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Acidimicrobiia, the actinomycetota of coastal marine sediments: Abundance, taxonomy and genomic potential

Sebastián Silva-Solar, Tomeu Viver, Yueqing Wang, Luis H. Orellana, Katrin Knittel, Rudolf Amann *

Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, Celsius Str 1, 28359 Bremen, Germany

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ABSTRACT

Microbial communities in marine sediments represent some of the densest and most diverse biological communities known, with up to a billion cells and thousands of species per milliliter. Among this taxonomic diversity, the class *Acidimicrobiia*, within the phylum *Actinomycetota*, stands out for its consistent presence, yet its limited taxonomic understanding obscures its ecological role. We used metagenome-assembled genomes from a 5-year Arctic fjord sampling campaign and compared them to publicly available *Acidimicrobiia* genomes using 16S rRNA gene and whole-genome phylogenies, alongside gene prediction and annotation to study their taxonomy and genomic potential. Overall, we provide a taxonomic overview of the class *Acidimicrobiia* and show its significant prevalence in Isfjorden and Helgoland coastal sediments, representing over 90% of *Actinomycetota* 16S rRNA gene sequences, and 3–7% of *Bacteria*. We propose *Benthobacter isfjordensis* gen. nov., sp. nov., *Hadalibacter litoralis* gen. nov., sp. nov., and two new species from *Ilumatobacter*, following SeqCode guidelines. In addition, we report the first *in situ* quantification of the family *Ilumatobacteraceae*, revealing its substantial presence (1–6%) in coastal sediments. This work highlights the need of refining the taxonomy of *Acidimicrobiia* to better understand their ecological contributions.

Introduction

Marine sediments harbor some of the most dense and diverse microbial communities on Earth, comprising up to a billion cells and thousands of species per milliliter (Probandt et al., 2018; Probandt et al., 2017). Within these diverse microbial communities, the phylum *Actinomycetota* emerges as a consistently prominent group (Balmonte et al., 2019; Gobet et al., 2012; Hoshino et al., 2020; Li et al., 2009; Miksch et al., 2021; Probandt et al., 2017; Ravenschlag et al., 1999; Seo et al., 2017; Teske et al., 2011). *Actinomycetota* (represented almost uniquely by the class *Acidimicrobiia*), were shown to account for approximately 30% of bacterial 16S rRNA gene sequences and 10% of total cell counts in Isfjorden, Svalbard (Miksch et al., 2021). Similar observations have been documented in the South Sea of Korea, where up to 10% of the community belongs to *Actinomycetota*, and almost all *Actinomycetota* sequences belong to *Acidimicrobiia.* In the North Sea, *Acidimicrobiia* account for up to 10% of the bacterial community in coarse grain sand, and approximately 20% in bottom waters (3–5 m above the seabed, Probandt et al., 2017). Other studies have documented similar abundances for the phylum *Actinomycetota* (referred to as *Actinobacteria* in older literature) without a more specific taxonomic classification (Gobet et al., 2012; Hoshino et al., 2020; Li et al., 2009). The repeated identification of *Acidimicrobiia* highlighted its persistence, suggesting it as possibly the most dominant representative of *Actinomycetota* in benthic marine ecosystems. However, the significance of distinct and more specific clades has not been analyzed, likely due to the absence of a robust taxonomic structure and a stable nomenclature. Unclear taxonomy and nomenclature hampers, in particular, quantifications of defined acidimicrobial taxa and the autecological assignment of functions.

The type species of the class *Acidimicrobiia, Acidimicrobium ferrooxidans*, was first described in 1996 (Clark and Norris, 1996). One year later, Stackebrandt et al. (1997) proposed the new family *Acidimicrobiaceae* (with *Acidimicrobium* as type genus), the order *Acidimicrobiales*, and the subclass *Acidimicrobidae* within the class *Actinobacteria* based on comparative sequence analysis of 16S rRNA genes. In 2012, in an update of Volume Five: "The Actinobacteria", Part B of the Bergey's Manual of Systematic Bacteriology (Parte et al., 2012), Goodfellow and colleagues proposed the elevation of the class

* Corresponding author.
E-mail address: ramann@mpi-bremen.de (R. Amann).

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Actinobacteria to phylum level following the taxonomic analysis of Stackebrandt et al. (1997) and Zhi et al. (2009). As a consequence, this elevated the subclass *Acidimicrobidae* to the class level. In the same volume, Norris (2012) revised the taxonomy of the new class and proposed the name *Acidimicrobiia,* which at that time only contained the order *Acidimicrobiales*, with two families and five genera: the family *Acidimicrobiaceae* comprising the genera *Acidimicrobium*, *Ferrimicrobium*, *Ferrithrix*, and *Ilumatobacter*; and the family *Iamiaceae* with the genus *Iamia*. *'Microtrichaceae'*, originally proposed as 'Microthrixaceae', (Joseph et al., 2003), although broadly used, is not an accepted name. The waste water bacterium '*Candidatus* Microthrix parvicella' was first isolated in 1973 (van Veen, 1973), and again in 1975 (Eikelboom, 1975), but due to not enough phenotypic data, it was not validly named. In 1996, '*Candidatus* Microthrix parvicella' was proposed under the *Candidatus* nomenclature for the first time (Blackall et al., 1996), since there was still not enough evidence to propose a valid name. Another important group of *Acidimicrobiia* whose taxonomy is not yet solved is '*Actinomarinales'* (for detailed information, see Mizuno et al., 2015). In 2018, the family *Ilumatobacteraceae* was proposed (Asem et al., 2018), which includes the genera *Ilumatobacter* and *Desertimonas*. In the same year, the ending *-ota* was proposed for prokaryotic phyla (Whitman et al., 2018) and '*Actinobacteriota'* was suggested. *Actinobacteria* was formally renamed as *Actinomycetota* in 2021 (Oren and Garrity, 2021).

In the last two decades, cultivation-independent molecular analysis of environmental microbial communities, including metagenomics, has expanded the tree of life beyond strain collections. The International Code of Nomenclature of Prokaryotes considers only pure cultures as type material (Sutcliffe et al., 2020), which in the class *Acidimicrobiia* gives a rather narrow view of its diversity. Today, the Genome Taxonomy Database (GTDB) comprises six orders within the class *Acidimicrobiia* with more than 50 families (Parks et al., 2022). Most of these families have an alphanumerical name and less than 5 genome representatives per family. Thus, the taxonomy of yet uncultivated members of the class *Acidimicrobiia* needs further curation.

This study provides a taxonomic overview of the class *Acidimicrobiia* following genome-based and 16S rRNA gene-based taxonomy. We report the abundance, diversity and importance of defined clades in the environment by adding and analysing high-quality metagenome-assembled genomes (MAGs) from coastal marine sediments, comparing them to *Acidimicrobiia* genomes available in public databases. Furthermore, we here describe four new species and two new genera according to the guidelines of The Code of Nomenclature of Prokaryotes Described from Sequence Data (SeqCode) (Hedlund et al., 2022): *Benthobacter isfjordensis* gen. nov. sp. nov., *Hadalibacter litoralis* gen. nov. sp. nov., *Ilumatobacter isfjordensis* sp. nov., and *Ilumatobacter herschelensis* sp. nov. We argue that high abundance of *Acidimicrobiia* in marine sediments is not the exception and that a large part of the sequences classified as *Actinomycetota* (or previously as *Actinobacteria*) in marine sediments also correspond to *Acidimicrobiia*.

Methods

Sampling

A 5-year sampling campaign was conducted in Isfjorden, a shallow fjord in Svalbard (78.11°N 14.35°E) between 2017 and 2022. Water depths at the sampling site varied between 3 and 9 m (for further detail see Miksch et al., 2021). Sediment samples were collected using a van Veen grab for samples from 2017 to 2019, and an Ellrott grab (Moncada et al., 2024) for samples from 2021 onwards, deployed from the R/V Farm. Sampling dates were selected based on light conditions: 20th of December 2017 (polar night), 1st of May 2018 (polar day), 25th of April 2019 (polar day), 13th of September 2019 (15 h daylight, 9 h of twilight), 9th of December 2021 (polar night), and 29th of April 2022 (polar day). Metagenomes and formaldehyde-fixed sediment samples from other sampling campaigns in coastal subtidal sediments from the German Wadden Sea were also included in our analysis, i.e. sediments from Helgoland (Miksch et al., 2021; Probandt et al., 2017), Wilhelmshaven (53.67°N 8.12°E; sampled on 14th of July 2021; Shipek grab and Elrott grab) as well as Königshafen (55.024°N 8.44°E; sampled on 15th of June 2021 and 24th of October 2021) and Hausstrand (55.015°N 8.436°E; 24th of October 2021) at Sylt.

Sequencing

DNA from Isfjorden coastal sandy sediments was extracted following a modified protocol from Zhou (Zhou et al., 1996). The quantity of the DNA was measured by fluorometry (Quantus, Promega, Wisconsin, United States) and the quality of it was assessed by capillary electrophoresis (Agilent FEMTOpulse, Santa Clara, United States). Next, libraries were prepared according to the recommendations by a protocol of the vendor (SMRTbell® Libraries for Ultra-Low DNA Input, Pacific Biosciences) followed by HiFi SMRT sequencing for 30 h on a Sequel IIe device at the Max Planck Genome center, Cologne, Germany.

