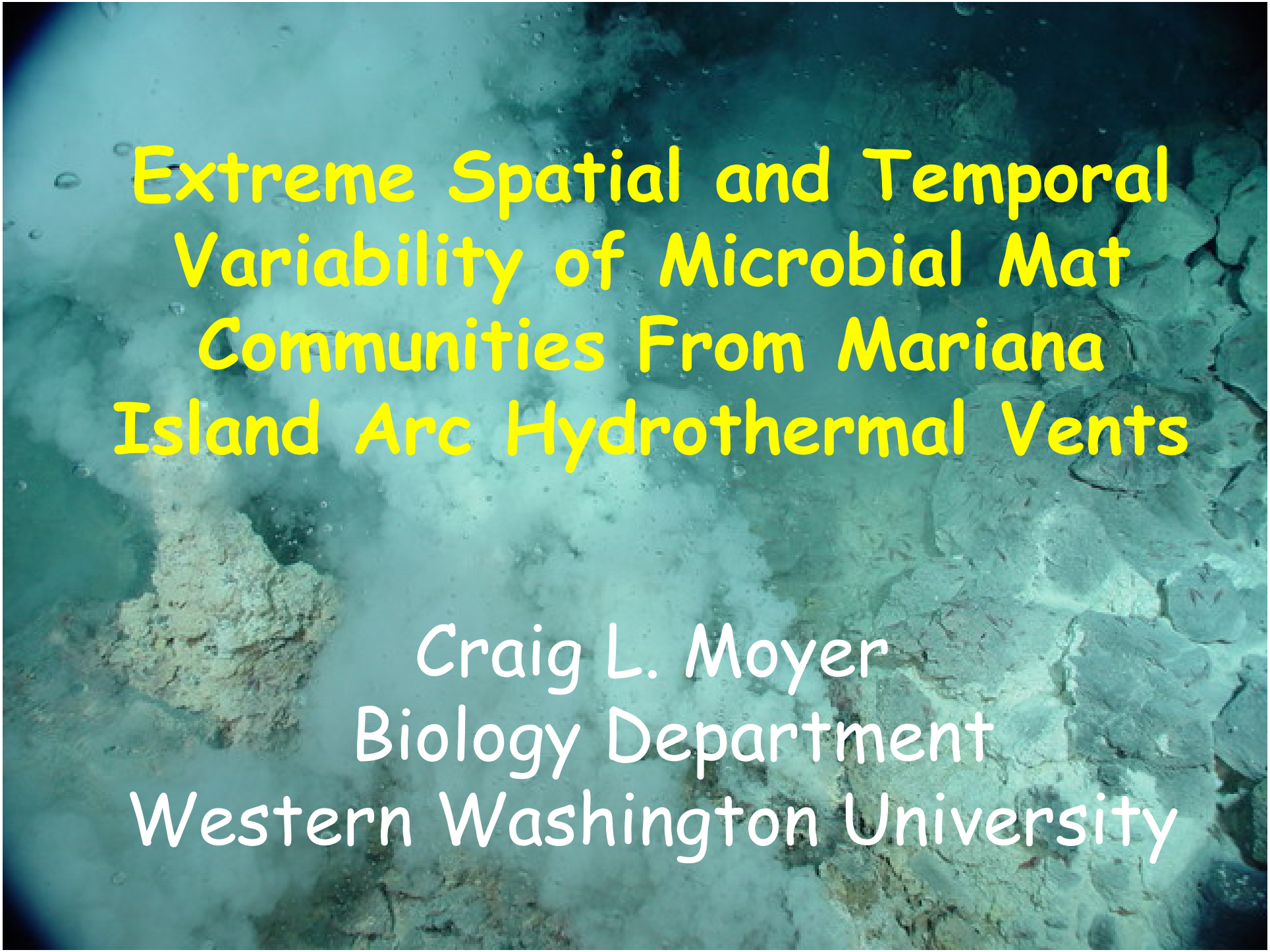


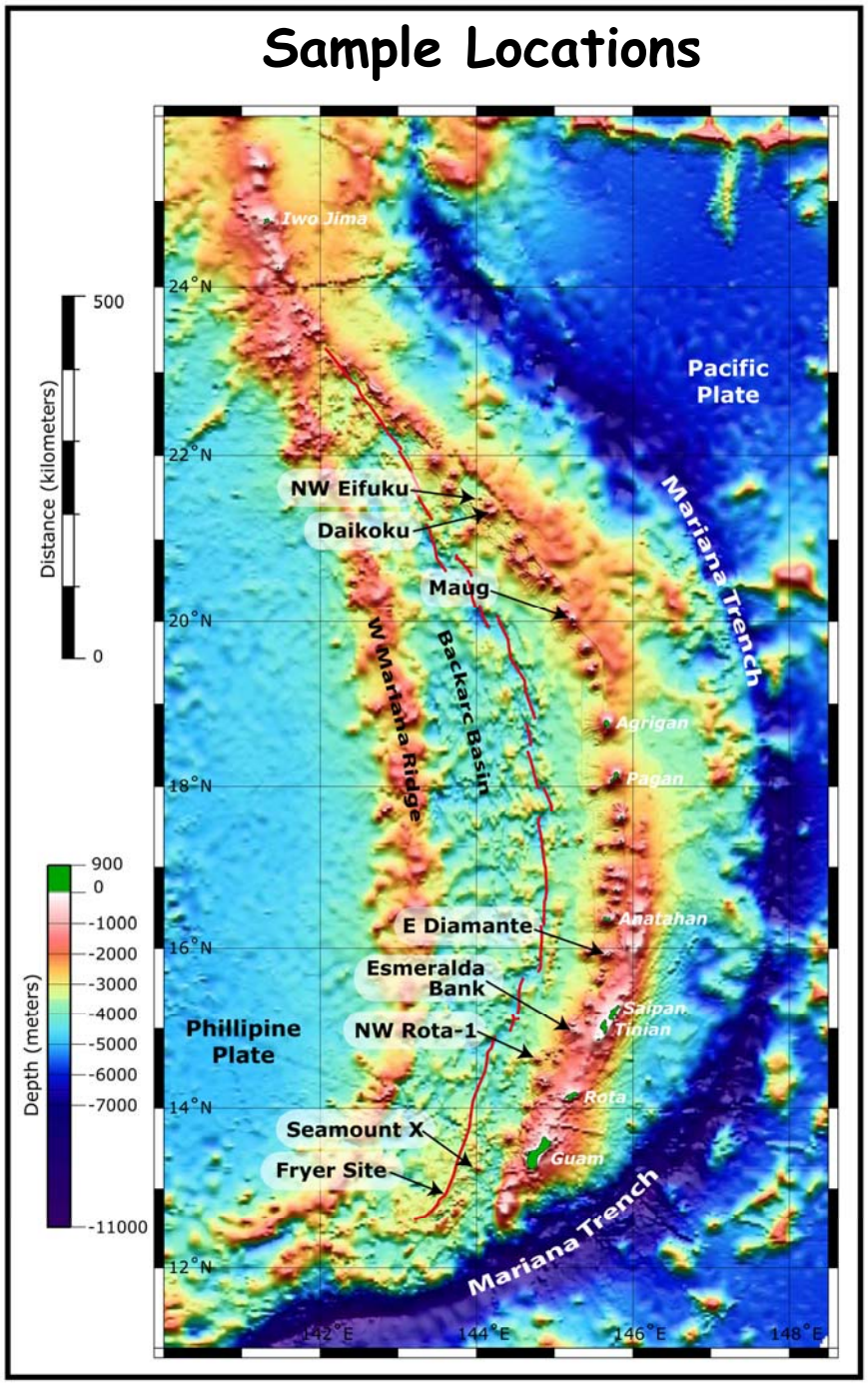
Extreme Spatial and Temporal Variability of Microbial Mat Communities From Mariana Island Arc Hydrothermal Vents

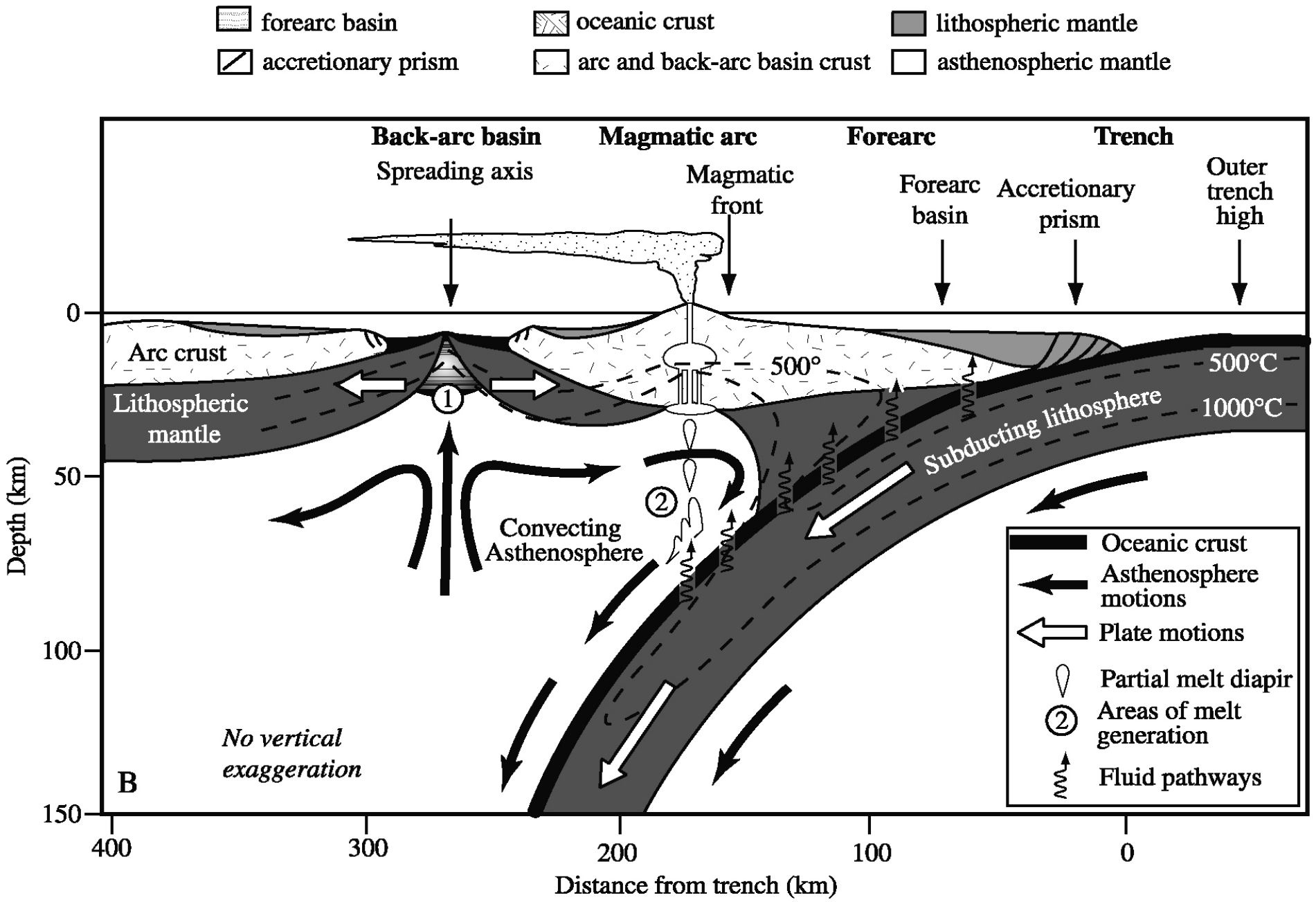
Craig L. Moyer
Biology Department
Western Washington University

