1 Ultra-small cells and DPANN genome unveiled inside an extinct vent chimney

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3 Hinako Takamiya¹, Mariko Kouduka¹, Hitoshi Furutani¹, Hiroki Mukai², Takushi
4 Yamamoto³, Shingo Kato⁴, Yu Kodama⁵, Naotaka Tomioka⁵, Motoo Ito⁵, and Yohey
5 Suzuki^{1*}

6

7 ¹Department of Earth and Planetary Science, The University of Tokyo, 7-3-1 Hongo,
8 Bunkyo-ku, Tokyo 113-0033, Japan

9 ²Faculty of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1,
10 Tsukuba, Ibaraki 305-8572, Japan

11 ³Global Application Development Center, Shimadzu Corporation, 1, Nishinokyo
12 Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan

13 ⁴Japan Collection of Microorganisms (JCM), RIKEN BioResource Research Center,
14 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

15 ⁵Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science
16 and Technology (JAMSTEC), Monobe B200, Nankoku, Kochi 783-8502, Japan

17

18 *Corresponding author. E-mail: yohey-suzuki@eps.s.u-tokyo.ac.jp (Y.S)

19

20 Abstract

21 Chemosynthetic organisms flourish around deep-sea hydrothermal vents where
22 energy-rich fluids are emitted from metal sulfide chimneys. In contrast
23 to actively venting chimneys, the nature of microbial life in extinct chimneys
24 without fluid venting remains largely unknown. Here, the occurrence of
25 ultra-small cells in silica-filled grain boundaries inside an extinct chimney is
26 demonstrated by high-resolution bio-signature mapping. The ultra-small cells
27 are associated with extracellularly precipitated Cu₂O nanocrystals. Single-gene

1 analysis shows that the chimney interior is dominated by a member of
 2 **Pacearchaeota** known as a major phylum of DPANN. Genome-resolved
 3 metagenomic analysis reveals that the chimney **Pacearchaeota** member is
 4 equipped with a nearly full set of genes for fermentation-based energy
 5 generation from nucleic acids, in contrast to previously characterized
 6 **Pacearchaeota** members lacking many genes for nucleic acid fermentation. We
 7 infer that the ultra-small cells associated with silica and extracellular Cu_2O
 8 nanocrystals in the grain boundaries are **Pacearchaeota**, on the basis of the
 9 experimentally demonstrated capability of silica to concentrate nucleic acids
 10 from seawater and the presence of Cu-exporting genes in a reconstructed
 11 **Pacearchaeota** genome. Given the existence of ~3-billion-year-old submarine
 12 hydrothermally deposited silica, proliferation of microbial life using
 13 silica-bound nucleic acids might be relevant to the primitive vent biosphere.

14

15 Keywords: **Pacearchaeota** fermentation pathways, silica-bound nucleic acids,
 16 extracellular cuprite nanoparticles, seafloor metal sulfide deposit, grain boundary
 17 habitability, deep-sea rocky biosphere

18

19 **Introduction**

20 The oldest record of pillow lava basalt, a geologic evidence of deep-sea hydrothermal
 21 activity, is found in the 3.8-billion-year-old Isua Supracrustal Belt in Greenland¹.
 22 Deep-sea hydrothermal fluid venting by “black smokers” is associated with the
 23 extensive eruption of pillow lava on the deep seafloor². Seawater is recharged,
 24 interacts with extrusive and intrusive basaltic rocks, and is then discharged as
 25 high-temperature hydrothermal fluid enriched with heavy metals and chemicals that

1 can be used for microbial energy generation, such as HS^- , Fe(II) , CH_4 , and H_2 . As
 2 abiotic synthesis of nucleic acids and amino acids appears to be favorable from the
 3 fluid-borne chemicals, deep-sea hydrothermal vents are considered to be one of the
 4 potential habitats for primitive cellular life on Earth³.

5 As a result of rapid cooling of hydrothermal fluid, vent chimneys tend to form
 6 with an internal zonation of mineral assemblages⁴. Decreasing temperature and
 7 increasing pH cause the sequential deposition of minerals. In general, chalcopyrite
 8 (CuFeS_2) precipitated from high-temperatures fluid ($>300^\circ\text{C}$) tends to form the inner
 9 massive wall, which is surrounded by marcacite (FeS_2), pyrite (FeS_2), and sphalerite
 10 (ZnS) precipitated from lower temperatures in outer porous layers. Since the discovery
 11 of deep-sea hydrothermal vents⁵, microbial populations thriving in actively venting
 12 chimneys have been studied intensively⁶. However, in contrast to active chimneys
 13 with excess energy supplies from hydrothermal fluids, extinct chimneys without fluid
 14 venting appear to be hostile for chemolithotrophic microbes with scarce energy
 15 supplies available solely from metal sulfide minerals.

16

17 **Results and Discussion**

18 **Extinct chimney inner wall with microbial signals**

19 The Pika site is a recently discovered deep-sea hydrothermal field with black smokers
 20 ($> 300^\circ\text{C}$) in the southern Mariana Trough, about 140 km east of Guam (**Figure 1a**)⁷.
 21 Chimney samples were collected by the remotely operated vehicle Hyper-Dolphin at a
 22 water depth and temperature of 2787 m and 1.7°C , respectively (**Figure 1b**). Zonation

1 characteristic of metal sulfide chimneys was found in a thin section of one of the
2 chimney samples (**Figure 1c**). There was an unaltered gold-colored part on the inner
3 wall of chalcopyrite directly deposited from black smokers (**Figure 1d**). The chimney
4 is referred to as IPdc in this manuscript.

