 1966
- 581 – Female antenna 2 without calceoli ... 19
- 582 19. Inner ramus of uropod 2 with marginal robust seta ... *P. daejeonensis* Lee,
- 583 Tomikawa, Nakano & Min, 2018
- 584 – Inner ramus of uropod 2 with 4 marginal robust setae ... *P. gudariensis* Tomikawa
- 585 & Sato in Tomikawa *et al.*, 2016
- 586 20. Outer plate of maxilla 1 with 5 serrate teeth ... *P. bohaensis* (Derzhavin, 1927)
- 587 – Outer plate of maxilla 1 with 7 serrate teeth ... 21
- 588 21. Telson cleft along 6.2% of length ... *P. birsteini* Labay, 1999
- 589 – Telson cleft along 16.3–20% of length ... 22
- 590 22. Outer ramus of uropod 2 with robust setae ... *P. relictata* Labay, 1999
- 591 – Outer ramus of uropod 2 without robust setae ... *P. susanaensis* Labay, 1999

592

593

## SUPPLEMENTARY MATERIAL

594 Supplementary material is available at *Journal of Crustacean Biology* online.  
595 S1 Table. Samples used for the molecular phylogenetic analyses, with voucher or isolate  
596 numbers, collection locality, and INSDC accession numbers.  
597 S2 Figure. Distributions of two *Pseudocrangonyx* phylogroups in western Japan.

598

599

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608

609

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735

## FIGURE LEGENDS

736

**Figure 1.** *Pseudocrangonyx akatsukai* n. sp., holotype female (10.2 mm), KUZ Z1980

737

(A); *Pseudocrangonyx komaii* n. sp., holotype male (5.8 mm), KUZ Z1988 (B).



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

747 **Figure 3.** *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980.  
748 Maxilla 1, dorsal view (**A**); apical robust setae on outer plate of maxilla 1 (**B**); maxilla  
749 2, dorsal view (**C**); maxilliped, dorsal view (**D**); apical setae on inner plate of  
750 maxilliped, dorsal view (**E**); gnathopod 1, lateral view (**F**); palmar margin of propodus  
751 and dactylus of gnathopod 1, medial view (**G**); serrate setae on posterodistal corner of  
752 carpus of gnathopod 1 (**H**); gnathopod 2, lateral view (**I**); palmar margin of propodus  
753 and dactylus of gnathopod 2, medial view (**J**); serrate setae on posterodistal corner of  
754 carpus of gnathopod 2 (**K**).

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

761 **Figure 5.** *Pseudocrangonyx akatsukai* **n. sp.**, holotype female (10.2 mm), KUZ Z1980  
 762 (A–J); paratype, male (7.7 mm), KUZ Z1981 (K–U). Pleopods 1–3, medial views,  
 763 plumose setae on rami omitted (A, D, E); retinacula on peduncle of pleopod 1, medial  
 764 view (B); bifid plumose seta (clothes-pin seta) on inner basal margin of inner ramus of  
 765 pleopod 1, medial view (C); uropods 1–3, dorsal views (F–H); terminal article of  
 766 uropod 3, dorsal view (I); telson, dorsal view (J); antenna 1, medial view, some distal  
 767 articles of main flagellum omitted (K); aesthetasc and associate setae on main flagellum  
 768 of antenna 1, medial view (L); antenna 2, medial view (M); calceolus on flagellum of  
 769 antenna 2, medial view (N); palmar margins of propodi and dactyli of gnathopods 1 and  
 770 2, medial views (O–P); uropod 1, dorsal view (Q); uropod 2, dorsal view (R); distal  
 771 setae on inner ramus of uropod 2, dorsal view (S); uropod 3, ventral view (T); terminal  
 772 article of uropod 3, ventral view (U).

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

781 **Figure 7.** *Pseudocrangonyx komaii* **n. sp.**, holotype male (5.8 mm), KUZ Z1988.  
 782 Maxilla 1, dorsal view (A); apical robust setae on outer plate of maxilla 1 (B); maxilla  
 783 2, dorsal view (C); maxilliped, dorsal view (D); apical setae on inner plate of  
 784 maxilliped, dorsal view (E); gnathopod 1, lateral view (F); palmar margin of propodus

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

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

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

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

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