Microbial Deep Subsurface

How does it stack up regarding:

Biomass?

Metabolism?

Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

*Calculated as described in the text.

Pg = Petagram or 10^{15}grams

Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

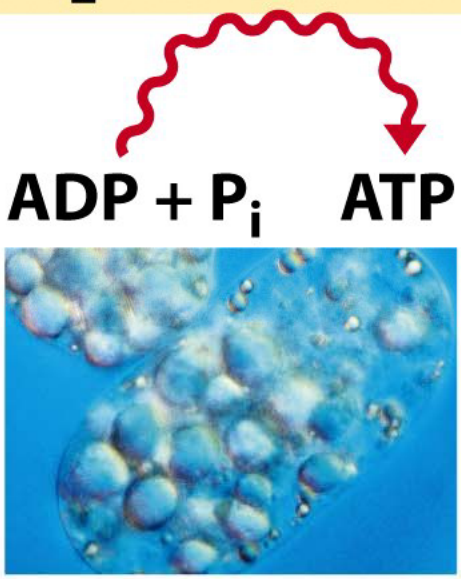
	Total C (Pg)	Total N (Pg)	Total P (Pg)
Plants:	560	12-20	1-2
Prokaryotes:	350-550	70-120	7-12

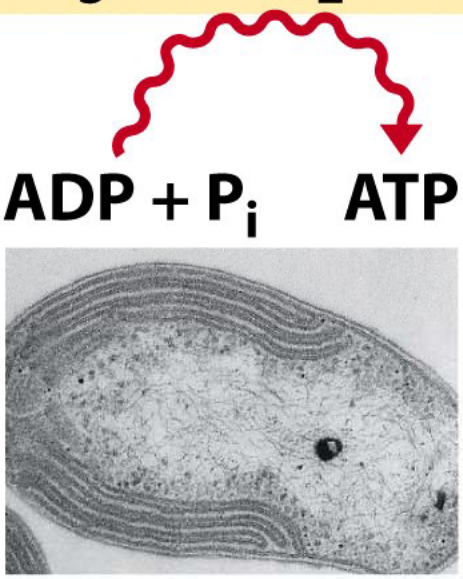
Take Home Message: Prokaryotes contain 60 to 100% the cellular carbon of all plants along with ~10x the N and P of plants!

Chemolithotrophy

$H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$

$NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$





Chemoautotrophy

Microbial Metabolic Menu

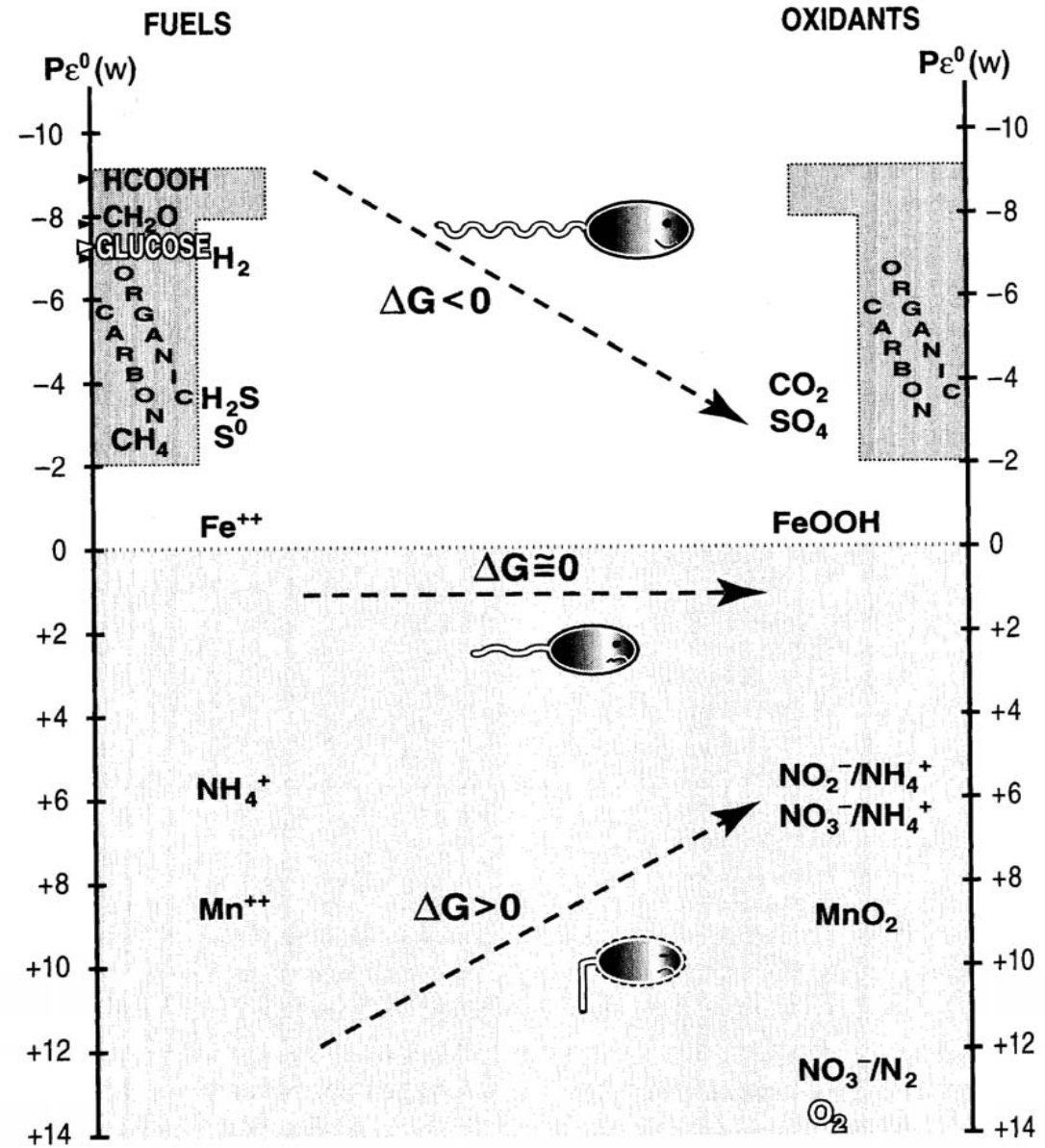
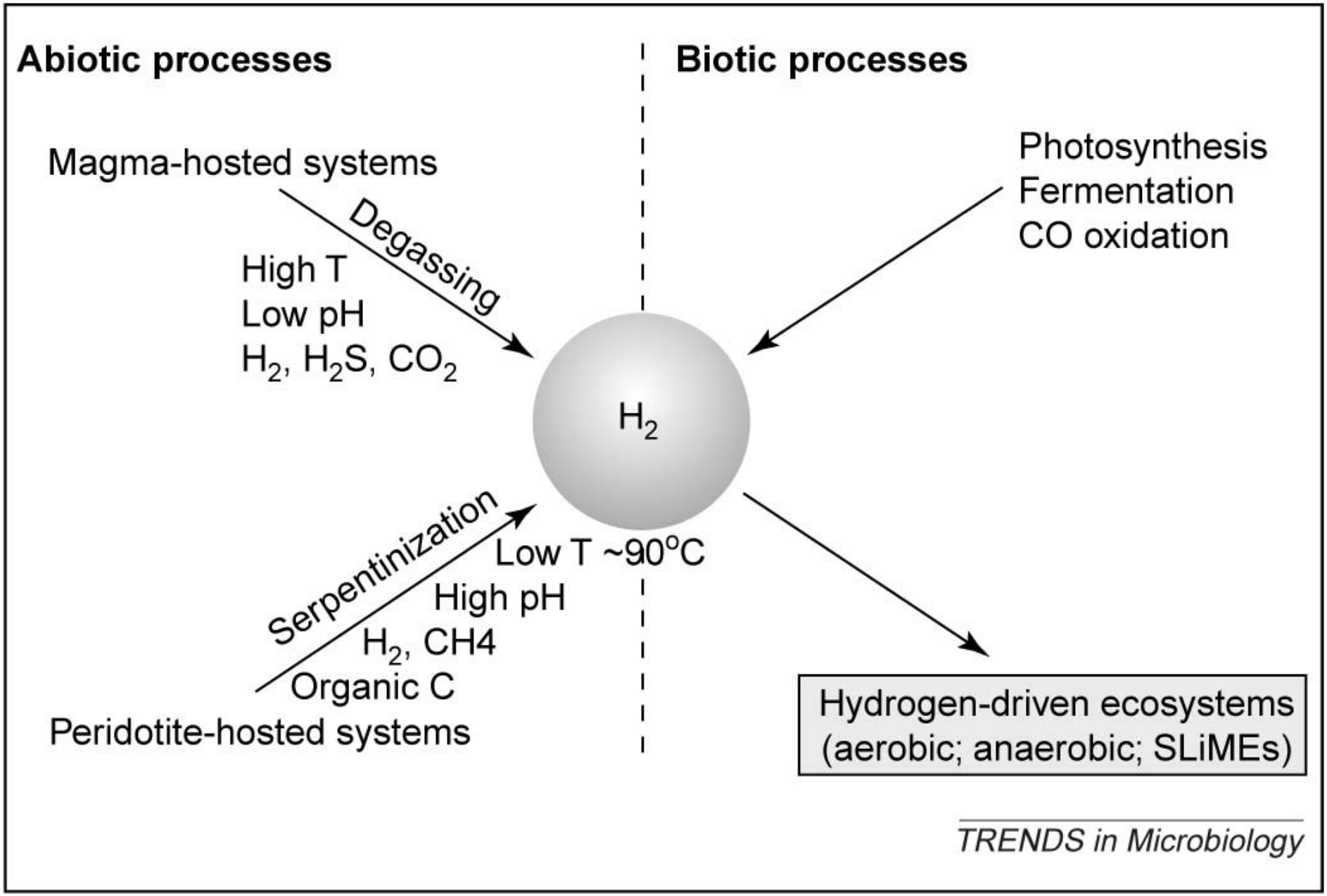
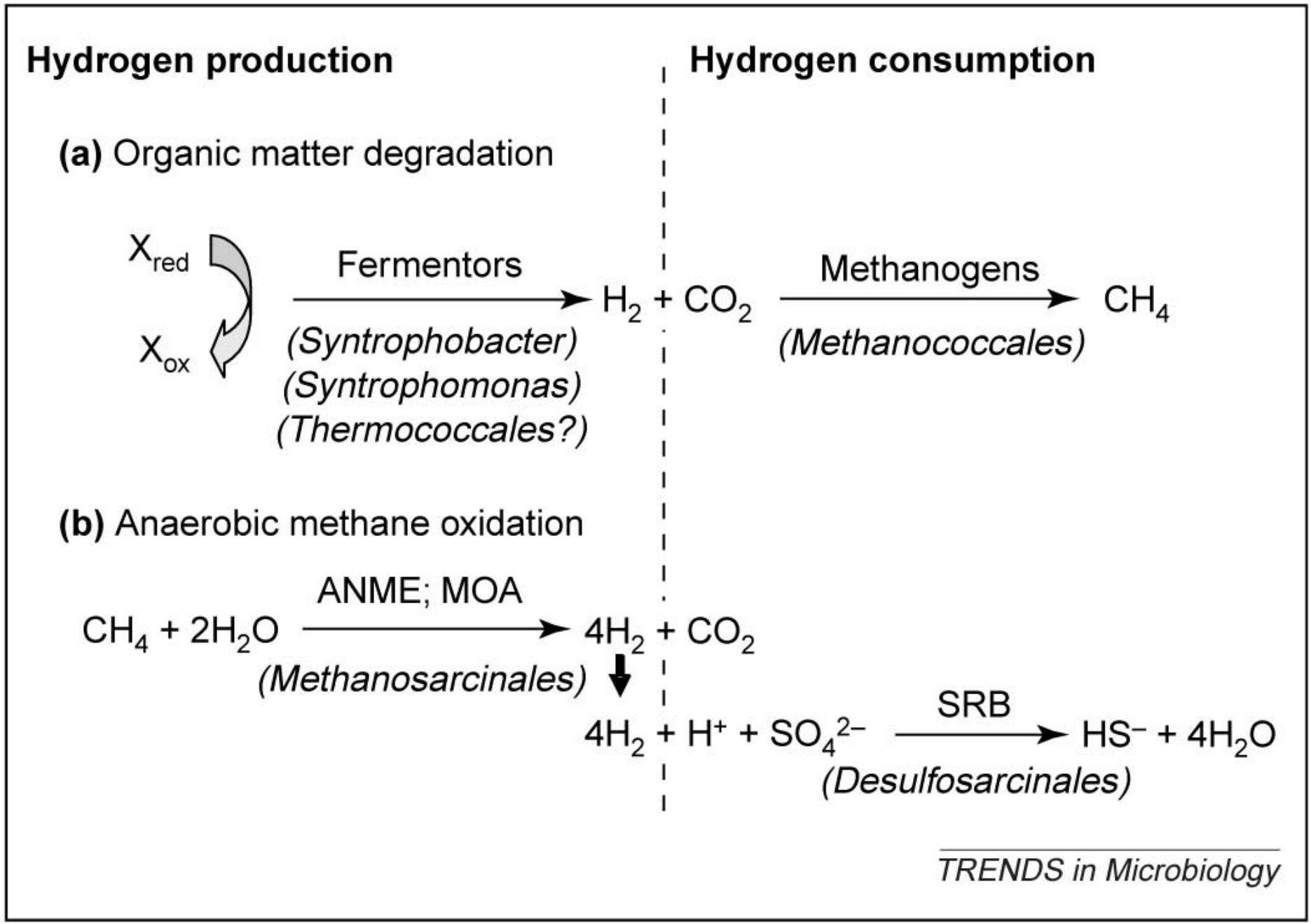
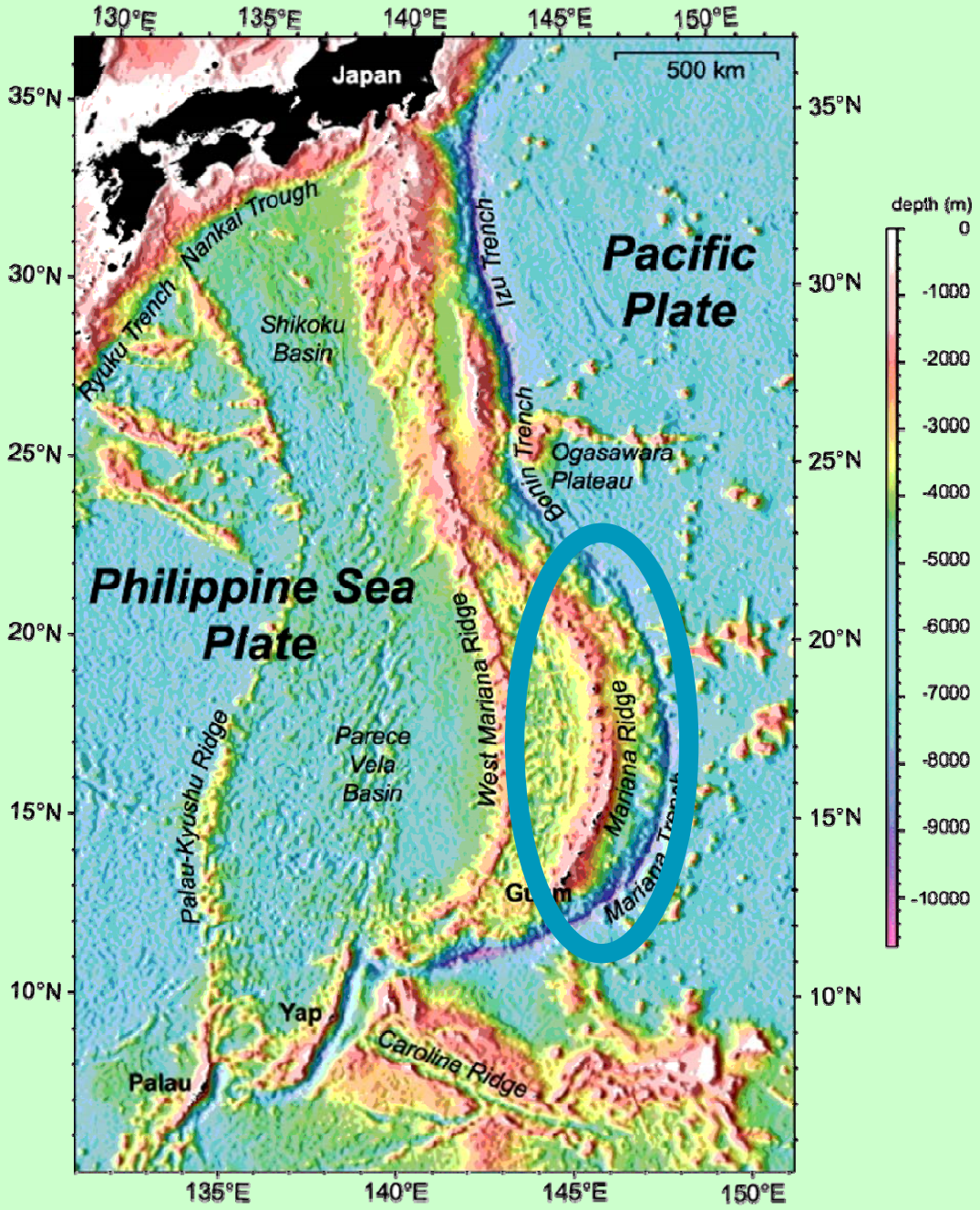
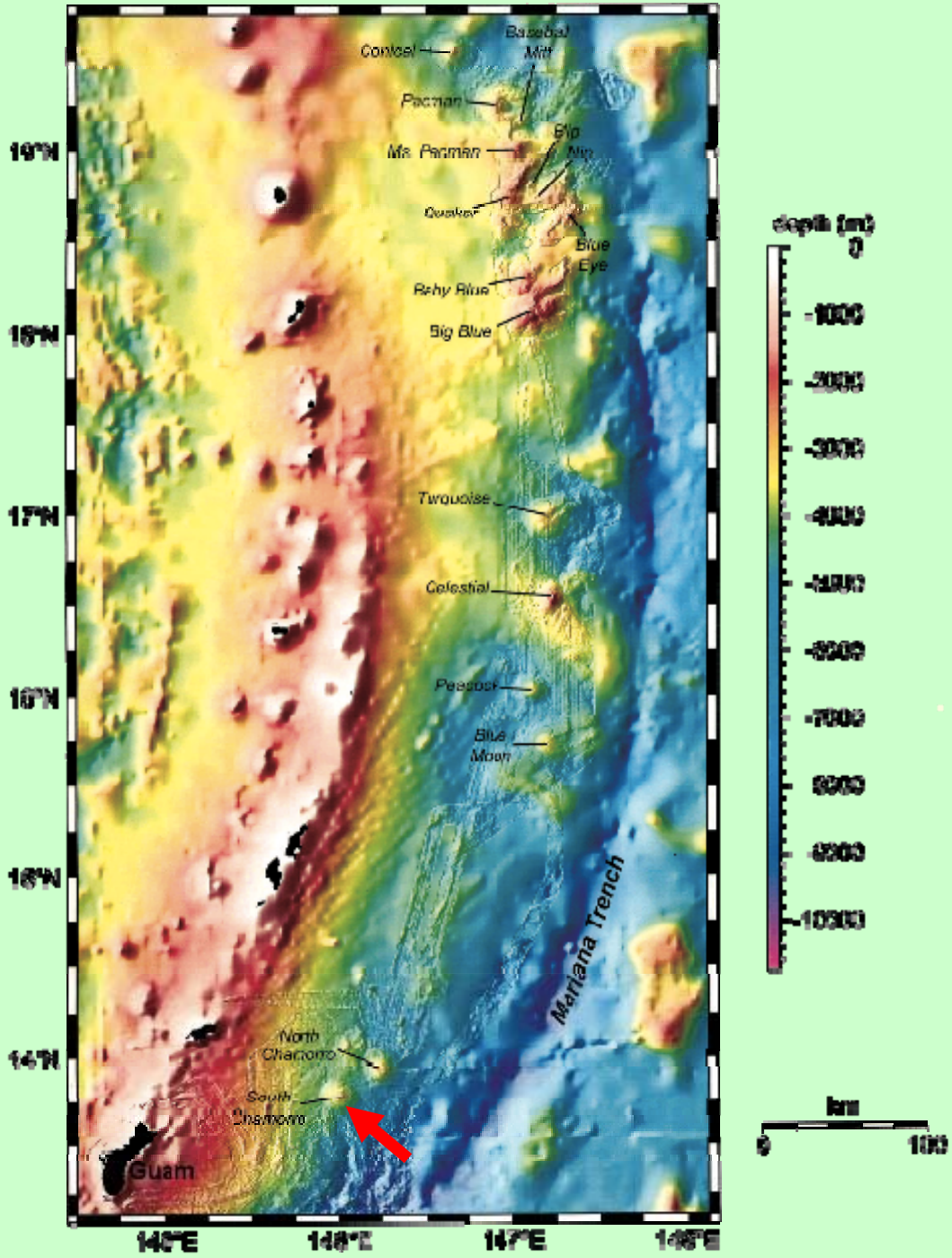


