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Reassessment of the Groundwater Amphipod *Paramoera relicta* Synonymizes the Genus *Relictomoera* with *Paramoera* (Crustacea: Amphipoda: Pontogeneiidae)

Takafumi Nakano[1,2] and Ko Tomikawa[1]*

[1]*Department of Science Education, Graduate School of Education, Hiroshima University,*
Higashihiroshima, Hiroshima 739-8524, Japan
[2]*Department of Zoology, Graduate School of Science, Kyoto University,*
Kyoto 606-8502, Japan

The systematic status of the stygobitic amphipod, *Paramoera relicta* Uéno, 1971, was revisited based on morphological examination of the paratypes, and molecular phylogenetic analyses using a newly collected specimen from its type locality. The paratypes clearly showed that this species does not possess the "sinusoid" antennal sinus in the head, which was erroneously described in the original description of this species; *P. relicta* is unquestionably classified within the genus *Paramoera* Miers, 1875. Molecular phylogenetic trees obtained with nuclear histone H3 and mitochondrial 16S rRNA demonstrated that *P. relicta* clearly belongs to the clade consisting of *Paramoera* species. Since the genus *Relictomoera* Barnard and Karaman, 1982 was erected according to the "sinusoid" characteristic of its type species *P. relicta*, this genus is no longer valid, and should be treated as a junior subjective synonym of *Paramoera*. One of the paratypes of *P. relicta* is herein designated as a neotype for the species to clarify its taxonomic status and emend the morphological characteristics of this pontogeneiid amphipod.

Key words: Japan, subterranean fauna, redescription, molecular phylogeny, neotypification

INTRODUCTION

Subterranean habitats in the Japanese Archipelago and adjacent islets have harbored diverse stygobitic crustacean amphipods; these were classified into the eight genera by Tomikawa et al. (2017). Two of them, *Awacaris* Uéno, 1971a and *Relictomoera* Barnard and Karaman, 1982, belong to the family Pontogeneiidae, and had been considered as enigmatic taxa (see Tomikawa et al., 2017). The taxonomic status of *Awacaris* was recently revisited synonymizing with *Sternomoera* Barnard and Karaman, 1982, and *Awacaris* now contains three subterranean and four epigean species distributed in the Japanese Archipelago and adjacent islets (Tomikawa et al., 2017). A previous study of *Awacaris* highlighted that the morphological features, which were defined as the diagnostic characteristics of this genus (Barnard and Karaman, 1982), might simply represent autoapomorphies of its type species (Tomikawa et al., 2017). The diagnosis of *Awacaris* is thus now expanded to contain the other congeners that previously classified within *Sternomoera*.

In contrast, the taxonomic status of *Relictomoera* has remained uncertain, although its diagnostic key character was based on the incomplete original description of the species assigned to this genus. *Relictomoera* was originally established based on the "sinusoid" antennal sinus of the type species, *Paramoera relicta* Uéno, 1971b, and another congener, *Paramoera tsushimana* Uéno, 1971c (Barnard and Karaman, 1982). However, Hirayama (1990) stated that the original descriptions and figures of their antennal sinuses were based on insufficient observations of the type specimens, and concluded that *Relictomoera* should be synonymized with the pontogeneiid *Paramoera* Miers, 1875. Based on the examination of the holotype, moreover, the recent redescription of *P. tsushimana* classified the species within *Awacaris* (as *Sternomoera* in Tomikawa et al. [2014]).

Although Hirayama (1990) already concluded that *Relictomoera* should be treated as a junior synonym of *Paramoera*, his taxonomic conclusion has not been followed by the major taxonomic studies (e.g., Lowry and Myers, 2013). In this study, the full morphological description of *Paramoera relicta* is provided based on its paratypes. Additionally, the molecular phylogenetic position of *P. relicta* is estimated based on a newly collected specimen using nuclear histone H3 and mitochondrial 16S rRNA markers. The present results signal an end to *Relictomoera* as a valid genus within Pontogeneiidae.

MATERIALS AND METHODS

Sampling and morphological examination

A specimen of *Paramoera relicta* was newly collected from its type locality, Iana Cave, in Fukuejima Island, Goto Islands, Kyushu, Japan, in 2010. The specimen was directly fixed and then preserved in ~80%. The geographical coordinates for the cave entrance was obtained using a Garmin eTrex® GPS unit. The pereon muscle was removed from the specimen under a stereomi-

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* Corresponding author. E-mail: tomikawa@hiroshima-u.ac.jp
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croscope (Olympus SZX7), and preserved in absolute ethanol for DNA extraction.

All appendages of the examined specimen were dissected in 70% ethanol and mounted in gum-chloral medium on glass slides under the stereomicroscope. Specimens were examined using a light microscope (Olympus BH2) and illustrated with the aid of a camera lucida. The body length from the tip of the rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm. The nomenclature of the setal patterns on the mandibular palp follows Stock (1974). The paratypes of *P. relicta* have been kept at the National Museum of Nature and Science, Tokyo (NSMT). The newly collected specimen has been deposited in the Zoological Collection of Kyoto University (KUZ).

DNA extraction, PCR and DNA sequencing

Genomic DNA was extracted from the pereon muscle preserved in absolute ethanol following the method described by Nakano (2012a). In addition to a *P. relicta* specimen, molecular data were obtained from 26 eusiroidean OTUs (Table 1); genomic DNA was extracted from appendage muscles applying the method described by Tomikawa et al. (2014) and Tomikawa et al. (2017). Primer sets for the polymerase chain reaction (PCR) and cycle sequencing reaction (CS) for the nuclear histone H3 (H3) and mitochondrial 16S rRNA (16S) used in this study are shown in Tomikawa et al. (2016). The PCR reaction and DNA sequencing were performed using a modified version of a method presented in Nakano (2012b), and the detailed conditions were identical to those in Tomikawa et al. (2016); the PCR and CS reactions were conducted using a Bio-Rad T-100 Thermal Cycler. In total, 54 sequences were obtained in this study and deposited with the International Nucleotide Sequence Database Collaboration (INSDC) though the DNA Data Bank of Japan (Table 1).

