

# Microbiology of Deep-Sea Hydrothermal Vents

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<http://marine.rutgers.edu/deep-seamicrobiology/>

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deep sea  
microbiology lab

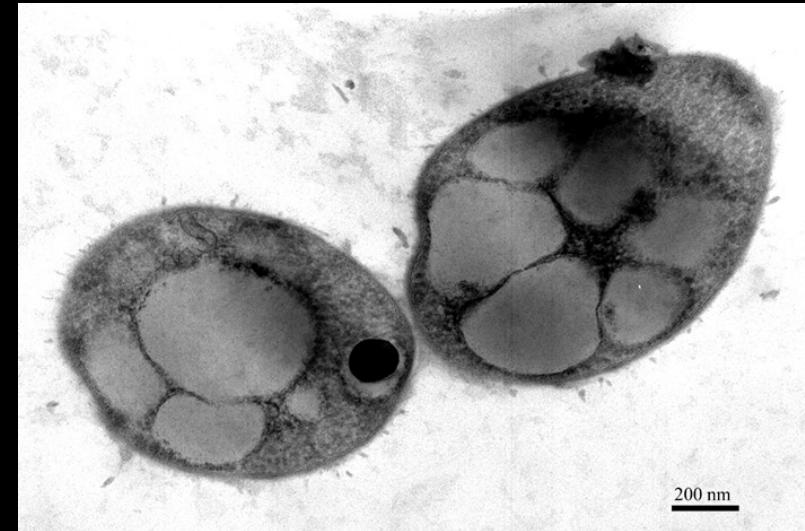
# deep sea microbiology lab



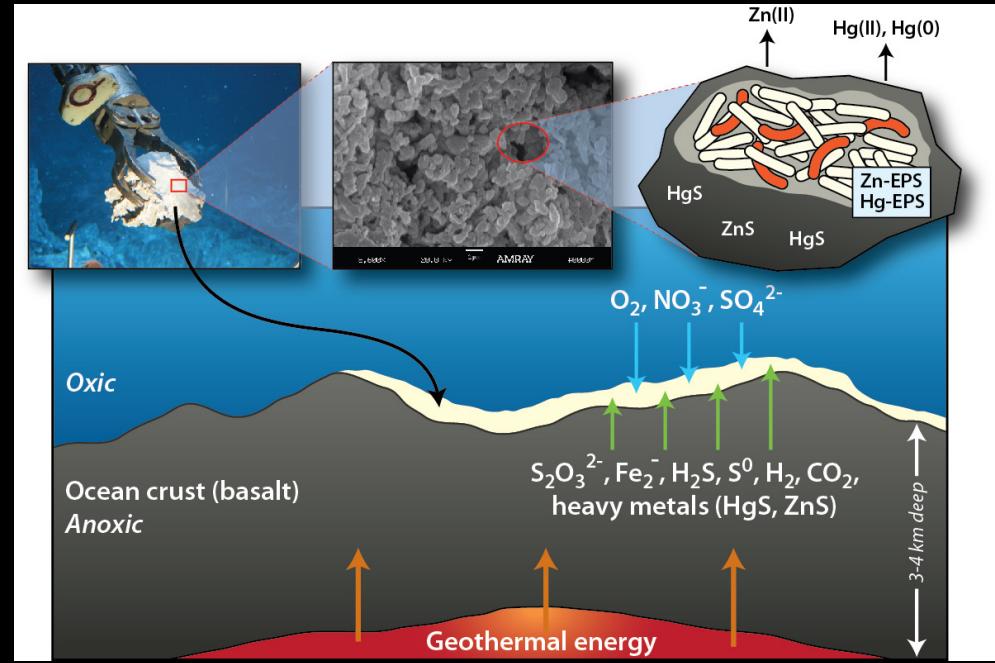
Microbiology of shallow-water hydrothermal systems...



...and of deep-sea hydrothermal vents

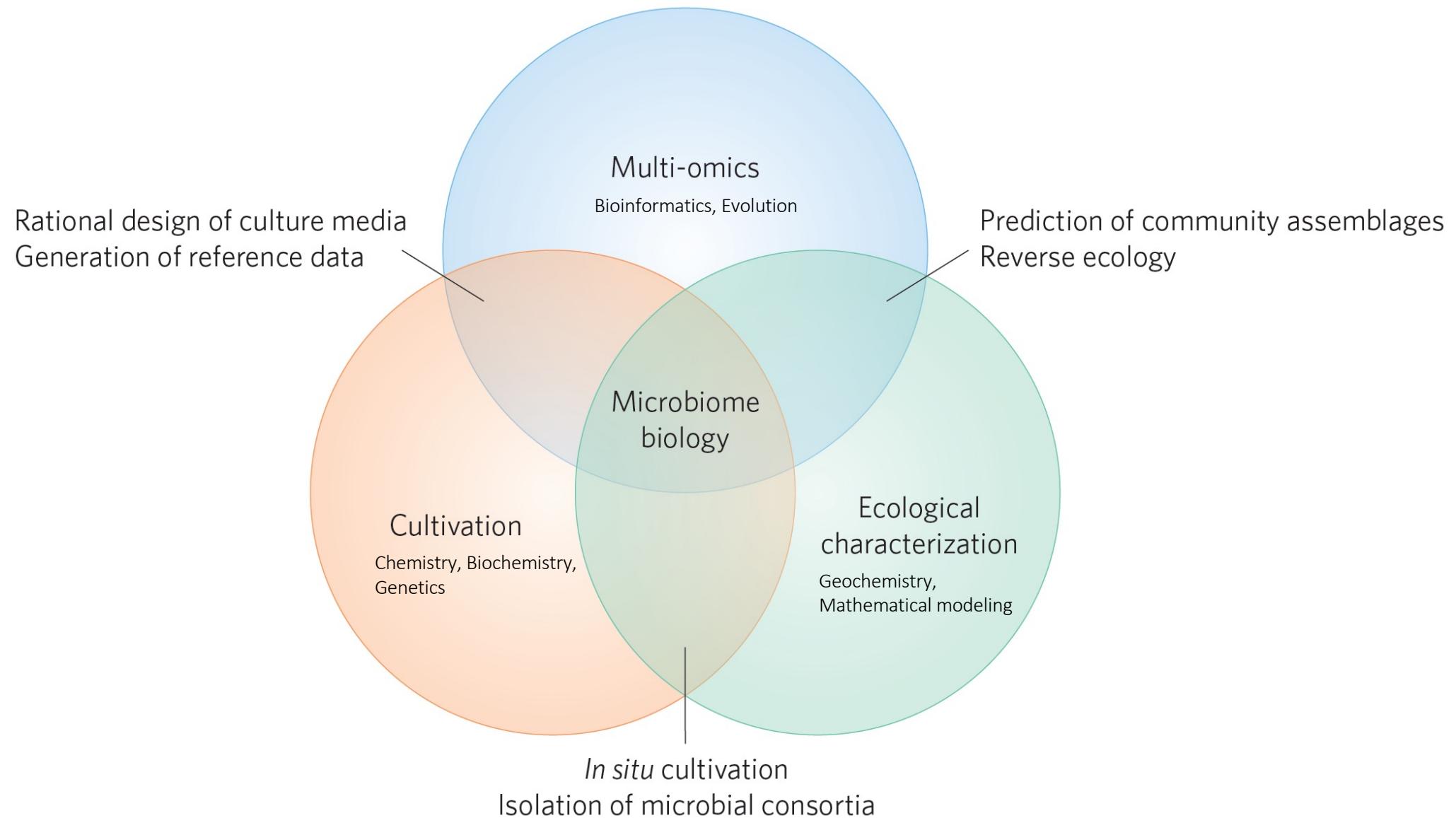


Physiology, ecology and evolution of marine microorganisms...



...and ecology of natural microbial communities

# Integration of physiological, molecular and geochemical approaches in environmental microbiology



# Outline

- **Geological and chemical characteristics of deep-sea vents**
- **Life without light: Chemosynthetically sustained ecosystems**
- Epsilonproteobacteria: Primary producers at deep-sea vents
- Symbiosis: How the vent invertebrates make a living
- Anaerobic thermophiles as “time capsules” to investigate early microbial metabolism and life on other planets

# Deep-sea biological communities are typically poor in terms of biomass

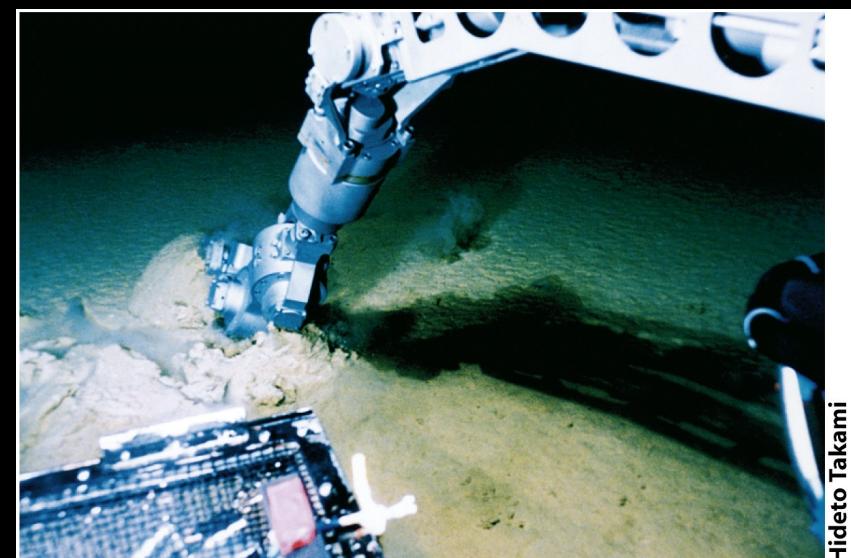
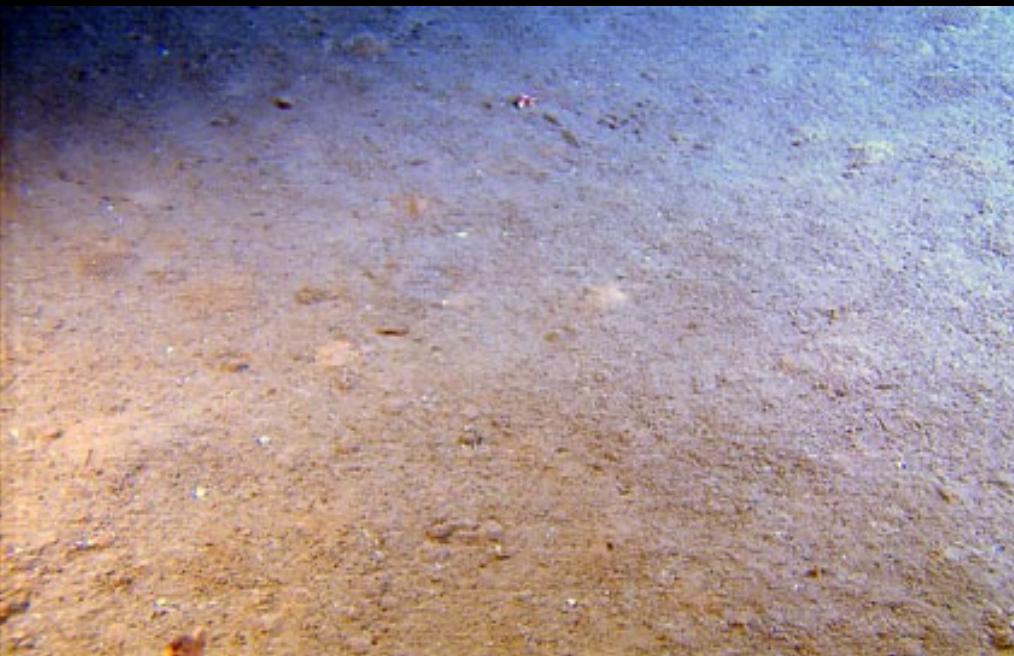
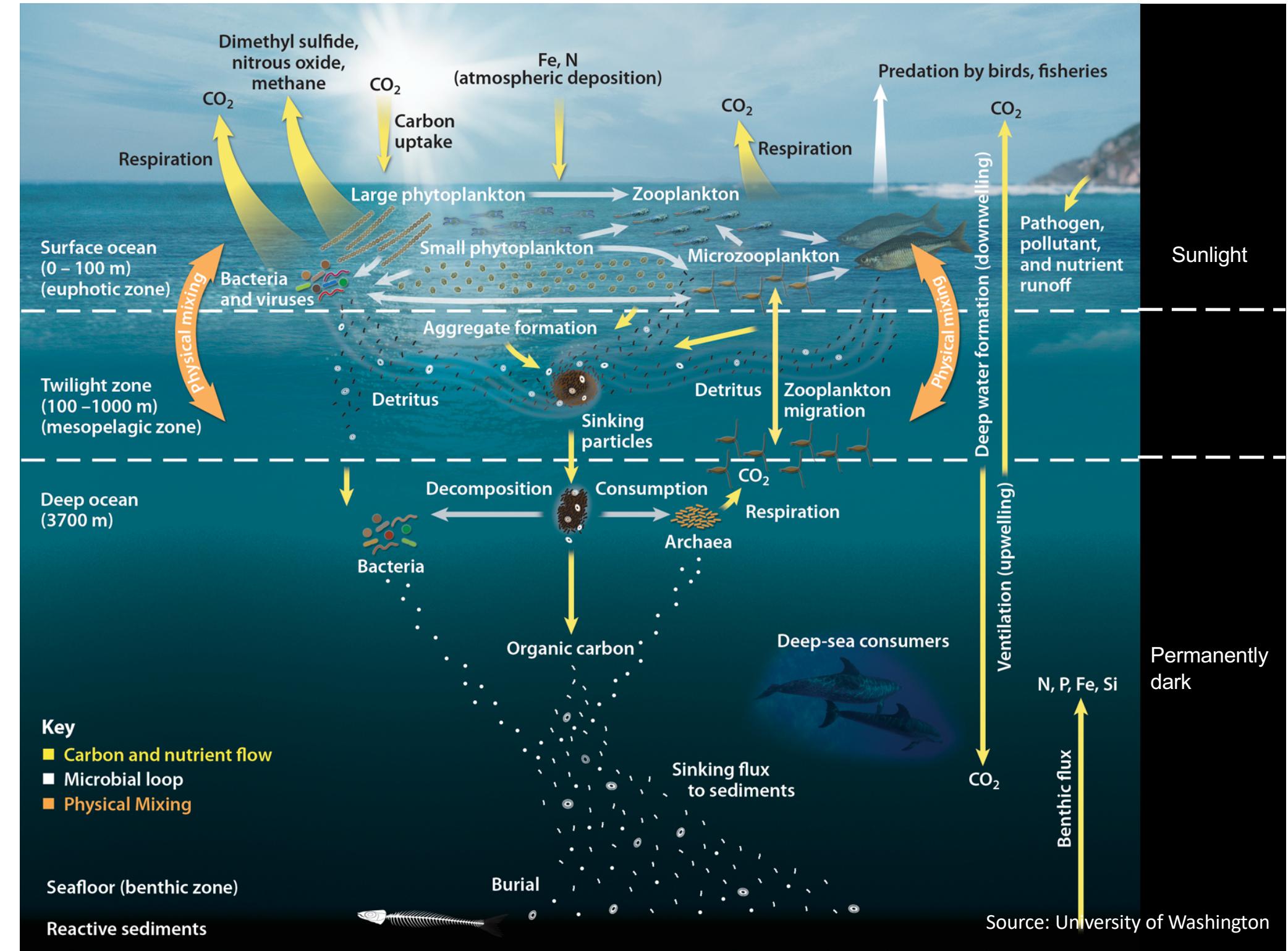
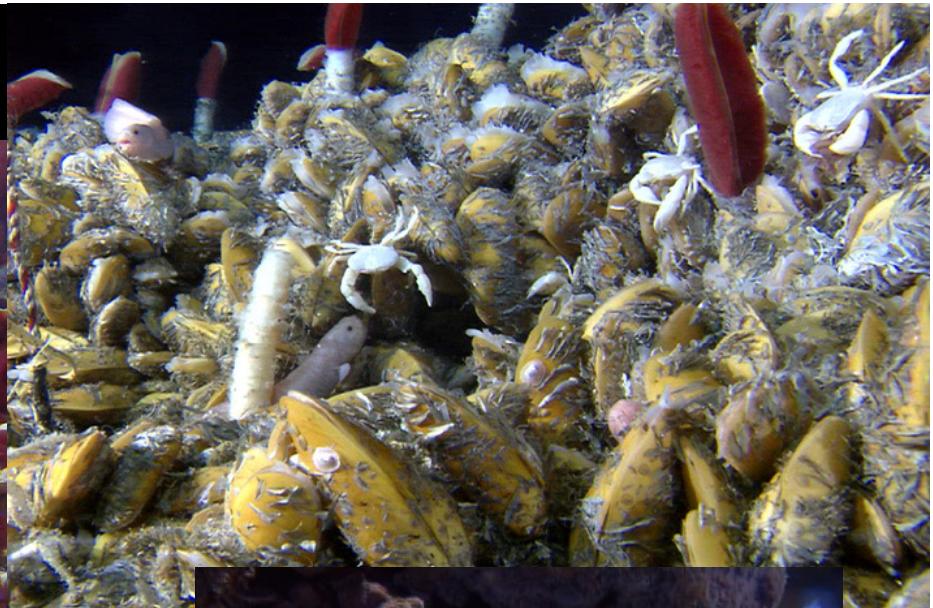


Figure 19-15 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

Hideto Takami



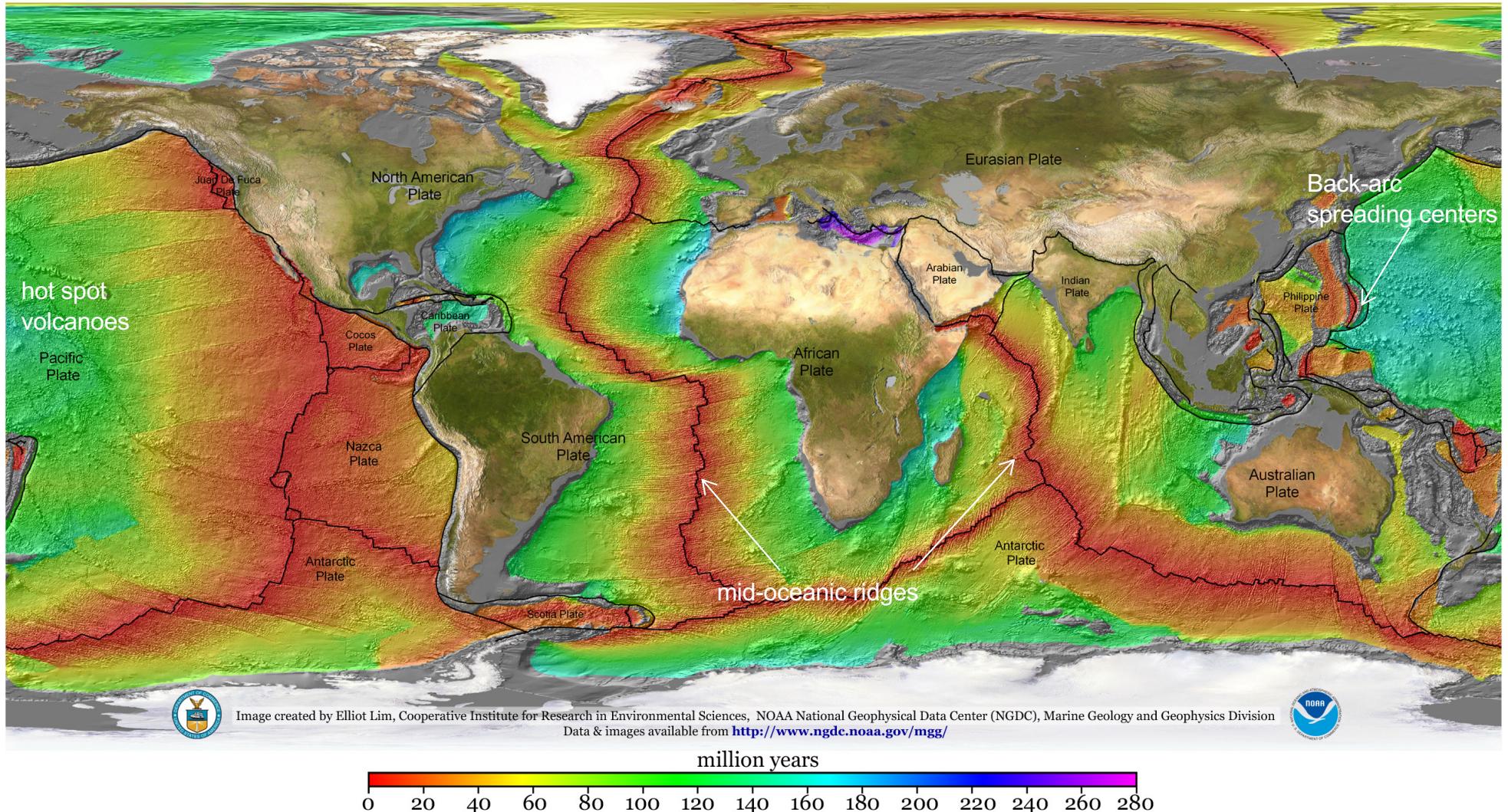
In contrast, hydrothermal vents host very rich biological communities



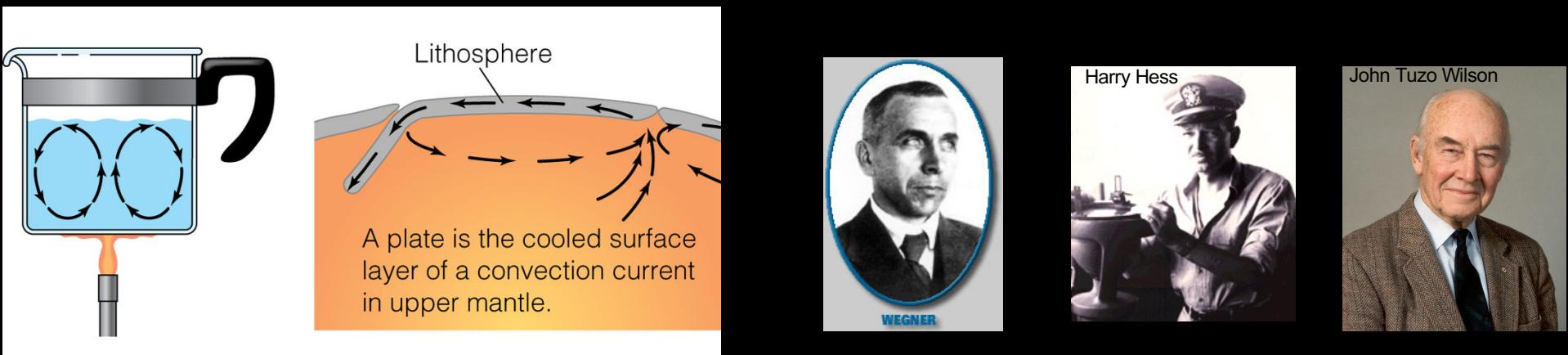
# Age of Oceanic Lithosphere (m.y.)

