An Ancient Respiratory System in the Widespread Sedimentary Archaea Thermoprofundales

Xinxu Zhang,1,2 Yuhan Huang,1,2 Yang Liu,1,2 Wei Xu,3 Jie Pan,1,2 Xiaowei Zheng,4 Huan Du,1,2 Cuijing Zhang,1,2 Zhongyi Lu,1,2 Dayu Zou,1,2 Zongbao Liu,1,2 Mingwei Cai,5 Jinbo Xiong,6 Yaxin Zhu,4 Zhiyang Dong,4 Hongchen Jiang,7 Hai liang Dong,8 Juquan Jiang,9 Zhuhua Luo,3 Li Huang,4 and Meng Li*1,2

1Archaeal Biology Center, Institute for Advanced Study, Shenzhen University, Shenzhen, Guangdong, China
2Shenzhen Key Laboratory of Marine Microbiome Engineering, Institute for Advanced Study, Shenzhen University, Shenzhen, Guangdong, China
3Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen, Fujian, China
4State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
5Institute of Chemical Biology, Shenzhen Bay Laboratory, Shenzhen, Guangdong, China
6State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Ningbo University, Ningbo, Zhejiang, China
7State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, Hubei, China
8State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Beijing, China
9Department of Microbiology and Biotechnology, College of Life Sciences, Northeast Agricultural University, Harbin, Heilongjiang, China

*Corresponding author: E-mail: limeng848@szu.edu.cn.
Associate editor: Fabia Ursula Battistuzzi

Abstract

Thermoprofundales, formerly Marine Benthic Group D (MBG-D), is a ubiquitous archaeal lineage found in sedimentary environments worldwide. However, its taxonomic classification, metabolic pathways, and evolutionary history are largely unexplored because of its uncultivability and limited number of sequenced genomes. In this study, phylogenomic analysis and average amino acid identity values of a collection of 146 Thermoprofundales genomes revealed five Thermoprofundales subgroups (A–E) with distinct habitat preferences. Most of the microorganisms from Subgroups B and D were thermophiles inhabiting hydrothermal vents and hot spring sediments, whereas those from Subgroup E were adapted to surface environments where sunlight is available. H₂ production may be featured in Thermoprofundales as evidenced by a gene cluster encoding the ancient membrane-bound hydrogenase (MBH) complex. Interestingly, a unique structure separating the MBH gene cluster into two modular units was observed exclusively in the genomes of Subgroup E, which included a peripheral arm encoding the [NiFe] hydrogenase domain and a membrane arm encoding the Na⁺/H⁺ antiporter domain. These two modular structures were confirmed to function independently by detecting the H₂-evolving activity in vitro and salt tolerance to 0.2 M NaCl in vivo, respectively. The peripheral arm of Subgroup E resembles the proposed common ancestral respiratory complex of modern respiratory systems, which plays a key role in the early evolution of life. In addition, molecular dating analysis revealed that Thermoprofundales is an early emerging archaeal lineage among the extant MBH-containing microorganisms, indicating new insights into the evolution of this ubiquitous archaea lineage.

Key words: archaea, evolution, horizontal gene transfer, hydrogenase, marine benthic group d, metagenomics.

Introduction

Archaeal cells are abundant and widespread in diverse habitats on Earth (∼1.1×10²⁹ cells), where they are estimated to constitute 37% and 87% of all prokaryotes in the global ocean and deep subsurface marine sediments, respectively (Lipp et al. 2008; Hoshino and Inagaki 2019). However, our understanding of the mechanisms underlying archaeal life is still limited when compared with that of other microorganisms (Baker et al. 2020). With the rapid expansion of high-quality archaeal genomes from pure isolates, metagenome-assembled genomes (MAGs) and single-cell amplified genomes (SAGs), the archaeal tree of life has expanded dramatically to at least 30 archaeal phyla (Tahon et al. 2021).

Thermoprofundales is an archaeal order within the class Thermoplas mata, which is formerly known as MBG-D (Marine Benthic Group D) (Vetriani et al. 1999),
An Abundant Archaeal Lineage in Sedimentary Environments Worldwide

A total of 146 Thermoprofundales genomes (>50% completeness and <5% contamination) were retrieved from metagenomes and single cells sequenced in this study (55 MAGs), and downloaded from the NCBI Assembly database (53 MAGs and 2 SAGs) (Coordinators 2018) and the genomic catalog of Earth’s microbiomes (36 MAGs) (Nayfach et al. 2020). A total of 63 genomes are more than 80% complete (supplementary table S1, Supplementary Material online). The phylogenomic tree based on 55 conserved single-copy ubiquitous archaeal genes (Dombrowski et al. 2020) showed that Thermoprofundales forms a monophyletic lineage in the Candidatus phylum Thermoplasmatota and its ancestral node is adjacent to the orders Methanomassiliicoccales, Thermoplasmatales, Acidiprostanales, Poseidonionales, and MGIII (fig. 1) (Rinke et al. 2021). The average amino acid identity (AAI) value between each genome in Thermoprofundales and that from its adjacent orders was <50% (supplementary fig. S1 and table S2, Supplementary Material online), confirming that Thermoprofundales is an order-level lineage (Zhou et al. 2018) according to the proposed standards for high-quality taxa descriptions of uncultivated microorganisms (Konstantinidis et al. 2017; Rinke et al. 2021).

Global distribution analysis as revealed by the 16S rRNA genes and MAGs both showed that Thermoprofundales is distributed in various sedimentary environments with wide temperature and salinity ranges (supplementary fig. S2, Supplementary Material online), presumably contributing to global carbon biogeochemical cycles as reported previously (Lloyd et al. 2013; Zhou et al. 2018). Based on the 16S rRNA gene, the relative abundances of Thermoprofundales were 0.1–6.3% in freshwater sediment, 0.3–8.6% in saline lake sediment, 0.1–17.1% in intertidal sediment, and 0.1–4.3% in marine sediment, which was consistent with those based on the MAGs abundance (supplementary table S3, Supplementary Material online). The highest relative abundances of Thermoprofundales as revealed by 16S rRNA genes and MAGs were found in sedimentary environments, such as an intertidal sediment from the southern coast of the United States (17.1%) and a hydrothermal sediment from the Guaymas Basin in the Gulf of California (24.5%), respectively. When compared with the upper water column which is dominated by free-living microorganisms, sedimentary habitats generally have low dissolved oxygen levels and high organic matter content, where abundant anaerobic heterotrophs adopt nonmotile or particle-attached lifestyles (Orcutt et al. 2011; Zhou et al. 2018).

