

Supplemental data

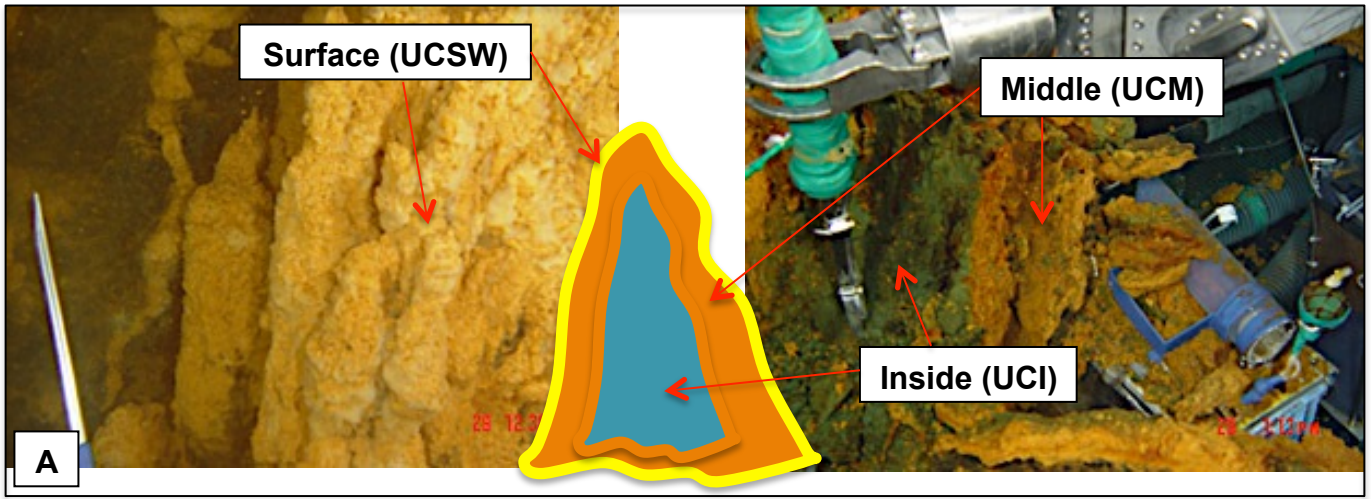
Figure captions

Figure S1. Photographs, conceptual diagram, SEM and EDX images of Fe-(oxyhydr)oxide chimneys collected from Urashima site. Conceptual diagram and photographs of Fe-rich chimney structure at Urashima site are shown in (A).

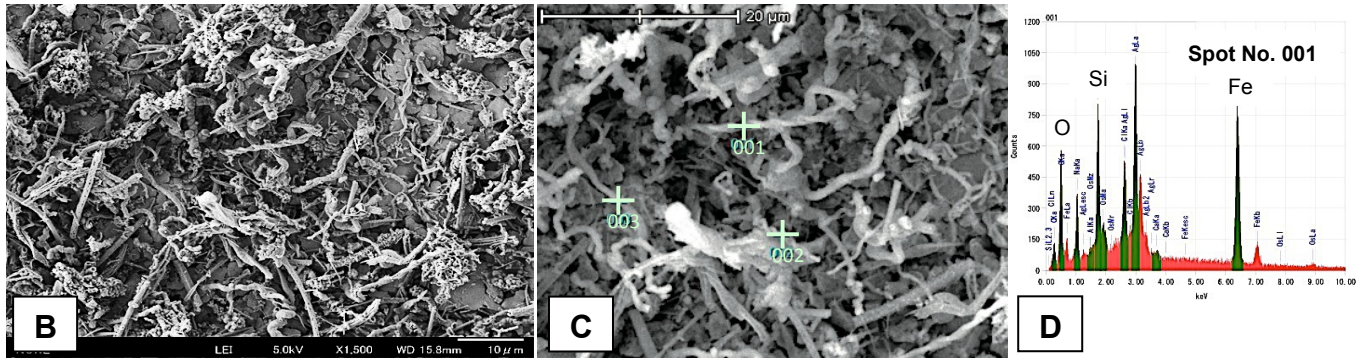
SEM images (B, C, E, F, H, I) and EDS spectrums (D, G, J) of the Fe-(oxyhydr)oxide samples collected from chimney of Urashima site are shown. Mint Green crosses indicate the EDS analysis areas in (C), (F) and (I). Only one spot (spot no. 001) data of each EDS analysis are shown in (D), (G) and (J). (B), (E) and (H) are same view. Scale bars, 10 μm . (C), (F) and (I) are same view. Scale bars, 20 μm .

