Subterranean Biology 44: 85–101 (2022)
doi: 10.3897/subtbiol.44.85517
https://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

A new species of *Allobathynella* (Crustacea, Bathynellacea, Parabathynellidae) from the hyporheic zone of the Hangang River, South Korea

Su-Jung Ji[1], Gi-Sik Min[1]

1 *Department of Biological Sciences and Bioengineering, Inha University, Incheon 22212, Republic of Korea*

Corresponding author: Gi-Sik Min (mingisik@inha.ac.kr)

Academic editor: Oana Teodora Moldovan | Received 19 April 2022 | Accepted 18 August 2022 | Published 1 September 2022

https://zoobank.org/9E30433F-8285-4437-BCEC-B1A56BDCFBFA

Citation: Ji S-J, Min G-S (2022) A new species of *Allobathynella* (Crustacea, Bathynellacea, Parabathynellidae) from the hyporheic zone of the Hangang River, South Korea. Subterranean Biology 44: 85–101. https://doi.org/10.3897/subtbiol.44.85517

Abstract

Bathynellacea including the parabathynellid genus *Allobathynella* Morimoto & Miura, 1957 is commonly found across the subterranean environment. The genus *Allobathynella* is the most species-rich genus known in Korea, and it now contains 23 species and one subspecies from South Korea and Japan. In this paper, we described a new species of *Allobathynella* from Danyang, South Korea. *Allobathynella danyangensis* **sp. nov.** can be distinguished from its congeners by the presence of five simple setae on the antennule, seven spines on the maxillule and 3-5-10-6 setal formula of the maxilla. We describe the new species with molecular diagnosis based on the mitochondrial *c* oxidase subunit 1, the mitochondrial 16S rDNA, and the nuclear 18S rDNA gene sequences and morphological study.

Keywords

Crustacea, Korean peninsula, molecular data, stygofauna, taxonomy

Introduction

Parabathynellidae Noodt, 1965 belongs to the order of Bathynellacea, which is a common group of the stygofauna (Park and Cho 2013). They live in groundwater (caves, aquifers, wells, springs, and interstitial spaces between sand grains in riverbeds), on all continents except Antarctica and mechanisms and lack planktonic larvae (Camacho et al. 2012, 2014). These isolated habitats and the lack of active dispersal mechanisms

have led to decreased dispersal distance, and hence, they consequentially show a high degree of endemism (Guzik 2008; Schminke 2014).

The genus *Allobathynella* has been considered as a primitive group of family Parabathynellidae in East Asia (Schminke 1973; Cho 2005; Park and Cho 2016). They have morphologically complex characters such as relatively large body lengths (1.28 mm–3.3 mm), multi-segmented antennule, antenna and thoracopodal exopod and wealth of appendicular ornamentation (Morimoto 1963; Fuchs et al. 2012; Park and Cho 2016). In particular, the multi-segmented exopod of thoracopods with more than two segments is a characteristic that occurs only in *Allobathynella* among the Korean parabathynellid genera.

The genus *Allobathynella* Morimoto & Miura, 1957 has been proposed for *A. japonica* from Japan (Morimoto 1957). It contains 23 species and one subspecies so far, including four species and one subspecies (*A. carinata* (Ueno, 1952), *A. kuma* (Uéno, 1956), *A. yaye* (Uéno, 1956), *A. gigantea* (Morimoto, 1959) and *A. gigantea pluto* (Morimoto, 1963)) incorporated into the genus *Parabathynella* Chappuis, 1926 (Uéno 1952, 1956, 1961; Morimoto 1959, 1963; Schminke 1973; Park and Cho 2008, 2016; Shin 2014). *Allobathynella* can be morphologically distinguished from *Parabathynella* by presence of one segmented thoracopod VIII in female and the presence of pleopod (Fuchs et al. 2012). All known species of *Allobathynella* are distributed in the Korean peninsula and Japan, and 17 species have been described in South Korea (Morimoto 1970; Park and Cho 2008, 2016; Shin 2014).

Based on the morphological examination of the specimens, here we report on a new species of *Allobathynella* found in the Hangang River in South Korea. In addition, we collected two related species, *A. hongcheonensis* Park & Cho, 2016 and *A. wonjuensis* Park & Cho, 2016, from type localities in the tributary of the Hangang River. We obtained mitochondrial cytochrome *c* oxidase subunit 1 (CO1), mitochondrial 16S rDNA and the nuclear 18S rDNA gene sequences from the new species and the two collected species and compared their morphological and molecular characteristics.

Materials and methods

Study area and groundwater sampling method

The samples were collected from the interstitial hyporheic zone of the Hangang River at three sites in South Korea: Danyang-gun, Hongcheon-gun, and Wonju-si, South Korea (Fig. 1B). The type locality of the new species, Danyang-gun, was a gravelly and rocky area rather than fine sandbanks (Fig. 1C, D). For sampling groundwater from the hyporheic zone, a 1 m core was driven into the points using a hammer, and water was collected using a manual pump. Approximately 80–100 L of the water sample was filtered through a 50 μm fine-mesh net. All the collected specimens were immediately preserved in 95% ethanol.

