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Extreme Spatial and Temporal Variability of Hydrothermal Microbial Mat Communities Along the Mariana Island Arc and Southern Mariana Back-Arc System

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Extreme spatial and temporal variability of hydrothermal microbial mat communities along the Mariana Island Arc and southern Mariana back-arc system

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[1] Twenty-five microbial communities were sampled from 18 different hydrothermal systems located at seven different sites along the Mariana Island Arc and at a single site from the southern Mariana Spreading Center over a 3-year period. Terminal restriction fragment length polymorphism (T-RFLP) analysis of the small subunit rRNA gene revealed that the microbial community diversity is much greater along the Mariana Arc/back-arc than at either hot spot volcanoes or mid-ocean ridges along the same spatial scale. Cluster analysis of T-RFLP fingerprints reveals the microbial communities formed three distinct clusters designated Mariana clusters I, II, and III. Microbial communities in Mariana Cluster I are all associated with iron-rich microbial mats and are dominated by members of the ζ-Proteobacteria and by unique phylotypes clustering deeply in the δ-Proteobacteria and within the Nitrospira division. Mariana Cluster II communities are all from shallow hydrothermal systems and mostly from colder sediments or microbial mats that are dominated by putative heterotrophic phylotypes usually associated with seawater and sediments not generally associated with hydrothermal fluid inputs. Mariana Cluster III is generally from much hotter vent sites and is dominated by sulfur-oxidizing ε-Proteobacteria. Quantitative-polymerase chain reaction (Q-PCR) of Archaeal abundance reveal that all of the microbial communities are dominated by members of the Bacterial domain. Sampling of microbial mats from Iceberg Vent at NW Rota-1 in 2004 and again in 2006 reveal the community has shown a transition from Caminibacter group ε-Proteobacteria phylotypes to a mixed population of Caminibacter, Sulfurovum, and Sulfurimonas group ε-Proteobacteria.

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1. Introduction

[2] The Mariana region is among the most volcanically active areas in the world. The Mariana Arc/back-arc system was formed as the converging northwest Pacific Plate subducted below the overriding Philippine Plate, beginning at least 50 Ma ago [*Seno and Maruyama*, 1984]. The Pacific Plate dives deep into the Earth forming the Mariana Trench and much of the plate is eventually melted and recycled within the mantle [*Tanimoto and Lay*, 2000]. Some of the subducted rock and sediment, however, are caught within a molten mantle wedge [*Hawkesworth et al.*, 1997]. The mantle wedge is a lens of convecting asthenosphere which melts and mixes the subducting pacific seafloor forming highly complex magmas [*Stern*, 2002]. Magma rapidly rises because it is less dense than the surrounding rock, forming active volcanoes and hydrothermal vents [*Turner et al.*, 2001]. The convecting asthenosphere also forms a back-arc spreading center within the Mariana Trough on the west side of the island arc [*Baker et al.*, 2005; *Kato et al.*, 2003].

[3] Hydrothermal discharge from volcanic arcs can be highly variable along the length of the arc because of the highly variable chemical composition of arc magma compared to magma from mid-ocean ridge systems. The arcs are constructed with chemically complex source materials [*Armstrong*, 1971; *Hawkesworth et al.*, 1993], and consequently, arc vent fluids often contain more variability and greater concentrations of gases and metals than hydrothermal fluids from mid-ocean ridges [*de Ronde et al.*, 2007; *Massoth et al.*, 2003; *Lupton et al.*, 2006]. Variable concentrations of chemicals used as microbial energy sources (e.g., Fe, S, CH_4) and physical conditions (e.g., temperature) coupled with the highly ephemeral nature of arc/back-arc hydrothermal systems should support more diverse microbial and macrofauna populations when compared to communities found at hot spot volcanoes and along mid-ocean ridges along a similar spatial scale.

[4] There are >6000 km of volcanic arcs in the western Pacific Ocean, of which only ~30% have been systematically surveyed [*Embley et al.*, 2006]. Submarine hydrothermal venting along these volcanic arcs therefore remains

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largely undetected and unexplored compared to mid-ocean ridge and hot spot systems where the vast majority of exploration and characterization resources have been concentrated. The lack of comprehensive knowledge of volcanic arcs is compounded by the incredible variability found in relatively short distances between active arc/back-arc volcanoes [*de Ronde et al.*, 2001, 2007; *Embley et al.*, 2004]. All together, these volcanoes represent a relatively unexplored hydrothermal source that may inject globally significant fluxes of heat and chemicals into the oceans [*Stein*, 1995].

[5] The submarine volcanoes of the Mariana Arc were systematically explored for the first time in 2003–2004 and again in 2006 as part of NOAA's Ocean Exploration Ring of Fire program (Figure 1). In 2003, much of the active volcanic seafloor was mapped and CTD Tow-Yo operations were performed at arc seamounts with suspected volcanic activity and along the southern back-arc spreading center to determine the presence of volcanic plumes. Areas with suspected active hydrothermal activity were identified for targeted remote operating vehicle (ROV) exploration and sampling in 2003–2004 and 2006.

[6] A potential hydrothermal plume on the southern Mariana Back-Arc Spreading Center (also known as the Malaguana-Gadao Spreading Center) was investigated in 2003 using the ROV Jason 2. A hydrothermal field named the Fryer Site was discovered with multiple high-temperature (77–248°C) hydrothermal vents with associated thick, iron-rich flocculate microbial mats [*Wheat et al.*, 2003].

[7] Twelve arc seamounts along the Mariana Island Arc with suspected hydrothermal activity were explored with the ROV ROPOS in 2004 and with the ROV Jason 2 in 2006. Most of these arc seamounts had multiple vent sites with vents ranging from white smokers with high concentrations of dissolved gases and gas bubbles (e.g., Champagne vent on NW Eifuku and Bubble Bath Vents on Daikoku) to black smokers (e.g., Five Fingers Vents, east Diamante) to low-temperature iron-rich vents (e.g., Yellow Cone/Top, NW Eifuku; Fe-Mounds, Esmeralda Bank; Fe-Mats, Seamount X).

[8] Microbial mats were observed growing at many of these vent fields. Microbial mats at hydrothermal vents are considered relatively large, variably thick (1 mm to 1 m), potentially complex biofilms that are usually dominated by chemoautotrophic bacteria metabolizing reduced metals and/or gases found in hydrothermal fluids [*Jannasch and Mottl*, 1985; *Jannasch and Wirsen*, 1981; *Moyer et al.*, 1995]. Hydrothermal sediments and chimneys are also habitats for chemoautotrophic microbial communities [*Teske et al.*, 2002; *Takai et al.*, 2001; *Schrenk et al.*, 2003; *López-García et al.*, 2003] that contribute to the primary production in these ecosystems. The microbial communities within these habitats can be highly variable between sites, largely dependent upon the heat and chemical composition of the hydrothermal fluids [*Moyer et al.*, 1995; *Sievert et al.*, 1999; *Taylor et al.*, 1999].

[9] The purpose of this study was to determine the variability of the microbial communities found in microbial mats, hydrothermal sediments, and within low-temperature hydrothermal chimneys from multiple seamounts and vent sites along the Mariana Arc/back-arc system. Terminal restriction fragment length polymorphism (T-RFLP) community fingerprinting [*Liu et al.*, 1997] of both Bacterial and Archaeal SSU rRNA genes was used to identify the similarity of the microbial communities within these habitats. T-RFLP fingerprinting is a sensitive genotyping method that is able to accurately resolve ribotypes in communities with low to intermediate richness [*Engebretson and Moyer*, 2003]. These fingerprints may also be compared using cluster analysis to determine the similarity between different microbial communities. Quantitative-polymerase chain reaction (Q-PCR) was used to calculate the ratio of Bacterial to Archaeal phylotypes within the microbial communities [*Takai and Horikoshi*, 2000]. Traditional clone library analysis of the SSU rRNA gene was then used to determine the phylogenetic relatedness of phylotypes found in the communities [*Moyer*, 2001]. This study represents the first community analysis of microbial mats and hydrothermal sediments from multiple volcanic centers on an arc/back-arc system.

2. Materials and Methods

2.1. Sample Collection

[10] Twenty-five discrete samples from the Mariana Arc/back-arc system were collected using either a push core or a suction sampler on either the ROV ROPOS or Jason 2 (Table 1). All samples were immediately frozen and maintained at −80°C until DNA extraction was performed on shore.

2.2. DNA Extraction

[11] Total genomic DNA (gDNA) was extracted from each sample in duplicate using the FastDNA Spin Kit for Soil following the manufacturer's protocol (Qbiogene, Irvine, California). Extracted gDNA from each sample was pooled, cleaned, and concentrated using Montáge PCR centrifugal filter devices (Millipore, Bedford, Massachusetts). The gDNAs were then quantified using a Nanodrop ND-1000 spectrophotometer and were diluted to 10 ng DNA per μL using filter sterilized 10 mM Tris (pH 8.0).

