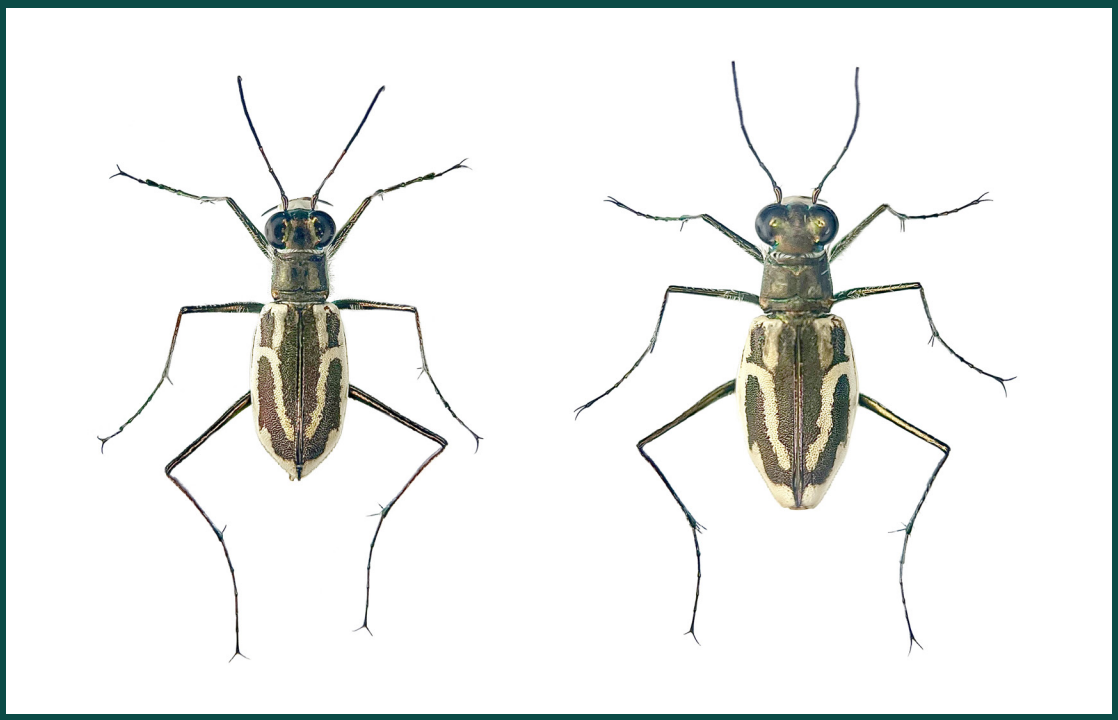
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# Brief study of endangered species, *Cicindela* (*Abroscelis*) *anchoralis* Chevrolat, 1845, based on DNA barcoding

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The Korean tiger beetle, *Cicindela* (*Abroscelis*) *anchoralis* Chevrolat, 1845, was collected from sandy beaches in Taean-gun, Chungcheongnam-do Province (TA), and Sinan-gun, Jeollanam-do Province (SA). This study describes the mitochondrial cytochrome c oxidase subunit I (COI) gene and 16S ribosomal RNA (16S) sequences of *C.* (*A.*) *anchoralis*, followed by an analysis of the genetic diversity and distance between the genus *Cicindela* and its congeners to determine the basic phylogenetic position and genetic diversity of the domestic population of *C.* (*A.*) *anchoralis*. The 16S sequences within the Korean population were found to be 100% identical, but the COI sequences exhibited three points with single nucleotide variations. Three haplotypes were observed in the SA population and one haplotype in the TA population. Intraspecies variation was relatively low in both the Japanese (JP) and Taiwanese (TW) populations, at 0−2.0% in COI and 0−0.5% in 16S. On the other hand, the genetic distance between closely related species was over 11.8% for COI and over 4.8% for 16S, showing high discrimination ability. In the gene tree, the *C.* (*A.*) *anchoralis* group was strongly supported by high supporting values, with the Korean population forming a clade separate from the JP and TW populations.

Keywords: DNA barcoding, endangered species, gene tree, genetic diversity, population decline

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## Introduction

Approximately 2,300 species of tiger beetles are known worldwide, and 18 species are currently recorded in South Korea (Kim *et al*., 2005; Gough *et al*., 2020). *Cicindela* (*Abroscelis*) *anchoralis* Chevrolat, 1845 is distributed from Southeast Asia to East Asia, Vietnam, China, Japan, Taiwan, and Korea (Satoh *et al*., 2004). In the 1980s, *Cicindela* (*Abroscelis*) *anchoralis* was distributed mainly in the coastal sand dunes along the West Sea, centered around Incheon and Taean-gun, Chungcheongnam-do Province but now it is only found intermittently in Taean-gun, Chungcheongnam-do Province and Sinan-gun, Jeollanam-do Province, and its population seems to have decreased significantly due to habitat reduction (Kim, 1978; National Institute of Ecology, 2021). Thus, this species is currently designated as an endangered species and has been protected in South Korea since 2012.

In 2023, the Research Center for Endangered Species, National Institute of Ecology developed a technique for artificially propagating *C.* (*A.*) *anchoralis* and in 2024 examined restoration strategies, including natural release in Taean-gun and Sinan-gun in 2024 (Cha, 2024; Cha and Jung, 2024). In connection with these efforts, this study aimed to identify the basic phylogenetic position and genetic diversity of the domestic population of *C.* (*A.*) *anchoralis*. Additionally, the study presents the DNA sequences of the domestic population to support further research.

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## Materials and Methods

### Sample collection and species identification

Specimens of *Cicindela* (*Abroscelis*) *anchoralis* were collected from sandy beach in Taean-gun (TA), Chungcheongnam-do Province and Sinan-gun (SA), Jeollanam-do Province, South Korea on July 7, 2023, June 21, 2023, respectively (Table 1). Specimens were observed under a stereomicroscope (SMZ 745T; Nikon, Tokyo, Japan) at magnifications ranging from 6.7× to 50×. Classification and terminology follow Kim *et al*. (2005) and the National Institute of Biological Resources (2024).

### DNA extraction, PCR amplification, and sequencing

To extract gDNA from the specimen, one of the legs was dissected from the specimen and the leg was added into the 1.5 mL tube with 180 μL ATL buffer and 20 μL proteinase K solution (DNeasy Blood & Tissue kit; Qiagen, Germany). The leg was then chopped into small pieces using curved-blade scissors, followed by incubation at 56°C for 1 h. The remaining steps were conducted in accordance with the manufacturer's instructions except for a final elution of 25 μL. All gDNA extracted from the leg was used as the PCR templated at a total concentration 20 ng. First, to identify as many sequence differences as possible within the COI gene, a primer pair was designed to amplify a PCR product with a size of approximately 900 base pairs (Anco_COI_F1, 5′-TTTGGTGCTTGATCA GGAATAG-3′; Anco_COI_R1, 5′-GTTGCGGATGTGA AGTAAGC-3′). Similar to the COI gene, a primer pair was designed to amplify a PCR product with a size of approximately 700 base pairs for the 16S gene (Anco_16S_F1, 5′-TTGACTGTGCAAAGGTAGC-3′; Anco_16S_R1, 5′-AGCAACACCCACTAATACAC-3′). Second, PCR amplification was performed using the AccuPower PCR premix (Bioneer, South Korea), with each of the COI and 16S primer pairs. The PCR consisted of 5 μL of the specimen's gDNA, 10 pmol of each primer pair, and 42.6 μL of nuclease-free water. PCR thermal cycling was conducted as follows: initial denaturation for 3 min at 95°C with 37 thermal cycles of 30 s at 95°C, 30 s at 57°C, and 1 min at 72°C, and a final extended period of 3 min at 72°C. Finally, PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced externally at Macrogen (Seoul, South Korea) to verify nucleotide variations among individual specimens.

### Phylogenetic analyses

The COI and 16S gene sequences determined in this study and those retrieved from GenBank were aligned using BioEdit (Hall, 1999). jModelTest 2.1.7 (Darriba *et al*., 2012) was used to determine the appropriate DNA substitution model for maximum likelihood (ML) and Bayesian inference (BI) analyses. For BI analyses, FASTA files were converted to NEXUS files using MEGA 7.0 (Tamura *et al*., 2011) and GTR + I (0.1770) + G (0.3590) model for COI and GTR + I (0.7570) model for 16S were used. ML was analyzed using MEGA 7 with 1,000 bootstrap replicates (Tamura *et al*., 2011). BI assessment was done on MrBayes 3.2.2 (Ronquist *et al*., 2012) with a chain length of 1,000,000 generations. Trees were sampled every 100 generations with a prior burn-in of 30%. Sequence dissimilarity was calculated using MEGA 7.0 (Tamura *et al*., 2011) with the Kimura2-parameter distance option (Kimura, 1980). Phylogenetic trees were visualized using the free software FigTree v1.4.1 (http://tree.bio.ed.ac.uk/spftware/figtree/, written by A. Rambaut).

## Results and Discussion

In this study, total 42 COI and 16S gene sequences, obtained from two populations of *Cicindela* (*Abroscelis*) *anchoralis*, were registered in GenBank (Table 1). The alignment length was 484 bp (32.0−37.8% of GC content and 131 polymorphic sites) in COI, and 669 bp (22.9−24.8% of GC content and 82 polymorphic sites) in 16S. Within species, there was a clear variation in COI of 0−2.0%, and 0−0.5% in 16S, similar to results obtained in the previous studies (Satoh and Hori, 2005). These sequences also showed clear variation between species of 11.8−17.4% in COI and 4.8−10.3% in 16S (Tables 2, 3). In both gene regions, *C*. (*A*.) *anchoralis* exhibited an intraspecific variation rate of less than 2%. Comparing the two gene sequences, COI exhibits clearer resolution in interspecies differences than 16S.

**Table 1.** Sequence information of *C*. (*A*.) *anchoralis* of the Korean population.

| DNA region | Sample number | Sampling site | GenBank accession number |
|---|---|---|---|
| COI | A1−16 | Sinan-gun, Jeollanam-do Province (SA) | PX472853−PX472868 |
| | A17−21 | Taean-gun, Chungcheongnam-do Province (TA) | PX472869−PX472873 |
| 16S | A1−16 | Sinan-gun, Jeollanam-do Province (SA) | PX472874−PX472889 |
| | A17−21 | Taean-gun, Chungcheongnam-do Province (TA) | PX472890−PX472894 |

**Table 2.** Genetic distances (*p*-distance) of COI gene sequence (484 base pairs) between *Cicindela* species and relatives (%).

| No. | Species (site) | Acc. No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *C. anchoralis* (SA) | PX472853 | | | | | | | | | | | | | |
| 2 | *C. anchoralis* (SA) | PX472866 | 0.0 | | | | | | | | | | | | |
| 3 | *C. anchoralis* (SA) | PX472854 | 0.2 | 0.2 | | | | | | | | | | | |
| 4 | *C. anchoralis* (TA) | PX472869 | 0.2 | 0.2 | 0.0 | | | | | | | | | | |
| 5 | *C. anchoralis* (TA) | NC038191 | 0.2 | 0.2 | 0.0 | 0.0 | | | | | | | | | |
| 6 | *C. anchoralis* (TA) | AB109898 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | | | | | | | | |
| 7 | *C. anchoralis* (TW) | AB109901 | 1.3 | 1.3 | 1.2 | 1.2 | 1.2 | 1.2 | | | | | | | |
| 8 | *C. anchoralis* (TW) | AB109902 | 1.5 | 1.5 | 1.3 | 1.3 | 1.3 | 1.3 | 0.2 | | | | | | |
| 9 | *C. anchoralis* (JP) | AB109900 | 1.7 | 1.7 | 1.5 | 1.5 | 1.5 | 1.5 | 1.7 | 1.8 | | | | | |
| 10 | *C. anchoralis* (JP) | AB109899 | 2.0 | 2.0 | 1.8 | 1.8 | 1.8 | 1.8 | 1.7 | 1.8 | 1.0 | | | | |
| 11 | *C. elisae* | PX472895 | 13.9 | 13.9 | 13.9 | 13.9 | 13.9 | 13.9 | 13.5 | 13.7 | 13.9 | 14.6 | | | |
| 12 | *C. laetescripta* | AB118736 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | 14.8 | 15.0 | 14.9 | 15.6 | 12.7 | | |
| 13 | *C. lewisi* | AB118721 | 14.7 | 14.7 | 14.7 | 14.7 | 14.7 | 14.7 | 14.9 | 15.2 | 14.7 | 14.9 | 14.0 | 12.1 | |
| 14 | *Calomera angulata* | AB118566 | 17.4 | 17.4 | 17.2 | 17.2 | 17.2 | 17.2 | 16.9 | 17.2 | 16.9 | 17.4 | 15.2 | 14.5 | 11.8 |

**Table 3.** Genetic distances (*p*-distance) of 16S gene sequence (668 base pairs) between *Cicindela* species and relatives (%).

| No. | Species (site) | Acc. No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *C. anchoralis* (SA) | PX472889 | | | | | | | | | | | | |
| 2 | *C. anchoralis* (TA) | PX472890 | 0.0 | | | | | | | | | | | |
| 3 | *C. anchoralis* (JP) | AB118689 | 0.2 | 0.2 | | | | | | | | | | |
| 4 | *C. anchoralis* (JP) | AB118688 | 0.3 | 0.3 | 0.2 | | | | | | | | | |
| 5 | *C. anchoralis* (JP) | AB118687 | 0.3 | 0.3 | 0.2 | 0.3 | | | | | | | | |
| 6 | *C. anchoralis* (JP) | AB118686 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | | | | | | | |
| 7 | *C. anchoralis* (JP) | AB118685 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | | | | | | |
| 8 | *C. anchoralis* (TW) | AB118691 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | | | | | |
| 9 | *C. anchoralis* (TW) | AB118692 | 0.5 | 0.5 | 0.3 | 0.5 | 0.5 | 0.5 | 0.5 | 0.2 | | | | |
| 10 | *C. elisae* | PX472897 | 6.3 | 6.3 | 6.5 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 7.0 | | | |
| 11 | *C. laetescripta* | AB118713 | 7.4 | 7.4 | 7.6 | 7.8 | 7.6 | 7.8 | 7.4 | 7.8 | 8.1 | 6.5 | | |
| 12 | *C. lewisi* | AB118708 | 9.6 | 9.6 | 9.8 | 10.1 | 10.1 | 10.1 | 9.6 | 10.1 | 10.3 | 4.8 | 7.2 | |
| 13 | *Calomera angulata* | AB118699 | 9.3 | 9.3 | 9.5 | 9.7 | 9.7 | 9.7 | 9.7 | 9.5 | 9.8 | 4.8 | 6.8 | 5.6 |

Currently, the tiger beetle *C.* (*A.*) *anchoralis* is only be observed in two regions in South Korea: Taean-gun (TA) and Sinan-gun (SA). Within the Korean population, COI varied by 0–0.2%, and no differences in 16S were exhibited (Tables 2, 3). Three single nucleotide variations were identified in COI gene analysis, and four types could be distinguished through pattern observations in the Korean population (Fig. 2). The presence of 'G' at Site 1 was a characteristic observed only in the TA group, suggesting that the TA group has a single type (Fig. 2). In contrast, the SA group exhibited three types, G-C, A-T, and A-C at Site 2-3 (Fig. 2). Therefore, the TA and SA groups can be distinguished by Site 1, but they share nucleotide sequence characteristics at Sites 2 and 3. Additionally, since the sample size of the TA group (five individuals) was smaller than that of the SA group (16 individuals), there is insufficient evidences to conclude that the two groups are genetically completely independent populations.

In the phylogenetic tree, both COI and 16S show similar results. *Cicindela* (*Abroscelis*) *anchoralis* is divided into a single branch, distinct from other species within the genus *Cicindela* (Figs. 3, 4). And it can be confirmed that *C.* (*A.*) *anchoralis* branch is clearly composed of three groups, Korean, Japanese, and the Taiwanese populations (Figs. 3, 4). According to Kim *et al*. (2005), the Korean population almost follows morphological characteristics of the Japanese population, but differs in having thicker and more distinct yellow stripes on the forewings. This is consistent with the results of our genetic analyses.

Genetic diversity was compared based on COI, which has

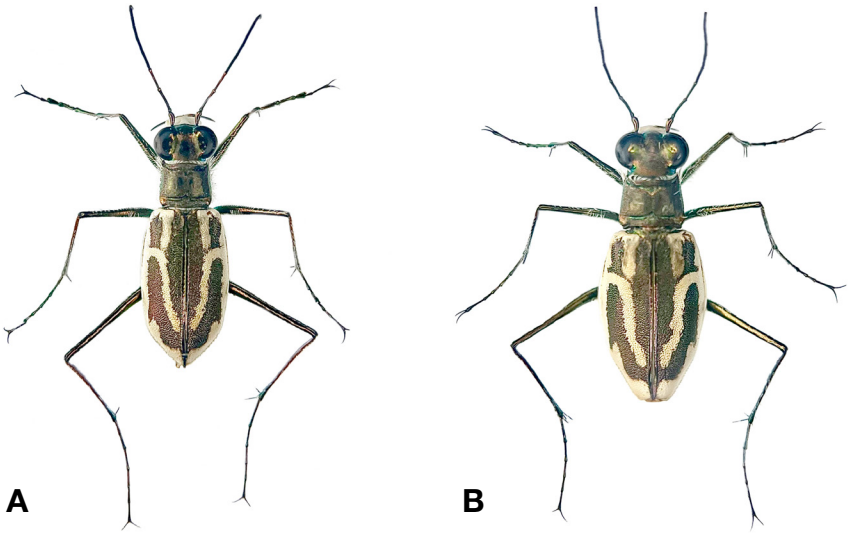



**Fig. 1.** Photographs of Korean *Cicindela* (*Abroscelis*) *anchoralis*: A. male adult, dorsal; B. female adult, dorsal.

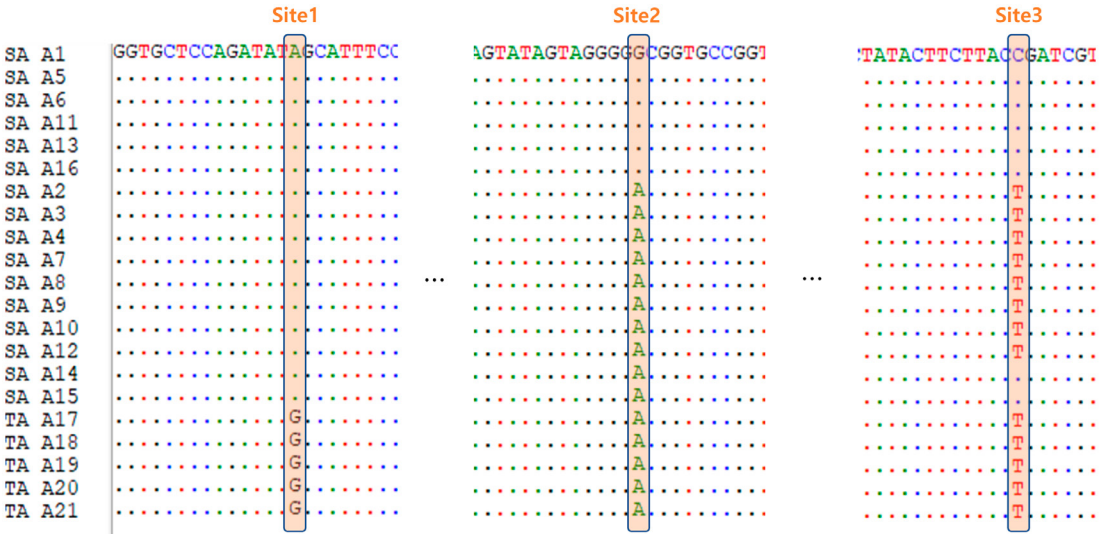



**Fig. 2.** The three regions of the COI gene sequence which exhibit variation within the Korean population of *Cicindela* (*Abroscelis*) *anchoralis*.

higher resolution than 16S the Korean population exhibited lower genetic diversity values than the Japanese populations (maximum variation value, 0.2% vs. 1%) (Table 2). In previous studies, specimens from Japan were collected from more than five habitat sites (Satoh and Hori, 2005), whereas in the present study, Korean individuals were collected from only two sites. Historical records indicate that they were once commonly found along sandy beaches on the west coast of South Korea (Kim, 1978), but their habitat has decreased significantly since then. Thus, it is likely that genetic diversity has also declined.

This study provides an analysis of the genetic diversity and phylogenetic position of *Cicindela* (*Abroscelis*) *anchoralis* populations in South Korea. The results show that this species possesses distinct genetic characteristics compared to other species within the genus *Cicindela*, with notable differences in both the COI and 16S gene regions. While intra-specific genetic variation within the South Korean population was low, clear genetic differentiation was observed between the Taean-gun and Sinan-gun populations. The differences in genetic diversity between the Korean and Japanese populations appear to reflect the effects of habitat reduction and isolation, which may have contributed to the decline in genetic diversity in the Korean population.

These findings underscore the importance of continued

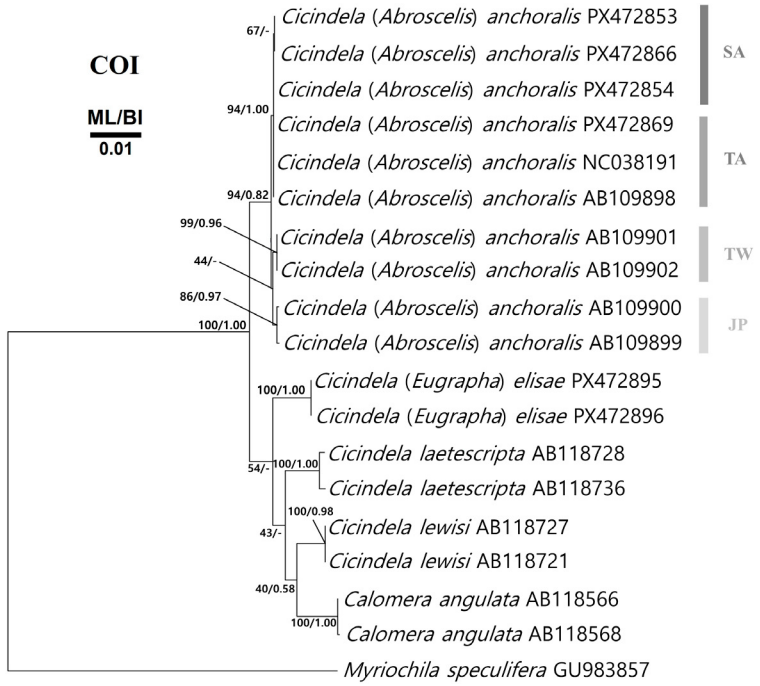



**Fig. 3.** Maximum likelihood and Bayesian inference analyses based on COI gene sequences. The new sequence provided in the present work is that of samples from SA, TA, and *C*. (*Eugrapha*) *elisae*. Numbers at nodes indicate the bootstrap values of ML out of 1,000 replicates and the posterior probability of BI. The scale bar corresponds to one substitution per 100 nucleotide positions. JP, Japan; SA, Sinan-gun; Korea; TA; Taean, Korea; TW, Taiwan.

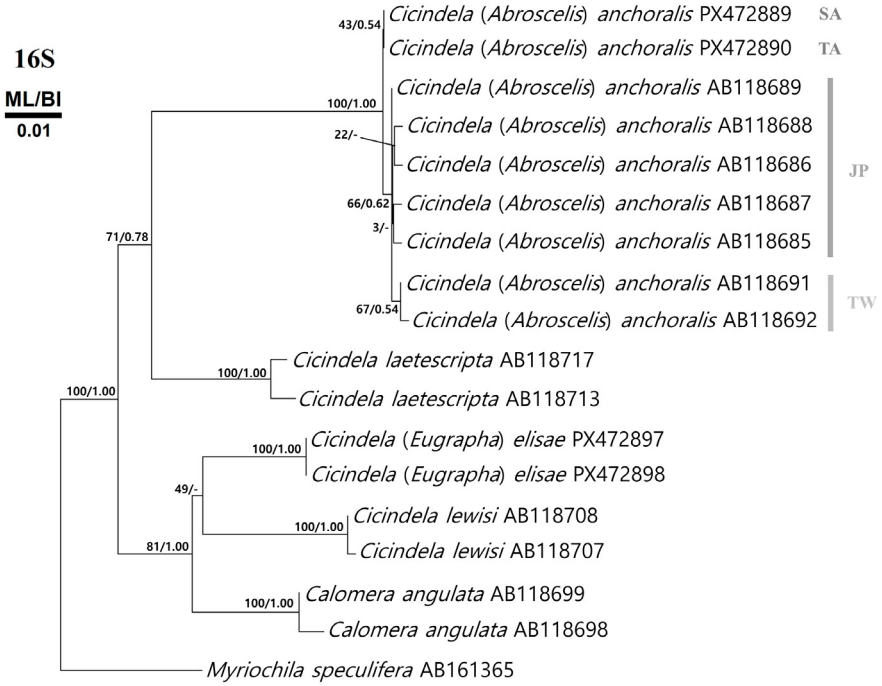



**Fig. 4.** Maximum likelihood and Bayesian inference analyses based on 16S gene sequences. The new sequence provided in the present work is that of samples from SA, TA, and *C*. (*Eugrapha*) *elisae*. Numbers at nodes indicate the bootstrap values of ML out of 1,000 replicates and the posterior probability of BI. The scale bar cForresponds to one substitution per 100 nucleotide positions. JP, Japan; SA, Sinan-gun; Korea; TA; Taean, Korea; TW, Taiwan.

monitoring and conservation efforts for *C.* (*A.*) *anchoralis*, particularly as its population remains limited to a few regions in South Korea. The genetic data presented here provide valuable insights that can guide future restoration strategies and help inform conservation policies aimed at protecting this endangered species.

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# A report of 19 unrecorded bacterial species isolated from the coastal regions of Korean islands in 2024

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Islands serve as unique biogeographic environments that support distinct microbial communities, yet the bacterial diversity of Korean islands remains largely unexplored. To bridge this gap, we investigated bacterial strains obtained from coastal seawater samples collected in 2024 from four Korean islands: Somaemuldo, Donggeomdo, Gyodongdo, and Udo. Approximately 1,400 bacterial isolates were obtained using standard dilution plating, and their taxonomic identities were determined through 16S rRNA gene sequence analysis. A total of 19 strains exhibited ≥98.7% sequence similarity to validly published species not previously reported in Korea and were thus recognized as newly recorded species. These strains exhibited broad phylogenetic diversity, spanning three phyla, four classes, seven orders, 12 families, and 17 genera. These previously unreported species were classified into *Epibacterium* and *Thalassospira* within the class *Alphaproteobacteria*; *Paraglaciecola*, *Colwellia*, *Thalassomonas*, *Psychromonas*, *Photobacterium*, *Vibrio*, *Neptunomonas*, and *Alkalimarinus* within the class *Gammaproteobacteria*; *Cyclobacterium*, *Flammeovirga*, *Antarcticibacterium*, *Wenyingzhuangia*, and *Zunongwangia* within the class *Bacteroidia*; *Peribacillus* and *Oceanobacillus* within the class *Bacilli*. Detailed descriptions of the morphological, biochemical, and phylogenetic characteristics of these newly recorded species are provided.

Keywords: 16S rRNA, coast, island, prokaryote, seawater, unrecorded bacterial species

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## Introduction

Islands, naturally formed landmasses rising above water at high tide, act as critical ecological bridges between terrestrial and marine environments. The Korean Peninsula is home to approximately 4,400 islands (Armstrong, 2023), predominantly distributed along the South and West Seas, each exhibiting unique biodiversity and ecological significance. Among Korea's five major ecological zones, which include forests, freshwater, coastal and marine areas, islands, and the Demilitarized Zone (DMZ), islands and coastal regions function as vital ecological connectors that link diverse ecosystems.

Despite the ecological importance of these regions, many islands in Korea remain unexplored, presenting valuable opportunities for biological discoveries. Island and coastal ecosystems, which function as transitional interfaces between terrestrial and marine environments, often exhibit heightened ecological complexity and species richness (Li *et al*., 2020). Notably, estuarine zones, which act as vital ecotones, facilitate nutrient exchange, provide essential spawning and nursery grounds, and support diverse faunal assemblages (Ward *et al*., 1999). These areas are recognized for their exceptional ecological productivity and intrinsic conservation value, while also contributing substantially to economic sustainability through fisheries and various ecosystem services.

Comprehensive surveys are crucial for uncovering the

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extensive microbial diversity present in island and coastal ecosystems and for enhancing our understanding of these ecologically significant environments. Recognizing this need, the Honam National Institute of Biological Resources (HNIBR) launched the 'Survey of Island-Coastal Indigenous Organisms (Prokaryotes)' research program in 2024 to assess microbial diversity across Korean island and coastal environments. Previous investigations (e.g., Joung *et al*., 2018, 2019; Kim *et al*., 2021; Cho *et al*., 2023; Lim *et al*., 2024) have successfully identified numerous previously unrecorded bacterial species from various Korean island ecosystems, highlighting the importance of continued exploration. As part of the ongoing HNIBR initiative, this study aims to isolate and characterize bacterial species that have not been previously recorded in Korea, enhancing our knowledge of the microbial diversity present in these ecologically significant regions.

During 2024, bacterial strains were isolated from seawater samples collected from four geographically distinct Korean islands: Somaemuldo, Donggeomdo, Gyodongdo, and Udo. Phylogenetic analyses based on 16S rRNA gene sequences identified 19 bacterial strains belonging to the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, and *Bacilli*. These strains represent newly documented bacterial species in Korea, and detailed taxonomic descriptions along with phenotypic characterizations are provided in this study.

## Materials and Methods

Coastal seawater samples were collected in 2024 from various island sites located in the southwestern region of Korea, including Somaemuldo, Donggeomdo, Gyodongdo, and Udo. The collected seawater samples were immediately refrigerated and transported to the laboratory within six hours. The collected seawater samples were subjected to serial dilution, and 200 µL aliquots from each dilution level were spread onto three different types of culture media: marine agar 2216 (MA; Difco), M-R2A (R2A agar prepared with aged seawater), and a 1/10 dilution of M-R2A. The plates were incubated aerobically at 20−25°C for 3 to 7 days. Individual colonies were subsequently subcultured on MA to obtain pure isolates. A total of 1,416 bacterial isolates were successfully purified. These cultures were preserved at −80°C in 20% glycerol (v/v) stocks and lyophilized for long-term storage. Detailed information on each strain and its respective origin are listed in Table 1.

Colony characteristics were documented at the stationary growth phase under low magnification. Cellular features, including size, morphology, and presence of flagella, were observed using transmission electron microscopy (CM200; Philips) following staining with 1% uranyl acetate. The Gram reaction was determined using both a standard staining kit (bioMérieux) and the KOH string test. Physiological and biochemical characteristics were assessed using API 20NE strips (bioMérieux) according to the manufacturer's instructions.

Genomic DNA was extracted from each isolate, and the 16S rRNA gene was amplified using the 27F and 1492R primers (Weisburg *et al*., 1991). Sanger sequencing was performed using 518F and 805R primers. The resulting sequences were compared against validly published species listed in EzBioCloud (Chalita *et al*., 2024). A similarity threshold of 98.7% was applied to define species-level matches (Chun *et al*., 2018). Strains exhibiting $\geq$98.7% similarity to known species that had not been previously reported in Korea were considered newly recorded species.

Multiple sequence alignment was conducted using the SILVA Incremental Aligner in ARB (Pruesse *et al*., 2007). Phylogenetic trees were reconstructed using maximum-likelihood, neighbor-joining, and minimum-evolution methods with 1,000 bootstrap replicates, implemented in MEGA X (Kumar *et al*., 2018). These analyses were based on the aligned 16S rRNA gene sequences exported from ARB.

## Results and Discussion

Comprehensive analysis of 16S rRNA gene sequences from 1,416 bacterial isolates led to the identification of 19 strains exhibiting $\geq$98.7% sequence similarity to validly published species. None of these strains had been previously documented in Korea, and therefore, they were classified as newly recorded bacterial species within the national microbial inventory. Detailed taxonomic assignments, strain identifiers, and accession numbers from both the HNIBR culture repository and GenBank are provided in Table 1.

The phylogenetic relationships of these strains were inferred from 16S rRNA gene sequence alignments and evaluated using multiple tree-building algorithms, including maximum-likelihood, neighbor-joining, and minimum-evolution methods. In all cases, each strain clustered within a well-supported clade alongside the type strain of its closest phylogenetic relative, with 99−100% bootstrap values exceeding conventional confidence thresholds (Fig. 1).

The 19 newly recorded taxa were distributed across three bacterial phyla, encompassing a broad taxonomic range that included four classes, seven orders, 12 families, and 17 genera (Table 1). Specifically, the 19 bacterial species were classified into the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, and *Bacilli*. In the class *Alphaproteobacteria*, the taxa were represented by *Epibacterium* and *Thalassospira*. The most diverse class,

**Table 1.** Taxonomic affiliation of 19 bacterial strains isolated from seawater samples collected off the coast of Korean islands in 2024.

| Class | Order | Family | Genus | Strain ID | HNIBR ID | Accession number | Closest species | 16S rRNA similarity | Isolation source | Isolation medium | Incubation conditions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Alphaproteobacteria* | *Rhodobacterales* | *Rhodobacteraceae* | *Epibacterium* | IMCC45479 | HNIBRBA13699 | PQ454709 | *Epibacterium ulvae* | 100 | Seawater | MA | 20°C, 3 days |
| | *Rhodospirillales* | *Thalassospiraceae* | *Thalassospira* | IMCC45448 | HNIBRBA13688 | PQ454698 | *Thalassospira xiamenensis* | 99.93 | Seawater | M-R2A | 20°C, 3 days |
| *Gammaproteobacteria* | *Enterobacterales* | *Alteromonadaceae* | *Paraglaciecola* | IMCC44831 | HNIBRBA13691 | PQ454701 | *Paraglaciecola hydrolytica* | 98.81 | Seawater | MA | 20°C, 3 days |
| | | *Colwelliaceae* | *Colwellia* | IMCC44789 | HNIBRBA13687 | PQ454697 | *Colwellia psychrerythraea* | 99.31 | Seawater | M-1/10R2A | 20°C, 3 days |
| | | | *Thalassomonas* | IMCC44975 | HNIBRBA13693 | PQ454703 | *Thalassomonas haliotis* | 98.97 | Seawater | M-R2A | 20°C, 3 days |
| | | *Psychromonadaceae* | *Psychromonas* | IMCC44965 | HNIBRBA13698 | PQ454708 | *Psychromonas japonica* | 98.90 | Seawater | MA | 20°C, 3 days |
| | | *Vibrionaceae* | *Photobacterium* | IMCC44890 | HNIBRBA13694 | PQ454704 | *Photobacterium lucens* | 99.57 | Seawater | M-1/10R2A | 20°C, 3 days |
| | | | *Vibrio* | IMCC45542 | HNIBRBA13701 | PQ454711 | *Vibrio harveyi* | 99.64 | Seawater | M-1/10R2A | 20°C, 3 days |
| | | | | IMCC45600 | HNIBRBA13702 | PQ454712 | *Vibrio tetraodonis* subsp. *pristinus* | 99.59 | Seawater | MA | 20°C, 3 days |
| | | | | IMCC45637 | HNIBRBA13704 | PQ454714 | *Vibrio algivorus* | 99.93 | Seawater | MA | 20°C, 3 days |
| | *Pseudomonadales* | *Nitrincolaceae* | *Neptunomonas* | IMCC44816 | HNIBRBA13689 | PQ454699 | *Neptunomonas antarctica* | 99.72 | Seawater | M-1/10R2A | 20°C, 3 days |
| | | *Oleiphilaceae* | *Alkalimarinus* | IMCC44926 | HNIBRBA13696 | PQ454706 | *Alkalimarinus coralli* | 99.03 | Seawater | MA | 20°C, 3 days |
| *Bacteroidia* | *Cytophagales* | *Cyclobacteriaceae* | *Cyclobacterium* | IMCC44859 | HNIBRBA13692 | PQ454702 | *Cyclobacterium plantarum* | 100 | Seawater | M-R2A | 20°C, 3 days |
| | | *Flammeovirgaceae* | *Flammeovirga* | IMCC45622 | HNIBRBA13703 | PQ454713 | *Flammeovirga arenaria* | 99.57 | Seawater | MA | 20°C, 3 days |
| | *Flavobacteriales* | *Flavobacteriaceae* | *Antarcticibacterium* | IMCC44908 | HNIBRBA13695 | PQ454705 | *Antarcticibacterium arcticum* | 99.86 | Seawater | M-1/10R2A | 20°C, 3 days |
| | | | *Wenyingzhuangia* | IMCC45590 | HNIBRBA13700 | PQ454710 | *Wenyingzhuangia gracilariae* | 99.79 | Seawater | MA | 20°C, 3 days |
| | | | *Zunongwangia* | IMCC44821 | HNIBRBA13690 | PQ454700 | *Zunongwangia mangrovi* | 99.45 | Seawater | MA | 20°C, 3 days |
| *Bacilli* | *Bacillales* | *Bacillaceae* | *Peribacillus* | IMCC44954 | HNIBRBA13697 | PQ454707 | *Peribacillus saganii* | 99.09 | Seawater | MA | 20°C, 3 days |
| | | | *Oceanobacillus* | IMCC45405 | HNIBRBA13705 | PQ454715 | *Oceanobacillus massiliensis* | 99.66 | Seawater | MA | 20°C, 3 days |

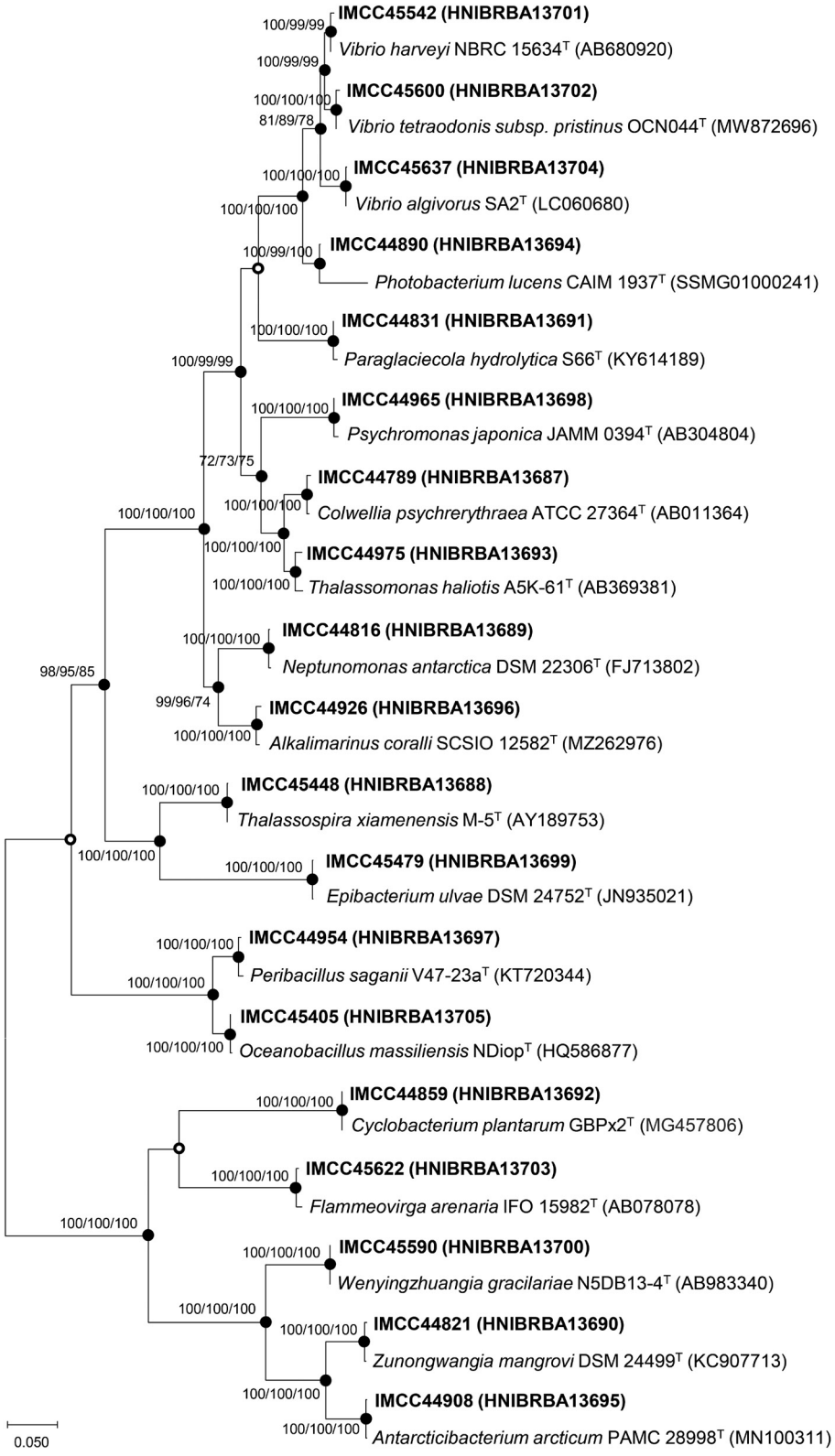



**Fig. 1.** Maximum-likelihood (ML) phylogenetic tree constructed from 16S rRNA gene sequences, illustrating the relationships between the 19 bacterial strains isolated in this study and their closest validly published species. Bootstrap support values above 70% are indicated at nodes in the order ML/neighbor-joining (NJ)/minimum evolution (ME); "–" denotes values below 70%. Filled circles represent nodes supported by both NJ and ME analyses, while open circles indicate support from only one of the two methods. The scale bar represents 0.05 nucleotide substitutions per site.