5 Analytical procedures have been developed to visualize and quantify
6 microbial cells hosted in crack-infilling minerals in the oceanic crust by preparing thin
7 sections of rocks embedded in hydrophilic resin called LR White^{8,9}. Microbial cells
8 embedded in the resin are stainable with a DNA dye called SYBR-Green I.
9 Microscopic examinations of the inner wall of the chimney prepared as described
10 above revealed the presence of patches with cell-like fluorescent signals where visible
11 light was not transmitted (**Supplementary Figure 1**). Focused ion beam (FIB)
12 sections with thicknesses of 3 μm (**Figure 1e, f, g**) and 300 nm (**Figure 1h, i, j**) were
13 fabricated to characterize patches associated with the cell-like signals by nanoscale
14 secondary ion mass spectrometry (NanoSIMS). In the 3- μm thick FIB section, signals
15 from ^{32}S , $^{12}\text{C}^{14}\text{N}$, and ^{31}P were detected in a silica-bearing layer with sub-micron voids
16 (**Figure 1g, Supplementary Figure 2**). Similar results were obtained from the
17 300-nm thick FIB section (**Figure 1j**). A selected area electron diffraction (SAED)
18 pattern showed that the silica-bearing layer also observed by transmission electron
19 microscopy (TEM) was composed of amorphous material (**Supplementary Figure**
20 **3**).

21

22 **Microbial cells visualized in chalcopyrite grain boundaries**

1 The greenish cell-like signals from the DNA dye and the overlapped NanoSIMS
 2 mapping of P and CN are consistent with results from previous study of microbial cells
 3 in mineral-filled cracks⁹. However, the appearance of individual cells was not clear, in
 4 contrast to the earlier work. To clearly observe individual cells, a 150-nm thick FIB
 5 section was fabricated from a chalcopyrite grain boundary associated with silica
 6 (**Supplementary Figure 4**) where greenish cell-like signals without light transmission
 7 were observed after SYBR-Green I staining (**Figure 2a**). It was revealed by
 8 NanoSIMS analysis that $^{12}\text{C}^{14}\text{N}$, ^{28}Si , and ^{31}P were overlapped in a ribbon-shaped
 9 grain boundary (**Figure 2b, c**). TEM observations of the region with overlapping ^{31}P ,
 10 $^{12}\text{C}^{14}\text{N}$, and ^{28}Si showed small spheres with diameters of <200 nm (**Figure 2d, e**).
 11 Cuprite (Cu_2O) was identified by a SAED pattern (**Figure 2f**) and an energy dispersive
 12 X-ray spectrum (EDS) (**Figure 2g**). High-resolution TEM observations revealed that
 13 ~5-nm-diameter particles of cuprite were spatially associated with the small spheres
 14 (**Figure 2h**).

15 To help interpret the TEM image of the small spheres associated with cuprite
 16 nanoparticles (**Figure 2e, Supplementary Figure 5a**), TEM observations were
 17 performed for cultured cells of *Geobacter sulfureducens* associated with
 18 extracellularly precipitated nanocrystals of uraninite (UO_2) (**Supplementary Figure**
 19 **5b, c**) and those of *Desulfovibrio desulfuricans* with UO_2 nanoparticles in the
 20 periplasmic space (**Supplementary Figure 5d, e, f**). These observations clarified that
 21 the dark contrast is derived from the presence of uraninite nanoparticles, with
 22 transparent contrast from cellular materials and resin. The small spheres associated
 23 with cuprite nanoparticles in the chimney sample appear to be very similar to
 24 microbial cells associated with extracellular uraninite precipitation (**Supplementary**

1 **Figure 5b)**^{10,11}. The image contrast of the small spheres observed in the 150-nm thick
 2 FIB section was not transparent (**Figure 2e, Supplementary Figure 5a**). This is
 3 explained by the effect of small cell size. In sections with a thickness of 150 nm,
 4 transparent contrast is expected for cells with large cells, regardless of the cell shape
 5 (**Supplementary Figure 5g**). However, small coccoid cells are visualized with
 6 slightly dark contrast (**Supplementary Figure 5g**). The image contrast of the small
 7 spheres with extracellular Cu₂O nanoparticles is therefore inferred to be derived from
 8 coccoid cells with a small size range. Small greenish spots visualized after DNA
 9 staining and the overlapped enrichment of P and CN revealed by NanoSIMS analysis
 10 support the conclusion that the small spheres are microbial cells.

11

12 ***In situ* biosignature analyses**

13 To strengthen the evidence that the small spheres are indeed microbial cells, we
 14 performed *in situ* biosignature analyses. The spatial distributions of biomolecules such
 15 as proteins and lipids in chalcopyrite grain boundaries were characterized by an
 16 imaging mass spectrometry using a thin section after SYBR-Green I staining and
 17 observations by fluorescence microscopy. Spot analysis of grain boundaries revealed
 18 the presence of a macromolecule with $m/z = 805.2$ (**Supplementary Figure 6**). The
 19 mass spectrum of resin was found to lack this macromolecule. Mapping of the
 20 macromolecule in the chimney inner wall confirmed its ubiquitous distribution in
 21 grain boundaries (**Figure 3a, b**).

