Microbiologia degli Ambienti Marini Idrotermali

PARTE A

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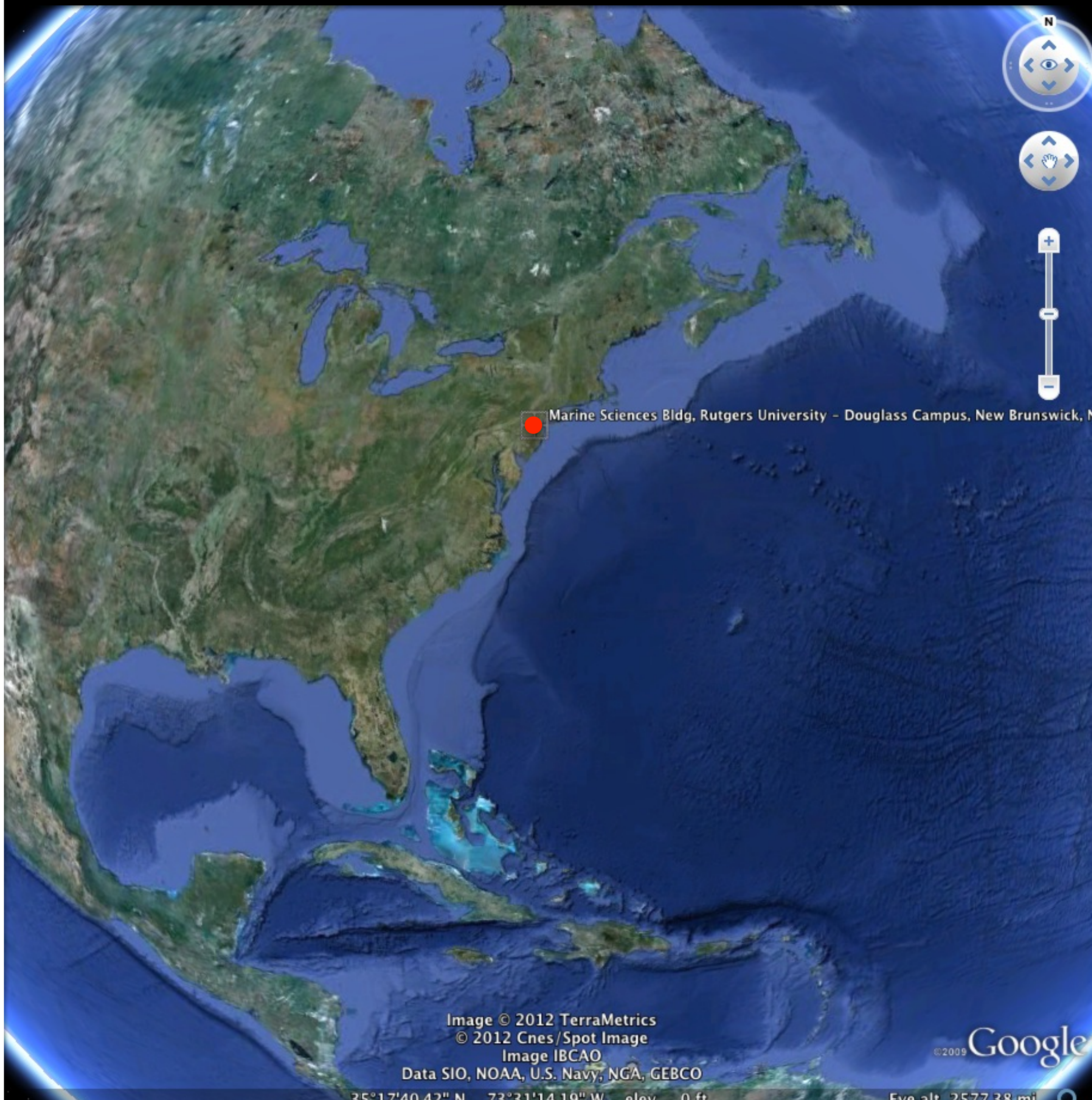
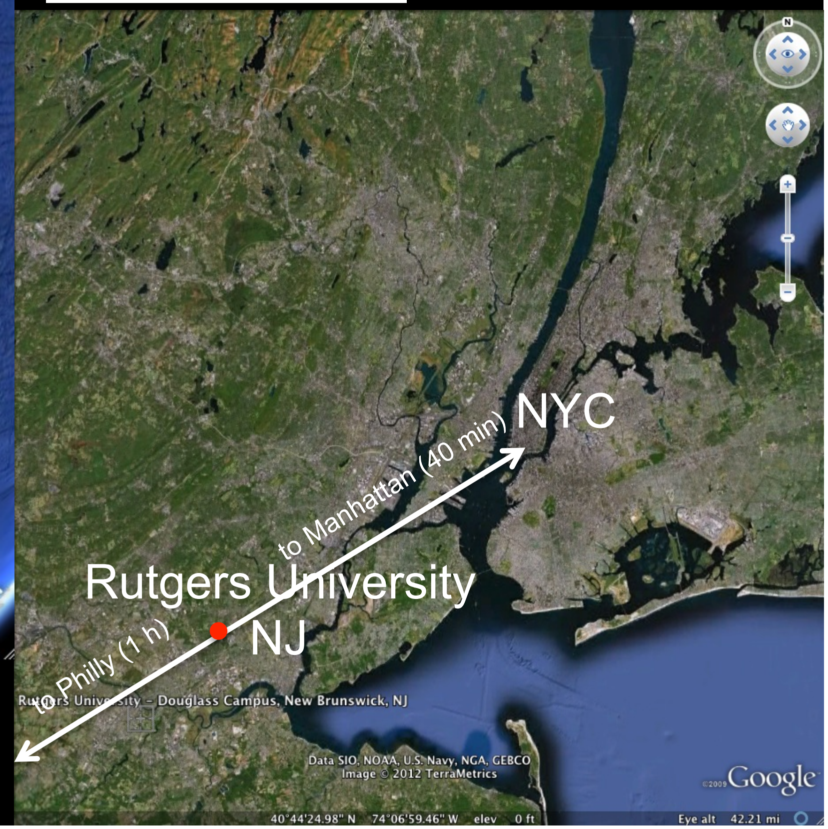


RUTGERS UNIVERSITY

Established in 1766



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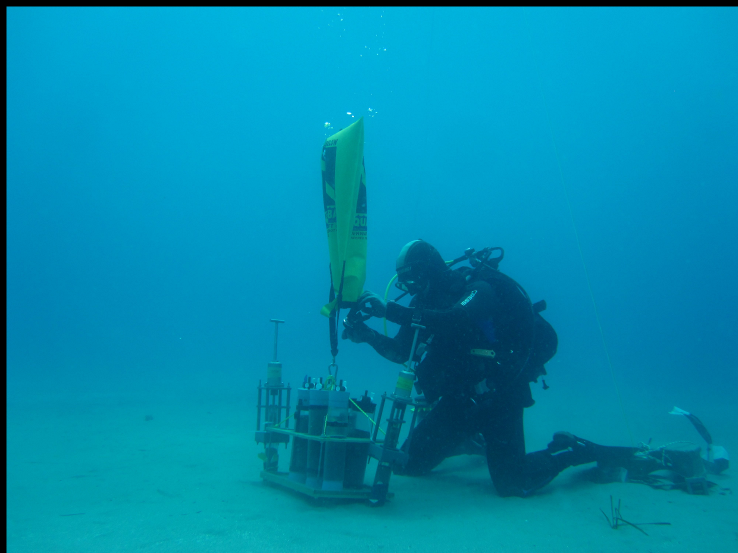
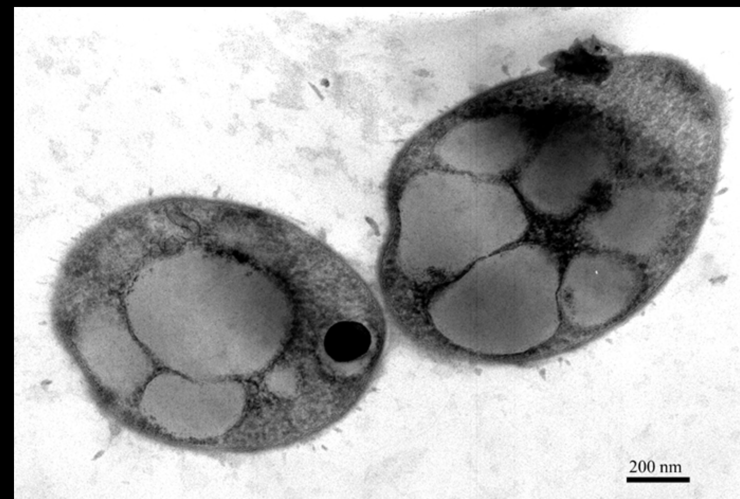
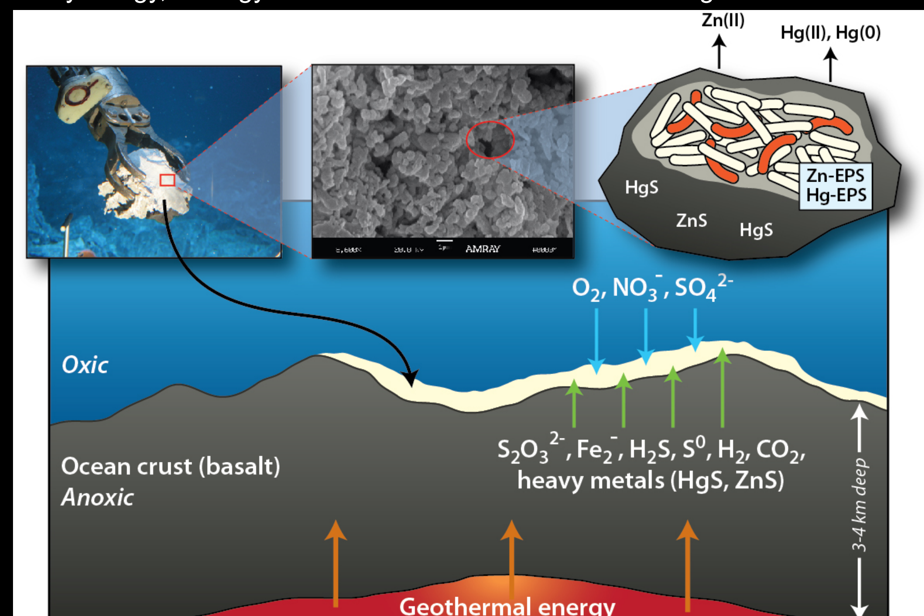


