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Genome-based classification and phylogenetic revision of the family Colwelliaceae with proposals for new genera and species

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Introduction: Psychrophilic marine bacteria belonging to the family Colwelliaceae have garnered increasing industrial and ecological interest. However, their phylogenetic positions remain ambiguous when classified solely based on 16S rRNA gene sequences, limiting accurate taxonomic resolution.

Methods: To resolve these ambiguities, we characterized four newly isolated species using a comprehensive taxogenomic framework. We analyzed genome-based indices, including average nucleotide identity (ANI), digital DNA–DNA hybridization (dDDH), and average amino acid identity (AAI), across all publicly available Colwelliaceae genomes. Genus-level AAI thresholds were established through repetitive clustering and evaluation strategies.

Results: Our analysis revealed that genus-level AAI values within Colwelliaceae ranged from 74.07% to 75.11%. Based on these thresholds, we re-evaluated 47 species, including the four novel isolates, and proposed the establishment of 18 new genera, expanding the current taxonomy from 6 to 24 genera. The four novel species were assigned to three of these newly proposed genera.

Discussion: This study presents a revised classification of the family Colwelliaceae grounded in genome-based taxonomic metrics. Our findings highlight the limitations of traditional 16S rRNA-based phylogenies and support the use of taxogenomic approaches to achieve higher taxonomic resolution in marine microbial lineages.

KEYWORDS

amino acid identity, *Colwelliaceae*, genome-based classification, *in silico* analysis, phylogenetic revision, marine microbes

1 Introduction

The family Colwelliaceae (order Alteromonadales, class Gammaproteobacteria, phylum Pseudomonadota) (Ivanova et al., 2004; Ivanova and Mikhailov, 2001) is primarily found in marine environments, such as marine sediments (Nogi et al., 2004; Xu et al., 2016), sea ice (Bowman et al., 1998; Zhang et al., 2008), seawater (Kristyanto et al., 2022; Wang et al., 2018a), and marine animals (Kim et al., 2017; Zhang et al., 2014). Genome sequencing confirmed that strains of the genus *Colwellia* (Deming et al., 1988) are specifically adapted to low temperatures (5°C) (Methé et al., 2005). The family Colwelliaceae is a typical marine secondary producer and plays a role in the decomposition of organic substances (hydrocarbons, lipids, proteins, and polysaccharides). They also ferment simple compounds (Bowman, 2014). In addition, *ligA* genes from some *Colwellia* species can be used to develop temperature-sensitive versions of the pathogen, which can induce an immune response while mitigating the potential for disease caused by the vaccine (such as Polio, viral disease; flu) (Duplantis et al., 2010). Like the related group *Pseudoalteromonas* (Gauthier et al., 1995), *Colwellia* can synthesize bioactive compounds (Bowman, 2007). Eicosapentaenoic acid production by members of the family Colwelliaceae has been demonstrated (Tatsumi et al., 2019), suggesting their potential application in biotechnology (Hashimoto et al., 2015). Accordingly, Colwelliaceae may be useful in various industrial and medical contexts.

The family Colwelliaceae currently consists of six genera: *Colwellia* (Deming et al., 1988), *Thalassomonas* (*Tsm*) (Macián et al., 2001), *Litorilituus* (Wang et al., 2013), *Thalassotalea* (*Tst*) (Zhang et al., 2014), *Cognaticolwellia* (Liu et al., 2020a), and *Pseudocolwellia* (Liu et al., 2020b). Initially, *Colwellia psychrerythraea* was assigned to the family Vibrionaceae (Deming et al., 1988), but it was later reclassified into a new genus based on 5S rRNA gene analysis (D'Aoust and Kushner, 1972). Based on DNA G + C content, the genus *Thalassomonas* formed a distinct clade from *Colwellia* (Macián et al., 2001), which led to the proposal of the family Colwelliaceae (Ivanova et al., 2004). The genus *Litorilituus* was introduced based on a strain isolated from an amphibian breeding area (Wang et al., 2013), while the genus *Thalassotalea* was established from the reclassification of several species in the genus *Thalassomonas* based on phylogenetic differences and DNA G + C content (Zhang et al., 2014). The taxonomic history has continued up to the present, with recent extensive reclassification based on genomic data. In this reclassification (Liu et al., 2020b), emphasized the limitations of using 16S rRNA gene for classifying this group.

The family Colwelliaceae is an important taxonomic group both industrially and academically because it produces a variety of substances, but the phylogenetic position of microorganisms belonging to the family Colwelliaceae based on the 16S rRNA gene is still unclear. While a bacterial species can be defined based on 70% genome identity, the genus definition is still ambiguous and this makes it difficult to identify position of species. Despite the 16S rRNA gene sequences have been used as a supplementary indicator, the criteria for defining genera vary across taxonomic groups (Caudill and Brayton, 2022). In particular, unlike *Colwellia*, which was recently reclassified based on genomic data, *Thalassomonas* and *Thalassotalea* have not yet undergone genome-based reclassification (Li et al., 2024; Rey-Velasco et al., 2024). A key limitation in the genome-wide analysis of certain genera is insufficient information on metabolic activity, hindering comprehensive genome-based analysis. Thus, both genomic and metabolic analyses are necessary to establish accurate phylogenetic relationships.

Advances in technology have made genome sequencing and *in silico* analysis commonplace, enabling the analysis of genomic differences and the original definition of species as well as applying it to taxa where taxonomic relationships are unclear. Recently, taxogenomics—focusing on whole-genome analyses—has gained attention as a method for addressing taxonomic ambiguities in various groups. Taxogenomics also reveals the unique characteristics of each group influenced by environmental and evolutionary factors. Therefore, the aim of this study was to perform a phylogenetic classification of all members of the family Colwelliaceae based on genomes to correct taxonomic errors, as well as to perform genome-based metabolic activity analysis.

2 Materials and methods

2.1 Isolation and cultivation

Four strains were isolated from different locations and dates. Strain MEBiC14330^T was isolated from sediment on April 5, 2020, from the Gordin Guyot region in the North-West Pacific Ocean (16° 50' 50" N, 150° 04' 36" E) at a depth of 1,300 m. Strain MEBiC06441^T was isolated on February 25, 2009, from sediment in Incheon (37° 16' 12" N, 126° 26' 34" E). Strain MEBiC06471^T was isolated on February 10, 2009, from sediment in the Taean-gun mud flat (36° 46' 30" N, 126° 08' 01" E). Strain MEBiC02087^T (=BS250^T) was isolated from sediment collected at 680 m deep in the Barents Sea (73° 03' N, 50° 03' E) in Russia. The first three strains were cultured at 25°C on marine agar 2216 medium (MA; BD Biosciences, Franklin Lakes, NJ, USA) on April 5, 2020; March 2, 2009; and February 15, 2009, respectively. After one week, the strains were purified by subculturing on new MA plates. To isolate the fourth strain, 1 cm^2 of sediment was diluted with sterile seawater and spread on MA (BD Biosciences). Inoculated plates were cultivated at 10°C for 1 week. Individual colonies were isolated from MA, and the morphologically distinct strain MEBiC02087^T was selected. After primary isolation and purification, the strain was cultivated at 15°C on MA. All strains were routinely cultured on MA and stored at −80°C in marine broth (BD Biosciences) supplemented with 20% (v/v) glycerol.

2.2 Small subunit rRNA gene and whole-genome sequencing

For small subunit 16S rRNA gene sequencing by Sanger Sequencing, the genomic DNA of strains MEBiC14330^T, MEBiC06441^T,

MEBiC06471^T, and MEBiC02087^T were extracted using a LaboPass bacterial genomic DNA isolation kit (Cosmo Gentech, Seoul, Korea) according to the manufacturer's instructions. PCR amplification of the 16S rRNA gene was performed using TaKaRa Ex Taq mixture (Takara Bio, Shiga, Japan) and a prokaryote-universal primer set: 27F (5′–AGAGTTTGATCMTGGCTCAG–3′) and 1492R (5′–TACGGYTACCTTGTTACGACTT–3′). The PCR conditions were as follows: initial denaturation at 98°C for 30 seconds, followed by 30 cycles of denaturation at 98°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. The PCR product was purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sequenced by Macrogen Co., Ltd. (Seoul, Korea). The PCR-generated 1492R and 27F sequences were processed using EditSeq version 7.0.0 (DNAstar, Madison, WI, USA). Each sequence was saved in SEQ file format and imported into SeqMan version 7.0.0 (DNAstar) (Hall, 1999) for assembly. In SeqMan, overlapping regions were automatically identified and merged into a single contig. The resulting contig was saved in text file format and subjected to BLAST analysis for further comparison and identification. Closely related strains were identified through BLAST, and their 16S rRNA gene sequences were obtained from the GenBank database and EzBioCloud server (http://www.ezbiocloud.net). (Yoon et al., 2017a). Multiple alignments were performed using Clustal W embedded in MEGA 11 (Tamura et al., 2021) and BioEdit (Hall, 1999). The distances were calculated using the Kimura 2-parameter model (Kimura, 1980) to construct a phylogenetic tree with neighbor-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981), and minimum-evolution (Rzhetsky and Nei, 1992) methods in MEGA 11. The outgroups downloaded from the NCBI were *Idiomarina baltica* OS145^T (AJ440214) and *Psychromonas antarctica* star-1^T (Y14697). The 16S rRNA gene sequences were deposited in the NCBI GenBank database under the accession numbers MEBiC14330^T (OR958774), MEBiC06441^T (OR958780), MEBiC06471^T (OR958775), and MEBiC02087^T (PQ119528).

For whole-genome sequencing, genomic DNA was extracted using the LaboPass Bacterial Genomic DNA Isolation Kit (Cosmo Gentech). The extracted DNA concentrations were confirmed using a NanoDrop and Qubit 4 (Thermo Fisher Scientific, Waltham, MA, USA) (García-Alegría et al., 2020). For whole-genome sequencing, an Illumina iSeq 100 was used (Khan et al., 2018). The quality of the sequencing data was confirmed by visualization using FastQC version 0.12.1 (FastQC Quality Control, San Diego, CA, USA) (Leggett et al., 2013). Poly-G trimming is essential to eliminate errors caused by sequencing artifacts, which commonly occur in Illumina sequencing platforms. To address this issue, Poly-G trimming was performed using Cutadapt version 4.4 (Martin, 2011). Specifically, consecutive G bases (≥10) at the start of the forward and reverse reads were removed to ensure the accuracy of the sequencing data and reduce the impact of sequencing biases. Trimming was performed using Trimmomatic version 0.39 (Bolger et al., 2014) with a quality cutoff of Q20 for both forward and reverse reads. Illumina universal adapter sequences (AGATCGGAAGAGC) were removed using the ILLUMINACLIP option, and sequences shorter than 50 bp after trimming were discarded using the MINLEN parameter. Additional parameters included LEADING:3, TRAILING:3, and SLIDINGWINDOW:4:20 to trim low-quality bases at the ends and sliding window-based quality trimming. The forward and reverse reads were processed separately and saved as paired output files. Subsequently, assembly was performed using SPAdes version 3.15.5 (Bankevich et al., 2012), with k-mer sizes of 21, 33, 55, 77, and 99, the –careful mode enabled, and the –cov-cutoff set to 'auto' to optimize assembly accuracy and perform error correction. The quality of the genome assembly was evaluated using the Quality Assessment Tool version 5.2.0 (Mikheenko et al., 2018) and was visually confirmed. The assessment was based on metrics such as N50, total length, and the number of contigs, with assemblies being selected based on a lower number of contigs, a higher N50 value, and a larger total genome size to ensure accuracy. Additionally, genome completeness and contamination levels were evaluated using CheckM version 1.2.3 (Parks et al., 2015) to further confirm the quality of the assembly. Annotation was performed using Prokka version 1.14.6 (Seemann, 2014), which predicts putative coding sequences and provides basic annotations of the predicted protein-coding regions. The genomes from type materials (n = 47) belonging to the family Colwelliaceae available in the NCBI database were collected for subsequent analysis. Their basic genomic information is described in Supplementary Table S1.

2.3 Genome-based analysis

The core gene phylogeny was performed using PhyloPhlAn version 3.1.68 (Asnicar et al., 2020). FAA result files were obtained from Prokka. The phylogenetic tree was constructed using RAxML version 8.2.12 (Stamatakis, 2014) with 400 core universal protein markers (Segata et al., 2013). The PROTCATLG model was applied, utilizing the LG amino acid substitution matrix with a CAT approximation to model rate heterogeneity across sites. Phylogenetic inference was conducted with 1,000 bootstrap replicates using rapid bootstrapping and a thorough maximum likelihood (ML) search (-f a), and final branch support values were computed using the '-f b' option. To assess the reliability of the RAxML tree's internal branches, further validation was performed using IQ-TREE2 version 2.3.5 (Minh et al., 2020). The optimal substitution model in IQ-TREE2 was selected using the ModelFinder feature, and the tree was constructed according to the selected Q.insect+F+R5 model. Bootstrap support for internal branches was assessed with 1,000 replicates, and approximate likelihood ratio tests (aLRT) were performed to validate the tree topology with 1,000 iterations.

