Earth's natural laboratories: What can reclamation science learn from geothermal systems in Yellowstone?

Inflated Plain,
Yellowstone Lake
Depth = 30 m
T = 70 – 90 ^{o}C
pH 5.6

Permits: YELL-SCI-5568, 5068

Funding provided by: National Science Foundation

Montana State University Thermal Biology Institute

Pacific Northwest National Laboratory
Proudly Operated by Battelle Since 1965

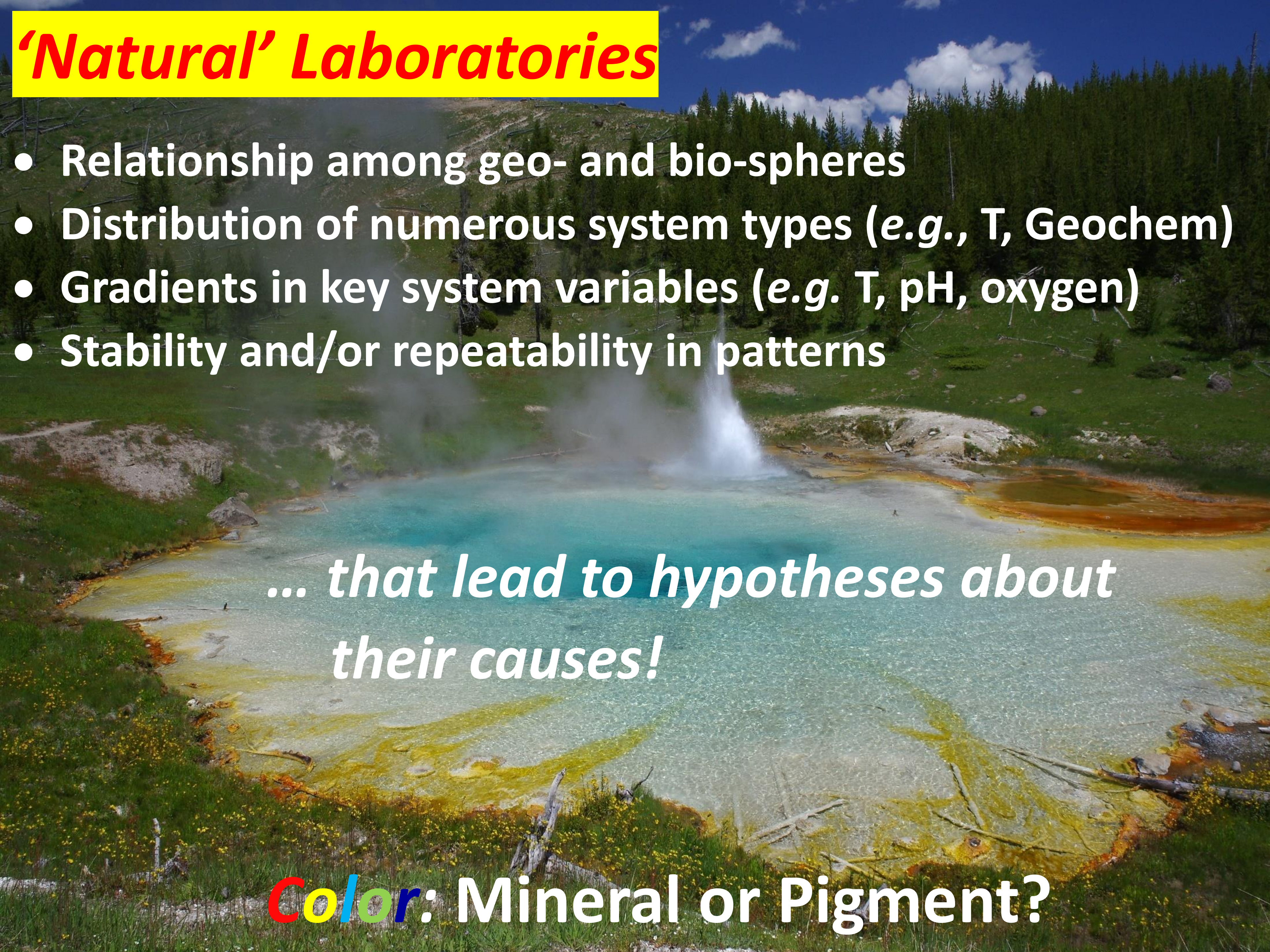
JGI DOE Joint Genome Institute
US Department of Energy Office of Science

Gordon and Betty Moore Foundation

National Park Service

American Society Mining & Reclamation
June 3-7, 2019

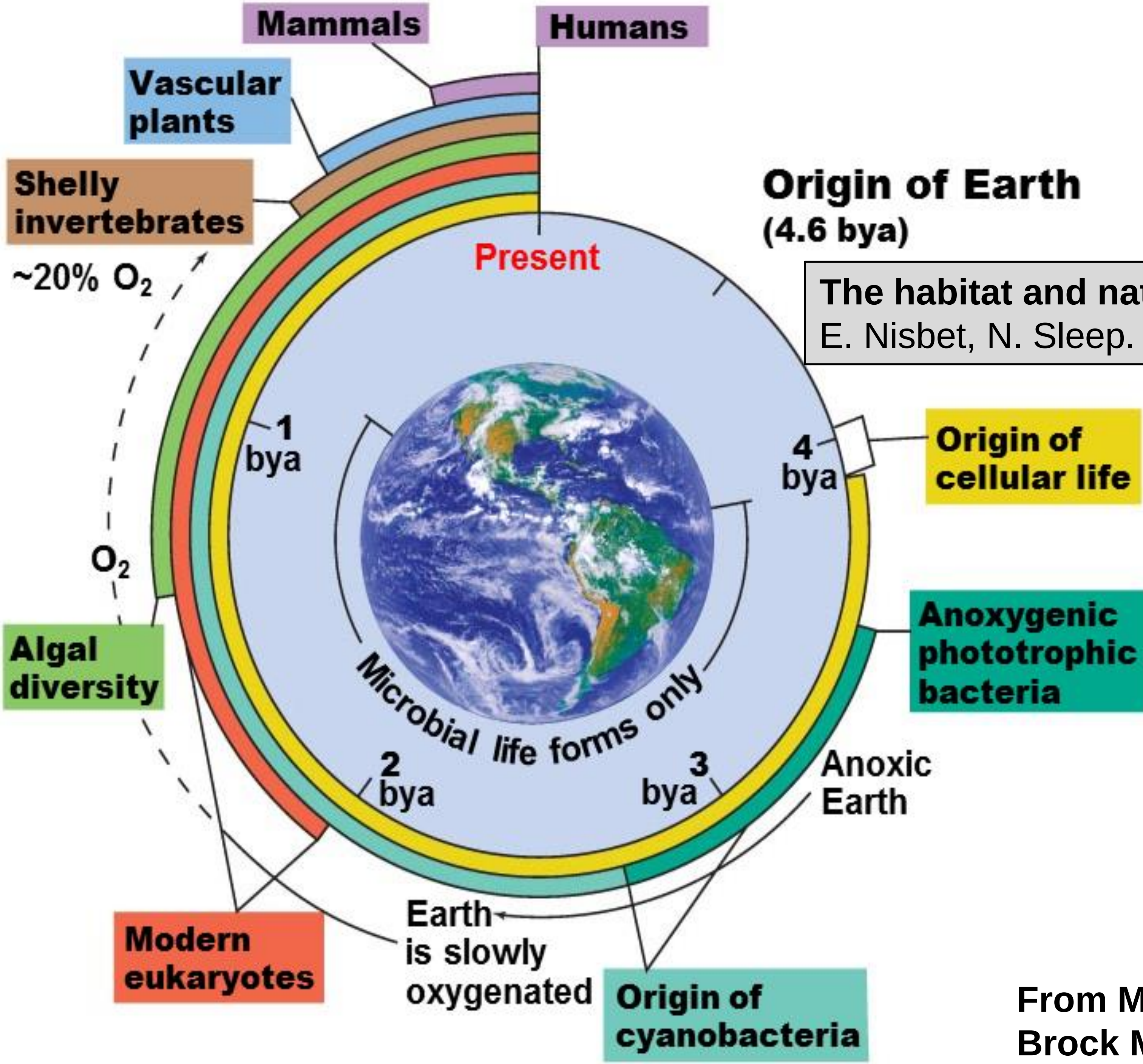
'Natural' Laboratories



- Relationship among geo- and bio-spheres
- Distribution of numerous system types (*e.g.*, T, Geochem)
- Gradients in key system variables (*e.g.* T, pH, oxygen)
- Stability and/or repeatability in patterns

… that lead to hypotheses about their causes!

Color: Mineral or Pigment?



The habitat and nature of early life.
E. Nisbet, N. Sleep. 2001. *Nature*

From Madigan, M. et al. Brock Microbiology

‘Geochemists’ Workbench’

<u>e- donors</u>

CH_4, H_2, H_2S, S, S_2O_3,

As(III), Fe(II), NH_4, CO, C

<u>e- acceptors</u>

O_2 , NO_3^- , NO_2^- , Fe(III)

As(V), SO_4^{2-}, S_2O_3 ,S,

CO_2

How much energy is available in a redox couple:

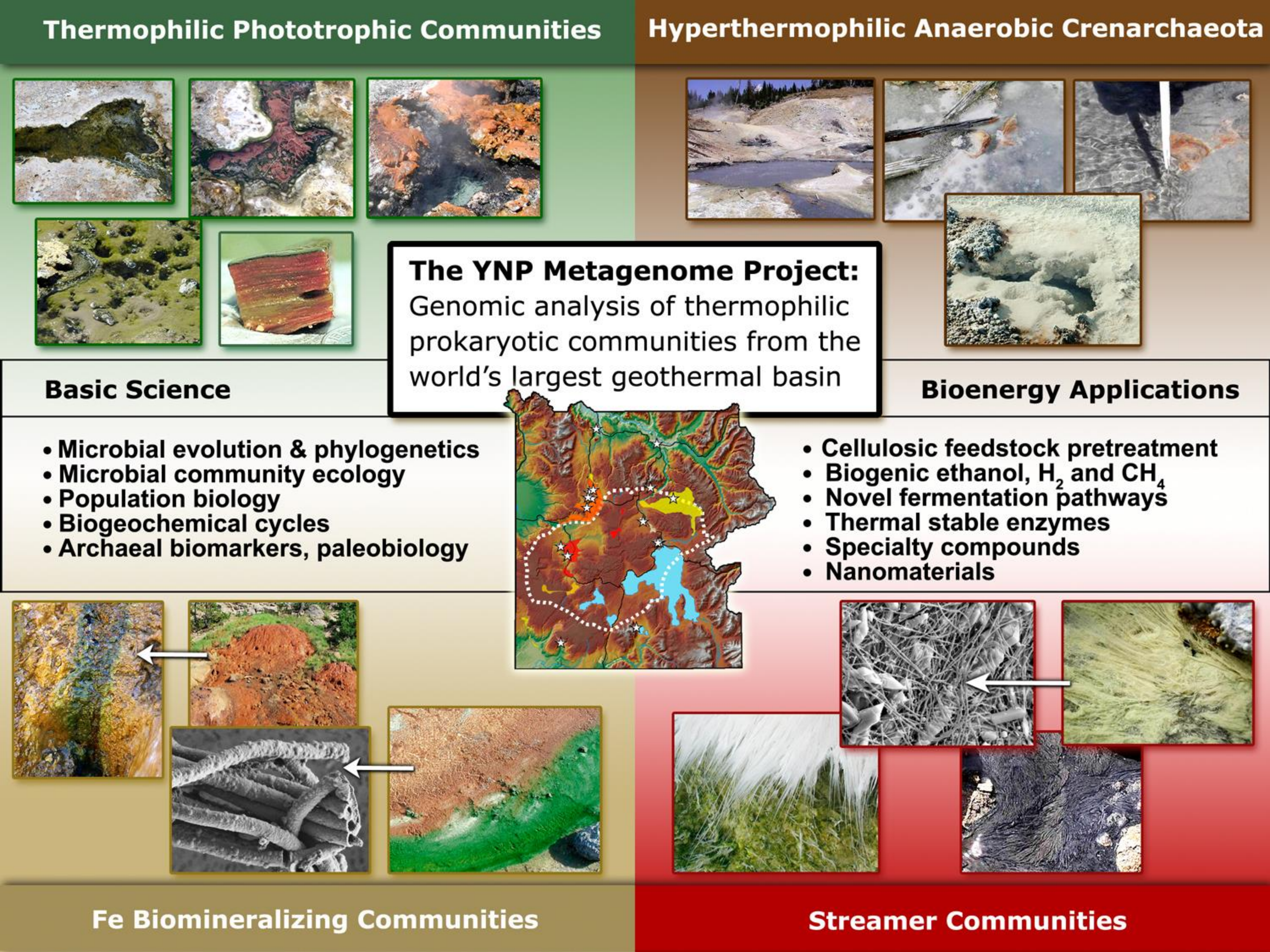
H_2(aq) + ½ O_2 (aq) → $H_2$0(l)

