

## DNA Barcoding of *Allobathynella yecheonensis* (Bathynellacea: Parabathynellidae) from Interstitial Groundwater, South Korea

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### ABSTRACT

Bathynellacea is a stygobitic group that is commonly associated with subterranean habitats. *Allobathynella* Morimoto and Miura, 1957, which is a genus of parabathynellid Bathynellacea, has the widest range of distribution and is rich in species from the Korean peninsula among Korean Bathynellacea genera. The material of *Allobathynella yecheonensis* Park and Cho, 2016, was found in the type locality of interstitial groundwater in the Nakdonggang River in South Korea. In this study, we determined the sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) and nuclear 18S ribosomal DNA of *A. yecheonensis* and compared them with those of *A. hongcheonensis*, *A. wonjuensis*, and *A. danyangensis*. In addition, we provided photographs of *A. yecheonensis* and diagnosis including a note on morphological observations.

**Keywords:** Korean peninsula, morphology, stygofauna, Syncarida

### INTRODUCTION

*Allobathynella* Morimoto and Miura, 1957, a genus of parabathynellid Bathynellacea, is a small interstitial crustacean that inhabits subterranean environments (Camacho et al., 2018). The genus *Allobathynella* is distributed across South Korea and Japan, with 24 valid species (Uéno, 1952, 1956, 1961; Morimoto, 1959, 1963; Schminke, 1973; Park and Cho, 2008, 2016; Shin, 2014; Ji and Min, 2022). It is the most species-rich genus in the Korean Bathynellacea fauna, including 18 species that are endemic to South Korea (Morimoto, 1970; Shin, 2014; Park and Cho, 2016; Ji and Min, 2022). Species of *Allobathynella* are characterized by six or seven segmented antennule, thoracopodal exopod with three or more segments and the presence of pleopod in the form of a stalk-like process having two setae (Park and Cho, 2008, 2016).

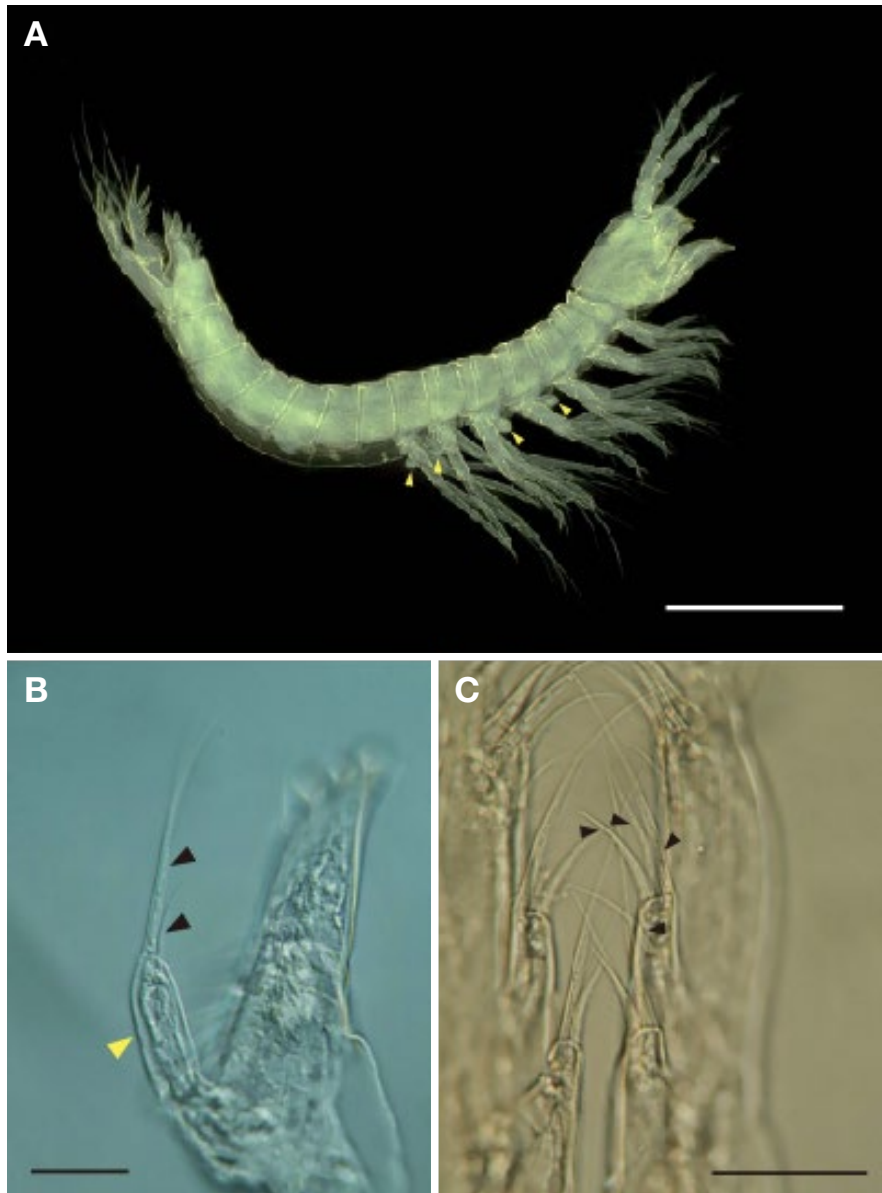
Identification of species in subterranean taxa is difficult owing to the convergent evolution and morphological simplification related to their underground habitat compared with other surface crustaceans (Camacho et al., 2011). Thus, a single morphological study is sometimes insufficient to distinguish related species. Hence, molecular diagnosis that supports morphological studies is needed (Camacho et al., 2011). How-