2.3. T-RFLP Preparation

[12] Three replicate PCRs were performed, each using 50 ng of total gDNA and domain specific primers 68F (TNANACATGCAAGTCGRRCG) and 1492R (RGYTACCTTGTTACGACTT) for Bacteria and 21F (TTCYGGTTGATCCYGCCRGA) and 922R (YCCGGCGTTGANTCCAATT) for Archaea, where R is purine analog K, Y is pyrimidine analog P, and N is an equal mixture of both nucleotide analogs at a single position (Glen Research, Sterling, Virginia). PCR conditions were as previously described [*Emerson and Moyer*, 2002]. The forward primers were labeled with 6-FAM (6-carboxyfluorescein) on the 5′ end. The PCR products were visually assayed for size by 1% agarose gel electrophoresis against a 1-kbase ladder DNA size standard. Only reaction mixtures yielding the expected DNA fragment size with corresponding no amplification of negative controls were used. The remaining fluorescently labeled PCR products were desalted using Montáge PCR centrifugal filter device (Millipore). Bacterial PCR products (15 μL) were then partitioned into eight aliquots and separately digested overnight with 5 U of *Hae* III, *Hha* I, *Alu* I, *Mbo* I, *Msp* I, *Rsa* I, *Hinf* I, and *Bst*U I (New England Biolabs, Berverly, Massachusetts) in a total volume

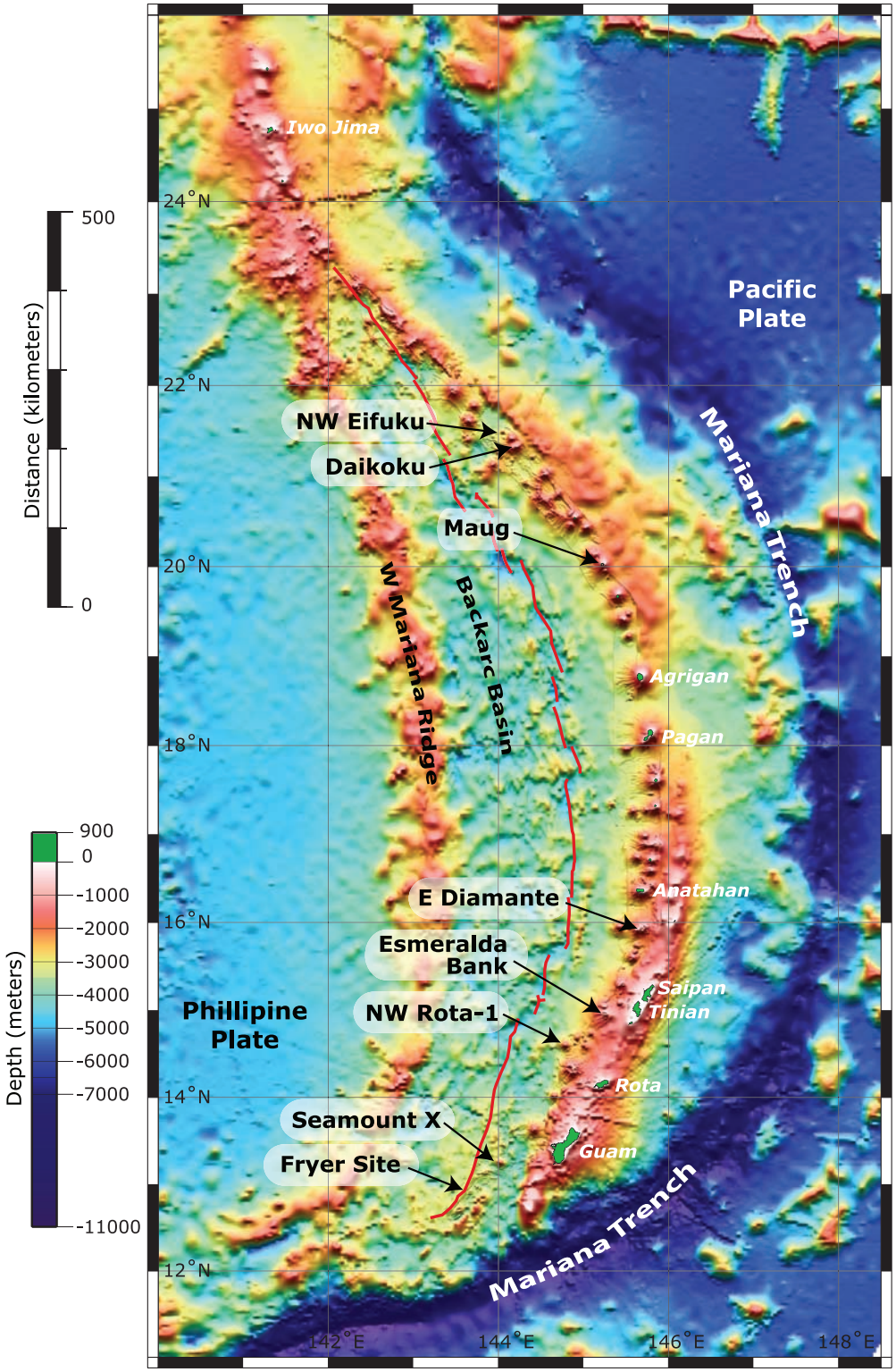

Figure 1. Bathymetric map of the Mariana Arc/back-arc system. Names in black with arrows indicate locations where sampling was conducted. (Map courtesy of R. Embley and S. Merle, NOAA.)

of 30 μL at 37°C, with the exception of the *Bst*UI reaction which was incubated at 60°C. Archaeal PCR products were treated the same, with the exception that *Sau*96 I was substituted for *Mbo* I. The restriction fragments were then desalted using Sephadex G-75 (Amersham Biosciences, Uppsala, Sweden) and dehydrated. The fragments were resuspended in 15 μL formamide and 0.33 μL Genescan ROX-500 internal size standard (Applied Biosystems, Foster City, California), denatured by heating for 5 min at 95°C, and separated by capillary electrophoresis using an ABI 3100 genetic analyzer with a 50 cm capillary array using POP6 polymer (Applied Biosystems). Each T-RFLP digestion was

Table 1. Description and Location of Sample Sites

Year	Sample	Vent Site	Location	Spreading Center/ Arc Seamount	Latitude/Longitude	Sample Type	Sample Description	Depth (m)	Temp (°C)
2003	J2-42-1W	Fryer Site	Back arc	SMBSC[a]	12°57.190′N/143°37.125′E	Push Core	Yellow/White mat	2860	77
2003	J2-42-2W	Fryer Site	Back arc	SMBSC[a]	12°57.190′N/143°37.125′E	Push Core	Yellow/Green mat	2860	77
2004	R782-b5	Shimmering Vent	Volcanic Arc	NW Rota-1	14°36.072′N/144°46.530′E	Suction Sample	Orange mat	516	15
2004	R782-b7	Shrimp Mound	Volcanic Arc	NW Rota-1	14°36.072′N/144°46.530′E	Suction Sample	Yellow mat	518	15
2004	R783-b56	Iceberg	Volcanic Arc	NW Rota-1	14°36.048′N/144°46.578′E	Suction Sample	White mat	529	58
2004	R786-b567	Fault Shrimp	Volcanic Arc	NW Rota-1	14°36.036′N/144°46.644′E	Suction Sample	White mat	584	20
2004	R788-b7	Mat City	Volcanic Arc	E Diamante	15°56.322′N/145°40.518′E	Suction Sample	White mat	206	ambient
2004	R788-b5	Five Towers	Volcanic Arc	E Diamante	15°56.556′N/145°40.884′E	Suction Sample	Orange mat	344	220
2004	R788-cc	Five Towers	Volcanic Arc	E Diamante	15°56.556′N/145°40.884′E	Chimney Chunks	Multicolored rocks	344	220
2004	R789-b5	Egg Drop Soup	Volcanic Arc	Maug Crater	20°01.206′N/145°13.308′E	Suction Sample	Orange mat	149	ambient
2004	R790-b56	Cave Vent	Volcanic Arc	Maug Crater	20°01.404′N/145°13.356′E	Suction Sample	Orange mat	145	28
2004	R791-b56	Bacto Balls	Volcanic Arc	NW Eifuku	21°29.328′N/144°02.436′E	Suction Sample	Fe mat	1716	ambient
2004	R791-b7	Yellow Top	Volcanic Arc	NW Eifuku	21°29.304′N/144°02.424′E	Suction Sample	Fe mat	1674	~6–8
2004	R792-b57	Champagne	Volcanic Arc	NW Eifuku	21°29.256′N/144°02.508′E	Suction Sample	White mat	1608	72–103
2004	R792-cc	Champagne	Volcanic Arc	NW Eifuku	21°29.256′N/144°02.508′E	Chimney Chunks	White rocks	1608	72–103
2004	R793-b1	Yellow Cone	Volcanic Arc	NW Eifuku	21°29.292′N/144°02.526′E	Suction Sample	Fe mat	1587	11
2004	R793-b57	Yellow Top	Volcanic Arc	NW Eifuku	21°29.310′N/144°02.424′E	Suction Sample	Fe mat	1678	~6–8
2004	R795-b56	Fish Spa	Volcanic Arc	Daikoku	21°19.476′N/144°11.532′E	Suction Sample	White sediment	390	ambient
2006	J2-184-W	Fe-Mats	Volcanic Arc	Seamount X	13°15.098′N/144°01.069′E	Suction Sample	Fe mat	1305	ND[b]
2006	J2-184-B	Snail Mat	Volcanic Arc	Seamount X	ND[b]	Suction Sample	White mat	1188	ND[b]
2006	J2-190-W	Fe-Mounds	Volcanic Arc	Esmeralda Bank	14°57.364′N/145°14.478′E	Suction Sample	Fe sediment	291	40
2006	J2-190-cc	Fe-Mounds	Volcanic Arc	Esmeralda Bank	14°57.364′N/145°14.478′E	Chimney Chunks	Fe crust	291	40
2006	J2-191-W	Iceberg	Volcanic Arc	NW Rota-1	14°36.052′N/144°46.579′E	Suction Sample	White mat	530	25
2006	J2-197-W	Bubble Bath	Volcanic Arc	Daikoku	21°19.505′N/144°11.488′E	Suction Sample	White mat	411	52
2006	J2-197-B	Fish Spa	Volcanic Arc	Daikoku	21°19.484′N/144°11.585′E	Suction Sample	Brown sediment	390	ambient

[a]Southern Mariana Back-Arc Spreading Center.
[b]ND, no data.

separated and visualized at least twice to ensure reproducibility of the analysis.

2.4. T-RFLP Analysis

[13] The fluorescently labeled 5′ terminal restriction fragments (T-RFs) were sized against the Genescan ROX-500 internal size standard using Genemapper v3.7 (Applied Biosystems). Only fragments between 50 and 500 nucleotides were included in the analysis. Electropherograms were then imported into the program BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Community fingerprints were compared in BioNumerics using average Pearson product moment correlation [*Häne et al.*, 1993] and unweighted pair group method with arithmetic mean (UPGMA; Applied Maths) cluster analysis of all eight restriction digests using the relative fluorescent proportions of each electropherogram. The cophenetic correlation coefficient was calculated to assess the robustness of the cluster analysis groupings. Peak detection was limited to peaks between 50 and 500 base pairs in size and with height at least 3% of the maximum value of the fingerprint.