## Data source:

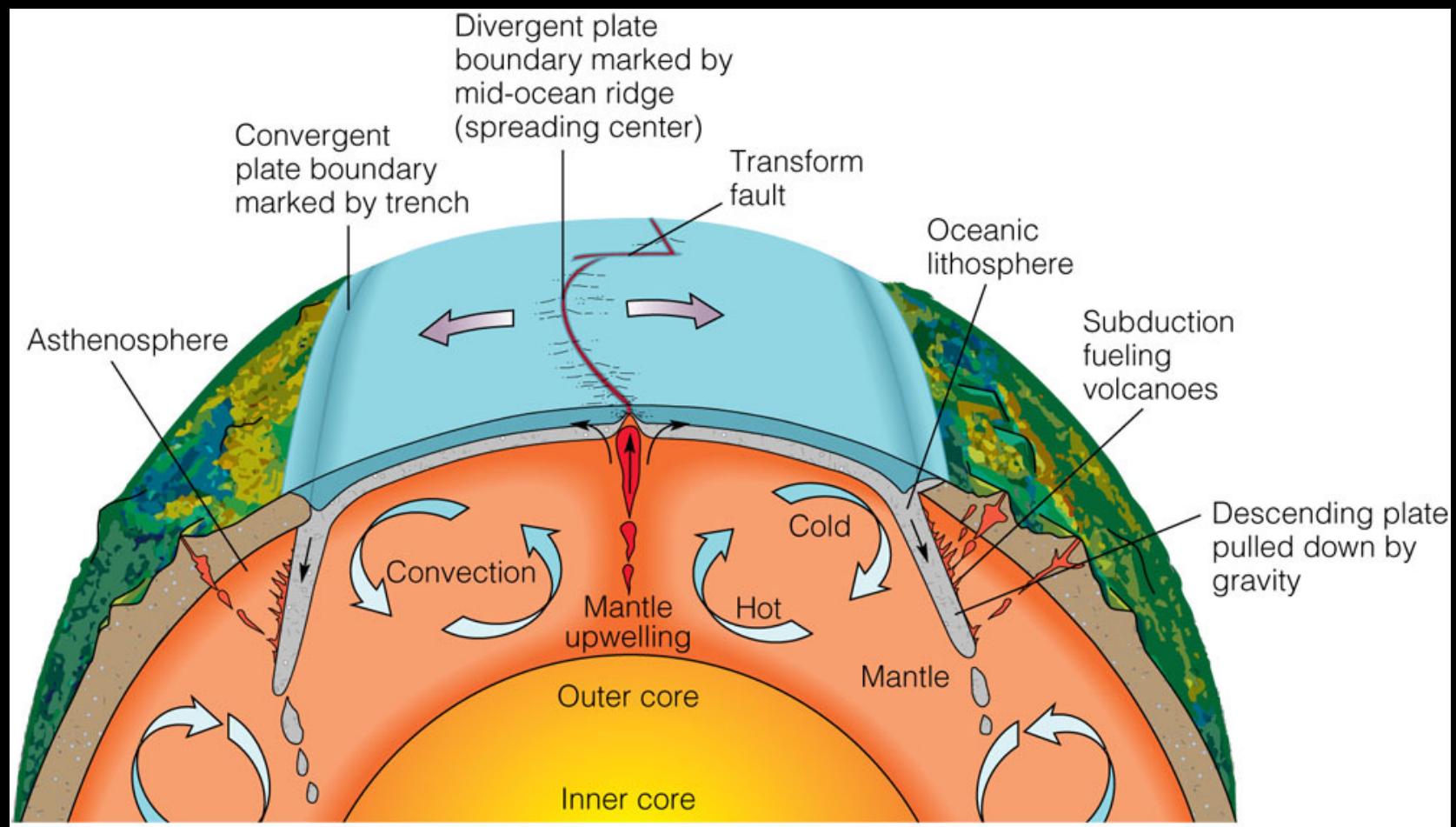
Muller, R.D., M. Sdrolias, C. Gaina, and W.R. Roest 2008. Age, spreading rates and spreading symmetry of the world's ocean crust, *Geochem. Geophys. Geosyst.*, 9, Q04006, doi:10.1029/2007GC001743.



# Mid-Oceanic Ridge Systems and Sea Floor Spreading



© 2006 Brooks/Cole - Thomson



km  
6  
4  
2  
0  
-2  
-4  
-6

America

Africa

500 Km

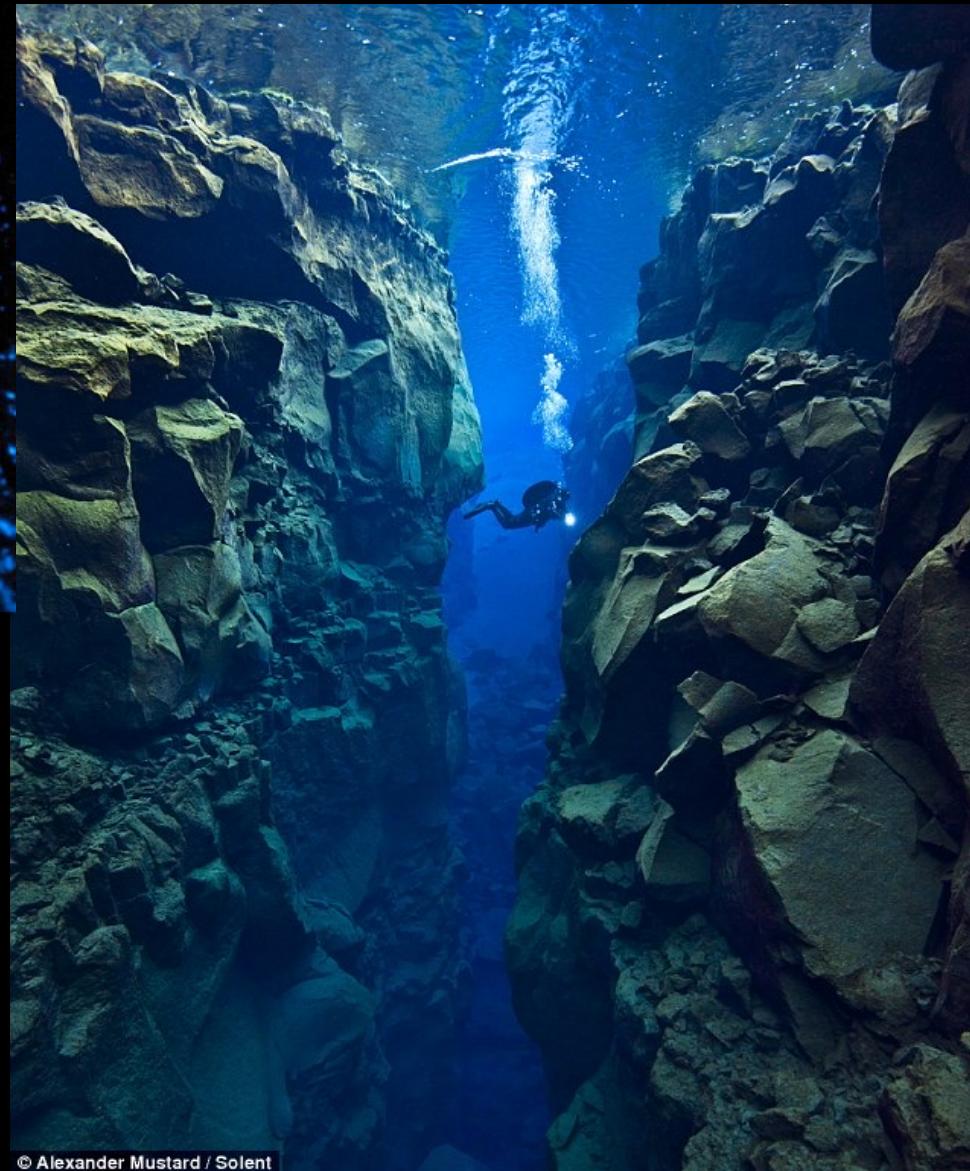


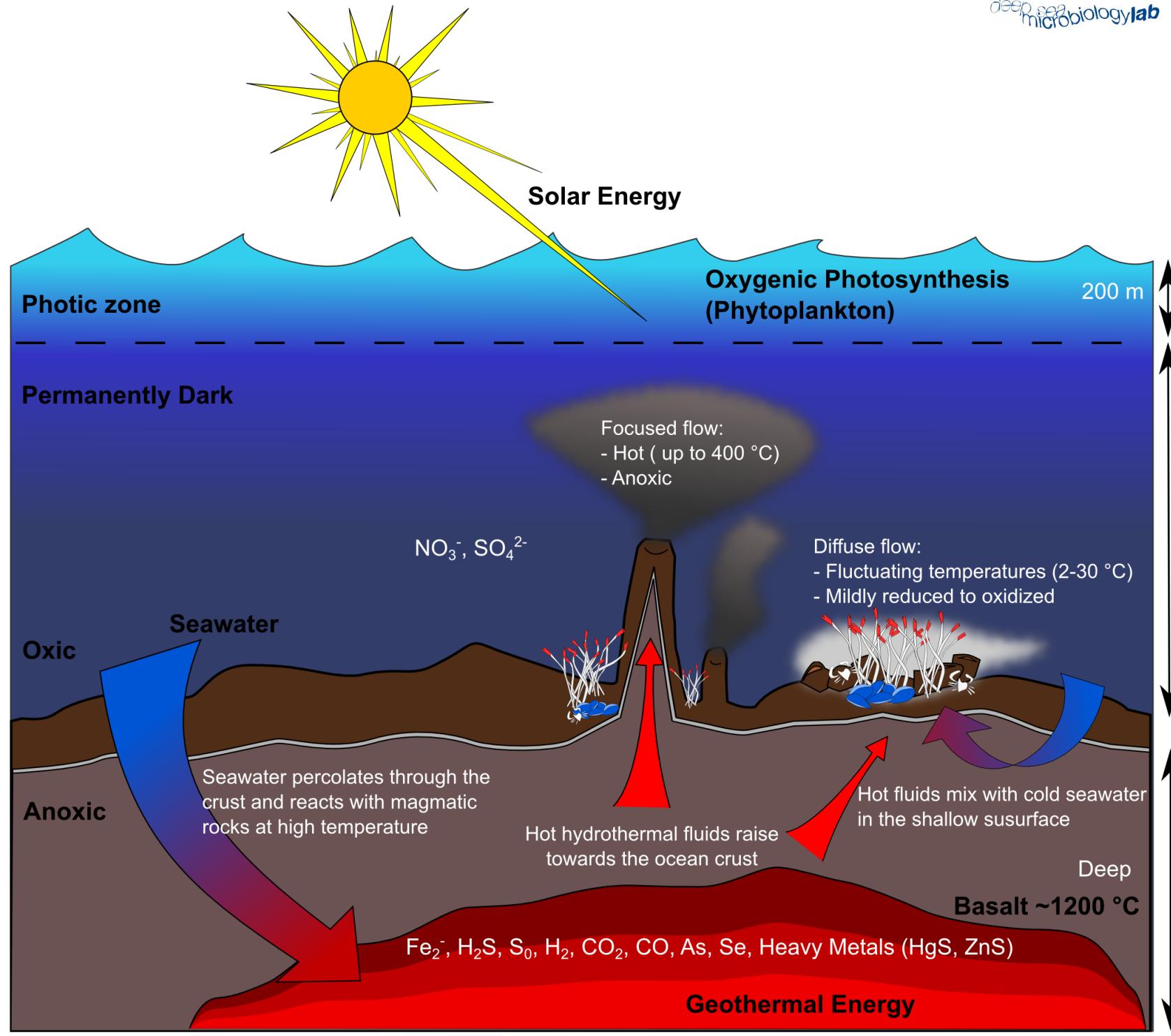
Rapid cooling of lava causes cracking of the seafloor along mid-oceanic ridges



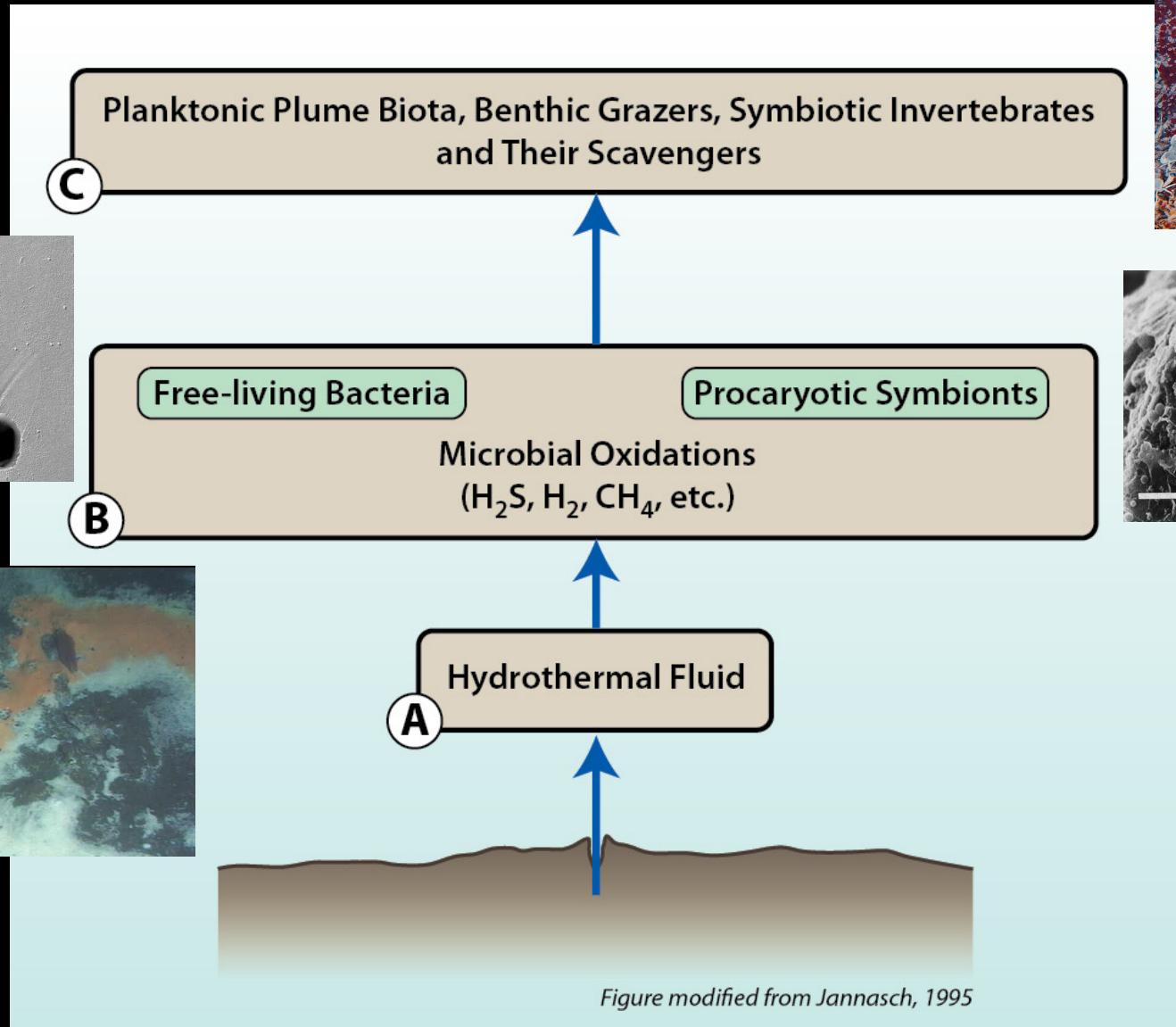
East Pacific Rise, 9° 50' N, 104° 17' W - depth 2,500 m

Mid-Atlantic Ridge between the Eurasian and North American plates in Iceland - bottom depth 25 m





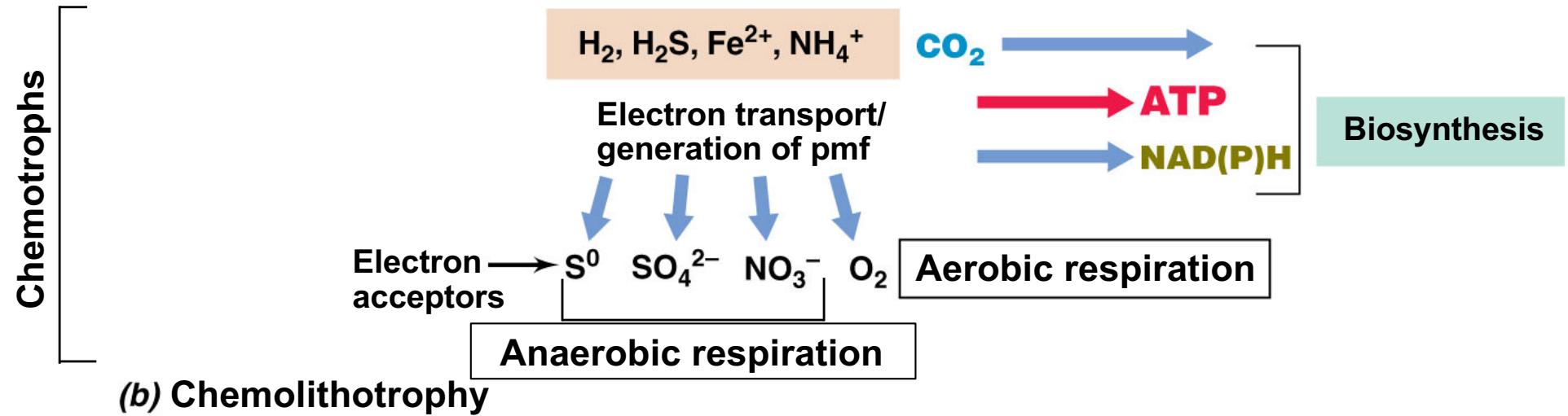
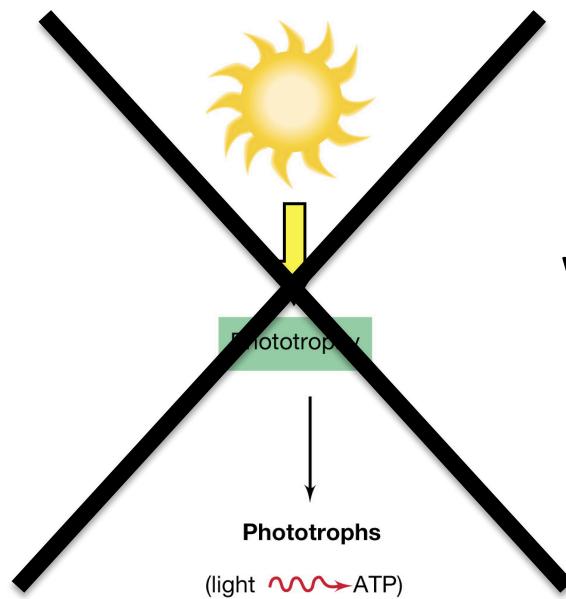
# Chemosynthetic microbes are the primary producers at deep-sea vents



Microorganisms mediate the transfer of energy from the geothermal source to the higher trophic levels

# Why do hydrothermal vents matter?

- Estimates of the amount of hydrothermal fluids flowing from these vents indicate that **the entire volume of the oceans could seep through the oceanic crust in about 10 million years.**
- Hydrothermal vents act as natural plumbing systems that transport heat and chemicals from the interior of the Earth to the lithosphere, hydrosphere, and biosphere. **Hydrothermal circulation influence the composition of the oceanic crust and help regulate global ocean chemistry.**
- Vents support complex organisms that have developed unique biochemical adaptations to high temperatures and environmental conditions we would consider toxic. **Learning about these organisms can teach us about the evolution of life on Earth.**

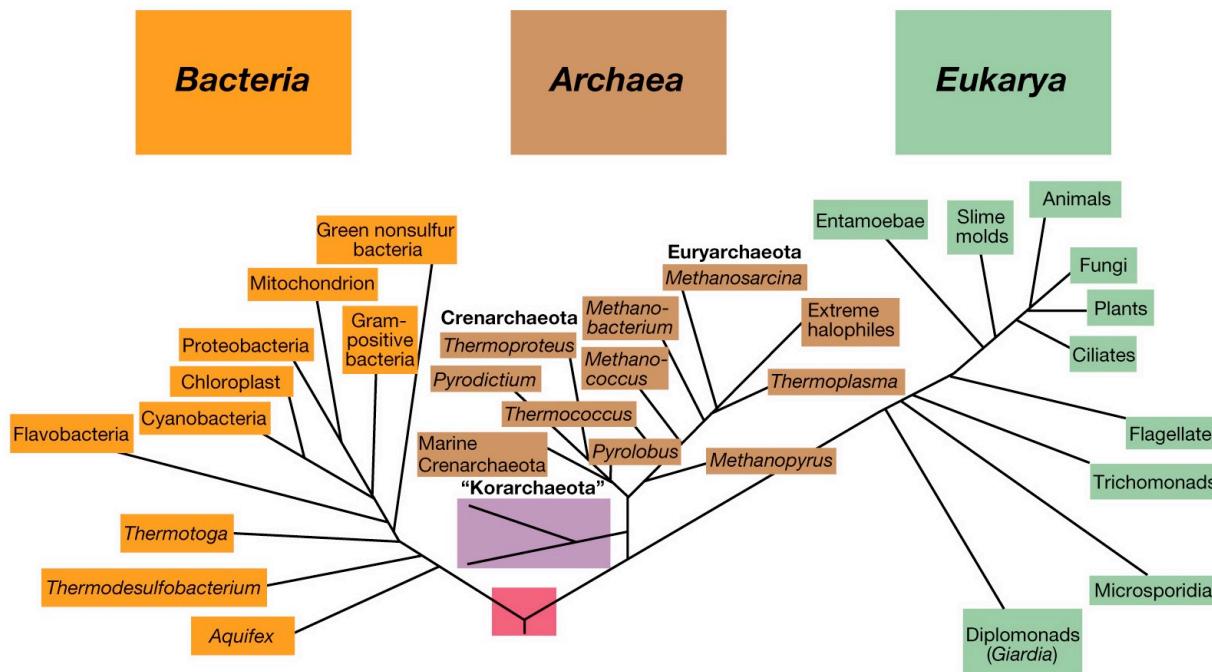


# Outline

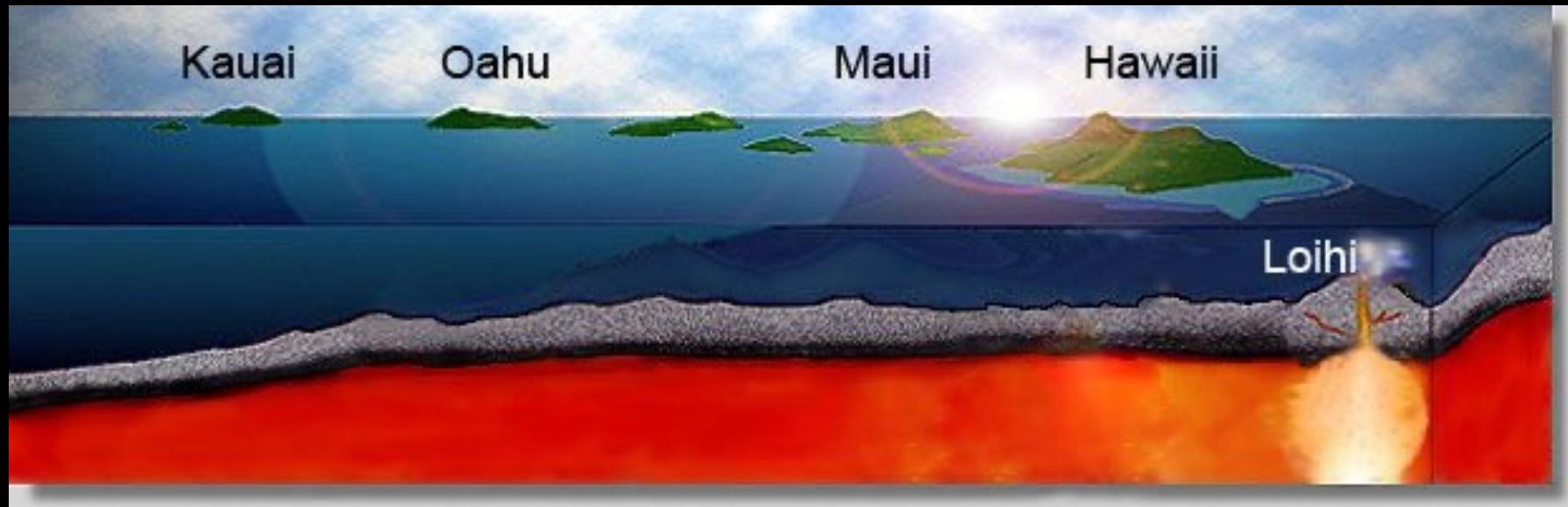
- Geological and chemical characteristics of deep-sea vents
- Life without light: Chemosynthetically sustained ecosystems
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# Which microorganisms are there?

