

Deep Earth Life and the Nevada Test Site Connection

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07/29/09

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Extremophiles



Clouds

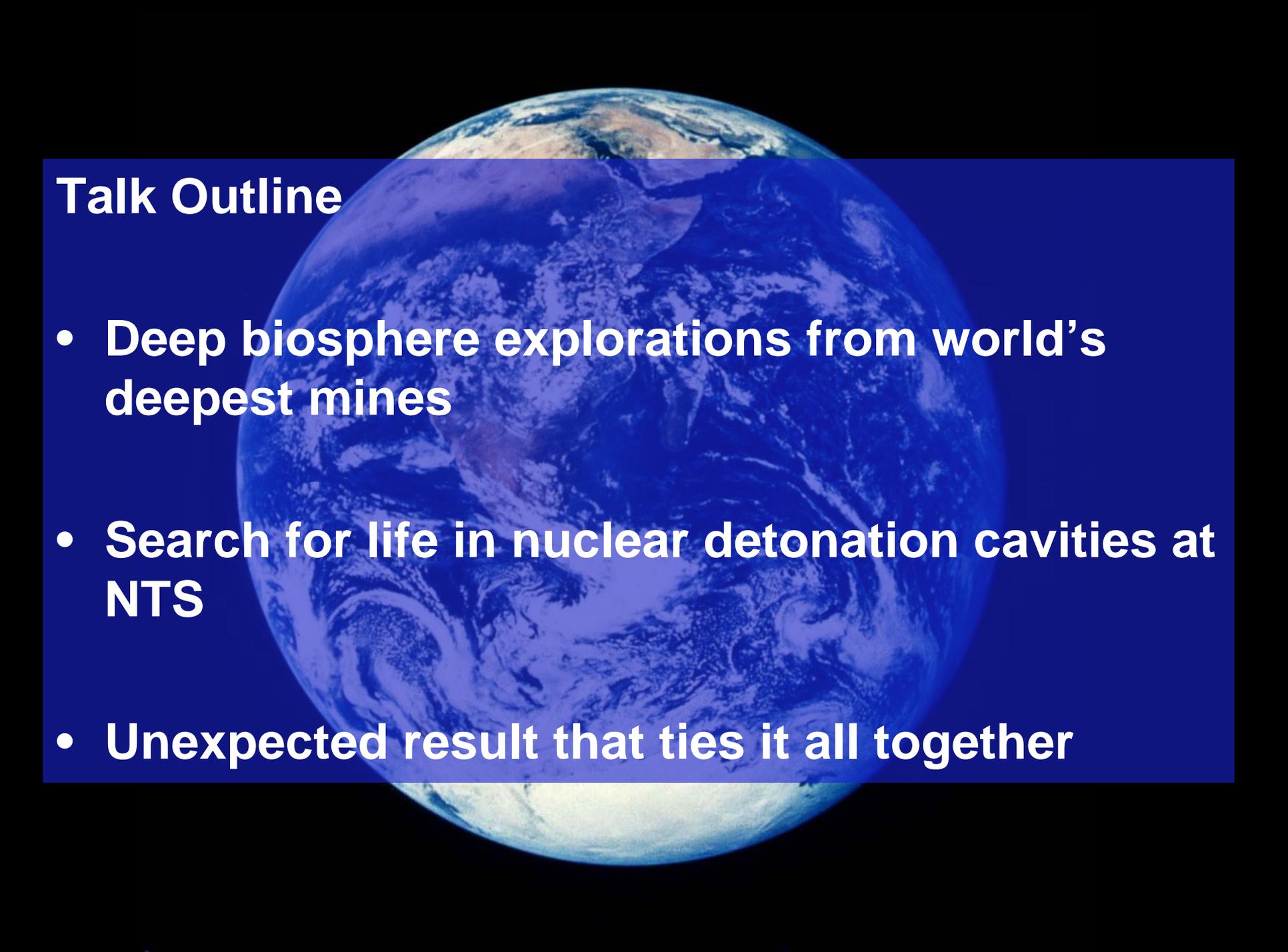


Atacama Desert



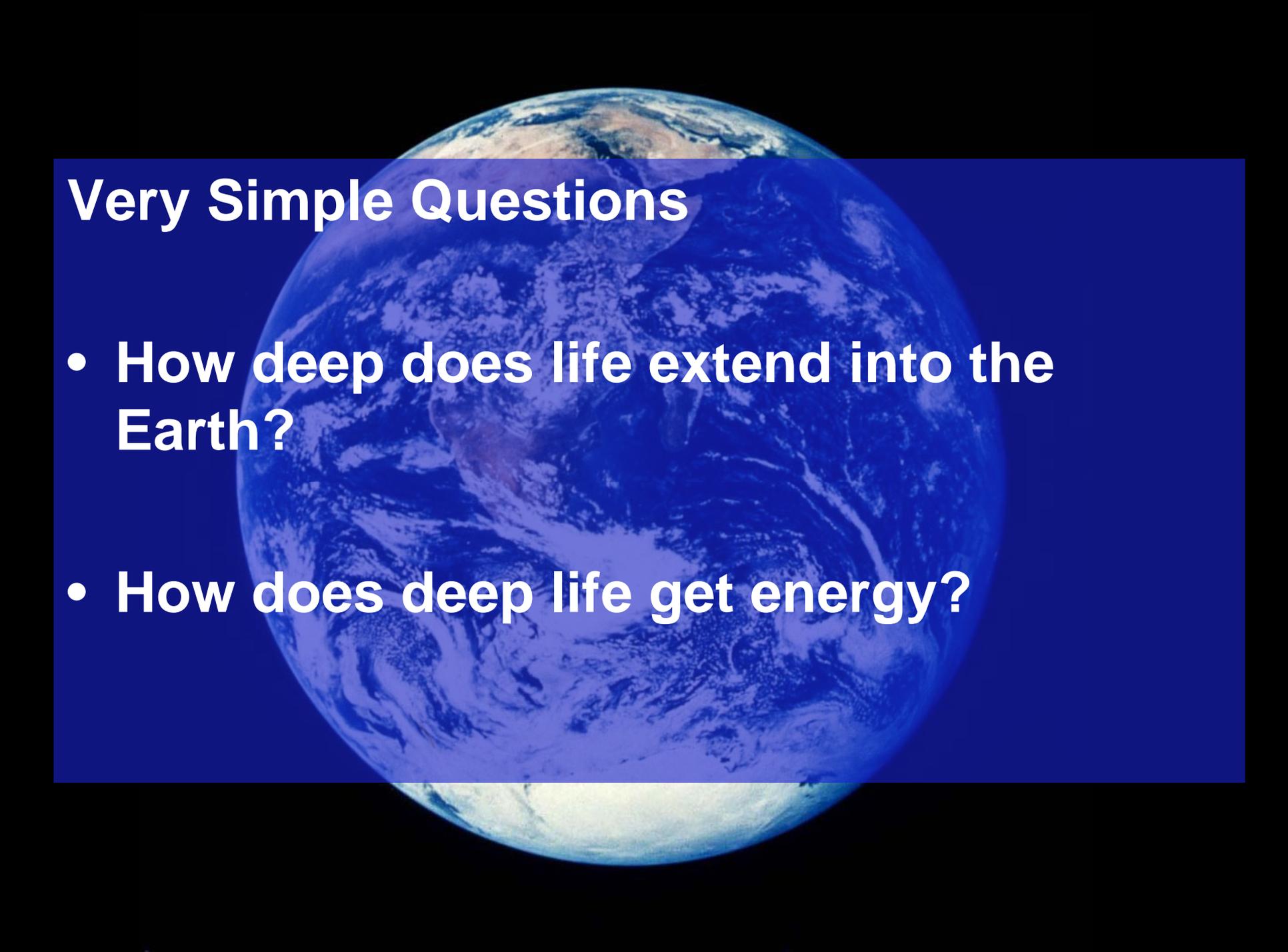
Ancient materials





Talk Outline