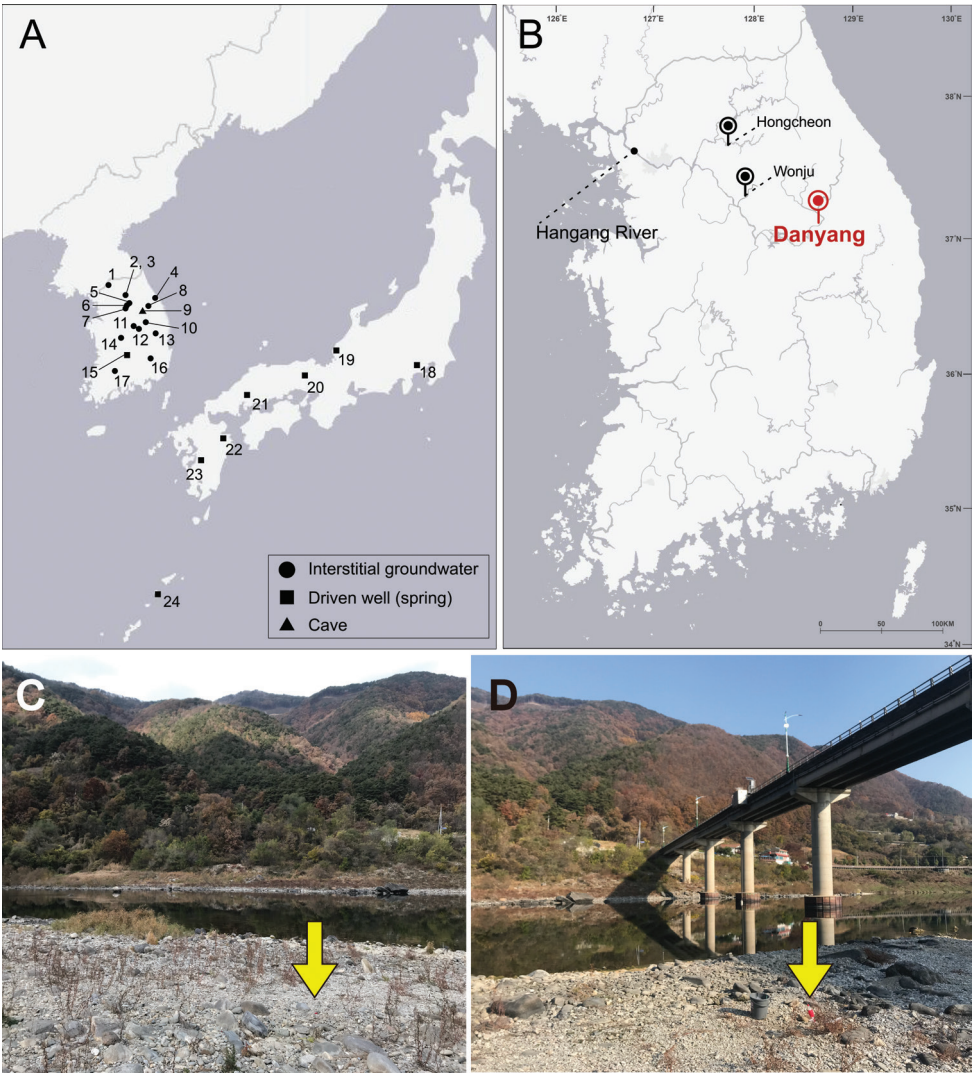

Figure 1. Map showing the type locality and habitat of the genus *Allobathynella* species and *A. danyangensis* sp. nov. **A** 1. *A. imjinensis*, 2. *A. hongcheonensis*, 3. *A. bangokensis*, 4. *A. gangneungensis*, 5. *A. wonjuensis*, 6. *A. munmakensis*, 7. *A. buronensis*, 8. *A. donggangensis*, 9. *A. coreana*, 10. *A. yeonjuensis*, 11. *A. maseongensis*, 12. *A. yecheonensis*, 13. *A. munsui*, 14. *A. okcheonensis*, 15. *A. shinjongieei*, 16. *A. cheongdoensis*, 17. *A. gureeyensis*, 18. *A. carinata*, 19. *A. mirabilis*, 20. *A. japonica*, 21. *A. yaye*, 22. *A. gigantea pluto*, 23. *A. kuma*, 24. *A. gigantea gigantea* **B** collection localities of the specimens used for the present study and the type locality of *A. danyangensis* sp. nov. **C, D** Collection sites of *A. danyangensis* sp. nov. at Danyang-gun, South Korea.

Morphological study

Specimens were dissected in glycerol under a stereo microscope (SZX12, Olympus, Japan). Dissected appendages were mounted using Eukitt Quick-hardening mounting medium (Sigma-Aldrich, St. Louis, MO, USA) for permanent slides. Observation and drawing were conducted using an optical microscope (DM2500, Leica, Germany). For scanning electron microscopy (SEM), the specimens were dehydrated in increasing concentrations of ethanol solutions, transferred into hexamethyldisilazane (Sigma-Aldrich, St. Louis, MO, USA), covered with platinum, and observed using a Hitachi S-4300SE (Hitachi, Japan). The type materials of the new species examined in this study were deposited in the collection at the National Institute of Biological Resources, Korea (NIBR).

Molecular analysis

The specimens used for the molecular study are listed in Table 1. Genomic DNA was extracted from the abdomens of specimens using the LaboPass Tissue Genomic DNA Isolation Kit Mini (Cosmo GENETECH, Seoul, South Korea) according to the manufacturer's instructions. Amplification by polymerase chain reaction (PCR) was conducted using the following primer sets: Bathy_F1 and Bathy_R1 for the CO1 mitochondrial gene (Ji et al. 2021); 16SarL F and 16SBathy-453R for the 16S mitochondrial gene (Palumbi et al. 1991; Perina et al. 2018); and two sets of 1F, 5R and 3F, 9R for the 18S nuclear gene (Giribet et al. 1996). These sequences were aligned using Clustal W (Thompson et al. 1994; Larkin et al. 2007) in Geneious v.8.1.9 (Biomatters, Auckland, New Zealand). The intra- and interspecific genetic distances were determined using MEGA X v.10.1.8 (Kumar et al. 2018).

Results

Taxonomy

Order Bathynellacea Chappuis, 1915
Family Parabathynellidae Noodt, 1965
Genus *Allobathynella* Morimoto & Miura, 1957

***Allobathynella danyangensis* sp. nov.**
https://zoobank.org/8146FEA6-E37B-4E4A-9535-307BD04992C5

Type locality. Danyang-gun (37°5'0.52"N, 128°28'57.11"E), South Korea. Collected by Su-Jung Ji, Chi-Woo Lee and Hee-Min Yang (19 June 2020 and 5 November 2021).

Type materials. ***Holotype*:** female (NIBRIV0000900570), dissected on six slides. Allotype: male (NIBRIV0000900577), dissected on five slides. ***Paratypes*:** Seven females (NIBRIV0000900571–3, NIBRIV0000900614–7) and five males (NIBRIV0000900574–6, NIBRIV0000900612–3).

Diagnosis. Antennule seven segmented with five simple setae on the inner distal margin of the third segment; antenna seven segmented with setal formula 0+0/0+0/1+0/1+1/0+1/1+1+1/5(1); labrum with 13 teeth; mandible palp one segmented with two apical setae; maxilla four segmented with a setal formula 3-5-10-6; thoracopods III–VII each with an epipod; uropod protopod with eight or nine spines and two distal spines slightly larger than other spines; furcal ramus with five spines; anal operculum slightly protruded.

Description of adult female (Figs 2–7). Body (Fig. 2) length 1.74 mm, head as long as three anterior thoracic segments combined.

Antennule (Fig. 3A) seven segmented, first segment with one small seta on inner distal margin, two simple dorsal setae of different sizes, and with four plumose setae on outer side; second segment with four simple setae on inner distal margin and one group of four plumose setae on outer margin; third segment with five simple setae on inner margin, with two simple lateral setae of different sizes and one lateral plumose seta; inner flagellum of third segment with three simple setae of different sizes; fourth segment with one stub seta and one plumose seta on dorsal margin and two plumose setae on the outer distal apophysis; fifth segment distally with four simple setae, two dorsal aesthetascs and one simple seta and medially with one simple seta on inner margin; sixth segment with four setae on inner margin, and with two aesthetascs, one simple seta, and one aesthetasc dorsally; and seventh segment with three subterminal aesthetascs and four simple setae.

Antenna (Fig. 3B) seven segmented; setal formula 0+0/0+0/1+0/1+1/0+0/1+1+1/5(1).

Labrum (Fig. 3C) with eight median teeth flanked by two (left) or three (right) teeth on lateral sides; ventral surface with one small round median projection, three pairs of teats and numerous combs of ctenidia.

Mandible (Fig. 3D, E) with incisor process of four teeth; tooth of ventral edge absent; spine row consisting of eight spines; palp one segmented with two apical setae of different sizes, longer one being basally barbed; with one or two bundles of ctenidia that look like chestnut bur near the base of the palp.