The average nucleotide identity values were calculated using the OrthoANI-usearch tool (Yoon et al., 2017b), and digital DNA-DNA hybridization (*d*DDH) was calculated using the Genome-to-Genome Distance Calculator provided by DSMZ (Braunschweig, Germany) (Meier-Kolthoff et al., 2013). The AAI calculator by EzAAI version 1.2.3 (Kim et al., 2021) was used to compare two genomes with amino

acids with a 20% minimum identity and 50% minimum query coverage threshold. The POCPs were calculated according to the method previously described (Qin et al., 2014). The POCP is a comparison of the amino acid sequences of the genomes of two strains using BLASTp. Roary 5.26.2 version (Page et al., 2015) was used to summarize the unique genes and features of the distinct genera. KofamKOALA (Aramaki et al., 2020) was used based on the Prokka numbers and protein sequences of 47 strains in the family Colwelliaceae. The codon usage bias of each group was analyzed to understand their evolutionary relationships and clustering. This was done by calculating the relative synonymous codon usage (RSCU), which reflects evolutionary processes influenced by factors such as G + C content, replication strand skew, and gene expression (Sharp et al., 2005). Codon usage was determined using the tool available at https://www.bioinformatics.org/sms2/codon_usage.html. The resulting values were then visualized using Plotly version 4.10.4 (Sievert, 2020) on the RStudio server (Carpenter et al., 2021).

2.4 Biochemical and morphological tests

The cell growth of strains MEBiC14330^T, MEBiC06441^T, MEBiC06471^T, and MEBiC02087^T were observed for 5 days on MA plates at 4, 10, 15, 20, 25, 28, 30, 35, and 45 °C. The pH tolerance range was determined at pH 3, 4, 5, 6, 7, 8, 9, and 10 in marine broth, with the pH adjusted using 10 mM MES (pH 4–6), 10 mM HEPES (pH 6–8), or 10 mM AMPSO (pH 8–10) as biological buffers. The salinity range for growth was tested in MB prepared from distilled water with 0, 0.5, and 1–10% (w/v) NaCl at intervals of 1%. The pH and NaCl growth tolerance test were observed for up to 5 days at 25°C in a 180 rpm shaking incubator and measured at OD_{600}. Catalase and oxidase activities were determined as previously described (Cappuccino and Sherman, 2014). Growth under anaerobic conditions was investigated using the GasPak Ez anaerobic pouch system (BD Biosciences) over 7 days on MA and MA supplemented with potassium nitrate at a concentration of 0.1%. Biochemical tests were performed using API kits (API 20NE, API 50CH, and API ZYM) according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). The API 20NE and 50CH kit results were observed after 24 and 48 h at 25°C. Gram staining was performed as previously described (Buck, 1982). Morphological characteristics were determined using transmission electron microscopy (Hitachi HT7800, Hitachi, Tokyo, Japan) of cells negatively stained with 2% uracil.

Fatty acid profiles were analyzed by the Korea Culture Center of Microorganisms (KCCM), using MIDI with Sherlock version 6.3 and the RTSBA6 database (Sasser, 1990). The polar lipids of the four strains were extracted and analyzed using two-dimensional ascending thin-layer chromatography (Minnikin et al., 1984). The major respiratory quinones were determined by high-performance liquid chromatography, as previously described (Collins et al., 1979). Cellular quinone analysis was performed by high-performance liquid chromatography using a reversed-phase C18 column as previously described (Minnikin et al., 1984).

3 Results

3.1 16S rRNA gene sequence-based phylogenetic analysis

To assess the taxonomic relationships of the four novel strains, 16S rRNA gene sequencing and phylogenetic analysis were conducted. The 16S rRNA gene sequence lengths obtained by Sanger sequencing were 1,415, 1,411, 1,411, and 1,431 bp, respectively. Phylogenetic analysis based on 16S rRNA gene sequences was conducted to elucidate the relationships among the four novel strains; however, low bootstrap values indicated weak support for these relationships (Supplementary Figure S1). The similarities between MEBiC14330^T, MEBiC06441^T, MEBiC06471^T, and MEBiC02087^T and other strains were as follows: strain MEBiC14330^T: *Colwellia asteriadis* KMD 002^T (96.75%), *Litorilituus lipolyticus* RZ04^T (96.26%), and *C. psychrerythraea* ATCC 27364^T (95.91%); strain MEBiC06441^T: *Thalassotalea piscium* T202^T (96.21%), *C. hornerae* ACAM 607^T (95.35%), and *C. piezophile* Y223G^T (95.13%); strain MEBiC06471^T: *Tst. profundi* YM155^T (96.01%), *Tsm. viridans* XOM25^T (95.63%), and *C. meonggei* MA1-3^T (95.49%); strain MEBiC02087^T: *Tsm. haliotis* A5K-61^T (95.60%), *C. hornerae* ACAM 607^T (95.35%), and *Cognaticolwellia beringensis* NB097-1^T (95.13%). All similarity values were calculated based on the valid names, and the top three strains with a similarity of over 95% were described.

In the phylogenetic tree, *Litroilituus* was located within the *Colwellia* clade, and some strains of *Pseudocolwellia* and *Colwellia* were located within the *Cognaticolwellia* clade, making the boundary between these genera ambiguous. This suggests the need for whole-genome analysis to clarify the phylogenetic relationships.

3.2 Genomic characteristics and genome-based phylogeny

The genomes of the strains MEBiC14330^T, MEBiC06441^T, MEBiC06471^T, and MEBiC02087^T were assembled *de novo* from 234, 136, 27, and 121 contigs, respectively. The genome size of new strains ranged from 4.19 to 4.46 Mb, concurrent with those of current Colwelliaceae members (3.30–7.95 Mb). Other genomic features are detailed in Supplementary Table S1. The 16S rRNA gene results obtained by Sanger sequencing were compared with those of the 16S rRNA gene sequences in the whole-genome assembly to verify the accuracy of the assembly. The lengths of the 16S rRNA extracted from the genome were 1,539, 1,537, 1,539, and 1,541 bp, respectively. The results were 99.01, 98.94, 98.31, and 99.72% for MEBiC14330^T, MEBiC06441^T, MEBiC06471^T, and MEBiC02087^T, respectively. The completeness of the assembled genomes was 97.81, 99.66, 99.66, and 98.94% for the four strains, respectively, and contamination was 1.32, 2.35, 1.62, and 0.52%, respectively. The six genera in the Colwelliaceae family have genome sizes of 3.72–5.72 Mb (*Colwellia*), 4.14–4.28 Mb (*Litorilituus*), 3.85–4.66 Mb (*Cognaticolwellia*), 4.72 Mb

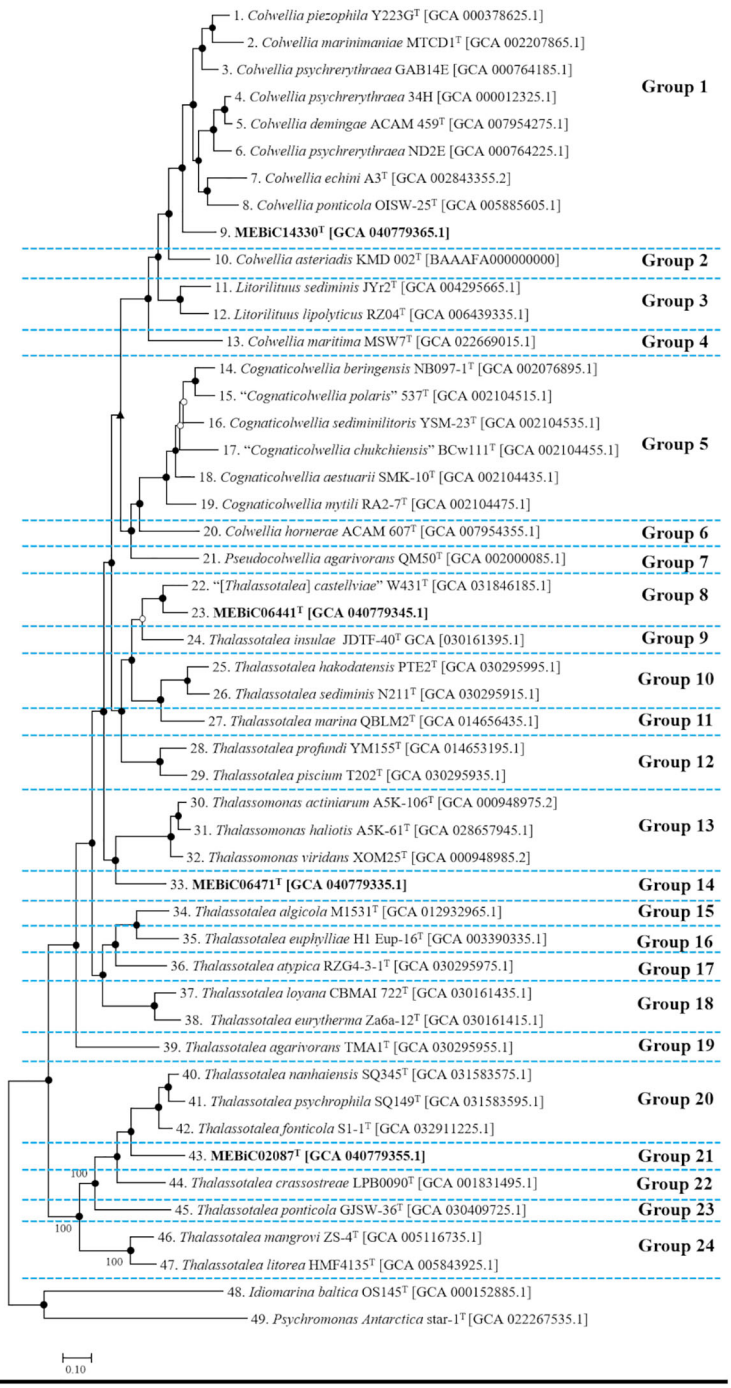

FIGURE 1
Phylogenomic tree of strains belonging to the family Colwelliaceae. A total of 47 strains within the family Colwelliaceae were subdivided into 24 groups based on a branch length threshold of 0.2. Two strains from the order Alteromonadales were selected as outgroups. The numbers preceding each scientific name correspond to the identical organisms in subsequent analyses. The scale bar represents 10.0% estimated sequence divergence. Node support values were determined using bootstrap values from PhyloPhlAn, approximate likelihood ratio test (aLRT) values from IQ-TREE, and additional bootstrap values. Node support is indicated as follows: (▲) for nodes with >90% support across all methods, (○) for nodes with 70–90% support, and (▲) for nodes where at least one method yielded <70% support.

(*Pseudocolwellia*), 3.30–4.90 Mb (*Thalassotalea*), and 6.45–7.95 Mb (*Thalassomonas*). The G + C contents are 36.8% to 39.6%, 37.4% to 39.4%, 37.5% to 41.9%, 35.8%, 36.3% to 45.9%, and 47.3% to 48.9%, respectively. Genera classifications before reclassification are presented in Supplementary Table S1.

In contrast to 16S rRNA gene-based phylogeny (Supplementary Figure S1), the phylogenomic tree provided a clear relationship (Figure 1); therefore, references for the four novel strains were selected from the phylogenomic tree, and the strain designation numbers are shown in Supplementary Table S1. The closest

relatives of each novel strain were as follows: MEBiC14330^T: *C. ponticola* OISW-25^T; MEBiC06441^T: *Tst. castellviae* W431^T and *Tst. insulae* JDTF-40^T; MEBiC06471^T: *Tsm. viridans* XOM25^T; and MEBiC02087^T: *Tst. psychrophile* SQ149^T, and *Tst. crassostreae* LPB0090^T. Therefore, relatives were selected based on Figure 1, and strains that were easily obtainable and for which direct experiments could be conducted were selected. The reference relationships for each strain are indicated by numbers in Supplementary Table S1 for all subsequent descriptions.

Most target genomes have been isolated from marine environments. Additionally, some species have been isolated from cold environments (Arctic sea ice, Arctic marine sediments, and the deep sea). However, their genomic characteristics differed. The genome size ranged from 3.30–7.95 Mb, and the G + C content ranged from 35.8% to 48.9%. Other information regarding the genome (contig number, N50, coding DNA, rRNA, tRNA, tmRNA, and CRISPR repeat regions) is summarized with the GenBank assembly accession numbers in Supplementary Table S1.