- **Deep biosphere explorations from world's deepest mines**
- **Search for life in nuclear detonation cavities at NTS**
- **Unexpected result that ties it all together**



Very Simple Questions

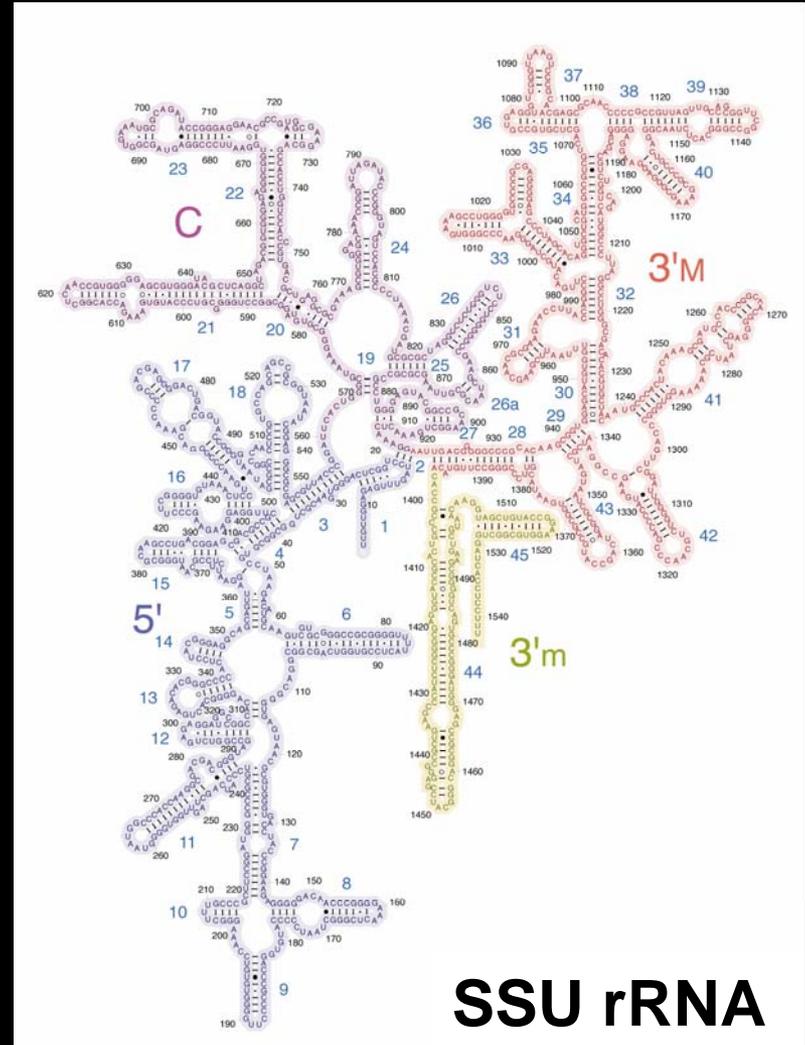
- **How deep does life extend into the Earth?**
- **How does deep life get energy?**