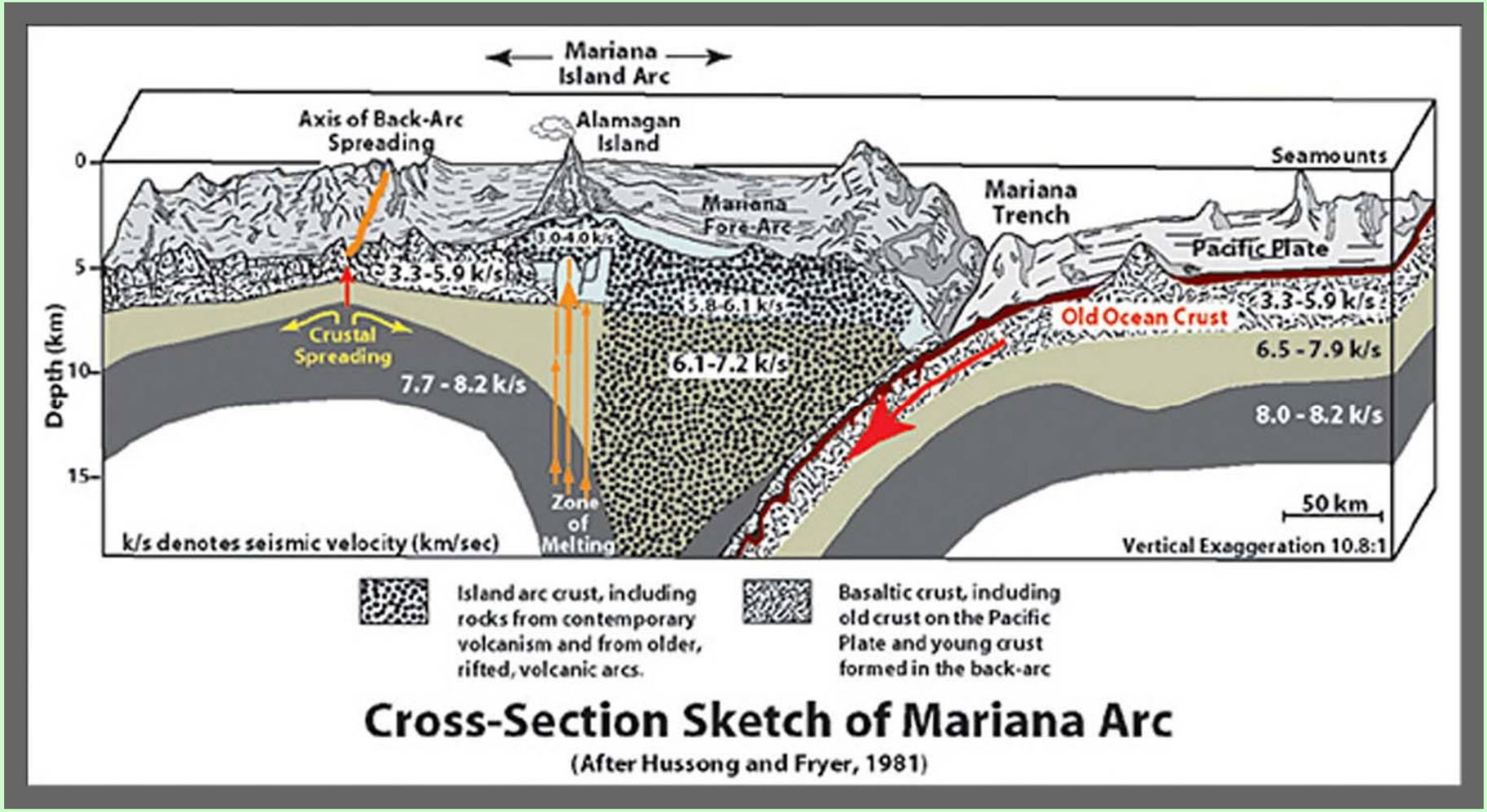
Figure courtesy of Ken Nealson


Abiotic processes
Biotic processes
Magma-hosted systems
Degassing
High T
Low pH
H_2, H_2S, CO_2
Photosynthesis
Fermentation
CO oxidation
H_2
Serpentinization
Low T ~90°C
High pH
H_2, CH4
Organic C
Peridotite-hosted systems
Hydrogen-driven ecosystems
(aerobic; anaerobic; SLiMEs)
TRENDS in Microbiology


Hydrogen production
Hydrogen consumption
(a) Organic matter degradation
X_red
X_ox
Fermentors
(Syntrophobacter)
(Syntrophomonas)
(Thermococcales?)
H2 + CO2
Methanogens
(Methanococcales)
CH4
(b) Anaerobic methane oxidation
ANME; MOA
CH4 + 2H2O
(Methanosarcinales)
4H2 + CO2
4H2 + H+ + SO4 2–
SRB
HS– + 4H2O
(Desulfosarcinales)
TRENDS in Microbiology


130°E
135°E
140°E
145°E
150°E
35°N
30°N
25°N
20°N
15°N
10°N
500 km
Japan
Pacific Plate
Philippine Sea Plate
Nankai Trough
Ryuku Trench
Shikoku Basin
Izu Trench
Bonin Trench
Ogasawara Plateau
West Mariana Ridge
Mariana Ridge
Mariana Trench
Parece Vela Basin
Palau-Kyushu Ridge
Guam
Yap
Palau
Caroline Ridge
depth (m)
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
-8000
-9000
-10000


Baseball Mtn
Comcel
Pacman
Ms. Pacman
Big Blue Eye
Quasker
Blue Eye
Itchy Blue
Big Blue
Turquoise
Celestial
Peacock
Blue Moon
Mariana Trench
North Chamorro
South Chamorro
Guam
19°N
18°N
17°N
16°N
15°N
14°N
144°E
146°E
147°E
148°E
depth (m)
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
-8000
-9000
-10000
km
0
100

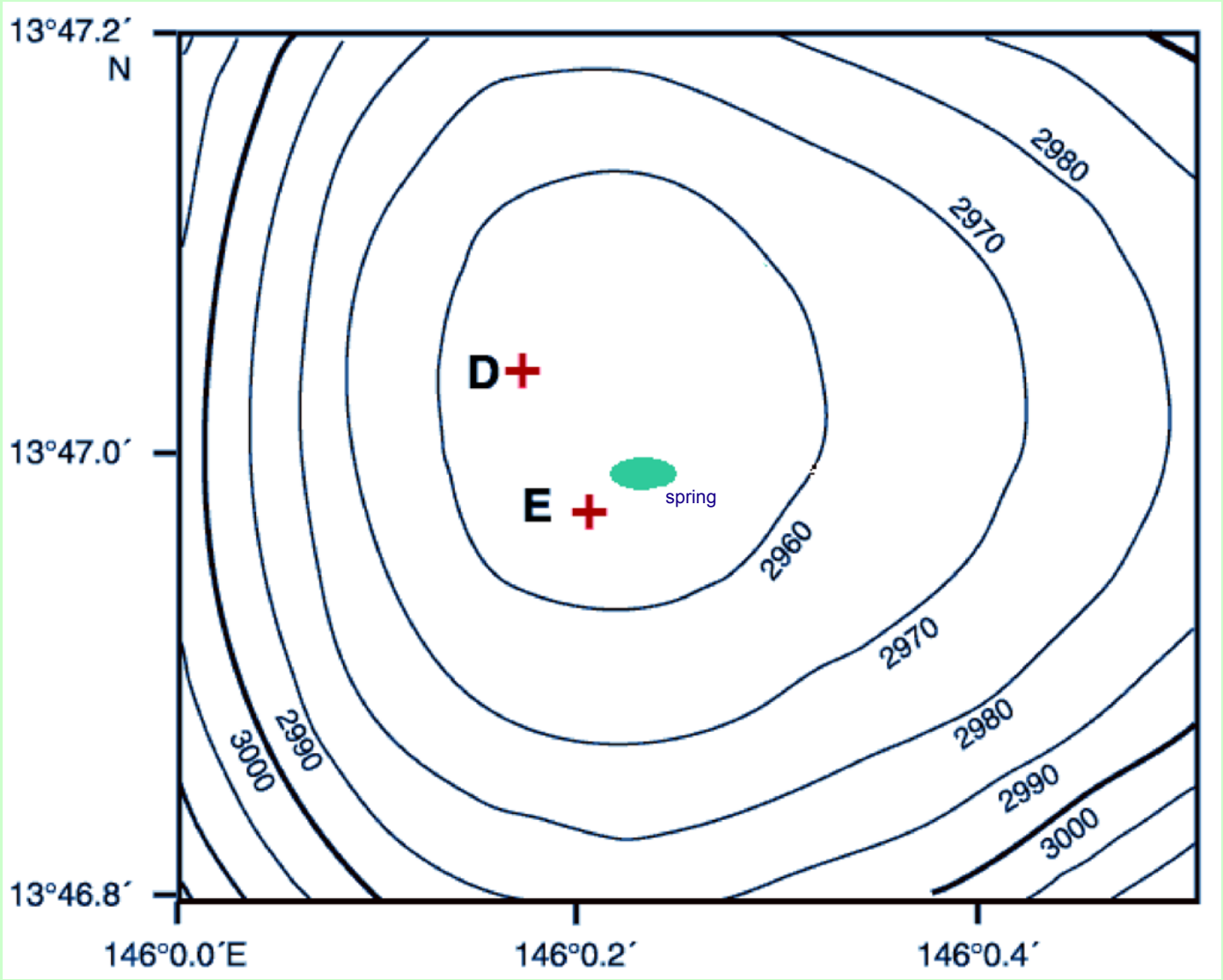


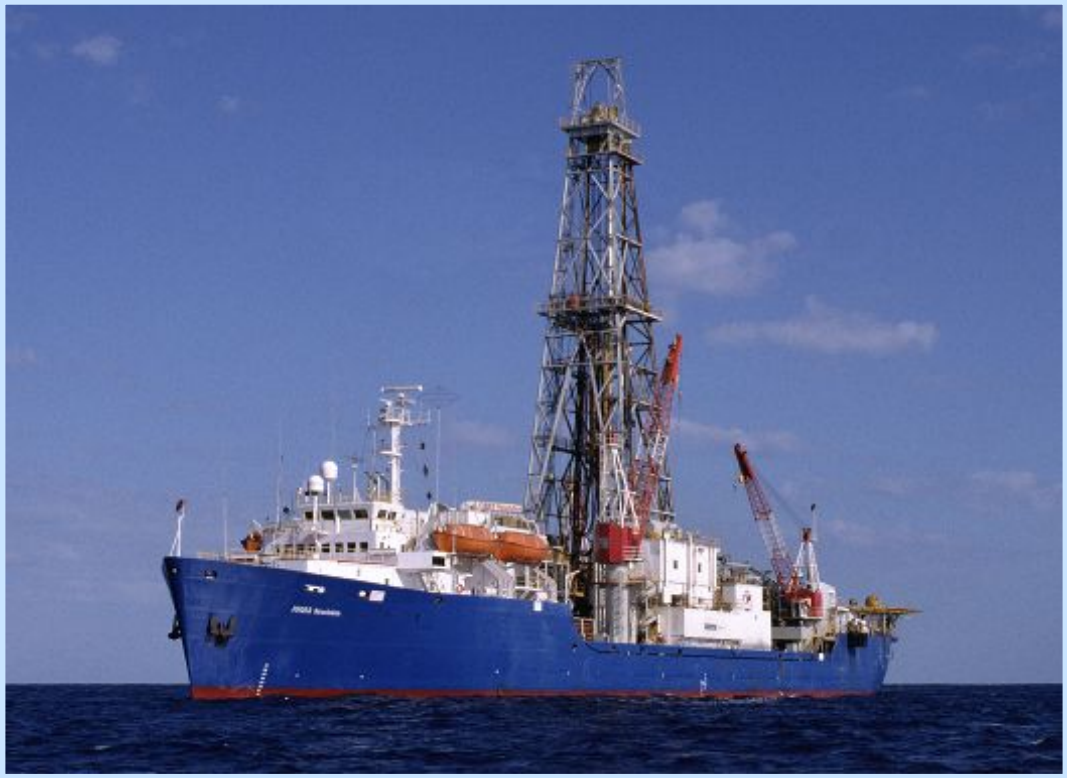
Cross-Section Sketch of Mariana Arc

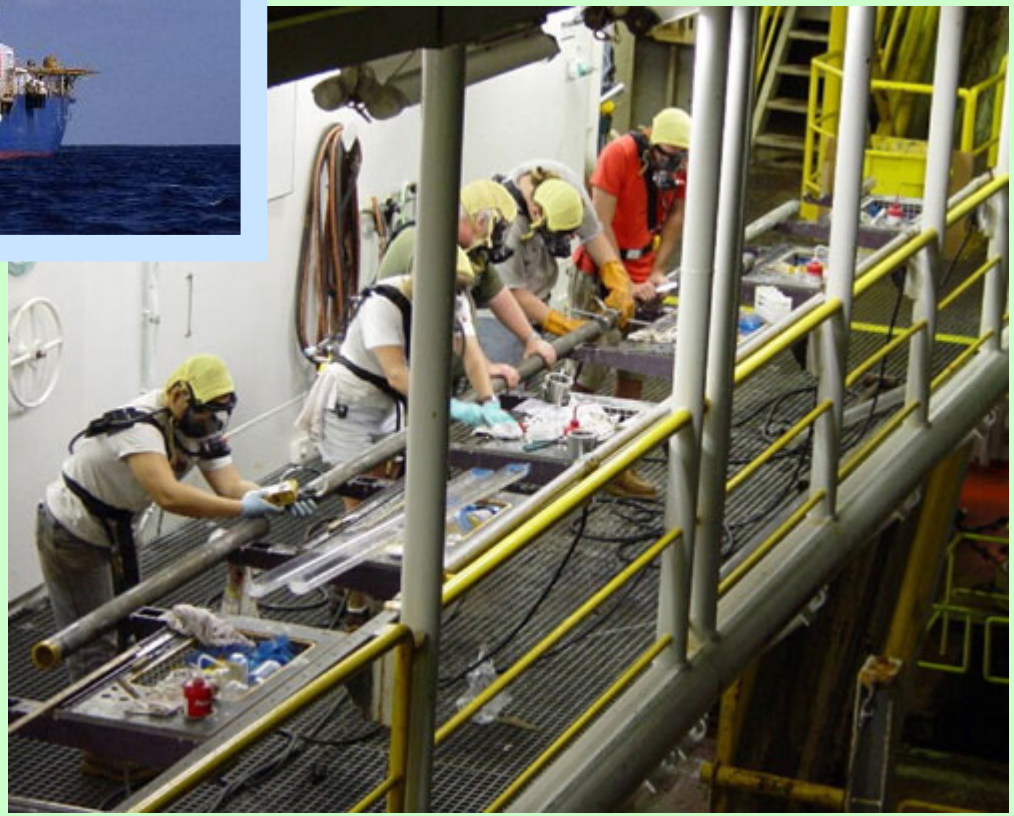
(After Hussong and Fryer, 1981)