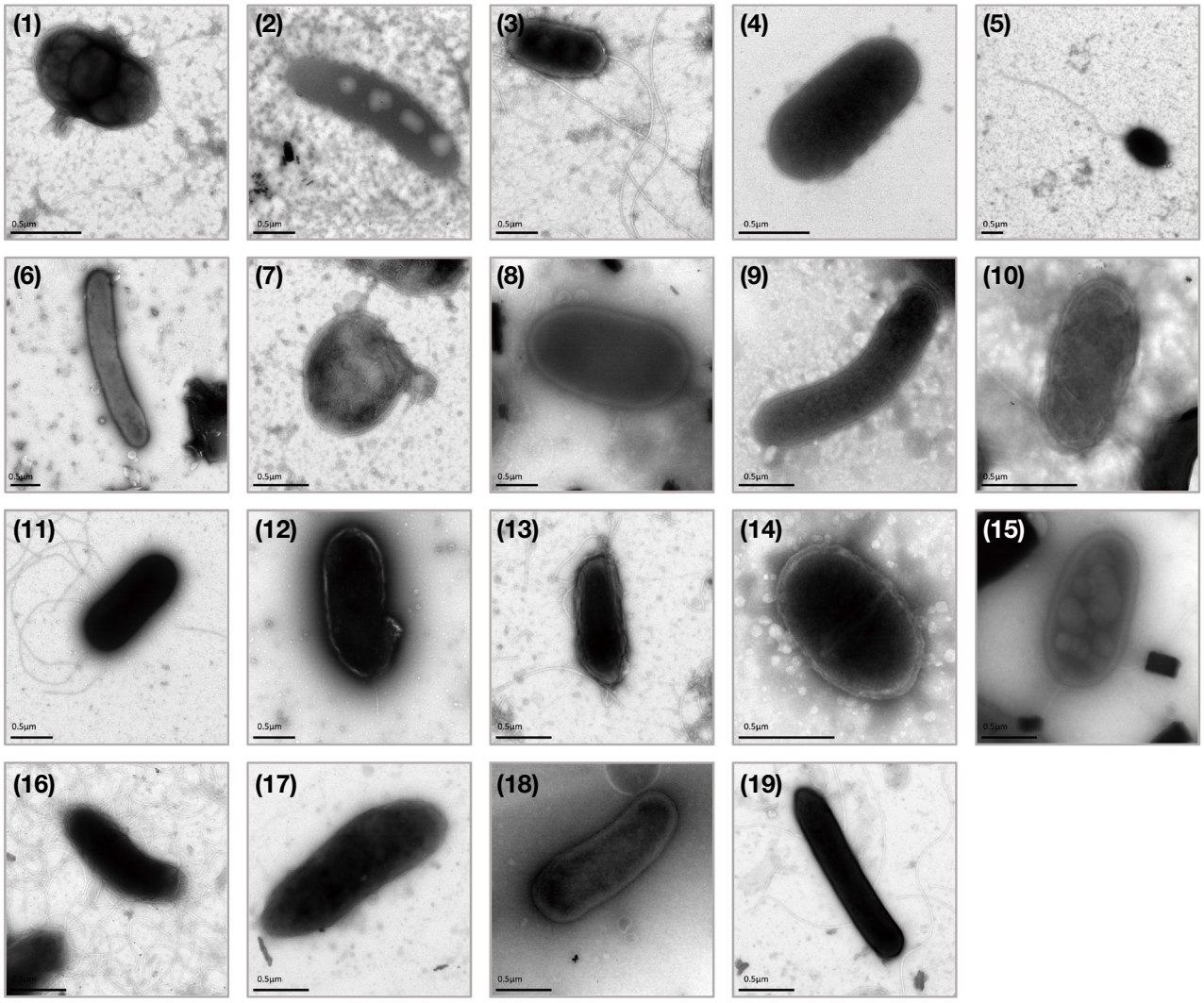



**Fig. 2.** Transmission electron micrographs of the 19 bacterial strains isolated in this study. Strain numbers: 1, IMCC44789; 2, IMCC45448; 3, IMCC44816; 4, IMCC44821; 5, IMCC44831; 6, IMCC44859; 7, IMCC44975; 8, IMCC44890; 9, IMCC44908; 10, IMCC44926; 11, IMCC44954; 12, IMCC44965; 13, IMCC45479; 14, IMCC45590; 15, IMCC45542; 16, IMCC45600; 17, IMCC45622; 18, IMCC45637; 19, IMCC45405. Scale bars = 0.5 μm.

*Gammaproteobacteria*, included the genera *Paraglaciecola*, *Colwellia*, *Thalassomonas*, *Psychromonas*, *Photobacterium*, *Vibrio*, *Neptunomonas*, and *Alkalimarinus*. Within the class *Bacteroidia*, the genera identified included *Cyclobacterium*, *Flammeovirga*, *Antarcticibacterium*, *Wenyingzhuangia*, and *Zunongwangia*. The class *Bacilli* comprised two genera: *Peribacillus* and *Oceanobacillus*.

The newly isolated strains displayed notable phenotypic diversity, encompassing both Gram-negative and Gram-positive profiles, presence or absence of flagellar structures, and varied cellular morphologies ranging from coccoid to rod-shaped forms (Fig. 2). A comprehensive description of each strain's morphological, physiological, and biochemical traits is provided in the subsequent species descriptions.

## Description of *Colwellia psychrerythraea* IMCC44789

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for glucose fermentation and esculin hydrolysis, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44789 (= HNIBRBA13687) was isolated from a coastal seawater sample Donggeomdo, Ganghwa, Incheon, Korea.

**Description of *Thalassospira xiamenensis* IMCC45448**

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and ivory-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, and malic acid are utilized as sole carbon sources, while capric acid, adipic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC 45448 (=HNIBRBA13688) was isolated from a coastal seawater sample, Juksanpo tidal flat in Gyodongdo, Ganghwa, Incheon, Korea.

**Description of *Neptunomonas antarctica* IMCC44816**

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44816 (=HNIBR BA13689) was isolated from a coastal seawater sample, Donggeomdo, Ganghwa, Incheon, Korea.

**Description of *Zunongwangia mangrovi* IMCC44821**

Cells are Gram-stain-negative, aerobic, non-flagellated, and short rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on MA at 20°C. Positive for glucose fermentation, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase but negative for nitrate reduction, indole production, arginine dihydrolase, urease, and esculin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose are utilized as sole carbon sources, while D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC44821 (=HNIBRBA13690) was isolated from a coastal seawater sample, Donggeomdo, Ganghwa, Incheon, Korea.

**Description of *Paraglaciecola hydrolytica* IMCC44831**

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for esculin hydrolysis, $\beta$-galactosidase and cytochrome oxidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, *N*-acetyl-glucosamine, and D-maltose are utilized as sole carbon sources, while D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC44831 (=HNIBRBA13691) was isolated from a coastal tidal flat sample, Donggeomdo, Ganghwa, Incheon, Korea.

**Description of *Cyclobacterium plantarum* IMCC44859**

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and pink-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, urease, esculin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44859 (=HNIBRBA13692) was isolated from a coastal tidal flat sample, Donggeomdo, Ganghwa, Incheon, Korea.

**Description of *Thalassomonas haliotis* IMCC44975**

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are circular, raised, entire, and pale lemon-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, and gelatin hydrolysis, but negative for indole production, arginine dihydrolase, urease, $\beta$-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44975 (=HNIBRBA13693) was isolated from a coastal seawater sample, Juksanpo in Gyodongdo, Ganghwa, Incheon, Korea.

**Description of *Photobacterium lucens* IMCC44890**

Cells are Gram-stain-negative, aerobic, non-flagellated, and short rod-shaped. Colonies are circular, convex, entire, and pale lemon-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase, but negative for urease and esculin hydrolysis in API 20NE. D-Glucose, D-mannose, *N*-acetyl-glucosamine, and potassium gluconate are utilized as sole carbon sources, while

L-arabinose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC44890 (=HNIBRBA13694) was isolated from a coastal seawater sample, Gyodongdo dock, Ganghwa, Incheon, Korea.

**Description of *Antarcticibacterium arcticum* IMCC44908**

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase but negative for arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44908 (=HNIBRBA13695) was isolated from a coastal seawater sample, Gyodongdo dock, Ganghwa, Incheon, Korea.

**Description of *Alkalimarinus coralli* IMCC44926**

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are punctiform, flat, entire, and white-colored after incubation for 3 days on MA at 20°C. Positive for urease and cytochrome oxidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC44926 (=HNIBRBA13696) was isolated from a coastal seawater sample, Namsanpo in Gyodongdo, Ganghwa, Incheon, Korea.

**Description of *Peribacillus saganii* IMCC44954**

Cells are Gram-stain-positive, aerobic, flagellated, and rod-shaped. Colonies are circular, flat, entire, and white-colored after incubation for 3 days on MA at 20°C. Positive for glucose fermentation, gelatin hydrolysis, and cytochrome oxidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, and $\beta$-galactosidase in API 20NE. D-Glucose and D-maltose are utilized as sole carbon sources, while L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC 44954 (=HNIBRBA13697) was isolated from a coastal seawater sample, Namsanpo in Gyodongdo, Ganghwa, Incheon, Korea.

**Description of *Psychromonas japonica* IMCC44965**

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are punctiform, flat, entire, and translucent-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction and glucose fermentation, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC 44965 (=HNIBRBA13698) was isolated from a coastal seawater sample, Juksanpo in Gyodongdo, Ganghwa, Incheon, Korea.

**Description of *Epibacterium ulvae* IMCC45479**

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and black-colored after incubation for 3 days on MA at 20°C. Positive for urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, $\beta$-galactosidase and cytochrome oxidase, in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC45479 (=HNIBRBA13699) was isolated from a coastal seawater sample, Cheonjin Port in Udo, Jeju, Korea.

**Description of *Wenyingzhuangia gracilariae* IMCC45590**

Cells are Gram-stain-negative, aerobic, non-flagellated, and short rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for urease, $\beta$-galactosidase, and cytochrome oxidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC 45590 (=HNIBRBA13700) was isolated from a coastal seawater sample, Haumokdong Port in Udo, Jeju, Korea.

**Description of *Vibrio harveyi* IMCC45542**

Cells are Gram-stain-negative, aerobic, flagellated, and short rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, indole production, glucose

fermentation, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase, and cytochrome oxidase, but negative for arginine dihydrolase and urease in API 20NE. Malic acid is utilized as sole carbon sources, while D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC 45542 (=HNIBRBA13701) was isolated from a coastal seawater sample, Cheonjin Port in Udo, Jeju, Korea.

**Description of *Vibrio tetraodonis* subsp. *pristinus* IMCC45600**

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are irregular, convex, undulate, and beige-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and cytochrome oxidase, but negative for indole production, arginine dihydrolase, urease, and $\beta$-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC45600 (=HNIBRBA13702) was isolated from a coastal seawater sample, Hagosudong Beach in Udo, Jeju, Korea.

**Description of *Flammeovirga arenaria* IMCC45622**

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are irregular, raised, curled, and beige-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase, but negative for indole production, arginine dihydrolase, urease, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC45622 (=HNIBRBA13703) was isolated from a coastal seawater sample, Biyangdo in Udo, Jeju, Korea.

**Description of *Vibrio algivorus* IMCC45637**

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on MA at 20°C. Positive for nitrate reduction, glucose fermentation, and cytochrome oxidase, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase in API 20NE. Potassium gluconate and malic acid are utilized as sole carbon sources, while D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid are not. Strain IMCC45637 (=HNIBRBA13704) was isolated from a coastal seawater sample, Biyangdo in Udo, Jeju, Korea.

**Description of *Oceanobacillus massiliensis* IMCC45405**

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and pale lemon-colored after incubation for 3 days on MA at 20°C. Positive for esculin hydrolysis, gelatin hydrolysis, and cytochrome oxidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and $\beta$-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC45405 (=HNIBRBA13705) was isolated from a coastal seawater sample, Juksanpo in Gyodongdo, Ganghwa, Incheon, Korea.

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# Report of 44 unrecorded bacterial species in Korea belonging to the class *Betaproteobacteria* and *Gammaproteobacteria* of the phylum *Pseudomonadota*, excavated during the survey in 2023

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The classes *Betaproteobacteria* and *Gammaproteobacteria* are two major classes within the phylum *Pseudomonadota*, encompassing a diverse range of Gram-negative, rod-shaped bacteria. Both classes are known for their metabolic versatility and ecological significance, inhabiting various environments. During a series of extensive surveys of indigenous prokaryotic species diversity in Korea, bacterial strains belonging to *Betaproteobacteria* and *Gammaproteobacteria* were isolated from various terrestrial environments. A total of 44 bacterial strains belonging to 30 genera in 17 families were identified as unrecorded species in Korea. The isolates assigned to *Betaproteobacteria* comprised 19 of the 44 unrecorded species, with *Burkholderiaceae* as the most abundant family. The most frequently isolated genus was *Burkholderia*. The isolates assigned to *Gammaproteobacteria* comprised 25 of the 44 unrecorded species, with *Lysobacteraceae* and *Pseudomonadaceae* as the most abundant families. The genera *Lysobacter* and *Pseudomonas* were the most frequently isolated genera. The detailed descriptions of each unrecorded species are provided.

Keywords: *Betaproteobacteria*, *Gammaproteobacteria*, *Pseudomonadota*, unrecorded species

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## Introduction

The phylum *Pseudomonadota* is one of the most diverse and ubiquitous bacterial groups (Oren and Garrity, 2021). The classes *Betaproteobacteria* and *Gammaproteobacteria* are among the largest classes of the phylum. Currently, *Betaproteobacteria* encompasses four valid orders, namely *Burkholderiales*, *Neisseriales*, *Rhodocyclales* and *Spirillales*, whereas *Gammaproteobacteria* encompasses 20 valid orders including *Pseudomonadales* and *Enterobacterales* (Parte *et al*., 2020). *Pseudomonadota* includes largest number of Gram-negative, rod-shaped species with diverse metabolic capabilities. Examples of such capabilities are degradation of polymers and recalcitrants, bioremediation,

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plant growth-promoting activities, and bioleaching (Oren and Garrity, 2021). They have been isolated from virtually everywhere in terrestrial and aquatic environments, and also in association with animals and plants.

In 2023, extensive surveys on prokaryotic diversity in Korea led to the isolation and taxonomic characterization of 44 novel strains within the classes *Betaproteobacteria* and *Gammaproteobacteria*, both belonging to the phylum *Pseudomonadota*. These strains were obtained from diverse environmental sources. As a result of this comprehensive analysis, 44 unrecorded species of *Pseudomonadota* in Korea were identified, and their detailed taxonomic properties are presented herein.

## Materials and Methods

A total of 44 bacterial strains were isolated from a variety of terrestrial and aquatic environmental sources, including agricultural soil, wetlands, sediments, sewage, tidal flat, seawater and various other soils (Table 1). The samples were put in sterile bottles or plastic bags, and transported to the labs under refrigeration. Each sample was processed individually, serially diluted, and the diluents spread onto various isolation media, including Luria-Bertani (LB) agar, marine agar (MA), peptone-tryptone-yeast extract-glucose (PTYG) agar, Reasoner's 2A (R2A), starch-casein agar (SCA) and tryptic soy agar (TSA). The inoculated plates were incubated at 25, 28 or 30°C for 2−7 days. Strain designations, isolation sources, growth media and growth conditions are summarized in Table 1 and also in the species descriptions. All isolates were purified through repeated subcultures to obtain single colonies. For long-term preservation, the strains were stored as suspensions in 20% (v/v) glycerol at −80°C and also as lyophilized ampoules. Colony morphology was observed on the same agar media used for cultivation. Cellular morphology and size dimensions were determined by either transmission electron microscopy (TEM) or scanning electron microscopy (SEM). Gram staining was performed according to standard protocols. Biochemical and physiological characteristics were determined using the API 20NE test kit (bioMérieux), following the instructions by the supplier.

The extraction of DNA, PCR amplification of the 16S rRNA gene, and sequencing were carried out as previously described (Joh *et al*., 2023). The obtained sequences were compared with those of the related type strains using the EzBioCloud database (Kim *et al*., 2012), and aligned together with related species using EzEditor2 (Jeon *et al*., 2014). Phylogenetic trees were constructed based on the Jukes-Cantor distance model (Jukes and Cantor, 1969) and the neighbor-joining method (Saitou and Nei, 1987) using MEGA version 7.0 (Kumar *et al*., 2016). The tree topologies were evaluated by bootstrap analyses with 1,000 resampled datasets (Felsenstein, 1985).

## Results and Discussion

A total of 44 bacterial strains belonging to the classes *Betaproteobacteria* and *Gammaproteobacteria* within the phylum *Pseudomonadota* were isolated from various sources of terrestrial and aquatic environments in Korea. These strains represented 44 unrecorded species of Korea, which were distributed across 25 genera and 17 families within the two classes (Table 1). Among them, 19 species were assigned to *Betaproteobacteria* (Table 1A), and the remaining 25 to *Gammaproteobacteria* (Table 1B).

At order level, *Burkholderiales* of *Betaproteobacteria* was the largest in size, encompassing 18 species, whereas *Lysobacterales* (5 species), *Pseudomonadales* (5) and *Enterobacterales* (4) formed the major groups within *Gammaproteobacteria*.

The most abundant families were *Burkholderiaceae* (11 species), *Comamonadaceae* (3 species), *Alcaligenaceae* (2 species) and *Oxalobacteraceae* (2 species) of *Betaproteobacteria*, and *Lysobacteraceae* (7 species), *Pseudomonadaceae* (4 species), *Shewanellaceae*, *Chromatiaceae*, *Enterobacteriaceae* and *Vibrionaceae* (2 species each) of *Gammaproteobacteria*. Single species was recorded for *Zoogloeaceae* of *Betaproteobacteria*, and *Marinobacteraceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Steroidobacteraceae*, *Alcanivoracaceae* and *Saccharospirillaceae*.

At genus level, *Burkholderia* (4 species) of *Betaproteobacteria*, and *Lysobacter* (4 species) and *Pseudomonas* (3 species) of *Gammaproteobacteria* were most abundant, while two species were recorded for *Pigmentiphaga*, *Pandoraea* and *Paraburkholderia* of *Betaproteobacteria*, and *Shewanella*, *Rheinheimera* and *Luteimonas* of *Gammaproteobacteria*. Single species were recorded for the remaining genera, *Cupriavidus*, *Limnobacter*, *Ralstonia*, *Comamonas*, *Ramlibacter*, *Rhodoferax*, *Herbaspirillum*, *Noviherbaspirillum* and *Azoarcus* of *Betaproteobacteria*, and *Marinobacter*, *Kluyvera*, *Siccibacter*, *Lonsdalea*, *Rahnella*, *Pseudoxanthomonas*, *Povalibacter*, *Alcanivorax*, *Salinibius*, *Azotobacter*, *Vibrio* and *Enterovibrio* of *Gammaproteobacteria*.

The detailed taxonomic affiliations of the obtained strains are listed in Table 1. These results would enhance our understanding on the bacterial diversity in Korea and also provide a basis for future studies in biotechnology and other applied sciences. Electron micrographs of the isolated strains are shown in Fig. 1, and phylogenetic relationships with closely related species are illustrated in Fig. 2 for *Betaproteobacteria* and Fig. 3 for *Gammaproteobacteria*. Detailed descriptions of each unrecorded species are provided below.

**Table 1.** List of unrecorded species belonging to the classes *Betaproteobacteria* and *Gammaproteobacteria*, and their taxonomic affiliations.

**A. *Betaproteobacteria***

| Affiliation | Strain code | NIBR ID[a] | Identification | Similarity (%) | Isolation source | Medium[b] | Culture conditions |
|---|---|---|---|---|---|---|---|
| *Burkholderiales* | | | | | | | |
| *Alcaligenaceae* | GDB4 | NIBRBAC000510584 | *Pigmentiphaga kullae* | 99.93 | Soil | R2A | 25°C, 2 days |
| | AN070 | NIBRBAC000510598 | *Pigmentiphaga litoralis* | 99.79 | Wetland | R2A | 28°C, 3 days |
| *Burkholderiaceae* | MAW7 | NIBRBAC000510847 | *Burkholderia australis* | 99.31 | Agricultural soil | R2A | 28°C, 3 days |
| | M34 | NIBRBAC000510732 | *Burkholderia diffusa* | 100 | Soil | R2A | 25°C, 3 days |
| | A6 | NIBRBAC000510740 | *Burkholderia pyrrocinia* | 99.38 | Soil | R2A | 25°C, 3 days |
| | MMS23-Y8 | NIBRBAC000510817 | *Burkholderia thailandensis* | 98.76 | Soil | R2A | 30°C, 3 days |
| | AN586 | NIBRBAC000510608 | *Cupriavidus neocaledonicus* | 99.14 | Wetland | R2A | 28°C, 3 days |
| | WK11 | NIBRBAC000510793 | *Limnobacter litoralis* | 99.45 | Sewage | R2A | 28°C, 3 days |
| | MMS23-hol29 | NIBRBAC000510829 | *Pandoraea fibrosis* | 99.86 | Soil | R2A | 30°C, 3 days |
| | MMS23-hol49 | NIBRBAC000510830 | *Pandoraea iniqua* | 100 | Soil | R2A | 30°C, 3 days |
| | B1W8 | NIBRBAC000510802 | *Paraburkholderia ribeironis* | 98.76 | Agricultural soil | R2A | 28°C, 3 days |
| | MMS23-S7 | NIBRBAC000510816 | *Paraburkholderia solitsugae* | 98.90 | Soil | SCA | 30°C, 7 days |
| | 17N1M7 | NIBRBAC000510543 | *Ralstonia insidiosa* | 99.72 | Sediment | R2A | 30°C, 2 days |
| *Comamonadaceae* | EM-0455 | NIBRBAC000510791 | *Comamonas aquaticum* | 99.93 | Agricultural soil | PTYG | 28°C, 4 days |
| | AN668 | NIBRBAC000510613 | *Ramlibacter tataouinensis* | 98.83 | Wetland | R2A | 28°C, 3 days |
| | BY2 | NIBRBAC000510795 | *Rhodoferax sediminis* | 100 | Agricultural soil | R2A | 28°C, 3 days |
| *Oxalobacteraceae* | AN613 | NIBRBAC000510609 | *Herbaspirillum denitrificans* | 98.76 | Wetland | R2A | 28°C, 3 days |
| | AMW7 | NIBRBAC000510842 | *Noviherbaspirillum autotrophicum* | 99.93 | Agricultural soil | R2A | 28°C, 3 days |
| *Rhodocyclales* | | | | | | | |
| *Zoogloeaceae* | GDB3 | NIBRBAC000510561 | *Azoarcus olearius* | 99.03 | Soil | R2A | 30°C, 2 days |

[a]National Institute of Biological Resources.
[b]MA, marine agar; R2A, Reasoner's 2A agar; LB, Luria-Bertani agar; PTYG, peptone-tryptone-yeast extract-glucose agar; TSA, tryptic soy agar; SCA, starch casein agar.

**Table 1.** Continued.

**B. *Gammaproteobacteria***

| Affiliation | Strain code | NIBR[a] ID | Identification | Similarity (%) | Isolation source | Medium[b] | Culture conditions |
|---|---|---|---|---|---|---|---|
| *Alteromonadales* | | | | | | | |
| *Marinobacteraceae* | HPW14 | NIBRBAC000510849 | *Marinobacter zhejiangensis* | 100 | Tidal flat | MA | 28°C, 3 days |
| *Shewanellaceae* | SBCC-1734 | NIBRBAC000510514 | *Shewanella jiangmenensis* | 100 | Seawater | MA | 25°C, 3−5 days |
| | MBLB4244 | NIBRBAC000510678 | *Shewanella oncorhynchi* | 98.87 | Soil | R2A | 30°C, 3 days |
| *Chromatiales* | | | | | | | |
| *Chromatiaceae* | gt1 | NIBRBAC000510643 | *Rheinheimera japonica* | 99.65 | Soil | MA | 30°C, 3 days |
| | DE95 | NIBRBAC000510644 | *Rheinheimera mesophila* | 99.03 | Soil | R2A | 30°C, 3 days |
| *Enterobacteriales* | | | | | | | |
| *Enterobacteriaceae* | MMS23-Ri17 | NIBRBAC000510834 | *Kluyvera georgiana* | 99.24 | Soil | LB | 30°C, 3 days |
| | 19H1E18 | NIBRBAC000510551 | *Siccibacter colletis* | 99.93 | Sediment soil | R2A | 30°C, 2 days |
| *Pectobacteriaceae* | 19N1E17 | NIBRBAC000510552 | *Lonsdalea populi* | 99.86 | Sediment | R2A | 30°C, 2 days |
| *Yersiniaceae* | 16S1T7 | NIBRBAC000510555 | *Rahnella inusitata* | 99.51 | Sediment | R2A | 30°C, 2 days |
| *Lysobacterales* | | | | | | | |
| *Lysobacteraceae* | MMS23-QF36 | NIBRBAC000510828 | *Lysobacter auxotrophicus* | 100 | Soil | R2A | 30°C, 3 days |
| | CAU 1721 | NIBRBAC000510778 | *Lysobacter erysipheiresistens* | 100 | Soil | MA | 30°C, 3 days |
| | BU5-26-2 | NIBRBAC000510749 | *Lysobacter prati* | 99.73 | Soil | R2A | 25°C, 3 days |
| | FY17 | NIBRBAC000510790 | *Lysobacter tabacisoli* | 99.73 | Agricultural soil | PTYG | 28°C, 4 days |
| | SBCC-2331 | NIBRBAC000510522 | *Luteimonas composti* | 99.05 | Seawater | MA | 25°C, 7 days |
| | DG1-10 | NIBRBAC000510731 | *Luteimonas gilva* | 100.00 | Soil | R2A | 25°C, 3 days |
| | MBLB3122 | NIBRBAC000510674 | *Pseudoxanthomonas mexicana* | 99.93 | Soil | TSA | 30°C, 3 days |
| *Nevskiales* | | | | | | | |
| *Steroidobacteraceae* | YT17 | NIBRBAC000510797 | *Povalibacter uvarum* | 99.45 | Agricultural soil | R2A | 28°C, 3 days |
| *Oceanospirillales* | | | | | | | |
| *Alcanivoracaceae* | CAU 1719 | NIBRBAC000510776 | *Alcanivorax jadensis* | 99.59 | Seawater | MA | 30°C, 3 days |
| *Saccharospirillaceae* | SBCC-2351 | NIBRBAC000510518 | *Salinibius halmophilus* | 100 | Tidal flat | MA | 30°C, 5 days |
| *Pseudomonadales* | | | | | | | |
| *Pseudomonadaceae* | SBCC-2184 | NIBRBAC000510520 | *Pseudomonas lalucatii* | 99.18 | Tidal flat | MA | 25°C, 5 days |
| | 16S4M11 | NIBRBAC000510542 | *Pseudomonas allii* | 100 | Sediment | R2A | 30°C, 2 days |
| | 18S3F2 | NIBRBAC000510553 | *Pseudomonas guariconensis* | 100 | Sediment | R2A | 30°C, 2 days |
| | MMS23-DY57 | NIBRBAC000510836 | *Azotobacter beijerinckii* | 100 | Soil | R2A | 30°C, 3 days |
| *Vibrionales* | | | | | | | |
| *Vibrionaceae* | SBCC-2261 | NIBRBAC000510509 | *Vibrio echinoideorum* | 99.93 | Tidal flat | MA | 25°C, 3 days |
| | SBCC-2194 | NIBRBAC000510508 | *Enterovibrio baiacu* | 99.73 | Seawater | MA | 25°C, 3 days |

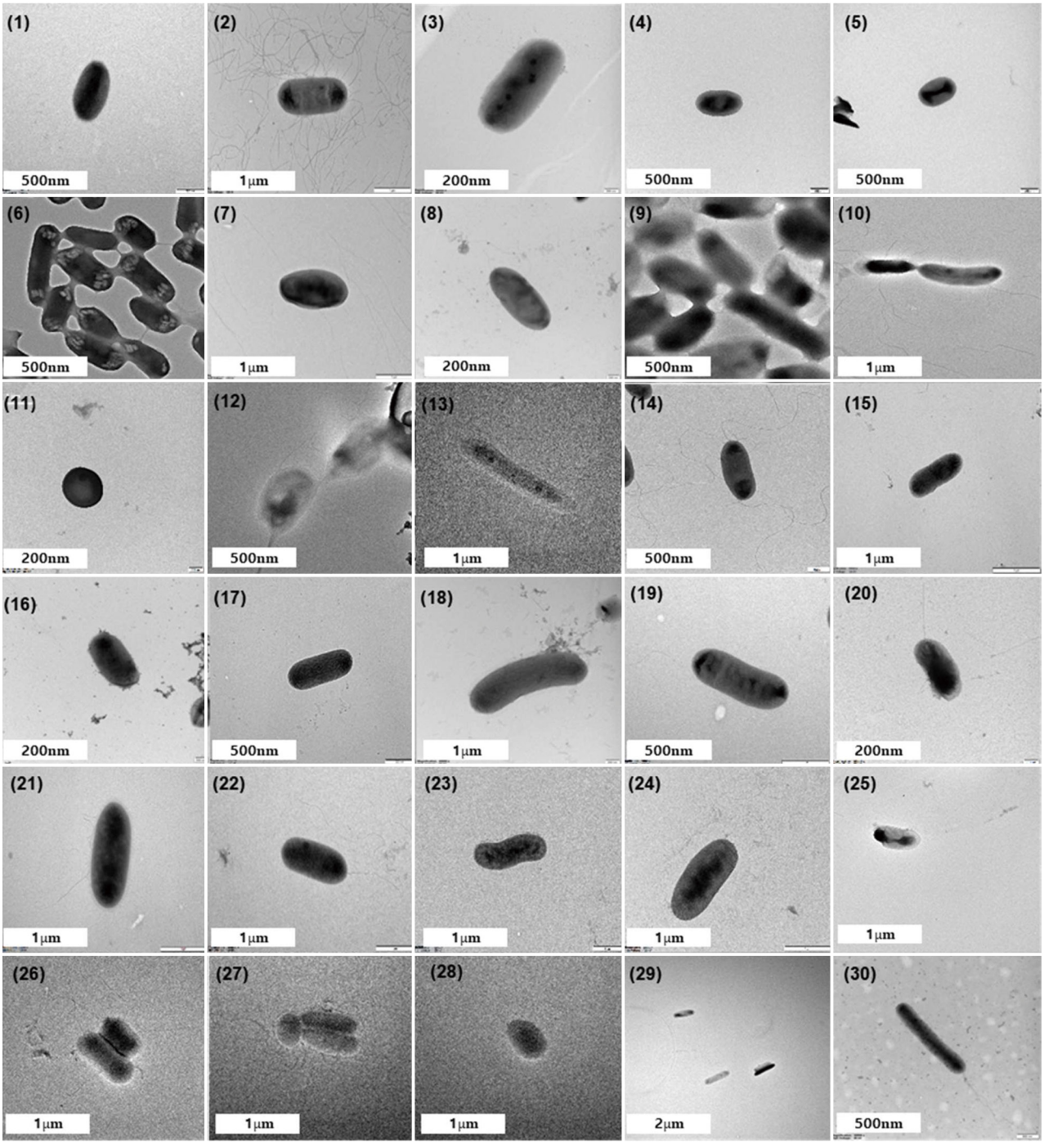



**Fig. 1.** Transmission electron or scanning electron micrographs of cells of the strains isolated in the study. Strains: 1, GDB4; 2, AN070; 3, MAW7; 4, M34; 5, A6; 6, MMS23-Y8; 7, AN586; 8, WK11; 9, MMS23-hol29; 10, MMS23-hol49; 11, B1W8; 12, MMS23-S7; 13, 17N1M7; 14, EM-0455; 15, AN668; 16, BY2; 17, AN613; 18, AMW7; 19, GDB3; 20, HPW14; 21, SBCC-1734; 22, MBLB4244; 23, gt1; 24, DE95; 25, MMS23-Ri17; 26, 19H1E18; 27, 19N1E17; 28, 16S1T7; 29, MMS23-QF36; 30, CAU 1721; 31, BU5-26-2; 32, FY17; 33, SBCC-2331; 34, DG1-10; 35, MBLB3122; 36, YT17; 37, CAU 1719; 38, SBCC-2351; 39, SBCC-2184; 40, 16S4M11; 41, 18S3F2; 42, MMS23-DY57; 43, SBCC-2261; 44, SBCC-2194.

**Description of *Pigmentiphaga kullae* GDB4**

Strain GDB4 (=NIBRBAC000510584), also isolated from soil in Oryu-dong, Seo-gu, Incheon, displays Gram-stain-negative, rod-shaped cellular morphology. Colonies grown on R2A medium at 25°C for 2 days are circular and white. Physiologically, strain GDB4 is positive for esculin

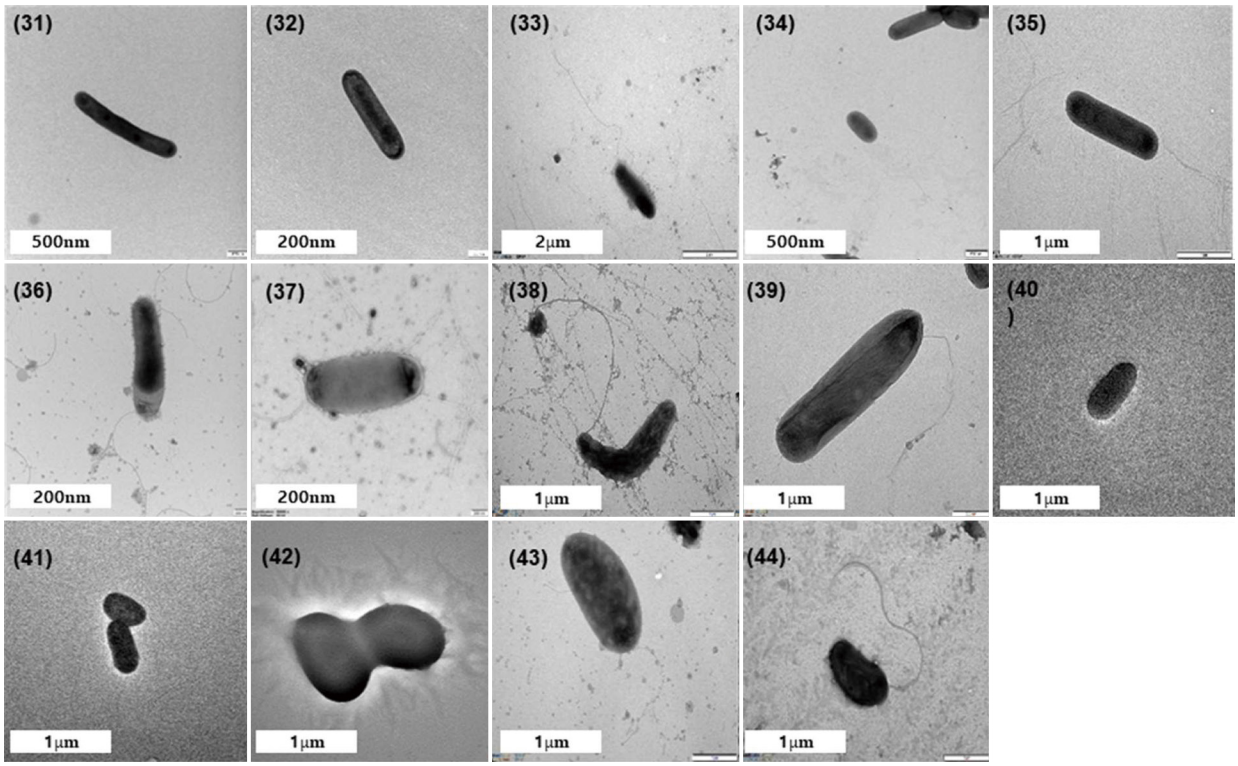



**Fig. 1.** Continued.

hydrolysis and cytochrome oxidase. It shows weakly positive results for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase. It utilizes D-mannitol, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid, but does not D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, or adipic acid. The 16S rRNA gene sequence for strain GDB4 has been deposited in GenBank under accession number OR492368.

### Description of *Pigmentiphaga litoralis* AN070

Strain AN070 (=NIBRBAC000510598), isolated from wetland sediment collected at Anseongcheon Stream, Anseong-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular with entire margins, flat, and yellow in color. The strain is negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase. It utilizes adipic acid, malic acid, and trisodium citrate as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, or phenylacetic acid. The 16S rRNA gene sequence of strain AN070 has been deposited in GenBank under the accession number OR527133.

### Description of *Burkholderia australis* MAW7

Strain MAW7 (=NIBRBAC000510847), isolated from agricultural soil sampled at Haksan-gil, Mureungdowon-myeon, Yeongwol-gun, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and white in color. The strain is positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, and malic acid as sole carbon sources, but does not utilize D-maltose, capric acid, adipic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain MAW7 has been deposited in GenBank under accession number OR759236.