DNA



Molecular Approach

- Extract total community DNA
- Amplify genes of interest
- Library construction
- DNA sequence analysis
- Community genomics

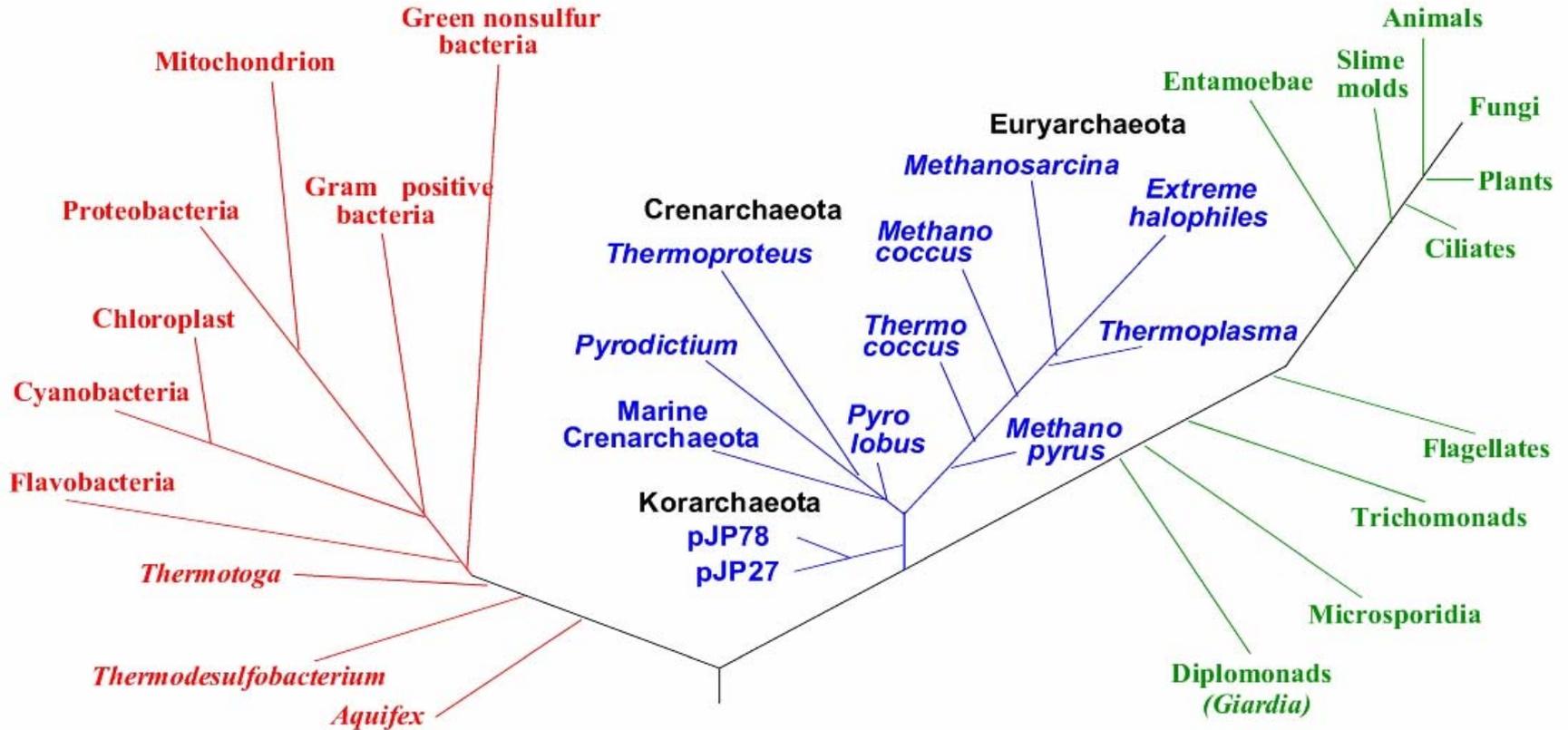


SSU rRNA

Bacteria

Archaea