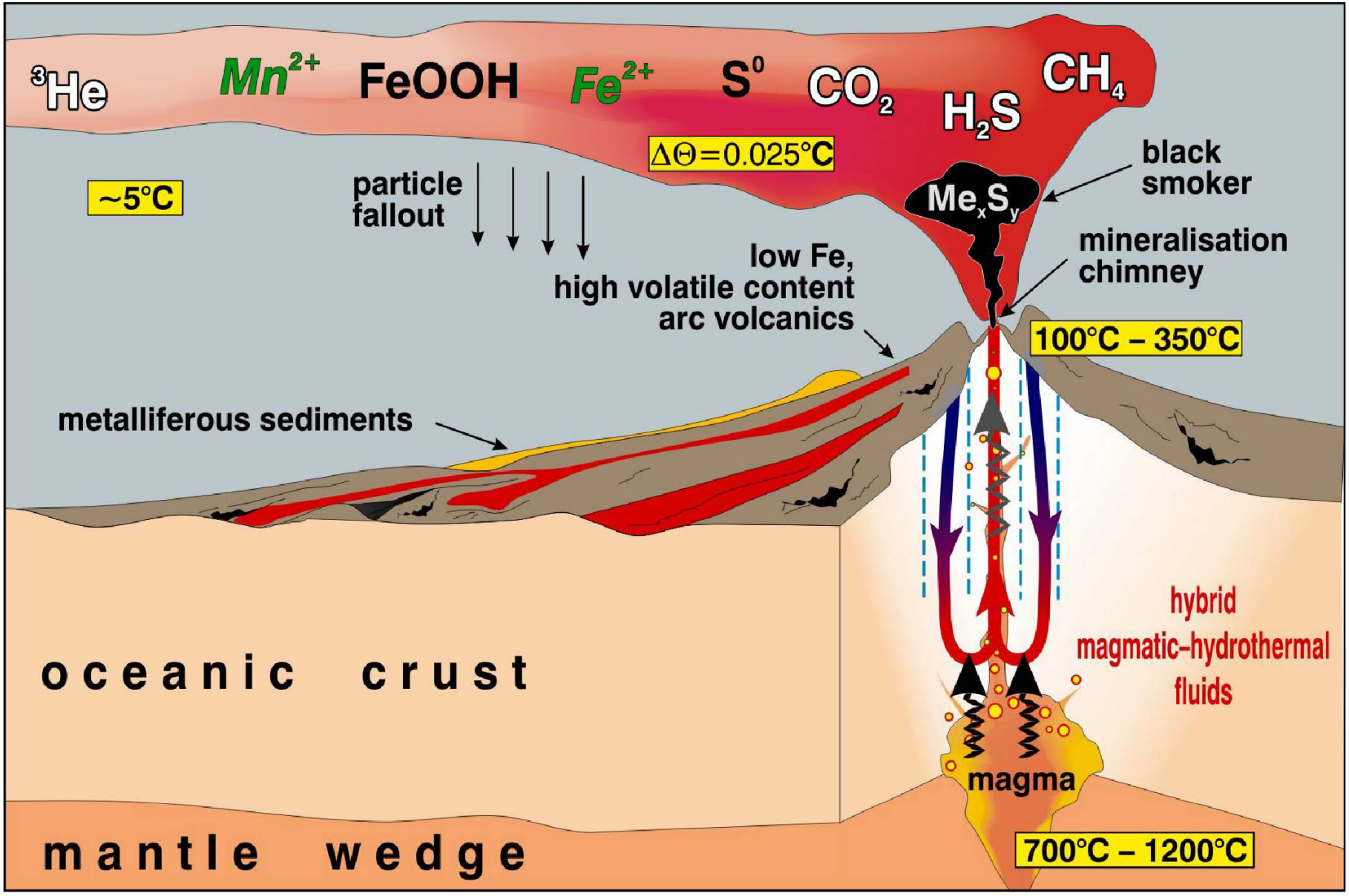
SROF Project
2003-2006

Unlike most vent systems, the Mariana arc/backarc is a convergent plate boundary

Sample Locations




forearc basin
accretionary prism
oceanic crust
arc and back-arc basin crust
lithospheric mantle
asthenospheric mantle
Back-arc basin
Spreading axis
Magmatic arc
Magmatic front
Forearc
Forearc basin
Accretionary prism
Trench
Outer trench high
Arc crust
Lithospheric mantle
500°
500°C
1000°C
Subducting lithosphere
Convecting Asthenosphere
No vertical exaggeration
B
Oceanic crust
Asthenosphere motions
Plate motions
Partial melt diapir
Areas of melt generation
Fluid pathways
Depth (km)
0
50
100
150
400
300
200
100
0
Distance from trench (km)



Schematic cartoon of a hybrid magmatic-hydrothermal venting system with water column plume. Note that the hydrothermal circulation is augmented by magmatic fluid in gaseous (yellow bubbles) and liquid (black squiggly arrows) phases. From "Fluids from Arcs: a submarine magmatic-hydrothermal perspective" G.J. MASSOTH, C.E.J. DE RONDE, E.T. BAKER, J.E. LUPTON, R.J. ARCULUS, & R.W. EMBLEY SOTA 2003

Microbial Metabolic Menu

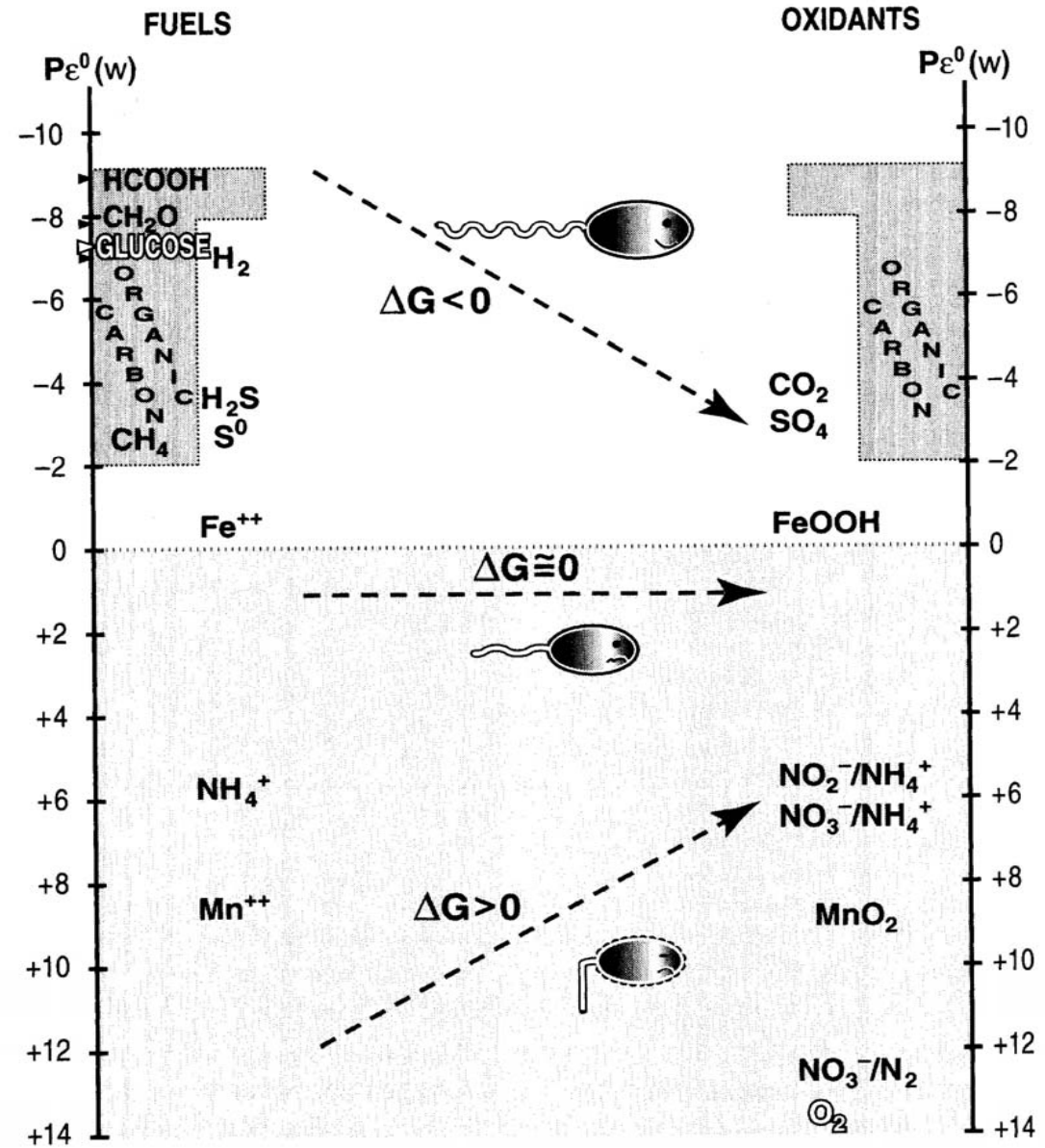
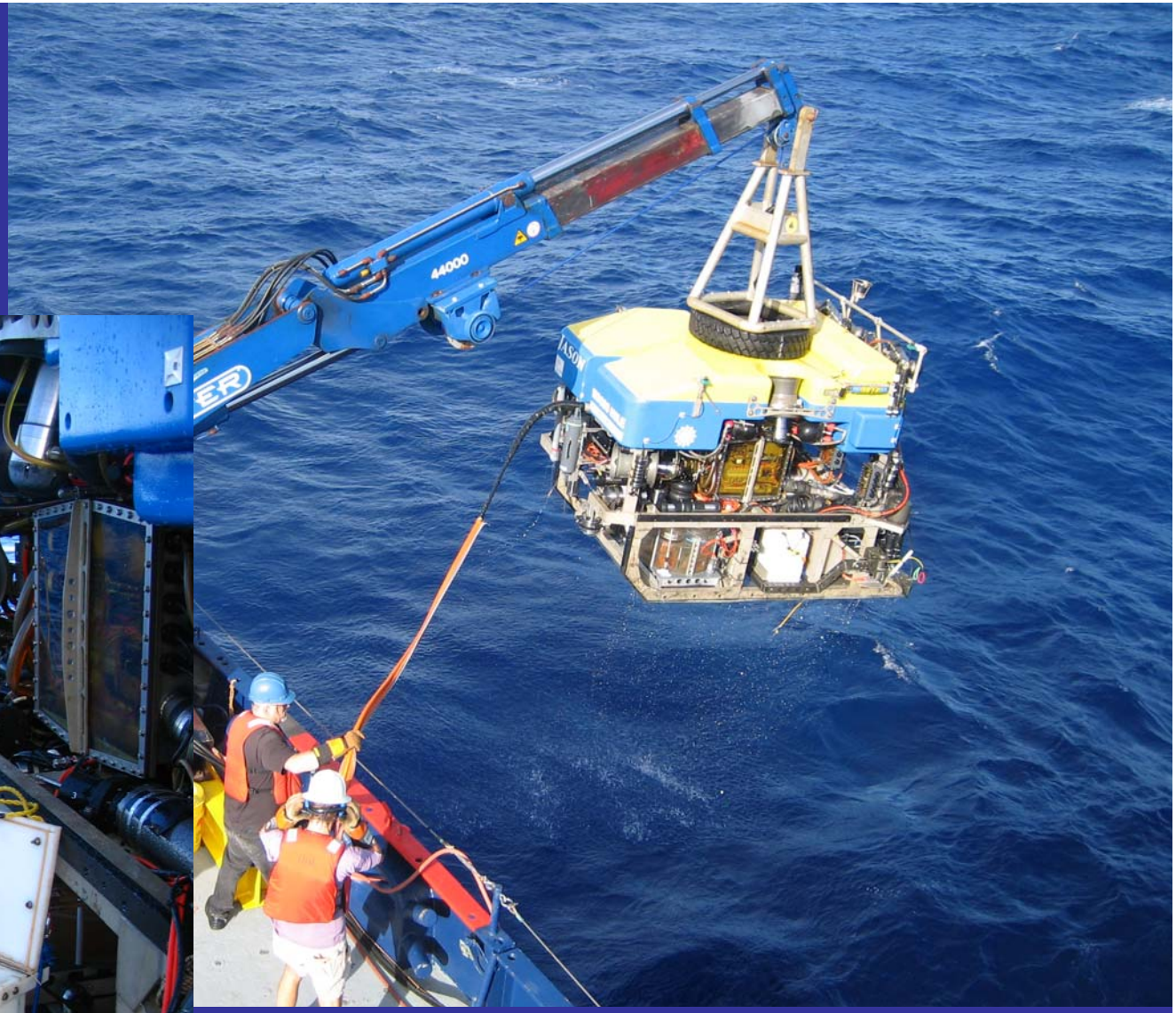


Figure courtesy of Ken Nealson


ROV ROPOS
2004
ROPOS.com
S G. THOMPSON
TTLE, WASHINGTON



ROV Jason II
2003 & 2006

Observing Active Volcanism at a Submarine Arc Volcano

Sulfur and Rocks Collected Inadvertently by ROPOS

Brimstone Pit, NW Rota-1

Submarine Ring of Fire
Major Results/Observations

- First Observations of Deep Submarine Arc Volcanic Event

- Photosynthetic/Chemosynthetic Ecosystems Interaction

- Shallowest Massive Sulfide Formation at 344 m (220^0 C)

- Champagne Vent, 1600 meter site with intense
  CO_2 Venting – Bubbles of Liquid CO_2