Molecular phylogenetic analyses

The phylogenetic position of *Paramoera relicta* was estimated based on H3 and 16S sequences. The 8 described species, i.e., *P. erimoensis* Kuribayashi and Kyono, 1995, *P. koysama* Kuribayashi and Kyono, 1995, *Awacaris japonica* (Tattersall, 1922), *A. kawasawai* Uéno, 1971a, *A. morinoi* (Tomikawa and Ishimaru) in Tomikawa et al. (2014), *A. rhyaca* (Kuribayashi et al., 1996), *A. yezoensis* (Uéno, 1933), and *Pontogeneia rostrata* Gurjanova, 1938 and 3 unidentified species, i.e., *Paramoera* spp. 1 and 2, and *Pontogeneia* sp., were included as pontogeneiid ingroup taxa. Additionally, two species, *Eusirus hirayamae* Bousfield and

Table 1. Samples used for the phylogenetic analyses. The information on the vouchers is accompanied by the collection locality, and the INSDC accession numbers. Acronyms: KUZ, the Zoological Collection of Kyoto University; and LBM, Lake Biwa Museum.

Species	Voucher	Locality	INSDC#	
			Histone H3	16S rRNA
Pontogeneiidae				
Paramoera relicta	KUZ Z1949	Iana Cave, Fukue Is, Nagasaki	LC331275	LC331276
Paramoera koysama	KUZ Z1928	Charatsunai Beach, Muroran, Hokakido	LC334140	LC334114
Paramoera erimoensis	KUZ Z1938	Yobetsu River, Shakotan, Hokkaido	LC334141	LC334115
Paramoera sp. 1	KUZ Z1939	Horonaifu River, Shakotan, Hokkaido	LC334142	LC334116
Paramoera sp. 2	KUZ Z1950	Cape Toyokuni, Misaki, Osaka	LC334139	LC334113
Awacaris japonica	KUZ Z1926	Wakitsuboikenomizu, Iwasaki, Aomori	LC334124	LC334098
Awacaris japonica	KUZ Z1929	Uchibera, Tsuruoka, Yamagata	LC334126	LC334100
Awacaris japonica	KUZ Z1936	Takinamicho, Fukui, Fukui	LC334130	LC334104
Awacaris japonica	KUZ Z1937	Kitakomatsu, Otsu, Shiga	LC334131	LC334105
Awacaris kawasawai	KUZ Z1947	Saruta Cave, Hidaka, Kochi	LC334123	LC334123
Awacaris morinoi	LBM1430005403 (paratype)	Gokasho-Kondo, Higashiomi, Shiga	LC334132	LC334106
Awacaris rhyaca	KUZ Z1927	Atsutaku-gokibiru, Ishikari, Hokkaido	LC334125	LC334099
Awacaris rhyaca	KUZ Z1930	Dondonbuchi, Goto, Nagasaki	LC334127	LC334101
Awacaris rhyaca	KUZ Z1934	Tonnai River, Rebun Is, Hokkaido	LC334128	LC334102
Awacaris rhyaca	KUZ Z1935	Shishizaki, Miyazu, Kyoto	LC334129	LC334103
Awacaris yezoensis	KUZ Z1940	Nishiku, Sapporo, Hokkaido	LC334133	LC334107
Awacaris yezoensis	KUZ Z1941	Horonai, Atsuma, Hokkaido	LC334134	LC334108
Awacaris yezoensis	KUZ Z1942	Torinuma, Furano, Hokkaido	LC334135	LC334109
Awacaris yezoensis	KUZ Z1943	Nokanancho, Ashibetsu, Hokkaido	LC334136	LC334110
Awacaris yezoensis	KUZ Z1944	Shumarinai, Horokanai, Hokkaido	LC334120	LC334094
Awacaris yezoensis	KUZ Z1945	Aoyamaoku, Tobetsu, Hokkaido	LC334121	LC334095
Awacaris yezoensis	KUZ Z1946	Uma, Fukagawa, Hokkaido	LC334122	LC334096
Pontogeneia rostrata	KUZ Z1933	Higashi Beach, Iwami, Tottori	LC334145	LC334119
Pontogeneia sp.	KUZ Z1931	Ogakichofukae, Etajima Is, Hiroshima	LC334143	LC334117
Pontogeneia sp.	KUZ Z1932	Ogakichofukae, Etajima Is, Hiroshima	LC334144	LC334118
Outgroup				
Eusirus hirayamae	KUZ Z1948	West Pacific, Cape Toi, Miyazaki	LC334143	LC334112
Calliopius sp.	KUZ Z1951	Daikoku Is, Akkeshi, Hokkaido	LC334144	LC334111

Hendrycks, 1995 and *Calliopius* sp., were selected as outgroup taxa.

The alignment of H3 was trivial, as no indels were observed. The sequences of 16S were aligned using MAFFT v. 7.310 L-INS-i (Katoh and Standley, 2013). The lengths of H3 and 16S sequences were 328 bp and 424 bp, respectively. The concatenated sequences yielded 752 bp of aligned positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). The ML phylogeny was reconstructed using RAxML v. 8.2.8 (Stamatakis, 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (BS) was conducted with 1000 replicates. The best-fit partition scheme was identified with the Akaike information criterion using PartitionFinder v. 2.1.2 (Lanfear et al., 2017) with the "all" algorithm: H3 1st and 2nd positions/H3 3rd position/16S.