Microbiology of shallow-water hydrothermal systems…



Physiology, ecology and and evolution of marine microorganisms…

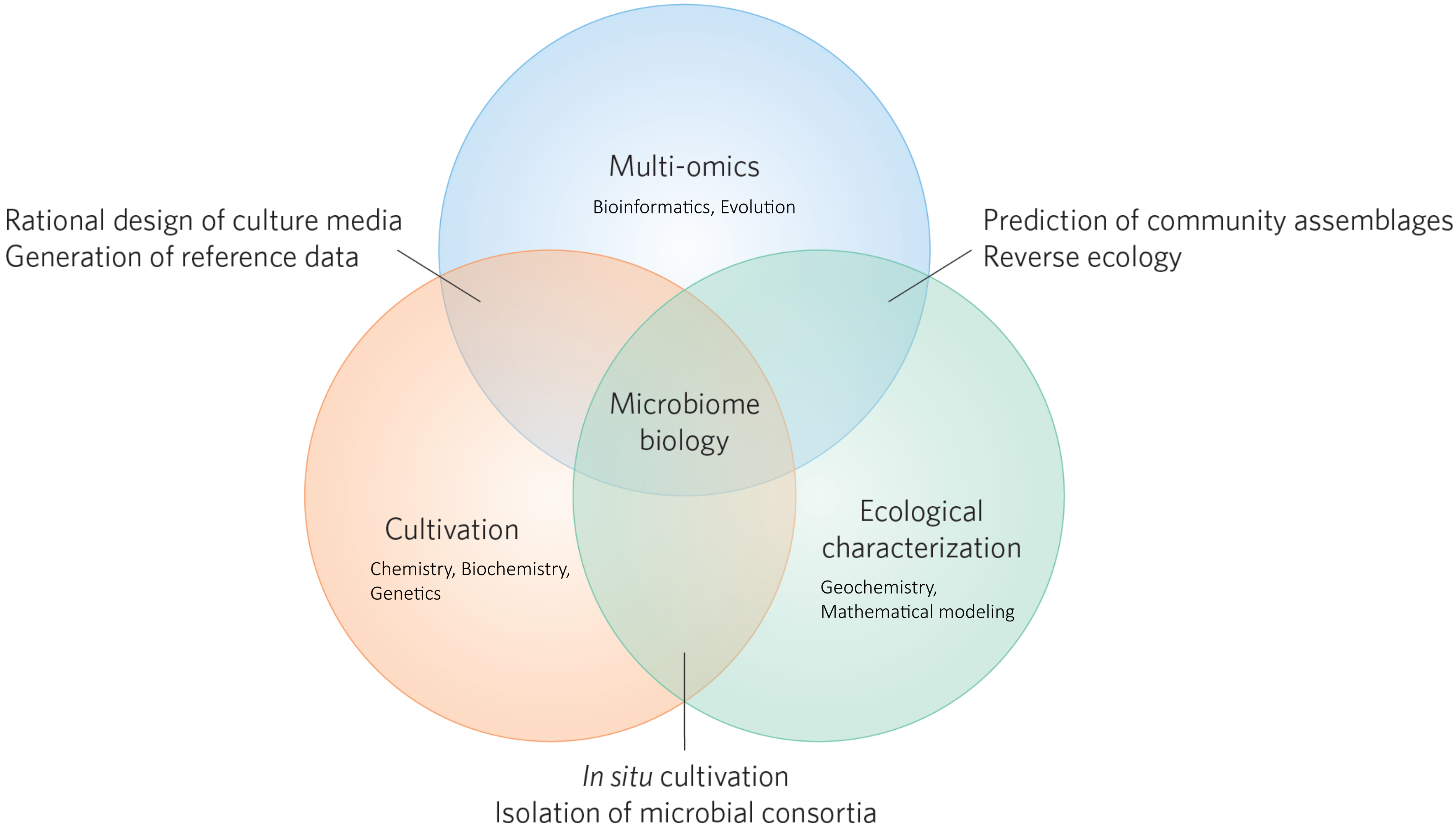
…and of deep-sea hydrothermal vents



…and ecology of natural microbial communities

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

Integration of physiological, molecular and geochemical approaches in environmental microbiology



Vilanova and Porcar, 2016. *Nature Microbiol.* 1:1

Struttura della Lezione

- **Background**

  Sorgenti idrotermali oceaniche e comunita` biologiche

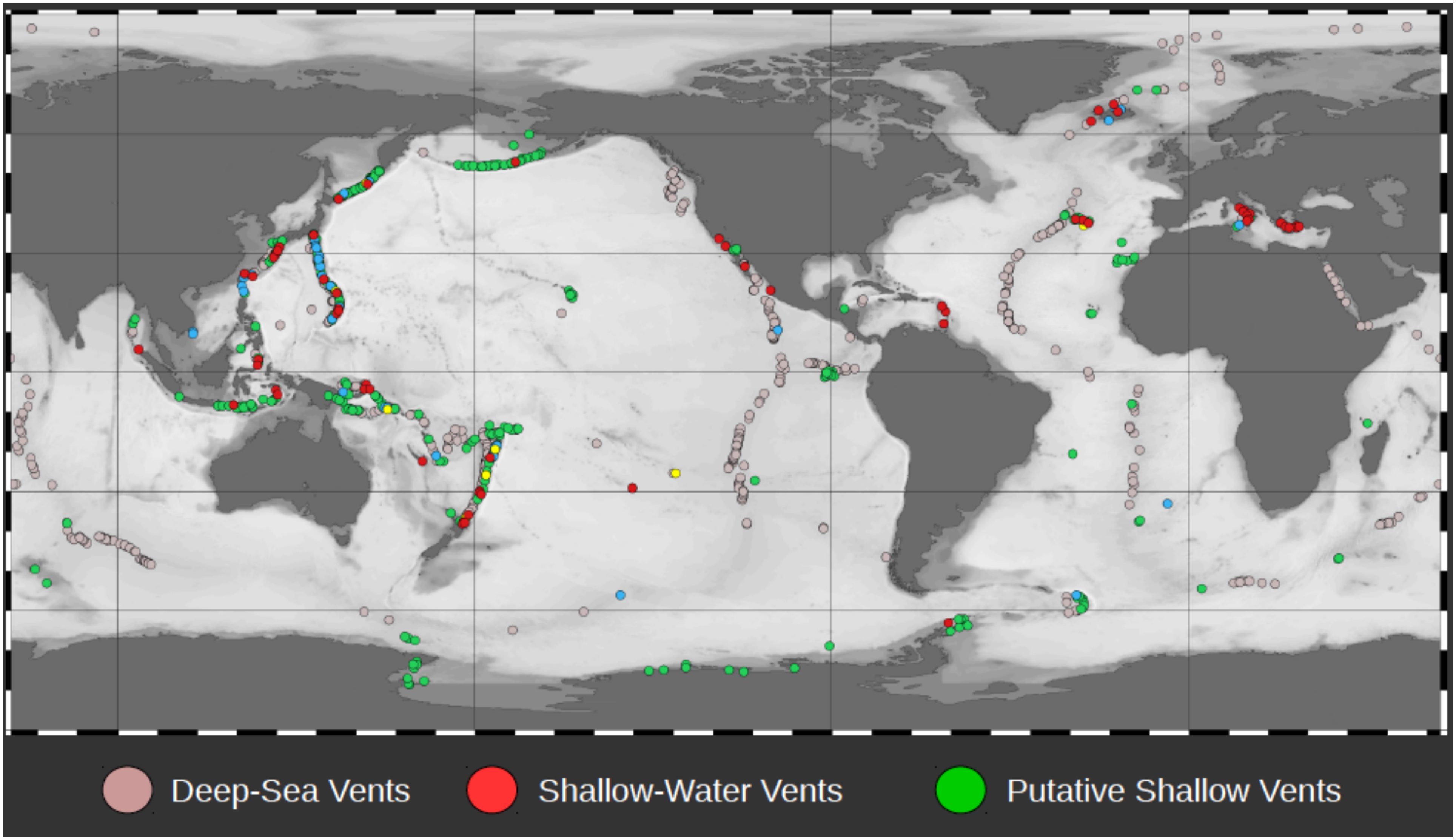
- **Microbiologia degli ambienti marini idrotermali**

  Comunita` microbiche chemosintetiche e rilevanza per il trasferimento di energia e carbonio dai fluidi idrotermali

  Fisiologia, genomica e proteomica di colture pure e di comunita` microbiche naturali

