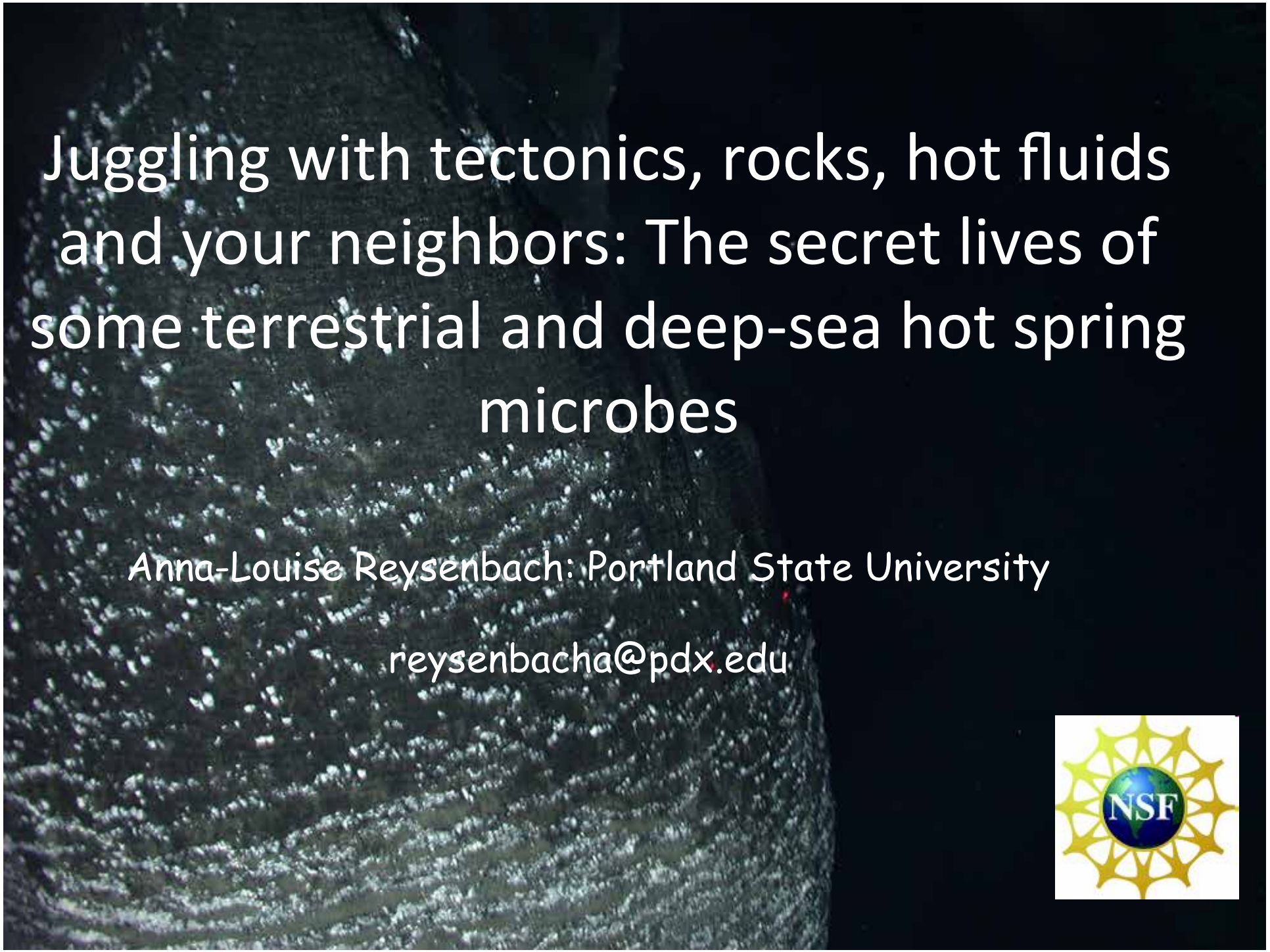
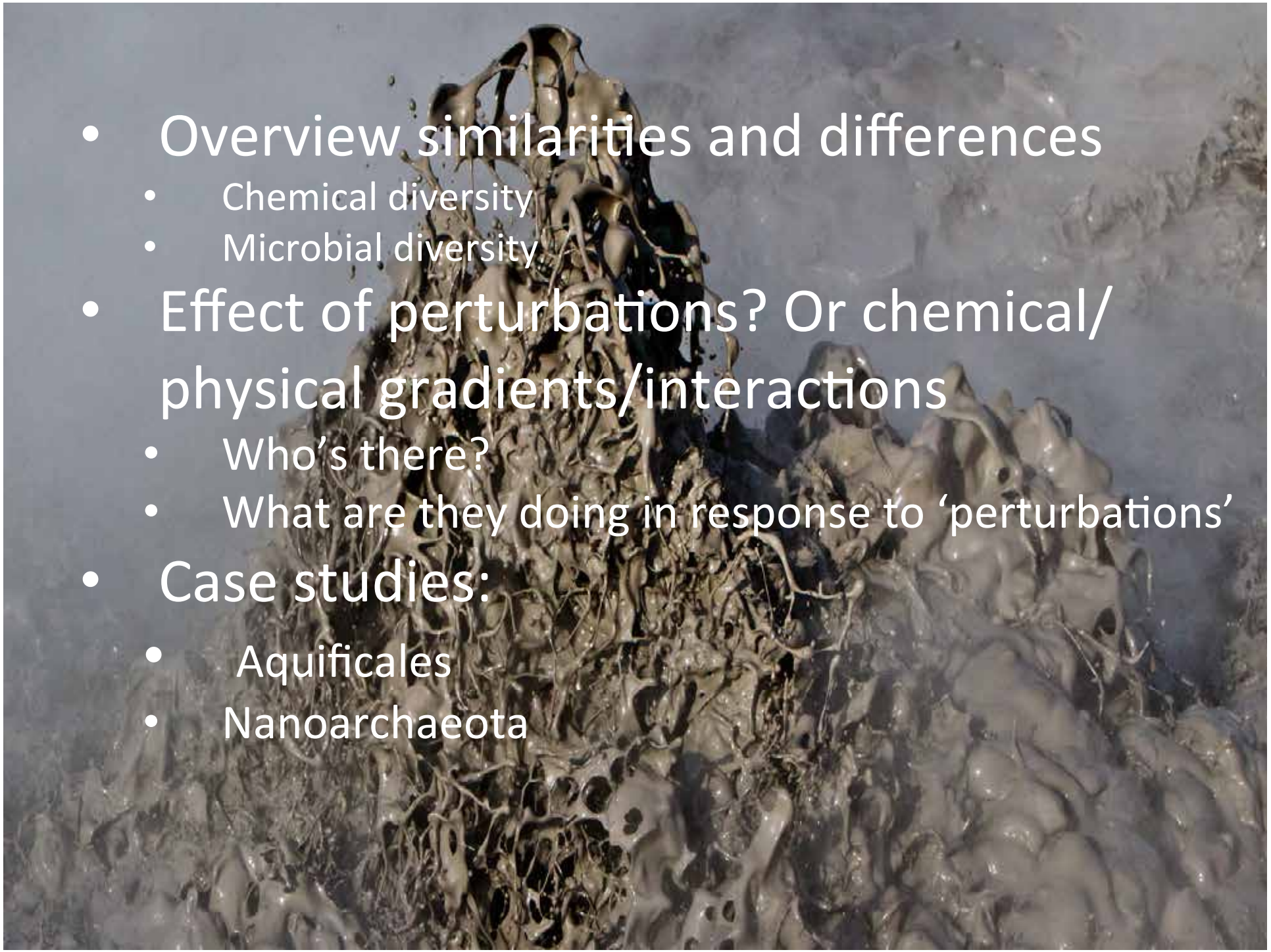


Juggling with tectonics, rocks, hot fluids and your neighbors: The secret lives of some terrestrial and deep-sea hot spring microbes

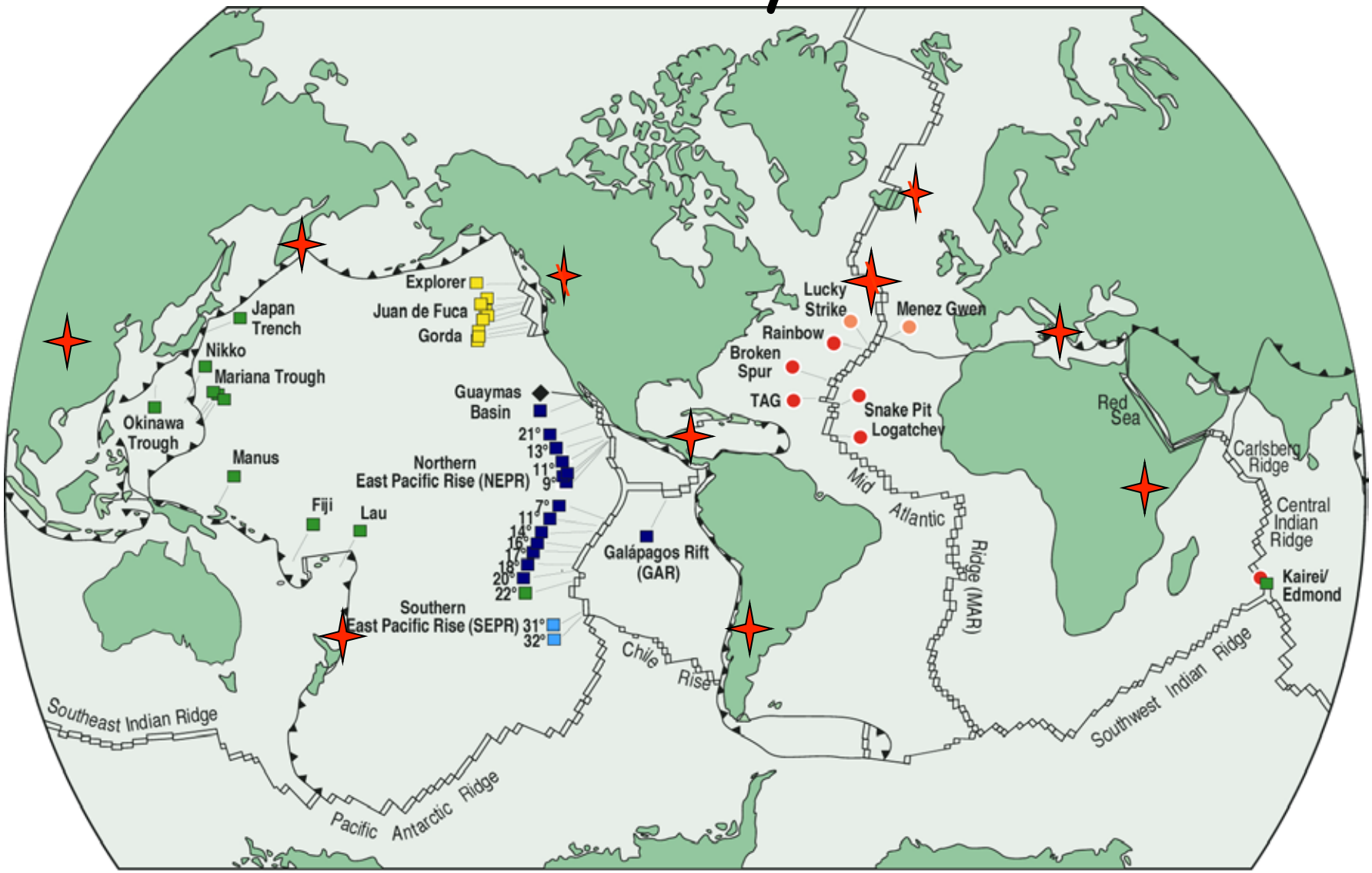
Anna-Louise Reysenbach: Portland State University