ever, collecting subterranean organisms can be challenging and obtaining molecular data can be difficult owing to their small size (Camacho et al., 2012).

In the present study, we obtained the sequences of cytochrome *c* oxidase subunit 1 (CO1) from newly collected specimens of *A. yecheonensis* Park and Cho, 2016 as a barcoding marker. Additionally, we obtained the sequences of nuclear 18S ribosomal DNA (18S rDNA) of the species. Two individuals of *A. yecheonensis* were collected from the interstitial hyporheic zone of Yecheon-gun, South Korea (36°34'44.9"N, 128°19'48.9"E). Each abdomen parts of the two specimens were used for extraction of genomic DNA. The remaining parts, except for the abdomen, were prepared as permanent slides after dissection and carried out a morphological examination under a stereomicroscope (SZX12, Olympus, Japan). Voucher specimens were deposited in the National Institute of Biological Resources (NIBRIV0000900867, 8), Korea. The primer pairs for polymerase chain reaction were as follows: Bathy\_F1 and Bathy\_R1 (Ji et al., 2021) for the partial CO1 mitochondrial gene and two primer sets, 1F, 5R and 3F, 9R (Giribet et al., 1996) for the 18S nuclear gene. Sequences were aligned and edited using Geneious v.8.1.9 (Biomatters, Auckland, New Zealand) and resulted in a common frame

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**Fig. 1.** *Allobathynella yecheonensis* Park and Cho, 2016, NIBRIV0000900868. A, Habitus; B, Palp and apical seta on mandible; C, Second segment of maxilla. Scale bars: A=0.5 mm, B, C=0.05 mm.

length of 717 bp for CO1 and 1,687 bp for 18S rDNA, which are shared by all of sequences. Intra- and inter-specific genetic distances were calculated by *p*-distance using MEGA X v.10.1.8 (Kumar et al., 2018). The results of this study provide molecular information to complement morphological knowledge for future studies.