- High Variability of Biological Communities among
  & between Volcanoes

Discovery of Active Smokers at only 344 meters

East Diamante Caldera

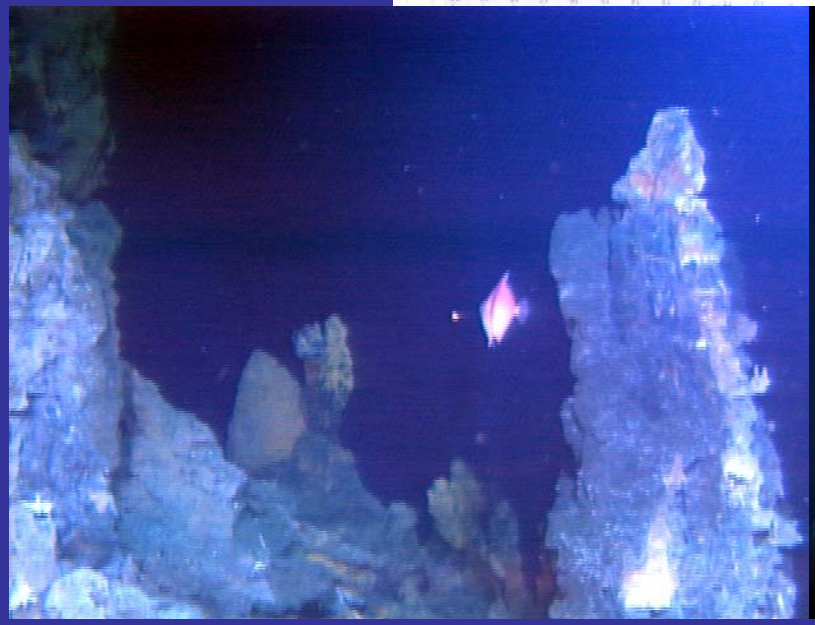


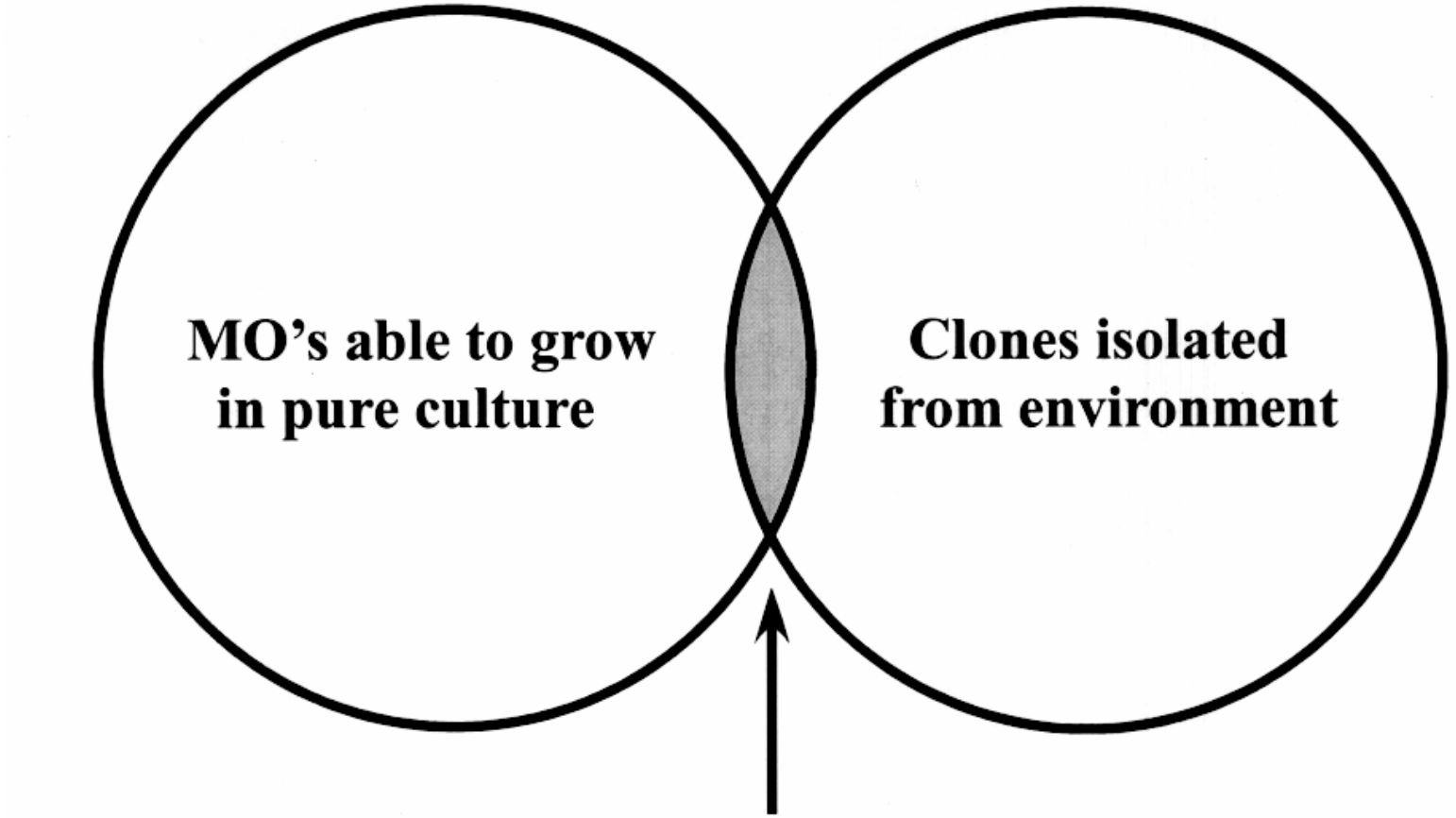
Table 1. Microbial mat samples (n=25) were collected and analyzed using T-RFLP, Q-PCR, and then targeted cloning & sequencing.

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	Backarc	SMBSC†	12°57.190 N 143°37.125 E	Push Core	Yellow/White mat	2860	77
2003	J2-42-2W	Fryer Site	Backarc	SMBSC†	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	Multi-colored 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
2006	J2-184-B	Snail Mat	Volcanic arc	Seamount X	nd	Suction Sample	White mat	1188	nd
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

† southern Mariana Backarc Spreading Center

Importance of a Molecular Microbiological Approach

- **Traditional culturing** techniques isolate ~1% of the total bacteria in marine ecosystems, thereby severely underestimating diversity and community structure.
- Because nutrient-rich **culture media** have been historically used during enrichment procedures, bacteria which may be dominant in natural communities are selected against in favor of copiotrophic (weedy) bacteria.
- **SSU rRNAs** and their respective genes are excellent descriptors of microbial taxa based on phylogeny.



>1% Crossover between these groups

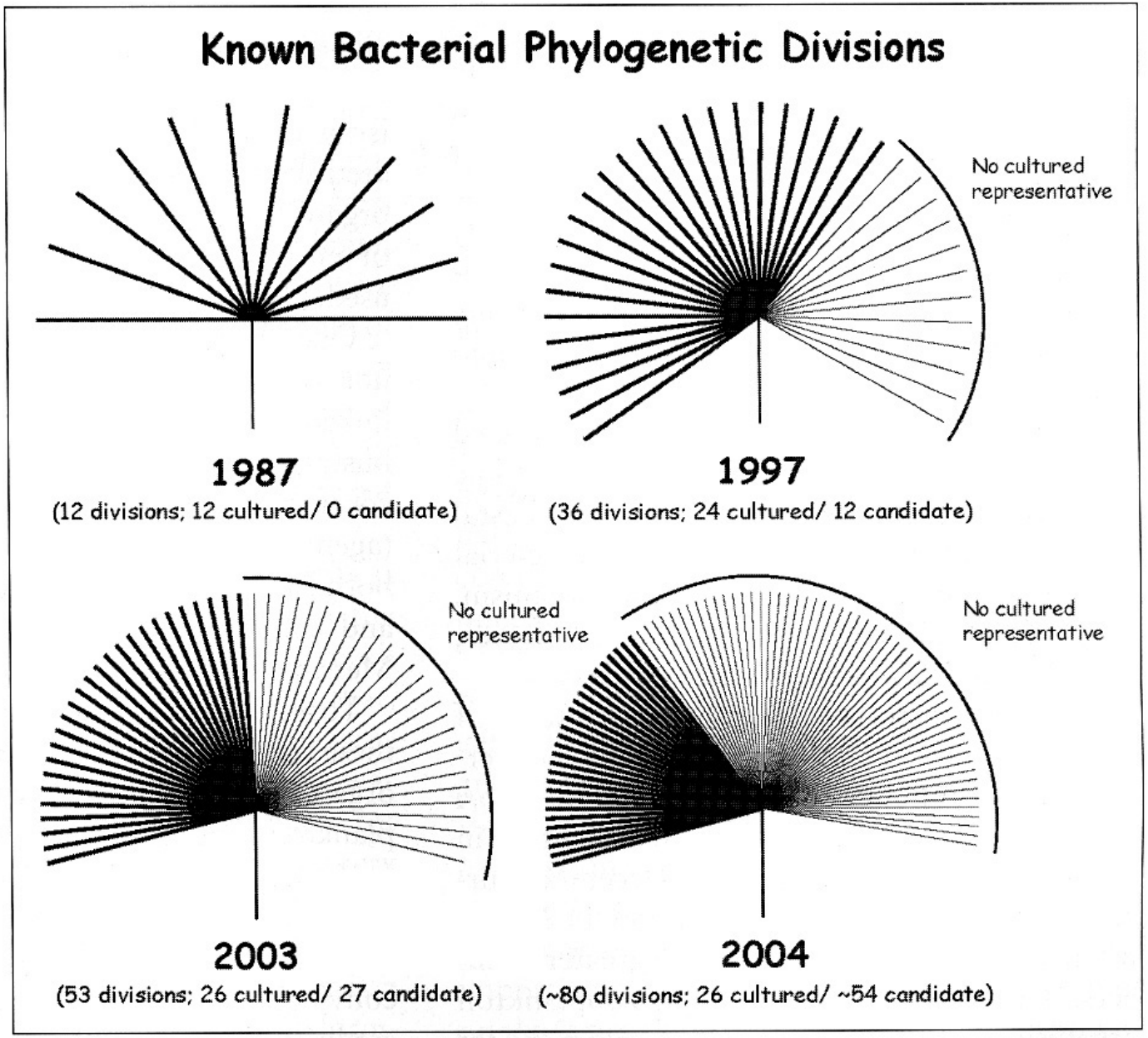
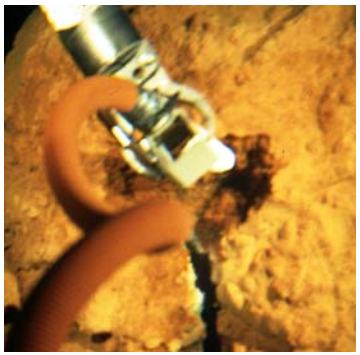


Diagram of the recent expansion in the number of known bacterial phylogenetic divisions. Currently, there is no resolution of any specific relationships between the bacterial divisions. The extent of known diversity is expanding rapidly due primarily to culture-independent environmental surveys. (Figure courtesy of Norman Pace and Kirk Harris.)

Why ribosomal RNAs?

- Found among **all** living organisms (for 3.8 of the last 4.5 billion years). Integral part of protein synthesis machinery.
- Cell component analyses provide culture-independent means of investigating questions in microbial ecology (lack of morphology).
- rRNAs offer a type of sequence information that makes them excellent descriptors of an organism's evolutionary history.
- No detectable horizontal gene transfer, especially important for *Bacteria* and *Archaea*.
- Large and growing database; RDP contains >480K SSU rRNA genes.