2.5. Bacteria/Archaea Q-PCR

[14] Bacteria to Archaea ratios were estimated by using a 5′ nuclease Q-PCR assay developed by *Takai and Horikoshi* [2000]. The probes were labeled with 6-FAM on the 5′ end and Iowa Black FQ quencher on the 3′ end (Integrated DNA Technologies, Coralville, Iowa). Reactions were carried out in triplicate in 30 μL reactions containing 20 ng gDNA, forward and reverse primer concentration at 800 nM, probe concentration at 200 nM, and 1X universal master mix (Applied Biosystems). The addition of 1 unit of Platinum Taq and 1X ROX (Invitrogen) were used to optimize the signal to noise of the reactions. Standard curves were constructed with equal mixtures either 3 different plasmids selected from the R792-b57 Archaeal clone library or equal mixtures of 6 different plasmids from the R792-b57 Archaeal and Bacterial clone libraries. The plasmids were diluted from 1 to 10^{-6} starting with a 10 ng/μL stock solution along with a negative control. All standard curves had R^2 values of >0.995.

2.6. Clone Library Construction

[15] Five replicate PCRs were performed on gDNA samples J2-42-1W, J2-42-2W, R783-b56, and R792-b57 using Bacterial specific primers, and R792-b57 using Archaeal specific primers. Identical PCR conditions and primers were used without 5′ fluorochrome labeling, and with 5′ phospho-link ends on the primers to enhance ligation reactions. The PCR amplicons were visually assayed for size and purity by gel electrophoresis as described above. The remaining sample was purified using a Montáge PCR centrifugal filter device and cloned with a TA cloning kit following the manufacturer's protocol (Invitrogen, Carlsbad, California). All putative clones were streaked for isolation and the insert was assayed for size using PCR with the primers M13F and M13R and running the products against a 1-kbase size standard by 1% agarose electrophoresis. The PCR products were then purified using Montáge PCR centrifugal filter device filters and diluted 2X with molecular biology grade water. The clones were then end-sequenced using M13F or M13R primers. Sequences were annotated to include 350 bases from the 5′ end of the SSU rRNA gene. The sequences were imported into the ARB software environment [*Ludwig et al.*, 2004] and aligned to the SSU_Jan04 database using the ARB fast aligner. A nucleotide similarity matrix was calculated using DNADIST from the PHYLIP package [*Felsenstein*, 2007] and the bacteria_rr5_dec04 ARB filter for Bacteria clone libraries and the Archaea_rr5_dec04 ARB filter for the Archaea clone library. Operational taxonomic units (OTUs) [*Moyer et al.*, 1994] were then defined as groups of at least two clones with a minimum of 97% similarity grouped using the furthest neighbor algorithm calculated with the program DOTUR [*Schloss and Handelsman*, 2005] using the similarity matrix from each clone library. Rarefaction curves were also calculated using the program DOTUR using a 97% similarity cutoff. Clones were checked for chimeras using the Bellerophon server [*Huber et al.*, 2004]. Putative chimeras were further screened with the program Pintail [*Ashelford et al.*, 2005] by comparing the putative chimera to any potential parent sequence.

2.7. Phylogenetic Analysis

[16] The full length clones were compared to sequences stored in GenBank using the BLAST algorithm [*Altschul et al.*, 1990]. These sequences and the full length clones were then imported into the ARB software environment and aligned to the SSU_Jan04 database using the ARB fast aligner [*Ludwig et al.*, 2004]. Additional sequences were selected in the database and exported to the sequence alignment program Bioedit [*Hall*, 1999] for additional alignment editing. Phylogenetic analyses were restricted to regions of moderately to highly conserved nucleotide positions that were unambiguously aligned for all sequences. Phylogenetic placements were calculated using fastDNAml version 1.2.2 [*Olsen et al.*, 1994] using the general two-parameter model of evolution [*Kishino and Hasegawa*, 1989] and allowing for the global swapping of branches. Using these parameters, the search for an optimal tree was repeated until the best log likelihood tree was calculated in at least three independent tree calculations. Each phylogenetic tree was bootstrapped 100 times. The search for each bootstrap was repeated until the best log likelihood score was calculated for at least two independent bootstrap calculations.

2.8. Nucleotide Sequence Accession Numbers

[17] The SSU rDNA sequences representing the OTUs used in this analysis have been submitted to GenBank and assigned accession numbers EU574647 through EU574679.

3. Results

3.1. Site Descriptions

[18] Twenty-five microbial communities were sampled from 18 different hydrothermal systems located at seven different arc seamounts along the Mariana Island Arc and at a single site on the southern back-arc spreading center over the course of 3 years (Table 1 and Figure 1). Depths ranged from 2860 m below sea level (mbsl) at the deepest sampling site on the southern back arc to 145 mbsl at the shallowest site in the Maug Crater (Table 1). Vent fluids ranged from black smokers venting at 220°C at Five Towers Vent Field

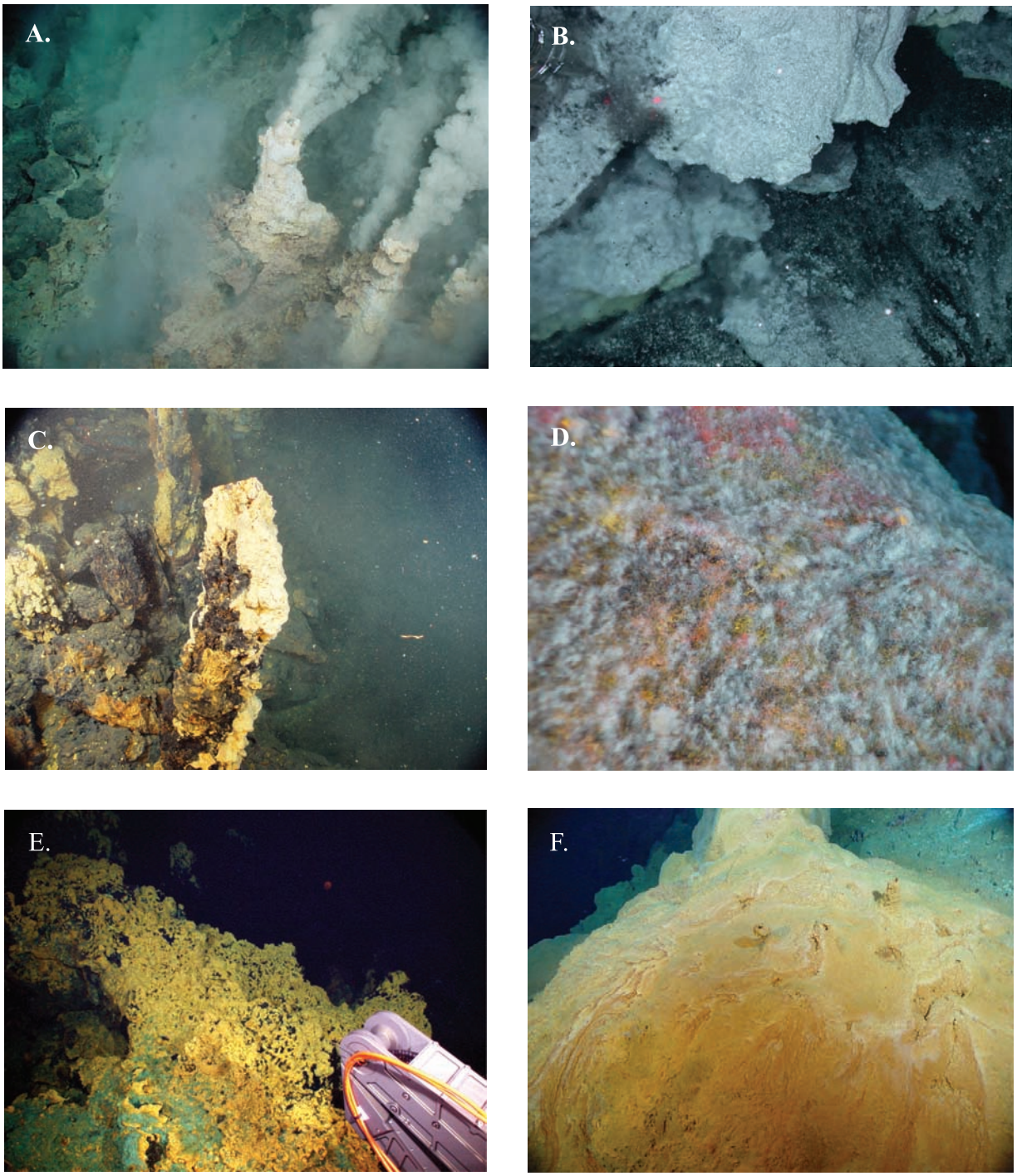

Figure 2. Photographs of microbial mats collected with the ROVs ROPOS and Jason 2. (a) White smoker vents covered with microbial mats at Champagne vents, NW Eifuku. (b) White microbial mats covering rocks at Iceberg Vent, NW Rota-1. (c) Yellow mats covering black smoker chimneys at Five Towers vents, east Diamante. (d) White microbial mats growing over photosynthetic algae at Mat City, east Diamante. (e) Orange and green microbial mat at the Fryer Site 2W Vent, southern Mariana Back-Arc Spreading Center. (f) Iron oxide encrusted microbial mat at Yellow Top Vent, NW Eifuku.

on east Diamante Seamount to seeps which were covered by microbial mats, but did not have a measurable temperature anomaly (Table 1).