Bayesian inference and posterior probabilities (PPs) were estimated using MrBayes v. 3.2.6 (Ronquist et al., 2012). The best-fit partition scheme and models for each partition were selected with the Bayesian information criterion using PartitionFinder with the "all" algorithm: for H3 1st and 2nd positions, K80 + I; for H3 3rd position, GTR + G; and for 16S, GTR + I + G. Two independent runs for four Markov chains were conducted for 2 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond, 2013), and the first 5001 trees were discarded based on the results.

RESULTS

Taxonomy

Genus ***Paramoera*** Miers, 1875

Relictomoera Barnard and Karaman, 1982, pp. 168–169; Hirayama, 1990, pp. 958–959 (in discussion; as a junior synonym of *Paramoera*); Barnard and Karaman, 1991, p.

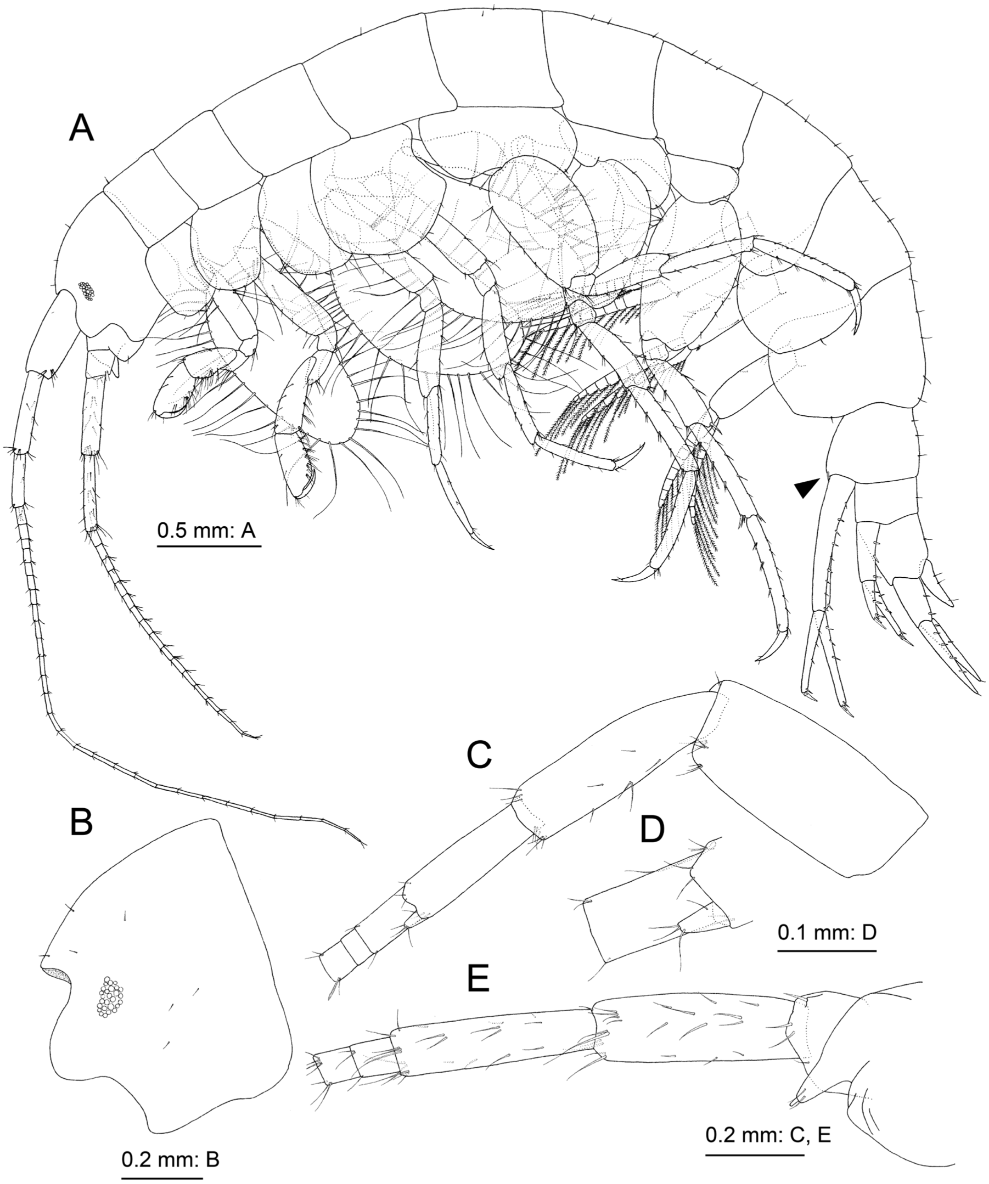

Fig. 1. *Paramoera relicta* Uéno, 1971b, female 6.6 mm, neotype (NSMT-Cr 26835). **(A)** Habitus, lateral view; an arrowhead indicates a robust seta on the posteroventral corner of urosomite 1. **(B)** Head, lateral view. **(C)** Antenna 1, medial view (some flagellar articles omitted). **(D)** Accessory flagellum of antenna 1, medial view. **(E)** Antenna 2, medial view (some flagellar articles omitted).

337; Sidorov, 2010, table 2; Tomikawa and Morino, 2012, p. 42; Lowry and Myers, 2013, p. 41.

Remarks. The examined specimens clearly showed that *Paramoera relicta* possesses the anterolateral part of its head without any distinctive sinuses. Since *P. relicta* clearly possesses the diagnostic characteristics of *Paramoera* defined by Staude (1995), the genus *Relictomoera* should be treated as a junior subjective synonym of *Paramoera*.