ΔG= -94 kJ/ e-transferred

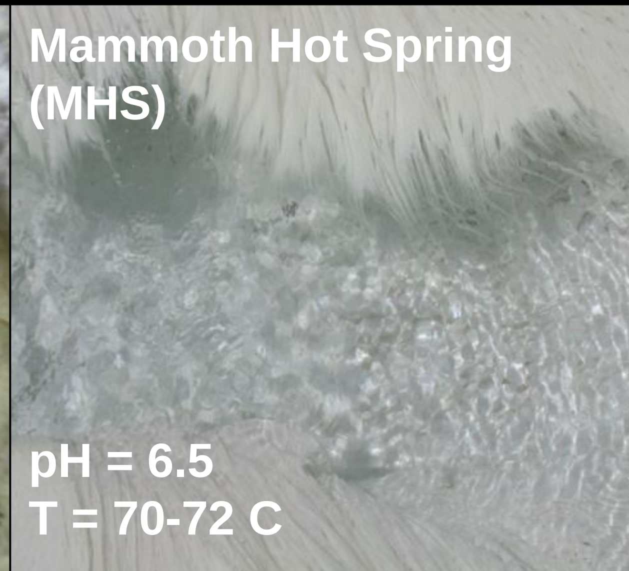
H_2(aq) + S^0 → H_2S (aq)

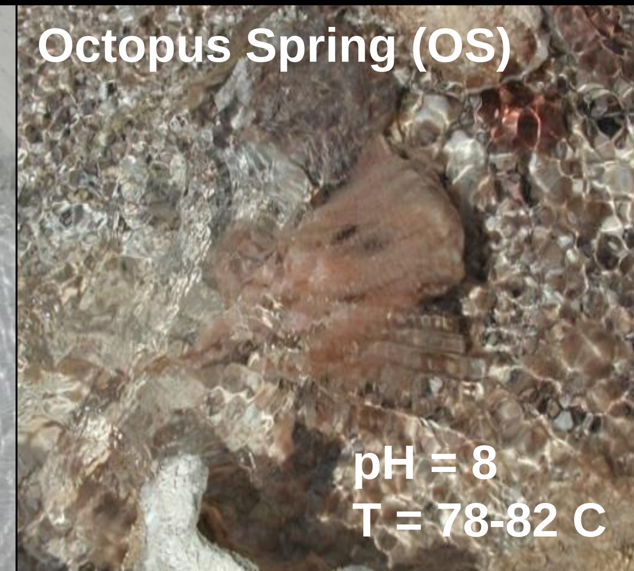
ΔG= -16 kJ/ e-transferred

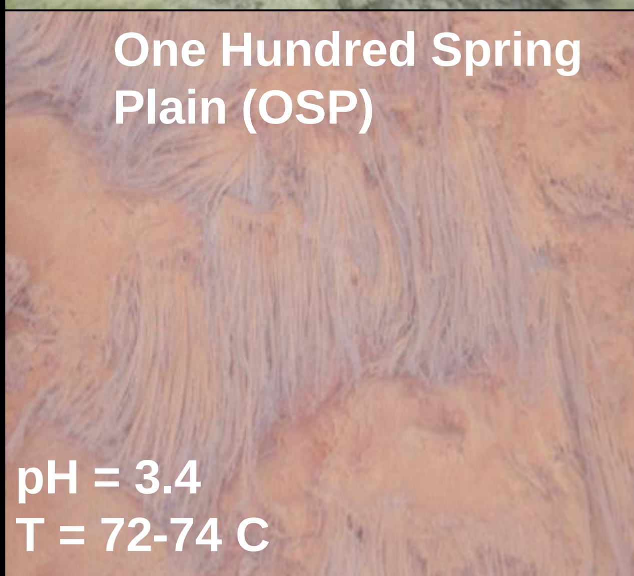
Inskeep et al., 2005, *Geobiology*

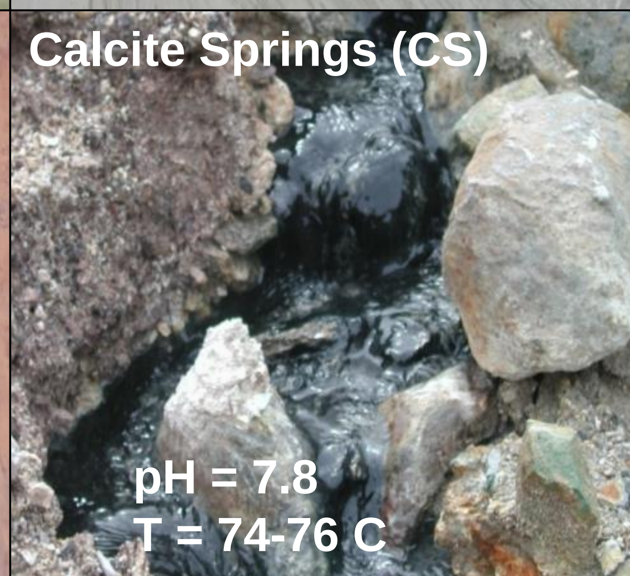

Thermophilic Phototrophic Communities
Hyperthermophilic Anaerobic Crenarchaeota
The YNP Metagenome Project:
Genomic analysis of thermophilic prokaryotic communities from the world's largest geothermal basin
Basic Science
• Microbial evolution & phylogenetics
• Microbial community ecology
• Population biology
• Biogeochemical cycles
• Archaeal biomarkers, paleobiology
Bioenergy Applications
• Cellulosic feedstock pretreatment
• Biogenic ethanol, H_2 and CH_4
• Novel fermentation pathways
• Thermal stable enzymes
• Specialty compounds
• Nanomaterials
Fe Biomineralizing Communities
Streamer Communities

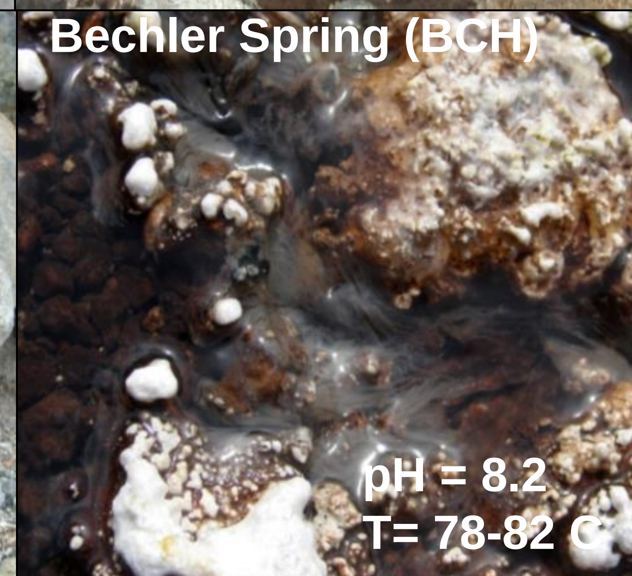
Filamentous 'Streamer' Communities in YNP:
Three Major Lineages

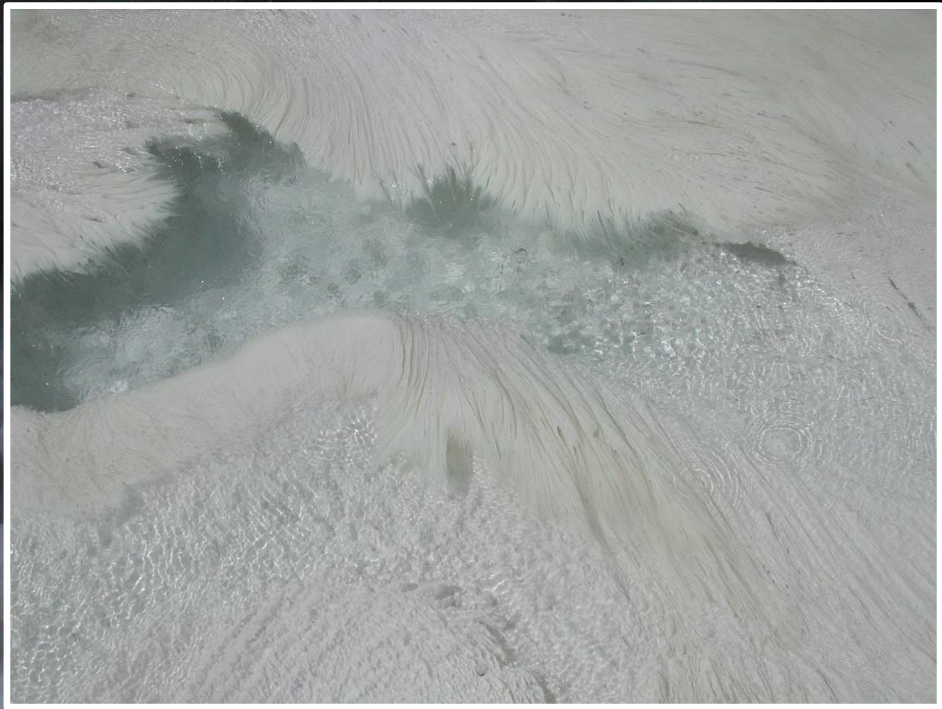










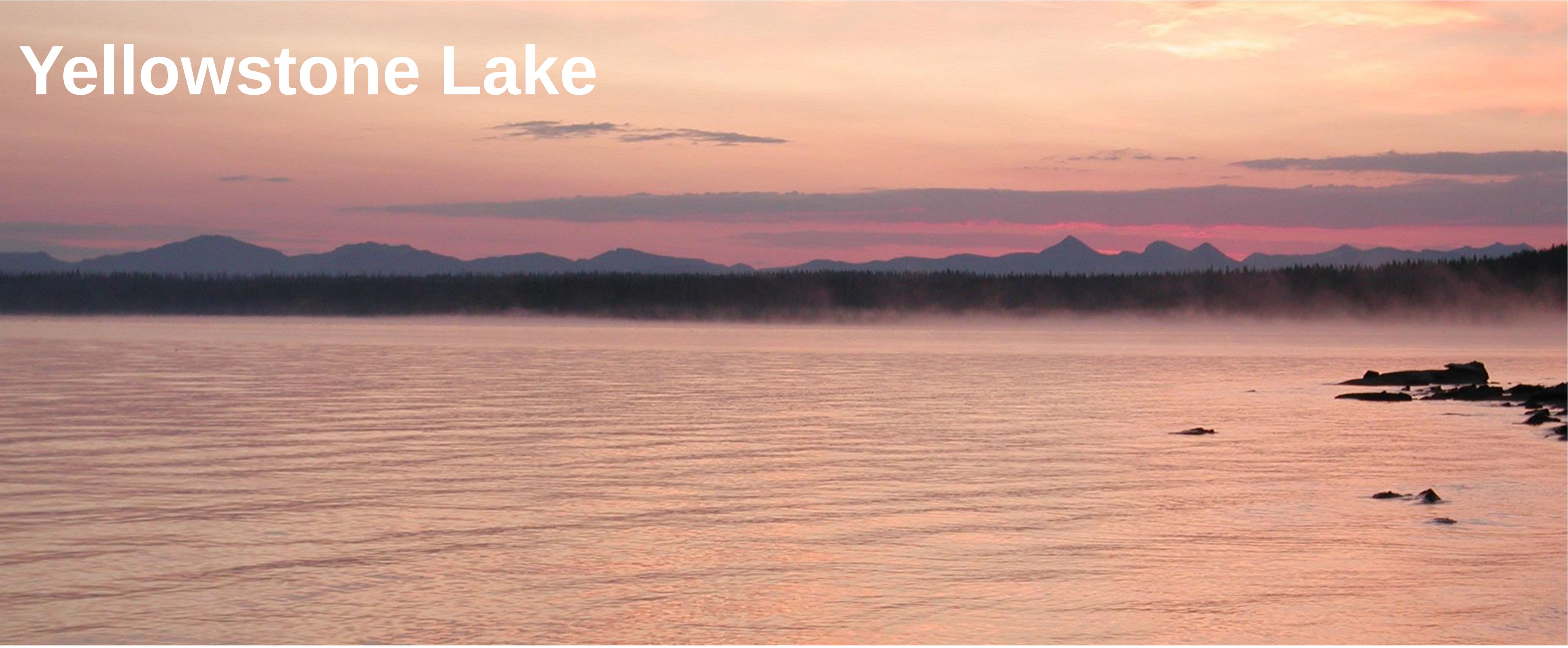


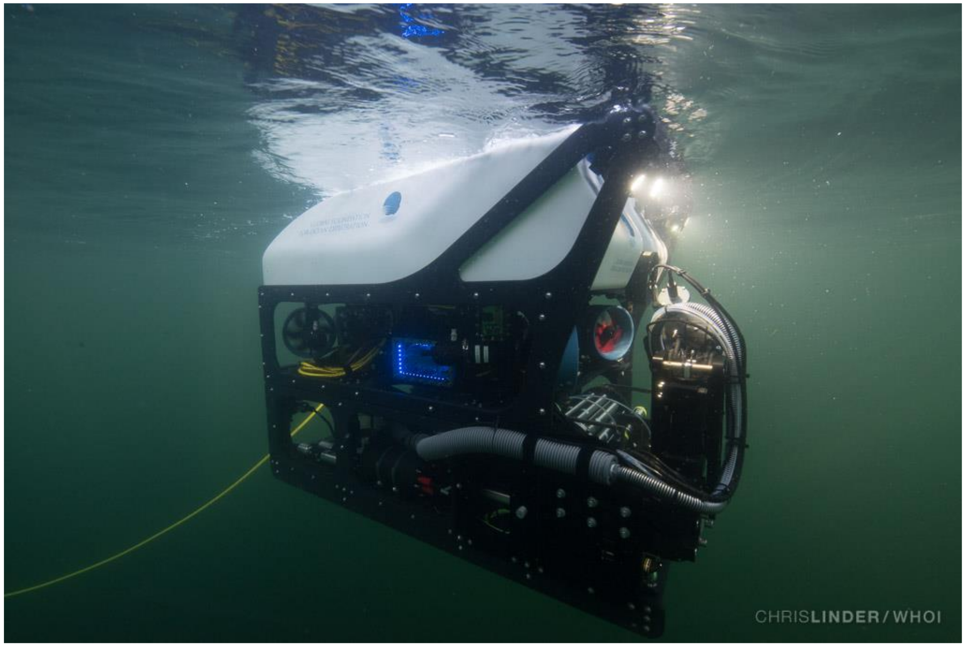
What's in a Wiggle?