reysenbacha@pdx.edu

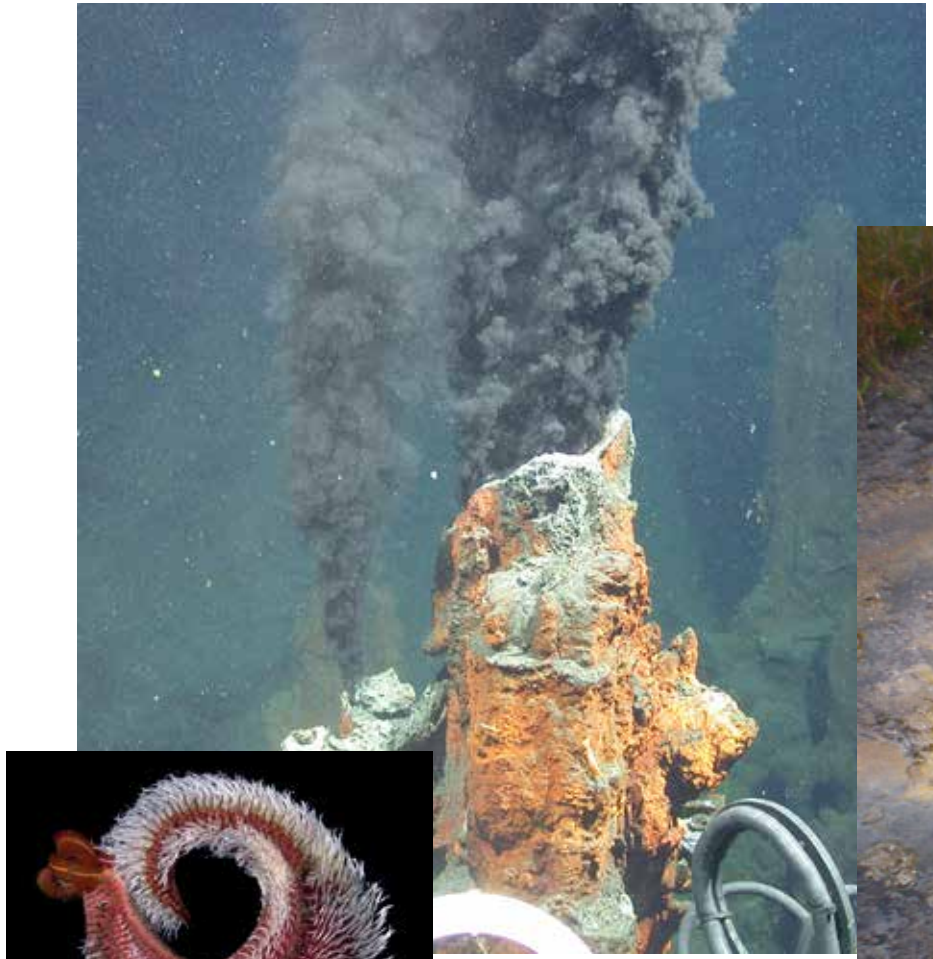


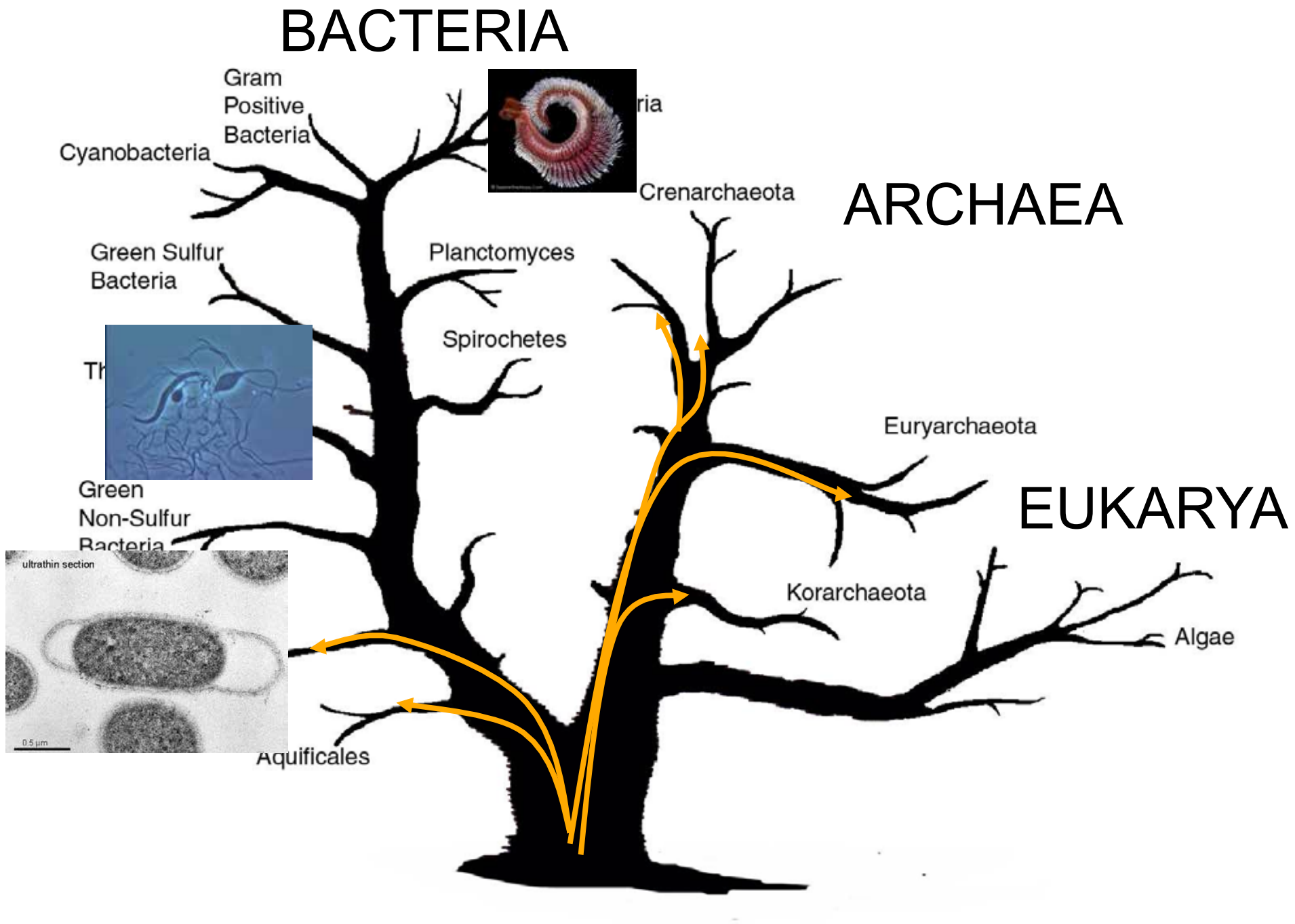
- Overview similarities and differences
  - Chemical diversity
  - Microbial diversity
- Effect of perturbations? Or chemical/ physical gradients/interactions
  - Who's there?
  - What are they doing in response to 'perturbations'
- Case studies:
  - Aquificales
  - Nanoarchaeota

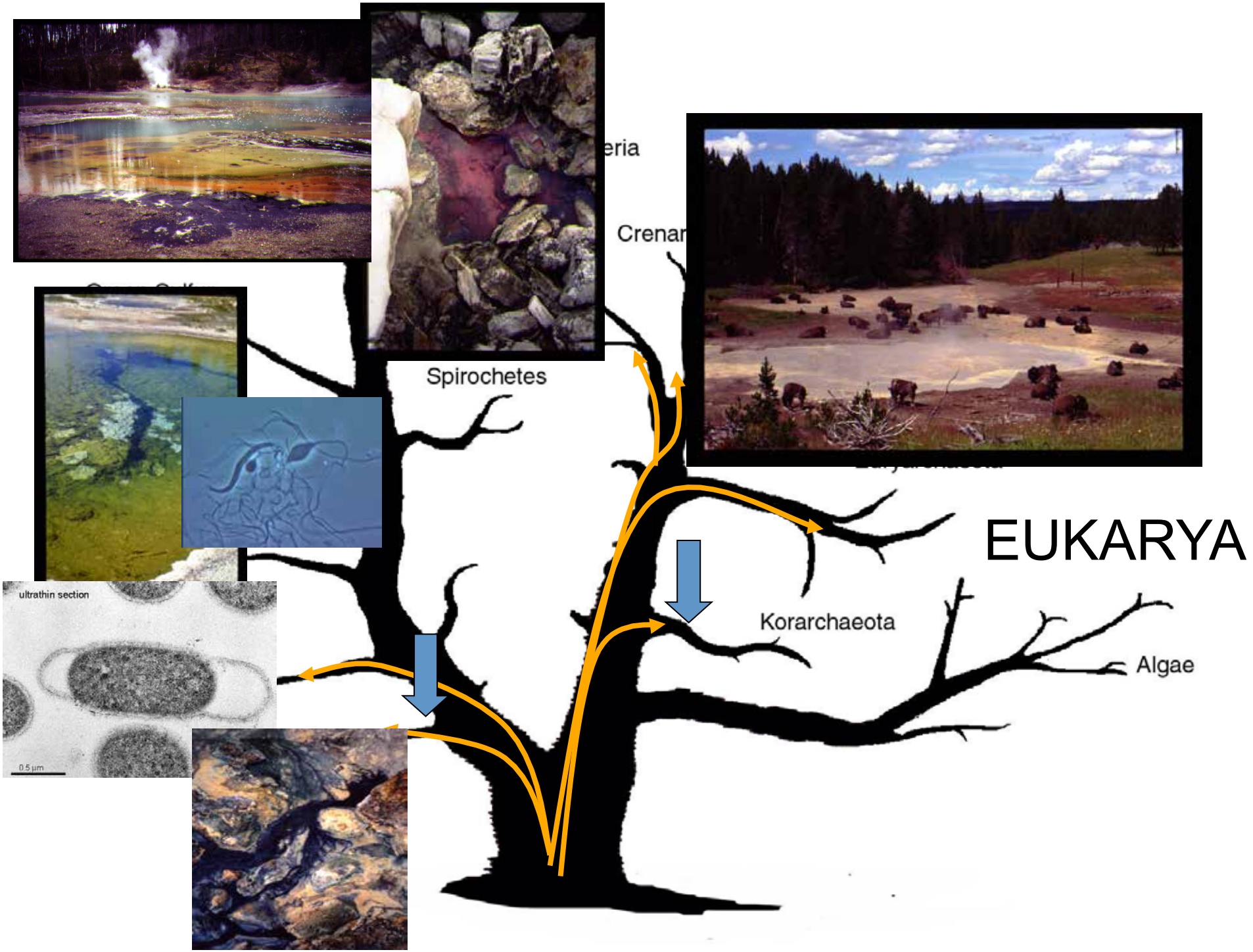
Overview: continental and marine microbial systems



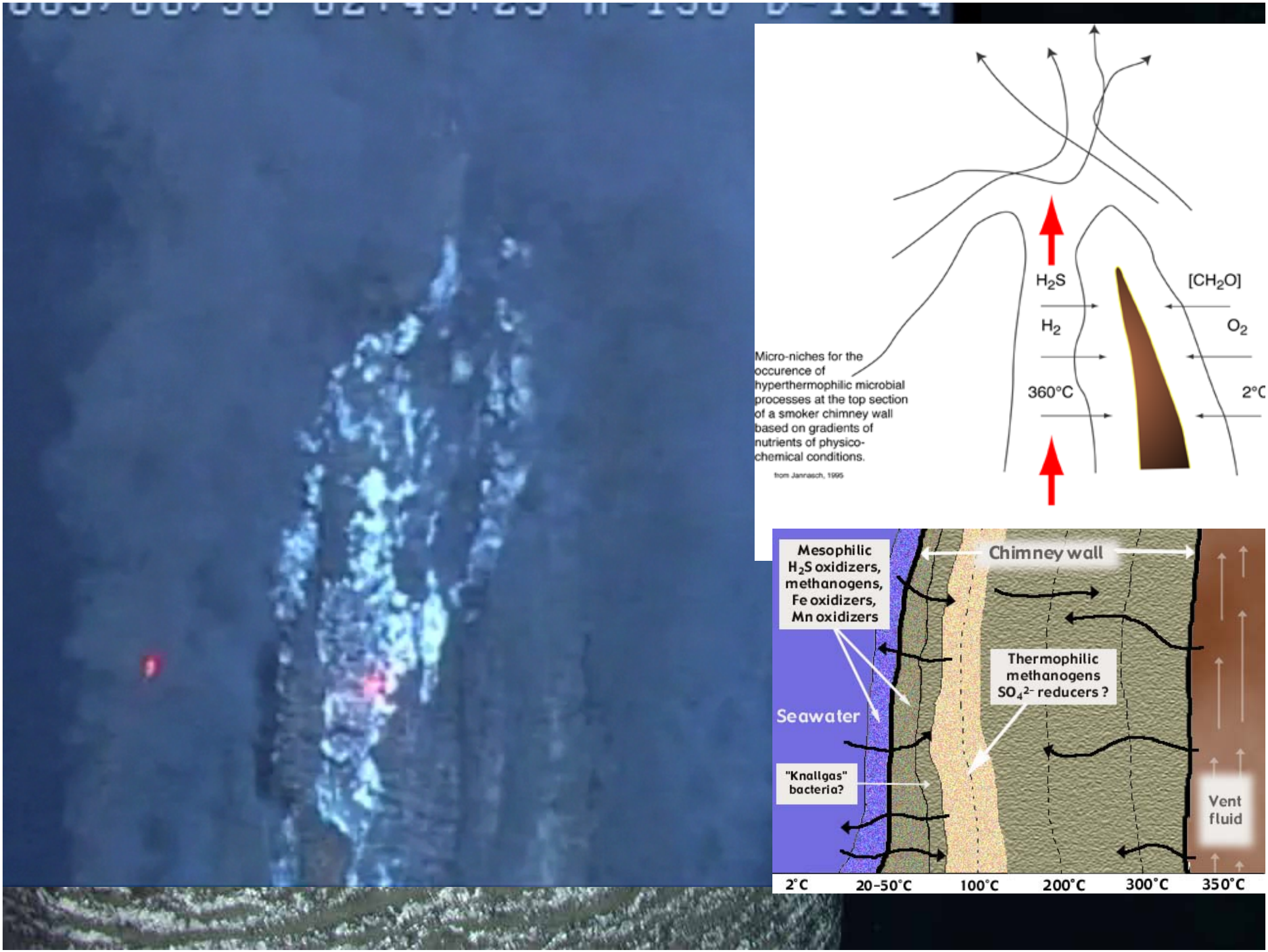
What do they share? How are they different from a microbial perspective?



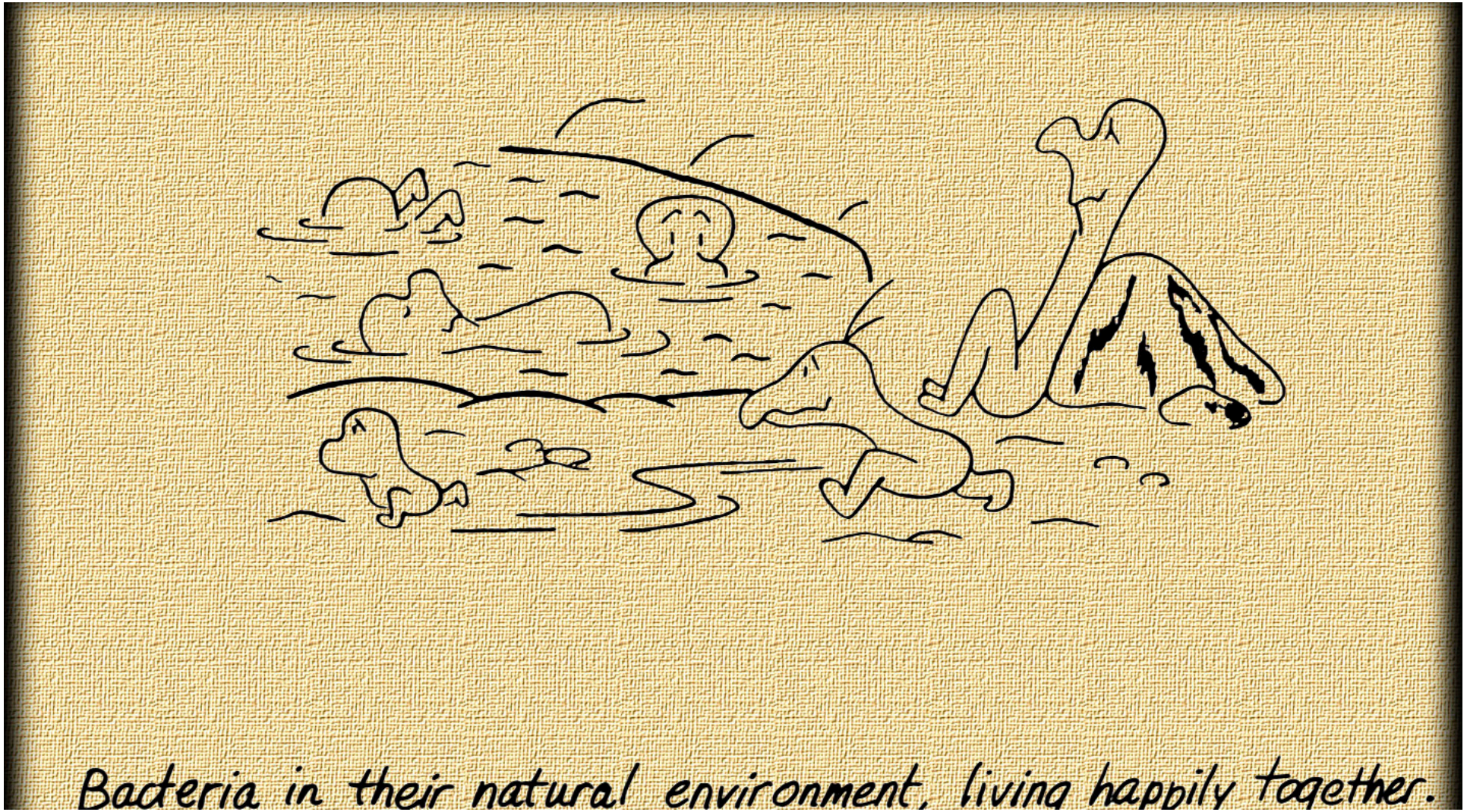

BACTERIA
Gram
Positive
Bacteria
Cyanobacteria
Green Sulfur
Bacteria
Green
Non-Sulfur
Bacteria
ultrathin section
0.5 µm
Aquificales
Planctomyces
Spirochetes
Crenarchaeota
ARCHAEA
Euryarchaeota
Korarchaeota
EUKARYA
Algae