Hydrothermal systems are widespread in the world's ocean

Estimated CO_2 flux from worldwide shallow-water vents: 3-17 Mt CO_2/year

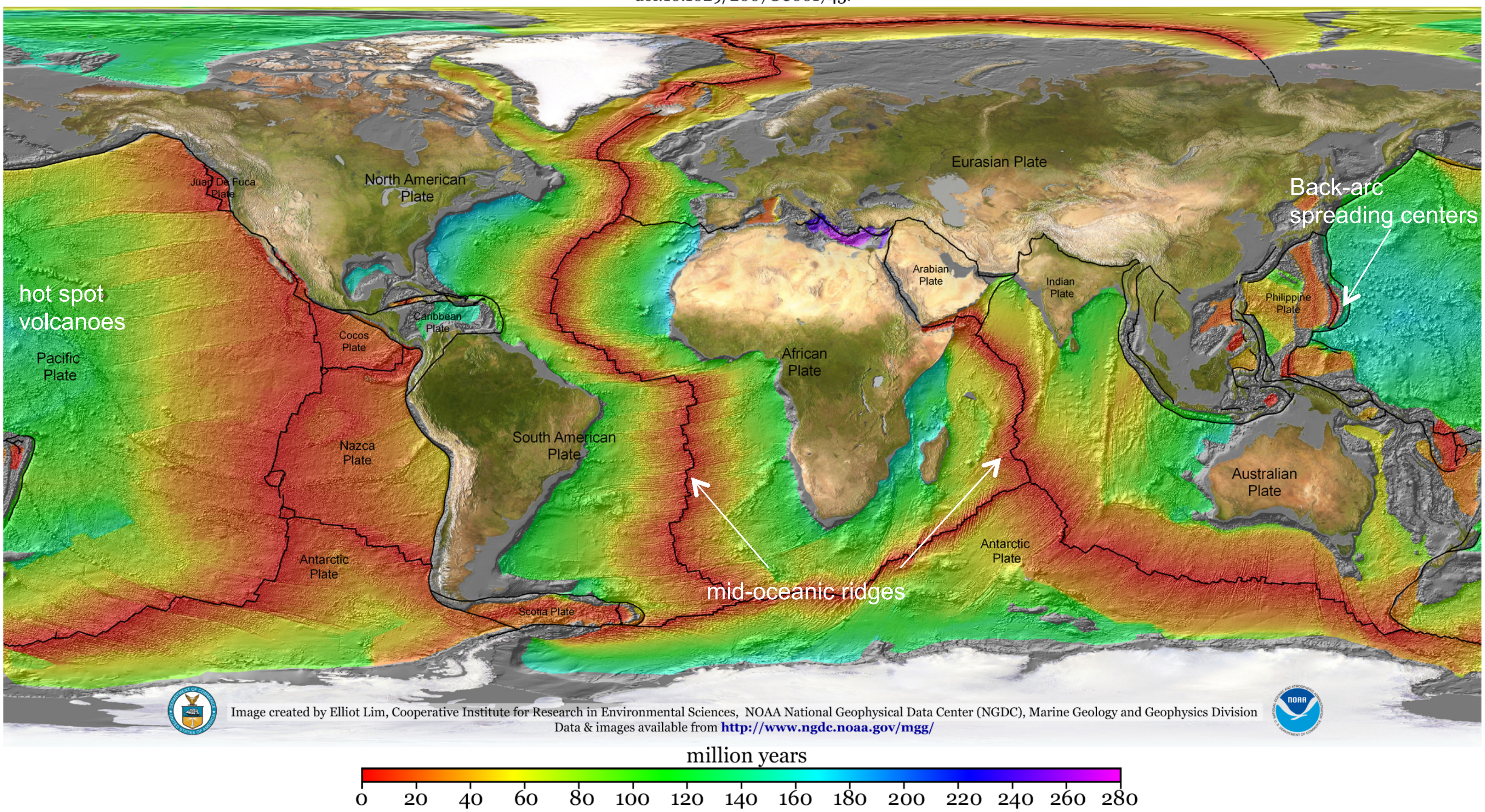


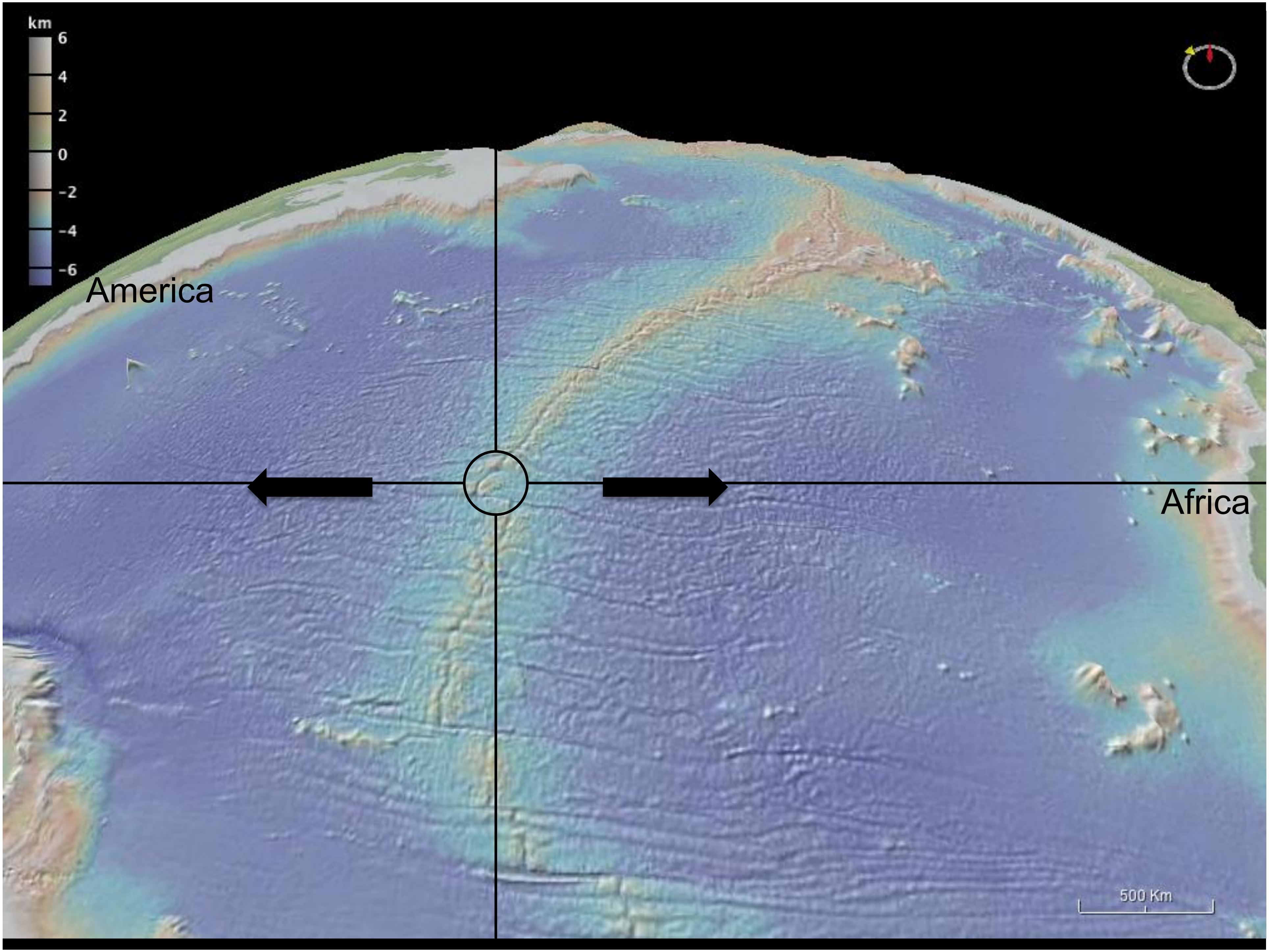
From: Price and Giovannelli, 2017. Reference Module in Earth Systems and Environmental Sciences

Age of Oceanic Lithosphere (m.y.)

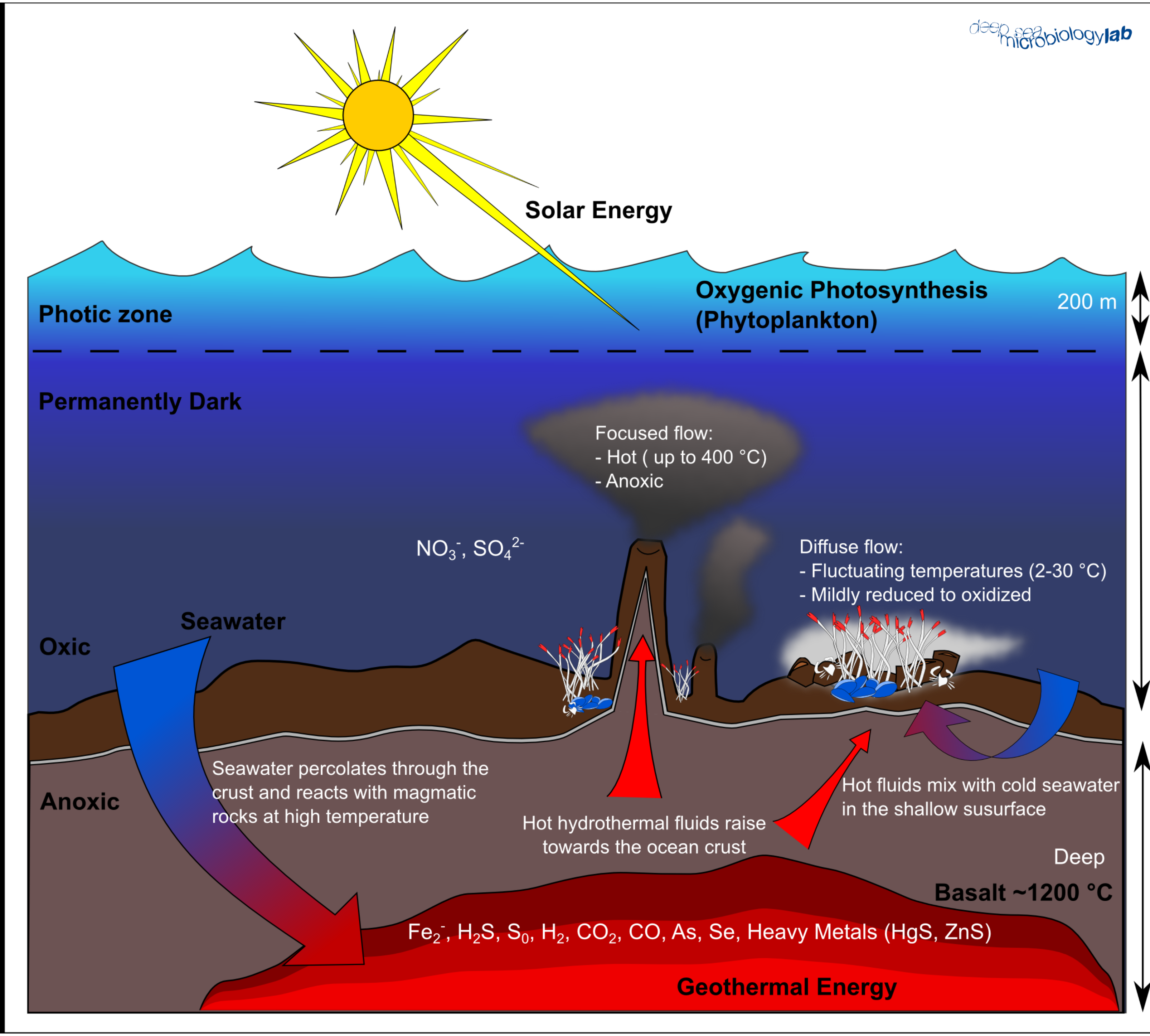
Data source:

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.





km
6
4
2
0
-2
-4
-6
America
Africa
500 Km



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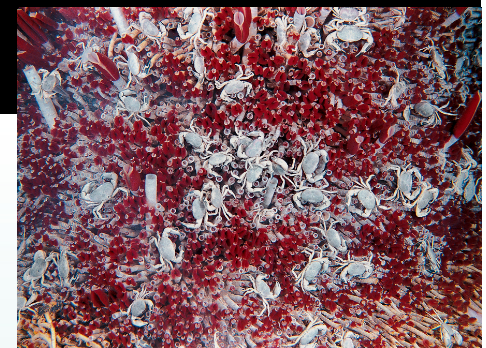
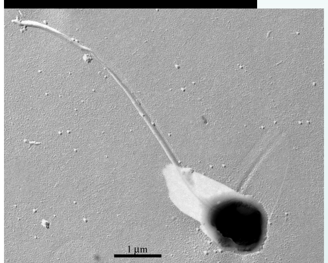
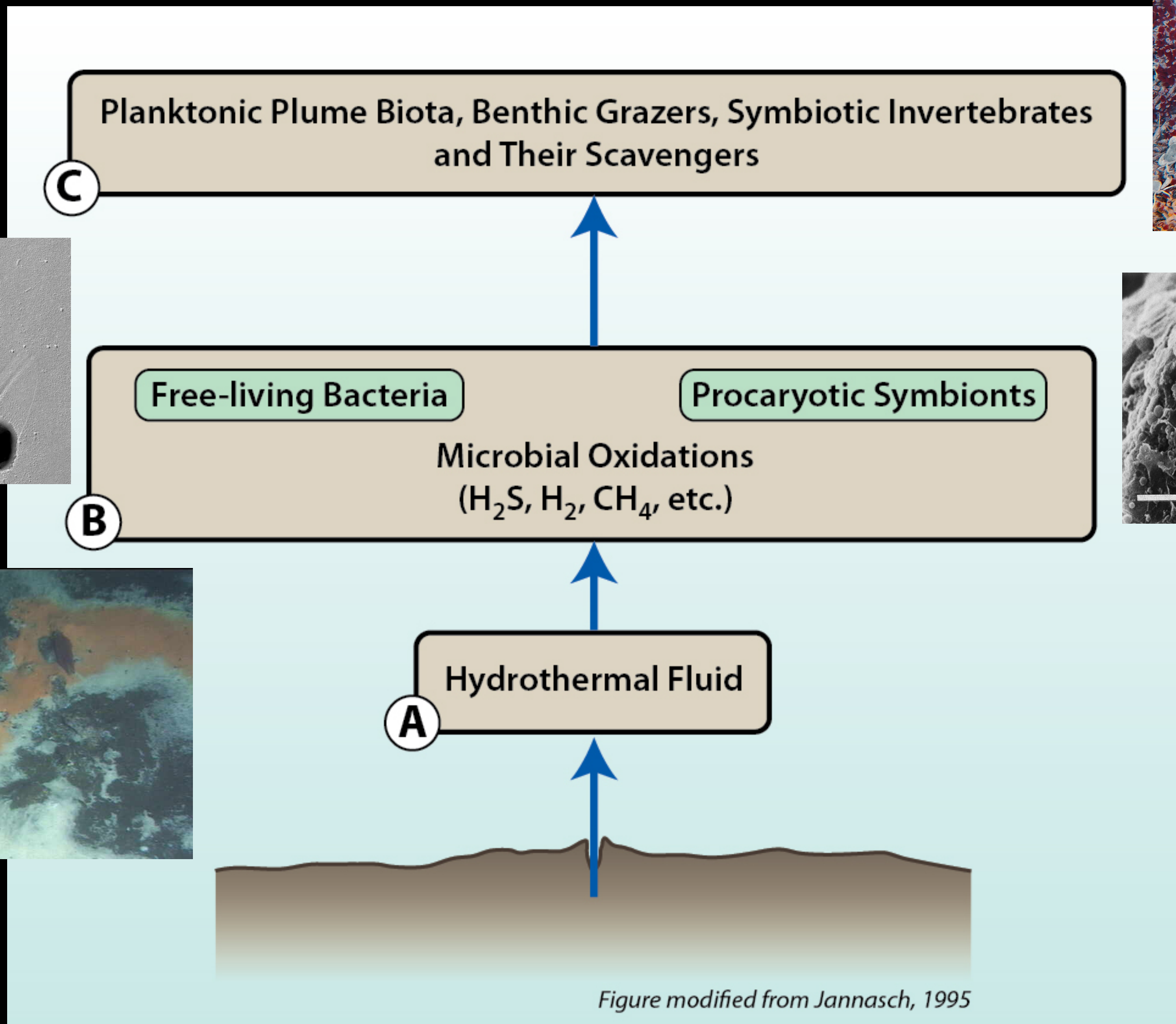
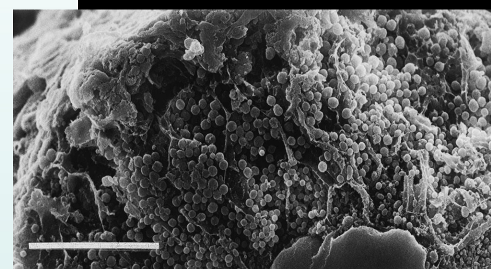
Solar Energy
Photic zone
Oxygenic Photosynthesis
(Phytoplankton)
200 m
Permanently Dark
Focused flow:
- Hot (up to 400 °C)
- Anoxic
NO_3^-, SO_4^{2-}
Diffuse flow:
- Fluctuating temperatures (2-30 °C)
- Mildly reduced to oxidized
Seawater
Oxic
Anoxic
Seawater percolates through the crust and reacts with magmatic rocks at high temperature
Hot fluids mix with cold seawater in the shallow susurface
Hot hydrothermal fluids raise towards the ocean crust
Deep
Basalt ~1200 °C
Fe_2^-, H_2S, S_0, H_2, CO_2, CO, As, Se, Heavy Metals (HgS, ZnS)
Geothermal Energy

