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Genomic comparison of deep-sea hydrothermal genera related to *Aeropyrum, Thermodiscus* and *Caldisphaera,* and proposed emended description of the family *Acidilobaceae*

Emily St. John [*], Anna-Louise Reysenbach [*]

[1] *Center for Life in Extreme Environments, Portland State University, Portland, OR 97201, USA*

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ABSTRACT

Deep-sea hydrothermal vents host archaeal and bacterial thermophilic communities, including taxonomically and functionally diverse *Thermoproteota.* Despite their prevalence in high-temperature submarine communities, *Thermoproteota* are chronically under-represented in genomic databases and issues have emerged regarding their nomenclature, particularly within the *Aeropyrum–Thermodiscus–Caldisphaera*. To resolve some of these problems, we identified 47 metagenome-assembled genomes (MAGs) within this clade, from 20 previously published deep-sea hydrothermal vent and submarine volcano metagenomes, and 24 MAGs from public databases. Using phylogenomic analysis, Genome Taxonomy Database Toolkit (GTDB-Tk) taxonomic assessment, 16S rRNA gene phylogeny, average amino acid identity (AAI) and functional gene patterns, we re-evaluated of the taxonomy of the *Aeropyrum–Thermodiscus–Caldisphaera.* At least nine genus-level clades were identified with two or more MAGs. In accordance with SeqCode requirements and recommendations, we propose names for three novel genera, viz. *Tiamatella incendiivivens, Hestiella acidicharens* and *Calypsonella navitae.* A fourth genus was also identified related to *Thermodiscus maritimus,* for which no available sequenced genome exists. We propose the novel species *Thermodiscus eudorianus* to describe our high-quality *Thermodiscus* MAG, which represents the type genome for the genus. All three novel genera and *T. eudorianus* are likely anaerobic heterotrophs, capable of fermenting protein-rich carbon sources, while some *Tiamatella, Calypsonella* and *T. eudorianus* may also reduce polysulfides, thiosulfate, sulfur and/or selenite, and the likely acidophile, *Hestiella,* may reduce nitrate and/or perchlorate. Based on phylogenomic evidence, we also propose the family *Acidilobaceae* be amended to include *Caldisphaera, Aeropyrum, Thermodiscus* and *Stetteria* and the novel genera described here.

Introduction

High temperature deep-sea vents support a rich phylogenetic and physiological diversity of thermophilic *Bacteria* and *Archaea*. Like in other ecosystems, the advent of 16S rRNA amplicon sequencing revealed how diverse the hydrothermal communities are and how many lineages have very few, if any, representatives in culture (Flores et al., 2011, 2012). For example, most of the characterized isolated archaeal cultures from deep-sea vents are *Methanobacteriota*, *Thermoplasmatota* and *Halobacteriota* (all syn. *Euryarchaeota;* Chuvochina et al., 2023), yet from amplicon sequencing, the *Thermoproteota* (syn. *Crenarchaeota*) are much more prevalent and more phylogenetically diverse than previously assumed. Further, the diversity and relative abundance of some community members from the *Thermoproteota* helped drive differences in the observed microbial community structures at Lucky Strike vent field versus Rainbow vent field along the Mid-Atlantic Ridge (Flores et al., 2011). The *Thermoproteota* are also noticeably underrepresented in genomic databases.

Within the *Thermoproteota*, prevalent orders associated with deep-sea vents (e.g. Flores et al., 2011, 2012; Zhou et al., 2022) include the *Thermofilales* (e.g. *Thermofilum*), *Thermoproteales* (e.g. *Pyrobaculum*), and the *Desulfurococcales* (includes the *Pyrodictiaceae*, *Desulfurococcaceae* families, e.g. *Desulfurococcus*, *Aeropyrum*, *Pyrodictium*). Metabolically, the *Desulfurococcales* are very diverse, encompassing the range of autotrophs (e.g. *Ignicoccus*; Huber et al., 2000; Paper et al., 2007) heterotrophs (e.g. *Desulfurococcus, Ignisphaera*; Niederberger et al., 2006; Zillig et al., 1982), aerobes (e.g. *Aeropyrum*; Nakagawa et al., 2004; Sako et al., 1996) anaerobes (e.g. *Desulfurococcus, Thermodiscus*; Stetter, 2001; Zillig

* Corresponding authors.
E-mail addresses: em9@pdx.edu (E. St. John), reysenbacha@pdx.edu (A.-L. Reysenbach).

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et al., 1982), fermenters (e.g. *Hyperthermus, Ignisphaera*; Niederberger et al., 2006; Zillig et al., 1991) and nitrate (e.g. *Pyrodictium*; Lin et al., 2016), sulfur (e.g. *Thermodiscus*; Stetter, 2001), iron (e.g. *Pyrodictium*; Lin et al., 2016), thiosulfate (e.g. *Zestosphaera*; St. John et al., 2019a) and perchlorate (e.g. *Aeropyrum*; Liebensteiner et al., 2015) respirers. Some also serve as hosts (e.g. *Ignicoccus*, *Zestosphaera;* Huber et al., 2002; St. John et al., 2019a) for the *Nanobdellota* (syn. *Nanoarchaeota*; Kato et al., 2022).

Taxonomically, considerable uncertainty exists within the *Desulfurococcales* as phylogenetic trees based on 16S rRNA gene sequences are often polyphyletic (Boyd et al., 2007; Jay et al., 2014; Niederberger et al., 2006; Prokofeva et al., 2000). In particular, the phylogenetic placement of members of the *Desulfurococcaceae* family has illustrated some of the taxonomic issues in this ecologically important group of *Archaea*. As more 16S rRNA genes have been sequenced, some incongruencies have emerged within the phylogeny of this family, namely the unstable position of the *Aeropyrum-Thermodiscus-Stetteria* clade. As genomes and metagenome assembled genomes (MAGs) have been added to the phylogenomic framework of the family however, more robust support for these issues has been realized, and whole genome analysis places this clade and *Caldisphaera* firmly within the family *Acidilobaceae* (Prokofeva et al., 2009), which is mostly represented by acid-tolerant thermophilic isolates from terrestrial hot springs (Boyd et al., 2007; Prokofeva et al., 2000, 2009). While the Genome Taxonomy Database (GTDB) further illuminated these issues by classifying this clade within the *Acidilobaceae*, no formal proposal has been published for including previously classified *Caldisphaeraceae* and *Desulfurococcaceae* into the *Acidilobaceae*. Using an extensive MAG dataset from deep-sea hydrothermal vents, we demonstrate that *Aeropyrum*, *Thermodiscus, Caldisphaera* and their relatives form a monophyletic clade with members of the *Acidilobaceae*. Here, we propose amending the description of the *Acidilobaceae* to include these *Desulfurococcaceae* and the *Caldisphaeraceae* and propose several new genera in this family based on high quality MAGs and metabolic criteria.

Materials and methods

Sample processing and metagenome sequencing, assembly and binning

Sample collection and DNA extraction were performed as previously described (Zhou et al., 2022). Metagenomic reads from Brothers volcano were sequenced and assembled as described in Reysenbach et al., 2020 with a 2000 bp minimum contig length (Table S1). DNA libraries from Eastern Lau Spreading Center (ELSC; 2005), Mid-Atlantic Ridge (MAR), East Pacific Rise (EPR) and Guaymas Basin were sequenced and assembled at the Department of Energy–Joint Genome Institute (JGI) as described in Zhou et al., 2022, and libraries from ELSC (2015) were sequenced and assembled as described in St. John et al., 2019b. Metagenomic assemblies generated by the JGI and assemblies of the ELSC (2015) data were both binned with a 1500 bp minimum contig length, as described in St. John et al., 2019b. Sequencing, assembly and binning methods are summarized in Table S1.

CheckM v.1.0.7 (Parks et al., 2015) was used to estimate completeness and contamination, and PhyloSift v.1.0.1 (Darling et al., 2014) was used to detect the presence of 16 ribosomal proteins (*rpL2-6, rpL14-16, rpL18, rpL22, rpL24, rpS3, rpS8, rpS10, rpS17, rpS19*; Anantharaman et al., 2016). To ensure continuity with the dataset described in Reysenbach et al., 2020, further analysis was restricted to MAGs with six or more of the listed ribosomal proteins, with ≥50% estimated completion and ≤10% estimated contamination. Re-assessment of MAG quality using the updated CheckM2 v.1.0.1 program (Chklovski et al., 2023; Table S2) confirmed 40 MAGs at medium (≥50% completion, <10% contamination) or high (>90% completion, <5% contamination) quality using CheckM2, while seven were assigned to low quality due to the relative low completion (44.45 to 49.83%; six MAGs) or high contamination (11.45%; one MAG). We elected to retain these seven MAGs in this study as they provide additional valuable genomic data.

Curation of type sequences

Preliminary phylogenetic analysis was performed as previously described (St. John et al., 2019a), using a maximum-likelihood concatenated phylogenetic tree of 16 ribosomal proteins (data not shown). MAGs clustering with the *Aeropyrum* and *Caldisphaera* were then curated with Emergent Self-Organizing Mapping (ESOM; Ultsch and Mörchen, 2005) using the tetramerFreq software package (Dick et al., 2009). Briefly, each individual MAG was visualized in ESOM with a suite of reference genomes based on tetranucleotide frequency. MAGs were manually defined in the ESOM map, and contigs below the 90% confidence threshold were removed. The four MAGs selected as type material under the SeqCode were also screened using CAT/BAT (von Meijenfeldt et al., 2019) and remaining contaminant contigs were removed. CheckM2 (Chklovski et al., 2023) was then used to re-estimate the completion and contamination of final MAGs.

Identification of MAGs related to Aeropyrum, Thermodiscus and Caldisphaera

To identify publicly available MAGs related to the *Aeropyrum, Thermodiscus* and *Caldisphaera*, a GTDB-Tk phylogenomic tree was used to screen MAGs from the Genomes of Earth's Microbiomes (GEM) catalog that were assigned by Nayfach et al. (2021) to the *Acidilobaceae* or to unclassified taxa within the *Thermoprotei*, *Desulfurococcales*, *Sulfolobales* and *Sulfolobaceae*. To prevent duplication, we excluded MAGs from the GEM catalog that were assembled from metagenomic read sets already present in this study. Within the GEM dataset, we identified two MAGs from the *Caldisphaera* and 14 from the DSZV01 group. We also included additional unique publicly available MAGs from the GTDB r214 database that were assigned to the *Acidilobaceae*. For functional analyses, however, the putative *Caldisphaera* MAG KMA_Bin23 (Genbank accession JAGDXI000000000.1) was excluded due to the presence of a 16S rRNA gene most closely related to *Thermoproteaceae* (*Caldivirga* and *Vulcanisaeta*). Additionally, *Acidilobus saccharovorans* was used as the sole genus representative in functional analyses, since none of the MAGs described in this study were assigned to the *Acidilobus*.