[19] Thick, luxuriant microbial mats were sampled at Champagne (Figure 2a), Iceberg (Figure 2b), Five Towers (Figure 2c), and the Bubble Bath Vent sites. These vents were all higher temperature (52–220°C) sites, with Champagne and Bubble Bath having multiple white smoker vents with vigorous CO_2 venting around the sites. Lower temperature white and salmon colored filamentous microbial mats were collected from Shimmering Vent, Shrimp Mound, Fault Shrimp, Mat City (Figure 2d), Egg Drop

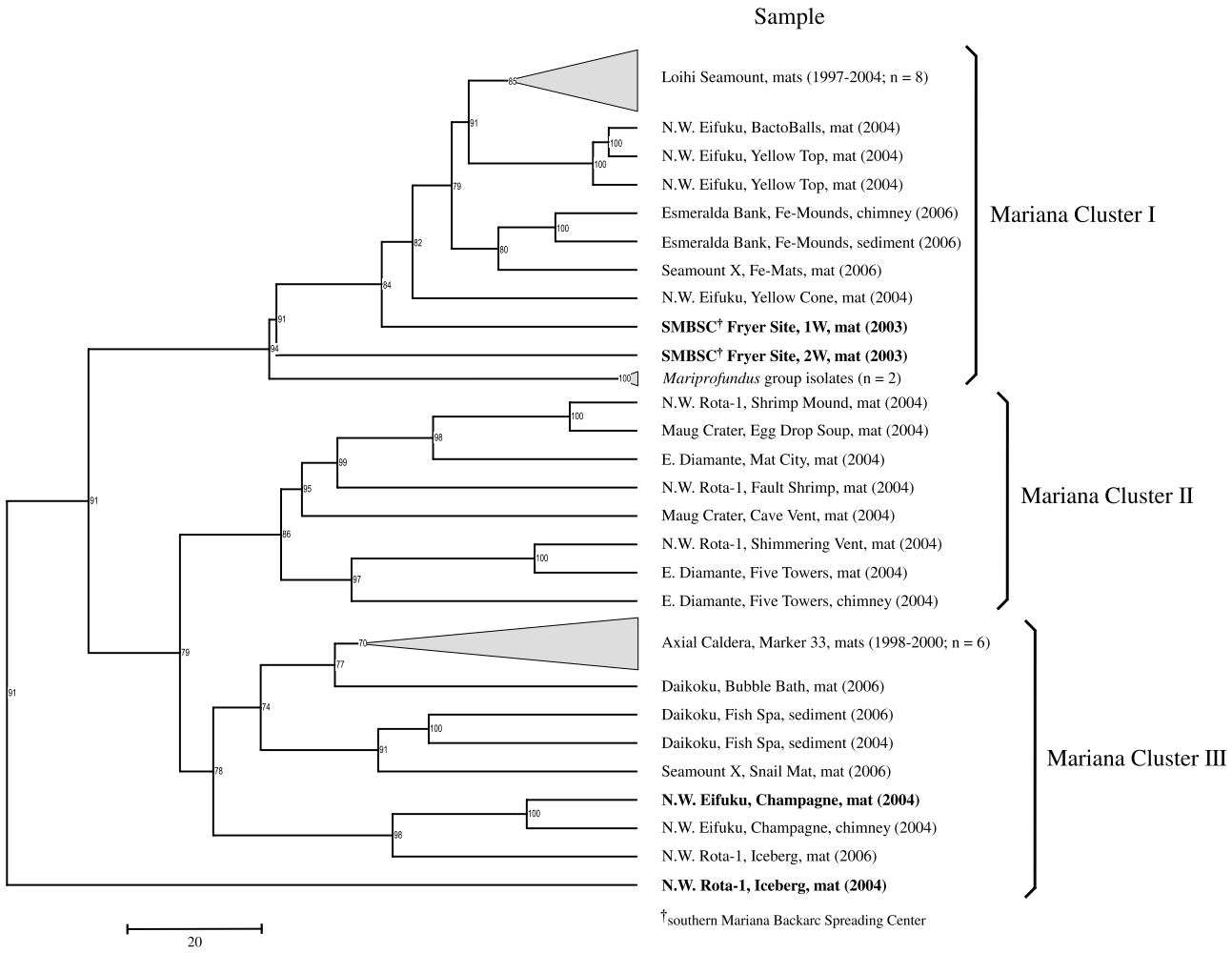

Figure 3. UPGMA/Pearson product moment correlation cluster analysis of T-RFLP Bacterial community fingerprints from 25 microbial communities from 18 different hydrothermal systems at the Mariana Arc/back-arc system. Microbial communities from Loihi Seamount, Axial Caldera, and isolates from the Mariprofundus group are also included. Scale bar is the Pearson product moment correlation r value X 100. Numbers at nodes are the cophenetic correlation coefficients. Samples in bold were examined by clone library analyses.

Soup, Cave Vent, and Snail Mat Vent sites. These microbial mats were all growing at near-ambient temperatures and are most likely being fed by volcanic iron and sulfide seeping through the sediments from a deeper source. Hydrothermal sediments were collected from the Fish Spa site on Diakoku Seamount. The Fish Spa site is inhabited by a dense population of flatfish which appeared to be feeding on polychaete worms which are supported by chemoautotrophic bacteria growing within the seafloor sediments [*Dower et al.*, 2006]. Iron-dominated microbial mats were collected from the Fryer Site (Figure 2e), Bacto Balls, Yellow Top, Yellow Cone (Figure 2f), Fe-Mats, and Fe-Mounds Vent sites. Microbial mats from the Fryer Site were perched upon a hydrothermal chimney venting iron-rich fluids at 77°C [*Wheat et al.*, 2003]. These mats were thick, flocculate, and multicolored. Crusty iron-dominated microbial mats were observed and collected from Yellow Cone Vent and the Fe-Mounds Vent Field. These mats were characterized by multiple small (5–7 cm) chimneys with slow (seeping) fluid flow. Highly flocculate, thick (10–100 cm deep) Fe-dominated microbial mats were collected from the Yellow Top and Fe-Mats Vent Field. Iron-rich microbial floc was also collected downslope of Yellow Top Vent at the Bacto Balls site, where gelatinous balls of iron oxide (2–4 cm) were seen rolling downslope of the microbial mats as they were exuding from near the Yellow Top Vents (Table 1).

3.2. T-RFLP Fingerprint Results

[20] T-RFLP fingerprint analysis of Bacterial SSU rDNA fragments shows the formation of three distinct clusters within the Mariana Arc/back-arc samples, designated Mariana clusters I, II, and III (Figure 3). All Archaeal T-RFLP fingerprints were universally dominated by a single ribotype (Figure 4) and averaged fewer T-RFs per digest than the Bacterial T-RFLPs (Table 2).

[21] Mariana T-RFLP Cluster I contains all of the Fe-dominated mat samples. Microbial mats from Loihi Seamount in Hawaii, which are known to be dominated

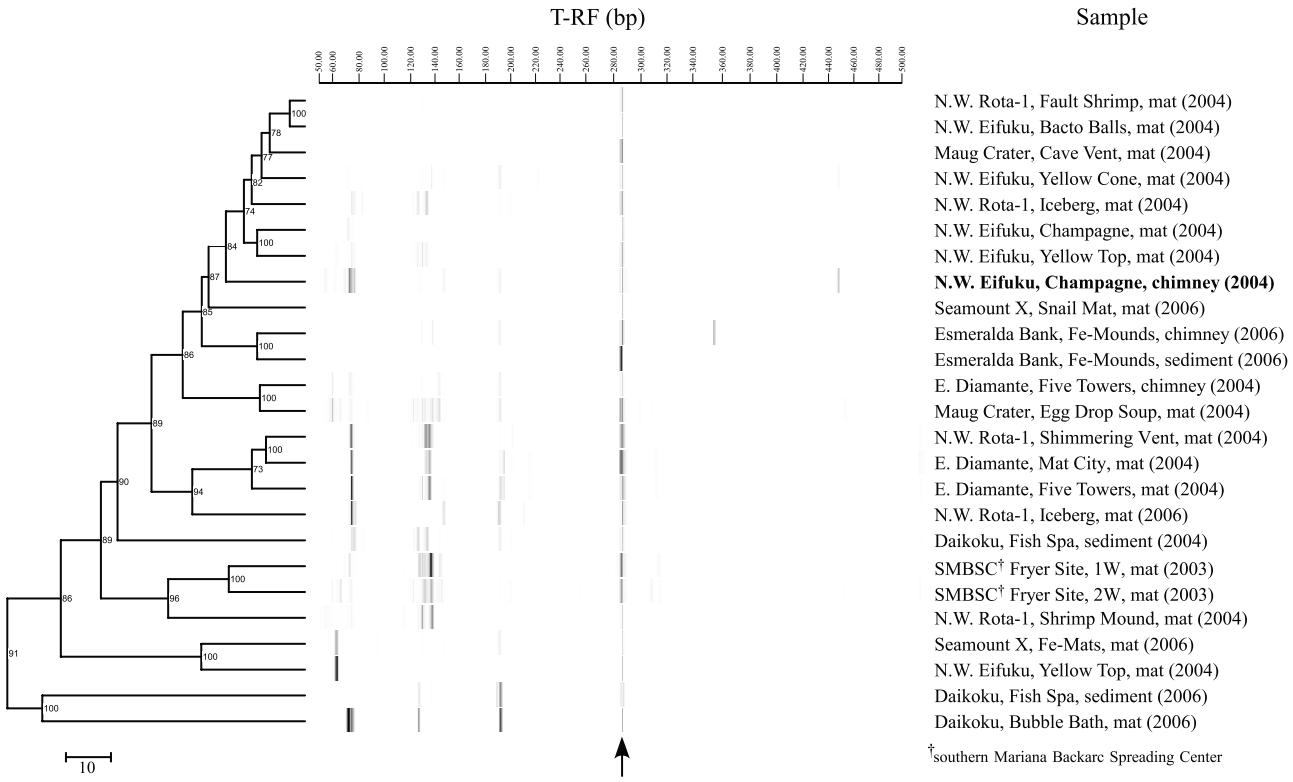

Figure 4. UPGMA/Pearson product moment correlation cluster analysis of T-RFLP Archaeal community fingerprints from 25 microbial communities from 18 different hydrothermal systems at the Mariana Arc/back-arc system. The center panel is a false-gel image of the *Msp* I digest showing the presence of a single ribotype peak in all samples. Scale bar is the Pearson product moment correlation r value X 100. Numbers at nodes are the cophenetic correlation coefficients. Arrow indicates ubiquitously occurring ribotype. Sample in bold was examined by clone library analysis.

by neutrophilic Fe-oxidizing bacteria [*Emerson and Moyer*, 2002; *Moyer et al.*, 1995; *Davis et al.*, 2005], also group within this cluster, although with much less community structure variability than the samples from the Mariana Island Arc/back arc (Figure 3). Isolates from the ζ-Proteobacteria [*Emerson et al.*, 2007] also group within this cluster.

