

DNA Barcoding of *Allobathynella cheongdoensis* and *Hangangbathynella taechooni* of Family Parabathynellidae (Crustacea, Bathynellacea)

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ABSTRACT

Members of *Allobathynella* Morimoto and Miura, 1957 and *Hangangbathynella* Park and Cho, 2013 belong to the family Parabathynellidae Noodt, 1965 exclusively inhabit subterranean environments including caves, springs, and interstitial groundwater. Among them, two parabathynellid species, *Allobathynella cheongdoensis* Park and Cho, 2016 and *Hangangbathynella taechooni* Park and Cho, 2013, have been reported in the hyporheic zones of the Nakdonggang and Namhangang Rivers in South Korea, respectively. To evaluate gene markers as diagnostic characters, we determined mitochondrial cytochrome *c* oxidase subunit 1 (CO1) and nuclear 18S rDNA from the topotypes of two Korean parabathynellid species, and then analyzed the genetic divergence among the two parabathynellids and their congeners. Additionally, we provided their habitus photographs and a brief discussion of the usefulness of the two marker regions for both genera as DNA barcodes.

Keywords: Korean peninsula, morphology, subterranean Crustacea, Syncarida

INTRODUCTION

The crustacean family Parabathynellidae Noodt, 1965 is amongst the most diverse groups of stygofauna in South Korea (Park and Cho, 2015). However, their classification has been considerably difficult because of the morphological convergence caused by lightless, hypoxia, and limited foods of subterranean environments including caves, springs, and interstitial groundwater (Danielopol et al., 1994; Malard and Hervant, 1999). To address these taxonomic problems, recent studies reported that molecular sequence data have been regarded as one of the important diagnostic characters (Camacho et al., 2012, 2021; Abrams et al., 2013; Matthews et al., 2020; Ji and Min, 2022a). In Korea, 34 parabathynellid species have been reported, but the cytochrome *c* oxidase subunit 1 (CO1) and/or 18S rDNA gene sequences have been known in only nine species (Park and Cho, 2016; Ji et al., 2021; Ji and Min, 2022a, 2022b, 2022c, 2023).

Two morphologically closely related Parabathynellid genera, *Allobathynella* Morimoto and Miura, 1957 and *Hangangbathynella* Park and Cho, 2013, are mainly distinguished by

the morphology of mandible proximal teeth, exopod of thoracopod, and pleopod (Park and Cho, 2013, 2016). In Korea, 18 species of *Allobathynella* Morimoto and Miura, 1957 and 5 species of *Hangangbathynella* Park and Cho, 2013, have been described to date. However, their sequence data were not enough to use gene markers as diagnostic characters and to infer phylogenetic relationship (Ji et al., 2021; Ji and Min, 2022a, 2022b, 2022c).

In this study, we collected specimens of two parabathynellid species, *Allobathynella cheongdoensis* Park and Cho, 2016 from Nakdonggang River (Cheongdo-gun, 35°40'28.5"N, 128°52'5.1"E) and *Hangangbathynella taechooni* Park and Cho, 2013 from Namhangang River (Yeoju-si, 37°13'54.6"N, 127°43'4.1"E), in South Korea. Their morphological and molecular examinations were performed as described by Ji and Min (2023). Sequences were aligned and edited using Geneious v.8.1.9 (Biomatters, Auckland, New Zealand). Genetic divergences were calculated by *p*-distance using MEGA X v.10.1.8 (Kumar et al., 2018) with the known sequences of the other *Allobathynella* and *Hangangbathynella* species (Tables 1, 2). All specimens used in this study were deposited