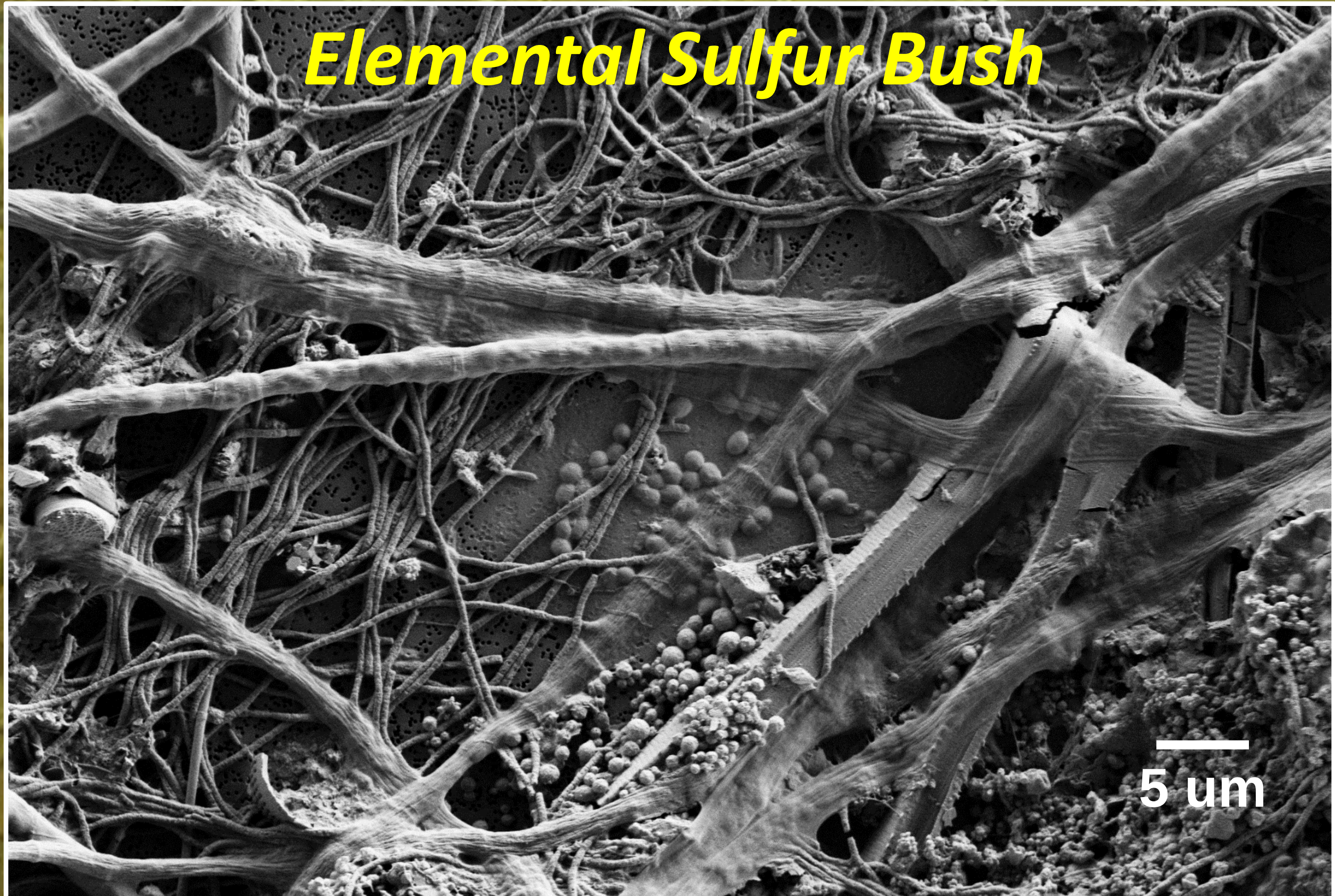
Mammoth Hot Springs

- Inskeep et al. 2010
- Inskeep et al. 2013
- Takacs-Vesbach/Inskeep et al. 2013
- Dong et al., 2019