[22] Mariana T-RFLP Cluster II contained only shallow and mostly lower temperature filamentous microbial mat samples collected in 2004. Preliminary clone library analysis of samples from this cluster (data not shown) revealed a highly diverse microbial community consisting primarily of phylotypes most closely related to heterotrophic isolates from seawater and sediments.

[23] Mariana T-RFLP Cluster III generally contained the samples from the warmer vent fluids (Table 1 and Figure 3). Multiple microbial mat samples from a time series study after an eruptive event at Marker 33 (Axial Caldera) also group within Marina Cluster III, but with far less community structure variability compared with the Mariana Island Arc samples (Figure 3). Hydrothermal fluid samples from posteruption Axial Caldera (Marker 33) have been shown to be dominated by putative sulfur-oxidizing ε-Proteobacteria [*Huber et al.*, 2003; *Sogin et al.*, 2006]. The Bubble Bath Vent grouped closest to the Axial vent samples, suggesting it contained a similar microbial mat community to the posteruptive Axial samples. Microbial mats from the Snail Mat site and sediments from the Fish Spa site also cluster within Mariana Group III, even though the samples are from seeps with nearly ambient fluid temperatures. Samples from Champagne and Iceberg Vents also group within the Mariana Cluster III. These samples had very thick growth of microbial mats and are also from warmer vent sites (25–103°C).

[24] The T-RFLP fingerprint from the Bacterial community at Iceberg vent on NW Rota-1 collected in 2004 did not cluster within any of the three Mariana T-RFLP clusters as this community was dominated by a single ribotype. This microbial mat community was again sampled in 2006 and clustered within the Mariana T-RFLP Cluster III (Figure 3).

3.3. Quantitative-PCR Results

[25] Quantitative-PCR (Q-PCR) was used to quantify the Archaea/Bacteria ratio of the microbial communities. All of the microbial communities were dominated by Bacteria, with a mean Archaeal community abundance of 4.5 ± 3.8% in the samples (Table 2). The sample with the greatest percent of Archaea was at the Fish Spa site in 2006 with 12.7 ± 0.62% Archaea, followed by the Fryer Site 1W Vent on the Mariana Back Arc and the Cave Vent site in the Maug Caldera, both having 11.3 ± 1.37% Archaea. The Chimney fragments from the Five Towers Vent site at east Diamante Seamount had the lowest ratio of Archaea/Bacteria with only 0.2 ± 0.02%, followed by Iceberg Vent on

Table 2. Biomass, Percent Archaea, and Estimated Microbial Richness of Each Sample

Year	Sample	Vent Site	ng DNA/g Sample (Wet Weight)	Percent Archaea	Average T-RFs Bacteria	Average T-RFs Archaea
2003	J2-42-1W	Fryer Site	2250	11.3 ± 0.49	18.4 ± 4.2	18.3 ± 6.5
2003	J2-42-2W	Fryer Site	2466	10.4 ± 0.26	17.0 ± 1.5	16.9 ± 5.4
2004	R782-b5	Shimmering Vent	147	2.2 ± 0.16	16.6 ± 3.6	9.8 ± 3.5
2004	R782-b7	Shrimp Mound	2450	4.4 ± 0.37	12.8 ± 4.0	8.3 ± 3.0
2004	R783-b56	Iceberg	2370	5.5 ± 0.45	6.6 ± 4.7	9.0 ± 3.8
2004	R786-b567	Fault Shrimp	6080	2.3 ± 0.34	12.8 ± 3.0	4.0 ± 1.4
2004	R788-b7	Mat City	11540	10.1 ± 1.10	13.5 ± 3.8	9.8 ± 2.3
2004	R788-b5	Five Towers	190	2.3 ± 0.17	16.8 ± 3.7	11.3 ± 4.0
2004	R788-cc	Five Towers	1044	0.2 ± 0.02	14.3 ± 3.3	10.3 ± 4.0
2004	R789-b5	Egg Drop Soup	156	2.3 ± 0.62	18.3 ± 4.8	5.5 ± 1.5
2004	R790-b56	Cave Vent	4380	11.3 ± 1.37	12.8 ± 2.4	3.3 ± 1.9
2004	R791-b56	Bacto Balls	477	4.0 ± 0.19	16.4 ± 5.2	2.0 ± 1.0
2004	R791-b7	Yellow Top	784	4.4 ± 0.78	17.1 ± 4.0	2.4 ± 0.9
2004	R792-b57	Champagne	3300	0.8 ± 0.07	9.3 ± 6.5	4.8 ± 2.3
2004	R792-cc	Champagne	1350	0.5 ± 0.03	6.4 ± 3.9	3.5 ± 2.3
2004	R793-b1	Yellow Cone	218	2.0 ± 0.24	15.4 ± 4.6	5.5 ± 2.3
2004	R793-b57	Yellow Top	517	2.9 ± 0.67	12.3 ± 6.8	7.0 ± 2.6
2004	R795-b56	Fish Spa	7106	2.8 ± 0.40	12.4 ± 3.6	11.8 ± 2.4
2006	J2-184-W	Fe-Mats	1486	2.9 ± 0.28	10.4 ± 4.3	5.9 ± 2.5
2006	J2-184-B	Snail Mat	5817	1.5 ± 0.16	12.9 ± 2.9	3.3 ± 1.7
2006	J2-190-W	Fe-Mounds	3722	6.0 ± 0.17	13.5 ± 5.5	6.1 ± 1.1
2006	J2-190-cc	Fe-Mounds	1631	6.3 ± 0.50	11.5 ± 4.3	6.1 ± 2.0
2006	J2-191-W	Iceberg	617	0.4 ± 0.15	6.0 ± 2.2	7.9 ± 2.6
2006	J2-197-W	Bubble Bath	1608	1.9 ± 0.32	8.4 ± 4.5	7.1 ± 4.7
2006	J2-197-B	Fish Spa	5240	12.7 ± 0.62	14.4 ± 4.3	10.1 ± 2.4

NW Rota-1 with 0.4 ± 0.15% Archaea in 2006, down from 5.5 ± 0.45% in 2004.

3.4. Clone Library Results

[26] On the basis of the T-RFLP cluster analysis and the Q-PCR results, five SSU rDNA clone libraries were constructed to identify and describe the phylotypes in these communities (e.g., sample names listed in bold, Figures 3 and 4). Clone libraries were constructed from Bacterial communities from Fryer Site on the southern Mariana Back-Arc Spreading Center (designated 1WB and 2WB, respectively), Champagne Vent on NW Eifuku (CPB), and Iceberg Vent on NW Rota-1 (IBB). Bacterial clone libraries were also constructed from Five Towers and Mat City on east Diamante and from Cave Vent in Maug Caldera, but were not included in any further analysis (data not shown) as they were highly diverse and dominated by putative heterotrophic phylotypes associated with seawater and sediments not generally associated with hydrothermal fluid inputs. An Archaeal SSU rDNA library was constructed from Champagne Vent on NW Eifuku (CPA). No chimeric sequences were detected in any of the full length SSU rRNA sequences analyzed.

[27] Rarefaction analysis shows the Bacterial clone libraries from sites 1W and 2W are more diverse in terms of richness than the clone libraries from either Champagne or Iceberg Vents (Figure 5). Though the communities detected in the clone libraries from 1W and 2W Vents had similar phylogenetic groupings, no identical phylotypes were found. The clone libraries from Champagne and Iceberg Vents were dominated by phylotypes belonging to the ε-Proteobacteria (Figure 6).

[28] The 2W Vent was dominated by phylotypes clustering within the Magnetobacterium group of the *Nitrospira* division (Figure 7). The only partially described member of this group is *Candidatus Magnetobacterium bavaricum*, a freshwater magnetotactic bacterium enriched from sediments from Lake Chiemsee, Germany [*Spring et al.*, 1993]. This bacterium contained up to 1000 magnetosomes per cell, and the cells also contained sulfur inclusion bodies which led the authors to theorize the bacterium couples sulfur oxidation and iron reduction [*Spring et al.*, 1993]. Other uncultured clones clustering within this group were found to dominate Bacterial communities in inactive hydrothermal chimney structures localized with metal sulfides [*Suzuki et al.*, 2004].

[29] Phylotypes from both 1W and 2W also clustered within the newly proposed [*Emerson et al.*, 2007] "Candidatus ζ-Proteobacteria" class (Figure 6). The obligate neutrophilic iron-oxidizing chemoautotroph *Mariprofundus ferrooxydans* is the first isolate in pure culture from this group and was enriched from Fe-rich microbial mats at Loihi Seamount, Hawaii [*Emerson et al.*, 2007] where it has been shown that phylotypes clustering within the ζ-Proteobacteria dominate low-temperature microbial mats [*Davis et al.*, 2005]. Other uncultured phylotypes within this group were found in Red Sea brine pools [*Eder et al.*, 2001] and dominated the microbial community in a sediment from a Fe-Si-Mn-rich hydrothermal mound found off-axis adjacent to the Cleft Segment of the Juan de Fuca Ridge (R. E. Davis et al., manuscript in preparation, 2008).

[30] The dominate phylotype within the 1WB clone library clustered within the Nitrospina group of the δ-Proteobacteria (Figure 6). Other clones from the 1WB and 2WB libraries clustered within the Thermomicrobia group (Figure 7) and the γ- and α-Proteobacteria (Figure 6).