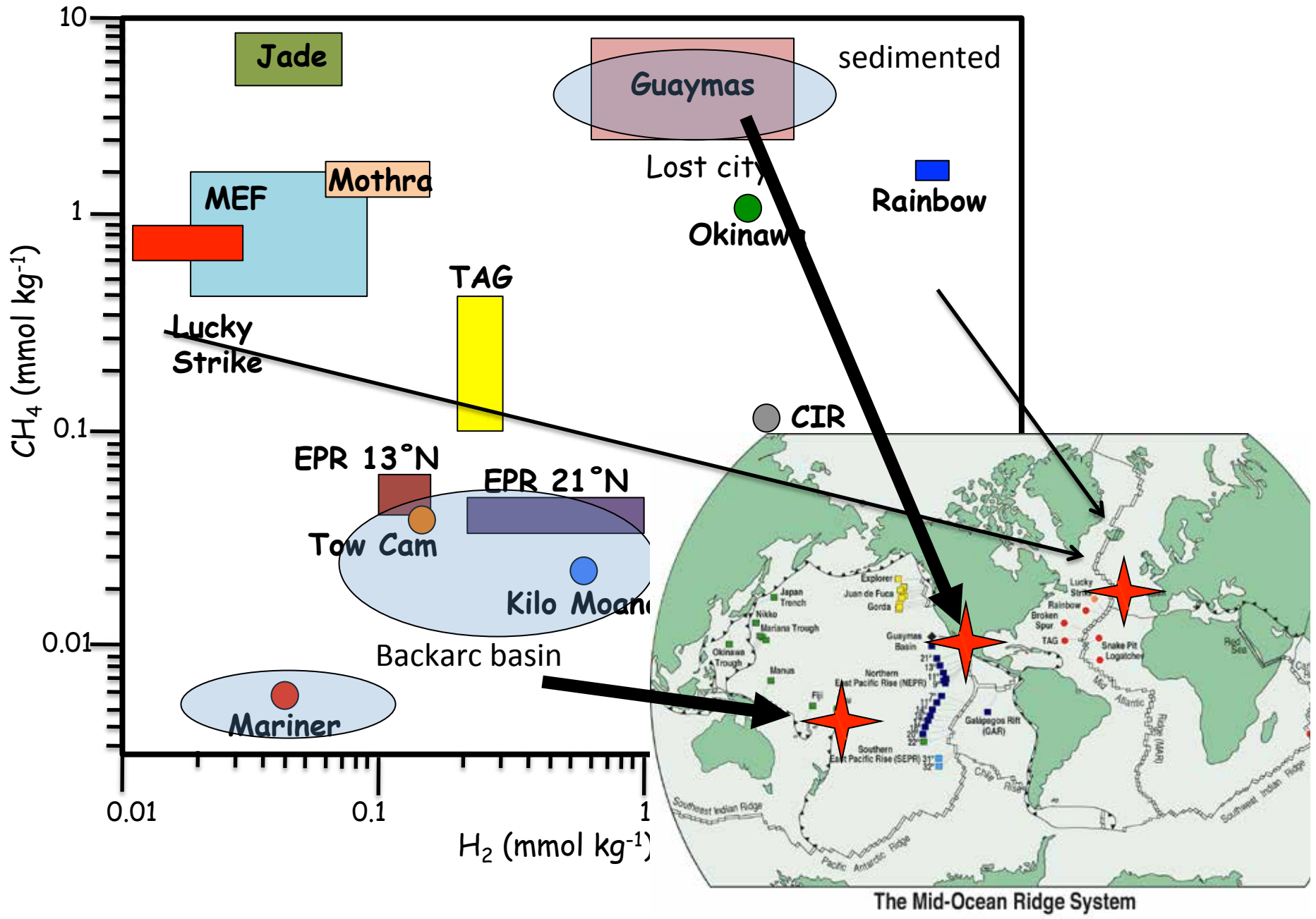

Spirochetes
Korarchaeota
Algae
EUKARYA
ultrathin section
0.5 µm

How do they respond to pertubations?
OR
what affects their distribution?


H_2S
H_2
360°C
$[CH_2O]$
O_2
2°C
Micro-niches for the occurence of hyperthermophilic microbial processes at the top section of a smoker chimney wall based on gradients of nutrients of physico-chemical conditions.
from Jannasch, 1995
Mesophilic H_2S oxidizers, methanogens, Fe oxidizers, Mn oxidizers
Chimney wall
Thermophilic methanogens SO_4^{2-} reducers ?
Seawater
"Knallgas" bacteria?
Vent fluid
2°C
20-50°C
100°C
200°C
300°C
350°C

Geochemical/geological ‘perturbations’?



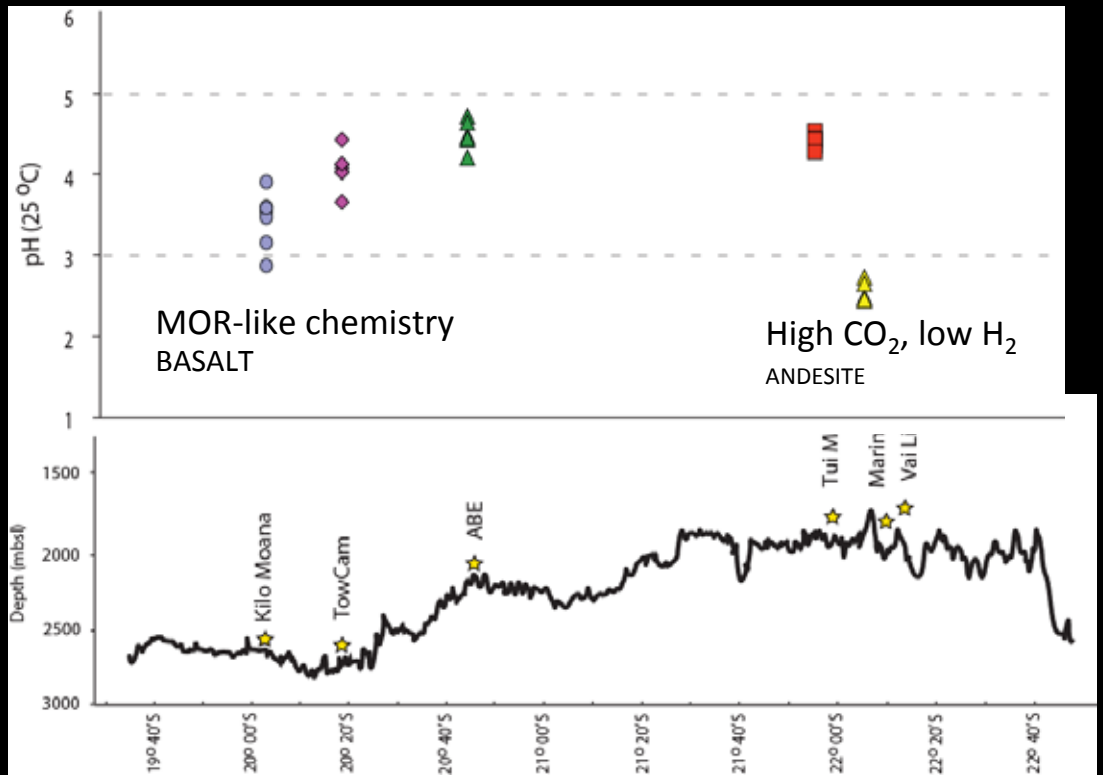

10
1
0.1
0.01
CH_4 (mmol kg^{-1})
0.01
0.1
1
H_2 (mmol kg^{-1})
Jade
Guaymas
sedimented
Lost city
Okinawa
Rainbow
MEF
Mothra
TAG
Lucky Strike
CIR
EPR 13°N
EPR 21°N
Tow Cam
Kilo Moana
Backarc basin
Mariner
Explorer
Juan de Fuca
Gorda
Japan Trench
Nikko
Mariana Trough
Okinawa Trough
Manus
Fiji
Northern East Pacific Rise (NEPR)
Guaymas Basin
Lucky Strike
Rainbow
Broken Spur
TAG
Snake Pit
Logatchev
Red Sea
Galapagos Rift (GAR)
Southern East Pacific Rise (SEPR)
Chile Rise
Mid Atlantic Ridge (MAR)
Southeast Indian Ridge
Southwest Indian Ridge
Pacific Antarctic Ridge
The Mid-Ocean Ridge System

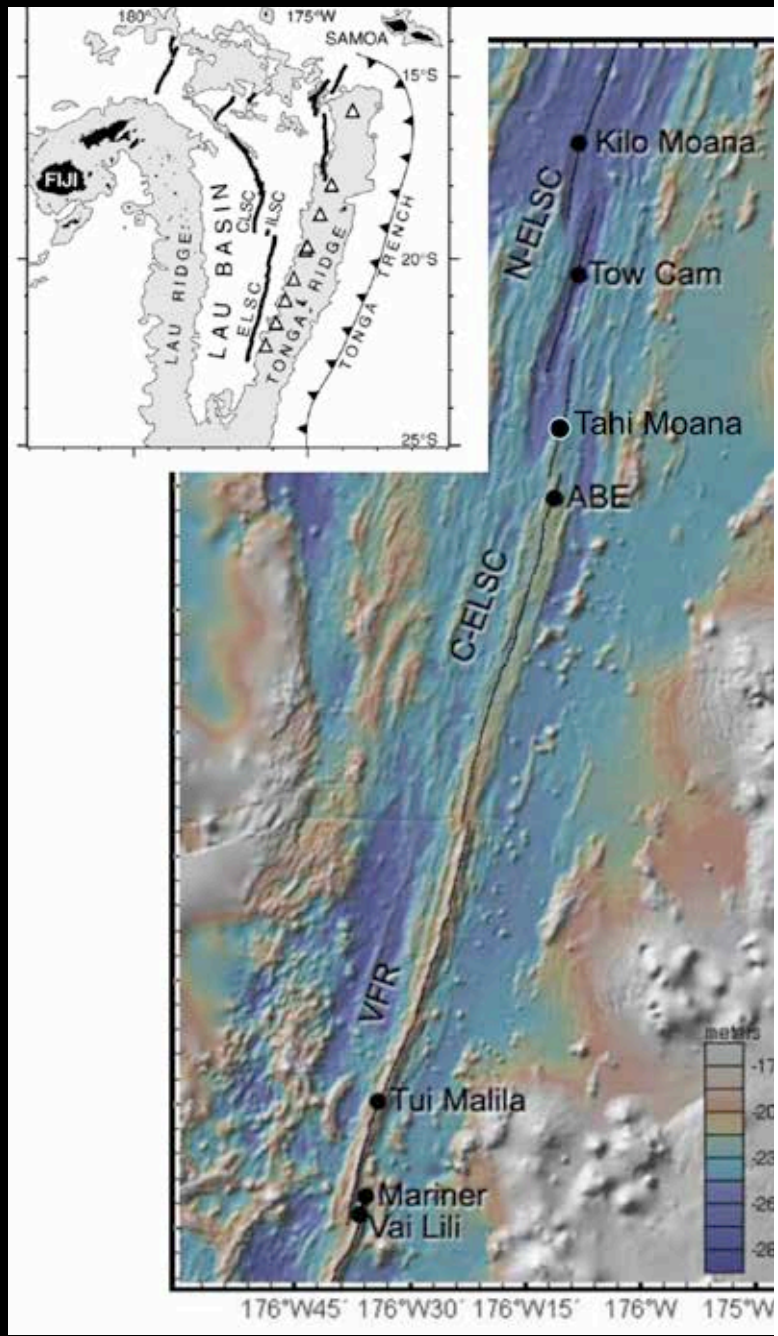
Back Arc Spreading Center (Eastern Lau Spreading Center)