Acquisition of metagenome-assembled genomes

Unassembled long-reads (LR) were evaluated using NanoPlot v1.32.1 (De Coster and Rademakers, 2023). The assembly was done following three different approaches: i) assembling individual datasets, ii) doing a co-assembly of all datasets, and iii) doing a co-assembly only with those reads which mapped onto a database of 937 public *Acidimicrobiia* genomes (Supplementary file) and 11 unpublished *Acidimicrobiia* bins. Assembly was done using flye-meta v2.9.1 (Kolmogorov et al., 2020; Kolmogorov et al., 2019) and hifiasm-meta vhamtv0.3 (Feng et al., 2022). Summary statistics, including contig length and GC-content, were calculated using scripts of the enveomics collection v1.9.0 (Rodriguez-R and Konstantinidis, 2016). Next, all six metagenomic samples were mapped to each assembly using MiniMap2 v2.24 (Li, 2018) excluding secondary read mapping, and filtered using and identity threshold of 0.95 (sam.filter.rb script with default settings). The average sequencing depth of contigs was determined using Samtools v1.16.1 (Danecek et al., 2021). The binning processes were performed using MaxBin2 v2.2.7 (Wu et al., 2016), MetaBAT2 v2.15 (Kang et al., 2019), and VAMB v3.0.9 (Nissen et al., 2021), and the outputs of these tools were integrated using DASTool v1.1.5 (Sieber et al., 2018). The completeness and contamination of the bins was assessed using the lineage workflow of checkM v1.2.2. Corrected genome size of each genome and MAG was calculated with the formula: $CorrectedGenomeSize = GenomeSize*(1 - Contamination/100)/(Completeness/100)$. The genomes were taxonomically annotated using GTDB-tk with the classify workflow command and default settings on the r214 database release. To this point, genomes classified as *Acidimicrobiia* from previous sampling campaigns in Herschel Island, Canada; Helgoland, Germany (Viver et al., 2024); and Isfjorden, Svalbard (Miksch et al., 2024), were added to the database. Finally, genomes with >50% completeness, <10% contamination and classified within the class *Acidimicrobiia* were dereplicated if they were obtained from the same metagenome using dRep v3.4.0 with an average nucleotide identity (ANI) threshold of 0.97. If present, the extraction of the 16S rRNA gene from the remaining genomes was done using barrnap v0.9 (https://github.com/tseemann/barrnap), with a rejection threshold of 20% of the full 16S rRNA gene sequence length (317 bp).

Acquisition of public genomes

For this study, we obtained a total of 937 annotated genomes from the class *Acidimicrobiia*, sourced from the NCBI genome repository on the 30th of June 2023. Additionally, we acquired three genomes from the order *Rubrobacterales* to serve as outgroup representatives within the phylum *Actinomycetota*, and three more genomes from the order Bacillales as outgroups from outside the phylum *Actinomycetota*. A workflow

similar to the one described in the previous section was applied to the public genomes, with the modifications outlined below. Our goal was to utilize only the most complete genomes within the class *Acidimicrobiia*, ensuring a high degree of distinctiveness up to the species level. Thus, we defined a completeness higher than 90% and performed replication with an ANI threshold of 0.95. Consequently, we compiled a total of 180 genomes, including outgroup representatives. Genomes from the order *Actinomarinales* usually show completeness values below our threshold, therefore, to represent this group in our analysis, three additional sequences from *Actinomarinales* were added after the filtration step. The 16S rRNA gene was extracted from public genomes using barrnap.

Phylogenetic analysis of acidimicrobial MAGs and genomes

Phylogenetic tree construction was derived from the alignment of bac120 generated by GTDB-tk. The tree was computed using IQTree v.2.0.3 (Minh et al., 2020), with the model selection tool to identify the appropriate substitution model (Kalyaanamoorthy et al., 2017) and the ultra-fast bootstrap tool (Hoang et al., 2018). The chosen model for substitution was LG + F + R10. Genomes from the order *Bacillales* were chosen as outgroup for rooting the tree. Trees were visualized in the interactive Tree of Life (iTol) (Letunic and Bork, 2021).

Phylogenetic analysis of 16S rRNA gene sequences

We initially selected all sequences corresponding to the class *Acidimicrobiia* from the SILVA SSU Ref138.1 NR99 database (Quast et al., 2013) (release June 2020), using the ARB software package v7.0.1 (Westram et al., 2011). Sequences were filtered based on a length greater than 1400 bp and an alignment quality exceeding 97%. This resulted in 1083 16S rRNA gene sequences. Additionally, 16S rRNA gene sequences recovered from genomes from the class *Acidimicrobiia* and the orders *Rubrobacterales* and *Bacillales* were incorporated into the analysis and aligned using the SINA aligner (Pruesse et al., 2012). After filtering by the same length criterion (1400 bp), 107 additional 16S rRNA gene sequences from genomes were added to the database. Furthermore, 14 sequences from isolates from the class *Acidimicrobiia* were included irrespective of their quality and length. A total of 1204 sequences were used for tree calculation.

Three distinct phylogenetic trees were constructed and compared using ARB, following the SOPPI guidelines (Peplies et al., 2008). A PHYML tree and a RAxML 7 tree were built using an *Actinomycetota* filter with minimum homology of 50%. The third tree was build using a Distance matrix Neighbor Joining method with Jukes-Cantor correction. A consensus tree was built from the three trees using the "Build consensus tree" option in ARB, and its branches were manually grouped based on sequences that showed coherent grouping between the three trees. Forty six partial 16S rRNA gene sequences from genomes were added to the tree using the ARB Parsimony method without allowing changes in the overall tree topology using the same filter mentioned above. For better visualization, sequences which did not belong to genomes and did not group coherently between trees were removed. Finally, the taxonomy of the 16S rRNA gene sequences was exported for subsequent comparison.

Relative abundance of 16S rRNA gene reads in long-read metagenomes

16S rRNA genes were extracted from LR metagenomes using barrnap v0.9. For Isfjorden metagenomes, sequences were processed by SILVAngs analysis pipeline (Quast et al., 2013). For Helgoland metagenomes, sequences were processed as previously described (Viver et al., 2024). In short, they were clustered in operational taxonomic units (OTUs) at 98.7%. Singleton and doubleton OTUs were removed from the analysis, and representative OTU sequences were imported into the SILVA Ref138 database. Sequences were aligned using the SINA aligner. Due to the big volume of this data, these 16S rRNA gene sequences were only used for relative abundance estimations.

Basic metabolism and functional annotation

The prediction of amino acid sequences was achieved using Prokka v1.14.6 (Seemann, 2014) on each individual genome. Basic metabolism was annotated using DRAM v1.2.2 (Shaffer et al., 2020) and BlastKoala v3.0 (Kanehisa et al., 2016). Functional annotation was done as previously described (Orellana et al., 2022). Annotation of glycoside hydrolases (GHs) was done using diamond v2.0.15 (Buchfink et al., 2021) against CAZy and hmmer v3.3.2 (Eddy, 2011) against DBCAN v11 (Yin et al., 2012). Annotations were only considered valid if they were confirmed by both methods. In addition, sulfatases were annotated using hmmer against the SulfAtlas database v1.3 (Barbeyron et al., 2016; Stam et al., 2023), while transporters were annotated by reference to the TCDB (Saier et al., 2021; Saier et al., 2006). To have a standardized way of comparing the number of functional genes per genome, GHs, sulfatases and transporters per Mbp were estimated.

Oligonucleotide probe design

Three probes for *Ilumatobacteraceae* were designed based on the database SILVA SSU Ref138.1 NR99 using the Probe Design tool from ARB (Westram et al., 2011). Sequences were filtered by lengths equal to or longer than 1250 nucleotides for *Bacteria* and 900 for *Archaea*. The database considered 431,145 sequences, of which 470 were within the family *Ilumatobacteraceae*. Competitor oligonucleotides (Manz et al., 1992) were designed when many non-target species had one or less weighted mismatches with the probe. Helper oligonucleotides (Fuchs et al., 2000) were targeted to be 5′ and 3′ immediately adjacent or at the opposite side of the helix. The new sets of oligonucleotide probes are shown in Supplementary Table S1.

Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) and cell counting

The protocol by Pernthaler and colleagues (Pernthaler et al., 2002) was applied with some modifications. In short, formaldehyde-fixed cells on polycarbonate filters were hybridized with a 300:1 ratio of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl pH 8.0, 1% Blocking reagent, 0.1 g ml^{-1} dextran sulfate, 0.02% SDS, and formamide concentration depending on each hybridization) and oligonucleotides working solution (8.4 pmol μl^{-1} each horseradish peroxidase-labeled oligonucleotide probe, helpers and competitors, from biomers.net [Ulm, Germany]) at 46 °C for 3 h. Filters were then washed at 48 °C for 15 min in preheated washing buffer (20 mM Tris-HCl pH 8.0, 0.01% SDS, NaCl concentration depending on the former hybridization formamide concentration, and 5 mM EDTA). For the catalyzed reporter deposition step, filters were incubated for 15 min at 20 °C in phosphate-buffered saline (1xPBS, pH 7.4) and then incubated for 45 min at 46 °C in detection solution under dark conditions (1 ml of amplification buffer: 1xPBS, 2 M NaCl, 0.1% Blocking reagent, 0.1 g ml^{-1} dextran sulfate; 10 µl of 0.15% H_2O_2 diluted in 1xPBS; and 1 µl of 1 mg ml^{-1} Alexa Fluor 488-labelled tyramides, reconstituted with 20 mg ml^{-1} 4-iodophenylboronoic diluted in N,N-dimethylformamide). Filters were then washed with 1xPBS for 5 min at 20 °C under dark conditions, washed with MilliQ deionized water for 1 min and quickly rinsed with 96% ethanol. After letting them dry, filters were placed on a microscopy glass slide and mounted with mounting solution containing 4′,6-diamidino-2-phenylindole (DAPI) (1 µg ml^{-1} DAPI in CitiFluorAF1 [CitiFluor Ltd., London, United Kingdom]: VECTASHIELD® Antifade Mounting Media [Vector Laboratories, Burlingame, CA, USA], 3:1).

Cell counts were done using the epifluorescence microscope Nikon Ci-S (Nikon, Düsseldorf, Germany), equipped with an objective Plan Apochromat Lambda 100x/1.45 Oil. Total cell numbers were determined by counting all DAPI stained cells in 20 randomly chosen

counting grids. For quantification of CARD-FISH signals, due to lower numbers, counting was continued for 50 grids to have a robust estimate.

Confocal Laser Scanning Microscopy and cell size measurements

Microscopy imaging and cell size measurements were done using the Confocal Laser Scanning Microscope Zeiss LSM 780 (Carl Zeiss, Jena, Germany) and the ZEISS ZEN software. Microscopy imaging was done with two track configurations: DAPI configuration, with an excitation wavelength of 405 nm, and an emission detection between 410 nm and 490 nm; and Alexa488 configuration, with an excitation wavelength of 488 nm, and an emission between 500 nm and 600 nm. Cell size measurements were done based on micrographs of CARD-FISH signals due to their location in the poles of the cell compared to DAPI signals located in the center.

Data availability

The metagenomes and MAGs from Isfjorden coastal sandy sediments were deposited in the European Nucleotide Archive (ENA), under the accession codes PRJEB71360 and PRJEB53193. The other MAGs can be found under the accession codes PRJEB66156 for Herschel Island, and PRJEB64856 for Helgoland (for specific accession codes, see Supplementary Table S1).