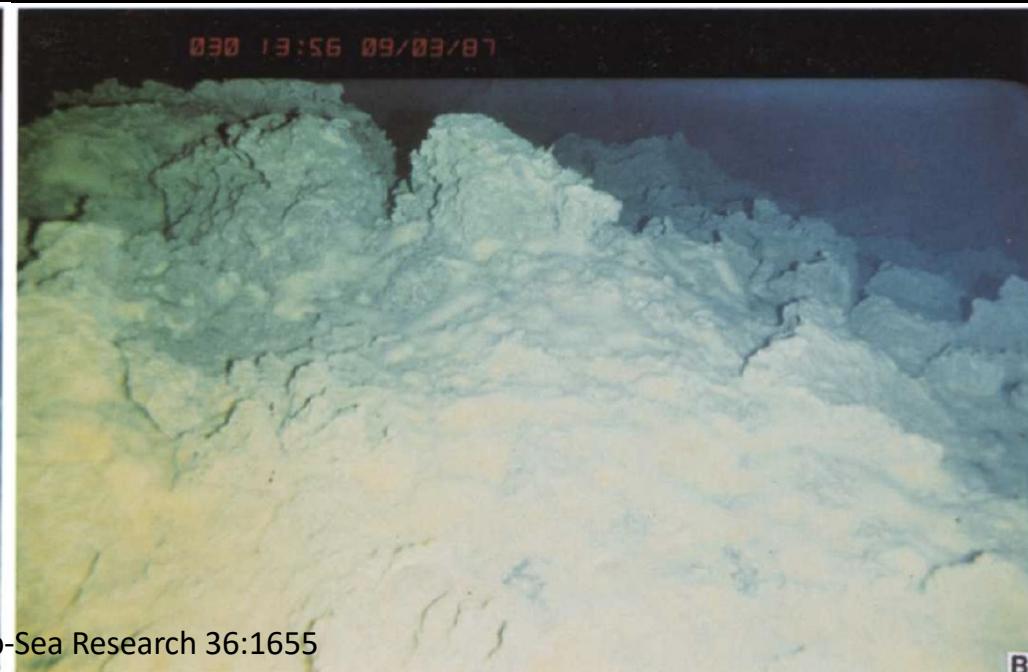
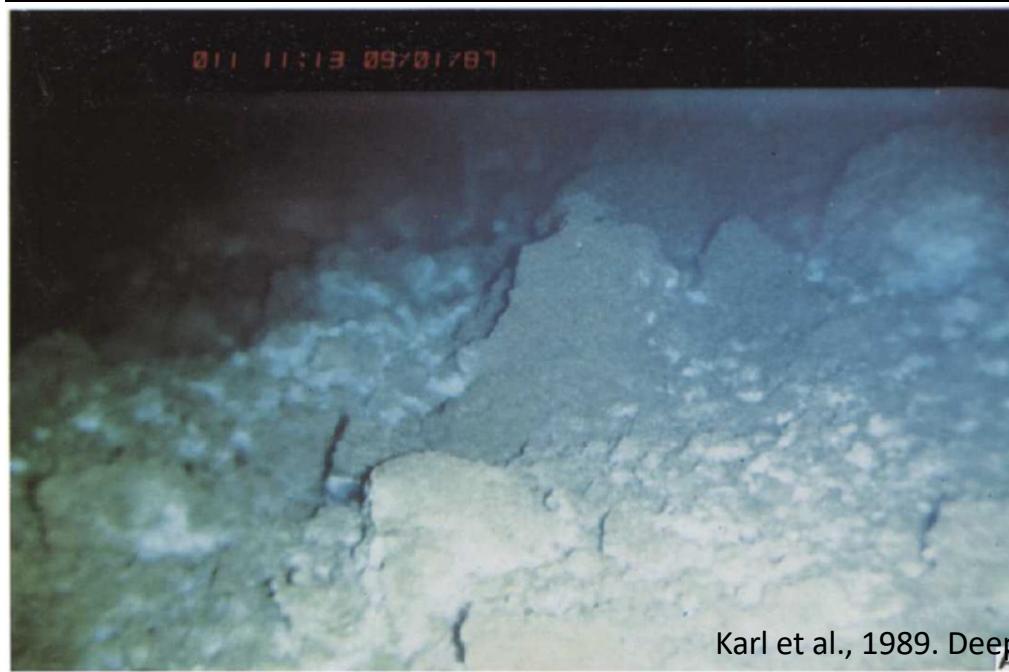
- Enrichment and isolation of pure cultures
  - Reveals the physiological and metabolic characteristics of microorganisms
  - Does not describe the true diversity of the microbial community
- Culture-independent analysis based on the 16S rRNA gene
  - Reveals the diversity of the microorganisms in the community
  - Does not reveal the physiological and metabolic characteristics of microorganisms



Pioneering work by Dave Karl and Craig Moyer at Pele Vent, Loihi seamount



Sulfur and iron oxidizing microbial mats at Pele Vent



Karl et al., 1989. Deep-Sea Research 36:1655

B

In 1995 Moyer et al. carried out a 16S rRNA gene-based investigation of vent microbial communities, revealing for the first time the importance of the Epsilonproteobacteria in marine geothermal environments

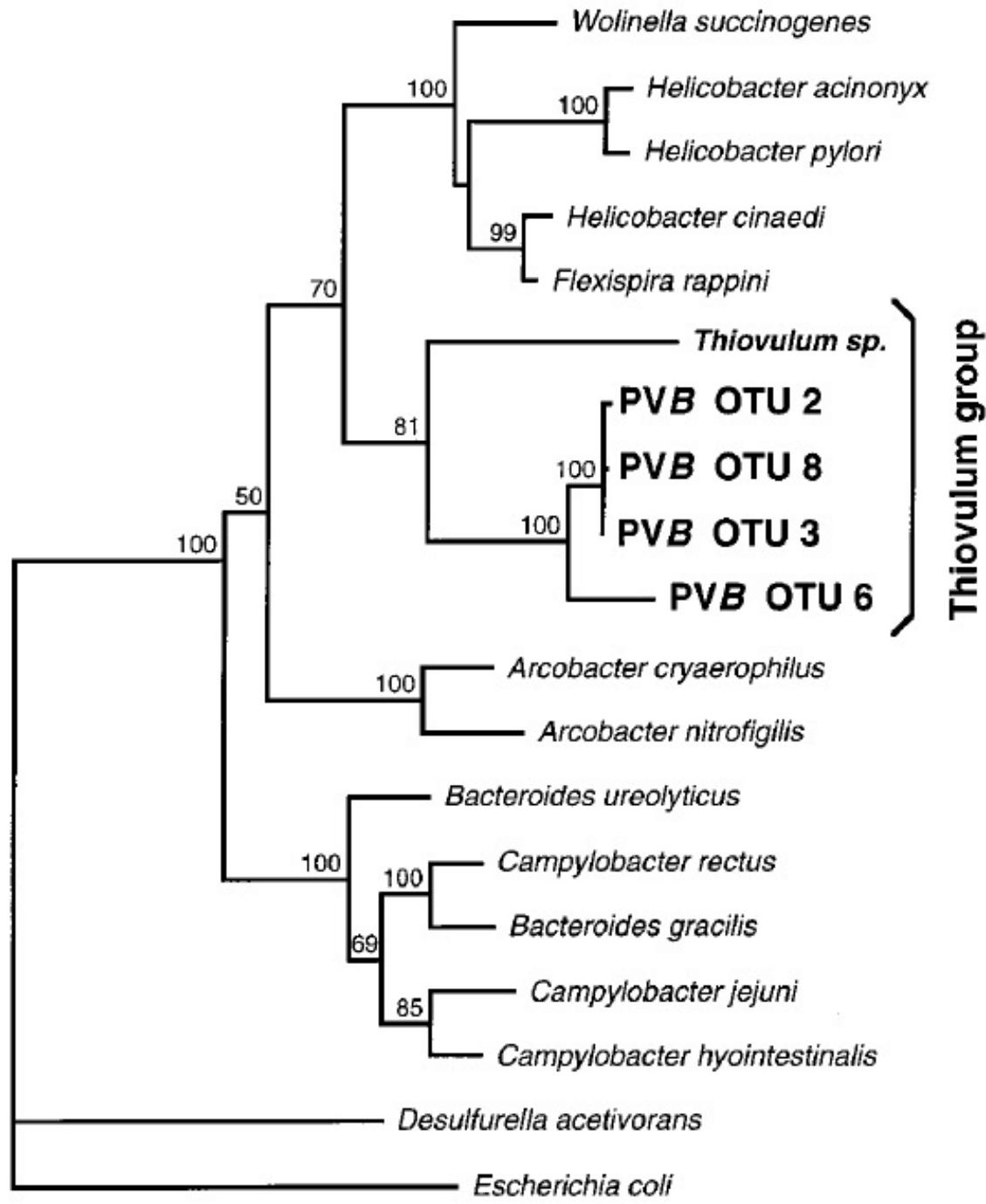
Moderate temperature (15-30°C) microbial mats

Amplification and cloning of 16S rRNA genes

**48 total 16S rRNA sequences were recovered**

**48% of these sequences were affiliated with the Epsilonproteobacteria**

Work by Polz and Cavanaugh (1995) and Haddad et al. (1995) on the episymbionts of *Rimicaris* and *Alvinella*, respectively, were also recognizing the importance of Epsilonproteobacteria at deep-sea vents



# In 1995, what could we infer about the physiology of vent Epsilonproteobacteria from related species?

- Many heterotrophic Epsilonproteobacteria, including human pathogen, were well known, but too distant from the vent strains
- In 1978 Wirsén and Jannasch established that enrichments of *Thiovulum* sp. grew at the interphase of H<sub>2</sub>S and O<sub>2</sub> and provided evidence that the bacterium was a chemoautotroph
- In 2000 Gevertz et al. isolated and described the *first* chemoautotrophic isolates of Epsilonproteobacteria (from an oil field brine). These bacteria were microaerophiles that used H<sub>2</sub>, H<sub>2</sub>S and S<sup>0</sup> as electron donors
- However, over time, the work of several laboratories that dedicated time to the isolation of these bacteria provided much needed information on their physiology

# Physiology: Characteristics of *Epsilonproteobacteria* isolated from deep-sea vents

	Isolation site	Optimum T (°C)	Electron donor(s)	Electron acceptor(s)	End product of nitrate respiration	Carbon source	Reference
<b><i>Epsilonproteobacteria</i></b>							
<i>Sulfurovum lithotrophicum</i>	MOT, Iheya, sediments	28–30	$\text{S}_2\text{O}_3^{\pm}, \text{S}^0$	$\text{NO}_3^-$ , $\text{O}_2$	$\text{N}_2$	$\text{CO}_2$	Inagaki et al. (2004)
<i>Sulfurimonas paralvinellae</i>	MOT, Iheya, <i>Paralvinella</i>	30	$\text{H}_2, \text{S}_2\text{O}_3^{\pm}, \text{S}^0$	$\text{NO}_3^-$ , $\text{O}_2$	$\text{N}_2$	$\text{CO}_2$	Takai et al. (2006b)
<i>Sulfurimonas autotrophica</i>	MOT, Hatoma Knoll, sediments	25	$\text{S}_2\text{O}_3^{\pm}, \text{S}^0, \text{H}_2\text{S}$	$\text{O}_2$		$\text{CO}_2$	Inagaki et al. (2003)
<i>Thioreductor micantisoli</i>	MOT, Iheya, sediments	32	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$	$\text{NH}_4^+$	$\text{CO}_2$	Nakagawa et al. (2005a)
<i>Nautilia lithotrophica</i>	EPR, 13°N, <i>Alvinella</i>	53	$\text{H}_2$ , Formate	$\text{S}^0$		$\text{CO}_2$ , Formate	Miroshnichenko et al. (2002)
<i>Nautilia nitratireducens</i>	EPR, 9°N, chimney	55	$\text{H}_2$ , Formate, acetate, complex organic substrates	$\text{NO}_3^-$ , $\text{S}^0$ , $\text{S}_2\text{O}_3^{\pm}$ , $\text{SeO}_4^{2-}$	$\text{NH}_4^+$	$\text{CO}_2$ , Formate	Pérez-Rodríguez et al. (2009)
<i>Nautilia profundicola</i>	EPR, 9°N, <i>Alvinella</i>	40	$\text{H}_2$ , Formate	$\text{S}^0$		$\text{CO}_2$ , Formate	Smith et al. (2008)
<i>Nautili abyssi</i>	EPR, 13°N, chimney	60	$\text{H}_2$	$\text{S}^0$		$\text{CO}_2$ , Yeast Extract, Peptone	Alain et al. (2009)
<i>Hydrogenimonas thermophila</i>	CIR, Kairei Field, colonizer	55	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$ , $\text{O}_2$	$\text{NH}_4^+$	$\text{CO}_2$	Takai et al. (2004c)
<i>Nitratiruptor tergarcus</i>	MOT, Iheya, chimney	55	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$ , $\text{O}_2$	$\text{N}_2$	$\text{CO}_2$	Nakagawa et al. (2005b)
<i>Nitratifractor salsuginis</i>	MOT, Iheya, chimney	37	$\text{H}_2$	$\text{NO}_3^-$ , $\text{O}_2$	$\text{N}_2$	$\text{CO}_2$	Nakagawa et al. (2005)
<i>Caminibacter profundus</i>	MAR, Rainbow, vent cap	55	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$ , $\text{O}_2$	$\text{NH}_4^+$	$\text{CO}_2$	Miroshnichenko et al. (2004)
<i>Caminibacter mediatlanticus</i>	MAR, Rainbow, chimney	55	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$	$\text{NH}_4^+$	$\text{CO}_2$	Voordeckers et al. (2005)
<i>Caminibacter hydrogenophilus</i>	EPR, 13°N, <i>Alvinella</i>	60	$\text{H}_2$	$\text{NO}_3^-$ , $\text{S}^0$	$\text{NH}_4^+$	$\text{CO}_2$ , complex organic substrates	Alain et al. (2002)
<i>Lebetimonas acidiphila</i>	Mariana Arc, colonizer	50	$\text{H}_2$	$\text{S}^0$		$\text{CO}_2$	Takai et al. (2005)

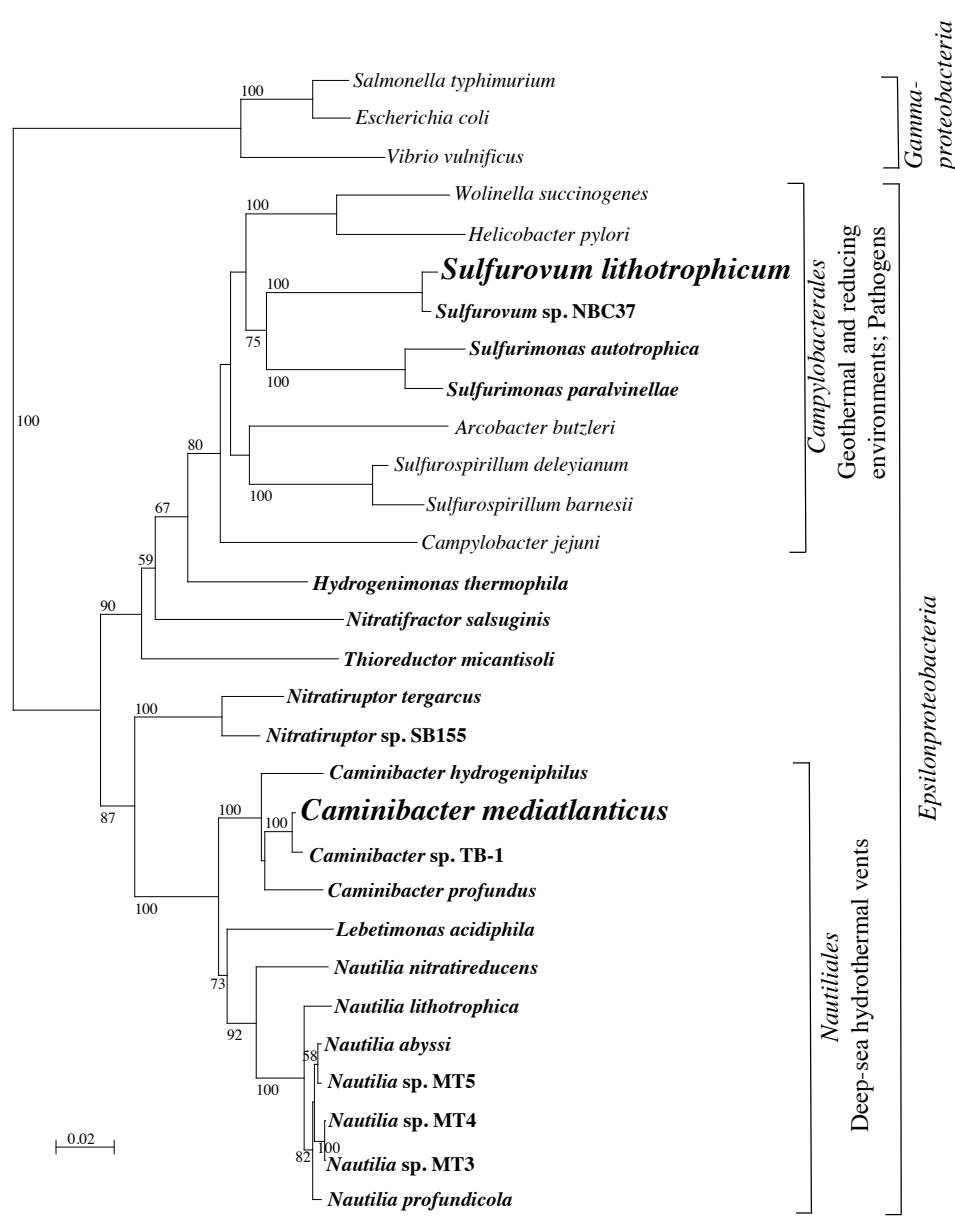
# *Epsilonproteobacteria* 101

*Epsilonproteobacteria* are one of the most abundant groups of microorganisms at deep-sea hydrothermal vents – possibly the most abundant.