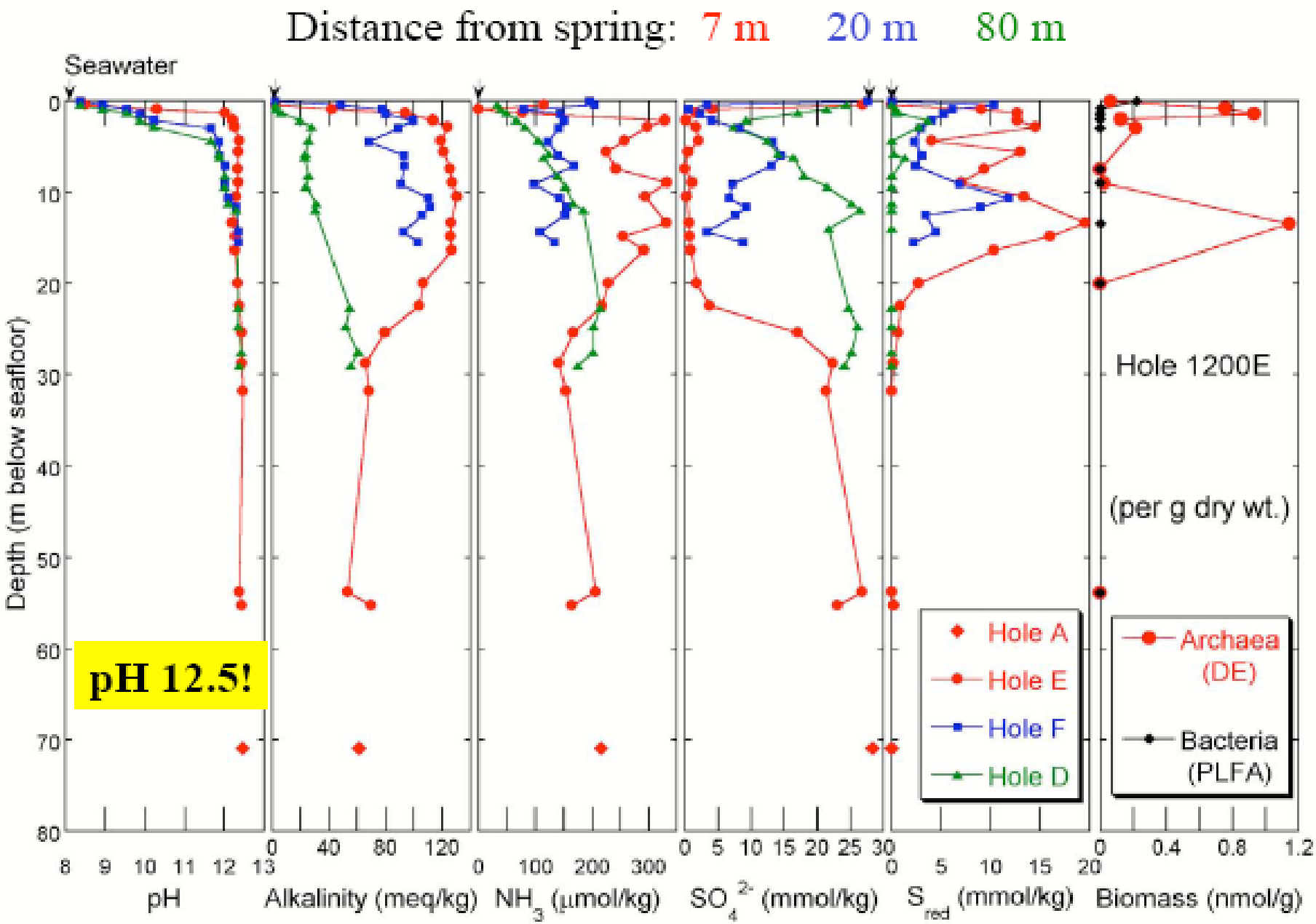
South Chamorro Summit

Site 1200, Holes D & E








Distance from spring: 7 m 20 m 80 m
Seawater
Depth (m below seafloor)
0
10
20
30
40
50
60
70
80
pH 12.5!
Hole 1200E
(per g dry wt.)
Hole A
Hole E
Hole F
Hole D
Archaea (DE)
Bacteria (PLFA)
8 9 10 11 12 13
pH
0 40 80 120
Alkalinity (meq/kg)
0 100 200 300
NH3 (µmol/kg)
0 5 10 15 20 25 30
SO4 2- (mmol/kg)
0 5 10 15 20
Sred (mmol/kg)
0 0.4 0.8 1.2
Biomass (nmol/g)
Anaerobic Methane Oxidation: CH4 + SO4 2- + 2OH- = CO3 2- + S= + 3H2O

Serpentinization and methane generation at high pH

$$2Mg_2SiO_4 + 3H_2O \rightarrow Mg_3Si_2O_5(OH)_4 + Mg(OH)_2$$

(*Peridotite) (water) (serpentine) (**brucite)

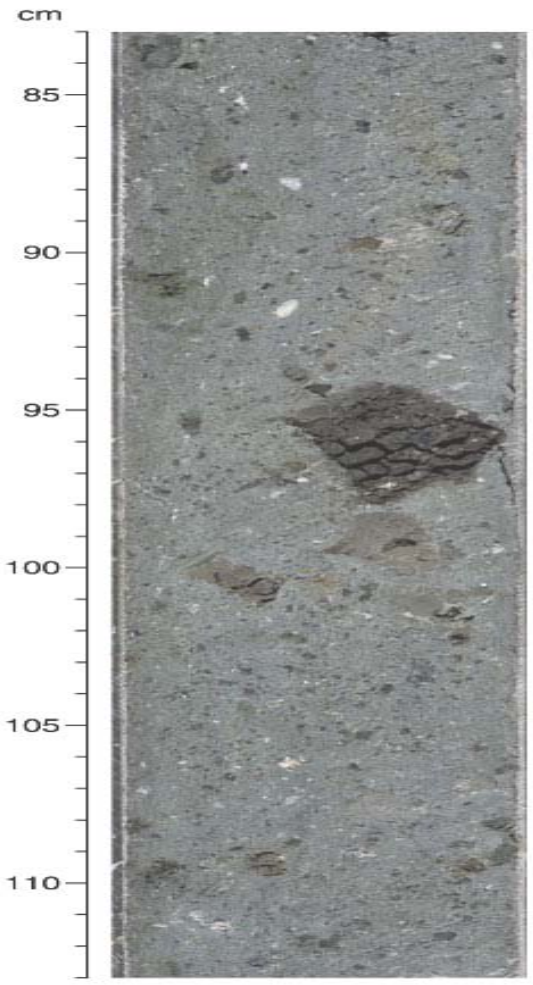
Methane production based on Fischer-Tropsch reaction:

$$4H_2 + CO_3^{2-} \rightarrow CH_4 + H_2O + 2OH^-$$

This reaction converts carbonate alkalinity to hydroxyl alkalinity, increasing surrounding pH

Hydrogen and methane provide energy sources for deep sea chemotrophic microorganisms

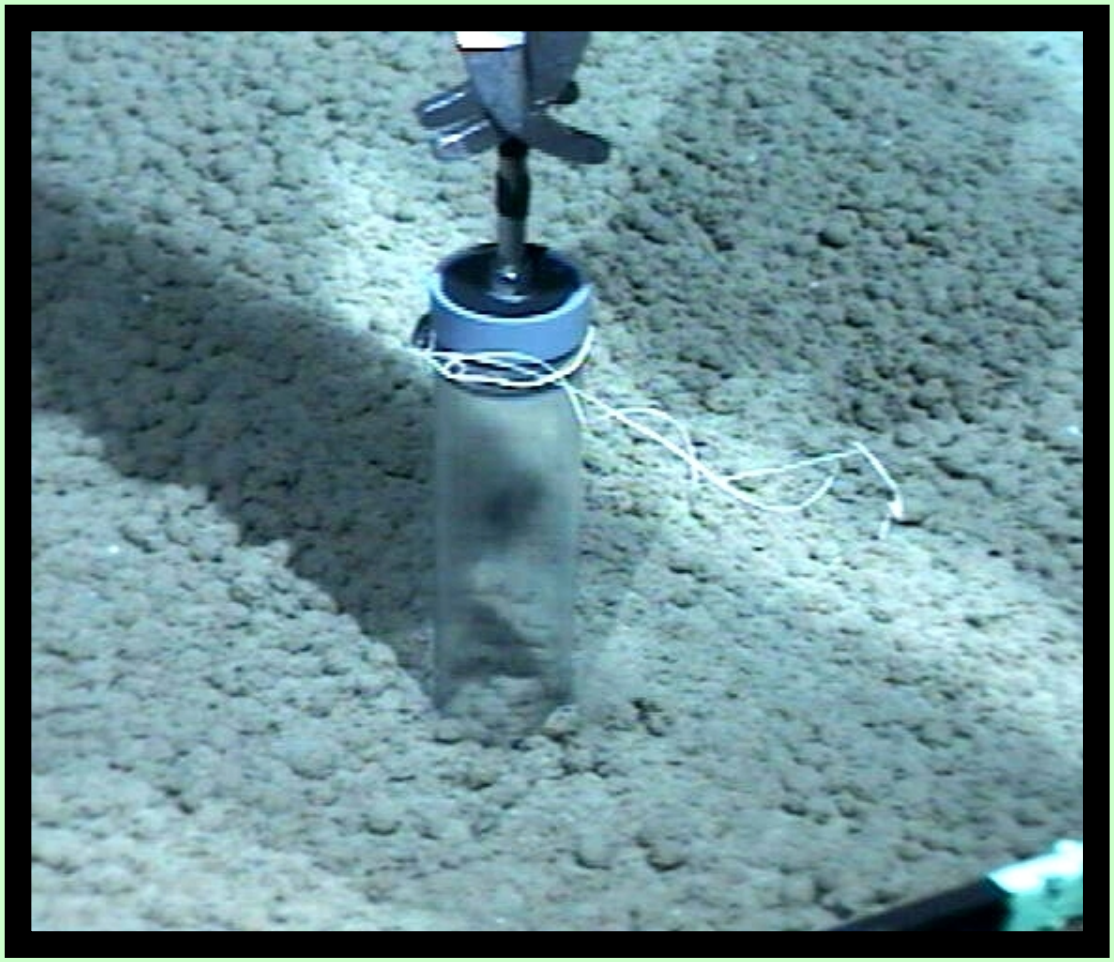
Mariana Forearc
South Chamorro Summit
Site 1200, Hole E
55.3 meters below sea floor

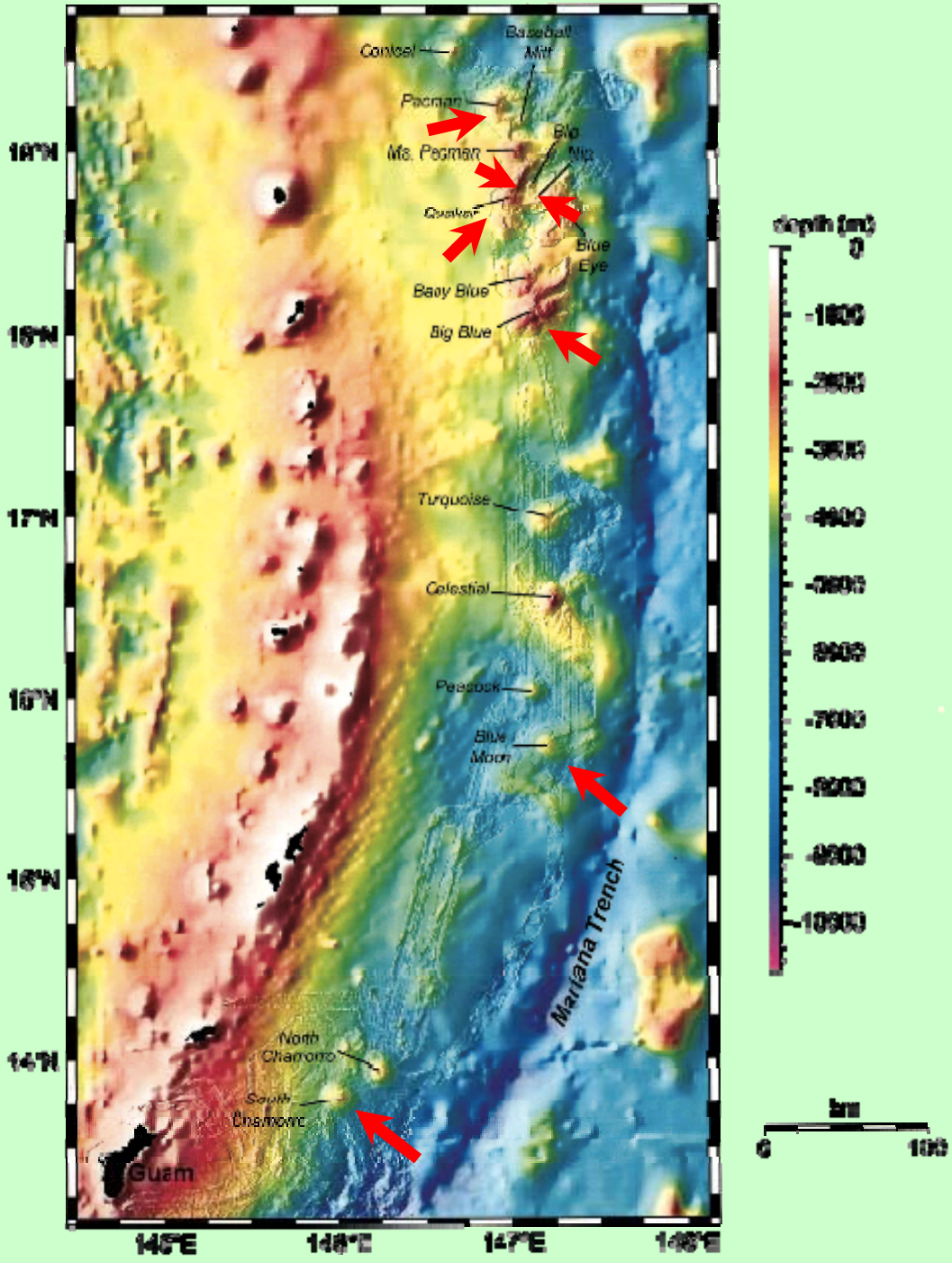


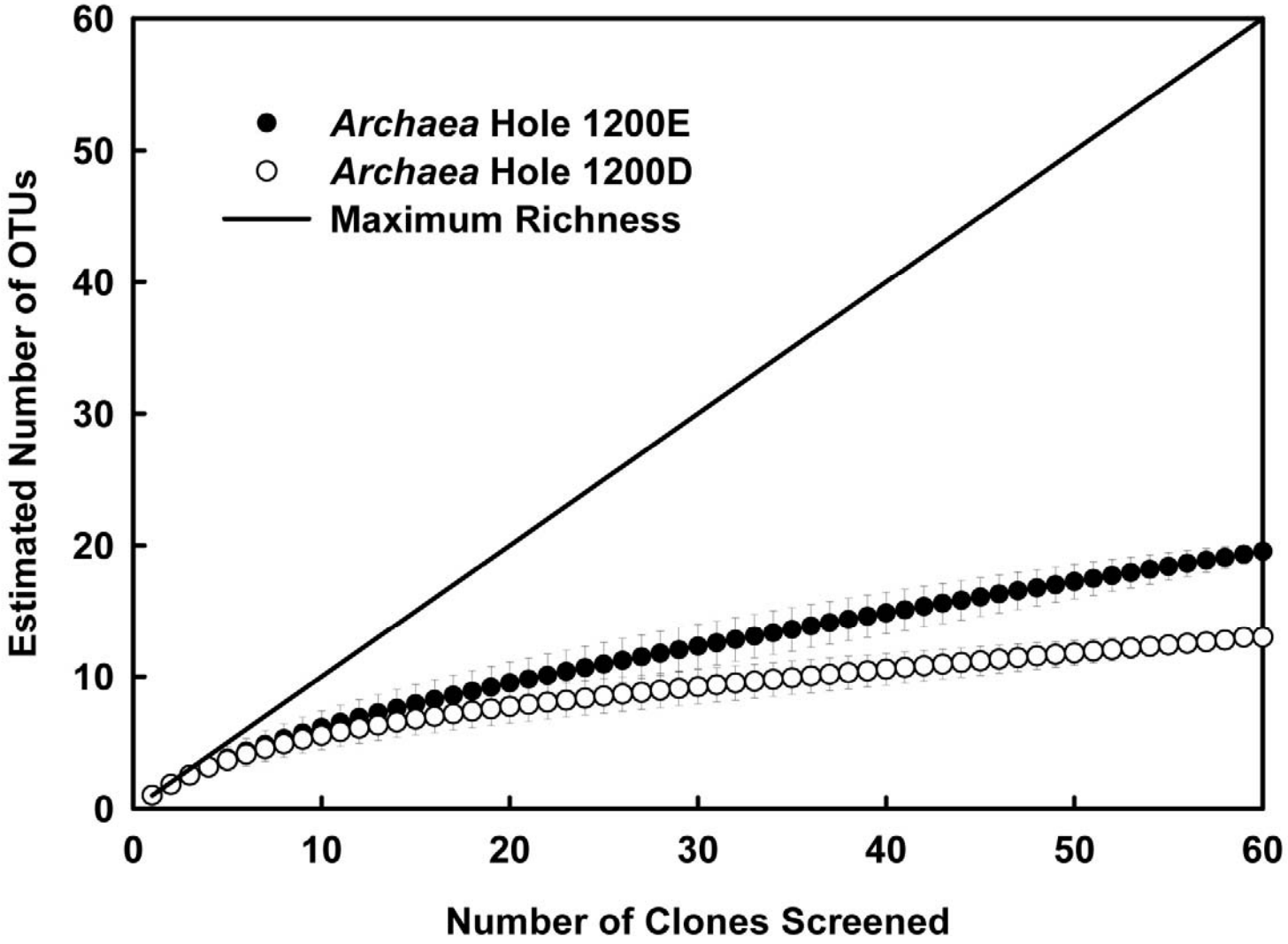
* Peridotite: native rock consisting mostly of the minerals olivine and pyroxene
** Brucite: magnesium hydroxide, found in veins of serpentine