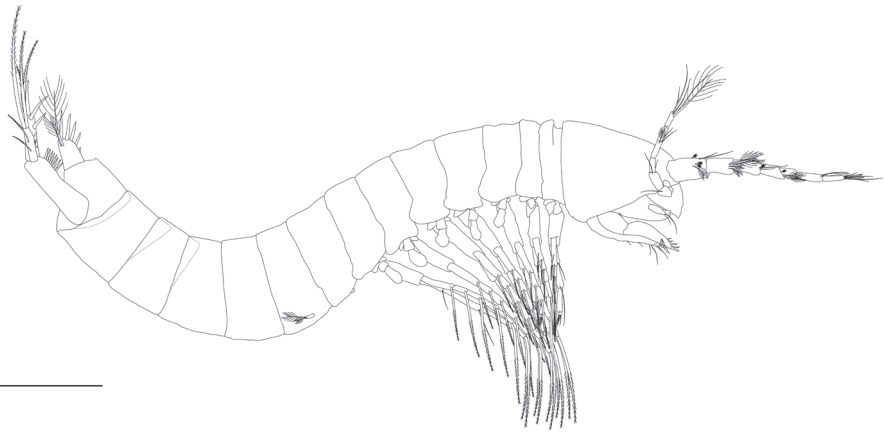

Figure 2. *Allobathynella danyangensis* sp. nov., paratype female, NIBRIV0000900571. Scale bar: 0.5 mm.

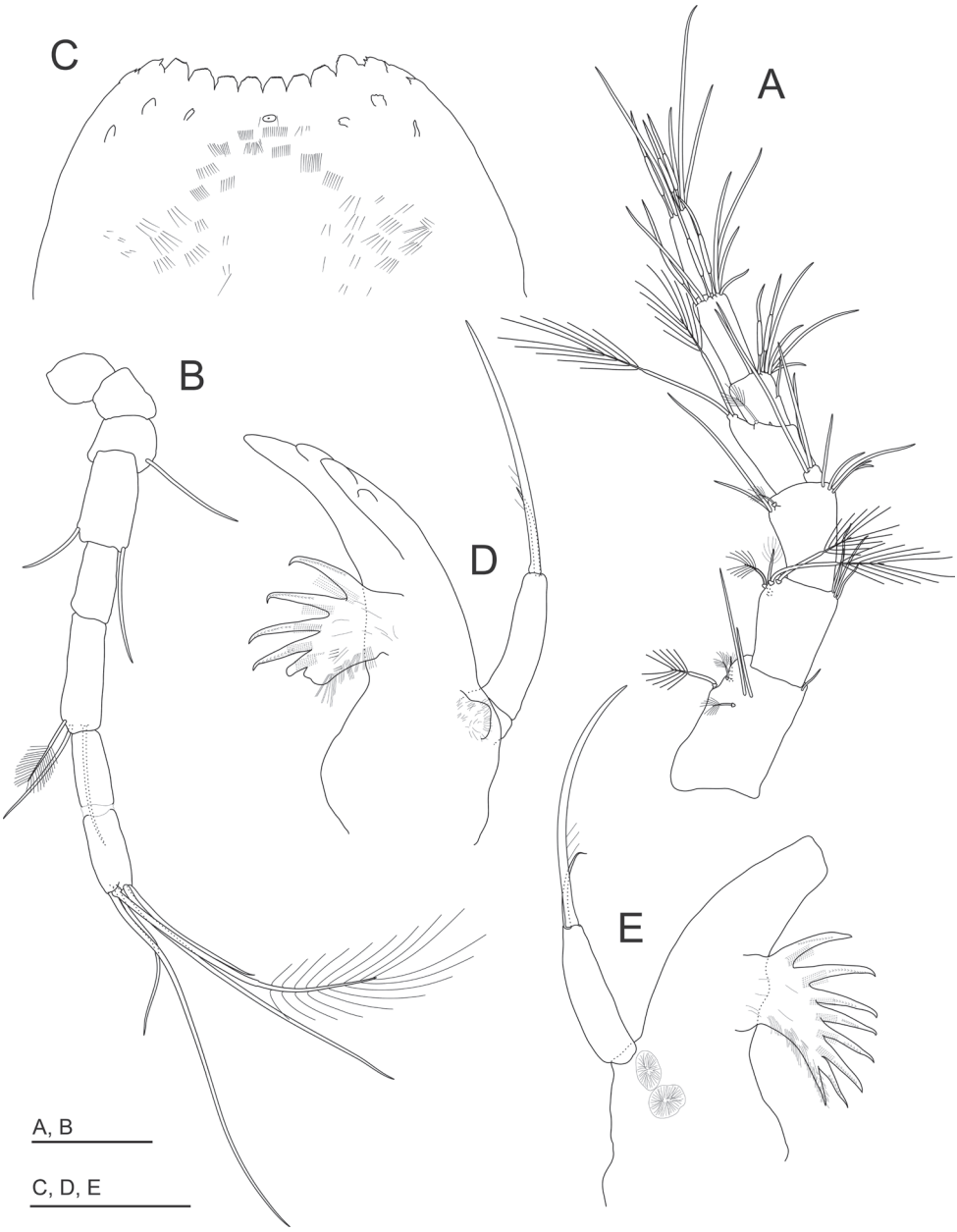

Figure 3. *Allobathynella danyangensis* sp. nov., holotype female **A** antennule **B** antenna **C** labrum **D** mandible (dorsal, right one) **E** mandible (dorsal, left one). Scale bars: 0.5 mm.

Maxillule (Fig. 4A) two segmented, proximal segment with four setae on distal margin; distal segment with two terminal smooth spines; five dentated spines on inner edge, and three simple setae of different length on outer distal margin.