MAG taxonomy and phylogeny

After curation, preliminary taxonomy was assigned to MAGs with GTDB-Tk v.2.3.0 r214 (Chaumeil et al., 2020). A *de novo* concatenated protein tree was generated from 53 archaeal marker genes with FastTree v.2.1.10 (Price et al., 2010) via GTDB-Tk "infer" (parameter "–gamma"), with support values calculated using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999). AAI comparisons were generated with CompareM v.0.1.2 (Parks, 2014; Table S3), and intra-genus ANI was calculated with pyANI (Pritchard et al., 2015) using the parameter "-m ANIb" (Table S4). AAI data was then visualized using Morpheus (Broad Institute, 2022), and hierarchical clustering was performed using default settings. AAI and ANI are effective tools for assessing relatedness between genomes since they are strongly correlated with shared gene content between genomes (Konstantinidis et al., 2017; Konstantinidis and Tiedje, 2005). They are also effective for comparing incomplete genomes, and thresholds have been proposed for delineating novel species and higher taxonomic ranks using these tools (Konstantinidis et al., 2017).

16S rRNA gene identification, classification, and phylogeny

Ribosomal RNA genes were identified using the Prokaryotic Genome Annotation Pipeline (PGAP; Tatusova et al., 2016), and rRNA genes from reference genomes were downloaded from Genbank or the JGI Genome Portal. Rfam v.14.1 (Kalvari et al., 2021) and Infernal v.1.1.4 (Nawrocki

and Eddy, 2013) were used to predict rRNA genes in the UBA158 MAG (Genbank accession DAXV00000000.1), as no annotation data was available. 16S rRNA gene sequences (≥1400 bp) were compared to their closest relatives using EZBioCloud (Yoon et al., 2017; Table S5). Additional 16S rRNA genes were also reconstructed from trimmed reads using phyloFlash (Gruber-Vodicka et al., 2020) with the parameter "-read_length 150". For phylogenetic analyses, 16S rRNA genes were aligned with MAFFT v.7.450 (Katoh and Standley, 2013) in Geneious v.10.2.6 (Kearse et al., 2012). The alignment was curated manually, and hypervariable regions were removed. A maximum-likelihood phylogeny was inferred in RaxML v.8.2.8 using the GTR + GAMMA model with 1000 rapid bootstraps (Stamatakis, 2014). The tree was rooted with the *Methanobacteriota, Halobacteriota* and *Thermoplasmatota* (syn. *Euryarchaeota*) and viewed using the Interactive Tree of Life v.6 (Letunic and Bork, 2021). Putative intervening sequences were also identified in full-length and partial 16S rRNA genes using alignments in Geneious (Kearse et al., 2012), and secondary structures were predicted for these intervening sequences using the Mfold RNA folding form with default options (Zuker, 2003).

Prediction of genome features and metabolic functions

Open reading frames (ORFs), RNAs and repeat regions were predicted for MAGs in this study using PGAP (Tatusova et al., 2016). When available, ORFs from reference genomes and MAGs were downloaded directly from Genbank or the JGI Genome Portal. Open reading frames for UBA158 were predicted using Prodigal v.2.6.3 via Prokka v.1.14.6 with the parameters "–kingdom Archaea" and "–gcode 11" (Data S1; Hyatt et al., 2010; Seemann, 2014). Signal peptides were predicted with the SignalP 5.0b command line tool with the parameter "-org arch" (Almagro Armenteros et al., 2019). CRISPR regions were identified in UBA158 and genomes from the GEM database using the CRISPRCas-Finder release 4.2.20 using the parameter "-def General" (Couvin et al., 2018).

Functional gene annotations were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database via GhostKoala (Kanehisa et al., 2016; Kanehisa and Goto, 2000). Additional annotations were done using a local BlastP search of the arCOG database with an E-value cutoff of 1E-5 (Altschul et al., 1990; Makarova et al., 2015). Glycoside hydrolases and glycosyltransferases were predicted with dbCAN3 using the HMMER: dbCAN, HMMER: dbCAN_sub and DIAMOND: CAZy tools (Zheng et al., 2023). We only considered annotations from the dbCAN3 database that were detected by at least two of the three dbCAN3 tools. The Dsr-dependent Dissimilatory Sulfur Metabolism Classification Tool (DiSCo) was also used to identify genes used in sulfur metabolism, including putative heterodisulfide reductase-like (*hdrA*) genes (Neukirchen and Sousa, 2021). Hydrogenases were first predicted using KEGG annotations as described previously (Reysenbach et al., 2020), followed by verification and additional classification with HydDB (Søndergaard et al., 2016). FeGenie (Garber et al., 2020) was used to screen predicted open reading frames for iron metabolism, with the parameter "–meta" for MAG data. However, no putative iron oxidation and/or reduction genes were identified. Additionally, open reading frames were compared to manganese oxidation genes (*mnxG,* Genbank accessions AAB06489.1, ABP68890.1, ABP68899.1 (Dick et al., 2008; van Waasbergen et al., 1996); *cotA,* AFL56752.1 (Su et al., 2013); *cumA*, AAD24211.1 (Brouwers et al., 1999); *mofA*, CAA81037.2 (Corstjens et al., 1997); *moxA*, CAJ19378.1 (Ridge et al., 2007) using a local BlastP search, but no genes were identified that passed the E-value threshold (1E-5).

Annotations were confirmed as needed using the CD-Search tool (Marchler-Bauer and Bryant, 2004) and/or BlastP searches against the NCBI nr database (Altschul et al., 1990; Sayers et al., 2022). Archaella, predicted major pili and associated genes were identified using arCOG annotations previously described (Makarova et al., 2016). Heme biosynthesis genes *ahbA* and *ahbB* were annotated using local BlastP searches (Altschul et al., 1990) against previously described genes from *Methanosarcina barkeri* Mbar_A1459 and Mbar_A1460 (Genbank accessions AAZ70416.1 and AAZ70417.1; Kühner et al., 2014), with an E-value cutoff of 1E-6. Although our annotations were unable to positively identify phosphofructokinase (*pfk*) genes, this gene is known to be present in *A. pernix* (Hansen and Schönheit, 2001) and has here been annotated as K26208. Annotations are presented in Table S6, with selected metabolic genes summarized in Tables S7 and S8.

DMSO reductase family gene tree

Putative dimethyl sulfoxide (DMSO) reductase family genes were identified via arCOG and KO annotations (K00123, K00370, K07306, K08352, K08356, K08357, K22516, arCOG01491, arCOG01492, arCOG01493, arCOG01495, arCOG01497 and arCOG02674). A reference dataset of DMSO reductase genes was then constructed using selected sequences from a previously published guide tree (Jay et al., 2014; Table S9) and additional genes from the arsenite oxidases *aioA* and *arxA* (Branco et al., 2009; Ellis et al., 2001; Lett et al., 2012; Muller et al., 2003; Santini and vanden Hoven, 2004; Warelow et al., 2017; Wells et al., 2020; Zargar et al., 2010), arsenate reductase *arrA* (Krafft and Macy, 1998; Malasarn et al., 2004; Wells et al., 2020), chlorate reductase *clrA* (Miralles-Robledillo et al., 2019; Thorell et al., 2003; Wolterink et al., 2003), periplasmic nitrate reductase *napA* (Siddiqui et al., 1993), respiratory nitrate reductase *narG* (Afshar et al., 2001; Liebensteiner et al., 2015), assimilatory nitrate reductases *narB/nasA* and *nasC* (Gangeswaran et al., 1993; Ogawa et al., 1995; Suzuki et al., 1993; Wells et al., 2020), perchlorate reductase *pcrA* (Bender et al., 2005), selenate reductase *srdA* (Kuroda et al., 2011), selenite reductase *srrA* (Wells et al., 2019, 2020) and tetrathionate reductase *ttrA* (Cozen et al., 2009; Hensel et al., 1999; Oltmann et al., 1974) families. Gene sequences ≥550 amino acids were aligned with MAFFT (Katoh and Standley, 2013) via Geneious (Kearse et al., 2012), and regions with ≥90% gaps were removed, generating a final alignment with 1550 amino acid positions. A maximum-likelihood phylogenetic tree was inferred in RAxML with the PROTGAMMALG model and 1000 rapid bootstraps (Stamatakis, 2014). Signal peptides were detected in reference genes using SignalP (Almagro Armenteros et al., 2019) with the appropriate organism model for each gene.

Results and discussion

Recovery of metagenome-assembled genomes

In order to further resolve the issues within the taxonomy of the *Desulfurococcales*, we screened medium to high quality MAGs from 42 previously reported deep-sea vent and submarine volcano metagenomes (Reysenbach et al., 2020; Zhou et al., 2022; Table S1). We selected 47 MAGs from 20 of these metagenomes to classify more fully, in addition to 24 MAGs from public databases (Fig. 1, Fig. S1, Table S2). These MAGs are focused on the *Aeropyrum, Thermodiscus* and *Caldisphaera* clades, as they represent a substantial phylogenetic diversity in the metagenomes, but they have very few genome representatives in public databases. MAGs in this study greatly increase both the genomic and geographical representation of the *Aeropyrum, Thermodiscus* and *Caldisphaera* clades, with representatives from ABE, Tui Malila and Mariner deep-sea hydrothermal vent fields along the Eastern Lau Spreading Center (ELSC)-Valu Fa Ridge in the southwestern Pacific Ocean, Lucky Strike and Rainbow vent fields on the Mid-Atlantic Ridge (MAR), and the undersea Brothers volcano along the Kermadec arc north of New Zealand (Table S1). Publicly available MAGs were also recovered from a deep-sea vent on the East Pacific Rise (EPR) and terrestrial geothermal springs in China, Taiwan, Russia, Malaysia and the USA.