[31] The majority of the Bacterial phylotypes from Champagne Vent and all of the phylotypes from the Iceberg Vent clustered within the ε-Proteobacteria (Figure 6). Culture-independent methods have shown that ε-Proteobacteria can dominate hydrothermal vent microbial mats [*Moyer et al.*, 1995] and hydrothermal fluids [*Corre et al.*, 2001] from

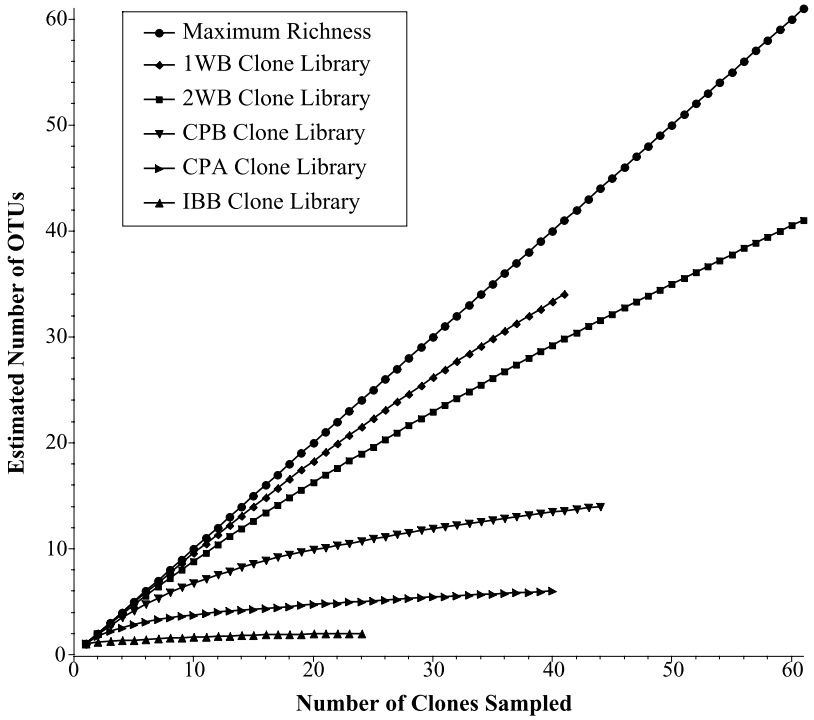

Figure 5. Rarefaction curves as indicators of OTU richness from five clone libraries. Curves were calculated using the furthest neighbor approach with a 97% similarity cutoff.

vents with very different temperature and chemical compositions. Multiple isolates from the ε-Proteobacteria have recently been cultured from hydrothermal vents [*Alain et al.*, 2002; *Miroshnichenko et al.*, 2002; *Nakagawa et al.*, 2006; *Inagaki et al.*, 2003; *Takai et al.*, 2003, 2006]. All deep-sea hydrothermal vent-associated ε-Proteobacteria isolates are either oxidizing reduced sulfur for energy or reducing sulfur while oxidizing hydrogen or formate; they can be aerobic, microaerophilic, and/or anaerobic [*Campbell et al.*, 2006].

[32] All of the clones from the Iceberg vent grouped into two OTUs, which clustered within the Caminibacter group within the ε-Proteobacteria. The Caminibacter group contains primarily cultured isolates rather than culture-independent environmentally derived sequences, as opposed to all of the other groups of ε-Proteobacteria that are dominated by phylotypes from environmental samples, and contain only a few cultured representatives. All cultured isolates from Caminibacter group are autotrophic anaerobes which either oxidize hydrogen gas or formate, reducing elemental sulfur, nitrate, or cystine. IBB OTUs 1 and 2 are most closely related to *Lebetimonas acidiphila*, a thermophile which oxidizes hydrogen and reduces elemental sulfur [*Takai et al.*, 2005].

[33] Champagne phylotypes CPB OTU 1, 4, and 7 all cluster within the Sufurimonas group of the ε-Proteobacteria. Sufurimonas group isolates are all mesophilic sulfur or hydrogen oxidizing chemoautotrophs which can grow in anaerobic to aerobic conditions, with all isolates being capable of growth under microaerophilic conditions [*Takai et al.*, 2006]. Environmental phylotypes from diverse microbial communities have been found to cluster within the Sufurimonas group, such as from deep-sea cold sediments [*Li et al.*, 1999], hydrothermal sediments [*López-García et al.*, 2003; *Teske et al.*, 2002] and from microbial mats from a hydrothermal vent [*Moyer et al.*, 1995] where they were found to dominate the microbial community [*Moyer et al.*, 1994].

[34] CPB OTU 6 clustered within the Sulfurovum group of the ε-Proteobacteria (Figure 6). The closest cultured representative of this group is *Sulfurovum lithotrophicum*, which is a mesophilic anaerobic or microaerophilic chemoautotroph which can oxidize elemental sulfur and thiosulfate and reduce oxygen or nitrate [*Inagaki et al.*, 2003].

[35] Other OTUs from Champagne Vent clustered within either an unclassified group most closely related to the candidate division GN02 (CPB OTU 2), the Thermotoga division (CPB OTU 3), or the Bacteroidetes Division (CPB OTU 5; Figure 7). None of these OTUs cluster with any known cultured isolates.

[36] The Archaeal clone library from Champagne Vent was dominated by phylotypes clustering within both the Euryarchaeota and the Crenarchaeota phyla (Figure 8). Although T-RFLP analysis showed the ubiquity of the Crenarchaeota ribotype (i.e., arrow in Figure 4), clone library analysis resulted in the ultimate detection of more phylotypes related to the Euryarchaeota than the Crenarchaeota (26 and 22 phylotypes, respectively).

[37] The most abundant group found in the Champagne Vent Archaeal clone library clustered within the Thermococcus group in the Euryarchaeota phylum (Figure 8). Isolates within the Thermococcus group includes thermophilic and hyperthermophilic anaerobes which grow by fermenting sugars and/or peptides, and whose growth can be stimulated by sulfur reduction [*Holden et al.*, 2001]. Phylotype CPA OTU 5 clusters within the deep-sea hydrothermal vent Euryarchaeotic

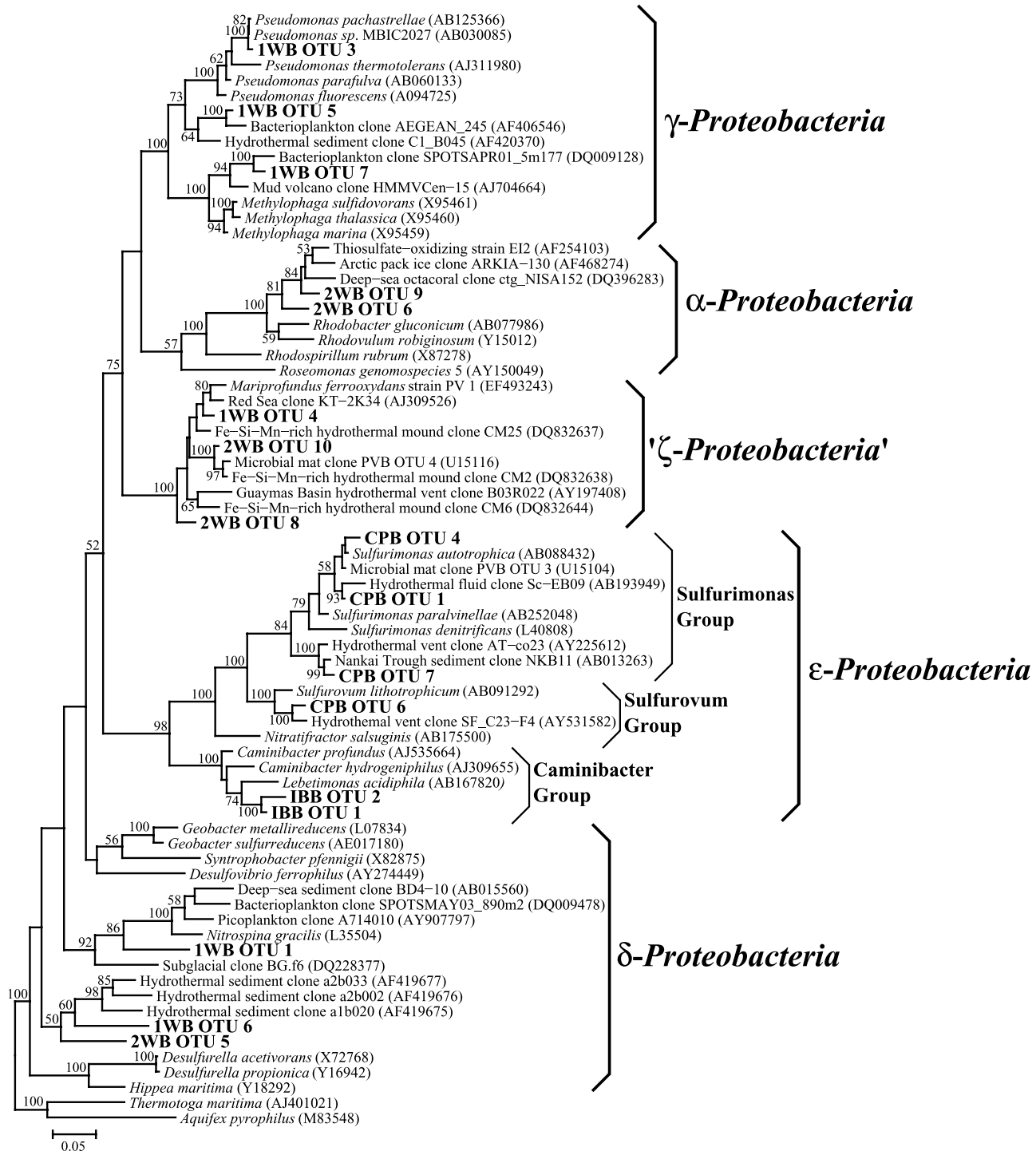

Figure 6. Maximum-likelihood phylogenetic tree showing the evolutionary placement of OTUs belonging to the Proteobacteria division of Bacteria. Scale bar represents 5 nucleotide substitutions per 100 positions.

2 (DHVE2) group. The only isolate from this group is the obligate thermoacidophilic heterotrophic iron- and sulfur-reducing archaeon *Aciduliprofundum boonei*, which was isolated from hydrothermal sediments [*Reysenbach et al.*, 2006].