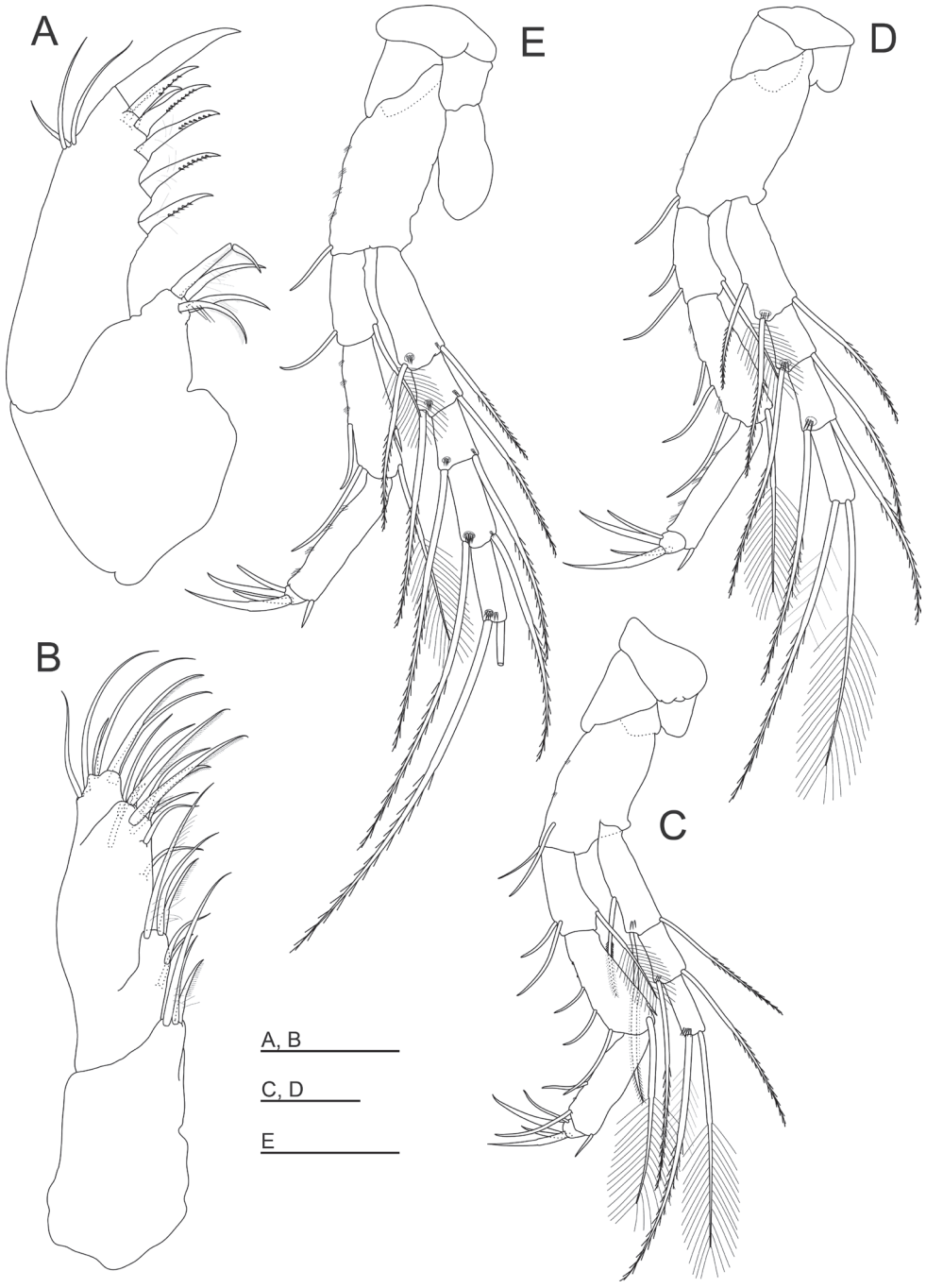

Figure 4. *Allobathynella danyangensis* sp. nov., holotype female **A** maxillule **B** maxilla **C** thoracopod I **D** thoracopod II **E** thoracopod III. Scale bars: 0.05 mm.

Maxilla (Fig. 4B) four segmented, setal formula 3-5-10-6.

Thoracopods I–VII (Figs 4C–E, 5) slightly increased in size up to thoracopod IV, thoracopods IV–VII similar in size; thoracopods III–VII each bearing one epipod on protopod; basipod with two distal setae in thoracopod I, with one distal seta in thoracopods II and III, and one distal and one median seta in thoracopods IV–VII; number of exopod segments of thoracopods I–VII: 3-4-5-6-6-6-6, with two setae on each segment, three in first segment of Th I; endopod of the thoracopods I–VII four-segmented, inner setae of first segment always plumose and all others smooth, setal formulae:

Thoracopod I	2 + 1/3 + 2/2 + 1/4(2)
Thoracopod II	2 + 1/3 + 2/0 + 1/4(2)
Thoracopods III–V	1 + 1/2 + 2/0 + 1/4(2)
Thoracopod VI	0 + 1/2 + 2/0 + 1/4(2)
Thoracopods VII	0 + 1/1 + 2/0 + 1/4(2)

Thoracopod VIII (Fig. 6A) conical in ventral view, with two sharp distal projections like teeth.

First pleopod (Fig. 6B) in form of stub bearing two distal plumose setae of different length.

Uropod (Figs 6C, 7D) bearing eight or nine spines on inner margin of sympod and two distal spines slightly larger than other spines; exopod 38% as long as the sympod length, with one outer seta, two terminal setae and one inner medial seta; inner setae strong, longer, and thicker than outer terminal seta; endopod longer than exopod, 52.8% as long as sympod with two dorsal plumose setae near base, two terminal setae and one subterminal plumose setae and with one terminal, and one subterminal spines and four additional spines.

Pleotelson (Fig. 6D) without seta; anal operculum slightly protruded.

Furcal rami (Fig. 6D) 1.3 times as long as wide, with two large distal spines and three smaller spines on inner margin, and with two dorsal plumose setae of different sizes.

Description of adult male (Fig. 7A–C). The male differs from the female in thoracopod VIII. Thoracopod VIII of male perpendicular to body, in the form of a bell in lateral view, 1.2 times longer than wide; protopod with a prominent penial region bearing distal opening; inner margin of penial region (dentate lobe) with five teeth (Fig. 7C, white arrow); epipod flat, with flat round distal part not reaching lower margin of the exopod; basipod with one seta near base of endopod, inner margin of basipod with distally drawn out into one projection, and basipodal seta as long as endopod; exopod one- third of basipod, round, with two distal lobes; the two lobes with tiny denticles (Fig. 7C, yellow arrow); endopod small, round, with two distal setae of different sizes.

Remarks. *Allobathynella danyangensis* sp. nov. is morphologically most similar to *A. coreana sensu* Park and Cho (2016) as follows: 1) the antennule third segment has two simple setae and one plumose seta on the outer distal margin, 2) the last segment of the antenna has five setae, 3) mandibular palp is one segmented, and 4) male thora-