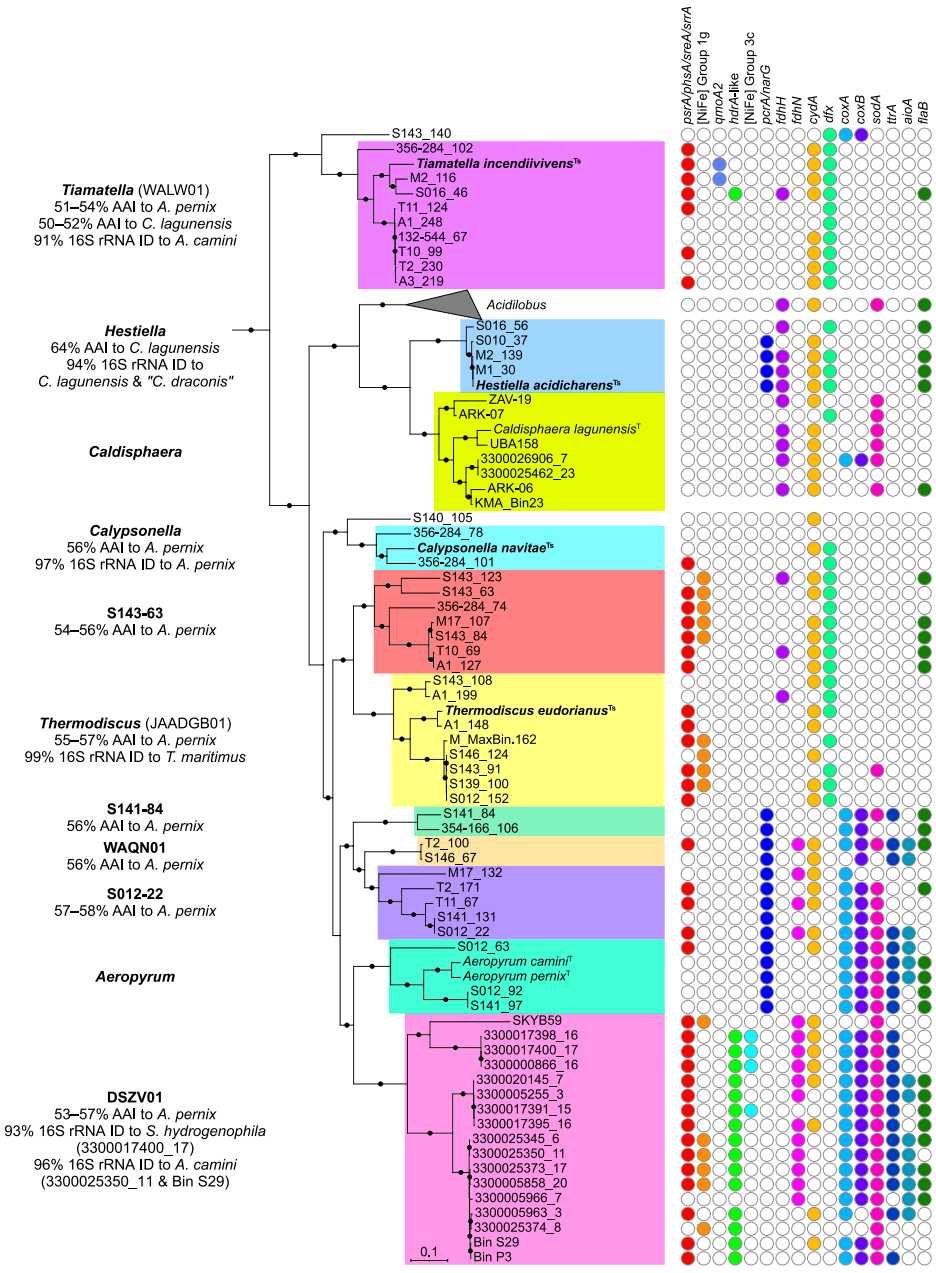

Fig. 1. Concatenated phylogenomic protein tree with proposed novel genera related to *Aeropyrum, Thermodiscus* and *Caldisphaera.* Type genomes proposed under SeqCode guidelines are in bold with the superscript "Ts". SH-like branch support (0.8–1.0) is indicated with filled black circles, and the scale bar represents 0.1 substitutions per amino acid. The presence of key functional genes is indicated with filled colorful circles, with *Acidilobus saccharovorans* representing the entire genus *Acidilobus*. Gene names are shown in Table S7, and the complete archaeal tree used to generate this figure is available in Fig. S1.

Metagenomic phylogeny of the Desulfurococcales and Acidilobales and identification of new genera

Phylogenetic reconstruction based on a concatenated alignment of 53 conserved archaeal proteins confirmed the polyphyletic distribution of the *Desulfurococcaceae* in the archaeal phylogenetic tree, namely in six separate clades (Fig. S2). Most notably the *Acidilobaceae* and *Caldisphaeraceae* (order *Acidilobales*; Prokofeva et al., 2009) were closely associated with *Aeropyrum* from the *Desulfurococcaceae* (order *Desulfurococcales*; Huber and Stetter, 2001), and they formed a highly supported monophyletic clade that included our large dataset of newly sequenced MAGs (Fig. 1, S1).

Within the *Aeropyrum-Caldisphaera-Acidilobus* cluster, we used a combination of phylogenomic analysis, 16S rRNA gene phylogeny, classification via the GTDB-Tk pipeline and average amino acid identity (AAI, Fig. 2) to identify distinct genera. Average nucleotide identity (ANI) was used to delineate individual species within the genera. While each tool has its own limitations, a synthesis of multiple approaches has proven effective for resolving issues between nomenclature and phylogenomics (Waite et al., 2020, 2017), and for defining new genera (Buessecker et al., 2022).

We identified nine potential genus-level clusters within the *Aeropyrum-Caldisphaera-Acidilobus* clade, each with at least two representative MAGs (Fig. 1, Table 1, Table S2). Several of these genera have already been identified using alphanumeric identifiers in the GTDB, but none have been formally proposed as novel genera. Within these nine putative genera, only five contained at least one MAG with a complete or near complete (>1400 bp) 16S rRNA gene, which is unsurprising given the difficulty assembling 16S rRNA genes from metagenomic data (Yuan et al., 2015). However, we were able to assemble several additional 16S

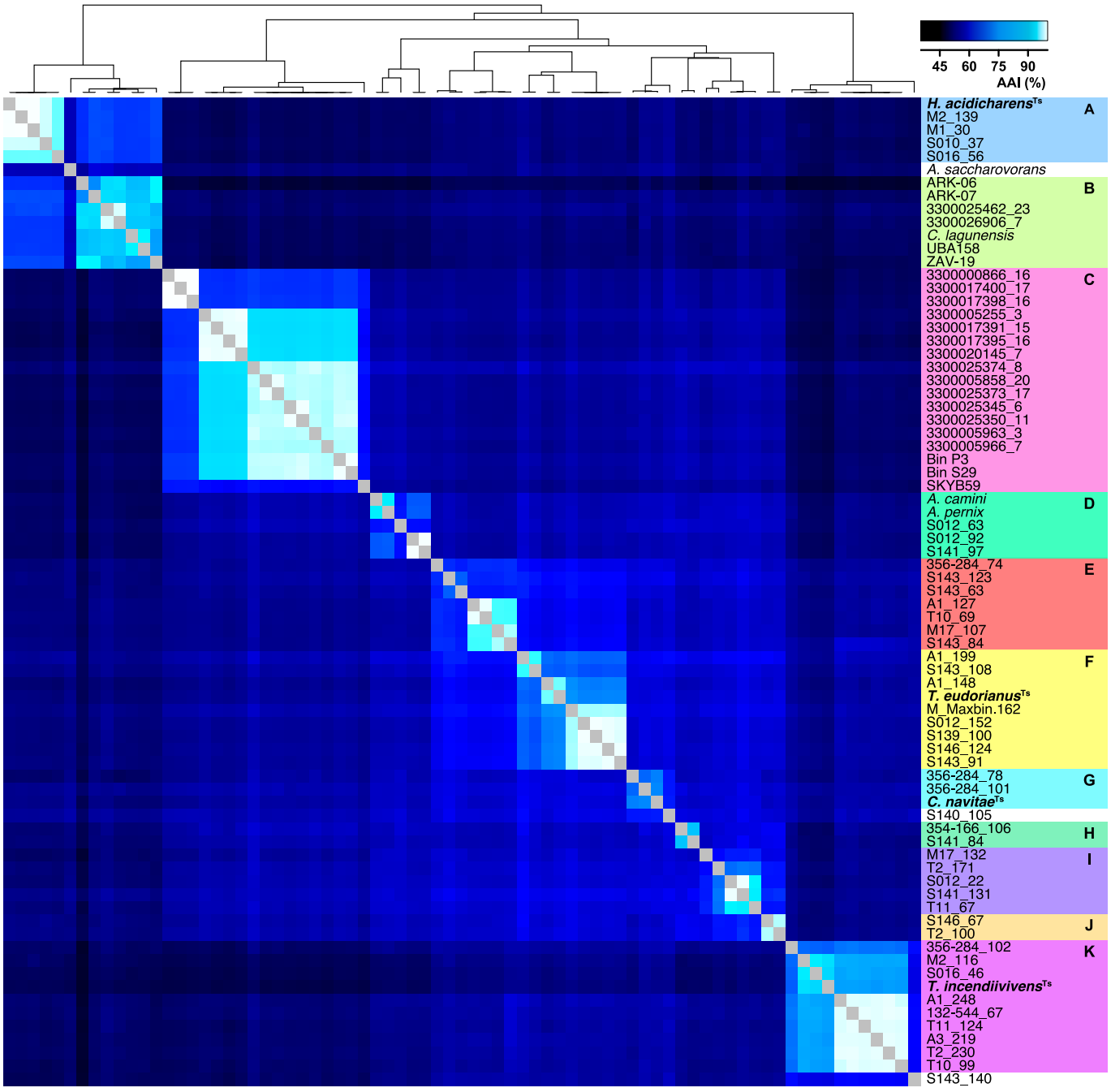

Fig. 2. Average amino acid identity (AAI) comparisons of MAGs from this study and previously published reference genomes. Shaded regions correspond to genera identified in Fig. 1. (A) *Hestiella*, (B) *Caldisphaera*, (C) DSZV01, (D) *Aeropyrum,* (E) S143-63, (F) *Thermodiscus,* (G) *Calypsonella*, (H) S141-84, (I) S012-22, (J) WAQN01, (K) *Tiamatella*.

rRNA genes from metagenomic reads using phyloFlash (≥1391 bp; Gruber-Vodicka et al., 2020). In some cases, these 16S rRNA genes were 100% similar to partial 16S rRNA genes (374 to 416 bp) found in MAGs assembled from the same metagenome, allowing us to tentatively place additional MAGs within the 16S rRNA gene phylogeny (Fig. S3). The topology of the small subunit rRNA gene tree was relatively consistent with that of the concatenated protein tree, and it demonstrated the close relationship between *Aeropyrum* and two cultivated isolates without sequenced genomes, *Stetteria hydrogenophila* (Jochimsen et al., 1997) and *Thermodiscus maritimus* (Stetter, 1986, 2001). However, bootstrap support was quite low for most clades related to the *Acidilobaceae,* which is further evidence that 16S rRNA gene analysis alone is not sufficient to determine the phylogenetic relationships within this group.