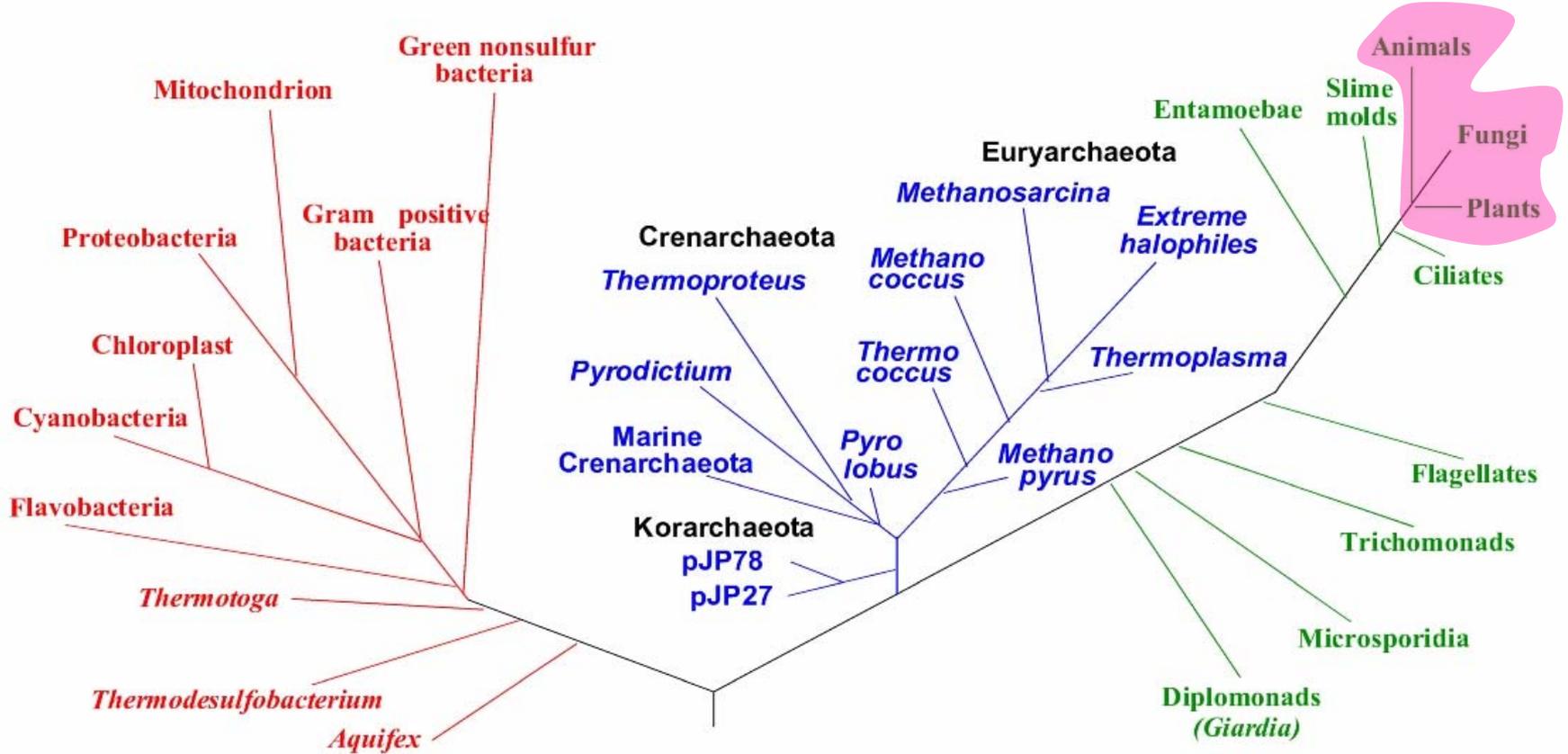
Eucarya



Bacteria

Archaea

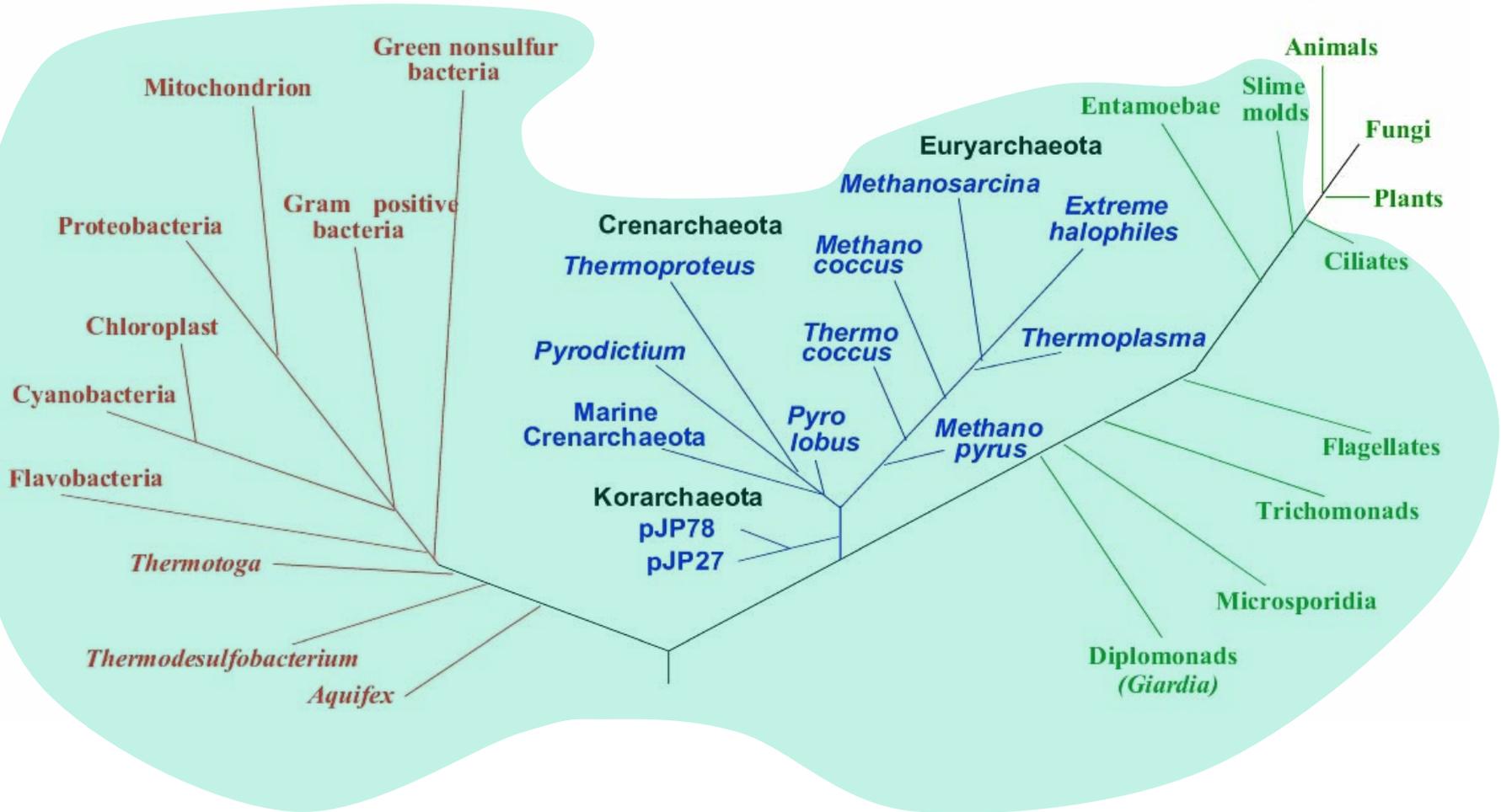
Eucarya

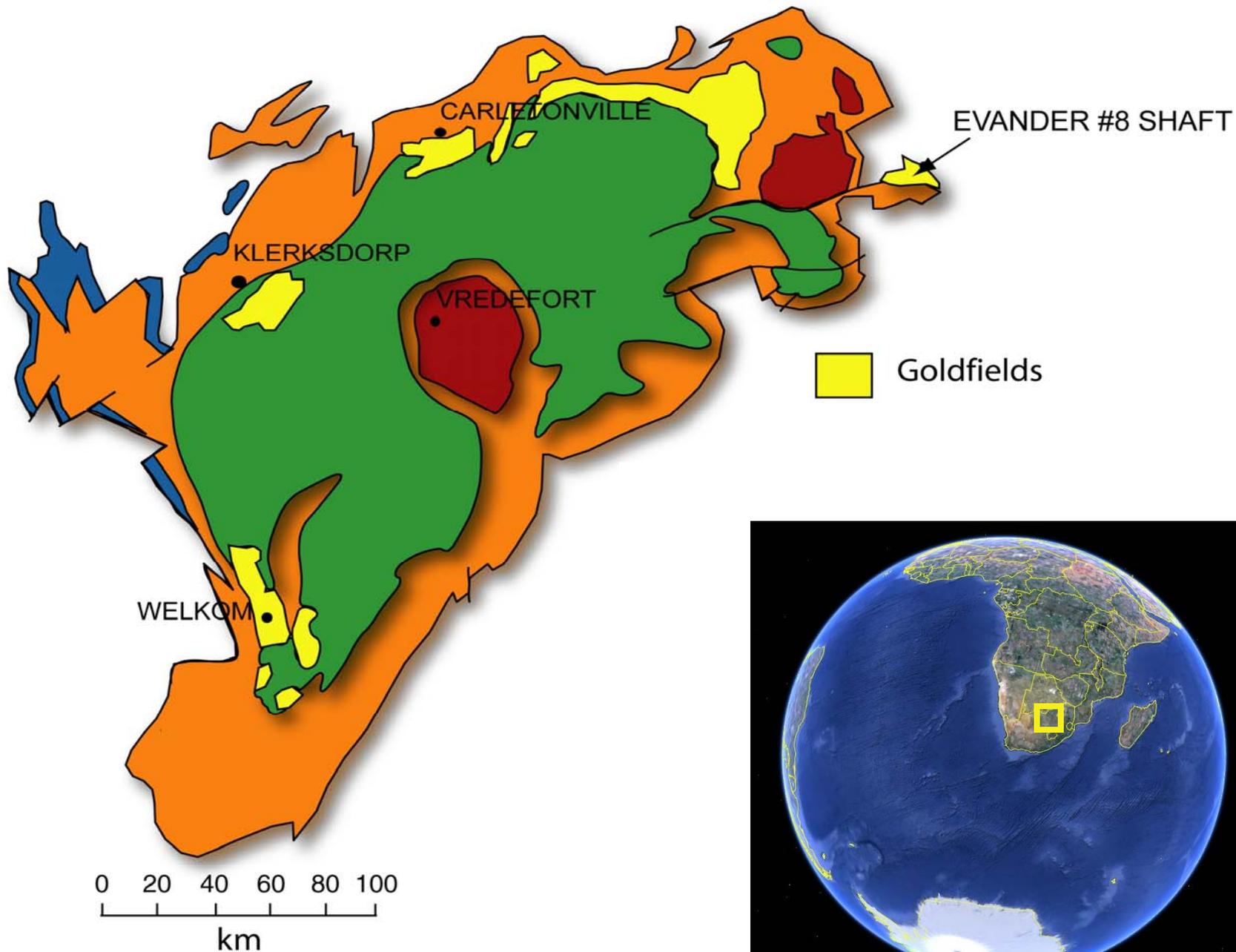


Bacteria

Archaea

Eucarya







Anglogold Ashanti Mponeng

Witwatersrand Deep Microbiology Project

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2005, p. 8773–8783
0099-2240/05/\$08.00+0 doi:10.1128/AEM.71.12.8773-8783.2005
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Vol. 71, No. 12