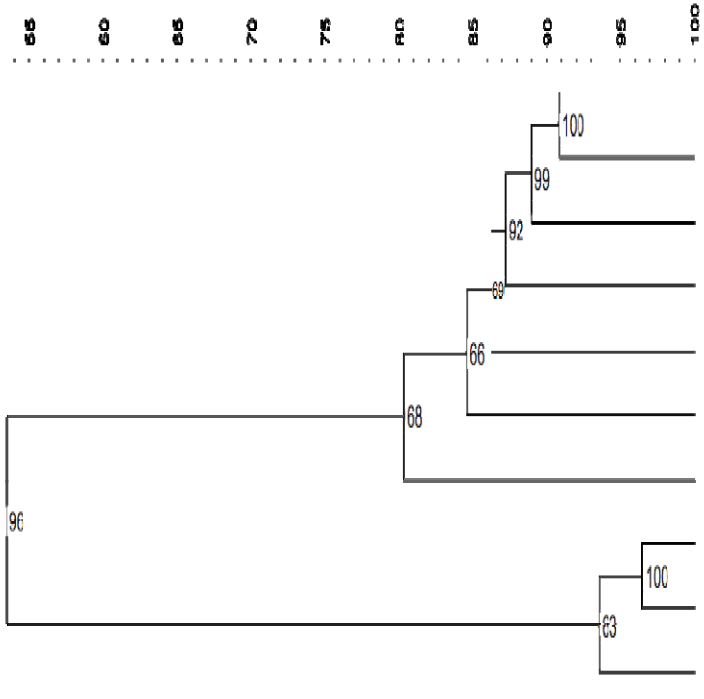

THOMAS G. THOMPSON
SEATTLE, WASHINGTON


JASON
WOODS HOLE
OCEANOGRAPHIC INSTITUTION

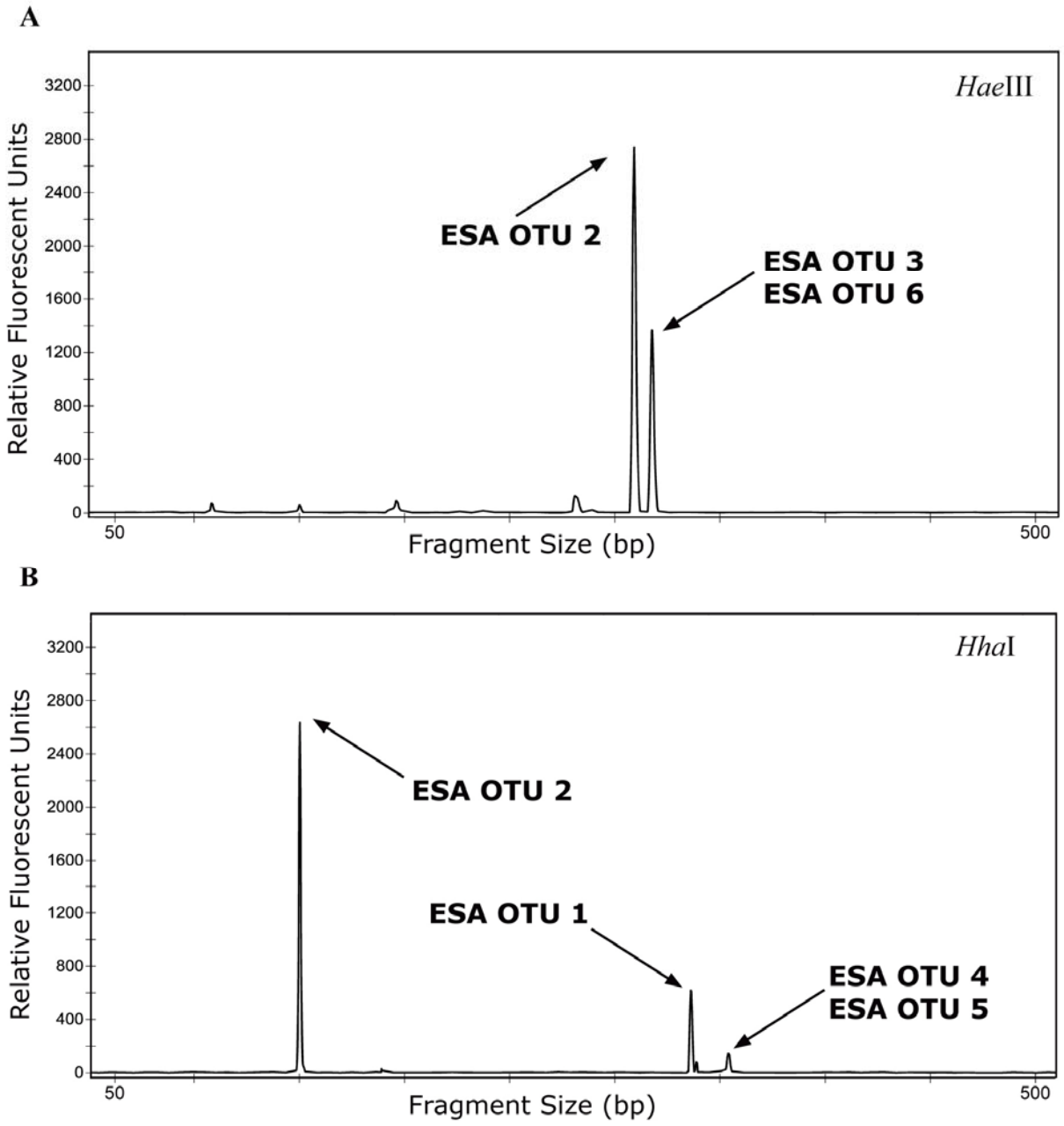


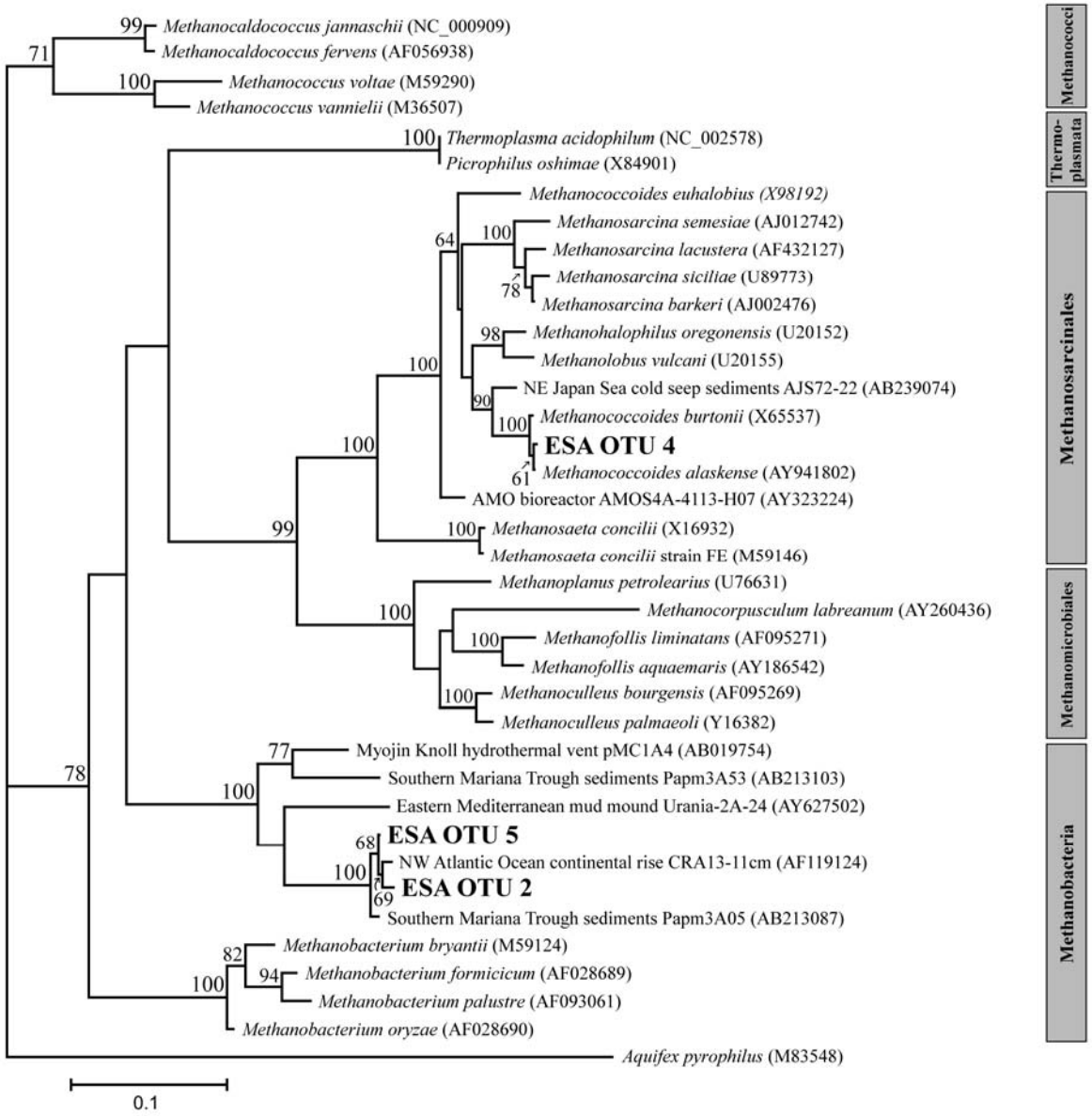

Conical
Baseball Mtn
Pacman
Ms. Pacman
Big Blue Nip
Quaker
Blue Eye
Baby Blue
Big Blue
Turquoise
Celestial
Peacock
Blue Moon
Mariana Trench
North Chamorro
South Chamorro
Guam
19°N
18°N
17°N
16°N
15°N
14°N
145°E
146°E
147°E
148°E
depth (m)
0
-1000
-2000
-3000
-4000
-5000
-6000
-7000
-8000
-9000
-10000
km
0
100

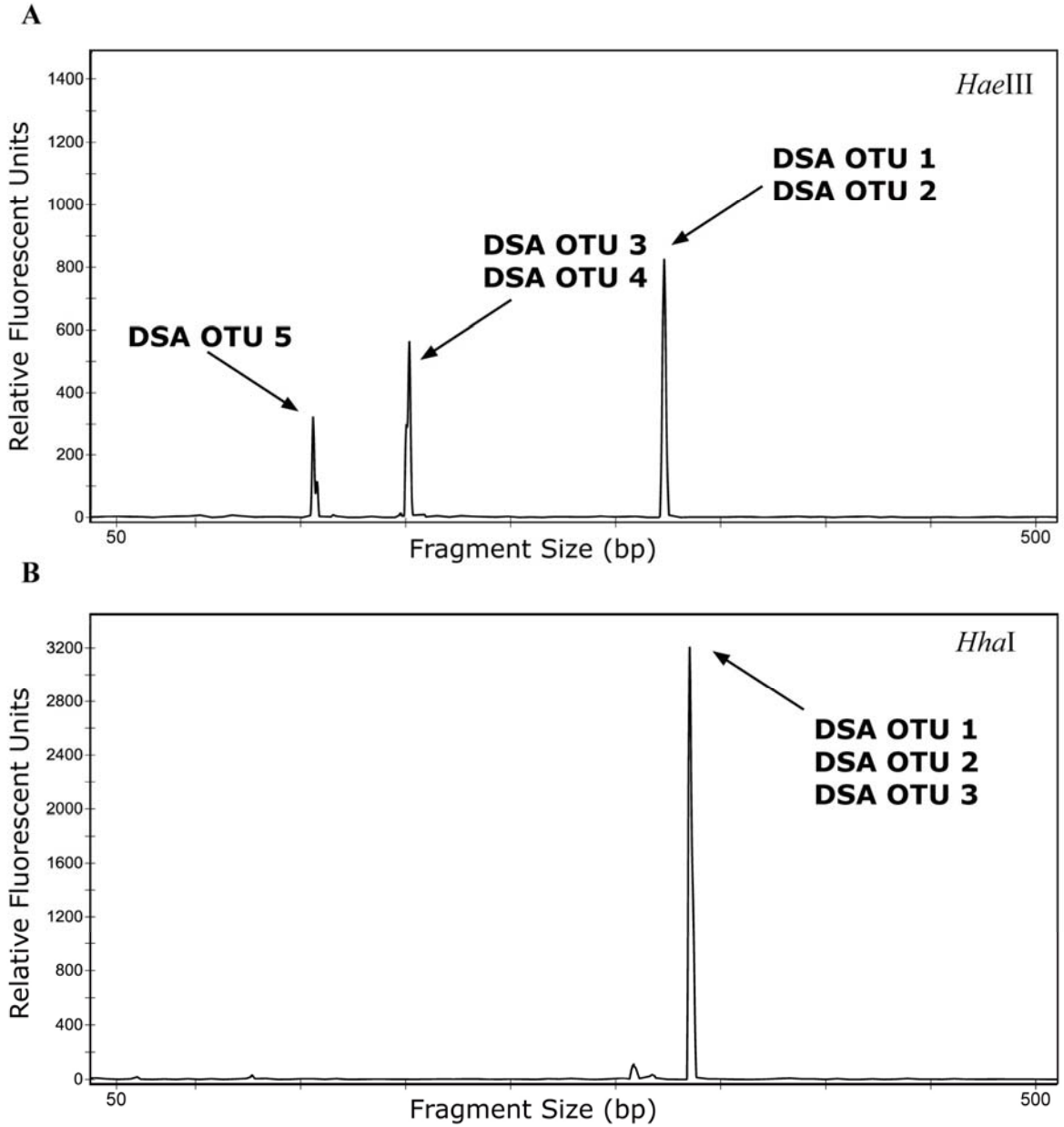

Estimated Number of OTUs
Number of Clones Screened
Archaea Hole 1200E
Archaea Hole 1200D
Maximum Richness
0
10
20
30
40
50
60

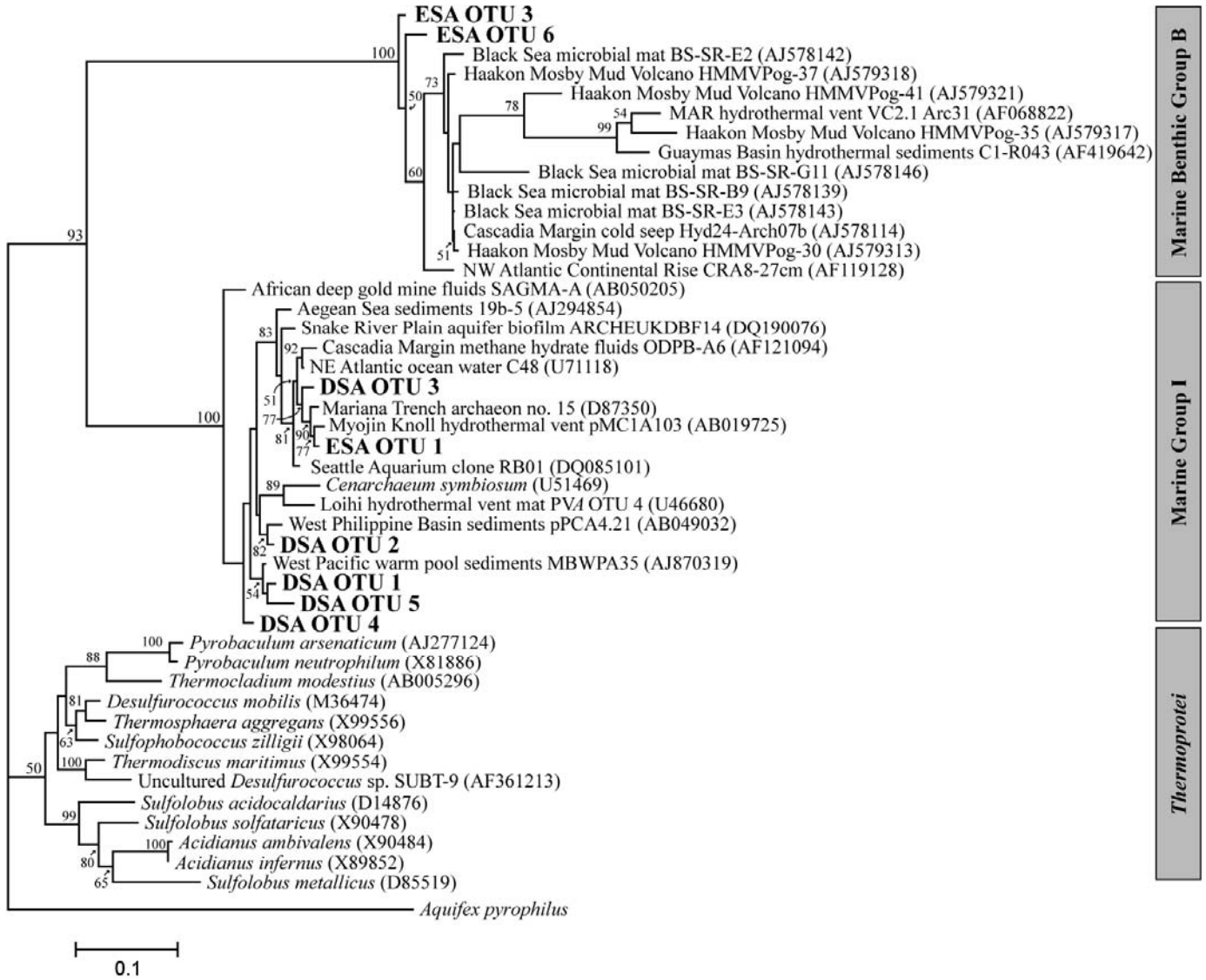


Site/Dive	Cruise	Sample Type	Year
Dip; TC 16	TN154	Trigger Core	2002
South Chamorro; J41-7	TN154	Push Core	2001
South Chamorro; 1200D	Leg 195	Whole-round Core	2001
Nip; TC 15	TN154	Trigger Core	2002
Quaker; J33-5	TN154	Push Core	2002
Pacman; PC 14	TN154	Piston Core	2002
South Chamorro; J41-4	TN154	Push Core	2002
South Chamorro; 1200E	Leg 195	Whole-round Core	2001
Big Blue; GC 19	TN154	Gravity Core	2002
Blue Moon; PC 25	TN154	Piston Core	2002


A
HaeIII
Relative Fluorescent Units
3200
2800
2400
2000
1600
1200
800
400
0
ESA OTU 2
ESA OTU 3
ESA OTU 6
50
500
Fragment Size (bp)
B
HhaI
Relative Fluorescent Units
3200
2800
2400
2000
1600
1200
800
400
0
ESA OTU 2
ESA OTU 1
ESA OTU 4
ESA OTU 5
50
500
Fragment Size (bp)


Methanocaldococcus jannaschii (NC_000909)
Methanocaldococcus fervens (AF056938)
Methanococcus voltae (M59290)
Methanococcus vannielii (M36507)
Thermoplasma acidophilum (NC_002578)
Picrophilus oshimae (X84901)
Methanococcoides euhalobius (X98192)
Methanosarcina semesiae (AJ012742)
Methanosarcina lacustera (AF432127)
Methanosarcina siciliae (U89773)
Methanosarcina barkeri (AJ002476)
Methanohalophilus oregonensis (U20152)
Methanolobus vulcani (U20155)
NE Japan Sea cold seep sediments AJS72-22 (AB239074)
Methanococcoides burtonii (X65537)
ESA OTU 4
Methanococcoides alaskense (AY941802)
AMO bioreactor AMOS4A-4113-H07 (AY323224)
Methanosaeta concilii (X16932)
Methanosaeta concilii strain FE (M59146)
Methanoplanus petrolearius (U76631)
Methanocorpusculum labreanum (AY260436)
Methanofollis liminatans (AF095271)
Methanofollis aquaemaris (AY186542)
Methanoculleus bourgensis (AF095269)
Methanoculleus palmaeoli (Y16382)
Myojin Knoll hydrothermal vent pMC1A4 (AB019754)
Southern Mariana Trough sediments Papm3A53 (AB213103)
Eastern Mediterranean mud mound Urania-2A-24 (AY627502)
ESA OTU 5
NW Atlantic Ocean continental rise CRA13-11cm (AF119124)
ESA OTU 2
Southern Mariana Trough sediments Papm3A05 (AB213087)
Methanobacterium bryantii (M59124)
Methanobacterium formicicum (AF028689)
Methanobacterium palustre (AF093061)
Methanobacterium oryzae (AF028690)
Aquifex pyrophilus (M83548)
0.1
Methanococci
Thermo-plasmata
Methanosarcinales
Methanomicrobiales
Methanobacteria