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

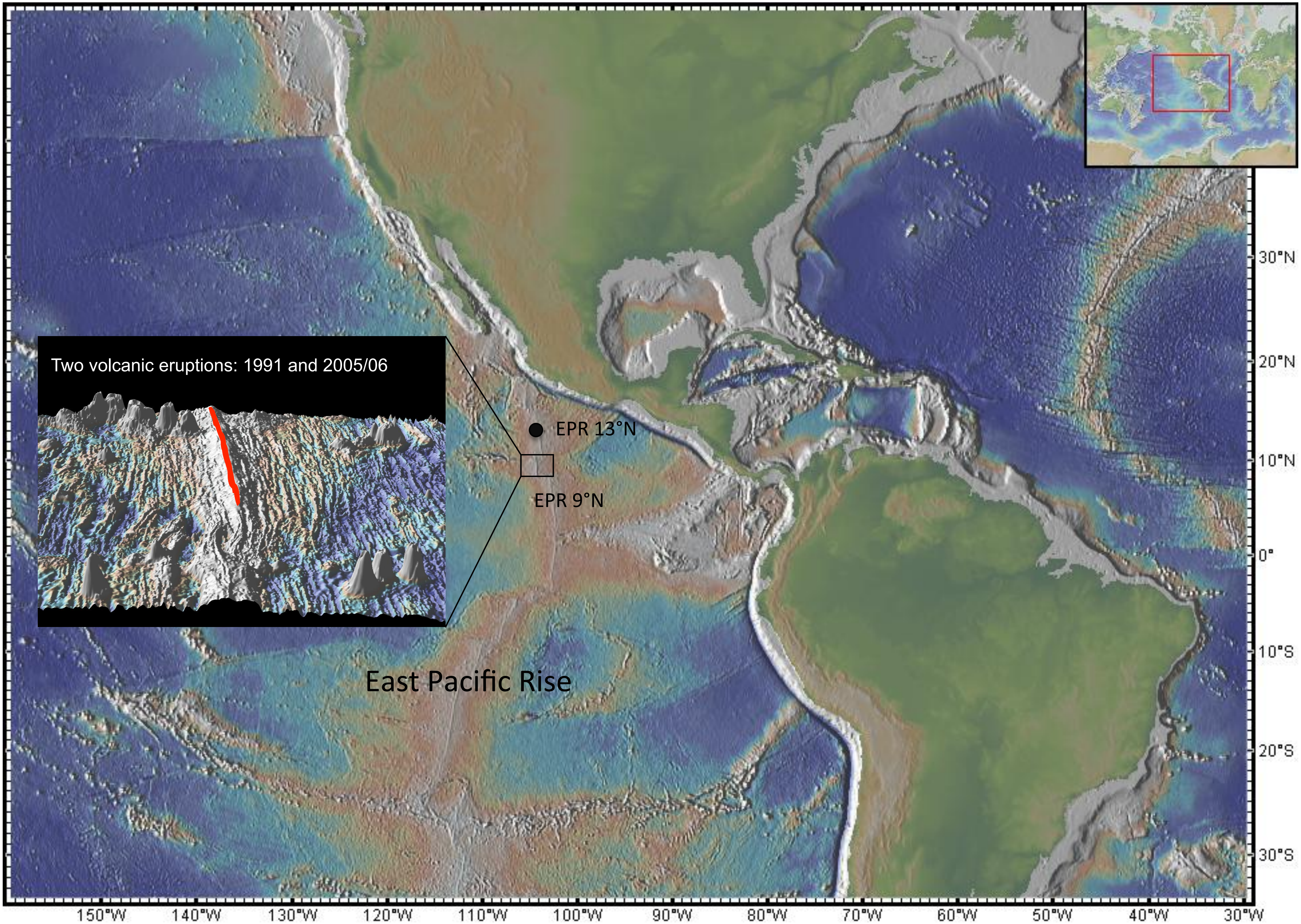
Adapted from: Sievert and Vetriani, 2012. *Oceanography* 25:219-233

Rilevanza delle sorgenti idrotermali oceaniche

- Stime sui flussi di fluidi idrotermali indicano che **l'intero volme degli oceani passa attraverso la crosta oceanica in circa 10 milioni di anni.**
- Le sorgenti idrotermali oceaniche funzionano come sistemi idraulici naturali per il trasporto di calore dall'interno del pianeta alla litosfera, idrosfera e biosfera. **La circolazione idrotermale influenza la composizione della crosta oceanica e regola la chimica globale degli oceani.**
- Le sorgenti idrotermali oceaniche supportano organismi che hanno sviluppato adattamenti biochimici unici alle alte temperature e a condizioni ambientali che noi consideriamo tossiche. **Lo studio di questi organismi ci puo` rivelare informazioni sull'evoluzione della vita sulla terra.**

Conclusioni

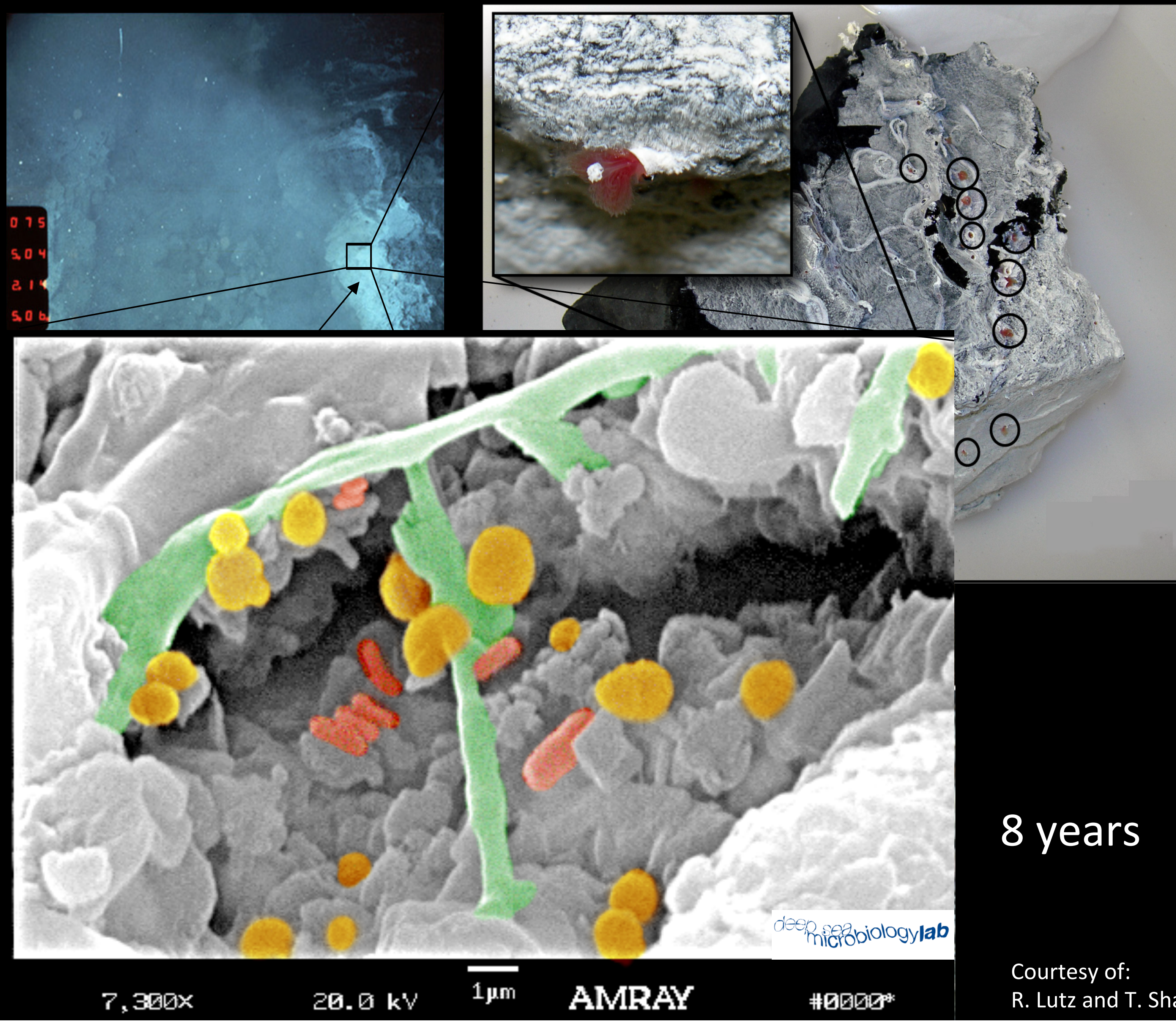
La fonte di energia per la vita alle sorgenti idrotermali oceaniche e` di origine geotermale. I microorganismi in questi ambienti utilizzano sostanze chimiche ridotte come fonte di energia (chemolitotrofi) al posto dei fotoni (fototrofi) e fissano la CO_2 di origine vulcanica (chemolitoautotrofi).



Two volcanic eruptions: 1991 and 2005/06
EPR 13°N
EPR 9°N
East Pacific Rise
30°N
20°N
10°N
0°
10°S
20°S
30°S
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

MkF “Crab Spa” 9°N EPR - Alvin Dive 4301, January 2007

Resetting the Clock: Biological Community Succession at 9°N Following the Eruption

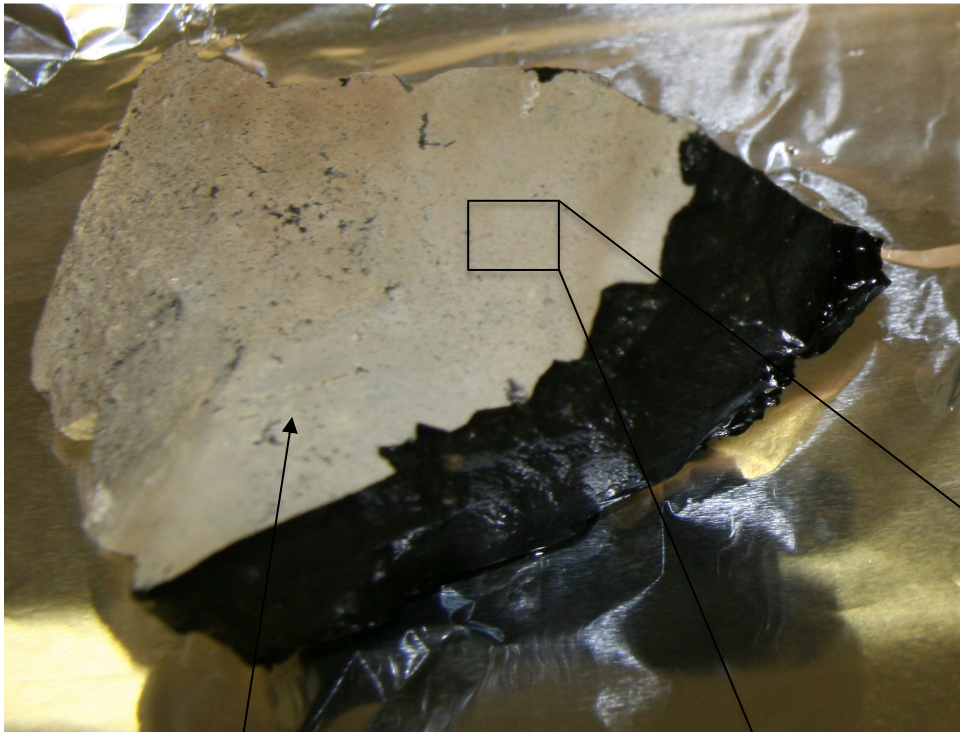


Courtesy of:
R. Lutz and T. Shank

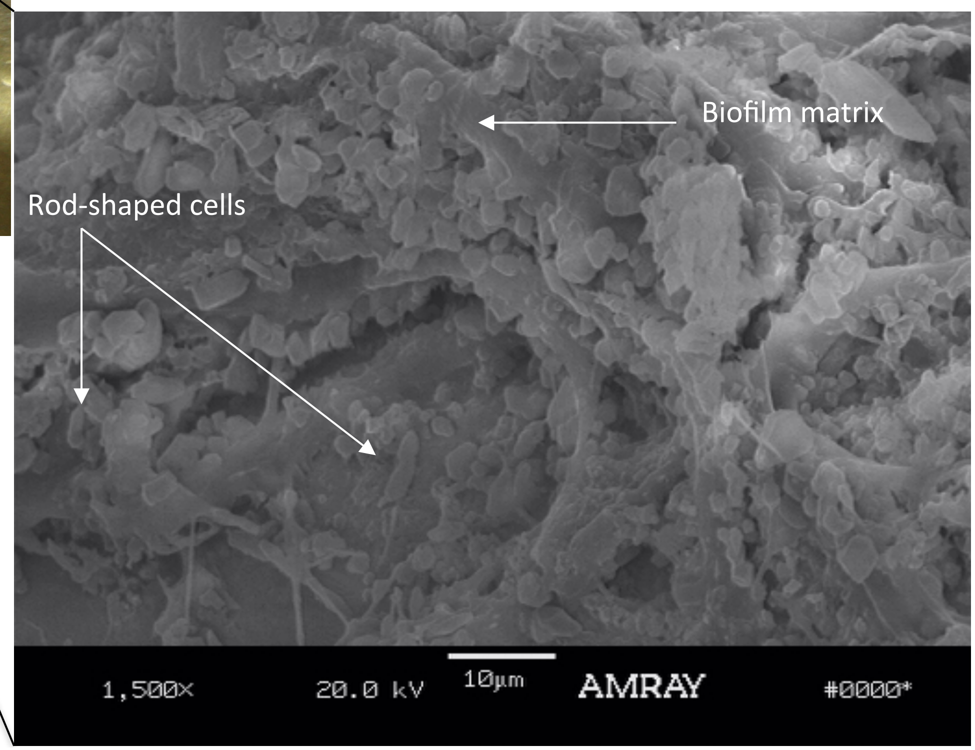
Chemosynthetic biofilms dominate newly formed diffuse-flow vents

Conditions in proximity of newly formed vents:

- Fluctuating temperatures (~2-60°C)
- Fluctuating redox state (mildly reduced to oxidized)
- High turbulence, vigorous flow
- Elevated H_2S



White microbial films attach to native basalt



Quali microorganismi ci sono?