When the amino acid sequence similarity between two genomes exceeds 80%, they can be classified into the same genus (Luo et al., 2014). This threshold has been widely applied in taxonomic studies (Liu, Zhang, Cheng, et al., 2020; On et al., 2020; Soutar and Stavrinides, 2022; Suresh et al., 2019). To reflect this classification threshold (corresponding to an estimated 20% sequence divergence), we identified groups with a phylogenetic distance of 0.2 or greater. The 47 Colwelliaceae genomes were divided into 24 groups, excluding the outgroup (Figure 1). Furthermore, strains 2, 4, and 6, numbered based on Figure 1, were not included because of the lack of genomic information. Therefore, genome-based classification was limited compared with the 16S rRNA gene-based phylogenetic tree. Groups 1, 2, 4, and 6 (Figure 1) were previously in the genus *Colwellia*; however, only Group 1 was retained in the genus *Colwellia*, and the newly reported novel strain MEBiC14330^T was included in Group 1. In addition, Groups 2, 4, and 6 should be classified as different genera, and genome-based phylogenetic results revealed new genera. Groups 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 22, 23, and 24 were assigned to the genus *Thalassotalea;* however, like *Colwellia*, they formed different clades, suggesting that they were different species. These results indicate that the family Colwelliaceae should be reorganized into 24 genera, including three genera comprising new strains from the existing six genera. A more detailed phylogenomic tree of these strains, including bootstrap values and aLRT, support values, is provided in Supplementary Figure S2.

3.3 Phenotypic and chemotaxonomic characterization

The four novel strains are described according to type, along with each reference strain (Figure 1). Type 1: strains 9 and 8; Type 2: strains 32 and 33; Type 3: strains 22, 23, and 24; Type 4: strains 41, 43, and 44. The reference species selected for the direct comparison were as follows: *C. ponticola* OISW-25^T, *Tst. insulae* JDTF-40^T (Korean Collection for Type Cultures, Jeollabuk-do, Korea), and *Tst. crassostreae* LPB0090^T (Korean Agricultural Culture Collection). The criteria for classifying these types were selected based on a genome-based phylogenetic tree. The criteria were selected based on strains that were easy to obtain, could be directly experimented on, and were published at the time of the comparative experiment. Therefore, strain 42 was not compared to Type 4.

MEBiC14330^T, MEBiC06471^T, MEBiC06441^T, and MEBiC02087^T are aerobic, motile, gram-negative, and catalase- and oxidase-positive bacteria. Microscopic images showed that all four strains were rod–shaped. All strains also had flagella except for MEBiC02087^T (Supplementary Figure S3). The optimal temperature, pH, and NaCl concentration for growth of strain MEBiC14330^T was 28°C, pH 7, and 2.0%, respectively. The optimal growth values for MEBiC06471^T were 25°C, pH 7, and 1.0% NaCl, respectively. The optimal growth values for MEBiC06441^T were 20°C, pH 7, and 1.0% NaCl, respectively. The optimal growth values for MEBiC02087^T were 9°C, pH 7–8, and 2.0–3.0% (Table 1). The optimal temperature, pH, and concentration of NaCl for Type 1 were similar to members in the genus *Colwellia*. Types 2 and 4 were strains belonging to different genera with large differences in their growth ranges. In Type 2, MEBiC06471^T grew at a higher temperature than strain 32, and a difference in the presence or absence of nitrate reduction was observed (strain 32 is a psychrophile). In Type 3, MEBiC06441^T and strain 22 were of the same genus; however, unlike strain 24, they hydrolyze starch and have yellow or brown pigments. Type 4 strains showed large differences in growth ranges, and MEBiC02087^T is a psychrophilic strain with a low growth temperature.

The chemotaxonomic data showed similar basic characteristics with the exception of MEBiC02087^T. All Type 1 strains showed activity toward esculin ferric citrate and inulin and used the naphthol-AS-BI-phosphohydrolase enzyme. However, MEBiC14330^T showed negative activities toward arabinose, adonitol, and arabitol and did not use L-arginine and urea enzymes. MEBiC06471^T was positive for nitrate reduction and negative for starch and urea hydrolysis and D-glucose use. MEBiC06441^T differed from the other two strains as it was negative for casein and gelatin hydrolysis, whereas MEBiC06441^T and strain 22 of the same genus did not hydrolyze starch. MEBiC02087^T was motile and positive for maltose use (Table 1).

In Type 1, the major fatty acids of the two strains were the same; however, $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) showed a difference of > 10%, and MEBiC14330^T also showed a high value of 9.5% in summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*). Type 2 had the same $C_{16:0}$ and summed feature 3 as their major fatty acids; however, $C_{17:1}$ *ω8c* in strain 32 was 19.7%, which was >10% higher than that in MEBiC06471^T. Strain 22 was excluded owing to lack of fatty acid information, and Type 3 had $C_{15:1}$ *ω8c*, $C_{17:1}$ *ω8c*, and summed feature 3 as major fatty acids. Additionally, MEBiC06441^T had $C_{16:0}$ as a major fatty acid. Type 4 showed substantially different fatty acid compositions, and MEBiC02087^T showed a high proportion of $C_{16:1}$ *ω9c*, $C_{12:0}$ 3OH, and summed feature 3 (Supplementary Table S2).

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TABLE 1 Main phenotypic characteristics of the novel strains.

Characteristics	Type1		Type2		Type3			Type4		
	9	8	33	32[1]	23	22[2]	24	43	44	41[3]
Isolation source	sediment	seawater	sediment	oysters	sediment	sediment	seawater	sediment	oyster	sediment
Motility	+	+	+	+	+	+	+	+	–	–
Colony color	Ivory	white-yellowish	Ecru	Green	Light brown	moderate yellow	Cream	Beige	white-yellowish	white
Cell size (lengh × width; μm)	1.2–8.0×0.4–1.0	0.2–0.6×0.5–6.0	1.1–3.3×0.4–1.0	1.5–2.0×0.8–1.0	0.9–3.4×0.4–0.9	0.3–1.0×0.5–6.0	Nr	0.5–0.7×1.1–2.5	2.2–2.6×0.3–0.6	1.8–3.1×0.2–0.4
Cell shape	rod-shaped	rod-shaped	rod-shaped	rod-shaped	rod-shaped	rod-shaped or ovoid	rod-shaped	rod-shaped	rod-shaped	rod-shaped
Catalase	+	+	+	+	+	+	+	+	+	–
Nitrate reduction	+	+	+	–	+	+	+	+	+	–
Growth range of										
pH(optimum)	6.0–8.0 (7.0)	5.5–8 (7.0–8.0)	5.0–8.0 (7.0)	Nr	5.0–9.0 (7.0)	5.5–8.0 (7.0–8.0)	Nr	6.0–10.0 (7.0–8.0)	5.0–7.5 (7.0)	7.0–9.0 (7.5)
Temperature (optimum) °C	25–30 (28)	4–30 (25)	10–30 (25)	13–37 (7.0)	10–25 (20)	10–37 (30)	4–37 (30)	0.0–16.0 (9.0)	15–37 (25)	4–25 (15)
NaCl (optimum) %	1.0–4.0 (2.0)	0.5–7.0 (2.0)	0.05–2.0 (1.0)	2.0–4.0 (Nr)	0.05–2.0 (1.0)	0–6.0 (2.0)	1.5–8.0 (Nr)	0.0–4.0 (2.0–3.0)	0–5.0 (2.0–3.0)	2.0–3.0 (3.0)
Respiratory quinones	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	MK-6	Q-8	MK-7, Q-8
Major Polar Lipids	PE, PG, AL3, L3	PE, PG	PE, PG, AL4, L4	Nr	PE, PG, AL5, L4	PE, PG	PE, PG, AL1, GL, L3	PE, PG	PE, PG	PE, PG
Hydrolyses of										
Casein	+	–	+	+	–	+	+	–	–	–
Starch	–	–	–	+	–	–	+	–	–	–
Gelatin	–	–	+	+	–	+	+	–	–	–
Urea	–	+	–	Nr	–	–	–	–	+	–
Utilization of										
D-Galactose	–	–	–	–	–	+	Nr	w+	–	–
D-Glucose	–	–	–	+	–	w+	+	+	–	+
Maltose	–	–	–	w+	–	+	+	+	–	–
D-Mannose	–	–	–	–	–	+	+	–	–	–
Sucrose	–	–	+	w+	–	+	Nr	+	–	+
Trehalose	w+	–	w+	Nr	–	+	Nr	–	–	+
Enzyme activity (API ZYM)										
Trypsin	+	+	–	Nr	–	+	Nr	w+	–	w+
α-Chymotrypsin	+	–	–	Nr	w+	–	Nr	+	+	w+
α-Glucosidase	–	–	–	Nr	–	w+	Nr	–	+	–
N-Acetyl-β-Glucosaminidase	+	+	w+	Nr	w+	–	Nr	–	+	–
α-Mannosidase	–	–	–	Nr	–	w+	Nr	–	+	–

Strain numbers are referenced in Supplementary Table S1. Gram-negative staining and Oxidase-positive are common characteristics shared by all the strains, and all strains are aerobic. +, positive –, negative; w+, weakly positive; Nr, Not reported. PE, phosphatidylethanolamine; PG, phosphatidylglycero; AL, unidentified aminolipids; L, unidentified lipids; GL, glycolipid. [1]Data taken from (Macián et al., 2001); [2]Data taken from (Rey-Velasco et al., 2024); [3]Data taken from (Li et al., 2024; Rey-Velasco et al., 2024)

Except for the undocumented strains, all strains contained the same major polar lipids, namely phosphatidylethanolamine and phosphatidylglycerol. MEBiC14330^T, MEBiC06471^T, and MEBiC06441^T contained phosphatidylethanolamine, phosphatidylglycerol, and unidentified amino lipids and lipids. Accordingly, the major polar lipid composition of Type 1 was different. Type 3 strain 24 differed because it had a glycolipid. Type 4 had the same composition, and all contained phosphatidylethanolamine and phosphatidylglycerol (Supplementary Figure S4).

All strains, except Type 4, had Q-8 as the major quinone. Type 4 strains 44 and 41 contained Q-8; however, strain 43 also contained MK-7 as the major quinone. MK-6 of MEBiC02087^T is species-specific. These characteristics differed among clade members and could be used to distinguish clade members from the four novel strains. However, the classification of the four novel strains was challenging because of the complexity of Colwelliaceae; therefore, additional taxogenomics was performed.

3.4 Matrix of genome indices

The AAI value is used as a standard to distinguish genera within a certain range, which varies from 60–80% (Konstantinidis and Tiedje, 2005; Rodriguez-R and Konstantinidis, 2014). AAI of 80–85% or 79% is used to distinguish species of the genus *Thalassotalea* within the family Colwelliaceae (Liu et al., 2020a). However, distinguishing between the genera of the entire family Colwelliaceae was not possible, and whether this is an appropriate AAI standard must be confirmed. To avoid subjective intervention, a previously published method of determining the thresholds was followed using clustering and scoring (Park et al., 2022). In this approach, the initial threshold was set to 60.00, and clustering was performed accordingly. A stringent penalty was applied for violations of monophyly, while a milder penalty was imposed for the formation of singleton groups. The threshold was then incrementally increased by 0.01 to determine the optimal value. When calculating the AAI values, the outgroups (48 and 49) value was removed before proceeding. The EzAAI value was 2.16 ± 0.53 higher than that of the Kostas Lab AAI calculator previously used (Park et al., 2022). Applying the EzAAI values, the AAI threshold range was calculated as 74.07–75.11%. Based on the determined threshold range, the separated groups in the column were represented by one square, resulting in 24 squares (Figure 2) identical to the genome tree (Figure 1).

In addition to the AAI value, POCP is also applied as an auxiliary standard, and 50% was recommended as the genus classification value for a specific group (Qin et al., 2014). However, applying a POCP threshold of 50% is not an appropriate standard for classifying species into many taxa. For example, a 65% POCP value for the genome-based classification of Geobacterales was used to separate the genus *Geotalea* into two lineages; however, phylogenomic analysis and AAI values did not support these results (Xu et al., 2021). The AAI and POCP results for each genome are displayed as a heat map in Figure 2, and detailed values can be found in Supplementary Tables S3, S4. The average nucleotide identity and digital DNA–DNA hybridization results, used as criteria for species differentiation between genomes, are summarized in a heatmap (Supplementary Figure S5). Detailed values are provided in Supplementary Tables S5, S6. Therefore, these results indicate that it is appropriate to reclassify Colwelliaceae into 24 genera based on their AAI values and phylogenomic results, excluding the POCP values.