A
HaeIII
Relative Fluorescent Units
1400
1200
1000
800
600
400
200
0
DSA OTU 1
DSA OTU 2
DSA OTU 3
DSA OTU 4
DSA OTU 5
50
500
Fragment Size (bp)
B
HhaI
Relative Fluorescent Units
3200
2800
2400
2000
1600
1200
800
400
0
DSA OTU 1
DSA OTU 2
DSA OTU 3
50
500
Fragment Size (bp)


ESA OTU 3
ESA OTU 6
Black Sea microbial mat BS-SR-E2 (AJ578142)
Haakon Mosby Mud Volcano HMMVPog-37 (AJ579318)
Haakon Mosby Mud Volcano HMMVPog-41 (AJ579321)
MAR hydrothermal vent VC2.1 Arc31 (AF068822)
Haakon Mosby Mud Volcano HMMVPog-35 (AJ579317)
Guaymas Basin hydrothermal sediments C1-R043 (AF419642)
Black Sea microbial mat BS-SR-G11 (AJ578146)
Black Sea microbial mat BS-SR-B9 (AJ578139)
Black Sea microbial mat BS-SR-E3 (AJ578143)
Cascadia Margin cold seep Hyd24-Arch07b (AJ578114)
Haakon Mosby Mud Volcano HMMVPog-30 (AJ579313)
NW Atlantic Continental Rise CRA8-27cm (AF119128)
African deep gold mine fluids SAGMA-A (AB050205)
Aegean Sea sediments 19b-5 (AJ294854)
Snake River Plain aquifer biofilm ARCHEUKDBF14 (DQ190076)
Cascadia Margin methane hydrate fluids ODPB-A6 (AF121094)
NE Atlantic ocean water C48 (U71118)
DSA OTU 3
Mariana Trench archaeon no. 15 (D87350)
Myojin Knoll hydrothermal vent pMC1A103 (AB019725)
ESA OTU 1
Seattle Aquarium clone RB01 (DQ085101)
Cenarchaeum symbiosum (U51469)
Loihi hydrothermal vent mat PVA OTU 4 (U46680)
West Philippine Basin sediments pPCA4.21 (AB049032)
DSA OTU 2
West Pacific warm pool sediments MBWPA35 (AJ870319)
DSA OTU 1
DSA OTU 5
DSA OTU 4
Pyrobaculum arsenaticum (AJ277124)
Pyrobaculum neutrophilum (X81886)
Thermocladium modestius (AB005296)
Desulfurococcus mobilis (M36474)
Thermosphaera aggregans (X99556)
Sulfophobococcus zilligii (X98064)
Thermodiscus maritimus (X99554)
Uncultured Desulfurococcus sp. SUBT-9 (AF361213)
Sulfolobus acidocaldarius (D14876)
Sulfolobus solfataricus (X90478)
Acidianus ambivalens (X90484)
Acidianus infernus (X89852)
Sulfolobus metallicus (D85519)
Aquifex pyrophilus
0.1
Marine Benthic Group B
Marine Group I
Thermoprotei

Marine Microbiology

How does it stack up regarding:

Diversity?

Metabolism?

Genetic diversity in Sargasso Sea bacterioplankton

Stephen J. Giovannoni, Theresa B. Britschgi, Craig L. Moyer & Katharine G. Field

Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, USA

BACTERIOPLANKTON are recognized as important agents of biogeochemical change in marine ecosystems, yet relatively little is known about the species that make up these communities. Uncertainties about the genetic structure and diversity of natural bacterioplankton populations stem from the traditional difficulties associated with microbial cultivation techniques. Discrepancies between direct counts and plate counts are typically several orders of magnitude, raising doubts as to whether cultivated marine bacteria are actually representative of dominant planktonic species[1–3]. We have phylogenetically analysed clone libraries of eubacterial 16S ribosomal RNA genes amplified from natural populations of Sargasso Sea picoplankton by the polymerase chain reaction[4]. The analysis indicates the presence of a novel microbial group, the SAR11 cluster, which appears to be a significant component of this oligotrophic bacterioplankton community. A second cluster of lineages related to the oxygenic phototrophs—cyanobacteria, prochlorophytes and chloroplasts—was also observed. However, none of the genes matched the small subunit rRNA sequences of cultivated marine cyanobacteria from similar habitats. The diversity of 16S rRNA genes observed within the clusters suggests that these bacterioplankton may be consortia of independent lineages sharing surprisingly distant common ancestors.

(Nature, 1990)

TABLE 1 Analysis of microbial population rRNA by the hybridization of kingdom, universal, and SAR11 cluster oligodeoxynucleotide probes

	Population composition (%)			
	Eubacterial	Eukaryotic	Archaebacterial	SAR11 cluster
Sargasso Sea	81.0	19.0	0.0	12.50
Ferry Reach	66.6	13.7	0.0	1.38
Key Biscayne	74.6	25.4	0.0	0.53
Yaquina Head	85.2	6.7	0.0	0.00

Binding of the phylogenetic group-specific probes was determined relative to universal probe binding using arrays of known RNAs (0, 2.5, 5, 10, 20, 30, 40, 50 ng) on nylon membranes[13]. DNA oligodeoxynucleotide probes were labelled with T4 polynucleotide kinase and γ-[^{32}P]ATP to a specific activity of between 1×10^8–2×10^8 c.p.m. μg^{-1}. The amount of bound probe was measured using a gas ionization scanner (Ambis systems). The compositions of the populations were determined from the binding of the kingdom-specific probes according to the following equation:

$$\%X = \frac{\delta C/\delta N \times (\delta U/\delta N)^{-1} - \sum_{i=1}^{r}[\delta R_i/\delta N \times (\delta U/\delta N)^{-1}]r^{-1}}{\sum_{i=1}^{p}[\delta P_i/\delta N \times (\delta U/\delta N)^{-1}]p^{-1} - \sum_{i=1}^{r}[\delta R_i/\delta N \times (\delta U/\delta N)^{-1}]r^{-1}} \times 100$$

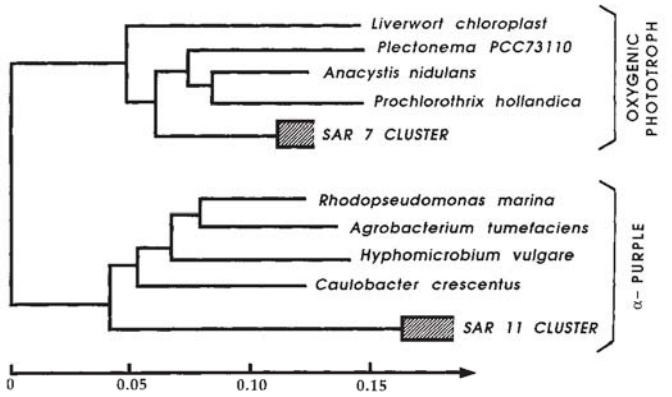


FIG. 3 Phylogenetic relationships of SAR7 and SAR11 16S rDNA sequence clusters to a collection of 16S rRNA sequences representing the oxygenic phototroph[8,28] and α-purple eubacterial phyla[29]. Four clones were sequenced completely (SAR6, SAR7, SAR1 and SAR11) and used for the inference of distant relationships. The tree was rooted using the sequences of *Bacillus subtilis* and *Heliobacterium chlorum*[8,30]. The analysis was restricted to 900 sequence positions. Regions of uncertain homology between phyla, including hypervariable domains, were excluded from this analysis. Hence, the variability within the clusters (indicated by the hatched boxes) is about 0.01 similarity units less in this figure than in Fig. 1. The 3′-terminal domain of the 16S rRNAs was excluded from the analysis because of an internal *Bam*HI restriction site in clones SAR1 and SAR11 at position 1,190.

Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

Oded Béjà,[1] L. Aravind,[2] Eugene V. Koonin,[2]
Marcelino T. Suzuki,[1] Andrew Hadd,[3] Linh P. Nguyen,[3]
Stevan B. Jovanovich,[3] Christian M. Gates,[3] Robert A. Feldman,[3]
John L. Spudich,[4] Elena N. Spudich,[4] Edward F. DeLong[1]*

Extremely halophilic archaea contain retinal-binding integral membrane proteins called bacteriorhodopsins that function as light-driven proton pumps. So far, bacteriorhodopsins capable of generating a chemiosmotic membrane potential in response to light have been demonstrated only in halophilic archaea. We describe here a type of rhodopsin derived from bacteria that was discovered through genomic analyses of naturally occuring marine bacterioplankton. The bacterial rhodopsin was encoded in the genome of an uncultivated γ-proteobacterium and shared highest amino acid sequence similarity with archaeal rhodopsins. The protein was functionally expressed in *Escherichia coli* and bound retinal to form an active, light-driven proton pump. The new rhodopsin exhibited a photochemical reaction cycle with intermediates and kinetics characteristic of archaeal proton-pumping rhodopsins. Our results demonstrate that archaeal-like rhodopsins are broadly distributed among different taxa, including members of the domain *Bacteria*. Our data also indicate that a previously unsuspected mode of bacterially mediated light-driven energy generation may commonly occur in oceanic surface waters worldwide.

(Science, 2000)

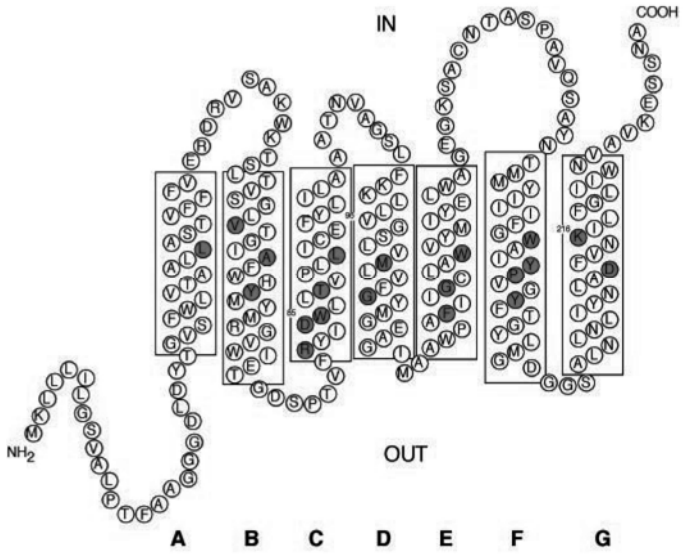
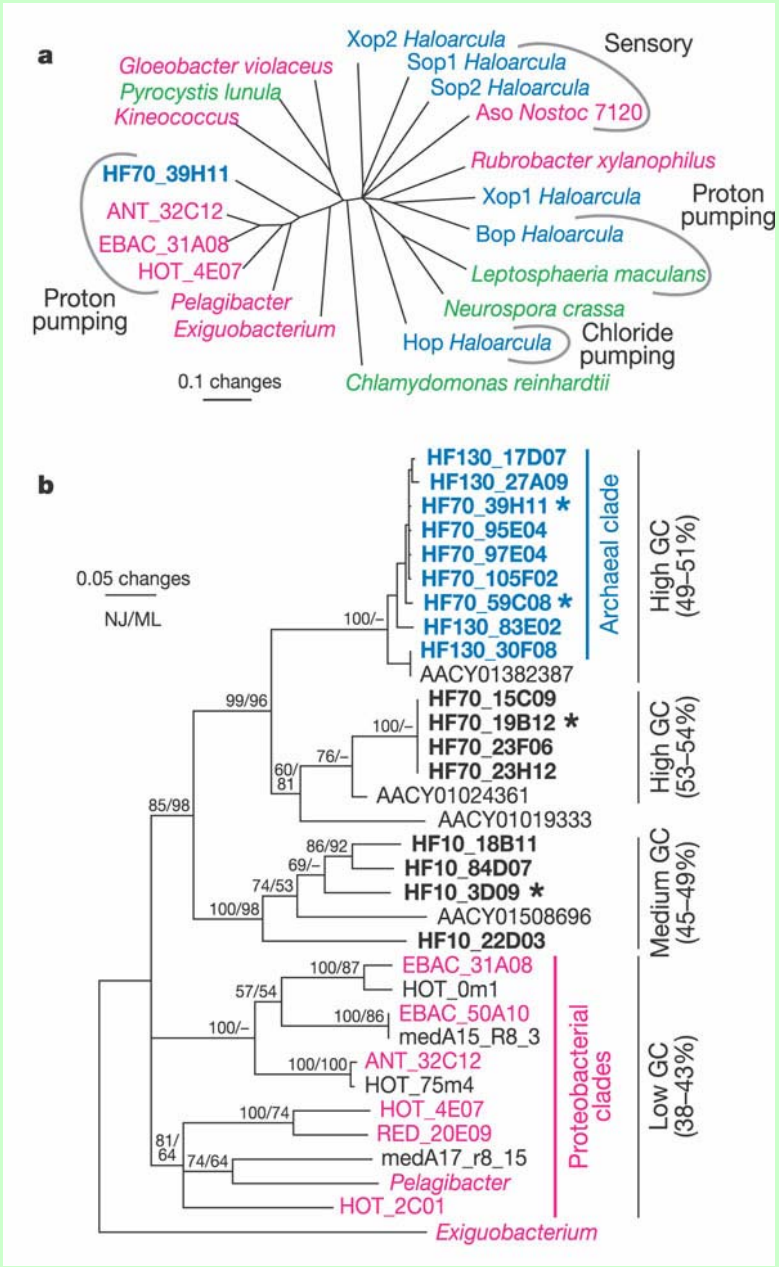
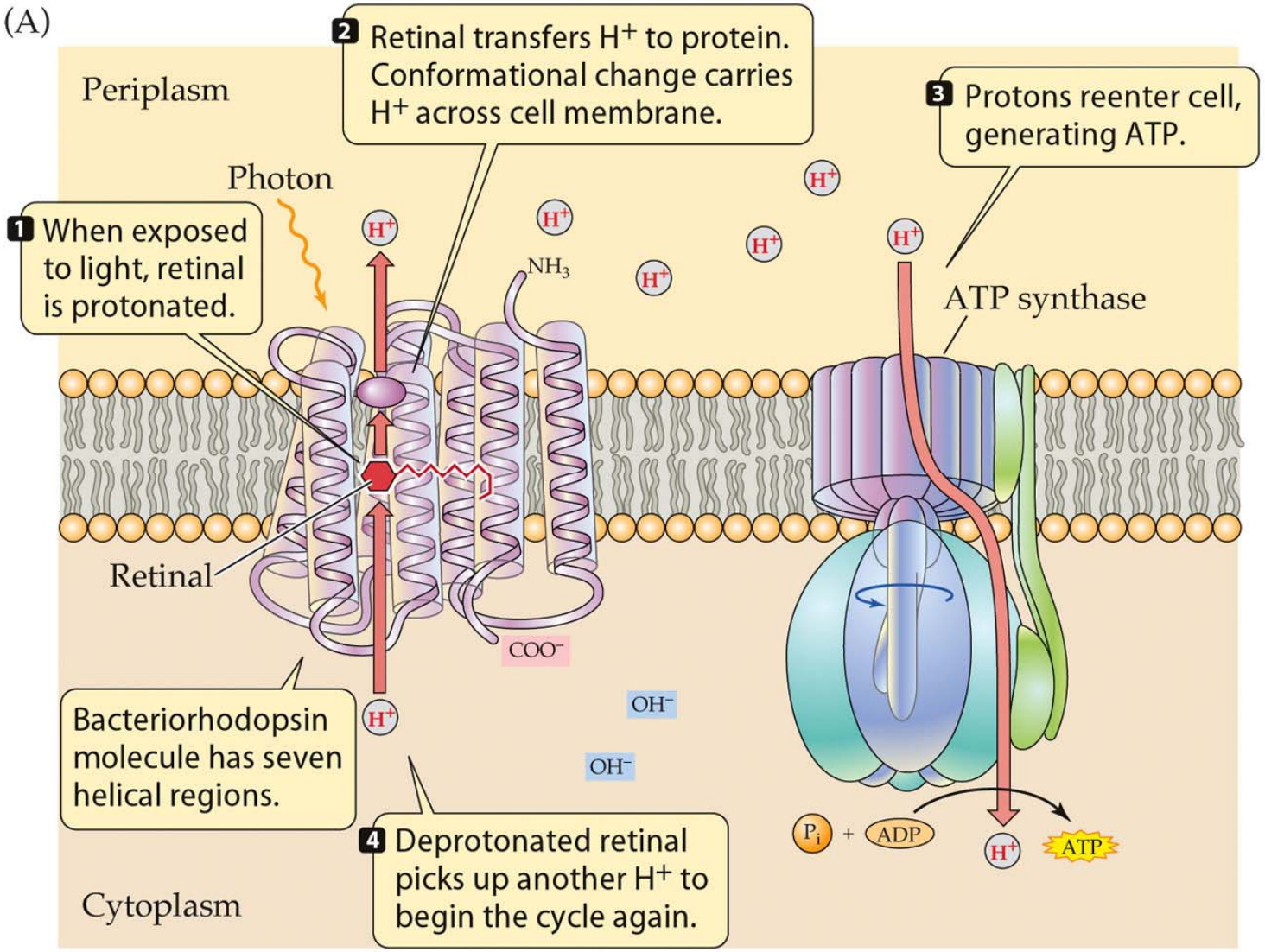


Fig. 2. Secondary structure of proteorhodopsin. Single-letter amino acid codes are used (*33*), and the numbering is as in bacteriorhodopsin. Predicted retinal binding pocket residues are marked in red.