### Description of *Burkholderia diffusa* M34

Strain M34 (=NIBRBAC000510732), isolated from soil at Nowon-gu, Seoul, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 25°C after 3 days of incubation are circular, slightly convex, glistening, and cream in color. The strain is positive for nitrate reduction, urease activity, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fer-

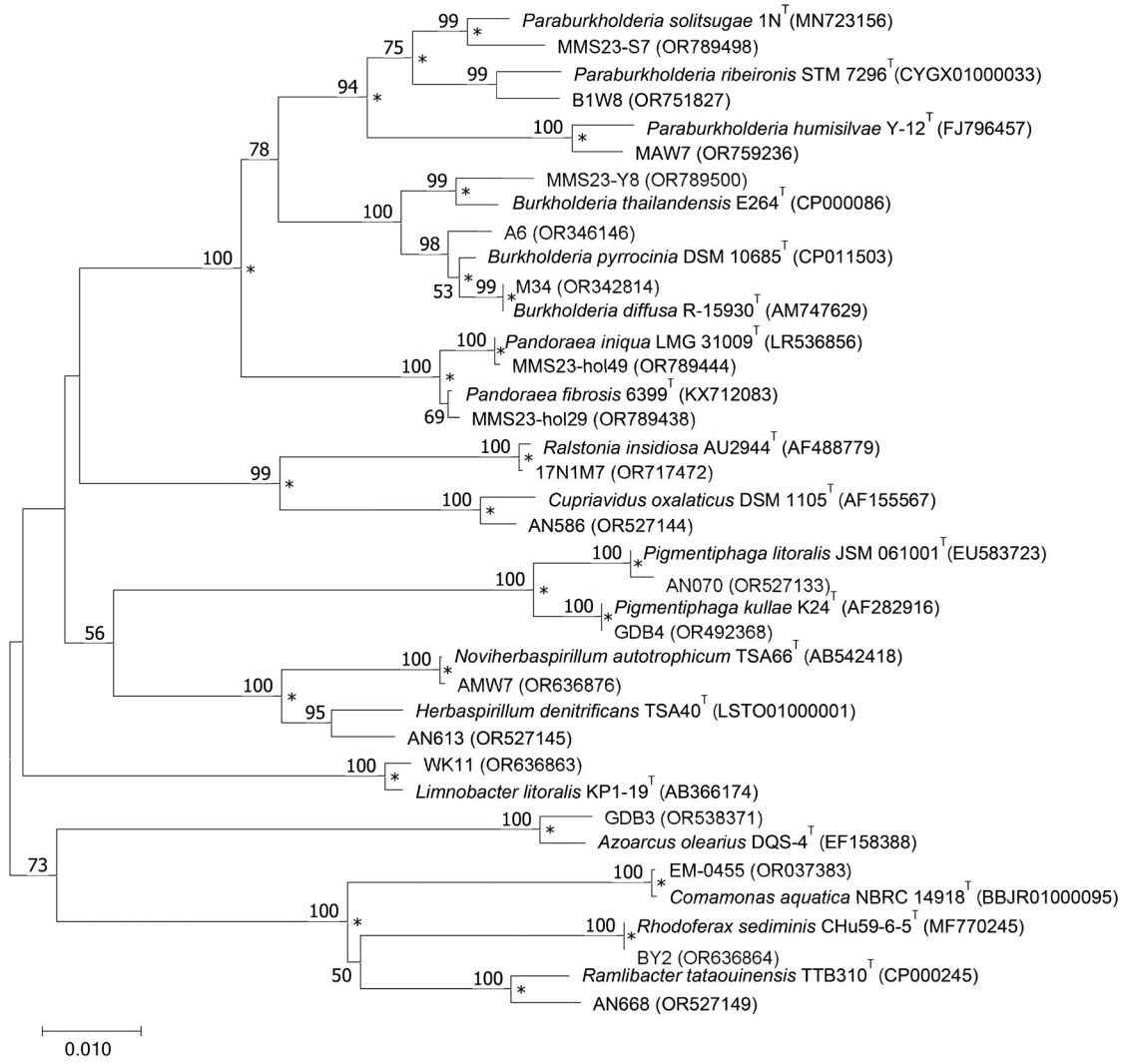



**Fig. 2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between the strains isolated in this study and their relatives of the class *Betaproteobacteria* in the phylum *Pseudomonadota*. Bootstrap values (>50%) are shown at branching points. Asterisks indicate the nodes also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

mentation, arginine dihydrolase, and gelatin hydrolysis. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain M34 has been deposited in Gen Bank under accession number OR342814.

### Description of *Burkholderia pyrrocinia* A6

Strain A6 (=NIBRBAC000510740), isolated from soil sampled at Nowon-gu, Seoul, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 25°C after 3 days of incubation are circular, slightly convex, glistening, and cream in color. The strain is positive for nitrate reduction, urease activity, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain A6 has been deposited in GenBank under accession number OR346146.

### Description of *Burkholderia thailandensis* MMS23-Y8

Strain MMS23-Y8 (=NIBRBAC000510817), isolated from soil collected at Singawon-dong, Seo-gu, Daejeon, Korea, is a Gram-stain-negative, rod-shaped bacterium.

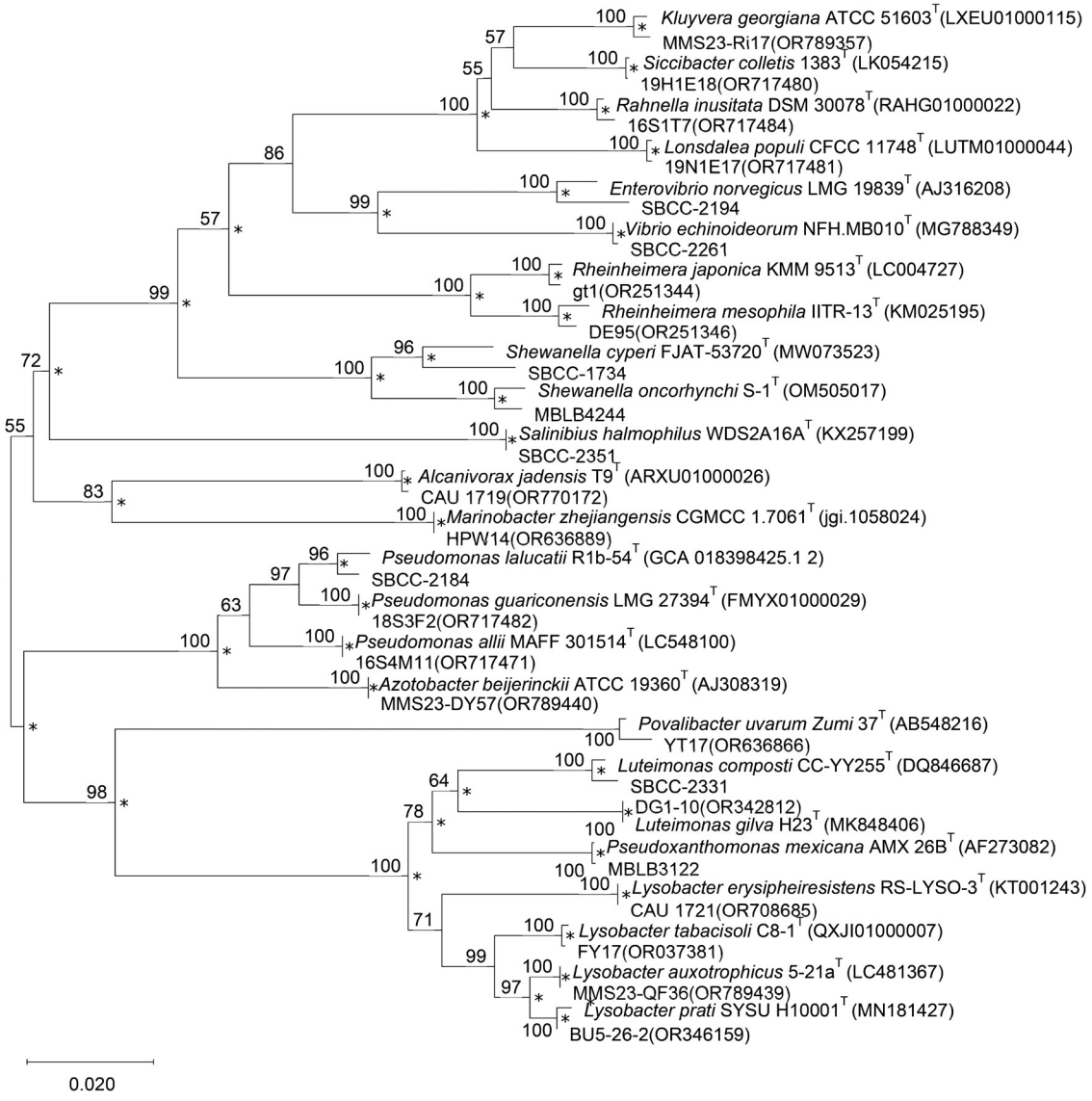



**Fig. 3.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between the strains isolated in this study and their relatives of the class *Gammaproteobacteria* in the phylum *Pseudomonadota*. Bootstrap values (>50%) are shown at branching points. Asterisks indicate the nodes also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

Colonies formed on R2A agar at 30°C after 7 days of incubation are circular, flat to slightly convex, smooth, glistening, and cream-colored. The strain is positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease activity, and gelatin hydrolysis, but negative for indole production, esculin hydrolysis, and β-galactosidase. It utilizes D-glucose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, and trisodium citrate as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-maltose, adipic acid, malic acid, or trisodium citrate. The 16S rRNA gene sequence of strain MMS23-Y8 has been deposited in GenBank under the accession number OR789500.

### Description of *Cupriavidus neocaledonicus* AN586

Strain AN586 (=NIBRBAC000510608), isolated from wetland sediment collected at Anseongcheon Stream, Anseong-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies grown on R2A agar at 28°C for 3 days are circular with entire margins, raised, and white in color. The strain is positive for glucose fermentation, arginine dihydrolase, and urease activity, but negative for nitrate reduction, indole production, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It utilizes D-glucose, potassium gluconate, capric acid, adipic acid, and malic acid as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-

mannitol, *N*-acetyl-glucosamine, D-maltose, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence of strain AN586 has been deposited in GenBank under the accession number OR527144.

**Description of *Limnobacter litoralis* WK11**

Strain WK11 (=NIBRBAC000510793), isolated from sewage sampled at Sinun-ri, Buan-eup, Jeollabuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and white in color. The strain is positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase. It utilizes malic acid as a sole carbon source, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain WK11 has been deposited in GenBank under accession number OR636863.

**Description of *Pandoraea fibrosis* MMS23-hol29**

Strain MMS23-hol29 (=NIBRBAC000510829), isolated from soil collected at Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon State, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are circular, flat, and white in color. The strain is positive for arginine dihydrolase, urease activity, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, and gelatin hydrolysis. It utilizes D-glucose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-mannitol, capric acid, or adipic acid. The 16S rRNA gene sequence of strain MMS23-hol29 has been deposited in GenBank under the accession number OR789438.

**Description of *Pandoraea iniqua* MMS23-hol49**

Strain MMS23-hol49 (=NIBRBAC000510830), isolated from soil sampled at Okcheon-gun, Chungcheongbuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are circular, flat, and white in color. The strain is positive for arginine dihydrolase, urease activity, and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, and β-galactosidase. It utilizes potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid as sole carbon sources, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, or adipic acid. The 16S rRNA gene sequence for strain MMS23-hol49 has been deposited in GenBank under accession number OR789444.

**Description of *Paraburkholderia ribeironis* B1W8**

Strain B1W8 (=NIBRBAC000510802), isolated from agricultural soil sampled at 979-1 Geoje-dong, Yeonje-gu, Busan, Korea, is a Gram-stain-negative, coccus-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and white in color. The strain is positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain B1W8 has been deposited in GenBank under accession number OR75182.

**Description of *Paraburkholderia solitsugae* MMS23-S7**

Strain MMS23-S7 (=NIBRBAC000510816), isolated from soil collected at Songnisan Mountain, Boeun, North Chungcheong Province, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies grown on starch-casein agar (SCA) at 30°C for 7 days are circular, flat to slightly convex, smooth, glistening, and cream-colored. The strain is positive for β-galactosidase activity, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, and gelatin hydrolysis. It utilizes *N*-acetyl-glucosamine as a sole carbon source, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence of strain MMS23-S7 has been deposited in GenBank under the accession number OR789498.

**Description of *Ralstonia insidiosa* 17N1M7**

Strain 17N1M7 (=NIBRBAC000510543), isolated from sediment soil collected at Guman-ri, Gandong-myeon, Hwacheon-gun, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies grown on R2A agar at 30°C for 2 days are circular with entire margins, glistening, and cream-colored. The strain is positive for urease activity, esculin hydrolysis, and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, β-galactosidase, and cytochrome oxidase. It utilizes D-glucose, *N*-acetyl-glucosa-

mine, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-mannitol, D-maltose, or phenylacetic acid. The 16S rRNA gene sequence of strain 17N1M7 has been deposited in GenBank under the accession number OR717472.

**Description of *Comamonas aquaticum* EM-0455**

Strain EM-0455 (=NIBRBAC000510791), isolated from agricultural soil sampled at 185 Okrim-ri, Ganghwa-eup, Ganghwa-gun, Incheon, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on PTYG agar at 28°C after 4 days of incubation are circular, entire, convex, smooth, and white in color. The strain is positive for nitrate reduction and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It utilizes capric acid, adipic acid, and malic acid as sole carbon sources, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain EM-0455 has been deposited in GenBank under accession number OR037383.

**Description of *Ramlibacter tataouinensis* AN668**

Strain AN668 (=NIBRBAC000510613), isolated from wetland sediment collected at Anseongcheon Stream, Anseong-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular with entire margins, flat, and white in color. The strain is negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence of strain AN668 has been deposited in GenBank under the accession number OR527149.

**Description of *Rhodoferax sediminis* BY2**

Strain BY2 (=NIBRBAC000510795), isolated from agricultural soil sampled at 699-2 Heungup-ri, Heungup-myeon, Wonju-si, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and yellow in color. The strain is positive for nitrate reduction and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain BY2 has been deposited in GenBank under accession number OR636864.

**Description of *Herbaspirillum denitrificans* AN613**

Strain AN613 (=NIBRBAC000510609), isolated from wetland sediment collected at Anseongcheon Stream, Anseong-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 5 days of incubation are circular with entire margins, flat, and pale yellow in color. The strain is positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase. It utilizes D-glucose, D-mannose, D-mannitol, and adipic acid as sole carbon sources, but does not utilize L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence of strain AN613 has been deposited in GenBank under the accession number OR527145.

**Description of *Noviherbaspirillum autotrophicum* AMW7**

Strain AMW7 (=NIBRBAC000510842), isolated from agricultural soil sampled at 1260-18 Changgi-ri, Anmyeon-eup, Chungcheongnam-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and brown in color. The strain is positive for nitrate reduction and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain AMW7 has been deposited in GenBank under accession number OR636876.

**Description of *Azoarcus olearius* GDB3**

Strain GDB3 (=NIBRBAC000510561), isolated from soil in Oryu-dong, Seo-gu, Incheon, exhibits Gram-stain-negative, rod-shaped cellular morphology. Colonies cultured on R2A medium at 30°C for 2 days are circular, convex, smooth, and yellowish. Physiologically, strain GDB3 is positive for esculin hydrolysis but negative for nitrate re-

duction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It utilizes D-mannose, D-mannitol, D-maltose, potassium gluconate, adipic acid, and trisodium citrate as sole carbon sources, but does not D-glucose, L-arabinose, *N*-acetyl-glucosamine, capric acid, malic acid, or phenylacetic acid. The 16S rRNA gene sequence for strain GDB3 has been deposited in GenBank under accession number OR538371.

**Description of *Marinobacter zhejiangensis* HPW14**

Strain HPW14 (=NIBRBAC000510849), isolated from mudflat sampled at Samsan-myeon, Ganghwa-gun, Incheon, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 28°C after 3 days of incubation are circular, entire, convex, smooth, and white in color. The strain is positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain HPW14 has been deposited in GenBank under accession number OR636889.

**Description of *Shewanella jiangmenensis* SBCC-1734**

Strain SBCC-1734 (=NIBRBAC000510514), isolated from seawater sampled at Seocheon-gun, Chungcheongnam-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 25°C after 3 days of incubation are circular, slightly convex, smooth, glistening, and light orange in color. The strain is positive for nitrate reduction, esculin hydrolysis, and gelatin hydrolysis, and weakly positive for β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, and urease. It utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and malic acid as sole carbon sources, but does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, or phenylacetic acid.

**Description of *Shewanella oncorhynchi* MBLB4244**

Strain MBLB4244 (=NIBRBAC000510678), isolated from soil sampled at Jeodong, Gangneung-si, Gangwon State, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are round, umbonate, with entire margins, and cream in color. The strain is positive for nitrate reduction, urease activity, esculin hydrolysis, and gelatin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, and β-galactosidase. It utilizes *N*-acetyl-glucosamine, D-maltose, capric acid, and malic acid as sole carbon sources, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid, trisodium citrate, or phenylacetic acid.

**Description of *Rheinheimera japonica* gt1**

Strain gt1 (=NIBRBAC000510643), isolated from soil sampled at Gaetgol Ecological Park in Janggok-dong, Siheung-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 30°C after 3 days of incubation are circular, convex, opaque, and yellow in color. The strain is positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase. It utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain gt1 has been deposited in GenBank under accession number OR251344.

**Description of *Rheinheimera mesophila* DE95**

Strain DE95 (=NIBRBAC000510644), isolated from soil sampled at Dongguk University Biomedical Campus, Ilsan-dong-gu, Goyang-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are circular, convex, with brick-colored mycelium and yellow pigmentation. The strain is positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. It utilizes *N*-acetyl-glucosamine and D-maltose as sole carbon sources, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain DE95 has been deposited in GenBank under accession number OR251346.

**Description of *Kluyvera georgiana* MMS23-Ri17**

Strain MMS23-Ri17 (=NIBRBAC000510834), isolated from soil sampled at Jang-dong, Daedeok-gu, Daejeon, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on LB agar at 30°C after 3 days of incubation are circular, smooth, glistening, and cream in color. The strain is positive for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase, but negative for gelatin hydrolysis. It utilizes D-glucose, L-arabinose, D-

mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid as sole carbon sources, but does not utilize capric acid or adipic acid. The 16S rRNA gene sequence for strain MMS23-Ri17 has been deposited in GenBank under accession number OR789357.

**Description of *Siccibacter colletis* 19H1E18**

Strain 19H1E18 (=NIBRBAC000510551), isolated from sediment soil sampled at Wabu-eup, Namyangju-si, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on ISP2 agar at 30°C after 3 days of incubation are opaque, circular, and pale yellow in color. The strain is positive for nitrate reduction, glucose fermentation, esculin hydrolysis, and β-galactosidase, but negative for indole production, arginine dihydrolase, urease, gelatin hydrolysis, and cytochrome oxidase. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate as sole carbon sources, but does not utilize capric acid, adipic acid, or phenylacetic acid. The 16S rRNA gene sequence for strain 19H1E18 has been deposited in GenBank under accession number OR71 7480.

**Description of *Lonsdalea populi* 19N1E17**

Strain 19N1E17 (=NIBRBAC000510552), isolated from sediment soil sampled at Okchun-gun, Guman-ri, Gandong-myeon, Hwacheon-gun, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 2 days of incubation are round, convex, and light cream in color. The strain is positive for glucose fermentation, arginine dihydrolase, urease, and esculin hydrolysis, but negative for nitrate reduction, indole production, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and potassium gluconate as sole carbon sources, but does not utilize L-arabinose, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain 19N1E17 has been deposited in Gen Bank under accession number OR717481.

**Description of *Rahnella inusitata* 16S1T7**

Strain 16S1T7 (=NIBRBAC000510555), isolated from sediment soil sampled at Nampyeong-ri, Bukpyeong-myeon, Jeongseon-gun, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 2 days of incubation are round, convex, smooth with entire margins, and cream in color. The strain is positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for indole production, arginine dihydrolase, urease, and cytochrome oxidase. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate as sole carbon sources, but does not utilize capric acid, adipic acid, or phenylacetic acid. The 16S rRNA gene sequence for strain 16S1T7 has been deposited in GenBank under accession number OR717484.

**Description of *Lysobacter auxotrophicus* MMS23-QF36**

Strain MMS23-QF36 (=NIBRBAC000510828), isolated from soil sampled at Heukseok-dong, Seo-gu, Daejeon, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are circular, flat, and pale yellow in color. The strain is positive for nitrate reduction, esculin hydrolysis, and gelatin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase. It utilizes *N*-acetyl-glucosamine and D-maltose as sole carbon sources, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid. The 16S rRNA gene sequence for strain MMS23-QF36 has been deposited in GenBank under accession number OR789439.

**Description of *Lysobacter erysipheiresistens* CAU 1721**

Strain CAU 1721 (=NIBRBAC000510778), isolated from soil sampled at Cheongwon-gu, Cheongju-si, Chungcheongbuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 30°C after 3 days of incubation are circular, smooth, entire, convex, translucent, and yellow in color. The strain is positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain CAU 1721 has been deposited in GenBank under accession number OR708685.

**Description of *Lysobacter prati* BU5-26-2**

Strain BU5-26-2 (=NIBRBAC000510749), isolated from soil sampled at Nowon-gu, Seoul, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 25°C after 3 days of incubation are circular, slightly convex, glistening, and pale orange yellow in color. The strain is positive for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, and urease. It does not utilize D-glu-

cose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain BU5-26-2 has been deposited in GenBank under accession number OR346159.

**Description of *Lysobacter tabacisoli* FY17**

Strain FY17 (=NIBRBAC000510790), isolated from agricultural soil sampled at 644-3 Geumsan-ri, Seo-myeon, Chuncheon-si, Gangwon-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on PTYG agar at 28°C after 4 days of incubation are circular, undulate, convex, smooth, and yellow in color. The strain is positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain FY17 has been deposited in GenBank under accession number OR037381.

**Description of *Luteimonas composti* SBCC-2331**

Strain SBCC-2331 (=NIBRBAC000510522), isolated from seawater sampled at Seogwipo-si, Jeju Island, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 7°C after 7 days of incubation are circular, convex, smooth, glistening, and yellow in color. The strain is positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. It utilizes D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, and adipic acid as sole carbon sources, but does not utilize D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, or phenylacetic acid.

**Description of *Luteimonas gilva* DG1-10**

Strain DG1-10 (=NIBRBAC000510731), isolated from soil sampled at Suseong-gu, Daegu, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 25°C after 3 days of incubation are circular, slightly convex, glistening, and cream yellow in color. The strain is positive for esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain DG1-10 has been deposited in GenBank under accession number OR342812.

**Description of *Pseudoxanthomonas mexicana* MBLB3122**

Strain MBLB3122 (=NIBRBAC000510674), isolated from soil sampled at Seo-gu, Incheon, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on ISP2 agar at 30°C after 3 days of incubation are circular, flat, translucent, and pale yellow in color. The strain is positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. It utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and malic acid as sole carbon sources, but does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, or phenylacetic acid.

**Description of *Povalibacter uvarum* YT17**

Strain YT17 (=NIBRBAC000510797), isolated from agricultural soil sampled at 979-1 Geoje-dong, Yeonje-gu, Busan, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on PTYG agar at 28°C after 4 days of incubation are circular, entire, convex, smooth, and brown in color. The strain is positive for nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain YT17 has been deposited in GenBank under accession number OR636866.

**Description of *Alcanivorax jadensis* CAU 1719**

Strain CAU 1719 (=NIBRBAC000510776), isolated from seawater sampled at Unchon Port, Haeundae-gu, Busan, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 30°C after 3 days of incubation are filamentous, raised, undulate, and white in color. The strain is negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain CAU 1719 has been deposited in GenBank under accession number OR770172.

**Description of *Salinibius halmophilus* SBCC-2351**

Strain SBCC-2351 (=NIBRBAC000510518), isolated from tidal flat sampled at Taean-gun, Chungcheongnam-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 30°C after 5 days of incubation are circular, slightly convex, smooth, glistening, and light yellow in color. The strain is negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources.

**Description of *Pseudomonas lalucatii* SBCC-2184**

Strain SBCC-2184 (=NIBRBAC000510520), isolated from tidal flat sampled at Gunsan-si, Jeonbuk State, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 25°C after 5 days of incubation are filamentous, raised, undulate, and light yellow in color. The strain is positive for arginine dihydrolase and weakly positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, urease, esculin hydrolysis, and gelatin hydrolysis. It utilizes D-glucose, D-mannitol, D-maltose, potassium gluconate, capric acid, malic acid, and trisodium citrate as sole carbon sources, but does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, adipic acid, or phenylacetic acid.

**Description of *Pseudomonas alii* 16S4M11**

Strain 16S4M11 (=NIBRBAC000506170), isolated from sediment soil sampled at Gangsang-myeon, Yangpyeong-gun, Gyeonggi-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 2 days of incubation are opaque, round with entire margin, raised, smooth, glistening, and light cream in color. The strain is positive for nitrate reduction, arginine dihydrolase, esculin hydrolysis, and gelatin hydrolysis, but negative for indole production, glucose fermentation, urease, β-galactosidase, and cytochrome oxidase. It utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, and trisodium citrate as sole carbon sources, but not adipic acid or phenylacetic acid. The 16S rRNA gene sequence for strain 16S4M11 has been deposited in GenBank under accession number OR717471.

**Description of *Pseudomonas guariconensis* 18S3F2**

Strain 18S3F2 (=NIBRBAC000510553), isolated from soil sampled at Okchun-gun, Chungcheongbuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 2 days of incubation are circular, convex, translucent, and light cream in color. The strain is positive for arginine dihydrolase, urease, and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase. It utilizes D-glucose, D-maltose, potassium gluconate, capric acid, malic acid, and trisodium citrate as sole carbon sources, but does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, adipic acid, or phenylacetic acid. The 16S rRNA gene sequence for strain 18S3F2 has been deposited in GenBank under accession number OR717482.

**Description of *Azotobacter beijerinckii* MMS23-DY57**

Strain MMS23-DY57 (=NIBRBAC000510836), isolated from soil sampled at Okchun-gun, Chungcheongbuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on R2A agar at 30°C after 3 days of incubation are circular, smooth, glistening, and cream in color. The strain is positive for urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and β-galactosidase. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources. The 16S rRNA gene sequence for strain MMS23-DY57 has been deposited in GenBank under accession number OR789440.

**Description of *Vibrio echinoideorum* SBCC-2261**

Strain SBCC-2261 (=NIBRBAC000510509), isolated from tidal flat sampled at Taean-gun, Chungcheongnam-do, Korea, is a Gram-stain-negative, ovoid and rod-shaped bacterium. Colonies formed on MA agar at 25°C after 3 days of incubation are circular, flat to slightly convex, smooth, glistening, and cream in color. The strain is positive for nitrate reduction, glucose fermentation, and β-galactosidase, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, and gelatin hydrolysis. It does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, or phenylacetic acid as sole carbon sources.

**Description of *Enterovibrio baiacu* SBCC-2194**

Strain SBCC-2194 (=NIBRBAC000510508), isolated from seawater sampled at Gampo in Gyeongju, Gyeongsangbuk-do, Korea, is a Gram-stain-negative, rod-shaped bacterium. Colonies formed on MA agar at 25°C after

3 days of incubation are circular, flat to slightly convex, smooth, glistening, and cream in color. The strain is positive for nitrate reduction, glucose fermentation, and β-galactosidase, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, and gelatin hydrolysis. It utilizes malic acid as a sole carbon source, but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, or phenylacetic acid.

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# Report on 34 unrecorded filamentous and sporulating bacterial species isolated from Korea in 2023

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In 2023, as research of discover native prokaryotic species in Korea, 34 filamentous and sporulating bacterial strains were affiliated to the phylum *Actinomycetota*. The strains formed monophyletic clades with the most closely related species with ≥98.7% of the 16S rRNA gene similarity. The 34 filamentous and sporulating *Actinomycetota* strains belonged to the families *Micromonosporaceae*, *Nocardiaceae*, *Pseudonocardiaceae*, *Streptomycetaceae*, and *Streptosporangiaceae* (of the orders *Micromonosporales*, *Mycobacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales*). 34 species of the phylum *Actinomycetota* have not been reported in Korea, suggesting unrecorded species in Korea. Information on strains such as Gram staining reaction, colony and cell morphology, biochemical characteristics, and isolation sources were provided in the species description.

Keywords: 16S rRNA, *Actinomycetota*, bacterial diversity, unrecorded species

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## Introduction

In 2023, 34 unrecorded bacteria were isolated from agricultural soil samples from various regions in Korea and identified as phylum of *Actinomycetes*. The taxonomic information of 34 unrecorded species is based on the nomenclature described in List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de) and consists of *Actinomycetota*. The phylum *Actinomycetota*, one of the largest phyla within the domain Bacteria, is widely distributed in aquatic and terrestrial environments (Lawson *et al*., 2018). The phylum is comprised of Gram-stain-positive, non-sporeforming, rod-shaped bacteria with a high G+C content (Gao and Gupta, 2005). The phylum *Actinomycetota* includes multicellular filamentous bacteria such as *Streptomyces* species (Feeney *et al*., 2022). These filamentous bacteria have garnered significant attention due to their intricate developmental life cycles, where growth and differentiation frequently coincide with the production of specialized metabolites (Gerwick and Moore, 2012). Among filamentous actinobacteria, the genus *Streptomyces* has been the most extensively studied, primarily because its species are the most prolific producers of specialized metabolites (Hutchings *et al*., 2019).

In 2023, a total of 192 unrecorded species were isolated from various samples during the research of discover native prokaryotic species in Korea, of which 34 belong to the phylum *Actinomycetota* (filamentous and sporulating species). In this study, we report these species and describe their characteristics.

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**Table 1.** The taxonomic affiliations of isolated strains belonging to the phylum *Actinomycetota*.

| Phylum | Order | Family | Strain ID | NIBR ID | Most closely related species | Similarity (%) | Isolation source | Medium | Incubation conditions |
|---|---|---|---|---|---|---|---|---|---|
| *Actinomycetota* | *Micromonosporales* | *Micromonosporaceae* | AN1124 | NIBRBAC000510621 | *Micromonospora provocatoris* | 99.86 | Wetland | R2A | 28°C, 3 d |
| | *Mycobacteriales* | *Nocardiaceae* | AN003 | NIBRBAC000510592 | *Nocardia aurea* | 100.00 | Wetland | R2A | 28°C, 3 d |
| | | | SBCC-2246 | NIBRBAC000510516 | *Nocardia higoensis* | 99.51 | Tidal Flat | MA | 25°C, 7 d |
| | | | AN064 | NIBRBAC000510597 | *Nocardia salmonicida* subsp. *cummidelens* | 99.02 | Wetland | R2A | 28°C, 3 d |
| | | | AN043 | NIBRBAC000510595 | *Nocardia salmonicida* subsp. *salmonicida* | 99.44 | Wetland | R2A | 28°C, 3 d |
| | | | AN051 | NIBRBAC000510596 | *Nocardia sungurluensis* | 98.96 | Wetland | R2A | 28°C, 3 d |
| | *Pseudonocardiales* | *Pseudonocardiaceae* | MMS23-KA4 | NIBRBAC000510822 | *Amycolatopsis vastitatis* | 99.71 | Soil | AA | 30°C, 3 d |
| | | | AN625 | NIBRBAC000510611 | *Lentzea pudingi* | 99.16 | Wetland | R2A | 28°C, 3 d |
| | | | AN040 | NIBRBAC000510594 | *Saccharothrix espanaensis* | 99.79 | Wetland | R2A | 28°C, 3 d |
| | | | MMS23-KR2 | NIBRBAC000510823 | *Saccharothrix sytingae* | 98.96 | Soil | R2A | 30°C, 3 d |
| | | | AN189 | NIBRBAC000510601 | *Umezawaea tangerina* | 99.30 | Wetland | R2A | 28°C, 3 d |
| | *Streptomycetales* | *Streptomycetaceae* | AN696 | NIBRBAC000510615 | *Actinacidiphila paucisporea* | 98.76 | Wetland | R2A | 28°C, 3 d |
| | | | SBCC-2341 | NIBRBAC000510527 | *Streptomyces althioticus* | 99.86 | Tidal Flat | R2A | 25–30°C, 7 d |
| | | | AN214 | NIBRBAC000510603 | *Streptomyces amritsarensis* | 100.00 | Wetland | R2A | 28°C, 3 d |
| | | | AN286 | NIBRBAC000510604 | *Streptomyces atrovirens* | 98.75 | Wetland | R2A | 28°C, 3 d |
| | | | AN001 | NIBRBAC000510591 | *Streptomyces bottropensis* | 100.00 | Wetland | R2A | 28°C, 3 d |
| | | | SBCC-2337 | NIBRBAC000510525 | *Streptomyces caniferus* | 99.93 | Tidal Flat | R2A | 25–30°C, 7 d |
| | | | MMS23-LS6 | NIBRBAC000510813 | *Streptomyces cerasinus* | 99.13 | Soil | SCA | 30°C, 7 d |
| | | | MMS23-IK53 | NIBRBAC000510833 | *Streptomyces chlorus* | 98.86 | Soil | R2A | 30°C, 3 d |
| | | | AN1078 | NIBRBAC000510620 | *Streptomyces daghestanicus* | 99.72 | Wetland | R2A | 28°C, 3 d |
| | | | AN016 | NIBRBAC000510593 | *Streptomyces endophyticus* | 99.79 | Wetland | R2A | 28°C, 3 d |
| | | | MMS23-CK42 | NIBRBAC000510832 | *Streptomyces erringtonii* | 98.67 | Soil | R2A | 30°C, 3 d |
| | | | MMS23-KR22 | NIBRBAC000510825 | *Streptomyces fractus* | 99.79 | Soil | R2A | 30°C, 3 d |
| | | | MMS23-GM15 | NIBRBAC000510820 | *Streptomyces hirsutus* | 99.86 | Soil | R2A | 30°C, 3 d |
| | | | AN679 | NIBRBAC000510614 | *Streptomyces hyaluromycini* | 99.24 | Wetland | R2A | 28°C, 3 d |
| | | | MMS23-LE10 | NIBRBAC000510814 | *Streptomyces kronopolitis* | 99.55 | Soil | SCA | 30°C, 7 d |
| | | | MMS23-CK28 | NIBRBAC000510831 | *Streptomyces lanatus* | 98.89 | Soil | R2A | 30°C, 3 d |
| | | | CAU 1726 | NIBRBAC000510783 | *Streptomyces naganishii* | 100 | Soil | GYE | 30°C, 3 d |
| | | | AN211 | NIBRBAC000510602 | *Streptomyces racemochromogenes* | 99.79 | Wetland | R2A | 28°C, 3 d |
| | | | SBCC-2338 | NIBRBAC000510526 | *Streptomyces sparsus* | 99.72 | Tidal flat | R2A | 25–30°C, 7 d |
| | | | SBCC-2348 | NIBRBAC000510528 | *Streptomyces sundarbansensis* | 99.79 | Tidal flat | R2A | 25–30°C, 7 d |
| | | | AN074 | NIBRBAC000510599 | *Streptomyces violaceus* | 99.59 | Wetland | R2A | 28°C, 3 d |
| | *Streptosporangiales* | *Streptosporanglaceae* | MMS23-TB5 | NIBRBAC000510818 | *Nonomuraea rhodomycinica* | 99.10 | Soil | SCA | 30°C, 7 d |
| | | | AR0 | NIBRBAC000510799 | *Streptosporangium sandarakinum* | 99.84 | Agricultural soil | R2A | 28°C, 7 d |

## Materials and Methods

A total of 34 bacterial strains assigned to the phylum *Actinomycetota* were isolated from various samples. The collected samples from each region were independently processed serial dilution and spread onto diverse culture media Reasoner's 2A agar (R2A; BD Difco), marine agar (MA; BD Difco), glucose yeast extract agar (GYE) actinomycete isolation agar (AA) and starch-casein agar (SCA) and incubated at 20−30°C for 3−7 days (Table 1). All strains were purified as single colonies and stored as 15−17% (v/v) glycerol suspension at −80°C as well as lyophilized ampoules.

The morphology and cell size of colonies for each strain were determined by using a scanning electron microscopy and transmission electron microscopy. The electron micrographs of each strain are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions. After extracting genomic DNA and amplify the 16S rRNA gene using (universal) primers (Weisburg *et al*., 1991), and the sequence database of each strain was submission in NCBI GenBank. The 16S rRNA gene sequences of the related taxa were obtained from EzBioCloud server (Yoon *et al*., 2017). 34 bacterial strains and related taxa (retrieved from the NCBI database) were aligned with SINA (v1.2.12) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse *et al*., 2012). Evolutionary distances applied during phylogenetic analysis were calculated through a two-parameter model (Kimura, 1983). A phylogenetic tree was constructed using bootstrap values (Felsenstein, 1985) based on 1,000 replicates using neighbor joining (Saitou and Nei, 1987) in the MEGA 11 program (Tamura *et al*., 2021).

## Results and Discussion

The 34 strains were distributed into 1 phylum: *Actinomycetota* (Table 1). All strains filamentous and sporulating bacteria (Figs. 1, 2). Unrecorded bacteria were identified as 10 genera of *Actinacidiphila*, *Amycolatopsis*, *Lentzea*, *Micromonospora*, *Nocardia*, *Nonomuraea*, *Saccharothrix*, *Streptomyces*, *Streptosporangium* and *Umezawaea* (Figs. 3, 4). Here we report 34 unrecorded bacterial species in Korea belonging to 5 orders, which were isolated in Korea; 1 strain of the *Micromonosporales*, 5 strains of the *Mycobacteriales*, 5 strains of the *Pseudonocardiales*, 21 strains of the *Streptomycetales*, and 2 strains of the *Streptosporangiales*.

### Description of *Micromonospora provocatoris* AN1124

Cells are Gram-staining-positive and spherical to oval. Colonies are filamentous, unbonate and orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the

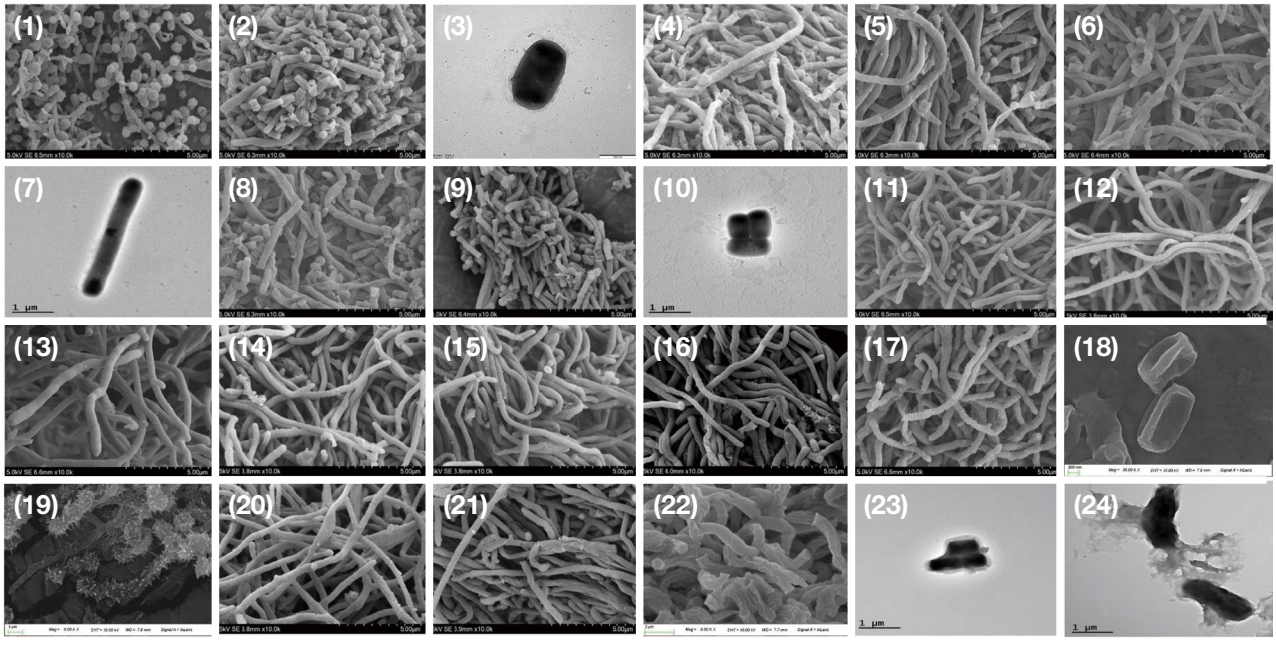



**Fig. 1.** Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, AN1124; 2, AN003; 3, SBCC-2246; 4, AN064; 5, AN043; 6, AN051; 7, MMS23-KA4; 8, AN625; 9, AN040; 10, MMS23-KR2; 11, AN189; 12, AN696; 13, SBCC-2341; 14, AN214; 15, AN286; 16, AN001; 17, SBCC-2337; 18, MMS23-LS6; 19, MMS23-IK53; 20, AN1078; 21, AN016; 22, MMS23-CK42; 23, MMS23-KR22; 24, MMS23-GM15.

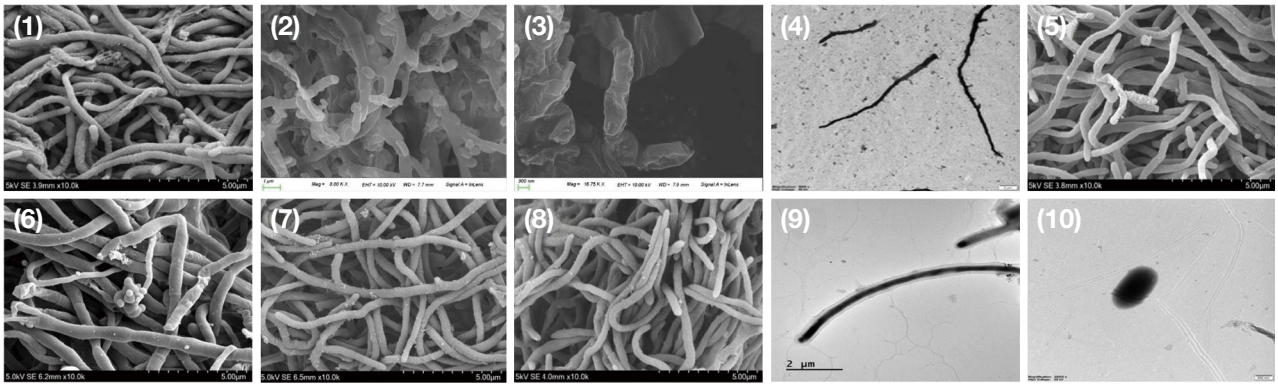



**Fig. 2.** Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, AN679; 2, MMS23-LE10; 3, MMS23-CK28; 4, CAU 1726; 5, AN211; 6, SBCC-2338; 7, SBCC-2348; 8, AN074; 9, MMS23-TB5; 10, AR0.

reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-maltose and adipate are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN1124 (=NIBRBAC000510621) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

### Description of *Nocardia aurea* AN003

Cells are Gram-staining-positive and rod shape. Colonies are filamentous, unbonate and pale-yellow color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, potassium gluconate and adipate are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN003 (=NIBRBAC000510592) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

### Description of *Nocardia higoensis* SBCC-2246

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat, smooth and pale light pink color after 7 days of incubation at 25°C on MA. In API 20NE, assimilation of potassium gluconate and adipate are positive, but the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain SBCC-2246 (=NIBRBAC000510516) was isolated a tidal flat sample, Chungcheong-do, Korea (36°53′17″N 126°19′39″E).