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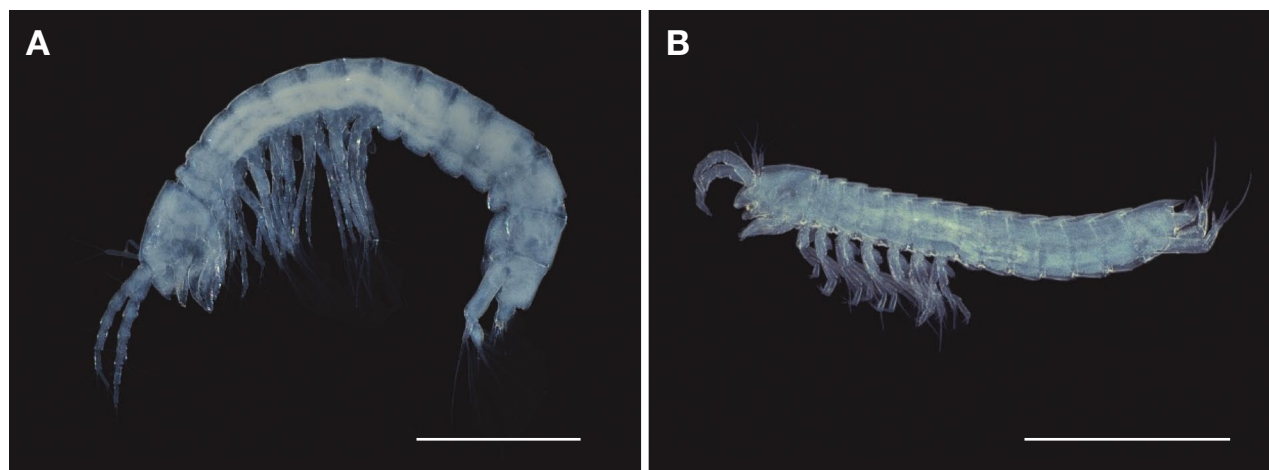


Fig. 1. Habitus photographs of *Allobathynella cheongdoensis* and *Hangangbathynella taechooni*. A, *A. cheongdoensis* (NIBRIV0000904532); B, *H. taechooni* (NIBRIV0000904533). Scale bars=0.5 mm.

at the National Institute of Biological Resources, Korea: NIBRIV0000904531 and NIBRIV0000904532 for *A. cheongdoensis*; NIBRIV0000904533–5 for *H. taechooni*.

RESULTS AND DISCUSSION

Phylum Arthropoda von Siebold, 1848
Class Malacostraca Latreille, 1802
Order Bathynellacea Chappuis, 1915
Family Parabathynellidae Noodt, 1965
Genus *Allobathynella* Morimoto and Miura, 1957

Allobathynella cheongdoensis Park and Cho, 2016 (Fig. 1A)

Allobathynella cheongdoensis Park and Cho, 2016: 122–128, figs. 63–67.

Diagnosis. Antennule seven segmented with two 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 + 0/1 + 1 + 1/5 (1); mandible palp two segmented with two apical setae; maxilla four segmented with setal formula 3–4–10–6; thoracopods III–VII each with an epipod; thoracopod VIII of female conical-shaped with two distal protrusions; thoracopod VIII of male having two basipod setae; most distal spine on uropod protopod is largest; furcal ramus with seven spines; anal operculum slightly protruded.

Genus *Hangangbathynella* Park and Cho, 2013

Hangangbathynella taechooni Park and Cho, 2013 (Fig. 1B)

Hangangbathynella taechooni Park and Cho, 2013: 867–872, figs. 1–5.

Diagnosis. Antennule seven segmented with three aesthetascs and four simple setae on the sixth segment; antenna seven segmented with setal formula 0 + 0/0 + 0/1 + 0/1 + 1/0 + 0/0 + 2/4 (1); mandibular spine row with seven spines; maxilla four segment with setal formula 2–3–10–6; thoracopod II–VII having one epipod on the protopod; thoracopod VIII of male with gourd-shaped epipod and with 4 spinules on the exopod; uropod protopod with 11 spines; furcal ramus with 5 spines; anal operculum sharply protruded.