Desulfotomaculum and *Methanobacterium* spp. Dominate a 4- to 5-Kilometer-Deep Fault

Duane P. Moser,^{1,*} Thomas M. Gihring,^{1,†} Fred J. Brockman,¹ James K. Fredrickson,¹ David L. Balkwill,² Michael E. Dollhopf,^{2,‡} Barbara Sherwood Lollar,³ Lisa M. Pratt,⁴ Erik Boice,⁴ Gordon Southam,⁵ Greg Wanger,⁵ Brett J. Baker,⁶ Susan M. Pfiffner,⁷ Li-Hung Lin,^{8,§} and T. C. Onstott^{1,2,¶}

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Alkaline, sulfidic, 54 to 60°C, 4 to 53 million-year-old meteoric water emanating from quartzite-hosted fractures >3.3 km beneath the surface supported a microbial bacterial species affiliated with *Desulfotomaculum* spp. and an archaeal species. The geochemical homogeneity over the 650-m length of the borehole, the absence of these microorganisms in mine service water support an indigenous community. The coexistence of these two microorganisms is consistent with a low and SO₄²⁻ in the presence of high pH, high concentrations of H₂ and CH₄, autotrophic methanogenesis. Sulfide isotopic compositions were highly enriched SO₂⁻ reduction under hydrologic isolation. An analogous microbial couple and isry have been reported recently for hydrothermal carbonate vents of the Los Ridge (D. S. Kelly et al., Science 307:1428-1434, 2005), suggesting that these heat subsurface habitats (continental and marine) bearing this geochemical signature microbial communities described here are notably different from microbial ecosystems of continental subsurface environments.

Numerous studies have revealed the presence of microbial communities occupying oceanic and terrestrial deep subsurface settings (12, 14, 23, 27, 53, 54). Due to its enormous volume, this habitat may host the majority of Earth's prokaryotes (76), and according to some estimates (25, 76), the collective biomass of subsurface microbiota may rival that of flora and fauna at the surface. It is generally accepted that life on Earth requires liquid water (9). The upper 4 km of the terrestrial crust contains 9.5 × 10¹⁶ km³ of groundwater, of which about 56% lies below 0.75 km in depth (6). Since reports of microorganisms over this depth interval continue (4, 53, 54, 70) and the basic requirements for life appear to be met (e.g., liquid water, habitable space, and permissive temperatures), it follows that a significant proportion of the biosphere may be microbial and associated with deep terrestrial hydrologic systems (76).

Although a reasonable picture of life on Earth is possible by extracting and sequencing DNA from an environmental sample, a process called environmental genomics or metagenomics (1–8). This approach allows us to identify members of microbial communities and to characterize the abilities of the dominant members even when isolation of those organisms has proven intractable. However, with a few exceptions (5, 7), assembling complete or even near-complete genomes for a substantial portion of the member species is usually hampered by the complexity of natural microbial communities.

In addition to elevated temperatures and a lack of O₂, conditions within Earth's crust at depths >1 km are fundamentally different from those of the surface and deep ocean environments. Severe nutrient limitation is believed to result in cell doubling times ranging from 100s to 1000s of years (9–11), and as a result subsurface microorganisms might be expected to reduce their reproductive burden and exhibit the streamlined genomes of specialists or spend most of their time in a state of semi-senescence, waiting for the return of favorable conditions.

Environmental Genomics Reveals a Single-Species Ecosystem Deep Within Earth

Dylan Chivian,^{1,2,*} Eoin L. Brodie,^{2,3} Eric J. Alm,^{2,4} David E. Culley,⁵ Paramvir S. Dehal,^{1,2} Todd Z. DeSantis,^{2,3} Thomas M. Gihring,⁶ Alla Lapidus,⁷ Li-Hung Lin,⁸ Stephen R. Lowry,⁷ Duane P. Moser,⁹ Paul M. Richardson,⁷ Gordon Southam,¹⁰ Greg Wanger,¹⁰ Lisa M. Pratt,^{11,12} Gary L. Andersen,^{2,3} Terry C. Hazen,^{2,3,12} Fred J. Brockman,¹³ Adam P. Arkin,^{1,2,14} Tullis C. Onstott^{12,15}

DNA from low-biodiversity fracture water collected at 2.8-kilometer depth in a South African gold mine was sequenced and assembled into a single, complete genome. This bacterium, *Candidatus Desulfuris aadviatoris*, composes >99.9% of the microorganisms inhabiting the fluid phase of this particular fracture. Its genome indicates a motile, sporulating, sulfate-reducing, chemoautotrophic thermophile that can fix its own nitrogen and carbon by using machinery shared with archaea. *Candidatus Desulfuris aadviatoris* is capable of an independent life-style well suited to long-term isolation from the photosphere deep within Earth's crust and offers an example of a natural ecosystem that appears to have its biological component entirely encoded within a single genome.