- Arricchimenti e isolamenti di colture pure

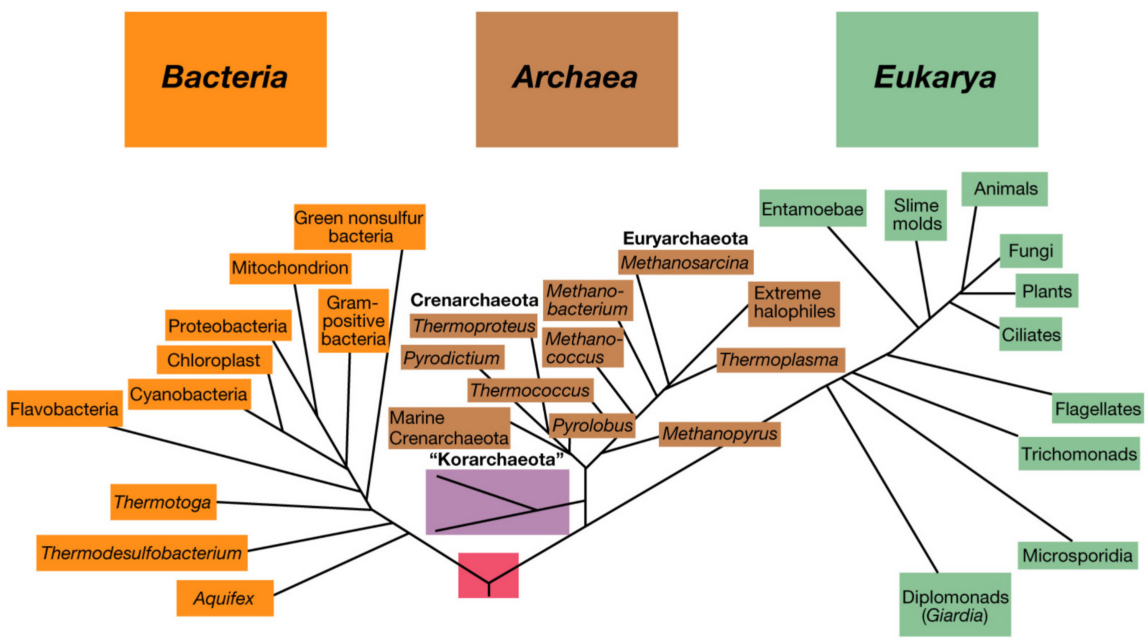
  Rivela le caratterisiche metaboliche e fisiologiche dei microorganismi

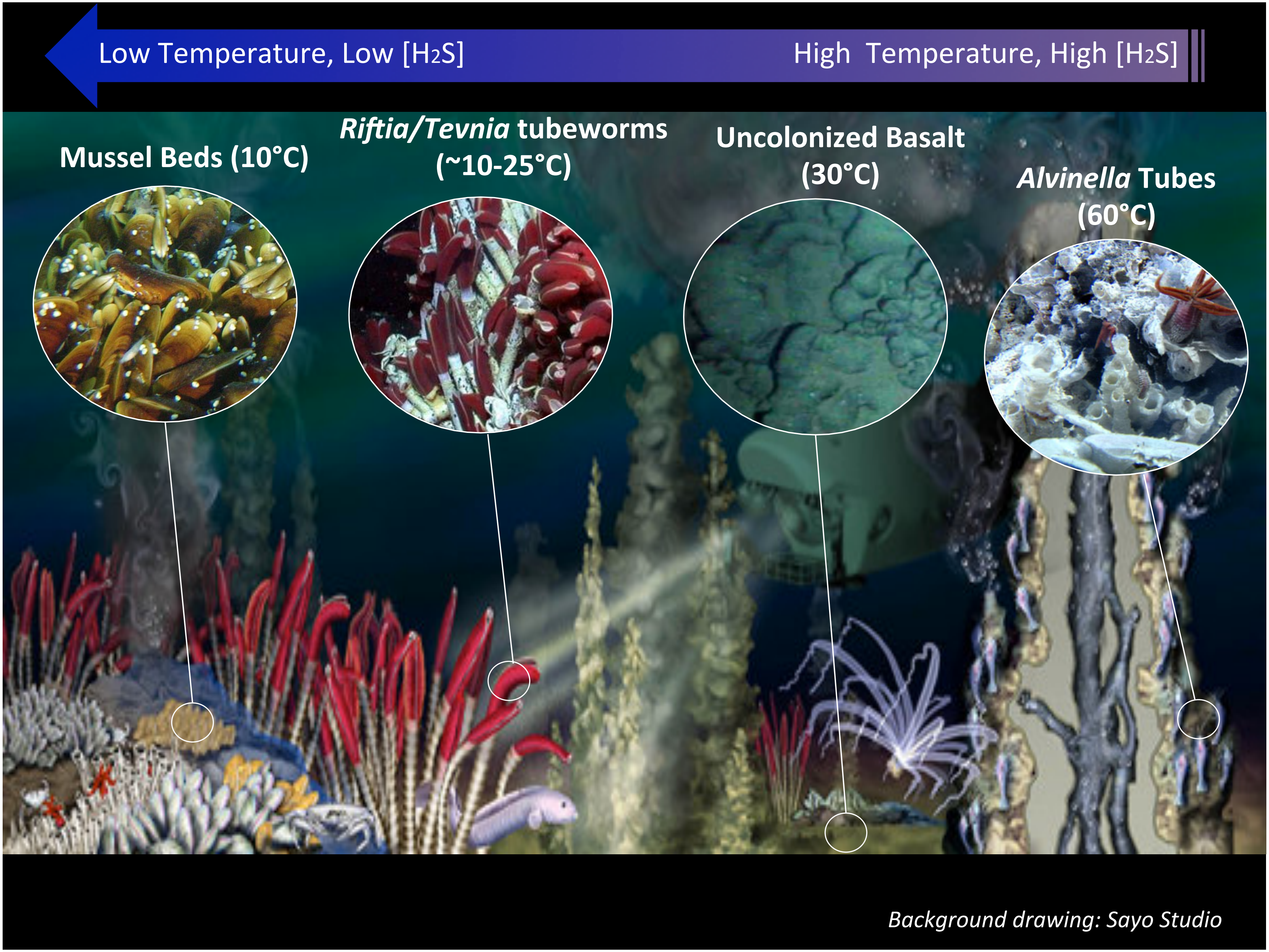
  Non descrive la vera diversita` delle comunita` microbiche naturali

- Analisi delle sequenze del gene per il 16S rRNA

  Rivela la diversita` delle comunita` microbiche naturali

  Non rivela le caratterisiche metaboliche e fisiologiche dei microorganismi

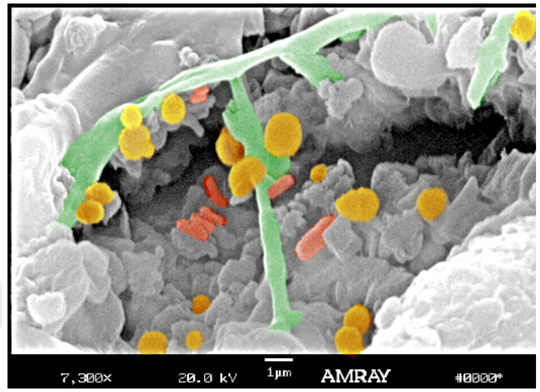




Low Temperature, Low [H2S]
High Temperature, High [H2S]
Mussel Beds (10°C)
Riftia/Tevnia tubeworms (~10-25°C)
Uncolonized Basalt (30°C)
Alvinella Tubes (60°C)
Background drawing: Sayo Studio

Assess the community composition of the total and active fractions of the bacterial biofilms

In situ collection and preservation of the natural community



Total community

DNA extraction

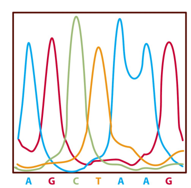
PCR amplification of the 16S rRNA gene followed by parallel sequencing (Pyrotag)

Active fraction of the community

RNA extraction

Reverse transcription to generate cDNA

PCR amplification of the 16S rRNA transcript followed by parallel sequencing (Pyrotag)