Influence of subduction on melting, crustal composition/structure

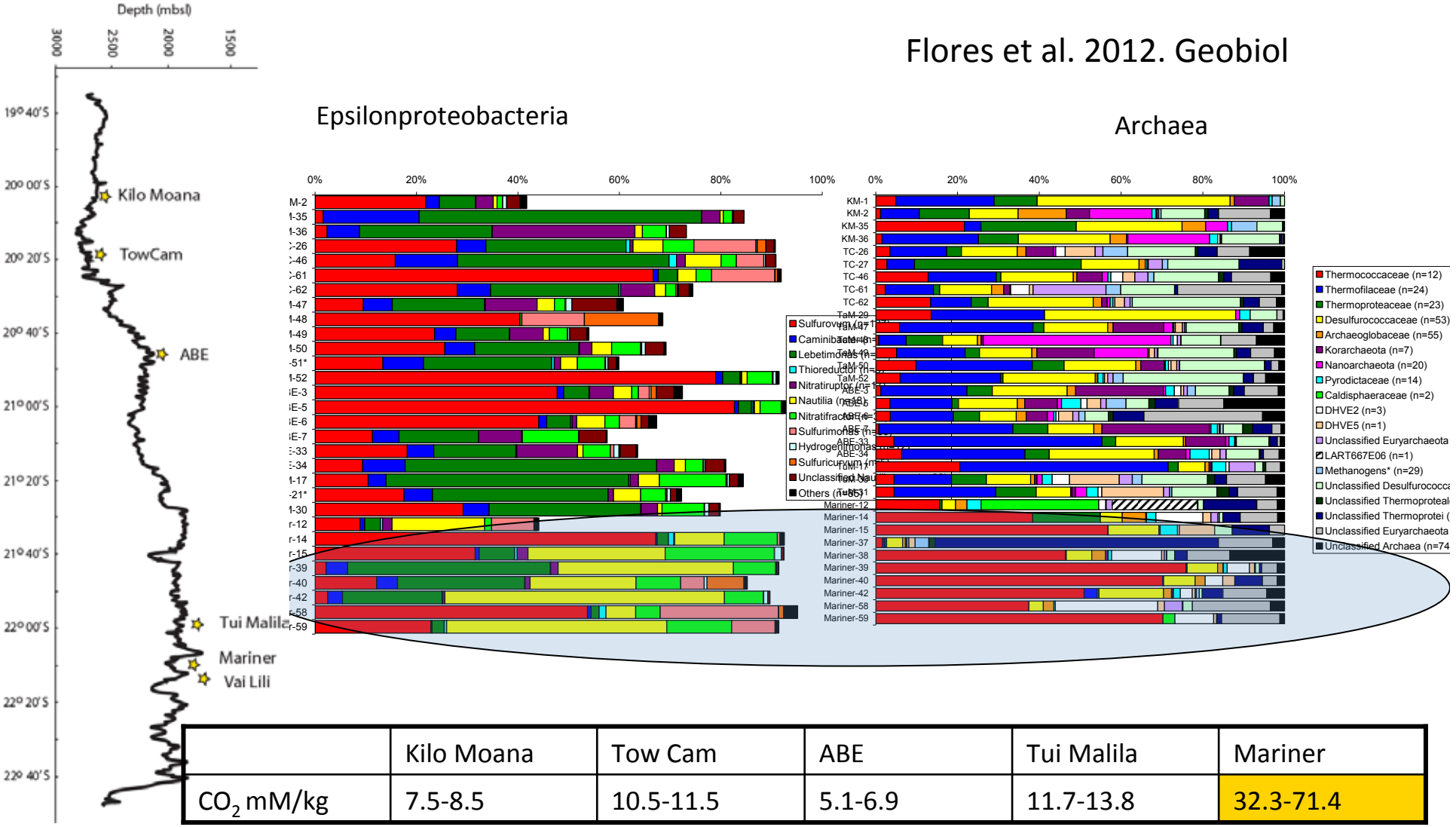
Variable distance between spreading axis and arc volcanism

Effects of geographic isolation on biology



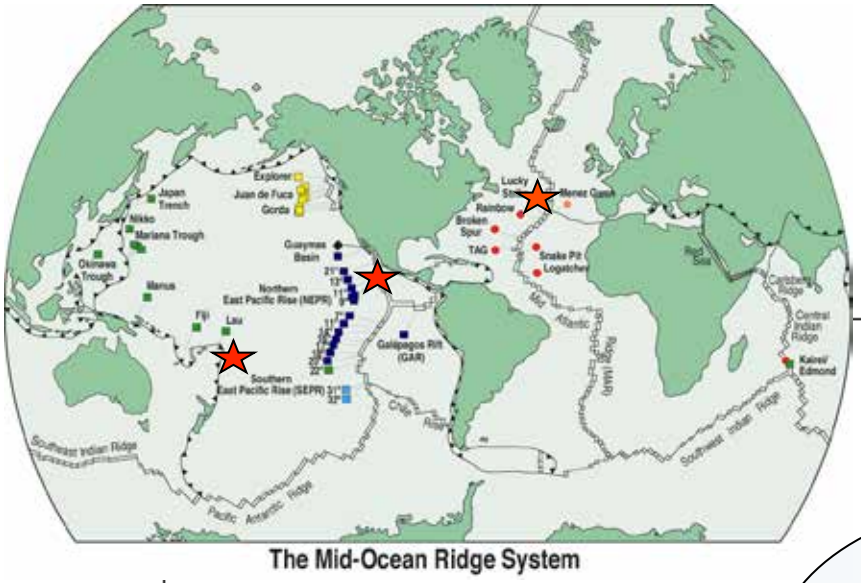


ELSC: bacterial and archaeal communities



Flores et al. 2012. Geobiol

	Kilo Moana	Tow Cam	ABE	Tui Malila	Mariner
CO_2 mM/kg	7.5-8.5	10.5-11.5	5.1-6.9	11.7-13.8	32.3-71.4



Standardise Samples by Total
Transform: Square root
Resemblance: S17 Bray Curtis similarity

2D Stress: 0.19

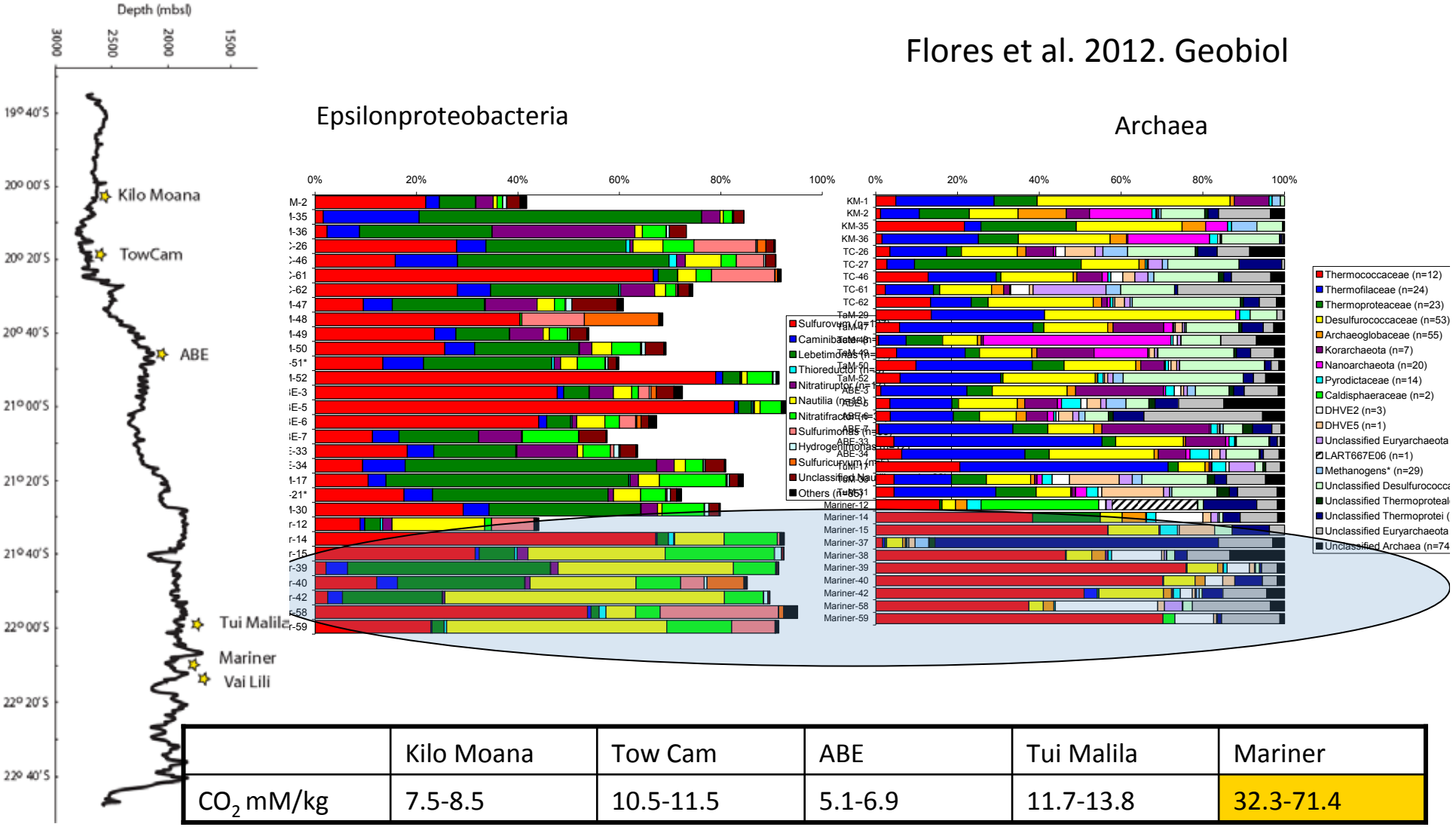
Low H_2

high H_2

Vent Field
- GB
- KM
- TC
- TAMO
- ABE
- TUMA
- MAR
- RB
- LS
- TAG

Flores et al.,2012
Geobiology

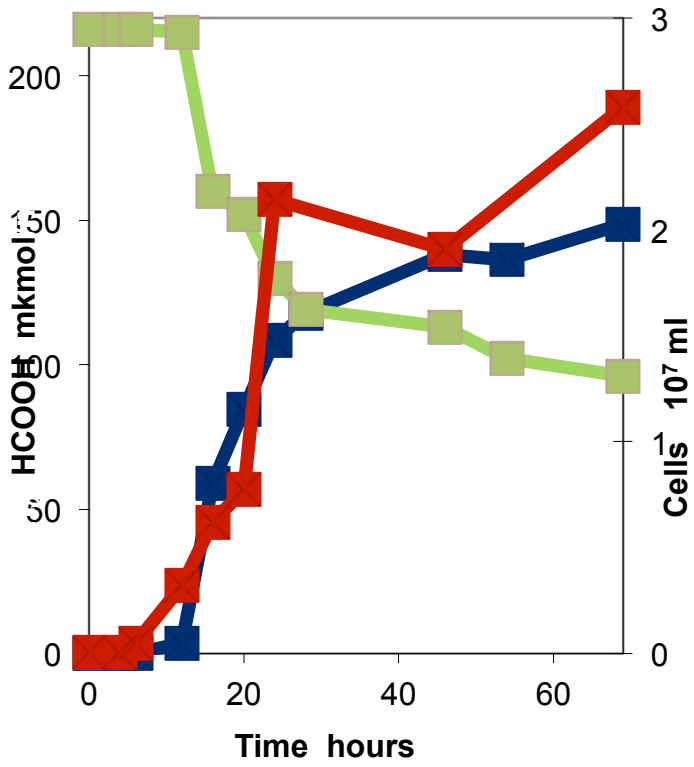
ELSC: bacterial and archaeal communities



Flores et al. 2012. Geobiol

	Kilo Moana	Tow Cam	ABE	Tui Malila	Mariner
CO_2 mM/kg	7.5-8.5	10.5-11.5	5.1-6.9	11.7-13.8	32.3-71.4

Anaerobic formate oxidation by Thermotogales



The energy of reaction:

$HCOO^- + H_2O \rightarrow HCO_3^- + H_2$

$\Delta G^{0'} = +1.3$ kJ/mol

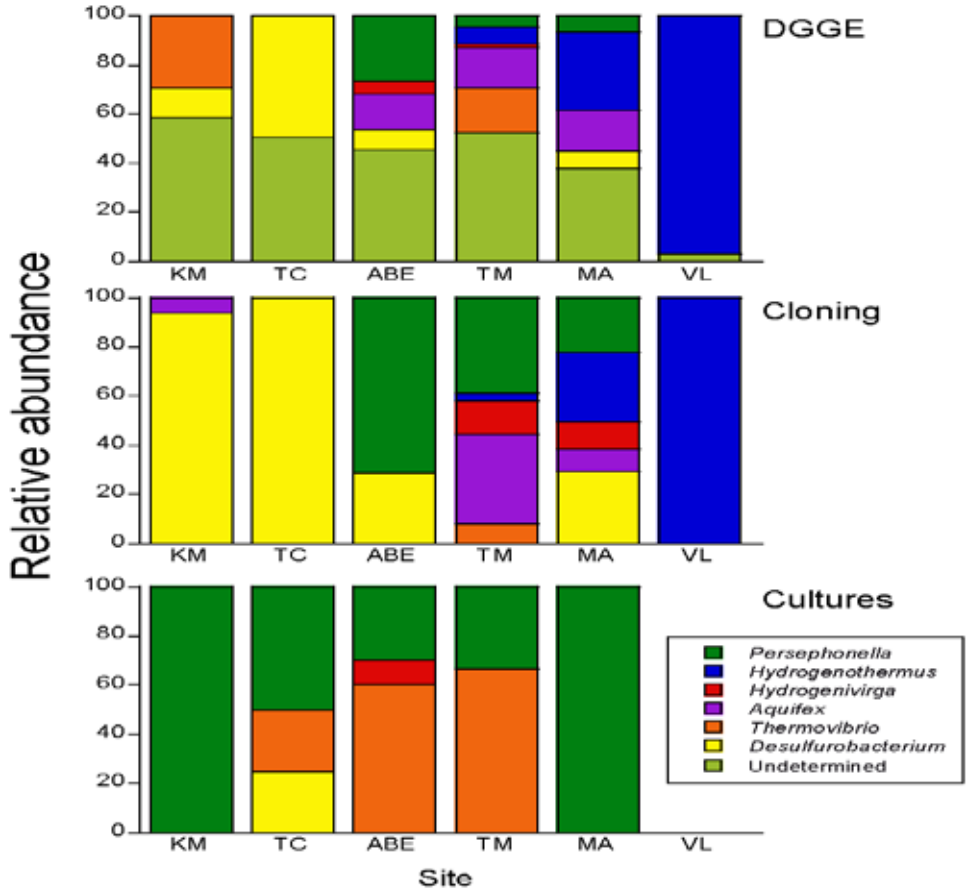
was always considered to be insufficient to support microbial growth

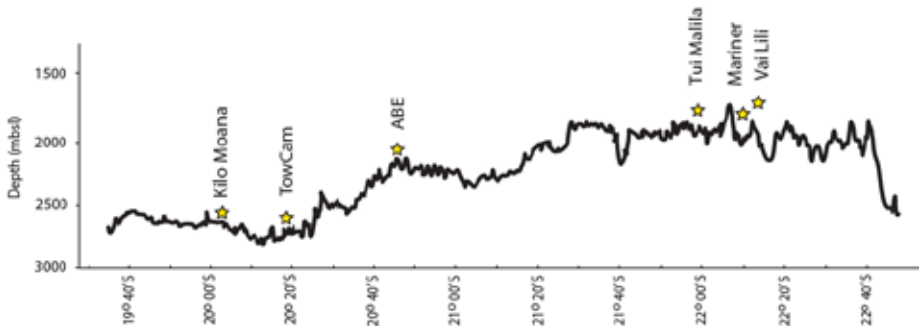
But at 80C….ΔG^{0} ' varied from -8 to -20 kJ/mol