Such microorganisms are of particular interest because they permit insight into a mode of life independent of the photosphere. One bacterium belonging to the *Firmicutes* phylum (Fig. 1A), which we herein name *Candidatus Desulfuris aadviatoris*, is prominent in small subunit (SSU or 16S) ribosomal RNA (rRNA) gene clone libraries (11–14) from almost all fracture fluids sampled to date from depths greater than 1.5 km across the Witwatersrand basin (covering 150 km by 300 km near Johannesburg, South Africa). This bacterium was shown in a previous geochemical and 16S rRNA gene study (11) to dominate the indigenous microorganisms found in a fracture zone at 2.8 km below land surface at level 104 of the Mponeng mine (MP104). Although Lin et al. (11) discovered that this fracture zone contained the least-diverse natural free-living microbial community reported at that time, exceeding the ~80% dominance by the methanogenic anaerobe IUAS-6 of a comparatively shallow subsurface community in Idaho (15), we were nonetheless surprised when the current environmental genomics study revealed only one species was actually present within the fracture fluid. Furthermore, we found that the

Long-Term Sustainability of a High-Energy, Low-Diversity Crustal Biome

Li-Hung Lin,^{1,2,*} Pei-Ling Wang,³ Douglas Rumble,⁴ Johanna Lippmann-Pipke,⁵ Erik Boice,⁶ Lisa M. Pratt,⁶ Barbara Sherwood Lollar,⁷ Eoin L. Brodie,⁸ Terry C. Hazen,⁸ Gary L. Andersen,⁹ Todd Z. DeSantis,⁹ Duane P. Moser,⁹ Dave Kershaw,¹⁰ T. C. Onstott¹

Geochemical, microbiological, and molecular analyses of alkaline saline groundwater at 2.8 kilometers depth in Archean metabasalt revealed a microbial biome dominated by a single phylotype affiliated with thermophilic sulfate reducers belonging to *Firmicutes*. These sulfate

genome of this organism appeared to possess all of the metabolic capabilities necessary for an independent life-style. This gene complement was consistent with the previous geochemical and thermodynamic analyses at the ambient ~60°C temperature and pH of 9.3, which indicated radiolically generated chemical species as providing the energy and nutrients to the system (11), with formate and H₂ as possessing the greatest potential among candidate electron donors, and sulfate (SO₄²⁻) reduction as the dominant electron-accepting process (11).

DNA was extracted from ~5600 liters of filtered fracture water by using a protocol that has been demonstrated to be effective on a broad range of bacterial and archaeal species, including recalcitrant organisms (16). A single, complete, 2.35-megabase pair (Mbp) genome was assembled with a combination of shotgun Sanger sequencing and 454 pyrosequencing (16). Similar to other studies that obtained near-complete consensus genomes from environmental samples (5, 17), heterogeneity in the population of the dominant species as measured with single-nucleotide polymorphisms (SNP) was quite low, showing only 32 positions with a SNP observed

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sequences, aqueous and gas geochemistry, and stable and noble gas isotopic signatures of moderately saline groundwater emanating from a fracture zone 2.825 km below the land surface (kmbls) in the Mponeng gold mine, South Africa. This high-pressure water-bearing fracture was intersected during exploratory drilling ahead of a tunnel advancing into an unmined zone ~100 m above the Ventersdorp Contact Reef (VCR) ore zone. The fracture water was initially sampled as soon as it was safe to do so (4 days after the fracture intersection), and three subsequent samples were obtained over a 54-day interval (Table 1) to monitor drilling contamination and possible changes of community structure and geochemistry as the fracture was de-watered and before being sealed by the mine's cementation team.

Fracture water samples yielded a uniform community structure dominated by a single phylotype (MP104-0916-b1) that constituted

characteristics of fracture water and mining water. NA, which is 1 μM for O₂ and 0.5 μg ml⁻¹ for archaeal DNA;

2 (7)	3 (15)	4 (58)	5
10:46E6X	MP104E65X	MP104E65X	MP104E65X
991902	C-099202	C-110902	SW-091602
Fracture water	Fracture water	Fracture water	Mining water
9/0.8 (55)	8/21.2 (64)	2.3/1.7 (96)	NA
9.3	9.3	9.2	NA
-350	-340	-263	NA
52	52	52	20
NA	7.1	8.9	1.4
NA	22.5	35.7	5.9
NA	71.9	84.9	0.42
NA	177	218	3.8
NA	900	1860	171
1390	NA	1060	NA
2600	2090	3715	NA
11800	9320	16600	NA
<d.l.	<d.l.	<d.l.	285
-684	-688	-695	NA
-31.7	-32.8	-33.2	NA
-367	-366	-390	NA
1.4 ± 10.2	15.8 ± 7.9	NA	NA
1.3 ± 10.6	16.9 ± 8.4	NA	NA
1.4 ± 3.8	21.0 ± 6.0	NA	NA
1.0 ± 3.8	23.8 ± 4.6	NA	NA
1.6 ± 8	30 ± 15	30 ± 15	3 ± 1.5 × 10 ⁵
<d.l.	~5 × 10 ⁻⁴	~5 × 10 ⁻⁴	206 ± 100
NA	NA	3.3 ± 0.3 × 10 ⁴	NA

1 μM sample 1, 27.6 mM for sample 3 and 50.6 mM for sample 4. [†]The error corrected for diffusive loss (D). Diffusive correction was not applied to the positive O₂ content for sample 1 as it may be derived from incomplete red by the extremely high water pressure and flow rate. [‡]The carbon ¹³C (‰) uncertainties for aqueous and gas are ±30% and ±10%, respectively. [§]The uncertainties for aqueous and gas are ±30% and ±10%, respectively.