Yellowstone Lake



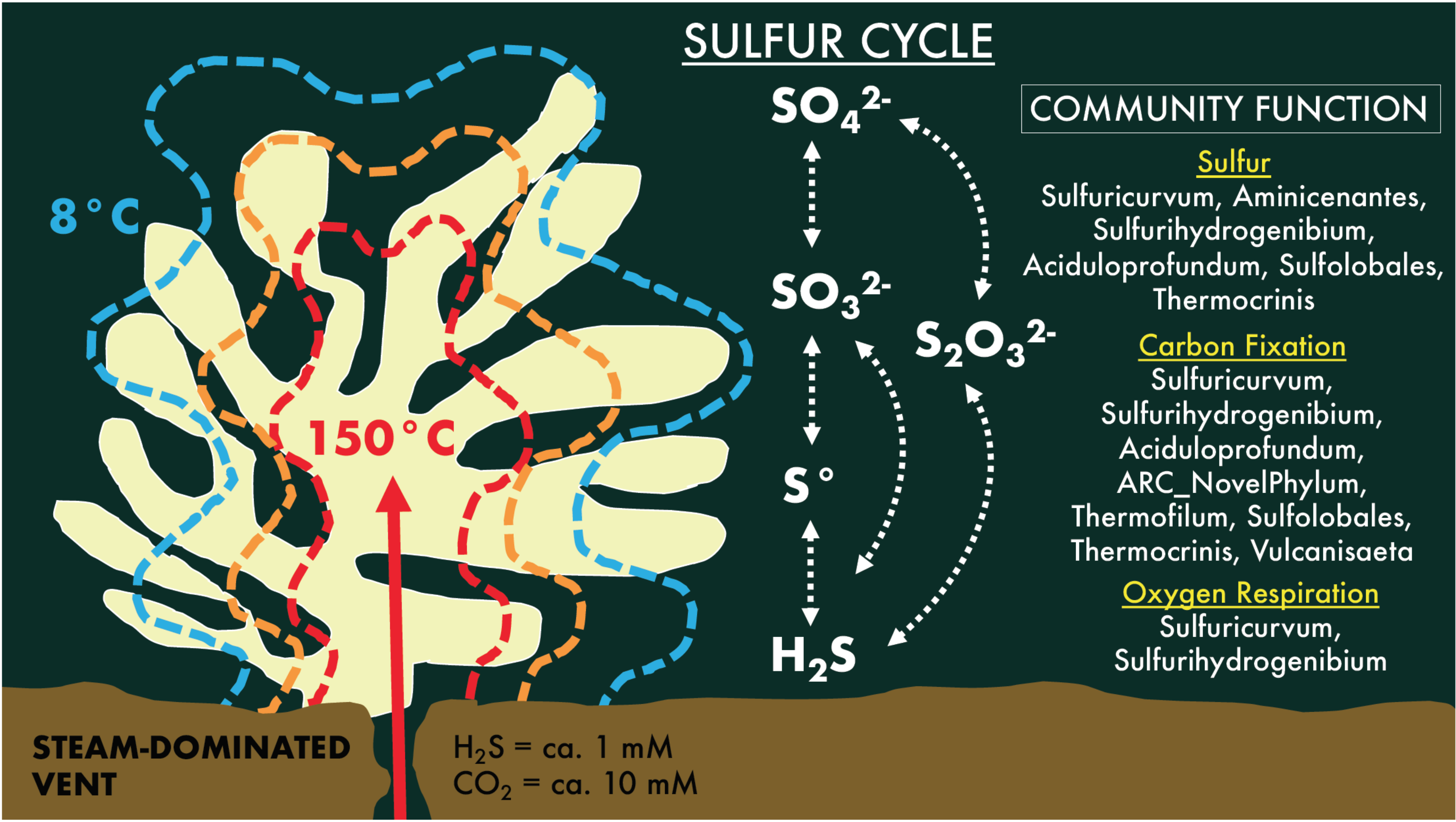




Yellowstone Lake Thermal Vents

Inskeep et al., 2015

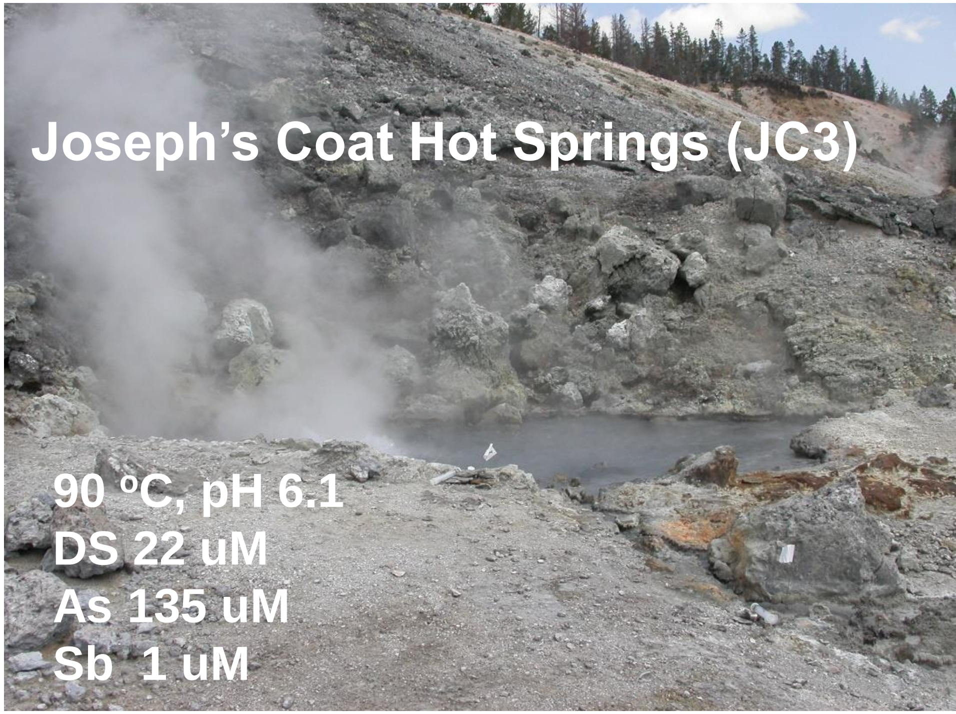
McKay et al., 2019

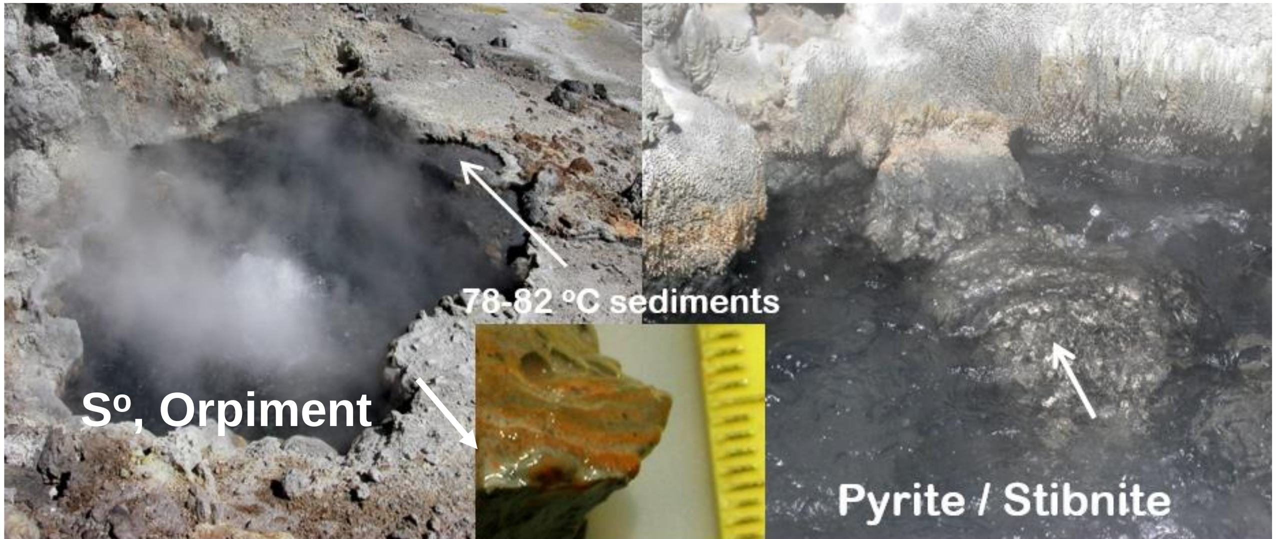


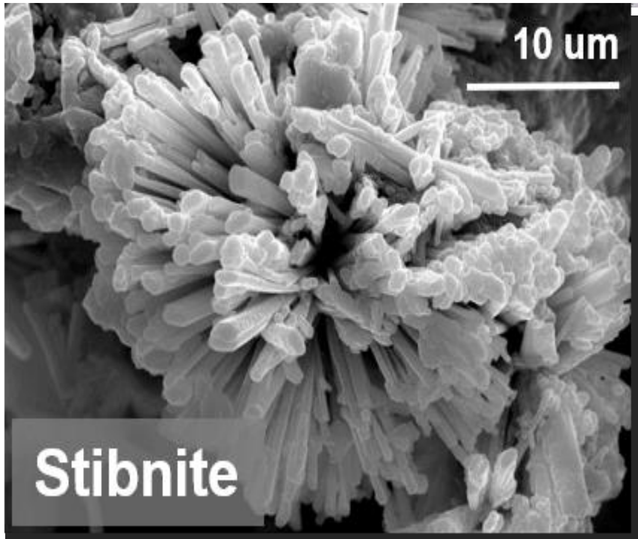
SULFUR CYCLE
SO_4^{2-}
SO_3^{2-}
$S_2O_3^{2-}$
S°
H_2S
8°C
150°C
STEAM-DOMINATED VENT
H_2S = ca. 1 mM
CO_2 = ca. 10 mM
COMMUNITY FUNCTION
Sulfur
Sulfuricurvum, Aminicenantes, Sulfurihydrogenibium, Aciduloprofundum, Sulfolobales, Thermocrinis
Carbon Fixation
Sulfuricurvum, Sulfurihydrogenibium, Aciduloprofundum, ARC_NovelPhylum, Thermofilum, Sulfolobales, Thermocrinis, Vulcanisaeta
Oxygen Respiration
Sulfuricurvum, Sulfurihydrogenibium

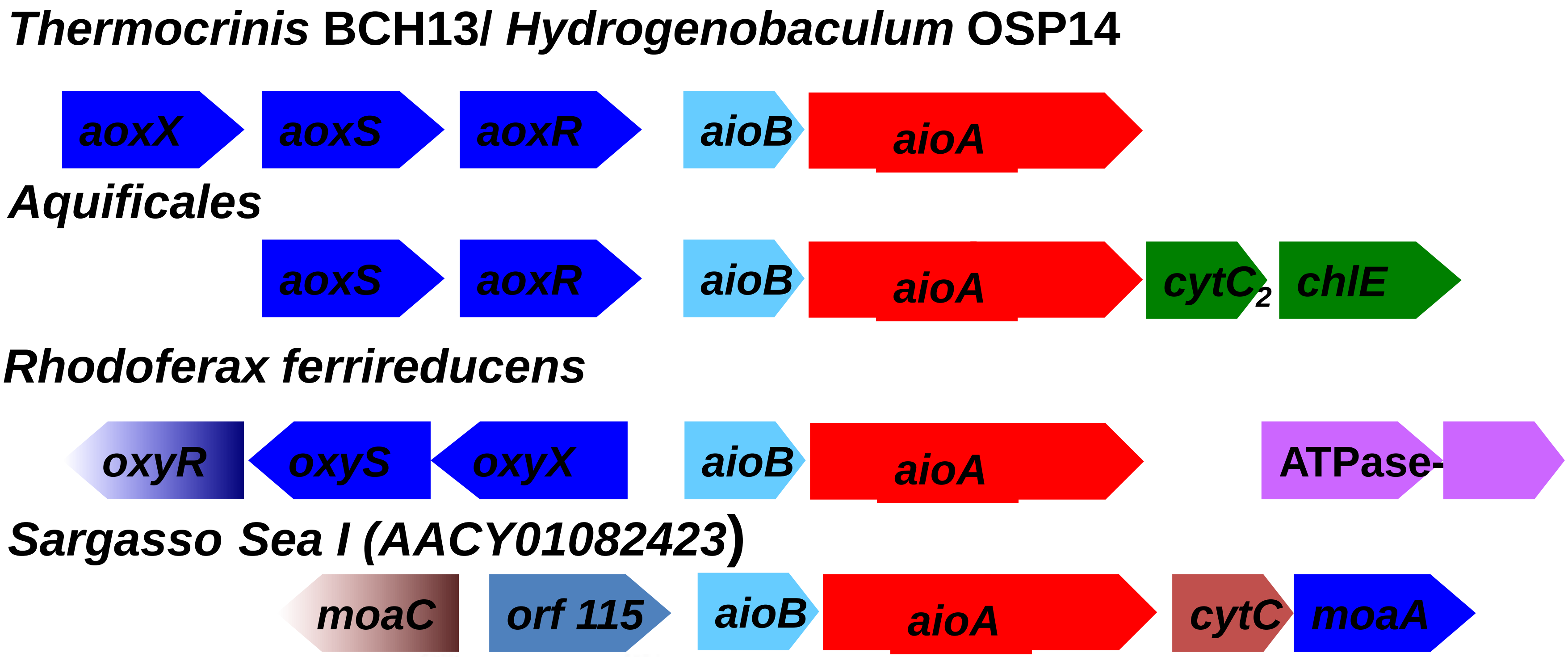
As and Sb cycling: As^{III} and As^{V} (Sb^{III} and Sb^{V})

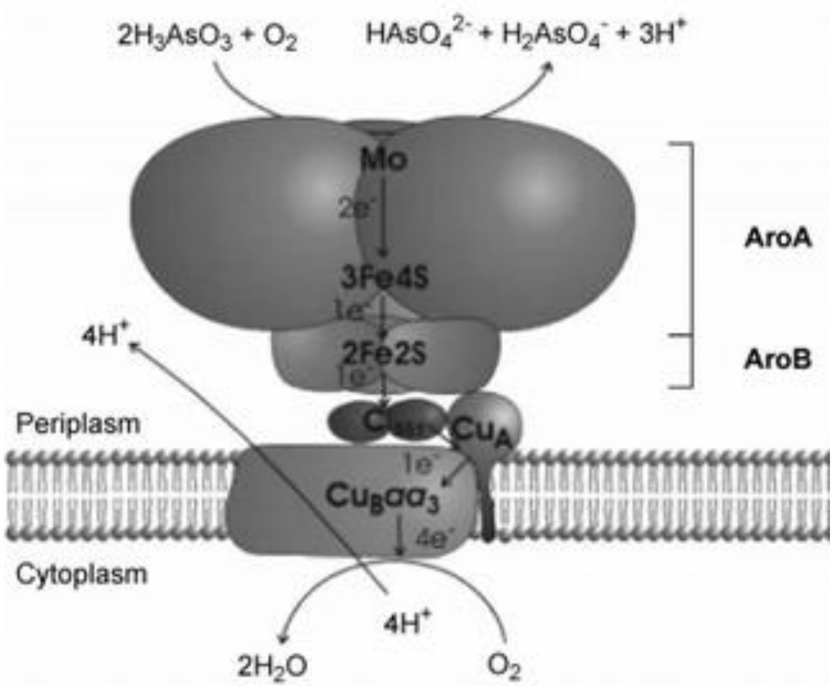
- Arsenite oxidation ~ energy gain using oxygen in some thermophiles (aroA, asoB)
- Arsenate reduction ~ dissimilatory reduction occurring in some archaea (arrA)