Thermotogales are not at continental hotsprings (with an exception)

Kim et al., Nature, 2010, 467:352-355

SE study: Aquificales

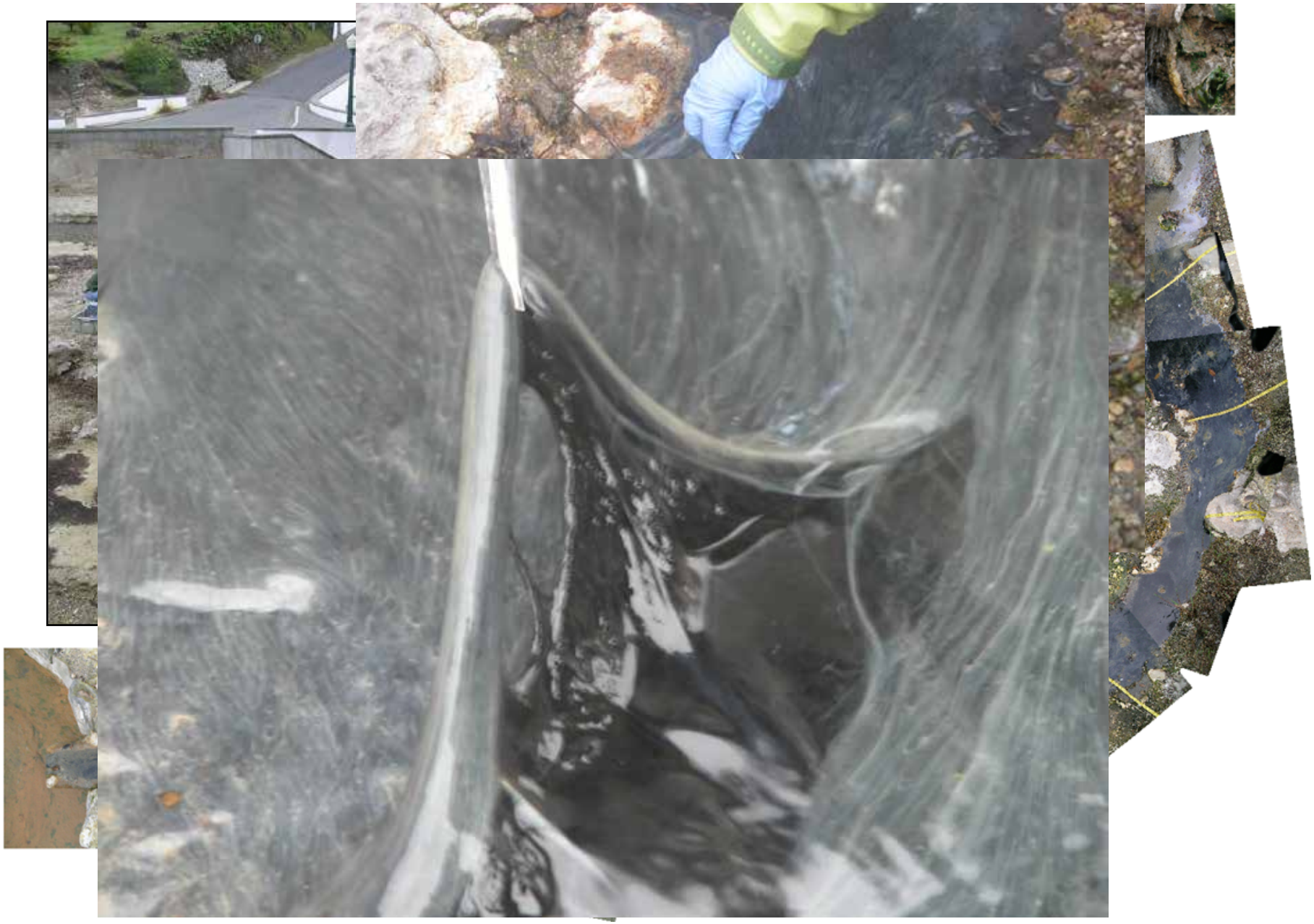




Like other groups…..
See their distribution at LAU trending with the sites.

Ferrera et al., 2014, Syst Appl Micro.

Case Study: Aquificales in continental systems

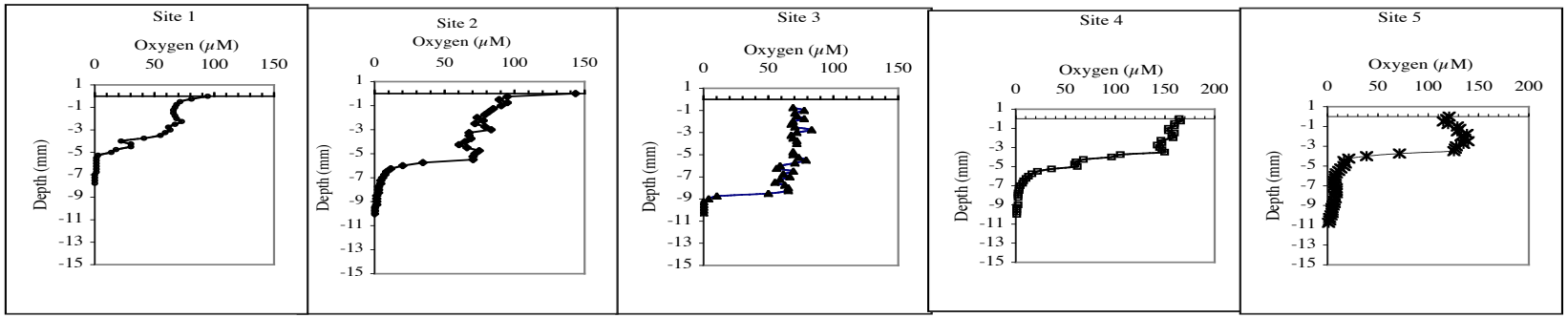


Responding to perturbations: Metabolic flexibility ..

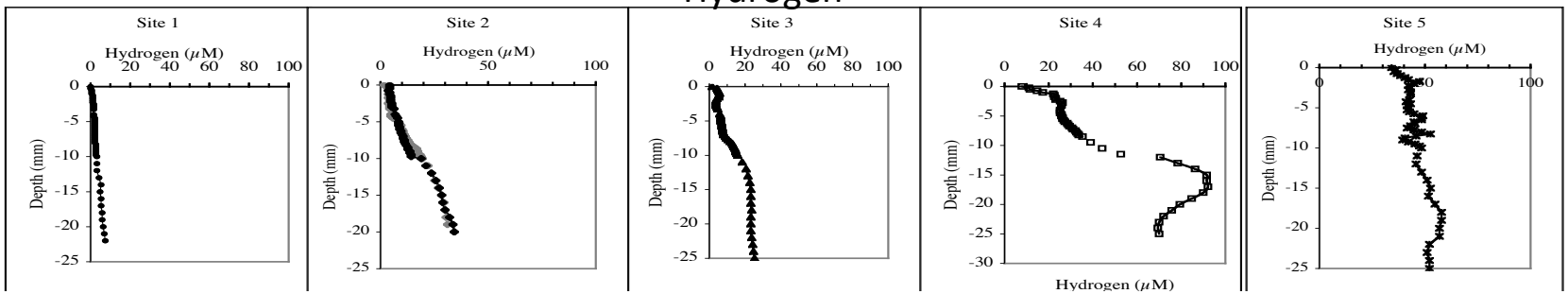
Hamamura et al., Environ Micro
Takacs-Vesbach et al, Frontiers in Extreme micro

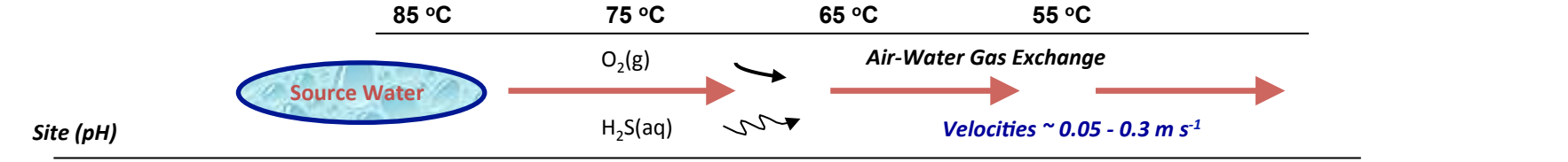
MICROPROFILES

Oxygen



Hydrogen



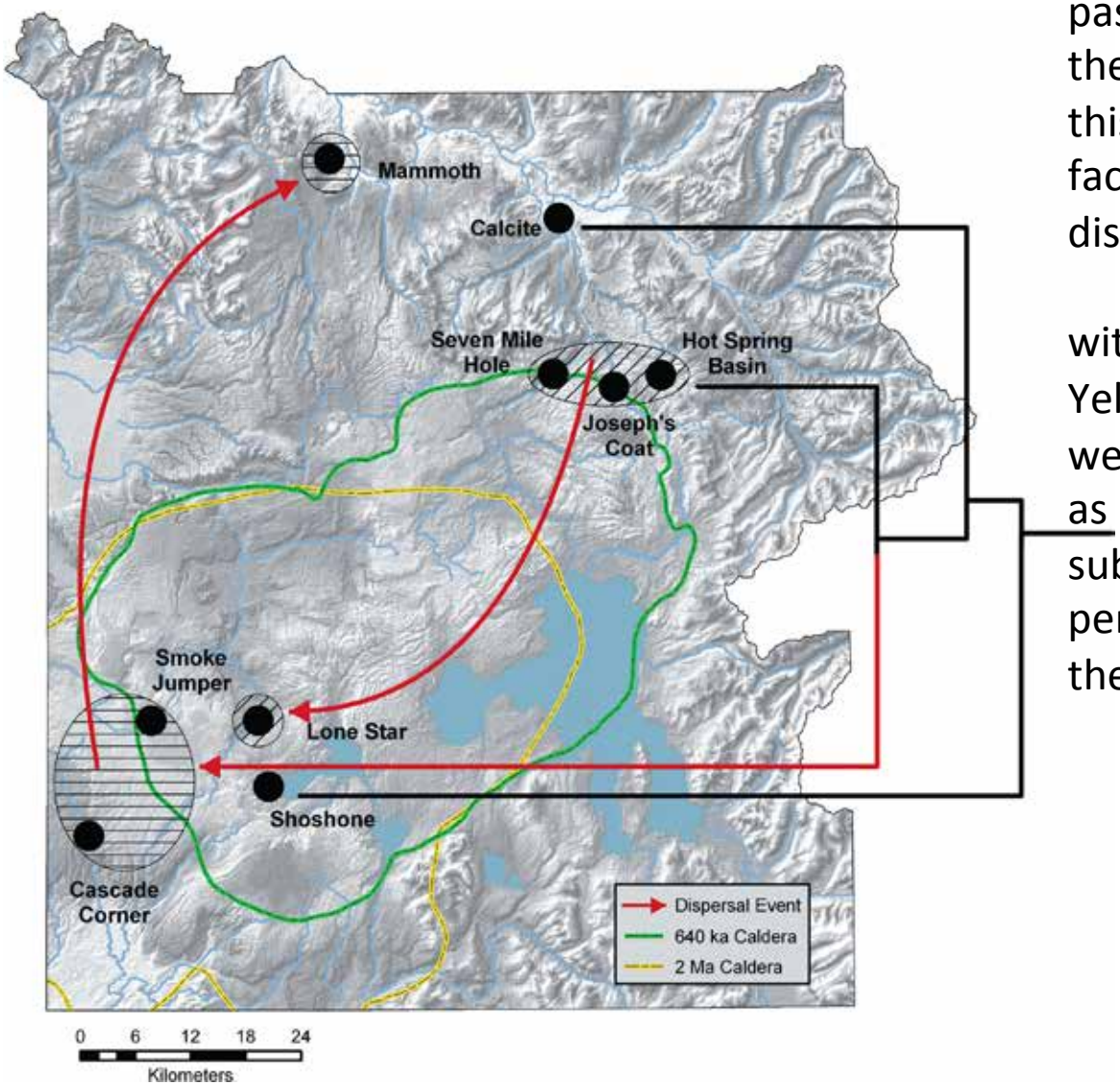


NGB-SS (3.1)	*Stygiolobus, Caldisphaera, Hydrogenobaculum, Metallosphaera, Acidimicrobium*
JC3 (6.2)	*Geothermobacterium, Caldococcus, Desulfurococcus, Sulfurihydrogenibium, Thermus*
NGB-PS (7)	*Thermofilum, Thermocrinis, Thermus, Thermomicrobium, Rhodothermus*
CS (8)	*Sulfurihydrogenibium*

Figure x. A conceptual summary of the distribution of 16S rRNA genes indicated using genus names of closest cultivated relatives as a function of distance from geothermal source for representative geothermal sites included in this study (Table 1). The exact position of each microbial population with respect to temperature is not absolute, but the trends in phylogenetic change down-gradient of geothermal discharge are represented along an O_2 gradient ranging from below detection (green) to saturation (white). Members of the Aquificales are highlited in brown font.