3.5 Comparison of codon bias

The relative synonymous codon usage values were calculated, and the codon usage bias was measured to test the evolutionary relationships and clustering (Supplementary Table S7; Supplementary Figure S6). Groups 1, 5, and 12 were considered individual genera; however, the plot distances of each species were relatively large compared to the distances of species in other groups (Supplementary Figure S7). In particular, species 3, 4, and 6 in Group 1 were the same species, namely *C. psychrerythraea*; however, the plot results showed different codon usage. Among the *Colwellia* strains belonging to Group 1, *C. psychrerythraea* formed different clades, including *C. psychrerythraea* GAB14E, *C. psychrerythraea* 34H^T, and *C. psychrerythraea* ND2E and should be separated into different strains. The three *C. psychrerythraea* strains differed, with values below the cutoff value (Supplementary Table S8). In addition, strains 3, 4, and 6 were classified as the same species, *C. psychrerythraea*. However, strains 3, 4, and 6 were separated by a distance of 0.38 and 0.22, respectively, and strains 4 and 6 were also related to each other by a distance of 0.2 (Supplementary Figures S8, S9). Additionally, the genomes of three *C. psychrerythraea* species show significant differences in genomic and phenotypic diversity (Techtmann et al., 2016). Group 5 was also divided into the same genus but formed a long distance. Codon usage across species can vary greatly, depending on mutations or genetic patterns. Therefore, they are limited to certain taxa and classes (Plotkin and Kudla, 2011). The average nucleotide identity (Supplementary Table S5) and digital DNA–DNA hybridization (Supplementary Table S6) values were also used to distinguish between species, and different *C. psychrerythraea* strains (strains 3, 4, and 6) were distinguished as independent species.

3.6 Defining phenotype and genotype characteristics for each group

Physiological and biochemical characteristics of all species within the Colwelliaceae family were collected by reviewing novel species descriptions and substrate utilization studies available in PubMed. The collected data were then organized in Table 2 according to the newly reclassified genera. Based on the summarized characteristics, a new genus name was determined, and the corresponding generic name was adopted and described for accurate distinction in the subsequent description. Most cells were rod-shaped (*bacillus*) and motile with

TABLE 2 Phenotype and genotype information of the genera of the family Colwelliaceae.

Genus	*Colwellia*	*Novicolwellia*	*Litorilituus*	*Paracolwellia*	*Cognaticolwellia*	*Allocolwellia*
Morphology	B/V	B	B/V	B	B/V	V
Flagellation	+ (Nr)	+	+	+	+ (Nr)	+
Motility	+	+	+	+	+	+
G+C content (%)	36.8 – 39.6	38.0	37.4 – 39.4	37.3	37.5 – 41.9	38.1
Quinone	Q-8 (Nr)	Q-8	Q-8	Q-8	Q-8	Nr
Respiration Mode	FA (A)	FA	A	A	A (FA)	FA
Nitrate reduction	†	+	†	+	+ (–)	+
Essential ions for growth	Mg^{2+}, NaCl				Mg^{2+}, NaCl	NaCl
Optimal pH	6 – 9	Nr	6.5 – 9	7	6 – 10	Nr
Optimal Temp (°C)	6 – 28	15	25 – 37	25	10 – 30	10
Optimal Salinity (w/v) %	2 – 4	Nr	2 – 3	3	1 – 4.3	Nr
Hydrolysis of						
Starch	†	+	+	–	+ (–)	+
Gelatin	†	–	+	+	+ (–)	–
Casein	†	–	+	+	+ (–)	+
Urea	†	–	Nr	+	– (+)	–
Isolation Source	sediment, sea ice, marine organisms (amphipod, sea urchin), sea water	sea organism (starfish *Asterias amurensis*)	sediment	sea water	sediment, sea ice, sea water, marine organism (mussel)	sea ice
Genome Size (Mb)	3.72 – 5.72	4.10	4.14 – 4.28	5.23	3.85 – 4.66	4.42
References	(Bowman et al., 1998; Christiansen et al., 2018; D'Aoust and Kushner, 1972; Kusube et al., 2017; Nogi et al., 2004; Park et al., 2019)	(Choi et al., 2010)	(Liu et al., 2020a; Wang et al., 2013)	(Kristyanto et al., 2022)	(Jung et al., 2006; Kim et al., 2017; Park et al., 2016; Yu et al., 2011; Zhang et al., 2017, Zhang et al., 2008)	(Bowman et al., 1998)
Genus	*Pseudocolwellia*	*Petrothalassotalea*	*Litorithalassotalea*	*Aliithalassotalea*	*Parathalassotalea*	*Thalassotalea*
Morphology	B	B/V	B	B	B	B
Flagellation	+	+	+	+	+	+
Motility	+	+	+	+	+	+
G+C content (%)	35.8	37.2 – 37.4	40.3	38.4 – 38.6	39.4	36.3 – 37.5

(Continued)

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TABLE 2 Continued

Genus	*Pseudocolwellia*	*Petrothalassotalea*	*Litorithalassotalea*	*Aliithalassotalea*	*Parathalassotalea*	*Thalassotalea*
Quinone	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8
Respiration Mode	FA	A (Nr)	A	FA/A	FA	A/SA
Nitrate reduction	+	+	+	+	Nr	+
Essential ions for growth			Nitrate, Mg^{2+}			Na^{+}
Optimal pH	7	5 (Nr)	7 – 8	7.5 – 8.5 (Nr)	7.5 – 8.5	7 – 8.5
Optimal Temp (°C)	28 – 30	20 (Nr)	30	33 (Nr)	30	25 – 30
Optimal Salinity (w/v) %	2 – 3	2	2	3 (Nr)	3	2 – 4
Hydrolysis of						
Starch	+	– (Nr)	–	†	–	†
Gelatin	+	– (Nr)	+	+	Nr	+
Casein	+	+ (Nr)	+	Nr	+	+
Urea	–	– (Nr)	+	– (Nr)	Nr	–
Isolation Source	sea water	sea water, sediment	tidal flat	marine organism (sea cucumber larvae), sediment	marine recirculating aquaculture system	deep sea seamount, marine organisms (gill of cultured flounder)
Genome Size	4.72	3.96 – 4.20	4.39	3.90 – 4.28	4.87	3.90 – 3.99
References	(Xu et al., 2017)	(Rey-Velasco et al., 2024)	(Park et al., 2018)	(Xu et al., 2016; Yamano et al., 2023)	(Hou et al., 2015)	(Liu et al., 2017; Zhang et al., 2014)
Genus	*Thalassomonas*	*Parathalassomonas*	*Algithalassotalea*	*Vitithalassotalea*	*Aminithalassotalea*	*Variithalassotalea*
Morphology	B	B	B	B	B/V	B
Flagellation	+	+	+	+	–	+
Motility	+	+	+	+	+	+
G+C content (%)	47.3 – 48.9	39.6	39.1	44.3	40.2	39.7 – 40.4
Quinone	Q-8 (Nr)	Q-8	Q-8	Q-8	Q-8	Q-8 (Nr)
Respiration Mode	A (SA)	A	FA	A	SA	A
Nitrate reduction	+ (–)	+	+	+	+	†

(Continued)

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TABLE 2 Continued

Genus	*Thalassomonas*	*Parathalassomonas*	*Algithalassotalea*	*Vitithalassotalea*	*Aminithalassotalea*	*Variithalassotalea*
Essential ions for growth	0.4–1.4× ASW , 2 ± 4% NaCl + $MgCl_2$				seawater or artificial seawater	
Optimal pH	8	4	6.5 – 7	7.5 – 9	6 – 9	7.5 – 8
Optimal Temp (°C)	24 – 30	25	30	25 – 30	28	25 – 28
Optimal Salinity (w/v) %	2 – 4	2	2	1	2	2.5 – 3
Hydrolysis of						
Starch	+	–	+	+	–	+
Gelatin	+	+	–	+	–	+
Casein	+	+	–	+	–	+
Urea	–	–	Nr	–	–	†
Isolation Source	marine organisms (abalone, sea anemone, oyster)	sediment	marine organism (red alga)	marine organism (coral)	sea water	marine organism (coral), seawater
Genome Size	6.45 – 7.49	4.13	4.06	4.77	4.39	3.55 – 4.04
References	(Hosoya et al., 2009; Macián et al., 2001)	This study	(Lian et al., 2021)	(Sheu et al., 2016)	(Wang et al., 2018)	(Sun et al., 2014; Thompson et al., 2006)
Genus	*Saccharothalassotalea*	*Psychrothalassomonas*	*Arcticithalassomonas*	*Molluscithalassomonas*	*Aquithalassomonas*	*Solithalassomonas*
Morphology	B/V	B	B	B	B/V	B
Flagellation	–	–	–	–	–	†
Motility	–	–	+	–	Nr	†
G+C content (%)	41.9	38.1 – 39	38.3	38.8	44.4	43.9 – 45.9
Quinone	Nr	Q-8 (MK-7)	MK-6	Q-8	Q-8	Q-8
Respiration Mode	A	A	A	A	A	SA, A
Nitrate reduction	+	– (+)	+	+	+	+
Essential ions for growth			Mg^{2+}, K^{+}		Mg^{2+}	
Optimal pH	7 – 9	7 – 7.5	7 – 8	7	7 – 8	6 – 8
Optimal Temp (°C)	25	15 – 25	9	25	25	30 – 37
Optimal Salinity (w/v) %	3	2 – 3	2 – 3	2 – 3	2	2 – 4

(Continued)

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TABLE 2 Continued

Genus	*Saccharothalassotalea*	*Psychrothalassomonas*	*Arcticithalassomonas*	*Molluscithalassomonas*	*Aquithalassomonas*	*Solithalassomonas*
Hydrolysis of						
Starch	–	–	–	–	+	– (Nr)
Gelatin	+	–	–	–	–	†
Casein	+	–	–	–	–	– (Nr)
Urea	+	–	–	+	–	–
Isolation Source	sea water	sediment	sediment	marine organism (oyster)	sea water	sediment
Genome Size	3.35	4.31 – 4.90	4.46	3.86	3.30	3.71 – 3.88
References	(Jean et al., 2006)	(Li et al., 2024)	This study	(Choi et al., 2017)	(Park et al., 2014)	(Kang et al., 2017; Zheng et al., 2019)

Nr, Not reported; +, Positive; –, Negative; FA, Facultative anaerobic; A, Aerobic; SA, Strictly aerobic; B, Bacilli; V, Vibrioid; †, Both positive and negative; + (–), Predominantly positive over negative; – (+), Predominantly negative over positive; (), Some results within parentheses.

flagella. However, the genera "*Colwellia*," "*Litorilithuus*," "*Cognaticolwellia*," "*Petrothalassotalea*," "*Aminothalassotalea*," "*Saccharothalassotalea,*" and "*Aquiithalassomonas*" include strains with a curved shape (*vibroid*), suggesting that a single genus can exhibit various forms beyond *bacillus*. Regarding the growth conditions, the characteristics of each group became clear. Differences in optimal growth temperatures were observed depending on the isolation environment and source. Because all species in the genera were isolated from marine environments, they required at least 1.0% (w/v) NaCl. On the genome tree (Figure 3), strains in the clade formed by "*Petrothalassotalea*" to "*Thalassotalea*" hydrolyzed casein and were positive for nitrate reduction. All strains included in "*Thalassomonas*" require artificial seawater containing NaCl, Mg^{2+}, Ca^{2+}, and K^{+} for growth. Strains in the clade formed by "*Algithalassotalea*" to "*Saccharothalassotalea*" had a relatively high optimal growth temperature of 25 °C or higher, and all strains were rod–shaped. No flagellation was observed from "*Saccharothalassotalea*" onwards. They were also classified based on the presence or absence of motility. Strains were negative for casein hydrolysis and aerobic from "*Psychrothalassomonas*" onwards. Strains in the clade formed by "*Psychrothalassomonas*" and "*Arcticithalassomona*" had ubiquinone and menaquinone as quinones. The genus "*Litorithalassotalea*" requires nitrate as an essential growth factor. Unlike other groups that have Q-8, "*Psychrothalassomonas*" and "*Arcticithalassomonas*" have MK-7 and MK-6 as their major quinones, respectively. The difference in DNA G + C content shows that all species in the "*Litorithalassotalea*," "*Thalassomonas*," "*Vitithalassotalea*," "*Aminothalassotalea*," "*Saccharothalassotalea*," "*Aquiithalassomonas*," and "*Solithalassomonas*" groups have DNA G + C content of >40%.

3.7 Potential metabolic differences between groups

Although a distinction between the genera was also observed in the phenotype, the potential metabolic differences were compared based on genome annotation to perform a more specific comparison between the genera (Figure 4). The methanogenesis, coenzyme, methane and methanol, photosystem, and nitrogen fixation pathways were absent in the family Colwelliaceae. Among the 20 amino acids, cysteine, tyrosine, and histidine were absent or were species-specific.