Proteorhodopsin
in marine *Bacteria*
and *Archaea*



A light-driven proton pump



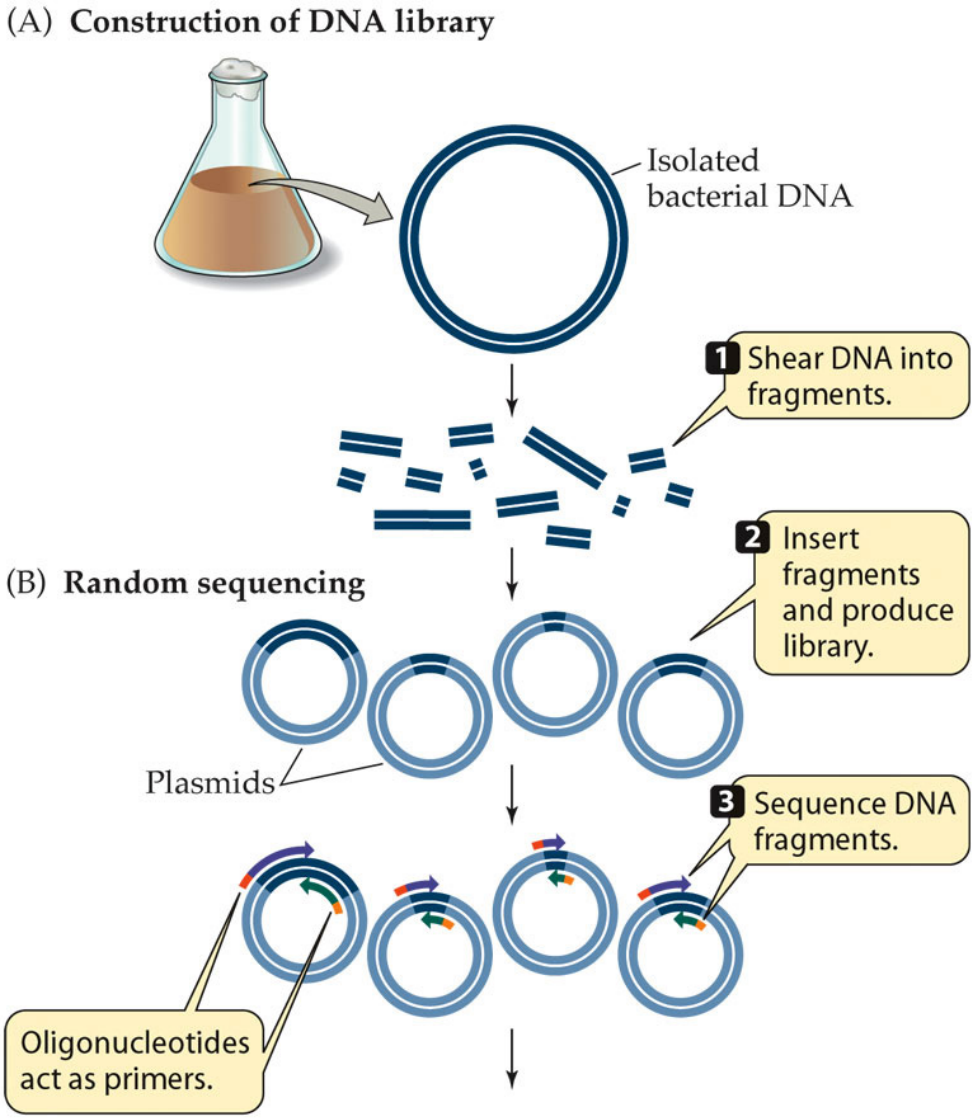
Environmental Genome Shotgun Sequencing of the Sargasso Sea

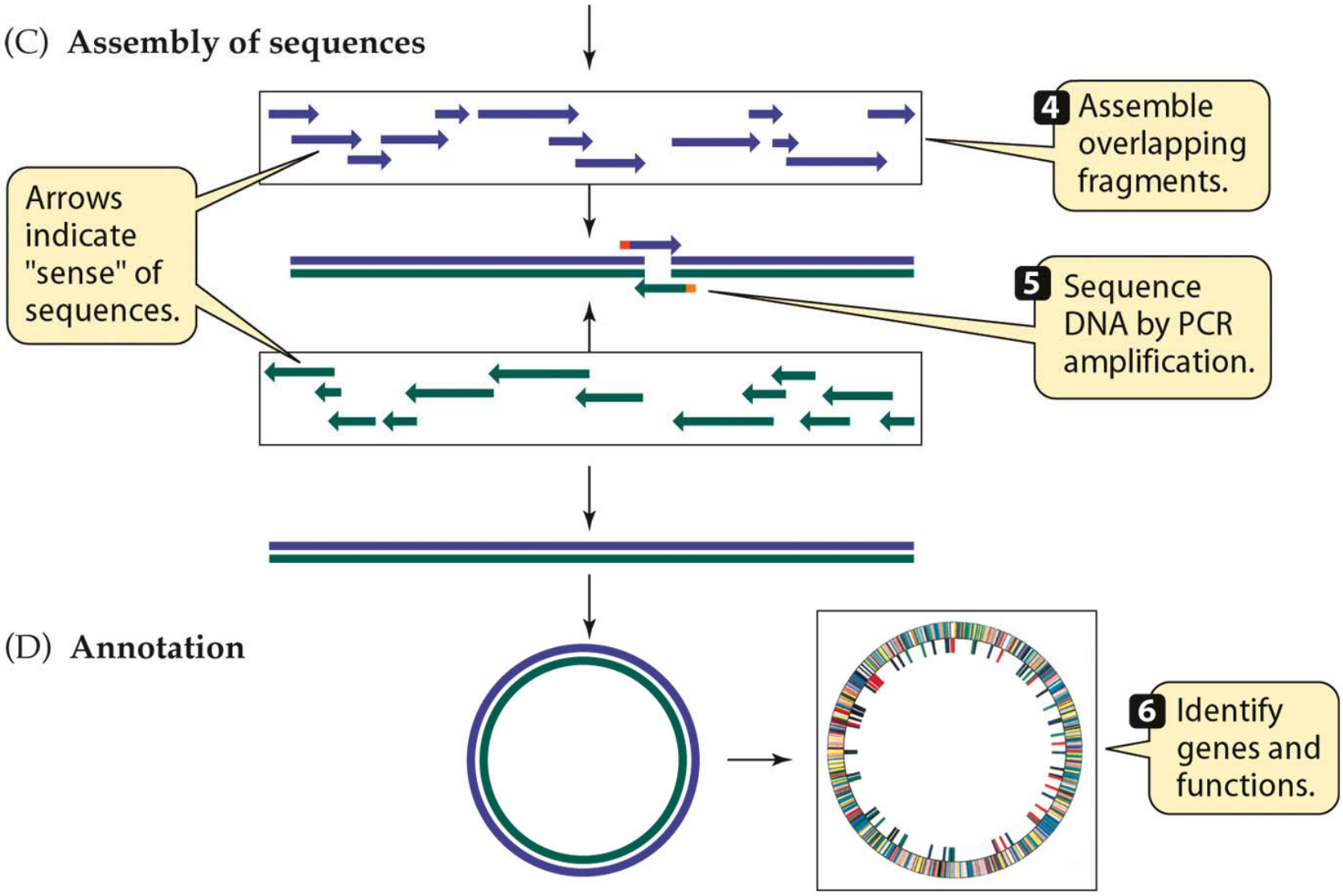
J. Craig Venter,[1]* Karin Remington,[1] John F. Heidelberg,[3] Aaron L. Halpern,[2] Doug Rusch,[2] Jonathan A. Eisen,[3] Dongying Wu,[3] Ian Paulsen,[3] Karen E. Nelson,[3] William Nelson,[3] Derrick E. Fouts,[3] Samuel Levy,[2] Anthony H. Knap,[6] Michael W. Lomas,[6] Ken Nealson,[5] Owen White,[3] Jeremy Peterson,[3] Jeff Hoffman,[1] Rachel Parsons,[6] Holly Baden-Tillson,[1] Cynthia Pfannkoch,[1] Yu-Hui Rogers,[4] Hamilton O. Smith[1]

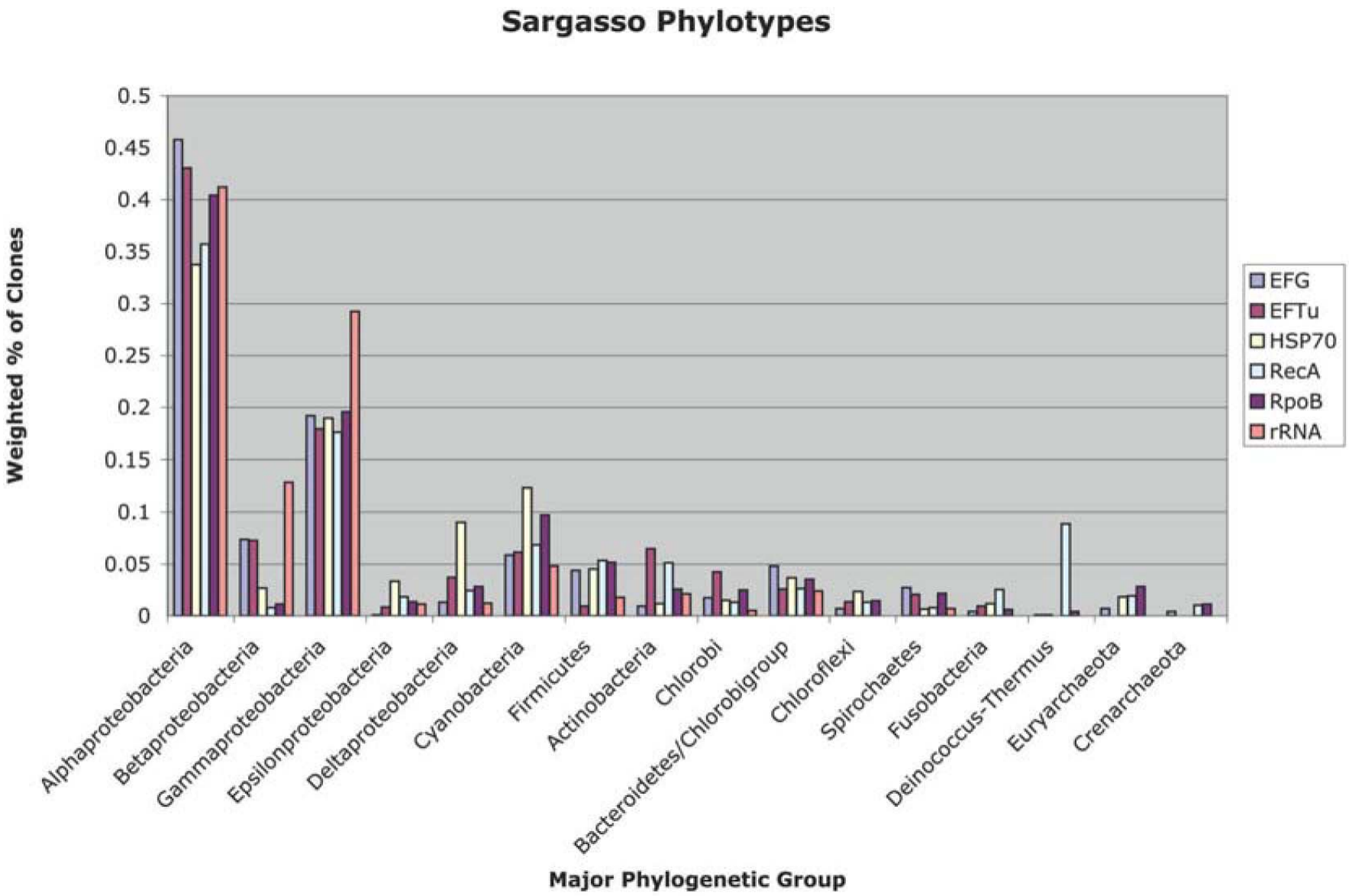
We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.

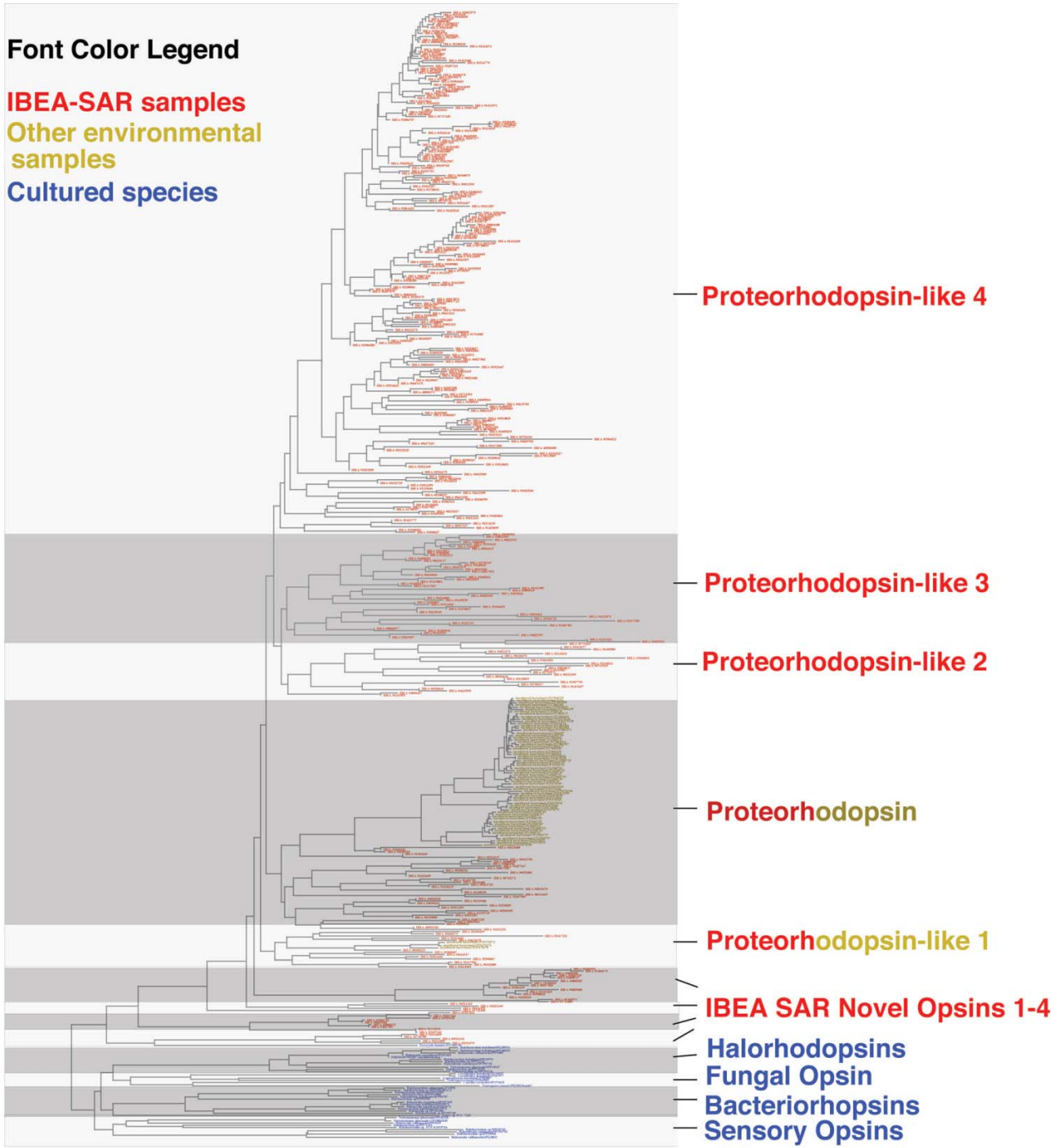
(Science, 2004)

Whole-genome shotgun sequencing




(C) Assembly of sequences
4 Assemble overlapping fragments.
Arrows indicate "sense" of sequences.
5 Sequence DNA by PCR amplification.
(D) Annotation
6 Identify genes and functions.


Sargasso Phylotypes
Weighted % of Clones
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
EFG
EFTu
HSP70
RecA
RpoB
rRNA
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Epsilonproteobacteria
Deltaproteobacteria
Cyanobacteria
Firmicutes
Actinobacteria
Chlorobi
Bacteroidetes/Chlorobigroup
Chloroflexi
Spirochaetes
Fusobacteria
Deinococcus-Thermus
Euryarchaeota
Crenarchaeota
Major Phylogenetic Group


Font Color Legend
IBEA-SAR samples
Other environmental samples
Cultured species
Proteorhodopsin-like 4
Proteorhodopsin-like 3
Proteorhodopsin-like 2
Proteorhodopsin
Proteorhodopsin-like 1
IBEA SAR Novel Opsins 1-4
Halorhodopsins
Fungal Opsin
Bacteriorhopsins
Sensory Opsins

Proteorhodopsin in the ubiquitous marine bacterium SAR11

Stephen J. Giovannoni[1], Lisa Bibbs[4], Jang-Cheon Cho[1]†, Martha D. Stapels[2]†, Russell Desiderio[3], Kevin L. Vergin[1], Michael S. Rappé[1]†, Samuel Laney[3], Lawrence J. Wilhelm[1], H. James Tripp[1], Eric J. Mathur[4] & Douglas F. Barofsky[2]

Proteorhodopsins are light-dependent proton pumps that are predicted to have an important role in the ecology of the oceans by supplying energy for microbial metabolism[1,2]. Proteorhodopsin genes were first discovered through the cloning and sequencing of large genomic DNA fragments from seawater[1]. They were later shown to be widely distributed, phylogenetically diverse, and active in the oceans[3–7]. Proteorhodopsin genes have not been found in cultured bacteria, and on the basis of environmental sequence data, it has not yet been possible to reconstruct the genomes of uncultured bacterial strains that have proteorhodopsin genes. Although the metabolic effect of proteorhodopsins is uncertain, they are thought to function in cells for which the primary mode of metabolism is the heterotrophic assimilation of dissolved organic carbon. Here we report that SAR11 strain HTCC1062 (‘*Pelagibacter ubique*’)[8], the first cultivated member of the extraordinarily abundant SAR11 clade, expresses a proteorhodopsin gene when cultured in autoclaved seawater and in its natural environment, the ocean. The *Pelagibacter* proteorhodopsin functions as a light-dependent proton pump. The gene is expressed by cells grown in either diurnal light or in darkness, and there is no difference between the growth rates or cell yields of cultures grown in light or darkness.