Paramoera relicta Uéno, 1971b
(Figs. 1–3)

Paramoera relicta Uéno, 1971b, pp. 161–167, figs. 1–3.

Relictomoera relicta: Barnard and Karaman, 1982, p. 168; Hirayama, 1990, p. 958 (in discussion); Barnard and Karaman, 1991, p. 337; Tomikawa and Morino, 2012, p. 42.

Diagnosis. Head with reduced eyes; antennal sinus rounded; mandibular palp article 3 with A-, D-, and E-setae; maxilla 1, inner plate with 5 plumose setae, outer plate with 10 serrate robust setae, palp article 2 without outer marginal setae; maxilla 2 inner plate with oblique inner row of 5 plumose setae; gnathopod 2 propodus shorter than carpus; coxa of pereopod 4 with posterior concavity; coxal gill on pereopod 7 lacking; sternal gill and hump absent; uropod 3 without plumose setae on rami; telson longer than wide.

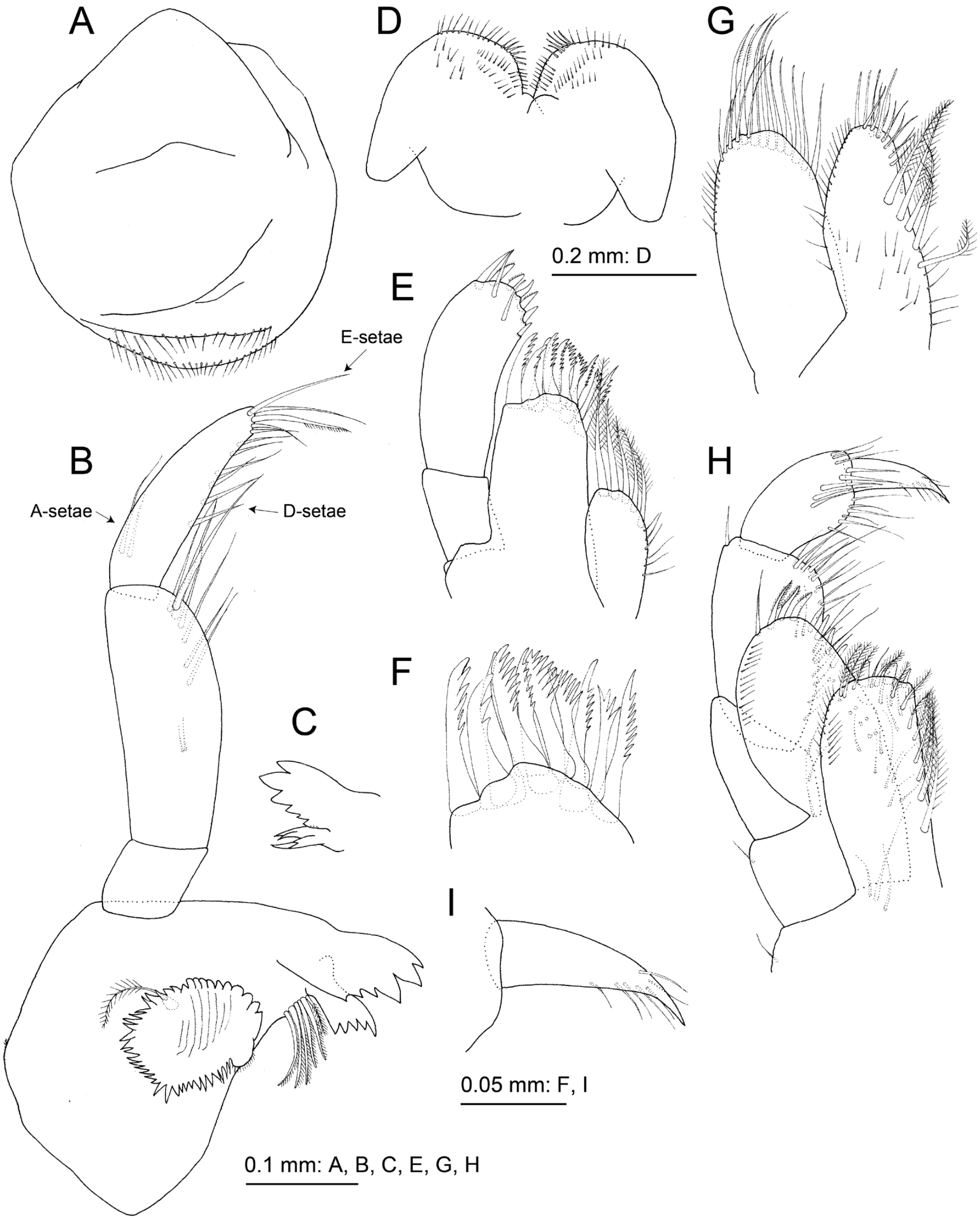

Fig. 2. *Paramoera relicta* Uéno, 1971b, female 6.6 mm, neotype (NSMT-Cr 26835). **(A)** Upper lip, anterior view. **(B)** Left mandible, medial view. **(C)** Incisor and lacinia mobilis of right mandible. **(D)** Lower lip, anterior view. **(E)** Maxilla 1, dorsal view. **(F)** Apical part of outer plate of maxilla 1, dorsal view. **(G)** Maxilla 2, dorsal view. **(H)** Maxilliped, dorsal view. **(I)** Dactylus of palp of maxilliped, dorsal view.