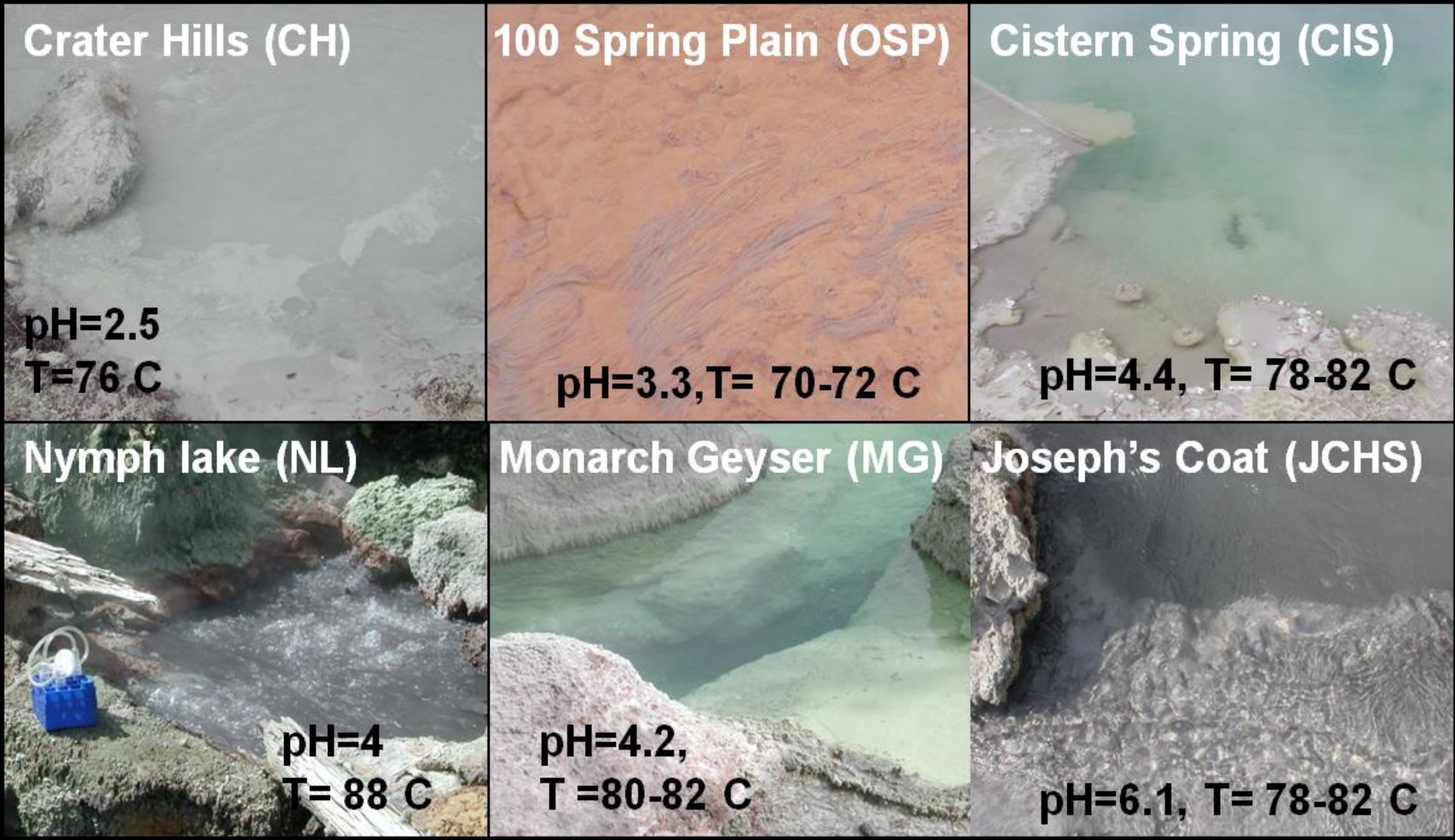


Van den Hoven & Santini, 2004. *Biochimica et Biophysica Acta*

D'Imperio *et al.*, 2007. *AEM*

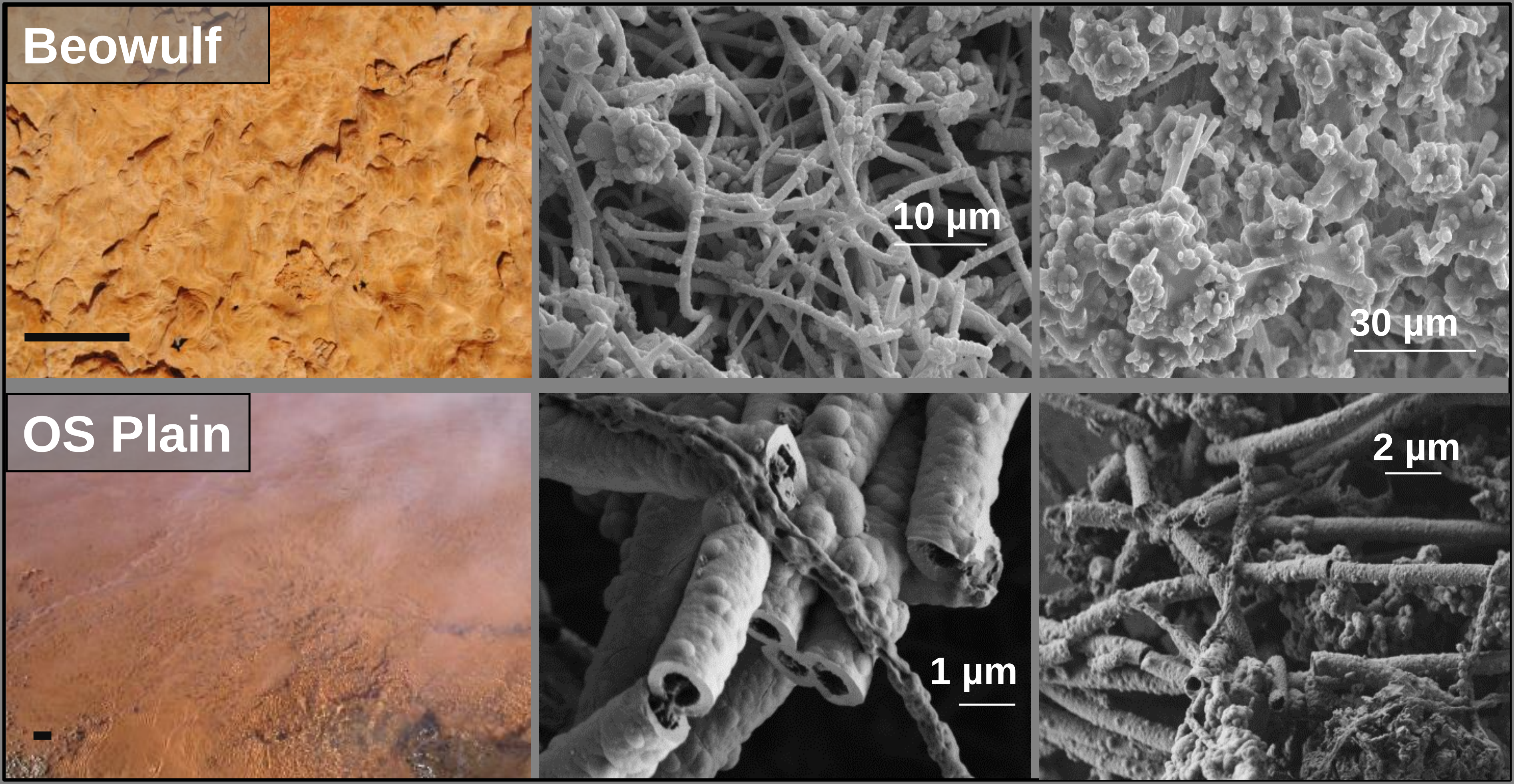
Santini *et al.*, 2007. *Biochimica et Biophysica Acta*

Archaeal-dominated sites and geochemical context



High-temperature acidic systems: low sulfide, high ferrous Fe, low oxygen

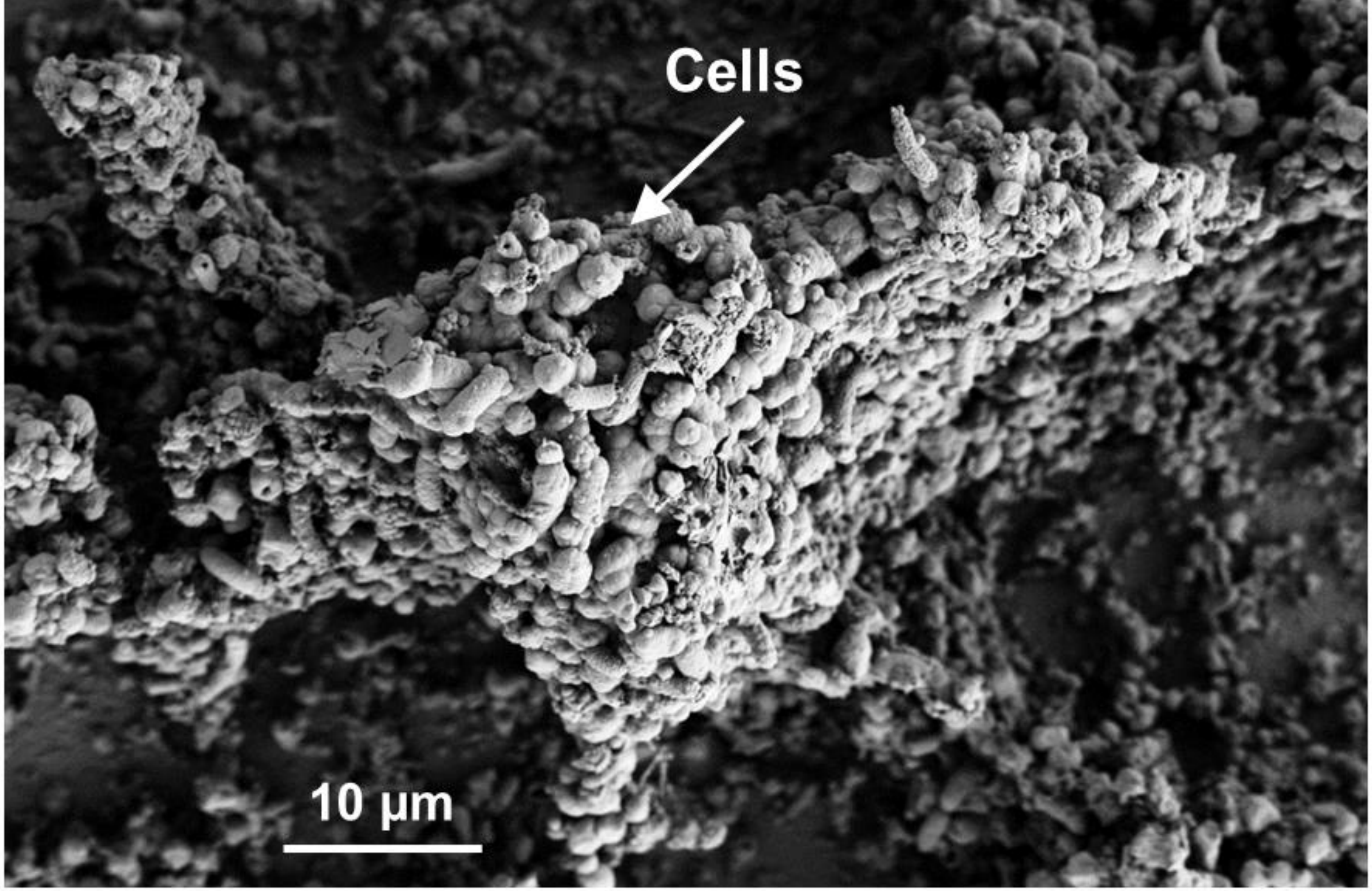
- Gas exchange, oxygen diffusion, Fe(III)-oxide biomineralization



Kozubal et al. 2012a, Frontiers in Microbiology


Echinus Geyser
NGB, YNP
A series of thin-film bioreactors, organized as alternating pools and terraces.
O_2(aq) ~ 20 – 60 µM.
ECH_B Nycodenz Prep (11/12)
1 µm

Microbial Beaver Dams

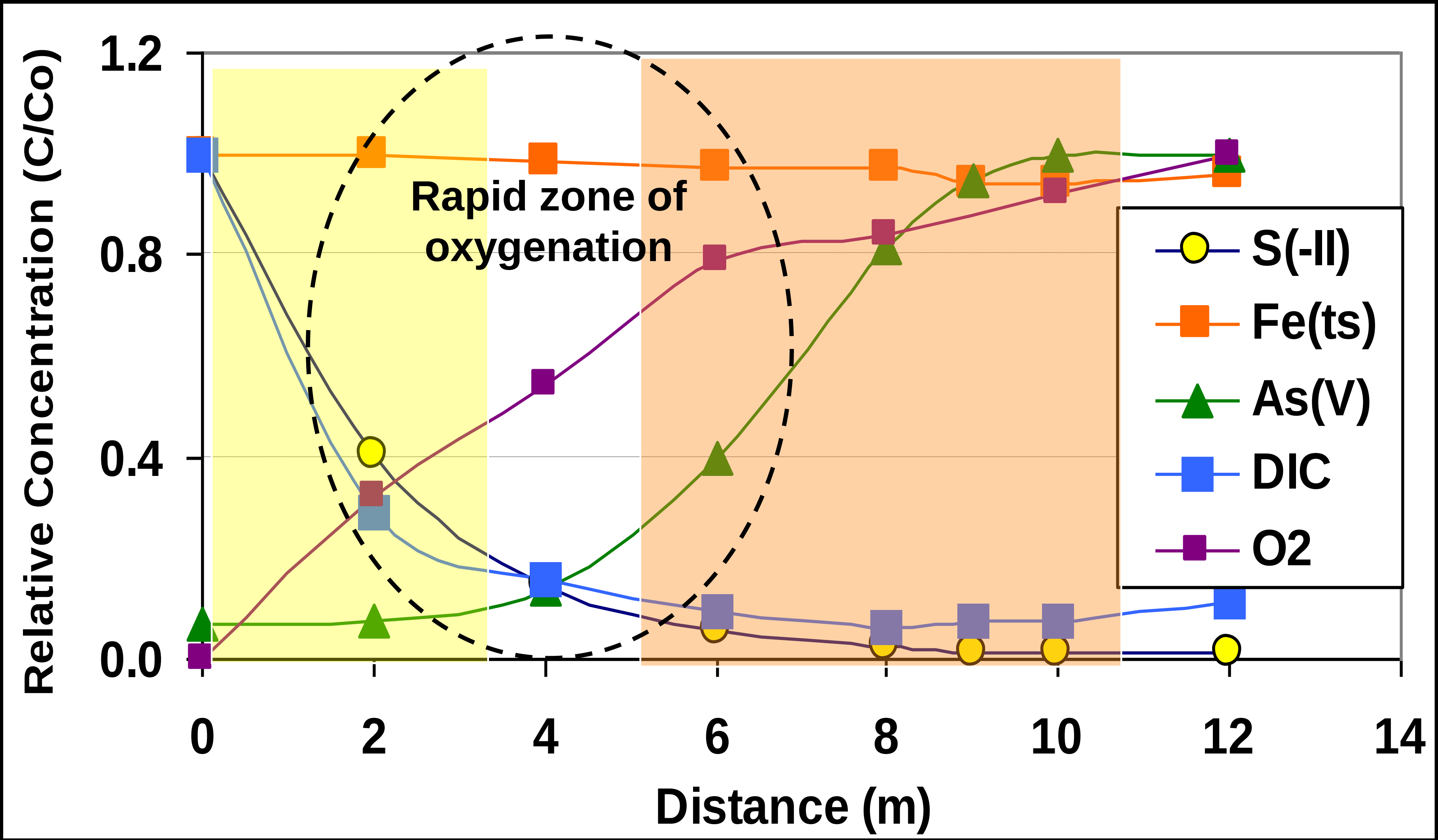


Echinus Geyser (Norris Geyser Basin)

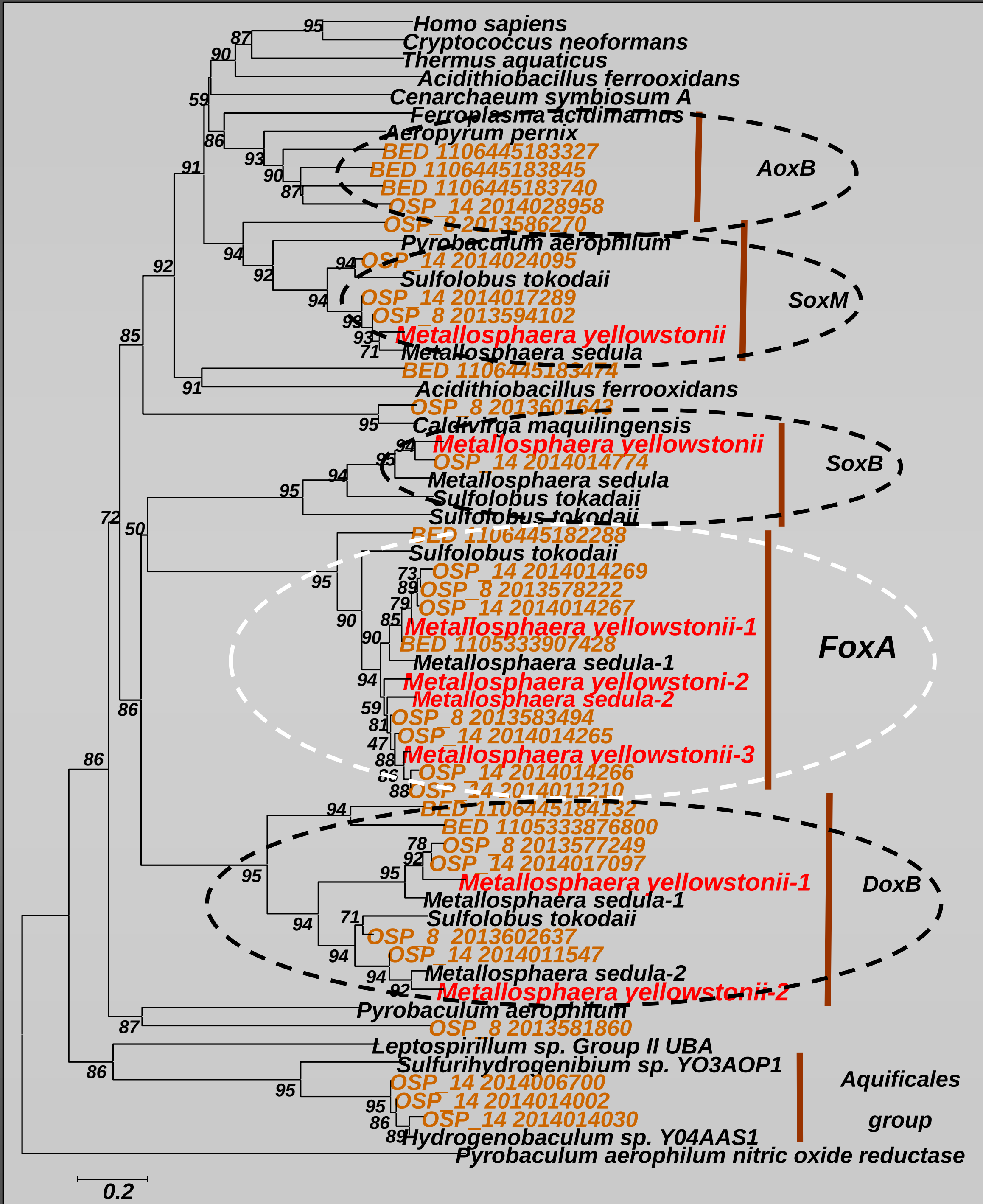
Kozubal et al., 2012
Beam et al., 2016
Jay et al., 2019