Hamamura et al, Inskeep et al,

The calderas delineate biogeographical provinces for Aquificales in Yellowstone

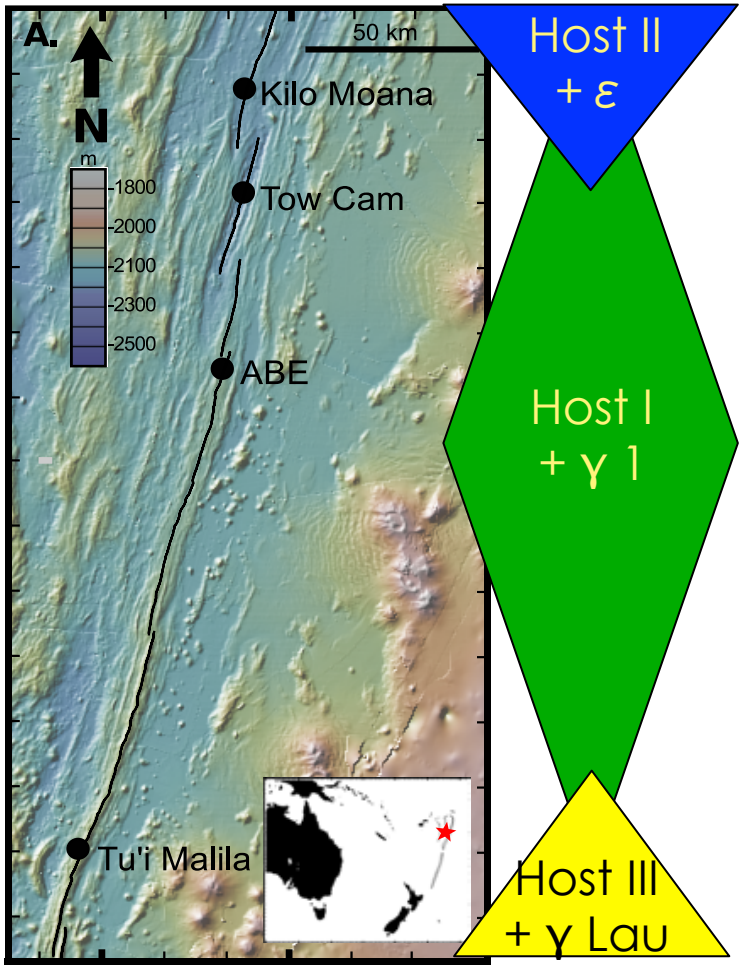


The pattern of distribution suggests that major geological events in the past 2 million years explain more of the variation in sequence diversity in this system than do contemporary factors such as habitat or geographic distance.

with each of the volcanic eruptions in Yellowstone, ancestral thermophiles went extinct within the calderas and as new springs formed, they were subsequently colonized from peripheral sites that survived outside the calderas.

Takacs-Vesbach et al, 2008

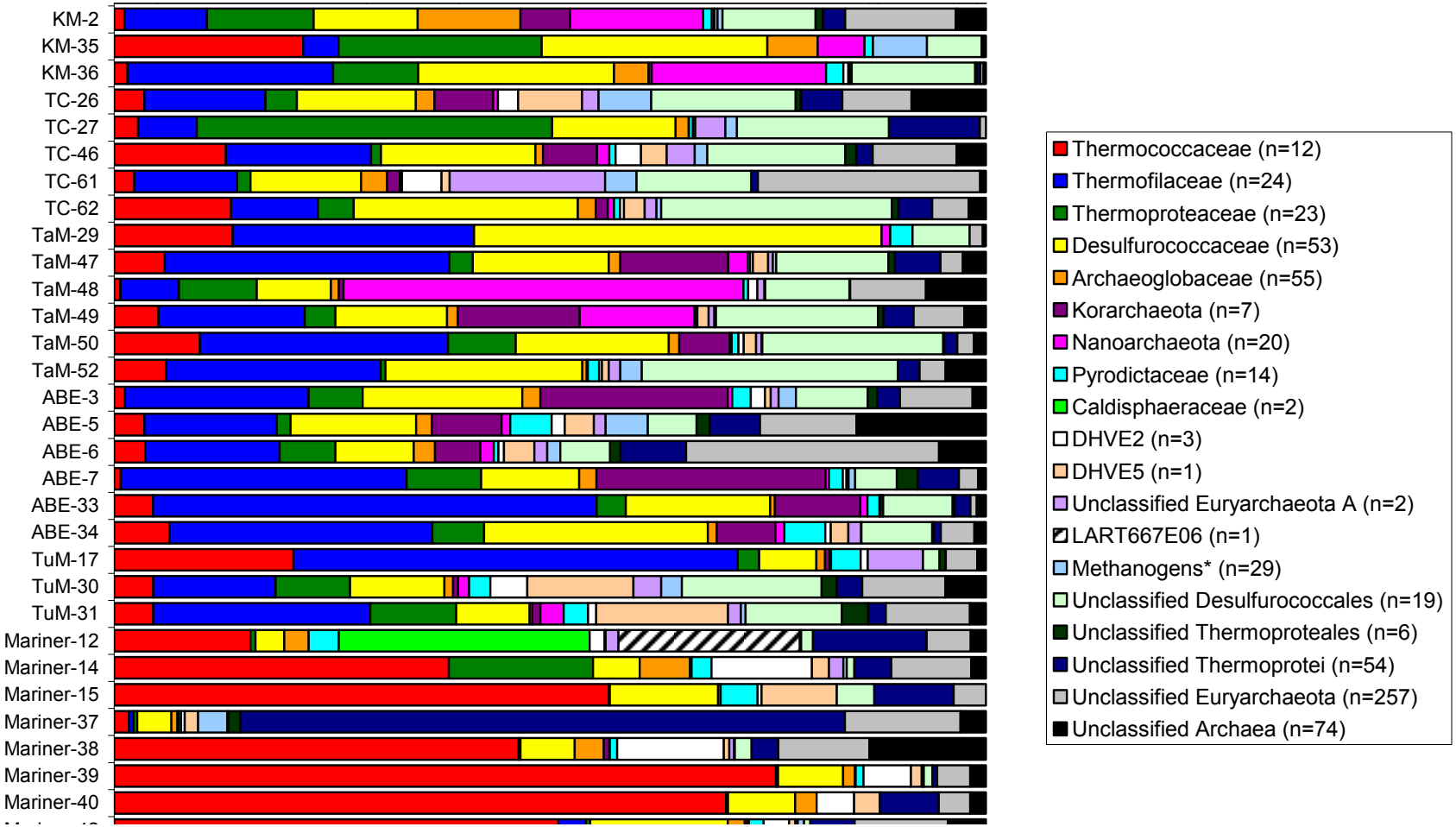
Not only chemistry – interspecies interactions— snails and endosymbionts at LAU

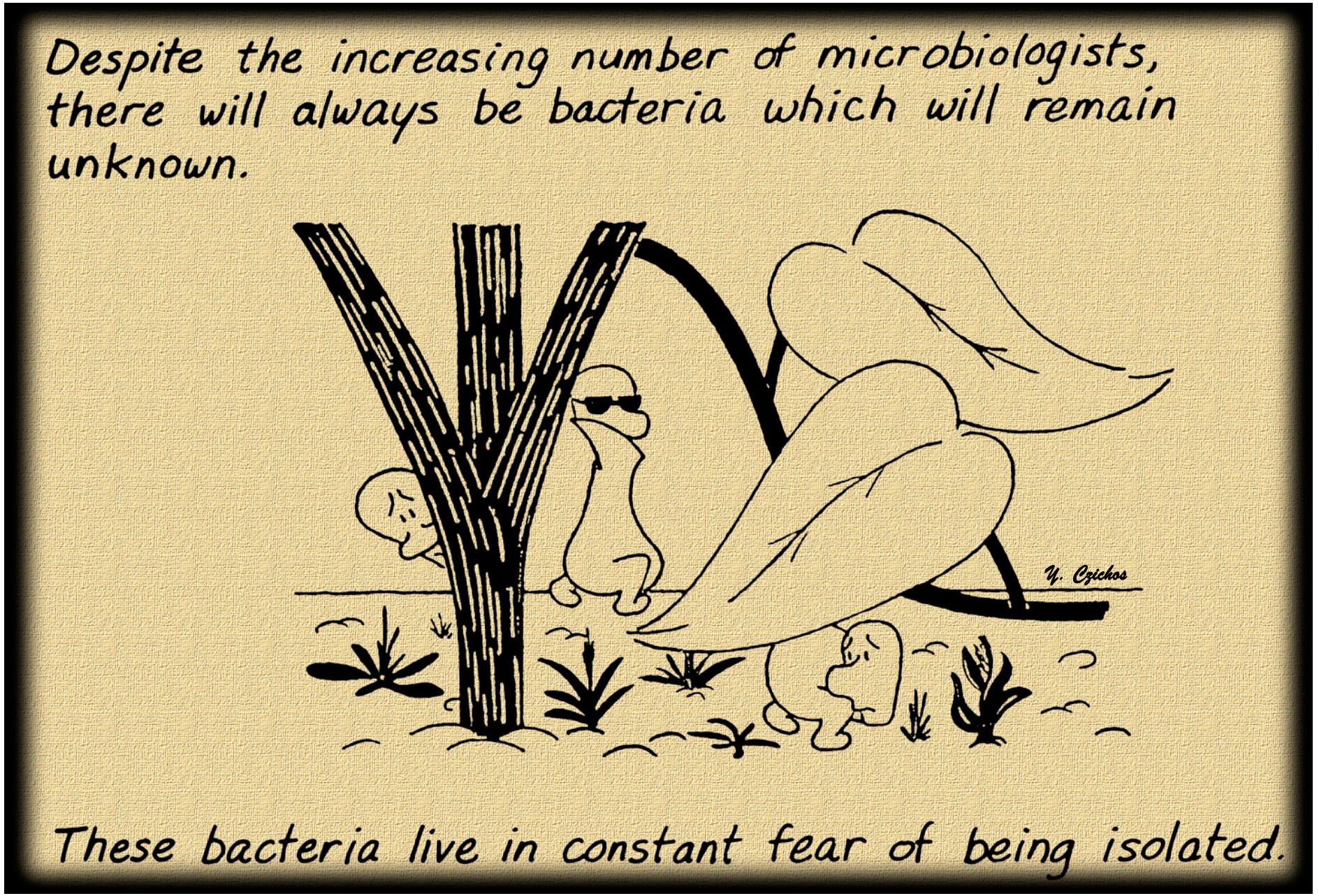


Courtesy Pete Girguis

Beinart et al, 2012 PNAS

Not only chemistry – interspecies interactions— Case study: **Nanoarchaeota and their hosts**





The illusive Nanoarchaeota

H. Huber et al. (2002) Nature, 417, 63.

A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont

Harald Huber*, Michael J. Hohn*, Reinhard Rachel*, Tanja Fuchs*†, Verena C. Wimmer‡ & Karl O. Stetter*

* *Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg, Germany*
‡ *Max Planck Institute for Medical Research, Department of Cell Physiology, Jahnstrasse 29, 69120 Heidelberg, Germany*

According to small subunit ribosomal RNA (ss rRNA) sequence comparisons all known Archaea belong to the phyla Crenarchaeota, Euryarchaeota, and—indicated only by environmental DNA sequences—to the 'Korarchaeota'[1,2]. Here we report the cultivation of a new nanosized hyperthermophilic archaeon from

† Present address: AstraZeneca GmbH, Tinsdaler Weg 183, D-22876 Wedel, Germany.

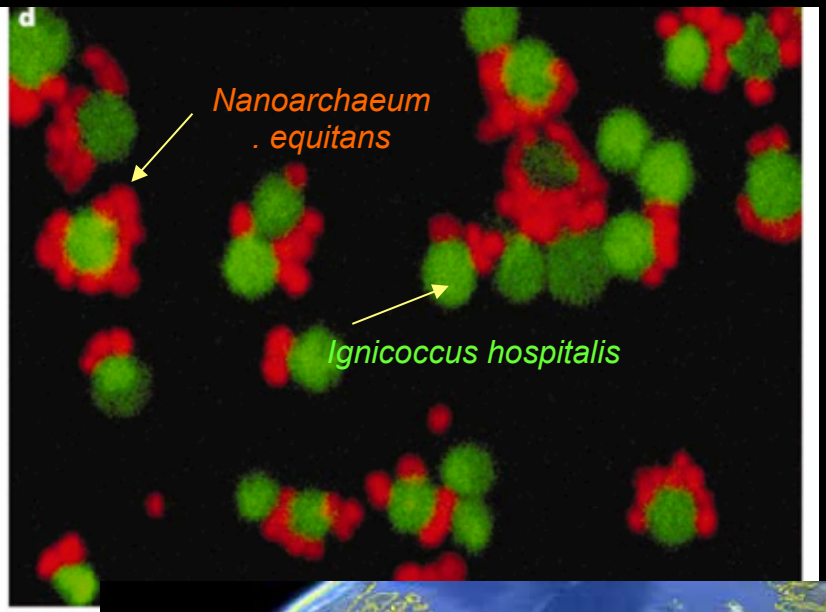


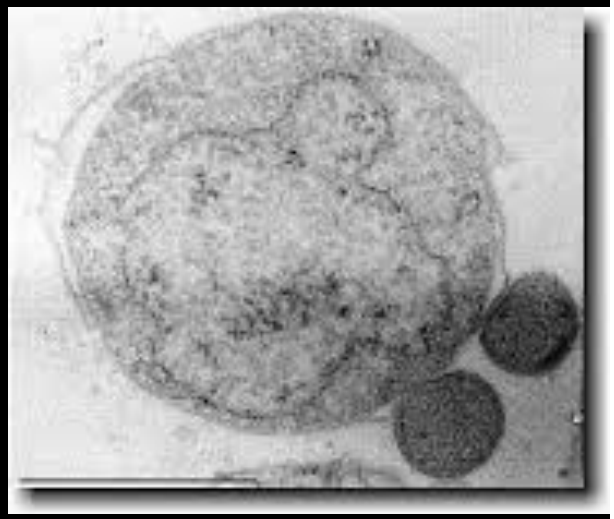
Figure 1 E
equitans'–I
cells of '*Na*
b, Ultrathin
Ignicoccus.
side; platinu
the CY3-lab
CREN499R

McClimment et al., first colonizer of deep-sea vent chimney

Ignicoccus hospitalis

- 90^{o}C, marine, obligate chemolithoautotroph (CO_2, H_2/S^0)

Huber et al. (2000) Paper et al. (2007)



Nanoarchaeum equitans:

- Strict dependence on attachment to *Ignicoccus hospitalis* surface Huber et al. (2002) and direct evidence of small molecule acquisition from host Jahn et al (2005, 2008)
- Little effect on *Ignicoccus* lab co-cultures (commensal/parasite?) Jahn et al (2008)
- Apparent strict host specificity Jahn et al (2008)
- Representative of a distinct archaeal phylum ?