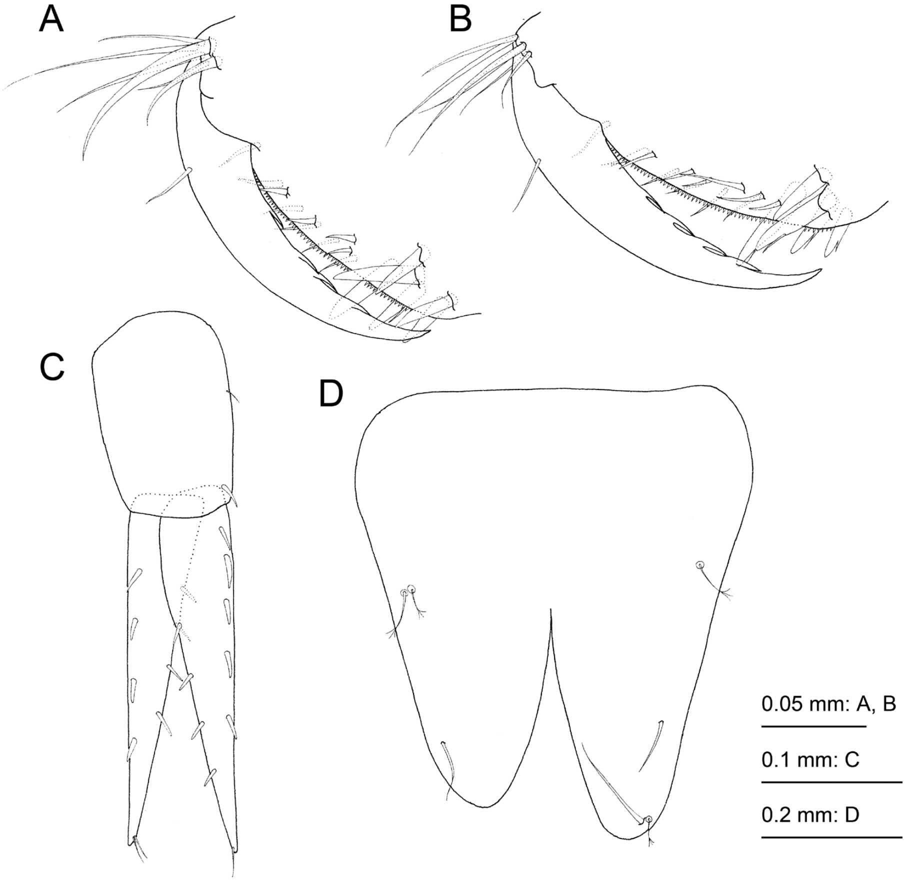

Fig. 3. *Paramoera relicta* Uéno, 1971b, female 6.6 mm, neotype (NSMT-Cr 26835). **(A)** Palmar margin of propodus and dactylus of gnathopod 1, medial view. **(B)** Palmar margin of propodus and dactylus of gnathopod 2, medial view. **(C)** Uropod 3, dorsal view. **(D)** Telson, dorsal view.

Designation of neotype. One paratype, NSMT-Cr 26835, collected from Iana Cave in Fukuejima Island, was chosen as the neotype for *P. relicta* in this study, because the erroneous original description of the species should be emended by an appropriate description based on a newly fixed name-bearing type for this species. The present neotype clarifies the taxonomic status of *P. relicta* revealing that this species does not possess a "sinusoid" antennal sinus.

The one female individual bearing a brood pouch was designated as the holotype for *P. relicta* by Uéno (1971b); the other 26 female specimens were thus automatically treated as its paratypes. These specimens were collected by the speleobiological survey by NSMT (as National Science Museum, Tokyo in Uéno [1971b]). Although the 26 paratypes have been kept in the zoological collection at NSMT, the holotype was missing in the collection at NSMT. Since the second author (KT) could not confirm that the holotype of *P. relicta* had been present in the collection at NSMT, the holotype is believed to be lost or destroyed in the past.

The neotype for *P. relicta* newly designated in this study is a female specimen, which is the consistent sex as for the holotype, and was collected from the type locality along with the other specimens of the original type series. Accordingly, the present neotypification does not violate Article 75.3 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999).

Material examined. *Neotype*: NSMT-Cr 26835, female 6.6 mm (slides), Iana Cave (as Sakishirazu-no-i-ana in Uéno [1971b]), Fukuejima Island, Goto Islands, Nagasaki Pref., Japan by Shun-Ichi Uéno and Yoshinobu Morimoto on 3 April 1970. *Additional materials*: NSMT-Cr 4137, remaining paratypes in the original type series, 25 females (not dissected), data as for neotype. KUZ Z1949, female, by Naoki Koike on 16 December 2010, locality as for neotype (Cave entrance: 32.594875°N, 128.782956°E).

Redescription. *Female* [neotype, NSMT-Cr 26835]. Body (Fig. 1A) dorsally smooth, with fine setae; urosomite 1 (Fig. 1A) with robust seta on posteroventral corner; head (Fig. 1A, B) shorter than pereonites 1 and 2 combined; rostrum short; eyes reduced, representing several ommatidia; lateral cephalic lobe rounded; antennal sinus rounded. Epimeral plates 1–3 (Fig. 1A) posterodistal corner not pointed; posterior margins of epimeral plates 2 and 3 each with seta.

Antenna 1 (Fig. 1A) half body length; peduncular articles 1–3 in length ratio 1.0 : 1.0 : 0.6; primary flagellum 28-articulate; accessory flagellum (Fig. 1D) 1-articulate, scale like, with three apical and one lateral setae. Antenna 2 (Fig. 1A, E) length 0.6 times that of antenna 1; peduncular article 2 gland cone with two subapical setae; peduncular article 5 slightly shorter than peduncular article 4; flagellum 17-articulate; calceolus absent.