### Description of *Nocardia salmonicida* subsp. *cummidelens* AN064

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, assimilation of D-glucose and *N*-acetyl-D-glucosamine are positive and $\beta$-glucosidase (esculin hydrolysis), potassium gluconate and adipate are weakly positive but protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-mannose, D-maltose, D-mannitol, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN064 (=NIBRBAC000510597) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

### Description of *Nocardia salmonicida* subsp. *salmonicida* AN043

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, $\beta$-glucosidase (esculin hydrolysis), assimilation of D-glucose, D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate, adipate and trisodium citrate are positive and $\beta$-galactosidase

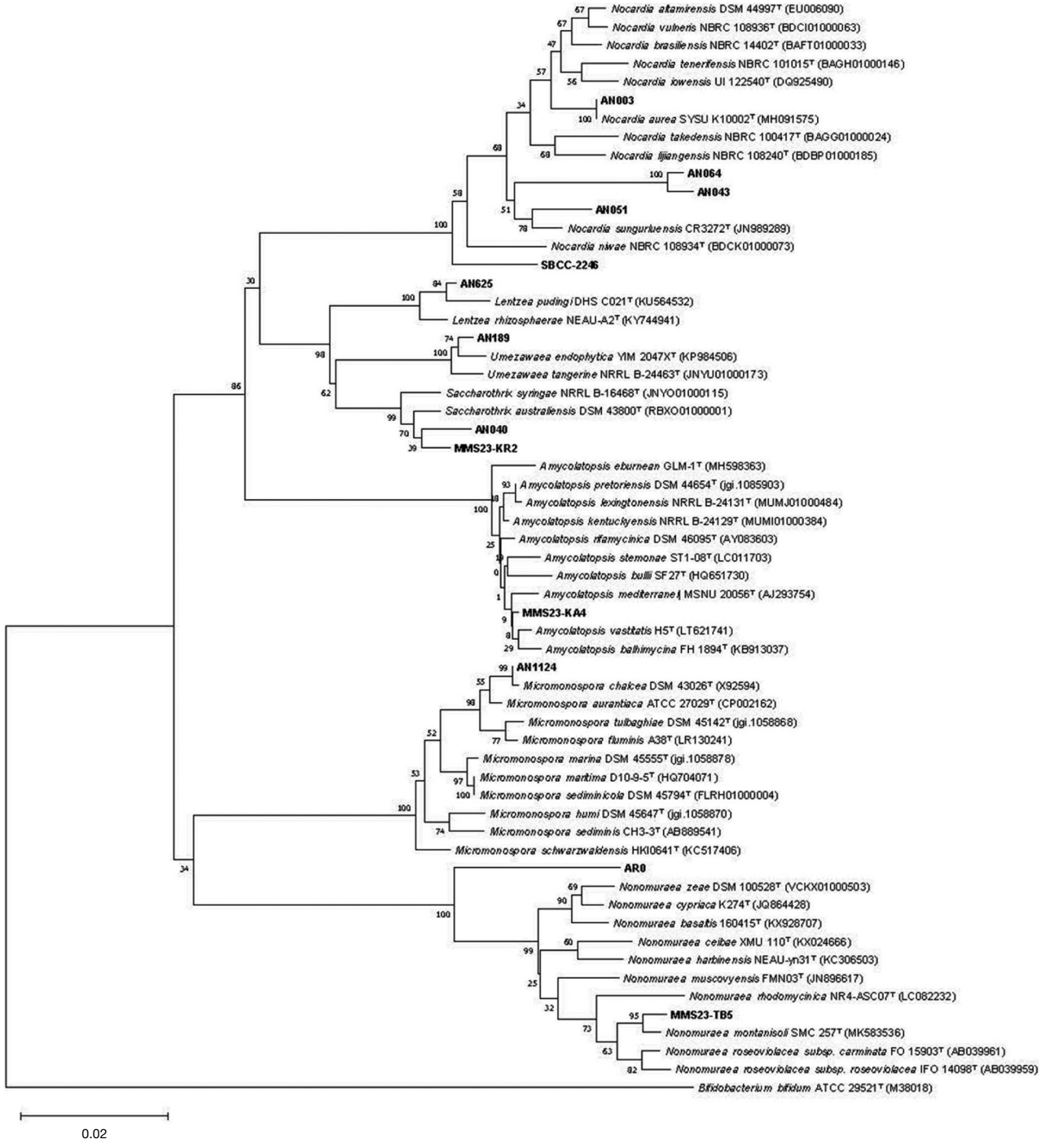



**Fig. 3.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family *Micromonosporaceae*, *Nocardiaceae*, *Pseudonocardiaceae* and *Streptosporanglaceae*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. *Bifidobacterium bifidum* ATCC 29521$^T$ used as an outgroup. Bar: 0.01 substitutions per nucleotide position.

(PNPG) are weakly positive but protease (gelatin hydrolysis), indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-maltose, D-mannitol, capric acid, malic acid and phenylacetic acid are negative. Strain AN043 (=NIBRBAC000510595) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′ 02.6″N 127°15′48.9″E).

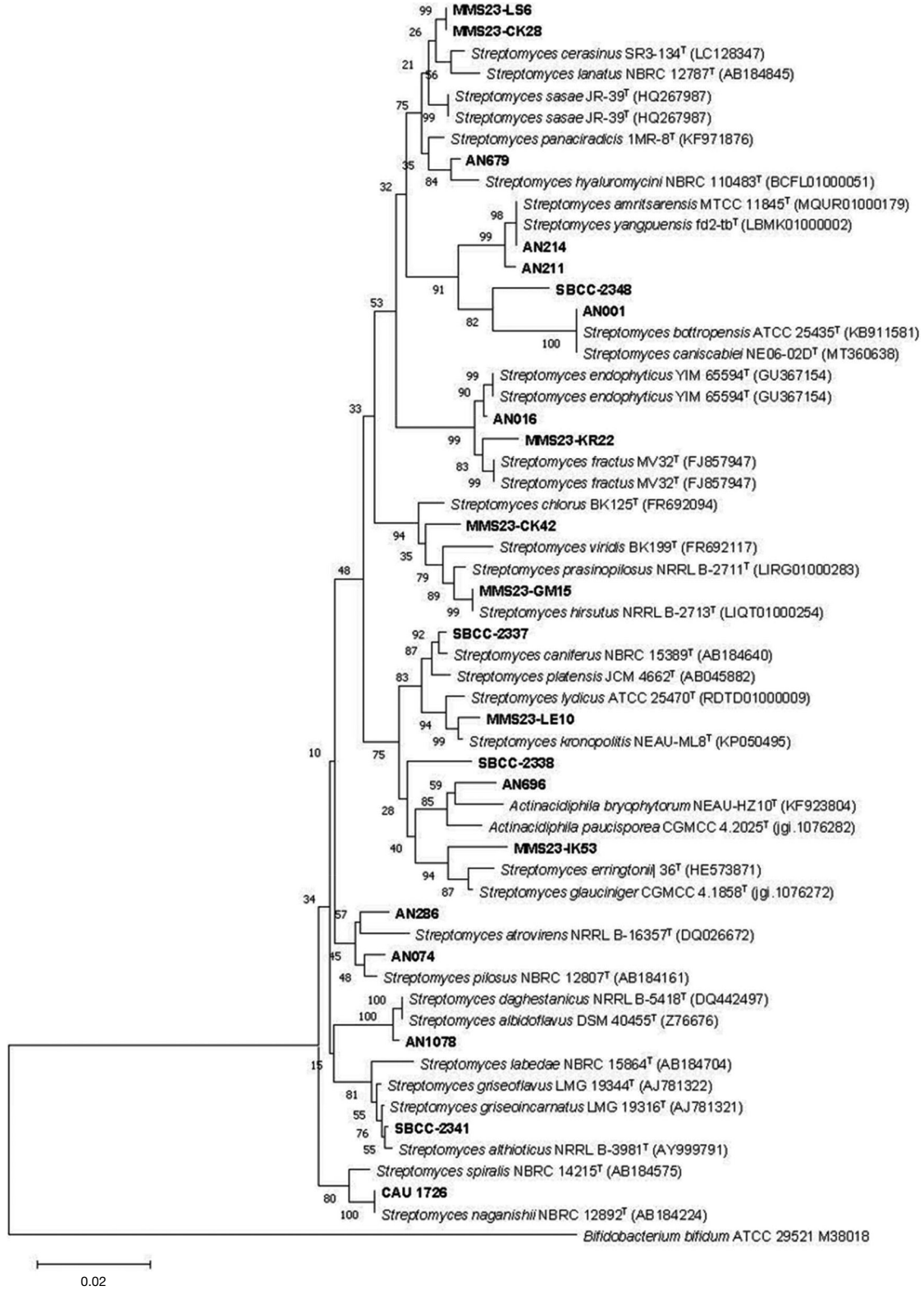



**Fig. 4.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family *Streptomycetaceae*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. *Bifidobacterium bifidum* ATCC 29521$^T$ used as an outgroup. Bar: 0.1 substitutions per nucleotide position.

**Description of *Nocardia sungurluensis* AN051**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of L-arabinose, potassium gluconate, adipate and malic acid are positive and assimilation of D-glucose are weakly positive. But protease (gelatin hydrolysis), indole production, glucose fermentation, arginine dihydrolase, assimilation of D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN051 (=NIBRBAC000510595) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Amycolatopsis vastitatis* MMS23-KA4**

Cells are Gram-staining-positive and rod shape. Colonies are circular, erose, irregular pale orange color after 3 days of incubation at 30°C on AA. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of potassium gluconate are positive. But urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KA4 (=NIBRBAC000510822) was isolated a soil sample, Gyeongsang-do, Korea (35°50′17″N 129°17′11″E).

**Description of *Amycolatopsis vastitatis* MMS23-KA4**

Cells are Gram-staining-positive and rod shape. Colonies are circular, erose, irregular pale orange color after 3 days of incubation at 30°C on AA. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of potassium gluconate are positive. But urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KA4 (=NIBRBAC000510822) was isolated a soil sample, Gyeongsang-do, Korea (35°50′17″N 129°17′11″E).

**Description of *Lentzea pudingi* AN625**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, potassium gluconate adipate and malic acid are positive and $\beta$-glucosidase (esculin hydrolysis) is weakly positive. But the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate, phenylacetic acid are negative. Strain AN625 (=NIBRBAC000510611) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Saccharothrix espanaensis* AN040**

Cells are Gram-staining-positive and rod shape. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in $\beta$-galactosidase (PNPG), assimilation of D-glucose, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid are positive and $\beta$-glucosidase (esculin hydrolysis) is weakly positive. But the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate, L-arabinose, D-mannose, D-maltose, D-mannitol, adipate, phenylacetic acid are negative. Strain AN040 (=NIBRBAC000510594) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Saccharothrix syringae* MMS23-KR2**

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire white color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in $\beta$-glucosidase (esculin hydrolysis) and protease (gelatin hydrolysis) are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, potassium gluconate, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KR2 (=NIBRBAC000510823) was isolated a soil sample, Gyeongsang-do, Korea (35°50′17″N 129°17′11″E).

**Description of *Umezawaea tangerine* AN189**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, and malic acid are positive. But the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of potassium glu-

conate, capric acid, phenylacetic acid are negative. Strain AN189 (=NIBRBAC000510601) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′ 48.9″E).

**Description of *Actinacidiphila paucisporea* AN696**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate brown color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of potassium gluconate, capric acid, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, and malic acid are negative. Strain AN696 (=NIBRBAC000510615) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127° 15′48.9″E).

**Description of *Streptomyces althioticus* SBCC-2341**

Cells are Gram-staining-positive and filamentous. Colonies are Circular to irregular, flat, forming aerial and substrate mycelia white to gray color after 7 days of incubation at 25−30°C on R2A. In API 20NE reactions in $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG) (weak), assimilation of D-glucose, L-arabinose (weak), D-mannose (weak), D-mannitol, potassium gluconate and malic acid are positive. But the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-maltose, capric acid, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, phenylacetic acid are negative. Strain SBCC-2341 (=NIBRBAC000510527) was isolated a wetland sample, Jeolla-do, Korea (35°40′45″N 126°31′47″E).

**Description of *Streptomyces amritsarensis* AN214**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale purple to white color after 3 days of incubation at 28°C on R2A. In API 20NE assimilation of D-glucose, D-mannose (weak), *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate, malic acid and trisodium citrate are positive. But reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of capric acid, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, and adipate are negative. Strain AN214 (=NIBRBAC 000510603) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Streptomyces atrovirens* AN286**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate and malic acid are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN286 (=NIBRBAC000510604) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′ 02.6″N 127°15′48.9″E).

**Description of *Streptomyces bottropensis* AN001**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate (weak) and malic acid are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN001 (=NIBRBAC000510 591) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Streptomyces caniferus* SBCC-2337**

Cells are Gram-staining-positive and filamentous. Colonies are Circular to irregular, umbonate, forming aerial and substrate mycelia white to dark gray color after 7 days of incubation at 25−30°C on R2A. In API 20NE reactions in $\beta$-galactosidase (PNPG), assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are positive. But the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, capric acid, adipate, phenylacetic acid are negative. Strain SBCC-2337 (=NIBRBAC000510525) was isolated a tidal flat sample, Jeolla-do, Korea (35°40′45″N 126°31′ 47″E).

**Description of *Streptomyces cerasinus* MMS23-LS6**

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex brown color after

7 days of incubation at 30°C on SCA. In API 20NE, the reactions in $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-LS6 (=NIBRBAC000510813) was isolated a soil sample, Gyeongsang-do, Korea (36°19′41″N 127°21′44″E).

**Description of *Streptomyces cerasinus* MMS23-IK53**

Cells are Gram-staining-positive and filamentous. Colonies are irregular, Convex green color after 7 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate are positive. But $\beta$-glucosidase (esculin hydrolysis), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of malic acid capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-IK53 (=NIBRBAC000510833) was isolated a soil sample, Chungcheong-do, Korea (36°03′25″N 127°35′59″E).

**Description of *Streptomyces daghestanicus* AN1078**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate ivory color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in urease, $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid and trisodium citrate (weak) are positive. But the reaction in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), reduction of indole production, glucose fermentation, arginine dihydrolase, assimilation of capric acid, and phenylacetic acid are negative. Strain AN1078 (=NIBRBAC000510620) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Streptomyces endophyticus* AN016**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate, adipate (weak) and malic acid are positive. But the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, assimilation of capric acid, and trisodium citrate and phenylacetic acid are negative. Strain AN016 (=NIBRBAC000510593) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Streptomyces cerasinus* MMS23-CK42**

Cells are Gram-staining-positive and filamentous. Colonies are irregular, convex white to gray color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and assimilation of D-glucose are positive. But $\beta$-galactosidase (PNPG), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate malic acid capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-CK42 (=NIBRBAC000510832) was isolated a soil sample, Chungcheong-do, Korea (36°03′25″N 127°35′59″E).

**Description of *Streptomyces fractus* MMS23-KR22**

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire ivory color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), arginine dihydrolase, urease, $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-mannose, *N*-acetyl-D-glucosamine and potassium gluconate are positive. But indole production, glucose fermentation, assimilation of D-glucose, L-arabinose, D-mannitol, D-maltose, malic acid, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-KR22 (=NIBRBAC000510825) was isolated a soil sample, Gyeongsang-do, Korea (35°50′17″N 129°17′11″E).

**Description of *Streptomyces hirsutus* MMS23-GM15**

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire light brown color after 3 days of incubation at 30°C on MA. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and $\beta$-galactosidase (PNPG), are positive. But arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate D-glucose, L-arabinose, D-mannitol, D-maltose, malic acid, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-GM15 (=NIBRBAC000510820) was isolated a soil sample, Jeolla-do, Korea (35°54′31″N 127°46′48″E).

**Description of *Streptomyces hyaluromycini* AN679**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale brown color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid and trisodium citrate are positive. But the reaction in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid and phenylacetic acid are negative. Strain AN679 (=NIBRBAC000510614) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127° 15′48.9″E).

**Description of *Streptomyces kronopolitis* MMS23-LE10**

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex brown color after 7 days of incubation at 30°C on SCA. In API 20NE, the reactions in arginine dihydrolase, urease, $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-galactosidase (PNPG), indole production, glucose fermentation, assimilation of L-arabinose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-LE10 (=NIBRBAC000510814) was isolated a soil sample, Gyeongsang-do, Korea (36°19′41″N 127°21′44″E).

**Description of *Streptomyces lanatus* MMS23-CK28**

Cells are Gram-staining-positive and filamentous. Colonies are circular, umbonate brown color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-maltose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-CK28 (=NIBRBAC000510831) was isolated a soil sample, Chungcheong-do, Korea (36°03′25″N 127°35′59″E).

**Description of *Streptomyces naganishii* CAU 1726**

Cells are Gram-staining-positive and filamentous. Colonies are circular, entire, pulvinate, opaques cream color after 3 days of incubation at 30°C on GYE agar. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), arginine dihydrolase, protease (gelatin hydrolysis), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid, trisodium citrate and phenylacetic acid are positive. But urease, $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), indole production, glucose fermentation amd assimilation of capric acid are negative. Strain CAU 1726 (=NIBRBAC000510783) was isolated a soil sample, Gyeongsang-do, Korea (35°10′03.2″N 129° 05′42.7″E).

**Description of *Streptomyces racemochromogenes* AN211**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate orange to white color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), assimilation of D-glucose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate (weak), malic acid and trisodium citrate are positive. But the reaction in $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of L-arabinose, D-mannitol, capric acid, adipate and phenylacetic acid are negative. Strain AN211 (=NIBRBAC 000510602) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Streptomyces sparsus* SBCC-2338**

Cells are Gram-staining-positive and filamentous. Colonies are circular to slightly irregular, flat, forming substrate mycelium dark greyish yellow color after 7 days of incubation at 25−30°C on R2A. In API 20NE, the reactions in $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG), assimilation of D-glucose, L-arabinose (weak), D-mannose (weak), D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate (weak), malic acid and trisodium citrate are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), protease (gelatin hydrolysis), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of capric acid and phenylacetic acid are negative. Strain SBCC-2338 (=NIBRBAC000510526) was isolated a tidal flat sample, Jeolla-do, Korea (35°40′45″N 126° 31′47″E).

**Description of *Streptomyces sundarbansensis* SBCC-2348**

Cells are Gram-staining-positive and filamentous. Colonies are circular to irregular, umbonate, forming aerial and substrate mycelia yellowish whitecolor after 7 days of incubation at 25−30°C on R2A. In API 20NE, the reactions in urease (weak), $\beta$-glucosidase (esculin hydrolysis), $\beta$-galactosidase (PNPG) (weak), assimilation of D-glu-

cose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate, adipate (weak), malic acid and trisodium citrate are positive. But nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), protease (gelatin hydrolysis), arginine dihydrolase, indole production, glucose fermentation, assimilation of L-arabinose, capric acid and phenylacetic acid are negative. Strain SBCC-2348 (=NIBRBAC000 510528) was isolated a tidal flat sample, Chungcheong-do, Korea (36°35′51″N 126°22′38″E).

**Description of *Streptomyces violaceus* AN074**

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate yellow color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in $\beta$-glucosidase (esculin hydrolysis) (weak), $\beta$-galactosidase (PNPG), assimilation of D-glucose, *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate (weak), adipate, malic acid, trisodium citrate and phenylacetic acid are positive. But the reaction in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of D-mannose, L-arabinose, D-mannitol and capric acid are negative. Strain AN074 (=NIBRBAC000510 599) was isolated a wetland sample, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

**Description of *Nonomuraea rhodomycinica* MMS23-TB5**

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex pink color after 7 days of incubation at 30°C on SCA. In API 20NE, the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis) and $\beta$-galactosidase (PNPG) are positive. But protease (gelatin hydrolysis), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid, D-maltose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-TB5 (=NIBRBAC000510818) was isolated a soil sample, Chungcheong-do, Korea (36°17′32″N 127°21′00″E).

**Description of *Streptosporangium sandarakinum* AR0**

Cells are Gram-staining-positive and rod shape. Colonies are circular, entire, convex, rough red color after 7 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($NO^{3-} \rightarrow NO^{2-}$), $\beta$-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and $\beta$-galactosidase (PNPG), are positive. But the reaction in urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-mannose, L-arabinose, D-mannitol D-glucose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid, trisodium citrate, phenylacetic acid and capric acid are negative. Strain AR0 (=NIBR BAC000510799) was isolated a agricultural soil sample, Gyeonggi-do, Korea (37°44′05″N 126°42′67″E).

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# First record of the family Eupronoidae (Crustacea, Amphipoda, Hyperiidea) from the Korea Strait, with description of a newly recorded species, *Parapronoe parva*

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This study aims to discover and document amphipods from Korea, contributing to the understanding of Korean amphipod taxonomy. During a pelagic zooplankton survey conducted in the Korea Strait, a species belonging to the eupronoid amphipod genus *Parapronoe* Claus, 1879 was collected and identified as *Parapronoe parva* Claus, 1879. The newly recorded species, *Parapronoe parva* Claus, 1879, is characterized by the morphology of the carpal process on gnathopod 2, the rami of uropod 3, and the telson. *Parapronoe parva* is morphologically similar to *P. campbelli* Stebbing, 1888, *P. elongata* Semenova, 1981, and *P. crustulum* Claus, 1879, but can be distinguished by differences in the structure of the epimeral plate 1, carpus of gnathopod 2, basis of pereopod 6, both rami of uropod 3, and telson. This study represents the first record of both the genus *Parapronoe* and the family Eupronoidae from Korean waters. Additionally, identification keys to the genera of the family Eupronoidae, and to the species of the genus *Parapronoe* are provided.

Keywords: Crustacea, hyperiid, plankton, Platysceloidea, taxonomy

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## Introduction

The superfamily Platysceloidea Bate, 1863 is one of the most taxonomically challenging groups within the suborder Hyperiidea (Shih and Chen, 1995). Members of Platysceloidea are characterized by extremely reduced mouthparts and a distinctive position of antennae 1 and 2. To date, the superfamily comprises 12 families worldwide, Amphithyridae Zeidler, 2016, Anapronoidae Bowman & Gruner, 1973, Brachyscelidae Stephensen, 1923, Eupronoidae Zeidler, 2016, Lycaeidae Claus, 1879, Lycaeopsidae Chevreux, 1913, Oxycephalidae Bate, 1863, Parascelidae Bovallius, 1887, Platyscelidae Bate, 1863, Pronoidae Dana, 1852, Thamneidae Zeidler, 2016, and Tryphanidae Boeck, 1871 (Horton *et al*., 2025). However, a comprehensive systematic revision of these families is still required to clarify the phylogenetic relationships among them (Zeidler, 2016).

Among these families, Eupronoidae Zeidler, 2016 can be distinguished from other families of superfamily Platysceloidea by its subchelate gnathopod 2 and articulated rami of all uropods (Zeidler, 2016). This family was proposed by Zeidler (2016) and comprises two genera, *Eupronoe* Claus, 1879 and *Parapronoe* Claus, 1879, previously placed in the family Pronoidae Claus, 1879 along with *Paralycaea* Claus, 1879. These two genera are clearly distinguished from other related genera by the morphological characteristics of the gnathopods and uropods. Based on these diagnostic features, Eupronoidae was established as an independent family. The genus *Pronoe* remains as a

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monotypic genus within Pronoidae, whereas *Paralycaea* was transferred to the newly established family Amphithyridae Zeidler, 2016, due to the morphological similarity of pereopods 5 and 6 to those of *Amphithyrus* Claus, 1879.

Currently, nine species belonging to two genera are recorded worldwide within the family Eupronoidae (Horton *et al*., 2025). Among these, the genus *Parapronoe* comprises four recognized species, *P. campbelli* Stebbing, 1888, *P. crustulum* Claus, 1879, *P. elongata* Semenova, 1981, and *P. parva* Claus, 1879, whereas the genus *Eupronoe* comprises five valid species, *E. armata* Claus, 1879, *E. intermedia* Stebbing, 1888, *E. laticarpa* Stephensen, 1925, *E. maculata* Claus, 1879, and *E. minuta* Claus, 1879. The genus *Parapronoe* was established by Claus (1879), with *P. crustulum* designated as the type species. This genus is characterized by the sub-terminally inserted articles on the basis of pereopod 5 and lanceolate rami of uropod 2.

To date, neither the genus *Parapronoe* nor the family Eupronoidae has previously been recorded from Korean waters. However, six species belonging to the two genera have been reported from Japanese waters adjacent to Korea, including three species of *Eupronoe* (*E. armata*, *E. maculata*, and *E. minuta*) and three species of *Parapronoe* (*P. campbelli*, *P. crustulum*, and *P. parva*) (Mori *et al*., 2010). In Chinese waters, nine species belonging to the two genera have been reported, including five species of *Eupronoe* (*E. armata*, *E. intermedia*, *E. laticarpa*, *E. maculata*, and *E. minuta*) and four species of *Parapronoe* (*P. campbelli*, *P. crustulum*, *P. elongata*, and *P. parva*) (Shih and Chen, 1995; Lowry, 2001). Although the genus *Parapronoe* had not previously been recorded in South Korea, *Parapronoe parva* Claus, 1879 was collected during a pelagic zooplankton survey conducted in the Korea Strait (Fig. 1). This study therefore represents the first record of both the genus *Parapronoe* and the family Eupronoidae from Korean waters, adding a newly recorded species to the Korean hyperiid amphipod fauna. In addition, keys to the genera of the family Eupronoidae and the species of the genus *Parapronoe* are provided.

## Materials and Methods

Specimens were collected with a MOCNESS net from subtidal waters of the South Sea, Korea (Fig. 1). The specimens were fixed in 95% ethanol and dissected in glycerol on Cobb's aluminum hole slides. The materials were examined under a stereomicroscope (Olympus SZX 10) and a compound microscopes (Olympus BX 51), and drawings and measurements were made with the aid of a drawing tube. Line drawings were produced using the program Clip Studio Paint (Celsys, Japan). Body length was measured from the end of the rostrum to the end of the urosome, along the dorsal parabolic line of the body. The examined specimens are deposited at the Marine Biodiversity institute of Korea (MABIK) in Seocheon, Korea and the Department of Biological Science, Dankook University (DKU), Cheonan, Korea.

## Systematic Accounts

Order Amphipoda Latreille, 1816
Suborder Hyperiidea Edwards, 1830
Superfamily Platysceloidea Bate, 1863
Family Eupronoidae Zeidler, 2016
표범옆새우과 (신칭)
Genus *Parapronoe* Claus, 1879 측표범옆새우속 (신칭)

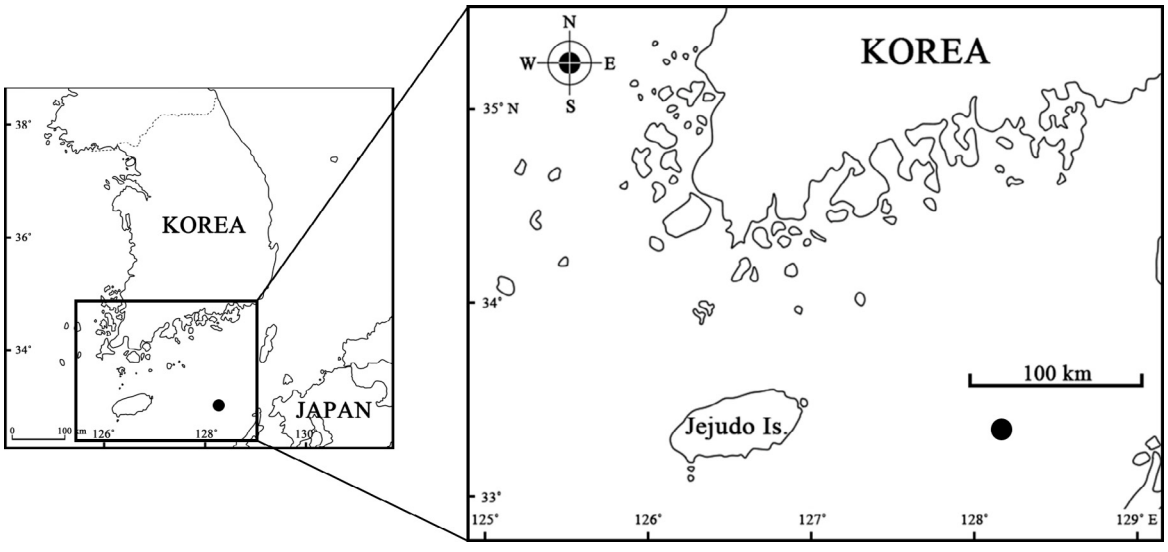



**Fig. 1.** Distribution of the *Parapronoe parva* Claus, 1879 (●: Korea Strait).

***Parapronoe parva* Claus, 1879 (Figs. 2, 3)**
꼬마표범옆새우 (신칭)
*Parapronoe parva* Claus, 1879: 177.
*Sympronoe parva* Stebbing, 1888: 1533, pl. 192; Zeidler, 1978: 24.
*Sympronoe propinqua* Stebbing, 1888: 1537, pl. 193.

**Material examined.** 1♀, dissected (appendages on one slide), MABIK CR00259793, 1♀, DKUAMP202516, Korea Strait, 33°22′06″N, 128°16′42″E, 16 May 2019, by MOCNESS net, collected by SH Choo.

**Description. Adult female** (MABIK CR00259793), body (Figs. 2, 3A) 7.7 mm long, dorsally smooth, covered with pigment fleck. Head subovate, slightly longer than deep, slightly longer than pereonites 1–3 combined; eyes well developed, covering most of head. Pereonites 1–7 (Fig. 3A), posterolateral margin produced. Urosomite 2 (Fig. 3L) fused with urosomite 3, length less than twice width, 1.29× longer than wide. Antenna 1 (Fig. 3B), peduncular article 2 subrectangular, anterior margin slightly concave, flagellum 3-articulate, flagellum articles 1–2 with 5 and 3 anterodistal setae each, terminal article with 2 setae.

Gnathopod 1 (Fig. 3C) simple; coxa subrectangular, longer than width; basis longish ovate, relatively long, widening distally, 1.13× longer than wide anterior margin with serration; ischium trapezoidal, 0.25× basis, posterior margin slightly convex; merus trapezoidal, expanded, 1.14× ischium; carpus longish ovate, 1.56× merus, anterodistal and posterodistal corner slightly produced; propodus subrectangular, relatively slender, 0.72× carpus, anterior margin slightly convex, posterior margin with 5 setae; dactylus falcate, short, 0.35× propodus.

Gnathopod 2 (Fig. 3D) subchelate; coxa subquadrate; basis subretangular, relatively long, 2.07× longer than wide, anterodistal corner slightly produced, posterior margin curved; ischium trapezoidal, short, 0.23× basis, posterior margin slightly convex; merus trapezoidal, widening distally, 1.64× ischium; carpus characteristic in form, subquadrate, 0.95× merus, anterodistal corner with serration, two-thirds of posterior margin with serration, posterodistal corner produced, carpal process rounded; propodus subrectangular, relatively slender, 0.85× carpus, anterior margin slightly convex, posterior margin with serration; dactylus falcate, short, 0.27× propodus.

Pereopod 3 (Fig. 3E), coxa subquadrate; basis longish ovate, widening distally, relatively long, 2.01× longer than wide; ischium trapezoidal, short, 0.22× basis; merus longish ovate, widening distally, 2.34× ischium; carpus subrectangular, 1.01× merus; propodus relatively slender, narrowing distally, 0.81× carpus; dactylus narrow, falcate, 0.34× propodus.

Pereopod 4 (Fig. 3F) similar to pereopod 3, but elongated.

Pereopod 5 (Fig. 3G), basis elongate, expanded, 1.59× longer than wide, midanterior margin convex, ischium inserted sub-terminally; ischium trapezoidal, relatively short, 0.13× basis, posterior margin concave; merus subrectangular, 2.62× ischium, anterodistal margin with serration, posterodistal corner slightly produced; carpus longish ovate, 0.98× merus, half of anterior margin with serration; propodus relatively slender, 0.97× carpus; dactylus narrow, falcate, 0.26× propodus.




**Fig. 2.** *Parapronoe parva* Claus, 1879, adult female, 7.7 mm, habitus. Scale bar = 0.5 mm.

Pereopod 6 (Fig. 3H), coxa trapezoidal; basis elongate-ovate, expanded, 1.81× longer than wide, narrowing distally, anterodistal corner produced, process concave medially; ischium trapezoidal, short, 0.07× basis, posterior margin concave; merus longish ovate, 4.56× ischium, anterior margin with serration, anterodistal corner produced, covering half of carpus; carpus subrectangular, 0.56× merus, anterior margin with serration; propodus slender, 0.57× carpus, anterior margin with serration; dactylus narrow, falcate, 0.23× propodus.

Pereopod 7 (Fig. 3I), coxa subrectangular; basis elongate-ovate, expanded, 3.02× longer than wide, narrowing distally; ischium ovate, short, 0.08× basis; merus longish ovate, 2.41× ischium; carpus subrectangular, slender, 1.19× merus; propodus subrectangular, 0.36× carpus; dactylus narrow, falcate, 0.48× propodus.

Uropod 1 (Fig. 3J), peduncle subrectangular, widening distally, laterodistal margin with serration; outer ramus lanceolate, 0.99× peduncle, lateral and two-thirds of medial margin with serration; inner ramus lanceolate, slightly broader than outer ramus, 0.99× outer ramus, two-thirds of lateral and medial margins with serration.

Uropod 2 (Fig. 3K) shorter than uropod 1; peduncle subrectangular, widening distally, laterodistal margin with serration; outer ramus lanceolate, 1.17× peduncle, lateral and two-thirds of medial margin with serration, terminal corner slightly rounded; inner ramus lanceolate, slightly

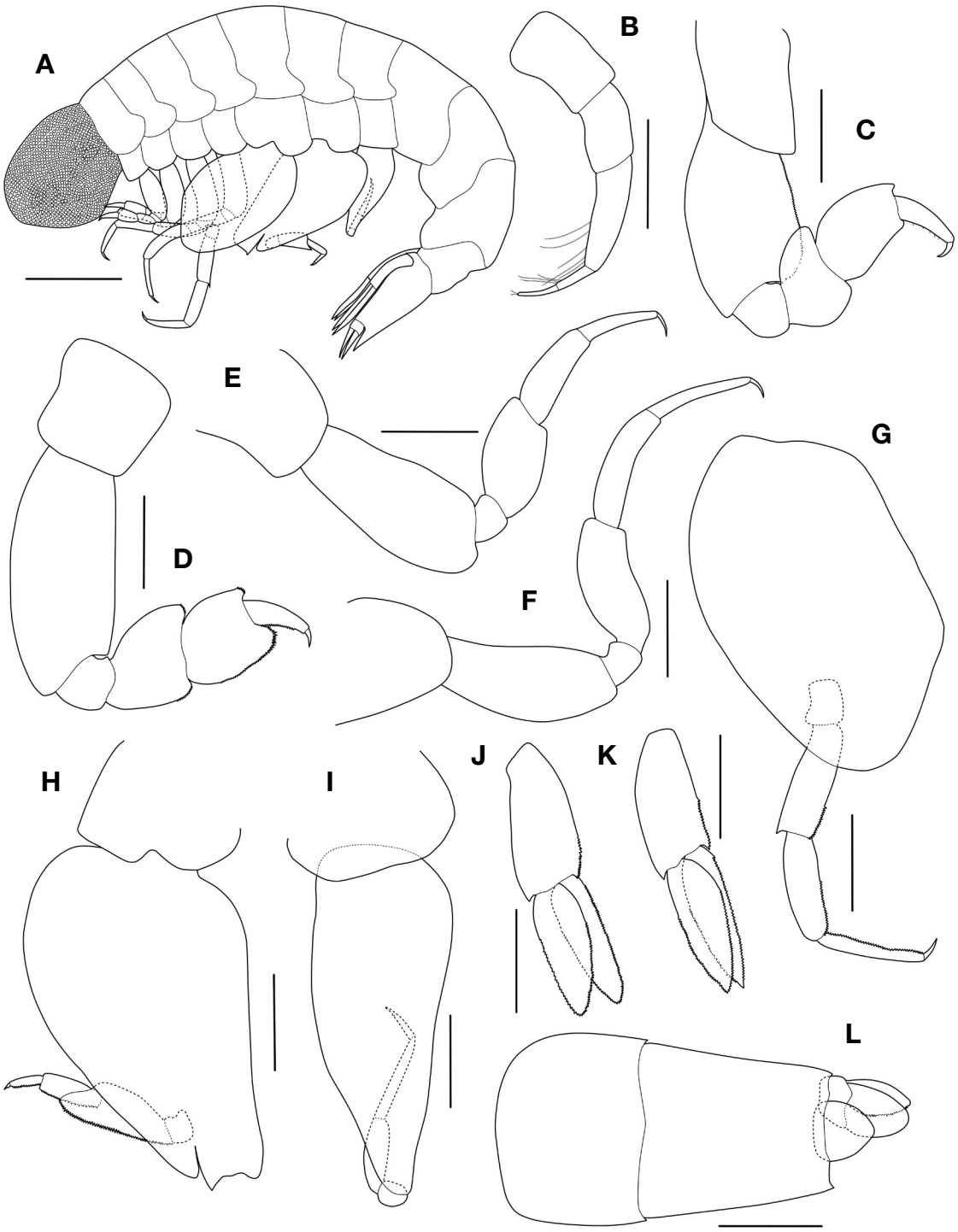



**Fig. 3.** *Parapronoe parva* Claus, 1879, adult female, 7.7 mm. A, habitus, lateral; B, antenna 1; C, gnathopod 1; D, gnathopod 2; E, pereopod 3; F, pereopod 4; G, pereopod 5; H, pereopod 6; I, pereopod 7; J, uropod 1; K, uropod 2; L, urosomites, uropod 3, telson. Scale bars: A = 1.0 mm; J–L = 0.5 mm; E–H = 0.4 mm; C, D, I = 0.3 mm; B = 0.2 mm.

broader than outer ramus, 0.97× outer ramus, two-thirds of lateral and medial margins with serration, terminal corner slightly rounded.

Uropod 3 (Fig. 3L) characteristic in form, shorter than uropod 2; peduncle trapezoidal; outer ramus relatively slender, 2.16× peduncle, medial margin with serrulation, terminal margin rounded; inner ramus expanded, broader than outer ramus, 1.06× outer ramus, terminal margin

rounded.

Telson (Fig. 3L) characteristic in form, articulated with urosomites 2–3 combined, 0.59× uropod 3, entire, short, subtriangular, unarmed.

**Distribution.** Cosmopolitan (Korea, China, Japan, Mediterranean Sea, Atlantic, Pacific, and Indian Oceans).

**Remarks.** *Parapronoe parva* Claus, 1879 is similar to *P. campbelli* Stebbing, 1888, *P. elongata* Semenova, 1981, and *P. crustulum* Claus, 1879 in that all four species share the following features: the ischium of pereopod 5 inserted subterminally on the basis and lanceolate rami of uropod 2. However, *P. parva* can be distinguished from its congeners by the following features (different characteristics of *P. campbelli*, *P. elongata*, and *P. crustulum* in brackets): 1) gnathopod 2, carpus with rounded process (vs. pointed in *P. campbelli*, *P. elongata*, and *P. crustulum*); 2) uropod 3, rami with rounded terminal margins (vs. pointed in *P. campbelli*, *P. elongata*, and *P. crustulum*); 3) urosomites 2–3 combined shorter than twice their width (vs. longer in *P. elongata*); and 4) telson about half the length of uropod 3 (vs. subequal in *P. campbelli* and *P. crustulum*, and shorter in *P. elongata*). Although only a limited number of specimens were available, the presence of rounded carpal process on gnathopod 2 and rounded terminal margin on uropod 3 indicates that our specimens agree well with the original description of *P. parva* provided by Claus (1879).