T-RFLP FLOWCHART



Environmental Sample

Extract genomic DNA

Genomic DNA

PCR w/ fluorescent primers

CGGTACCGGGCAGCTGAATTACG

CGGAGCTCTAAGCTAACCGGACG

CGTCCGGCAGCTCTAACGTTACG

CGGCCGGCTAAGCAGCTGTTACG

Community of PCR amplicons

Cut with *AluI*

A CGGTACCGGGCAG E CTGAATTACG

B CGGAG F CTCTAAGCTAACCGGACG

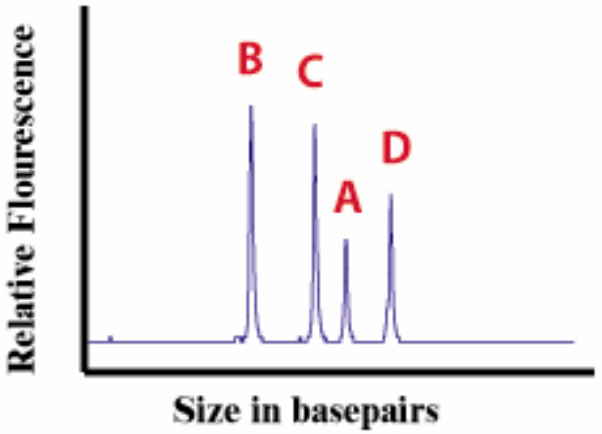
C CGTCCGGCAG G CTCTAACGTTACG

D CGGCCGGCTAAGCAG H CTGTTACG

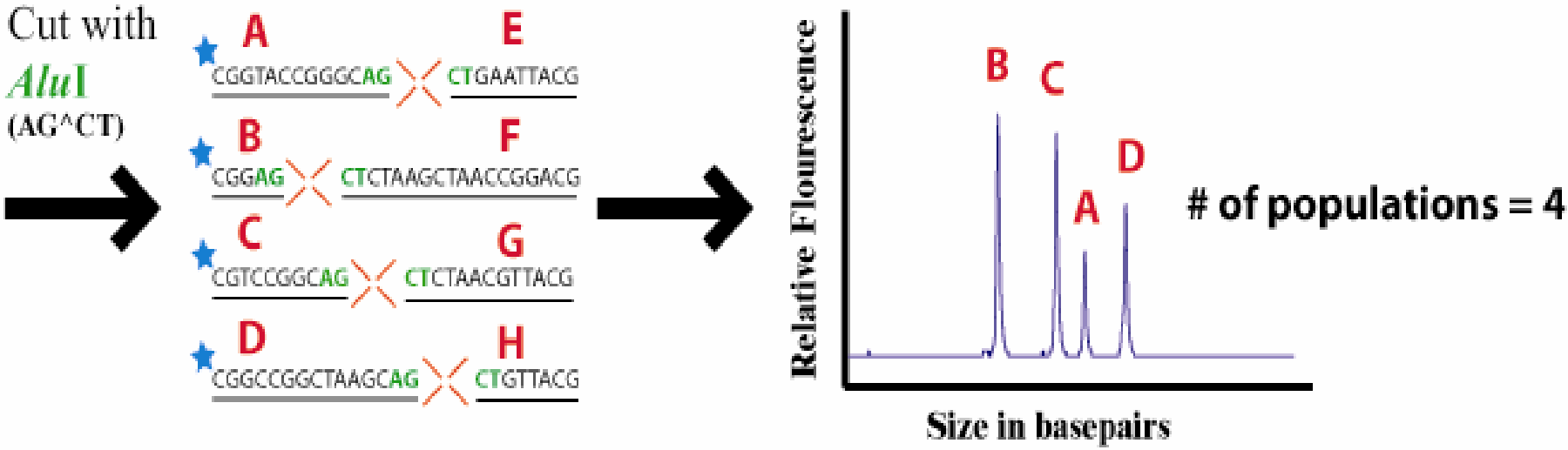
Community of RFs

LIF-CE

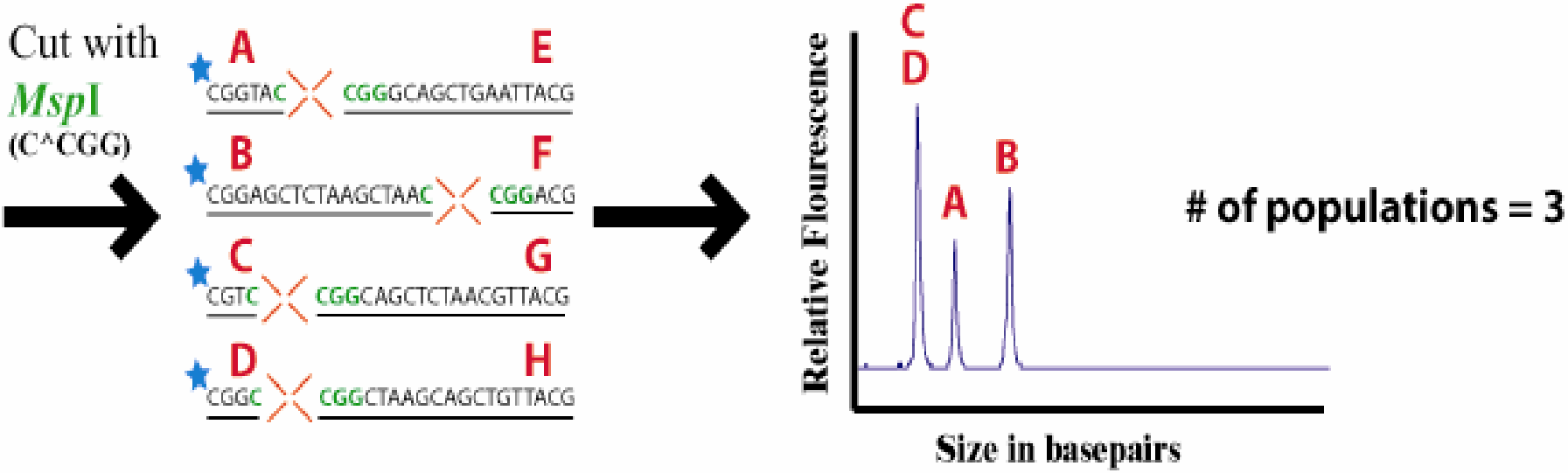
Separated fragments

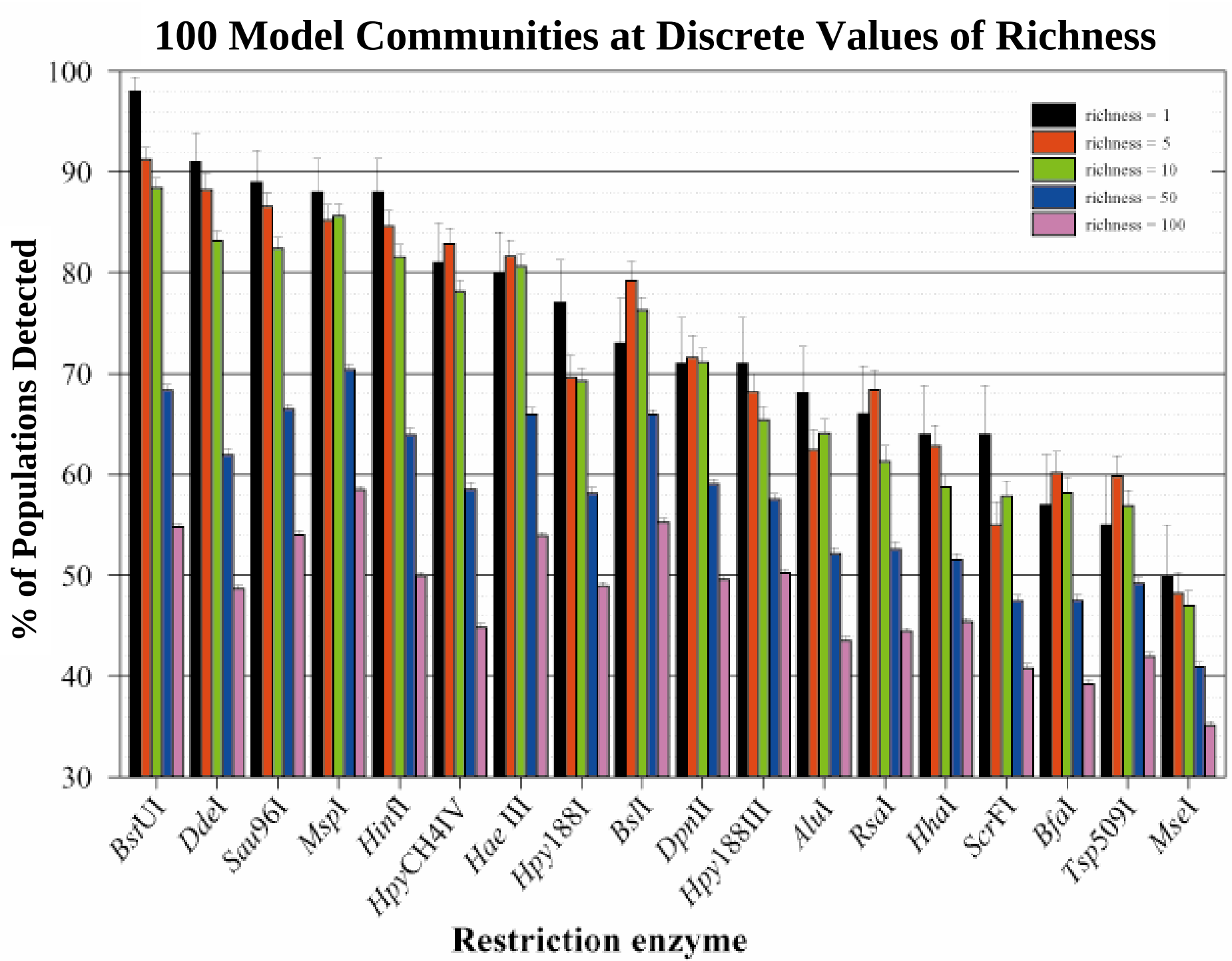


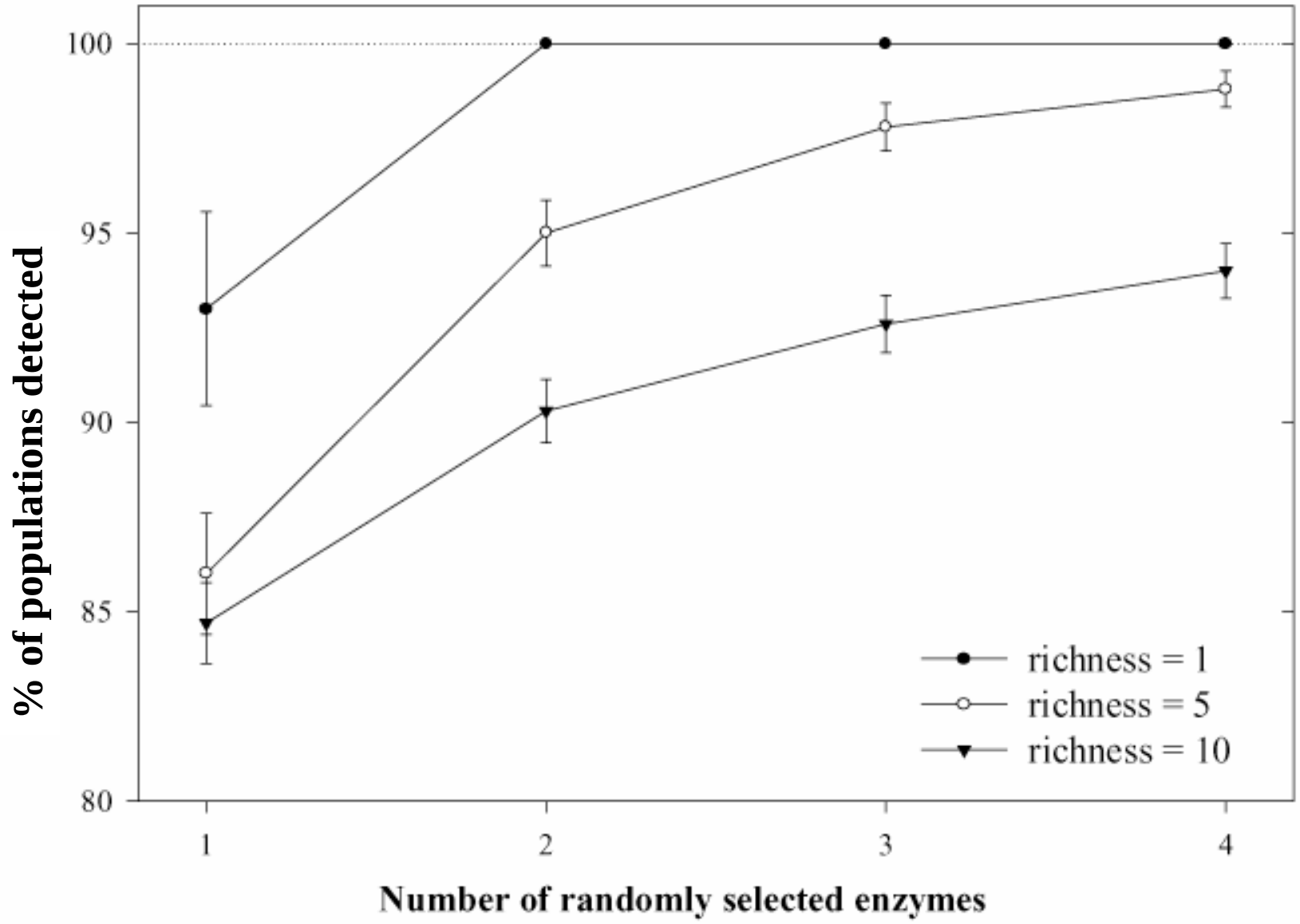
Chromatogram of peak heights



Size is limited to 50-500 basepairs



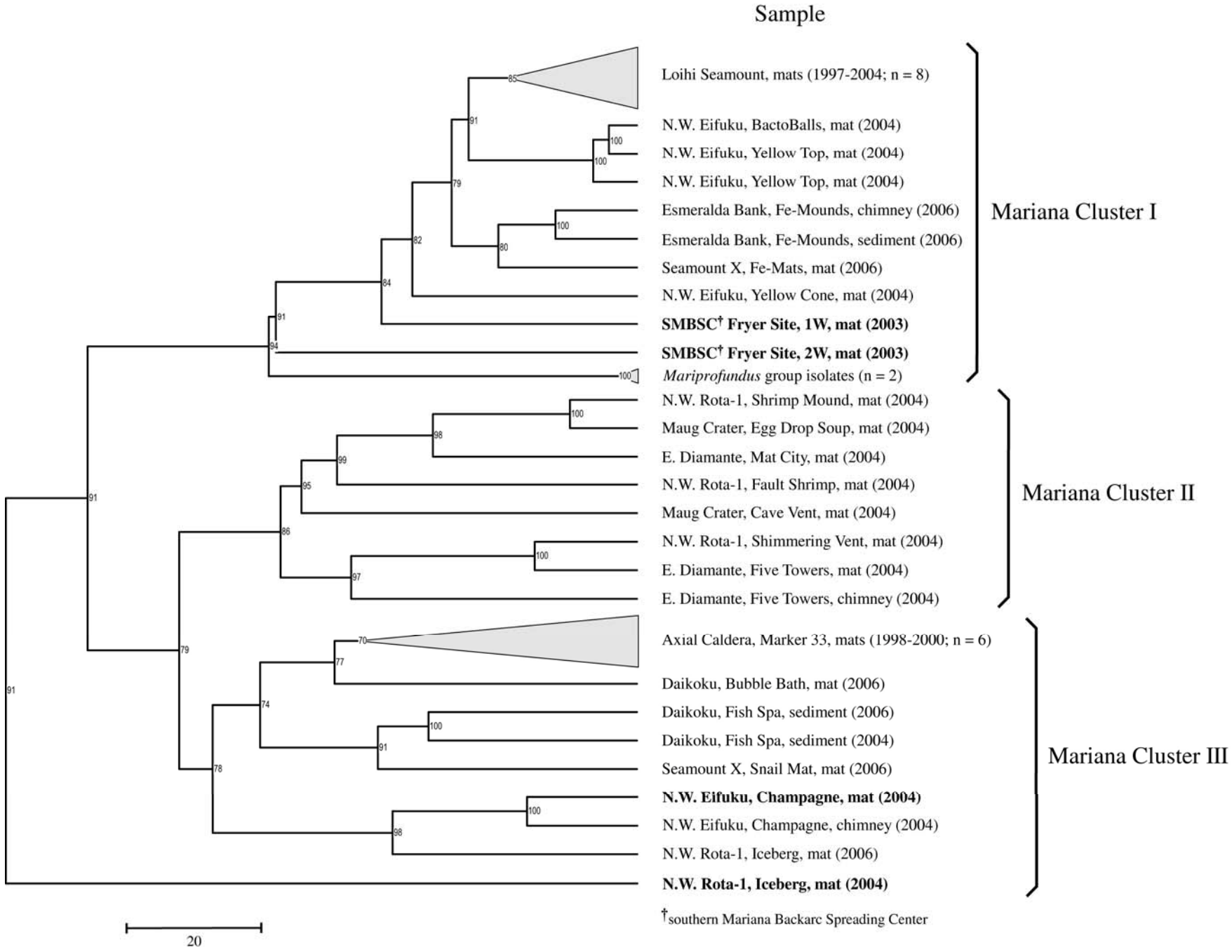

100 Model Communities at Discrete Values of Richness
% of Populations Detected
100
90
80
70
60
50
40
30
richness = 1
richness = 5
richness = 10
richness = 50
richness = 100
BsrUI
DdeI
Sau96I
MspI
HinfI
HpyCH4IV
Hae III
Hpy188I
BslI
DpnII
Hpy188III
AluI
RsaI
HhaI
ScrFI
BfaI
Tsp509I
MseI
Restriction enzyme

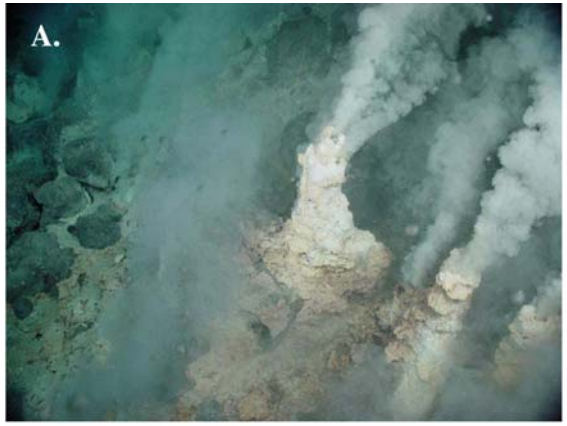


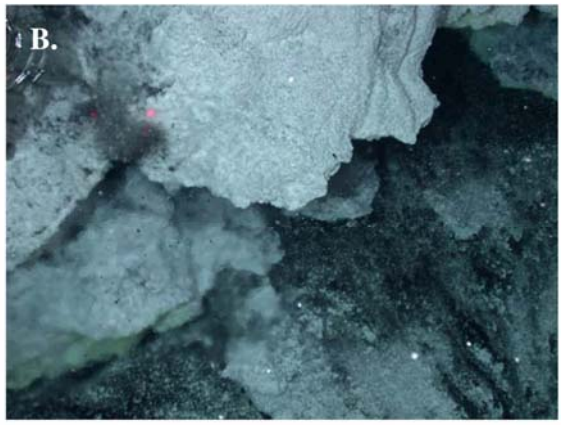
Take Home Message:
Accurately resolve ribotypes in communities with low to intermediate richness!

Integrating Molecular Methods

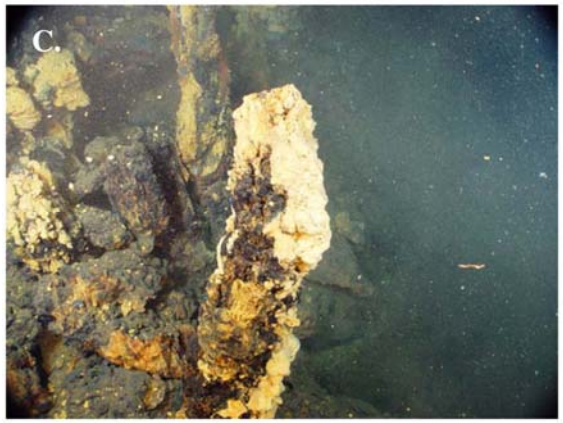
- Terminal-restriction fragment length polymorphisms (T-RFLPs) were used to track populations based on ribotypes (in lots of samples).