Upper lip (Fig. 2A) ventral margin convex, rounded, with minute setae. Left and right mandibular incisors (Fig. 2B, C) 6-dentate, with left lacinia mobilis 5-dentate and right bifid, bearing five teeth; molar process triturative with plumose seta; palp 3-articulate, articles 1–3 in length ratio 1.0 : 3.3 : 2.9, article 1 bare, article 2 with 9 marginal and submarginal setae, article 3 with 2 A-, 6 D-, 5-E setae. Lower lip (Fig. 2D) outer lobes broad, setulose, mandibular lobes narrow; inner lobes indistinct. Maxilla 1 (Fig. 2E, F) inner plate narrow with five plumose setae; outer plate rectangular with 10 serrate robust setae; palp 2-articulate; article 1 rectangular, lacking setae; article 2 outer margin without setae, apical margin with robust setae and submarginally with slender setae. Maxilla 2 (Fig. 2G) inner plate with oblique inner row of five plumose setae. Maxilliped (Fig. 2H, I) with subrectangular inner plate, bearing two blunt and one slender robust setae apically; outer plate ovate, exceeding half of palp article 2, apical margin with plumose setae, inner part with robust setae submarginally; palp 4-articulate, inner part of article 4 with submarginal setae.

Gnathopod 1 (Figs. 1A, 3A) basis with long setae on anterior and posterior margins; propodus slightly longer than carpus, palmar margin oblique, posterodistal corner of palm with 3 inner and 3 outer robust setae; posterior margin of dactylus serrated. Gnathopod 2 (Figs. 1A, 3B) longer than gnathopod 1; basis with long setae on anterior and posterior margins; propodus shorter than carpus, palmar margin oblique, posterodistal corner of palm with two inner and three outer robust setae; posterior margin of dactylus serrated.

Pereopods 3 and 4 (Fig. 1A) slender; coxa 4 expanded with posterior concavity; bases linear; dactyli of pereopods 3 and 4 length 0.4 and 0.5 times as long as propodi, respectively. Pereopod 5 (Fig. 1A) with weakly bilobed coxa; basis weakly expanded posteriorly, with posterodistal lobe; dactylus length 0.4 times that of propodus. Pereopod 6 (Fig. 1A) with bilobed coxa, posterior lobe elongate downward; basis expanded posteroproximally, with posterodistal lobe; dactylus length 0.5 times that of propodus. Pereopod 7 (Fig. 1A) with rounded coxa; basis linear-oblong, with weak posterodistal lobe; dactylus length 0.4 times that of propodus.

Coxal gills (Fig. 1A) on gnathopod 2 and pereopods 3–4 slender, gills on pereopods 5–6 pyriform. Sternal gill and hump absent. Brood plates (Fig. 1A) large, broad, with numerous long setae, present on gnathopod 2 and pereopods 3–5.

Pleopods 1–3 (Fig. 1A) peduncles with marginal setae; rami developed.

Uropod 1 (Fig. 1A) peduncle elongate, outer margin with eight robust setae, inner and outer distal corners each with robust seta; inner ramus with three inner marginal robust setae; outer ramus slightly shorter than inner ramus, with three robust setae on inner margin. Uropod 2 (Fig. 1A) with peduncle bearing two robust setae on outer margin, inner and outer distal corners each with robust seta; inner ramus with two inner and one outer marginal robust setae; outer ramus shorter than inner ramus, with two outer marginal robust setae. Uropod 3 (Fig. 3C) inner ramus slightly shorter than outer ramus, with marginal robust setae and two subapical slender setae; outer ramus 1-articulate, with marginal robust setae and subapical slender seta.

Telson (Fig. 3D) length 1.1 times longer than wide, cleft for 52% of length; apex of right lobe with small penicillate seta and slender seta; left lobe shorter than right lobe, without penicillate seta nor slender seta on apex, and thus may be regenerated; each lateral margin with slender seta plus one or one pair of penicillate setae.

Distribution. Known only from the type locality.