Novicolwellia (Group 2) had complete pathways for dimethylamine, trimethylamine dehydrogenase, and chitinase, distinguishing it from other groups in the same clade. *Litorilituus* (Group 3) had complete pathways for all metabolic pathways related to nitrate reduction, distinguishing it from other groups in the same clade. *Paracolwellia* (Group 4) had complete pathways for nitrate oxidation but no pathways for the glyoxylate shunt and flagellum. Strains in *Cognaticolwellia* (Group 5) had complete pathways for ferric iron AGC types substrate binding to AfuA, glyoxylate shunt, flagellum, chemotaxis, cytochrome bd complex, and cobalamin biosynthesis in all six strains. *Allocolwellia* (Group 6) had the same pathways as *Cognaticolwellia* (Group 5) but had a different metabolic pathway for the transporter phosphonate. The metabolism of phosphonate transporters is a characteristic feature

of *Allocolwellia* (Group 6) isolated from Antarctica, reflecting the high abundance of phosphonate-producing genes in Antarctic seawater (Lockwood et al., 2022). *Pseudocolwellia* (Group 7) had the same pathway as *Cognaticolwellia* (Group 5); however, starch glycogen synthesis, mixed acid—ethanol, acetyl-CoA to acetaldehyde (reversible)—transporter urea, and type VI sections were the only pathways in *Pseudocolwellia* (Group 7). *Petrothalassotalea* (Group 8) and *Litorithalassotalea* (Group 9) are groups within the same clade with complete chitinase and nitrate pathways; however, *Petrothalassotalea* had the cobalt transporter CorA, whereas *Litorithalassotalea* had dimethylamine trimethylamine dehydrogenase. *Aliithalassotalea* (Group 10) and *Parathalassotalea* (Group 11) were grouped within the same clade, which did not have a nitrate pathway and contained chitinase. However, *Aliithalassotalea* had the cobalt transporter CorA, whereas *Parathalassotalea* had a mixed acid-ethanol, acetyl-CoA to acetaldehyde (reversible). *Thalassotalea* (Group 12) completely metabolizes nitrate, and some species contain sulfur dioxygenase. Sulfur dioxygenase metabolism is associated with the common sulfide toxins found in marine animals and is a prominent feature of *Thalassotalea* isolated from marine organisms (Zhang et al., 2013). *Thalassomonas* (Group 13) had complete pathways for nitrite reduction, nitrous oxide reduction, nitric oxide reduction, cobalt transporter CorA, chitinase, curli fimbriae biosynthesis, and type II secretion. *Thalassomonas* is isolated from marine organisms inhabiting intertidal zones and metabolizes the cobalt transporter CorA, which is associated with osmotic stress (Guskov and Eshaghi, 2012). *Parathalassomonas* (Group 14) had nitrate oxidation and the cobalt transporter CorA metabolic pathways, distinguishing it from other species forming the same clade. *Algithalassotalea* (Group 15) to *Variithalassotalea* (Group 18) formed a single clade and showed complete pathways for ferric iron ABC-type substrate binding of AfuA, glyoxylate shunt, and chemotaxis. *Vitithalassotalea* (Group 16) did not have a pathway for nitrate but had a pathway for Type VI secretion. The Type VI secretion system is a defense mechanism against coral disease outbreaks and is a characteristic feature of strains isolated from symbiotic coral environments (Wang et al., 2024). *Aminithalassotalea* (group 17) was the only group in this clade with a nitrate pathway. *Variithalassotalea* (group 18) had complete pathways for alpha-amylase and starch glycogen synthesis. *Saccharothalassotalea* (Group 19) had a pathway for biofilm PGA synthesis, different from *Algithalassotalea* (Group 15) to *Variithalassotalea* (Group 18). *Psychrothalassomonas* (Group 20) and *Solithalassomonas* (Group 24) formed a separate clade and did not exhibit metabolism in the glyoxylate shunt, flagellum, or chemotaxis. In addition, they possess a pathway for biofilm PGA synthesis, unlike other groups.

These findings indicate that the groups that are phylogenetically distinguished into clades have specific metabolic pathways. Many shared characteristics exist among members in the family Colwelliaceae, yet each genus exhibits sufficiently distinctive features for clear differentiation (Supplementary Figure S10).

4 Discussion

Although a genome-based reclassification of the Colwelliaceae, which was widely distributed in marine environments, performed in 2020 (Liu et al., 2020b), it was based on only 14 publicly available genomes at the time, failing to provide clear taxonomic criteria for many newly isolated Colwelliaceae species. Consequently, determining the precise phylogenetic position of four newly isolated Colwelliaceae strains based on 16S rRNA gene sequences was also challenging. Therefore, constructing a whole-genome phylogenetic tree was necessary to clarify their phylogenetic placement. To address this, we conducted an extensive reclassification using all available Colwelliaceae genomes, following a taxogenomic approach. This approach was inspired by previous studies that resolved taxonomic discrepancies and corrected misidentifications through genome-based reclassification, preventing future errors (Liang et al., 2021). Furthermore, genome-based reclassifications of the family Desulfovibrionaceae (Park et al., 2022) have revealed clear taxonomic trends, which guided our adoption of AAI-based genus delineation thresholds. Using this approach, we incorporated the genomes of 47 available strains, expanding the previously recognized six genera and reclassifying the family Colwelliaceae into 24 genera. The 24 genera were isolated from marine environments and shared many features, such as their Gram staining properties and requirement for NaCl for growth; however, they were distinguished from each other by their DNA G + C content, range, optimal growth temperature, respiration mode, and fatty acid composition. Differences were also observed in the metabolic potential predicted by genome annotation. The two genera (*Psychrothalassomonas*, *Arcticithalassomonas*) can be distinguished from each other by their markedly different major quinones (Groups 20 and 21), essential ion requirements for growth (Groups 1, 5, 13, 17, 20, and 22), and growth temperature ranges (Group 21 is psychrophilic). However, to propose a new taxon or change an existing taxon, Rule 30-3b of the International Code of Nomenclature of Prokaryotes must be followed, which indicates that the type strain must be deposited in two publicly recognized public culture collections. The classification cannot be ideally completed because some taxa of Colwelliaceae do not meet the mentioned conditions, owing to deposits remaining in only one culture collection or the loss of resources (*Allocolwellia hornerae* ACAM 607 and the *Variithalassotalea loyana* CBMAI 722). However, based on these data, 16 new genera, four new species, namely *Colwellia multivorans* MEBiC14330^T (=KCCM 43508^T = MCCC 1K09162^T), *Petrothalassotalea aciditolerans* MEBiC06441^T (=KCCM 43506^T = MCCC 1K09573^T), *Parathalassomonas gelatinilyticus* MEBiC06471^T (=KCCM 43507^T = MCCC 1K09163^T), and *Arcticithalassomonas sediminis* MEBiC02087^T (=KCCM 42335^T = JCM 14072^T), and three additional genera, namely *Petrothalassotalea*, *Parathalassomonas*, and *Arcticithalassomonas*, were proposed by reclassifying members of the family Colwelliaceae. The details of this process are described below.

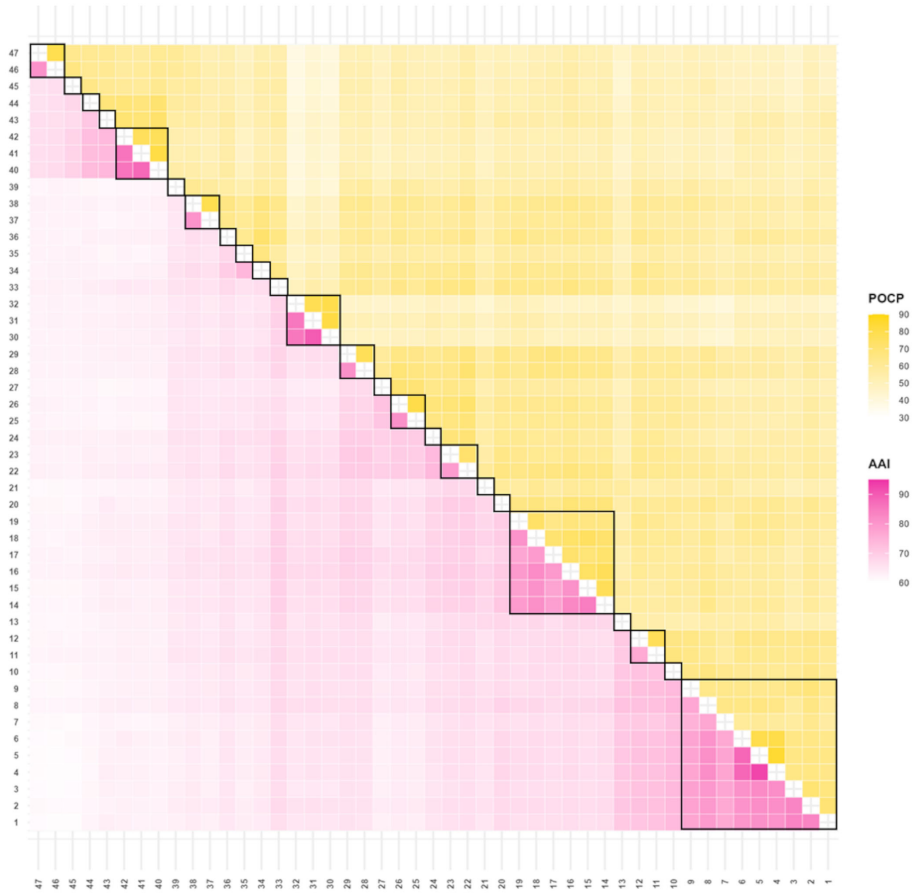

FIGURE 2
AAI and Percentage of Conserved Protein (POCP) from pairwise whole-genome comparisons. The AAI and POCP values are expressed as heat maps. The lower left triangle in pink indicates AAI, and POCP is indicated in yellow in the upper right triangle. The group was subdivided into 24 groups when it was divided based on the AAI value alone, which are separately surrounded by a black square on the figure.

5 Description

5.1 Emended description of the genus *Colwellia* Deming et al., 1988 emend. Liu et al., 2020

Colwellia [Colwel'li'a. M. L. ending -ia. M. L. fem.n. *Colwellia*; named after Rita Colwell to honor her work in the systematics of marine bacteria]

This emended description is based on the original description by Deming et al. (1988) and Liu et al. (2020a), with the following additions: Cells are Gram-stain negative, curved or straight rods. Most species are motile by means of a single polar flagellum. Catalase and oxidase positive. The predominant ubiquinone is Q-8. The DNA G + C content is 36.8% to 39.6%. The most abundant fatty acids and polar lipids are summarized as summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), and $C_{16:0}$, and phosphatidylethanolamine and phosphatidylglycerol, respectively. The Type species is *Colwellia psychrerythraea*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.2 Description of *Colwellia multivorans* sp. nov.