- A/B Q-PCR needed to determine domain level proportions.
- Clone library and phylogenetic analyses needed to identify these phylotypes within the community (in few samples).

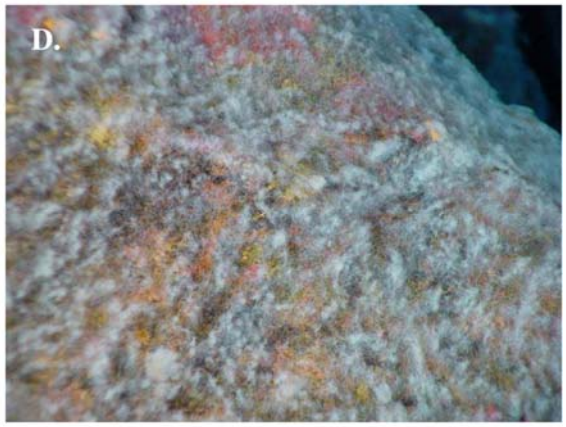

Sample
Loihi Seamount, mats (1997-2004; n = 8)
N.W. Eifuku, BactoBalls, mat (2004)
N.W. Eifuku, Yellow Top, mat (2004)
N.W. Eifuku, Yellow Top, mat (2004)
Esmeralda Bank, Fe-Mounds, chimney (2006)
Esmeralda Bank, Fe-Mounds, sediment (2006)
Seamount X, Fe-Mats, mat (2006)
N.W. Eifuku, Yellow Cone, mat (2004)
SMBSC† Fryer Site, 1W, mat (2003)
SMBSC† Fryer Site, 2W, mat (2003)
Mariprofundus group isolates (n = 2)
Mariana Cluster I
N.W. Rota-1, Shrimp Mound, mat (2004)
Maug Crater, Egg Drop Soup, mat (2004)
E. Diamante, Mat City, mat (2004)
N.W. Rota-1, Fault Shrimp, mat (2004)
Maug Crater, Cave Vent, mat (2004)
N.W. Rota-1, Shimmering Vent, mat (2004)
E. Diamante, Five Towers, mat (2004)
E. Diamante, Five Towers, chimney (2004)
Mariana Cluster II
Axial Caldera, Marker 33, mats (1998-2000; n = 6)
Daikoku, Bubble Bath, mat (2006)
Daikoku, Fish Spa, sediment (2006)
Daikoku, Fish Spa, sediment (2004)
Seamount X, Snail Mat, mat (2006)
N.W. Eifuku, Champagne, mat (2004)
N.W. Eifuku, Champagne, chimney (2004)
N.W. Rota-1, Iceberg, mat (2006)
N.W. Rota-1, Iceberg, mat (2004)
Mariana Cluster III
†southern Mariana Backarc Spreading Center
20





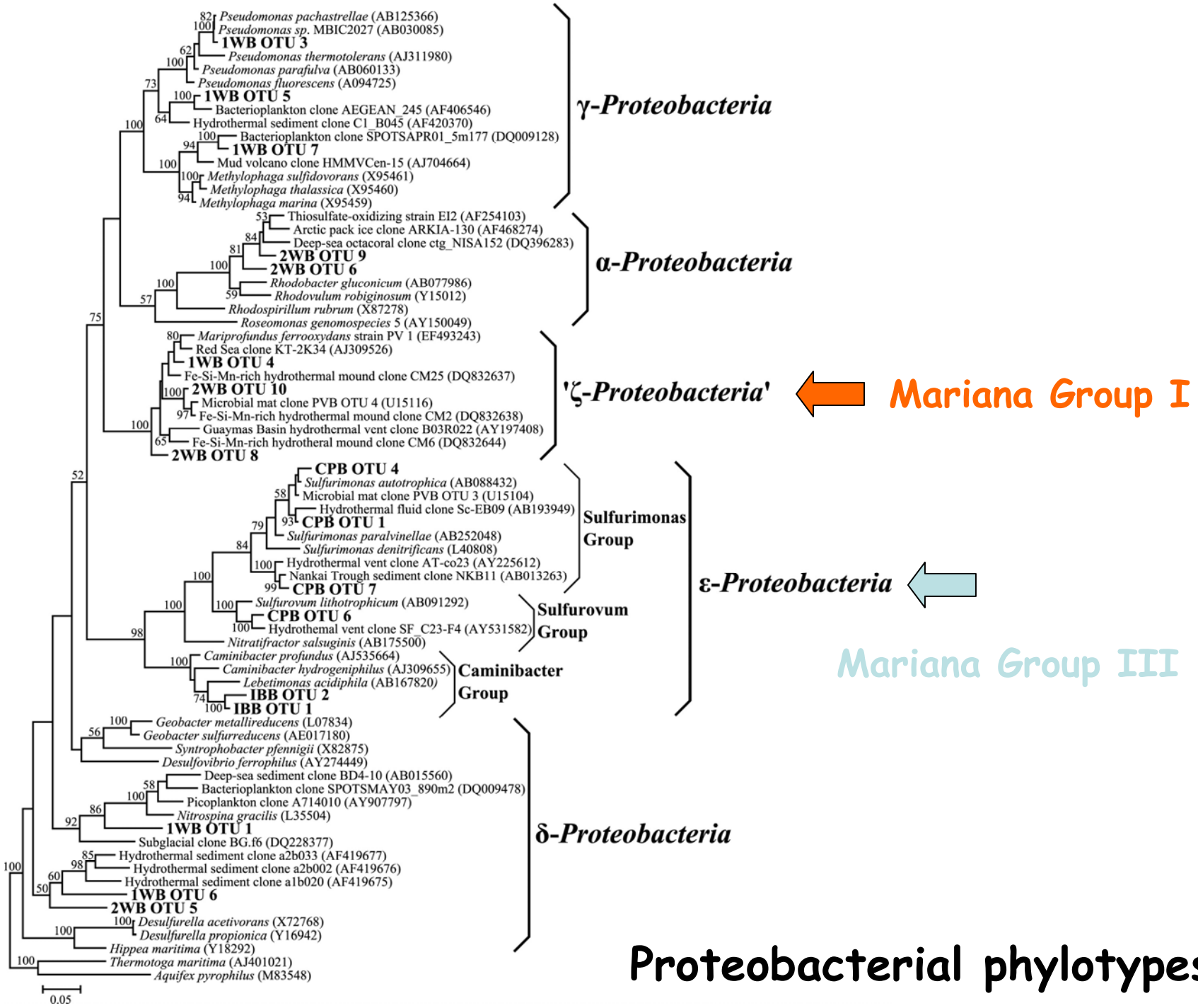
Mariana Group III
Mostly Sulfur-cycling
ε-*Proteobacteria*