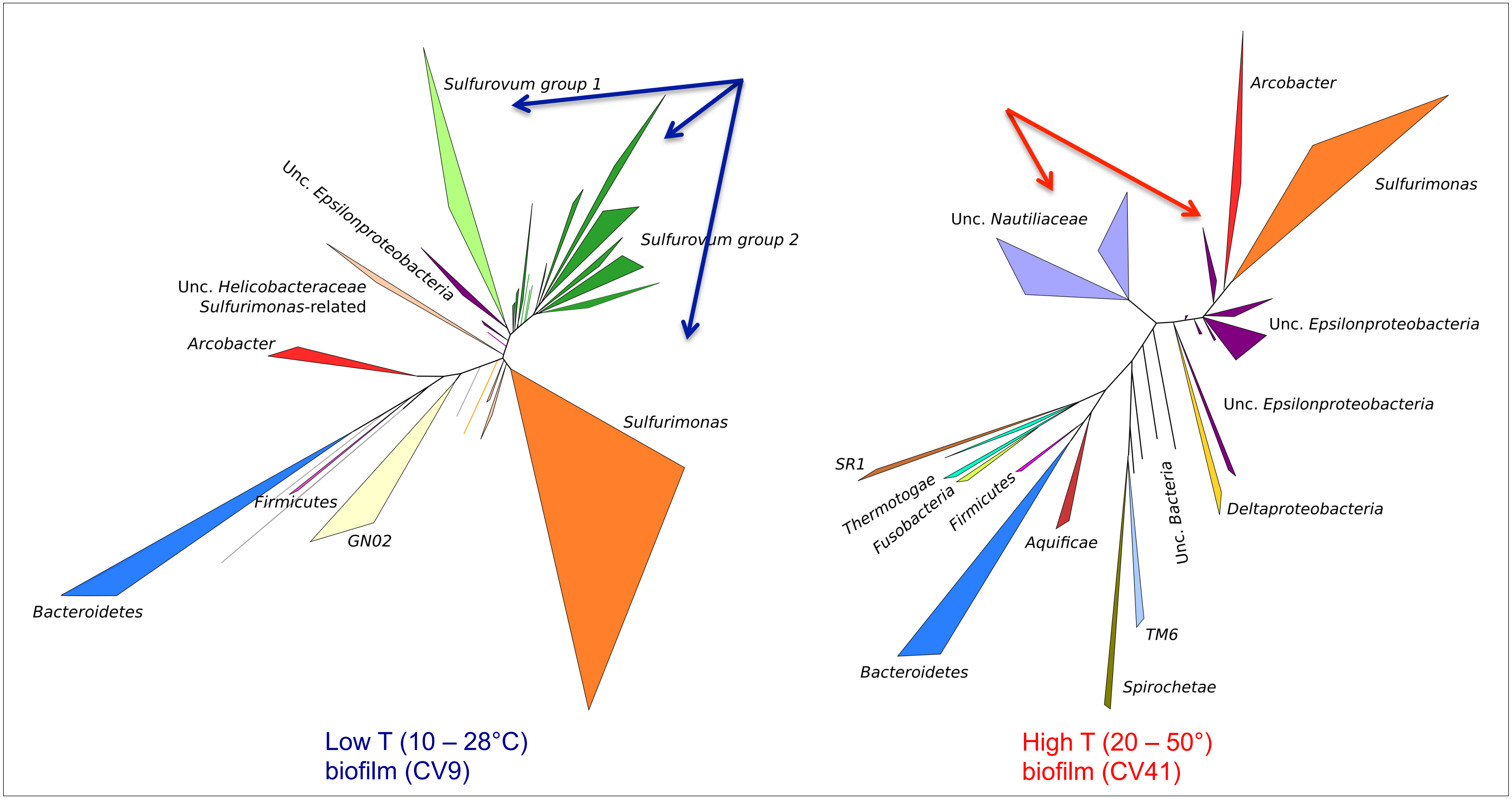


Sequence data

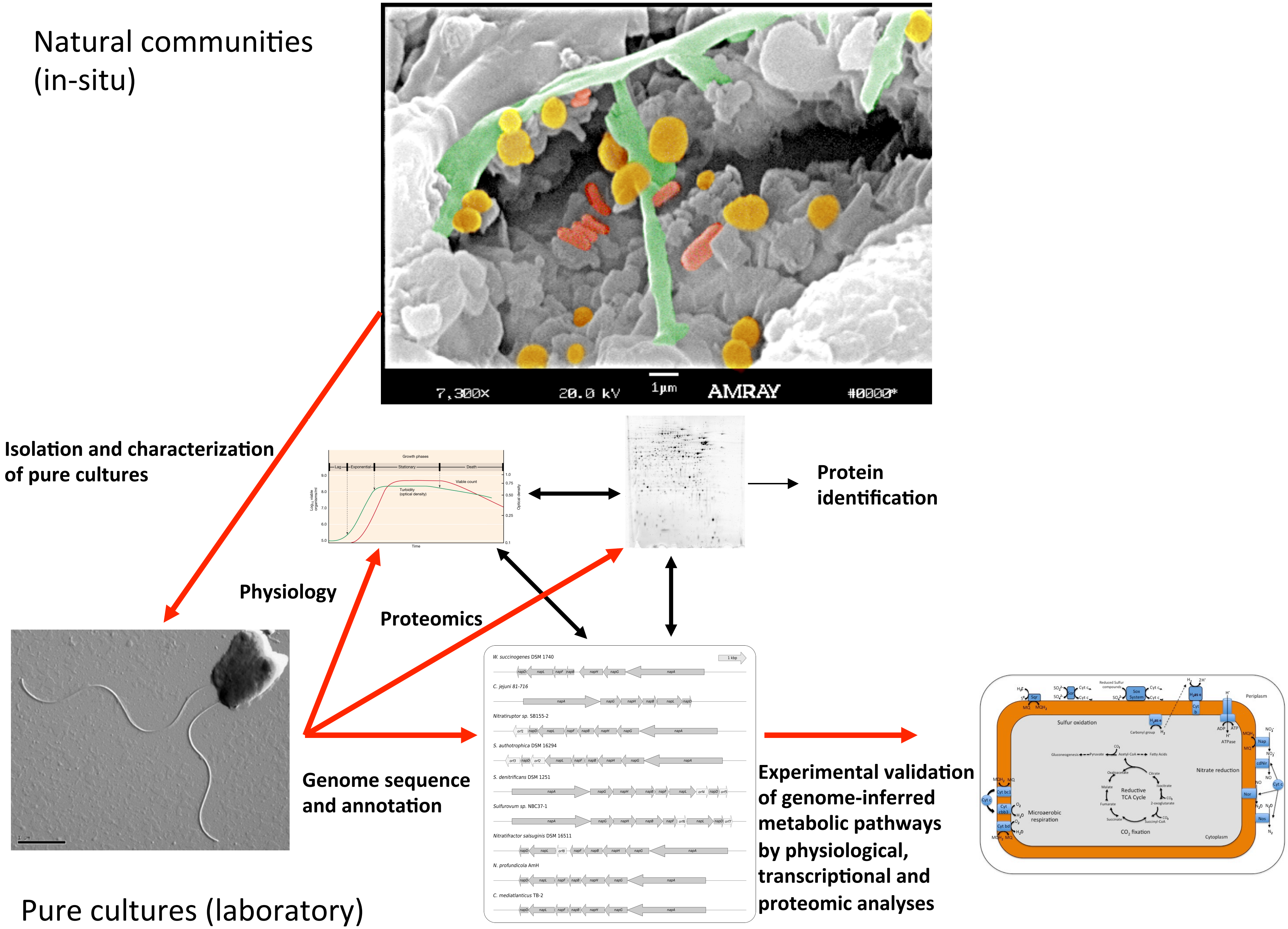
Analysis via the QIIME (Quantitative Insights Into Microbial Ecology) pipeline

Prokaryotic universal primers for PCR V4 amplicons (~ 300 bp)
Ion Torrent Platform
Average reads/sample: 72584
Analysis run at 97% identity
Total No. of unique OTUs: 38432

Epsilonproteobacteria dominate the active fraction of bacteria of the low (CV9) and high (CV41) temperature biofilm communities

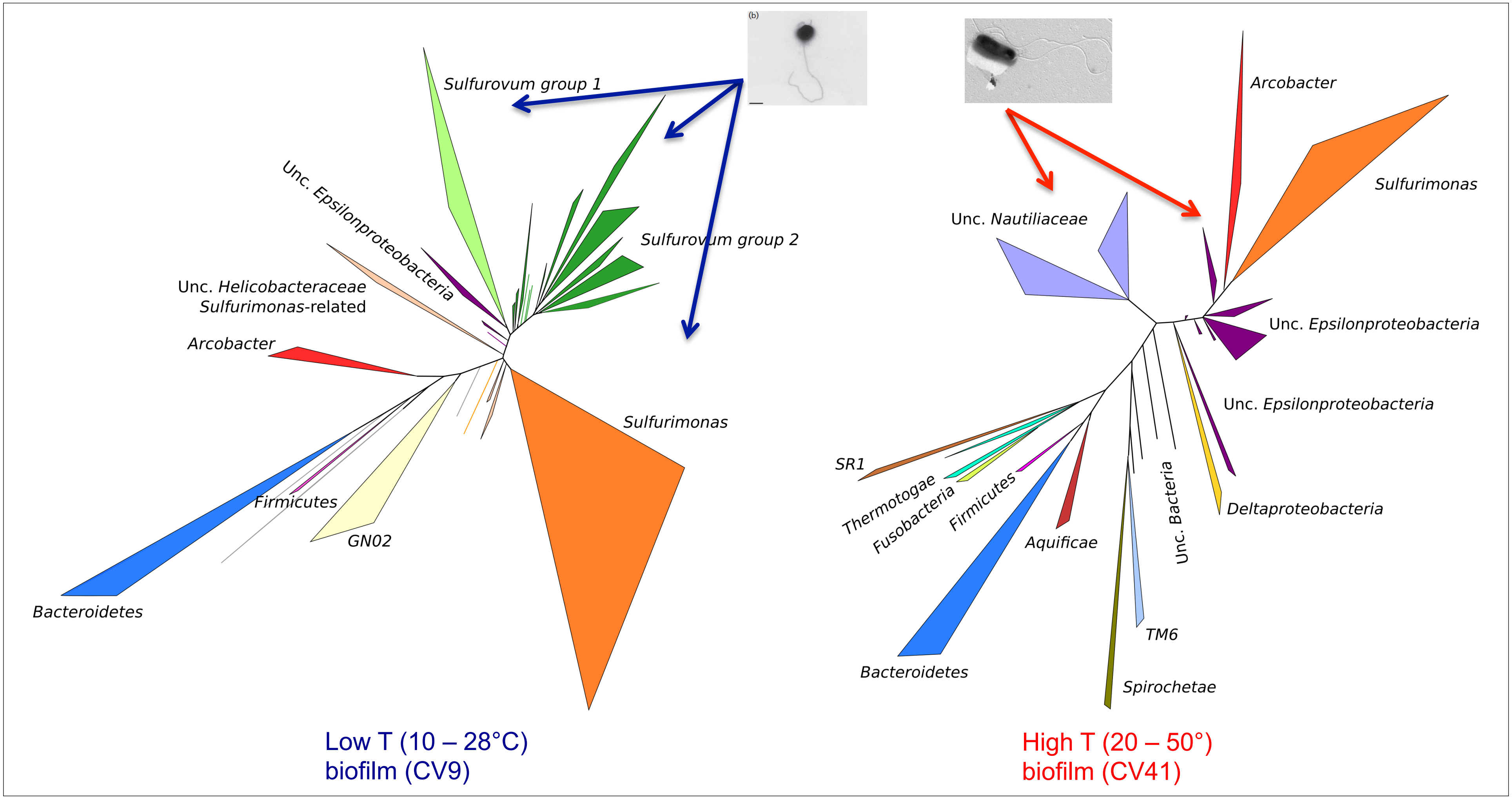


Vetriani, Borin, Giovannelli et al., in preparation



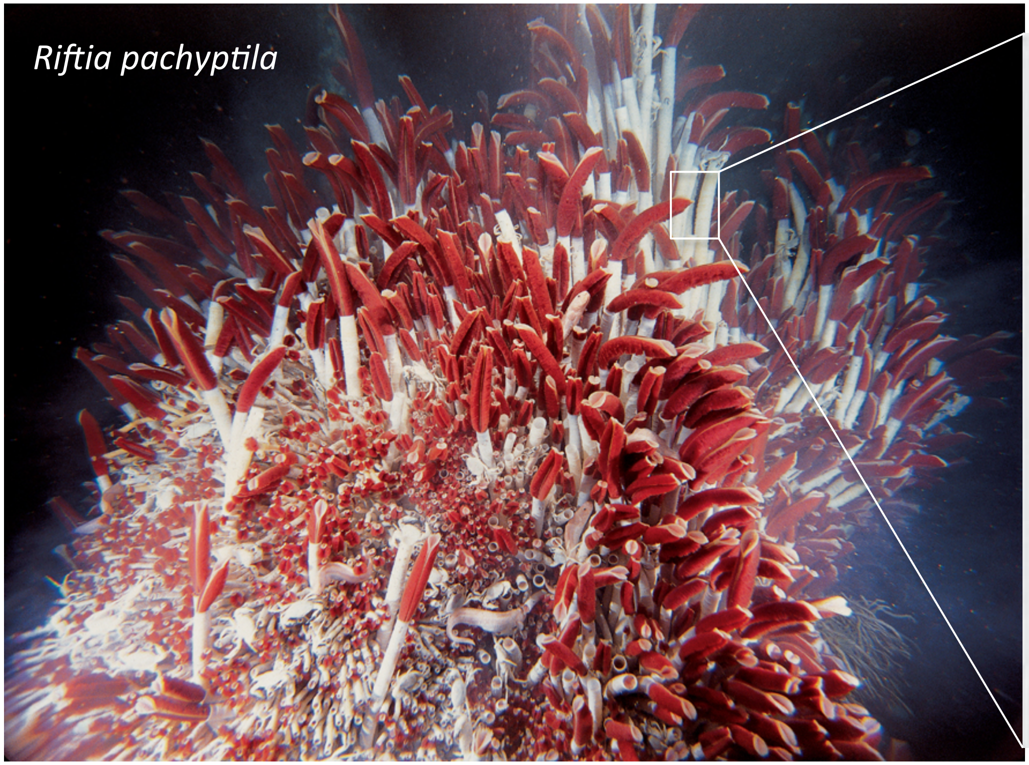
Natural communities (in-situ)
7,300x
20.0 kV
1µm
AMRAY
#0000*
Isolation and characterization of pure cultures
Protein identification
Physiology
Proteomics
Genome sequence and annotation
Experimental validation of genome-inferred metabolic pathways by physiological, transcriptional and proteomic analyses
Pure cultures (laboratory)