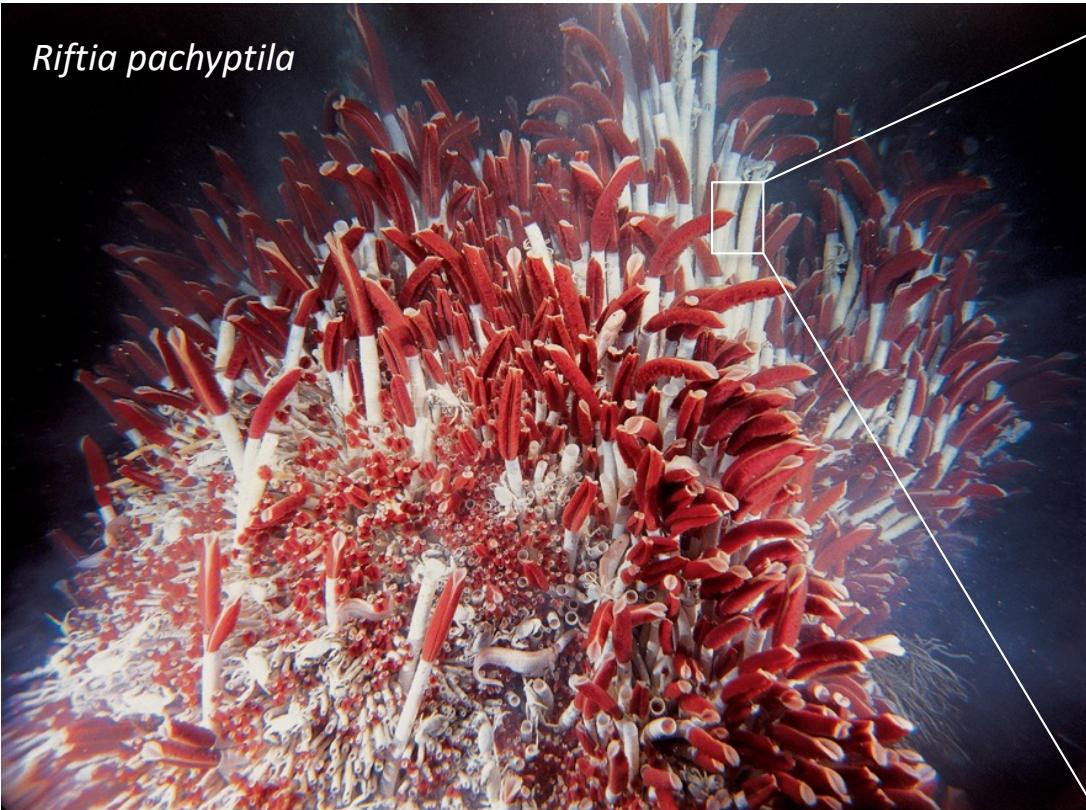
*Epsilonproteobacteria* are the pioneer colonizers of newly formed diffuse flow vents

*Epsilonproteobacteria* include organisms that live in geothermal environments as well as human pathogens

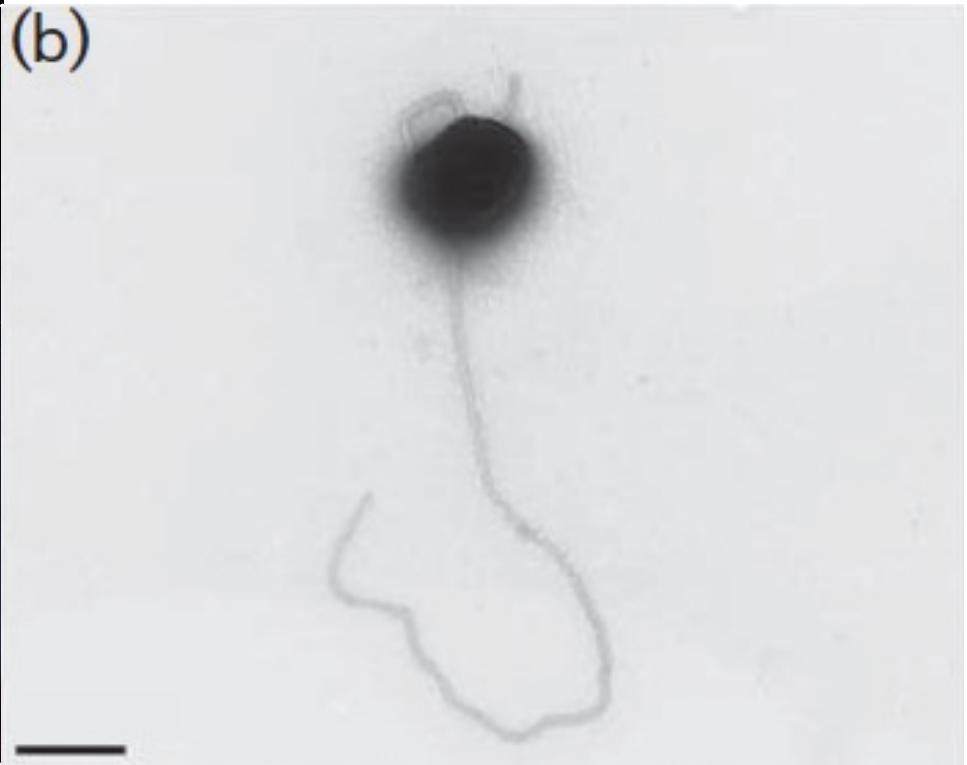
The deepest lineage within the *Epsilonproteobacteria* is represented by the *Nautiliales*, which include obligate anaerobes isolated from deep-sea hydrothermal vents. The other species from either vent or non-vent environments emerged later in evolution, along with the emergence of host-associated species.



# *Sulfurovum riftiae* DSM 101780<sup>T</sup>



(b)



*Epsilonproteobacterium*

$T_{opt.}$  35°C,  $t_g$ : 180 min

Gram negative

Anaerobic

Obligate chemolithoautotroph

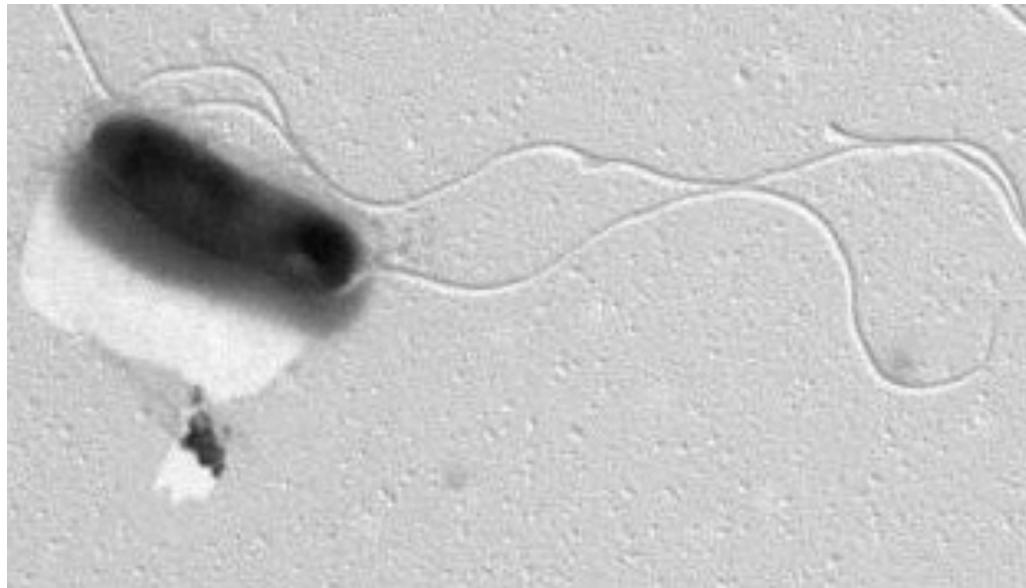
Electron donor: Thiosulfate, S<sup>0</sup>

Electron acceptors: NO<sub>3</sub><sup>-</sup>; reduced to N<sub>2</sub>

deep sea  
microbiology lab

Culture Collection

# *Caminibacter mediatlanticus* DSM 16658<sup>T</sup>



*Epsilonproteobacterium*

T<sub>opt.</sub> 55°C

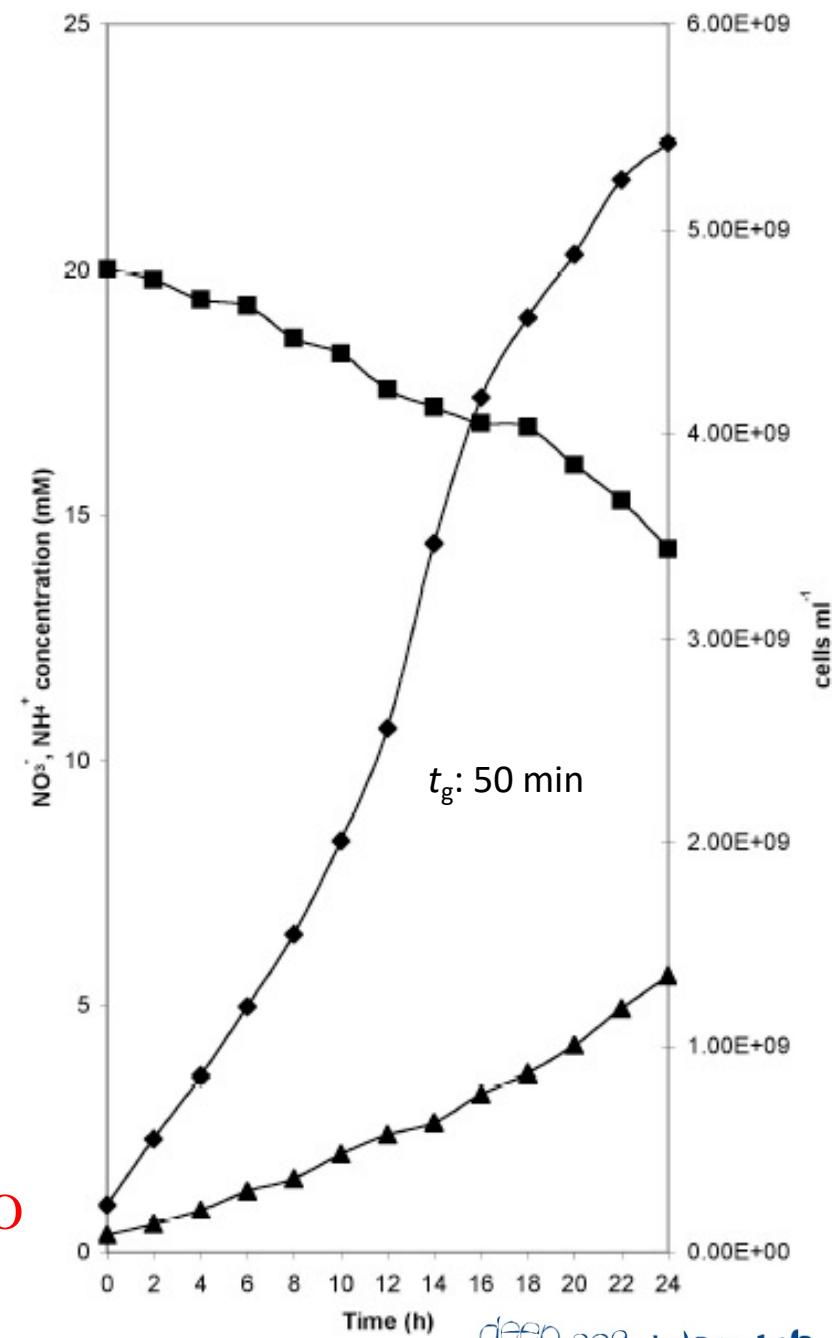
Gram negative

Strictly anaerobic

Obligate chemolithoautotroph

Electron donor: H<sub>2</sub>

Electron acceptors: NO<sub>3</sub><sup>-</sup>; reduced to NH<sub>4</sub><sup>+</sup>  
S<sup>0</sup>; reduced to H<sub>2</sub>S



# Genome analysis: Reconstruction of carbon and energy metabolism in vent *Epsilonproteobacteria*

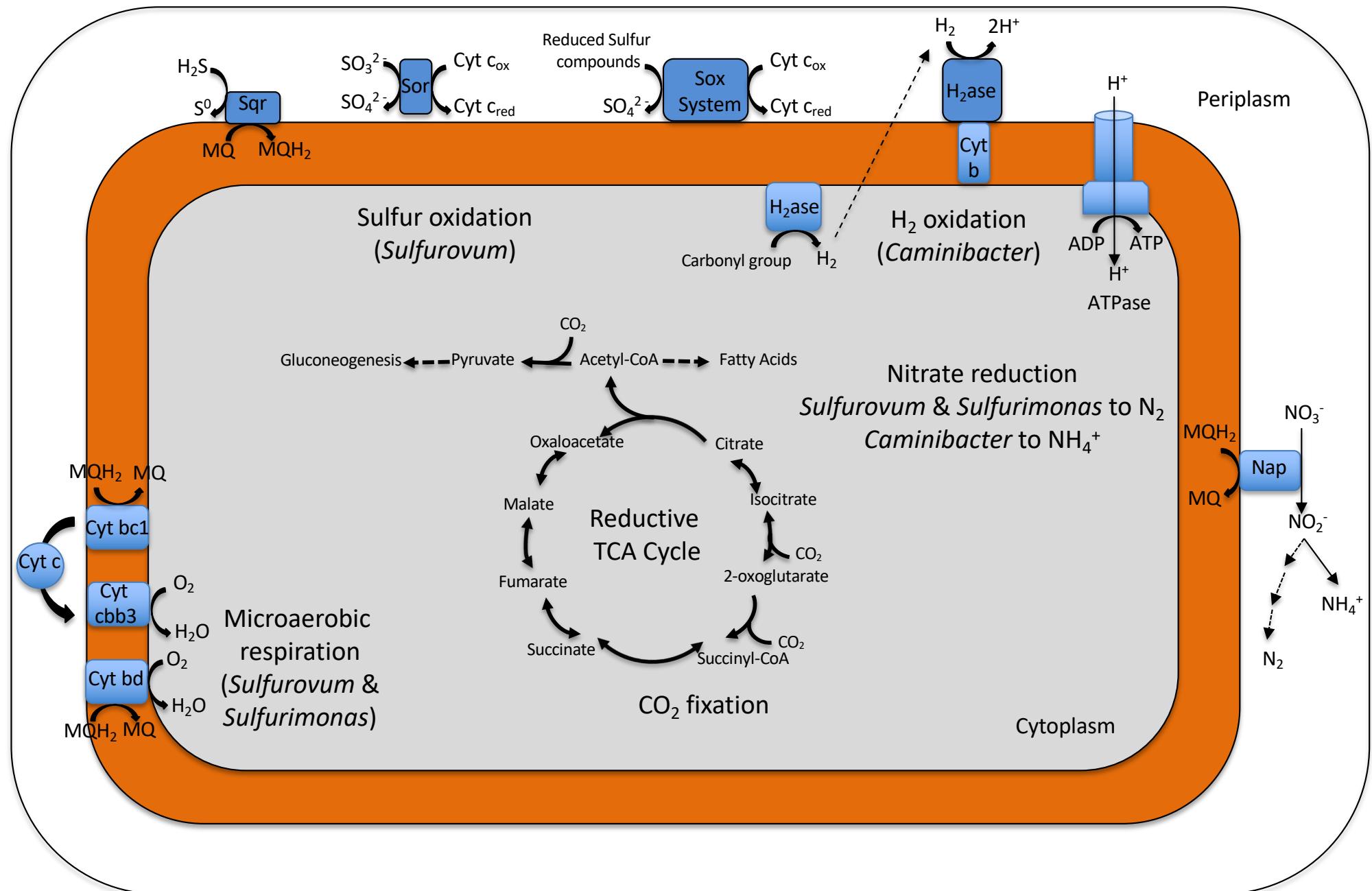


Figure created with data from Nakagawa et al., 2007, PNAS 104:12146-12150 and Giovannelli et al., 2011, S/IS 5:135-143

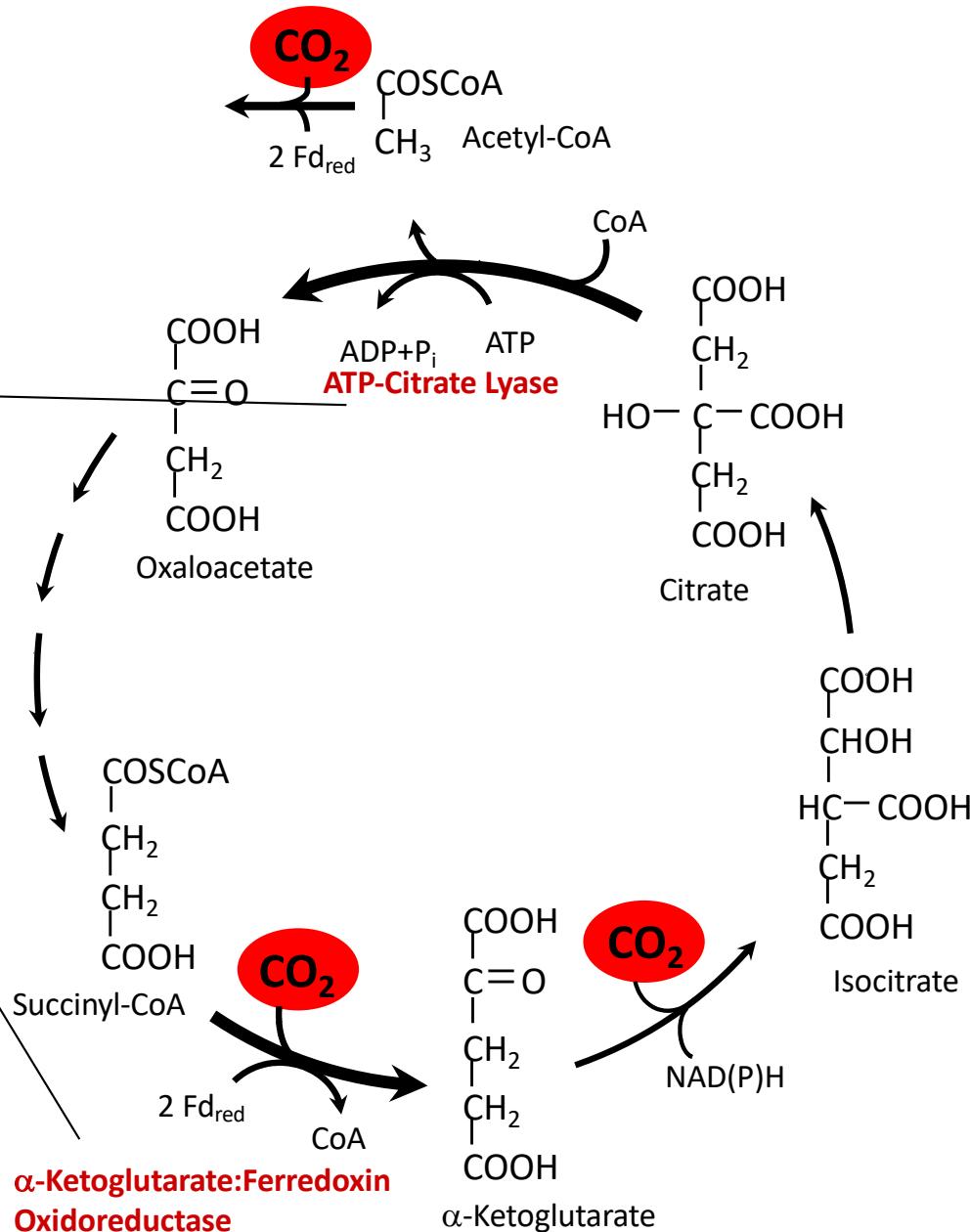
# Metabolism:

## $\text{CO}_2$ Fixation in *Caminibacter mediatlanticus* occurs via the Reverse TCA Cycle

**Table 2** Specific activities [nmol min<sup>-1</sup> (mg cell protein)<sup>-1</sup>] of enzymes of the reductive TCA cycle in *C. mediatlanticus*

Enzyme activity tested	<i>Caminibacter mediatlanticus</i>
Assay temperature (°C)	55
ATP citrate lyase	275
2-Oxoglutarate:BV oxidoreductase	330
Pyruvate:BV oxidoreductase	160
Fumarate reductase (BV)	710
Isocitrate dehydrogenase (NAD)	45
Isocitrate dehydrogenase (NADP)	7,800
Malate dehydrogenase (NADH)	4,080
2-Oxoglutarate dehydrogenase (NAD)	n.d.
2-Oxoglutarate dehydrogenase (NADP)	n.d.
Pyruvate dehydrogenase (NAD)	n.d.
Pyruvate dehydrogenase (NADP)	n.d.