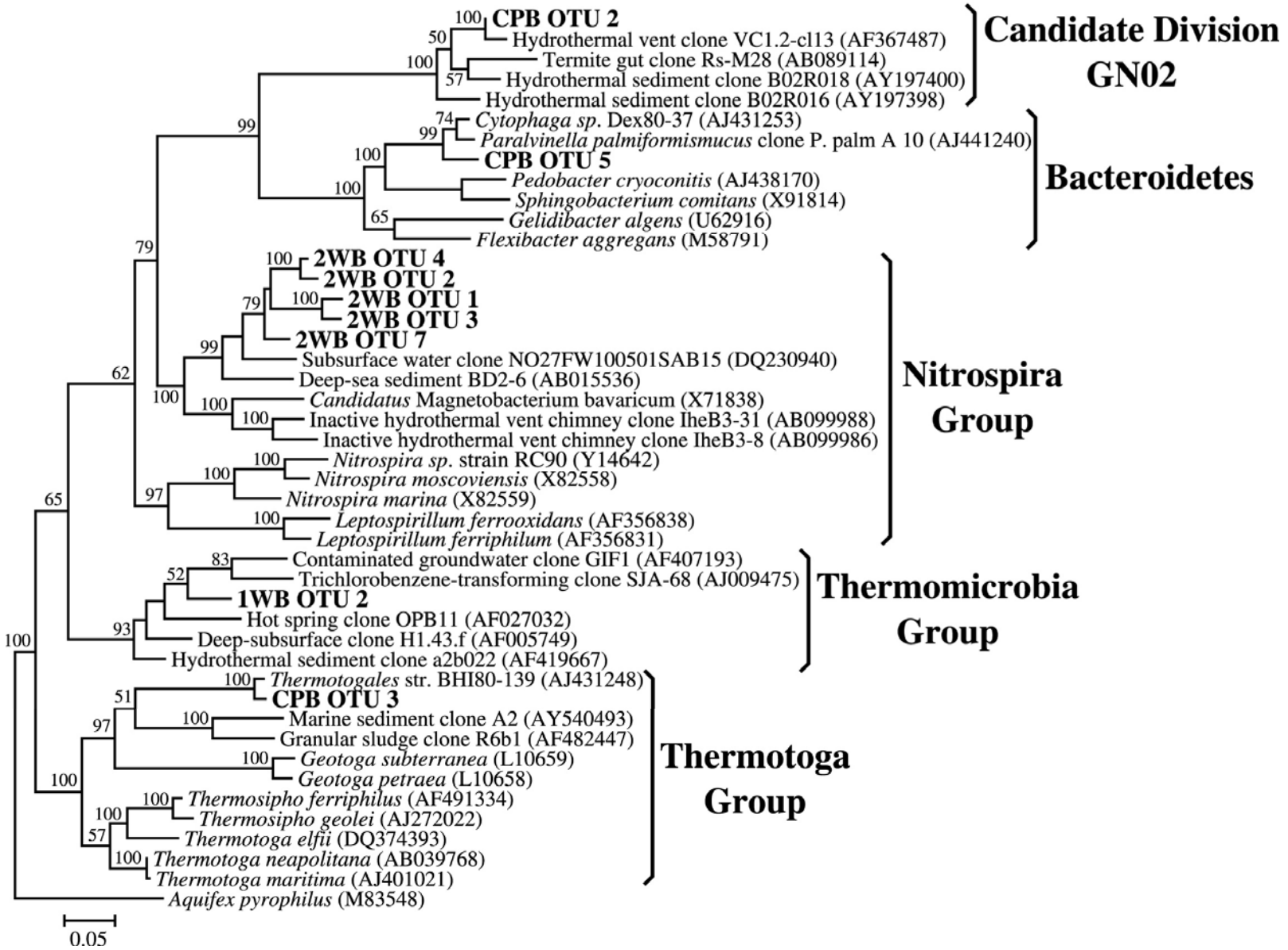




Mariana Group II
Mostly Heterotrophs
like CFBs

Mariana Group I
Mostly Iron-cycling
ζ-*Proteobacteria*


Pseudomonas pachastrellae (AB125366)
Pseudomonas sp. MBIC2027 (AB030085)
1WB OTU 3
Pseudomonas thermotolerans (AJ311980)
Pseudomonas parafulva (AB060133)
Pseudomonas fluorescens (A094725)
1WB OTU 5
Bacterioplankton clone AEGEAN_245 (AF406546)
Hydrothermal sediment clone C1_B045 (AF420370)
Bacterioplankton clone SPOTSAPR01_5m177 (DQ009128)
1WB OTU 7
Mud volcano clone HMMVCen-15 (AJ704664)
Methylophaga sulfidovorans (X95461)
Methylophaga thalassica (X95460)
Methylophaga marina (X95459)
γ-Proteobacteria
Thiosulfate-oxidizing strain EI2 (AF254103)
Arctic pack ice clone ARKIA-130 (AF468274)
Deep-sea octacoral clone ctg_NISA152 (DQ396283)
2WB OTU 9
2WB OTU 6
Rhodobacter gluconicum (AB077986)
Rhodovulum robiginosum (Y15012)
Rhodospirillum rubrum (X87278)
Roseomonas genomospecies 5 (AY150049)
α-Proteobacteria
Mariprofundus ferrooxydans strain PV 1 (EF493243)
Red Sea clone KT-2K34 (AJ309526)
1WB OTU 4
Fe-Si-Mn-rich hydrothermal mound clone CM25 (DQ832637)
2WB OTU 10
Microbial mat clone PVB OTU 4 (U15116)
Fe-Si-Mn-rich hydrothermal mound clone CM2 (DQ832638)
Guaymas Basin hydrothermal vent clone B03R022 (AY197408)
Fe-Si-Mn-rich hydrotheral mound clone CM6 (DQ832644)
2WB OTU 8
'ζ-Proteobacteria'
Mariana Group I
CPB OTU 4
Sulfurimonas autotrophica (AB088432)
Microbial mat clone PVB OTU 3 (U15104)
Hydrothermal fluid clone Sc-EB09 (AB193949)
CPB OTU 1
Sulfurimonas paralvinellae (AB252048)
Sulfurimonas denitrificans (L40808)
Hydrothermal vent clone AT-co23 (AY225612)
Nankai Trough sediment clone NKB11 (AB013263)
CPB OTU 7
Sulfurimonas Group
Sulfurovum lithotrophicum (AB091292)
CPB OTU 6
Hydrothemal vent clone SF_C23-F4 (AY531582)
Nitratifractor salsuginis (AB175500)
Sulfurovum Group
Caminibacter profundus (AJ535664)
Caminibacter hydrogeniphilus (AJ309655)
Lebetimonas acidiphila (AB167820)
IBB OTU 2
IBB OTU 1
Caminibacter Group
ε-Proteobacteria
Mariana Group III
Geobacter metallireducens (L07834)
Geobacter sulfurreducens (AE017180)
Syntrophobacter pfennigii (X82875)
Desulfovibrio ferrophilus (AY274449)
Deep-sea sediment clone BD4-10 (AB015560)
Bacterioplankton clone SPOTSMAY03_890m2 (DQ009478)
Picoplankton clone A714010 (AY907797)
Nitrospina gracilis (L35504)
1WB OTU 1
Subglacial clone BG.f6 (DQ228377)
Hydrothermal sediment clone a2b033 (AF419677)
Hydrothermal sediment clone a2b002 (AF419676)
Hydrothermal sediment clone a1b020 (AF419675)
1WB OTU 6
2WB OTU 5
Desulfurella acetivorans (X72768)
Desulfurella propionica (Y16942)
Hippea maritima (Y18292)
Thermotoga maritima (AJ401021)
Aquifex pyrophilus (M83548)
δ-Proteobacteria
0.05
Proteobacterial phylotypes



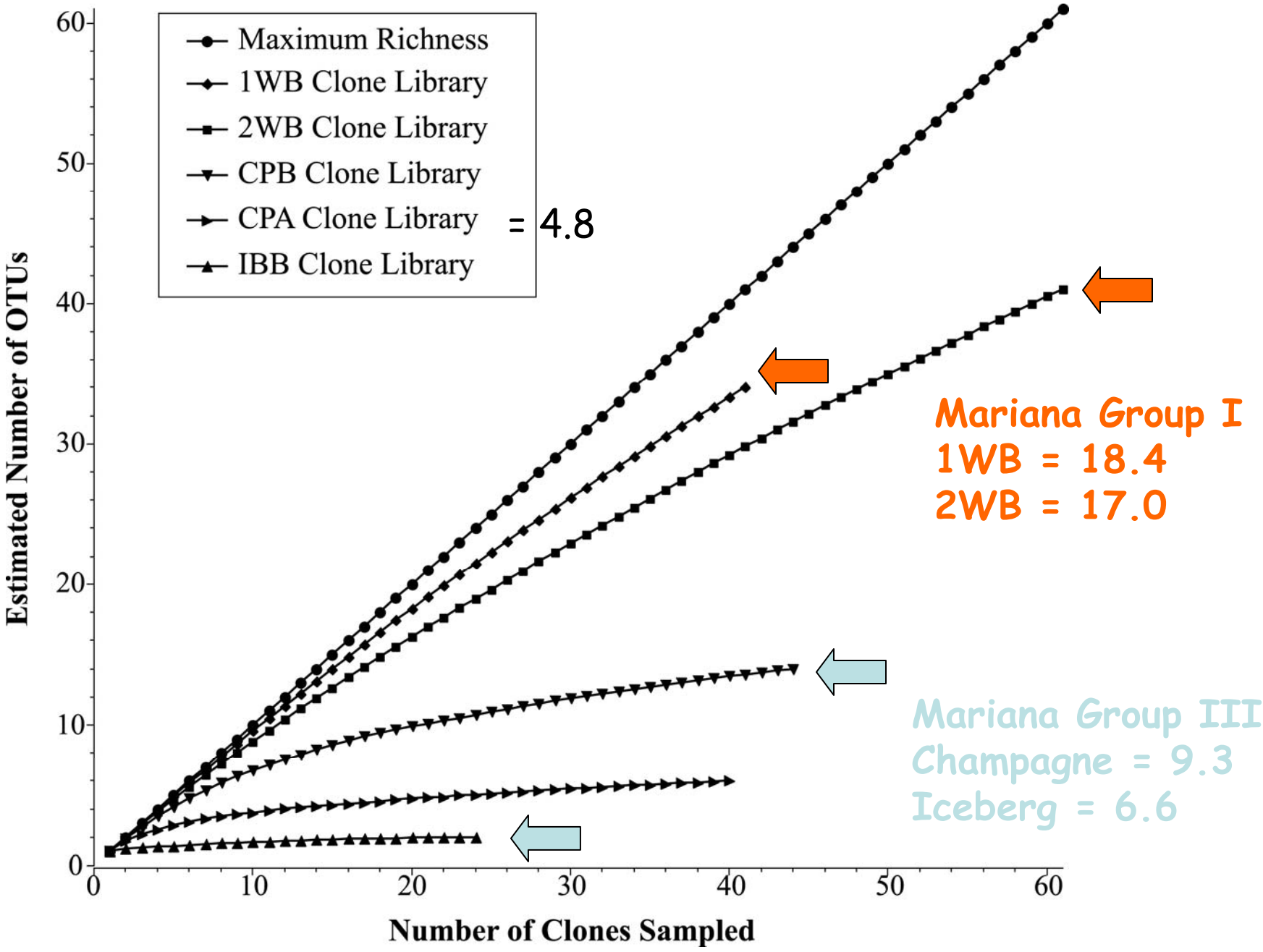
Non-Proteobacterial phylotypes

Estimate of Complexity (aka Richness)