**Key to genera of the family Eupronoidae**

– Pereopod 5, ischium inserted terminally on basis ..... *Eupronoe* Claus, 1879

– Pereopod 5, ischium inserted subterminally on basis ..... *Parapronoe* Claus, 1879

**Key to species of the genus *Parapronoe***

1. Telson subequal in length to uropod 3 ..... 2

– Telson about half as long as, or shorter than uropod 3 ... 4

2. Pereopod 6, basis with distinct locking mechanism ..... *P. crustulum* Claus, 1879

– Pereopod 6; basis without distinct locking mechanism ... *P. campbelli* Stebbing, 1888

3. Gnathopod 2 with rounded carpal process; uropod 3, both rami with rounded terminal margins ..... *P. parva* Claus, 1879

– Gnathopod 2 with pointed carpal process; uropod 3, both rami with pointed terminal margins ..... *P. elongata* Semenova, 1981

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# A report on 22 unrecorded freshwater bacterial species isolated from the Seomjin River, Republic of Korea

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The Seomjin River in South Korea has been recognized for its rich biodiversity, which is attributed to its excellent water quality. Given its potential to harbor diverse oligotrophic bacterial species, a biodiversity monitoring program has been conducted since 2020. This study catalogs 22 previously unreported bacterial species from the Seomjin River basin in South Korea, identified based on 16S rRNA gene sequencing, and further characterized by their Gram staining properties, colony and cellular morphology, biochemical traits, and phylogenetic affiliation. The 22 bacterial species exhibited 16S rRNA gene sequence similarities of at least 98.7% and were classified into 4 phyla, 6 classes, 10 orders, 17 families, and 19 genera. The identified genera were classified into the following classes: *Methylorubrum*, *Aureimonas*, *Bosea*, *Tsuneonella*, *Erythrobacter*, *Sphingomonas*, *Parablastomonas*, and *Asticcacaulis* (*Alphaproteobacteria*); *Rivibacter*, *Sheuella*, and *Duganella* (*Betaproteobacteria*); *Pseudomonas* and *Dickeya* (*Gammaproteobacteria*); *Mycobacterium*, *Nocardia*, and *Brachybacterium* (*Actinomycetes*); *Paenibacillus* and *Neobacillus* (*Bacilli*); and *Chitinophaga* (*Chitinophagia*). This study expands the understanding of bacterial diversity in the Seomjin River ecosystem and contributes to national species conservation and sustainable utilization efforts.

Keywords: freshwater, Seomjin River, unreported bacterial species

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## Introduction

The Seomjin River, situated in the southwestern region of South Korea, serves as an important geographical, ecological, cultural, and historical relevance. Originating from the Demi Spring at the foot of the Palgong Mountain and extending to the Korean Strait, this 223 km long river with a 4,911 km$^2$ basin area encompasses diverse landscapes and a rich biodiversity, potentially housing numerous oligotrophic bacterial species. Celebrated for its immaculate water quality, the river may play a central role in both environmental conservation and regional sustainability (Savio *et al*., 2015). The complexity of freshwater ecosystems could present diverse research opportunities, reflecting broader ecological dynamics and human-environment interactions (Bae *et al*., 2023). Considering the status of the Seomjin River as a habitat with great biodiversity, there is a need to expand biodiversity monitoring of this area and to study the assemblages of aquatic microorganisms connected to the food web and biogeochemical cycles for the functional diversity of freshwater organisms (i.e., plants and animals) (Falkowski *et al*., 2008, Battin *et al*., 2016).

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This study aimed to enhance understanding of bacterial diversity in lotic ecosystems, resulting in the discovery of 22 unrecorded bacterial species from the Seomjin River basin that had not been previously identified in South Korea. The discovered bacterial species belonged to the phyla *Pseudomonadota*, *Actinomycetota*, *Bacillota*, and *Bacteroidota*, respectively. By reporting the unrecorded bacterial species inhabiting the Seomjin River basin, it is expected to contribute to national species conservation efforts and support their sustainable utilization.

## Materials and Methods

The isolation and cultivation of 22 freshwater bacterial strains from the Seomjin River were conducted with slight modifications based on a previously reported method (Park *et al*., 2024). To facilitate the isolation of bacterial strains with diverse physiological characteristics, the standard dilution plating method was applied on multiple culture media, including Reasoner's 2A (R2A), International Streptomyces Project medium 4 (ISP4), tryptic soy agar (TSA), marine agar 2211 (MA), and nutrient agar (NA). The plates were incubated at 20 and 25°C for 7 days, which correspond to the water temperatures recorded at the time of sample collection, facilitating the cultivation of bacterial strains adapted to their native conditions. Following isolation, each isolate was purified as a single colony via serial dilution spreading, and the pure cultures were preserved in 20% glycerol suspensions at −80°C.

Genomic DNA was extracted, and the 16S rRNA gene was amplified using the universal primers 27F and 1492R. The resulting sequences were analyzed by comparison with reference bacterial strains available in the EzBioCloud database (Chalita *et al*., 2024), following standard molecular protocols. A similarity threshold of 98.7% was applied, and bacterial strains showing ≥98.7% sequence similarity to known species yet to be recorded in Korea were classified as unreported species. For phylogenetic analysis, 16S rRNA gene sequences from the isolates were aligned with those of type strains using Clustal_W, and the alignments were manually refined with EzEditor (Jeon *et al*., 2014). Phylogenetic trees were constructed using MEGA 7.0 (Kumar *et al*., 2016) with the neighbor-joining method (Saitou and Nei, 1987) and the Kimura 2-parameter evolutionary model (Kimura, 1980). The reliability of the constructed trees was evaluated through bootstrap analysis with 1,000 replicates (Felsenstein, 1985).

Colony morphology was examined on agar plates using a magnifying glass once cultures reached the stationary phase. Cellular morphology and dimensions were further examined by transmission electron microscopy. Gram staining was conducted using a Gram-staining kit (bioMérieux), while biochemical characteristics were evaluated with API 20NE galleries (bioMérieux) following the manufacturer's protocols. The strain identifiers, sources of isolation, culture media used, and incubation conditions are detailed in Table 1.

## Results and Discussion

A total of 22 bacterial strains were isolated from the Seomjin River, all representing species not previously recorded in Korea. These strains were classified into 4 phyla, 6 classes, 10 orders, 17 families, and 19 genera. The taxonomic distribution and identification details of these species are summarized in Table 1. At the genus level, the newly identified species belong to *Methylorubrum*, *Aureimonas*, *Bosea*, *Tsuneonella*, *Erythrobacter*, *Sphingomonas*, *Paracoccus*, *Blastomonas*, and *Asticcacaulis* within the class *Alphaproteobacteria*; *Rivibacter*, *Sheuella*, and *Duganella* within the class *Betaproteobacteria*; *Pseudomonas* and *Dickeya* within the class *Gammaproteobacteria*; *Mycobacterium*, *Nocardia*, and *Brachybacterium* within the class *Actinomycetes*; *Paenibacillus* and *Neobacillus* within the class *Bacilli*; and *Chitinophaga* within the class *Chitinophagia*. The phylogenetic relationships between these isolates and their closest relatives are shown in Fig. 1 (*Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*), Fig. 2 (*Actinomycetes*) and Fig. 3 (*Bacilli* and *Chitinophagia*). Transmission electron micrographs of the isolates are presented in Fig. 4, while their morphological, physiological, and biochemical characteristics are described in detail below.

### Description of *Methylorubrum rhodinum* 20SJ16W-72

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and bright red colored after 7 days of incubation at 20°C on R2A. Positive for nitrate reduction, urease, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and $\beta$-galactosidase. Potassium gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 20SJ16W-72 displays the highest 16S rRNA gene sequence similarity with *Methylorubrum rhodinum* JCM 2811$^T$ (99.9%). Strain 20SJ 16W-72 (=NNIBR2021641BA1641) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912 965.

### Description of *Aureimonas glaciei* 21SJ13W-57

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are irregular, dry and bright yellow colored after 7 days of incubation at 20°C on ISP4. Posi-

**Table 1.** Summary of unrecorded freshwater bacterial strains isolated from the Seomjin River and their taxonomic affiliations.

| Phylum | Class | Order | Family | Strain ID | GPS | NNIBR ID | Accession number | Most closely related species | Similarity (%) | Medium | Incubation conditions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonadota* | *Alphaproteobacteria* | *Hyphomicrobiales* | *Methylobacteriaceae* | 20SJ16W-72 | 35°00′4.07″N 127°07′6.20″E | NNIBR2021641BA1641 | OL912965 | *Methylorubrum rhodinum* | 99.9 | R2A | 20°C, 7 d |
| | | | *Aurantimonadaceae* | 21SJ13W-57 | 35°0′1.65″N 127°7′4.99″E | NNIBR2021641BA1652 | OL912976 | *Aureimonas glaciei* | 99.6 | ISP4 | 20°C, 7 d |
| | | | *Boseaceae* | 21SJ14S-33 | 35°04′16.83″N 127°14′09.42″E | NNIBR2021641BA1589 | OK562845 | *Bosea psychrotolerans* | 99.8 | R2A | 25°C, 7 d |
| | | *Sphingomonadales* | *Erythrobacteraceae* | 21SJ02S-50 | 35°39′19.96″N 127°13′30.70″E | NNIBR2021641BA1654 | OL677209 | *Tsuneonella troitsensis* | 99.2 | R2A | 25°C, 7d |
| | | | | 21SJ10S-S20 | 35°3′46.92″N 127°44′27.91″E | NNIBR2021641BA1661 | OL677216 | *Tsuneonella amylolytica* | 99.1 | R2A | 25°C, 7 d |
| | | | | 21SJ02W-27 | 35°39′19.96″N 127°13′30.70″E | NNIBR2021641BA1656 | OL677211 | *Erythrobacter ramosus* | 98.9 | R2A | 25°C, 7 d |
| | | | *Sphingomonadaceae* | 21SJ08W-1 | 35°9′57.43″N 127°27′16.10″E | NNIBR2021641BA1648 | OL912972 | *Sphingomonas aerolata* | 98.8 | ISP4 | 20°C, 7 d |
| | | | | 21SJ09W-S21 | 35°11′58.47″N 127°36′0.88″E | NNIBR2021641BA1660 | OL677215 | *Sphingomonas fonticola* | 99.7 | R2A | 25°C, 7 d |
| | | | | 21SJ09S-26 | 35°11′58.47″N 127°36′0.88″E | NNIBR2021641BA1653 | OL677208 | *Parablastomonas arctica* | 99.4 | R2A | 25°C, 7 d |
| | | *Caulobacterales* | *Caulobacteraceae* | 21SJ04S-1 | 35°31′12.67″N 127°7′4.86″E | NNIBR2022641BA2257 | OP847229 | *Asticcacaulis biprosthecium* | 99.7 | R2A | 25°C, 7 d |
| | *Betaproteobacteria* | *Burkholderiales* | *Sphaerotilaceae* | 21SJ04S-8 | 35°31′12.67″N 127°7′4.86″E | NNIBR2022641BA2258 | OP847230 | *Rivibacter subsaxonicus* | 99.9 | R2A | 25°C, 7 d |
| | | | *Alcaligenaceae* | 21SJ08W-16 | 35°9′57.43″N 127°27′16.10″E | NNIBR2021641BA1650 | OL912974 | *Sheuella amnicola* | 98.9 | R2A | 20°C, 7 d |
| | | | *Oxalobacteraceae* | SJ13W-44 | 35°0′4.44″N 127°7′1.04″E | NNIBR2022641BA2291 | OQ073732 | *Duganella margarita* | 99.5 | ISP4 | 25°C, 7 d |
| | *Gammaproteobacteria* | *Pseudomonadales* | *Pseudomonadaceae* | 20SJ16W-91 | 35°00′4.07″N 127°07′6.20″E | NNIBR2021641BA1642 | OL912966 | *Pseudomonas otitidis* | 99.6 | TSA | 20°C, 7 d |
| | | *Enterobacterales* | *Pectobacteriaceae* | 20SJ17W-63 | 35°4′17.55″N 127°14′9.13″E | NNIBR2021641BA1643 | OL912967 | *Dickeya lacustris* | 99.5 | MA | 20°C, 7 d |

**Table 1.** Continued.

| Phylum | Class | Order | Family | Strain ID | GPS | NNIBR ID | Accession number | Most closely related species | Similarity (%) | Medium | Incubation conditions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Actinomycetota* | *Actinomycetes* | *Mycobacteriales* | *Mycobacteriaceae* | 21SJ03S-24 | 35°35'14.90"N 127°6'18.07"E | NNIBR2021641BA1657 | OL677212 | *Mycobacterium pallens* | 98.9 | R2A | 25°C, 7 d |
| | | | *Nocardiaceae* | 21SJ08S-113 | 35°9'57.43"N 127°27'16.10"E | NNIBR2021641BA1645 | OL912969 | *Nocardia aobensis* | 99.2 | MA | 20°C, 7 d |
| | | *Micrococcales* | *Dermabacteraceae* | 21SJ08W-46 | 35°9'57.43"N 127°27'16.10"E | NNIBR2021641BA1651 | OL912975 | *Brachybacterium tyrofermentans* | 99.2 | MA | 20°C, 7 d |
| *Bacillota* | *Bacilli* | *Caryophanales* | *Paenibacillaceae* | 21SJ08S-108 | 35°9'57.43"N 127°27'16.10"E | NNIBR2021641BA1644 | OL912968 | *Paenibacillus susongensis* | 98.8 | MA | 20°C, 7 d |
| | | | | 21SJ08S-65 | 35°9'57.43"N 127°27'16.10"E | NNIBR2021641BA1647 | OL912971 | *Paenibacillus rhizoplanae* | 99.7 | TSA | 20°C, 7 d |
| | | | *Bacillaceae* | 21SJ08S-43 | 35°9'57.43"N 127°27'16.10"E | NNIBR2021641BA1646 | OL912970 | *Neobacillus bataviensis* | 99.4 | NA | 20°C, 7 d |
| *Bacteroidota* | *Chitinophagia* | *Chitinophagales* | *Chitinophagaceae* | 21SJ14W-13 | 35°04'16.8"N 127°14'09.4"E | NNIBR2021641BA1590 | OK562846 | *Chitinophaga sancti* | 99.0 | R2A | 25°C, 7 d |

tive for nitrate reduction, glucose fermentation, urease, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, arginine dihydrolase, gelatin hydrolysis and $\beta$-galactosidase. D-glucose, D-mannose and *N*-acetyl-glucosamine are utilized. Does not utilize L-arabinose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ13W-57 displays the highest 16S rRNA gene sequence similarity with *Aureimonas glaciei* B5-2$^T$ (99.6%). Strain 21SJ13W-57 (=NNIBR2021641BA1652) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912976.

### Description of *Bosea psychrotolerans* 21SJ14S-33

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on R2A. Positive for arginine dihydrolase, urease, and esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, $\beta$-galactosidase and oxidase. L-arabinose, D-mannose, adipic acid, malic acid, and trisodium citrate are utilized. Does not utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid and phenylacetic acid. Strain 21SJ14S-33 displays the highest 16S rRNA gene sequence similarity with *Bosea psychrotolerans* 1131$^T$ (99.8%). Strain 21SJ14S-33 (=NNIBR2021641BA1589) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OK562845.

### Description of *Tsuneonella troitsensis* 21SJ02S-50

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and yellow colored after 7 days of incubation at 25°C on R2A. Positive for $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis and oxidase. Potassium gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ02S-50 displays the highest 16S rRNA gene sequence similarity with *Tsuneonella troitsensis* JCM 17037$^T$ (99.2%). Strain 21SJ02S-50 (=NNIBR2021641BA1654) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677209.

### Description of *Tsuneonella amylolytica* 21SJ10S-S20

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yel-

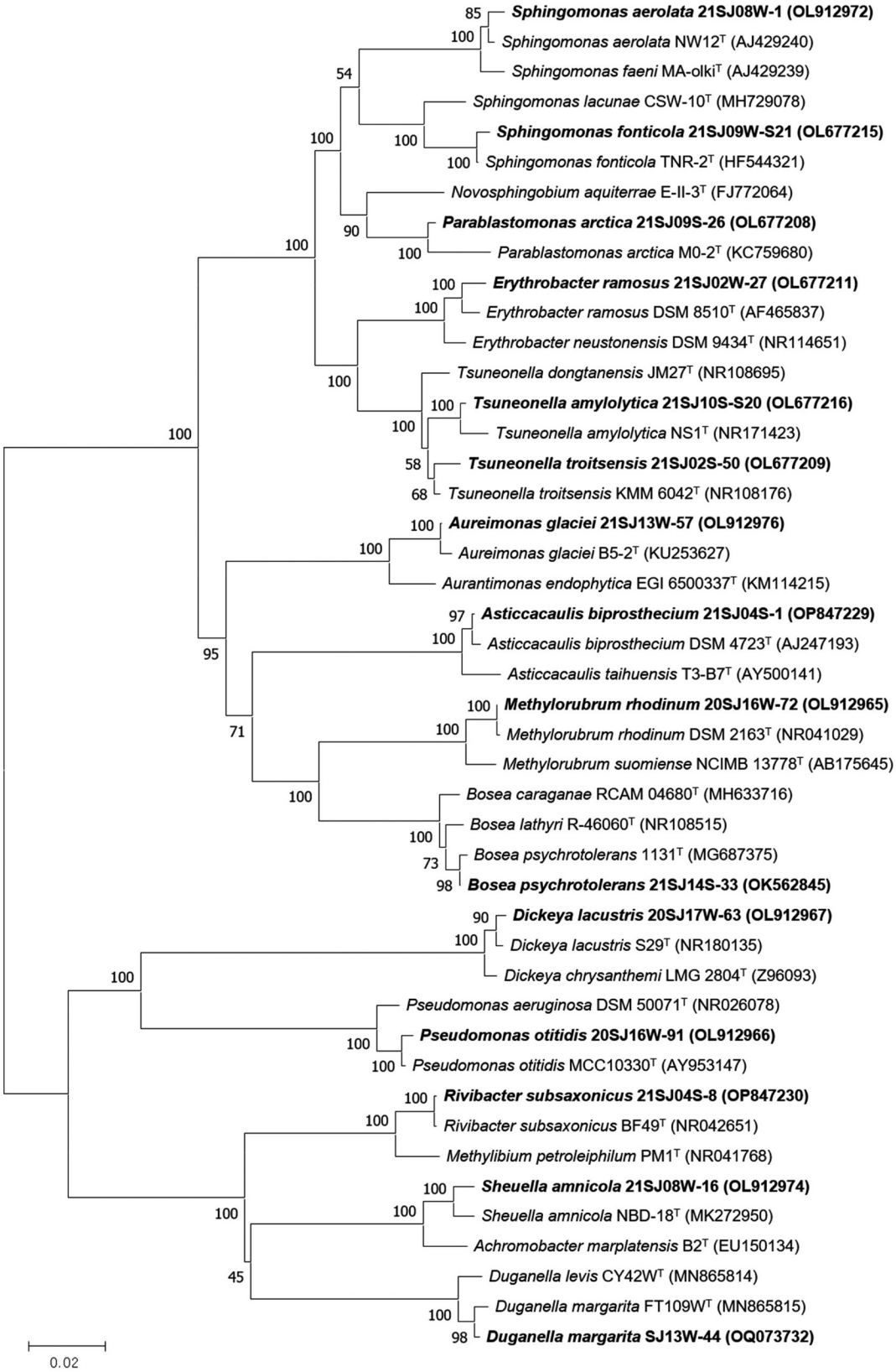



**Fig. 1.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. Bootstrap values (>45%) are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

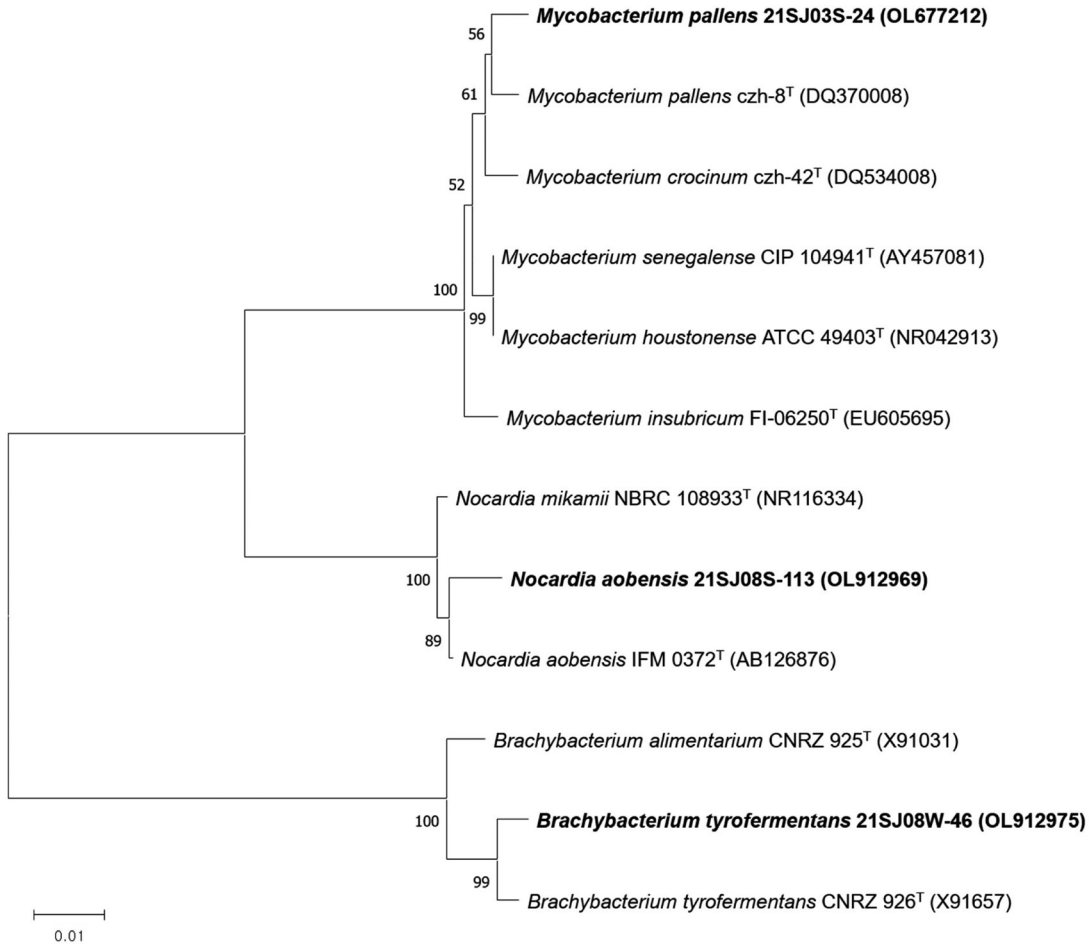



**Fig. 2.** Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Actinomycetes*. Bootstrap values (>50%) are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.

low colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, $\beta$-galactosidase and oxidase. D-glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ10S-S20 displays the highest 16S rRNA gene sequence similarity with *Tsuneonella amylolytica* NS1$^T$ (99.1%). Strain 21SJ10S-S20 (=NNIBR 2021641BA1661) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677216.

### Description of *Erythrobacter ramosus* 21SJ02W-27

Cells are Gram-staining-negative, non-flagellated and short, rod-shaped. Colonies are convex, circular, smooth and red colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis, gelatin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and oxidase. D-glucose, D-maltose and adipic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 21SJ02W-27 displays the highest 16S rRNA gene sequence similarity with *Erythrobacter ramosus* DSM 8510$^T$ (98.9%). Strain 21SJ02W-27 (=NNIBR 2021641BA1656) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677211.

### Description of *Sphingomonas aerolata* 21SJ08W-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and orange colored after 7 days of incubation at 20°C on ISP4. Positive for esculin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease,

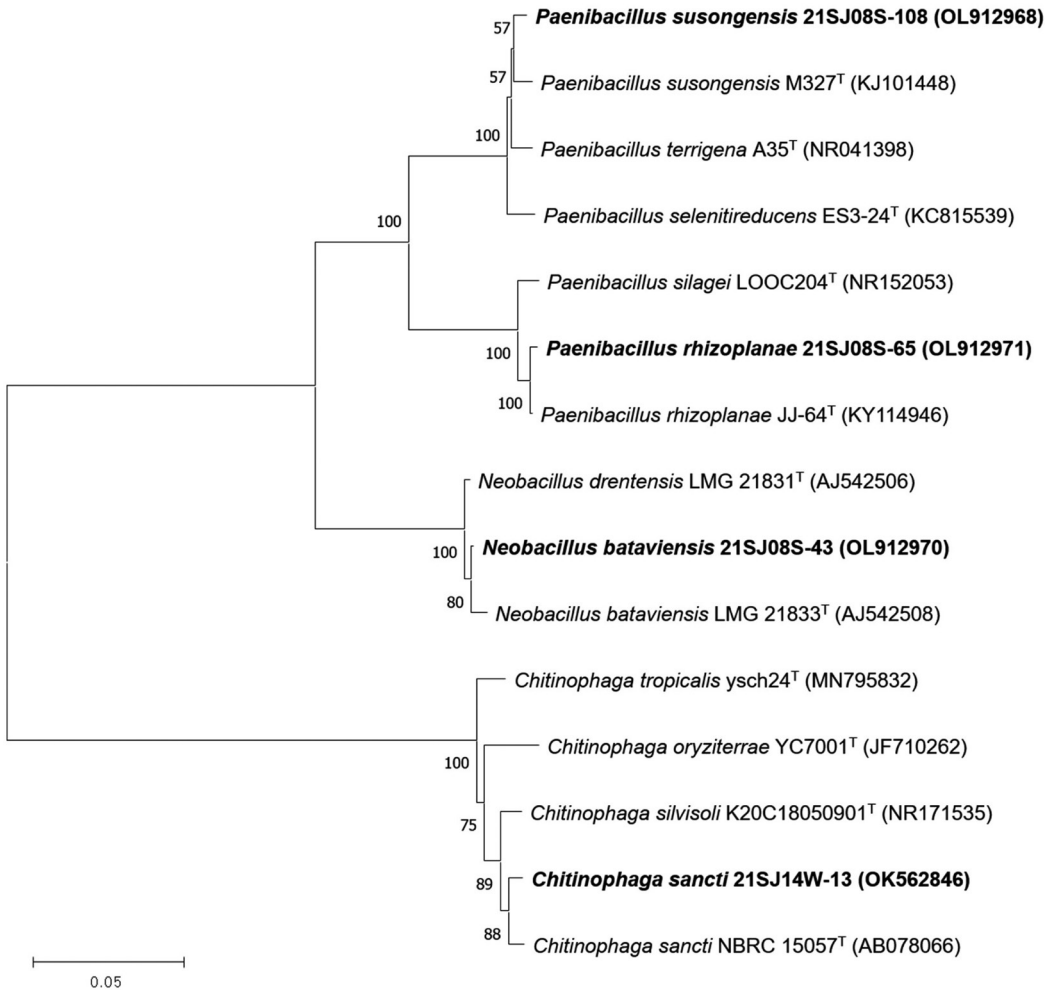



**Fig. 3.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Bacilli* and *Chitinophagia*. Bootstrap values (>50%) are shown at nodes. Bar, 0.05 substitutions per nucleotide position.

gelatin hydrolysis and oxidase. D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-mannitol, capric acid, adipic acid and phenylacetic acid. Strain 21SJ08W-1 displays the highest 16S rRNA gene sequence similarity with *Sphingomonas aerolata* NW12$^T$ (98.8%). Strain 21SJ08W-1 (=NNIBR 2021641BA1648) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912972.

### Description of *Sphingomonas fonticola* 21SJ09W-S21

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-maltose is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ09W-S21 displays the highest 16S rRNA gene sequence similarity with *Sphingomonas fonticola* TNR-2$^T$ (99.7%). Strain 21SJ09W-S21 (=NNIBR 2021641BA1660) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677215.

### Description of *Parablastomonas arctica* 21SJ09S-26

Cells are Gram-staining-negative, non-flagellated and spherical-shaped. Colonies are circular, convex and white colored after 7 days of incubation at 25°C on R2A. Positive for $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydro-

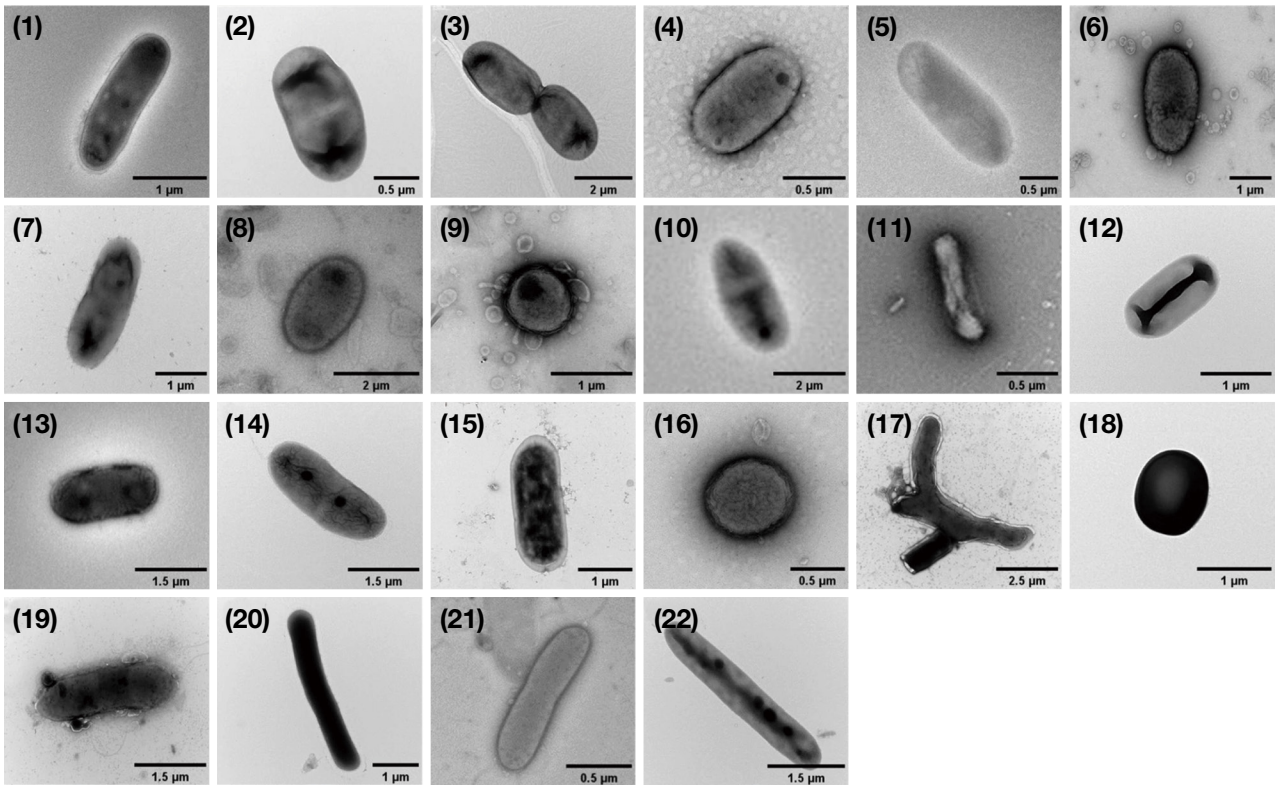



**Fig. 4.** Transmission electron micrographs of cells of the strains. Strains: 1, 20SJ16W-72; 2, 21SJ13W-57; 3, 21SJ14S-33; 4, 21SJ02S-50; 5, 21SJ10S-S20; 6, 21SJ02W-27; 7, 21SJ08W-1; 8, 21SJ09W-S21; 9, 21SJ09S-26; 10, 21SJ04S-1; 11, 21SJ04S-8; 12, 21SJ08W-16; 13, SJ13W-44; 14, 20SJ16W-91; 15, 20SJ17W-63; 16, 21SJ03S-24; 17, 21SJ08S-113; 18, 21SJ08W-46; 19, 21SJ08S-108; 20, 21SJ08S-65; 21, 21SJ08S-43; 22, 21SJ14W-13.

lysis and oxidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ 09S-26 displays the highest 16S rRNA gene sequence similarity with *Parablastomonas arctica* M0-2$^T$ (99.4%). Strain 21SJ09S-26 (=NNIBR2021641BA1653) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677208.

### Description of *Asticcacaulis biprosthecium* 21SJ04S-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-mannitol is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ04S-1 displays the highest 16S rRNA gene sequence similarity with *Asticcacaulis biprosthecium* DSM 4723$^T$ (99.7%). Strain 21SJ04S-1 (=NNIBR2022 641BA2257) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OP847229.

### Description of *Rivibacter subsaxonicus* 21SJ04S-8

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase and oxidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ04S-8 displays the highest 16S rRNA gene sequence similarity with *Rivibacter subsaxonicus* DSM 19570$^T$ (99.9%). Strain 21SJ04S-8 (=NNIBR2022641BA2258) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OP847230.

**Description of *Sheuella amnicola* 21SJ08W-16**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and dark-beige colored after 7 days of incubation at 20°C on R2A. Positive for nitrate reduction, urease and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis and $\beta$-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ08W-16 displays the highest 16S rRNA gene sequence similarity with *Sheuella amnicola* NBD-18$^T$ (98.9%). Strain 21SJ08W-16 (=NNIBR2021641BA1650) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912974.

**Description of *Duganella margarita* SJ13W-44**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on ISP4. Positive for nitrate reduction, urease, esculin hydrolysis, $\beta$-galactosidase and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis. *N*-acetyl-glucosamine is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SJ13W-44 displays the highest 16S rRNA gene sequence similarity with *Duganella margarita* FT109W$^T$ (99.5%). Strain SJ13W-44 (=NNIBR2022641BA2291) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OQ073732.

**Description of *Pseudomonas otitidis* 20SJ16W-91**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and ivory colored after 7 days of incubation at 20°C on TSA. Positive for nitrate reduction, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and $\beta$-galactosidase. D-glucose, L-arabinose, D-mannitol, capric acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Does not utilize D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and adipic acid. Strain 20SJ16W-91 displays the highest 16S rRNA gene sequence similarity with *Pseudomonas otitidis* MCC 10330$^T$ (99.6%). Strain 20SJ16W-91 (=NNIBR2021641 BA1642) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912966.

**Description of *Dickeya lacustris* 20SJ17W-63**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and ivory colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, indole production, esculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for glucose fermentation, arginine dihydrolase, urease and oxidase. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-maltose, capric acid, adipic acid and phenylacetic acid. Strain 20SJ17W-63 displays the highest 16S rRNA gene sequence similarity with *Dickeya lacustris* S29$^T$ (99.5%). Strain 20SJ17W-63 (=NNIBR2021641BA1643) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912967.

**Description of *Mycobacterium pallens* 21SJ03S-24**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and yellow colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase and oxidase. D-mannitol and potassium gluconate are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ03S-24 displays the highest 16S rRNA gene sequence similarity with *Mycobacterium pallens* czh-8$^T$ (98.9%). Strain 21SJ03S-24 (=NNIBR2021641 BA1657) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677212.

**Description of *Nocardia aobensis* 21SJ08S-113**

Cells are Gram-staining-positive, non-flagellated and hyphae-shaped. Colonies are irregular, dry and bright yellow colored after 7 days of incubation at 20°C on MA. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, $\beta$-galactosidase and oxidase. D-glucose, capric acid, malic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and phenylacetic acid. Strain 21SJ08S-113 displays the highest 16S rRNA gene sequence similarity with *Nocardia aobensis* IFM 0372$^T$ (99.2%). Strain 21SJ08S-113 (=NNIBR20216 41BA1645) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912969.

**Description of *Brachybacterium tyrofermentans* 21SJ08W-46**

Cells are Gram-staining-positive, non-flagellated and spherical-shaped. Colonies are convex, smooth and bright yellow colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, $\beta$-galactosidase and oxidase in API 20NE, but negative for indole production, arginine dihydrolase, urease and gelatin hydrolysis. D-glucose, L-arabinose, D-mannose, D-maltose, potassium gluconate, capric acid, and trisodium citrate are utilized. Does not utilize D-mannitol, *N*-acetyl-glucosamine, adipic acid, malic acid and phenylacetic acid. Strain 21SJ08W-46 displays the highest 16S rRNA gene sequence similarity with *Brachybacterium tyrofermentans* CNRZ 926$^T$ (99.2%). Strain 21SJ08W-46 (=NNIBR2021641BA1651) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912975.

**Description of *Paenibacillus susongensis* 21SJ08S-108**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, smooth and ivory colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase and oxidase in API 20NE, but negative for indole production and urease. L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-glucose, capric acid, adipic acid and phenylacetic acid. Strain 21SJ08S-108 displays the highest 16S rRNA gene sequence similarity with *Paenibacillus susongensis* M327$^T$ (98.8%). Strain 21SJ08S-108 (=NNIBR2021641BA1644) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912968.

**Description of *Paenibacillus rhizoplanae* 21SJ08S-65**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and ivory colored after 7 days of incubation at 20°C on TSA. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-mannose and D-maltose are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ08S-65 displays the highest 16S rRNA gene sequence similarity with *Paenibacillus rhizoplanae* JJ-64$^T$ (99.7%). Strain 21SJ08S-65 (=NNIBR 2021641BA1647) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912971.

**Description of *Neobacillus bataviensis* 21SJ08S-43**

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, convex, smooth and ivory colored after 7 days of incubation at 20°C on NA. Positive for glucose fermentation, esculin hydrolysis, gelatin hydrolysis, $\beta$-galactosidase and oxidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and urease. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate are utilized. Does not utilize capric acid, adipic acid and phenylacetic acid. Strain 21SJ08S-43 displays the highest 16S rRNA gene sequence similarity with *Neobacillus bataviensis* LMG 21833$^T$ (99.4%). Strain 21SJ08S-43 (=NNIBR2021 641BA1646) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912970.

**Description of *Chitinophaga sancti* 21SJ14W-13**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on R2A. Positive for glucose fermentation, urease, esculin hydrolysis, gelatin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and oxidase. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and D-maltose are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ14W-13 displays the highest 16S rRNA gene sequence similarity with *Chitinophaga sancti* NBRC 15057$^T$ (99.0%). Strain 21SJ14W-13 (=NNIBR 2021641BA1590) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OK562846.

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# Three newly recorded species of Lepidoptera (Argyresthiidae, Gracillariidae, Tortricidae) from Korea

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Three species of microlepidoptera belonging to the families Argyresthiidae, Gracillariidae, and Tortricidae are newly recorded from Korea: *Argyresthia rara* Moriuti, 1969, *Caloptilia querci* (Kumata, 1982), and *Pammene gallicana* (Guenée, 1845). Specimens were collected using light traps and examined in detail based on external morphology and genitalia structures. Diagnostic characters of adults and genitalia are described and illustrated comprehensively, and brief notes on their distribution and host plants are provided. The discovery of these species expands the known range of each taxon within East Asia and contributes to a better understanding of the diversity and biogeographical composition of the Korean microlepidopteran fauna.

Keywords: Argyresthiidae, Gracillariidae, Korea, Lepidoptera, new records, Tortricidae

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## Introduction

The Microlepidoptera comprise an artificial assemblage of small-sized moths representing diverse and relatively primitive lineages within Lepidoptera (Pogue, 2009). Despite their remarkable diversity and ecological importance, the fauna of microlepidopteran species in Korea remains insufficiently studied compared with that of larger moths. The first comprehensive checklist of Korean microlepidoptera was published by Park (1983) and later revised by Byun *et al.* (2009), enumerating approximately 1,200 species across several families. However, continued field surveys have revealed additional unrecorded taxa, suggesting that the Korean microlepidopteran fauna is still far from being completely known.