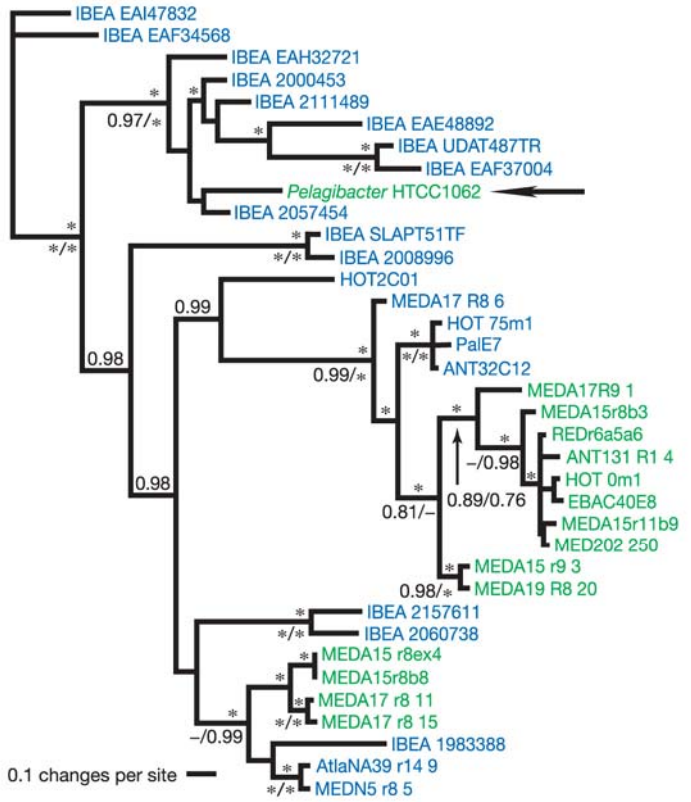


Figure 1 | Phylogenetic relationships between proteorhodopsin amino acid sequences. Shown is the relationship between the *Pelagibacter* strain HTCC1062 proteorhodopsin amino acid sequence and selected representatives of proteorhodopsin genes cloned from seawater DNA. The sequences most similar to *Pelagibacter* proteorhodopsin are included in the tree. Green text indicates proteorhodopsin genes encoding a leucine residue at position 105, blue text indicates a glutamine residue at this position. The tree was rooted with the sequences of rhodopsins from *Gloeobacter violaceus* and *Pyrocystis lunula*. Numbers above nodes are posterior probabilities; numbers below nodes are parsimony bootstrap values and neighbour-joining bootstrap values (separated by a slash). Asterisks indicate a value of 1.0.

(Nature, 2005)

Genome Streamlining in a Cosmopolitan Oceanic Bacterium

Stephen J. Giovannoni,[1*] H. James Tripp,[1] Scott Givan,[2] Mircea Podar,[3] Kevin L. Vergin,[1] Damon Baptista,[3] Lisa Bibbs,[3] Jonathan Eads,[3] Toby H. Richardson,[3] Michiel Noordewier,[3] Michael S. Rappé,[4] Jay M. Short,[3] James C. Carrington,[2] Eric J. Mathur[3]

The SAR11 clade consists of very small, heterotrophic marine α-proteobacteria that are found throughout the oceans, where they account for about 25% of all microbial cells. *Pelagibacter ubique*, the first cultured member of this clade, has the smallest genome and encodes the smallest number of predicted open reading frames known for a free-living microorganism. In contrast to parasitic bacteria and archaea with small genomes, *P. ubique* has complete biosynthetic pathways for all 20 amino acids and all but a few cofactors. *P. ubique* has no pseudogenes, introns, transposons, extrachromosomal elements, or inteins; few paralogs; and the shortest intergenic spacers yet observed for any cell.

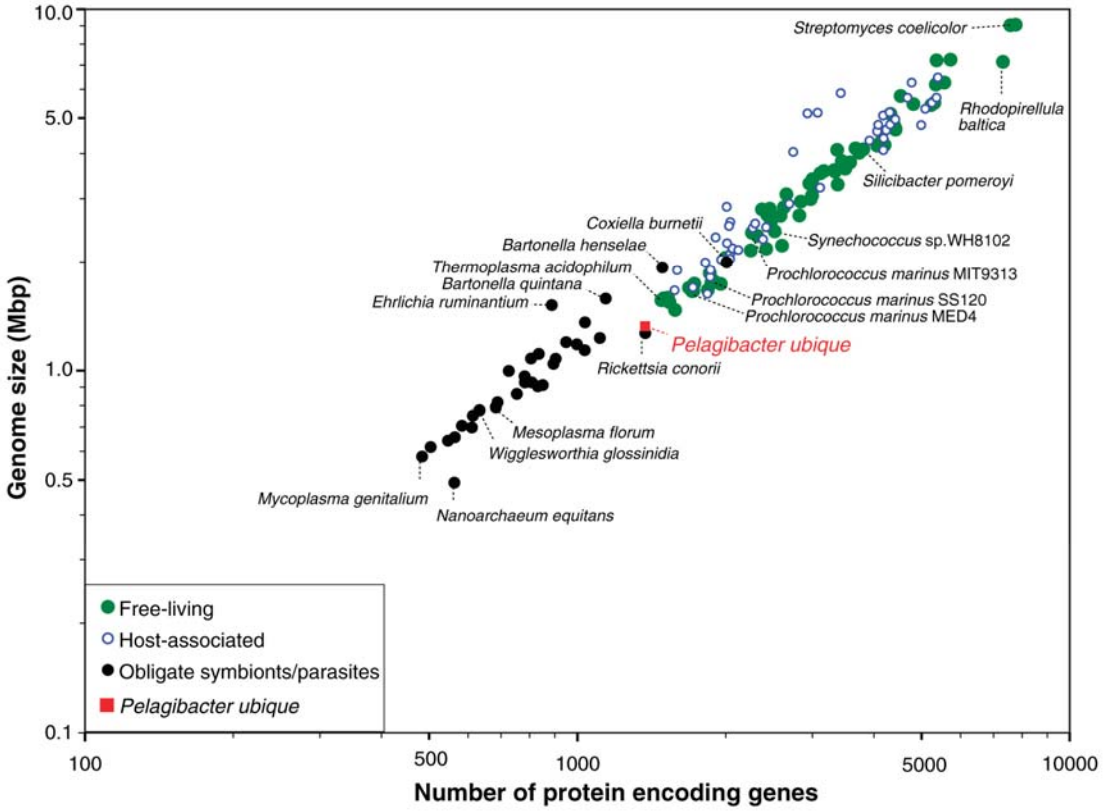


Fig. 1. Number of predicted protein-encoding genes versus genome size for 244 complete published genomes from bacteria and archaea. *P. ubique* has the smallest number of genes (1354 open reading frames) for any free-living organism.

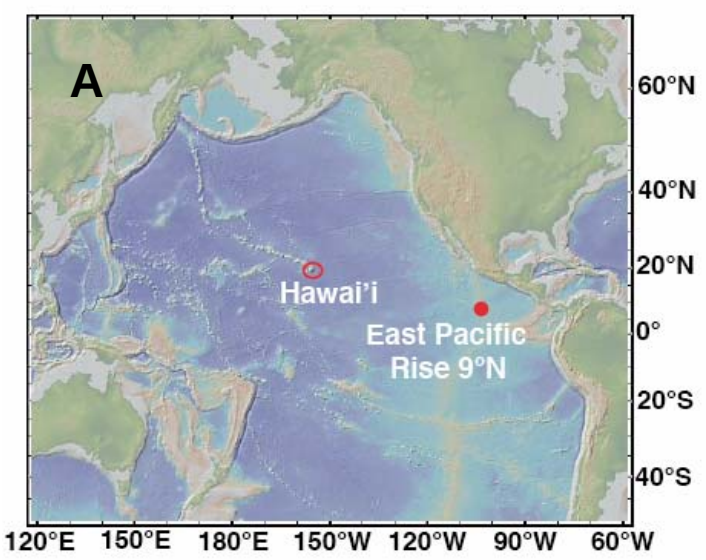
(Science, 2005)

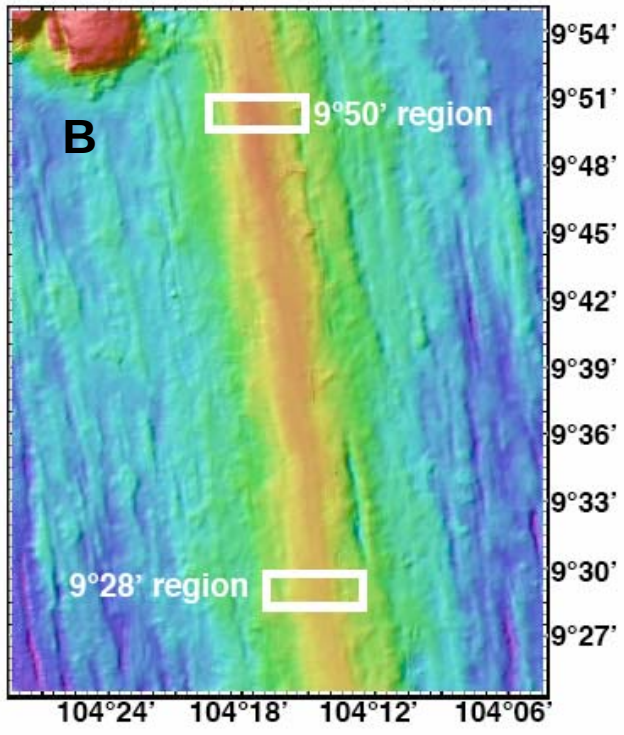
Abundance and diversity of microbial life in ocean crust

Cara M. Santelli[1,2], Beth N. Orcutt[3], Erin Banning[1], Wolfgang Bach[2,4], Craig L. Moyer[5], Mitchell L. Sogin[6], Hubert Staudigel[7] & Katrina J. Edwards[2,3]

Oceanic lithosphere exposed at the sea floor undergoes seawater–rock alteration reactions involving the oxidation and hydration of glassy basalt. Basalt alteration reactions are theoretically capable of supplying sufficient energy for chemolithoautotrophic growth[1]. Such reactions have been shown to generate microbial biomass in the laboratory[2], but field-based support for the existence of microbes that are supported by basalt alteration is lacking. Here, using quantitative polymerase chain reaction, *in situ* hybridization and microscopy, we demonstrate that prokaryotic cell abundances on sea floor-exposed basalts are 3–4 orders of magnitude greater than in overlying deep sea water. Phylogenetic analyses of basaltic lavas from the East Pacific Rise (9° N) and around Hawaii reveal that the basalt-hosted biosphere harbours high bacterial community richness and that community membership is shared between these sites. We hypothesize that alteration reactions fuel chemolithoautotrophic microorganisms, which constitute a trophic base of the basalt habitat, with important implications for deep-sea carbon cycling and chemical exchange between basalt and sea water.

(Nature, 2008)





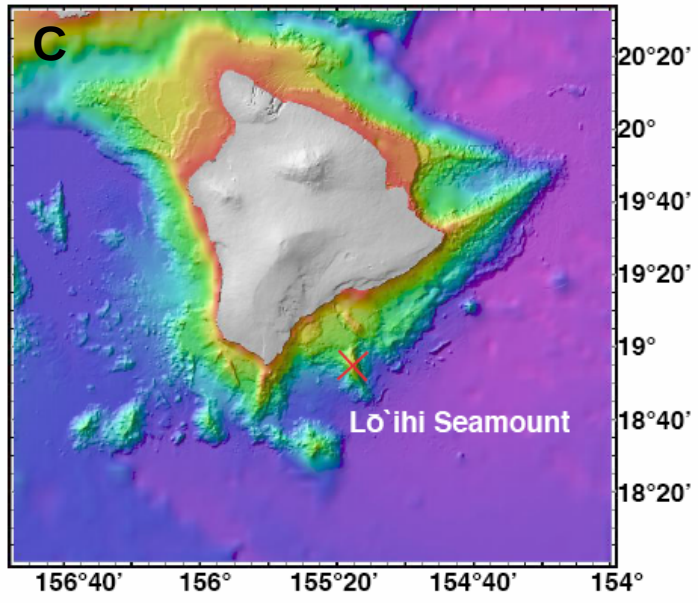


Fig. S1 Maps of the locations of study sites where seafloor lavas were collected in the Pacific Ocean (A). Basalt samples from the East Pacific Rise (B) were collected on and near the ridge axis up to 3 km off-axis between approximately 9°28'N and 9°50'N (white boxes). Basalt samples from around the big island of Hawaii (C) were collected from the Pisces Peak and South Rift sites on the Loihi seamount and from the South Point location.

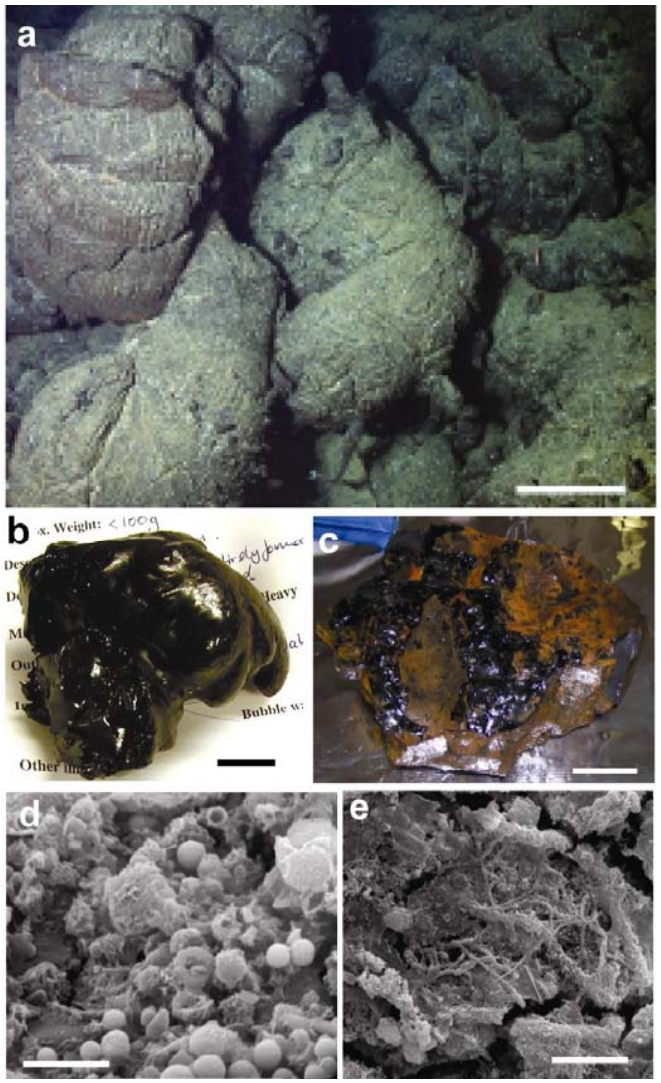


Figure 1 | A highly diverse and abundant epi- and endo-lithic microbial community exists on basaltic lavas from the East Pacific Rise. a, The East Pacific Rise at 9°N is characterized by lava flows, such as pillow basalts shown here, directly exposed at the seafloor. Scale bar, 40 cm. **b,c,** Photographs showing the range of volcanic samples used in this study from fresh and glassy **(b)** to more altered and oxide coated **(c)**. Scale bars, 2 cm **(b)** and 4 cm **(c)**. **d,e,** SEM images of different presumed cellular morphologies, such as coccoidal **(d)** and filamentous **(e)** structures that were observed on ferromanganese oxide encrusted and Fe-oxide-coated samples. Scale bars, 5 μm.

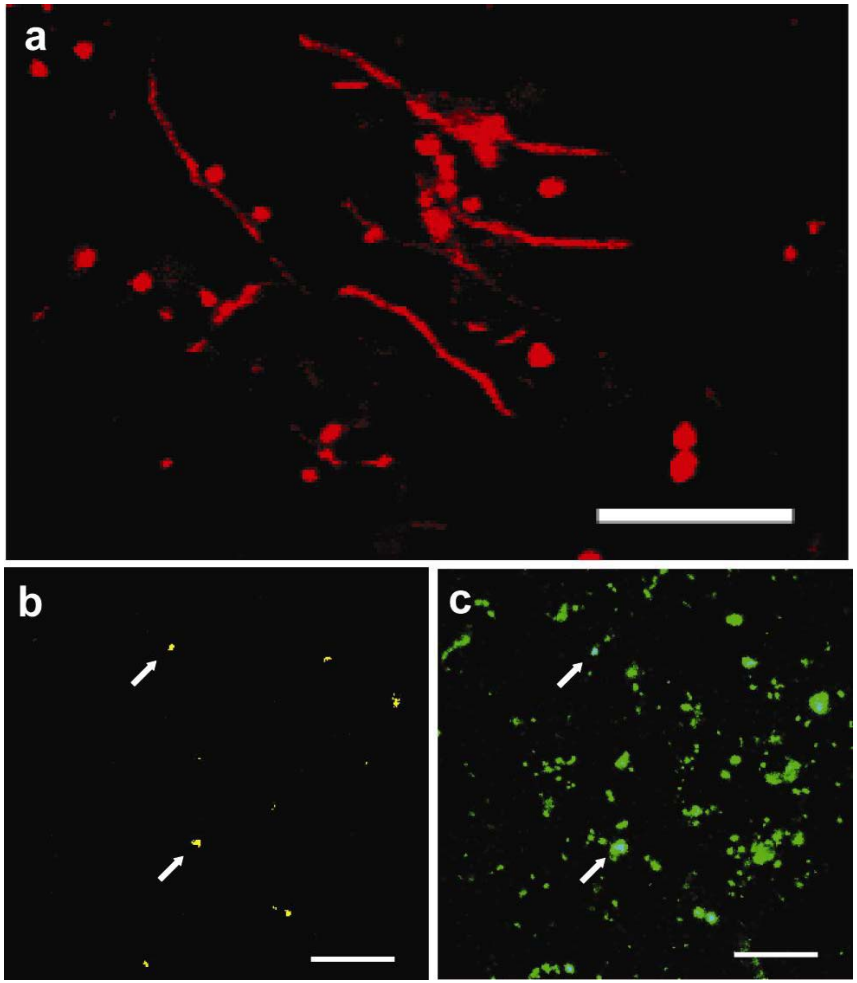


Figure 2 | CARD-FISH analyses. a,b,c, Confocal laser scanning micrographs depicting CARD-FISH on lava surfaces from the EPR. Prokaryotic cells were hybridized with either probe EUB338(I-III) mix **(a)** or probe ARCH915 **(b)** to target *Bacteria*, or *Archaea* respectively. A variety of bacterial cell morphologies such as filaments, cocci, and rods were confirmed with hybridizations **(a)**. Side-by-side comparisons of *Archaea* **(b)** versus total cells **(c)** reveals that *Archaea* account for only a small portion of the total cells. Total cells were identified with the general DNA stain SYBR Green. The arrows point to some cells that overlap in each frame. Scale bars, 10 µm **(a)** and 20 µm **(b,c)**.