Results and Discussion

Five Subgroups with Distinct Habitat Preferences and Metabolic Features

The Thermoprofundales MAGs were classified into five subgroups according to their phylogenetic positions (fig. 1)
and AAI values (supplementary fig. S1, Supplementary Material online), whose average values between each pair of genomes in the same subgroup were higher than 63.0% (supplementary table S2, Supplementary Material online). Genomes from Subgroups B, D, and E showed distinct habitat preferences (fig. 1). For example, 76.0% and 100% of the genomes from Subgroup B and Subgroup D were identified in sediments from deep-sea hydrothermal vents, which are characterized by extremely high temperatures for microorganisms (e.g., >60 °C) (Dombrowski et al. 2018). This is further supported by the identification of a reverse gyrase gene (the hallmark gene of some hyperthermophiles and/or moderate thermophiles (Campbell et al. 2009; Lipscomb et al. 2017; Feng et al. 2019)) exclusively in genomes of the Subgroups B and D (supplementary fig. S3 and table S4, Supplementary Material online). Therefore, these two subgroups seem to comprise thermophiles, and the result is consistent with a recent report in which Subgroups B and D were assigned as JdFR-43 and HyVt, respectively (Liu et al. 2022). However, the preferred habitats for Subgroups A and C were not revealed because of the underrepresentation of genomes in these two subgroups (two for Subgroup A and one for Subgroup C) (fig. 1).

A survey combining the 16S rRNA gene sequences identified in the MAGs of this study with those from a previous report (Zhou et al. 2018) showed that the sequences of Subgroup E clustered in a monophyletic lineage, which included clades from 1 to 11 as classified previously (Zhou et al. 2018) (supplementary fig. S4, Supplementary Material online). The lineage of Subgroup E had the highest diversity among the five subgroups based on the MAGs. A high percentage (84.2%) of the MAGs were obtained from terrestrial or marine surface environments (e.g., freshwater river, intertidal zone, shrimp pond, saline lake, and seawater), which are characterized by high sunlight incidence and mild temperatures (15–40 °C) (fig. 1; supplementary...
table S1, Supplementary Material online). Interestingly, a gene encoding the light-sensing heliorhodopsin was identified exclusively in Subgroup E (supplementary fig. S5, Supplementary Material online). However, phototrophy might not be supported in Thermoprofundales because heliorhodopsin has not yet to be confirmed to display proton-pumping activity (Pushkarev et al. 2018), and the genomes of Thermoprofundales lack the key genes for photosynthesis (PsAB and PsbAD). Nevertheless, the heliorhodopsin gene in Thermoprofundales was always accompanied by several protein-encoding genes involved in carotenoid biosynthesis (CrtZ, CrtB, CrtD, CruA, and CrtU) and repair of DNA damage caused by light-induced oxidative stress (PhrB and UvsE) (Meulenbroek et al. 2013; Zhang et al. 2017) in the same gene cluster (supplementary fig. S6, Supplementary Material online). Carotenoid production occurs often in phototrophic microorganisms, such as photosynthetic bacteria (e.g., Arthrobacter, Rhodobacter, and Rhodospirillum), extremely halophilic archaea (Halobacteria), microalgae, and some yeast (Mussagy et al. 2019). In Subgroup E, some genomes possess complete gene sets for the de novo biosynthesis of bacterioruberin and zeaxanthin (fig. 2), and key genes such as bisanhydrobacterioruberin hydratase (CrfA) and beta-carotene 3-hydroxylase (CrtZ) were first detected in the Candidatus phylum Thermoplasmata (Rinke et al. 2021) (supplementary figs. S7 and S8, Supplementary Material online). Carotenoids produced by these pathways might act as antioxidants and protect cells against oxidative damage, such as that resulting from exposure to sunlight, UV radiation, and/or H2O2 (Giani et al. 2019). Due to the adjacent gene arrangement among heliorhodopsin, carotenoid biosynthesis, and DNA damage repair, we speculate that the heliorhodopsin in Subgroup E functions as a light sensor to mitigate light-induced oxidative stress, probably by regulating the expression of genes for carotenoid biosynthesis and light-induced DNA damage repair (Pushkarev et al. 2018; Bulzu et al. 2021). Evolutionary History of Thermoprofundales To shed light on the evolutionary history of Thermoprofundales, gene gain and loss events were predicted by mapping all the orthologous genes found in high-quality genomes (>80% completeness and <5% contamination) to a phylogenomic tree. In general, 132 and 29 genes were gained and lost at the ancestral node of Thermoprofundales, respectively (Node 1 in fig. 3). For example, multiple genes involved in protein degradation were gained at this node (supplementary table S5, Supplementary Material online), making Thermoprofundales an important protein degrader in global sedimentary environments (Lloyd et al. 2013; Lazar et al. 2017; Zhou et al. 2018). Two genes related to survival in fluctuating environmental conditions were gained: a gene for cold shock protein CspA and a gene for glucosylglycerate synthase (GGS). These two genes might help Thermoprofundales to cope with multiple stressful conditions, such as harsh temperatures, osmotic pressure, oxidative stress, starvation, and pH extremes (Ketominen et al. 2016; Nunes-Costa et al. 2017). Of note, the evolution of Subgroup E genomes had unique features when compared with other subgroups (104 gains and 43 losses; Node 2 in fig. 3). Among the 104 gained genes at the ancestral node of Subgroup E, a heliorhodopsin gene and two genes involved in chlorophyll synthesis (BchE and BchG) possibly facilitate the utilization of sunlight (supplementary table S6, Supplementary Material online). Meanwhile, four DNA repair genes (UvrABC and XthA), which might participate in the removal of damaged bases caused by strong sunlight (Verhoeven et al. 2002), were also gained at Node 2. This suggests that microorganisms in Subgroup E evolved strategies to both use light energy and prevent its damage, which may be driving forces to separate this subgroup from others during the evolution of Thermoprofundales. HGT is the process of acquiring foreign DNA from other organisms through transformation, transduction, and conjugation (e.g., conjugative plasmids) (Douglas and Langille 2019). It is a major driving force for microbial evolution and plays important roles in archaeal speciation, metabolism, and adaptation (Wagner et al. 2017; Brito 2021). On average, 3.5% of the genes in a Thermoprofundales genome were identified as HGT genes, and 70.4% of these HGTs were derived from other distantly related archaeal genomes rather than bacterial ones (fig. 3; supplementary table S7, Supplementary Material online). Halobacteriota and Thermoproteota (GTDB taxonomy (Rinke et al. 2021), formerly a group of archaea including Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota) were the major HGT donors. This result is in contrast with the hypothesis that most of the HGTs in some mesophilic archaea originated from bacteria (López-García et al. 2015), and previous reports showed that gene transfer from bacteria to archaea occurred five times more frequently than from archaea to bacteria (Nelson-Sathi et al. 2015). The functional patterns between archaeal and bacterial-derived HGTs were further compared using the Thermoprofundales genomes at Nodes 1 and 2 in figure 3, respectively. The result revealed that archaeal donors contributed more HGTs in the processes of transcription, and carbohydrate transport and metabolism. However, bacterial donors contributed more HGTs related to energy production and conversion, cell wall/membrane/envelope biogenesis, and translation, ribosomal structure, and biogenesis (supplementary fig. S9, Supplementary Material online). In addition, we examined the HGT donors in genomes from other orders in the Candidatus phylum Thermoplasmata as proposed elsewhere (Rinke et al. 2021), including Acidiphilales, Thermoplasmatales, Methanomassiliicoccales, Poseidionales, and MGIII (supplementary fig. S10, Supplementary Material online). More than 53% of the HGTs identified in Acidiphilales, Thermoplasmatales, and Methanomassiliicoccales were derived from other archaeal genomes. However, more than 92% of the HGTs in Poseidionales and MGIII were from bacterial genomes (supplementary fig. S10, Supplementary Material online). This result minimizes the potential biases caused by the overall dominance of bacterial
genomes in the reference database (191,527 bacterial vs. 3,073 archaeal genomes in GTDB Release 95; Rinke et al. 2021), and it further confirms that Thermoprofundales acquired most of its HGTs from other distantly related archaeal species.