Colwellia multivorans [mul.ti.vo'rans. L. masc. adj. *multus* many; L. part. adj. *vorans* devouring, eating; N.L. part. adj. *multivorans* devouring different kinds of carbon source]

Cells are Gram-stain-negative, aerobic, motile, and catalase- and oxidase-positive. Colonies grown on MA are smooth, convex and punctiform and the cell size was 1.2–8.0 μm × 0.4–1.0 μm. Growth is observed at 25–30°C (optimal temperature 28°C), pH 6–8 (optimal pH 7), and 1.0–4.0% (optimum 2.0%) NaCl. The major fatty acids and quinones are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and Q-8, respectively. Polar lipids mainly included phosphatidylethanolamine, phosphatidylglycerol, three unidentified aminolipids, and three unidentified lipids. In the API kit result, nitrate reduction is negative. In the API 50CH and 20NE system, acid is produced from the following substrates; Glycerol, D-Glucose, Methyl-α D-Mannopyranoside, N-Acetyl Glucosamine, Esculin ferric citrate, D-Melibiose, Inulin, and D-Tagatose. But negative for Erythritol, Arabinose, D-Adonitol, Methyl-β D-Xylopyranoside, Xylose, Galactose, Inositol, Cellobiose, D-Lactose, Glycogen, Xylitol,

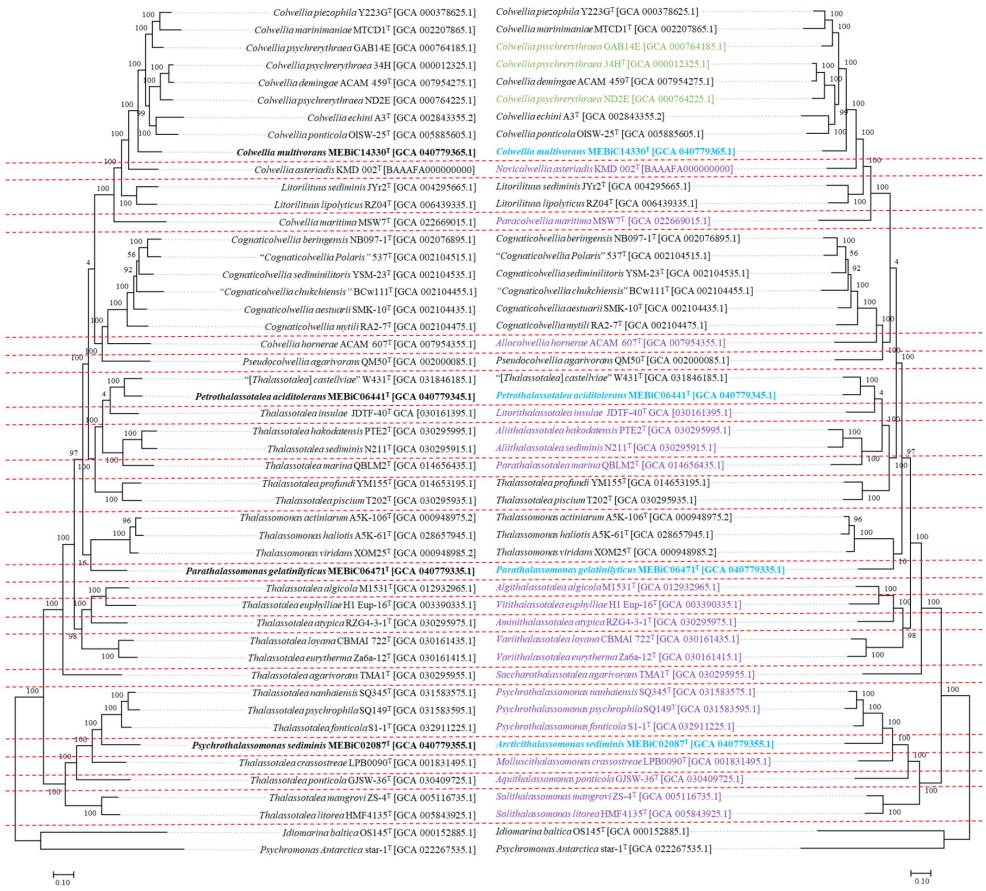

FIGURE 3
Reclassification of Colwelliaceae using whole-genome sequence similarity inferred by a genomic tree. This figure includes a mirror image. The left-sided tree represents before reclassifications, and the right-sided tree represents after reclassifications. Purple labels indicate that a new genus has been proposed in this study, blue labels indicate that a species or genus has been newly proposed in this study, and green labels indicate that a species is currently in need of further research as it may be classified as a different species. The tree is based on Maximum Likelihood with 1,000 bootstraps.

Gentiobiose, D-Turanose, D-Lyxose, L- Fucose, L-Arabitol, Urea, Gellatine, Capric acid, Adipic acid, Malic acid, and phenylacetic acid. Enzymes that exhibited activity include Alkaline Phosphatase, Leucine Arylamidase, Valine Arylamidase, Trypsin, α-Chymotrypsin, Acid Phosphatase, Naphtol-AS-BI-Phosphohydrolase, and N-acetyl-β-Glucosaminidase. In contrast, α-Galactosidase, β-Glucuronidase, β-Glucosidase, α-Glucosidase, α-Mannosidase, and α- Fucosidase are negative in enzyme activity. The DNA G + C content is 38.2%.

The type strain is MEBiC14330^T (=KCCM 43508^T = MCCC 1K09162^T), isolated from 1,300m depth sediment collected at Gordin Guyot (seamount). The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is OR958774 and that of the genome is JBFONR000000000.

5.3 Description of *Novicolwellia* gen. nov.

Novicolwellia [No.vi.col.well'i.a. L. masc. adj. *novus*, new; N.L. fem. n. *Colwellia*, a prokaryotic genus from the American microbiologist Rita R. Colwell. N.L. fem. n. *Novicolwellia* a new *Colwellia*]

Cells are Gram-stain-negative, facultative anaerobic, rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and $C_{16:0}$. The DNA G + C content of type species is 38.0%. The type species is *Novicolwellia asteriadis*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.4 Description of *Novicolwellia asteriadis* comb. nov.

Novicolwellia asteriadis [as.te'ri.adis. N.L. n. *Asterias* a zoological genus name; N.L. gen. n. *asteriadis* of *Asterias*, referring to the isolation of the type strain from the starfish *Asterias amurensis*]

Basonym: *Colwellia asteriadis* Choi et al., 2010

The description is identical to that of Choi et al. (2010).

The type strain KMD 002^T (=KCCM 90077^T = JCM 15608^T) was isolated from the skin of a specimen of the starfish *Asterias amurensis* collected at the Im-Won port in Samcheok on the East Sea of Korea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain KMD 002^T is EU599214 and NZ_BAAAFA000000000, respectively.

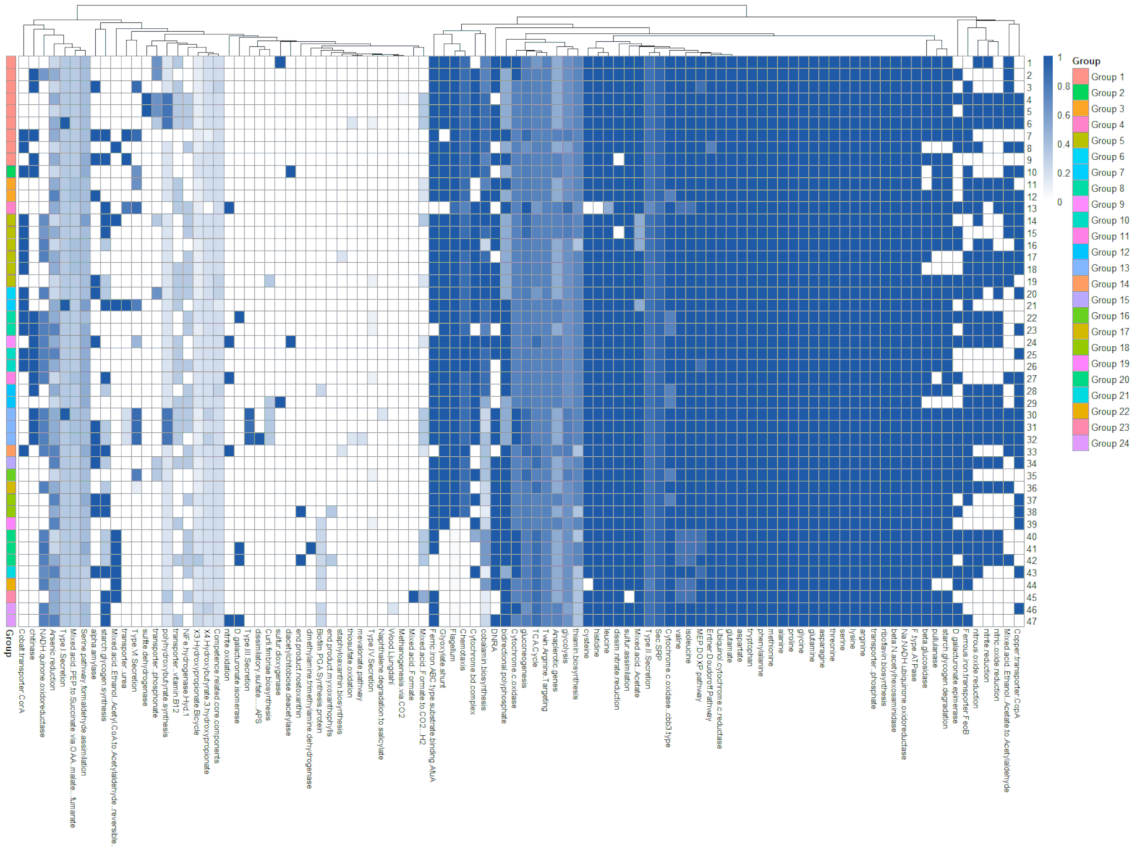

FIGURE 4
A heatmap based on the Kyoto Encyclopedia of Genes and Genomes metabolic pathways, created by extracting only active categories from the Kofamscan results. The same color on the left distinguishes groups of the same type. Relative abundance (%) is indicated by the blue bar.

5.5 Description of *Paracolwellia* gen. nov.

Paracolwellia [Pa.ra.col.well'i.a. Gr. prep. para, next to; N.L. fem. n. *Colwellia*, a prokaryotic genus from the American microbiologist Rita R. Colwell. N.L. fem. n. *Paracolwellia* a new genus near the genus *Colwellia*]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$, $C_{15:1}$ $\omega 8c$, $C_{17:1}$ $\omega 8c$, and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$). The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of type species is 37.3%. The type species is *Paracolwellia maritima*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.6 Description of *Paracolwellia maritima* comb. nov.

Paracolwellia maritima [ma.ri'ti.ma. L. fem. adj. *maritima*, of the marine environment, maritime]

Basonym: *Colwellia maritima* Kristyanto et al., 2022

The description is identical to that of Kristyanto et al (2022).

The type strain MSW7^T (=KACC 22339^T = JCM 35001^T) was isolated from seawater of Yellow Sea in South Korea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain MSW7^T is MZ310521 and JAKKSL000000000, respectively.

5.7 Emended description of the genus *Cognaticolwellia* Liu et al., 2020

Cognaticolwellia [Cog.na.ti.col.wel'li.a. L. masc. adj. cognatus relative, related, kindred; N.L. fem. n. *Colwellia* a bacteria generic name; N.L. fem. n. *Cognaticolwellia* related to *Colwellia*]

This emended description is based on the original description by Liu et al. (2020a), with the following additions: Some members require Mg^{2+} and NaCl for survival.

5.8 Description of *Allocolwellia* gen. nov.

Allocolwellia [Al.lo.col.well'i.a. Gr. masc. adj. allos, other; N.L. fem. n. *Colwellia*, a prokaryotic genus from the American microbiologist Rita R. Colwell. N.L. fem. n. *Allocolwellia* another *Colwellia*]

Cells are Gram-stain-negative, facultatively anaerobic, curved-rod, and motile. Psychrophilic and chemoheterotroph. The predominant ubiquinone is Q-8. In liquid media, T_{opt} is approximately 12°C and T_{max} is about 23–24°C. The DNA G + C content of type species is 38.1%. The type species is *Allocolwellia hornerae*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.9 Description of *Allocolwellia hornerae* comb. nov.

Allocolwellia hornerae [hor.ner′ae. L. adj. *hornerae* in honor of Rita Horner, an American biologist who pioneered studies on sea-ice microbiota]

Basonym: *Colwellia hornerae* Bowman et al., 1998

The description is identical to that of Bowman et al. (1998).

The type strain ACAM 607^T (=CIP 105821^T = ACAM 607^T) was isolated from fast sea ice of the Prydz Bay coast, Antarctica. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain ACAM 607^T is JN175346 and VOLR00000000, respectively.

5.10 Description of *Petrothalassotalea* gen. nov.

Petrothalassotalea [Pe.tro.tha.las.so.ta'le.a. Gr. pem. n. petra, rock, stone (referring to petroleum); N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Petrothalassotalea*; a *Thalassotalea* isolated from petroleum contaminated coastal sediments]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are $C_{15:1}$ *ω8c*, $C_{16:0}$, $C_{17:1}$ *ω8c*, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, five unidentified aminolipids, and four unidentified lipids. The DNA G + C content of type species is 37.4%. The type species is *Petrothalassotalea aciditolerans*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.11 Description of *Petrothalassotalea aciditolerans* sp. nov.

Petrothalassotalea aciditolerans [a.ci.di.to'le.rans. L. neut. adj. *acidum*, an acid; L. pres. part. *Tolerans*, tolerating; N.L. neut. part. adj. *aciditolerans*, acid-tolerating]

In addition to the genus description, the following are added: Colonies grown on MA are rough, flat, and irregular. The cell size was 0.9–3.4 μm × 0.4–0.9 μm. Growth is observed at 10–25°C (optimal temperature 20°C), pH 5–7 (optimal pH 7), and 0.5–2.0% (optimum 1.0%) NaCl. In the API 50CH and 20NE system, acid is produced from the following substrates: D-Ribose, L-Xylose, L-Rhamnose, D-Mannitol, Esculin ferric citrate, L-Arabitol, L-arginine and Urea. L-Arabinose, Dulcitol, D-Maltose, D-Lactose, D-Melezitose, D-Raffinose, D-Tagatose, and L-Fucose are weakly positive. Glycerol, Erythritol, D-Arabinose, D-Xylose, D-Adonitol, Methyl-β D-Xylopyranoside, Glucose, Inositol, Methyl-α D-Mannopyranoside, Methyl-α D-Glucopyranoside, N-AcetylGlucosamine, Arbutin, Salicin, Cellobiose, Inulin, Starch, Glycogen, Xylitol, and Gentiobiose are negative. Alkaline phosphatase, Leucine arylamidase, and Valine arylamidase activites are positive, but Esterase, Lipase, Trypsin, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase activities are negative. Cystine arylamidase, α-chymotrypsin, acid phosphatase, Naphthol-AS-BI-phosphohydrolase, and N-acetyl-β-glucosaminidase activities are weak.