Results

Genomes from Isfjorden sediment covered two major orders of Acidimicrobiia

We focused our study on *Acidimicrobiia* MAGs from coastal marine environments: 17 from coastal sandy sediments of Isfjorden, 1 from

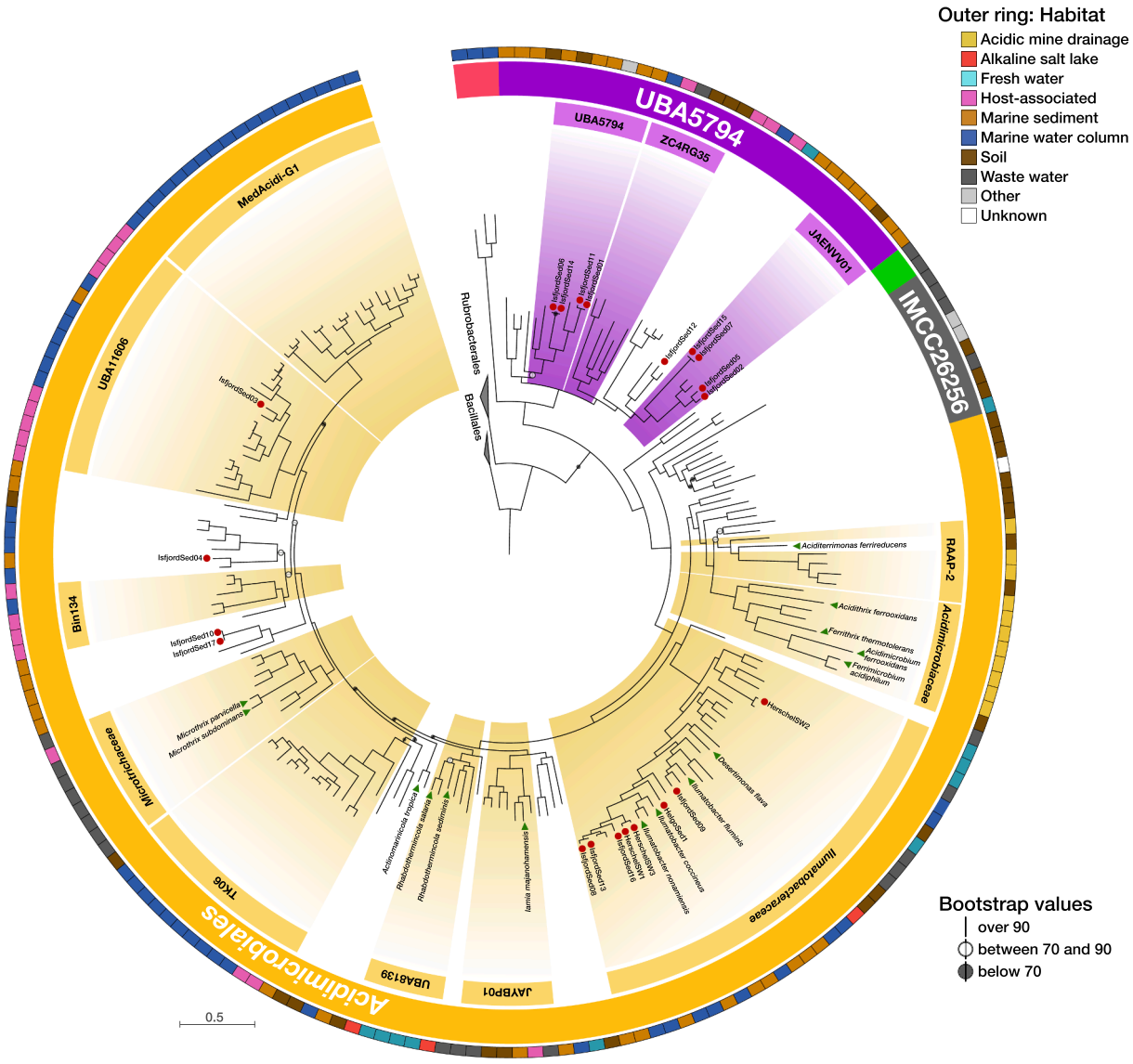

Fig. 1. Phylogenetic tree of 198 *Acidimicrobiia* genomes including MAGs recovered from Helgoland (North Sea), Herschel Island (Canada) and Isfjorden (Svalbard). The maximum-likelihood tree is based on the GTDB-tk bac120 alignment and the taxonomy is based on GTDB database r214. *Bacillales* and *Rubrobacterales* were used as outgroups. The colored middle ring indicates orders within the class *Acidimicrobiia*: order *Actinomarinales* is labelled in red, UBA5794 in purple, UBA2766 in green, IMCC26256 in light grey, and *Acidimicrobiales* in yellow. The inner ring and the gradient-colored area highlight families containing five or more genomes. Green triangles indicate genomes representing isolates, and red circles indicate the sequences recovered from MAGs coming from Helgoland sediments (HelgoSed), seawater surrounding Herschel Island (HerschelSW) and Isfjorden coastal sediments (IsfjordSed). Bootstrap values are indicated in the branches. The outer ring depicts the habitat from which each genome was recovered. The scale bar indicates the average number of substitutions per site.

coastal sandy sediments of Helgoland, and 3 from seawater surrounding Herschel Island (Supplementary Table S2). From Isfjorden, 5 MAGs were recovered from a co-assembly of LR metagenomes, 5 MAGs were recovered from a co-assembly of *Acidimicrobiia* LR PacBio sequences (see methods), and 7 MAGs were obtained from Illumina short reads metagenomes from a previous study (Miksch et al., 2024). Half of the MAGs contained a full 16S rRNA gene sequence, and 3 had a partial gene sequence. IsfjordSed15 contained 3 copies of the 16S rRNA gene, with similarity value below 90%, suggesting contamination.

To describe the taxonomy and phylogeny of the class *Acidimicrobiia*, publicly available genomes of this taxon were analyzed together with our own MAGs. Five orders could be identified in the genome-based tree: *Actinomarinales*, UBA5794, UBA2766, IMCC26256, and *Acidimicrobiales* (Fig. 1). MAGs retrieved from Helgoland sediments, Isfjorden coastal sediments and seawater surrounding Herschel Island were classified in four major families (defined by comprising at least five representatives) according to GTDB taxonomy: four MAGs were classified as family UBA5794 (IsfjordSed01, IsfjordSed06, IsfjordSed11, and IsfjordSed14), four MAGs were classified as JAENVV01 (IsfjordSed02, IsfjordSed05, IsfjordSed07, and IsfjordSed15), eight MAGs were classified as *Illumatobacteraceae* (IsfjordSed08, IsfjordSed09, IsfjordSed13, IsfjordSed16, HerschelSW1-3, and HelgoSed1), and one MAG was classified as