Genera delineation

Using the recent recommendations for naming organisms under the SeqCode (Hedlund et al., 2022), we have chosen to propose names only for new genera containing at least one MAG with a complete or near complete 16S rRNA gene, although all the putative genera identified are discussed below. All type MAGs for named genera also conform to additional SeqCode requirements (>90% completion, <5% contamination) and recommendations, including high genome integrity, >80% of tRNAs, read coverage ≥10x and agreement of taxonomy based on 16S

Table 1
Summary of nine putative genera described in this study. The number of species in each genus cluster was determined using ANI and MAG % completion was estimated with CheckM2. Shaded boxes indicate the presence of MAG(s) at a geographic location. BV, Brothers volcano; ELSC, Eastern Lau Spreading Center and Valu Fa Ridge; MAR, Mid-Atlantic Ridge; EPR, East Pacific Rise; Terr, terrestrial hot spring.

Genus	# MAGs	# Species	Maximum completion (%)	Average completion (%)	BV	ELSC	MAR	EPR	Terr
Tiamatella	10	5	97	70	■	■	■		
Hestiella	5	2	96	91	■	■			
Calypsonella	3	3	99	68		■	■		
Thermodiscus	9	6	95	77	■	■		■	
S143-63	7	5	100	94	■	■	■		
S141-84	2	2	83	65	■		■		
WAQN01	2	1	100	82	■	■			
S012-22	5	4	92	75	■	■			
DSZV01	17	4	96	86					■

rRNA genes and whole-genome analysis (Table S2).

A new genus (WALW01, herein *Tiamatella*) represented by 10 MAGs forms a clear monophyletic sister clade to the *Acidilobus*, *Caldisphaera* and *Aeropyrum* groups (Fig. 1), and AAI analysis supports its designation as a distinct genus (50–54% AAI similarity to *Caldisphaera lagunensis* and *Aeropyrum pernix;* Fig. 2, Table S3). A full-length 16S rRNA gene (Genbank accession OR964487.1) was identified in the type MAG S016_52_esom. Based on EZBioCloud analysis (Yoon et al., 2017) it is only 91% similar to its closest cultivated relatives *Aeropyrum camini* and *S. hydrogenophila* and members of the *Pyrodictiaceae* (Table S5). Megablast searches against the NCBI nr/nt database (Morgulis et al., 2008; Sayers et al., 2022) and EZBioCloud analysis also revealed distantly related PCR and clone sequences (~94–96% identity) from deep-sea vents along the EPR (Ehrhardt et al., 2007; Genbank accession DQ417487.1), Juan de Fuca Ridge (Wang et al., 2009; EU428003.1) and Southern Okinawa Trough (Nunoura and Takai, 2009; AB235336.1 & AB235331.1). The type genome represents a distinct species based on ANI (Table S4) and is related to two other potentially acidophilic species from low-pH vents at Mariner vent field on the Valu Fa Ridge in the Lau Basin (M2_116_esom), and the Lower Cone, Brothers volcano (S016_46_esom; Fig. 1). Six MAGs from Tui Malila and ABE, ELSC, also form a fourth distinct *Tiamatella* species. Although none of the MAGs in this fourth species have a full-length 16S rRNA gene, we were able to assign a partial 16S rRNA gene (374 bp) from the T11_124_esom MAG to a phyloFlash-reconstructed gene from the T11 metagenome (T11. PFspades_9). This reconstructed T11 gene clusters with the type species in the 16S rRNA gene tree (Fig. S3), and it shows strong similarity (~99%) to a clone sequence from the Southern Mariana Trough (Kato et al., 2010; AB293241.1). A single MAG from Lucky Strike, MAR (356-284_102_esom), also represents a fifth species in *Tiamatella,* demonstrating that members of this genus are widespread in deep-sea hydrothermal vents.

An additional monophyletic clade most closely related to *Caldisphaera* (herein *Hestiella;* Fig. 1) was obtained from acidic and lower temperature sites such as the Mariner microbial mats (pH 5.1, 59 °C; Mottl et al., 2011) and the highly mixed, mildly acidic Lower Cone (pH 5.14, 45 °C) and more acidic Upper Cone (pH 2.08, 200 °C) at Brothers volcano (Reysenbach et al., 2020); and they are probably thermoacidophiles, like their sister clade *Caldisphaera*. While GTDB taxonomic assignments placed this clade in *Caldisphaera* (sp015523575 and sp015522625), we propose that these genomes represent a distinct genus based on phylogenomic tree topology, AAI, 16S rRNA gene similarity, environment and functional gene data. *Hestiella* forms a monophyletic clade in the phylogenomic tree (Fig. 1), and AAI comparisons with *C. lagunensis,* the only cultivated member of *Caldisphaera* with a sequenced genome (64.05 to 64.29%; Fig. 2, Table S3), fall within the recommended range for new genera, albeit close to the cutoff (45–65%; Konstantinidis et al., 2017). The full-length 16S rRNA gene (Genbank accession OR964506.1) identified in *Hestiella* type MAG 131-447_51_esom is only 94.07–94.48% similar to the cultivated isolates *C. lagunensis* and *"Caldisphaera draconis"* (Table S5), consistent with suggested guidelines for a new genus (Konstantinidis et al., 2017). Additionally, the level of 16S rRNA gene divergence between *Hestiella* and *Caldisphaera* is much higher than the divergence between cultivated *Caldisphaera* taxa (~97%). Using functional gene analysis (see below), we also identified multiple key metabolic differences in *Hestiella* compared to *Caldisphaera.*

Hestiella appear to be unique to deep-sea hydrothermal vent environments, while members of *Caldisphaera* have only been identified in terrestrial geothermal springs. Nearly identical MAGs (131-447_51_esom, M2_139_esom and M1_30_esom; 99.6–99.8% ANI; Table S4) from *Hestiella* were recovered from Mariner, along the Valu Fa Ridge in the Lau Basin, in samples collected a decade apart (2005 and 2015), suggesting they are a stable, persisting member of the microbial community in this acidic system. The MAGs obtained from Mariner and the Upper Cone at Brothers volcano form a single species even though they are geographically distant, while one MAG from the Lower Cone at Brothers volcano represents a second unique species of *Hestiella.*

A small clade, representing another new genus (herein *Calypsonella*), was composed of three MAGs from Lucky Strike, MAR, and Mariner. Based on ANI analysis, each genome likely represents a distinct species (~73 to 75% ANI; Table S4). The monophyletic *Calypsonella* clade has strong support in the phylogenomic tree (Fig. 1), and AAI comparisons support its placement as a new genus (Fig. 2, Table S3). The GTDB-Tk pipeline was also unable to assign *Calypsonella* to any previously described genus (Table S2). The near complete 16S rRNA gene (Genbank accession OR964486.1) from the type genome M2_131_esom is ~96 to 97% similar to members of *Aeropyrum, Stetteria, Thermodiscus* and related *Pyrodictiaceae* (Table S5), similar to the 16S rRNA gene divergence between the previously described lineages *T. maritimus, A. pernix,* and *S. hydrogenophila* (~96 to 97%).

Fortuitously, 16S rRNA gene phylogenetic analysis assigned two MAGs (S143_76_esom and M_Maxbin.162) to the previously described isolate, *Thermodiscus maritimus* (~99.1% 16S rRNA gene sequence similarity; Table S5). However, to our knowledge, the genome of the disc-shaped anaerobic hyperthermophile (Stetter, 1986, 2001) has never been sequenced. Although several *Thermodiscus* MAGs described here are in GTDB, the current GTDB system of classification does not incorporate 16S rRNA phylogenies, resulting in members of this genus being assigned an alphanumeric identifier that is not associated with the legacy isolate, *T. maritimus*. Here, we positively identify the GTDB genus JAADGB01 as *Thermodiscus* and report nine genomes associated with the genus that represent approximately six species. Both (near) full-length *Thermodiscus* 16S rRNA genes described here (Genbank accessions

PP060619.1 and JAADGB000000000.1) show >99% similarity to the *T. maritimus* 16S rRNA gene and to each other. However, ANI analysis suggests the two sequenced MAGs belonged to distinct species (~72% ANI; Table S4). This is similar to the pattern seen in cultivated *Aeropyrum* spp., which have near-identical 16S rRNA gene sequences (99.86% similar) but divergent genomes (~81% ANI). Although there is no sequenced genome available for *T. maritimus* for comparison, the pattern of 16S rRNA gene and whole-genome divergence in the *Thermodiscus* MAGs suggests that they are likely distinct species, separate from *T. maritimus*. Additionally, the *Thermodiscus* MAGs described here were identified at deep-sea hydrothermal vents (ELSC and EPR) and from the submarine Brothers volcano, while the original *T. maritimus* isolate was cultivated from hot shallow marine sediment off the coast of Vulcano Island, Italy (Stetter, 1986). Given this distinction in geographic location and the pattern of genomic divergence, we propose the name *Thermodiscus eudorianus* to describe the type sequence S143_76_esom. In the absence of a *T. maritimus* genome sequence, the high-quality type MAG of *T. eudorianus* (95.13% completion, 0.11% contamination, 10 contigs) will provide a valuable resource for future comparative research in the *Thermoprotei*.

Further, a second genus closely related to *Thermodiscus,* S143-63, was identified. Although none of the MAGs contain a full-length 16S rRNA gene sequence, we were able to reconstruct a 16S rRNA gene from phyloFlash (356–284.PFspades_53; Genbank accession OP605402.2) that was associated with the 356-284_74_esom MAG (378 bp; Fig. S3). This reconstructed 16S rRNA gene shows ~99% similarity to a clone sequence from the EPR (Nercessian et al., 2003; AF526978.1). Members of S143-63 were distributed across the Tui Malila and ABE vent fields at ELSC, Mariner vent field on the Valu Fa Ridge in the Lau Basin, and Lucky Strike, MAR. Genomes from three distinct species of S143-63 were also found in a single metagenome from the NW Caldera Wall, at Brothers volcano.