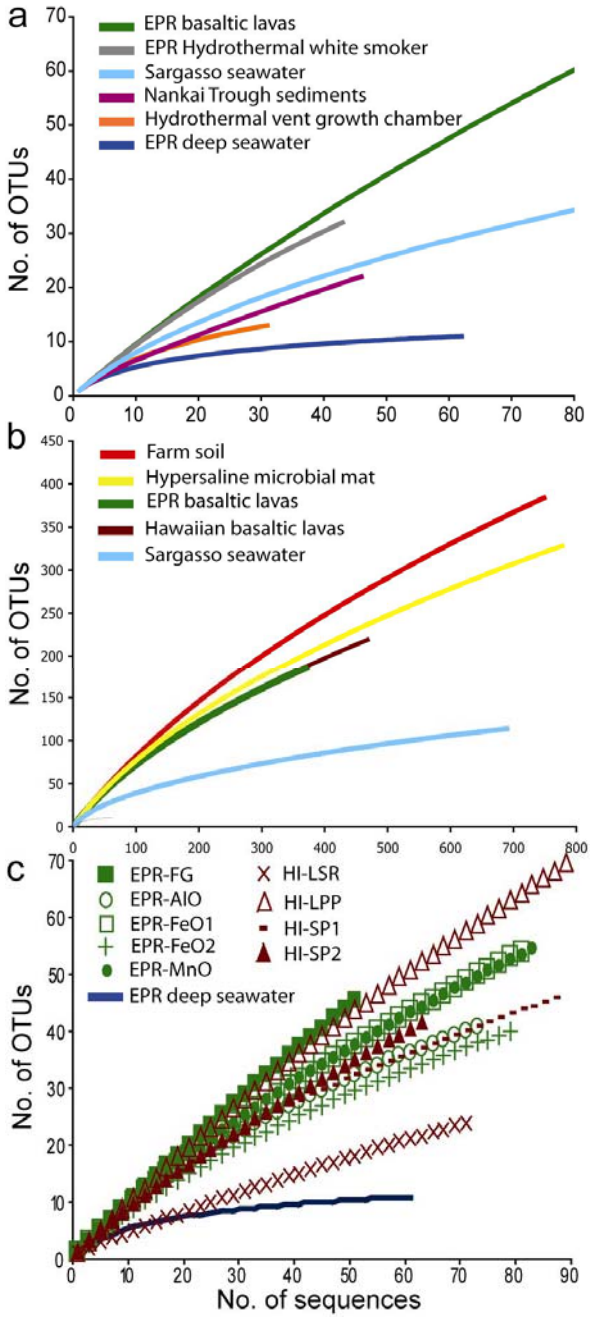


Figure 3 | Relative bacterial richness from several environmental studies shown through rarefaction analyses. The observed species richness of *Bacteria* inhabiting seafloor lavas from the EPR is compared to the richness found in other ocean environments **(a)**, such as clone library surveys from the Sargasso Sea (partial curve shown), a MAR hydrothermal vent *in situ* growth chamber, an EPR hydrothermal white smoker spire, deep-sea sediments from the Nankai Trough, and ambient bottom seawater from the EPR. Here the five basalt clone library results are summed and treated as a single ecosystem. The observed elevated bacterial richness of the EPR basalts (cumulative community) is also compared to another basalt-hosted community from the Hawaiian Islands (HI) and other known high richness environments **(b)**, such as a farm soil (partial curve shown) and a hypersaline microbial mat from the Guerrero Negro Only (partial curve shown). Partial rarefaction curves are shown for visualization purposes, however complete data sets were used in calculating the curve projections. **c,** Rarefaction curves for the individual EPR and HI basalt clone libraries and the EPR deep seawater library. Only partial curves for HI-LPP is shown (total clones = 246). Each comparative study in **(a)** and **(b)** is based on near full-length 16S rRNA gene sequences, and most studies are the sum of several environmental samples. OTUs are defined at a sequence similarity of ≥ 97%.

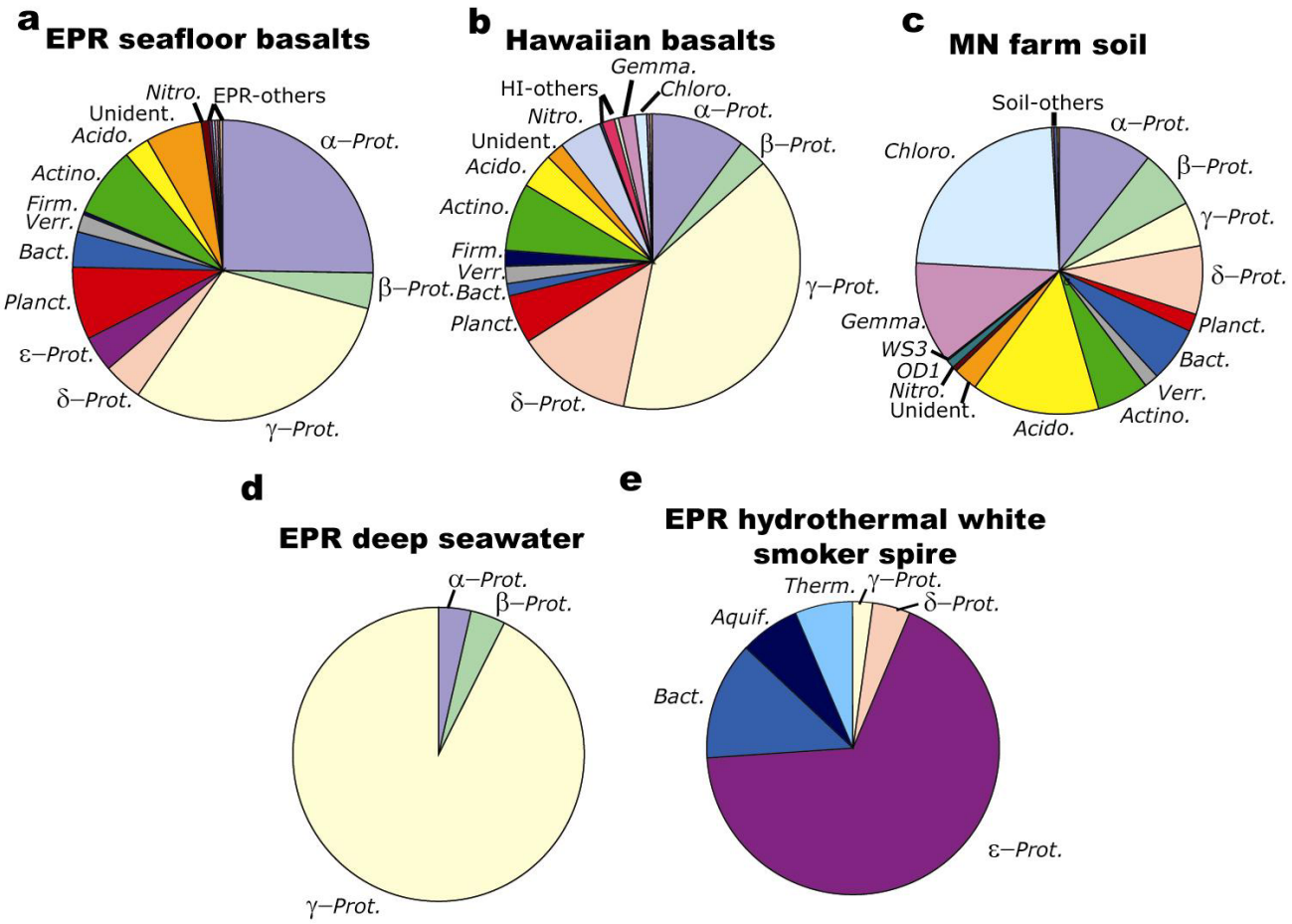


Fig. S2 Phylogenetic identity of *Bacteria* from environmental studies**. a-e,** Distribution of bacterial 16S rRNA sequences into major taxonomic groups from the cumulative EPR basalt clone library **(a)**, the cumulative Hawaiian basalt clone library **(b)** Minnesota farm soil **(c)**, ambient bottom seawater from the EPR **(d)**, and an EPR hydrothermal white smoker spire **(e)**. EPRbasalt-others(<0.5% each): *Gemmatimonadetes*, *Spirochaetes,* Candidatus Scalindua brodae, and Cand. div. OP11. HI-others (<1%): *Deferribacteres,* Spirochaetes, Cand. divs. OD1, TM6, WS3. Soil-others (<0.5% each): *Firmicutes, Cyanobacteria, Deinococcus-Thermus,* Cand. divs. OP11, OP10, SPAM, WS6, TM7, and BRC1. Haw Abbreviations: Prot., *Proteobacteria;* Bact., *Bacteroidetes;* Firm, *Firmicutes*; Verr., *Verrucomicrobia*; Planct., *Planctomycetes*; Chloro, *Chloroflexi*; Actino., *Actinobacteria*; Acido., *Acidobacteria*; Gemma., *Gemmatimonadetes*; Aquif., *Aquificae*; Nitro., *Nitrospira;* Therm., *Thermales*; Unident., Unidentified.

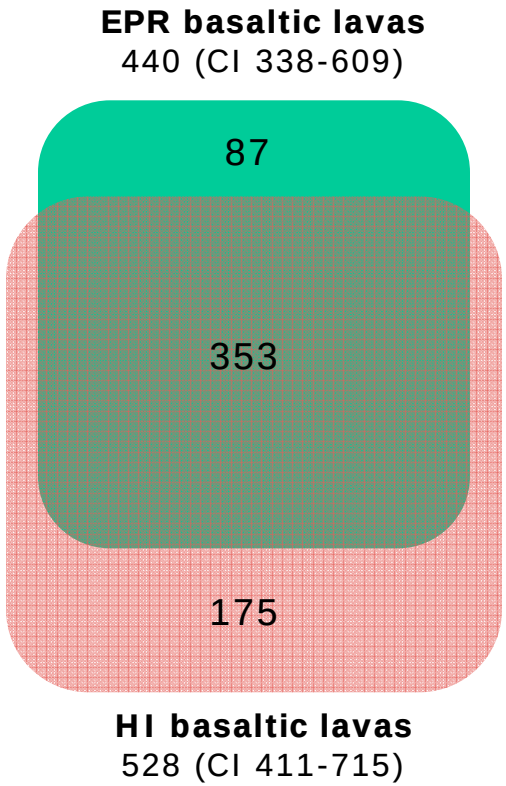


Fig. S3 Venn diagram depicting the estimated OTU richness that is shared between the EPR basalts community [n (# of sequences) = 370] and the HI basalts community (n = 472). The Chao1 richness estimates (determined by DOTUR) for each community are shown under label with 95% confidence intervals. Shared OTU richness estimates (numbers within the lenses) were determined using the computer program SONS13. The object sizes represent the approximate OTU memberships but are not drawn to scale. OTUs are defined at a distance level of 0.03.

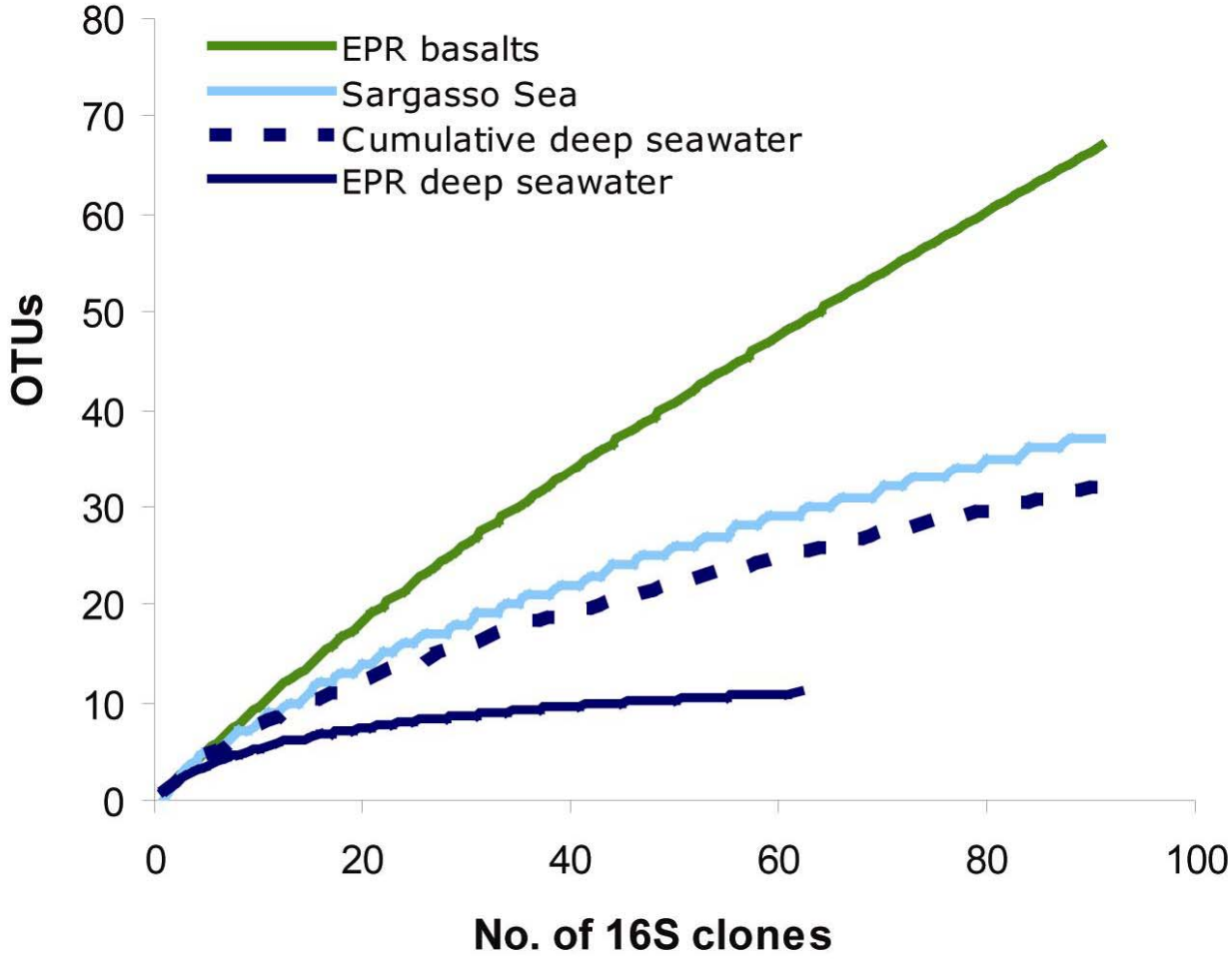


Fig. S4 Rarefaction analyses comparing the diversity between EPR deep seawater and cumulative deep seawater (combination of clone libraries from 3 different studies including EPR deep seawater and two studies by Huber et al.1,2). OTUs are defined at a distance level of 0.03 as assigned by DOTUR. Analyses show that the addition of clones from the Huber et al. deep seawater libraries increases species diversity approximately equal to that of Sargasso Sea surface waters. However, the bacterial diversity found in deep seawater relative to that found in EPR basalt is still the same.

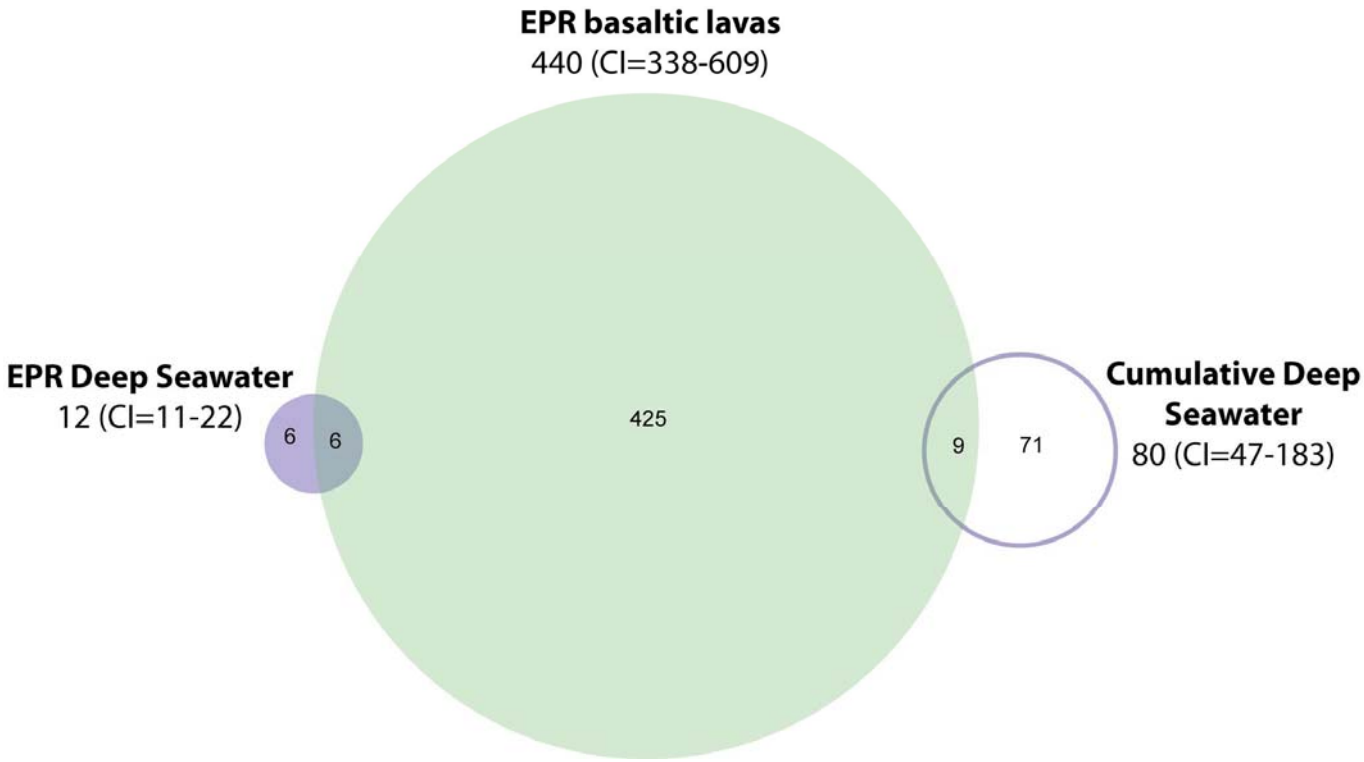


Fig. S5 Venn diagram depicting the estimated OTU richness that is shared between the EPR basalts community [n (# of sequences) = 370] and the EPR deep seawater community [n = 62] or the EPR basalts and the cumulative deep seawater communities [n = 91 (described in Fig. S3)]. The Chao1 richness estimates (determined by DOTUR) are shown below each circle label for each community with 95% confidence intervals. Shared OTU estimates (numbers within the lenses) were determined using the computer program SONS13. The circle sizes represent the approximate OTU memberships but are not drawn to scale. OTUs are defined at a distance level of 0.03.