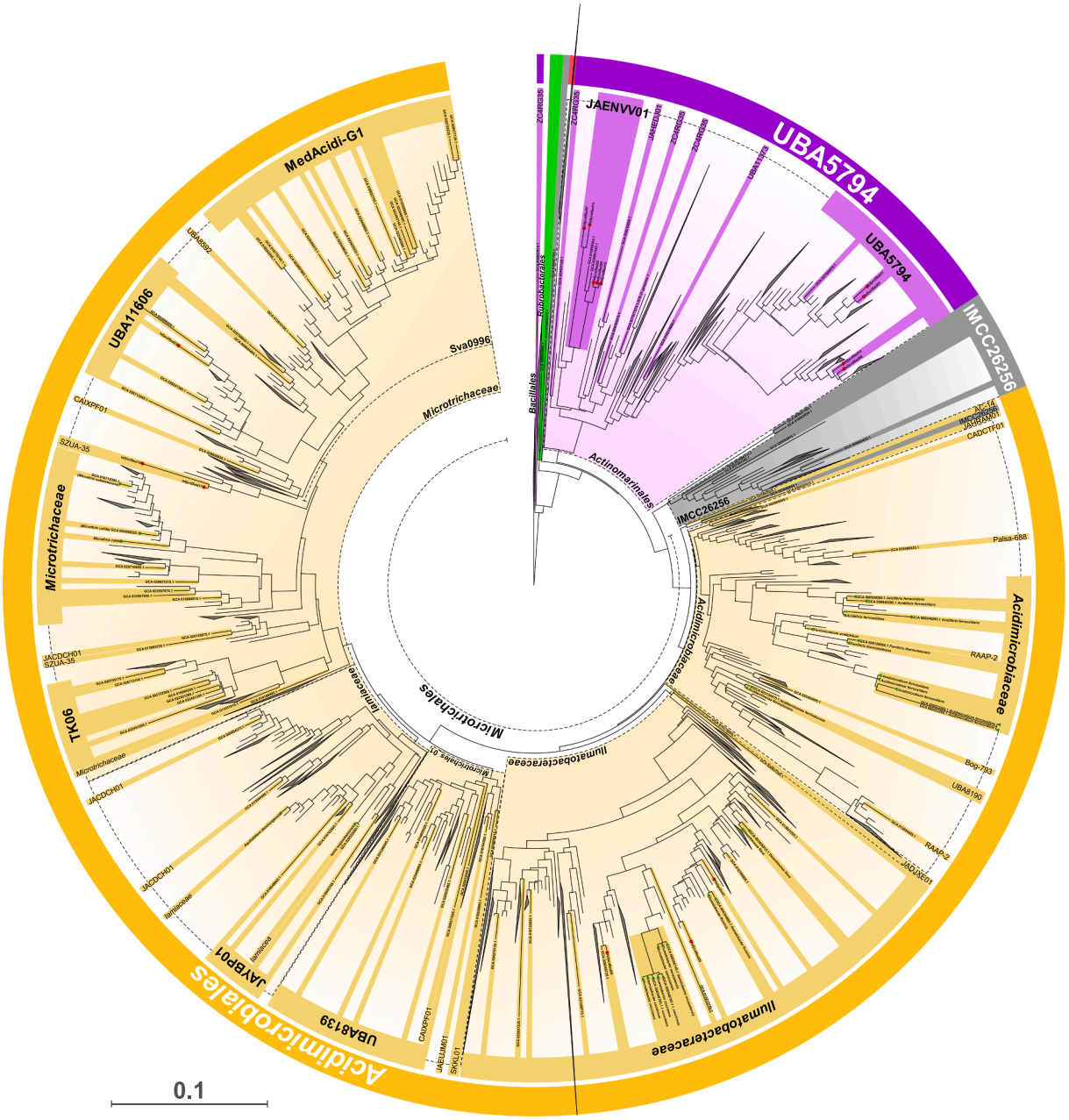

Fig. 2. Phylogenetic reconstruction of *Acidimicrobiia* full-length 16S rRNA gene sequences. The tree contains 1,083 full-length 16S rRNA gene sequences from the SILVA database SSU Ref138.1 NR99 (release June 2020) and 153 sequences recovered from MAGs. *Bacillales* and *Rubrobacterales* were used as outgroups. The tree is a consensus between three trees: PHYML, RAxML 7 and Distance matrix with Neighbor Joining. The colored rings highlight the overlap of SILVA and GTDB taxonomies. The center of the tree shows SILVA taxonomy with dashed lines and light-colored areas, and the two outer rings show GTDB taxonomy: order *Actinomarinales* is labelled in red, UBA5794 in purple, UBA2766 in green, IMCC26256 in light grey, and *Acidimicrobiales* in yellow. The second outer ring highlights GTDB-families from Fig. 1, and some other smaller families. Green triangles indicate sequences representing isolates, and red circles indicate the sequences recovered from MAGs from Helgoland sediments (HelgoSed), seawater surrounding Herschel Island (HerschelSW) and Isfjorden coastal sediments (IsfjordSed). Branches with no genome representatives were collapsed and depicted as grey triangles. Scale bar indicates 10% estimated sequence divergence.

UBA11606 (IsfjordSed03). Four MAGs from Isfjorden were classified as other minor families (families with less than five representatives in our database) of the order *Acidimicrobiales*: IsfjordSed04 in UBA10347, IsfjordSed10 and IsfjordSed17 in SZUA-35, and IsfjordSed12 in UBA4744.

Genomes from isolates belonged to four families in the order *Acidimicrobiales*: *Acidimicrobiaceae*, *Ilumatobacteraceae*, UBA8139, and '*Microtrichaceae*'. Only *Ilumatobacteraceae* was represented by genomes from isolates and MAGs from our environmental samples.

Habitats from which the acidimicrobial genomes originated were categorized as: acidic mine drainage, alkaline salt lake, fresh water, host-associated, marine sediments, marine water column, soil, waste water, other, and unknown. Our data suggests trends in the habitat preferences of certain families (outer ring of tree in Fig. 1). In clockwise order, the families JAENVV01, *Acidimicrobiaceae*, TK06, *Microtrichaceae,* and MedAcidi-G1 were isolated from marine sediment, acidic mine drainage, marine water column, waste water, and marine water column, respectively. Although the genomes from the order UBA5794 did not show a specific isolation source, 16S rRNA gene sequences from this group showed to be mostly isolated from marine sediments (Supplementary Fig. S1). Examples for diverse isolation sources are also present in our data. Diversity in habitat was most obvious in the family *Ilumatobacteraceae*, with more than half of the genomes obtained from seawater and marine sediments, and the rest from fresh water, waste water, and alkaline salt lakes.

Comparison of 16S rRNA gene-based and genome-based taxonomies

We investigated the phylogenetic relationships within the class *Acidimicrobiia* by comparing SILVA and GTDB taxonomy. For that, GTDB taxonomy was overlaid onto a 16S rRNA gene consensus tree with SILVA taxonomy (Fig. 2). The trees used to build the consensus tree are shown in Supplementary Fig. S2. Both taxonomies agreed on phylogenetic relations on the family and order level. Four of the five orders found in the class *Acidimicrobiia* following GTDB taxonomy had an analogue in SILVA taxonomy: *Actinomarinales* and UBA5794 are analogous to *'Actinomarinales'*, IMCC26256 is present in both taxonomies with the same nomenclature, and *Acidimicrobiales* is analogous to '*Microtrichales*'. UBA2766 did not correspond to any order in SILVA taxonomy.

The SILVA taxonomy was not as detailed as GTDB taxonomy for almost every family. In other words, within one SILVA-family it was common to find more than one GTDB-family. The SILVA-family *Acidimicrobiaceae* included the GTDB families RAAP-2, CADCTF01, Palsa-688, Bog-793, and UBA8190. The family *'Microtrichaceae'* in SILVA taxonomy included TK06, '*Microtrichaceae*', SZUA-35, JACDCH01, CAIXPF01, UBA11606, UBA8592, MedAcidi-G1. The clade Sva0996 in SILVA taxonomy comprised the GTDB families CAIXPF01, UBA11606, UBA8592, and MedAcidi-G1. The SILVA-family *Iamiaceae* corresponded to JAYBP01, *Iamiaceae*, and JACDCH01. The only exceptions to greater span in SILVA taxonomy was *Ilumatobacteraceae*, which was similar in both taxonomic systems.

Conserved genome characteristics within Acidimicrobiia families

To understand common genome characteristics and physiological capabilities of *Acidimicrobiia*, we analyzed and compared our MAGs with 174 genomes of the class *Acidimicrobiia* from public databases. The genome size, GC-content, and an overview of selected annotated genes for the class *Acidimicrobiia* (families with five or more representatives in our database) is shown in Fig. 3.

In general, the order *Acidimicrobiales* showed greater diversity in their characteristics than the order UBA5794. Even more, *Ilumatobacteraceae* is the most heterogeneous family among *Acidimicrobiia* with respect to genome size, GC-content and number of functional annotations. Particularly, the corrected genome sizes of the class *Acidimicrobiia* varied between 1.7 and 8.5 Mbp, both values from *Ilumatobacteraceae* (Fig. 3A). The order *Acidimicrobiales* showed mean corrected genome size of 3.4 ± 1.2 (standard deviation) Mbp, being more diverse in their sizes than UBA5794 with a mean genome size of 3 ± 0.5 Mbp (F-test, $P < 0.001$). The family *Ilumatobacteraceae* had a mean corrected genome size of 3.9 ± 1.7 Mbp and the family UBA5794 a mean corrected genome size of 2.9 ± 0.5 Mbp, both similar to their respective orders. The GC-content of the whole class was high, with three quarters of the genomes having a GC-content of ≥60% (Fig. 3B). The order *Acidimicrobiales* showed a mean GC-content of 63 ± 8%, while the order UBA5794 showed a mean GC-content of 63 ± 3%. The families *Ilumatobacteraceae* and UBA5794 had a GC-content of 64 ± 6% and 62 ± 4%, respectively.

Regarding the functional annotation of the MAGs, the number of glycoside hydrolases per Mbp in class *Acidimicrobiia* ranged from 0 to 7.9 (Fig. 3C; the highest value does not belong to major families, for more detail see "Others" in Supplementary Fig. S3). The mean number of glycoside hydrolases per Mbp for the orders *Acidimicrobiales* and UBA5794 was 2.5 ± 1.4 and 2.2 ± 1.4, respectively (Fig. 3C). The families *Ilumatobacteraceae* and UBA5794 showed similar mean glycoside hydrolases per Mbp, with 3.1 ± 1.2 and 3.0 ± 1.2, respectively. The number of sulfatases per Mbp in the class *Acidimicrobiia* ranged from 0 to 7.6 (Fig. 3D). The mean number of sulfatases per Mbp was 2.9 ± 1.8 for the order *Acidimicrobiales* and 1.3 ± 0.7 for the order UBA5794. *Ilumatobacteraceae* showed a relatively high mean number of sulfatases per Mbp, with an average value of 3.6 ± 1.6. The family UBA5794 showed an average number of sulfatases per Mbp similar to other MAGs in its order, with 1.2 ± 0.6. The number of transporters per Mbp ranged from 8.7 to 27.3 (both values coming from *Ilumatobacteraceae*), with 85% of the MAGs having less than 20 (Fig. 3E). The order *Acidimicrobiales* showed an average number of transporters per Mbp of 15.4 ± 3.5, and the order UBA5794 showed an average number of transporters per Mbp of 18 ± 2.9. Finally, the MAGs from databases and the MAGs from this study showed values within similar ranges.

Acidimicrobiia accounts for 3–7% of bacterial 16S rRNA gene sequences in long-read metagenomes

To estimate the relative contribution of the class *Acidimicrobiia* to the bacterial community in Isfjorden sediments, we extracted 16S rRNA gene sequences from our six LR metagenomes. In general, we extracted between 2087 and 2842 bacterial 16S rRNA gene sequences, with the exception of samples from May 2018 and April 2019 from which we only retrieved 832 and 1236 bacterial 16S rRNA gene sequences, respectively (Table 1). Similar results were obtained for Helgoland sediments (Supplementary Table S4).

The class *Acidimicrobiia* represented between 3.5% and 6.8% of the total bacterial 16S rRNA gene sequences in Isfjorden, and between 3.2% and 5.9% in Helgoland. In both, *Acidimicrobiia* sequences were more than 90% of the phylum *Actinomycetota* (or *'Actinobacteriota'* in SILVA taxonomy). More specifically, the SILVA-order *'Actinomarinales'* constituted between 2.1% and 4.1% of the bacterial 16S rRNA gene sequences in Isfjorden, the highest value in December 2017, and 1.1% and 3.2% in Helgoland. All sequences classified as '*Actinomarinales*' corresponded to uncultured (and unclassified) '*Actinomarinales*'. On the other hand, 16S rRNA gene sequences classified as '*Microtrichales*' constituted between 1.3% and 2.9% of the bacterial sequences in Isfjorden, and between 1.3% and 3.6% in Helgoland, with roughly one half of them classified as *Ilumatobacteraceae* and the other half as '*Microtrichaceae*'. For Isfjorden and Helgoland, all 16S rRNA gene sequences classified as *Ilumatobacteraceae* were classified within the genus *Ilumatobacter*. Furthermore, more than half of the 16S rRNA gene sequences classified as '*Microtrichaceae*' were classified as group Sva0996, with the rest being uncultured (and unclassified) '*Microtrichaceae*'. Only one sequence from Isfjorden was classified as part of the order IMCC26256.