Figure S2. Rarefaction curves from 16S rRNA gene clone libraries.

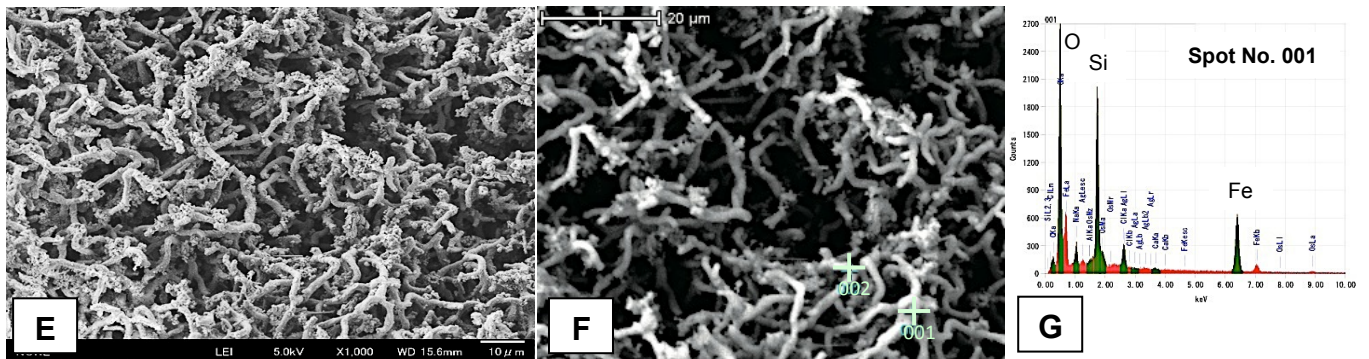
Phylotypes were defined at 97% sequence similarity. Rarefaction analysis (Heck et al., 1975 (1)) was performed using the DOTUR program (Schloss and Handelsman, 2005 (2)) to evaluate the phylotype richness of microbial communities in the iron mats.



Chimney surface at Urashima site (UCSW)



Chimney interior (middle) part at Urashima site (UCM)



Chimney most interior (inside) part at Urashima site (UCI)