Mean values were obtained from at least five measurements. Standard errors were less than  $\pm 20\%$ . n.d., no activity detected, detection limit  $<1 \text{ nmol min}^{-1} (\text{mg cell protein})^{-1}$



Courtesy of M. Hügler

# “Next-Generation Sequencing” Technologies

At the same time, next generation sequencing platforms were becoming available to environmental microbiologists

Such platforms allow for parallel sequencing of DNA molecules, providing a number of sequences several orders of magnitude higher than 1<sup>st</sup> generation sequencers

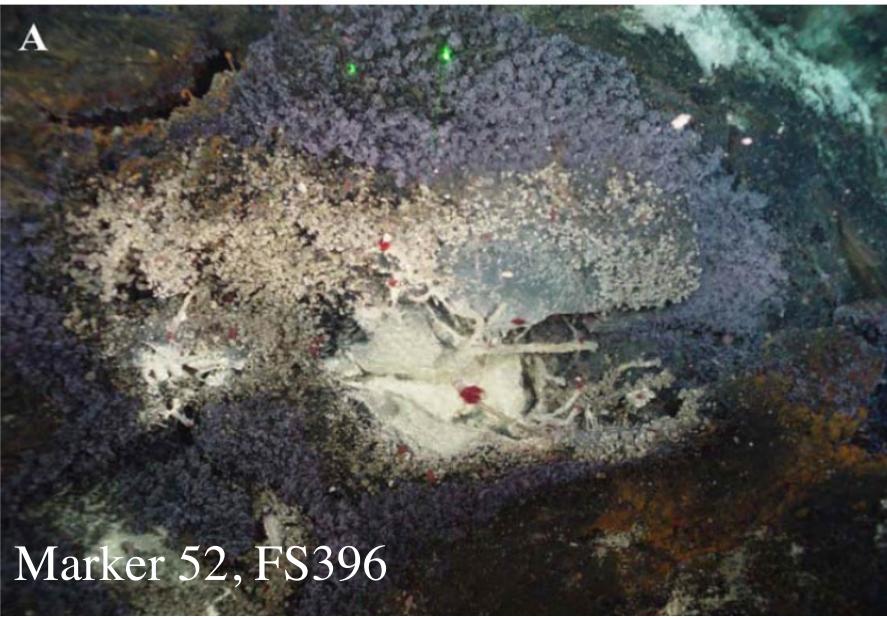
2<sup>nd</sup> generation: Roche 454



4<sup>th</sup> generation: Ion Torrent



Juan de Fuca Ridge



Mid-Atlantic Ridge

150°W 140°W 130°W 120°W 110°W 100°W 90°W 80°W 70°W 60°W 50°W 40°W 30°W

30°N

20°N

10°N

0°

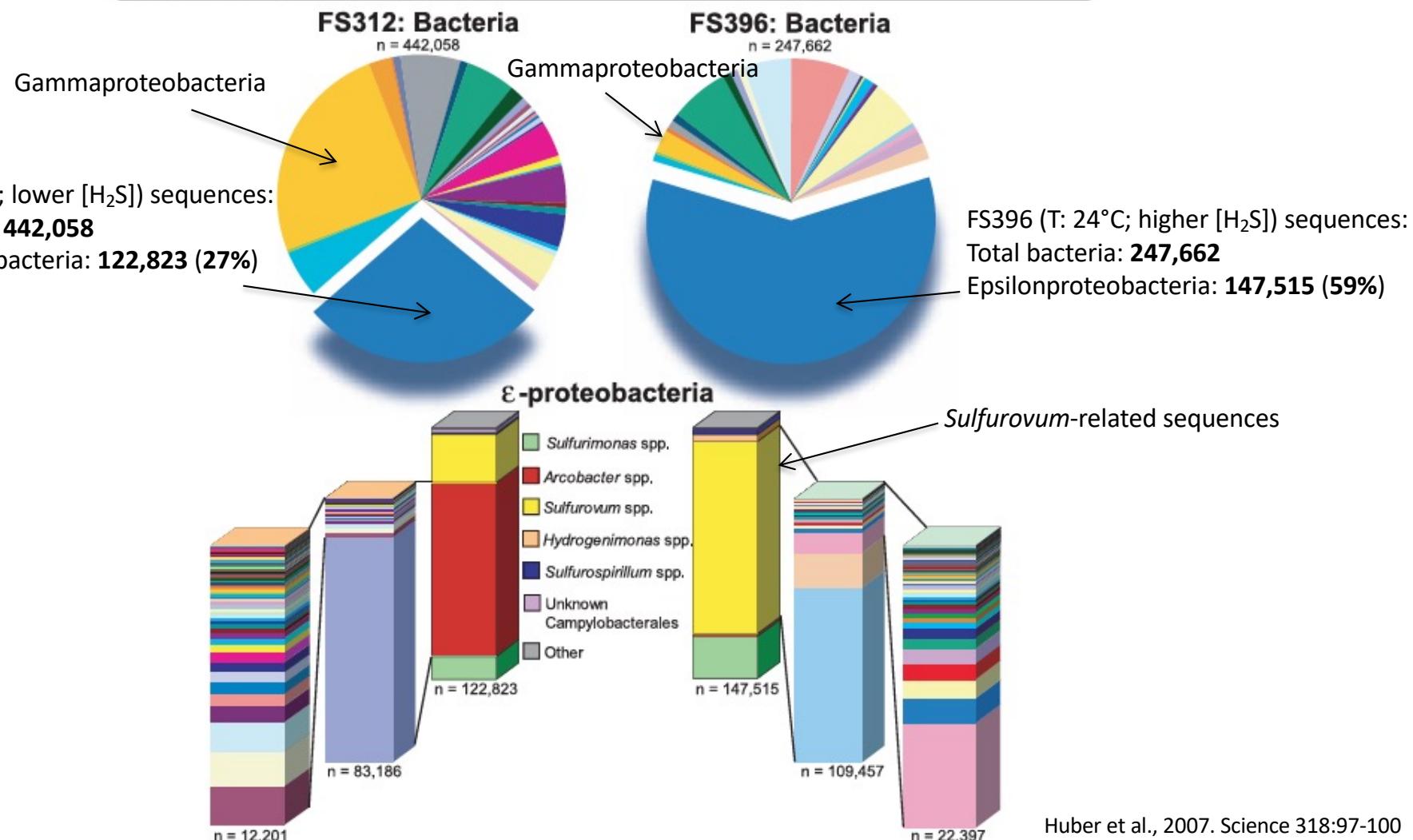
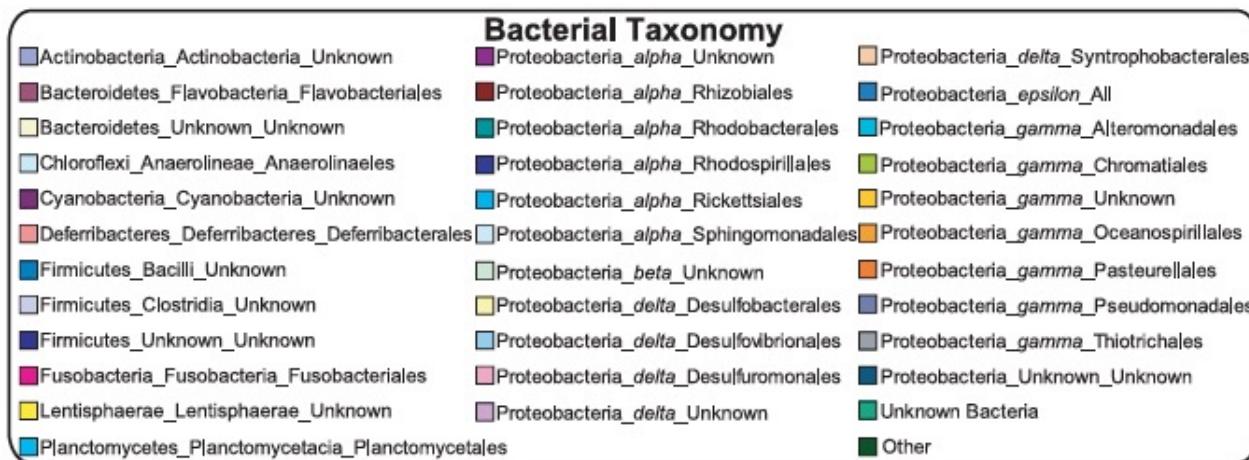
10°S

20°S

30°S

## Deep sequencing of the 16S rRNA gene from deep-sea vent microbial communities (Pyrotag sequencing)

- Collection of fluids from two active deep-sea hydrothermal vents (Fluid T: 31 and 24 °C)
- Concentration of microbial biomass from the fluid samples
- Extraction of genomic DNA from the microbial biomass
- PCR amplification of the V6 region of the 16S rRNA gene
- Deep sequencing of the V6 amplicons (454 platform, **689,720** bacterial sequences)
- Sequencing and assembly of 270,000 sequence reads
- Bioinformatic analyses and taxonomic assignment



# What about in-situ microbial function?

What are the genes expressed by the biofilm communities?

Analysis of all the transcripts in a microbial community (metatranscriptomics)

RNA is the starting material that gets analyzed. We obtain information about the genes expressed by the microbial community

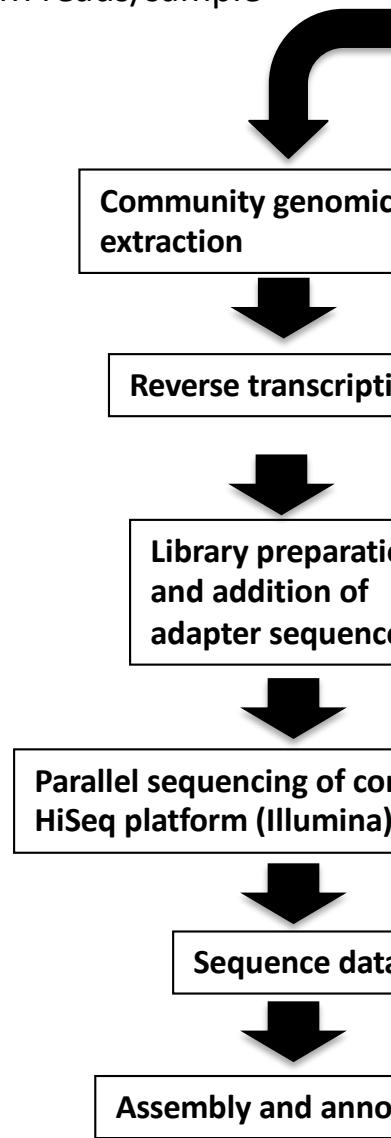
# What are the genes expressed by the microbes?

Assess the transcript composition (metatranscriptome)

Metatranscriptomics

Total community

Over 10M reads/sample



*In situ* collection and preservation of the natural community



Chemosynthetic microbial biofilms



Reverse transcription

Library preparation  
and addition of  
adapter sequences

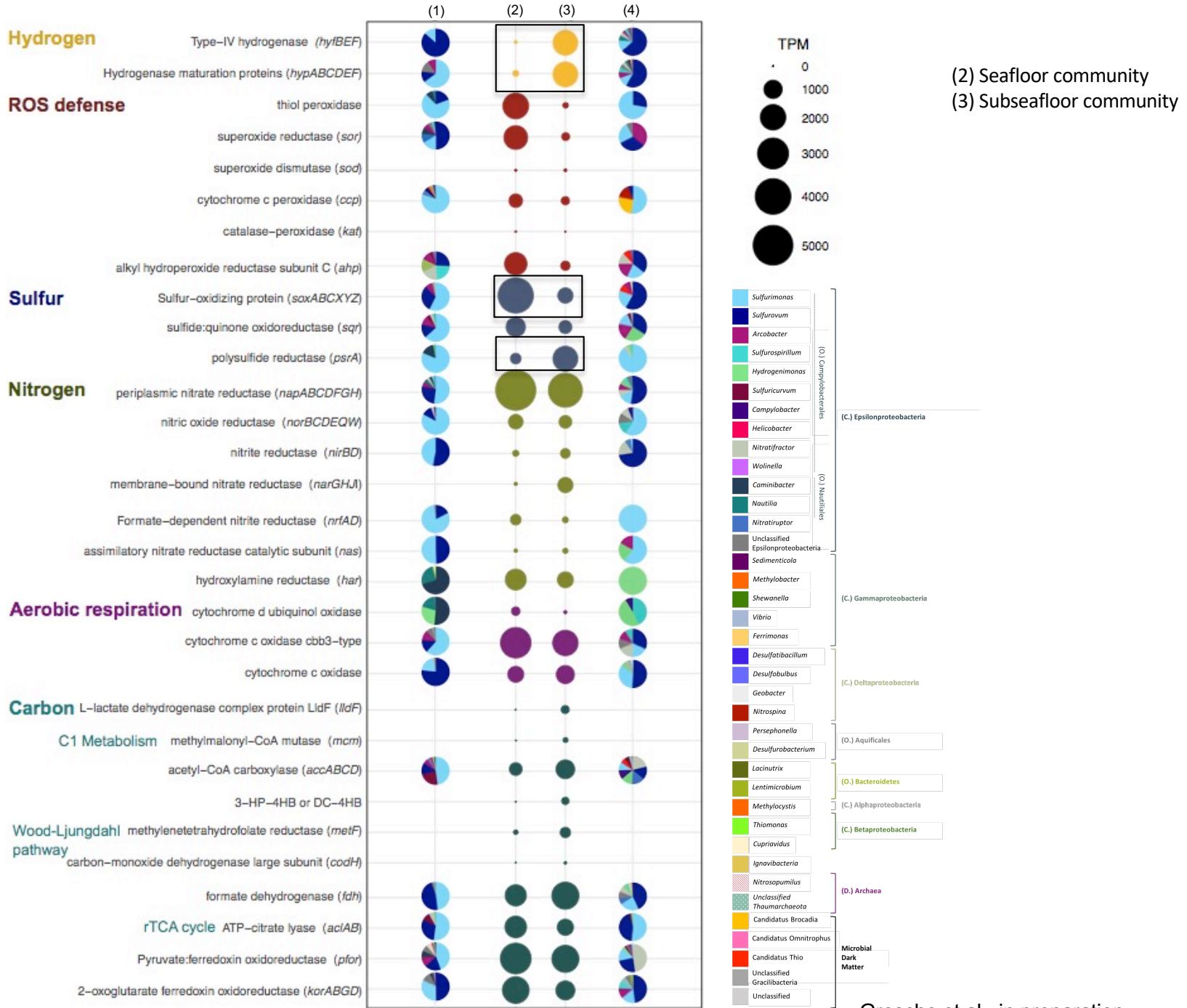
Parallel sequencing of community DNA on  
HiSeq platform (Illumina)

Sequence data

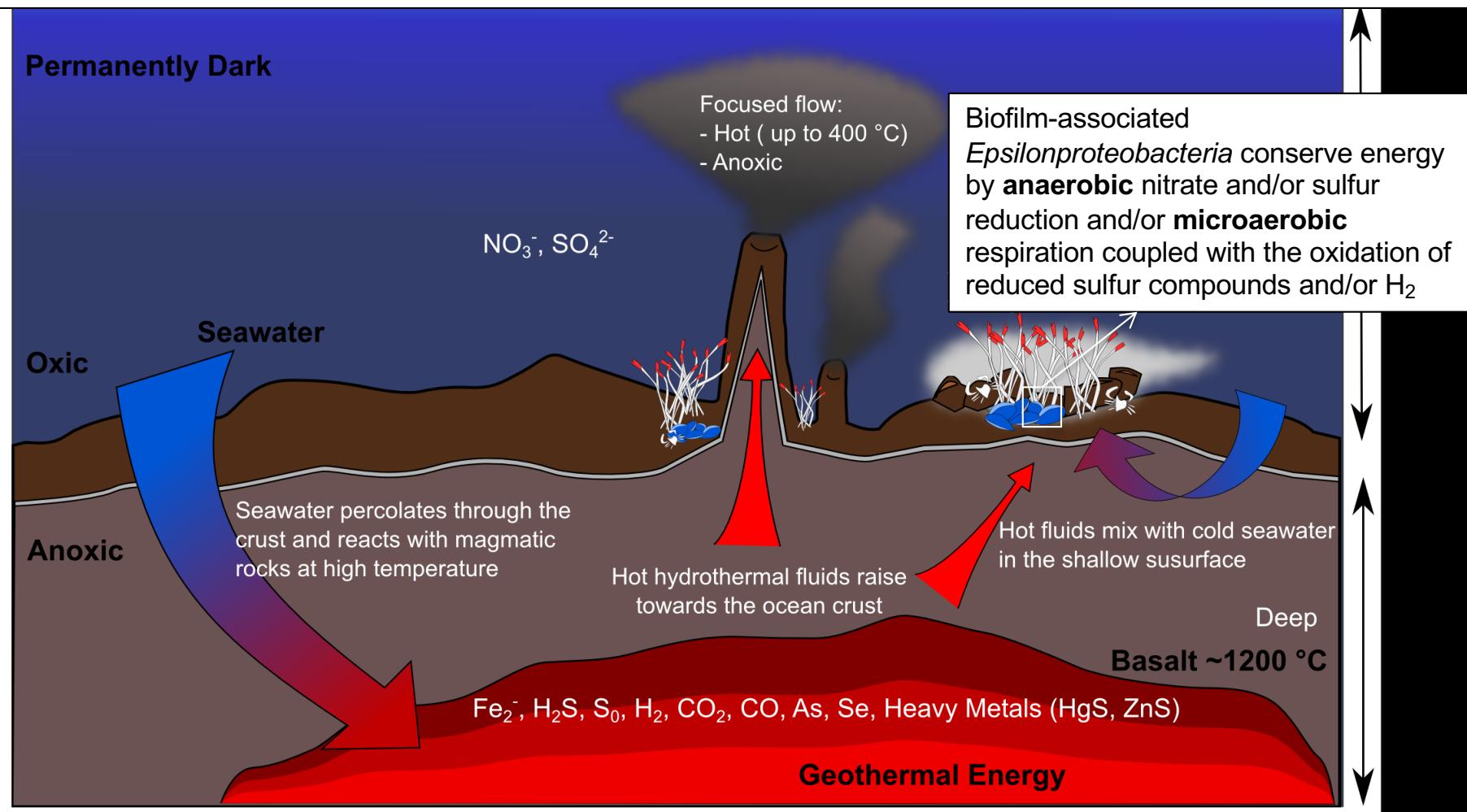
Assembly and annotation

Transcript analysis

# Metatranscriptome analysis: *In situ* gene expression of deep-sea vent seafloor and subseafloor biofilm communities



- Chemosynthetic *Epsilonproteobacteria* dominate the active fraction of the biofilm communities at deep-sea vents over a 10 – 55°C temperature range.
- Mesophilic *Epsilonproteobacteria* are more abundant in the lower temperature biofilms.
- Thermophilic *Epsilonproteobacteria* dominate the higher temperature biofilms and they are not found in the lower temperature ones.
- *Epsilonproteobacteria* fix CO<sub>2</sub> *in situ* via the reductive TCA cycle. Measurements of rates of carbon fixation in laboratory strains and *in situ* are consistent.



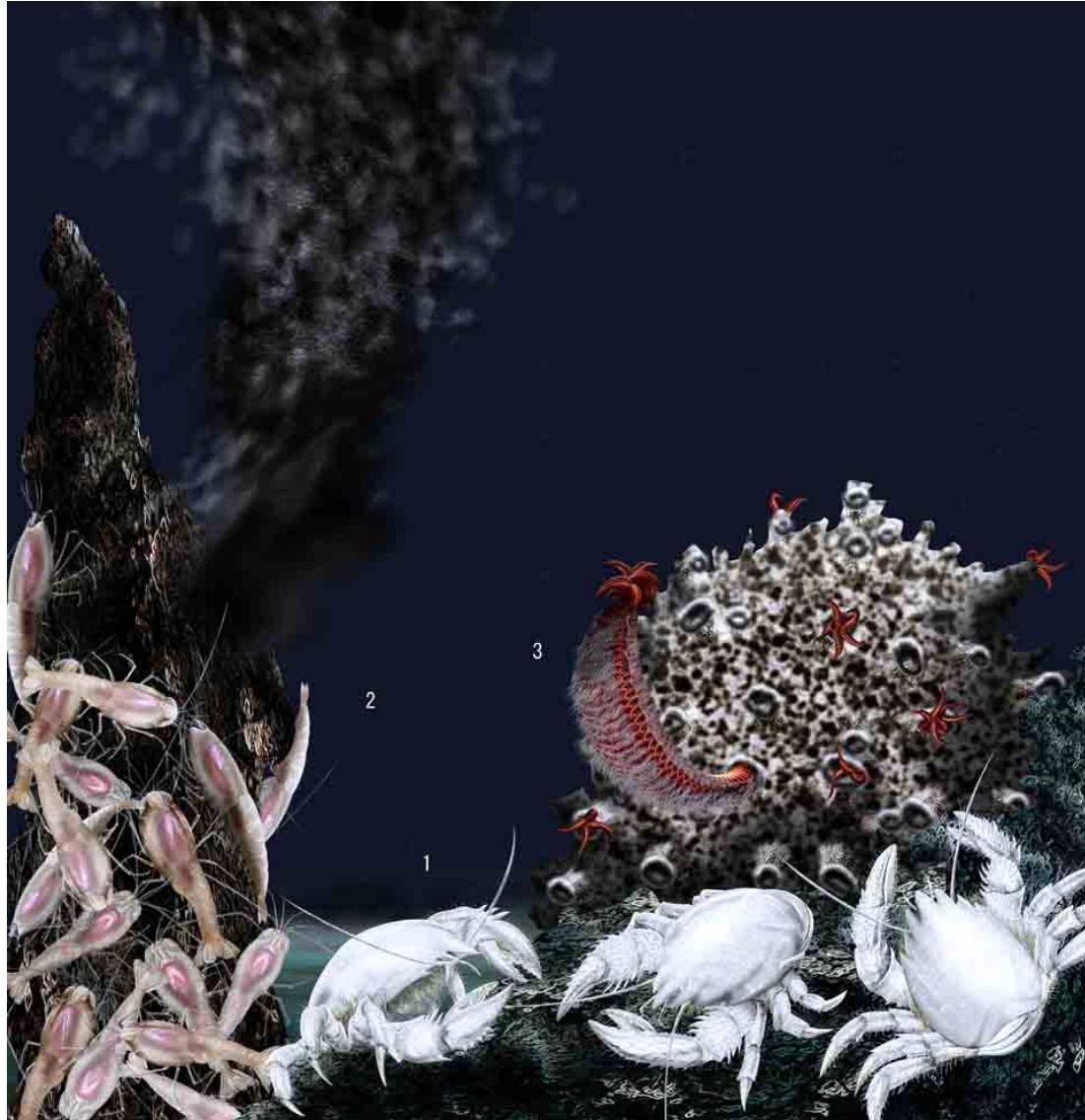
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- **Symbiosis: How the vent invertebrates make a living**
- Anaerobic thermophiles as “time capsules” to investigate early microbial metabolism and life on other planets

# “Episymbiosis”

Invertebrates with chemosynthetic ‘episymbionts’

MAR & CIR  
Shrimps  
(*Rimicaris* spp.)

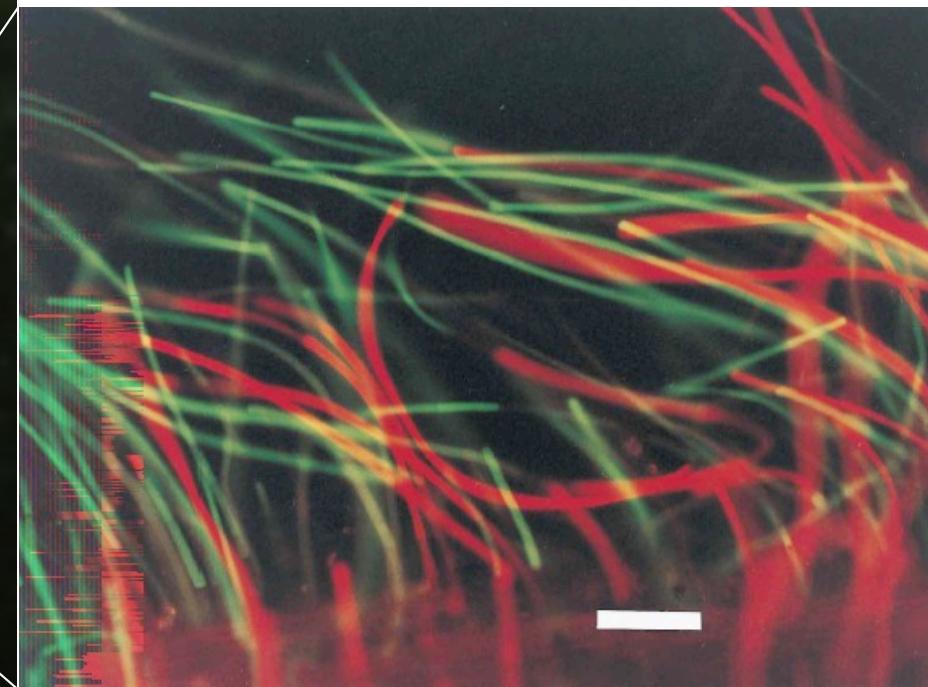


East Pacific  
Polychaetes  
(*Alvinella* spp.)

West Pacific  
Galetheids  
(*Shinkaia* spp.)

Courtesy of Ken Takai, Jamstec

## Cooperative Associations: *Alvinella pompejana* and *Epsilonproteobacteria* episymbionts



Cary et al., 1997. Appl. Environ. Microbiol. 63:1124-1130

Filamentous bacteria can provide protection from high levels of toxic metals (arsenic, cadmium, copper) and nutrient.