22 To obtain another line of convincing evidence for the biogenicity of the small
 23 spheres¹², Raman spectroscopy was used to characterize the same region where

1 NanoSIMS analysis was performed on the ribbon-shaped grain boundary (**Figure 2c**).
 2 High-resolution mapping was performed for the peak intensity at 2800–3000 cm^{-1} ,
 3 which is attributed to CH_{2-3} typically found in microbial lipids (**Figure 3c**)¹³. The peak
 4 intensity was strong along grain boundaries. Raman spectra obtained from the grain
 5 boundaries with the high CH_{2-3} signal were different from those of the resin and
 6 minerals spatially associated with the small spheres (**Figure 3d, Supplementary**
 7 **Figure 7**). The main spectral difference is explained by the presence of CH_3 and amide
 8 groups (C–N and N–H; indicated by red arrows), which is consistent with the
 9 distribution of $^{12}\text{C}^{14}\text{N}$ determined by NanoSIMS analysis (**Figure 2c**). We exclude the
 10 possibility that the small spheres are abiotic carbonaceous particles such as recently
 11 discovered particulate graphite (C) in hot and cold vent fluids¹⁴. In addition to the
 12 circularity and narrow size distribution of the spheres, the lack of sharp edges typically
 13 found in abiotic carbonaceous particles further supports the biogenicity of the small
 14 spheres¹⁵. Taken together, we conclude that the small spheres are ultra-small cells.

15

16 **Microbial abundance and composition in the extinct chimney**

17 To understand microbial life in the chalcopyrite inner wall, the interior and exterior of
 18 the extinct chimney was carefully separated for cell counting and 16S rRNA gene
 19 amplicon analysis in combination with bulk mineralogical analysis. Cell densities in
 20 the chimney interior and exterior were determined by microscopic observations to be
 21 1.9×10^8 and 1.4×10^8 cells/ cm^3 , respectively, similar to those in extinct chimneys from
 22 Central Indian Ridge and western Pacific¹⁶. Single-gene analysis based on 16S rRNA
 23 gene sequences revealed that members of Pacearchaeota formerly referred to as Deep

1 Sea Hydrothermal Vent Euryarchaeotal Group 6 (DHVE-6) were predominant in the
 2 chimney interior, remarkably different from the chimney exterior, which was
 3 dominated by members of the bacterial phylum Nitrospirae (**Supplementary Figure 8**
 4 **and Supplementary Table 1**). Powder X-ray diffraction (XRD) analysis revealed
 5 both subsamples were composed of chalcopyrite, marcasite and pyrite
 6 (**Supplementary Figure 9**). It is likely that the remarkable difference in microbial
 7 composition can be attributed to textural features of the chimney interior (massive)
 8 and exterior (porous) (**Figure 1d**). This notion is supported by very minor occurrences
 9 of Pacearchaeota in previous studies of porous parts of extinct chimneys mainly
 10 containing chalcopyrite^{16,17}. The absence of Epsilonproteobacteria, indicator
 11 organisms of active fluid venting in deep-sea hydrothermal fields¹⁷, is consistent with
 12 the extinct nature of the chimney sample.

13

14 **Genomic features of the chimney Pacearchaeota**

15 As 16S rRNA gene amplicon analysis supported the idea that the Pacearchaeota
 16 members are predominant in the inner chalcopyrite wall, a near-complete genome of
 17 Pacearchaeota representative of 16S rRNA gene sequences was reconstructed by
 18 metagenomic analysis. In addition to analysis of a 16 rRNA gene sequence in the
 19 reconstructed genome (**Figure 4a**), ribosomal protein S3 region (rpS3) gene sequence
 20 analysis further confirmed phylogenetically affiliation with the Pacearchaeota phylum
 21 (**Figure 4b**). The genome size was very small (0.81 Mbp), with a genomic
 22 completeness of 90.4%, based on 52 single copy genes (**Supplementary Tables 2, 3**)

1 ¹⁸. The small genome is consistent with genome sizes of Pacearchaeota members from
2 previous studies (**Figure 4c**)¹⁸⁻²⁰.

3

4 With respect to carbon metabolism, nearly full sets of genes involved in
5 glycolysis and the non-oxidative pentose phosphate pathway (PPP) were encoded in
6 the IPdc Pacearchaeota genome, indicating adenosine triphosphate (ATP) generation
7 via fermentation of sugar-based biomolecules (**Figure 4c, Supplementary Figure 10,**
8 **Supplementary Table 4**). Pacearchaeota-related genes for the nucleotide salvage
9 pathway, including those encoding adenosine monophosphate (AMP) phosphorylase
10 (*deoA*) and ribulose 1,5-bisphosphate carboxylase/oxygenase (*RuBisCO*), were found
11 in IPdc contigs without being binned into genomes (**Figure 4c, Supplementary Table**
12 **5**). Phylogenetic analysis revealed that Pacearchaeota-related *RuBisCO* from IPdc
13 contigs was classified as Type III-b and closely related *RuBisCO* genes in the
14 Pacearchaeota genomes from the previous studies (**Supplementary Figure 11**)¹⁸⁻²⁰.
15 Amino acid sequence motifs that control catalytic activity and substrate binding were
16 identical to those found in *Methanocaldococcus jannaschii*, the purified *RuBisCO* of
17 which has carboxylase and oxygenase activities²¹.

18 Archaeal type III *RuBisCO*s are not associated with the typical role of
19 *RuBisCO* in CO₂ fixation in the Calvin-Benson-Bassham cycle; instead, they have
20 recently been demonstrated to be involved in AMP metabolism^{22,23}. In this pathway,
21 AMP is converted to ribulose-1,5-bisphosphate (RuBP), and then the generation of
22 two molecules of glycerate 3-phosphate from RuBP and CO₂ is catalysed by the type

1 III archaeal RuBisCOs (**Supplementary Figure 11**). These reactions are important to
2 feed glycerate 3-phosphate into glycolysis for extra ATP generation.