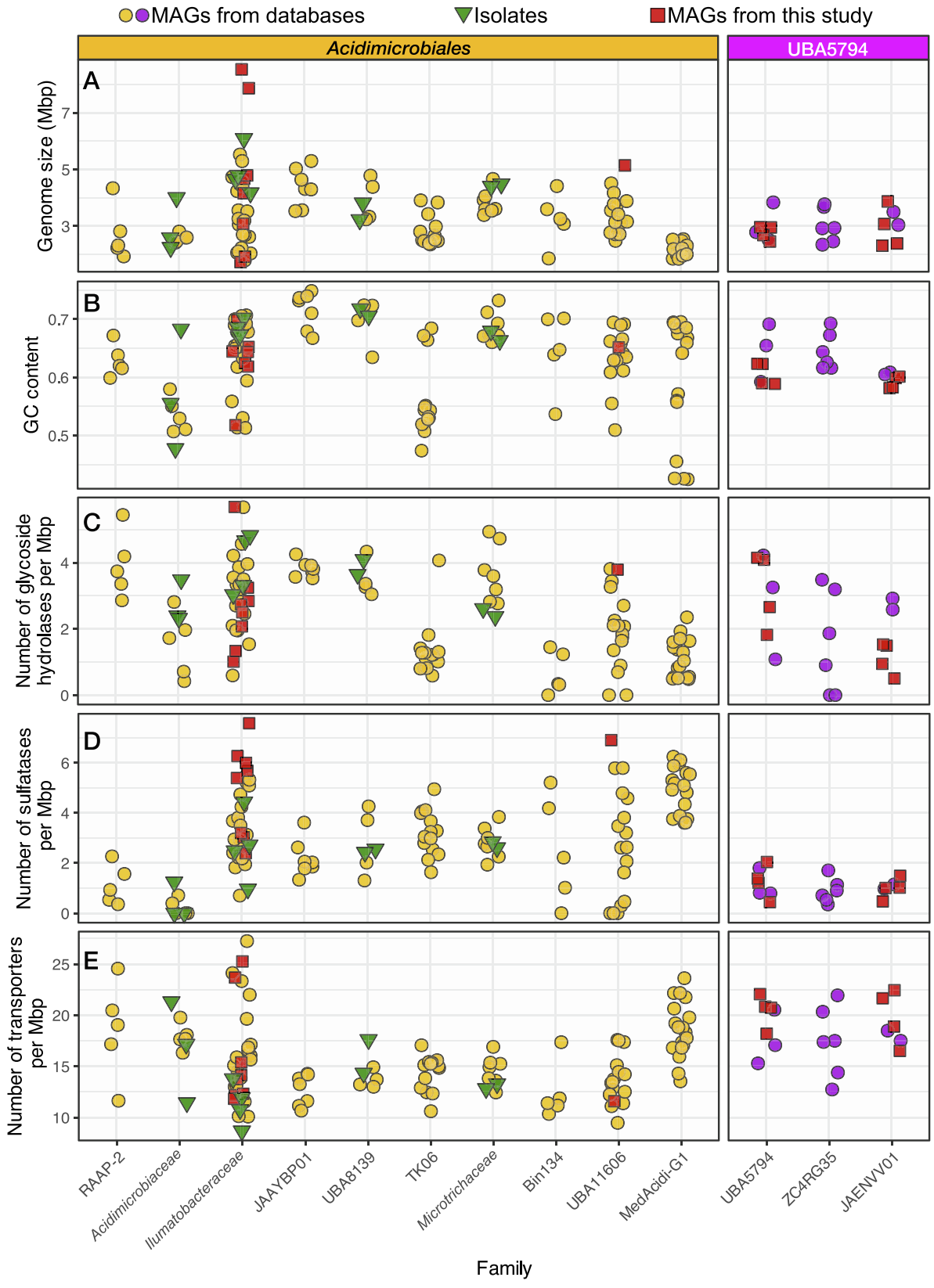

Fig. 3. Summary of MAG characteristics and gene annotations of the major families in the class *Acidimicrobiia*. Genome size (A) and GC-content (B) of genomes from class *Acidimicrobiia* grouped by order and family. (C) Number of glycoside hydrolases per Mbp. (D) Number of sulfatases per Mbp pairs. (E) Number of transporters per Mbp. Yellow and purple circles represent public genomes from Acidimicrobiales and UBA5794, respectively. Green triangles indicate MAGs representing isolates, and red squares indicate MAGs coming from Helgoland sediments (HelgoSed), surrounding seawater of Herschel Island (HerschelSW) and Isfjorden coastal sediments (IsfjordSed). The x-coordinate of each point was perturbed with uniform noise to avoid overlap and achieve better visualization.

Table 1
Read frequencies of *Acidimicrobiia* OTUs in Isfjorden sediments based on SSU rRNA gene sequences extracted from six metagenomes.

Sampling date	December 2017		May 2018		April 2019		September 2019		December 2021		April 2022	
Taxa	No. reads	%	No. reads	%	No. reads	%	No. reads	%	No. reads	%	No. reads	%
All	3169		4015		1772		3351		4319		5316	
Bacteria	2674	100	832	100	1236	100	2087	100	2683	100	2842	100
Actinobacteriota	185	6.9	43	5.2	48	3.9	99	4.7	115	4.3	151	5.3
Acidimicrobiia	183	6.8	42	5.0	43	3.5	93	4.5	112	4.2	146	5.1
Actinomarinales	109	4.1	18	2.2	26	2.1	45	2.2	76	2.8	70	2.5
Uncultured *Actinomarinales*	109	4.1	18	2.2	26	2.1	45	2.2	76	2.8	69	2.4
Other *Actinomarinales*	0	–	0	–	0	–	0	–	0	–	1	0.04
Microtrichales	74	2.8	24	2.9	17	1.4	48	2.3	35	1.3	76	2.7
Ilumatobacteraceae	35	1.3	16	1.9	11	0.89	30	1.4	15	0.56	36	1.3
Ilumatobacter	35	1.3	16	1.9	11	0.89	30	1.4	15	0.56	36	1.3
Microtrichaceae	38	1.4	8	1.0	6	0.49	18	0.86	20	0.75	40	1.4
Sva0996	28	1.0	4	0.48	4	0.32	10	0.48	6	0.22	22	0.77
Uncultured *Microtrichaceae*	10	0.37	4	0.48	2	0.16	8	0.38	14	0.52	18	0.63
Uncultured *Microtrichales*	1	0.04	0	–	0	–	0	–	0	–	0	–
IMCC26256	0	–	0	–	0	–	0	–	1	0.04	0	–

Ilumatobacteraceae has a high in situ abundance in coastal sandy sediments

Probes specifically targeting *Ilumatobacteraceae* were developed for *in situ* quantification and visualization. Probes ILU498, ILU523, and ILU940 were tested in coastal sandy sediment samples from Isfjorden. Cell numbers were similar for the three probes with *in situ* abundance ranging from 2.1×10^6 to 4.8×10^7 cells per ml of sediment. The signals of ILU498, ILU523 and ILU940 were bright and of similar shape (Fig. 4A–C). *Ilumatobacteraceae* were mostly found as free-living short rods, and exceptionally as colonies of cocci or rods (Fig. 4A–D). The CARD-FISH signal was commonly found at the poles of the cells, whereas the DAPI signal was found in the center. Cells labelled with ILU498, ILU523, ILU940 had similar cell dimensions and agreed with those previously reported for *Ilumatobacter* (Matsumoto et al., 2009; Matsumoto et al., 2013) (Supplementary Table S4). MANOVA test showed no difference between the probes ($P = 0.1$).

Based on highest specificity and coverage, probe ILU498 was selected for *in situ* quantification of *Ilumatobacteraceae*. Besides Isfjorden sediments, it was applied on three other coastal sandy sediment samples from different sites located in the German Bight of the North Sea: Sylt, Wilhelmshaven, and Helgoland. *Ilumatobacteraceae* showed high *in situ* abundance with values between 2.5×10^6 and 1.4×10^8 cells per ml of sediment, and a median value of 8.4×10^6 (Fig. 4E–F). Relative abundances ranged from 0.8% to 6.2%, and an overall mean of 2.8 ± 1.8%. Coastal sandy sediments from Isfjorden, Svalbard, showed the highest mean relative abundance of *Ilumatobacteraceae* with 4.5 ± 2.2% of total cell counts. Helgoland and Wilhelmshaven showed the lowest mean relative abundances for *Ilumatobacteraceae*, with a mean of 1.4 ± 0.5% and 1.1 ± 0.3%, respectively.

Proposal of Benthobacter gen. nov.

The MAG IsfjordSed06 recovered from Isfjorden coastal sandy sediments fulfills the criteria to be assigned to a new species following SeqCode guidelines. It is currently classified as part of the genus JAHEEL01, family UBA5794, and order UBA5794 according to GTDB taxonomy; and according to SILVA taxonomy, it is classified as part of the order '*Actinomarinales*'. We checked the isolation source of other genomes in the genus JAHEEL01 and close relatives in SILVA taxonomy, and marine sediments appears to be a common habitat for these bacteria (Supplementary Fig. S1). Therefore, we propose *Benthobacter* gen. nov. (Ben.tho.bac'ter. Gr. masc. adj. *benthos*, sea floor; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Benthobacter*, a rod-shaped bacterium found in benthic samples, based on rod-shaped signals reported by Miksch et al., 2021). Average amino acid identity and 16S rRNA gene sequences similarity between this MAG and the closest relative, *Spongiisocius variivorans*, were 47% and 87%, respectively, supporting the delineation of this