Simplified Natural Communities (*Yellowstone National Park)*

- ***Physicochemical processes establish changes in state variables as a function of distance and/or depth in geothermal outflow channels***



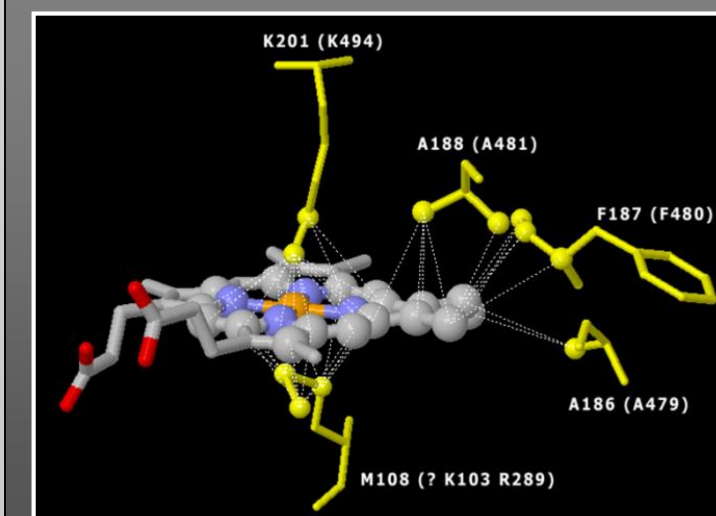
Langner et al. 2001; Inskeep et al. 2004, Macur et al. 2004; Kozubal et al., 2012a



Sulfolobales Heme Cu oxidases

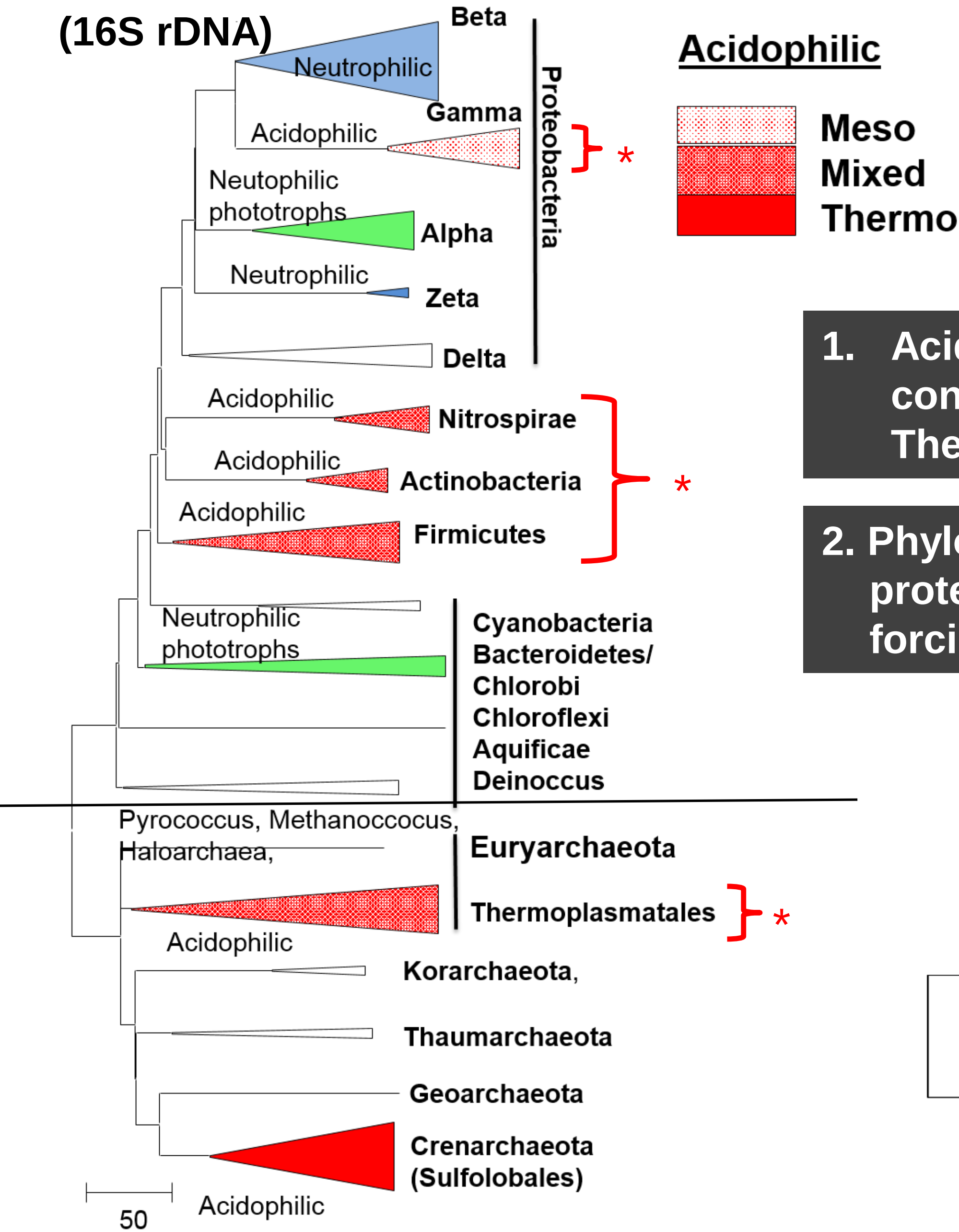
HCO subunit I of the *fox* complex, specific to Fe(II)-oxidizing Sulfolobales

← *Expressed under Fe(II) /O_2*



Kozubal et al. 2011, AEM

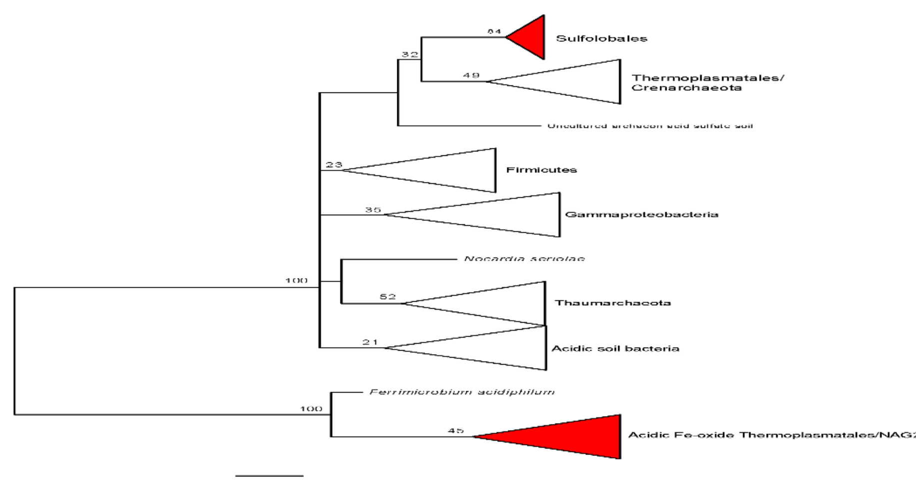
Phylogenetic Location of Fe(II)-oxidizing Microorganisms



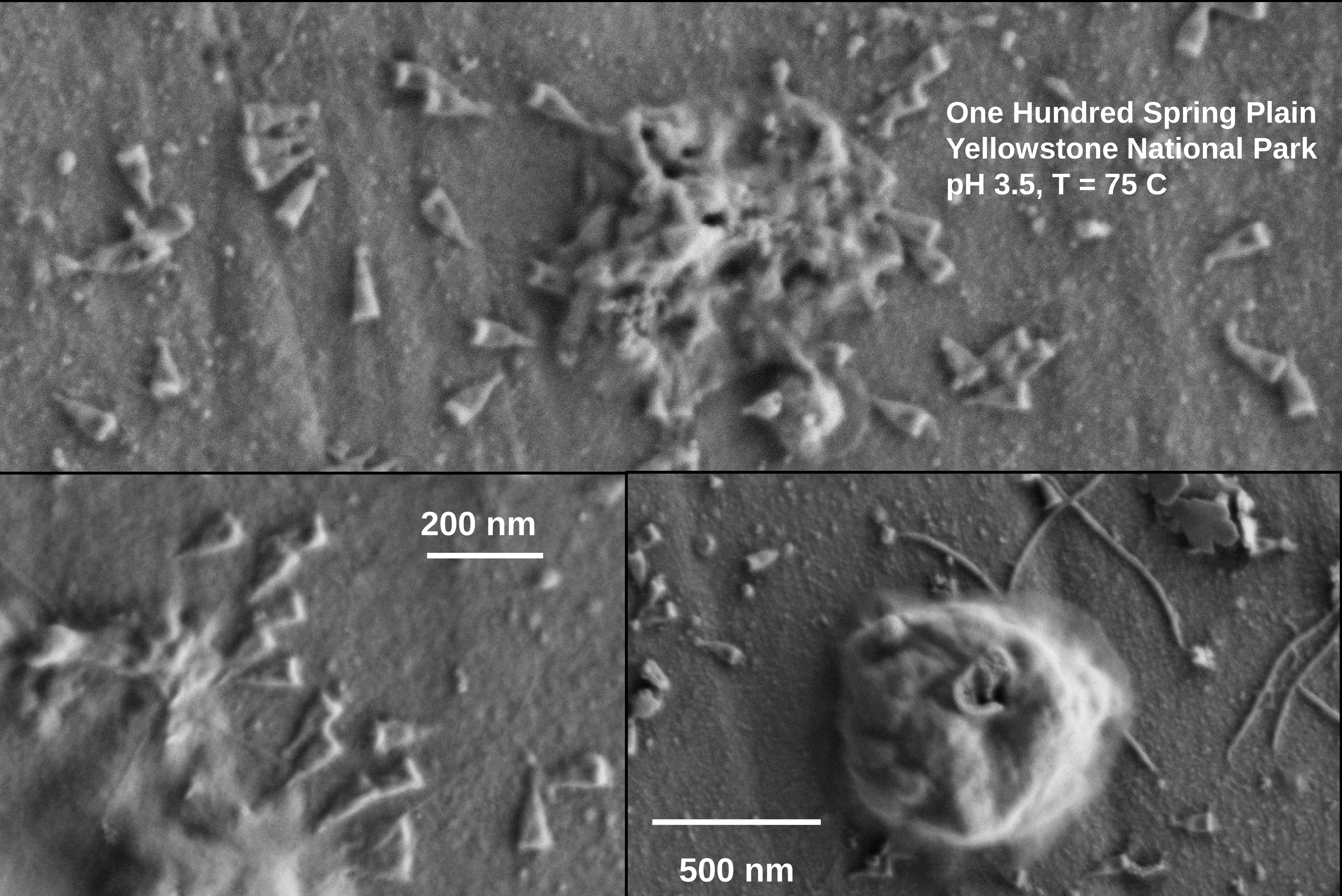
Significant niche overlap among the Thermoplasmatales and Fe(III)-oxidizing bacteria

1. Acidophilic bacteria in 4 unrelated phyla contain HCOs that are more similar to the Thermoplasmatales

2. Phylogeny of HCO-associated small blue Cu proteins (mco) also suggest environmental forcing and HGT to different bacterial phyla



Evidence of virions and virocells within 15 days of *in situ* incubation; viral predation and turnover must be incorporated into 'microbiome' analyses