Remarks. Examination of the type specimens of *P.*

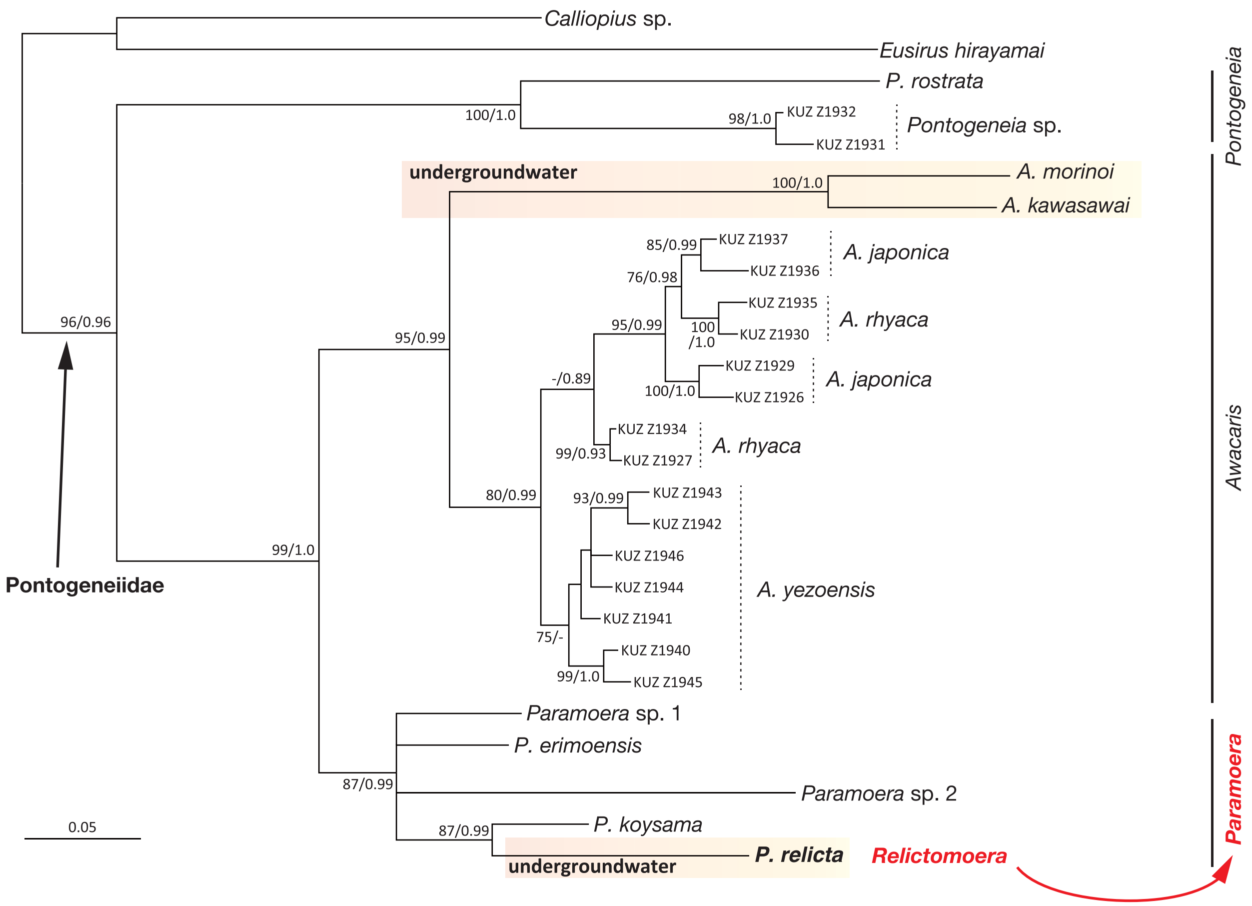

Fig. 4. Bayesian inference tree for 752 bp of nuclear H3 and mitochondrial 16S markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities.

relicta has revealed some features that were not mentioned and/or erroneously described in its original description (Uéno, 1971b). Newly confirmed diagnostic features are as follows: antennal sinus rounded, mandibular palp article 3 with A-setae, outer plate of maxilla 1 with 10 serrate robust setae, maxilliped palp article 4 with several setae along inner margin, gnathopod 2 carpus longer than its propodus, coxal gill absent on pereopod 7, and sternal gills and humps absent.

Paramoera relicta can be distinguished from the species within the subgenus *Ganigamoera* Sidorov, 2010 under *Paramoera* by the following features (features of *Ganigamoera* in parentheses; Sidorov, 2010): epimeral plates without (with) notches on posterior margins; mandibular palp article 3 shorter than article 2 (equal in length); sternal humps are absent (present); pereopod 7 without (with) coxal gill; and telson lobe entire (notched) apically.

The remaining type series does not contain any male amphipods of *P. relicta*. Since the newly collected material, as well as its missing holotype, were also female specimens (Uéno, 1971b), male morphological characteristics of *P. relicta* remain unknown. Four *Paramoera* species inhabiting Northwestern Pacific exhibit sexual dimorphisms in their pleopods 2 (Kuribayashi and Kyono, 1995; Sidorov, 2010); *P. koysama*, which is a phylogenetically close congener revealed by the present analyses (see below), is one of the four species possessing the sexually dimorphic pleopod 2. Accordingly, it is possible that a male individual of *P. relicta* also bears this dimorphic characteristic. Further field survey at its type locality should be conducted to obtain male individuals of *P. relicta* unveiling their morphological characteristics.

Molecular phylogenies

The obtained BI tree (mean ln $L = -5227.657$; Fig. 4) for estimating the phylogenetic position of *Paramoera relicta* had an identical topology to that of the ML tree (ln $L = -5245.115$; not shown). The monophyly of the ingroup taxa was well supported in the both analyses (BS = 96%, PP = 0.96). Each of the monophyly of *Awacaris* (BS = 95%, PP = 0.99), that of *Paramoera* (BS = 87%, PP = 0.99), and that of *Pontogeneia* Boeck, 1871 (BS = 100%, PP = 1.0) was well supported. Additionally, *Awacaris* and *Paramoera* formed a well-supported clade in the both analyses (BS = 99%, PP = 1.0).

Phylogenetic relationships within *Awacaris* were almost concordant with those shown by Tomikawa et al. (2017). The fully-supported monophyletic group consisting of two stygobitic species, *A. kawasawai* and *A. morinoi*, formed a sister lineage to a clade (BS = 80%, PP = 0.99) comprising the other epigean congeners. Contrary to *Awacaris*, the present analyses failed to obtain the robust phylogenetic relationships within *Paramoera*. However, the monophyly of *P. koysama* and *P. relicta* was well-supported in the both analyses (BS = 87%, PP = 0.99).

DISCUSSION

Taxonomic account of *Relictomoera*

The obtained phylogenies clearly recovered the systematic conclusion that *Paramoera relicta* is a member of *Paramoera*; *Relictomoera* cannot receive the taxonomic account as a distinct genus within Pontogeneiidae anymore. However, the taxonomic status of this genus-group taxon “under” *Paramoera* remains subject to discussion.

The genus *Paramoera* is the largest group within the family, and now comprises more than 50 species (see Jung et al., 2016). Although most of the *Paramoera* species including its type species *P. australis* Miers, 1875 are epigean, several species distributed in North Pacific have been known as being hyogean amphipods, in which eyes are vestigial or completely lacking (Barnard, 1977; Staude, 1995; Sidorov, 2010). As vehicles for those species, four subgenera, i.e., *Ganigamoera*, *Humilomoera* Staude, 1995, *Moanamoera* Staude, 1995, and *Rhithromoera* Staude, 1995, have been erected to highlight their characteristics within *Paramoera*, which were considered to adopt to subterranean or interstitial habitats (Staude, 1995; Sidorov, 2010).