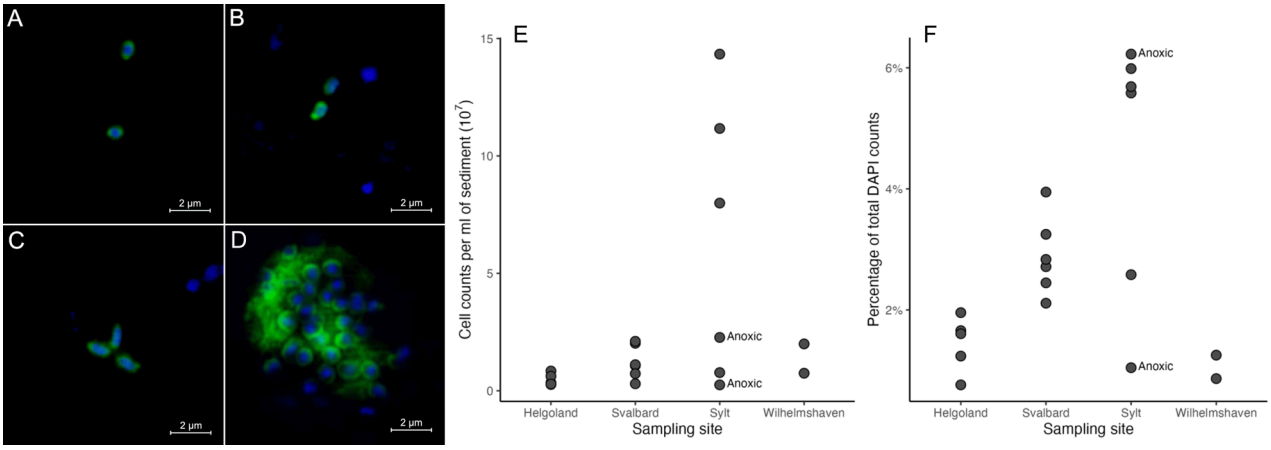

Fig. 4. Confocal laser scanning micrographs of CARD-FISH-stained *Ilumatobacteraceae* (A-D) and their abundance (E-F) in coastal sandy sediments. Probes used to target *Ilumatobacteraceae* were (A) ILU498, (B) ILU523, and (C) ILU940. Panel (D) shows a maximum intensity projection of a z-stack micrograph of an *Ilumatobacteraceae* colony labelled with ILU498. CARD-FISH signals of *Ilumatobacteraceae* were usually concentrated in the poles and DAPI signal in the center. Scale bar is 2 µm. (E) Absolute abundance and (F) relative abundance of *Ilumatobacteraceae* in oxic (0–2 cm) sediments from Helgoland, Svalbard, Sylt and Wilhelmshaven. For Sylt, oxic and anoxic sediments were studied.

taxon as a unique and distinct order from the class *Acidimicrobiia*. We propose the order *Benthobacterales* (family *Benthobacteraceae*) for the taxonomic placement of this group of bacteria.

Description of Benthobacter isfjordensis gen. nov. sp. nov.

Benthobacter isfjordensis (is.fjor.den'sis. N.L. masc. adj. *isfjordensis*, from or belonging to Isfjorden, Svalbard). The type genome sequence is IsfjordSed06 and was recovered from a concatenated file of raw reads from six PacBio long-read metagenomes coming from a 5-years (2017, 2018, 2019, 2021 and 2022) sampling campaign in coastal sandy sediments of Isfjorden, Svalbard. It has a completeness of 94% and a contamination of 3.8% according to checkM, corrected genome size of 2.96 Mbp, GC-content of 62%, a full-length 16S rRNA gene sequence, and tRNAs for all 20 amino acids. It is composed by 31 contigs, and showed an average sequencing depth of 20x in the metagenome from December 2017. The MAG IsfjordSed14 represents the same species and here we use it to support the description of *Benthobacter isfjordensis*. It has a completeness of 87.6% and a contamination of 3.9%, a corrected genome size of 2.68 Mbp, GC-content of 62%, a partial 16S rRNA gene sequence, and tRNAs for 17 amino acids. Its average sequencing depth in the metagenome from December 2017 was 25x. The formal protologue is given in Table 2.

Benthobacter isfjordensis showed, based on MAG IsfjordSed06 and MAG IsfjordSed14, most of the genes involved in basic carbon metabolism of an aerobic heterotrophic bacterium. Glycolysis was almost complete. The only step missing was the phosphorylation of D-glyceraldehyde 3-phosphate to 1,3-biphosphateglycerate, by the enzyme glyceraldehyde 3-phosphate dehydrogenase. Citrate cycle and pentose phosphate pathway were complete. The majority of electron transport chain was present, but the complex III (also known as cytochrome bc1 complex) was incomplete. Glycogen, nucleotide sugar, and UDP-N-acetyl-D-glucosamine biosynthesis pathways were complete. Entner-Doudoroff pathway was almost complete; the only step missing was the dehydration of 6-phospho-D-gluconate to 2-dehydro-3-deoxy-6-phospho-D-gluconate, by the enzyme phosphogluconate dehydratase. Although the MAG had genes identified as part of carbon fixation pathways in prokaryotes, none of those pathways was complete. Genes encoding for flagella or pili were not detected. No complete pathways for secondary metabolites were identified. The steps of denitrification pathway present in the genome were the import of extracellular nitrate, reduction of nitrate to nitrite, and dissimilatory reduction of nitrite to nitric oxide. Also, a nitrilase was found in the genome. No pathway for sulfate reduction or methanogenesis was detected. The F420 biosynthesis pathways was almost complete.

Glycoside hydrolases present in MAGs IsfjordSed06 and IsfjordSed14 were GH1, GH3, GH13 (sub-families 9, 11, 16, 20, and 23), GH32, GH36, GH57, GH77, GH133, and GH149 (Supplementary Fig. S4). Sulfatases from families S1_4, S1_13, and S1_16 were also encoded in the MAGs (Supplementary Fig. S5).

Proposal of Hadalibacter gen. nov.

The MAG IsfjordSed11 recovered from Isfjorden coastal sandy sediments fulfills almost all criteria (16S rRNA gene missing) to be assigned to a new species following SeqCode guidelines. The MAG is currently classified as part of the genus B3-G11, family UBA5794, and order UBA5794 in GTDB taxonomy; according to SILVA taxonomy, they are classified as part of the order '*Actinomarinales*'. Most of the MAGs of the genus B3-G11 were isolated from hydrothermal vents or the deep ocean. So, to give a hint (and some priority) to those genomes we decided to name the genus *Hadalibacter* (Ha.da.li.bac'ter. N.L. gen. n. hadalis, from the deepest regions of the ocean; N.L. masc. n. bacter, rod; N.L. masc. n. Hadalibacter, A rod-shaped bacterium from the deepest regions of the ocean). IsfjordSed11 shares a 59% average amino acid identity and a 16S rRNA gene sequence similarity of 93% with *Benthobacter isfjordensis*, indicating that they belong to the same family, i.e., *Benthobacteraceae*, yet different genera.

Description of Hadalibacter litoralis gen. nov. sp. nov.

Hadalibacter litoralis (li.to.ra'lis. L. masc. adj. litoralis, of the shore, a shallow-water dweller) corresponds to the MAG IsfjordSed11. The MAG IsfjordSed01 represents the same species and here we use it to support the description of *Hadalibacter litoralis*. IsfjordSed01 has a completeness of 54.3% and a contamination of 2.8%, a corrected genome size of 2.95 Mbp, GC-content of 59%, a full-length 16S rRNA gene sequence, and tRNAs for 11 amino acids. IsfjordSed11 has a completeness of 87.3% and a contamination of 4.9%, a corrected genome size of 2.46 Mbp, GC-content of 59%, a partial 16S rRNA gene sequence, and tRNAs for 13 amino acids. The average sequencing depth for both MAGs was 4x in the metagenome from December 2017.

Hadalibacter litoralis showed, based on MAG IsfjordSed01 and MAG IsfjordSed11, most of the genes involved in basic carbon metabolism. Glycolysis and pentose phosphate pathway were almost complete. Citrate cycle and glyoxylate cycle were complete. The majority of electron transport chain was present, but the complex III was incomplete. Glycogen biosynthesis pathway was complete. Nucleotide sugar and UDP-N-acetyl-D-glucosamine biosynthesis pathways were almost complete. Although the MAG has genes identified as part of carbon fixation pathways in prokaryotes, none of those pathways is complete. Genes encoding for flagella or pili were not detected. No complete pathways for secondary metabolites were identified. The steps of denitrification pathway present in the genome were the import of extracellular nitrate, and the dissimilatory reduction of nitrate to nitrite. No pathway for sulfate reduction or methanogenesis was detected. The F420 biosynthesis pathways was almost complete.

Glycoside hydrolases present in MAGs IsfjordSed01 and IsfjordSed11 were GH1, GH3, GH13 (sub-families 9, 11, 18, and 23), and GH149 (Supplementary Fig. S4). Sulfatases from families S1_4, S1_13, and S1_6 were also encoded in the MAGs (Supplementary Fig. S5).

Description of Ilumatobacter isfjordensis sp. nov.

Ilumatobacter isfjordensis (is.fjor.den'sis. N.L. masc. adj. *isfjordensis*, from or belonging to Isfjorden, Svalbard). Its type genome is the MAG IsfjordSed13. IsfjordSed08 belongs to the same species and it is here used to support the description of *Ilumatobacter isfjordensis.* IsfjordSed13 has a completeness of 92.5% and a contamination of 8.1%, a corrected genome size of 4.79 Mbp, GC-content of 64%, no 16S rRNA gene sequence, and tRNAs for 19 amino acids. IsfjordSed08 has a completeness of 50% and a contamination of 1.7%, a corrected genome size of 7.86 Mbp, GC-content of 64%, a full-length 16S rRNA gene sequence, and tRNAs for 17 amino acids. *Ilumatobacter isfjordensis* shares a 66% average amino acid identity, and a 16S gene sequence similarity of 95% with *Ilumatobacter coccineus*, indicating that they belong to the same genus, i.e., *Ilumatobacter*. The average sequencing depth for IsfjordSed13 and IsfjordSed08 was 6x and 5x, respectively, in the metagenome from December 2017.

Ilumatobacter isfjordensis showed, based on MAG IsfjordSed08 and MAG IsfjordSed13, most of the genes involved in basic carbon metabolism. Pyruvate oxidation and pentose phosphate pathway was complete. Glycolysis and the citrate cycle were almost complete. Galactose degradation pathway and nucleotide sugar biosynthesis pathways were complete. Most of the electron transport chain was present, but complex III was incomplete. Glycogen pathway was complete. Although the MAG encoded genes identified as part of carbon fixation pathways in prokaryotes, most pathways were complete. The dark part of the Crassulacean Acid Metabolism (CAM) was complete. Genes encoding flagella or pili were not detected. Pantothenate, heme, C10-C20 isoprenoid, and dTDP-L-rhamnose biosynthesis pathways were almost complete. The steps of the denitrification pathway present in the genome were the

Table 2
Protologue of the new species descriptions.