# What is the metabolic potential of the *Alvinella* episymbionts?

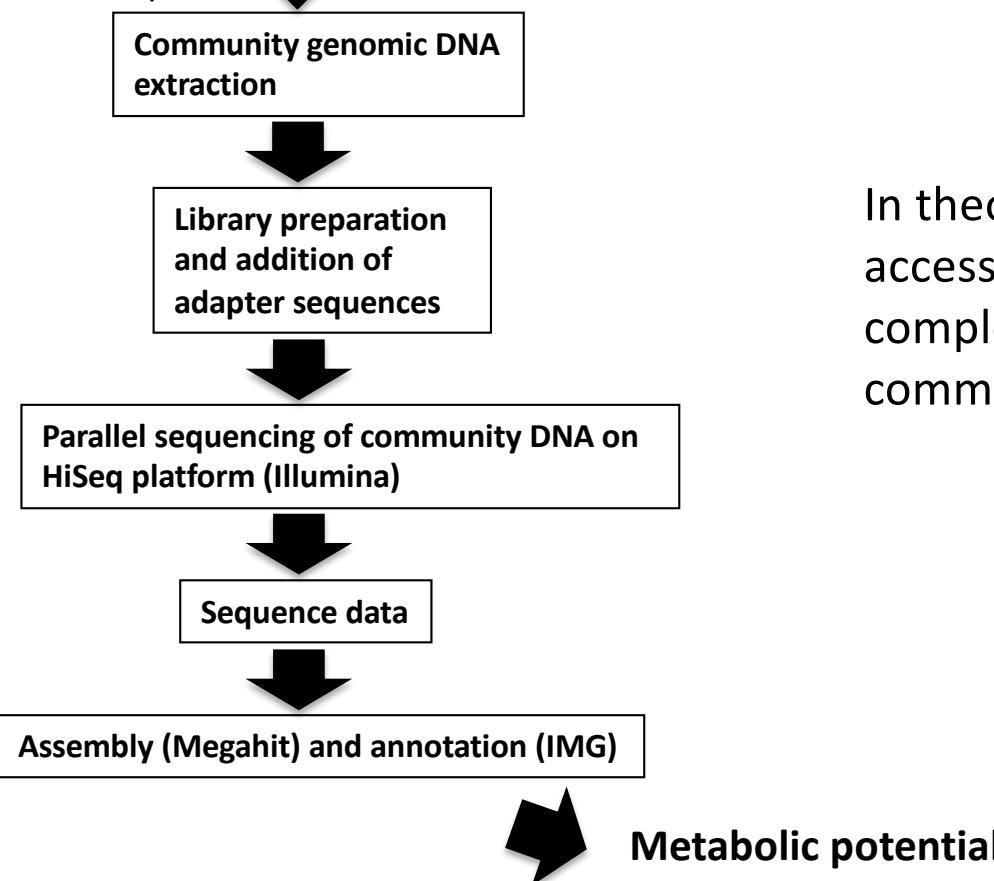
Analysis of all the genes in a microbial community  
(metagenomics)

DNA is the starting material that gets analyzed. We obtain information about the metabolic potential of the microbial community

# Assess the metabolic potential (metagenome: what can they do?)

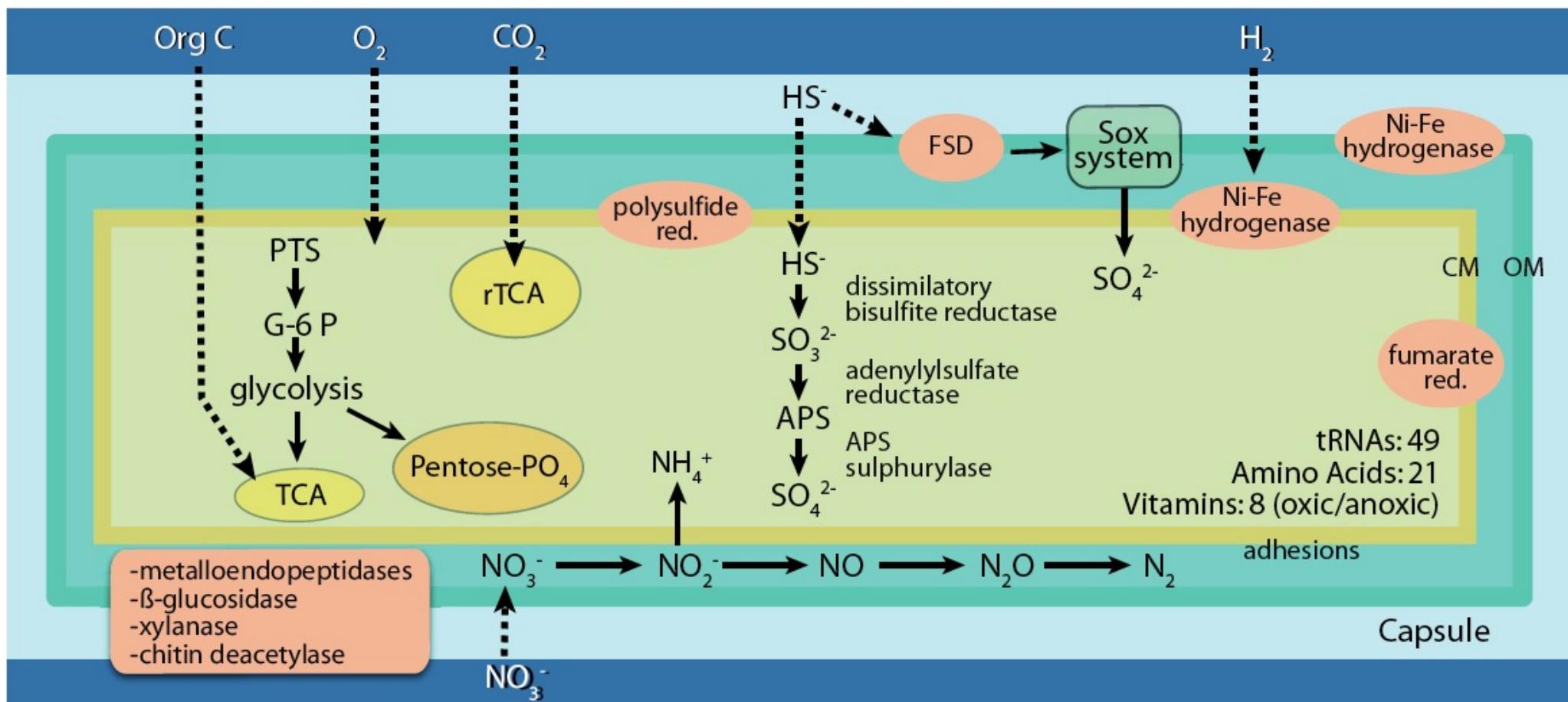
*In situ* collection and  
preservation of the natural  
community

Metagenomics  
Total community  
Over 10M reads/sample



In theory the metagenome makes accessible the entire genetic complement of a given microbial community

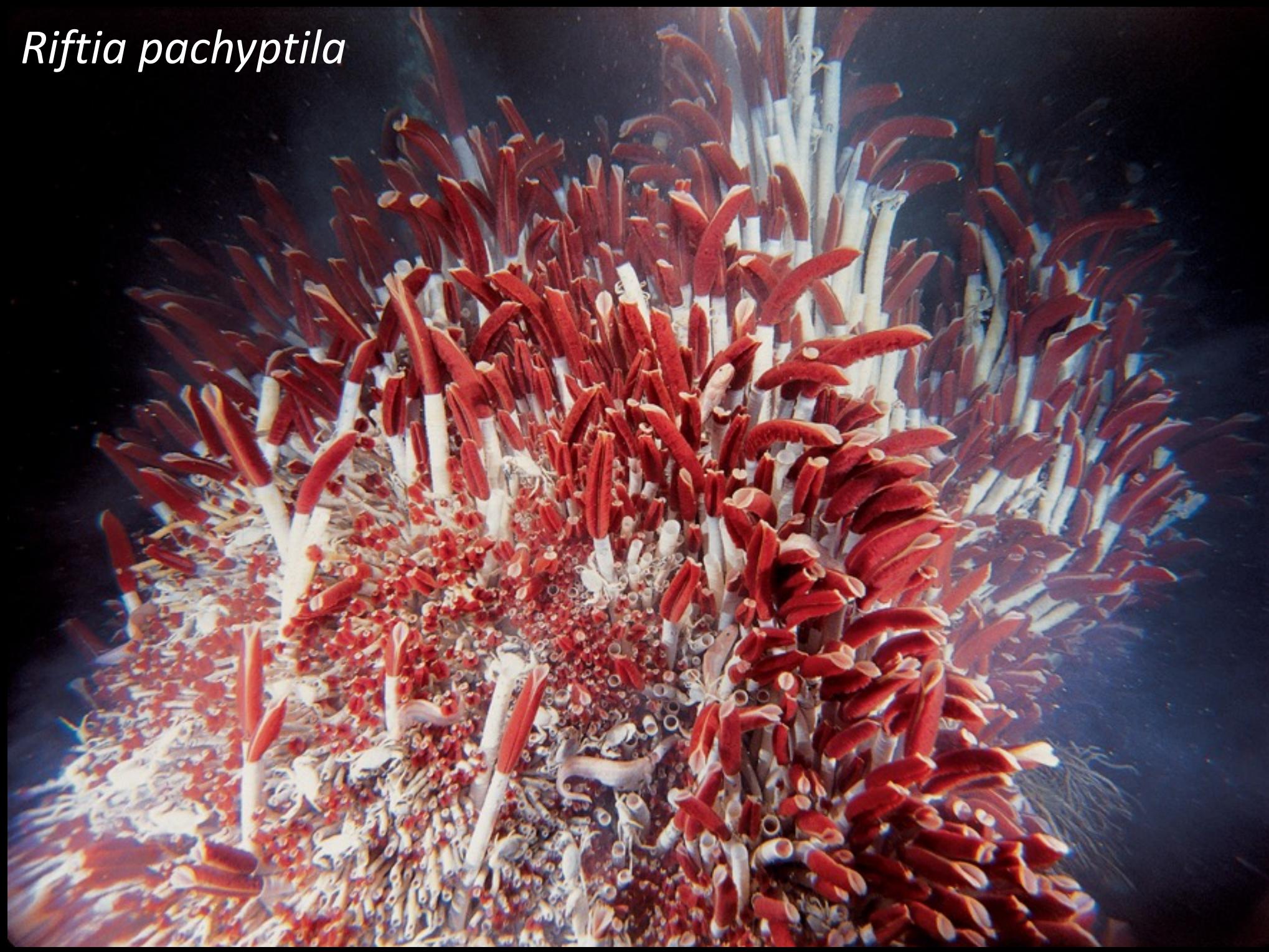
# Model of predicted metabolic processes in the episymbiont cell based on annotation of the episymbiont metagenome



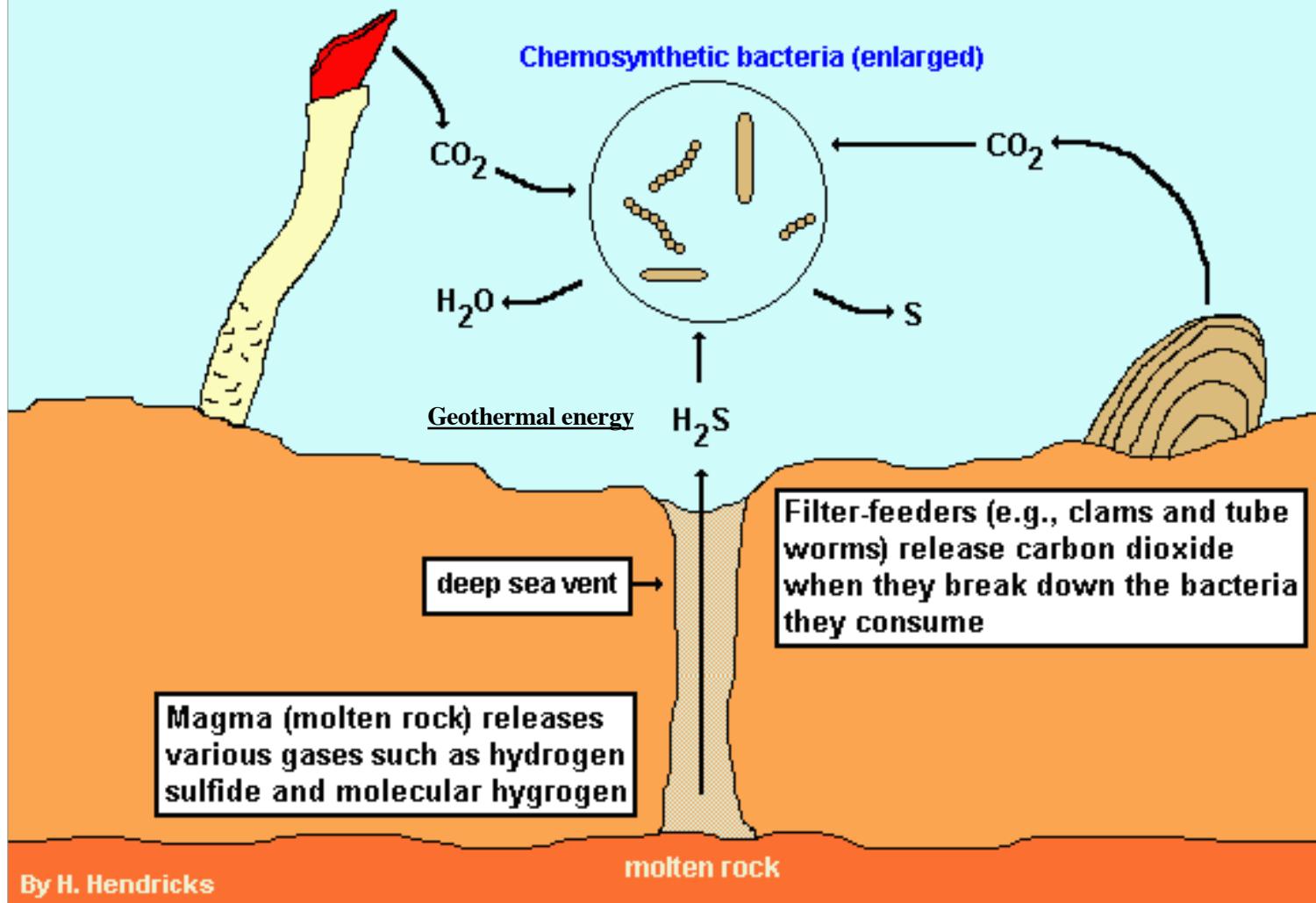
*Alvinella* does not depend completely  
on its episymbionts

What happens when the host depends  
completely on its symbionts?

*Riftia pachyptila*

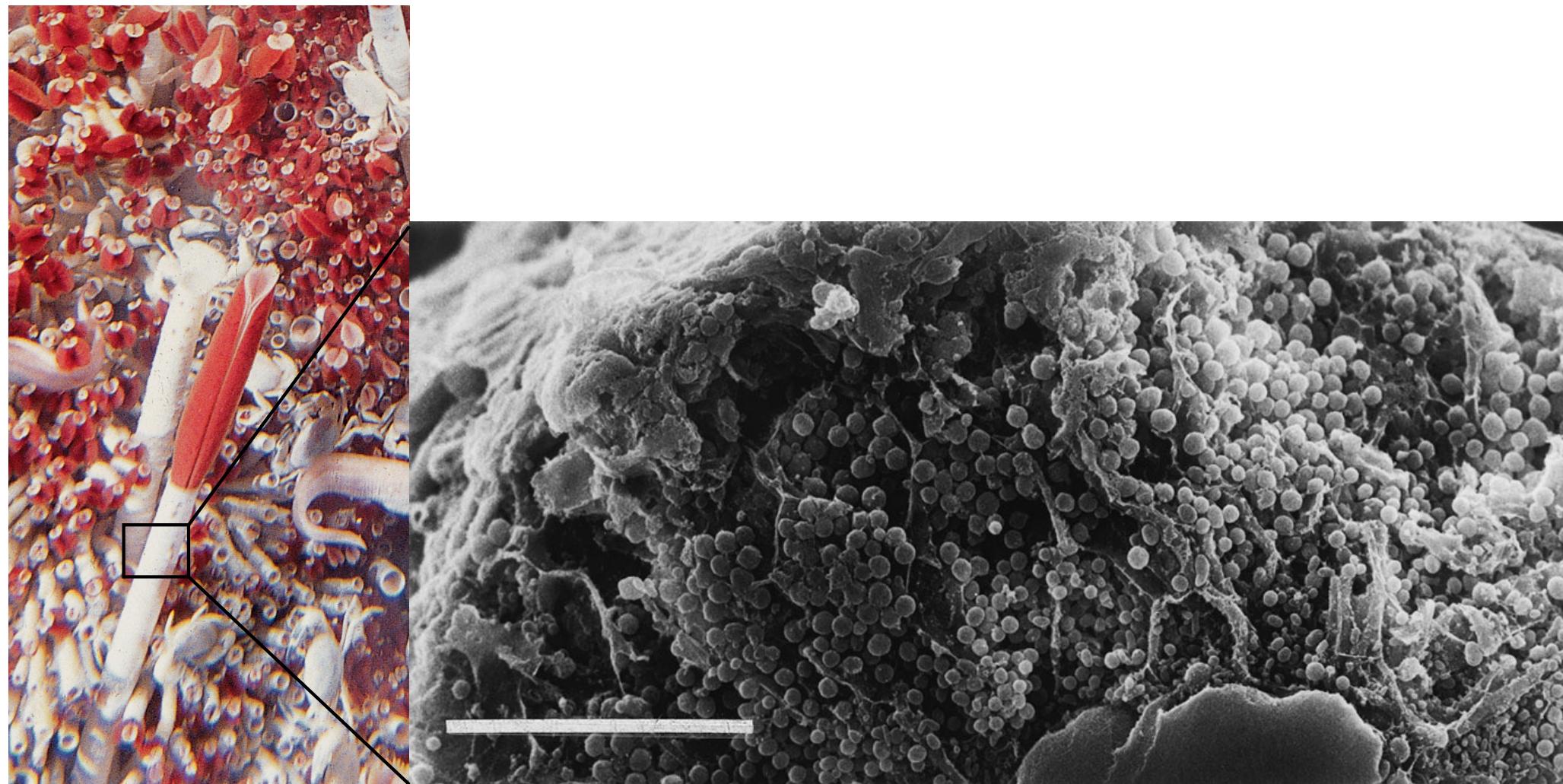


**Chemosynthetic bacteria use the energy stored in sulfur compounds to power the conversion of CO<sub>2</sub> to organic compounds**



Aerobic Chemosynthesis (e.g., Sulfide-oxidizing bacteria)

# Sulfur-oxidizing symbionts in *Riftia* trophosome



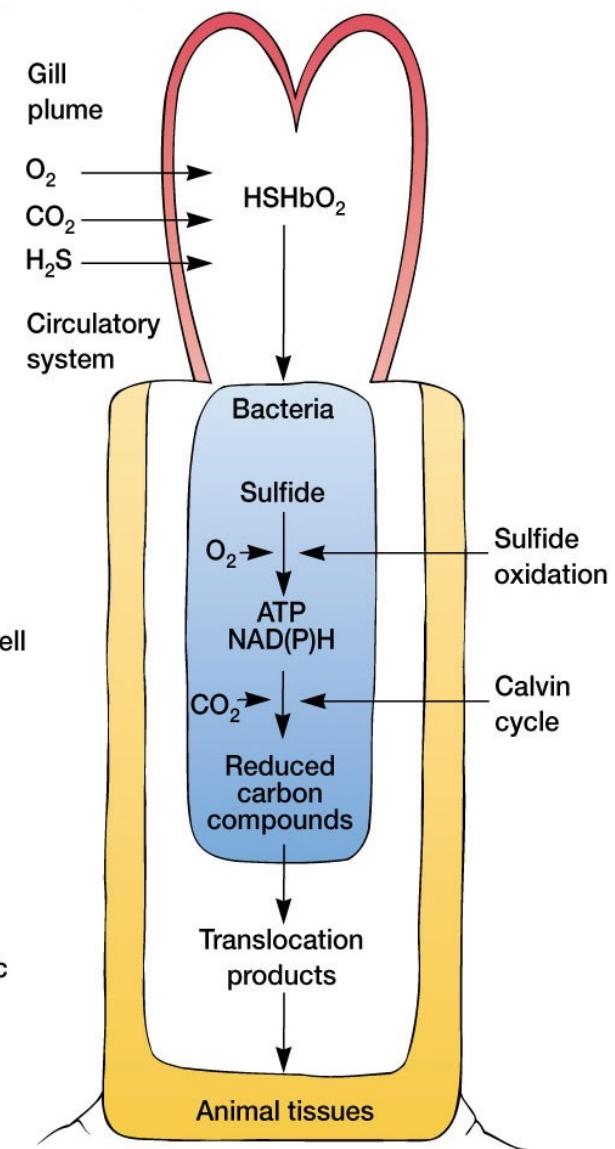
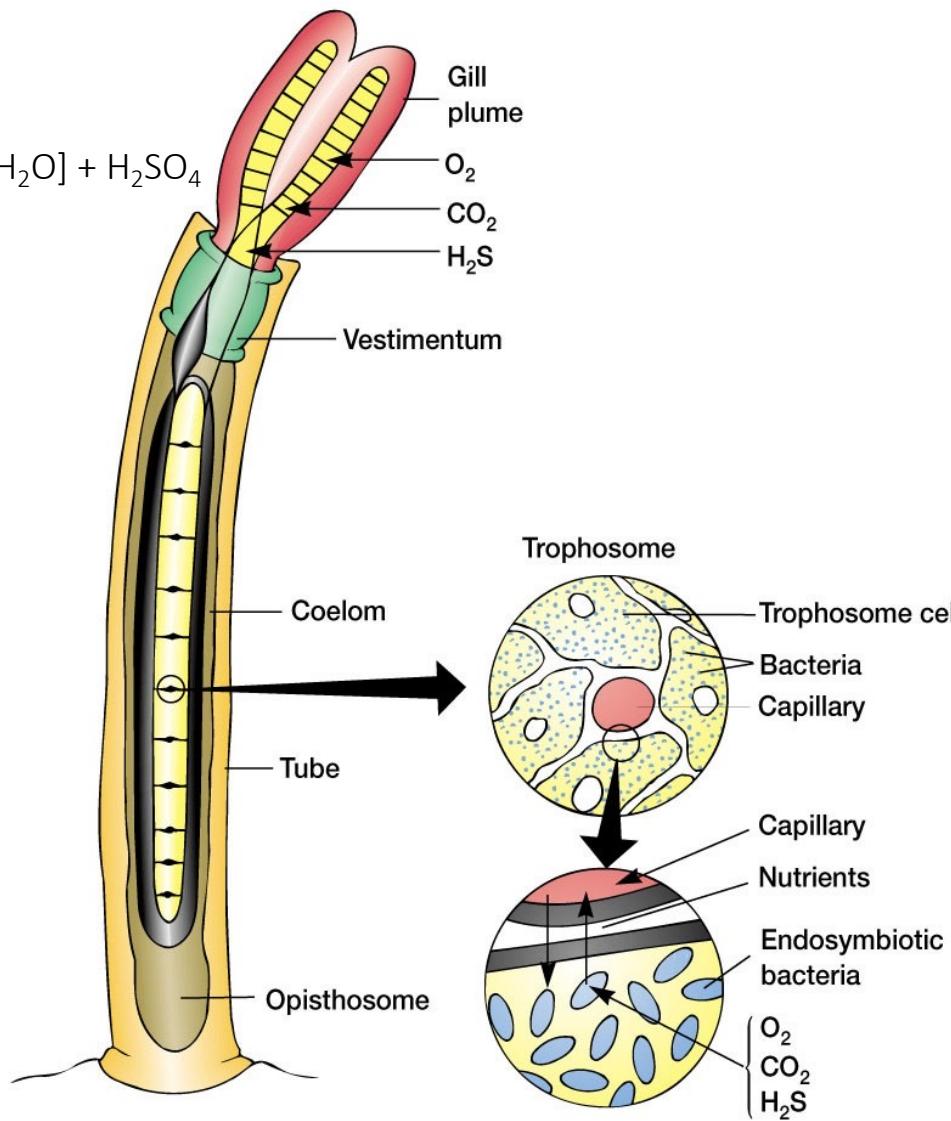
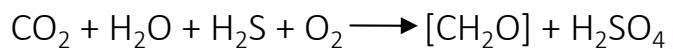
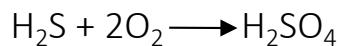
Symbionts contain high levels of the enzyme ATP sulfurylase