Summary Comments

- *Yellowstone geothermal 'microbiomes' are comprised of diverse thermophiles in a plethora of different natural laboratories*
- *Lineage-specific functional proteins involved in energy capture track with geochemical conditions (e.g., O_2, Fe(II), S, As, H_2)*
- *Oxygen is an important electron acceptor for many thermophilic organisms*
- *Gas-exchange, hydrodynamics and diffusion contribute as niche determinants*
- *Numerous 'model' systems inform key controls on biogeochemical cycling*

Acknowledgements

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- C. Hendrix and S. Gunther (YNP Center for Resources)
- Thermal Biology Institute (MSU)

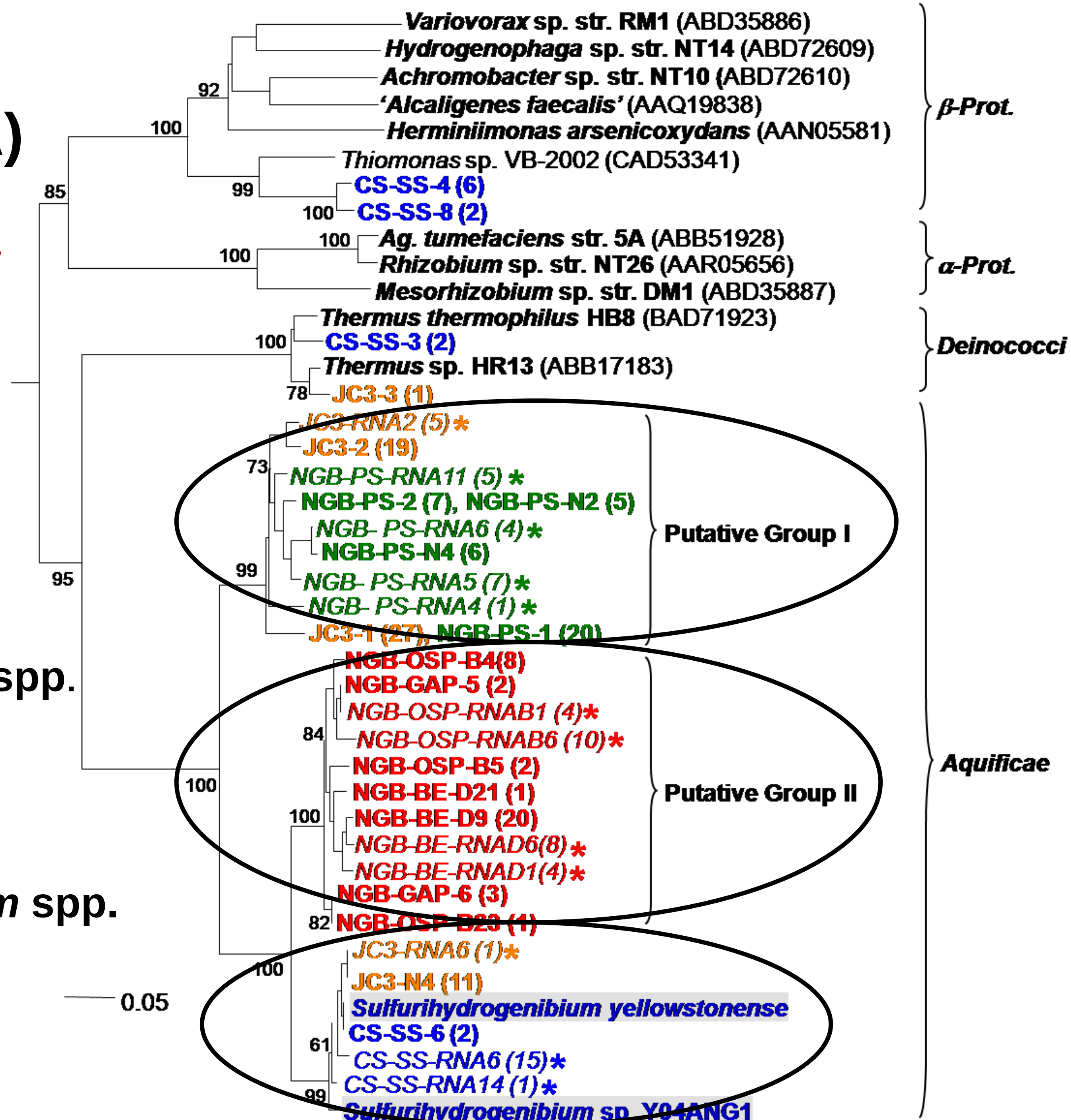
Arsenite oxidases (AroA)

Inskeep et al., 2007
Hamamura et al. 2009, 2010

***Thermocrinis* spp.**
pH 7, Fe^{III} Mats
⟶

***Hydrogenobaculum* spp.**
pH 2-4, Fe^{III} Mats
⟶

***Sulfurihydrogenibium* spp.**
pH 6-7, FeS_2, S Mats
⟶



The Oxygen Cycle

Source Water, pH ~ 3-4

O_2(g) O_2(g) O_2(g)

Dissolved Gases

H_2(aq), CH_4(aq), CO_2(aq), H_2S(aq)

Flow-Direction (2-20 cm/sec)

Air-Water Gas Exchange

- **Henry's Law: O_2(g) = O_2(aq)**
- **Kinetics: *f* (velocity, turbulence, air-water surface area)**

Biological Oxygen Demand (BOD)

Type A/B HCOs

O_2 + aa3 + ATPase = growth

e.g. Dox/Fox/Aox/Sox

Type C HCOs

O_2 + cbb3 + ATPase = growth

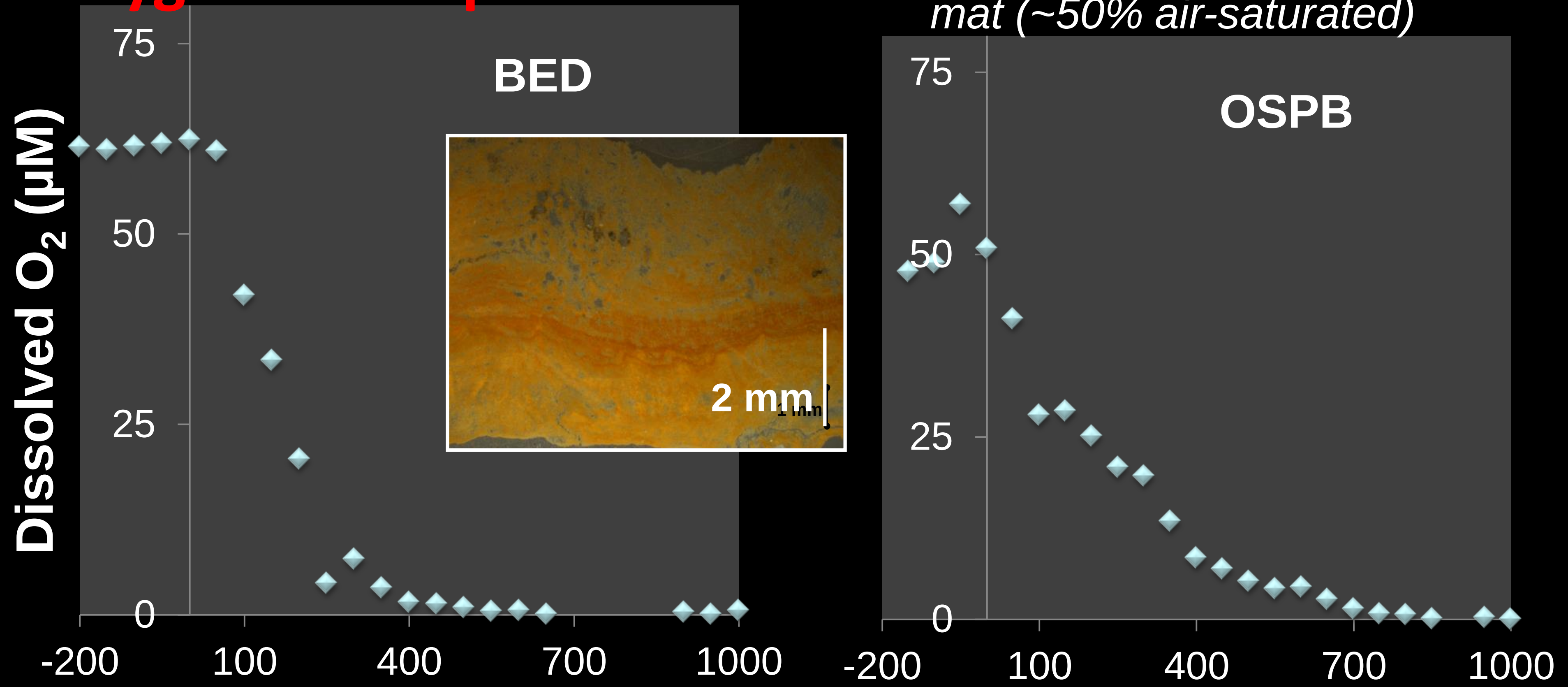
Chemical Oxygen Demand (COD)

e.g., Reactions with Reduced Sulfur

$O_2 + H_2S = S_2O_3 = S^0 + SO_4$

Oxygen micro-profiles:

Stream water overlaying mat (~50% air-saturated)

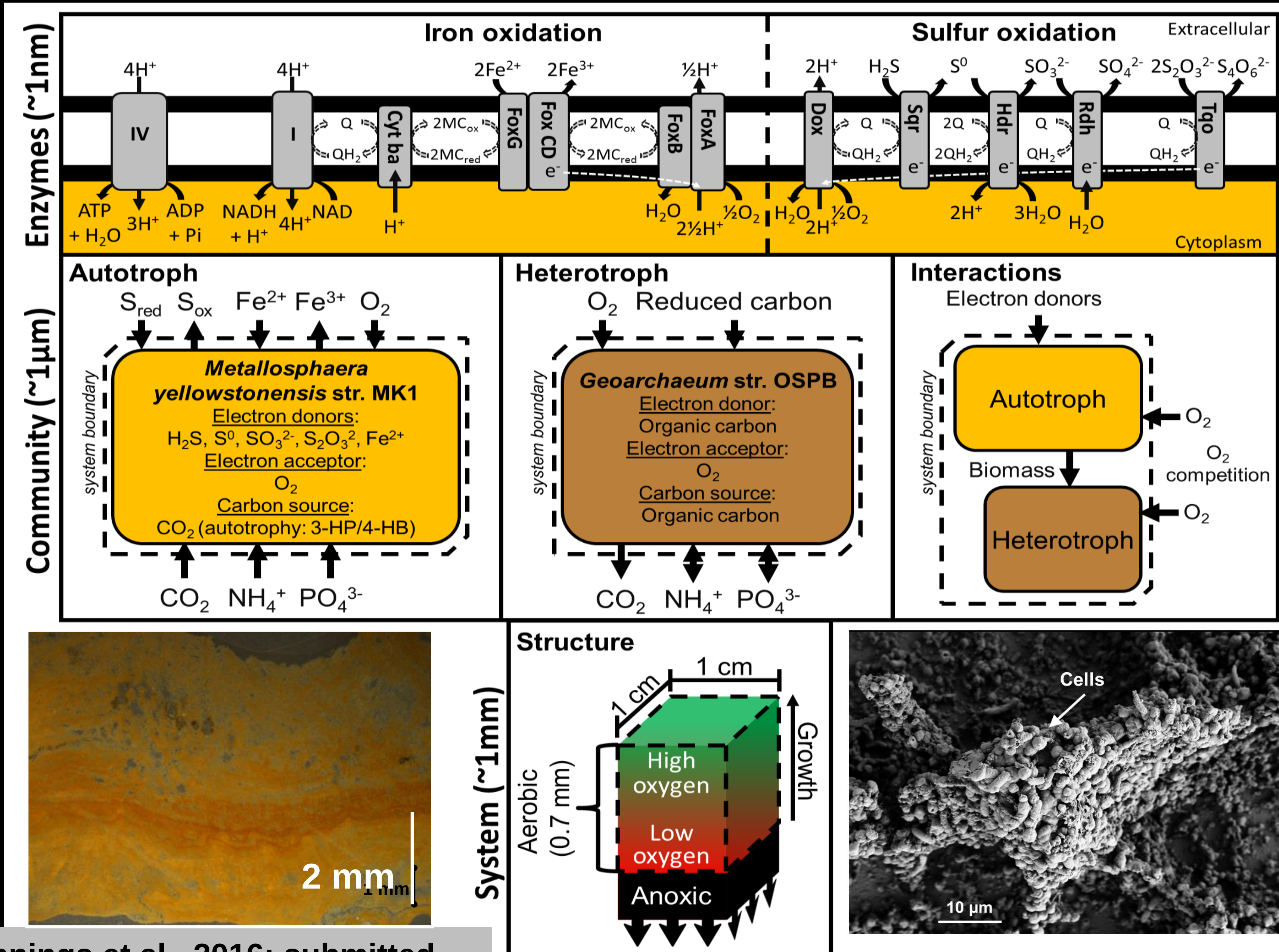


Depth (µm)

- **O_2 penetration depth ~700 ± 200 µm**
- ***Net O_2 Flux* = 1.5 *10^{-4} µmol cm^{-2} sec^{-1}**

Bernstein et al. 2013, *Environ. Microbiol.*
Beam et al. 2016, *Front. Microbiol.*

Genome-enabled Microbial Interaction Modeling



Jennings et al., 2016; submitted

Reference Microbial Community Types

Concept, Platform and Organizing Structure

- *Consistent themes for graduate training,*
- *Biotic, geochemical, hydrodynamic interactions,*
- *Profiling 'reference communities' (> 100 site metagenomes available from our group)*
- *Foundation for biotic (resource) inventory.*

For example (see videos):

Mammoth Hot Springs Filamentous Streamers

What's in a Wiggle?