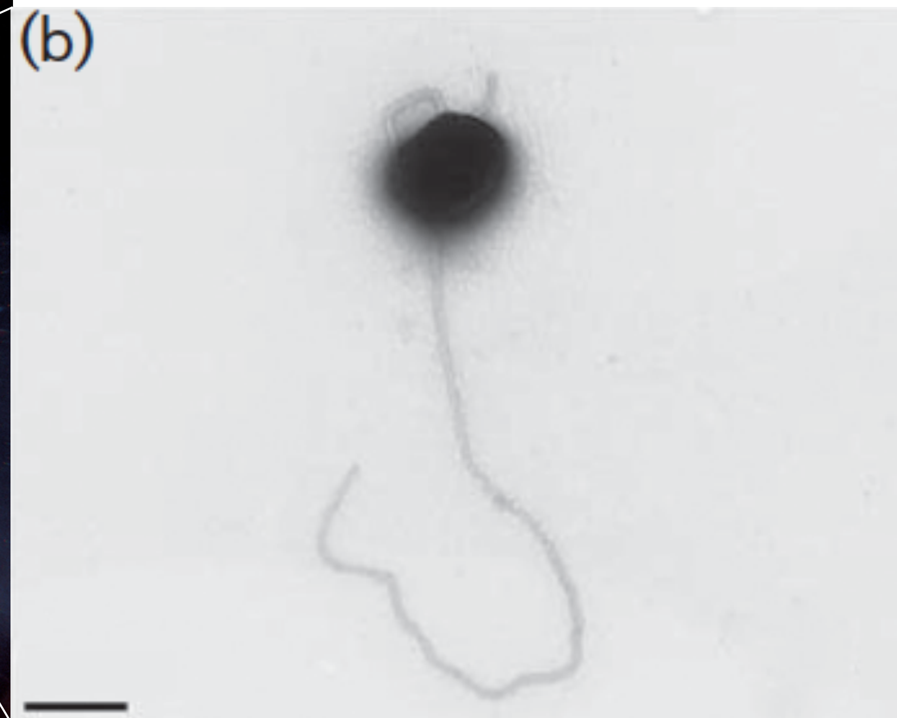
Active bacteria of the low (CV9) and high (CV41) temperature biofilm communities
Representative isolates were isolated and cultured in the laboratory



Vetriani, Borin, Giovannelli et al., in preparation

Sulfurovum riftiae DSM 101780^T





Epsilonproteobacterium
$T_{opt.}$35°C, t_g: 180 min
Gram negative
Anaerobic
Obligate chemolithoautotroph
Electron donor: Thiosulfate, S^0
Electron acceptors: NO_3^-; reduced to N_2

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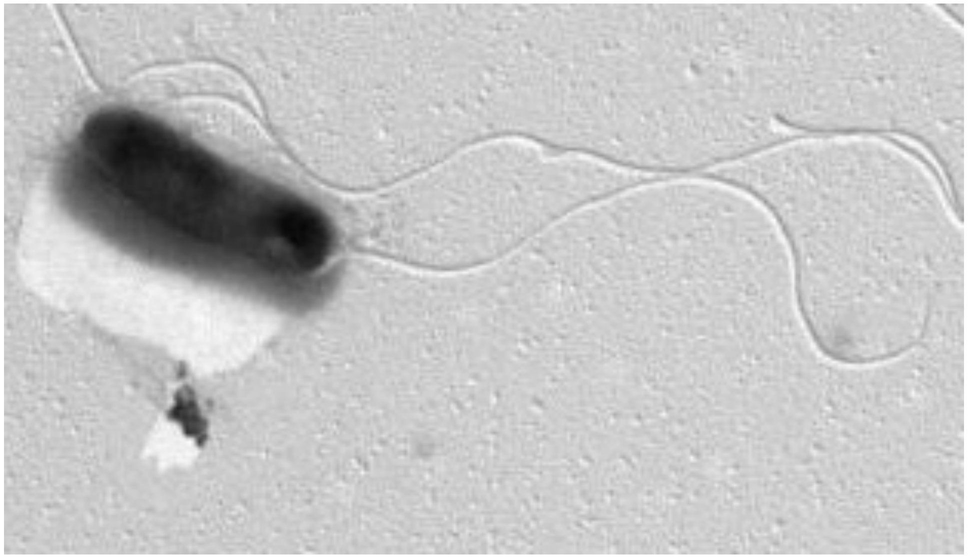
Culture Collection

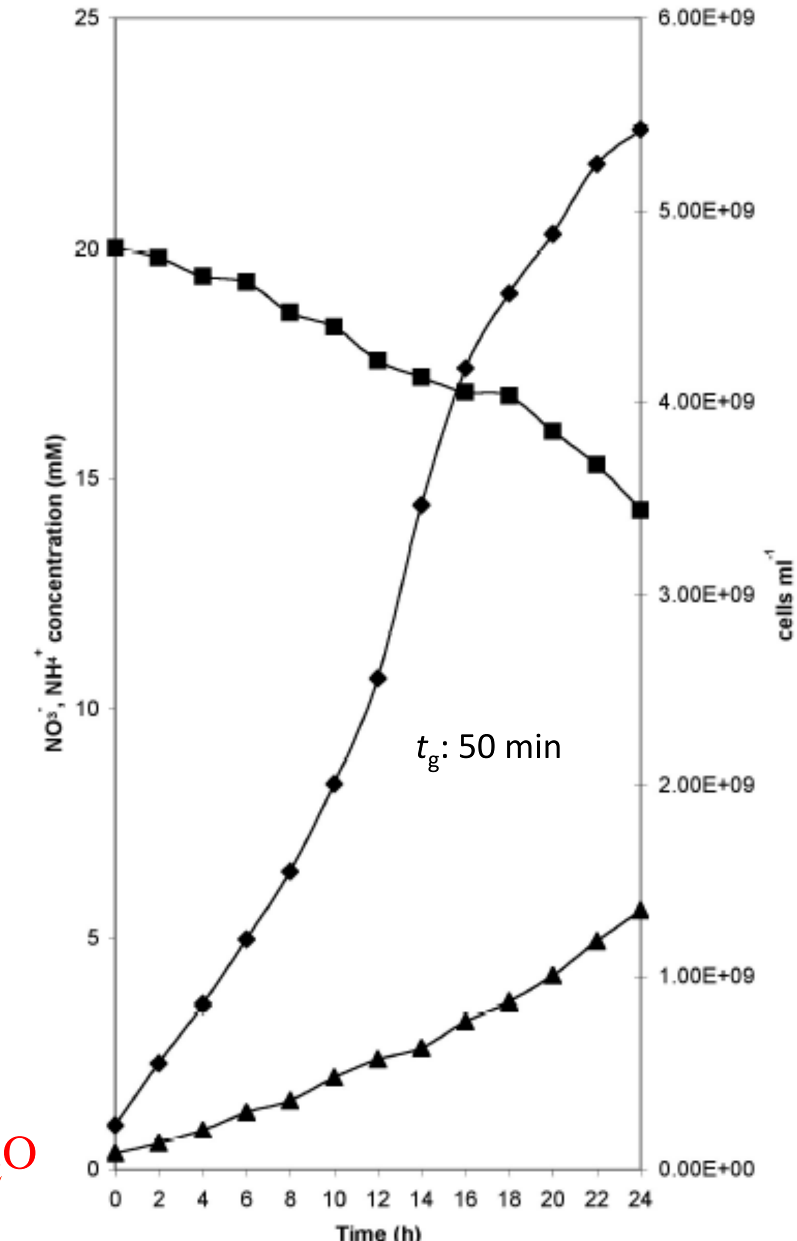
Giovannelli et al., 2016. *IJSEM* 54:1477

Caminibacter mediatlanticus DSM 16658^T



Epsilonproteobacterium
$T_{opt.}$55°C
Gram negative
Strictly anaerobic
Obligate chemolithoautotroph
Electron donor: H_2
Electron acceptors: NO_3^-; reduced to NH_4^+
S^0; reduced to H_2S

$$CO_2 + 6H_2 + H^+ + NO_3^- \rightarrow [CH_2O] + NH_4^+ + OH^- + 3H_2O$$
$$CO_2 + 3H_2 + S^0 \rightarrow [CH_2O] + H_2S + H_2O$$

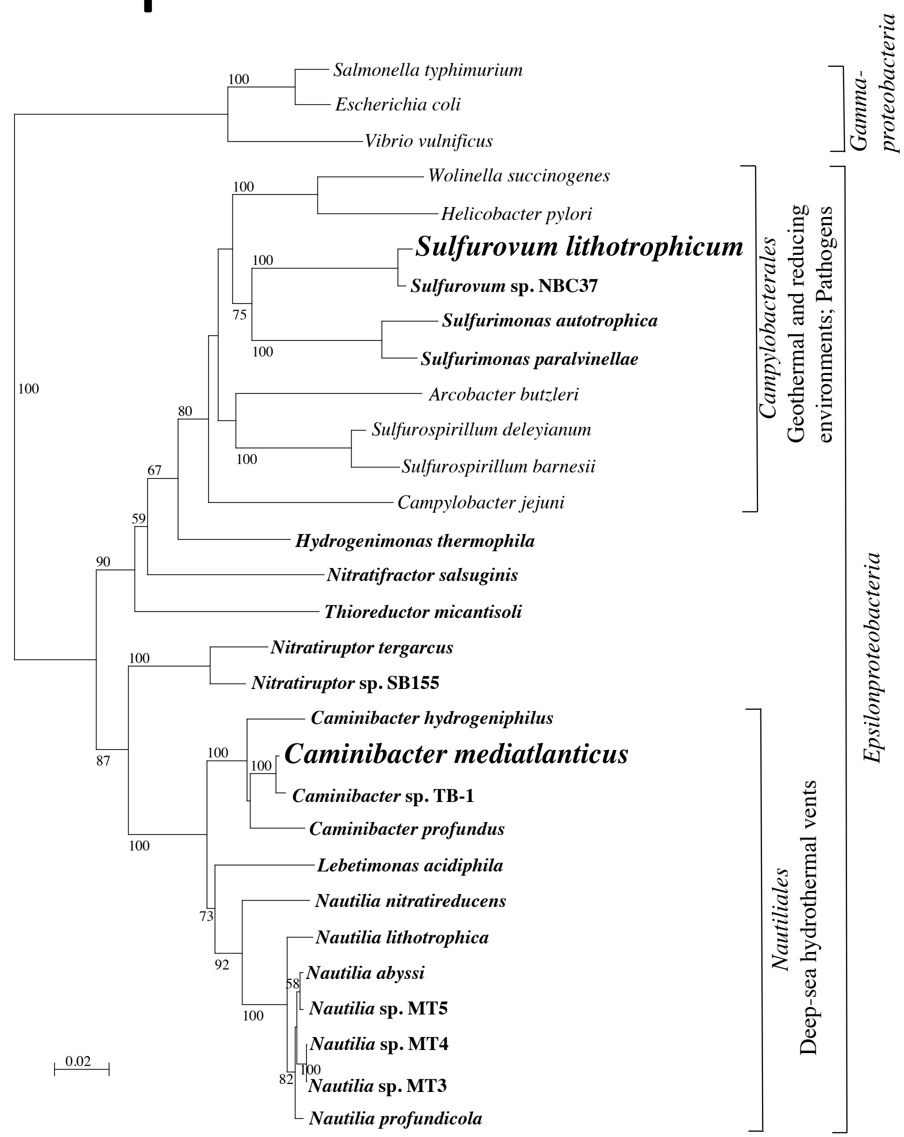


Voordeckers et al., 2005. *IJSEM* 55:773

deep sea microbiologylab
Culture Collection

Chi sono gli Epsilonproteobatteri

- Sono uno dei gruppi piu` abbondanti alle sorgenti idrotermali oceaniche
- Sono i primi colonizzatori quando si forma una nuova sorgente
- Includono organismi che vivono in ambienti sulfidici ma anche commensali di mammiferi e patogeni umani
- Gli Epsilonproteobatteri piu` antichi appartengono ai *Nautiliales*, che include anaerobi obbligati isolati esclusivamente dalle sorgenti idrotermali oceaniche. Le altre specie, non solo di provenienza marina, si sono evolute dai *Nautiliales* in tempi piu` recenti