Gene 421 (2008) 20–26

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

The split genes of *Nanoarchaeum equitans* are an ancestral character ☆

Massimo Di Giulio *

Laboratory for Molecular Evolution, Institute of Genetics and Biophysics 'Adriano Buzzati Traverso', CNR, Via P. Castellino, 111, 80131 Naples, Napoli, Italy

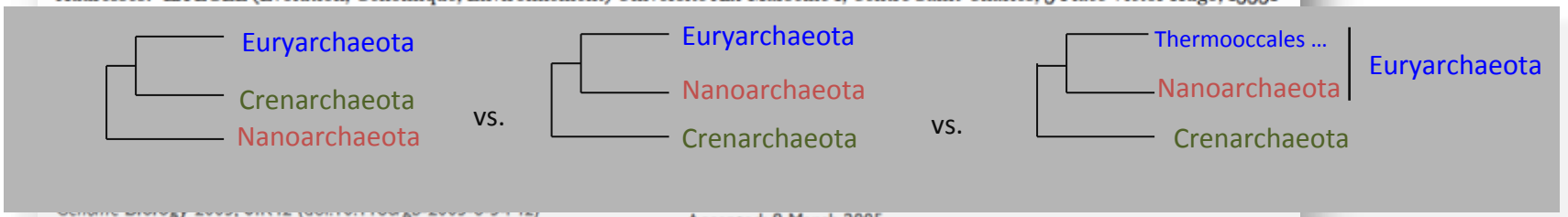
Research

Open Access

Nanoarchaea: representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to Thermococcales?

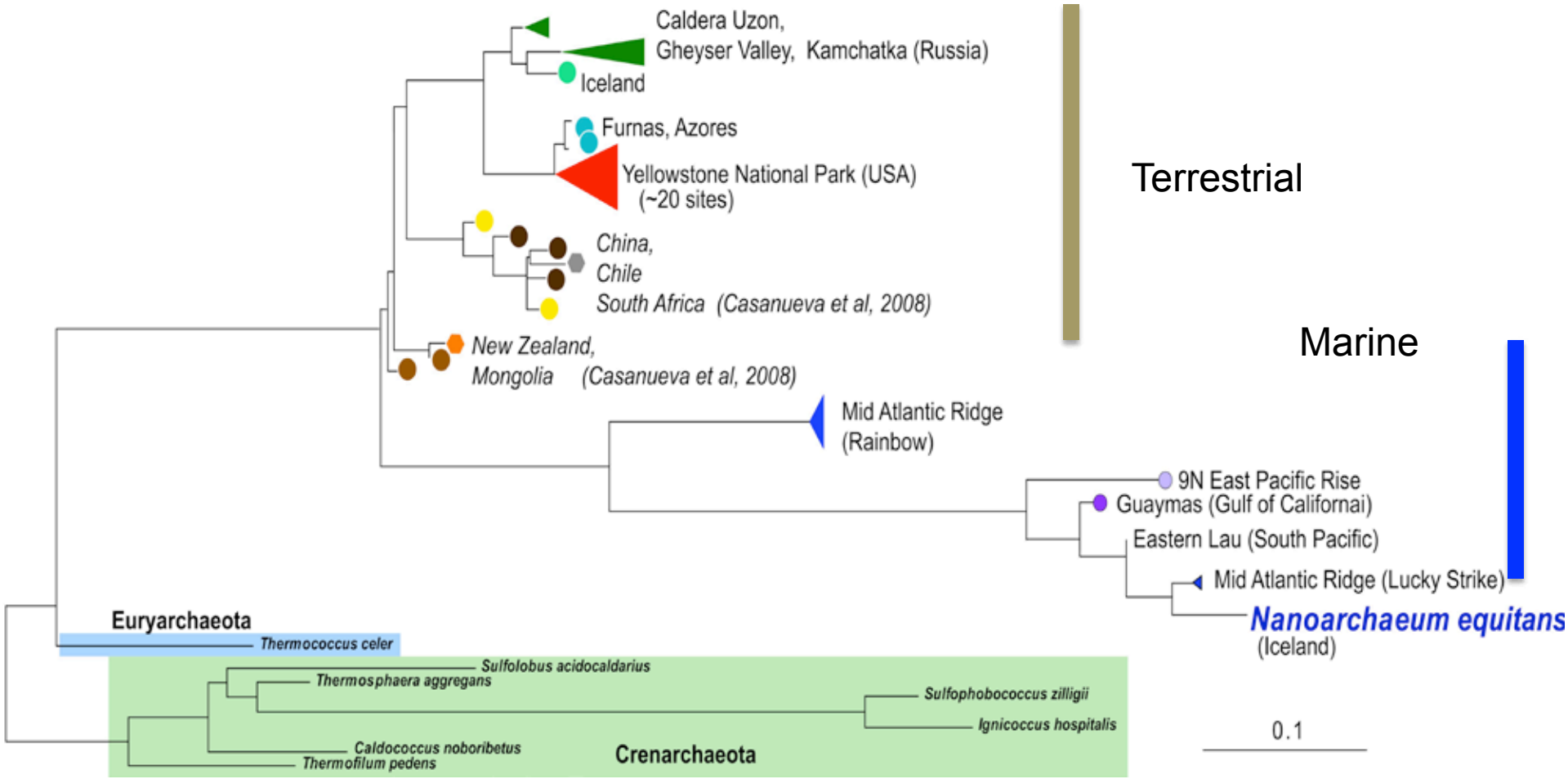
Celine Brochier*, Simonetta Gribaldo†, Yvan Zivanovic‡, Fabrice Confalonieri‡ and Patrick Forterre†‡

Addresses: *EA EGEE (Evolution, Génomique, Environnement) Université Aix-Marseille I, Centre Saint-Charles, 3 Place Victor Hugo, 13331



Accepted: 9 March 2005

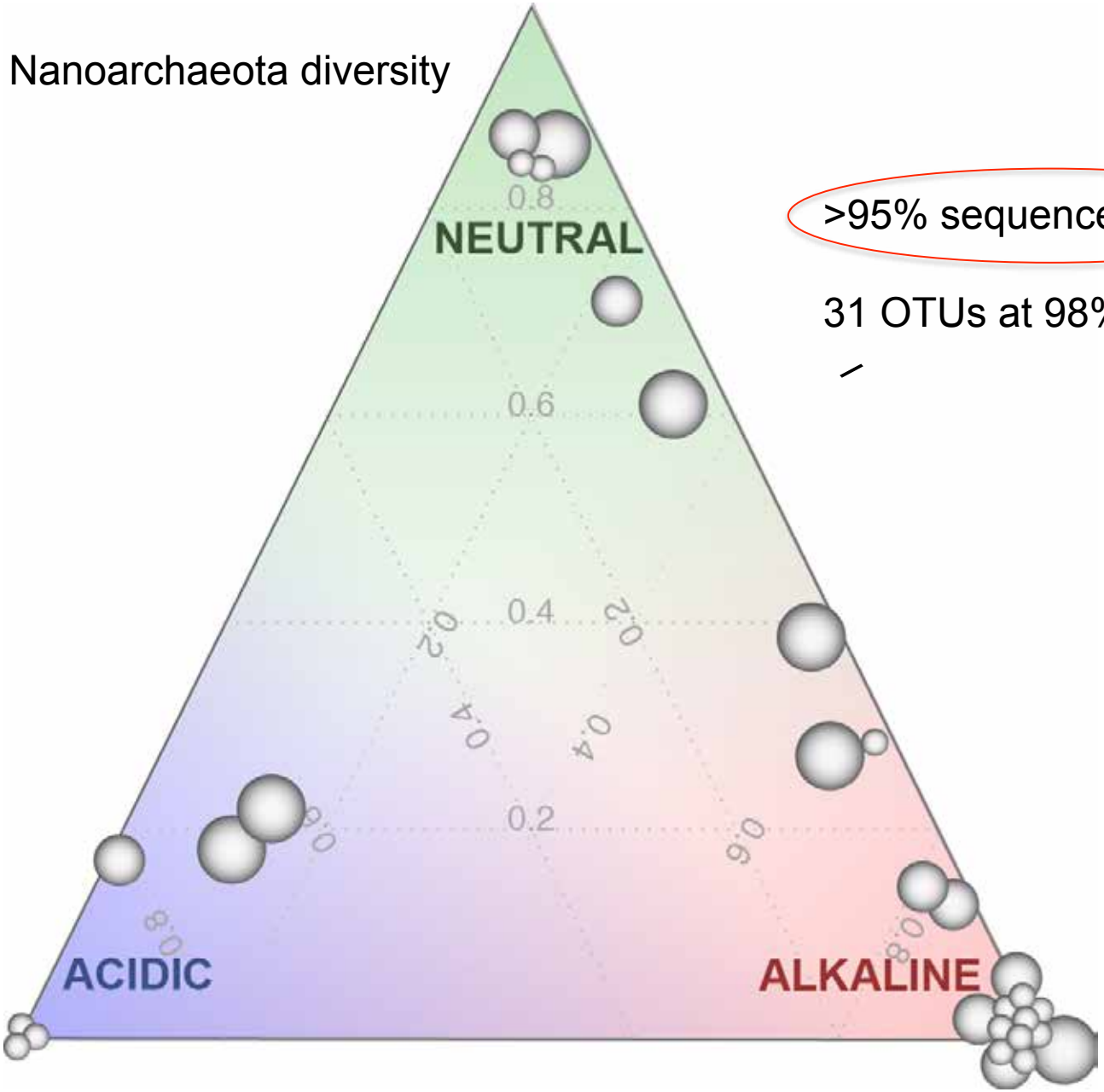
Global distribution: >500 SSU rRNA sequences



- *a few from non-thermal sites (saline) (Cassanueva et al 2008)*
- Jncluding more data from 454 pyrosequencing characterizations