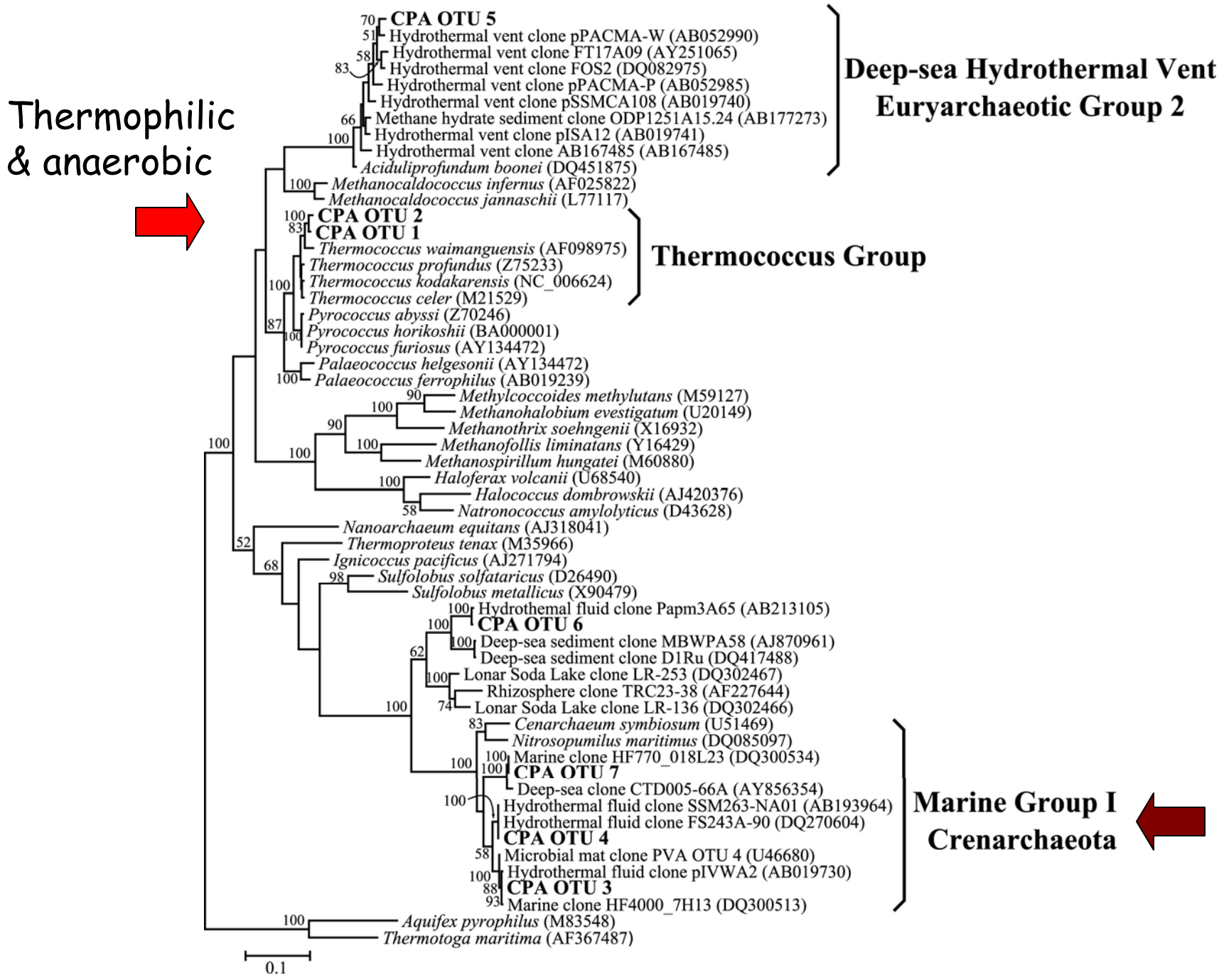
Biomass Proxy

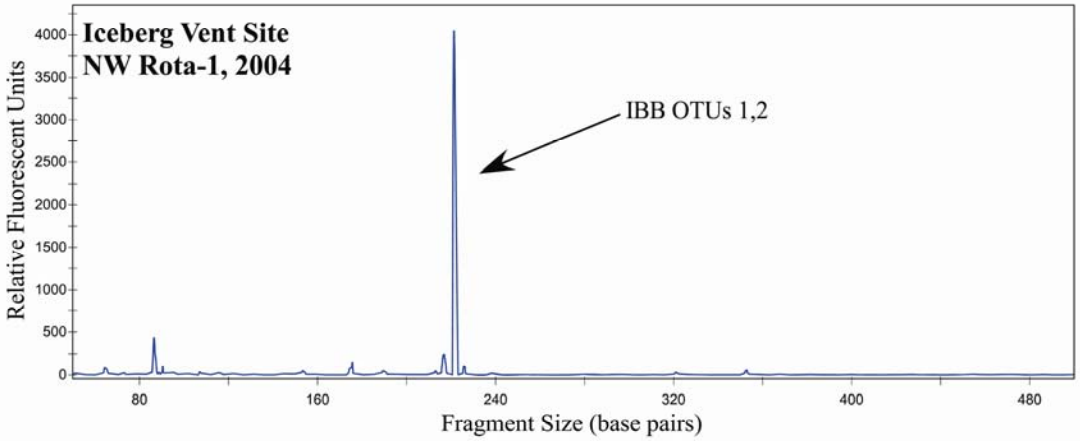
Year	Sample	Vent Site	ng DNA/g Sample (wet weight)	Percent Archaea	Ave. T-RFs Bacteria	Ave. T-RFs Archaea
2003	J2-42-1W	Fryer Site	2250	11.3±0.49	18.4±4.2	18.3±6.5
2003	J2-42-2W	Fryer Site	2466	10.4±0.26	17.0±1.5	16.9±5.4
2004	R782-b5	Shimmering Vent	147	2.2±0.16	16.6±3.6	9.8±3.5
2004	R782-b7	Shrimp 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

Difference is Bacteria


Maximum Richness
1WB Clone Library
2WB Clone Library
CPB Clone Library
CPA Clone Library
IBB Clone Library
= 4.8
Estimated Number of OTUs
Number of Clones Sampled
0
10
20
30
40
50
60
Mariana Group I
1WB = 18.4
2WB = 17.0
Mariana Group III
Champagne = 9.3
Iceberg = 6.6

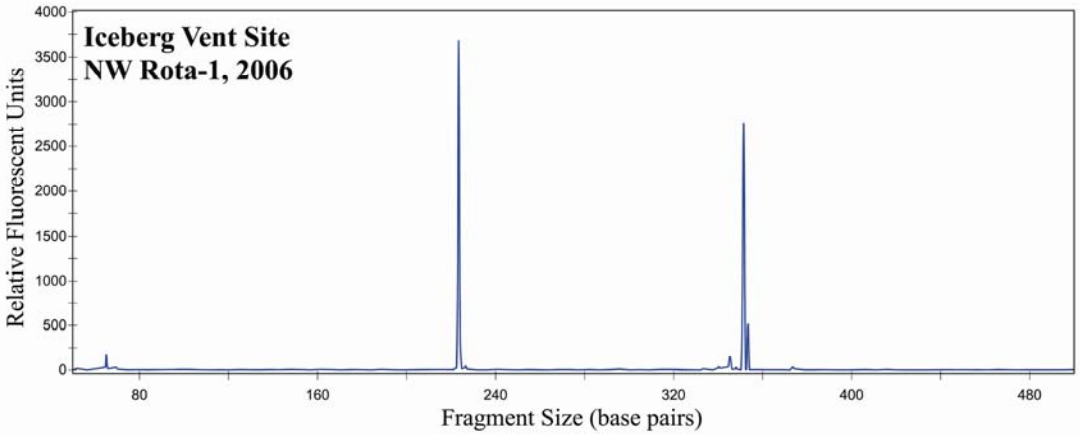
Marine Group I: Cosmopolitan Archaeoplankton


Thermophilic & anaerobic
CPA OTU 5
Hydrothermal vent clone pPACMA-W (AB052990)
Hydrothermal vent clone FT17A09 (AY251065)
Hydrothermal vent clone FOS2 (DQ082975)
Hydrothermal vent clone pPACMA-P (AB052985)
Hydrothermal vent clone pSSMCA108 (AB019740)
Methane hydrate sediment clone ODP1251A15.24 (AB177273)
Hydrothermal vent clone pISA12 (AB019741)
Hydrothermal vent clone AB167485 (AB167485)
Aciduliprofundum boonei (DQ451875)
Deep-sea Hydrothermal Vent Euryarchaeotic Group 2
Methanocaldococcus infernus (AF025822)
Methanocaldococcus jannaschii (L77117)
CPA OTU 2
CPA OTU 1
Thermococcus waimanguensis (AF098975)
Thermococcus profundus (Z75233)
Thermococcus kodakarensis (NC_006624)
Thermococcus celer (M21529)
Thermococcus Group
Pyrococcus abyssi (Z70246)
Pyrococcus horikoshii (BA000001)
Pyrococcus furiosus (AY134472)
Palaeococcus helgesonii (AY134472)
Palaeococcus ferrophilus (AB019239)
Methylcoccoides methylutans (M59127)
Methanohalobium evestigatum (U20149)
Methanothrix soehngenii (X16932)
Methanofollis liminatans (Y16429)
Methanospirillum hungatei (M60880)
Haloferax volcanii (U68540)
Halococcus dombrowskii (AJ420376)
Natronococcus amylolyticus (D43628)
Nanoarchaeum equitans (AJ318041)
Thermoproteus tenax (M35966)
Ignicoccus pacificus (AJ271794)
Sulfolobus solfataricus (D26490)
Sulfolobus metallicus (X90479)
Hydrothemal fluid clone Papm3A65 (AB213105)
CPA OTU 6
Deep-sea sediment clone MBWPA58 (AJ870961)
Deep-sea sediment clone D1Ru (DQ417488)
Lonar Soda Lake clone LR-253 (DQ302467)
Rhizosphere clone TRC23-38 (AF227644)
Lonar Soda Lake clone LR-136 (DQ302466)
Cenarchaeum symbiosum (U51469)
Nitrosopumilus maritimus (DQ085097)
Marine clone HF770_018L23 (DQ300534)
CPA OTU 7
Deep-sea clone CTD005-66A (AY856354)
Hydrothermal fluid clone SSM263-NA01 (AB193964)
Hydrothermal fluid clone FS243A-90 (DQ270604)
CPA OTU 4
Microbial mat clone PVA OTU 4 (U46680)
Hydrothermal fluid clone pIVWA2 (AB019730)
CPA OTU 3
Marine clone HF4000_7H13 (DQ300513)
Marine Group I Crenarchaeota
Aquifex pyrophilus (M83548)
Thermotoga maritima (AF367487)
0.1

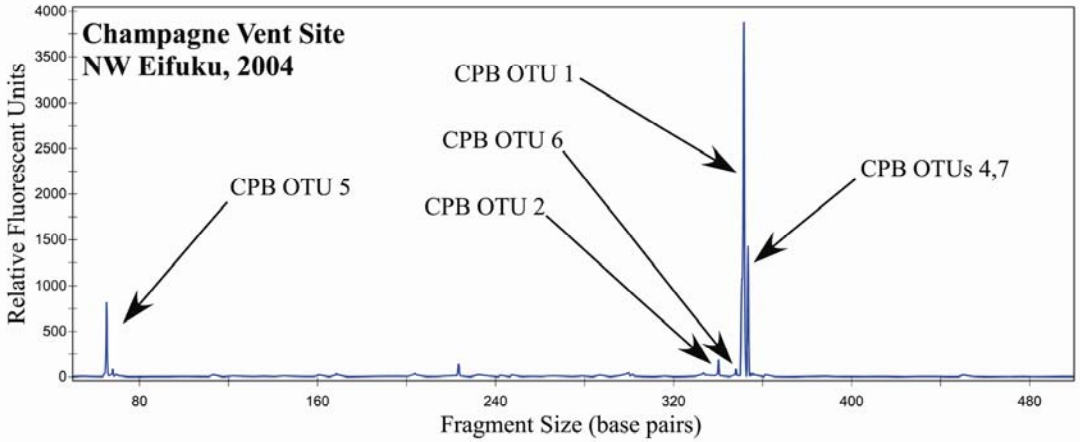


Mariana Group III
ε-*Proteobacteria*
Mostly Caminibacter Group

58 to 25°C Shift



Mariana Group III
ε-*Proteobacteria*
Both



Mariana Group III
ε-*Proteobacteria*
Mostly Sulfurimonas Group
~73°C

SUMMARY

- Microbial community diversity is much greater along the Mariana Arc/Backarc than at either a hotspot volcano or at mid-ocean ridge along similar same spatial scales.

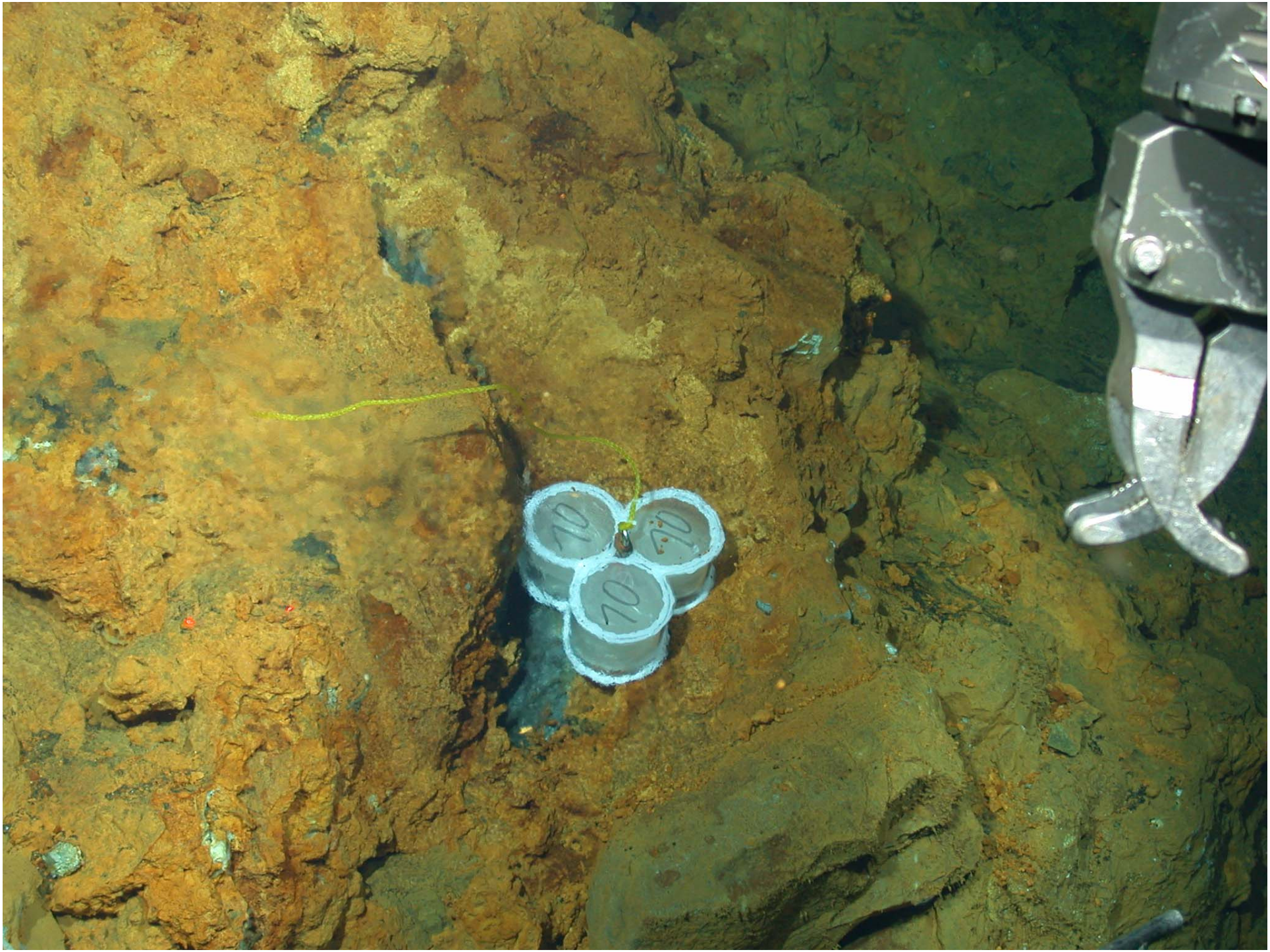
- This is most likely the result of the complexity of vent effluent chemistry and the ephemeral nature of hydrothermal venting along the Mariana Arc/Backarc.