In addition, 437 sequences from the Thermoprofundales genomes were predicted as plasmids using PlasFlow (Krawczyk et al. 2018) and PlasClass (Pellow et al. 2020) (supplementary table S8, Supplementary Material online). Plasmids are mobile genetic elements (MGEs), and they contain nonchromosomal fragments of DNA and facilitate the fast evolution and adaptation of microorganisms to diverse environments (Heuer and Smalla 2012). They typically lack essential genes for microbial growth, but possess genes involved in response to changes in environmental conditions or exposure to pollutants (e.g., heavy metals and antibiotics) (Carattoli 2013). Interestingly, the gene encoding an extracellular protein-degrading enzyme for gingipain (Merops family C25) was located in the plasmids of 27 genomes. This enzyme, which participates in the protein remineralization process, is abundant and active in samples from Aarhus Bay sediments of Denmark (Lloyd et al. 2013). Some structural genes encoding the archaellum were located in plasmids from 19 Thermoprofundales genomes, including some key genes for archaellum rotation and assembly (FlaI, FlaJ, and FlaB). As a motility structure peculiar to the Archaea domain (Albers and Jarrell 2018), the archaellum may enable Thermoprofundales to move toward favorable micro-niches (Herzog and Wirth 2012), and/or mediate its surface attachment, cell–cell communication, and extracellular electron exchange (Näther et al. 2006; Walker et al. 2019). Six Thermoprofundales plasmids also encoded the complete gene sets for riboflavin biosynthesis (RibBHE), a precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These two coenzymes are major electron carriers in multiple and ubiquitous redox reactions involved in energy conservation pathways and cellular processes (Thakur et al. 2017).

H₂ Production by an Ancient MBH Complex
Phylogenetic reconstruction revealed that 71.2% of the genomes from the five Thermoprofundales subgroups encoded a gene of the Group 4d [NiFe] hydrogenase, which is an MBH-type H₂-producing hydrogenase (Vignais and Billoud 2007) (fig. 4a; supplementary table S4, Supplementary Material online). The MBH complex is composed of 14 subunits, including a membrane arm
and a peripheral arm (Schut et al. 2013). The membrane arm is formed by ten subunits (MbhABCDEFGHIM) located in the cell membrane, where it functions as an Na⁺/H⁺ antiporter domain (fig. 2). The peripheral arm is formed by four subunits (MbhJKLN) exposed to the cytoplasm, where it functions as a [NiFe] hydrogenase domain (Yu et al. 2018). The cryo-EM structure of this enzyme from a hyperthermophilic archaeon, *Pyrococcus furiosus*, was recently obtained. It is reported that the H₂-evolving catalytic site is located at the E21 and the [NiFe] center (C68, C71, C374, and C377) of the large subunit MbhL (Yu et al. 2018). Sequence alignment and 3D structure reconstruction of the MbhL coding genes revealed that all the H₂-evolving catalytic sites are conserved in Thermoprofundales, when compared with *P. furiosus* (supplementary fig. S11, Supplementary Material online). Furthermore, the MbhL gene from Thermoprofundales FT_bin5.232 was expressed in *Escherichia coli* BL21 strain to test its activity. Consistent with previous reports (Sapra et al. 2000; McTernan et al. 2015), the purified...
enzyme showed catalytic activity for H₂ production as assayed by gas chromatography (fig. 4b).

Hydrogen metabolism (including H₂ oxidation and production) is one of the most ancient and widespread metabolic traits, whose metabolite (i.e., H₂) is widely used to generate energy to support microbial growth (Lane et al. 2010; Greening et al. 2016). Microorganisms produce H₂ by using the [NiFe]-hydrogenase (Groups 3b and 4d) or [FeFe]-hydrogenase (Greening et al. 2016; Søndergaard et al. 2016). These H₂ producers contribute to the major biogenic pool of H₂ on Earth (e.g., anoxic sediments and gastrointestinal tracts) (Greening and Boyd 2020). In Thermoprofundales, the production of H₂ is catalyzed by the MBH-type [NiFe] hydrogenase, and reduced ferredoxin is the natural electron donor of this enzyme (Yu et al. 2018). Various genes coding for the production of reduced ferredoxin were detected in the Thermoprofundales genomes, showing that other components needed for hydrogen production are not a limitation in this microorganism (fig. 2). For example, identified pathways that lead to the generation of reduced ferredoxin include pyruvate and 2-oxoglutarate oxidation (pyruvate ferredoxin oxidoreductase, 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase), and glyceraldehyde oxidation (aldehyde ferredoxin oxidoreductase) in the glycolysis pathway.