3

4 **Cross-linking cellular and genomic features**

5 We could not directly identify the ultra-small cells by performing fluorescence *in-situ*
6 hybridization in thin sections (**Supplementary Figure 12**)²⁴. Thus, we attempted to
7 correlate the IPdc Pacearchaeota genome with the ultra-small cells in the grain
8 boundary of the inner chalcopyrite wall. Given DPANN archaea with small cells have
9 small genomes²⁵⁻²⁷, the <200-nm sized cells observed by TEM (**Figure 2e**) are
10 consistent with the small genome size of the IPdc Pacearchaeota genome (**Figure 4c**).
11 Cuprite nanoparticles, which were extracellularly associated with the ultra-small cells
12 (**Figure 2f, g, h**), are thermodynamically stable in anoxic and neutral-to-alkaline
13 conditions²⁸. In the presence of O₂, the oxidation of cuprite nanoparticles is fast²⁹.
14 These cuprite characteristics support the inference that the grain boundary has been
15 anoxic since the termination of fluid venting. This inference is also supported by the
16 presence of a Pacearchaeota-affiliated ferredoxin gene, which is an indicator of
17 anaerobic metabolism³⁰. The hyperthermophilic archaeon *Archaeoglobus fulgidus* is
18 known to use Cu-exporting ATPase (CopA) and soluble Cu chaperone (CopZ) for
19 translocating intracellular Cu(I) through cytoplasmic membrane (**Supplementary**
20 **Figure 10**)³¹. Both genes encoded in the IPdc Pacearchaeota genome appear to play
21 key roles in the extracellular formation of Cu(I)-bearing cuprite nanoparticles. As
22 cuprite nanoparticles have anti-bacterial effects³², the close association with cuprite

1 nanoparticles could help the Pacearchaeota member to dominate against bacterial
2 competitors in the chimney interior.

3 Nanosolid analyses revealed that the grain boundaries of the inner
4 chalcopyrite wall were associated with amorphous silica. Amorphous silica is known
5 to adsorb nucleic acids and is widely used in molecular biology methods to purify
6 DNA and RNA from other cellular components such as proteins and lipids³³. We
7 hypothesize that the ability of amorphous silica to selectively concentrate nucleic
8 acids from seawater might explain the dominance of the Pacearchaeota member that
9 produces ATP from nucleic acid fermentation in the grain boundaries
10 (**Supplementary Figure 10**). To demonstrate binding of nucleic acids to amorphous
11 silica in seawater, DNA extracted from *Escherichia coli* was loaded into a silica-based
12 spin column. In slightly alkaline conditions (pH ~8), DNA was strongly bound to
13 amorphous silica in seawater, whereas DNA binding was very limited in a low-salinity
14 solution such as TE buffer (**Supplementary Figure 13**). Thus, the possibility that
15 silica-bound nucleic acids could serve as carbon and energy sources in the chimney
16 interior was experimentally confirmed.

17

18 **Implications**

19 In the chimney inner wall, most of organic matter such as nucleic acids is initially
20 destructed by black smoker (>300°C)³⁴. Regardless of venting low- and
21 high-temperature fluids, amorphous silica is ubiquitously formed inside the chimney
22 by cooling of hydrothermal fluids³⁵. By utilizing nucleic acids bound on amorphous

1 silica from seawater, microorganisms could obtain sufficient energy and carbon
2 sources to thrive inside extinct chimneys.

3 DPANN archaea are deep-branching and potentially diverged from
4 non-DPANN archaea at an early evolutionary stage³⁶. DPANN archaea are known to
5 have small genome sizes and lack many biosynthetic pathways^{18,37}. In particular, a
6 member of Pacearchaeota has the smallest genome among DPANN archaea³⁷. The
7 Pacearchaeota members from deep-sea metal sulfide deposits have relatively many
8 genes for glycolysis, the PPP and the nucleotide salvage pathway, whereas many
9 Pacearchaeota members from groundwater samples lack many genes involved in
10 nucleic acid fermentation in their reconstructed genomes^{18,37}. We suggest that these
11 genes are necessary for survival in the deep-sea rocky biosphere using nucleic acids
12 bound to amorphous silica and/or released from other microorganisms.

13 The interior of the extinct chimney, found to be dominated by Pacearchaeota,
14 appears to be environmentally similar to the early anoxic ocean on Earth. Salinity is an
15 important factor controlling binding of nucleic acids to silica. The salinity range of the
16 primitive ocean is estimated to be comparable to, or higher than, that of the modern
17 ocean³⁸. We therefore suggest that auxotrophy based on nucleic acid fermentation
18 encoded in Pacearchaeota and other DPANN genomes may have played important
19 roles in underpinning microbial ecosystems at hydrothermal vents on the early Earth.
20 This possibility is supported by silica-bearing filaments within massive chalcopyrite
21 textures in a 3.25-billion-year-old submarine hydrothermal deposit³⁹.

1   **Captions for Figures**

2   **Figure 1| Sampling of an extinct chimney and microbial signal detections. a,**

3   Bathymetric map of the southern Marina Trough showing the Pika site and Guam. **b,**

4   Photograph showing the extinct chimney sampled by the remotely operated vehicle

5   *Hyper Dolphin*. **c,** Light microscopy image of a thin section from the extinct chimney.