Among the microlepidopteran groups, the families Argyresthiidae, Gracillariidae, and Tortricidae include species of considerable economic and ecological significance. Members of Argyresthiidae are mostly host-specific shoot or fruit, Gracillariidae species are well known for their leaf-mining larvae, and Tortricidae, particularly the subfamily Olethreutinae, comprises several important agricultural pests. Although these families are well represented in the Palearctic Region, they have been poorly documented in Korea from limited localities.

During recent faunistic surveys conducted in various regions of Korea, three species belonging to these families were discovered for the first time in the country. In this paper, we report *Argyresthia rara* Moriuti, 1969, *Caloptilia querci* (Kumata, 1982), and *Pammene gallicana* (Guenée, 1845) for the first time from Korea. Diagnostic characteristics and genital structures of the species are described and illustrated, with brief notes on their distribution and host associations.

## Materials and Methods

All available specimens of microlepidoptera from Korea were examined in this study. All specimens examined for

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the present study were collected at night using light traps. Images of the adult specimens were taken using a Canon EOS 550D digital camera (Canon, Japan) and images of the head and antenna of specimens were taken using a digital camera attached to the Dhyana 400DC (Tucsen Photonics, Fuzhou, China) with a microscope (LEICA M205C © Leica Microsystems, Wetzlar, Hesse, Germany). The genitalia were dissected under a stereomicroscope (Nikon SMZ445; Nikon, Tokyo, Japan), using the method described by Holloway *et al.* (1987) and were mounted on a slide glass with Canada balsam as the mounting medium. Images of genitalia were taken using a digital camera attached to a microscope (LEICA M205C © Leica Microsystems, Wetzlar, Hesse, Germany).

Abbreviations used in this study are as follows:

TL (type locality), HNUSEL (Systematic Entomology Lab., Hannam University, Daejeon, Republic of Korea), HUNHM (Hannam University Natural History Museum, Daejeon, Republic of Korea) and NIBR (National Institute of Biological Resources, Incheon, Republic of Korea), GW (Gangwon state), JN (Jeollanam-do).

## Taxonomic Accounts

Superfamily Yponomeutoidea Stephens, 1829
Family Argyresthiidae Bruand, 1850
Genus *Argyresthia* Hübner, [1825]

***Argyresthia rara* Moriuti, 1969 (Figs. 1A, 2A)**
깜보라광택집나방 (신칭)

*Argyresthia rara* Moriuti, 1969: 22. TL: Honshu, Japan.

**Adult** (Fig. 1A). Wingspan 10 mm. Head covered with white scales; vertex white; face smooth and pale yellow; labial palpus creamy white; antenna filiform; scape of antenna yellowish white; flagellum white with brown scales covering the apices of each segment. Mesoscutum of thorax covered with white scales; tegula golden-brown. Legs ground color creamy white; femur creamy white; tarsus and tibia white, ringed with brown scales. Forewing ground color dark fuscous with purplish sheen; dorsal streak white, stretched from base to 1/3 of forewing. Hindwing and cilia dark gray.

**Male genitalia** (Fig. 2A). Tuba analis as long as valva width. Socius with 20 sclerotized special scales. Valva short linguiform, hairs along the costal, ventral, and apical margin; costal hairs rather slender. Aedeagus long, about 3 times longer than valva width; Cornuti with one long thick spine, surrounded by numerous minute spinules. 8th sternite V-shaped.

**Female genitalia.** Unknown in Korea (see Moriuti, 1969).

**Material examined.** [Korea] 1♂, Baekjeon-ri, Hwaam-myeon, Jeongseon-gun, GW, 24 viii 2024 (leg. BK Byun), gen. slide No. HNUSEL-1874-coll. NIBR (RORYIN0000 004459).

**Distribution.** Korea (new record), Japan (Moriuti, 1969).

**Host plants.** Unknown.

Superfamily Gracillarioidea Stainton, 1854
Family Gracillariidae Stainton, 1854
Subfamily Gracillariinae Stainton, 1854

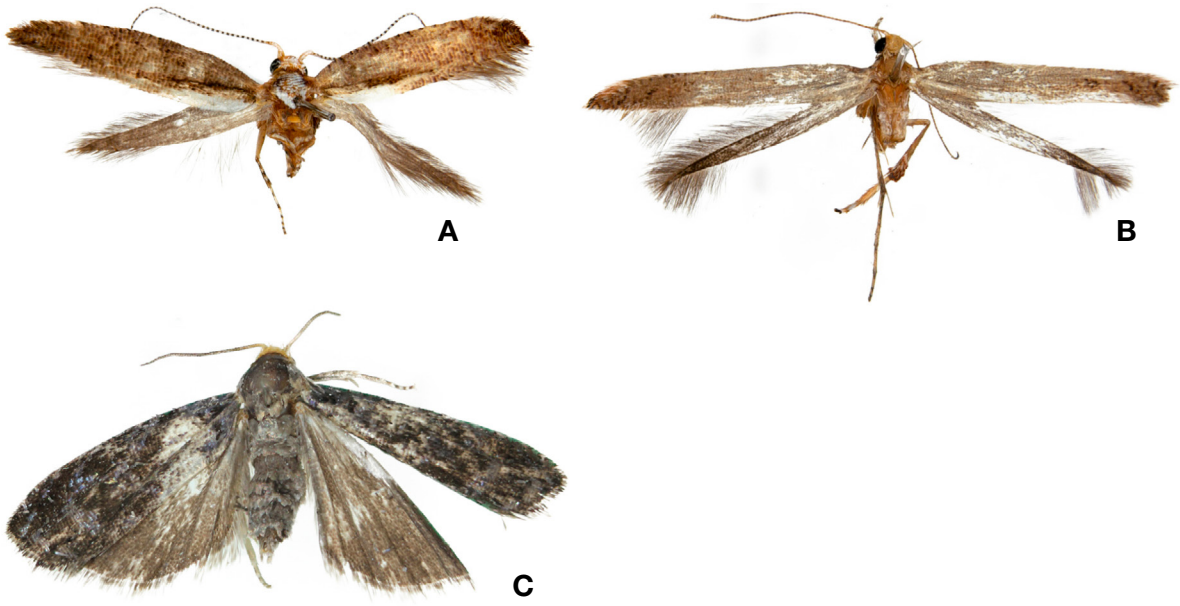



**Fig. 1.** Adult habitus. A, *Argyresthia rara* Moriuti, 1969; B, *Caloptilia querci* (Kumata, 1982); C, *Pammene gallicana* (Guenée, 1845).

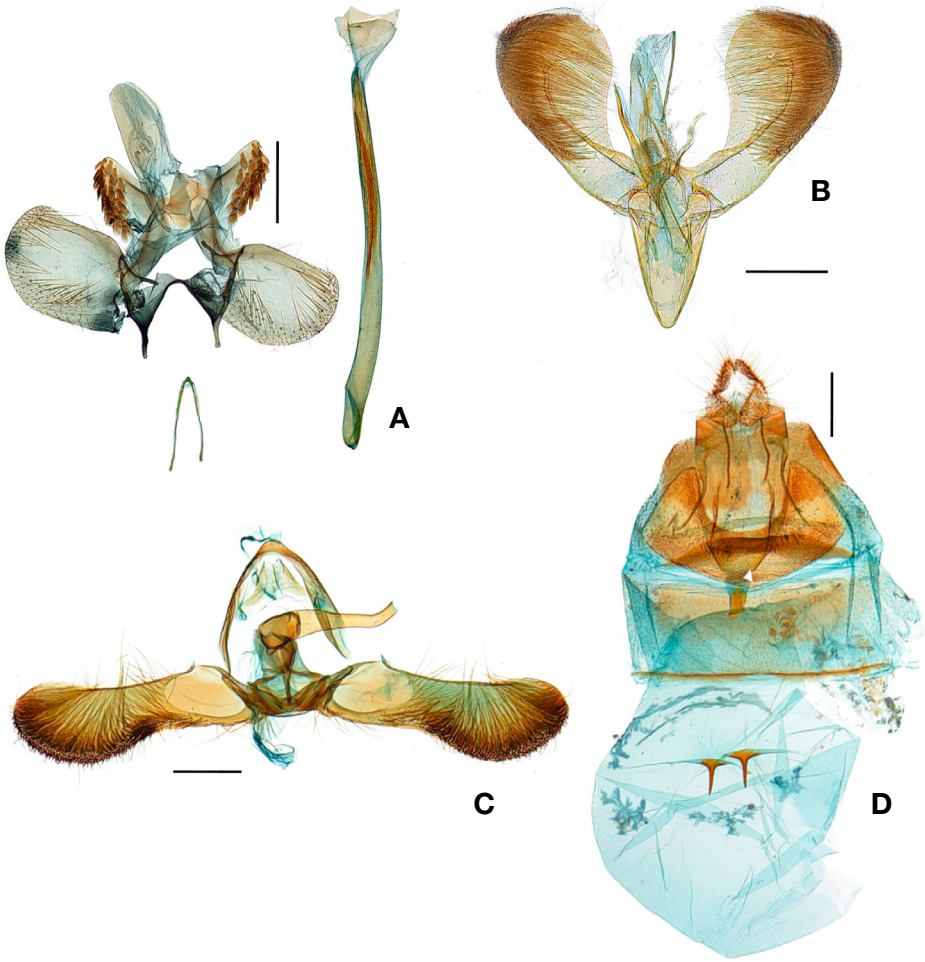



**Fig. 2.** Genitalia structures. A, *Argyresthia rara* Moriuti, 1969, male; B, *Caloptilia querci* (Kumata, 1982), male; C, *Pammene gallicana* (Guenée, 1845), male; D, *Ditto*, female. Scale bars = 0.2 mm.

Genus *Caloptilia* Hübner, 1816

***Caloptilia querci* (Kumata, 1982) (Figs. 1B, 2B)**
참나무가는나방 (신칭)
*Caloptilia querci* Kumata, 1982: 103. TL: Hokkaidō, Japan.

**Adult** (Fig. 1B). Wingspan 12 mm. Face and Head ocherous-yellow to yellowish-gray, sparsely irrorated with dark scales. Labial palpus whitish above, ochreous-yellow to reddish-brown below; second segment bearing a brownish elongate-triangular tuft slightly longer than the smooth apical segment, the latter with two faint oblique brown streaks ventrally. Antennae light ochre-yellow, annulated with dark brown; scape reddish-brown, paler above, without pecten. Thorax ochre-yellow to ochre-brown dorsally, sometimes slightly darkened anteriorly on tegulae; pleural surface reddish-brown with faint ochre-whitish streaks. Fore and mid legs dark reddish-brown; fore coxa with two ochre-whitish bands at base and middle.

Forewing ground color ocherous-brownish, irrorated with ocherous-yellowish or whitish scales; dark brown spots situated near apex. Two narrow dark brown to blackish fasciae near apex, oblique outwardly, often edged with fine whitish iridescent scales outwardly. Hindwing ground color dark brown; cilia reddish-brown around apex with a pale vertical subapical line.

**Male genitalia** (Fig. 2B). Subscaphium moderately sclerotized, slightly widened apically; tegumen simple, weakly arched dorsally; peniculi approximately one-third as long as valva, distinctly twisted and hook-shaped apically. Valva long and strongly upturned from the basal third, gradually widened toward apex; apical margin evenly rounded; long and slender setae densely arranged along the apical margin and extending posteriorly to about two-thirds of the ventral margin. Vinculum narrowly rounded apically, about half the length of valva. Aedeagus straight, slender, distinctly shorter than valva, needle-shaped, slightly narrowed api-

cally; cornuti not observed.
**Female genitalia.** Unknown in Korea (see Kumata, 1982).
**Material examined.** [Korea] 1♂, Wando-eup, Wando-gun, JN, 15 vii 2019 (leg. BK Byun), gen. slide no. HNUSEL-6022-coll. HUNHM.
**Distribution.** Korea (new record), Japan (Kumata, 1982).
**Host plants.** *Castanea crenata* Siebold & Zucc., *Castanopsis cuspidata* (Thunb.) Schottky, *Quercus acutissima* Carruth., *Q. glauca* Thumb., and *Q. mongolica* var. *crispula* (Blume) H. Ohashi. [Fagaceae] (Kumata, 1982).

Superfamily Tortricoidea Latreille, 1803
Family Tortricidae Latreille, 1803
Subfamily Olethreutinae Walsingham, 1895
Genus *Pammene* Hübner, [1825]

***Pammene gallicana* (Guenée, 1845) (Figs. 1C, 2C, 2D)**
참나무혹애기잎말이나방 (신칭)
*Orchemia gallicana* Guenée, 1845: 192. TL: France.
*Grapholitha pudicana* Herrich-Schäffer, 1851: 263.
*Grapholitha implicatana* Christoph, 1881: 417.

**Adult** (Fig. 1C). Wingspan 10–11 mm. Head ochreous; antenna yellowish brown; labial palpus grayish brown. Thorax and abdomen dark brown; legs yellowish brown. Forewing costa gently arched; termen without notch below apex; ground color grayish brown, median fascia violet-gray; costal strigulae ill-defined; dorsal blotch absent. Hindwing ground color grayish brown; cilia grayish white.
**Male genitalia** (Fig. 2C). Tegumen slightly protuberant at the top. Valva long, broad with rounded angle. basal cavity ovate; cucullus clavate, densely with setae at lower margin; sacculus well sclerotized. Aedeagus moderate in size, narrowed apically, cornuti absent.
**Female genitalia** (Fig. 2D). Papillae anales small and narrow, densely covered with setae. Apophyses anteriores slightly longer than apophyses posteriores. Sterigma subquadrate, well sclerotized, with 2 protrusions laterally. Ostium bursae cup-shaped, long and broad. Ductus bursae short and narrow, well sclerotized. Corpus bursae large and ovate, with two horn-shaped signum.
**Material examined.** [Korea] 1♀, Gurae-ri, Sangdong-eup, Yeongwol-gun, GW, 24 viii 2019 (leg. BK Byun), gen. slide no. HNUSEL-1023-coll. NIBR (RORYIN000000 4471); 1♂1♀, Gunae-ri, Sangdong-eup, Yeongwol-gun, GW, 24 viii 2019 (leg. BK Byun), gen. slide no. HNUSEL-1022, 1024-coll. HNUSEL.
**Distribution.** Korea (new record), Japan, Russia, Europe (Komai, 1999).
**Host plants.** Galls of *Quercus robur* L. [Fagaceae] (Disque, 1908), *Angelica sylvestris* L., *Daucus carota* L., *Heracleum sphondylium* L., *Pastinaca sativa* L., *Thysselinum palustre* (L.) Hoffm., *Silaum silaus* (L.) Schinz & Thell. [Apiaceae] (Bradley *et al*., 1979).

## Discussion

The three species newly recorded in this study represent valuable additions to the Korean microlepidopteran fauna, particularly within the families Argyresthiidae, Gracillariidae, and Tortricidae, which have received comparatively less attention in faunistic surveys. Their detection further supports the view that the diversity of small moths in Korea remains insufficiently documented.

*Argyresthia rara* was previously known only from Japan and can be identified by its distinctive forewing pattern with a white dorsal streak and purple sheen. *Caloptilia querci* is distributed in Japan and associated with several *Quercus* and *Castanea* species (Kumata, 1982). *Pammene gallicana* is known across the Palearctic Region and is associated with Apiaceae plants.

Additional sampling across different regions will be required to determine whether these occurrences indicate established populations, rare immigrant records, or undersampled taxa. The present findings suggest the possibility that the Korean Peninsula may function as a transitional zone for Palearctic microlepidopteran diversity, but further evidence is needed to test this hypothesis. Future studies incorporating more specimens, larval host associations, and detailed life histories will be essential for evaluating the ecological significance and potential economic impact of these species in Korea.

## Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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# A report of 7 unrecorded haloarchaea species isolated from solar salterns in Yellow Sea

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Solar saltern samples were collected from diverse regions near Yellow Sea to obtain unrecorded haloarchaea in Korea, including Siheung in Gyeonggi-do, Taean in Chungcheongnam-do, and Buan, Jeollabuk-do. Samples were suspended in a 20% (w/v) NaCl solution and enriched for one month at 37°C in freshly prepared DB Characterization Medium No. 2 broth with minor modifications. The enriched cultures were then spread onto agar plates to isolate single colonies of halophilic archaea. Phylogenetic analysis of the 16S rRNA gene revealed that the isolates exhibited at least 98.7% sequence similarity to previously described species. In total, seven haloarchaeal species, previously unreported in Korea but validly published under the International Code of Nomenclature of Prokaryotes, were obtained. These isolates were classified into the order *Halobacteriales*. The 7 *Halobacteriales* species were further categorized into the 6 species of the family *Haloferacaceae* and 1 species of the family *Halobacteriaceae*, comprising 4 genera, including *Halobellus* (4 species), *Haloplanus* (1 species), *Salinigranum* (1 species), and *Natronomonas* (1 species). Collectively, these unrecorded haloarchaeal species spanned 1 order, 2 families, and 3 genera. This research shows the potential for discovering previously unreported species within domestic solar saltern environments. Comprehensive analyses, including Gram staining, cell morphology, physiological characteristics, basic biochemical profiling, and phylogenetic analysis, were performed and presented for each species.

Keywords: comprehensive analyses, haloarchaea, solar saltern, unrecorded species

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## Introduction

Archaea possess distinctive biological characteristics that set them apart from bacteria and eukaryotes. In 1977, Carl Woese employed 16S rRNA sequence data to infer phylogenetic relationships among diverse prokaryotic groups, revealing that a cluster of aerobic, red-pigmented extreme thermophiles constituted a newly defined lineage now recognized as the Archaea (Woese and Fox, 1977). This group encompassed methanogens as well as thermophilic and hyperthermophilic prokaryotes, including organisms later classified within the *Halobacteria* (Oren, 2014). A seminal study published in 1978 examined the small-subunit rRNA of *Halobacterium halobium* and *Halobacterium cuticulumbrum*. These species were later reclassified as strains of *Halobacterium salinarum*, providing critical evidence that extreme halophiles were phylogenetically distinct from bacteria (Ventosa and Oren, 1996).

Haloarchaea are renowned for their exceptional tol-

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erance to high-salt environments, a capability achieved through the regulation of osmotic balance via the production of compatible solutes (DasSarma and DasSarma, 2015). They are typically small in size and display diverse cellular morphologies, including rods, cocci, and spirals (Hezayen *et al*., 2001). Haloarchaea generate energy through multiple metabolic pathways, encompassing aerobic and anaerobic respiration, fermentation, and phototrophic ATP production mediated by bacteriorhodopsin (Bickel-Sandkötter *et al*., 1996). Their vibrant pigmentation derives largely from carotenoids, which also protect against UV-induced cellular damage (Oren and Rodriguez-Valera, 2001). Additional adaptations, including specialized transport proteins and membrane structures composed of ether-linked lipids, enable these organisms to endure extreme salinity (van de Vossenberg *et al*., 2000). In high-salt environments, halophilic archaea depend on $Na^+$ ions to stabilize their cell walls, ribosomes, and enzymes. Unlike non-halophilic bacteria, which possess robust cross-linked peptidoglycan cell walls, halophilic archaea feature thick, rigid cell walls and cytoplasmic membranes that require high salt concentrations for structural integrity and survival (Kandler and König, 1993; DasSarma and DasSarma, 2015). These cells are further protected by envelopes containing highly glycosylated S-layers composed of negatively charged saccharides, contributing to surface stability under hypersaline conditions (Kandler and König, 1993).

The kingdom *Halobacteria* currently comprises 9 families, 82 genera, and 357 species with validly published names, representing an approximately 50% increase from the 6 families, 57 genera, and 233 species reported in 2017 (Arahal *et al*., 2017; Cui *et al*., 2023). This expansion is largely attributable to major taxonomic revisions informed by comprehensive phylogenomic analyses based on single-copy marker proteins. These analyses prompted the reorganization of orders within the kingdom *Halobacteria*, including the merger of the former orders *Haloferacales* and *Natrialbales* into *Halobacteriales* (Cui *et al*., 2023). As global diversity continues to be redefined, efforts to document regional microbial resources have become increasingly important.

To contribute to the understanding of domestic biodiversity for haloarchaea, this study focuses on unrecorded haloarchaeal species that have not yet been reported in Korea. Based on 16S rRNA gene sequence analysis, seven species from the order *Halobacteriales*, belonging to four genera (*Halobellus*, *Haloplanus*, *Natronomonas*, and *Salinigranum*) and the families *Haloferacaceae* and *Halobacteriaceae*, were identified. Their phenotypic characteristics and phylogenetic analyses further confirmed their status as previously unrecorded haloarchaea in Korea.

## Materials and Methods

Samples were collected from solar salterns located in Siheung (July 2018), Taean (January 2024), and Buan (January 2024). Each sample (1 g) was suspended in 10 mL of 20% (v/v) NaCl solution and thoroughly vortexed. The resulting suspensions were inoculated directly into DB Characterization Medium No. 2 (DBCM2) agar plates or into 100 mL of modified DBCM2 broth in 500 mL shaking flasks. The modified DBCM2 broth contained the following components (per liter): 833 mL MDS salt water (7 g of KCl, 35 g of $MgSO_4 \cdot 7H_2O$, 30 g of $MgCl_2 \cdot 6H_2O$, 240 g of NaCl, and 5 mL of 1 M $CaCl_2$ solution), 1 mL of $FeCl_2$ solution, 0.05 g of peptone (Oxoid, Hampshire, UK), 0.25 g of yeast extract (BD, Franklin Lakes, NJ, USA), 5 mL of 1 M $NH_4Cl$, 2 mL of potassium phosphate buffer, 1 mL of trace element solution (2.0 mg of $FeSO_4 \cdot 7H_2O$, 141.5 mg of $ZnSO_4 \cdot 7H_2O$, 57.6 mg of $MnSO_4 \cdot H_2O$, 6.0 mg of $H_3BO_3$, 190.0 mg of $CoCl_2 \cdot 6H_2O$, 2.0 mg of $CuCl_2 \cdot 2H_2O$, 24.0 mg of $NiCl_2 \cdot 6H_2O$, and 36.0 mg of $Na_2MoO_4 \cdot 2H_2O$ per liter), 3 mL of vitamin solution (3.0 mg of biotin, 4.0 mg of folic acid, 50.0 mg of pyridoxine·HCl, 33.0 mg of thiamine·HCl, 10.0 mg of riboflavin, 33.0 mg of nicotinic acid, 17.0 mg of DL-calcium pantothenate, 17.0 mg of vitamin $B_{12}$, 13.0 mg of *p*-aminobenzoic acid, and 10 mg of lipoic acid per liter), 10 mL of 1 M sodium pyruvate solution. The medium was further supplemented with 0.25 g of fish peptone (HiMedia, Maharashtra, India), 1 g of D-glucose (Samchun Chemical, Pyeongtaek, Korea), and 1 g of sucrose (Samchun Chemical, Pyeongtaek, Korea), and the pH was adjusted to 7.0–7.3 with 1 M Tris-base. DBCM2 agar plates were solidified with 2% (w/v) agarose (KisanBio, Seoul, Korea). Agar cultures were incubated at 37°C for one month, while broth cultures were incubated at 37°C with shaking at 200 rpm. Subsequently, agar and broth cultures were serially diluted and spread onto fresh DBCM2 agar plates. Distinct colonies were selected and repeatedly subcultured onto fresh media every 3–7 days. After at least three successive subculturing steps, pure isolates were obtained and preserved at −80°C in a cryoprotectant solution containing 20% (w/v) glycerol and 20% (w/v) NaCl.

Phenotypic characterization was performed using cultures grown at 37°C on DBCM2 agar plates. Cell morphology was examined by light microscopy (CX23; Olympus, Tokyo, Japan) and scanning electron microscopy (SUPRA 55VP; Carl Zeiss, Oberkochen, Germany). Gram staining was carried out using a commercial Gram stain kit (BioWORLD, Dublin, OH, USA) following the manufacturer's instructions. To assess salinity tolerance, each species was cultivated in DBCM2 broth containing NaCl concentrations ranging from 5% to 30% (w/v) in 5% increments and incubated for 3–7 days at 37°C and 200

rpm. The pH range for growth was evaluated by cultivating cells for 3−7 days at 37°C and 200 rpm in buffer-adjusted media with pH values from 4.0 to 11.0 in 1.0 unit increments. The following 100 mM buffer systems were used: 100 mM of $CH_3COOH/CH_3COONa$ buffer (pH 4.0−6.0), 100 mM of $NaH_2PO_4/Na_2HPO_4$ buffer (pH 7.0−8.0), 100 mM $NaHCO_3/Na2CO_3$ buffer (pH 9.0−10.0), and 100 mM of $Na_2CO_3$/NaOH buffer (pH 11.0−12.0). Growth temperature was assessed by incubating cultures on DBCM2 agar at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, and 55°C for 3−7 days. Catalase and oxidase activities were tested using 3% (v/v) $H_2O_2$ and 1% (w/v) tetramethyl-p-phenylenediamine, respectively.

Carbon and nitrogen source utilization was examined in modified DBCM2 broth lacking all carbon and nitrogen sources, supplemented individually (0.1%, w/v) with one of the following substrates: D-glucose, D-mannose, D-galactose, D-maltose, D-sucrose, D-lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, glycerol, L-sorbose, pyruvate, D-mannitol, succinate, DL-lactate, L-malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-alanine, L-glutamate, L-glutamic acid, L-arginine, L-lysine, and L-ornithine.

Genomic DNA was extracted using the LaboPass™ Tissue Genomic DNA Isolation Kit (Cosmogenetech, Seoul, Korea). The partial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using archaeal-specific primers described by Cui *et al.* (2009). PCR products were purified with the LaboPass™ PCR Purification Kit (Cosmogenetech, Seoul, Korea) and submitted to Macrogen Co., Ltd. (Seoul, Korea) for sequencing. Sequencing was performed using archaeal universal primers, including 18F (5′-ATTCCGGTTGATCCTGCC-3′), 344F (5′-CGGGYGCASCAGGCGCGAA-3′), 520R (5′-GWATTACCGCGGCKGCTG-3′), 787F (5′-ATTAGATACCCSBGTAGTCC-3′), and 1518R (5′-AGGAGGTGATCCAGCCGC-3′) (Abdallah *et al.*, 2018). Raw sequences were assembled using the SeqMan™ II expert sequence analysis software (Thombre *et al.*, 2016). Taxonomic identification of the assembled sequences was performed using the EzBioCloud 16S-based ID service (https://www.ezbiocloud.net/identify), and the closest related taxa were retrieved from the EzTaxon-e database (http://www.ezbiocloud.net/eztaxon) (Yoon *et al.*, 2017). Multiple sequence alignments were generated using the ClustalW algorithm implemented in BioEdit 7.2.6.1 (Thompson *et al.*, 1994; Hall, 1999).

Phylogenetic trees were constructed in MEGA 12 based on the aligned 16S rRNA gene sequences (Kumar *et al.*, 2024). Sequence relatedness was inferred using the maximum likelihood (ML) method (Felsenstein, 1981), and tree robustness was evaluated with 1,000 bootstrap replications. Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980).

## Results and Discussion

The 16S rRNA gene sequence analyses revealed that all seven isolates represented species not previously reported in Korea. Detailed strain information, including taxonomic assignment from species to class level, identification results, isolation sources, and sequence accession numbers from both the National Institute of Biological Resources (NIBR) in Korea and GenBank, is provided in Table 1. Seven species were classified within the order *Halobacteriales*. Among them, six species belonged to the family *Haloferacaceae*, and one species belonged to the family *Halobacteriaceae*. The *Haloferacaceae* species comprised members of the genera *Halobellus* (4 species), *Haloplanus* (1 species), and *Salinigranum* (1 species). The single species assigned to the *Halobacteriaceae* was identified as *Natronomonas*. The isolated unrecorded haloarchaeal species exhibited various morphologies, including pleomorphic and rod-shaped (Fig. 1). All seven species were Gram-stain-negative and lysed in distilled water. Some species required $Mg^{2+}$ for growth, and most exhibited mesophilic (20−40°C) and neutrophilic (pH 7.0) growth characteristics. All isolates grew at NaCl concentrations above 10% (w/v). Catalase activity was detected in all species, whereas oxidase activity varied among them. Growth was supported in all isolates when D-glucose or pyruvate was provided as the sole energy source. However, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, starch, L-lysine, and L-ornithine were not utilized as sole substrates.

The identification of the seven unrecorded species based on 16S rRNA gene sequence similarities was further supported by phylogenetic analysis using the ML algorithm (Fig. 2). Detailed morphological, physiological, and biochemical characteristics of the seven haloarchaeal species are presented in the following strain descriptions.

### Description of *Salinigranum marinum* MBLA0167

Cells are Gram-stain-negative and rod-shaped. Colonies are red, circular, and flat after 7 days of incubation on DBCM2 agar at 37°C. Catalase activity is positive, whereas oxidase activity is negative. Growth occurs at 25−45°C (optimum, 37°C) in 10−25% NaCl (optimum, 15%), and at pH 5.0−10.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is required for growth. D-glucose, sucrose, lactose, D-sorbitol, pyruvate, L-malate, glycine, and L-alanine are utilized as sole energy sources. In contrast, D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, starch, glycerol, D-mannitol, acetate, DL-lactate, succinate, fumarate, citrate, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PV549648. Strain MBLA0167

**Table 1.** Summary of all strains isolated in this study and their taxonomic affiliations.

| Order | Family | Strain ID | Accession/ NIBRARC number | Closest species | Similarity (%) | Isolation source | Medium |
|---|---|---|---|---|---|---|---|
| *Halobacteriales* | *Haloferacaceae* | MBLA0167 | PV549648 NIBRARC000513757 | *Salinigranum marinum* | 99.4 | Solar saltern in Siheung | DBCM2 |
| | | MBLA0307 | PP917501 NIBRARC000513548 | *Halobellus clavatus* | 99.7 | Solar saltern in Taean | |
| | | MBLA0308 | PP916780 NIBRARC000513549 | *Halobellus salinus* | 100.00 | Solar saltern in Taean | |
| | | MBLA0309 | PP917499 NIBRARC000513550 | *Halobellus ramosii* | 99.6 | Solar saltern in Taean | |
| | | MBLA0310 | PP917500 NIBRARC000513551 | *Halobellus inordinatus* | 99.6 | Solar saltern in Buan | |
| | | MBLA0311 | PP917502 NIBRARC000513552 | *Haloplanus salinus* | 99.0 | Solar saltern in Buan | |
| | *Halobacteriaceae* | MBLA0186 | PQ481849 NIBRARC000513553 | *Natronomonas aquatica* | 98.8 | Solar saltern in Siheung | |

(=NIBRARC000513757) was isolated from a solar saltern in Siheung, Gyeonggi-do, Korea (37°24′08.6″N 126° 45′22.7″E).

### Description of *Natronomonas aquatica* MBLA0186

Cells are Gram-stain-negative rod-shaped. Colonies are red, circular, and flat after 7 days of incubation on DBCM2 at 37°C. Catalase activity is positive, whereas oxidase activity is negative. Growth occurs at 25−50°C (optimum, 37°C) in 15−30% NaCl (optimum, 25%), and at pH 7.0−9.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is not required for growth. D-glucose, glycerol, pyruvate, L-alanine, and L-glutamate are utilized as sole energy sources. In contrast, D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, sucrose, lactose, starch, D-mannitol, D-sorbitol, acetate, DL-lactate, succinate, L-malate, fumarate, citrate, glycine, L-arginine, L-aspartate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PQ481849. Strain MBLA0186 (=NIBRARC000513553) was isolated from a solar saltern in Siheung, Gyeonggi-do, Korea (37°24′08.6″N 126°45′22.7″E).

### Description of *Halobellus clavatus* MBLA0307

Cells are Gram-stain-negative and rod-shaped. Colonies are red, circular, and convex after 7 days of incubation on DBCM2 at 37°C. Catalase and oxidase activity are positive. Growth occurs at 25−50°C (optimum, 37°C) at 10−30% NaCl (optimum, 20%), and at pH 6.0−9.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is not required for growth. D-glucose, D-mannose, D-galactose, sucrose, lactose, glycerol, pyruvate, DL-lactate, succinate, L-malate, and fumarate are utilized as sole energy sources. In contrast, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, starch, D-mannitol, D-sorbitol, acetate, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PP917501. Strain MBLA0307 (=NIBRARC000513548) was isolated from a solar saltern in Taean, Chungcheongnam-do, Korea (36°25′14.5″N 126°22′37.1″E).

### Description of *Haloferax namakaokahaiae* MBLA0308

Cells are Gram-stain-negative and pleomorphic shaped. Colonies are red, circular, and convex after 7 days of incubation on DBCM2 at 37°C. Catalase and oxidase activity are positive. Growth occurs at 25−50°C (optimum, 37°C) in 10−30% NaCl (optimum, 20%), and at pH 6.0−9.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is not required for growth. D-glucose, sucrose,

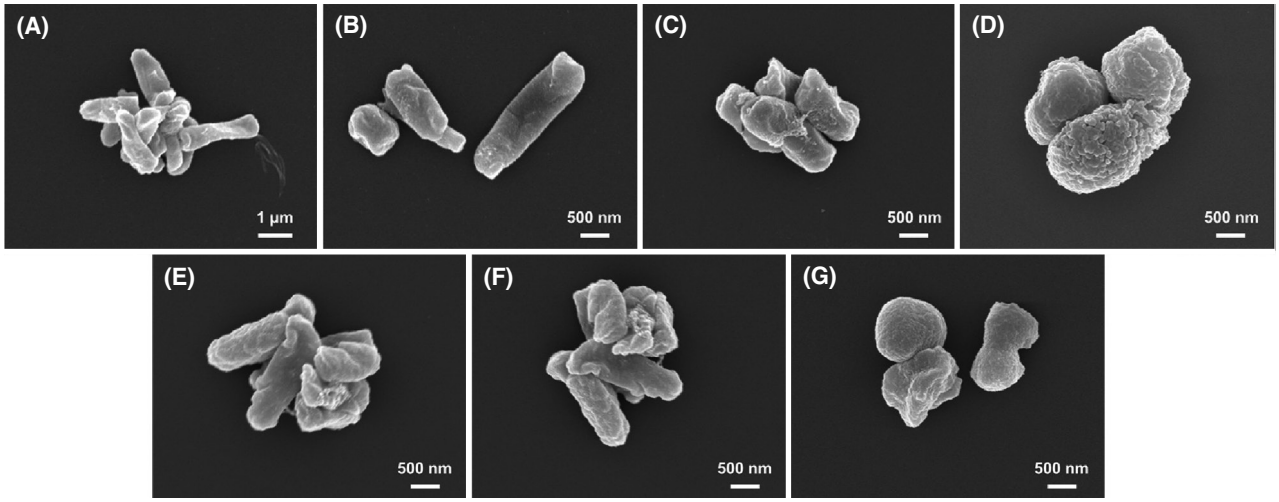



**Fig. 1.** Scanning electron micrographs of the 7 unrecorded species isolated in this study. Strains: A, MBLA0167; B, MBLA0186; C, MBLA0307; D, MBLA0308; E, MBLA0309; F, MBLA0310; G, MBLA0311.

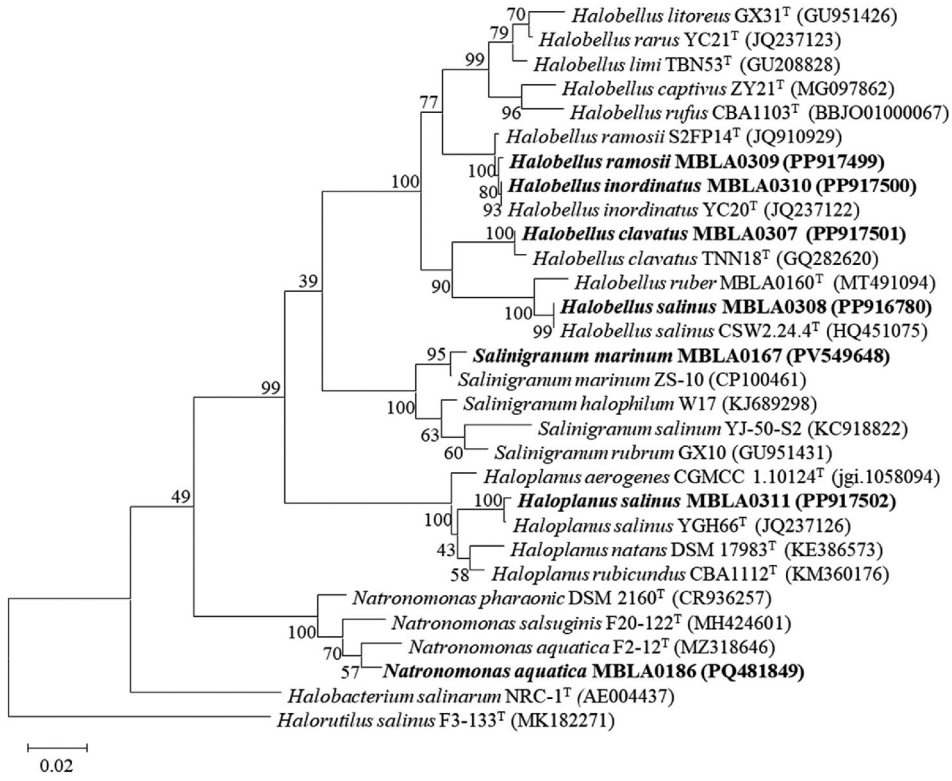



**Fig. 2.** Maximum likelihood (ML) phylogenetic tree based on the 16S rRNA gene sequences between the species isolated in this study. A phylogenetic tree was constructed with their relatives of the genera *Halobellus*, *Haloplanus*, *Natronomonas*, and *Salinigranum*. The numbers on the nodes indicate the bootstrap values. Bar, 0.02 accumulated changes per nucleotide, respectively.

glycerol, D-sorbitol, pyruvate, DL-lactate, fumarate, and citrate are utilized as sole energy sources. In contrast, D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, lactose, starch, D-mannitol, acetate, succinate, L-malate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PP916780. Strain MBLA0308

(=NIBRARC000513549) was isolated from a solar saltern in Taean, Chungcheongnam-do, Korea (36°25′14.5″N 126°22′37.1″E).

**Description of *Halobellus ramosii* MBLA0309**

Cells are Gram-stain-negative and rod-shaped. Colonies are red, circular, and convex after 7 days of incubation on DBCM2 at 37°C. Catalase activity is positive, whereas oxidase activity is negative. Growth occurs at 25−50°C (optimum, 37°C) in 15−30% NaCl (optimum, 25%), and at pH 7.0−9.0 (optimum, pH 7.0). Cell lyse in distilled water, and $Mg^{2+}$ is not required for growth. D-glucose, glycerol, pyruvate, and succinate are utilized as sole energy sources. In contrast, D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, sucrose, lactose, starch, D-mannitol, D-sorbitol, acetate, DL-lactate, L-malate, fumarate, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PP917499. Strain MBLA0309 (=NIBRARC000513550) was isolated from a solar saltern in Taean, Chungcheongnam-do, Korea (36°25′14.5″N 126°22′37.1″E).

**Description of *Halobellus inordinatus* MBLA0310**

Cells are Gram-stain-negative and rod-shaped. Colonies are red, circular, and convex after 7 days of incubation on DBCM2 at 37°C. Catalase activity is positive, whereas oxidase activity is negative. Growth occurs at 20−50°C (optimum, 37°C) in 10−30% NaCl (optimum, 20%), and at pH 6.0−9.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is required for growth. D-glucose, glycerol, acetate, pyruvate, DL-lactate, succinate, L-malate, and fumarate are utilized as sole energy sources. In contrast, D-mannose, D-galactose, D-fructose, L-sorbose, D-ribose, D-xylose, maltose, sucrose, lactose, starch, D-mannitol, D-sorbitol, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PP917500. Strain MBLA0310 (=NIBRARC000513551) was isolated from a solar saltern in Buan, Jeollabuk-do, Korea (35°35′50.7″N 126°36′46.8″E).

**Description of *Haloplanus salinus* MBLA0311**

Cells are Gram-stain-negative and pleomorphic shaped. Colonies are red, circular, and convex after 7 days of incubation on DBCM2 at 37°C. Catalase activity is positive, but oxidase activity is negative. Growth occurs at 20−50°C (optimum, 37°C) in 10−30% NaCl (optimum, 20%), and at pH 6.0−8.0 (optimum, pH 7.0). Cells lyse in distilled water, and $Mg^{2+}$ is required for growth. D-glucose, D-mannose, pyruvate, and DL-lactate are utilized as sole energy sources. In contrast, D-galactose, D-fructose, L-sorbose, D-ribose, maltose, sucrose, lactose, starch, glycerol, D-mannitol, D-sorbitol, acetate, succinate, L-malate, fumarate, citrate, glycine, L-alanine, L-arginine, L-aspartate, L-glutamate, L-lysine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is PP917502. Strain MBLA0311 (=NIBRARC000513552) was isolated from a solar saltern in Buan, Jeollabuk-do, Korea (35°35′50.7″N 126°36′46.8″E).