<5% sequence divergence

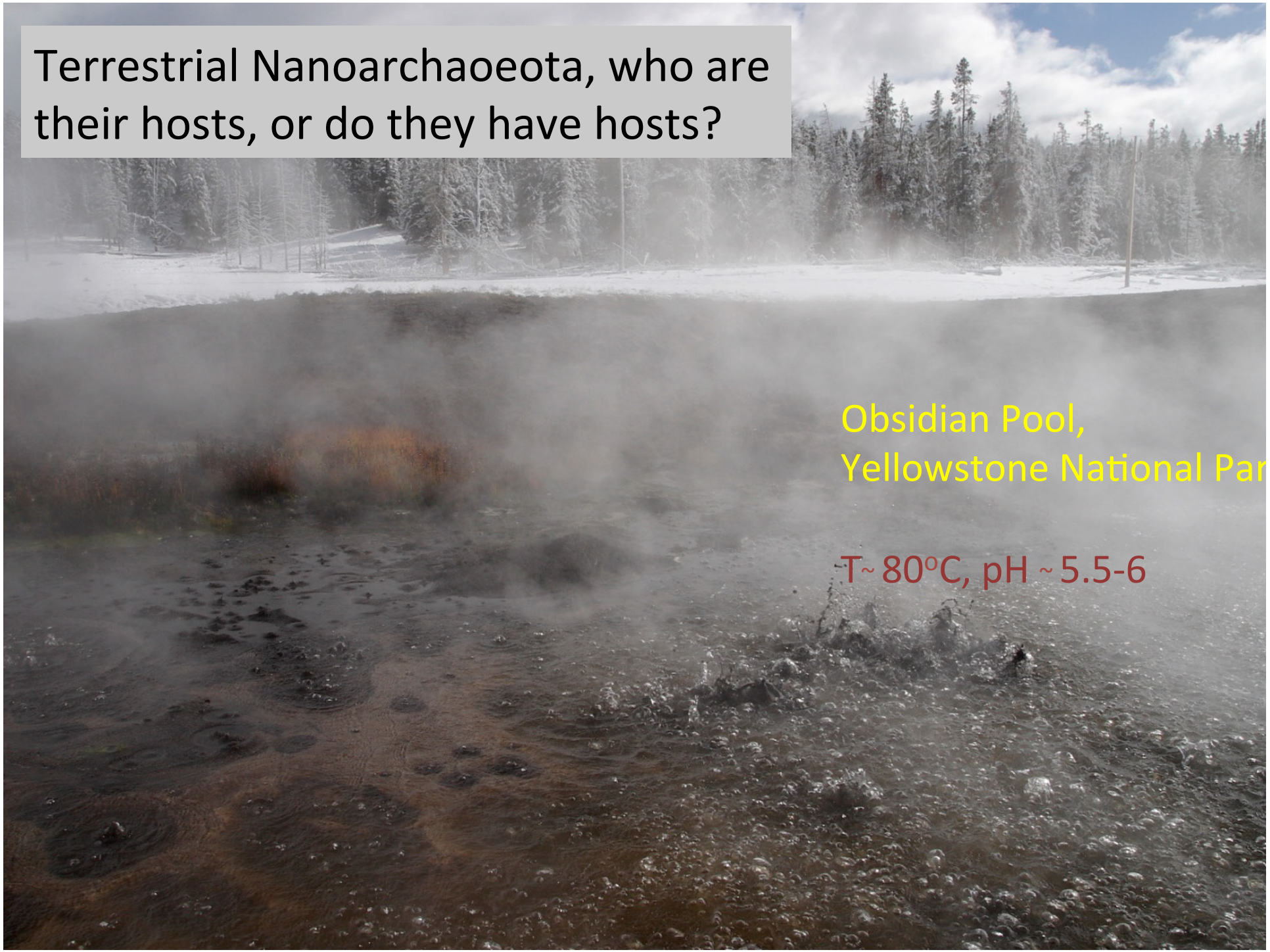
Yellowstone Nanoarchaeota diversity



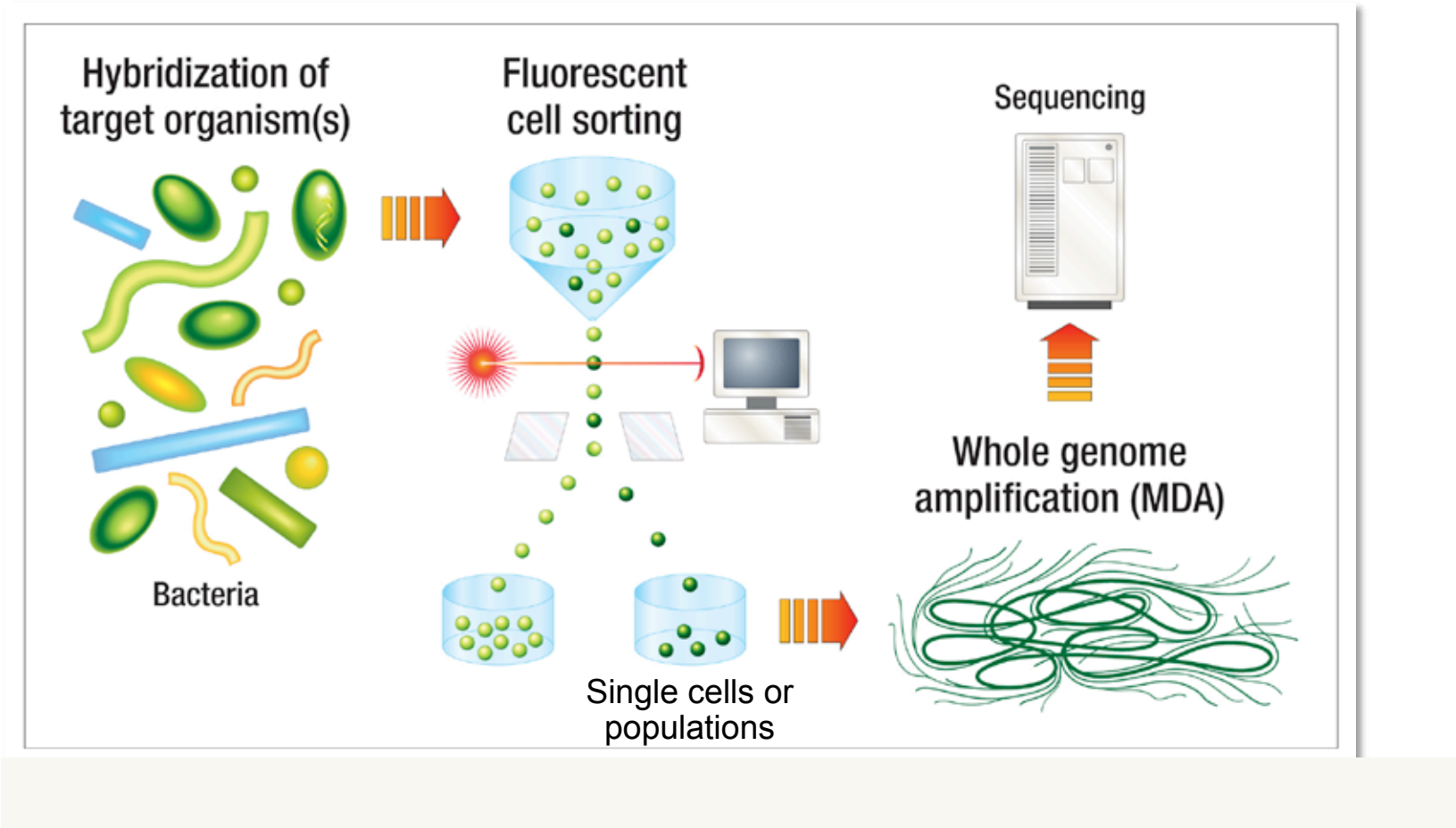
>95% sequence identity

31 OTUs at 98%

Mike Robeson


Terrestrial Nanoarchaoeota, who are their hosts, or do they have hosts?
Obsidian Pool,
Yellowstone National Par
T~ 80°C, pH ~ 5.5-6

Targeted single cell genomics and metagenomics



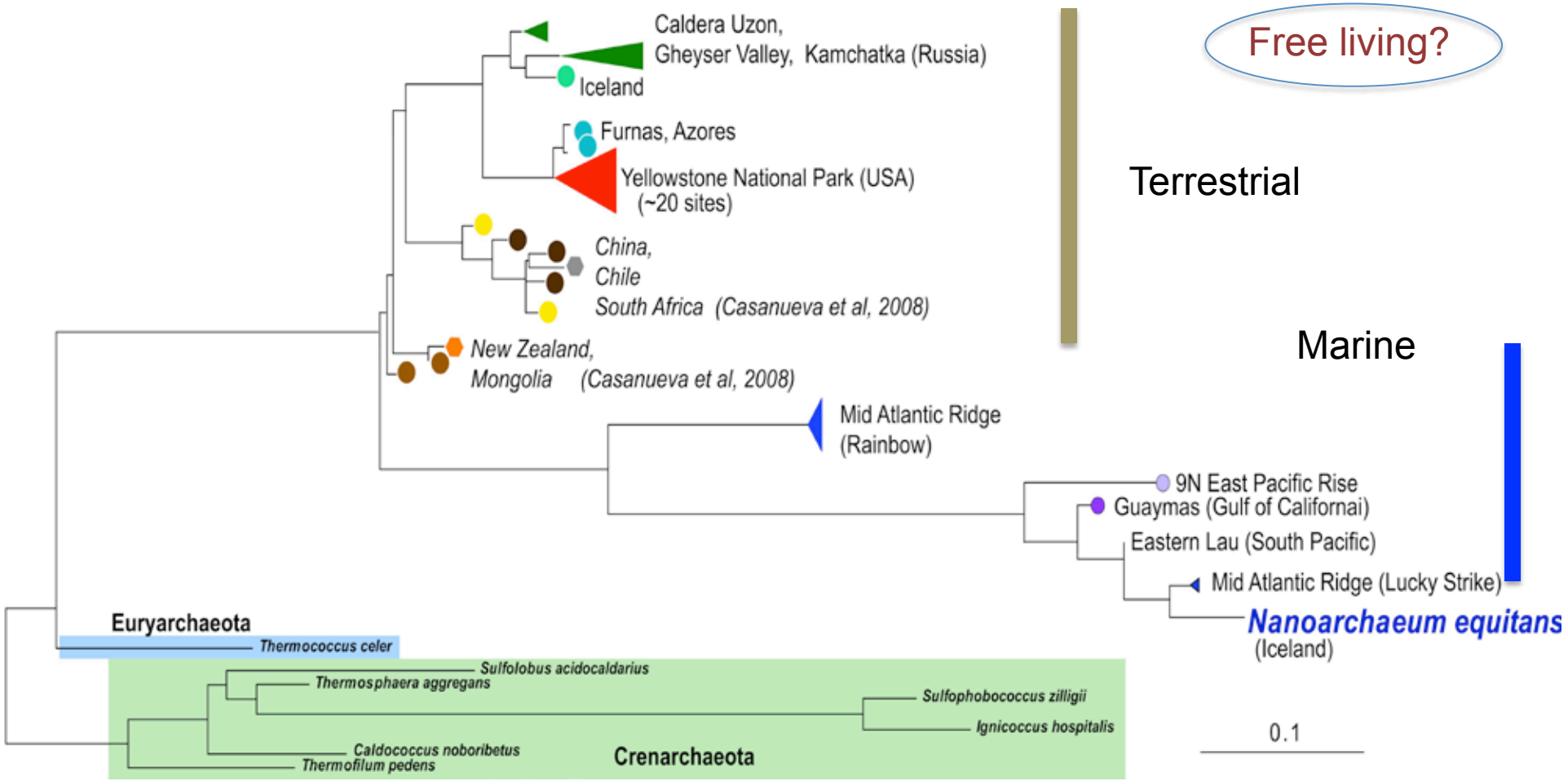
Features	N. equitans	Nst1	Note
Split Proteins			
Reverse gyrase	NEQ 318-434	Nst 337-402	Same site
Glu-tRNA^{Gln} amidotransferase	NEQ 245-396	Nst 197-449	Same site
Predicted RNA-binding protein	NEQ 438-506	Nst 176-251	Same site
RNA polymerase subunit B	NEQ 156-173	Nst 632-633	Same site*
Archaeosine tRNA-guanine transglycosylase	NEQ 124-305	Nst 096-232	Same site*
Large helicase-related protein	NEQ 003-409	Nst 172-239	Different site
DNA polymerase I	NEQ 068-528	Nst 417	Not split in *Nst1*
Topoisomerase I	NEQ 045-324	Nst 174	Not split in *Nst1*
P-loop ATPase-acetyltransferase fusion protein	NEQ 096-495	Nst 401	Not split in *Nst1*
Alanyl-tRNA synthetase	NEQ 211-547	Nst 054	Not split in *Nst1*
Diphthamide synthase DPH2	NEQ226	Nst 222-441	Not split in N.eq
Uncharacterized conserved protein (arCOG04253)	-	Nst 474-480	Absent in N.eq.
tRNAs			
cis-spliced tRNAs	Ile, Met, Trp, Tyr	Ile, Tyr	
trans-joined tRNAs	iMet, His, Lys, Gln, Glu (2)	none	
RNase P	Absent	Present	
Gluconeogenesis-Glycolysis	Absent	Present	
Polyamine biosynthesis	Absent	Present	
ATP synthase	Present	Absent	
Archaellum	Absent	Present	
Aminoacid metabolism	GluDH	AsnS, GATase	

! Split in other archaea also"
"

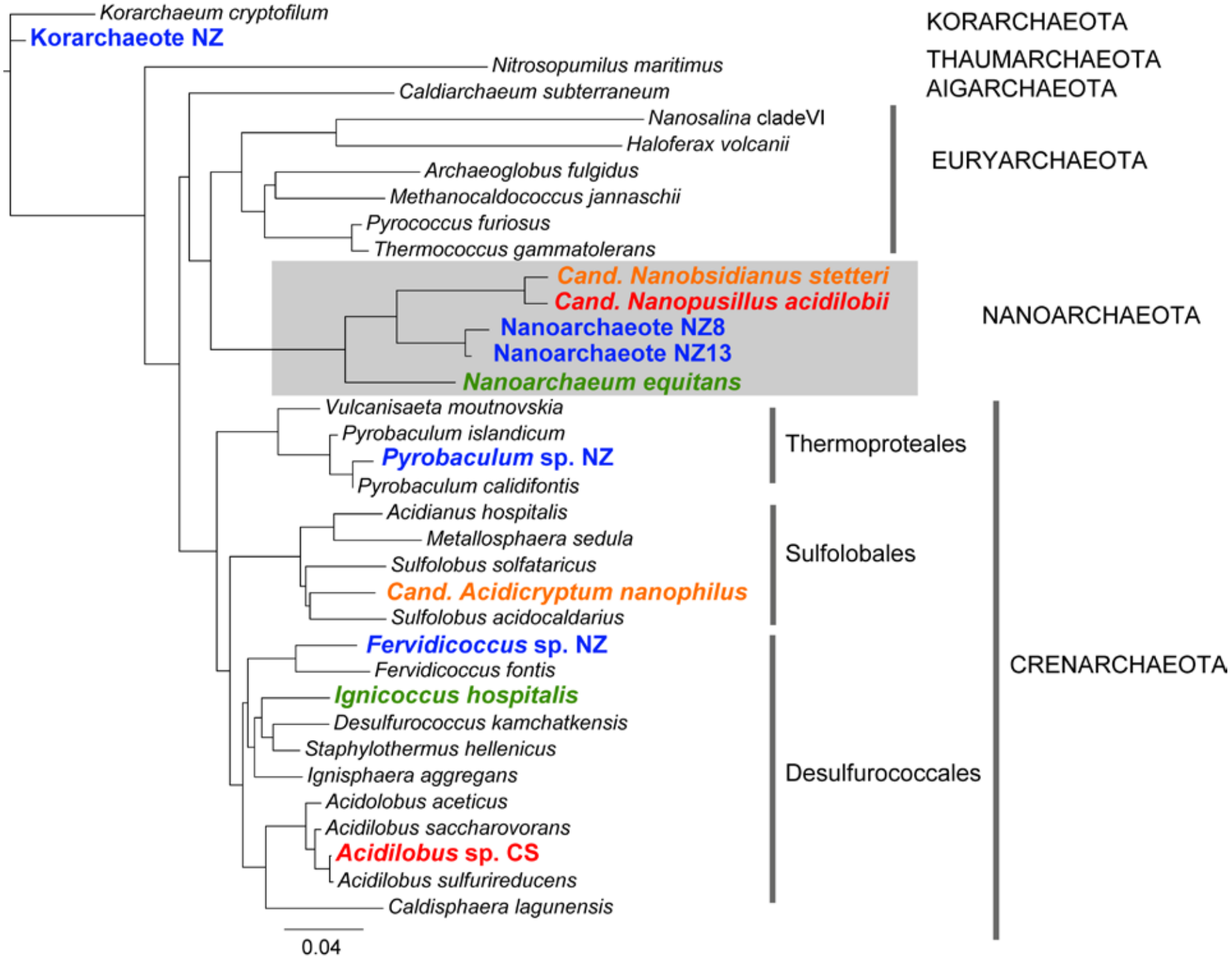
Table 1. Notable genomic differences between *N. equitans* and *Nst1*.

Podar et al 2013,

Global distribution: >500 SSU rRNA sequences



New Zealand Nanos
~74C, pH 6.45


Korarchaeum cryptofilum
Korarchaeote NZ
Nitrosopumilus maritimus
Caldiarchaeum subterraneum
Nanosalina cladeVI
Haloferax volcanii
Archaeoglobus fulgidus
Methanocaldococcus jannaschii
Pyrococcus furiosus
Thermococcus gammatolerans
Cand. Nanobsidianus stetteri
Cand. Nanopusillus acidilobii
Nanoarchaeote NZ8
Nanoarchaeote NZ13
Nanoarchaeum equitans
Vulcanisaeta moutnovskia
Pyrobaculum islandicum
Pyrobaculum sp. NZ
Pyrobaculum calidifontis
Acidianus hospitalis
Metallosphaera sedula
Sulfolobus solfataricus
Cand. Acidicryptum nanophilus
Sulfolobus acidocaldarius
Fervidicoccus sp. NZ
Fervidicoccus fontis
Ignicoccus hospitalis
Desulfurococcus kamchatkensis
Staphylothermus hellenicus
Ignisphaera aggregans
Acidolobus aceticus
Acidilobus saccharovorans
Acidilobus sp. CS
Acidilobus sulfurireducens
Caldisphaera lagunensis
0.04
KORARCHAEOTA
THAUMARCHAEOTA
AIGARCHAEOTA
EURYARCHAEOTA
NANOARCHAEOTA
Thermoproteales
Sulfolobales
Desulfurococcales
CRENARCHAEOTA

Conclusions

- Some commonalities between continental and marine microbial systems
- You are what you eat
- What you eat depends on host rock, mineralogy, fluid flowpaths, water-rock reactions, time, interactions with your Nanoarchaeotes etc.
- Just scratching the surface re. what drives microbial diversity and colonization in hot springs

but clearly the geology/geochemistry and biology are very deeply interconnected both in continental and marine systems

Thank-you

Gilberto Flores, Northridge, CA

Mircea Podar , Oakridge National Lab, USA

Meg Tivey and Jeff Seewald, WHOI

Matt Stott, Wiebke Ziebis, Pete Girguis

National Science Foundation (NSF), USA

The crew, pilots and scientific parties