6   **d,** Reflection light microscope image of the thin section. The inner chalcopyrite wall is

7   indicated with arrows. **e,** Fluorescence microscopy image of a grain boundary with

8   greenish cell-like signals where a 3- μ m thick focused ion beam (FIB) section was

9   fabricated. **f,** Ga ion image of a 3- μ m thick FIB section from the grain boundary shown

10   in Figure 1e. **g,** Nanoscale secondary ion mass spectrometry (NanoSIMS) images of

11   the FIB-fabricated grain boundary with intensity color contours. **h,** Fluorescence

12   microscopy image of a grain boundary with greenish cell-like signals where a 300-nm

13   thick FIB section was fabricated. **i,** Ga ion image of a 300-nm thick FIB section as

14   pointed in Figure 1i. **j,** NanoSIMS images of the FIB-fabricated grain boundary with

15   intensity color contours.

16

17   **Figure 2| Visualization of microbial cells associated with Cu-bearing**

18   **nanocrystals in chalcopyrite grain boundaries. a,** Fluorescence microscopy image

19   of a grain boundary with greenish cell-like signals where a 150-nm thick FIB section

20   was fabricated. **b,** Ga ion image of a 150-nm thick FIB section of the grain boundary

21   shown in Figure 2a. A green dotted line shows a hole corresponding to a chalcopyrite

22   grain in the FIB section. **c,** NanoSIMS images of the 150-nm thick FIB section with

23   intensity color contours. Yellow arrows indicate the region presented in Figure 2e. **d,**

24   TEM image of a ribbon-shaped grain boundary of a 150-nm thick FIB section. A

25   yellow square indicates the same region presented in Figure 2e. **e,** TEM image of small

1 spheres. **f**, Selected area electron diffraction pattern from the small spheres in Figure
 2 2e. Arrows indicate ring patterns with interplanar spacings. **g**, Energy dispersive X-ray
 3 spectrum from the small spheres. **h**, High-resolution TEM image of crystalline
 4 nanoparticles in the region pointed by a black arrow in Figure 2e. White lines indicate
 5 lattice fringes of a nanocrystal with the rim indicated by a pink dotted line.

6

7 **Figure 3| Biological signatures determined by imaging mass spectrometry and**
 8 **Raman spectroscopy from chalcopyrite grain boundaries. a**, Reflection light
 9 microscope image of inner chalcopyrite wall. The red square highlights the region
 10 observed at a higher magnification in Figure 3b. **b**, Mapping image of a
 11 macromolecule at with $m/z = 805.2$ by imaging mass spectrometry analysis with an
 12 intensity color contour. **c**, Optical microscope image overlapped with a Raman
 13 spectroscopy mapping image of the peak intensity at $2800\text{--}3000\text{ cm}^{-1}$, as highlighted
 14 by a yellow dotted rectangle. **d**, Raman spectrum obtained from the region indicated
 15 by a white arrow in Figure 3c. Red arrows indicate peaks not found in the resin or
 16 minerals spatially associated with the small spheres, while back arrows indicate peaks
 17 also found in the resin (**Supplementary Figure 7**).

18

19 **Figure 4| Comparative analysis of Picea crataegus-affiliated genomes from**
 20 **present and previous studies. a–b**, Neighbor-joining phylogenetic trees based on
 21 nucleotide sequences of 16S rRNA (left) and amino-acid sequences of ribosomal
 22 protein S3 (right). Branches with bootstrap values $>50\%$ are indicated with filled
 23 circles. Colors indicate sampling sources: deep-sea metal sulfide deposits (red)²⁰;
 24 shallow groundwater, Rifle Site, Colorado (blue)¹⁸; and deep groundwater, Crystal

Geyser Site, Utah (green)¹⁹. **c**, Overview of presence or lack of genes for metabolic pathways such as glycolysis, the pentose phosphate pathway, and nucleotide salvage pathway. The lack of genes does not indicate their absolute absence in the original genome, because of the incompleteness of genome reconstruction. KEGG Orthology (KO) numbers for these genes are listed in Supplementary Table 5. A circle indicates the presence of the gene in the near-complete IPdc Pacearchaeota genome, whereas a triangle indicates its presence in contig sequences assembled from IPdc sequence fragments.

Methods

Sample collection and subsampling. A metal sulfide chimney was collected from an active hydrothermal vent field at the Pika site (12°55.15'N, 143°36.96'E) in the Southern Mariana Trough during the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) Scientific Cruises NT12-24 of the R/V *Natsushima* in September of 2012. The Southern Mariana Trough is a back-arc basin where the Philippine Sea Plate is subducted (**Figure 1a**). The chimney structure visually confirmed for the lack of hydrothermal fluid venting was collected by the manipulator arm of remotely operated vehicle (ROV) *Hyper Dolphin* (**Figure 1b**). The metal sulfide chimney was placed into an enclosed container in order to minimize contamination from surrounding seawater during transportation to the surface.

The collected chimney was immediately subsampled onboard in a cold room at 4°C. Using sterile chisels and spatulas, the exterior portion of the sample was subsampled. For subsampling of the interior portion, the chimney surface was flamed by a gas torch to prevent contamination from the exterior portion. Some intact portions

1 of the chimney structure were preserved for light and electron microscopic
 2 observations, μ -Raman spectroscopy, imaging mass spectrometry and nanoscale
 3 secondary ion mass spectrometry (NanoSIMS) analysis for mineral and microbial
 4 distributions, while the rest of the chimney structure was ground into powder by using
 5 sterile pestles and mortars. Both the intact and the ground subsamples were fixed with
 6 3.7% formamide in seawater onboard. The rest of the subsamples were frozen at -80°C
 7 for DNA extraction and mineralogical characterizations. All solutions were filtered
 8 with a 0.22- μ m-pore-size filter (Millipore).