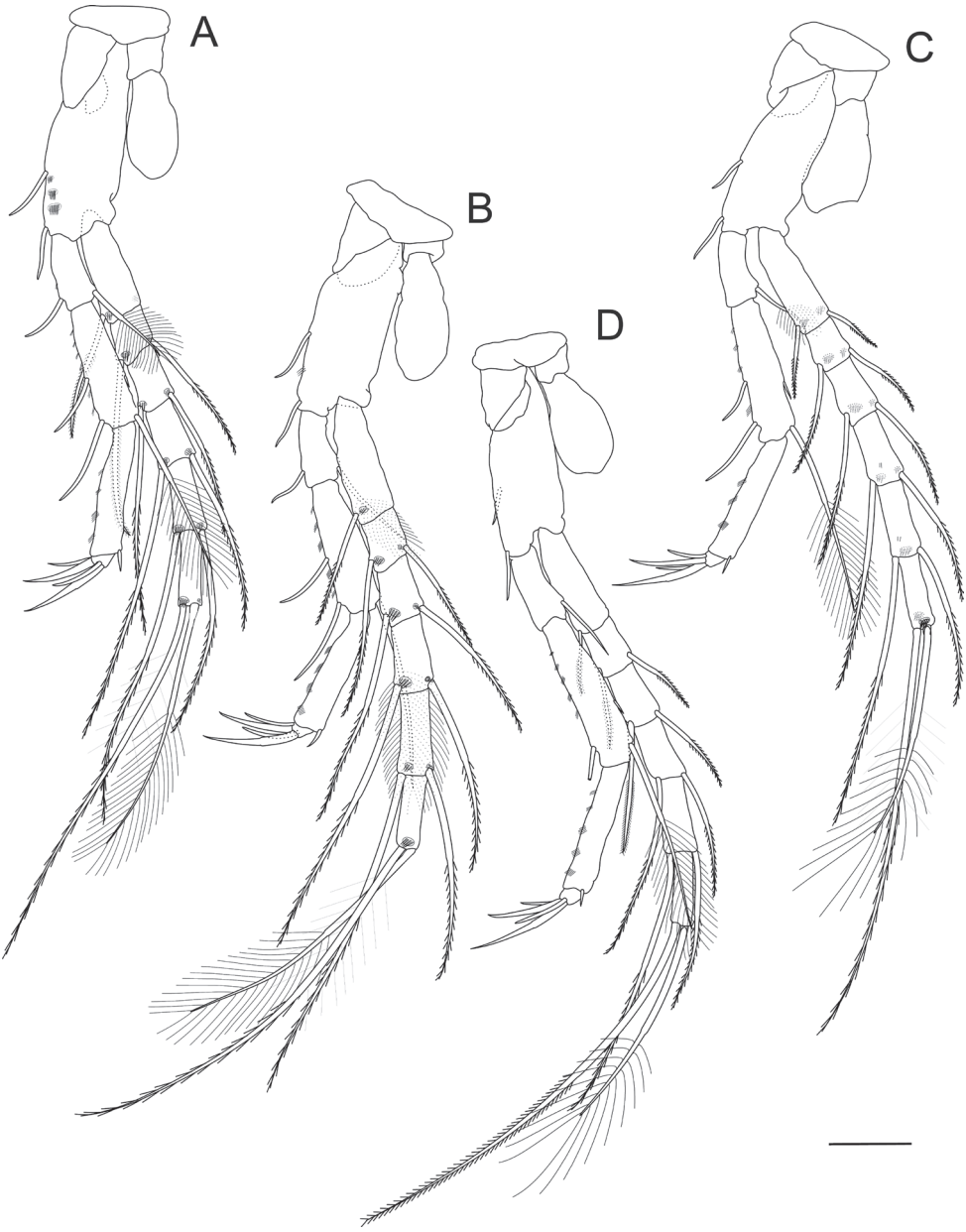

Figure 5. *Allobathynella danyangensis* sp. nov., holotype female **A** thoracopod IV **B** thoracopod V **C** thoracopod VI **D** thoracopod VII. Scale bars: 0.05 mm.

copod VIII has one long basipodal seta. However, the new species can be differentiated from *A. coreana* by the following characteristics (characters of *A. coreana* in parentheses): 1) the antennule third segment has five (four) simple setae, 2) the mandibular palp has two (one) apical setae, 3) the maxillule has seven (eight) spines on the distal segment and 4) the third segment of the maxilla has 10 (12) setae.

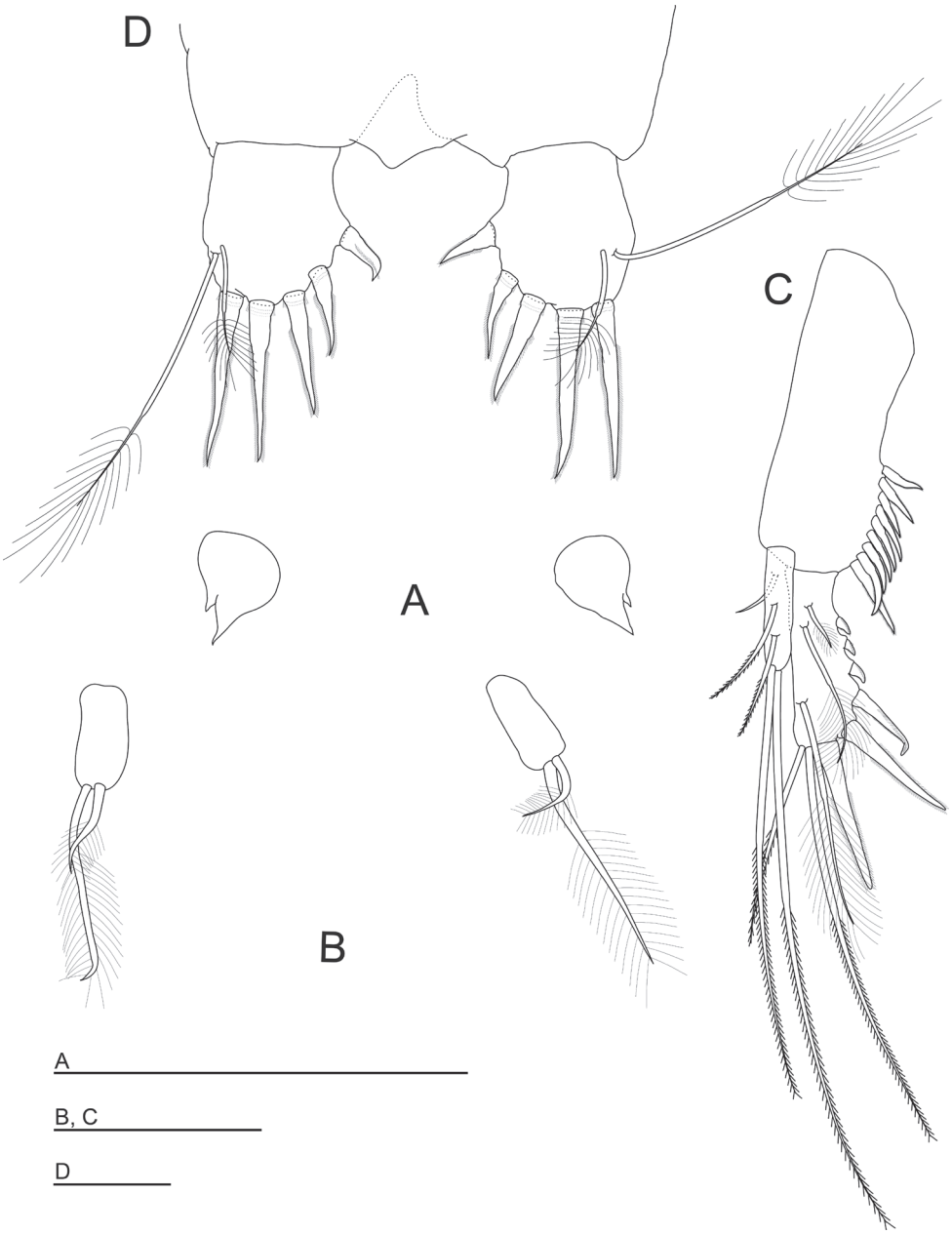

Figure 6. *Allobathynella danyangensis* sp. nov., holotype female **A** thoracopod VIII **B** pleopod **C** uropod **D** telson. Scale bars: 0.05 mm.