The type strain is MEBiC06441^T (=KCCM 43506^T = MCCC 1K09573^T), isolated from tidal sediment collected at Incheon City, Republic of Korea. The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is OR958780 and that of the genome is JBFONU000000000.

5.12 Description of *Litorithalassotalea* gen. nov.

Litorithalassotalea [Li.to.ri.tha.las.so.ta'le.a. L. neut. n. *litus*, shore; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Litorithalassotalea*, a relative of *Thalassotalea* from the shore]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. Some members require Mg^{2+} ions or Nitrate for growth. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid, one unidentified glycolipid, and three unidentified lipids. The DNA G + C content of type species is 40.3%. The type species is *Litorithalassotalea insulae*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.13 Description of *Litorithalassotalea insulae* comb. nov.

Litorithalassotalea insulae [in′su.lae. L. gen. n. *insulae* of an island, referring to the source of isolation of the type strain]

Basonym: *Thalassotalea insulae* Park et al., 2018

The description is identical to that of Park et al. (2018).

The type strain JDTF-40^T (=KACC 19433^T = KCTC 62186^T = NBRC 113040^T) was isolated from a tidal flat in Jindo Island, an island of the Republic of South Korea, in the Yellow Sea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain JDTF-40^T is MG592731 and BSST00000000, respectively.

5.14 Description of *Aliithalassotalea* gen. nov.

Aliithalassotalea [A.li.i.tha.las.so.ta'le.a. L. masc. adj. alius other, another; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Aliithalassotalea*, another *Thalassotalea*]

Cells are Gram-stain-negative, aerobic or facultatively anaerobic, rod-shaped and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$, $C_{17:1}$ *ω8c* and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content is 38.4% to 38.6%. The type species is *Aliithalassotalea sediminis*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.15 Description of *Aliithalassotalea hakodatensis* comb. nov.

Aliithalassotalea hakodatensis [ha.ko.da.ten'sis. N.L. fem. adj. *hakodatensis*, from Hakodate, referring to the isolation site of the strain]

Basonym: *Thalassotalea hakodatensis* Yamano et al., 2023

The description is identical to that of Yamano et al. (2023).

The type strain PTE2^T (=JCM 34608^T = KCTC 82592^T) was isolated from a pentactula larvae of *Apostichopus japonicus* reared in a laboratory aquarium at Hokkaido University, Hokkaido, Japan. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain PTE2^T is LC757706 and AP027365, respectively.

5.16 Description of *Aliithalassotalea sediminis* comb. nov.

Aliithalassotalea sediminis [se.di'mi.nis. L. gen. n. sediminis of sediment]

Basonym: *Thalassotalea sediminis* Xu et al., 2017

The description is identical to that of Xu et al. (2016).

The type strain N211^T (=KCTC 42588^T = MCCC 1H00116^T) was isolated from marine sediment collected off the coast of Weihai, China. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain N211^T is LC757706 and AP027361, respectively.

5.17 Description of *Parathalassotalea* gen. nov.

Parathalassotalea [Pa.ra.tha.las.so.ta'le.a. Gr. prep. para, next to; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Parathalassotalea*, a new genus near the genus *Thalassotalea*]

Cells are Gram-stain-negative, facultatively anaerobic, rod-shaped, and motile. Catalase- and oxidase-positive. The predominant ubiquinone is Q-8. The major fatty acids are $C_{17:1}$ *ω8c* and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content of type species is 39.4%. The type species is *Parathalassotalea marina*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.18 Description of *Parathalassotalea marina* comb. nov.

Parathalassotalea marina [se.di'mi.nis. L. gen. n. sediminis of sediment]

Basonym: *Thalassotalea marina* Hou et al., 2015

The description is identical to that of Hou et al. (2015).

The type strain QBLM2^T (=CGMCC 1.12814^T = KCTC 42731^T) was isolated from the rearing water of a marine recirculating aquaculture system for yellow grouper (*Epinephelus awoara*) in Tianjin, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain QBLM2^T are KT153611 and BNCK00000000, respectively.

5.19 Emended description of the genus *Thalassotalea* Zhang et al., 2014

Thalassotalea [Tha.las.so.ta'le.a. Gr. n. thalasso the sea; L. fem. n. talea a rod; N.L. fem. n. *Thalassotalea* a rod from the sea]

This emended description is based on the original description by Zhang et al. (2014), with the following additions: Cells are Gram-stain-negative, aerobic or strictly aerobic, chemo-organotrophic rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. Some members require Na^+ for growth. The predominant ubiquinone is Q-8. The major fatty acids are $C_{17:1}$ *ω8c* and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content is 36.3% to 37.5%. The type species is *Thalassotalea piscium*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.20 Emended description of the genus *Thalassomonas* Macián et al., 2001

Thalassomonas [Tha.las'so.mo.nas. Gr. n. Thalasso the sea; Gr. n. monas a unit; N.L. n. *Thalassomonas* a monad from the sea]

This emended description is based on the original description by Macián et al. (2001), with the following additions: Cells are Gram-stain-negative, aerobic or strictly aerobic, rod-shaped, and motile. Catalase- and oxidase-positive. some members require seawater components such as Mg^{2+} for growth in addition to the NaCl and heterotrophic. The predominant ubiquinone is Q-8. The major fatty

acids are $C_{16:0}$ and $C_{17:1}$ *ω8c*. The DNA G + C content is 47.3% to 48.9%. The type species is *Thalassomonas viridans*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.21 Description of *Parathalassomonas* gen. nov.

Parathalassomonas [Pa.ra.tha.las.so.mo.nas. Gr. prep. para, next to; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Parathalassomonas*; a genus adjacent to the genus *Thalassomonas*]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile. Catalase- and oxidase-positive. Nitrate reduction is negative. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, four unidentified aminolipids, and four unidentified lipids. The DNA G + C content of type species is 39.6%. The type species is *Petrothalassotalea aciditolerans*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.22 Description of *Parathalassomonas gelatinilyticus* sp. nov.

Parathalassomonas gelatinilyticus [ge.la.ti.ni.ly'ti.cus. N.L. neut. n. gelatinum, gelatin; N.L. masc. adj. lyticus (from Gr. masc. adj. lytikos), able to loosen, able to dissolve; N.L. masc. adj. *gelatinilyticus*, gelatin-dissolving]

In addition to the genus description, the following are added: Colonies grown on MA are smooth, convex, and circular, and the cell size was 1.1–3.3 μm × 0.4–1.0 μm. Growth is observed at 10–30° C (optimal temperature 25°C), pH 5–8 (optimal pH 7), and 0.5–2.0% (optimum 1.0%) NaCl. In the API 50CH and 20NE system, acid is produced from the following substrates; Erythritol, D-Ribose, D-Adonitol, Methyl-β D-Xylopyranoside, D-Glucose, D-Mannose, Inositol, D-Mannitol, N-Acetylglucosamine, Amygdalin, Arbutin, Esculin ferric citrate, Salicin, Cellobiose, Maltose, Lactose, D-Melibiose, D-Saccharose, D-Tagatose, and Gelatin. Acid from D-Fructose, D-Sorbitol, D-Trehalose, Inulin, D-Melezitose, Xylitol, and Arabitol is weakly positive. Glycerol, Arabinose, Xylose, Galactose, L-Sorbose, L-Rhamnose, Dulcitol, Methyl-α D-Mannopyranoside, Methyl-α D-Glucopyranoside, Starch, Glycogen, Gentiobiose, D-Turanose, D-Lyxose, Fucose, Urea, Esculin, Capric acid, Adipic acid, Malic acid, and trisodium citrate are negative for acid production. Alkaline phosphatase and Leucine arylamidase enzyme activities are positive, while Esterase, Lipase, Valine arylamidase, Naphthol-AS-BI-phosphohydrolase, α-glucosidase, and N-acetyl-β-glucosaminidase are weakly positive. Cystine arylamidase, Trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, β-glucosidase, α-mannosidase, and α-fucosidase activity is negative

The type strain is MEBiC06471^T (=KCCM 43507^T = MCCC 1K09163^T), isolated from tidal sediment collected at Taean-gun, Republic of Korea. The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is OR958775 and that of the genome is JBFONV000000000.

5.23 Description of *Algithalassotalea* gen. nov.

Algithalassotalea [Al.gi.tha.las.so.ta'le.a. L. fem. n. alga, a seaweed; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Algithalassotalea*; a relative of *Thalassotalea* from an alga]

Cells are Gram-stain-negative, facultatively anaerobic, rod-shaped, and motile by a single polar flagellum and gliding. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are summed features 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{16:0}$, and summed features 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content of type species is 39.1%. The type species is *Algithalassotalea algicola*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.24 Description of *Algithalassotalea algicola* comb. nov.

Algithalassotalea algicola [al.gi'co.la. L. n. alga -ae an alga (seaweed, dinoflagellate etc.); L. suff. -cola from L. n. *incola* an inhabitant; N.L. fem. n. *algicola* an inhabitant of algae]

Basonym: *Thalassotalea algicola* Lian et al., 2022

The description is identical to that of Lian et al. (2021).

The type strain M1531^T (=MCCC 1H00400^T = KCTC 72865^T) was isolated from a red alga at coastal water in Weihai, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain M1531^T are MN822801 and JABBXH000000000, respectively.

5.25 Description of *Vitithalassotalea* gen. nov.

Vitithalassotalea [Vi.ti.tha.las.so.ta'le.a. L. fem. n. vita, life; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Vitithalassotalea*; a *Thalassotalea* from marine live]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile by means of a single polar flagellum. Catalase- and oxidase-positive. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), $C_{18:1}$ *ω7c*, $C_{16:0}$, and $C_{17:1}$ *ω8c*. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, one uncharacterized aminophospholipid, five uncharacterized phospholipids, three

uncharacterized aminolipids, and two uncharacterized lipids. The DNA G + C content of type species is 44.3%. The type species is *Vitithalassotalea euphylliae*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.26 Description of *Vitithalassotalea euphylliae* comb. nov.

Vitithalassotalea euphylliae [eu.phyl'li.ae. N.L. gen. n. *euphylliae* of *Euphyllia*, isolated from a coral belonging to the genus *Euphyllia*]

Basonym: *Thalassotalea euphylliae* Sheu et al., 2016

The description is identical to that of Sheu et al. (2016).

The type strain Eup-16^T (=BCRC 80910^T = LMG 29001^T = KCTC 42743^T) was isolated from the torch coral *Euphyllia glabrescens*, collected off southern Taiwan. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain Eup-16^T are LN849949 and QUOU00000000, respectively.

5.27 Description of *Aminithalassotalea* gen. nov.

Aminithalassotalea [A.mi.ni.tha.las.so.ta'le.a. N.L. fem. n. *amina*, amine; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Aminithalassotalea*; a *Thalassotalea* loving amino acids]

Cells are Gram-stain-negative, strictly aerobic, straight or slightly curved rods, non-flagellated and non-gliding. Oxidase is positive, but catalase is negative. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The major fatty acids are summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$) and $C_{16:0}$. The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of type species is 40.2%. The type species is *Aminithalassotalea atypica*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.28 Description of *Aminithalassotalea atypica* comb. nov.

Aminithalassotalea atypica [a.ty'pi.ca. Gr. pref. a- not; L. adj. *typicus* typical; N.L. fem. adj. *atypica* atypical]

Basonym: *Thalassotalea atypica* Wang et al., 2018

The description is identical to that of Wang et al. (2018).

The type strain RZG4-3-1^T (=JCM 31894^T = KCTC 52745^T = MCCC 1K03276^T) was isolated from the surface seawater of the Yellow Sea in China (35° 31' 01" N, 119° 37' 30" E). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain RZG4-3-1^T is KY014437 and AP027364, respectively.