[38] Phylotypes CPA OTUs 3, 4, and 7 all cluster within the Marine group I Crenarchaeota (Figure 8). This group was discovered in 1992 [*DeLong*, 1992] and are now known to be ubiquitous in aquatic environments [*Kato et al.*, 1997; *Moyer et al.*, 1998; *MacGregor et al.*, 2001; *Takai et al.*, 2001], and are enriched in hydrothermal vent microbial mats and in hydrothermal plumes [*Moyer et al.*, 1998; *Takai et al.*, 2004b]. The only isolated member of this group is *Nitrosopumilus maritimus*, a chemoautrophic ammonia-

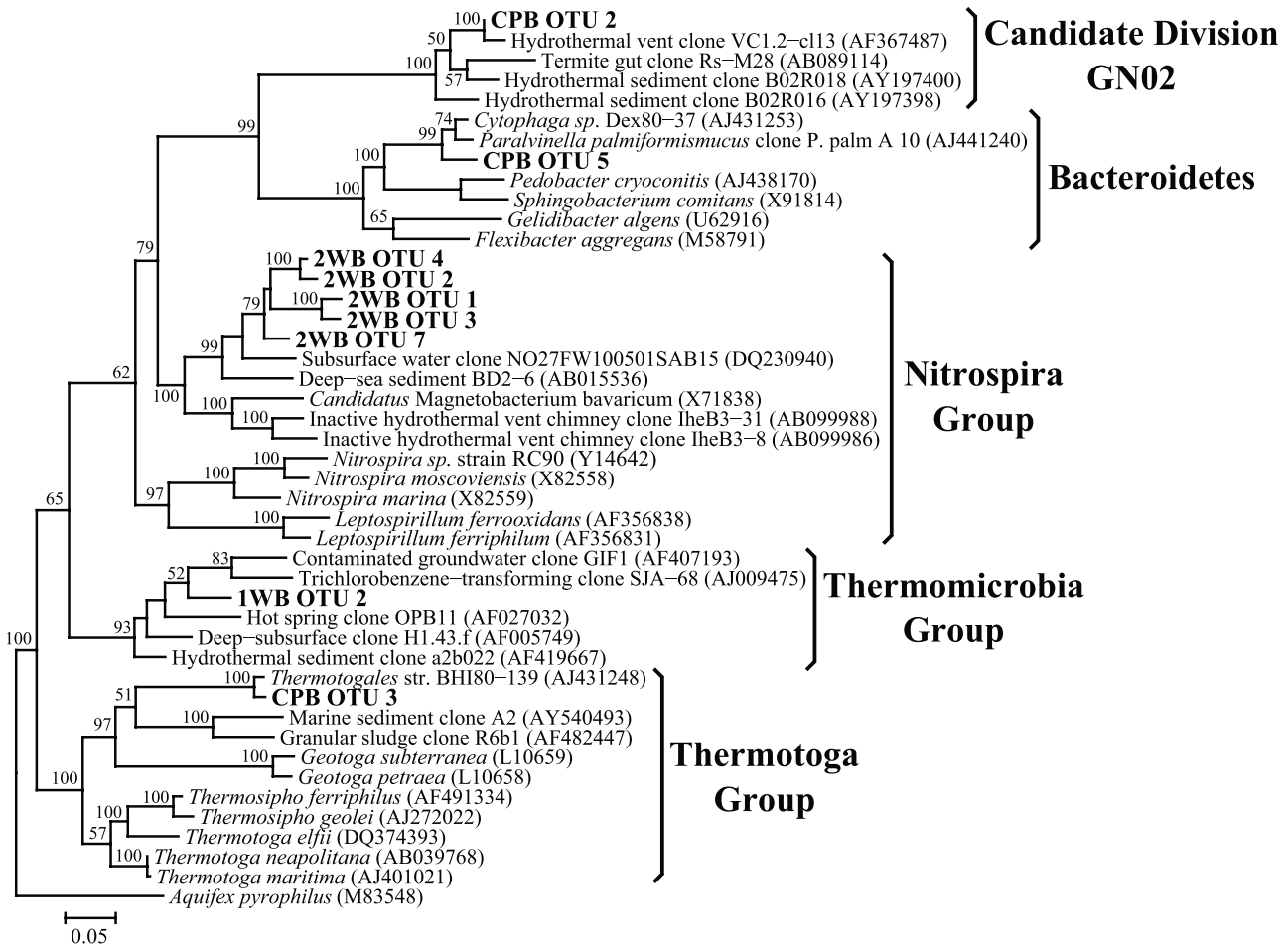

Figure 7. Maximum-likelihood phylogenetic tree showing the evolutionary placement of OTUs belonging to the Thermotoga, Thermomicrobia, Nitrospira, Bacteroidetes, and candidate division GN02. Scale bar represents 5 nucleotide substitutions per 100 positions.

oxidizing archaeon isolated from a marine aquarium [*Könneke et al.*, 2005]. Phylotype CPA OTU 6 clustered within an uncultured group within the Crenarchaeota phylum (Figure 8). This group is most closely related to the mesophilic soil Crenarchaota group [*Bintrim et al.*, 1997].

4. Discussion

[39] T-RFLP fingerprints showed much more diversity with respect to community structure variability among microbial mats along the Mariana Arc/back-arc system than from either microbial mats from a mid-ocean ridge (Axial Caldera, Figure 3) or from a hot spot hydrothermal system (Loihi Seamount, Figure 3). This extreme diversity found among microbial mat communities is most likely the result of the complexity of hydrothermal fluids and the ephemeral nature of hydrothermal vents that have been systematically surveyed at arc/back-arc systems [*de Ronde et al.*, 2001, 2007; *Embley et al.*, 2004; *Massoth et al.*, 2003] as compared to hot spot or mid-ocean ridge systems.

[40] The high gas concentrations of the hydrothermal fluids along presumably young hydrothermal vents form the unique habitats that support microbial mat communities at white smoker vent sites such as the Champagne Vent Field at NW Eifuku and Bubble Bath Vents at Daikoku Seamount found in Mariana Cluster III (Figure 3). Champagne Vents had the highest concentration of gas of any vent site that has thus far been discovered [*Lupton et al.*, 2006]. Many of the phylotypes found at the Champagne Vent site are associated with phylogenetic groups that obligately utilize anaerobic metabolisms. All known Thermococcus and Thermotoga isolates are anaerobic heterotrophs [*Holden et al.*, 2001] and phylotypes from the Bacteroidetes and some of the ε-Proteobacteria found at Champagne Vents are most closely related to phylotypes found in tubes or mucus secretions of hydrothermal vent annelids which are either from reduced oxygen or anaerobic environments [*López-García et al.*, 2002]. *Thermococcus*, *Thermotoga*, and ε-Proteobacteria related to phylotypes from Champagne Vents have all been found in presumably anaerobic hydrothermal fluids using a variety of sampling methods [*Corre et al.*, 2001; *Huber et al.*, 2003; *Nakagawa et al.*, 2006; *Takai et al.*, 2004a]. The microbial mat communities growing at this vent site are clearly influenced by the increased concentration of carbon dioxide and the low oxygen reducing conditions.

[41] The cold seep filamentous microbial mats and hydrothermal sediments clustering within Mariana Cluster

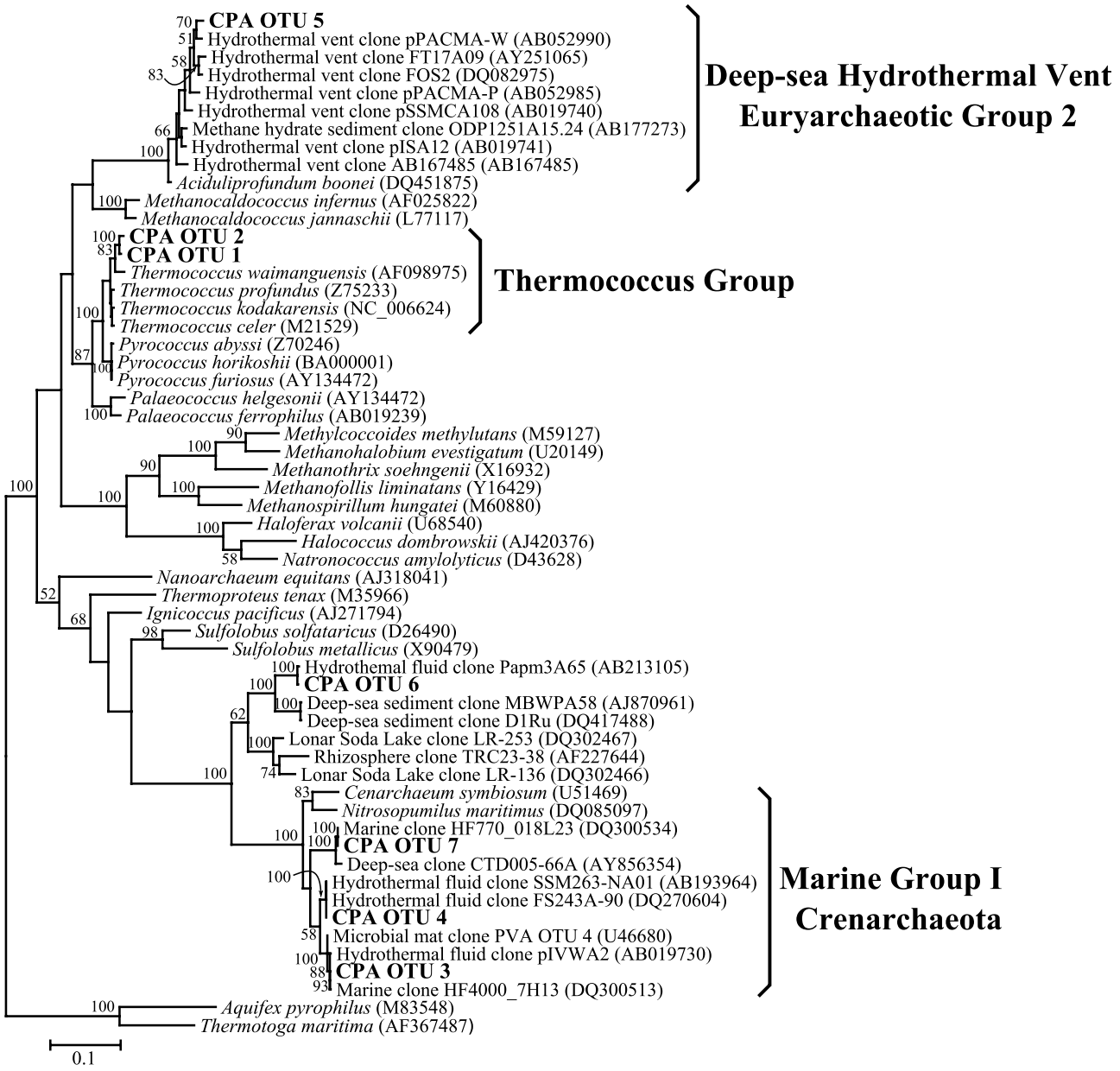

Figure 8. Maximum-likelihood phylogenetic tree showing the evolutionary placement of OTUs belonging to the Crenarchaeota and Euryarchaeota divisions of Archaea. Scale bar represents 5 nucleotide substitutions per 100 positions.