The new species is morphologically also closely resemble *A. hongcheonensis* Park & Cho, 2016 as follows: 1) the antennule third segment has two simple setae and one plumose seta on the outer distal margin, 2) the mandibular palp is one segmented and has two apical setae and 3) maxillule has seven spines on the distal segment. However,

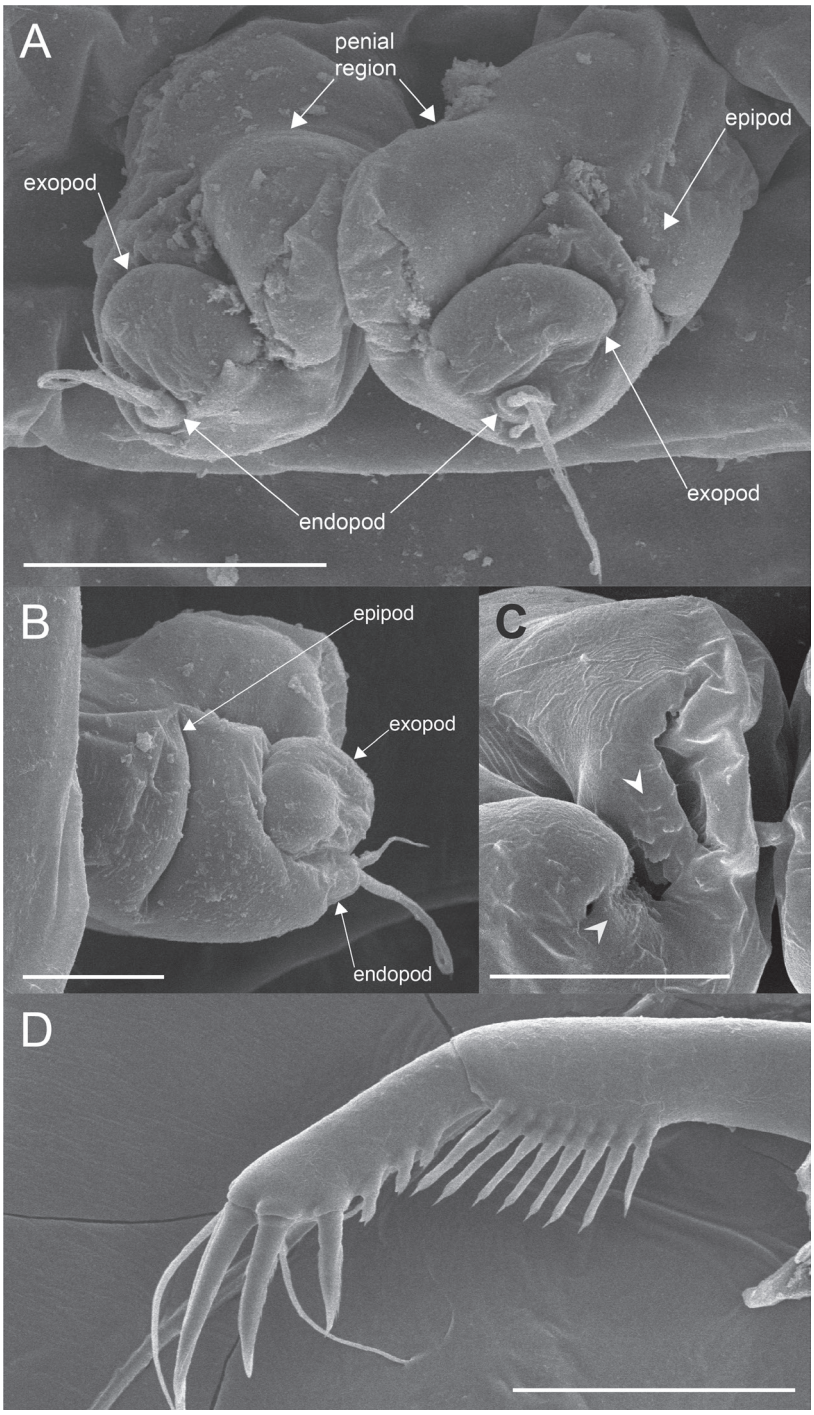

Figure 7. *Allobathynella danyangensis* sp. nov. (**A**) paratype male, NIBRIV0000900574, (**B**) paratype male, NIBRIV0000900575, (**C**) paratype male, NIBRIV0000900576, (**D**) paratype female, NIBRIV0000900617 **A** thoracopod VIII (ventral view) **B** thoracopod VIII (lateral view) **C** Thoracopod VIII (ventral view) **D** uropod. Scale bars: 0.05 mm (**A**); 0.02 mm (**B, C**); 0.1 mm (**D**).

the new species differs from *A. hongcheonensis* in the following characteristics (characters of *A. hongcheonensis* in parentheses): 1) the second segment of maxilla has five (four) setae, 2) thoracopod VIII of female has two sharp distal projections (two distal lobes with denticles) and 3) thoracopod VIII of male has one long (tiny) basipodal seta.

Etymology. The species name is derived from Danyang-gun, where the material was collected.

Allobathynella hongcheonensis Park & Cho, 2016

Material examined. Collected in the type locality (37°41'57.5"N, 127°40'10.4"E) by Chi-Woo Lee (25 March 2015). One female specimen was examined (NIBRIV0000900580). Although the specimen differs from the original description of the species in having eight spines on the mandible spine row instead of nine, five spines on the endopod of the uropod instead of six, and five spines on the furcal ramus instead of six, it is within the range of intraspecific variability. In addition, the present specimen morphologically differs from *A. bangokensis* Park & Cho, 2016, which is a sympatric species with *A. hongcheonensis*, in the antenna, maxillule and maxilla. Thus, we identified the studied specimen as *A. hongcheonensis*.

Allobathynella wonjuensis Park & Cho, 2016

Material examined. Collected in the type locality (37°22'34.1"N, 127°51'15.2"E) by Chi-Woo Lee (25 March 2015). Two female specimens were examined (NIBRIV0000900578–9). The two specimens are consistent with the original description of the species, except having nine spines on the uropod sympod instead of eight in NIBRIV0000900578. Therefore, we identified the studied specimens as *A. wonjuensis*.

Molecular analysis. We sequenced and analyzed DNA extracted from the new species and the two collected species (Table 1). A total of 786 bp for the mitochondrial CO1, 452 bp for 16S rDNA and 1704 bp for the 18S rDNA gene. The uncorrected pairwise distances within and among the species of the genus *Allobathynella* are shown in Table 2. In the analyzed species, the ranges of interspecific variation for CO1, 16S and 18S were 16.8–19.8%, 19.1–21.7% and 0.2%, respectively (Table 2).

Discussion

The species of the genus *Allobathynella* are distributed across South Korea and Japan and occurred mostly in interstitial groundwater habitats at the riverbanks in South Korea, and in spring or driven well habitats in Japan (Fig. 1A). Seven *Allobathynella* species were found distributed in the northwestern part of South Korea along the course of the Hangang River, a major Korean river (Fig. 1B). These species were *A. bangokensis*, *A. hongcheonensis*, *A. wonjuensis*, *A. munmakensis*, *A. buronensis*, *A. coreana*, and

Table 1. Samples used for the molecular analyses, with collection locality and date, voucher numbers and GenBank accession numbers.