Species name	*Benthobacter isfjordensis*	*Hadalibacter litoralis*	*Ilumatobacter isfjordensis*	*Ilumatobacter herschelensis*
Genus name	*Benthobacter*	*Hadalibacter*	*Ilumatobacter*	*Ilumatobacter*
Specific epithet	*isfjordensis*	*litoralis*	*isfjordensis*	*herschelensis*
Genus etymology	Ben.tho.bac'ter. Gr. masc. adj. benthos, sea floor; N.L. masc. n. bacter, rod; N.L. masc. n. *Benthobacter*, a rod-shaped bacterium found in benthic samples.	Ha.da.li.bac'ter. N.L gen. n. hadalis, related to the deepest regions of the ocean; N.L. masc. n. bacter, rod; N.L. masc. n. *Hadalibacter*, a rod-shaped bacterium found in the deep sea.	I.lu.ma.to.bac'ter. Gr. n. *iluma –atos*, sediment deposited in water; N.L. masc. n. bacter, a rod, a bacterium; N.L. masc. n.*Ilumatobacter*, a rod isolated from a sediment.	I.lu.ma.to.bac'ter. Gr. n. *iluma –atos*, sediment deposited in water; N.L. masc. n. bacter, a rod, a bacterium; N.L. masc. n. *Ilumatobacter*, a rod isolated from a sediment.
Genus status	gen. nov.	gen. nov.	validly published under the ICNP	validly published under the ICNP
Species etymology	is.fjor.den'sis. N.L. masc. adj. *isfjordensis*, from or belonging to Isfjorden, Svalbard.	li.to.ra'lis. L. masc. adj. *litoralis*, of the shore.	is.fjor.den'sis. N.L. masc. adj. *isfjordensis*, from or belonging to Isfjorden, Svalbard.	her.schel.en'sis. N.L. masc. adj. *herschelensis*, from or belonging to Herschel Island.
Species status	sp. nov.	sp. nov.	sp. nov.	sp. nov.
Description of the new taxon and diagnostic traits	The MAGs show basic carbon metabolism almost complete. The majority of electron transport chain was present but complex III was missing. No carbon fixation pathways, no flagella or pili. Denitrification pathways are present (only import of extracellular nitrate, reduction of nitrate to nitrite, and reduction of nitrite to nitric oxide), and nitrilase present. The pathway for F420 biosynthesis is almost complete. The glycoside hydrolases present in these MAGs are: GH1, GH3, GH13 (sub-families 9, 11, 16, 23, and 20), GH32, GH57, GH77, GH133, and GH149. Sulfatases from families S1_4, S1_13, and S1_16 were also encoded in the MAGs.	The MAGs show basic carbon metabolism. The majority of electron transport chain was present, but the complex III was missing. No carbon fixation pathways, no flagella or pili. Denitrification patways are present (only import of extracellular nitrate, reduction of nitrate to nitrite). The pathway for F420 biosynthesis is almost complete. No complete pathways for secondary metabolites were identified. The glycoside hydrolases present in these MAGs are: GH1, GH3, GH13 (sub-families 9, 11, 18, and 23), and GH149. Sulfatases from families S1_4, S1_13, and S1_6 were also encoded in the MAGs.	The MAGs show basic carbon metabolism. The majority of the electron transport chain was present, but complex III was missing. The dark part of the Crassulacean Acid Metabolism (CAM) was complete. No genes encoding flagella or pili were detected. Pantothenate, heme, C10-C20 isoprenoid, and dTDP-L-rhamnose biosynthesis pathways were almost complete. Denitrification pathway present (only import of extracellular nitrate and the reduction of nitrate to nitrite). Nitrilase was also encoded. No pathway for sulphate reduction or methanogenesis was detected. The F420 biosynthesis pathway was almost complete.The glycoside hydrolases present in these MAGs are: GH1, GH2, GH3, GH13 (sub-families 4, 9, 11, and 30), GH65, GH114, and GH149. Sulfatases from families S1_4, S1_5, S1_12, S1_13, S1_14, S1_27, S1_48, S1_63, S2, and S3 were also encoded in the MAGs.	The MAGs show most of the genes involved in basic carbon metabolism. The majority of the electron transport chain was present, but complex III was incomplete. No carbon fixation pathways, no flagella or pili. The riboflavin, siroheme and haem biosynthesis pathways were almost complete. The C5 isoprenoid and C10-C20 isoprenoid biosynthesis pathways were almost complete. No nitrogen metabolism was encoded. The assimilatory sulphate reduction pathway was complete. No methanogenesis was detected. The F420 biosynthetic pathway was present but incomplete. The glycoside hydrolases present in these MAGs are: GH2, GH3, GH13 (sub-families 9, 11, and 30), GH18, GH36, and GH65. Sulfatases from families S1_4, S1_5, S1_14, S2, and S3 were also encoded in the MAGs.
Country of origin	Norway	Norway	Norway	Canada
Region of origin	Isfjorden, Svalbard	Isfjorden, Svalbard	Isfjorden, Svalbard	Herschel Island
Date of isolation (mm/yyyy)	–	–	–	–
Source of isolation	Marine sediment of an Arctic fjord	Marine sediment of an Arctic fjord	Marine sediment of an Arctic fjord	Coastal surface seawater
Sampling date (mm/yyyy)	12/2017–05/2022	12/2017–05/2022	12/2017–05/2022	07/2022
Latitude (xx°xx′xx″ N/S)	78° 6′ 36″ N	78° 6′ 36″ N	78° 6′ 36″ N	69° 33′ 32.4″ N
Longitude (xx°xx′ E/W)	14° 21′ 0″ E	14° 21′ 0″ E	14° 21′ 0″ E	138° 54′ 24.84″ W
Depth (meters below sea level)	8–4	8–4	8–4	Surface seawater
Genome accession number	GCA_964014115.1^{TS}	GCA_964199975.1^{TS}	GCA_964199985.1^{TS}	GCA_963926495^{TS}
Genome status	Draft − 94% completeness	Draft − 87% completeness	Draft − 93% completeness	Draft − 91% completeness
Genome size (Mbp)	2.89	2.46	4.79	3.08
G + C mol%	62.35	58.99	64.38	62.44
Number of MAGs in study	2 (Isfjord06^{TS} and Isfjord14)	2 (Isfjord01 and Isfjord11^{TS})	2 (Isfjord08 and Isfjord13^{TS})	2 (HerschelSW1 and HerschelSW3^{TS})
Metagenomic raw data	SAMEA115040709 SAMEA115040710 SAMEA115040711 SAMEA115040712 SAMEA115040713 SAMEA115040714	SAMEA115040709 SAMEA115040710 SAMEA115040711 SAMEA115040712 SAMEA115040713 SAMEA115040714	SAMEA115040709 SAMEA115040710 SAMEA115040711 SAMEA115040712 SAMEA115040713 SAMEA115040714	SAMEA115135962
SeqCode registry URL	seqco.de/r:af2x99s7	seqco.de/r:ksp-zbyb	seqco.de/r:ksp-zbyb	seqco.de/r:ksp-zbyb

import of extracellular nitrate and the dissimilatory reduction of nitrate to nitrite. Nitrilase was also encoded in the MAG. No pathway for sulfate reduction or methanogenesis was detected. The F420 biosynthesis pathway was almost complete.

Glycoside hydrolases present in MAGs IsfjordSed08 and IsfjordSed13 were GH1, GH2, GH3, GH13 (sub-families 4, 9, 11, and 30), GH18, GH65, GH114, and GH149 (Supplementary Fig. S4). Sulfatases from families S1_4, S1_5, S1_12, S1_13, S1_14, S1_27, S1_48, S1_63, S2, and S3 were present in the genomes (Supplementary Fig. S5).

Description of Ilumatobacter herschelensis sp. nov.

Ilumatobacter herschelensis (her.schel.en'sis. N.L. masc. adj. *herschelensis*, from or belonging to Herschel Island). Its type genome is the MAG HerschelSW3. HerschelSW1 belongs to the same species and it is here used to support the description of *Ilumatobacter herschelensis*. HerschelSW1 has a completeness of 53.8% and a contamination of 2.1%, a corrected genome size of 1.92 Mbp, GC-content of 62%, no 16S rRNA gene sequence, and tRNAs for 9 amino acids. HerschelSW3 has a completeness of 90.5% and a contamination of 4.6%, a corrected genome size of 3.08 Mbp, GC-content of 62%, no 16S rRNA gene sequence, and tRNAs for 16 amino acids. *Ilumatobacter herschelensis* shares a 67.6% average amino acid identity with *Ilumatobacter coccineus*, indicating that they belong to the same genus, i.e., *Ilumatobacter*. HerschelSW3 had a coverage of 18x in its source metagenome.

Ilumatobacter herschelensis showed, based on MAG HerschelSW1 and HerschelSW3, most of the genes involved in basic carbon metabolism. The citrate cycle and the oxidative phase of the pentose phosphate pathway were complete. Glycolysis, gluconeogenesis, the non-oxidative phase of the pentose phosphate pathway and the Entner-Doudoroff pathway were incomplete. The glycogen biosynthesis and galactose degradation pathways were complete. Most of the electron transport chain was present, but complex III was incomplete. Although the MAG has genes identified as part of carbon fixation pathways in prokaryotes, none of these pathways is complete. Genes encoding flagella or pili were not detected. The riboflavin, siroheme and heme biosynthesis pathways were almost complete. The C5 isoprenoid and C10-C20 isoprenoid biosynthesis pathways were almost complete. No nitrogen metabolism was encoded. The assimilatory sulfate reduction pathway was complete. No methanogenesis was detected. The F420 biosynthetic pathway was present but incomplete.

Glycoside hydrolases present in MAGs HerschelSW1 and HerschelSW3 were GH2, GH3, GH13 (sub-families 9, 11, and 30), GH18, GH36, and GH65 (Supplementary Fig. S4). Sulfatases from families S1_4, S1_5, S1_14, S2, and S3 were also encoded (Supplementary Fig. S5).

Discussion

The class *Acidimicrobiia* represents one of the most prevalent clades of bacteria in the ocean, and the most abundant class of *Actinomycetota* in marine benthic environments, yet our understanding of their taxonomy and ecological functions is limited. Here, we reviewed the taxonomy of *Acidimicrobiia* and provide data on the abundance of specific acidimicrobial clades in marine benthic environments of polar and temperate regions.