5.29 Description of *Variithalassotalea* gen. nov.

Variithalassotalea [Va.ri.i.tha.las.so.ta'le.a. L. masc. adj. *varius*, varying; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Variithalassotalea*; a *Thalassotalea* from various origin]

Cells are Gram-stain-negative, aerobic, rod-shaped, and motile by a single polar flagellum. Catalase is positive. The major fatty acids are summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{14:0}$, and $C_{17:1}$ $\omega8c$. The DNA G + C content is 39.7% to 40.4%. The type species is *Variithalassotalea loyana*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.30 Description of *Variithalassotalea loyana* comb. nov.

Variithalassotalea loyana [loy'an.a. N.L. fem. adj. *loyana* named in honor of the Israeli biologist Y. Loya]

Basonym: *Thalassomonas loyana* Thompson et al., 2006

The description is identical to that of Thompson et al. (2006).

The type strain CBMAI 722^T (=LMG 22536^T = CBMAI 722^T) was isolated from diseased coral in Eilat, Israel. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain CBMAI 722^T is AY643537 and BSSV00000000, respectively.

5.31 Description of *Variithalassotalea eurytherma* comb. nov.

Variithalassotalea eurytherma [eu.ry.ther'ma. Gr. adj. eurys wide; Gr. adj. thermos hot; N.L. fem. adj. eurytherma able to tolerate a wide range of temperatures]

Basonym: *Thalassomonas eurytherma* Sun et al., 2014

The description is identical to that of Sun et al. (2014).

The type strain Za6a-12^T (=CGMCC 1.12116^T = JCM 18483^T) was isolated from the Zhoushan Islands in the East China Sea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain Za6a-12^T is JQ288724 and BSSU00000000, respectively.

5.32 Description of *Saccharothalassotalea* gen. nov.

Saccharothalassotalea [Sac.cha.ro.tha.las.so.ta'le.a. Gr. neut. n. *sakchar*, sugar; N.L. fem. n. *Thalassotalea*, a genus name meaning a rod from the sea; N.L. fem. n. *Saccharothalassotalea*; a *Thalassotalea* loving sugar]

Cells are Gram-stain-negative, aerobic, straight or curved rods, and non-motile. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. The major fatty acids are $C_{16:0}$, $C_{17:1}$ $\omega 8c$, $C_{17:0}$, $C_{15:0}$ iso 2-OH/ $C_{16:1}$ $\omega 7c$, and $C_{13:0}$. The DNA G + C content of type species is 41.9%. The type species is *Saccharothalassotalea agarivorans*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.33 Description of *Saccharothalassotalea agarivorans* comb. nov.

Saccharothalassotalea agarivorans [a.ga.ri.vo′rans. N.L. n. agarum agar; L. part. adj. *vorans* devouring, destroying; N.L. part. adj. *agarivorans* agar-devouring]

Basonym: *Thalassomonas agarivorans* Jean et al., 2006

The description is identical to that of Jean et al. (2006).

The type strain TMA1^T (= BCRC 17492^T = JCM 13379^T = DSM 19706^T) was isolated from shallow seawater of An-Ping Harbour, Tainan, Taiwan. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain TMA1^T is DQ212914 and AP027363, respectively.

5.34 Description of *Psychrothalassomonas* gen. nov.

Psychrothalassomonas [Psy.chro.tha.las.so.mo.nas. Gr. masc. adj. *psychros*, cold; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Psychrothalassomonas*; a *Thalassomonas* living in cold water]

Cells are Gram-stain-negative, aerobic, rod-shaped, and non-motile. Oxidase is positive, but catalase is negative. The strains are psychrophilic or psychrotrophic. Nitrate reduction is variable. The predominant quinone is Q-8, but some members additionally include MK-7. The major fatty acids are summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$) and $C_{16:0}$. The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content is 38.1% to 39.0%. The type species is *Psychrothalassomonas psychrophila*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.35 Description of *Psychrothalassomonas nanhaiensis* comb. nov.

Psychrothalassomonas nanhaiensis [nan.hai.en'sis. N.L. fem. adj. *nanhaiensis*, pertaining to NanHai, the Chinese name for the South China Sea, where the type strain was isolated]

Basonym: *Thalassotalea nanhaiensis* Li et al., 2024

The description is identical to that of Li et al. (2024).

The type strain SQ345^T (= MCCC 1K04232^T = JCM 33808^T) was isolated from deep-sea sediment of the South China Sea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain SQ345^T is MK801284 and CP134146, respectively.

5.36 Description of *Psychrothalassomonas psychrophila* comb. nov.

Psychrothalassomonas psychrophile [psy.chro'phi.la. Gr. masc. adj. *psychros* cold; Gr. masc. adj. *philos* loving; N.L. fem. adj. *psychrophila*, referring to loving low temperatures]

Basonym: *Thalassotalea psychrophila* Li et al., 2024

The description is identical to that of Li et al. (2024).

The type strain SQ149^T (= MCCC 1K04231^T = JCM 33807^T) was isolated from deep-sea sediment of the South China Sea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain SQ149^T is MK801283 and CP134145, respectively.

5.37 Description of *Psychrothalassomonas fonticola* comb. nov.

Psychrothalassomonas fonticola [fon.ti'co.la. L. fem. n. *fons*, spring; L. masc./fem. suff. *-cola*, inhabitant, dweller; N.L. fem. n. *fonticola*, an inhabitant of a seep]

Basonym: *Thalassotalea fonticola* Li et al., 2024

The description is identical to that of Li et al. (2024).

The type strain S1-1^T (= MCCC 1K06879^T = JCM 34824^T) was isolated from deep-sea sediment of the South China Sea. The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence and genome sequence of strain S1-1^T is OP303371 and CP136600, respectively.

5.38 Description of *Arcticithalassomonas* gen. nov.

Arcticithalassomonas [Arc.ti.ci.tha.las.so.mo.nas. L. masc. adj. *arcticus*, northern, arctic; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Arcticithalassomonas*; a *Thalassomonas* isolated from Arctic Sea]

Cells are Gram-stain-negative, aerobic, rod-shaped and motile. Catalase- and oxidase-positive. Nitrate reduction is positive. Some members require Mg^{2+} and K^+ ions in addition to the NaCl for growth. The predominant ubiquinone is MK-6. The major fatty acids are $C_{16:0}$ and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$). The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of type species is 39.3%. The type species is *Arcticithalassomonas sediminis*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.39 Description of *Arcticithalassomonas sediminis* sp. nov.

Arcticithalssomonas sediminis [se.di.mi' nis. L. gen. n. sediminis of sediment, the source of the type strain]

In addition to the genus description, followings are added; Colonies grown on MA are circular, opaque, convex with entire margin and the cell size was 0.5–0.7 μm × 1.1–2.5 μm. Growth is observed at 0–16°C (optimal temperature 9°C), pH 6–10 (optimal pH 7–8) and 0.0–4.0% (optimum 2.0–3.0%) NaCl. Require Mg^{2+} and K^{+} ions in addition to the NaCl for growth. β-galactosidase, and β-Glucosidase activities are present but arginine dihydrolase, protease and Urease activities are not. Indole is produced from tryptophan and nitrate reduction is negative. Alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, trypsin, α-Chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities and weak cystine arylamidase activities are present. Substrates utilized are include α-Cyclodextrin, dextrin, glycogen, N-acetyl-D-glucosamine, cellobiose, D-fructose, gentiobiose, α-D-glucose, α-D-lactose, maltose, D-mannitol, sucrose, D-trehalose, cis-aconitic acid, citric acid, D-glucuronic acid, D,L-lactic acid, bromosuccinic acid, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, L-serine, L-threonine, inosine, uridine, thymidine, glycerol, D,L-α-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate. D-galactose, β-methyl-D-glucoside, α-ketobutyric acid, α-ketoglutaric acid, and alaninamide is weakly utilize. In the API 50CH and 20NE system, acid produced from the following substrates; glycerol, glucose, fructose, mannose, esculin, cellobiose, maltose, melibiose, sucrose, trehalose, starch, and glycogen, and weakly for ribose, galactose, mannitol, and salicin. MEBiC02087^{T} is identical to BS250^{T}.

The type strain is MEBiC02087^{T} (=KCCM 42335^{T} = JCM 14072^{T}), isolated from 680 m depth sediment collected at Barents Sea, Russia. The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is PQ119528 and that of the genome is JBFONS000000000.

5.40 Description of *Molluscithalassomonas* gen. nov.

Molluscithalassomonas [Mol.lus.ci.tha.las.so.mo.nas. L. pl. neut. n. *Mollusca*, an animal phylum; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Molluscithalassomonas*; a *Thalassomonas* from a mollusks]

Cells are Gram-stain-negative, aerobic, rod-shaped and non-motile. Catalase- and oxidase-positive. Nitrate reduction is positive. Does not grow under microaerophilic or anaerobic conditions. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$, $C_{17:1}$ *ω8c*, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of type species is 38.8%. The type species is *Molluscithalassomonas crassostreae*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.41 Description of *Molluscithalassomonas crassostreae* comb. nov.

Molluscithalassomonas crassostreae [crass.os'tre.ae. N.L. gen. n. *crassostreae* of *Crassostrea gigas*, named after the generic name of the oyster from which the type strain was isolated]

Basonym: *Thalassotalea crassostreae* Choi et al., 2017

The description is identical to that of Choi et al. (2017).

The type strain LPB0090^{T} (=KACC 18695^{T} = JCM 31189^{T}) was isolated from an oyster species from Yeongheung Island, Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain LPB0090^{T} is KU160140 and CP017689, respectively.

5.42 Description of *Aquithalassomonas* gen. nov.

Aquithalassomonas [A.qui.tha.las.so.mo.nas. L. fem. n. *aqua*, water; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Aquithalassomonas*; a *Thalassomonas* live in water]

Cells are Gram-stain-negative, aerobic, rod-shaped or ovoid, non-motile, and non-flagellated. Catalase- and oxidase-positive. Nitrate reduction is positive. Some members require Mg^{2+} ions for growth. The predominant ubiquinone is Q-8. The major fatty acids are $C_{16:0}$, $C_{17:1}$ *ω8c*, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of type species is 44.4%. The type species is *Aquithalassomonas ponticola*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.43 Description of *Aquithalassomonas ponticola* comb. nov.

Aquithalassomonas ponticola [pon.ti'co.la. L. n. *pontus* the sea; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. fem. n. *ponticola* living in the sea]

Basonym: *Thalassotalea ponticola* Park et al., 2014

The description is identical to that of Park et al. (2014).

The type strain GJSW-36^{T} (=KCTC 42155^{T} = CECT 8656^{T}) was isolated from seawater collected from Geoje Island in the South Sea, South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain GJSW-36^{T} is KU160140 and CP017689, respectively.

5.44 Description of *Solithalassomonas* gen. nov.

Solithalassomonas [So.li.tha.las.so.mo.nas. L. neut. n. *solum*, soil; N.L. fem. n. *Thalassomonas*, a genus name meaning a rod from the sea; N.L. fem. n. *Solithalassomonas*; a *Thalassomonas* from a soil]

Cells are Gram-stain-negative, strictly aerobic, rod-shaped or ovoid, non-motile or motile by single polar flagellum. Oxidase is positive, but catalase varies depending on the strain. Nitrate reduction is positive. The predominant ubiquinone is Q-8. The main fatty acid in common is $C_{16:0}$. The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content is 43.9% to 45.9%. The type species is *Aquithalassomonas ponticola*. A member of the family Colwelliaceae, the class Gammaproteobacteria, according to 16S rRNA gene and whole genome sequence analysis.

5.45 Description of *Solithalassomonas mangrovi* comb. nov.

Solithalassomonas mangrovi [man.gro′vi. N.L. gen. n. mangrovi of or belonging to a mangrove wetland]

Basonym: *Thalassotalea mangrovi* Zheng et al., 2019

The description is identical to that of Zheng et al. (2019).

The type strain ZS-4^T (=KCTC 72399^T = MCCC 1K03630^T) was isolated from marine mangrove sediment collected at Beilun Estuary National Nature Reserve, Guangxi Province, PR China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain ZS-4^T are MK911729 and SWDB00000000, respectively.

5.46 Description of *Solithalassomonas litorea* comb. nov.

Solithalassomonas litorea [li.to′re.a. L. fem. adj. *litorea* of seashore]

Basonym: *Thalassotalea litorea* Kang et al., 2017

The description is identical to that of Kang et al. (2017).

The type strain HMF4135^T (=KCTC 52154^T = NBRC 112672^T) was isolated from seashore sand in Jeju, Republic of Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and genome sequence of strain HMF4135^T are KU853009 and VCBC00000000, respectively.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

ML: Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. S-HY: Formal Analysis, Writing – review & editing. YK: Formal Analysis, Resources, Validation, Writing – review & editing. YP: Formal Analysis, Methodology, Writing – review & editing. M-JP: Conceptualization, Data curation, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. KK: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2025.1532186/full#supplementary-material

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