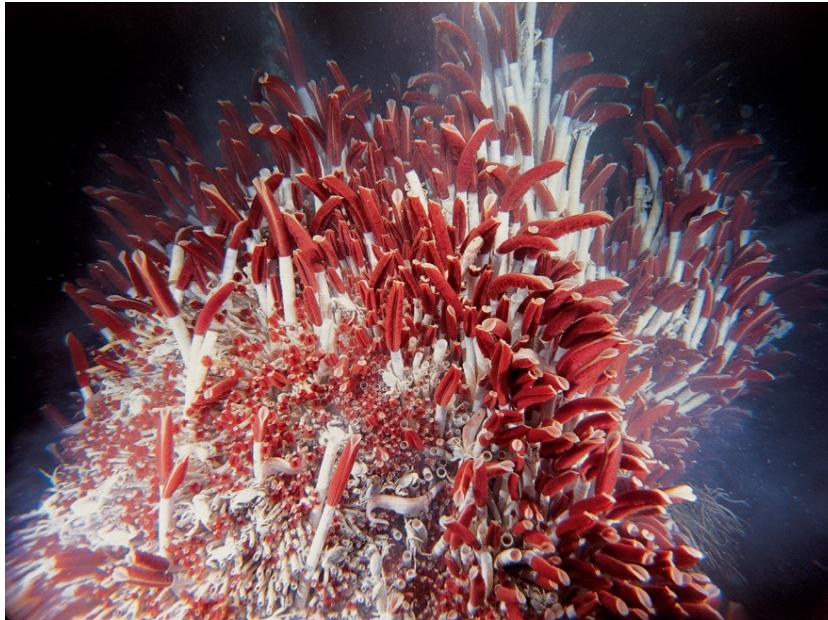
# Riftia-symbiont association

Thiotrophic symbionts:



Which processes are the *Riftia* symbionts mediating?

# Analysis of expressed proteins in a microbial community (metaproteome: what are they doing?)



Protein is the starting material that gets analyzed. We obtain information about the expressed function of the microbial community

Metaproteomics:  
Active fraction of the  
community  
2096 total proteins

Protein extraction

SDS-PAGE and in-gel  
tryptic digest

Samples analyzed by LC-MSMS on QExactive

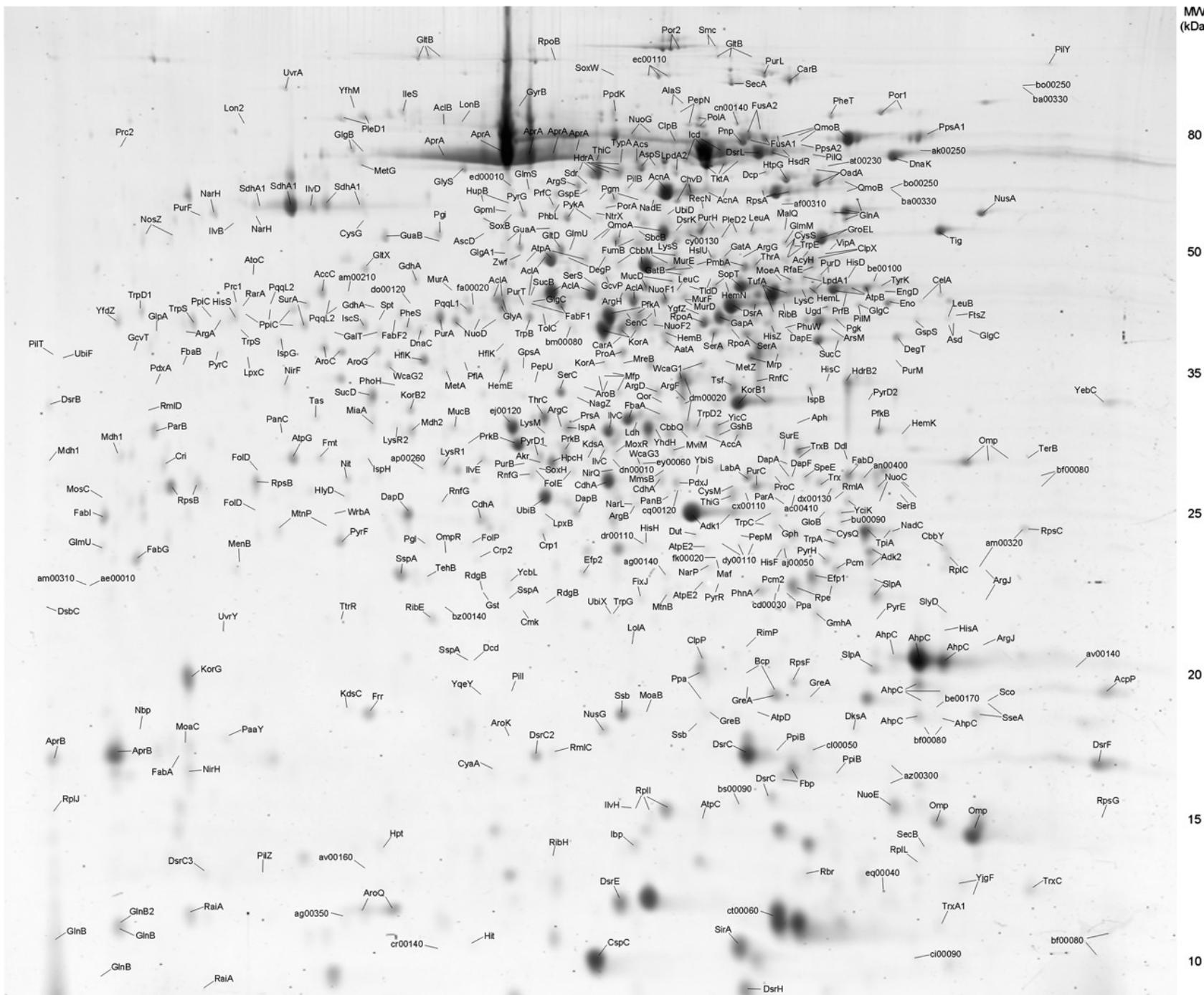
Analysis via X!Tandem  
Spectra were normalized to  
median of all qualified spectra

Main expressed pathways

# Proteome analysis of *Riftia pachyptila* endosymbionts

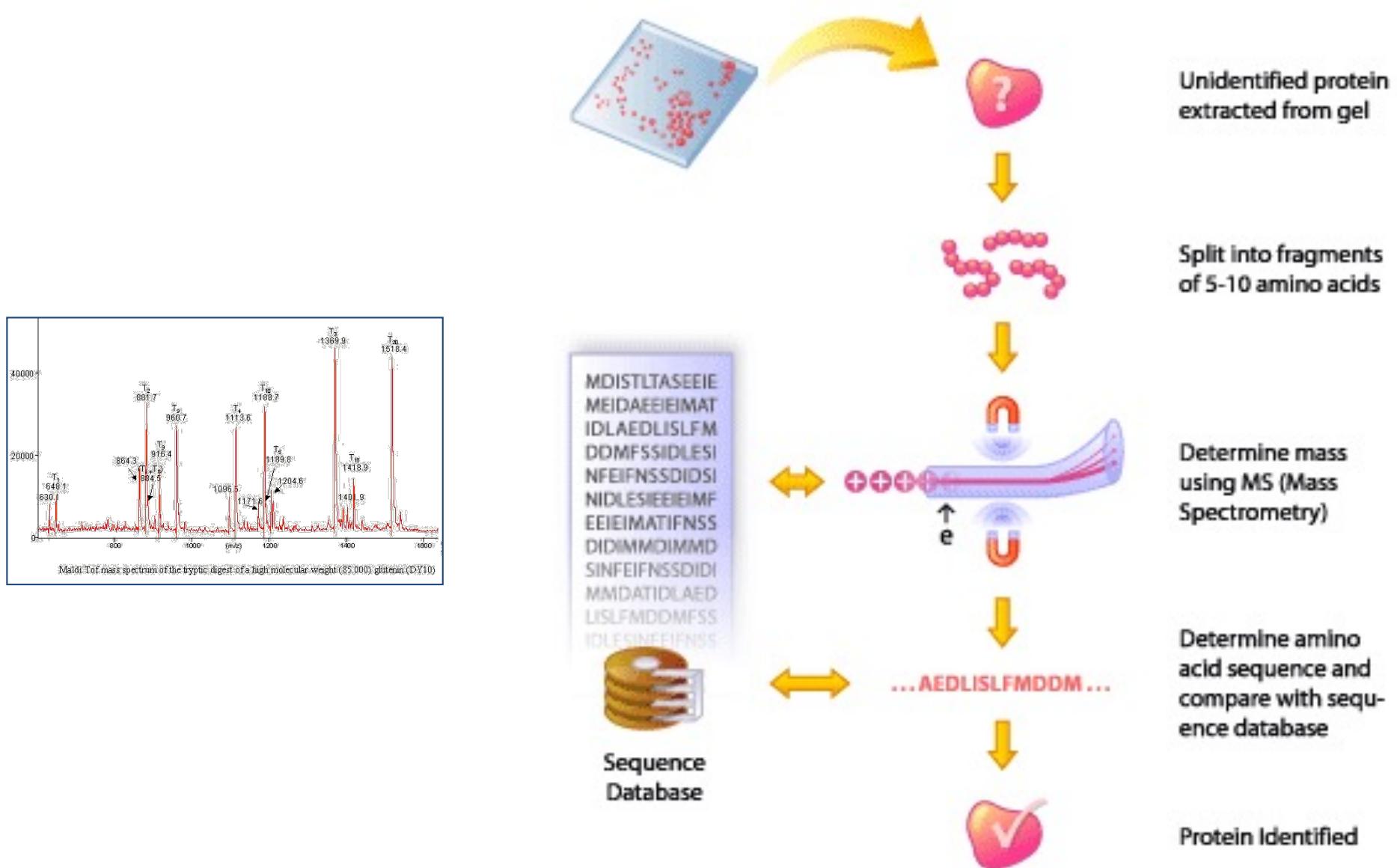
(the genome sequence of the symbionts was obtained previously)

- Collection of *R. pachyptila* tubeworms and storage at -80°C
- Removal of the endosymbionts from the worms by gradient centrifugation
- Extraction of total proteins from the symbionts
- Separation of proteins on two-dimensional polyacrylamide gels
- 800 protein spots were excised from the gel(s), digested and analyzed by mass spectrometry
- Comparison to the database and identification
- Reconstruction of metabolic pathways
- Quantitative analysis



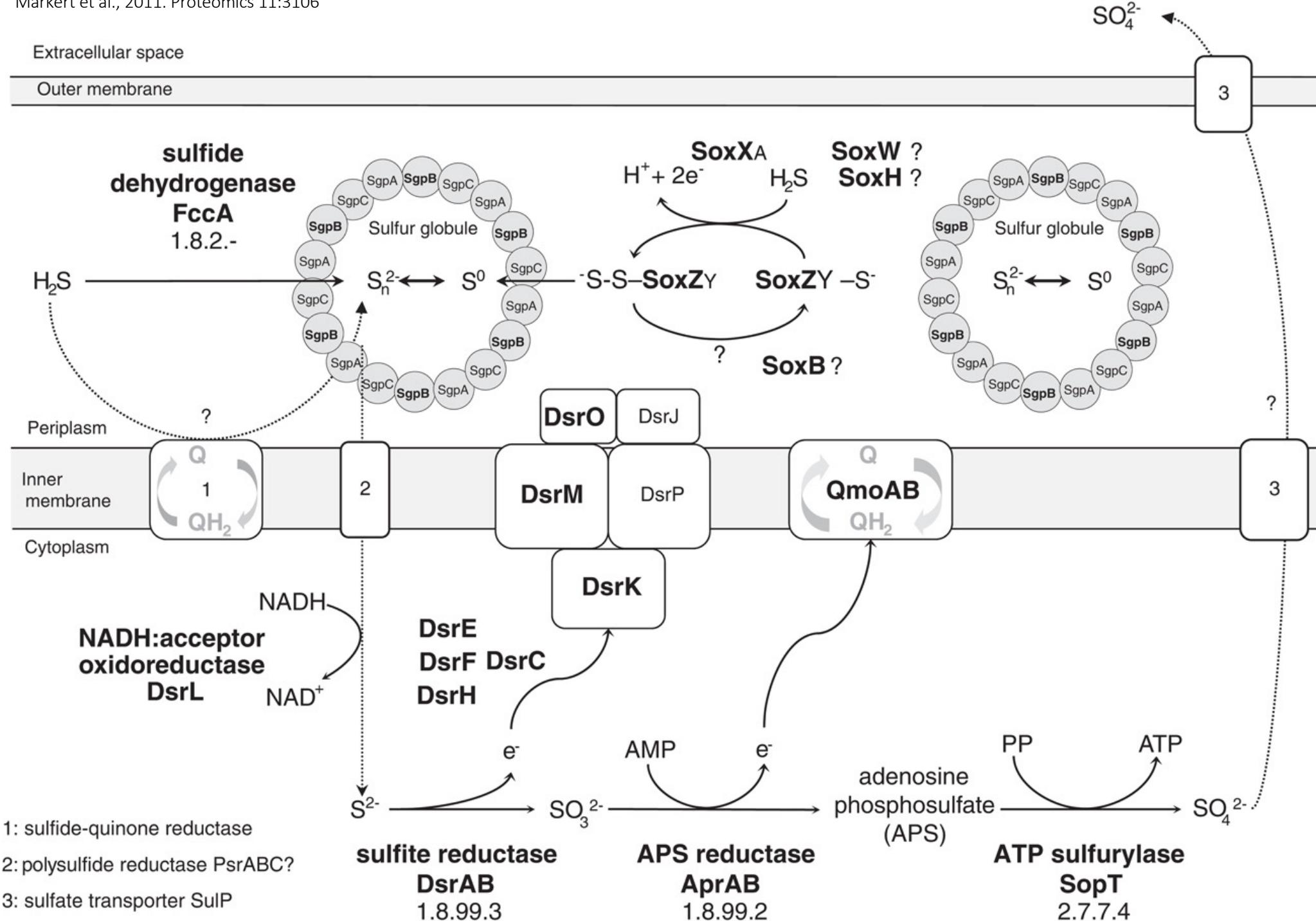
## Reference map of the *R. pachyptila* endosymbiont intracellular proteome

# Identification of a Protein by Mass Spectrometry



# Proteome-based reconstruction of the sulfide oxidation pathway in *R. pachyptila* symbionts

Markert et al., 2011. Proteomics 11:3106



# Outline

- Geological and chemical characteristics of deep-sea vents
- Life without light: Chemosynthetically sustained ecosystems
- Epsilonproteobacteria: Primary producers at deep-sea vents
- Symbiosis: How the vent invertebrates make a living
- **Anaerobic thermophiles as “time capsules” to investigate early microbial metabolism and life on other planets**

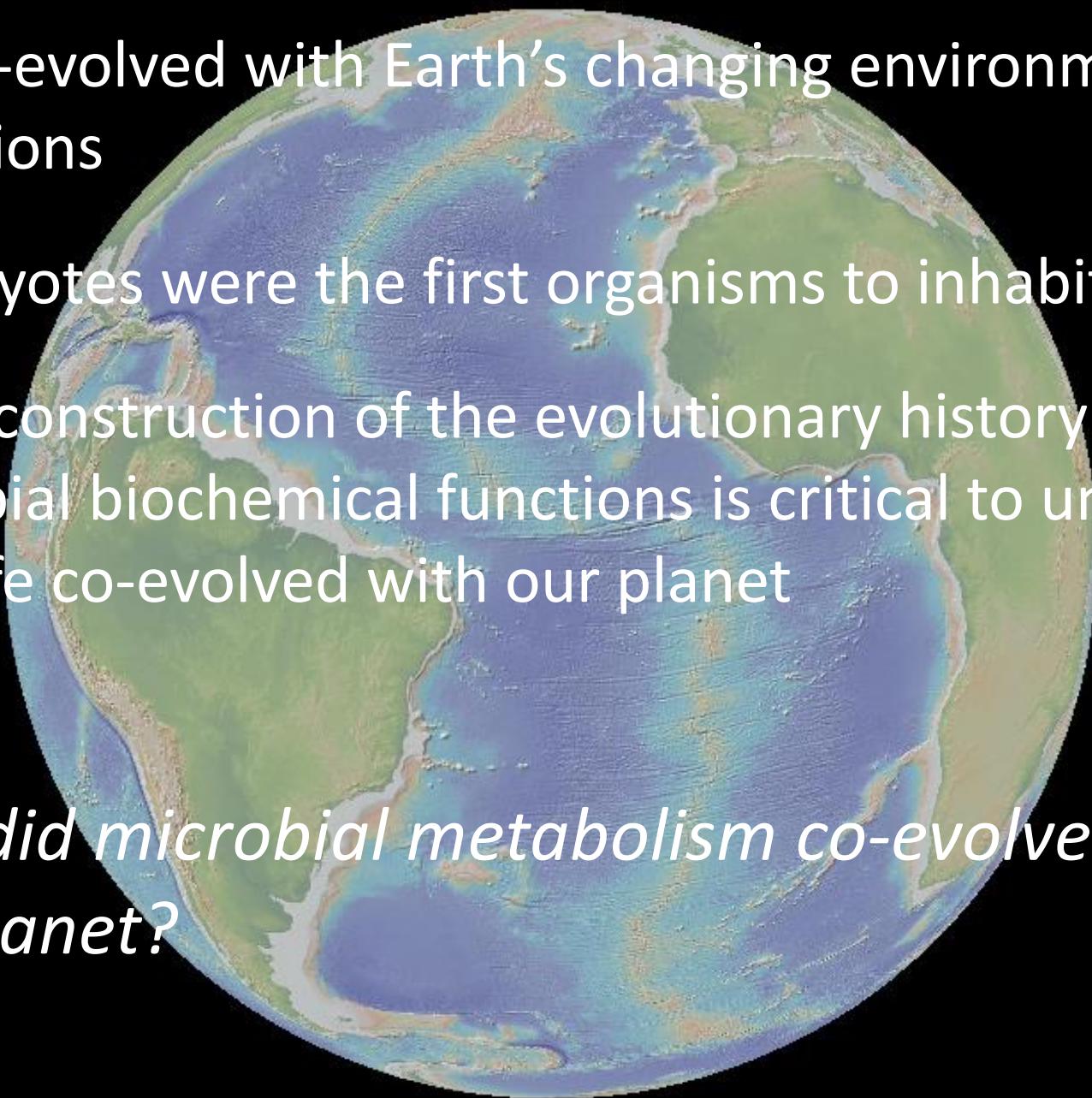


Life co-evolved with Earth's changing environmental conditions

Prokaryotes were the first organisms to inhabit Earth

The reconstruction of the evolutionary history of key microbial biochemical functions is critical to understand how life co-evolved with our planet

*How did microbial metabolism co-evolved with our planet?*



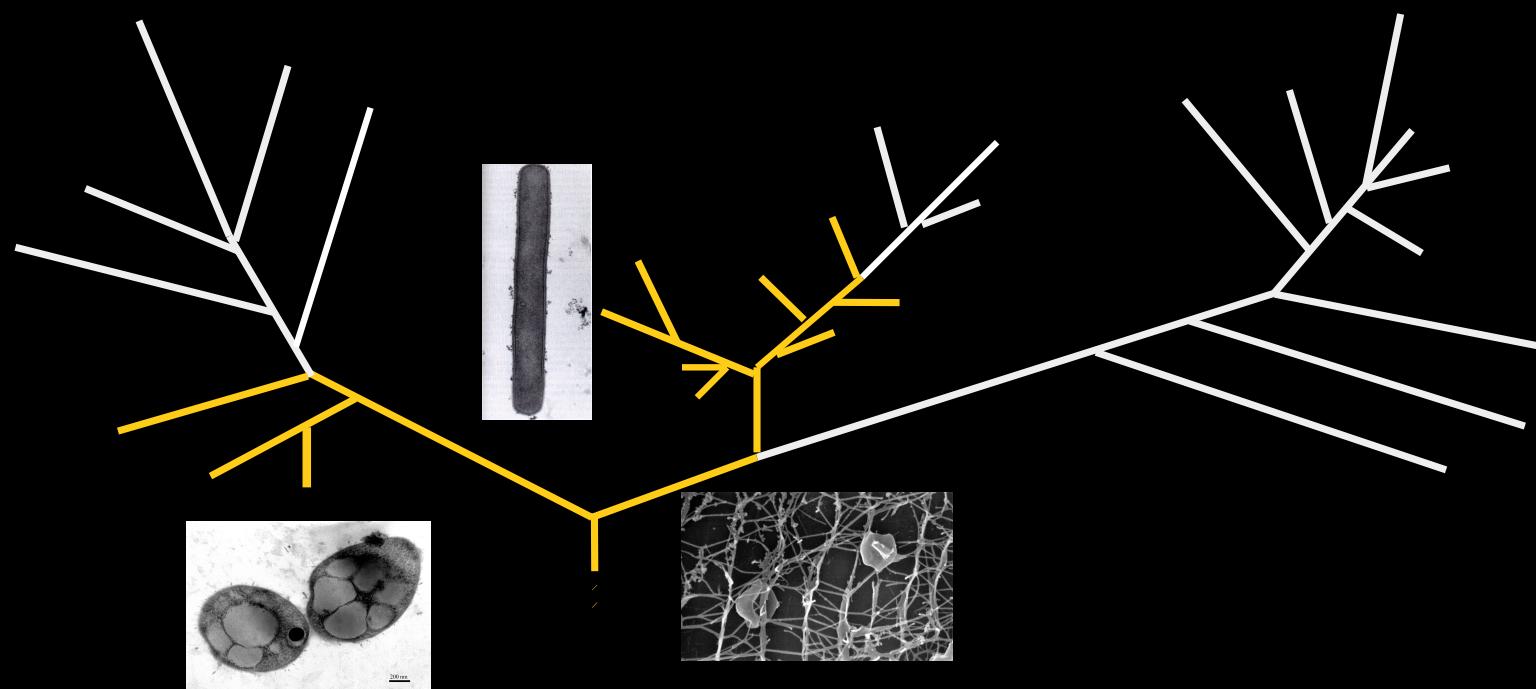
2000 Km



The Earth's early atmosphere was composed predominantly by gases released by volcanoes: ammonia, carbon dioxide, carbon monoxide, hydrogen, methane, nitrogen, sulfur dioxide, and water vapor

# Why geothermal environments?

- Modern geothermal habitats can be considered relic environments similar to the early Earth.
- Modern microorganisms that inhabit such relic habitats belong to lineages that co-evolved with our planet and carry both ancestral and more recently acquired traits (genes and enzymes).
- Hence, these microorganisms can be thought of as “time capsules” that might have maintained some ancestral genetic and biochemical traits and can be used as models to reconstruct the evolution of metabolism.

