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). However, 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 the mitochondrial cytochrome c oxidase subunit 1 (CO1) from newly collected specimens of Allobathynella 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 Allobathynella 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.
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 COI 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

![Image](image_url)
DNA Barcoding of Allobathynella yecheonensis

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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)

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+0/1+0/1+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 3-4-5-5-5-5-5 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 intersitial 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
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REFERENCES


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