- Cluster analysis of T-RFLP fingerprints reveals the microbial communities formed three distinct clusters designated Mariana Clusters I, II, and III.

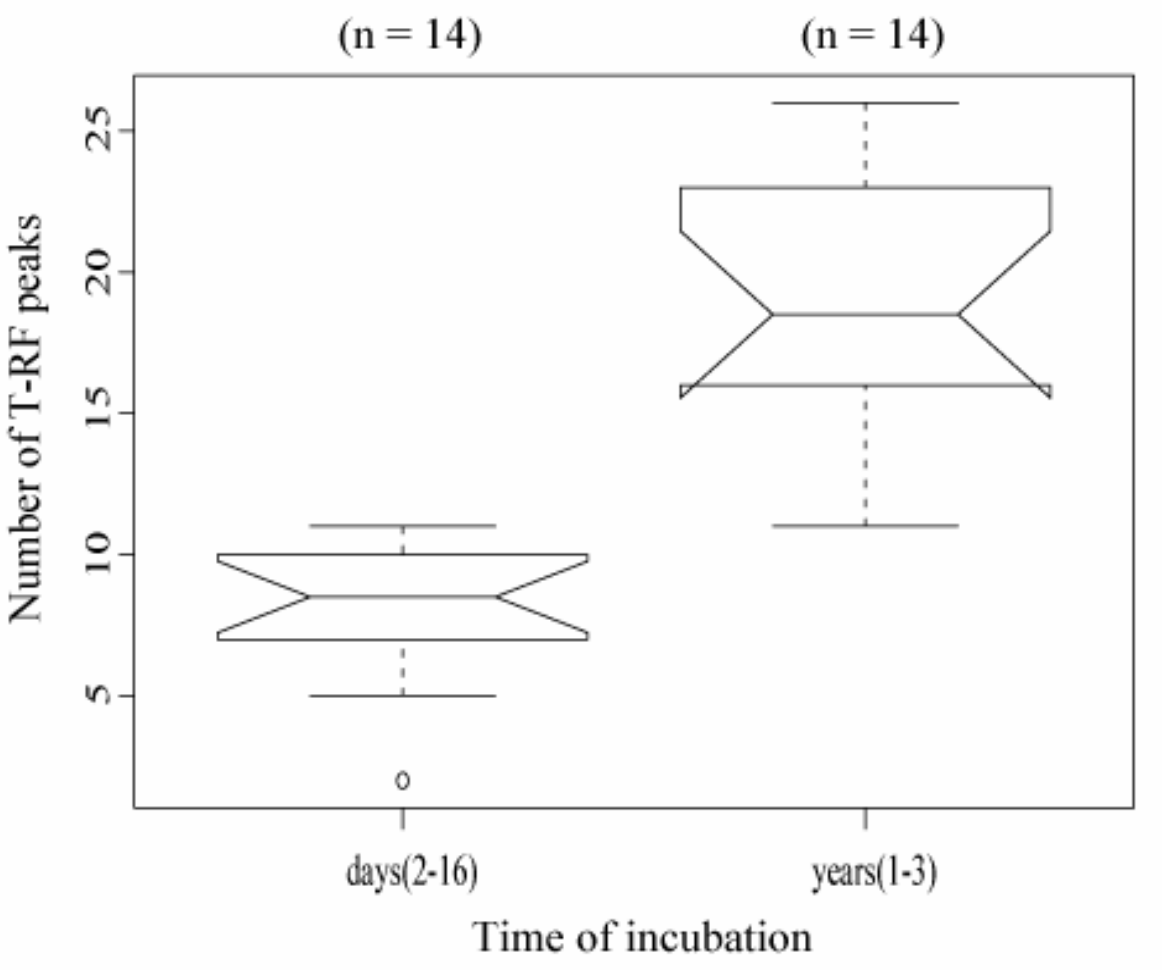
- The Mariana Arc/Backarc hydrothermal vent systems support a Bacterial biodiversity hotspot which may be indicative of convergent plates boundary vent systems worldwide.

Microbial Growth Chambers (MGCs)

- Silica wool as inert substrate with ~3m^2 surface area
- Enclosed by 202 micron Nytex filter
- Can withstand 100°C
- Placed within flow for short- and long-term durations


10
10
10

Notched Box-and-Whisker Plot: Axial Seamount



- Entire dataset pooled into 2 bins
- Notches do NOT overlap indicating that the differences are highly significant
- Yields a first order approximation for bacterial colonization rates.

Quote: Baas Becking (1934)

(Referring to bacteria) - “everything is everywhere:
but the milieu selects ... in nature and
in the laboratory”.

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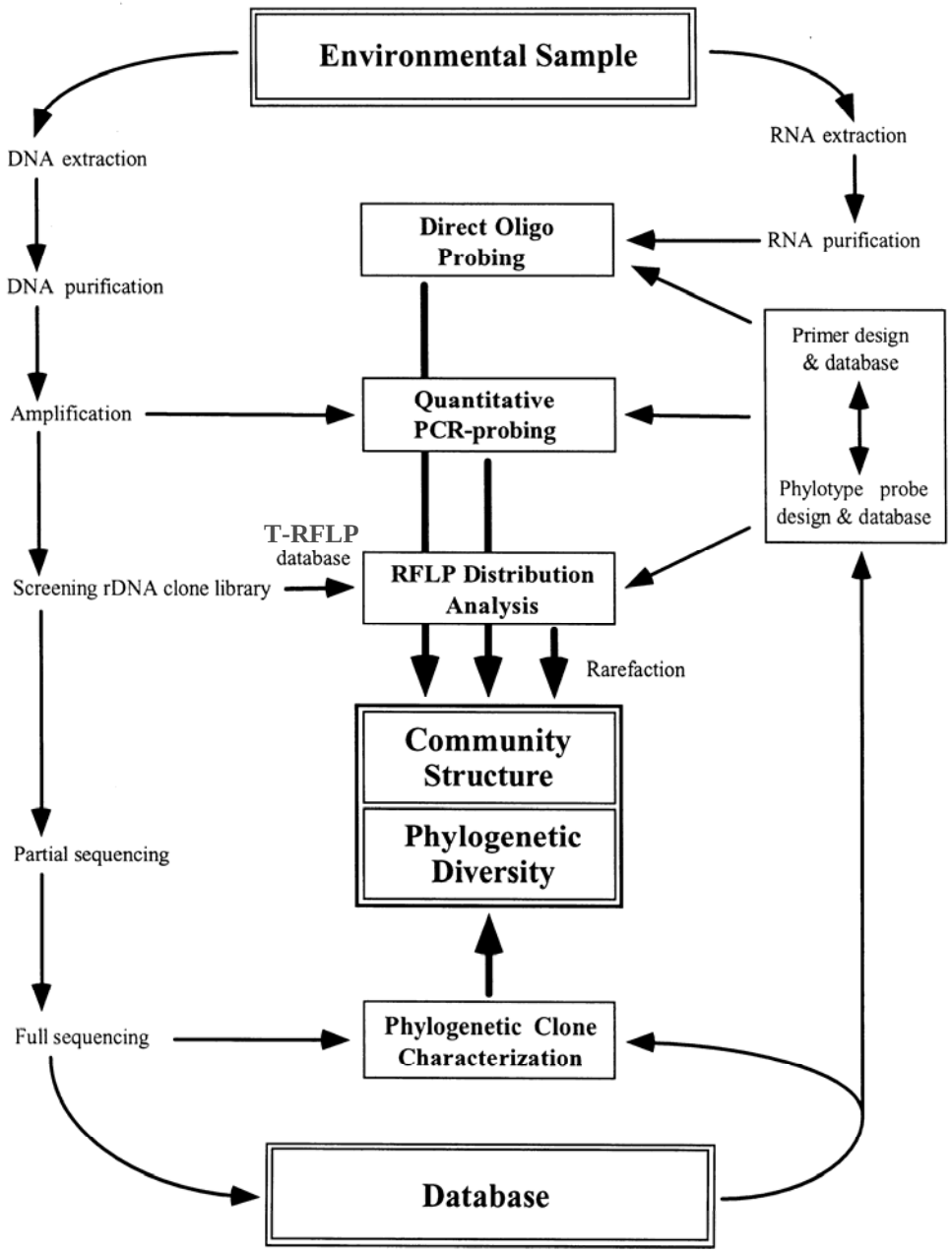
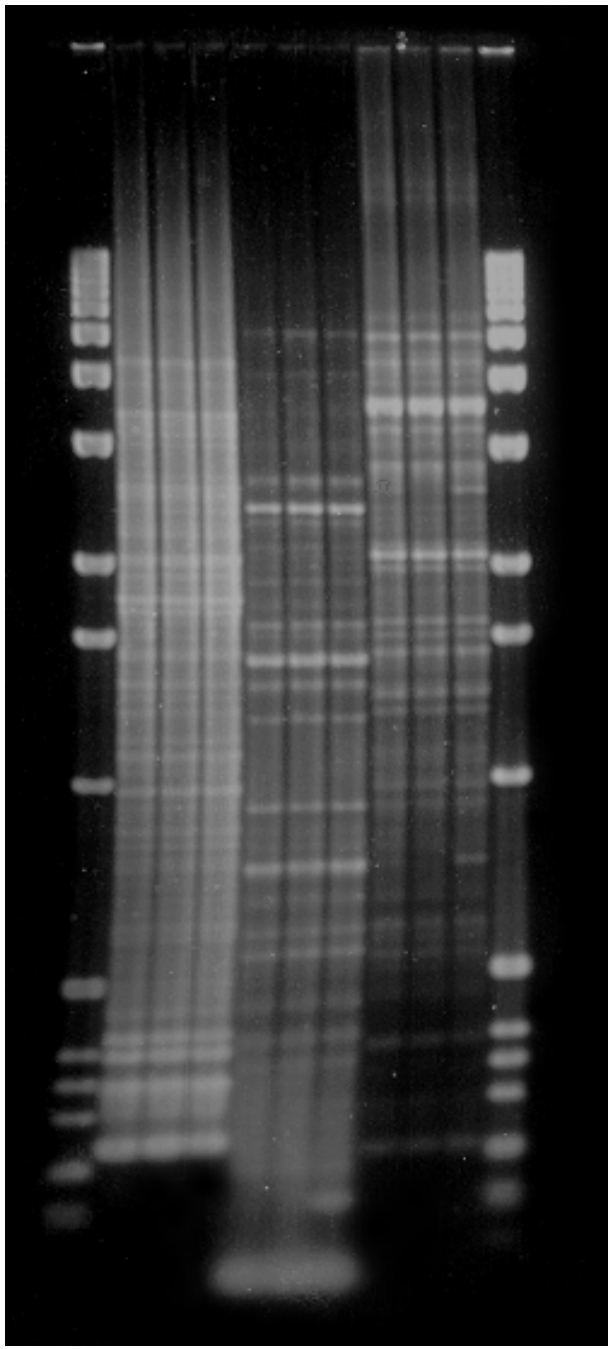
Submarine Ring of Fire
Diakoku Volcano 2006


Environmental Sample
DNA extraction
RNA extraction
DNA purification
RNA purification
Direct Oligo Probing
Primer design & database
Amplification
Quantitative PCR-probing
Phylotype probe design & database
T-RFLP database
Screening rDNA clone library
RFLP Distribution Analysis
Rarefaction
Community Structure
Phylogenetic Diversity
Partial sequencing
Full sequencing
Phylogenetic Clone Characterization
Database



BOX, ERIC, & REP PCR

Lanes represent: Strains RL1, ES1, & ES2

- Minimal genetic variability among strains of Fe-oxidizing bacteria.
- Additional strains will determine if endemic or cosmopolitan at genomic level.