## Conflicts of Interest

The author of this paper have no affiliation with any interests and are solely responsible for the paper.

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# Redescription on two calanoid copepods from Korean waters

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*Pseudocalanus minutus* (Clausocalanidae) and *Tortanus* (*Boreotortanus*) *discaudatus* (Tortanidae) are newly added in the Korean species list. The two species were collected from the East Sea under the influence of the southward cold current (North Korean Cold Current). *P. minutus* exhibits the following characteristics: in lateral view cephalosome of *P. minutus* is protruding, anterior rostrum is somewhat angular, and has spiniform processes on posteroventral margins of the second and third pedigerous somites. *T.* (*B.*) *discaudatus* is characterized by the following: the caudal rami of male and female are remarkably asymmetrical, the right caudal ramus is longer than the left one; in female, the outer marginal seta of right caudal ramus is modified into a swollen process; in male, thicker and longer than the left. In this study, we provide detailed descriptions of *P. minutus* and *T.* (*B.*) *discaudatus* due to their insufficient original descriptions, along with taxonomic keys to the species.

Keywords: Clausocalanidae, East Sea, Korean waters, *Pseudocalanus*, Tortanidae, *Tortanus*

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## Introduction

The East Sea is hydrographically distinct in the upper water column: in the coastal region, the East Korean Warm Current characterized by high salinity flows northward along the Korean coast (Hase *et al*., 1999), while the North Korean Cold Current characterized with low salinity flows southward (Huh and Shim, 1987; Ashjian *et al*., 2005). Fronts typically have high productivity and diverse plankton species composition (Bowman and Esaias, 1977). In the East Sea, which has these hydrographic characteristics, we obtained two species, *Pseudocalanus* and *Tortanus*, respectively.

The genus *Pseudocalanus* Boeck (1873) is one of the genera of the family Clausocalanidae Giesbrecht (1892) and is widely distributed in temperate-boreal neritic waters of the Northern Hemisphere (Corkett and McLaren, 1978). On the other hand, the genus *Tortanus* Giesbrecht in Giesbrecht & Schmeil, 1898 which comprises five subgenera (*Acutanus* Ohtsuka, 1992; *Atortus* Ohtsuka, 1992; *Boreotortanus* Ohtsuka & Reid, 1998; *Eutortanus* Smirnov, 1935; *Tortanus* Giesbrecht in Giesbrecht & Schmeil, 1898) is widely distributed throughout the coastal regions of the Indo-Pacific and Northwest Atlantic, but each subgenus has a restricted distribution (Ohtsuka, 1992; Ohtsuka *et al*., 1992). In Korean waters, so far, the genus *Pseudocalanus* has comprised only one species, *P. newmani* Frost, 1989, while the genus *Tortanus* did eight species (*T.* (*Eutortanus*) *derjugini* Smirnov, 1935; *T.* (*E.*) *dextrilobatus* Chen & Zhang, 1965; *T.* (*Tortanus*) *forcipatus* (Giesbrecht, 1889); *T.* (*T.*) *gracilis* (Brady, 1883); *T.* (*E.*) *komachi* Itoh, Ohtsuka & Sato, 2001; *T.* (*Atortus*) *longipes* Brodsky, 1948; *T.* (*E.*) *spinicaudatus* Shen & Bai, 1956; *T.* (*E.*) *vermiculus* Shen, 1955).

In this study, we redescribe *Pseudocalanus minutus*

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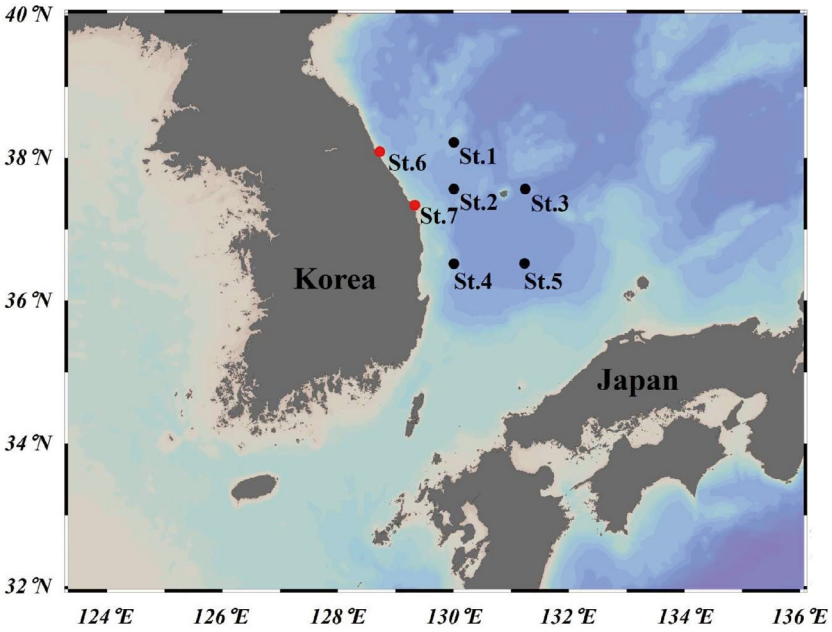



**Fig. 1.** Map of study area showing sampling location.

(Krøyer, 1845) and *Tortanus* (*Boreotortanus*) *discaudatus* (Thompson I.C. & Scott A., 1897) and these species are newly added to the Korean species list. Also, we provide key characteristics for species identification that were not described in previous records.

## Materials and Methods

Zooplankton were collected from coastal waters of the East Sea (Fig. 1), using a conical net (mesh size 200 μm). The samples were fixed to a final concentration of 5% with neutralized formaldehyde for morphological description. *Pseudocalanus minutus* and *Tortanus discaudatus* were sorted out and dissected under a dissecting microscope (SMZ745T; Nikon, Japan) in CMC-10 aqueous mounting medium (Masters, Wood Dale, IL, USA), mounted on slides, and then sealed with high-quality nail varnish. Drawings were generated using a differential interference contrast microscope (ECLIPSE 80i, Nikon, Tokyo, Japan) equipped with a drawing tube and digital pen display (Cintiq 22HD, Wacom, Kazo, Japan). Morphological terminology follows Huys and Boxshall (1991). Voucher specimens were deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon, South Korea.

## Systematic Accounts

Order Calanoida Sars G.O., 1903

Family Clausocalanidae Giesbrecht, 1892

Genus *Pseudocalanus* Boeck, 1873

***Pseudocalanus minutus* (Krøyer, 1845) (Figs. 2–4)**

Korean name: 미세가시헛노벌레 (신칭)

*Calanus minutus* Krøyer, 1845: pl. 41, fig. 4.

*Pseudocalanus gracilis* Sars, 1903: p. 154, suppl. pl. I; Mori, 1937: p. 36, pl. 14, figs. 12–17.

*Pseudocalanus elongatus* Brodsky, 1950: p. 112, fig. 34.

*Pseudocalanus minutus* Wilson, 1932: p. 43, fig. 25; Tanaka, 1956: p. 384; Geletin, 1977: p. 85, fig. 2; Brodsky *et al*., 1983: p. 221, fig. 103; Frost, 1989: p. 530, fig. 4; Samatov, 2001: p. 219, fig. 1; Markhaseva et. al., 2012: p. 65, figs. 7–9.

**Material examined.** 1♀ (MABIK CR00258688) dissected in 12 slides glasses, 37♀♀, East Sea (St. 1), 38°12′36.00″N, 130°0′0.00″E, Feb. 2023; 22♀♀, East Sea (St. 2), 37°33′11.88″N, 130°0′0.00′E, Feb. 2023; 33♀♀, East Sea (St. 3), 37°33′11.88″N, 131°14′35.88″E, Feb. 2023; 8♀♀, East Sea (St. 4), 36°30′18.00″N, 130°0′0.00″E, Feb. 2023; 7♀♀, East Sea (St. 5), 36°30′18.00″N, 131°13′58.80″E, Feb. 2023. 10 individuals were used for length measurement.

**Description.** Female. Body length 1.38–1.89 mm (n = 10). Body elongated; cephalosome somewhat triangular in dorsal view; cephalosome clearly fused with first pedigerous somite; cephalosome protruding anteriad of rostrum, with pair of filaments; fourth and fifth pedigerous somites fused; posterolateral corners of prosome symmetrical and rounded (Fig. 2A, B). Posteroventral margins of second

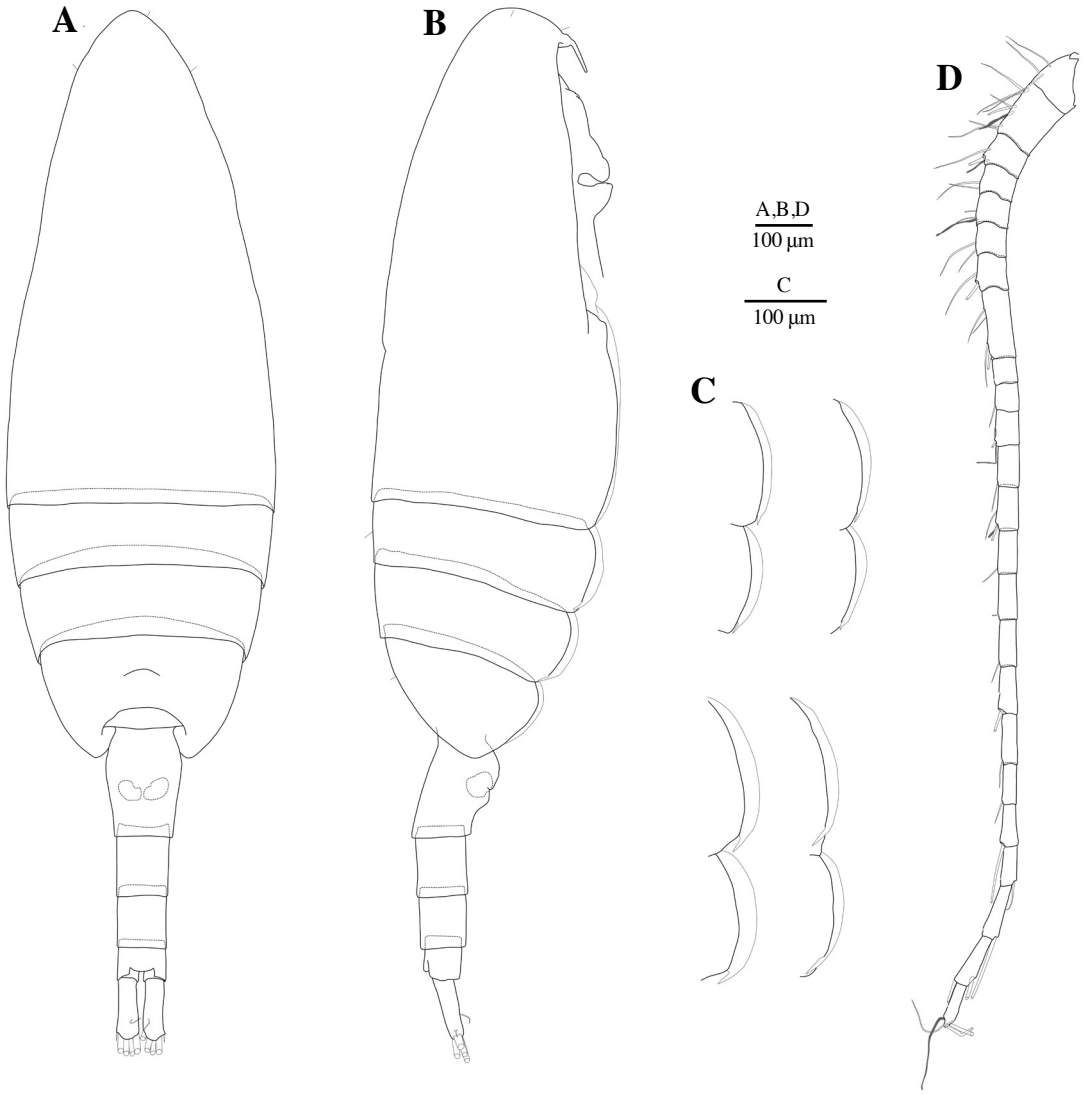



**Fig. 2.** *Pseudocalanus minutus* (Krøyer, 1845), female. A, Habitus, dorsal; B, Habitus, right lateral; C, Posteroventral margins of second and third pedigerous somites; D, Antennule. Scale bars in µm.

and third pedigerous somites with spiniform processes (Fig. 2B). Urosome 4-segmented; genital double somite symmetrical, spermathecae well visible in lateral view; anal segment shortest; anal segment and caudal rami separated (Fig. 2A, B). Caudal rami symmetrical; caudal rami with 1 small dorsolateral seta, 1 ventral seta and 4 setae (Fig. 2A, B).

Antennule symmetrical, 24-segmented; extends beyond anal segment (Fig. 2D). Fusion pattern and setal formula as follows: I-3, II-IV-6 + ae (aesthetasc), V-2 + ae, VI-2, VII-2 + ae, VIII-2, IX-2, X-XI-4 + ae, XII-1, XIII-1, XIV-2 + ae, XV-1, XVI-2 + ae, XVII-1, XVIII-1, XIX-1, XX-2, XXI-1 + ae, XXII-1, XXIII-1, XXIV-1 + 1, XXV-1 + 1, XXVI-1 + 1, XXVII-XXVIII-4 + ae.

Antenna biramous, exopod slightly larger than endopod; coxa with 1 seta; basis with 2 setae; endopod 2-segmented; first endopodal segment with 2 setae, row of posterior spinules on distal; second endopodal segment with 8 + 7 setae, row of posterior spinules on distal lobe; exopod incompletely 8-segmented, with setal formula 1-1, 1-1, 1, 1, 1, 1, 1, 3 (Fig. 3A).

Mandible with coxal gnathobase and biramous palp; gnathobase with about 8 teeth; basis of mandibular palp with 4 setae; endopod 2-segmented; first endopodal segment with 4 setae, row of posterior spinules on distal; second endopodal segment with 11 setae; exopod 5-segmented, setal formula 1, 1, 1, 1, 2 (Fig. 3B).

Maxillule praecoxal arthrite with 14 stout setae; coxal endite with 3 setae, epipodite with 9 setae; basis proximal and distal basal endite with each 4, 5 setae; endopod

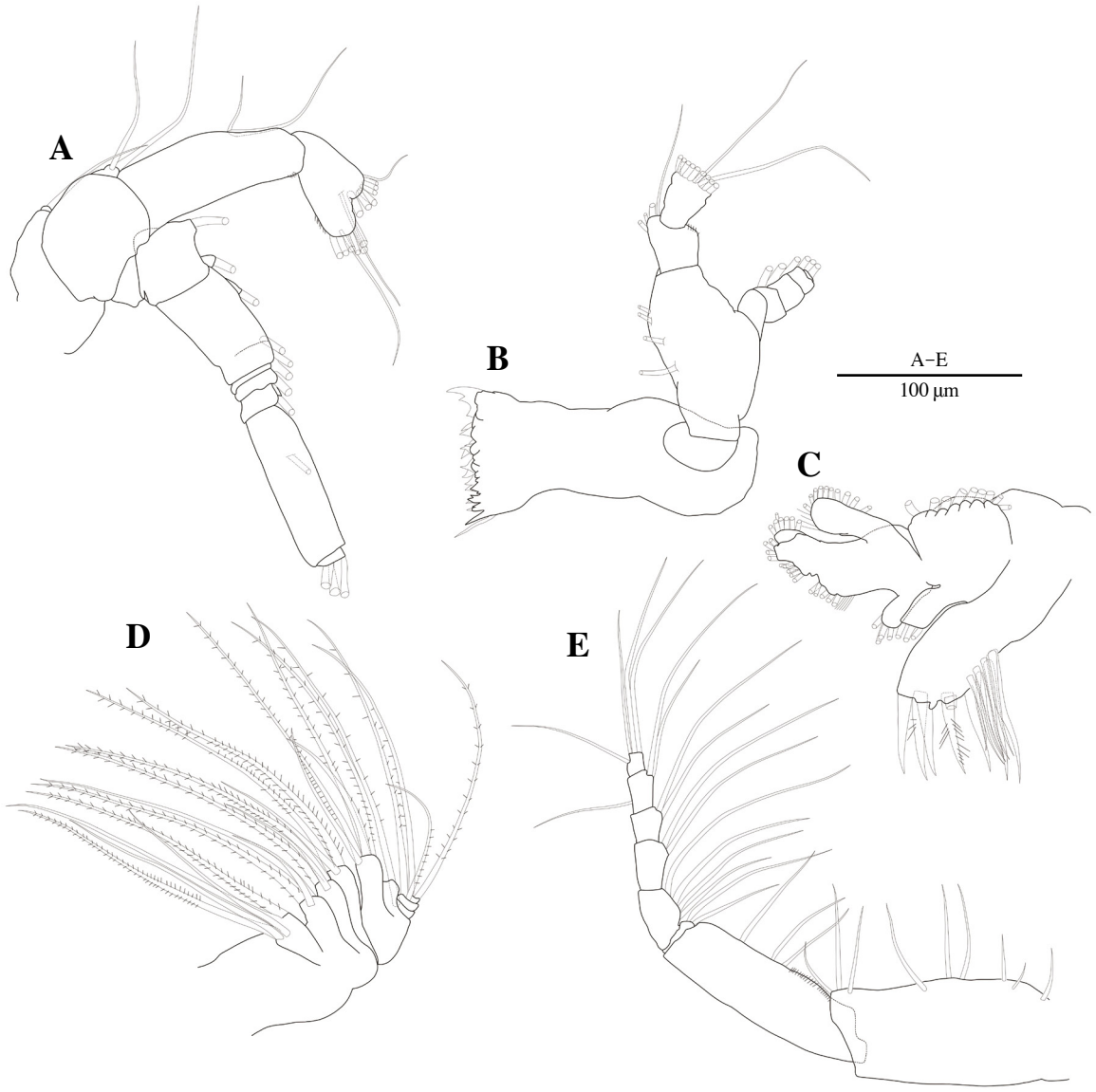



**Fig. 3.** *Pseudocalanus minutus* (Krøyer, 1845), female. A, Antenna; B, Mandible; C, Maxillule; D, Maxilla; E, Maxilliped. Scale bar in μm.

1-segmented, with 16 setae; exopod 1-segmented, with 10 setae (Fig. 3C).

Maxilla with praecoxa and coxa partly fused, endites setal formula 5, 3, 3, 3; basis endite with 4 setae; endopod 4-segmented, with 5 setae (Fig. 3D).

Maxilliped, syncoxa with endite setal formula 1, 2, 3, 3; basis with 3 setae, row of posterior spinules on proximal; 2 setae on incompletely incorporated first endopodal segment; endopod 5-segmented, segmental setal formula 4, 2, 2, 2 + 1, 4 (Fig. 3E).

Legs 1 to 4 biramous: leg 1 with 1-segmented endopod and 3-segmented exopod, leg 2 with 2-segmented endopod and 3-segmented exopod, legs 3 to 4 with 3-segmented endopod and exopod (Fig. 4A–D). Coxa of leg 1 without seta; basis with curved medial distal seta (Fig. 4A). Coxae of legs 2 to 4 with inner marginal seta (Fig. 4B–D). Seta and spine formula of legs 1 to 4 as follows (spines, Roman numerals; setae, Arabic numerals):

| | Coxa | Basis | Exopodal segment | Endopodal segment |
|---|---|---|---|---|
| Leg 1 | 0-0 | 0-1 | I-0; I-1; I,I,3 | 0,2,3 |
| Leg 2 | 0-1 | 0-0 | I-1; I-1; III,I,4 | 0-1; 1,2,2 |
| Leg 3 | 0-1 | 0-0 | I-1; I-1; III,I,4 | 0-1; 0-1; 1,2,2 |
| Leg 4 | 0-1 | 0-0 | I-1; I-1; III,I,4 | 0-1; 0-1; 1,2,2 |

Fifth leg of female absent.

Male not found in present study.

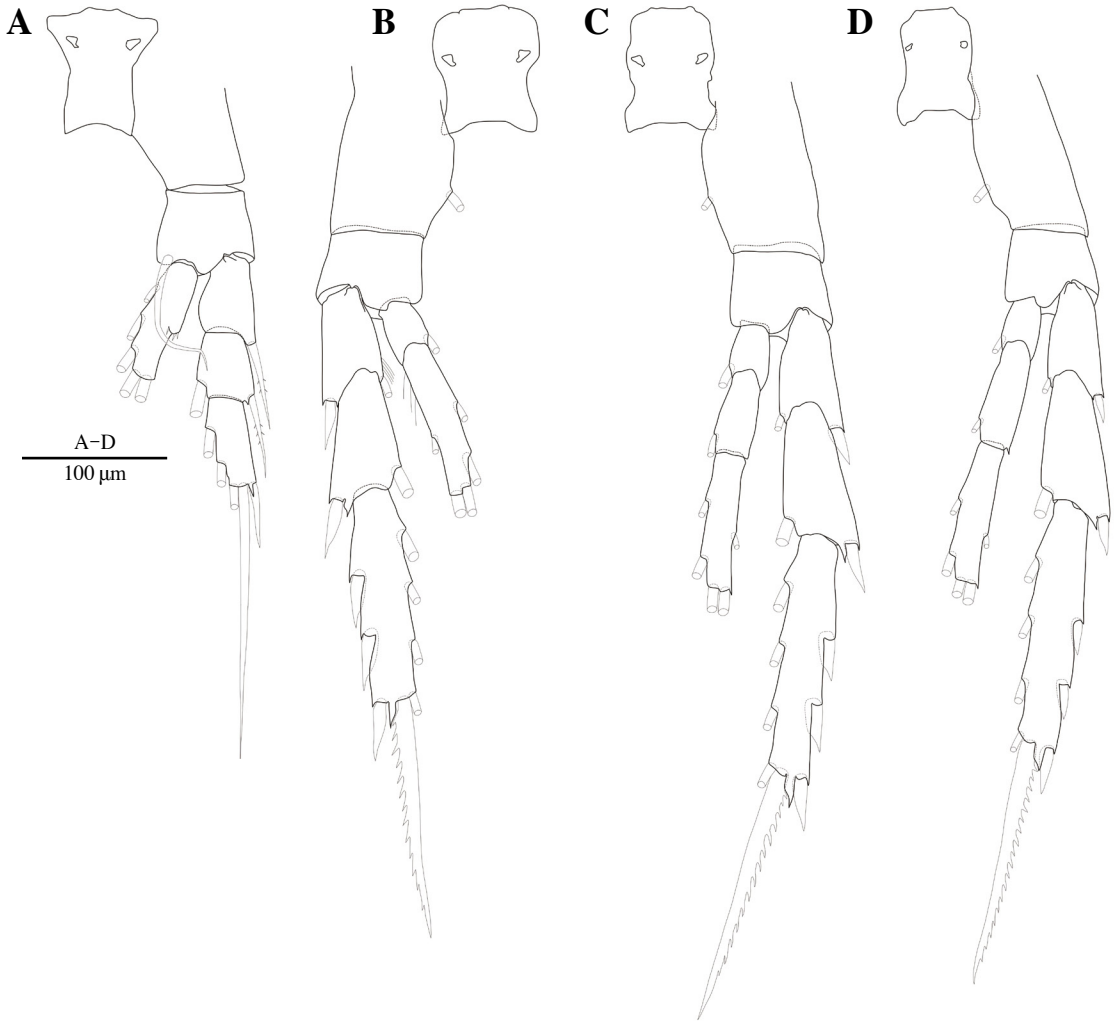



**Fig. 4.** *Pseudocalanus minutus* (Krøyer, 1845), female. A, Leg 1; B, Leg 2; C, Leg 3; D, Leg 4. Scale bar in μm.

**Distribution.** *Pseudocalanus minutus* was recorded in Norwegian coast (Sars, 1903), North Atlantic Ocean (Wilson, 1932), North Pacific Ocean (Mori, 1937), Sagami Bay (Tanaka, 1956), Black Sea (Brodsky, 1950), Northwestern Pacific (Geletin, 1977; Samatov, 2001), and White Sea (Markhaseva *et al*., 2012).

**Remarks.** *Pseudocalanus minutus* has wide geographical distribution, commonly co-existing with two or three other species of the genus. *P. minutus* and *P. newmani* are genus *Pseudocalanus* species that occur in the temperate-boreal coastal waters of the western Pacific. Compared to other *Pseudocalanus* species, the urosome of *P. minutus* tends to be shorter than its cephalosome (Frost, 1989). *P. minutus* exhibits the following morphological differences from *P. newmani*: 1) cephalosome of *P. minutus* in lateral view is protruding anteriad of rostrum and somewhat angular, but *P. newmani* is scarcely protruding anteriad of rostrum; 2) spiniform processes are present on posteroventral margins of second and third pedigerous somites in *P. minutus*, but not present in *P. newmani* (in *P. newmani*, spiniform processes may sometimes be present (Frost, 1989)). In *P. minutus*, the spiniform processes on the posteroventral margins of the second and third pedigerous somites show morphological variation. Spiniform processes may be long, short or absent (Markhaseva, 2012). In Korean waters, the long or short spiniform processes described by Markhaseva (2012) were also confirmed (Fig. 2C). However, *P. minutus* individuals without spiniform processes were not collected in Korean waters.

### Key to species of genus *Pseudocalanus* in Korean waters

1. Posteroventral margins of second and third pedigerous somites with spiniform processes·····*P. minutus*
2. Posteroventral margins of second and third pediger-

ous somites without spiniform processes ··· *P. newmani*

Order Calanoida Sars G.O., 1903
Family Tortanidae Sars G.O., 1902
Genus *Tortanus* Giesbrecht & Schmeil, 1898

***Tortanus* (*Boreotortanus*) *discaudatus***
**(Thompson I. C. & Scott A., 1897) (Figs. 5–8)**
Korean name: 한쪽꼬리부푼긴노벌레 (신칭)
*Corynura discaudata* Thompson and Scott, 1897: p. 80, pl. VI, figs. 1–11, pl. VII, figs. 1–2.
*Corynura bumpusii* Wheeler, 1901: p. 184, fig. 21.
*Tortanus discaudatus* Esterly, 1924: p. 109, fig. P; Brodsky, 1950: p. 429, fig. 304; Anraku and Omori, 1963: p. 119, figs. 5–10; Gardner and Szabo, 1982: p. 424; Ohtsuka and Reid, 1998: p. 776, figs. 1–4.

**Material examined.** 1♀ (MABIK CR00258690) dissected in 12 slides glasses, 1♂ (MABIK CR00258689) dissected in 12 slides glasses, 14♀♀, 12♂♂, East Sea (St. 6), 38°04′50.20″N, 128°42′32.90″E, Jul. 2022; 3♀♀, East Sea (St. 7), 37°19′21.10″N, 129°18′56.80″E, Jul. 2022. 10 individuals were used for length measurement.

**Description.** Male. Body length 1.74–1.87 mm (n = 10). Body elongated; cephalosome clearly separated from first pedigerous somite; large nauplius eye situated at anterior margin of cephalosome; rostrum absent; fourth and fifth pedigerous somites separated; posterolateral

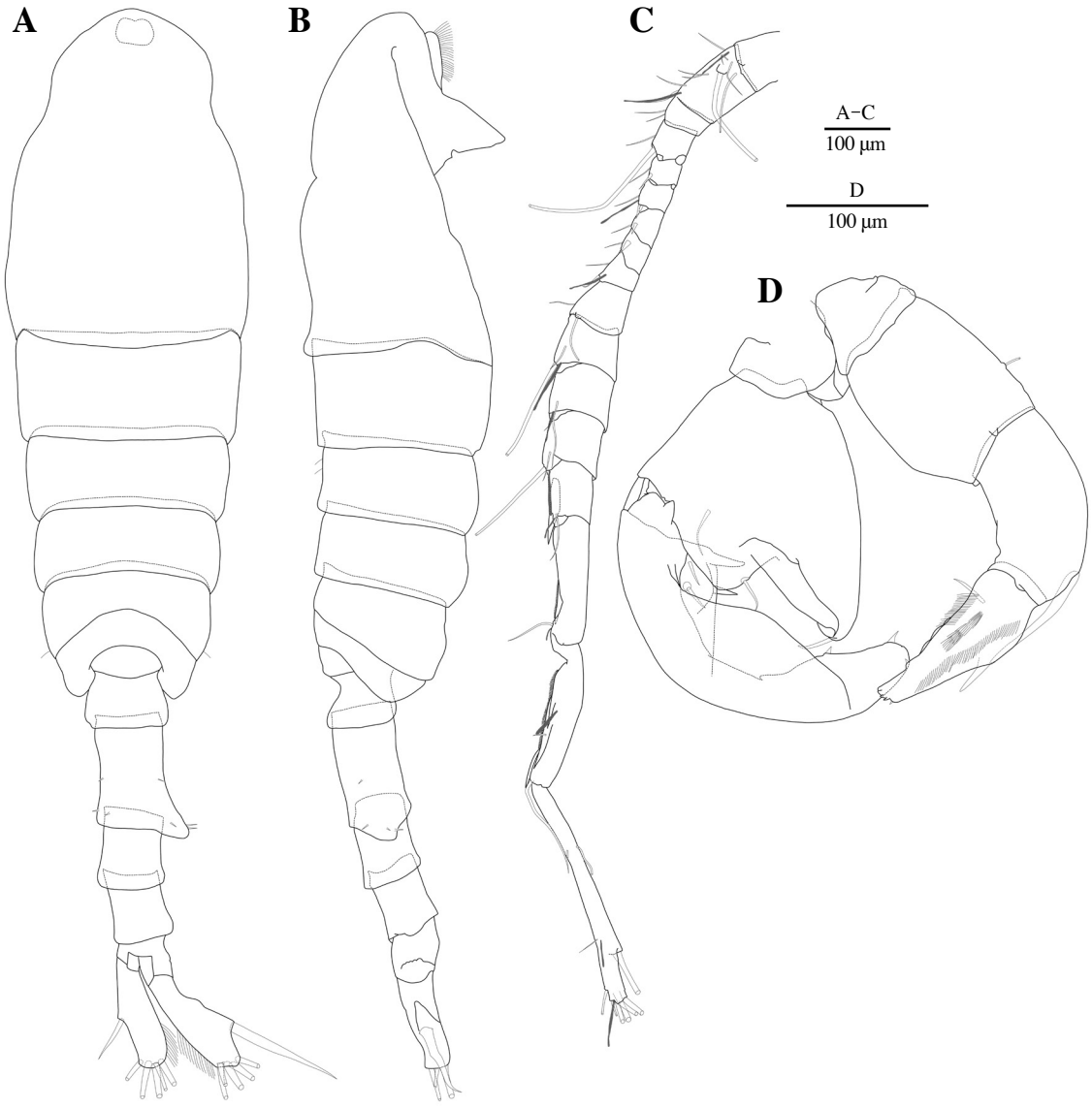



**Fig. 5.** *Tortanus* (*Boreotortanus*) *discaudatus* (Thompson I. C. & Scott A., 1897), male. A, Habitus, dorsal; B, Habitus, right lateral; C, Right antennule, posterior; D, Leg 5. Scale bars in μm.

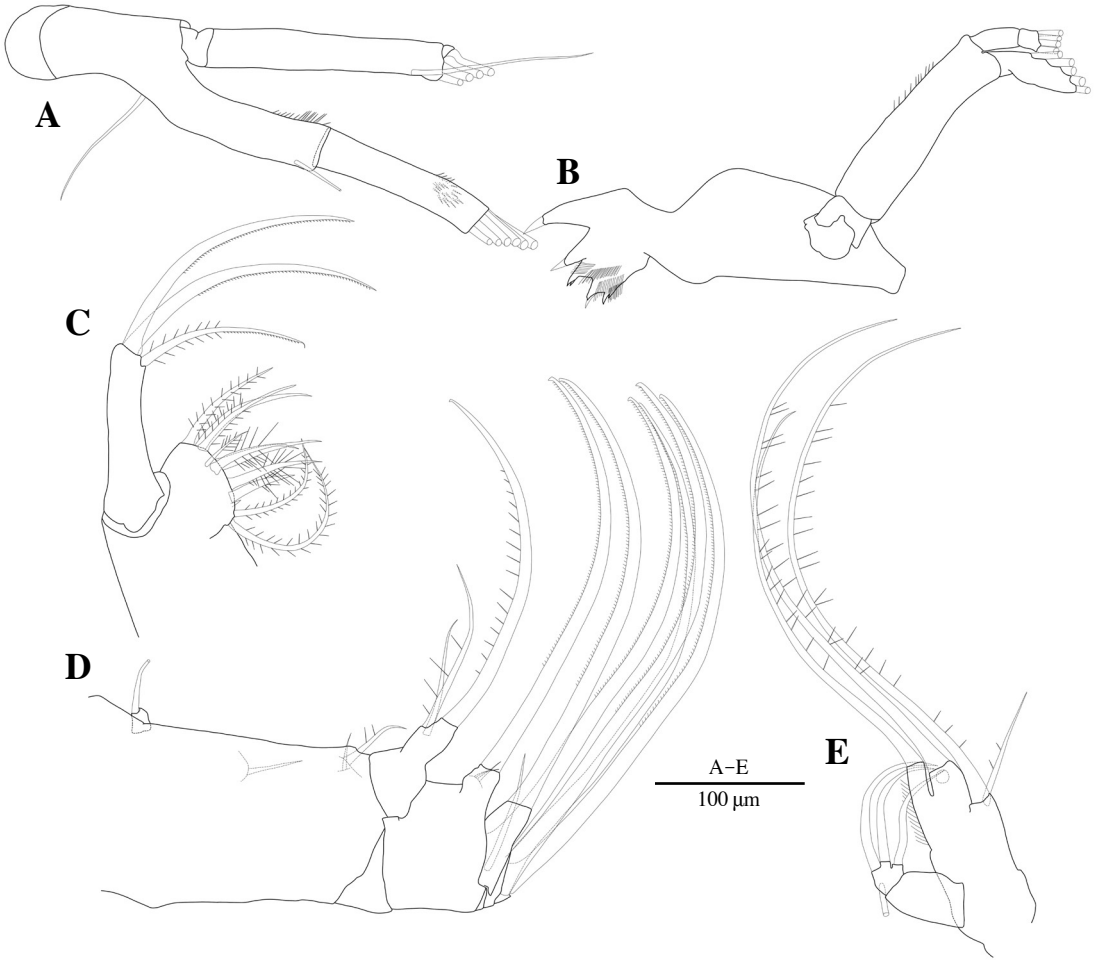



**Fig. 6.** *Tortanus* (*Boreotortanus*) *discaudatus* (Thompson I. C. & Scott A., 1897), male. A, Antenna; B, Mandible; C, Maxillule; D, Maxilla; E, Maxilliped. Scale bar in µm.

corners of fifth pedigerous somite rounded (Fig. 5A, B). Urosome 5-segmented; second urosomite produced laterally at right posterior corner; third urosomite and anal segment slightly asymmetrical; anal segment and caudal rami separated; caudal rami asymmetrical; right caudal ramus longer than left caudal ramus; right caudal ramus curved outward, with sclerotized right marginal seta (Fig. 5A, B).

Right antennule with segments II-XIV completely or incompletely fused (Fig. 5C). Fusion pattern and setal formula as follows: I-1, II-V-5 + ae, VI-2, VII-2 + ae, VIII-2, IX-2, X-2, XI-2 + ae, XII-2, XIII-2, XIV-2 + ae, XV-2, XVI-2 + ae, XVII-2 + ae, XVIII-2 + ae, XIX-1 + ae + 1 process, XX-1 + ae + 1 process, XXI-XXIII-2 + ae + 2 processes, XXIV-XXVIII-10 + 2ae.

Antenna biramous, endopod larger than exopod; coxa unarmed; basis fused with first endopodal segment, armed with 1 seta; endopod 2-segmented; proximal segment with 1 seta, row of posterior spinules on distal; second endopodal segment with 6 setae, row of spinules on distal; exopod 3-segmented; first exopodal segment unarmed; second exopodal segment with 3 setae; third exopodal segment with 2 setae (Fig. 6A).

Mandible with coxal gnathobase and biramous palp; gnathobase with 5 teeth; basis with row of spinules on distal; endopod 2-segmented; first endopodal segment without seta; second endopodal segment with 4 setae; exopod incompletely fused, with 5 setae (Fig. 6B).

Maxillule: basis, endopod and exopod absent; praecoxal arthrite with 10 setae and 1 minute seta, and 1 spinous process; coxal endite with 3 clawlike setae (Fig. 6C).

Maxilla developed; proximal and distal praecoxal endites with 1 and 2 setae; proximal and distal coxal endites with 2 and 3 setae; basal endite with 1 long clawlike and 2 short setae; endopod with 5 long clawlike and 1 rudimentary seta (Fig. 6D).

Maxilliped with setal formula of praecoxal and coxal

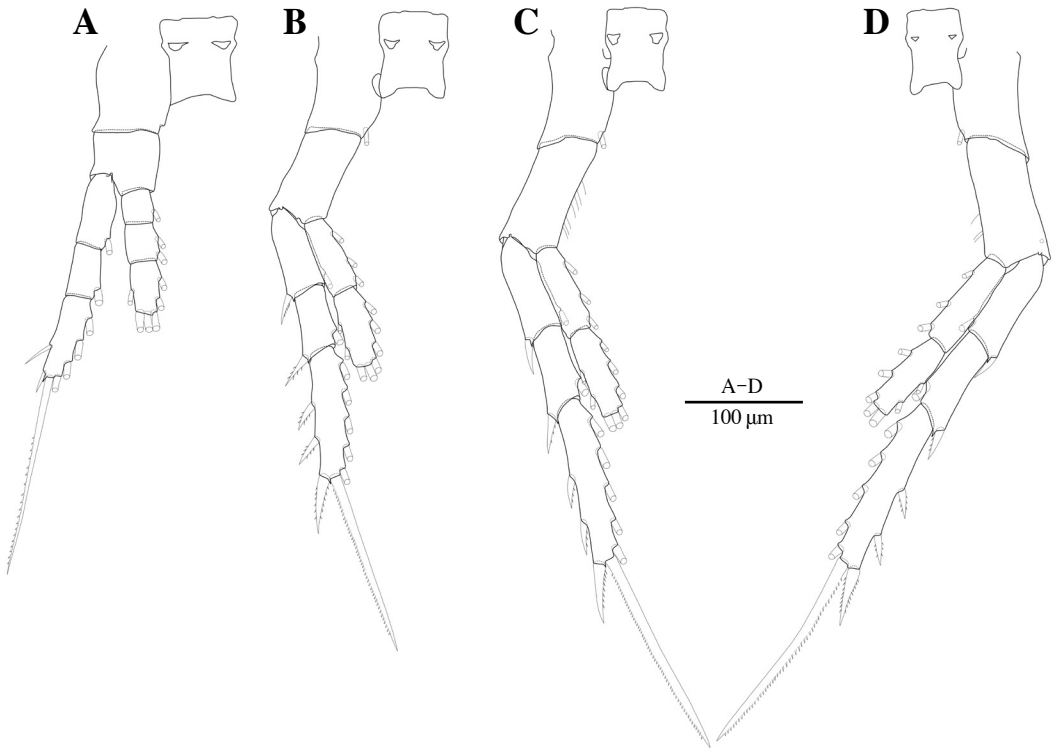



**Fig. 7.** *Tortanus* (*Boreotortanus*) *discaudatus* (Thompson I. C. & Scott A., 1897), male. A, Leg 1; B, Leg 2; C, Leg 3; D, Leg 4. Scale bar in μm.

endites: 0, 2, 2, 1. Basis unarmed; endopod 1-segmented, with 3 + 1 setae (Fig. 6E).