Three additional deep-sea associated genera (S141-84, S012-22, WAQN01) and a large terrestrial clade (DSZV01) with high quality genomes that most likely represent at least two genera from terrestrial geothermal springs in China (Nayfach et al., 2021), Malaysia (Liew et al., 2022) and the USA (Fernandes-Martins et al., 2021) were also identified. Since the former do not contain complete 16S rRNA gene sequences, we do not describe these further here. Several of the metagenome reads from the terrestrial clade are also not publicly available, and thus naming this lineage using the SeqCode guidelines (Hedlund et al., 2022) is not possible at this time.

Intervening sequences in 16S rRNA genes

Alignments of partial and complete 16S rRNA genes recovered from the *Aeropyrum-Thermodiscus-Caldisphaera* cluster revealed the presence of several intervening sequences (IVS; Fig. S4A–B), ranging from small hairpins to complex predicted secondary structures (Fig. S4C–P). IVS have been widely reported in 16S rRNA genes from the *Thermoprotei,* including members of the *Thermoproteaceae* (e.g. *Caldivirga, Pyrobaculum, Vulcanisaeta, Thermoproteus*; Coleman et al., 2015; Jay and Inskeep, 2015), the *Desulfurococcaceae* (*Staphylothermus, Zestosphaera*; Jay and Inskeep, 2015; St. John et al., 2019a) and both *A. pernix* and *A. camini* (Nakagawa et al., 2004; Nomura et al., 1998, 2002). Some of these IVS encode the LAGLIDADG domain, indicative of a homing endonuclease likely used in propagating the IVS (Jay and Inskeep, 2015; Nomura et al., 1998). Further, *A. pernix* strains from coastal geothermal springs and shallow submarine vents may harbor multiple patterns of IVS dispersal (Nomura et al., 2002). While the type strain *A. pernix* K1 encodes a large 699 bp IVS with a LAGLIDADG domain, eight *A. pernix* isolates from Tachibana Bay all encode two smaller IVS (62 and 122 bp in length), and strains isolated from Obama Spa do not encode any IVS (Nomura et al., 2002). In our analysis, although no IVS were found in the available *T. maritimus* 16S rRNA gene, both *Thermodiscus* 16S rRNA genes described here contained a single large IVS (453–586 bp) with a putative LAGLIDADG domain, identified using the CD-Search tool (Marchler-Bauer and Bryant, 2004), and both had complex predicted secondary structures (Fig. S4F–G). The two IVS had little sequence similarity and were inserted in the 16S rRNA gene at two distinct locations. However, the insertion point for the *T. eudorianus* S143_76_esom IVS was conserved in multiple other 16S rRNA genes from this study, and it has also been documented for several other genera, including *Pyrobaculum*, *Thermoproteus*, *Staphylothermus* and *Zestosphaera* (Jay and Inskeep, 2015; St. John et al., 2019a). Other 16S rRNA genes with IVS at this conserved insertion point included partial 16S rRNA genes from *Tiamatella* (A3_219_esom; Fig. S4H) and WAQN01 (T2_100_esom; Fig. S4I), and multiple 16S rRNA genes from the terrestrial DSZV01 group (3300025350_11, 3300005963_3 and Bin S29; Fig. S4J–P). Intervening sequences in 16S rRNA genes can cause significant issues for 16S rRNA gene-based diversity studies by interrupting primer binding sites, or by increasing the length of the 16S rRNA gene beyond the size selected during PCR amplification (Jay and Inskeep, 2015; Salman et al., 2012). In the case of *Thermodiscus,* populations may show different patterns of IVS dispersal, leading to patchy detection worldwide. Given these difficulties, it is likely that the relative abundance of many *Acidilobaceae*, and therefore their importance in high-temperature ecosystems worldwide, has been chronically underestimated or undetected entirely in 16S rRNA gene diversity studies.

Metabolic potential and distinguishing features of different genera

Identifying the carbon and energy sources that support growth has long been key to characterizing novel microbial taxa and provides insights into the functional roles that organisms may play in natural communities. In the era of genomics, functional gene patterns also provide a valuable starting point for directed cultivation efforts. To more fully characterize the metabolic potential of new genera described here, we screened genomes for functional genes involved in both heterotrophy and autotrophy, aerobic and anaerobic respiration, and oxygen tolerance (Table 2, Tables S6–S8). Further, we used Tome (Li et al., 2019) to predict the optimal temperature for growth of our newly proposed genera, a crucial characteristic used to define new microbial genera and species.

Carbon utilization

As previously reported for cultivated *Caldisphaera* (Boyd et al., 2007; Itoh et al., 2003), *Thermodiscus* (Stetter, 2001), *Stetteria* (Jochimsen et al., 1997) and *Aeropyrum* (Nakagawa et al., 2004; Sako et al., 1996), all the novel genera described in this study are likely capable of heterotrophic protein degradation. Genes for proteases and/or peptidases, transaminases, and transporters for di/oligopeptides and branched-chain amino acids were widespread across all the genera, and each novel genus encodes 2-oxoacid:ferredoxin oxidoreductase genes (*oorA, oorB*) similar to the characterized enzymes from *A. pernix* that oxidize a wide range of substrates including glyoxylate, 2-oxobutyrate and pyruvate (Nishizawa et al., 2005). Additional ferredoxin oxidoreductases specific for pyruvate (*porA, porB*) and indolepyruvate (*iorA, iorB*; Ma et al., 1997) were also identified in most novel genera. With the exception of S141-84, each new genus includes at least one MAG with both an acetyl-CoA synthetase (*acs*) and a complete tricarboxylic acid (TCA) cycle, suggesting that acetyl-CoA generated by 2-oxoacid:ferredoxin oxidoreductases can be converted to acetate via fermentation (Mai and Adams, 1996) or fully oxidized to CO_2 via the TCA cycle, as previously described in *A. saccharovorans* (Mardanov et al., 2010). Aldehydes generated by 2-oxoacid:ferredoxin oxidoreductases may also be converted to alcohols using alcohol dehydrogenases (*adhE, adhP*; Ma et al., 1997). As in *Acidilobus* genomes (Dibrova et al., 2014; Jay et al., 2014; Mardanov et al., 2010) we also identified putative genes for fatty acid degradation in all genera, including the cultivated lineages *Aeropyrum, Caldisphaera* and *Acidilobus*.

Table 2
Comparison of novel taxa and cultivated type strains in *Caldisphaera, Thermodiscus, Stetteria* and *Aeropyrum*. Carbon and energy usage, relationship to oxygen and motility were predicted for novel genera using functional gene occurrence in type sequences. Data for isolates were retrieved from Boyd et al., 2007 ("*C. draconis*"); Itoh et al., 2003 (*C. lagunensis*); Jochimsen et al., 1997 (*S. hydrogenophila*), Liebensteiner et al., 2015 (*A. pernix* and *A. camini*); Nakagawa et al., 2004 (*A. camini*); Sako et al., 1996 (*A. pernix*). ND, no data.

Organism	*Tiamatella incendiivivens*	*Caldisphaera lagunensis*	*"Caldisphaera draconis"*	*Hestiella acidicharens*	*Calypsonella navitae*	*Thermodiscus maritimus*	*Thermodiscus eudorianus*	*Aeropyrum pernix*	*Aeropyrum camini*	*Stetteria hydrogenophila*
Habitat	Deep-sea hydrothermal sulfide deposit	Hot spring	Hot spring	Deep-sea hydrothermal vent biofilm	Deep-sea hydrothermal sulfide deposit	Hot marine sediment	Deep-sea hydrothermal sulfide deposit	Coastal solfatara	Deep-sea hydrothermal vent	Shallow marine hydrothermal sediment
Location	Lower Cone, Brothers volcano	Mt Maquiling, Philippines	Yellowstone National Park, USA	Mariner, Valu Fa Ridge	Mariner, Valu Fa Ridge	Vulcano Island, Italy	NW Caldera Wall, Brothers volcano	Kodakara-Jima Island, Japan	Suiyo Seamount	Paleohori Bay, Milos, Greece
Motility and/ or archaella	Non-motile	Non-motile	ND	Motile	Non-motile	Non-motile	Non-motile	Motile	Motile	Archaellum present
Optimal temperature (°C)	80*	70–75 (74*)	70–72	82*	91*	90	90*	90–95 (89*)	85 (86*)	95
Optimal pH	ND	3.5–4.0	2.5–3.0	ND	ND	~5.5	ND	7.0	8.0	6
Carbon sources	Protein-rich compounds	Starch, glycogen, gelatin, peptone, beef extract, yeast extract	Tryptone, peptone, casamino acids, yeast extract, glycogen, gelatin, beef extract, pine needle extract	Protein-rich compounds, starch, glycogen	Protein-rich compounds	Yeast extract	Protein-rich compounds	Yeast extract, tryptone, trypticase peptone, nutrient broth	Yeast extract, tryptone	Yeast extract, tryptone, peptone, casamino acids, cell-free extracts
Relationship to oxygen	Anaerobe	Anaerobe; aerotolerant up to 2% O_2	Strict anaerobe	Anaerobe	Anaerobe	Strict anaerobe	Anaerobe	Facultative aerobe	Facultative aerobe**	Strict anaerobe
Electron acceptor	Organic compound(s), S°, thiosulfate, polysulfides and/or selenite	Organic compound(s), S°	Organic compound(s), S°	Organic compound(s), perchlorate and/ or nitrate	Organic compound(s)	Organic compound(s), S°	Organic compound (s), S°, thiosulfate, polysulfides and/or selenite	O_2, perchlorate	O_2, perchlorate**	S°, thiosulfate
Electron donor	Organic compound(s)	Organic compound(s)	Organic compound(s)	Organic compound(s)	Organic compound(s)	H_2, Organic compound(s)	Organic compound (s)	Organic compound(s)	Organic compound(s)	H_2
Comment(s)								Stimulated by thiosulfate, but no H_2S produced	Inhibited by S°, weakly stimulated by thiosulfate	Stimulated by CO_2

* Optimal growth temperature predicted with Tome (Li et al., 2019).
** Based on genomic evidence (Liebensteiner et al., 2015).