Echinus Geyser Fe-oxide Microbial Mats

Microbial 'Beaver' Dams

Field Laboratory for Graduate Training









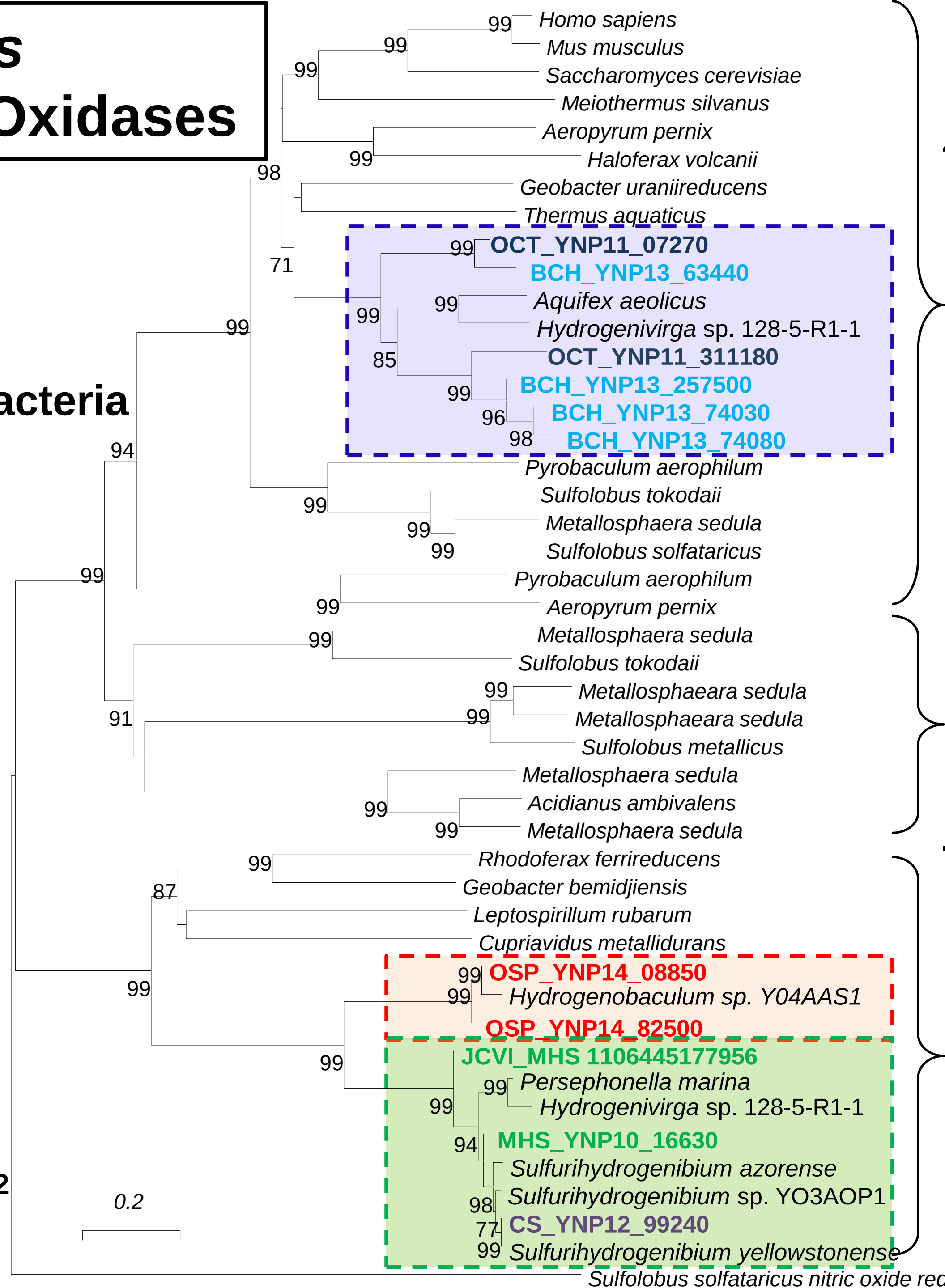
Aquificales
Heme Cu Oxidases

Type A-HCO

- **Majority of bacteria**

Type C-HCO

- **cbb3 HCO**
- **low K_m for O_2**



Takacs-Vesbach et al. 2013. Front. Microbiol.

Type A-HCO

Sites OCT_11, BCH_13

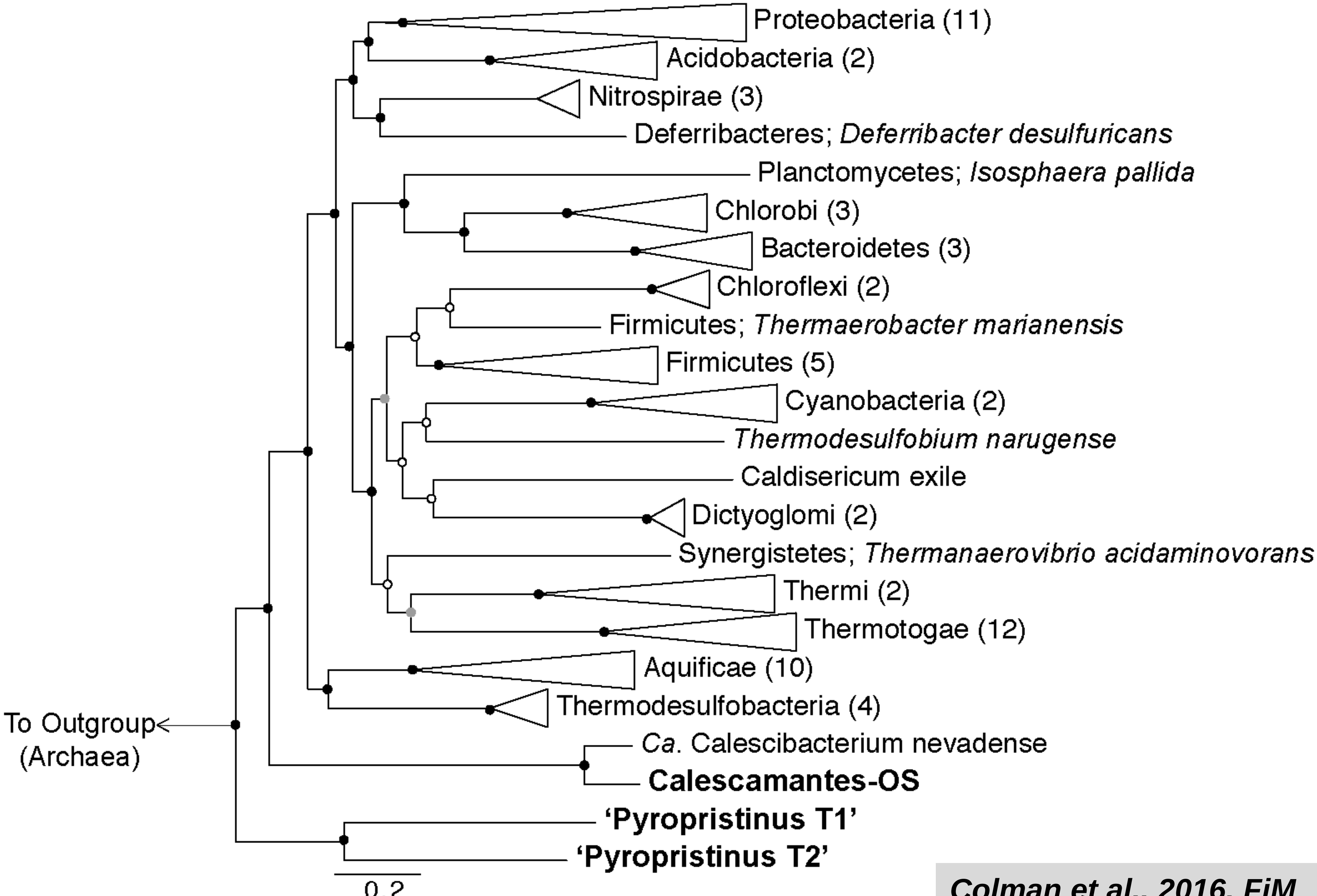
Type B-HCO

Type C-HCO

Sites DS_9, OSP_14

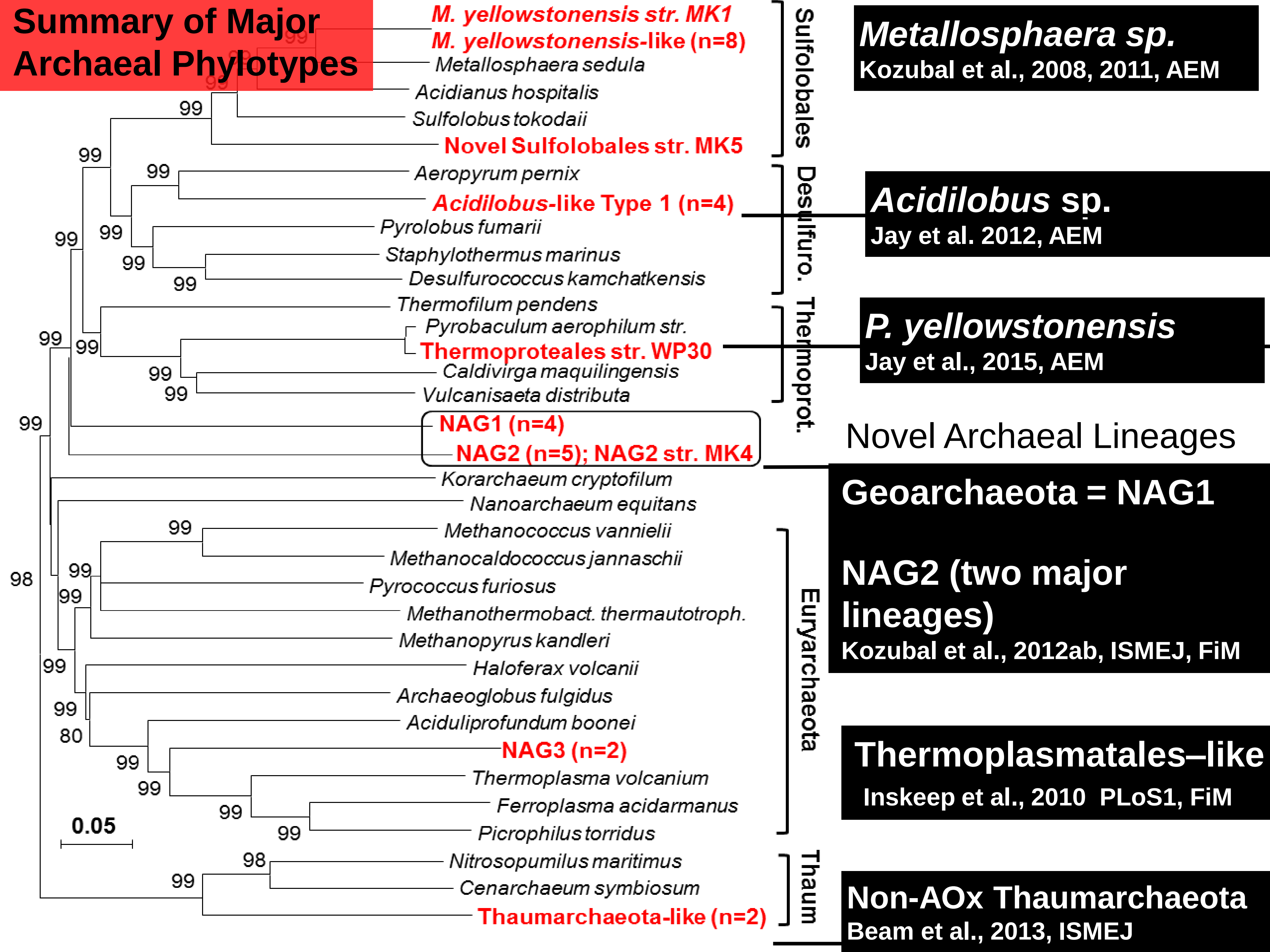
Sites MHS_10, CS_12

Phylogenetic Tree: *Bacteria*



Colman et al., 2016, FiM

Summary of Major Archaeal Phylotypes



Simplified Natural Communities (Yellowstone National Park):
One Hundred Springs Plain, Norris Geyser Basin (NGB)

Chemotrophic communities studied along main flow path

Geochemistry

pH = 3.4-3.5

Fe(II) = 45 µM

O_2(aq) = <1 to 100 µM (A to D)

H_2S(aq) = 10 to < 0.3 µM (A to D)