The stygobitic *Paramoera* species were first described from lave tubes and subterranean habitats in Hawaiian Islands (Barnard, 1977). These Hawaiian species may be assigned to a distinct subgenus within *Paramoera* (Barnard, 1977). However, such a specific subgenus for the species was not established due to the taxonomic uncertainty of the other *Paramoera* species, as well as the *Paramoera*-like genera within Pontogeneiidae (Barnard, 1977).

The subgenus *Moanamoera* was established for the Hawaiian hypogean species by Staude (1995). Along with *Moanamoera*, two subgenera, *Humilomoera* and *Rhithromoera* were erected for interstitial *Paramoera* species inhabiting Northeastern Pacific coast, whose eyes are also reduced or vestigial (Staude, 1995). Although Staude (1995) recognized that most epigean *Paramoera* species could not be assigned to any subgenera, it was emphasized that his establishment of the three subgenera could shed light on adaptive evolution to subterranean and interstitial habitats in the North Pacific *Paramoera* amphipods.

The other subgenus *Ganigamoera* was erected for two subterranean species inhabiting Primorsky Krai, Russian Far East, which were considered as the *Paramoera* amphipods bearing stygomorphic characteristics (Sidorov, 2010). It was mentioned that the diagnostic characteristics of *Ganigamoera* are almost concordant to those of *Relictomoera* defined by Barnard and Karaman (1982) (Sidorov, 2010); the former could be discriminated from the latter only by the “sinusoid” anterolateral margin of the head of “*Relictomoera*” species.

Although the “sinusoid” feature is not present on the heads of “*Relictomoera*” species as revealed by the previous (Hirayama, 1990; Tomikawa et al., 2014) and the present studies, *Paramoera relicta* can be discriminated from the *Paramoera* (*Ganigamoera*) species by the following morphological characteristics: smooth posterodistal margins of epimeral plates, shorter mandibular palp article 3, absence of sternal humps and coxal gill 7, and entire apexes of telson lobes. Moreover, it has been implied that *Ganigamoera* might not be a monophyletic taxon (Sidorov, 2010). It thus remains uncertain whether *P. relicta* and the Russian *Paramoera* (*Ganigamoera*) species form a unique phylogroup within *Paramoera*.

The present molecular phylogenies revealed the close relationship between the epigean *P. koysama* and the subterranean *P. relicta* (Fig. 4). As the present dataset contained only the Japanese *Paramoera* species, elucidation of the precise phylogenetic relationships among *P. relicta* and the species classified within the subgenus *Ganigamoera* remain unresolved. In this study, therefore, *Relictomoera* is not treated as a valid subgenus for the stygobitic *Paramoera* species indigenous to East Asia. The taxonomic account of the nominal genus-group *Relictomoera* within *Paramoera* await resolution in future studies. It is possible that *Relictomoera* will be resurrected as a subgenus within *Paramoera*, and deemed to be a senior subjective synonym of *Ganigamoera*.

Implications for evolutionary history of *P. relicta*

Although the phylogenetic relationships among the present *Paramoera* OTUs remain uncertain, the present results shed light onto the affinity between *P. relicta* and *P. koysama*, which is an epigean species indigenous to a coastal habitat in Hokkaido, Japan (Kuribayashi and Kyono, 1995). In addition to *P. koysama*, *P. koreana* Stephensen, 1945 was considered a close congener of *P. relicta* by Uéno (1971b); this species has been also deemd to be an epigean amphipod (Stephensen, 1945; Uéno, 1971b).

The phylogenetic position of the subterranean *P. relicta* indicates that pontogeneiid amphipods inhabiting East Asia have shifted their habitat preferences to the underground water habitats in parallel (Fig. 4). Contrary to the stygobitic *Awacaris* species, whose eyes are completely absent (Uéno, 1971a; Tomikawa et al., 2014; Tomikawa et al., 2017), *P. relicta* possesses the vestigial eyes though this species indigenous to the freshwater subterranean habitat in the cave of Fukuejima Island. Additionally, both of its genetically close congener, *P. koysama*, and morphologically similar species, *P. koreana*, are generally marine epigean and possess the well-developed eyes in their heads (Stephensen, 1945; Kuribayashi and Kyono, 1995).

The Iana Cave, which is the type locality of *P. relicta*, is an insular lava cave located in the Tomie region on Fukuejima Island (Matsui et al., 1977). The Tomie volcano geologically belongs to the Fukue Volcano Group consisting of the Quaternary volcanoes around Fukuejima Island (Nagaoka and Furuyama, 2004), and it is assumed that this volcano had been active until Early to Middle Pleistocene, i.e., > 0.3 million years ago (Hoang et al., 2013; Miyazaki et al., 2016; Kuritani et al., 2017). Since this species possesses vestigial eyes, and its phylogenetic/morphological close congeners are epigean taxa, *P. relicta* may possess the transitional morphological traits from the surface to subterranean water. The paleogeological history of Fukuejima Island implies that this amphipod's invasion of the underground habitat occurred recently, i.e., after Middle Pleistocene at the earliest. To depict the evolutionary history about the invasion of underground habitats among the East Asian pontogeneiid amphipods, phylogenetic relationships among *Paramoera* species should be assessed on the basis of a more broader OTU set including ex-Japanese *Paramoera* species.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

TN provided and analyzed the sequence data. KT prepared the taxonomic description and the figures of the amphipod. TN wrote the manuscript including the neotype designation along with KT.

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