9 **Mineralogical and microbiological characterizations of thin sections from the**
 10 **metal sulfide chimney.** To clarify mineral composition and microbial distribution
 11 within the chimney interior, thin sections were prepared using LR White resin. The
 12 intact chimney subsamples were dehydrated twice in 100% ethanol for 5 min, and then
 13 infiltrated four times with LR White resin (London Resin Co. Ltd.) for 30 min and
 14 solidified in an oven at 50°C for 48 h. Solidified blocks were trimmed into thin
 15 sections and polished with corundum powder and diamond paste. For the staining of
 16 microbial cells embedded in LR White resin, TE buffer with SYBR Green I
 17 (TaKaRa-Bio, Inc.) was mounted on thin sections and covered with cover glasses.
 18 After dark incubation for 5 min, thin sections rinsed with deionized water and
 19 mounted with the antifade reagent VECTASHIELD (Vector Laboratories) were
 20 observed using a fluorescence microscope (Olympus BX51) and a charge-coupled
 21 device (CCD) camera (Olympus DP71). Two ranges of fluorescence between
 22 540–570 nm and 570–600 nm were used to discriminate microbial cells from
 23 mineral-specific fluorescence signals.

1 Mineral assemblages and textures were observed using the same microscope
2 with the transmission light mode. Reflection light microscopy observations were
3 conducted by an optical microscope (Nikon ECLIPSE E600POL E6TP-M61) and a
4 CCD camera (Zeiss AxioCam MRc 5). Carbon-coated thin sections were
5 characterized using a scanning electron microscope (Hitachi S4500) at an accelerating
6 voltage of 15 kV. Back-scattered electron imaging coupled to energy-dispersive X-ray
7 spectroscopy (EDS) was used to analyze the chemical compositions of mineral phases
8 according to image contrasts.

9 To analyze microbial cells found in thin sections by NanoSIMS at the Kochi
10 Institute for Core Sample Research (KOCHI), JAMSTEC (CAMECA NanoSIMS
11 50L), 3- μ m- 300-nm- and 150-nm-thick sections were fabricated using focused ion
12 beam (FIB) sample-preparation and micro-sampling techniques using a Hitachi
13 FB-2100 instrument at the University of Tokyo or a Hitachi SMI-4050 at KOCHI. The
14 thin-section samples were locally coated with the deposition of W (100–500-nm thick)
15 for protection and trimmed using a Ga-ion beam at an accelerating voltage of 30 kV. A
16 focused primary Cs^+ ion beam of approximately 1.0 pA (100-nm beam diameter) was
17 rastered on the samples. Secondary ions of $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}_2^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{28}\text{Si}^-$, $^{31}\text{P}^-$ and
18 $^{32}\text{S}^-$ were acquired simultaneously with multidetection using seven electron
19 multipliers at a mass-resolving power of approximately 4500. Each run was initiated
20 after stabilization of the secondary ion-beam intensity following presputtering of <
21 approximately 2 min with a relatively strong primary ion-beam current (approximately
22 20 pA). Each imaging run was repeatedly scanned (15 to 20 times) over the same area,
23 with individual images comprising 256×256 pixels. The dwell times were 5,000
24 $\mu\text{s}/\text{pixel}$ for the measurements, and total acquisition time was approximately 2 h. The

1 images were processed using the NASA JSC imaging software for NanoSIMS
2 developed by the Interactive Data Language program⁴⁰.

3 Transmission electron microscopy (TEM) was used to examine microbial
4 distributions and the structure and composition of minerals at the nanometre scale.
5 JEOL JEM-2010 with energy dispersive X-ray spectrometry (EDS) was operated at
6 200 kV at the University of Tokyo. A JEOL JEM-ARM200F transmission electron
7 microscope was used at an accelerating voltage of 200 kV at KOCHI, JAMSTEC.

8 **Imaging mass spectrometry (MS).** An atmospheric pressure matrix-assisted laser
9 desorption/ionization system equipped with a quadrupole ion trap-time of flight
10 analyzer (MALDI-TOF) was used to obtain MS data from spots or regions observed
11 by microscopy (iMScope *TRIO*, Shimadzu). The thin section subjected to nanosolid
12 characterizations was further thinned by a mechanical polisher (Leica EM TXP Target
13 Preparation Device). The thin section was coated with 9-aminoacridine (Merck) by
14 iMLayer (Shimadzu) with a thickness of 1.0 μm and then irradiated by a 355 nm
15 Nd:YAG laser with a laser diameter of $\sim 5 \mu\text{m}$. The positive ion mode was used for
16 imaging of the thin section. Scanning was performed with a pitch of 5 μm . Operation
17 conditions were as follows: frequency, 1000 Hz; 50 shots per spot. To visualize the
18 ion images, Imaging MS Solution (Shimadzu) was used. For a reference, LR White
19 resin sectioned into a thickness of 20 μm by the ultramicrotome was mounted on a
20 carbon tape.