New Insights into the Evolution of Modern Respiratory Systems

In Subgroup E, gene arrangement of the 14 subunits of MBH differed from those in subgroups A, B, C, D, and P. furiosus (Yu et al. 2018) (fig. 5a). In the genomes of Subgroup E, the peripheral arm genes of the [NiFe] hydrogenase domain were separated from the membrane arm genes of the Na⁺/H⁺ antiporter domain. A detailed survey of all the genomes in the NCBI GenBank database (January 25, 2021) further revealed that the discontinuous gene arrangement of MBH was unique to Thermoprofundales Subgroup E in Bacteria and Archaea (fig. 6). Phylogenetic analysis of the concatenated 4 peripheral arm genes (MbhJKLN), the 10 membrane arm genes (MbhABCDEFGHIM), and the complete 14 genes (MbhA–N) showed a consistent placement of Thermoprofundales as a monophyletic lineage adjacent to some Aciduliprofundum genomes (fig. 6, supplementary fig. S12, Supplementary Material online). These results are also coherent with the genome tree of all the MBH-containing genomes, revealing that the genes for the peripheral and membrane arms in Thermoprofundales are evolutionary conserved.

Metatranscriptomic analysis of the FT mangrove sediments revealed distinct transcriptional patterns between the genes of the peripheral arm and the membrane arm in some MAGs from Subgroup E (fig. 5f), suggesting that these two arms could function independently. To further confirm this, a recombinant plasmid containing the membrane arm gene cluster MbhABCDEFGHIM from FT_bin5.232 was introduced into an antiporter-deficient strain E. coli KNabc, which is commonly used to test the function of Na⁺/H⁺ antiporters (Shao et al. 2021). In general, when cultured in LBK medium containing either 0.1 or 0.2 M NaCl, a much better growth performance was observed for KNabc transformed with the membrane arm plasmid than that for the same strain with an empty plasmid (fig. S5c–e). This showed that the membrane arm protein of Subgroup E partially compensated the deficiency of KNabc for growing at high salt concentrations, confirming its function as an Na⁺ transporter.

Molecular dating analysis revealed that Subgroup E was an early diverged lineage among all the MBH-containing microorganisms in Thermoplasmatota (fig. 7). The ancestor of Subgroup E might have emerged from a habitat that resembles the sediments of terrestrial surface (fig. 1), which are characterized by mild temperature (<40 °C),
low salinity (<2%), low oxygen availability, and intense sunlight incidence, when compared with the deep sea. The ancestor of Subgroup E possibly experienced extensive gene duplication, rearrangement, and HGT events throughout its evolutionary history, including changes in the region of the MBH gene cluster. The separation of MBH into two modules, as observed in Subgroup E, might facilitate the adaption to the aquatic environments on the terrestrial surface. This would be achieved through a more efficient but less energy-consuming processes for hydrogen production and proton pumping, respectively. For example, 1) the synthesis of a 4-subunits (MbhJKLN) instead of a 14-subunits (MbhA–N) hydrogenase complex is functional for hydrogen production; 2) Subgroup E microorganisms may have a reduced need to pump out excess sodium when compared with other subgroups, since most of the genomes in Subgroup E were retrieved from terrestrial habitats with low salinity. These speculations are supported by the much higher transcriptional abundances of the peripheral arm than those of the membrane arm in four MAGs from the FT mangrove sediments (fig. 5f), showing that hydrogen production is more transcriptionally active than proton pumping in Subgroup E.

Interestingly, the gene structure of the peripheral arm in Subgroup E resembles that of the proposed common ancestral respiratory complex (ARC) of modern respiratory systems such as MBH and complex I (fig. 5a). The ARC, encoded by 5-subunits genes (MbhJKLMN), is a core protein module that can be traced back to the Last Universal Common Ancestor (LUCA) of Bacteria and Archaea; it is thought to function independently as a hydrogen-producing enzyme, which plays a key role in the early evolution of life (Schut et al. 2016). It is hypothesized that MBH shares a common ancestor with Complex I, and both of them possibly evolve from the Mrp (multiple resistance and pH adaptation) antiporter by acquiring a membrane-anchored hydrogenase module (Yu et al. 2018). The Mrp antiporter is an Na⁺ (or K⁺)/H⁺ exchanger representing an ancestor of many essential redox-driven proton pumps including MBH and Complex I (Steiner and Sazanov 2020). The conserved structural features in the two complexes indicate a similar energy conservation mechanism between the peripheral and membrane arms. However, a major difference between the two complexes is the absence of a proposed sodium ion translocation unit in the membrane arm of Complex I, but it is shared between MBH and Mrp antiporter (Yu et al. 2018). This
indicates that the membrane arm of MBH evolves earlier than that of Complex I. Meanwhile, the phylogenetic tree of representative [NiFe]-hydrogenase groups also showed that the MbhL gene in the peripheral arm of MBH emerged earlier than the NuoD gene of complex I (fig. 4a). A primary driving force in the evolution of ARC to modern respiratory complexes may be the availability of oxygen and associated high redox potential compounds (Schut et al. 2016). However, when compared with ARC, the lack of an MbhM subunit in the peripheral arm of Subgroup E does not appear to impact its function as a hydrogenase, because the MbhM is reported to be a membrane anchor for the peripheral arm and it did not show any ion translocation activity in *P. furiosus* (Yu et al. 2018).

**Conclusion**

Our study provides a comprehensive insight into the abundant and widespread sedimentary archaeal group Thermoprofundales, and it expands the knowledge of Thermoprofundales’ diversity, evolution, and ecological roles in global biogeochemical cycles. The genomic evolution of Thermoprofundales is driven by HGT events, of which ~70.5% are derived from other distantly related archaeal lineages. Thermoprofundales possesses a separated
two modular gene structure of MBH with high similarity to the ARC system, and the two modules were confirmed to be functionally independent and active. Our findings uncover an unprecedented role of Thermoprostales as an H₂ producer in global sedimentary environments, and Thermoprostales may fuel other H₂ consumers and facilitate extensive microbe-microbe interactions in the community.

**Materials and Methods**

**Sample Collection, Nucleic Acid Extraction and Sequencing**

The seawater samples of the Yap metagenomes were obtained in the Yap Trench area of the western Pacific during the 37th Dayang cruise in 2016. Seawater samples were collected by using CTD SBE911plus (Sea-Bird Electronics, USA) at depth intervals of 4, 30, 75, 125, 200, and 2,000 mbsl. Detailed information is described elsewhere (Zhang et al. 2018; Liu et al. 2021). Briefly, 8 l of each seawater sample was filtered through a 0.22 μm-mesh membrane filter immediately after recovery onboard. The membrane was cut into ~0.2 cm² pieces with flame-sterilized scissors, and the DNA was extracted using PowerSoil® DNA Isolation Kit (MoBio, USA) following the manufacturer’s protocol. Sequencing was performed on Illumina Hiseq X Ten platform using 2 × 150 bp pair-end technology.