Species, sex	Locality (Coordinates)	Date	Voucher No.	GenBank accession No.		
				COI	16S	18S
A. danyangensis sp. nov., holotype female	Danyang-gun, South Korea (37°5'0.52"N, 128°28'57.11"E)	2021.11.05	NIBRIV0000900570	OP214600	OP214779	OP214784
A. danyangensis sp. nov., paratype female	"	2021.11.05	NIBRIV0000900571	OP214601	OP214780	OP214785
A. danyangensis sp. nov., paratype female (juvenile)	"	2020.06.19	NIBRIV0000900572	OP214602	–	–
A. hongcheonensis, female	Hongcheon-gun, South Korea (37°41'57.5"N, 127°40'10.4"E)	2015.03.25	NIBRIV0000900580	OP214603	OP214781	OP214786
A. wonjuensis, female	Wonju-si, South Korea (37°22'34.1"N, 127°51'15.2"E)	2015.03.25	NIBRIV0000900578	OP214604	OP214782	OP214787
A. wonjuensis, female	"		NIBRIV0000900579	OP214605	OP214783	OP214788

Table 2. Intra- and interspecific genetic distances of three molecular markers (CO1, 16S rDNA and 18S rDNA) (*p*-distance) among the new species and two *Allobathynella* species obtained in the present study.

CO1	Intraspecific (%)	Interspecific (%)		
Species name		**1**	**2**	**3**
A. danyangensis sp. nov.	0–0.5	–		
A. hongcheonensis	–	16.8–16.9	–	
A. wonjuensis	1	19.0–19.5	19.5–19.8	–
16S	**Intraspecific (%)**	**Interspecific (%)**		
Species name		**1**	**2**	**3**
A. danyangensis sp. nov.	0	–		
A. hongcheonensis	–	21.7	–	
A. wonjuensis	0	21.6	19.1	–
18S	**Intraspecific (%)**	**Interspecific (%)**		
Species name		**1**	**2**	**3**
A. danyangensis sp. nov.	0	–		
A. hongcheonensis	–	0.2	–	
A. wonjuensis	0	0.2	0.2	–

A. donggangensis (Morimoto 1970; Park and Cho 2016) and may be closely related to each other. Comparison of the morphological features of the eight *Allobathynella* species, including the two species collected in the present study and the new species, is provided in Table 3. On the other hand, *A. coreana* has been detected at four sites in South Korea to date: Yongdam-gul Cave, Kwangcheon-seon-gul Cave, driven well at Ka'eun-myeon and the hyporheic zone of Yeongwol-gun, South Korea (Morimoto 1970; Park and Cho 2016). Although the four distribution sites of *A. coreana* are geographically close to the type locality of the new species, the four forms have been described in the original paper without sufficient morphological details of important taxonomic characters, and the new species is morphologically distinct from all four forms of *A. coreana* (Table 3). Furthermore, considering their morphological differences based on available data, and that the populations from each locality are geographically isolated and may not interact with each other, the four forms of *A. coreana* may be separate species. Therefore, taxonomic re-examination and molecular data are required to estimate the true species diversity, with possible existence of cryptic species, and understand their distribution ranges.

Table 3. Morphological differences among eight species of *Allobathynella* from a tributary of Hangang River, South Korea. Characteristics of *A. coreana* include the four geographical forms.

		A. bangokensis	*A. buronensis*	*A. donggangensis*	*A. hongcheonensis*	*A. munmakensis*	*A. wonjuensis*	*A. coreana*				*A. danyangensis* sp. nov.
								Cave Yongdam	Cave Kwangcheon	Ka'eun-myeon	Yeongwol-gun	
Antennule	**No. of segment**	7	7	7	7	7	6	7	7	7	7	7
	Inner margin of 3rd segment	3 simple setae	5 simple setae	3 simple setae	4 simple setae	4 simple setae	4 simple setae	?	?	?	4 simple setae	5 simple setae
	Outer margin of 3rd segment	2 simple setae, 1 plumose seta	2 simple setae, 1 plumose seta	1 simple setae, 1 plumose seta	2 simple setae, 1 plumose seta	2 simple setae, 1 plumose seta	2 simple setae, 1 plumose seta	?	?	?	2 simple setae, 1 plumose seta	2 simple setae, 1 plumose seta
Antenna	**Setal formula**	0-0-1-2-0-3-5	0-0-1-2-0-3-5	0-0-1-2-1-2-4	0-0-1-2-0-3-5	0-0-1-2-0-3-5	0-0-1-2-0-3-4	0-0-0-0-1-1-4	0-0-1-2-1-2-4	0-0-0-1-1-3-5	0-0-1-2-0-3-5	0-0-1-2-0-3-5
	Ctenidia in 2nd segment	present	absent	absent	absent	absent	absent	?	?	?	absent	absent
Labrum	**No. of teeth**	14	10	17	13	14	14	11	9	10	14	13
Mandible	**Palp segment**	1	1	1	1	1	1	1	1	1	1	1
	No. of seta	2	2	1	2	1	1	2	2	2	1	2
Maxillule	**No. of spines on distal segment**	8	7	6	7	7	7	6	7	7	8	7
Maxilla	**Setal formula**	3-5-11-6	3-4-10-6	3-5-9-6	3-4-10-6	3-5-11-6	3-4-9-6	3-3-7-6	3-4-9-6	3-4-8-7	3-5-12-6	3-5-10-6
Thoracopod	**Epipod in**	IV-VII	III-VII	IV-VII	III-VII	III-VII	III-VII	III-VII	III-VII	IV-VII	III-VII	III-VII
Uropod	**No. of spines on sympod**	10	9	10	7	9	8	10	8	12	12	8–9
Furcal ramus	**No. of spines**	6	5	6–7	5–6	6	5	5	6	5	6	5–6
Ref.		Park & Cho, 2016	Park & Cho, 2016	Park & Cho, 2016	Park & Cho, 2016; this study	Park & Cho, 2016	Park & Cho, 2016, this study	Morimoto, 1970	Morimoto, 1970	Morimoto, 1970	Park & Cho, 2016	This study

In our specimens, the young individuals resembled adults and had thoracopod VIII acting as a reproductive organ. However, they still lacked segments on the exopods of the thoracopods. All the specimens that we considered as adults and described had the 3-4-5-6-6-6-6 exopod segment formula, but the juveniles had formulae of 3-4-4-5-5-5-4, 3-4-5-5-5-5-4 and 2-3-4-5-5-4-3. Observation of morphological traits and analysis of gene sequence data from a juvenile indicate that they are the same species as the present new species (Tables 1, 2). They seem to acquire the rest segments of the exopods of thoracopods through subsequent moulting (Schminke 1974). Progenesis is defined as the sexual maturation of organisms at a morphologically juvenile or larval stage (Gould 1977). In general, progenesis is regarded as an important role in the evolution of interstitial organisms (Schminke 1973; Westheide 1987). Pressure for small size in the interstitial space is estimated as the primary factor (Gould 1977), and this is also considered to be the most convincing opinion for the regressive morphological status of interstitial taxa (Westheide 1987). Thus, the segment formula of thoracopod exopods can be a taxonomically inadequate character when judging them as adults as their genital organs are mature or when describing them without examining enough specimens.