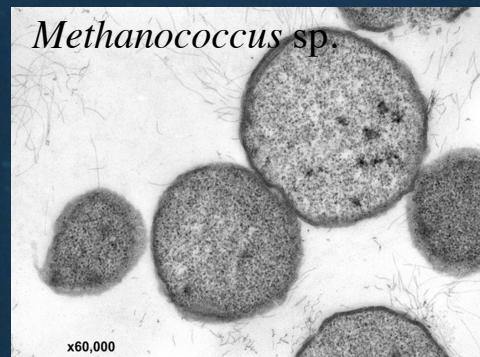


# Microbial Life at High Temperature

Thermophilic microorganisms take advantage of the chemical and temperature gradients associated with the emission of hydrothermal fluids

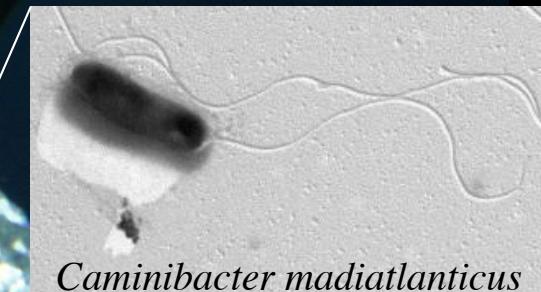
Some of these organisms are anaerobic, and can derive energy from the reduction of sulfur or carbon dioxide

Anaerobic metabolism is thought to be an ancestral trait, as oxygen was not available in the early Earth



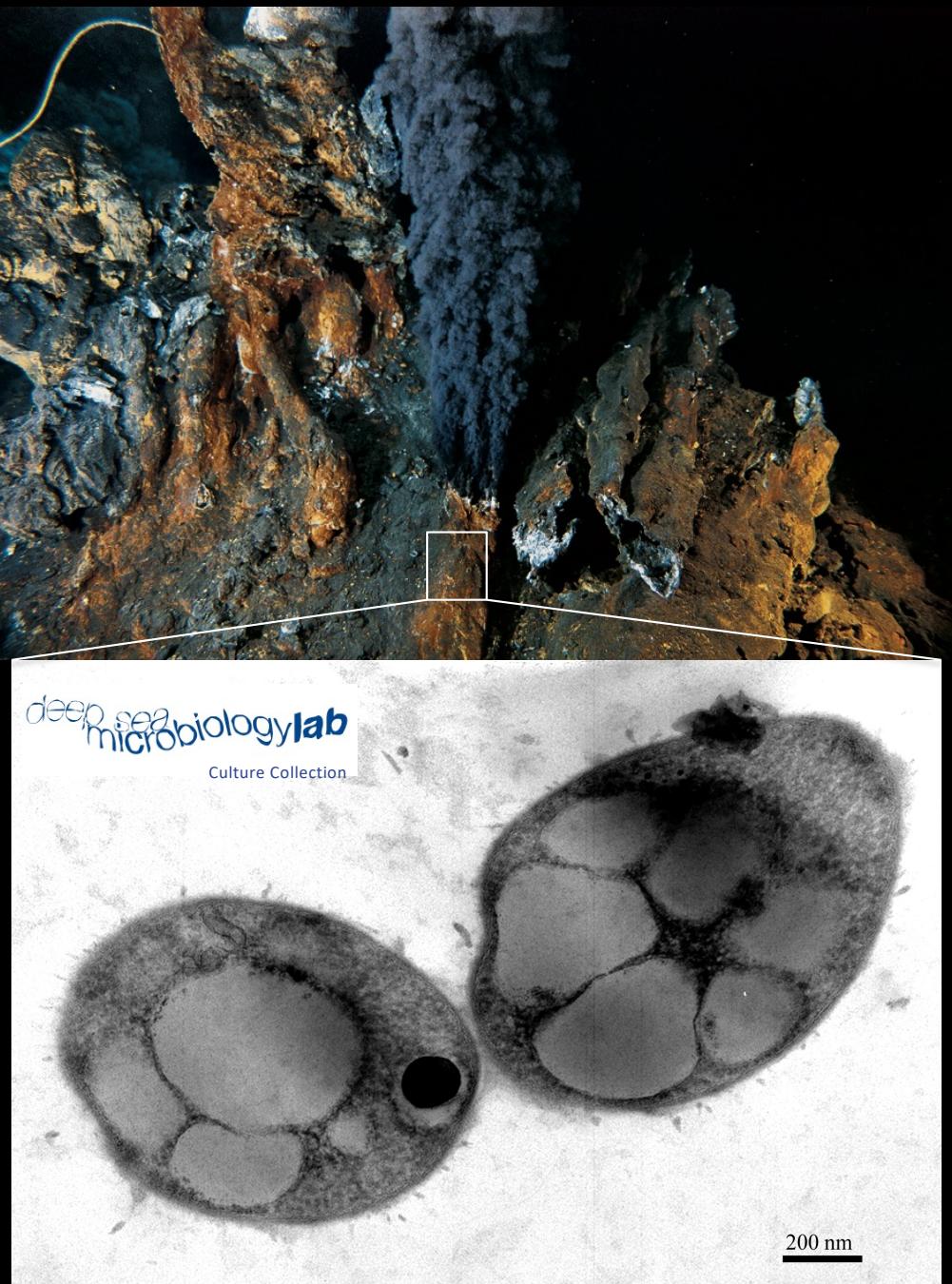
High temperature, reducing fluids

Cold, oxygenated sea water



*Caminibacter mediatlanticus*

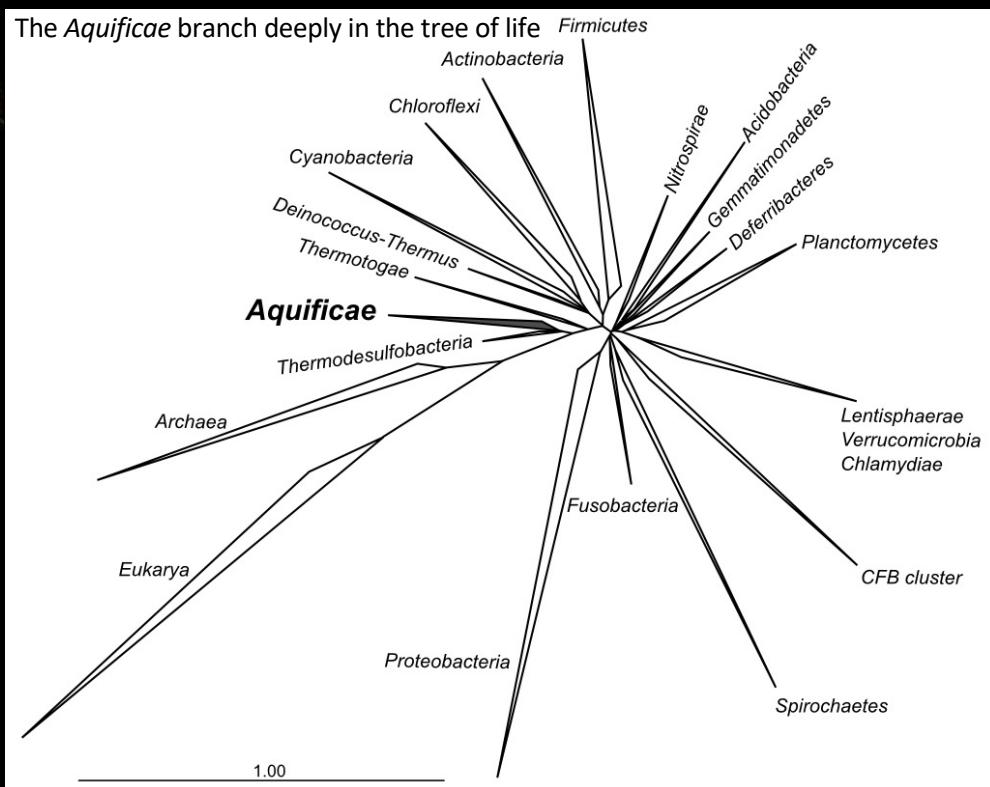
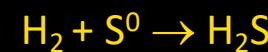
# *Thermovibrio ammonificans*, an anaerobic, thermophilic, chemosynthetic bacterium



*Thermovibrio ammonificans*

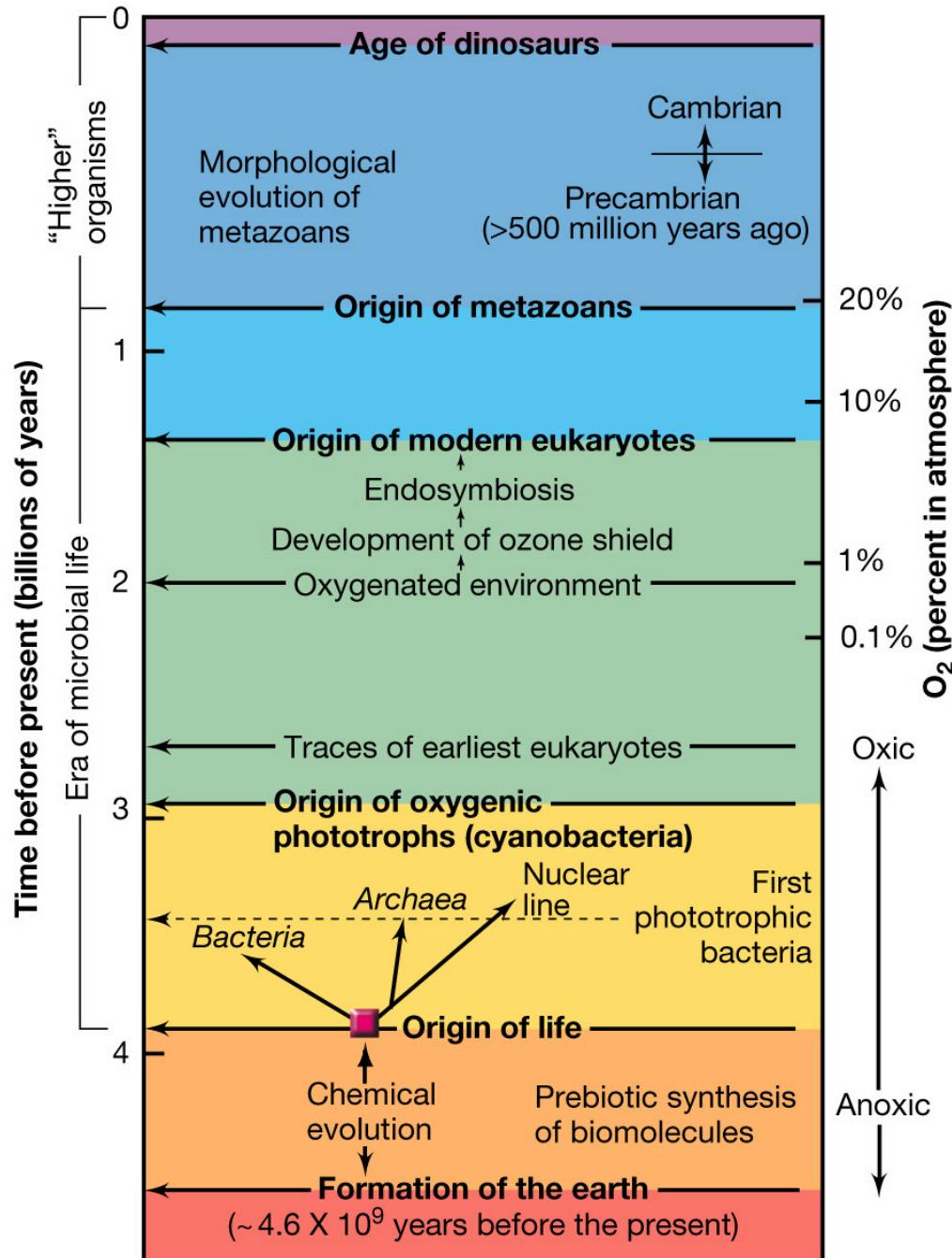
Opt. T: 75° C

Isolated from a deep-sea hydrothermal vent, EPR

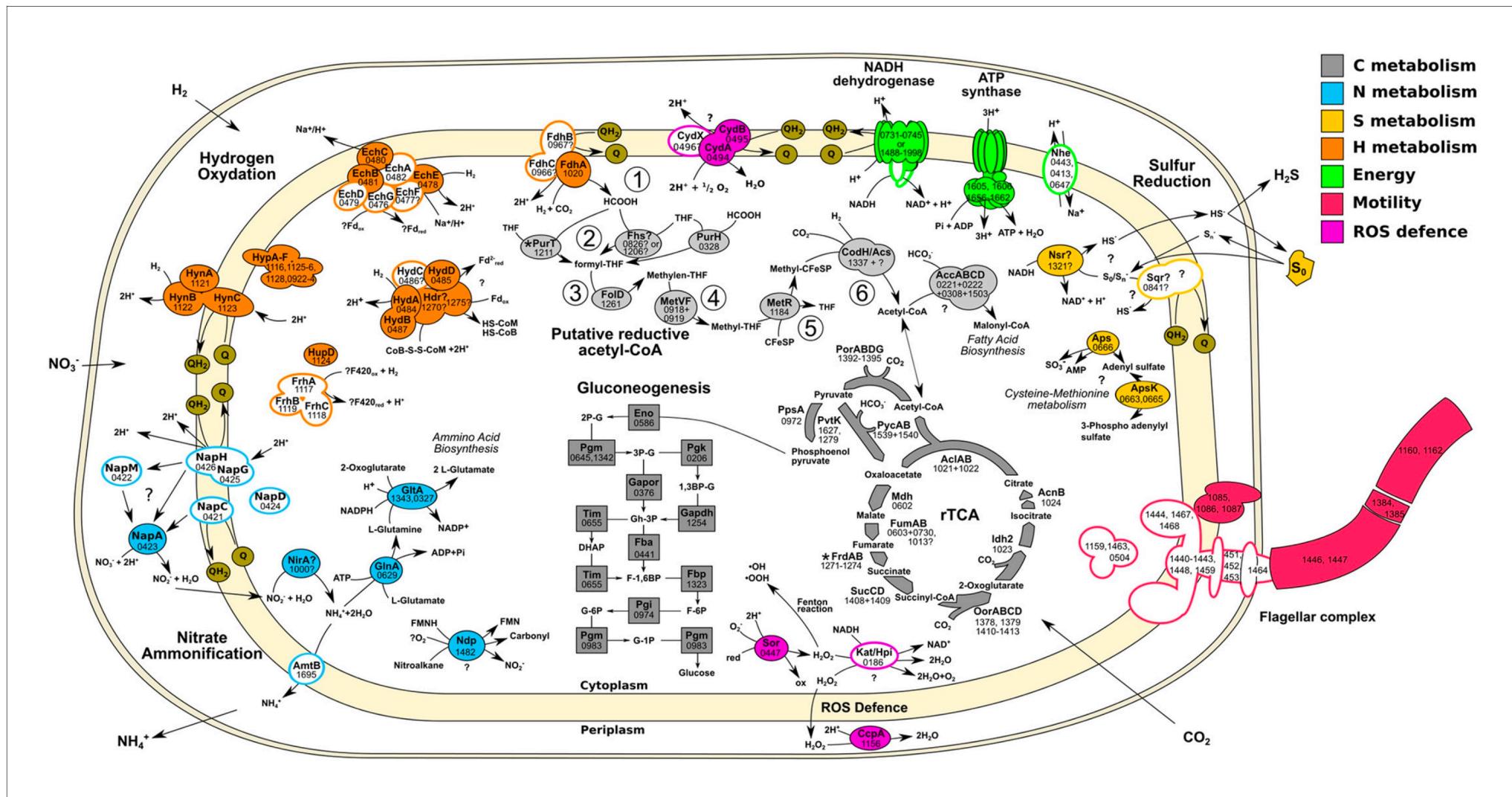


From: Vetriani et al., 2004. *Int. J. Syst. Evol. Microbiol.* 54:175-181 and Giovannelli et al., *eLife*, in revision

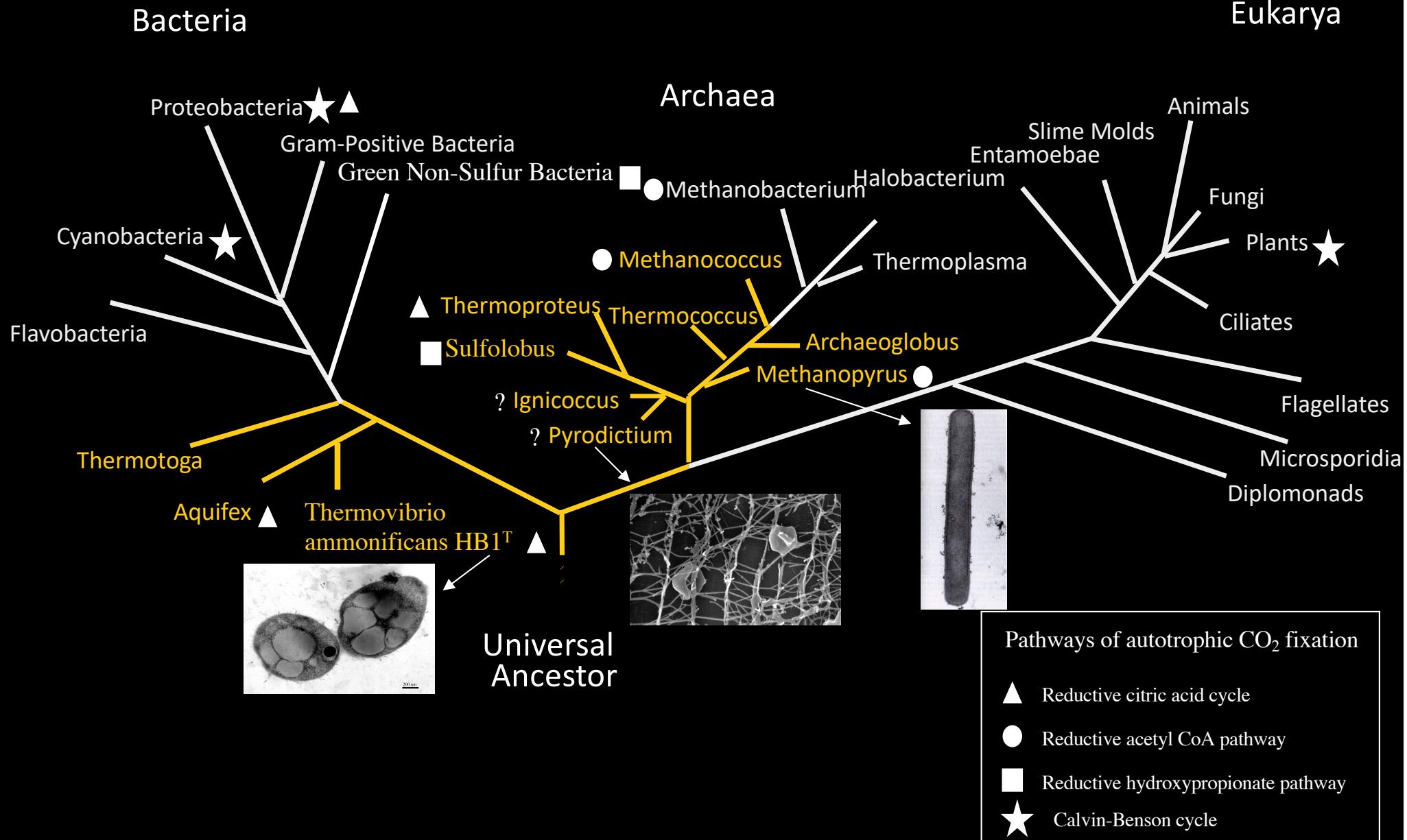
Anaerobic thermophiles may have evolved over 4 billion years ago, when oxygen of photosynthetic origin was not yet available in the Earth's atmosphere



# Genome-enabled reconstruction of the central metabolism of *Thermovibrio ammonificans*



# Molecules as Documents of Evolutionary History



# Cassini finds molecular hydrogen in the Enceladus plume: Evidence for hydrothermal processes

Waite et al., 2017. *Science* 356, 155–159



A picture of the ice plumes on Enceladus taken by the Cassini spacecraft. Photo by NASA

Model of possible hydrothermal processes at the bottom of Enceladus' ocean. Such processes might be responsible for the production of hydrogen