The sediment samples of DZ, XMD, YX, and FT were obtained from four representative mangrove nature reserves (China) in 2017, including Danzhou Xinyinggang Nature
Wu et al. (2016) modules
Peng et al. (2022) with the para

5. Taxonomic assignment of the genera

Sequencing was performed on Illumina HiSeq X Ten platform using 2 × 250 bp pair-end technology.

Metagenomic Assembly and Binning
The Yap4, Yap30, Yap75, Yap125, Yap 200, Yap2000, DZ, XMD, YX, JLS1, L30, and L100 sets of metagenomes were assembled and binned using the same method (Zhang et al. 2019). Briefly, raw reads of each sample were trimmed using Sickle (v.1.33) (https://github.com/najoshi/sickle) with default settings, and the reads were then de novo assembled into contigs using IDBA-UD (v.1.1.1) (Peng et al. 2012) with the parameters “-mink 65 -maxk 145 -step 10.” Metagenomic binning was performed using MetaBAT2 (v.2.12.1) (Kang et al. 2019) with nine different combinations of specificity and sensitivity parameters (–m 2,000, 2,500, or 3,000; –maxP 85, 90, or 95), and the bins were merged and refined by DAS Tool (v.1.1) (Sieber et al. 2018).

The FT and YT sets of metagenomes were assembled and binned using the same method. Raw reads were trimmed with the read_qc module from metaWRAP (v.1.1) (Uritskiy et al. 2018). Clean reads from the same sampling site were pooled together before de novo assembly to one co-assembly, and they were then assembled using MEGAHIT (v.1.1.2) (Li et al. 2015) with the flag “–meta-large.” Metagenomic binning was performed using MetaBAT2 (v.2.12.1) with eight different combinations of specificity and sensitivity parameters (–m 60 or 95; –minS 60 or 95; –maxEdges 200 or 500), and the bins were merged and refined by DAS Tool (v.1.1) (Sieber et al. 2018).

The N1, N4, N5, and QZM sets of metagenomes were assembled and binned using the same method. Raw reads were trimmed and filtered using fastp (v.0.20.0) with default parameters (Chen et al. 2018). Clean reads of each sample were separately assembled into contigs using SPAdes (v.3.13.0) (Pjibelski et al. 2020) with the parameters “--meta -only-assembler -k 21, 31, 55, 77, 99, 121.” Metagenomic binning was performed using MetaBAT2 (v.2.12.1) and MaxBin2 (v.2.2.5) (Wu et al. 2016) modules in the metaWRAP pipeline (v.1.0.3) (Uritskiy et al. 2018), and the bins were merged and refined by Binning_refiner (v.1.2) (Song and Thomas 2017).

The completeness, contamination, and heterogeneity of the MAGs were determined based on lineage-specific conserved marker gene sets in each genome by CheckM (v.1.0.7) (Parks et al. 2015).

Retrieval of Thermoprofundales Genomes
The collection of Thermoprofundales genomes was obtained from three sources, which included 55 MAGs retrieved from the metagenomes of this study, 55 MAGs/SAGs downloaded from the NCBI GenBank database (Coordinators 2018) (January 25, 2021), and 36 MAGs from the genomic catalog of Earth’s microbiomes (Nayfach et al. 2020). Taxonomic assignment of the genomes was performed by using “classify” workflow in the GTDB-Tk software (v.1.3.0) (Chaumeil et al. 2020), and

Sequencing was performed on Illumina NovaSeq 6000 platform using 2 × 150 bp pair-end technology.

The FT sediment samples were collected at the top 10 cm. The FT sediment samples were collected at depth intervals of 0–2, 6–8, 12–14, 20–22, and 28–30 cm. Detailed information on the processing of samples is described elsewhere (Zhang et al. 2019). Briefly, genomic DNA was extracted from 0.3 g of each sediment using a DNeasy PowerSoil kit (Qiagen, Germany) according to the manufacturer’s instructions. Total RNA was extracted from 2 g of each FT sediment using an RNeasy PowerSoil Total RNA Kit (Qiagen). A DNase Max Kit (Qiagen) and an RNeasy PowerClean Pro Cleanup Kit (Qiagen) were used to remove genomic DNA and purify the remaining RNA, respectively. Sequencing was performed on Illumina NovaSeq 6000 platform using 2 × 150 bp pair-end technology.

The YT samples were obtained from the Rongcheng Swan Lake Nature Reserve (China) in 2018. Details are described elsewhere (Liu et al. 2021). The sediment cores were collected using columnar samplers at depth intervals of 0–2, 21–26, and 36–41 cm in a seagrass meadow (YT_FC) and a nonseagrass-covered site nearby (YT_N), respectively. DNA was extracted from 10 g sediment of each sample using PowerMax® Soil DNA Isolation Kit (MoBio, Germany) according to the manufacturer’s protocol. Sequencing was performed on Illumina HiSeq 2500 platform using 2 × 150 bp pair-end technology.

The JLS1 sample was collected from the surface sediment of Jiulong River estuary (China) in 2018. Approximately 10 g of each sample was applied for DNA extraction with the procedure as described elsewhere (Chen et al. 2016; Natarajan et al. 2016).
candidate Thermoprofundales genomes were assigned to “c__E2” in the GTDB taxonomy. A phylogenomic tree based on 55 conserved single-copy ubiquitous archaeal genes (Dombrowski et al. 2020) was then constructed using IQ-TREE (v.1.6.3) (Nguyen et al. 2015) with ModelFinder (Kalyaanamoorthy et al. 2017), and the Thermoprofundales genomes formed a monophyletic clade adjacent to other orders from the Candidate phylum Thermoplasmataota (e.g., Methanomassiliicoccales, Thermoplasmatales, Acidoprofundales, Poseidoniales, and MGIII). The tree is visualized using the iTOL online tool (v.5) (Letunic and Bork 2021). The AAI value shared by any two genomes was calculated using CompareM software with default parameters (v.0.1.2) (https://github.com/dparks1134/CompareM), and the heatmap was drawn using TBtools (v.1.0.75) (Chen et al. 2020).