21 **Raman spectroscopy.** High-resolution confocal Raman system (Horiba LabRam HR)
22 equipped with a laser (532 nm) was used to characterize chalcopyrite grain boundaries
23 on a thin section. The incident laser was operated at 1.2–4 mW. Analytical uncertainty

1 in the Raman shift was $\sim 2 \text{ cm}^{-1}$, and the spatial resolution was $\sim 1 \text{ }\mu\text{m}$. Raman spectra
2 were compared with standard spectra obtained from RRUFF (<http://rruff.info>), and
3 peak assignments were based on references^{13,41-43}.

4 **Uranium reduction experiments and TEM observations of incubated cells.**

5 *Desulfovibrio desulfuricans* [ATCC#642] and *Geobacter sulfurreducens*
6 [ATCC#51573] were subjected to uranium reduction experiments previously
7 performed for *Desulfosporosinus* spp.⁴⁴ In an anaerobic glove box with a gas mixture
8 of $\text{N}_2\text{-CO}_2\text{-H}_2$ (90:5:5), cells enriched in media recommended by ATCC were
9 incubated in 0.25% bicarbonate solution at pH 7 containing 1 mM of uranyl acetate. 10
10 mM sodium lactate and 10 mM sodium acetate were added for electron donors of *D.*
11 *desulfuricans* and *G. sulfurreducens*, respectively. After 24-h incubation, cells were
12 harvested by centrifugation at $10,000\times g$ for 3 min. Whole mounts of the incubated
13 cells of *D. desulfuricans* and *G. sulfurreducens* were observed by TEM (JEOL
14 JEM-2010). The incubated cells of *D. desulfuricans* were embedded in LR White resin
15 as described above, and 100-nm thick sections prepared with an ultramicrotome
16 (Ultracut S, Reichert-Nissei, Tokyo, Japan) were also observed by TEM.

17 **Mineralogical and microbiological characterizations of powdered chimney**

18 **samples.** To clarify mineral compositions of the powdered subsamples, X-ray
19 diffraction (XRD) pattern analysis was performed by Rigaku RINT 2000 powder
20 diffractometer at 40 kV and 30 mA. The total cell numbers of the chimney interior and
21 exterior were measured by a direct count method with SYBR Green I. The
22 formaline-fixed powdered samples were suspended in 1:1 ethanol / phosphate
23 buffered saline (PBS) solution. Some portions of the suspensions were sonicated for

1 30 sec. A 0.22- μ m-pore-size, 25-mm-diameter polycarbonate filter (Millipore) was
2 used to collect the sonicated suspension. To stain microbial cells, the filter was
3 incubated in TAE buffer containing SYBR Green I for 5 min at room temperature. The
4 stained filter was briefly rinsed with deionized water and observed under
5 epifluorescence using the Olympus BX51 microscope with the Olympus DP71 CCD
6 camera.

7 **DNA extraction and 16S rRNA gene amplicon analysis.** Prokaryotic DNA was
8 extracted from 0.1 g of frozen powdered chimney subsamples as described
9 previously⁴⁵. In brief, the powdered chimney subsample was incubated at 65°C for 30
10 min in 300 μ L of alkaline solution consisting of 75 μ L of 0.5 N NaOH and 75 μ L of TE
11 buffer (Nippon Gene Co.) including 10 mM Tris-HCl and 1 mM EDTA. After
12 incubation, the aliquots were centrifuged at 5,000 $\times$ g for 30 sec. Then, the supernatant
13 was transferred into a new tube and neutralized with 150 μ L of 1 M Tris-HCl (pH 6.5;
14 Nippon Gene Co.). After neutralization, the DNA-bearing solution (pH 7.0–7.5) was
15 concentrated using a cold ethanol precipitation, and the DNA pellet was dissolved in
16 50 μ L of TE buffer. The purified DNA solution was stored at -4°C or -20°C for longer
17 storage. For the negative control, DNA extraction from the subsample was performed
18 in parallel with one extraction negative control to which no sample was added.

19 The 16S rRNA gene was amplified using LA Taq polymerase (TaKaRa-Bio,
20 Inc.). For pyrosequencing, the 454 GS-junior sequencer (Roche Applied Science) was
21 used. The primers Uni530F and Uni907R⁴⁶ were extended with adaptor sequences
22 (CCATCTCATCCCTGCGTGTCTCCGACTCAG for Uni530F and
23 CCTATCCCCTGTGTGCCTTGGCAGTCTCAG for Uni907). The forward primer
24 Uni530 was barcoded with 8-mer oligonucleotides⁴⁷. Thermal cycling was performed

1 with 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 45 sec, and
 2 extension at 72°C for 1 min. A PCR product with the expected size was excised from
 3 1.5% agarose gels after electrophoresis on TAE (40 mM Tris acetate, 1 mM EDTA, pH
 4 8.3), which was purified using MinElute Gel Extraction Kit (Qiagen, Inc.). A DNA
 5 concentration of the purified PCR product was measured by the Quant-iT dsDNA HS
 6 assay kit and the Qubit fluorometer (Invitrogen, Inc.). The concentration of total
 7 double-stranded DNA in each sample was adjusted to 5 ng/μL. Emulsion PCR was
 8 performed using the GS FLX Titanium emPCR Kit Lib-L (Roche Applied Science) to
 9 enrich DNA library beads for the 454 GS-junior sequencers. Amplified DNA
 10 fragments were sequenced according to the manufacturer's instructions.