All the genera described here contain at least a partial set of genes for glycolysis and gluconeogenesis via the Embden-Meyerhof-Parnas pathway (Bräsen et al., 2014). None of the novel genera encode the extensive suite of glycoside hydrolases found in *A. saccharovorans,* which degrades a broad range of carbohydrates and polysaccharides (Prokofeva et al., 2009). However, some MAGs from the *Hestiella* encode a group of glycoside hydrolases similar to those found in *C. lagunensis* (GH15, GH31, GH38 and GH57), which is capable of growth on starch and glycogen (Itoh et al., 2003). The genera S012-22 and WAQN01 have expanded suites of glycoside hydrolases compared to their relative *A. pernix,* which cannot grow on carbohydrates under laboratory conditions (Sako et al., 1996). Several members of the terrestrial DSZV01 lineage also contain glycoside hydrolase genes (GH13, GH57) with potential for alpha-amylase activity used for the hydrolysis of starch and glycogen (Janeček and Blesák, 2011; Pujadas and Palau, 2001).

In contrast, we recovered no evidence of autotrophic carbon fixation in any of the genera described here. We screened MAGs for key genes from the dicarboxylate/4-hydroxybutyrate (DC/HB) and 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) pathways found in other *Thermoproteota,* and for the reverse TCA, Wood-Ljungdahl and Calvin-Benson-Bassham (CBB) pathways (Berg 2011; Berg et al., 2007, 2010; Huber et al., 2008). As previously reported in *Acidilobus*-like genomes (Jay et al., 2014), we identified several 4-hydroxybutyryl-CoA dehydratase (*abfD*) genes in the MAGs but did not recover additional marker genes needed for a functional HP/HB or DC/HB pathway (Berg et al., 2010). While the TCA cycle was widespread in the MAGs, we did not recover ATP-citrate lyase genes that would suggest the cycle was operating in the autotrophic direction, and genes from the Wood-Ljungdahl pathway were almost entirely undetected. Two MAGs encode ribulose bisphosphate carboxylase (*rbcL*) genes, but we did not recover any additional evidence for a functional CBB pathway, further suggesting the genera described here are entirely reliant on heterotrophic carbon degradation.

Sulfur metabolism

Sulfur and thiosulfate metabolism have been widely reported in the *Thermoprotei* (e.g. Jochimsen et al., 1997; Prokofeva et al., 2000, 2009; Stetter, 2001; Zillig et al., 1982) and are known to be key functions in deep-sea hydrothermal vent communities (e.g. Dick, 2019; Reysenbach et al., 2020; Yamamoto and Takai, 2011). We were initially unable to identify sulfur or thiosulfate reductases in the MAGs using GhostKoala or the arCOG database (see Materials and Methods). However, phylogenetic analysis of DMSO reductase genes (Fig. S5, Table S9) revealed a large cluster of genes related to sulfur (*sreA*), polysulfide (*psrA*), thiosulfate (*phsA*) and selenite reductase (*srrA*) genes. Although branch support for the entire cluster is only moderate (61%), we identified two sub-clades with medium (71%) and high (92%) branch support (Fig. S5) that include previously described thiosulfate and sulfur reductase genes, respectively. Therefore, several *psrA/phsA/sreA/srrA* genes were tentatively assigned to the novel genera described here. Gene(s) from *Tiamatella, Calypsonella*, *Thermodiscus* and its sister genus S143-63 and DSZV01 were identified in the well-supported clade that includes a sulfur reductase from *Acidianus ambivalens* (Laska et al., 2003) and a sulfur reductase from *Chlorobaculum tepidum* that functions in the oxidative direction (Lyratzakis et al., 2023). Additional gene(s) from *Tiamatella*, *Thermodiscus*, S143-63, WAQN01, S012-22 and DSZV01 were also more closely related to a thiosulfate reductase from *Pyrobaculum aerophilum* (Haja et al., 2020). This supports a prior study showing that *T. maritimus* can grow via sulfur respiration (Stetter, 2001), which has not been investigated previously given the lack of genomic data. In some *Thermodiscus,* S143-63 and DSZV01 MAGs, we also identified hydrogenotrophic [NiFe] type 1g hydrogenases, which are unique to the *Thermoproteota* and are associated with sulfur reduction (Søndergaard et al., 2016), corroborating the report that hydrogen may stimulate growth via sulfur reduction in some *Thermodiscus* spp. (Stetter, 2001). Many genes from terrestrial DSZV01 MAGs were also identified in a third poorly supported clade (Fig. S5) that clusters with *phsA* and *psrA* genes from the known polysulfide and/or thiosulfate reducers, *Wolinella succinogenes* (Krafft et al., 1992), *Shewanella oneidensis* (Burns and DiChristina, 2009) and *Salmonella enterica* (Heinzinger et al., 1995), suggesting that metabolism of sulfur species is widespread across this terrestrial lineage.

Functional gene analysis also revealed a suite of heterodisulfide reductase-like genes in *Tiamatella* and terrestrial DSZV01. Genes related to heterodisulfide reductase are widespread across both *Bacteria* and *Archaea* and are associated with methanogens, sulfate reducers and sulfur oxidizers (Grein et al., 2013). Although our initial screening identified *hdrA*-like genes in four *Tiamatella* MAGs, we restricted our later analysis to the two *Tiamatella* MAGs with more extensive gene neighborhoods surrounding the *hdrA*-like genes, assisting in assessing gene cluster patterns. *Tiamatella* heterodisulfide reductase-like genes were arranged in pairs and were classified by the Dsr-dependent Dissimilatory Sulfur Metabolism Classification Tool (DiSCo; Neukirchen and Sousa, 2021) as reductive-type *qmoA2* and *qmoB2* genes, typically associated with sulfate reducing organisms and some sulfur oxidizing *Bacteria* (Grein et al., 2013). The gene clusters also included the *qmoC* subunit, a *tusA*-like sulfurtransferase gene, which may act as a trafficking system for persulfides (Appel et al., 2021; Dahl, 2015), and an iron-sulfur hydrogenase subunit (*mvhD*), all of which are commonly associated with heterodisulfide reductase-like systems (Appel et al., 2021). Although the *qmoABC* gene cluster is typically found in dissimilatory sulfate reducers and sulfur oxidizers, we were unable to identify the adenosine 5′-phosphosulfate reductase genes (*aprB, aprA*) used in both these processes (Appel et al., 2021). Screening with DiSCo also failed to recover any *hdrB* or *hdrC* genes, and hence the function of these *hdrA*-like genes in *Tiamatella* remains unresolved. However, given their involvement in sulfur metabolism in other taxa and the presence of *tusA*-like sulfurtransferase genes, it is likely the *qmoABC* system is involved in sulfur metabolism. Additional *hdrA*-like genes were also encoded in terrestrial DSZV01 MAGs, and were typically found in two distinct gene cluster patterns; one including a single nonspecific *hdrA* gene with a 2-oxoacid:ferredoxin oxidoreductase, and a second with a non-specific *hdrA,* a *qmoB2* and *qmoC* gene and a [NiFe] Group 3c hydrogenase complex often found in methanogens and sulfate reducers (Søndergaard et al., 2016). However, we were unable to identify any *apr, hdrB* or *hdrC* genes in the DSZV01 MAGs, and therefore the function of heterodisulfide reductase-like genes in these taxa remains ambiguous.

Nitrate and (per)chlorate metabolism

Within the DMSO reductase gene tree (Fig. S5), we also identified a clade of genes related to perchlorate (*pcrA*) and nitrate (*narG*) reductases from the *Bacteria* and *Archaea.* Although nitrate reduction has not been reported in the *Desulfurococcaceae* to our knowledge, it has been identified in the sister family *Pyrodictiaceae* (e.g. Lin et al., 2016). Conversely, *Aeropyrum* are known to facultatively respire perchlorate under anaerobic conditions, using a *nar*-like gene complex (Liebensteiner et al., 2015). Multiple genomes from *Hestiella* encoded *pcrA/narG* genes related to *Vulcanisaeta distributa,* which cannot reduce nitrate under laboratory conditions (Itoh et al., 2002), and to the known nitrate reducer *Bacillus subtilis* (Hoffmann et al., 1995). If functional, nitrate and/or perchlorate reduction would be a key physiological difference distinguishing *Hestiella* from terrestrial acidophilic relatives of *Caldisphaera*. A large clade of genes related to the perchlorate reductase from *A. pernix* (Liebensteiner et al., 2015) and the nitrate/chlorate reductase from *Pyrobaculum aerophilum* (Afshar et al., 2001) was recovered in all named *Aeropyrum* spp. and MAGs, and in S141-84, S012-22 and WAQN01 MAGs. Both MAGs from WAQN01 also contain a second divergent *pcrA/narG* gene, suggesting they may potentially respire different compounds with these enzyme complexes. Although we were unable to determine the substrate specificity of these *pcrA/narG* genes, they collectively point to widespread anaerobic metabolism across

Hestiella and several genera more closely related to *Aeropyrum*.

Formate metabolism

As previously reported for *Acidilobus*-like population genomes (Jay et al., 2014), we recovered several *fdhH* formate dehydrogenases from putatively acidophilic *Hestiella* MAGs and their acidophilic relatives *Acidilobus* and *Caldisphaera,* and from *Tiamatella, Thermodiscus* and S143-63 (Fig. S5). Formate dehydrogenases oxidize formate to carbon dioxide, releasing electrons to the quinone pool or to other enzymes involved in fermentation or the electron transport chain, thus contributing to energy conservation (Wang and Gunsalus, 2003). N-type formate dehydrogenase genes (*fdhN*) with signal peptides were also identified in S012-22, WAQN01 and DSZV01, which are all more closely related to *Aeropyrum*. In *Escherichia coli,* the N-type formate dehydrogenase is coupled with a nitrate reductase, with strongest expression under anaerobic conditions (Wang and Gunsalus, 2003). Because all WAQN01 and S012-22 MAGs with an *fdhN* gene also encode a perchlorate/nitrate reductase gene, they may be able to form a similar complex with the N-type formate dehydrogenase during anaerobic growth.