Remarks on DNA barcodes for genus *Allobathynella* and *Hangangbathynella*. The newly obtained five CO1 and four 18S rDNA sequences from two specimens of *A. cheongdoensis* and three specimens of *H. taechooni* were registered in GenBank (Tables 1, 2). The alignments length was 516 bp (30.0–40.3% GC content and 137 polymorphic sites) for CO1 and 1,654 bp (51.7–52.6% GC content and 56 polymorphic sites) for 18S rDNA. Within each species, the pairwise genetic distances were 0.2% in the CO1 and 0.1% in the 18S rDNA for *A. cheongdoensis*, and 0.0–2.7% in the CO1 and 0.0% in the 18S rDNA for *H. taechooni*. Between species of each genus, the pairwise genetic distances were 17.6–21.9% in the CO1 and 0.1–0.3% in the 18S rDNA for five *Allobathynella* species, and 6.0–6.2% in the CO1 and 0.1% in the 18S rDNA for two *Hangangbathynella* species. As a result, the CO1 gene for *Allobathynella* species is highly diagnostic and could be considered as a useful candidate marker for DNA barcoding than 18S rDNA. In the case of *Hangangbathynella*, the CO1 genetic distance between the two identified species showed a relatively low interspecific-

Table 1. Genetic distances (p -distance) of CO1 (516 bp) among species of *Allobathynella* and *Hangangbathynella*

No.	Species name	GenBank No.	1	2	3	4	5	6	7	8	9	Reference
1	<i>A. cheongdoensis</i>	OR147938										This study
2	<i>A. cheongdoensis</i>	OR147939	0.002									This study
3	<i>A. yecheonensis</i>	OP718746	0.190	0.192								Ji and Min (2022c)
4	<i>A. danyangensis</i>	OP214602	0.219	0.217	0.196							Ji and Min (2022b)
5	<i>A. hongcheonensis</i>	OP214603	0.213	0.215	0.192	0.176						Ji and Min (2022b)
6	<i>A. wonjuensis</i>	OP214605	0.182	0.184	0.180	0.180	0.209					Ji and Min (2022b)
7	<i>H. taechooni</i>	OR147940	0.244	0.246	0.229	0.215	0.219	0.233				This study
8	<i>H. taechooni</i>	OR147941	0.244	0.246	0.229	0.215	0.219	0.233	0.000			This study
9	<i>H. taechooni</i>	OR147942	0.258	0.260	0.238	0.219	0.223	0.227	0.027	0.027		This study
10	<i>H. mihoensis</i>	MW624440	0.238	0.240	0.227	0.223	0.207	0.236	0.060	0.060	0.062	Ji et al. (2021)

CO1, cytochrome c oxidase subunit 1.

Table 2. Genetic distances (p -distance) of 18S rDNA (1,654 bp) among species of *Allobathynella* and *Hangangbathynella*

No.	Species name	GenBank No.	1	2	3	4	5	6	7	8	Reference
1	<i>A. cheongdoensis</i>	OR149859									This study
2	<i>A. cheongdoensis</i>	OR149860	0.001								This study
3	<i>A. yecheonensis</i>	OP719281	0.001	0.001							Ji and Min (2022c)
4	<i>A. danyangensis</i>	OP214784	0.003	0.002	0.002						Ji and Min (2022b)
5	<i>A. hongcheonensis</i>	OP214786	0.001	0.001	0.000	0.002					Ji and Min (2022b)
6	<i>A. wonjuensis</i>	OP214787	0.003	0.002	0.002	0.002	0.002				Ji and Min (2022b)
7	<i>H. taechooni</i>	OR149861	0.030	0.030	0.030	0.029	0.030	0.030			This study
8	<i>H. taechooni</i>	OR149862	0.030	0.030	0.030	0.029	0.030	0.030	0.000		This study
9	<i>H. mihoensis</i>	ON062081	0.030	0.029	0.029	0.029	0.029	0.030	0.001	0.001	Ji and Min (2022a)

ic distances (6.0–6.2%) and high intraspecies distances (up to 2.7%), showing low resolution. Though, given the lack of molecular data within the genus *Hangangbathynella*, it is premature to assess the usefulness of the CO1 and 18S rDNA as barcodes for them at the moment.

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CONFLICTS OF INTEREST

Gi-Sik Min, a contributing editor of the *Animal Systematics, Evolution and Diversity*, was not involved in the editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

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