The phylogenetic tree reconstructions of *Acidimicrobiia* showed that the sequence grouping at the family and order level using the 16S rRNA gene-based phylogeny was in good agreement to the grouping obtained using the genome-based method. An earlier comparison of 16S rRNA-based phylogeny with genome-based phylogeny suggested consistency when analysing 20 genomes of *Acidimicrobiia* (Hu et al., 2018). Several molecular markers for the whole class and for specific groups were documented in the same study. Although, identifying molecular markers and describing in great depth the phylogeny of *Acidimicrobiia* was beyond the scope of this study, the quantity and quality of genomes used here (approximately 200 with completeness greater than 90% and contamination less than 10%) allowed for a robust taxonomic characterization of the class. However, it is worth noting that, besides three genomes used as reference, the *'Actinomarinales'* group in GTDB taxonomy was excluded from our analysis due to our completeness filtration step. Members of these marine bacterioplankton group were described as ultra-small, low-GC (33%), photoheterotrophs (Ghai et al., 2013), whose genome is streamlined and approximately 1.1 Mbp in length (López-Pérez et al., 2020). *'Actinomarinales'* comprises the previously known Marine Actinobacteria Clade (MAC), which in turn includes the groups OM1, SAR432, BDA1-5, and D92-32 (Ghai et al., 2013; Mizuno et al., 2015). Likely due to the difficulty that checkM has to accurately estimate completeness of streamlined genomes, most of *'Actinomarinales'* genomes in GTDB showed less than 80% completeness, therefore being removed in the early stages of our analysis. We do not exclude the possibility that other groups were also neglected due to the apparent low quality of their genomes, and we recommend that future studies relax filtration parameters and include a second tool for a more robust completeness estimation, e.g., checkM2 (Chklovski et al., 2023). Altogether, we report that *Acidimicrobiia* is a monophyletic class, whose taxonomy can be confidently addressed by 16S rRNA gene-based and genome-based methods.

Despite consistent phylogenies, we identified nomenclatural inconsistencies. The names used by SILVA database and GTDB were different for several taxonomic groups. We believe that this is mainly due to two factors, the first is that the guidelines used by both databases for naming entries are different when no official name is available (Parks et al., 2022; Quast et al., 2013). The second is that the entire class has less than 15 representative isolates, all from one order, *Acidimicrobiales*, and only from four families, *Ilumatobacteraceae*, *Acidimicrobiaceae*, *Iamiaceae* and *'Microtrichaceae'*. In the last 10 years, environmental microbiologists argued for officially naming important clades based on genomic information, despite having no representatives in culture collections (Hedlund et al., 2022; Konstantinidis et al., 2017; Murray et al., 2020; Pallen et al., 2022), pushing to advance from an alphanumerical nomenclature to a binominal, revised and meaningful nomenclature. Ultimately, together, proper nomenclature and a solid taxonomy are fundamental to understand the ecology of microorganisms.

Relative abundances of 16S rRNA gene sequences extracted from LR metagenomes showed that the class *Acidimicrobiia* comprised approximately 5% of the bacterial community in marine sediments from Isfjorden (an Arctic fjord) and Helgoland (a long-term sampling marine station in the German North Sea). In both regions, *Acidimicrobiia* comprised more than 90% of *Actinomycetota* sequences (referred to as *'Actinobacteriota'* in SILVA taxonomy). Relative abundances of the class *Acidimicrobiia* are also high in other marine benthic environments, although the estimations highly depend on the methods used. 16S rRNA amplicon analyses had indicated that relative abundance of *Acidimicrobiia* might be up to 25–34% of bacterial sequences in Isfjorden (Miksch et al., 2021), and 5–10% in German North Sea sediments (Probandt et al., 2017). When looking at single sand grains (Probandt et al., 2018), *Acidimicrobiia* also accounted for approximately 5% of bacteria. Similarly high values (5–10%) were reported in seawater from the South Korean Sea (Seo et al., 2017). Other studies had shown similar relative abundances for the phylum *Actinomycetota* without further taxonomic specification (Giner-Lamia and Huerta-Cepas, 2024; Gobet et al., 2012; Hoshino et al., 2020; Li et al., 2009). Since currently the number of deep-sequenced metagenomes from sediments is limited, we leave it up to future studies to confirm the estimates based on 16S rRNA amplicon on the genomic level, beyond the two sets of metagenomes from Isfjorden and Helgoland presented here. We hypothesize that members of the class *Acidimicrobiia* are abundant, the most common representative of the phylum *Actinomycetota*, and presumably important cosmopolitan players in marine benthic microbiomes.

The novel set of oligonucleotide probes presented in this study (Supplementary Table S1) will facilitate the *in situ* identification and absolute quantification of the family *Ilumatobacteraceae* in the

environment. Using CARD-FISH, it was previously shown that *Acidimicrobiia* account for 10–15% of the microbial community in the coastal sediments of Isfjorden (Miksch et al., 2021). However, *Ilumatobacteraceae* were not covered by those probes, and therefore, its contribution to the community had not been considered. Our FISH data suggests an average relative abundance of *Ilumatobacteraceae* of approximately 3% in Isfjorden sediments, and between 0.8% and 6.2% in sandy sediments of the German Bight. Relative read frequencies estimates obtained by extracting 16S rRNA gene sequences from PacBio metagenomes were slightly lower, but in the same range with 0.6–1.9%. We interpret this as independent proof of a relative abundance of *Ilumatobacteraceae* in the single digit percent range, which is substantial considering the high diversity and evenness of sandy marine sediments.

In the pursuit of moving from alphanumerical to binominal nomenclature for important clades, we propose four new species and two new genera following the SeqCode guidelines (Hedlund et al., 2022): *Benthobacter isfjordensis* gen. nov., sp. nov., *Hadalibacter litoralis* gen. nov. sp. nov., *Ilumatobacter isfjordensis* sp. nov., and *Ilumatobacter herschelensis* sp. nov. *Benthobacter Isfjordensis* and *Hadalibacter litoralis* have no close representative in culture collections, being this the first description of type material in their order. Previously, members of the GTDB-order UBA5794 were described as sponge symbionts (Nguyen et al., 2023), also following SeqCode guidelines. *Spongiisociales*, with the type species *Spongiisocius variivorans*, was proposed as a new order. *Benthobacter isfjordensis* showed low phylogenetic proximity to *Spongiisociales* (AAI of 47% and 16S rRNA gene similarity of 87%), leading us to suggest *Benthobacterales* as a new and distinct order. Also, our data suggests that *Hadalibacter litoralis* belongs to the family *Benthobacteraceae*. In the following section, we discuss the genomic potential of the new species, making emphasis in their energy source and motility.

The metabolic prediction of the four new (candidate) species suggests that they are aerobic organoheterotrophic bacteria. *Benthobacter isfjordensis*, *Hadalibacter litoralis*, and *Ilumatobacter isfjordensis*, showed facultative dissimilatory nitrate reduction to nitrite (*Benthobacter isfjordensis* also showed nitrite reduction to nitric oxide). Isolates of the class *Acidimicrobiia* have been shown to reduce inorganic nitrogenous compounds to varying degrees (Asem et al., 2018; Jin et al., 2013; Kurahashi et al., 2009; Rossetti et al., 2005). Naturally, an alternative electron acceptor provides members of the class *Acidimicrobiia* the potential to thrive in environments that experience sudden anoxia, such as coastal sediments that are constantly reworked by storms and tides. Also, several glycoside hydrolases were found to be encoded in the genomes of the new (candidate) species. GH1 (broad family with β-glucosidases and β-galactosidases), GH3 (broad family with β-glucosidases and other β-type linkages), GH13_9 (α-1,4-glucanase, amylase), GH13_11 (α-glucosidases and α-amylases), and GH149 (β-1,3-glucan phosphorylase, laminarinase) were found in all three species from Isfjorden (Supplementary Fig. S4). *Ilumatobacter isfjordensis* and *Ilumatobacter herschelensis* shared GH2 (broad family targeting β-type linkages), GH3, GH13_9, GH13_11, GH13_30 (α-amylase), GH18 (chitinase), and GH65 (α-glucan phosphorylase). High completeness and low contamination of the genomes suggest with high certainty that they do encode for these glycoside hydrolases, which gives species from *Acidimicrobiia* the potential to feed on common substrates in marine benthic environments, such as starch and glycogen (chains of glucose connected by α-1,4 and α-1,6 glycosidic linkages), laminarin (chain of glucose connected by β-1,3 and β-1,6 glycosidic linkages), and chitin (chain of N-acetylglucosamine connected by β-1,4 glycosidic linkages). Pure cultures from *Ilumatobacter* had shown to be positive for α-glucosidase, but negative for α-galactosidase, α-mannosidase, α-fucosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosamidase in biochemical tests (Matsumoto et al., 2013), despite coding for some of them. It is worth noting that all *Ilumatobacter* isolates were obtained from the coast of Japan, and we here present not only *Ilumatobacter* genomes recovered from polar areas, but also genomes from another order within the class *Acidimicrobiia* that is not represented in culture collections, i.e., *Benthobacterales*. Transcriptomic analysis of sediment samples from Isfjorden indicate that some of these glycoside hydrolases are highly expressed at a community level, without necessarily belonging to the class *Acidimicrobiia* (Miksch et al., 2024). Future studies should focus on experimentally testing the growth of *Acidimicrobiia* on culture media with nitrate as the only electron acceptor, and specific substrates, such as laminarin or chitin, as the electron donor.

None of the new (candidate) species encoded for flagella or pili, suggesting a lack of motility. Even more, detailed annotation of the class *Acidimicrobiia* (data not shown) showed that genes encoding for flagella or pili are rather rare. In line with this, it was recently documented that more than half of the isolates of the class *Acidimicrobiia* do not have a flagellar gene set (Zhu et al., 2023). Motility (or non-motility) has been proposed as a predictor of the stage in an ecological succession at which an organism settles on marine particles (Datta et al., 2016), where early-stage colonizers show flagella, and late-stage colonizers do not, therefore depending on other species to settle. Along the same line, we hypothesize that most organisms from the class *Acidimicrobiia* will appear at late stages of surface colonization in marine environments.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used DeepL Translator and DeepL Write in order to translate and improve readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Sebastián Silva-Solar: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tomeu Viver:** Writing – review & editing, Methodology, Formal analysis. **Yueqing Wang:** Formal analysis, Data curation. **Luis H. Orellana:** Writing – review & editing, Supervision. **Katrin Knittel:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rudolf Amann:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

All data was made public, and its accessibility is stated in the main text.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2024.126555.

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