III, such as the Snail Mat on Seamount X and the Fish Spa on Daikoku Seamount, are also likely dominated by sulfur-oxidizing ε-Proteobacteria. These microbial communities are most likely supported by slow seeping sulfide which is either in a small focused setting, such as at the Snail Mat, or over a scale of hundreds of meters, such as at the Fish Spa. These slow seeping systems can support extensive macrofauna populations because of their low-temperature and high-biomass production. This was especially seen in the Fish Spa site, where large populations of flatfish were supported by the microbial chemoautotrophic primary production within the hydrothermal sediment [*Dower et al.*, 2006].

[42] The microbial mats belonging to Mariana Cluster I are dominated by members of the δ-Proteobacteria, Nitrospira, as well as members of the ζ-Proteobacteria. All of these microbial groups have pure culture isolates capable of iron-cycling metabolisms [*Lovley*, 1997; *Emerson et al.*, 2007; *Bond et al.*, 2000; *Spring et al.*, 1993]. The presence of high concentrations of biogenic iron oxides within the Fryer Site 1W and 2W microbial mats [*Wheat et al.*, 2003], which are dominated with phylotypes related to iron-oxidizing bacteria, strongly suggests iron-dominated communities within these ecosystems. These thick iron-cycling microbial mats can substantially accelerate the rate of iron oxidation, with the biological oxidation reaction occurring up to 60× faster than the abiotic reaction [*Søgaard et al.*, 2000]. Iron oxide microbial mats also can act to accumulate and concentrate heavy metals by over 3 orders of magnitude compared to the surrounding rock and fluids [*Anderson and*

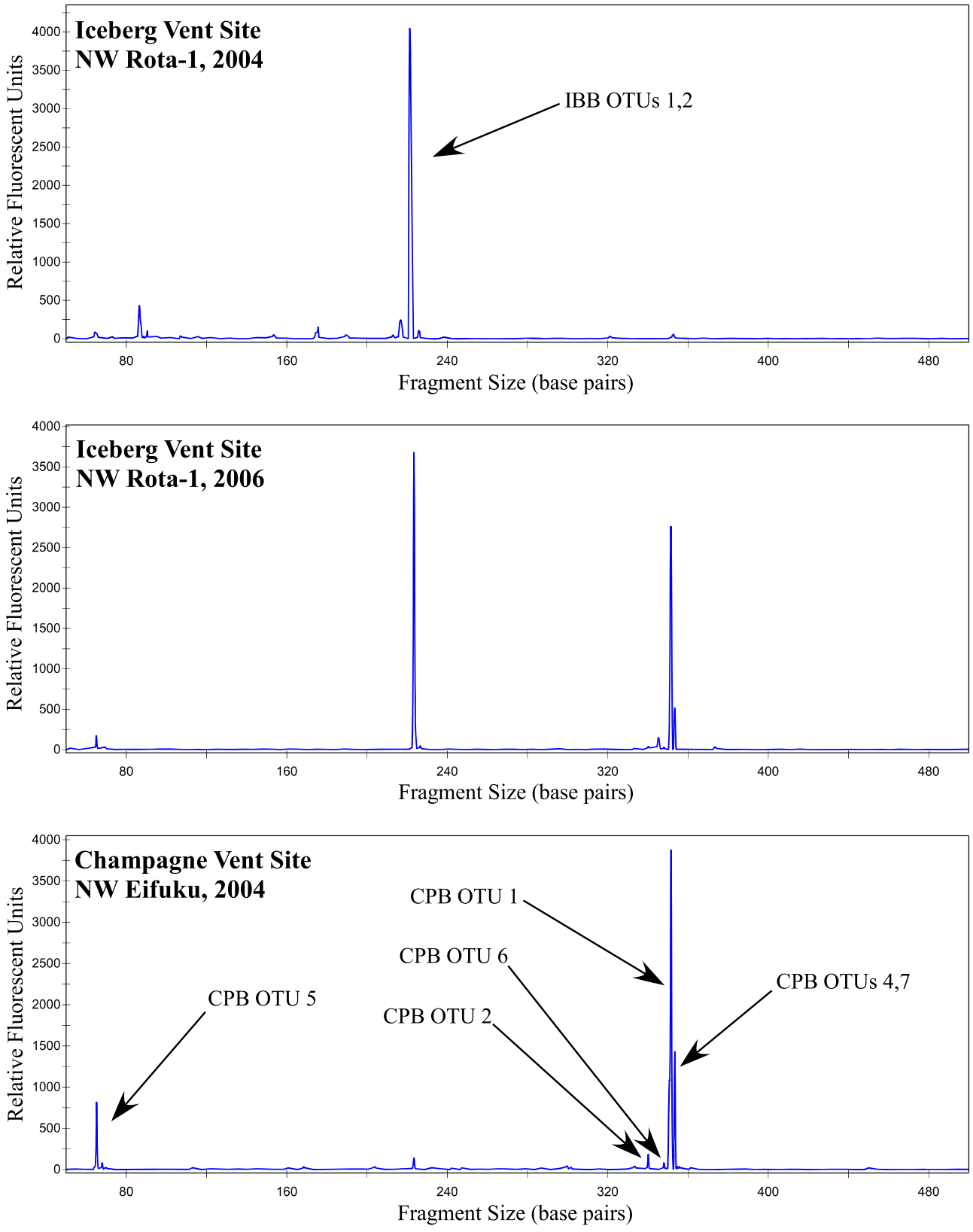

Figure 9. T-RFLP electropherogram showing the *Bst*UI digest representing the Bacterial community from Iceberg Vent in 2004 and 2006 and from Champagne Vent in 2004. Arrows indicate peaks corresponding to OTUs found in respective SSU rDNA clone libraries.

Pedersen, 2003]. This accumulation of iron oxides and heavy metals may form a zone of heavy metal deposition around these diffuse vents, which may be bioaccumulated and passed up the food chain, as chemoautotrophic growth via iron oxidation inputs a significant amount of organic carbon into the benthic food chain around these vent sites.

[43] The Archaeal T-RFLP fingerprints did not form clusters that correlated with the Bacterial T-RFLP fingerprints (Figure 4). Samples with radically different depths and temperatures grouped more closely with each other, suggesting that the Archaeal populations within the microbial mats are not directly influenced by the same hydrothermal fluid forcing functions as the more dominate Bacterial populations. In all samples, the dominate T-RFLP peak corresponded to populations from the Marine Group I Crenarchaeota (Figure 4), such as CPA OTUs 3, 4, and 7 (Figure 8). The ubiquitous presence of these phylotypes within hydrothermal vent habitats has been previously shown [*Moyer et al.*, 1998; *Takai et al.*, 2004b]. Although T-RFLP fingerprints show the dominance of the Marine Group I Crenarchaeota, Q-PCR analysis shows that these populations make up a very small portion of the overall microbial mat community when compared with Bacteria (Table 2).

[44] In addition to the extreme spatial variability found in microbial mat communities from vents at both the same and different seamounts, temporal variability was observed in some microbial mats sampled in 2004 and again in 2006 (Table 1). The microbial mat community from Iceberg vent on NW Rota-1 Seamount in 2004 was a clear outlier in the T-RFLP cluster analysis (Figure 3) due to the occurrence of a dominant single T-RFLP peak, and clone library analysis showed the community was dominated by ε-Proteobacteria phylotypes clustering most closely to cultured hydrogen-oxidizing Caminibacter group isolates (Figure 6). T-RFLP fingerprint analysis of the Iceberg Vent microbial mat community sampled in 2006 clustered closely with the microbial mat community from Champagne Vent from 2004 (Figure 3). Comparison of the T-RFLP fingerprints of the Champagne Vent microbial mats and the 2004 and 2006 microbial mats from Iceberg vent (Figure 9) shows the ribotypes associated with the Sulfurimonas and Sulfurovum groups of ε-Proteobacteria from the 2004 Champagne Vent microbial mat becoming more prevalent in the 2006 Iceberg vent microbial mat. This transition in the dominant microbial mat populations from the Caminibacter group to the Sulfurimonas and Sulfurovum group phylotypes may be a response to changing gas, fluid, and temperature composition of the vent over time. Models of the evolution of hydrothermal fluid composition after an eruptive event show a dramatic increase in volatiles immediately after the event, and the gradual evolution toward an increased brine-dominated vent composition, with decreased heat flux and increased concentrations of iron [*Butterfield et al.*, 1997]. Although it is unknown when the Iceberg vent became active, Brimstone Vent on NW Rota-1 is the site of an ongoing explosive eruption event [*Embley et al.*, 2006] which has been active for at least 2 years. The decreasing hydrothermal fluid temperature at Iceberg Vent (58°C in 2004 and 25°C in 2006; Table 1) certainly indicates the vent was in a state of cooling. The dynamic nature of the volcanic evolution of NW Rota-1 and the encouraging results from T-RFLPs from Iceberg Vent makes the Mariana Arc/back-arc system especially attractive for more in depth time series experiments to determine the potential for temporal variability within the microbial communities over longer time frames.

[45] This is the first study to describe the microbial communities of multiple hydrothermal sites along an arc/back-arc system. The diversity of microbial mat communities seems to be greater at subduction zones than at either hot spot volcanoes and along mid-ocean ridges. This enhanced community diversity seen in the T-RFLP fingerprint analyses is thought to be driven by complex subducting source material and the enhanced volcanic activity of arc systems when compared to other volcanic systems. Microbial mat communities were found to be extremely variable across the arc, and could even be extremely variable at a single arc seamount, such as what was observed on NW Eifuku, where the gassy, CO_2-dominated Champagne Vents were found in relative close proximity to the iron-cycling microbial mats at Yellow Top Vent Field and Yellow Cone Vent. The Mariana Arc/back-arc hydrothermal vent systems support a Bacterial biodiversity hot spot which may be indicative of these systems on a global scale.

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