(Micro)aerobic respiration and oxygen tolerance

Within *Tiamatella, Hestiella, Calypsonella, Thermodiscus* and S143-63, we did not identify any cytochrome *c* oxidase genes (*coxA*, *coxB*, *coxAC*, arCOG15028) used for aerobic respiration, confirming that these organisms are likely anaerobic. Superoxide dismutase (*sodA*), used to mitigate toxicity of reactive oxygen species (Johnson and Hug, 2019), was also undetected in these genera with a single exception in a *Thermodiscus* MAG (S143_91_esom). Conversely, most genomes of *Tiamatella, Hestiella, Calypsonella, Thermodiscus* and S143-63 encoded superoxide reductase (*dfx*), a widely distributed enzyme often associated with anaerobes (Nivière and Fontecave, 2004). Cytochrome *bd* quinol oxidase (*cydA*) genes were also recovered from these genera, typically in the *cydAA'* isoform commonly found in *Thermoprotei* which may be exclusive to thermophiles (Murali et al., 2021). The cytochrome *bd* quinol oxidase complex can be found in both microaerophiles and strict anaerobes, and it may contribute to proton motive force, provide resistance to oxidative stress, or scavenge oxygen molecules to protect aerosensitive enzymes (Borisov et al., 2011; Das et al., 2005; Murali et al., 2021). Given the apparent absence of cytochrome *c* oxidase genes, it is likely that these five genera are indeed anaerobic and utilize the *cydAA'* complex to detoxify and scavenge low levels of oxygen.

In contrast, several taxa more closely aligned with *Aeropyrum,* including the deep-sea genera S141-84, WAQN01 and S012-22 and terrestrial DSZV01, encode cytochrome *c* oxidase subunits and superoxide dismutase genes in most MAGs, suggesting they engage in (micro) aerobic respiration. We also identified *sodA* genes across *Caldisphaera* genomes, consistent with the mild aerotolerance previously reported for *C. lagunensis* (Itoh et al., 2003). The *cydAA'* complex was found in WAQN01, S012-22, DSZV01 and one *Aeropyrum* sp. MAG, where it may contribute to various stress responses and provide metabolic flexibility by allowing microbes to continue aerobic respiration at varying levels of oxygen availability, as has been described for *E. coli* (Borisov et al., 2011). The majority of MAGs in S141-84, WAQN01, DSZV01 and *Aeropyrum* also encoded a putative tetrathionate reductase-like gene (*ttrA*; Fig. S5), which may function in reverse as a thiosulfate oxidase, as demonstrated previously in marine bacteria (Whited and Tuttle, 1983). If functional, thiosulfate oxidation could provide electrons for aerobic respiration (Liebensteiner et al., 2015), accounting for the stimulatory effect of thiosulfate in growing *Aeropyrum* (Nakagawa et al., 2004; Sako et al., 1996; Table 2) and the upregulation of *ttrA* in *A. pernix* during aerobic growth (Liebensteiner et al., 2015). However, further validation would be necessary to determine the substrate specificity of these enzymes, as they are also closely related to a putative *ttrA*-like arsenate reductase from *Pyrobaculum aerophilum* (Cozen et al., 2009). Additionally, several WAQN01, DSZV01 and *Aeropyrum* MAGs and one S012-22 MAG also encode arsenite oxidase (*aioA*) genes, often found in aerobes using As(III) as an electron donor (van Lis et al., 2013). Deep-sea hydrothermal vent fluids, including those at ELSC, are known to contain detectable levels of arsenic (Breuer and Pichler, 2013), making this a potentially viable energy source for these microaerophiles.

Selected biosynthetic pathways

Many of the *Thermoprotei* are thought to be auxotrophic, requiring exogenous purines, amino acids, vitamins and/or cofactors for growth (e.g. Brown et al., 2011; Jay et al., 2014; Kim and Lee, 2003; Lebedinsky et al., 2014; St. John et al., 2019a). We screened the MAGs in this study for the presence or absence of different biosynthetic pathways, specifically seeking to identify biosynthetic capabilities or auxotrophies that would help distinguish new genera from their characterized relatives. Unlike most genera in this study, *Tiamatella* and S141-84 appear to lack an uridine monophosphate (UMP) biosynthesis pathway, suggesting they may require exogenous pyrimidines for growth. Additionally, we recovered a gene cluster in three *Hestiella* MAGs that encoded a near-complete pathway for inosine monophosphate (IMP) biosynthesis, which was undetectable in nearly all other MAGs in this study. If active, this pathway would allow *Hestiella* to grow without exogenous purines, further distinguishing *Hestiella* from its sister genus *Caldisphaera.* Although we were only able to identify a subset of the IMP biosynthesis genes in the *Hestiella* type genome (131-447_51_esom), the genes were found at the end of a contig, and the remaining IMP biosynthesis genes are likely encoded in the unsequenced genome fragments. Additionally, MAGs from *Hestiella* and several other genera encoded the two-gene pathway for *de novo* pyridoxal phosphate (PLP) biosynthesis (*pdxS, pdxT*; Wu et al., 2022), which was not detected in *Tiamatella, Caldisphaera, Thermodiscus,* S143-63 or WAQN01. Multiple *Hestiella* genomes and the *Calypsonella* type MAG also encoded genes for *de novo* NAD+ biosynthesis (*nadA, nadB, nadC;* Rodionov et al., 2008), in addition to the set of niacin salvage genes found in all other genera described here (Jay et al., 2014; Rodionov et al., 2008).

Consistent with a previous study of *A. pernix* (Kim and Lee, 2003), auxotrophies for amino acids appeared widespread, and in many cases, pathways for amino acid biosynthesis were incomplete in all MAGs, rendering a comparison of amino acid biosynthesis across the genera unfeasible. However, we identified clear patterns in the distribution of the Shikimate pathway, used to generate precursors for aromatic amino acid biosynthesis (Daugherty et al., 2001). While most novel genera included at least one MAG with a complete Shikimate pathway, genes for the pathway were either absent or nearly undetected in *Tiamatella, Calypsonella,* S143-63 and DSZV01.

Archaella and pili

Historically, motility has been a key distinguishing characteristic when delineating novel genera and species. In *Archaea*, the ATP-consuming motility is powered by archaella (archaeal flagella), which are typically encoded in small gene clusters comprised of archaellins and accessory proteins, including the characteristic *arlX* gene in the *Thermoproteota* (Jarrell et al., 2021). The archaellum is closely related to type IV pili, which are involved in adhesion, biofilm formation and regulation of swimming motility in archaeal model organisms (Pohlschroder and Esquivel, 2015). Using arCOGs identified by Makarova et al. (2016), we screened for the presence of archaellum-specific genes (*arlBXFGHJ*), secretion ATPases (*arlI*) and signal peptidases (*arlK/pulO*) used for assembly of archaella and/or type IV pili, and predicted major pilins (arCOG03871, arCOG03872) and pilin assembly genes (*tadC*). Major pilin and archaellum/pilin assembly genes were identified in nearly all MAGs, while coding potential for archaella was more patchily distributed. One *Tiamatella* MAG (S016_46_esom) encoded a complete suite of

archaella genes and is likely motile, lending additional support to its delineation as a distinct species based on ANI (Table S4), while archaellum genes were almost completely absent in all other *Tiamatella* MAGs. Nearly all *Hestiella* genomes encoded a cluster of archaellum genes and are likely capable of motility, in contrast to their non-motile relative *C. lagunensis* (Itoh et al., 2003). Although the *Thermoproteota* type *arlX* gene was not readily apparent in *Hestiella* MAGs, we identified an *arlX* candidate in the archaella gene clusters that was highly conserved across the MAGs and showed moderate similarity (~33% by BlastP; Altschul et al., 1990) to the *alrX* gene from *A. pernix*. Like *T. maritimus* (Stetter, 2001), both *Calypsonella* and *Thermodiscus* species reported here appear to be non-motile, a feature that may help distinguish *Thermodiscus* from the closely related genus S143-63. The genera most closely related to *Aeropyrum* each contained at least one MAG with a complete (S141-84, WAQN01) or partial (S012-22, DSZV01) suite of archaellum genes.

Conclusion

Despite their prevalence in high-temperature environments, the *Thermoproteota* historically have been under-represented in genome databases and plagued by inconsistencies in their taxonomic treatment. Here, we have leveraged MAG data from deep-sea hydrothermal vents worldwide to address these incongruencies in the clades related to *Aeropyrum, Thermodiscus* and *Caldisphaera.* Using a synthesis of 16S rRNA gene and concatenated protein phylogeny, GTDB assessment, AAI, and functional gene patterns, we have identified multiple highly supported genus-level clades within this portion of the *Thermoproteota* tree, and we link genomic data to the legacy isolate, *Thermodiscus maritimus*. Here, we propose that three of these novel genera be recognized and named under the SeqCode: viz. *Tiamatella, Hestiella* and *Calypsonella.* Given the historically close relationship between the *Aeropyrum-Stetteria-Thermodiscus* clade and the *Caldisphaera* and *Acidilobus* group, we also propose that the *Acidilobaceae* be amended to include these genera and the new genera described in this study.

Description of *Tiamatella* gen. nov.

Tiamatella gen. nov. (Ti.a.ma.tel'la. N.L. dim. fem. n. *Tiamatella*, little Tiamat, referring to an ancient Mesopotamian primordial sea goddess).

Delineation of this genus is supported by AAI, phylogenomic analysis, 16S rRNA gene comparison and taxonomic assignment in GTDB. MAGs assigned to this genus have been identified at two hydrothermal vent fields along the Eastern Lau Spreading Center (ABE and Tui Malila), Mariner vent field on the Valu Fa Ridge, Lau Basin, the Lower Cone of the deep-sea Brothers volcano along the Kermadec arc and Lucky Strike vent field along the Mid-Atlantic Ridge. Members of this taxon are approximately 51–54% similar to *Aeropyrum pernix* and 50–52% similar to *Caldisphaera lagunensis* by AAI. They form a well-supported monophyletic clade in a concatenated phylogenomic tree constructed using 53 archaeal genes. A 16S rRNA gene recovered from the type genome shows ~89 to 91% similarity to *Aeropyrum pernix*, *Aeropyrum camini*, *Thermodiscus maritimus* and *Stetteria hydrogenophila*. Based on functional genomic analysis, members of this genus are likely anaerobic and may utilize proteins or amino acids as carbon sources. They are auxotrophic for purines and pyrimidines. Several members of this genus also encode DMSO reductase family genes from the *psrA/phsA/sreA/srrA* clade and may utilize polysulfides, thiosulfate, sulfur, and/or selenite as energy sources. ANI analysis suggests this genus includes at least five distinct species, and motility is likely variable between species. The name proposed here is derived from a primordial Mesopotamian sea goddess depicted as both a chaotic and creative force, and it references the turbulent but biologically rich environments in which these *Archaea* inhabit. The SeqCode identifier for this genus is https://seqco.de/i:32112.

Description of *Tiamatella incendiivivens* sp. nov.