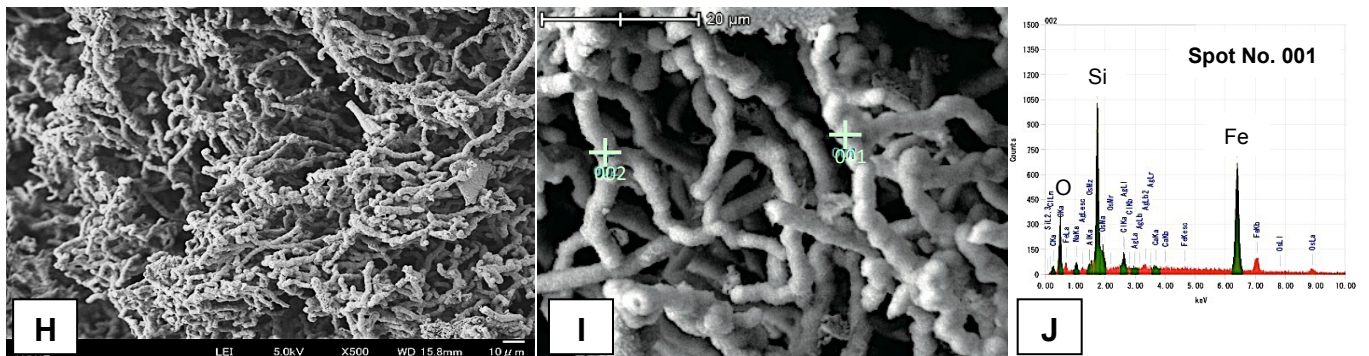


Figure S1

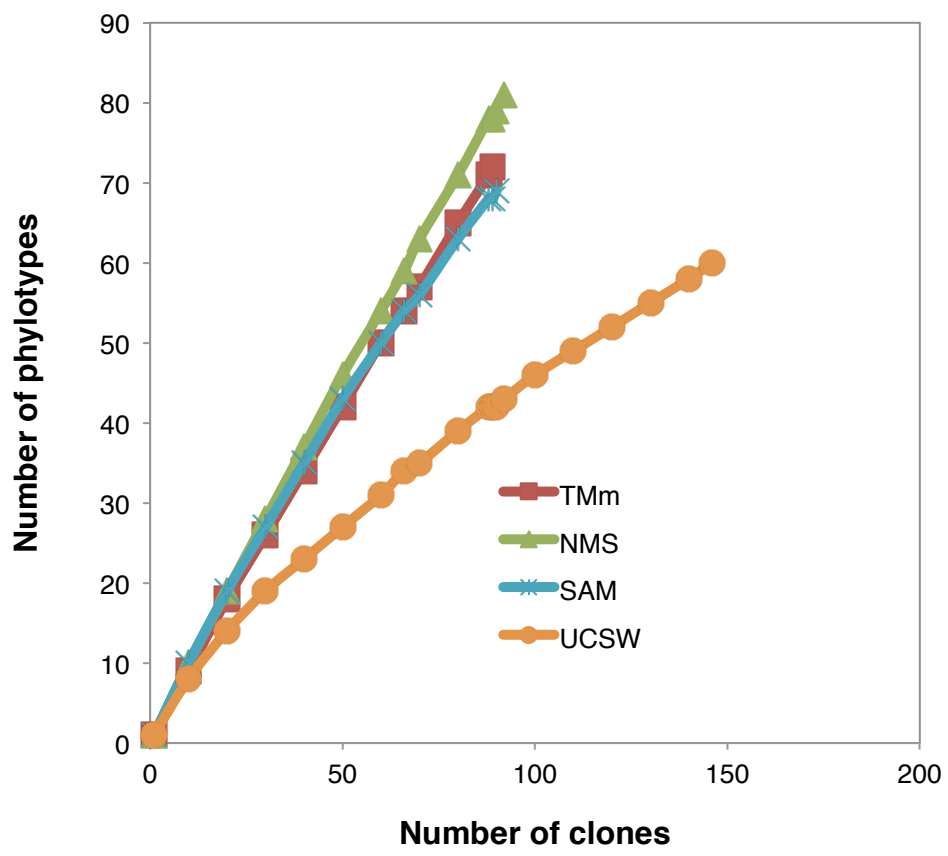


Figure S2

TABLE S1. A summary of the differences between genus *Mariprofundus* and genus *Gallionella*.

Genus	<i>Mariprofundus</i>	<i>Gallionella</i>	
Class	<i>Zetaproteobacteria</i>	<i>Betaproteobacteria</i>	
Species	<i>M. ferrooxydans</i>	<i>G. ferruginea</i> ,	<i>G. capsiferiformans</i>
Feature of Strains:	PV-1 (3, 4)	(5-8)	ES-2 (9)
Isolate source	Deep-sea Hydrothermal sediment	Well water, Groundwater	Groundwater
Morphology	Curved rods	Bean-shapes or curved rods	Bean-shapes or curved rods
Stalks	+	+	+
Length (µm)	1.0 - 4.0	1.5-2.5	~0.8
Width (µm)	0.3 - 0.6	0.3-1.0	ND
Motility	+	+	+
Temperature range (optimum) for growth (°C)	10-30 (25-30)	5-25 (20)	4-30 (ND)
pH range (optimum) for growth	5.5-7.2 (6.2-6.5)	5.0-6.5 (ND)	5.5-7.5 (6.0-6.5)
Salinity range (optimum) for growth (% NaCl)	1.5-4.5 (2.5)	ND	ND
Energy sources:			
Fe(II)	+	+	+
Thiosulfate, sulfide	—	+	—
Other lithotrophy	—	—	—
Organic matter	—	+	—
Doubling time (h)*	12	10	12
G+C content (mol%)	54	35.2-37.6	52.7

ND: not determined

*Doubling time: In the proliferation when using iron as an energy source.

Table S1

TABLE S2. Elemental compositions of iron mat samples determined by SEM-EDS analysis.

Showed relative element concentrations for the iron mat samples.