Gene Annotation, Metabolic Reconstruction, and Metatranscriptomic Activity
The protein-coding genes of each genome were predicted by Prodigal (v.2.6.3) (Hyatt et al. 2010) using “-p meta” option. Orthologous gene families were identified using OrthoFinder (v.2.2.1) (Emms and Kelly 2019) with the parameters “-S diamond -M msa.” Gene annotation was performed against multiple web servers and protein databases and using the BLASTP program (E-value cutoff ≤ 1 × 10\(^{-5}\)) (Altschul et al. 1990), including the Rapid Annotations using Subsystems Technology (RAST) server (Brettin et al. 2015), the Kyoto Encyclopedia of Genes and Genomes (KEGG) server (Kanehisa et al. 2021), the Clusters of Orthologous Genes (COG) (Galperin et al. 2021), the archaeal Clusters of Orthologous Genes (arCOG) (Makarova et al. 2015), the Protein families database (Pfam) (Mistry et al. 2021), the Conserved Domain Database (CDD) (Lu et al. 2020), the Protein Clusters (PRK) (https://www.ncbi.nlm.nih.gov/proteinclusters/), the Institute for Genomic Research’s database of protein Families (TIGRFAM) (Haft et al. 2013), the Carbohydrate-Active enZymes database (CAZy) (Lombard et al. 2014), and the MEROPS database (Rawlings et al. 2017). Signal peptides and extracellular peptides were predicted using SignalP (v.5.0) (Almagro Armenteros et al. 2019) and PSORTb (v.3.0.3) (Yu et al. 2010), respectively. Potential metabolic pathways were reconstructed based on the above predicted annotations and reference pathways depicted in KEGG (Kanehisa et al. 2021) and MetaCyc (Caspi et al. 2020). In FT mangrove sediments, transcriptomic abundances of the separated peripheral and membrane arms from Subgroup E were determined by mapping non-rRNA reads to the corresponding sequences using Bowtie 2 (v.2.3.4.1) (Langmead and Salzberg 2012) with default settings, and they were calculated using the RPKM (reads per kilobase per million sequenced reads) method (Robinson and Oshlack 2010; Zhang et al. 2018).

Construction of Phylogenetic Trees
Phylogenetic trees of all the key genes in this study (i.e., Mbhl, reverse gyrase, 16S rRNA, Cruf, Crtz, and the concatenated 14-subunits, 10-subunits, and 4-subunits MBH) were constructed using IQ-TREE (v.1.6.3) with ModelFinder, and ultrafast bootstrapping was used to estimate the reliability of each branch with 1,000 times resampling. The trees were visualized using iTOL (v.5).

Sequence Alignment and 3D Structure Reconstruction
The protein sequences of the Mbhl gene were aligned using free end gaps with the Blosum62 cost matrix in Geneious Prime® software (v.2020.2.4) (https://www.geneious.com). Three-dimensional structures of the Mbhl protein were reconstructed using the C-I-TASSER online tool with default options (Zheng et al. 2021).

Expression, Purification, and Enzymatic Assay of Mbhl
The Mbhl gene from Thermoprofundales FT_bin5.232 was fused with a PelB signal peptide and a maltose-binding protein (MBP) tag. The nucleotide sequences were then synthesized after codon optimization for E. coli, inserted into the Ndel–Xhol site of plasmid pET-22b(+), and transformed into an E. coli BL21(DE3) strain. Meanwhile, an empty plasmid pET-22b(+) was transformed into an E. coli BL21(DE3) as the negative control. The expression was induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) in a total volume of 2.0 l at 37 °C for 4 h when the OD\(_{600}\) was 0.6. The cells were harvested by centrifugation at 8,000 rpm for 5 min and then lysed by sonication in 1×PBS buffer (1.8 mM KH\(_2\)PO\(_4\), 8.1 mM Na\(_2\)HPO\(_4\), 136.9 mM NaCl, 2.7 mM KCl, pH = 7.4). The cell lysate was centrifuged at 12,000 rpm for 10 min to remove debris, and the supernatant was applied to an MBP column. After washing with 1×PBS buffer, the fusion protein was eluted with 1×PBS buffer containing 10 mM maltose, and then dialyzed into a storage buffer (1.8 mM KH\(_2\)PO\(_4\), 8.1 mM Na\(_2\)HPO\(_4\), 300 mM NaCl, 2.7 mM KCl, 10% glycerol, pH = 7.4).

Hydrogenase activity was examined by measuring H\(_2\) production at 37 °C over a period of 15 min in a 10 ml anaerobic culture tube, as described elsewhere (Adams and Mortenson 1984) with few modifications. Briefly, the air in the headspace of each tube was replaced with Argon before the experiment. The 2 ml assay mixture contained 25 μg/ml fusion protein, 1 mM methyl viologen, and 20 mM sodium dithionite in 50 mM EPPS buffer (pH = 8.0). The inactivated Mbhl protein was prepared by boiling the recombinant Mbhl at 100 °C for 30 min. The H\(_2\) concentration was measured by gas chromatography (Agilent 7890B, USA).

NaCl Tolerance Test
Nucleotide sequences of the gene cluster MbhABCDEFGHIM from Thermoprofundales FT_bin5.232 were synthesized after codon optimization for E. coli, inserted into the Ndel–Xhol site of plasmid pET-22b(+), and transformed into an antipporter-deficient strain E. coli KNabc. Meanwhile, an empty plasmid pET-22b(+) was transformed into an E. coli KNabc as the negative control. They were separately incubated in an LBK medium
Molecular Dating Analysis
Estimation of the species divergence time was performed using the MCMCtree tool (v.4.9j) (Yang 2007) with several temporal calibrations as described elsewhere (Ren et al. 2019). Briefly, the root of Archaea is about 4,380–3,460 Mya (Wolfe and Fournier 2018), Thermoproteales and Sulfolobales both originate after the GOE (about 2,330 Mya) (Blank 2009; Luo et al. 2016), and MRCA of Sulfolobus solfataricus and Sulfolobus islandicus originate after 475 Mya (Blank 2011). The parameters in MCMCtree were used as below: first, the codeml module was used to calculate the overall substitution rate for each gene family. Then, the mcmctree module (with usedata = 3) was used to calculate the maximum likelihood estimation of branch lengths, gradient, and Hessian (Reis and Yang 2011). Finally, the mcmctree module (with usedata = 2) was used to estimate the divergence times. The estimation has been repeated for two times, and the two replicates had a consistent performance as shown in supplementary figure S13, Supplementary Material online. The parameters of the mcmctree module were “burnin = 20,000, sampfreq = 10, nsample = 200,000.”

Gene Gain and Loss Analysis
High-quality (>80% completeness and <5% contamination) Thermoprofundales and reference genomes were selected (including the ancestral nodes, sister lineages and outgroups) to reconstruct the genome contents and phylogenomic tree using OrthoFinder (v.2.2.1) and IQ-TREE (v.1.6.3) (based on 55 conserved marker genes; Dombrowski et al. 2020), respectively. Processes governing the evolution of the five Thermoprofundales subgroups were inferred from predicted gene gain and loss events using BadiRate (v.1.35) (Librado et al. 2012) with options “-anc -bmodel FR -rmodel BDI -ep CWP.”