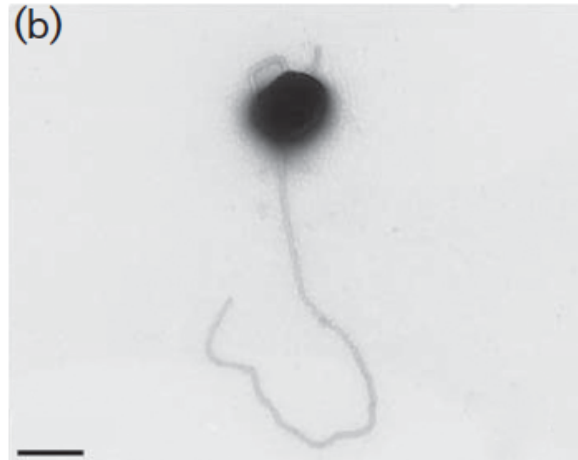
Vetriani et al., 2014. *ISME J.* 8:1510

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
Epsilonproteobacteria							
Sulfurovum lithotrophicum	MOT, Iheya, sediments	28–30	$S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Inagaki et al. (2004)
Sulfurimonas paralvinellae	MOT, Iheya, *Paralvinella*	30	H_2, $S_2O_3^=$, S^0	NO_3^-, O_2	N_2	CO_2	Takai et al. (2006b)
Sulfurimonas autotrophica	MOT, Hatoma Knoll, sediments	25	$S_2O_3^=$, S^0, H_2S	O_2		CO_2	Inagaki et al. (2003)
Thioreductor micantisoli	MOT, Iheya, sediments	32	H_2	NO_3^-, S^0	NH_4^+	CO_2	Nakagawa et al. (2005a)
Nautilia lithotrophica	EPR, 13°N, *Alvinella*	53	H_2, Formate	S^0		CO_2, Formate	Miroshnichenko et al. (2002)
Nautilia nitratireducens	EPR, 9°N, chimney	55	H_2, Formate, acetate, complex organic substrates	NO_3^-, S^0, $S_2O_3^=$, $SeO_4^=$	NH_4^+	CO_2, Formate	Pérez-Rodríguez et al. (2009)
Nautilia profundicola	EPR, 9°N, *Alvinella*	40	H_2, Formate	S^0		CO_2, Formate	Smith et al. (2008)
Nautili abyssi	EPR, 13°N, chimney	60	H_2	S^0		CO_2, Yeast Extract, Peptone	Alain et al. (2009)
Hydrogenimonas thermophila	CIR, Kairei Field, colonizer	55	H_2	NO_3^-, S^0, O_2	NH_4^+	CO_2	Takai et al. (2004c)
Nitratiruptor tergarcus	MOT, Iheya, chimney	55	H_2	NO_3^-, S^0, O_2	N_2	CO_2	Nakagawa et al. (2005b)
Nitratifractor salsuginis	MOT, Iheya, chimney	37	H_2	NO_3^-, O_2	N_2	CO_2	Nakagawa et al. (2005)
Caminibacter profundus	MAR, Rainbow, vent cap	55	H_2	NO_3^-, S^0, O_2	NH_4^+	CO_2	Miroshnichenko et al. (2004)
Caminibacter mediatlanticus	MAR, Rainbow, chimney	55	H_2	NO_3^-, S^0	NH_4^+	CO_2	Voordeckers et al. (2005)
Caminibacter hydrogeniphilus	EPR, 13°N, *Alvinella*	60	H_2	NO_3^-, S^0	NH_4^+	CO_2, complex organic substrates	Alain et al. (2002)
Lebetimonas acidiphila	Mariana Arc, colonizer	50	H_2	S^0		CO_2	Takai et al. (2005)

Sievert and Vetriani, 2012. *Oceanography* 25:219-233

Assess the metabolic potential (genome) of Epsilonproteobacteria



Genomic DNA extraction

↓

Library preparation and addition of adapter sequences

↓

Parallel sequencing of genomic DNA on HiSeq platform (Illumina)

↓

Sequence data

↓

Annotation

➔ **Metabolic potential**

Analisi genomiche rivelano il metabolismo centrale degli Epsilonproteobatteri delle sorgenti idrotermali oceaniche

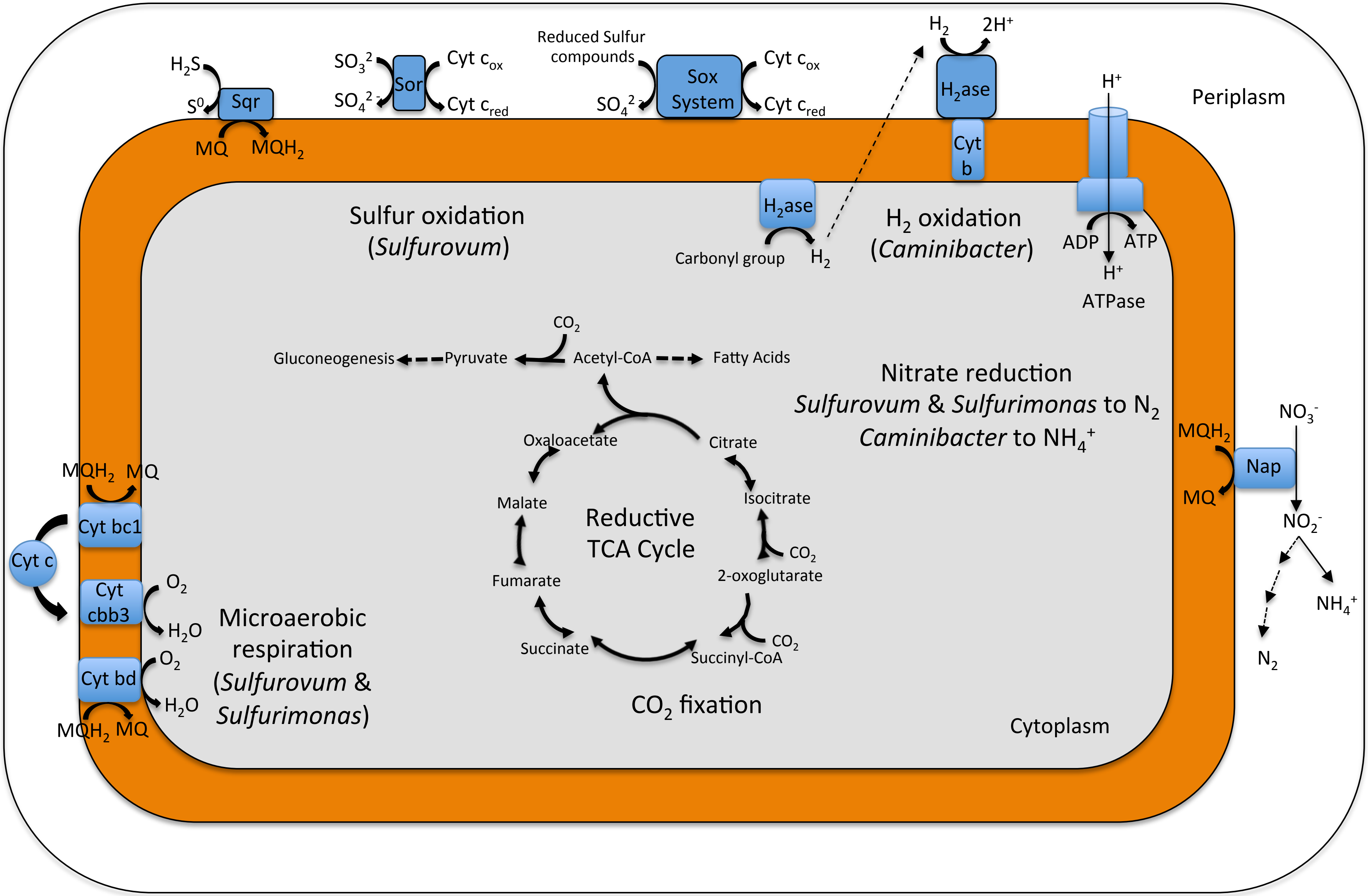


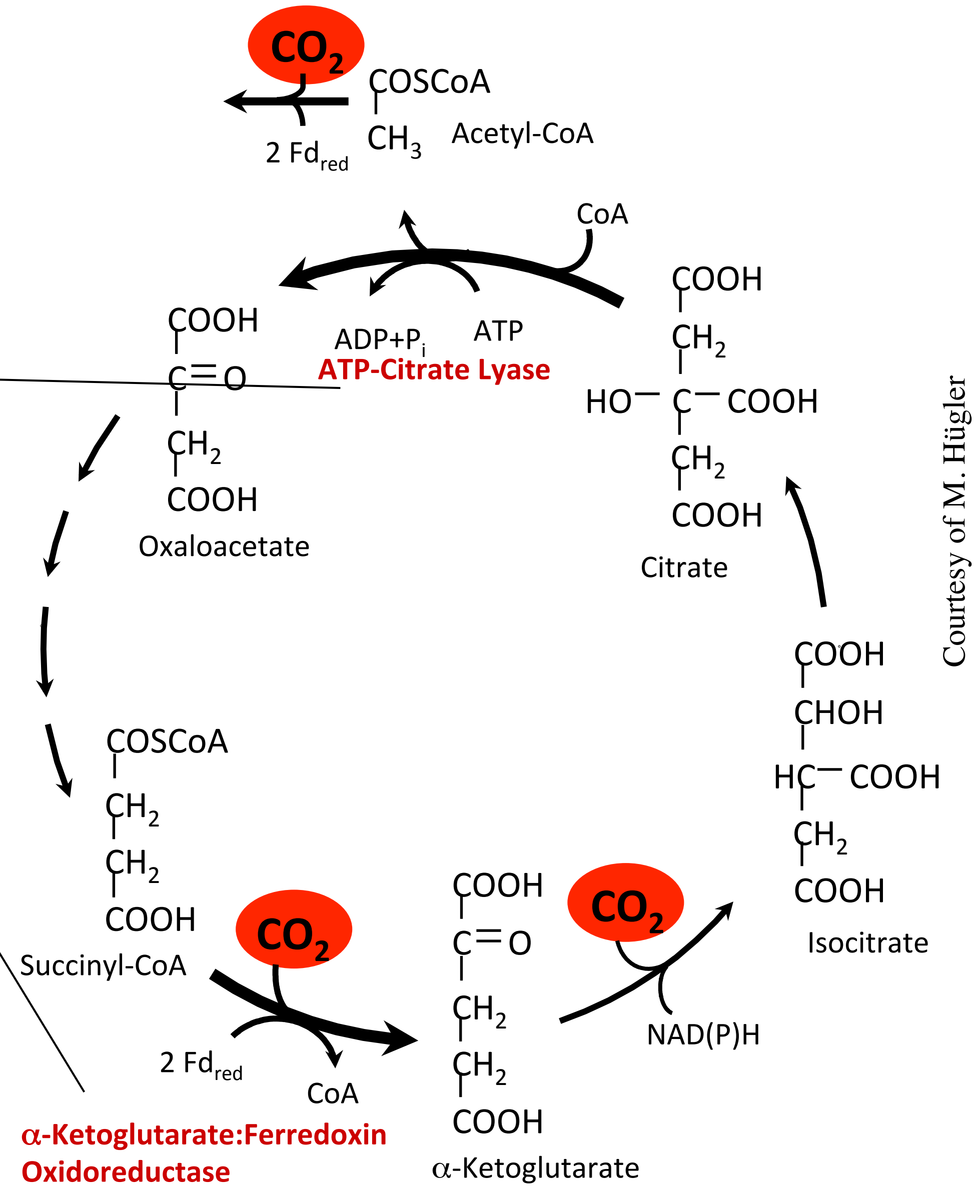
Figure created with data from Nakagawa et al., 2007, *PNAS* 104:12146-12150 and Giovannelli et al., 2011, *SIGS* 5:135-143

Metabolism:
CO_2 Fixation in *Caminibacter mediatlanticus* occurs via the Reverse TCA Cycle

Table 2 Specific activities [nmol min^{-1} (mg cell protein)$^{-1}$] of enzymes of the reductive TCA cycle in *C. mediatlanticus*

Enzyme activity tested	*Caminibacter mediatlanticus*
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 ±20%. n.d., no activity detected, detection limit <1 nmol min^{-1} (mg cell protein)$^{-1}$



Voordeckers et al., 2008. *Extremophiles* 12:627-640.