Legs 1 to 4 biramous: leg 1 with 3-segmented endopod and 3-segmented exopod, leg 2 to 4 with 2-segmented endopod and 3-segmented exopod (Fig. 7A–D). Coxae of legs 1 to 4 with inner marginal seta; basis of leg 4 with outer marginal seta (Fig. 7A–D). Seta and spine formula of legs 1 to 4 as follows (spines, Roman numerals; setae, Arabic numerals):

| | Coxa | Basis | Exopodal segment | Endopodal segment |
|---|---|---|---|---|
| Leg 1 | 0-1 | 0-0 | 0-1; 0-1; II,1,4 | 0-1; 0-2; 1,2,3 |
| Leg 2 | 0-1 | 0-0 | I-1; I-1; III,I,5 | 0-3; 2,2,4 |
| Leg 3 | 0-1 | 0-0 | I-1; I-1; III,I,5 | 0-3; 2,2,4 |
| Leg 4 | 0-1 | 1-0 | I-1; I-1; III,I,5 | 0-3; 2,2,3 |

Fifth leg with right and left coxal segments fused to form common base. Basis of right leg expanded inward; basis with 2 triangular processes on distal and inner margin, each with minute seta; basis with 1 seta on posterior surface; exopod curved inward, proximal with 1 pointed process, 2 setae and short spine; distal of exopod with 2 spines. Basis of left leg with 1 seta on outer margin; exopod 2-segmented; first exopodal segment with 1 spiniform seta on distal outer margin; second exopodal segment tapering distally, with 1 outer spine, 1 inner seta and 1 distal spine; fringed with setules of various length (Fig. 5D).

Female: Body length 1.86–2.07 mm (n = 10). Similar male habitus except urosome. Fourth and fifth pedigerous somites separated; posterolateral corners of fifth pedigerous somite triangular process pointing backward (Fig. 8A, B). Urosome 3-segmented, asymmetrical; second urosomite and anal segment adorned with spinules on right side; anal segment and caudal rami fused; right caudal ramus thicker and longer than left caudal ramus, right marginal seta modified as large acute process (Fig. 8A, B).

Antennule symmetrical, 19-segmented; segments II-XIV almost completely or incompletely fused (Fig. 8C). Fusion pattern and setal formula as follows: I-1, II-IX-9 + 2ae (aesthetasc), X-2, XI-2 + ae, XII-2, XIII-2, XIV-2 + ae, XV-2 + ae, XVI-2 + ae, XVII-2 + ae, XVIII-2 + ae, XIX-2 + ae, XX-2, XXI-2 + ae, XXII-1, XXIII-1, XXIV-1 + 1, XXV-1 + 1 + ae, XXVI-XXVIII-6 + ae.

Fifth leg 3-segmented; both coxae and intercoxal sclerite fused to form base; basis with 1 outer marginal seta; endopod absent; exopod 1-segmented, curved inwardly, left leg longer than right leg; outer margin with 4 minute spinules (Fig. 8D).

**Distribution.** *Tortanus discaudatus* was recorded in the

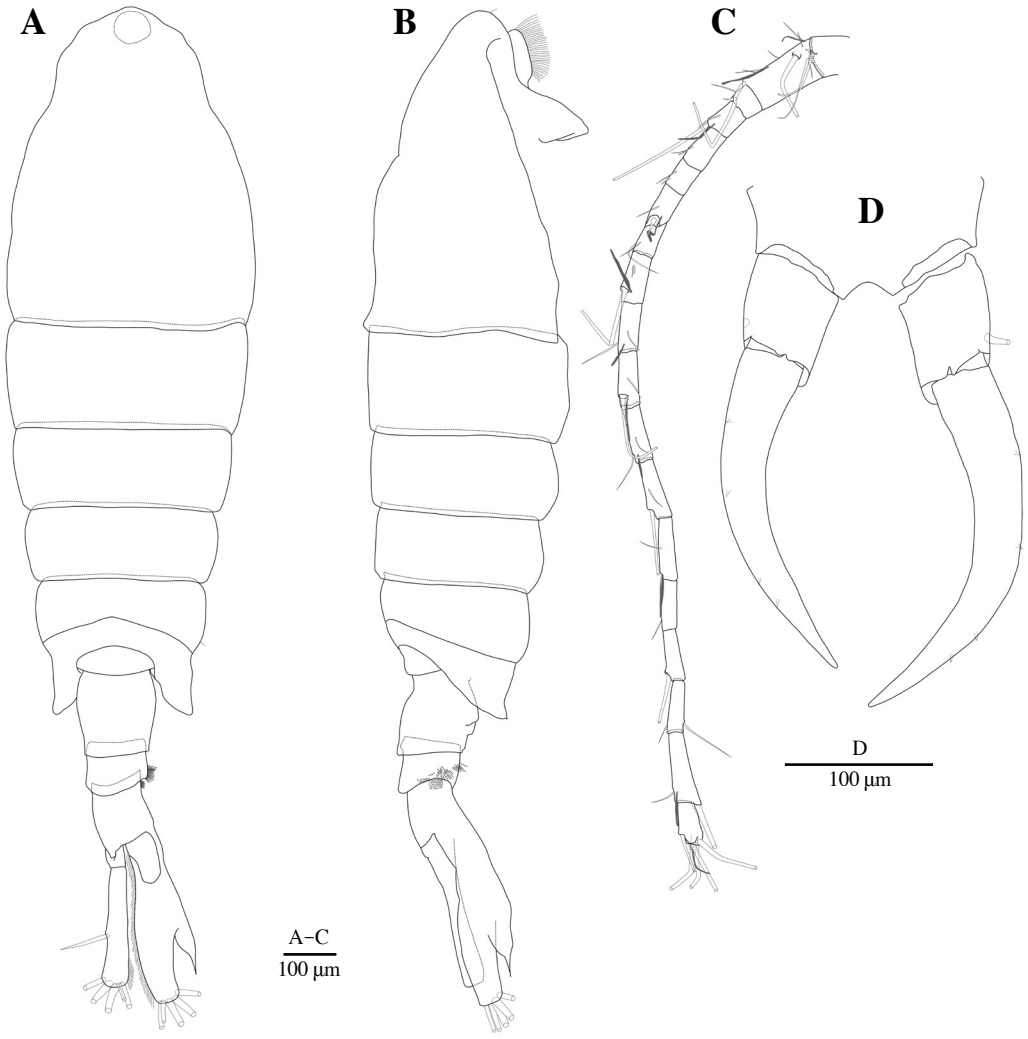



**Fig. 8.** *Tortanus* (*Boreotortanus*) *discaudatus* (Thompson I. C. & Scott A., 1897), female. A, Habitus, dorsal; B, Habitus, right lateral; C, Antennule, posterior; D, Leg 5. Scale bars in μm.

Woods Hole (Wheeler, 1901), San Francisco Bay (Esterly, 1924), Chukchi Sea, Bering Sea, Sea of Japan, shores of North Korea, Sea of Okhotsk, North Sea, Atlantic shore of North America (Brodsky, 1950), British Columbia coast (Gardner and Szabo, 1982).

**Remarks.** Ohtsuka and Reid (1998) established a new subgenus, *Boreotortanus* for *T. discaudatus*. The genus *Boreotortanus* is distinguished from other subgenera by the following morphological characteristics (Ohtsuka and Reid, 1998): 1) the caudal rami of males and females are remarkably asymmetrical, with the right caudal ramus being longer than the left caudal ramus. In female, the outer marginal seta of right caudal ramus is modified into a swollen process, and in male, it is thicker and longer than the left; 2) the characteristics of antennule segmentation and armature elements represent the most primitive state among all subgenera; 3) maxillule praecoxal arthrite with 11 setae; 4) second endopodal segment of leg 4 with 7 setae; and 5) structure of both exopods of male leg 5. The Korean specimen is consistent with the description of previous records, but there are some differences. In the illustration by Ohtsuka and Reid (1998), there are 3 minute outer spinules on the exopodal segment of the female fifth leg, but in the Korean specimen, there are 4 minute outer spinules. In males, the exopodal segment of fifth leg has bifurcate process on proximal inner margin, but in the Korean specimen, there is a pointed process on the proximal inner margin. *T.* (*B.*) *discaudatus* is distributed in the cold waters of the northwestern and northeastern Pacific coasts, extending into the southern part of the Arctic Sea and the northwestern Atlantic (Ohtsuka and Reid, 1998).

**Key to species of genus *Tortanus* in Korean waters**

1. Antennule asymmetrical····· 2 (Male)
1a. Antennule symmetrical····· 10 (Female)
2. Outer marginal seta of right caudal ramus much thicker and longer than left; second endopodal segment of leg 4 with 7 setae····· ····· *Tortanus* (*Boreotortanus*), *T.* (*B.*) *discaudatus*
2a. Caudal rami of male not as above; second endopodal segment of leg 4 with 6 setae ·····3
3. Leg 1 endopod 3-segmented, terminal endopodal segments of legs 2 and 3 with 8 setae ····· ····· *Tortanus* (*Eutortanus*), 4
3a. Leg 1 endopod 3-segmented, terminal endopodal segments of legs 2 and 3 with 6–7 setae ·····8
4. Fifth leg right exopod with pointed process on about 1/3 of exopodal segment···· *T.* (*E.*) *vermiculus*
4a. Fifth leg right exopod without pointed process of exopodal segment·····5
5. Fifth leg right exopod with fingerform process, wave on outer margin ····· *T.* (*E.*) *spinicaudatus*
5a. Fifth leg right exopod without fingerform process ···· ·····6
6. Fifth leg right basis with inwards long isosceles triangular proces····· *T.* (*E.*) *komachi*
6a. Fifth leg right basis without inwards long isosceles triangular process ·····7
7. Fifth leg right exopod relatively wider····· ····· *T.* (*E.*) *dextrilobatus*
7a. Fifth leg right exopod relatively narrower ····· ····· *T.* (*E.*) *derjugini*
8. Fourth and fifth pedigerous somites fused; terminal exopodal segment of leg 1 with 1 spine; terminal endopodal segments with 6 setae ····· ····· *Tortanus* (*Atortus*), *T.* (*A.*) *longipes*
8a. Fourth and fifth pedigerous somites separated; terminal exopodal segment of leg 1 with 2 spines; terminal endopodal segments with 7 setae····· ····· *Tortanus* (*Tortanus*), 9
9. Fifth leg right exopod with process on inner margin ····· *T.* (*T.*) *gracilis*
9a. Fifth leg right exopod without process on inner margin····· *T.* (*T.*) *forcipatus*
10. Outer marginal seta of right caudal ramus much thicker and longer than left; second endopodal segment of leg 4 with 7 setae····· ····· *Tortanus* (*Boreotortanus*), *T.* (*B.*) *discaudatus*
10a. Caudal rami of male not as above; second endopodal segment of leg 4 with 6 setae ·····11
11. Leg 1 endopod 3-segmented, terminal endopodal segments of legs 2 and 3 with 8 setae····· ····· *Tortanus* (*Eutortanus*), 12
11a. Leg 1 endopod 3-segmented, terminal endopodal segments of legs 2 and 3 with 6–7 setae ·····16
12. Right caudal ramus uneven dorsolateral process on anterior right side····· *T.* (*E.*) *vermiculus*
12a. Right caudal ramus unarmed on right side·····13
13. Anal segment with spiniform process on right side · ····· *T.* (*E.*) *spinicaudatus*
13a. Anal segment without spiniform process on right side·····14
14. Anal segment and caudal rami completely fused ····· ····· *T.* (*E.*) *komachi*
14a. Anal segment and caudal rami completely separated ·····15
15. Anal segment with anterolateral process ····· ····· *T.* (*E.*) *dextrilobatus*
15a. Anal segment without anterolateral process····· ····· *T.* (*E.*) *derjugini*
16. Fourth and fifth pedigerous somites fused; terminal exopodal segment of leg 1 with 1 spine; terminal endopodal segments with 6 setae ····· ····· *Tortanus* (*Atortus*), *T.* (*A.*) *longipes*
16a. Fourth and fifth pedigerous somites separated; terminal exopodal segment of leg 1 with 2 spines; terminal endopodal segments with 7 setae····· ····· *Tortanus* (*Tortanus*), 17
17. Right fifth leg about twice as long as left leg····· ····· *T.* (*T.*) *forcipatus*
17a. Right fifth leg slightly longer than left leg····· ····· *T.* (*T.*) *gracilis*

## Conflicts of Interest

The author of this paper have no affiliation with any interests and is solely responsible for the paper.

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# The application of Nanopore long-read sequencing to reveal an exceptionally large tandem repeat region in the *Remanea naksanensis* mitochondrial genome

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Animal mitochondrial genomes are widely used as genetic markers in evolutionary studies such as systematics, species identification, and population genetics. Mitochondrial DNA is generally compact, encodes 37 genes, and typically contains a major non-coding region involved in replication and transcription. This region often harbors repetitive elements that hinder complete mitochondrial genome reconstruction using primer walking or short-read sequencing. In the present study, we first attempted to reconstruct the mitochondrial genome of *Remanea naksanensis* using Illumina short reads generated from three long-range PCR amplicons. However, the assemblies remained discontinuous in two putative non-coding regions. We subsequently employed Oxford Nanopore long-read sequencing in order to span these unresolved regions, and short reads were used for polishing. The resulting circular mitochondrial genome was found to be approximately 22.9 kb and contained two copies of an inverted repeat separated by a 9-bp spacer and an 8.2-kb tandem repeat array that could not be recovered from the short reads alone. This study provides an important mitochondrial genome resource for harpacticoid copepods, and a hybrid long- and short-read workflow for assembling repeat-rich mitochondrial genomes.

Keywords: Harpacticoida, long-read sequencing, mitogenome

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## Introduction

Mitochondrial DNA has been widely used as a molecular marker in animal evolutionary studies, including systematics, species identification, phylogeography, and population genetics (Avise, 1994; Dasalle *et al*., 2017). With a few exceptions, it is a closed circular molecule, approximately 15−20 kb in length. Many animal mitochondrial genomes contain a major non-coding region, generally termed the "control region", that includes regulatory elements for replication and transcription (Boore, 1999). This region often harbors repetitive sequences and segmental duplications (Gan *et al*., 2019; Formenti *et al*., 2021), which may contribute to mitogenome size variation (Savolainen *et al*., 2000; Wang *et al*., 2015) and can also hinder the assembly of complete mitochondrial genomes (Le *et al*., 2001; Gan *et al*., 2019).

A common traditional strategy for sequencing metazoan mitochondrial genomes involves long-range PCR coupled with primer walking (Dotson and Beard, 2001; Machida *et al*., 2002; Miller *et al*., 2004; Burger *et al*., 2007). Although primer walking is highly specific and yields

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accurate sequences, it is relatively slow and expensive (Cameron, 2014). Recently, long-range PCR amplicons have been sequenced using next-generation (short-read) sequencing platforms rather than primer walking (Cameron, 2014; Gan *et al*., 2019). This time- and cost-efficient approach enables the high-coverage assembly of complete mitochondrial genomes (Shendure *et al*., 2017; Kinkar *et al*., 2020). However, short-read data often struggle to resolve the repetitive elements that exceed individual read lengths and lack unique flanking sequences on both sides (Sharbrough *et al*., 2023). Third-generation (long-read) sequencing (e.g., Oxford Nanopore technology) can generate reads longer than 10 kb without being affected by nucleotide composition (Rang *et al*. 2018; Gan *et al*., 2019). These extended reads can span several kilobases, revealing large repeat structures that cannot be assembled from short-read data (Shendure *et al*., 2017; Blom, 2021).

Copepoda is a small crustacean taxon that plays a pivotal role in aquatic ecosystems as a vital link between primary producers and higher trophic levels (Huys and Boxshall, 1991). With advances in sequencing technologies, the number of published copepod mitochondrial genomes has increased; however, the available data still represent only a small fraction of their extensive species diversity. In particular, although harpacticoids comprise about 30% of all known copepods (ca. 4,500 species; Walter and Boxshall, 2025) and are a key component of benthic communities (Wells, 1988; Boxshall and Halsey, 2004), complete harpacticoid mitochondrial genomes have been reported for only six species in three genera to date (*Amphiascoides atopus*, *Ikanecator primus*, *Tigriopus californicus*, *T. japonicus*, *T. kingsejongensis*, and *T. west*; Machida *et al*., 2002; Jung *et al*., 2006; Burton *et al*., 2007; Easton *et al*., 2014; Hwang *et al*., 2019; Zoral *et al*., 2024).

Herein, we present the complete mitochondrial genome of the harpacticoid copepod *Remanea naksanensis* Back, Lee & Huys, 2011. Initially, we attempted to assemble the mitochondrial genome using only the Illumina short reads generated from three long-range PCR amplicons; however, two putative non-coding regions remained unresolved. To overcome this limitation, we generated Oxford Nanopore long reads from the same long-range PCR products, enabling complete circularization and annotation of the mitogenome. We characterized two long non-coding regions, including two inverted-repeat copies separated by a 9-bp spacer, and an exceptionally large tandem repeat array with a terminal truncated repeat unit. Our study provides a validated mitochondrial genomic resource for harpacticoid copepods and describes the repeat-rich mitochondrial non-coding regions in harpacticoid copepods.

## Materials and Methods

### Sample collection and DNA extraction

A meiofaunal sample was collected on June 23, 2020, from a Karaman-Chappuis hole at the estuary of Munam Stream in Goseong, Korea (38°17′47″N, 128°32′56″E), with a fine-sand substrate. Paramesochrid individuals were isolated under a stereomicroscope (SApo, Leica microsystems, Wetzlar, Germany) and genomic DNA was extracted from each of several isolated individuals using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The remaining exoskeletons were retained for morphological species identification based on previous taxonomic studies (e.g., Huys *et al*., 1996; Wells, 2007).

### Conventional amplification

*Remanea naksanensis*, the most abundant species in the meiofaunal sample, was selected as the target for mitochondrial genome amplification. Three mitochondrial gene fragments, cytochrome c oxidase subunit I (*COXI*), small subunit ribosomal RNA gene (*srRNA*), and cytochrome b (*COB*), were amplified from a single individual as anchor regions for subsequent long-PCR. Fragment amplification was performed using Premix EX Taq HS (Takara Bio, Shiga, Japan) with the primer pairs listed in Table 1. PCR products were sequenced using the same primers by a commercial Sanger sequencing service provider (Bionics, Seoul, Korea). All of the newly obtained sequences were assigned GenBank accession numbers (*COXI*: PX884362; *srRNA*: PZ024137; *COB*: PX892115).

### Long-PCR and sequencing

Species-specific primer sets were designed to amplify the entire mitochondrial genome in three overlapping long-range PCR fragments based on anchor sequences (Table 1). Long-range PCR was performed in 25 μL reaction volumes using the same DNA template as in the anchor amplification, each primer pair, and LongAmp HS Taq Master Mix (New England Biolabs, Ipswich, MA, USA). The cycling conditions followed the manufacturer's recommendations, except that the extension step per cycle was set to 7−12 min depending on the estimated amplicon length, and the final extension step was 10 min. The PCR products were examined on a 1% agarose gel to confirm the expected amplicon size. The amplicons were sent to Bionics (Seoul, Korea) for paired-end sequencing on an Illumina MiSeq platform.

Despite the use of multiple strategies, including de novo assembly using SPAdes (v3.15.3; Prjibelski *et al*., 2020) and seed-based assembly using NOVOPlasty

**Table 1.** Information of the PCR primers used in this study.

| Name | Sequences (5′-3′) | Tm (°C) | Reference |
|---|---|---|---|
| *COXI* | | | |
| jgLCO1490 | TITCIACIAAYCAYAARGAYATTGG | 54.6 | Geller et al., 2013 |
| cop-coi-2189 | GGGTGACCAAAAAATCARAA | 55.2 | Bucklin et al., 2010 |
| *COB* | | | |
| ucytb151F | TGTGGRGCNACYGTWATYACTAA | 60.1 | Merritt et al., 1998 |
| ucytb270R | AANAGGAARTAYCAYTCNGGYTG | 60.0 | Merritt et al., 1998 |
| *srRNA* | | | |
| L13337-12S | YCTACTWTGYTACGACTTATCTC | 54.1 | Machida et al., 2002 |
| H13845-12S | GTGCCAGCAGCTGCGGTTA | 64.7 | Machida et al., 2002 |
| *COX1–COB* | | | |
| sed115-01-01 | AGCTTGATCAGGAATAATTGGCACAGGAC | 64.3 | This study |
| sed115-01-03 | GAGGAGGGTTTGCTGTTGACAATGCTACT | 66.1 | This study |
| *COB–srRNA* | | | |
| sed115-01-04 | CAGGAGTGACTAAAGGATTAGCTGGGATA | 63.4 | This study |
| sed115-01-06 | GGGTTAGAAGCCAGGTATTACTGAAGTAA | 61.5 | This study |
| *srRNA–COX1* | | | |
| sed115-01-05 | GAGGATTGTCTTCCTAACTCAACTTTCTC | 61.3 | This study |
| sed115-01-02 | AAGAGAAGAATAGCCGTGACTAGGACAGA | 64.1 | This study |

(v4.3.1; Dierckxsens *et al*., 2016), short-read assemblies remained discontinuous because of unresolved gaps within the two putative non-coding regions. In order to resolve these regions, additional long-range PCR amplifications were performed, and the amplicons were then sequenced on a MinION Mk1B (Oxford Nanopore Technologies, Oxford, UK). Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109) together with Native Barcoding Expansion 1-12 (EXP-NBD104). Equimolar amounts of barcoded libraries were pooled and loaded onto an R9.4.1 flowcell. Library preparation and sequencing steps were performed according to the manufacturer's instructions (NBA_9093_v109_revO_12NOV2019). Raw signals were basecalled with a super-accuracy model and demultiplexed using Guppy (v6.5.7; Oxford Nanopore Technologies, Oxford, UK).

### Assembly and annotation

The read length distribution was initially assessed using NanoPlot (v1.46.2; de Coster and Rademakers, 2023) with 100-bp bins. The basecalled reads for each amplicon were filtered using fastplong (v0.4.1; Chen *et al*., 2018) to retain reads within the modal read length bin, and reads with more than 40% of bases having a Phred score $\leq 10$ were discarded. The remaining reads were assembled de novo using Flye (v2.9.6; Kolmogorov *et al*., 2019) using the *--nano-hq* and *--asm-coverage 100* options and the estimated length of each amplicon. The minimum overlap length was set to approximately 30% of the estimated size. The assemblies of each amplicon were polished with four iterations of Racon (v1.5.0; Vaser *et al*., 2017) using the filtered reads, followed by Medaka (v2.1.1; Oxford Nanopore Technologies, Oxford, UK) using the *r941_min_sup_g507* model. Final polishing was performed with Pilon (v1.24, *--mindepth* 24; Walker *et al*., 2014) using Illumina short reads.

The three amplicon assemblies were manually stitched based on the overlapping regions guided by the anchor sequences. The complete mitochondrial sequence was annotated using the MITOS web server (v2.1.10; Bernt *et al*., 2013) and ARWEN (v1.2; Laslett and Canbäck, 2008), and protein-coding genes and ribosomal RNA gene boundaries were manually curated. Tandem and inverted repeats were identified using Tandem Repeat Finder (v4.0.9; Benson, 1999) and Inverted Repeat Finder (v3.09; Warburton *et al*., 2004), respectively. The mitochondrial genome of *R. naksanensis* was visualized using CIRCOS (v0.69-8; Krzywinski *et al*., 2009).

## Results and Discussion

A total of 333,847 paired-end reads were generated on an Illumina MiSeq using three long-range PCR amplicons to assemble the mitochondrial genome of *R. naksanensis* (*COXI-COB*: 113,667 reads, SRR36883791; *COB-srRNA*:

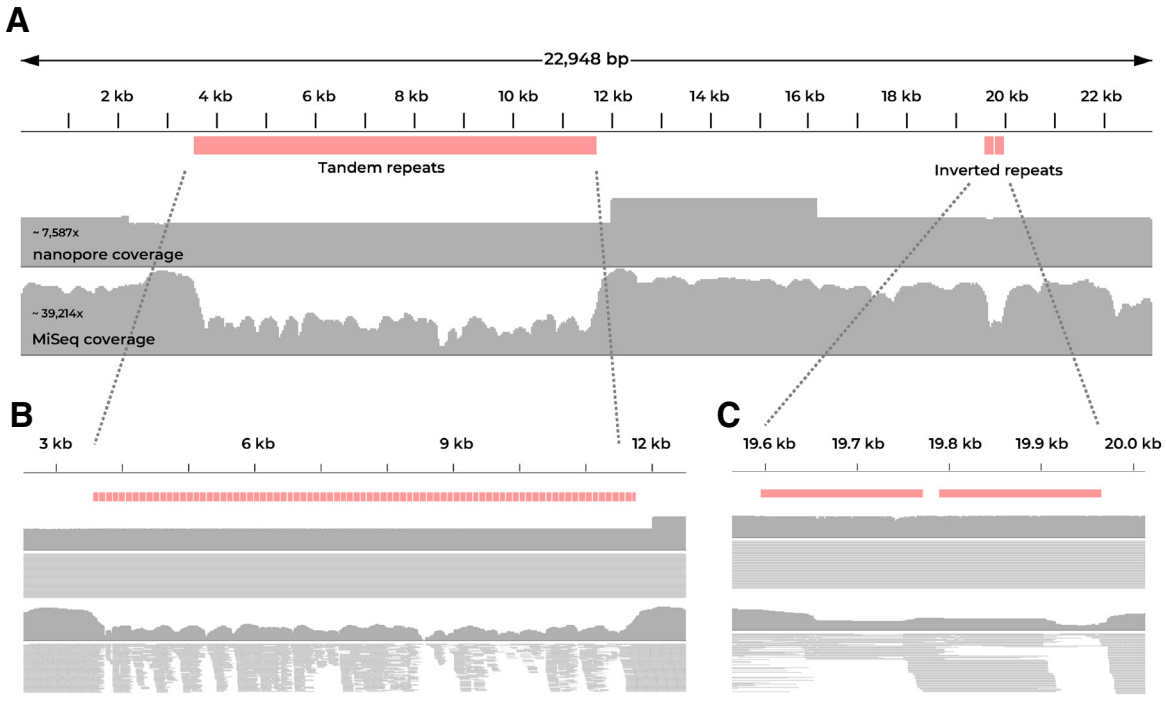



**Fig. 1.** IGV (Robinson et al., 2023) view showing the read-mapping evidence across repeat-rich non-coding regions of the *Remanea naksanensis* mitochondrial genome. A: Genome-wide overview of the read-depth profiles from Nanopore and MiSeq amplicon sequencing, highlighting the tandem repeat array and the inverted repeat (red blocks) together with coverage depth from Oxford Nanopore (top) and Illumina Miseq (bottom); B: Zoom-in of the tandem-repeat array showing the ambiguous short-read mapping across repetitive segments, whereas long reads support continuity across the array; C: Zoomed view of the inverted repeat region representing consistent long-read support across the repeat boundaries. Read alignments were cropped in IGV.

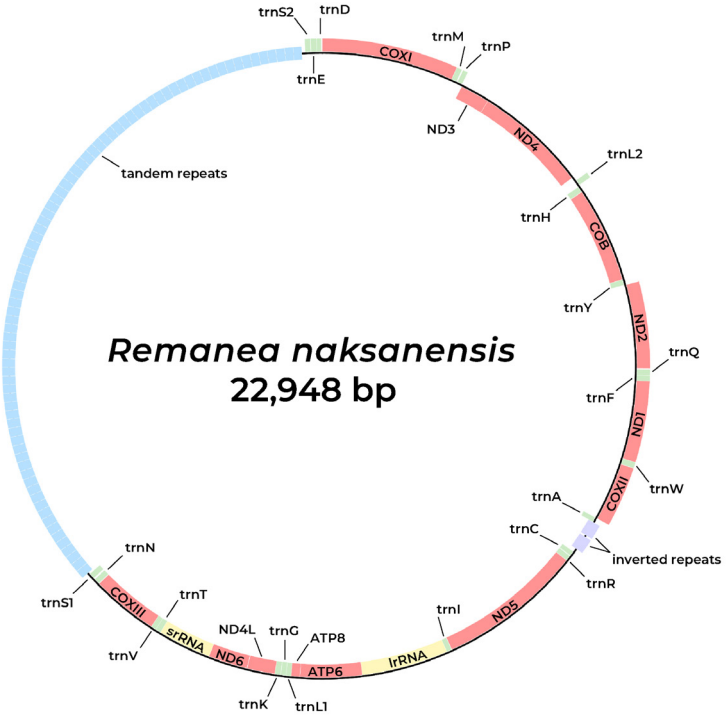



**Fig. 2.** Mitochondrial genome map of *Remanea naksanensis*. Genes encoded on the J- and N- strand are shown outer or inner circle, respectively.

105,883 reads, SRR36883787; *srRNA-COXI*: 114,297 reads, SRR36883789). Despite the high sequencing depth (>39,000×; Fig. 1A), the two regions remained discontinuous in both the seed-based and de novo assemblies. We inferred that the repetitive elements in these regions complicated assembly and were difficult to resolve using short-read data (Gan *et al*., 2019; Tørresen *et al*., 2019). Accordingly, we generated Oxford Nanopore long-read data from the same long-range PCR products in order to span the discontinuities. In total, 71,359 long reads were obtained (*COXI*-COB: 56,498 reads, SRR36883790; *COB-srRNA*: 8,318 reads, SRR36883786; *srRNA-COXI*: 6,543 reads, SRR36883788), yielding approximately 327.5 Mb of sequence data (*COXI-COB*: 223.6 Mb; *COB-srRNA*: 64.7 Mb; *srRNA-COXI*: 39.2 Mb). The overall read length N50 was 4,190 bp (*COXI-COB*: 4,169 bp; *COB-srRNA*: 9,217 bp; *srRNA-COXI*: 8,791 bp). Long-read data spanned both problematic regions (Fig. 1B, C), enabling the complete assembly of a single circular mitochondrial genome (Fig. 2).

The complete mitochondrial genome of *R. naksanensis* is 22,948 bp in length (accession number: PX893796; Fig. 2), with an A+T content of 76.2%. It contains a typical set of 37 mitochondrial genes (13 protein-coding genes, 22 tRNAs, and two rRNA genes). All protein-coding genes used ATN start codons and terminated with TAA stop codons, except for *COXI*, which ended with an incomplete stop codon (T) immediately preceding *trnM* (Table 2). The J-strand encoded 24 genes, whereas the N-strand encoded 13 genes. In addition to this canonical architecture, two non-coding regions longer than 100 bp were identified (Figs. 1, 2; Table 2), which together accounted for a substantial proportion of the increased genome size relative to other harpacticoid mitochondrial genomes. At the boundary of each non-coding region, a repetitive unit overlaps the adjacent tRNA by a few nucleotides.

One non-coding region located between *trnA* and *trnC* harbored two inverted repeat copies (Fig. 2; Table 2). This region was A+T rich (approximately 80%). The two copies were 181 bp each, separated by a 9-bp spacer, and showed approximately 99% sequence identity with two mismatches and no indels. The other non-coding region, located between *trnS1* and *trnS2*, contained a long tandem repeat array composed of approximately 100-bp repeat units (A+T content = ~90%). Long-read sequencing enabled the characterization of this region, identifying 81 repeat units (97–105 bp) and a terminally truncated repeat unit (41 bp) adjacent to *trnS2*. Overall, the tandem repeat array exceeded 8 kb, representing, to our knowledge, the longest single non-coding region reported in copepod mitochondrial genomes to date (the former longest: 4,341 bp in *Eurytemora affinis*, NC_046694; Choi *et al*., 2019).

The alignment of the repeat units revealed five conserved sequence blocks with only minor variations (1–3 mismatches) across the otherwise conserved sites (Fig. 3). The 41-bp truncated repeat unit contained the first three conserved blocks but terminated prematurely relative to the full-length units. The presence of this truncated unit at the array boundary suggests that it was generated via replication slippage. Such boundary-imperfect units commonly attributed to slipped-strand mispairing, a mecha-

**Table 2.** Mitochondrial genome organization of *Remanea naksanensis*.

| Name | Location | Strand | Size | Codon | | Intergenic nucleotides |
|---|---|---|---|---|---|---|
| | | | | Start | Stop | |
| *COXI* | 1-1546 | N | 1,546 | ATC | T | 0 |
| *trnM* | 1547-1608 | N | 62 | | | 10 |
| *trnP* | 1619-1682 | N | 60 | | | 6 |
| *ND3* | 1689-2039 | J | 351 | ATA | TAA | −1 |
| *ND4* | 2039-3379 | J | 1,341 | ATA | TAA | 50 |
| *trnL2* | 3430-3496 | N | 67 | | | 11 |
| *trnH* | 3508-3578 | J | 71 | | | 2 |
| *COB* | 3581-4714 | J | 1,134 | ATG | TAA | 1 |
| *trnY* | 4716-4782 | J | 67 | | | 1 |
| *ND2* | 4784-5767 | N | 984 | ATT | TAA | 11 |
| *trnQ* | 5779-5846 | N | 68 | | | 1 |
| *trnF* | 5848-5909 | N | 62 | | | −3 |
| *ND1* | 5907-6833 | N | 927 | ATA | TAA | −1 |
| *trnW* | 6833-6899 | N | 67 | | | 1 |
| *COXII* | 6901-7596 | N | 696 | ATG | TAA | −5 |
| *trnA* | 7592-7653 | J | 62 | | | 417 |
| *trnC* | 8069-8127 | J | 59 | | | 5 |
| *trnR* | 8133-8196 | J | 64 | | | 1 |
| *ND5* | 8198-9913 | J | 1,716 | ATA | TAA | 2 |
| *trnI* | 9916-9977 | J | 62 | | | 0 |
| *lrRNA* | 9978-11005 | J | 1,028 | | | 0 |
| *ATP6* | 11006-11746 | J | 741 | ATG | TAA | −8 |
| *ATP8* | 11739-11843 | J | 105 | ATA | TAA | −1 |
| *trnL1* | 11843-11904 | J | 62 | | | −1 |
| *trnG* | 11904-11964 | J | 61 | | | 2 |
| *trnK* | 11967-12034 | J | 68 | | | 0 |
| *ND4L* | 12035-12364 | J | 330 | ATT | TAA | 3 |
| *ND6* | 12368-12832 | J | 465 | ATA | TAA | 0 |
| *srRNA* | 12833-13466 | J | 634 | | | 0 |
| *trnT* | 13467-13530 | J | 64 | | | 0 |
| *trnV* | 13531-13592 | J | 62 | | | 0 |
| *COXIII* | 13593-14384 | J | 792 | ATG | TAA | 5 |
| *trnN* | 14390-14458 | J | 69 | | | 18 |
| *trnS1* | 14477-14542 | J | 66 | | | 8205 |
| *trnS2* | 22748-22813 | N | 66 | | | 3 |
| *trnE* | 22817-22881 | N | 65 | | | 0 |
| *trnD* | 22882-22944 | N | 63 | | | 4 |

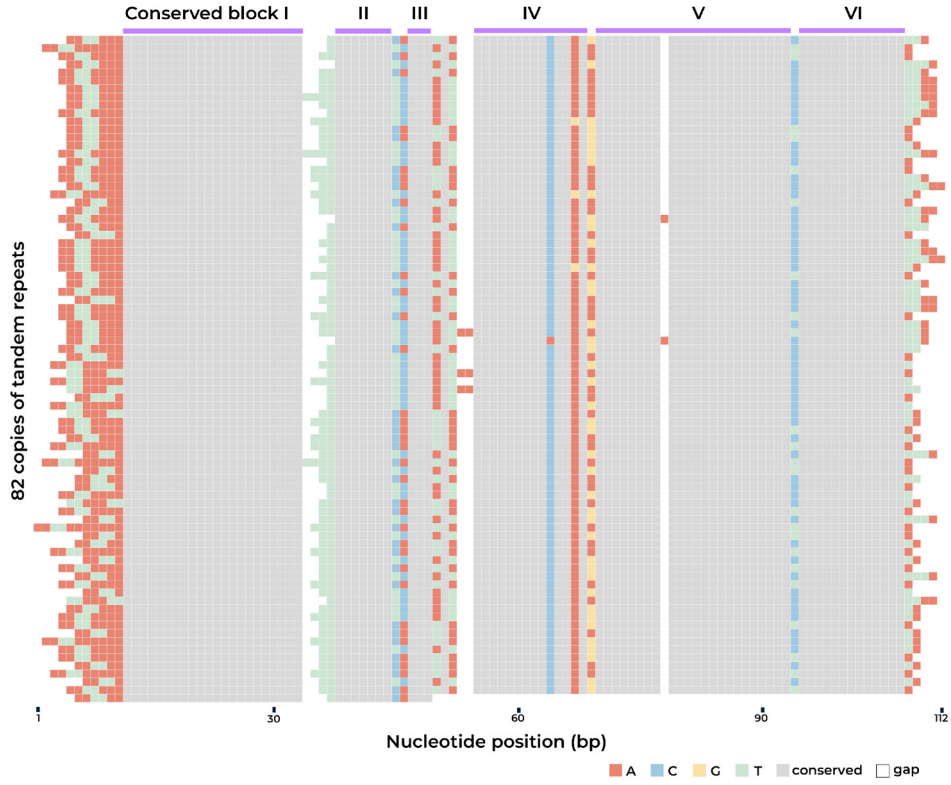



**Fig. 3.** Sequence conservation across the 82 tandem repeat copies extracted from the expanded non-coding region.

nism widely invoked for imprecise or pre-mature termination of replication, recombination during replication, and/or maintenance (Levinson and Gutman, 1987; Lunt and Hyman, 1997; Pâques *et al*., 1998; Rokas *et al*., 2003).

In the non-coding regions of animal mitochondrial genomes, sequence and length variations are commonly observed within species, and length heteroplasmy has been widely reported (Rand, 1993; Lunt *et al*., 1998). Such variations in length have important implications for evolutionary studies (Zhang and Hewitt, 1997). Although similar variability may occur in the two non-coding regions of *R. naksanensis*, our data were generated from long-range amplicons derived from a single individual, which precluded direct assessment of intraspecific variation. The read-mapping results did not reveal any obvious evidence of repeated copy number heterogeneity in this individual (Fig. 1). However, additional sequencing without PCR amplification and read-length binning is required to evaluate repeat-length polymorphisms and repeat-unit diversity at both the individual and population levels.

In the present study, we have assembled the complete mitochondrial genome of *R. naksanensis* de novo using a combination of short (Illumina MiSeq) and long (Oxford Nanopore sequencing) reads generated from three long-range PCR amplicons. This mitochondrial genome provides a validated resource for further comparative and phylogenetic studies on harpacticoid copepods, including paramesochid lineages, for which mitochondrial genomic data remain limited. Furthermore, our long-read approach revealed an approximately 8-kb tandem repeat region that was initially undetected in short-read assemblies.

Most of the reported copepod mitochondrial genomes have been sequenced using Sanger sequencing or short-read platforms, and some remain only partially characterized (e.g. *Calanus sinicus* (GU355641), Minxiao *et al*., 2011; *Calanus fimarchicus* (MG001887) and *Calanus glacialis* (MG001883), Weydmann *et al*., 2017; *Schizopera knabeni* (KF667527), Easton *et al*., 2014). Recently, concerns have been raised that mitochondrial genomes assembled solely from short, even when reported as complete, may omit repeat-rich regions and thus remain in complete (Filipović *et al*., 2021; Formenti *et al*., 2021; Kinkar *et al*., 2021). Consistent with this, several complete mitochondrial genomes based on short reads have later been revised using long-read sequencing, which enabled the detection of previously unrecognized structures within non-coding regions (Kinkar *et al*., 2020; Sharbrough *et al*., 2023). Therefore, long-read sequencing is an important strategy for accurately characterizing repeat-rich mitochondrial genomes and improving assembly completeness.

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## Conflicts of Interest

The author of this paper have no affiliation with any interests and are solely responsible for the paper.

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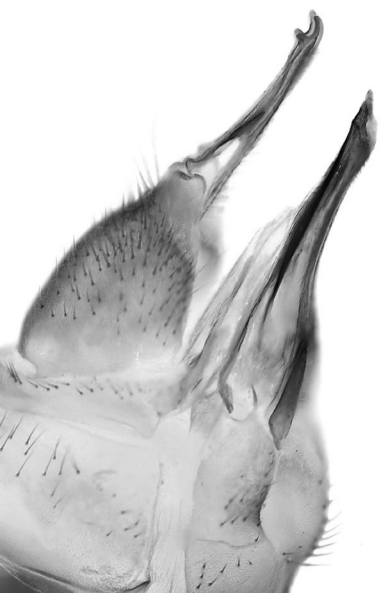
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**Front Cover**

This cover shows photographs of tiger beetle, *Cicindela* (*Abroscelis*) *anchoralis* Chevrolat, 1845, collected in the South Korea. The figure on the left is a male, and the one on the right is a female. The related article and detailed information can be found on pages 117–122. Photos by Deokjea Cha.