Tiamatella incendiivivens sp. nov. (in.cen.di.i.vi'vens. L. neut. n. *incendium*, fire; L. pres. part. *vivens* living; N.L. fem. adj. *incendiivivens*, living in or near the fire, referring to the active volcanic environment of Lower Cone, Brothers volcano).

The MAG representing this species was obtained from a sample from the Lower Cone of the deep-sea Brothers volcano along the Kermadec arc. The genome consists of 27 contigs, totaling 1,454,292 bp in length, and has a GC content of 41.6%. Based on CheckM2, the MAG is approximately 95.79% complete with 1.74% contamination. It encodes a complete 16S rRNA gene and tRNA genes for 19 standard amino acids. Phylogenomic analysis places this genome within *Tiamatella.* Based on functional genomic analysis, this organism is likely a non-motile anaerobe that utilizes protein-rich carbon sources and may derive energy from reduction of sulfur, polysulfides, thiosulfate or selenite. It is predicted to be a hyperthermophile, growing best at approximately 80 °C.

The type genome for this species is S016_52_esom^{Ts} (available under Genbank WGS accession number WALW00000000.1 and BioSample accession number SAMN12837229), and the 16S rRNA gene is available under Genbank accession number OR964487.1. Metagenome short reads are available under Sequence Read Archive accession SRR10312493. The SeqCode identifier for this species is https://seqco.de/i:32611.

Description of *Hestiella* gen. nov.

Hestiella gen. nov. (He.sti.el'la. N.L. dim. fem. n. *Hestiella*, little Hestia, named for the Greek goddess of the hearth and home).

Identification of this genus is supported by phylogenomic analysis, 16S rRNA comparison, AAI, environmental distribution and functional genomic differences. MAGs belonging to this genus were identified from multiple samples, over several years, from the Mariner deep-sea vent field on the Valu Fa Ridge in the Lau Basin, and from Upper and Lower Cone at the deep-sea Brothers volcano along the Kermadec arc. Based on ANI analysis, the genus includes two distinct species (92% ANI between spp.). In a concatenated gene tree using 53 archaeal marker genes, this genus forms a highly supported monophyletic clade most closely related to terrestrial *Caldisphaera.* AAI between members of this genus and *Caldisphaera lagunensis* is approximately 64.05 to 64.29%, and a full-length 16S rRNA gene recovered from the type species is 94.07% similar to *C. lagunensis*. Functional genomic analysis suggests this genus are motile anaerobic heterotrophs that degrade protein-rich carbon sources and potentially starch and/or glycogen. It may be distinguished from *Caldisphaera* by coding potential for a perchlorate/nitrate reductase gene likely used in anaerobic respiration, NAD(P)+ and pyridoxal 5-phosphate biosynthesis and, in some cases, inosine monophosphate biosynthesis. The apparent absence of superoxide dismutase genes also suggests members of this genus may be strictly anaerobic unlike their mildly aero-tolerant relative *C. lagunensis*. The name proposed for this genus references the lifestyle of these *Archaea* which thrive in the 'home-like' oasis of warmth and nourishment provided by deep-sea vents. The SeqCode identifier for this genus is https://seqco.de/i:32607.

Description of *Hestiella acidicharens* sp. nov.

Hestiella acidicharens sp. nov. (*a.ci.di.char'ens.* L. neut. adj. *acidum*, acid; N.L. part. adj. *charens* delighting in, from Gr. v. *chairo* to rejoice or delight in; N.L. part. adj. *acidicharens,* delighting in acid).

Genomes of members of this species were recovered from an acidic hydrothermal vent at Mariner on the Valu Fa Ridge in the Lau Basin and the Upper Cone of the deep-sea Brothers volcano along the Kermadec arc. MAGs range in size from approximately 1.06 to 1.48 Mbp and are in 77 to 157 contigs, with a G + C content of 39.9 to 40.2%. Based on CheckM2 estimates, MAG completeness ranges from 76.15 to 96.42%,

while contamination is 0.03 to 0.39%. ANI between members of this species is >97%, and phylogenomic analysis with 53 archaeal marker genes places this species in *Hestiella*. Based on functional gene analysis, members of this species are likely motile anaerobic nitrate and/or perchlorate reducers that degrade protein-rich carbon sources, starch and/or glycogen, growing best at approximately 82 to 83 °C.

The MAG 131-447_51_esom^{Ts} is the nomenclatural type for the species (available under Genbank WGS accession number JAALXO000000000.1 and BioSample accession number SAMN14146171), and the 16S rRNA gene is available under Genbank accession number OR964506.1. The type MAG was identified from a microbial mat sample from the Mariner vent field. Short reads used to assemble the metagenome are available under Sequence Read Archive accession number SRR7968105, and the SeqCode identifier for this genus is https://seqco.de/i:32609.

Description of *Calypsonella* gen. nov.

Calypsonella gen. nov. (Ca.lyp.so.nel'la. N.L. dim. fem. n. *Calypsonella*, little Calypso, referring to the Nereid water spirit in Greek mythology).

Members of this taxon were identified at Mariner on the Valu Fa Ridge in the Lau Basin and Lucky Strike, Mid-Atlantic Ridge. A phylogenomic reconstruction using 53 archaeal marker genes places MAGs of this genus in a well-supported monophyletic clade. Using AAI, MAGs are approximately 56% similar to *Aeropyrum pernix*, and they could not be related to any previously described genus using GTDB-Tk taxonomic analysis. The 16S rRNA gene recovered from the type genome is approximately 96 to 97% similar to the 16S rRNA genes of *Aeropyrum pernix, Aeropyrum camini, Thermodiscus maritimus* and *Stetteria hydrogenophila,* consistent with the level of 16S rRNA gene sequence divergence seen between the genera *Aeropyrum, Stetteria* and *Thermodiscus* (~96–97%). Based on ANI analysis, each of the three MAGs in this genus represents a distinct species (~73–75% similarity). Functional gene analysis suggests that members of this genus are likely non-motile anaerobes, and they may utilize protein-rich carbon sources and at least one member may reduce sulfur, thiosulfate, polysulfides or selenite. The SeqCode identifier for this genus is https://seqco.de/i:33278.

Description of *Calypsonella navitae* sp. nov.

Calypsonella navitae sp. nov. (na'vi.tae. L. gen. n. *navitae*, of a mariner, sailor; referring both to the marine habitat and to the Mariner deep sea vent field along the Valu Fa Ridge in the Lau Basin).

The MAG belonging to this species was assembled and binned from a hydrothermal metagenome from the 'Toilet Bowl' at Mariner deep sea vent field along the Valu Fa Ridge in the Lau Basin. The genome consists of 65 contigs totaling 1,604,348 bp in length, with a GC content of 62.4%. It encodes a near-complete 16S rRNA gene (1403 bp), a partial 23S rRNA gene, and tRNA genes for all 20 standard amino acids. Analysis with CheckM2 suggests this MAG is 98.84% complete with 0.61% contamination. Phylogenomic reconstruction places this species within *Calypsonella*. Based on functional gene analysis, this lineage is likely a non-motile anaerobe that degrades protein-rich carbon sources, and it likely grows best at ~91 °C.

The nomenclatural type for this species is M2_131_esom^{Ts} (available under Genbank WGS accession number WWDU00000000.1 and BioSample accession number SAMN13706328), and the 16S rRNA gene is available under Genbank accession number OR964486.1. The SeqCode identifier for this species is https://seqco.de/i:32612, and short reads used to assemble the metagenome are available under Sequence Read Archive accession number SRR13853571.

Description of *Thermodiscus eudorianus* sp. nov.

Thermodiscus eudorianus sp. nov. (eu.dor.i.a'nus N.L. masc. adj. *eudorianus,* pertaining to Eudora, the Nereid water spirit in Greek mythology).

The MAG representing this species was obtained from the Northwest Caldera Wall at the deep-sea Brothers volcano along the Kermadec arc. The MAG is composed of 10 contigs, totaling 1,522,352 bp in length, with a GC content of 54.2%. Based on CheckM2 analysis, the genome is about 95.13% complete and 0.11% contaminated, and it includes a complete 16S rRNA gene, a partial 23S rRNA gene and tRNA genes for all 20 standard amino acids. Inclusion of this MAG in *Thermodiscus* is supported by 16S rRNA gene sequence identity, and its distinction as a unique species is based on geographical location at a deep-sea volcano, and ANI-based divergence between *Thermodiscus* MAGs. Based on functional genomic analysis, this species is likely a non-motile, piliated anaerobic heterotroph that degrades protein-rich carbon sources and may reduce sulfur, thiosulfate, polysulfides or selenite. It is predicted to grow best at approximately 90 °C. The name proposed for this species refers to an ocean-associated Nereid spirit who is the sister of Calypso, referencing the phylogenetic relationship between the *Calypsonella* and *Thermodiscus.*

The MAG S143_76_esom^{Ts} is the nomenclatural type for the species (available under Genbank WGS accession number WAOW00000000.1 and BioSample accession number SAMN12837307), and the 16S rRNA gene is available under the Genbank accession number PP060619.1. Short reads used to assemble the metagenome are available under Sequence Read Archive accession number SRR10312501 and the SeqCode identifier for this genus is https://seqco.de/i:33329.

Emended description of the family *Acidilobaceae* (Prokofeva et al., 2009)

Members of the *Acidilobaceae* are associated with marine and terrestrial geothermal environments. Cultivated taxa are thermophiles or hyperthermophiles, ranging from acidophilic to neutrophilic, and they display disc or coccoid morphology. Based on cultivation data and genomic analysis, genera in this family typically utilize carbohydrates and/or protein-rich carbon sources for growth. Growth may be stimulated by sulfur and/or thiosulfate, although in some cases, it may either inhibit or be required for growth. The members of this family form a distinct clade in 16S rRNA gene and concatenated protein phylogenetic trees, and they can be distinguished from other families on the basis of AAI, phylogenomic and phylogenetic analysis, and 16S rRNA gene sequence similarity.

The emended family includes the type genus *Acidilobus* (Prokofeva et al., 2000) and the genera *Caldisphaera* (Itoh et al., 2003), *Aeropyrum* (Sako et al., 1996), *Thermodiscus* (Stetter, 2001), *Stetteria* (Jochimsen et al., 1997), *Hestiella, Tiamatella* and *Calypsonella* (this study).

CRediT authorship contribution statement

Emily St. John: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Anna-Louise Reysenbach:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Genome sequences and metagenome reads described in this study are publicly available in Genbank (Table S2) and the Sequence Read Archive (Table S1).

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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.2024.126507.

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