Supplementary material
Supplementary data are available at Molecular Biology and Evolution online.

Acknowledgments
This work was supported by the National Natural Science Foundation of China (grant nos 91951102, 32225003, 31970105, 32061133009), the Innovation Team Project of Universities in Guangdong Province (grant no. 2020KCG TD023), the Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDB42000000), and the Science and Technology Innovation Committee of Shenzhen (grant nos JCYJ20190808152403587, JCY J20200109105010363). We thank Qianjun He, Chao Xia, and Zhaokui Jin for help with the measurement of H2. We thank Lin Meng for preparing the E. coli KNabc strain. We thank Wan Liu for the genome submission to eLMSG.

Data Availability
The genome sequences from the current study have been deposited in eLMSG (an eLibrary of Microbial Systematics and Genomics, https://www.biosino.org/elmsg/) under accession numbers LMSG_G000004137.1–LMSG_G00000 4191.1. The accession numbers of the metagenomic raw reads of each sample used in this study are provided in supplementary table S1, Supplementary Material online. Metatranscriptomic raw reads of the five FT samples have been deposited in NODE (National Omics Data Encyclopedia, https://www.biosino.org/node/) with accession numbers OES078050–OES078054.
References

Coordinators NR. 2018. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 46:D8–D13.

Adam PS, Borrel G, Brochier-Armanet C, Gribaldo S. 2017. The growing tree of archaea: new perspectives on their diversity, evolution and ecology. *ISME J.* 11:2407–2425.

Adams MWW, Morton LE. 1984. The purification of hydrogenase II (uptake hydrogenase) from the anaerobic N2-fixing bacterium *Clostridium pasteurianum*. *Biochim Biophys Acta Bioenerg.* 766:51–61.

Albers S-V, Jarrell KF. 2018. The archaellum: an update on the unique archaeal motility structure. *Trends Microbiol.* 26:351–360.

Almagro Armenteros JJ, Tsirigos KD, Senderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. 2019. Signalp 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol.* 37:420–423.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol.* 215:403–410.

Baker BJ, De Anda V, Seitz KW, Dombrowski N, Santoro AE, Lloyd KG. 2020. Diversity, evolution and archaea of *Clostridium*. *Nat Microbiol.* 5:878–900.

Blank CE. 2009. Phylogenomic dating—a method of constraining the age of microbial taxa that lack a conventional fossil record. *Astrobiology.* 9:173–191.

Blank CE. 2011. An expansion of age constraints for microbial clades that lack a conventional fossil record using phylogenomic dating. *J Mol Evol.* 73:188–208.

Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Puschen GD, et al. 2015. RASTTk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep.* 5:8365.

Brito IL. 2021. Examining horizontal gene transfer in microbial communities. *Nat Rev Microbiol.* 19:442–453.

Bulzu P-A, Kavagutti Vinicius S, Chiriac M-C, Vavourakis Charlotte D, Galperin MY, Wolf YI, Makarova KS, Vera Alvarez R, Landsman D, Koonin EV. 2021. COG database update: focus on microbial diversity, model organisms, and widespread pathogens. *Nucleic Acids Res.* 49:D274–D281.

Giani M, Garbayo I, Vilchez C, Martinez-Espinosa RM. 2019. Haloarchaeal carotenoids: healthy novel compounds from extreme environments. *Mar Drugs.* 17:524.

Greening C, Biswas A, Carere CR, Jackson CJ, Taylor MC, Stott MB, Cook GM, Morales SE. 2016. Genomic and metagenomic surveys of hydrogenase distribution indicate *H*. is a widely utilised energy source for microbial growth and survival. *ISME J.* 10:761–777.

Greening C, Boyd E. 2020. Editorial: microbial hydrogen metabolism. *Front Microbiol.* 11:56.

Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. 2013. TIGRFAMs and genome properties in 2013. *Nucleic Acids Res.* 41:D387–D395.

Herzog B, Wirth R. 2012. Swimming behavior of selected species of archaea. *Appl Environ Microbiol.* 78:1670–1674.

Heuer H, Smalla K. 2012. Plasmids foster diversification and adaptation of bacterial populations in soil. *FEMS Microbiol Rev.* 36:1083–1104.

Hoshino T, Inagaki F. 2019. Abundance and distribution of archaea in the subseafloor sedimentary biosphere. *ISME J.* 13:227–231.

Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser JS. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics.* 11:119.

Inagaki F, Kuyper MMM, Tsunogai U, Ishibashi J, Nakamura K, Treude T, Ohkubo S, Nakaseama M, Gena K, Chiba H, et al. 2006. Microbial community in a sediment-hosted CO2 lake of the southern Okinawa Trough hydrothermal system. *Proc Natl Acad Sci U S A.* 103:14164–14169.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ProtTest-3: fast model selection for accurate phylogenetic estimators. *NAT Methods.* 14:587–589.

Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. 2021. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res.* 49:D454–D551.

Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, Wang Z. 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ.* 7:e7359.

Kato-Timonen R, Hietala N, Palonen E, Hakakorpi A, Lindström M, Koonin EV. 2016. Diversity, biogeography, and biodegradation of big biological data. *ISME J.* 10:1340.

Koonin EV. 2021. COG database update: focus on microbial diversity, model organisms, and widespread pathogens. *Science.* 371:351–362.

Konstantinidis KT, Rosselló-Móra R, Amann R. 2017. Uncultivated microbes in need of their own taxonomy. *ISME J.* 11:2399–2406.

Krawczyk PS, Lipinski L, Dziembowski A. 2018. Plasflow: predicting plasmid sequences in metagenomic data using genome signatures. *Nucleic Acids Res.* 46:e35.

Lagkouvardos I, Joseph D, Kapflhammer M, Giritli S, Horn M, Haller D, Clavel T. 2016. IMNGS: a comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. *Sci Rep.* 6:33721.

Lane N, Allen JF, Martin W. 2010. How did LUCA make a living? *Chemosmiosis in the origin of life. Bioessays.* 32:271–280.
Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar Gustavo A, Meulenbroek EM, Peron Cane C, Jala I, Iwai S, Moolenaar GF, Goosen McTernan PM, Chandrayan SK, Wu C-H, Vaccaro BJ, Lancaster WA, Makarova KS, Wolf YI, Koonin EV. 2015. Archaeal clusters of orthologous groups (arCOGs): an update and application for analysis of ancient metagenomes. *Mol Biol Evol* 32:1674–1676.