## RESULTS AND DISCUSSION

Partial mitochondrial CO1 and nuclear 18S rDNA sequences

from two specimens (NIBRIV0000900867, 8) of *A. yecheonensis* were obtained. The newly determined sequences were registered in GenBank (accession Nos. OP718746, 7 for CO1 and OP719281, 2 for 18S rDNA). Within the species, the pairwise genetic distance of *A. yecheonensis* was 0.4% in the CO1 (717 bp) and all identical in 18S rDNA (1,687 bp) sequences (Table 1). Compared with available molecular data within the same genus, *A. yecheonensis* showed a genetic distance of 16.3% to 20.4% for CO1: the minimum was with *A. wonjuensis* and the maximum was with *A. danyangensis*. In the case of 18S rDNA, the genetic distance between the species ranged

**Table 1.** Intra- and interspecific genetic distances of CO1 (717 bp) (*p*-distance) among *Allobathynella* species

No.	CO1 Species name	Intraspecific distance (%)	Interspecific (%)				Accession No.	Data source
			1	2	3	4		
1	<i>A. yecheonensis</i> (n=2)	0.4	–	–	–	–	OP718746, OP718747	Present study Ji and Min (2022) Ji and Min (2022) Ji and Min (2022) Unpublished
2	<i>A. hongcheonensis</i> (n=1)	–	18.8	–	–	OP214603		
3	<i>A. worjuensis</i> (n=2)	1.1	16.3–17.6	19.9–20.4	–	OP214604, OP214605		
4	<i>A. danyangensis</i> (n=3)	0–0.6	20.1–20.4	17.9–18.0	19.5–20.2	OP214600, OP214601, OP214602		
5	<i>Allobathynella</i> sp.	–	18.7–18.8	19.0	19.1–19.8	KY310669	18.7–18.8	

**Table 2.** Intra- and interspecific genetic distances of 18S rDNA (1,687 bp) (*p*-distance) among *Allobathynella* species

No.	18S rDNA Species name	Intraspecific distance (%)	Interspecific (%)				Accession No.	Data source
			1	2	3	4		
1	<i>A. yecheonensis</i> (n=2)	0	–	–	–	–	OP719281, OP719282	Present study Ji and Min (2022) Ji and Min (2022) Ji and Min (2022)
2	<i>A. hongcheonensis</i> (n=1)	–	0.1	–	–	OP214786		
3	<i>A. worjuensis</i> (n=2)	0	0.2	0.2	–	OP214787, OP214788		
4	<i>A. danyangensis</i> (n=2)	0	0.2	0.2	0.1	OP214784, OP214785		

from 0.1% to 0.2%.

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

***Allobathynella yecheonensis* Park and Cho, 2016 (Fig. 1)**  
*Allobathynella yecheonensis* Park and Cho, 2016: 116–122,  
figs. 58–62.

**Diagnosis.** Antennule seven segmented with four 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 one segmented with two apical setae (Fig. 1B, black arrow and yellow arrows); maxilla four segmented with four setae on the second segment (Fig. 1C, black arrows); thoracopods IV–VII each with an epipod (Fig. 1A, yellow arrows); thoracopod VIII of female tiny and conical in ventral view with two sharp distal protrusions; uropod sympod with two most distal spines significantly larger than others; anal operculum slightly protruded.

**Note on morphological observation.** These two specimens coincided well with the diagnostic characteristics of *A. yecheonensis* described by Park and Cho (2016). However, the two specimens that we examined have the 3-4-5-5-5-5 and 3-4-5-6-6-6-6 exopod segment formulas whereas described as the formula of 3-4-4-5-5-5-4 in the original description. As the molecular results based on CO1 sequences confirm that the two specimens are the same species (Tables 1, 2), the differences in thoracopodal exopod can be interpreted as intraspecific morphological variation. This variation could be due to progenesis due to pressure for the small size of the interstitial space. It is known that progenesis is a significant role in the evolution of interstitial species (Gould, 1977; Westheide, 1987; Ji and Min, 2022). In conclusion, molecular information is useful for resolving taxonomic problems, which occur when morphological observations are solely relied on.

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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. Remaining author has declared no conflicts of interest.

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