Sample Element	TMm	NMS	SAM	SIM Mean % Weight	UCSW	UCM*	UCI**
C	4.2	9.6	6.9	10.1	5.6	9.1	5.1
O	18.9	21.3	36.3	42.1	14.1	39.4	19.0
Na	12.6	3.0	5.7	3.4	5.4	3.4	13.3
Mg	0.0	0.9	0.7	0.5	0.2	0.4	0.7
Al	0.4	0.1	0.0	0.1	0.1	0.1	0.1
Si	6.8	7.4	21.8	22.8	5.4	14.2	20.2
S	1.0	0.4	0.0	0.0	0.0	0.0	0.0
Cl	12.5	5.2	10.6	2.5	3.6	2.5	11.7
Ca	1.6	1.1	0.6	0.4	0.3	0.2	0.2
Fe	41.7	51.1	17.4	18.2	38.3	30.9	29.7
Ag	0.4	0.0	0.0	0.0	27.1	0.0	0.0

*Chimney interior part at Urashima site

**Chimney most interior parts at Urashima site

Table S2

TABLE S3. Further taxonomic affiliation of gammaproteobacterial 16S rRNA gene clones from iron mat samples in different deep-sea hydrothermal systems.

	TMm	NMI	NMS	SAM	SIM	UCSW
<i>Aciditibacillus</i>	1		3		1	
<i>Alteromonadales</i>	5	6	1	1	1	1
BD-8 marine group			1			
<i>Chromatiales</i>	1	14	3	2		
<i>Escherichia shigella</i>			1			
<i>Coxiellaceae</i>				1		1
<i>Methylococcales</i>				5	1	19
<i>Halomonadaceae</i>		1				1
<i>Pseudospirillum</i>		2				
<i>Salinicola</i>		1				
<i>Actinobacter</i>	3	2				
<i>Piscirickettsiaceae</i>		16	1			
<i>Leucothrix</i>			1			
Other gamma	3		9		3	
<i>Vibrio</i>		1	1			
<i>Sinobacteraceae</i>	1		1	2	6	
Total	14	43	22	11	12	23

Table S3

TABLE S4. Further taxonomic affiliation of deltaproteobacterial 16S rRNA gene clones from iron mat samples in different deep-sea hydrothermal systems.

	TMm	NMI	NMS	SAM	SIM	UCSW
43F-1404R	1			2	1	
<i>Bacteriovoraceae</i>		1			1	1
<i>Bdellovibrionaceae</i>				1	1	1
<i>Desulfobulbaceae</i>				1		7
<i>Desulfurellaceae</i>				2		3
DTB120	21		1	1	4	1
GR-WP33-30	5		1		2	
<i>Myxococcales</i>			4	2	4	2
SAR324 clad			2			
Sh765B-TzT-29			2	1		
Total	33	1	12	12	14	16

Table S4

TABLE S5. Further taxonomic affiliation of alphaproteobacterial 16S rRNA gene clones from iron mat samples in different deep-sea hydrothermal systems.

	TMm	NMI	NMS	SAM	SIM	UCSW
<i>Caulobacteraceae</i>	1	3				
MNG3					1	
Uncultured					3	
<i>Parvulaculaceae</i>					1	
<i>Rhodobacteraceae</i>	2		1	1		5
<i>Rhodospirillaceae</i>	2	7	6	1	8	1
<i>Rickettsiaceae</i>	1					1
S26-47			1			
SAR11 crade		2		1	2	
SB1-18					1	
<i>Sphingomonadacea</i>				2		
Total	6	12	8	5	16	7

Table S5

TABLE S6. Chemical compositions^I of hydrothermal fluid and/or pore-water of iron mat samples in the Fox site of Tarama knoll, the Yellow Top site and Champagne vent site of NW Eifuku field, the Snail and Urashima site of the southern Mariana Trough.

Sampling site	Sampling year	T _{max} ^{II} °C	pH	Mg (mM)	Ca (mM)	NH ₄ ⁺ (μM)	SO ₄ ²⁻ (mM)	H ₂ S (mM)	H ₂ (μM)	CO ₂ (mM)	CH ₄ (μM)
Fox site ^{II}	2010	24	6.4	48.8	12.0	98.2	25.6	ND	2.9	12.7	5.2
Yellow Top site	2010	2.8	7.4	ND ^{IV}	ND	<100	ND	0.026	ND	ND	ND
Champagne vent site ^V	2004	103	ND	43.0	ND	ND	ND	12.5	<0.2	3000	<0.2
Champagne vent site ^V	2005	108	ND	43.0	ND	ND	ND	30.0	<0.2	800	<0.2
Urashima site ^{VI}	2010	76.0	5.7	39.2	13.3	13.1	22.2	0.0	0.5	11.4	4.9
Snail site ^{VI}	2010	64.5	6.9	42.5	11.1	8.9	25.3	0.0	0	3.6	0.0