Librado P, Vieira FG, Rozas J. 2012. Badirate: estimating family turnover rates by likelihood-based methods. *Bioinformatics* 28: 279–281.

Lipp JS, Morono Y, Inagaki F, Hinrichs K-U. 2008. Significant contribution of archaea to extant biomass in marine subsurface sediments. *Nature* 454:991–994.

Lipscomb GL, Hahn EM, Crowley AT, Adams MWW. 2017. Reverse gyrase is essential for microbial growth at 95°C. *Extremophiles* 21:603–608.

Liu Y, Makarova KS, Huang W-C, Wolf YI, Nikolskaya AN, Zhang X, Cai M, Zhang C-J, Xu W, Luo Z, et al. 2021. Expanded diversity of Asgard archaea and their relationships with eukaryotes. *Nature* 593:553–557.

Liu Y-F, Yang L, Liu Z-L, Fang B, Zhou L, Liu J-F, Yang S-Z, Gu J-D, Mu B-Z. 2022. Discovery of the non-cosmopolitan lineages in Candidatus thermoprofundales. *Environ Microbiol* 24: 3063–3080.

Lloyd KG, Schreiber L, Petersen DG, Kjeldsen KU, Lever MA, Steen S. 2017. Predominant archaea in marine sediments degrade detrital DNA lesions. *Glycobiology* 27:213–227.

Orcutt BN, Sylvan JB, Knab NJ, Edwards KJ. 2011. Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiol Mol Biol Rev* 75:361–422.

Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. Checkm: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055.

Pellow D, Mizrahi I, Shamir R. 2020. Plasclass improves plasmid sequence classification. *PloS Comp Biol* 16:e1007781.

Peng Y, Leung HCM, Yiu SM, Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* 28:1420–1428.

Prijibelski A, Antipov D, Meleshko D, Lapidus A, Korobeinikov A. 2020. Using SPAdes de novo assembler. *Curr Protoc Bioinformatics*. 70:e102.

Pushkarvev A, Inoue K, Larom S, Flores-Uribe J, Singh M, Konno M, Tomida S, Ito S, Nakamura R, Tsunoda SP, et al. 2018. A distinct abundant group of microbial rhodopsins discovered using functional metagenomics. *Nature* 558:595–599.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–D596.

Rawlins JD, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. 2017. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res* 46:D624–D632.

Reis Md, Yang Z. 2011. Approximate likelihood calculation on a phylogeny for Bayesian estimation of divergence times. *Mol Biol Evol* 28:161–2172.

Ren M, Feng X, Huang Y, Wang H, Hu Z, Clingenpeel S, Swan BK, Fonseca MM, Posada D, Stepanauskas R, et al. 2019. Phylogenomics suggests oxygen availability as a driving force in Thaumarchaeota evolution. *ISME J* 13:2150–2161.

Rinke C, Chuvchina M, Mussig AJ, Chaumeil P-A, Davin AA, Waite DW, Whitman WB, Parks DH, Hugenholtz P. 2021. A standardized archaean taxonomy for the genome taxonomy database. *Nat Microbiol* 6:946–959.

Robinson MD, Oshlack A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol* 11: R25.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584.

Sapra R, Verhagen MFJM, Adams MWW. 2000. Purification and characterization of a membrane-bound hydrogenase from the thermophilic archaeon Pyrococcus furiosus: multifunctional organelles, made for swimming, adhesion to various surfaces, and cell-cell contacts. *J Bacteriol* 188: 6915–6923.

Nayfach S, Roux S, Seshadri R, Udwary D, Varghese N, Schulz F, Wu D, Paez-Espino D, Chen IM, Hunttemann M, et al. 2020. A genomic catalog of Earth’s microbiomes. *Nat Biotechnol* 39:499–509.

Nelson-Sathi S, Sousa FL, Roettger M, Lozada-Chávez N, Thiergart T, Janssen A, Bryant D, Landan G, Schönheit P, Siebers B, et al. 2015. Origins of major archaeal clades correspond to gene acquisitions from bacteria. *Nature* 517:77–80.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32:268–274.

Nunes-Costa D, Maranha A, Costa M, Alarico S, Empadinhas N. 2017. Glucosylglucosyl esterase metabolism, bioversatility and mycobacterial survival. *Glycobiology* 27:213–227.

Yeung CHC, Yiu SM, Hen F, Yim CM, Fung MS, Leung HCM, Chin FYL. 2011. Predictive meta-carbohydrate database (CDB) in *Nucleic Acids Res*. 42:D490–D495.

López-García P, Zivanovic Y, Deschamps P, Moreira D. 2015. Bacterial gene import and morphospecies adaptation in archaea. *Nat Rev Microbiol* 13:447–456.

Luo S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS, et al. 2020. CDD/SPARCLE: the conserved domain database in 2020. *Nucleic Acids Res*. 48:D265–D268.

Lu J, Zhang X, Wang C, Li M, Chen J, Xiong J. 2021. Responses of sediment resistosome, virulence factors and potential pathogens to decades of antibiotics pollution in a shrimp aquaculture. *Sci Total Environ* 794:148760.

Luo G, Oso S, Beukes Nicolas J, Wang David T, Xie S, Summons Roger E. 2016. Rapid oxygenation of Earth’s atmosphere 2.33 billion years ago. *Sci Adv* 2:e1600134.

Makarova KS, Wolf YI, Koonin EV. 2015. Archaeal clusters of orthologous genes (arCOGs): an update and application for analysis of shared features between Thermococcales, Methanococcales, and Methanobacteriales. *Life* 5:818–840.

McTernan PM, Chandrayan SK, Wu C-H, Vaccaro BJ, Lancaster WA, Adams MWW. 2015. Engineering the respiratory membrane-bound hydrogenase of the hyperthermophilic archaeon *Pyrococcus furiosus* and characterization of the catalytically active cytoplasmic subcomplex. *Protein Eng Des Sel*. 28:1–8.

Meulenbroek EM, Peron Cane C, Jala I, Iwai S, Moolenaar GF, Goosen N, Pannu NS. 2013. UV damage endonuclease employs a novel dual-dinucleotide flipping mechanism to recognize different DNA lesions. *Nucleic Acids Res*. 41:1363–1371.

Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar Gustavo A, Sonnhammer ELL, Tosatto SCE, Paladini L, Raj S, Richardson LJ, et al. 2021. Pfam: the protein families database in 2021. *Nucleic Acids Res*. 49:D412–D419.