11 Raw reads were demultiplexed, trimmed and filtered based on their 8-bp
 12 sample-specific tag sequences, quality values (QV) and lengths using Mothur v. 1.
 13 31⁴⁸ to obtain unique reads more than 250 base pairs (bp), and an average quality score
 14 >27. Filtered sequences were aligned with Mothur to the Greengenes reference
 15 database⁴⁹, and chimeric sequence reads were removed with Chimera Uchime in
 16 Mothur. Sequence reads were clustered into operational taxonomic units (OTU)
 17 sharing 97% identity within each OTU. Phylogenetic affiliations of the OTUs were
 18 assigned by the neighbor joining method in the ARB software package⁵⁰, along with
 19 closely related sequences retrieved from GenBank
 20 (<http://www.ncbi.nlm.nih.gov/genbank/>) through BLASTn searches (somewhat
 21 similar sequences).

22 **Genome-resolved metagenomic analysis.** From the subsample of the chimney
 23 interior, genomic DNA was extracted by a using an UltraClean Soil DNA Isolation Kit
 24 (MoBio Laboratories). As described previously⁵¹, shotgun library construction was

1 performed for the extracted DNA using a KAPA Hyper Prep kit for Illumina (KAPA
2 Biosystems), and sequencing of the library was performed on an Illumina MiSeq
3 platform (MiSeq PE300). The trimming and filtering of the reads were performed
4 using CLC Genomics Workbench (QIAGEN Aarhus A/S)¹⁶. After the high-quality
5 reads were assembled using SPAdes⁵², the produced contigs were binned into a
6 near-complete genome using Metabat⁵³. The gene prediction and annotation of the
7 contigs and the near-complete genome were annotated using Prokka⁵⁴ and the Kyoto
8 Encyclopedia of Genes and Genomes (KEGG) pathway tool⁵⁵ with the Blast-KOALA
9 tool⁵⁶. Initial analyses of near-complete genomes from Crystal Gysler¹⁵ were
10 performed by ggKbase (<https://ggkbase.berkeley.edu>). For selected functional genes,
11 closely related sequences and their source organisms were initially accessed using
12 NCBI Protein BLAST Program⁵⁷. Amino-acid sequences were aligned using Muscle⁵⁸.
13 To construct a neighbor-joining tree, the ARB software package was used⁵⁰.

14 **Fluorescence *in-situ* hybridization analysis.** Whole-cell hybridization was
15 performed for thin sections of the intact chimney subsample embedded in LR-White
16 resin. Hybridization was conducted at 46°C in a solution containing 20 mM Tris–HCl
17 (pH 7.4), 0.9 M NaCl, 0.1% sodium dodecyl sulfate, 30% (v/v) formamide and 50
18 ng/μL of each probe labeled at the 5' end with fluorescence dye. A Cy-5 labeled probe
19 targeting the domain Archaea (Arch915: 5'-GTGCTCCCCCGCCAATTCCT-3')⁵⁹
20 and a Cy-5 labeled probe targeting Pacearchaeta (Pace915:
21 5'-GTGTCTCCCCGCCAATTCCT-3') were used for hybridization of the positive
22 control and the chimney thin sections, respectively. After hybridization, the specimens
23 were washed at 48°C in a solution lacking the probes and formamide at the same
24 stringency, adjusted by NaCl concentration. After staining with

1 4',6-diamidino-2-phenylindole (DAPI) at 0.4 µg/ ml, the slides were examined using
2 the Olympus BX51 microscope. For the positive control, cultured archaeal cells of
3 *Methanocaldococcus* sp. Mc-365-70 were used. For the negative control, a
4 bacteria-specific probe named EUB338 (5'-GCT GCC TCC CGT AGG AGT-3')⁶⁰
5 was used to check the absence of non-specific binding of the EUB338 probe on the
6 cultured archaeal cells under the same hybridization conditions.

7 **DNA binding experiments to amorphous silica.** A silica-based spin column was
8 used to bind genomic DNA extracted from *Escherichia coli* (NBRC#20004). *E. coli*
9 was grown at 30°C in LB liquid medium and harvested by centrifugation at 8,000×g
10 before reaching the stationary growth stage. The *E. coli* cells were subjected to DNA
11 extraction as described above for metagenomic analysis. Some portions of the DNA
12 extract diluted in seawater and TE buffer were loaded on silica-based spin columns
13 (MoBio Laboratories). After loading of the diluted DNA extracts, the silica-based
14 spin columns were centrifuged at 10,000×g to recover the loaded DNA extracts. DNA
15 concentrations in the DNA extracts before and after loading to the silica-based spin
16 columns were measured with by the Quant-iT dsDNA HS assay kit and the Qubit
17 fluorometers. For the seawater-based experiment, the DNA-loaded spin column was
18 eluted with TE buffer. The recovered DNA was quantified as described above. Fourier
19 transformed infrared-ray (FT-IR) spectrometry (Shimadzu IRSprite) was used to clarify
20 the crystallinity of silica. Powder materials were analyzed by a mode of attenuated
21 total reflection (ATR) through KBr beam-splitter with temperature-controlled
22 DLATGS detector. Representative FT-IR spectra were collected by averaging 45
23 individual spectra. Amorphous silica (Aldrich) was used for a reference.

24

1   **Data and Code availability**

2   All data needed to evaluate the conclusions in the paper are present in the paper and/or
3   the Supplementary Materials. The 16S rRNA gene sequences in this study were all
4   deposited in the DDBJ nucleotide sequence database with accession numbers
5   LC554901-LC555740. The metagenome-assembled genome sequences were
6   deposited with accession numbers BH XO01000001–BH XO01000035 under the
7   BioProject accession number, PRJDB6687. The Pacearchaeota genome constructed in
8   this study was named as IPdc11 in the database.

9

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20   **References**

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