^I Measured at 25°C

^{II} T_{max}: Maximum temperature

^{III} Gas data was collected in 2011 (NT11-18 cruise; Makita et al. (10))

^{IV} ND: No data

^V Estimated end-member composition by Lupton et al. (11), based on extrapolating to Mg concentration 43 mM. The Champagne vent fluids had pH ranging from 3.4 to 4.8. The low end of measured pH of Champagne vent fluids was consistent with CO₂ buffering in the end-member fluid.

^{VI} A detailed discussion of Snail and Urashima site can be found in Toki et al. (12).

Table S6

References

1. Heck, K.L., Jr, Van Belle, G., and Simberloff, D. (1975) Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. *Ecology* **56**: 1459–1461.
2. Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71**: 1501–1506.
3. Emerson, D. and Moyer, C.L. (2002) Neutrophilic Fe-oxidizing bacteria are abundant at the Loihi Seamount hydrothermal vents and play a major role in Fe oxide deposition. *Appl. Environ. Microbiol.* **68**: 3085-3093.
4. Emerson, D., Rentz, J.A., Lilburn, T.G., Davis, R.E., Aldrich, H., Chan, C. and Moyer, C.L. (2007) A novel lineage of *Proteobacteria* involved in formation of marine Fe- oxidizing microbial mat communities. *PLoS ONE* **2**: e667. DOI: 10.1371/journal.pone.0000667.
5. Hallbeck, L., Pedersen, K. (1990) Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. *Journal of General Microbiology* **136**: 1675-1680.
6. Lütters-Czekalla, S. (1990) Lithoautotrophic growth of the iron bacterium *Gallionella ferruginea* with thiosulfate or sulfide as energy source. *Archives of Microbiology* **154**(5): 417-421
7. Hallbeck, L., Pedersen, K. (1991) Autotrophic and mixotrophic growth of *Gallionella ferruginea*. *Journal of General Microbiology* **137**: 2657-2661
8. Hallbeck, L., Ståhl, F., Pedersen, k. (1993) phylogeny and phenotypic characterization of the stalk-forming and iron-oxidizing bacterium *Gallionella ferruginea*. *Microbiology* **139**(7) 1531-1535
9. Emerson, D., Field, E.K., Chertkov, O., Davenport, KW., Goodwin, L., Munk, C., Nolan, M., Woyke, T. (2013) Comparative genomics of freshwater Fe-oxidizing bacteria: implications for physiology, ecology, and systematics. *Front Microbiol.* **4**:254. doi: 10.3389/fmicb.2013.00254. eCollection 2013.
10. Makita, H., Reimer, J.D., Yamanaka, T., Nunoura, T., Watanabe, H., Miyazaki, M., Toki, T., Ise, Y., White, K., Yanagawa, K., Shikiba, H., Fukumoto, N., Nagashio, H., Kikuchi, S., Kawai, E., Mochizuki, Y. (2011) *Natsushima NT11-18 Cruise report*. http://www.godac.jamstec.go.jp/catalog/data/doc_catalog/media/NT11-18_all.pdf
11. Lupton, J., Butterfield, D., Lilley, M., Evans, L., Nakamura, K., Chadwick Jr, W., Resing, J., Embley, R., Olson, E., Proskurowski, G., Baker, E., de Ronde, C., Roe, K., Greene, R., Lebon, G., Young, C. (2006) Submarine venting of liquid carbon dioxide on a Mariana Arc volcano. *Geochem. Geophys. Geosyst.*, **7**(8), 1-20, Q08007, DOI: 10.1029/2005GC001152, 2006.
12. Toki, T., Ishibashi, J., Noguchi, T., Tawata, M., Tsunogai, U., Yamanaka, T. and Nakamura, K. (2015) Chemical and Isotopic Compositions of Hydrothermal Fluids at Snail, Archaean, Pika, and Urashima Sites in the Southern Mariana Trough. In: *Subseafloor Biosphere Linked to Hydrothermal Systems: TAIGA Concept*, Ishibashi, J., Okino, K., Sunamura, M. (Eds.), pp.587-602, Springer Japan, DOI: 10.1007/978-4-431-54865-2_45.