The previous classification of the order Bathynellacea from Korea has been investigated using only a morphological approach while the recent studies suggest combining both morphological and genetic analysis to characterize genera and species or reveal their phylogenetic relationships (Camacho et al. 2013; González-Miguéns et al. 2020). To examine the genetic divergence within and between the present new species and the two collected species, we sequenced 786 bp of the mitochondrial CO1, 452 bp of 16S and 1704 bp of the 18S gene (Tables 1, 2). The small distance found in the 18S gene tells us that the three species definitely belong to the same genus and the distances of the COI and 16S mitochondrial genes, undoubtedly show us that they are three different species. It is necessary to sequence genes from the type localities of nominal species, well characterized morphologically, in order to make adequate comparisons with morphologically similar species from other localities. Only in this way, combining molecular and morphological data, will it be possible to understand the true diversity of the group. Our result provides a basis for future comparison with other Bathynellacea species and contributes to phylogenetic studies.

Acknowledgements

This study work was supported by a grant from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202130202, NIBR202203202).

References

Camacho AI, Dorda BA, Rey I (2012) Undisclosed taxonomic diversity of Bathynellacea (Malacostraca: Syncarida) in the Iberian Peninsula revealed by molecular data. Journal of Crustacean Biology 32(5): 816–826. https://doi.org/10.1163/193724012X638473

Camacho AI, Dorda BA, Rey I (2013) Old and new taxonomic tools: description of a new genus and two new species of Bathynellidae from Spain with morphological and molecular characters. Journal of Natural History 47(21–22): 1393–1420. https://doi.org/10.1080/00222933.2013.768361

Camacho AI, Dorda BA, Rey I (2014) Iberian Peninsula and Balearic Island Bathynellacea (Crustacea, Syncarida) database. ZooKeys (386): 1–20. https://doi.org/10.3897/zookeys.386.6296

Cho JL (2005) A primitive representative of the Parabathynellidae (Bathynellacea, Syncarida) from the Yilgarn Craton of Western Australia. Journal of Natural History 39(39): 3423–3433. https://doi.org/10.1080/00222930500345806

Fuchs A, Hahn HJ, Cho JL (2012) *Parabathynella badenwuerttembergensis* sp. nov., the first record of Parabathynellidae Noodt, 1965 (Malacostraca: Bathynellacea) from Germany. Journal of Crustacean Biology 32(4): 655–663. https://doi.org/10.1163/193724012X630651

Giribet G, Carranza S, Baguna J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada+ Arthropoda clade. Molecular Biology And Evolution 13(1): 76–84. https://doi.org/10.1093/oxfordjournals.molbev.a025573

González-Miguéns R, Muñoz-Nozal E, Jiménez-Ruiz Y, Mas-Peinado P, Ghanavi HR, García-París M (2020) Speciation patterns in the *Forficula auricularia* species complex: cryptic and not so cryptic taxa across the western Palaearctic region. Zoological Journal of the Linnean Society 190(3): 788–823. https://doi.org/10.1093/zoolinnean/zlaa070

Gould SJ (1977) Ontogeny and phylogeny. The Belknap Press of Harvard University Press, Cambridge, Mass. & London, 1–8.

Guzik MT, Abrams KM, Cooper SJB, Humphreys WF, Cho JL, Austin AD (2008) Phylogeography of the ancient Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western Australia. Invertebrate Systematics 22(2): 205–216. https://doi.org/10.1071/IS07040

Ji SJ, Lee CW, Min GS (2021) A new species of *Hangangbathynella* (Crustacea, Bathynellacea, Parabathynellidae) from South Korea. ZooKeys 1046: 143–155. https://doi.org/10.3897/zookeys.1046.66141

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallance IM, Wilm A, Lopez R, Thopson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Morimoto Y (1957) *Allobathynella japonica* gen. et sp. nov., a new bathynellid from Japan. Proceedings of the Japan Academy 33(3): 145–148. https://doi.org/10.2183/pjab1945.33.145

Morimoto Y (1959) Bathynellids of the Amami group of the Ryu-kyu Islands (Syncarida, Bathynellacea). Memoirs of the College of Science, University of Kyoto, Series B 26: 269–280.

Morimoto Y (1963) Occurrence of a giant *Parabathynella* in Southeastern Kyushu, Japan. Annotationes Zoologicae Japonenses 36(3): 161–165.

Morimoto Y (1970) Results of the speleological survey in South Korea 1966. XXI Bathynellid crustaceans (Syncarida) from South Korea. Bulletin of the National Science Museum, Tokyo 13: 149–184.

Palumbi SR, Martin A, Roman S, McMillan M, Stice G, Grabowski G (1991) Simple fool's guide to PCR. University of Hawaii, Honolulu.

Park JG, Cho JL (2008) A preliminary inquiry into an Asian topsy-turvy Dom, *Allobathynella* with the description of a new species from South Korea and redescription of *A. japonica* (Crustacea, Syncarida, Parabathynellidae). Journal of Natural History 42(3–4): 223–238. https://doi.org/10.1080/00222930701850018

Park JG, Cho JL (2013) New genus and two new species of Parabathynellidae (Malacostraca: Syncarida) from South Korea. Journal of Crustacean Biology 33(6): 866–881. https://doi.org/10.1163/1937240X-00002184

Park JG, Cho JL (2016) Fourteen new species of *Allobathynella* Morimoto & Miura, 1957 from South Korea: with a redescription of *A. coreana* Morimoto, 1970 (Crustacea, Bathynellacea, Parabathynellidae). Journal of Species Research 5(1): 49–156. https://doi.org/10.12651/JSR.2016.5.1.049

Perina G, Camacho AI, Huey J, Horwitz P, Koenders A (2018) Understanding subterranean variability: the first genus of Bathynellidae (Bathynellacea, Crustacea) from Western Australia described through a morphological and multigene approach. Invertebrate Systematics 32(2): 423–447. https://doi.org/10.1071/IS17004

Schminke HK (1973) Evolution, System und Verbreitungsgeschichte der Familie Parabathynellidae (Bathynellacea, Malacostraca). Akademie der Wissenschaften und der Literatur Mainz, Mathematisch-Naturwissenschaftliche Klasse, Mikrofauna des Meeresbodens 24: 1–192.

Schminke HK (1974) Mesozoic intercontinental relationships as evidenced by bathynellid Crustacea (Syncarida: Malacostraca). Systematic Biology 23(2): 157–164. https://doi.org/10.1093/sysbio/23.2.157

Schminke HK (2014) Freshwater origin of Bathynellacea (Malacostraca). Crustaceana 87(10): 1225–1242. https://doi.org/10.1163/15685403-00003322

Shin S (2014) Invertebrate Fauna of Korea Volume 21, Number 38. Arthropoda: Malacostraca: Bathynellacea: Parabathynellidae. National Institute of Biological Resources, Ministry of Environment, Korea, 1–195.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Uéno M (1952) Three new species of Bathynellidae (Syncarida) found in subterranean waters of Japan. Annotationes Zoologicae Japonenses 25(1): 317–328.

Uéno M (1956) More species of *Parabathynella* from Japan. Annotationes Zoologicae Japonenses 29(2): 109–115.

Uéno M (1961) A new Japanese bathynellid. Crustaceana 2(2): 85–88. https://doi.org/10.1163/156854061X00266

Westheide W (1987) Progenesis as a principle in meiofauna evolution. Journal of Natural History 21(4): 843–854. https://doi.org/10.1080/00222938700770501