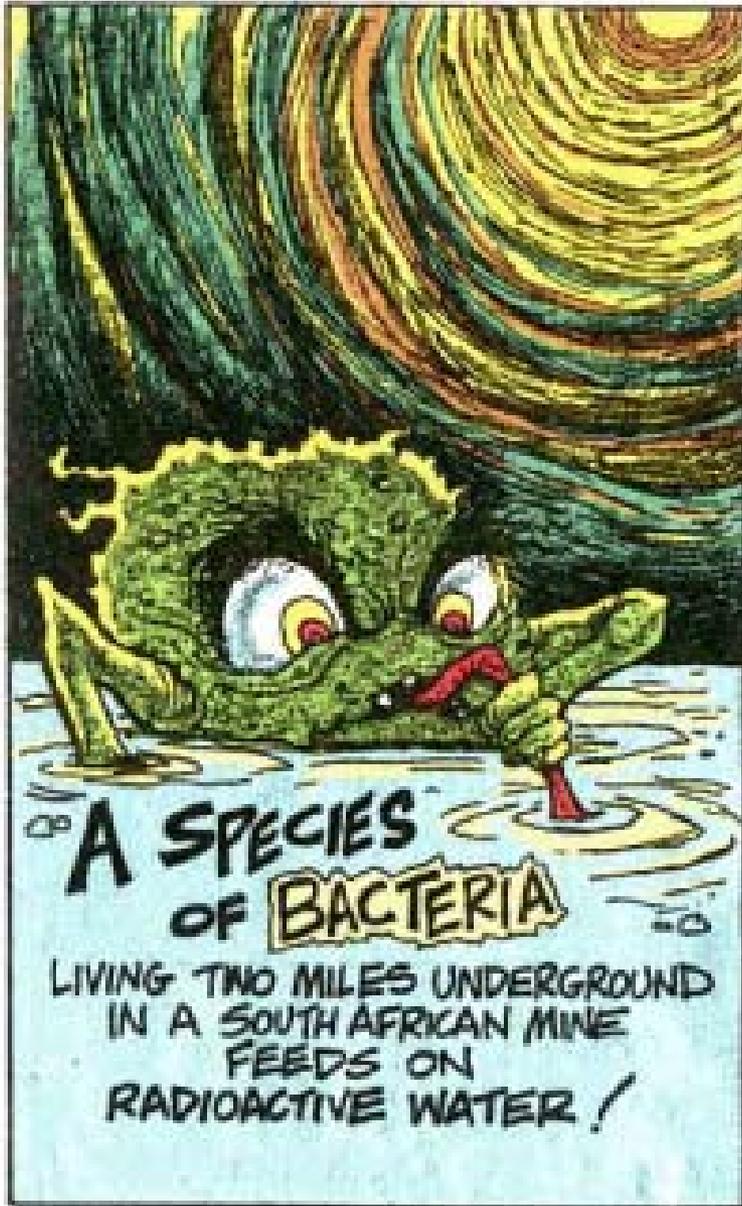
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Roller's — Believe It or Not!



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The TIME 100

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ENTERTAINERS

LEADERS &
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HEROES &
PIONEERS

SCIENTISTS
& THINKERS

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TITANS

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Tullis Onstott

By Carolyn Sayre

Wondering
whether

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BRIAN FINKE / LEVINE AND LEAVITT FOR TIME
Tullis Onstott

extraterrestrial life exists? Tullis Onstott, 53, has found it—sort of. The fact that the living things he's discovered make their

homes nearly two miles below the earth's surface may disqualify them from the extra part of extraterrestrial. But dig them up and fly them off, and there's no limit to the cosmic places they might survive. That's the beauty of the work Onstott, a professor of geosciences at Princeton University, is doing. Until we collect living aliens, he's showing us they're probably there to be found. Scientists once despaired of discovering even simple otherworldly organisms because they assumed that all life should be like earthly life—built around warmth, light and liquid water. In recent years, however, biology has shown itself to be tougher than that. Investigators have discovered organisms—simple ones—frozen in polar ice and thriving in scalding ocean vents.

Experts have investigated less thoroughly the regions deep within the earth—places where living things could establish a safe redoubt. Uncover such critters on Earth, and you would prove that they might exist elsewhere too. Last year Onstott and his team published such a find. After years of dust and dirt, piggybacking off gold miners in South Africa, they identified self-sustaining bacteria living in rock deep below the surface that draw their energy from chemicals produced by the radioactive splitting of water, essentially feasting on hydrogen and sulfur compounds. So when we do finally uncover the first alien life-form, you may get the sense that you've seen it before.

Next: Svante Pääbo >>

The People Who Shape Our World

Here's our list of the 100 men and women whose power, talent or moral example is transforming the world



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Sara Gaines and the “kibble”

Follow the Water



Evander Mine 8 Shaft 18 Level, 2.1 kmbls

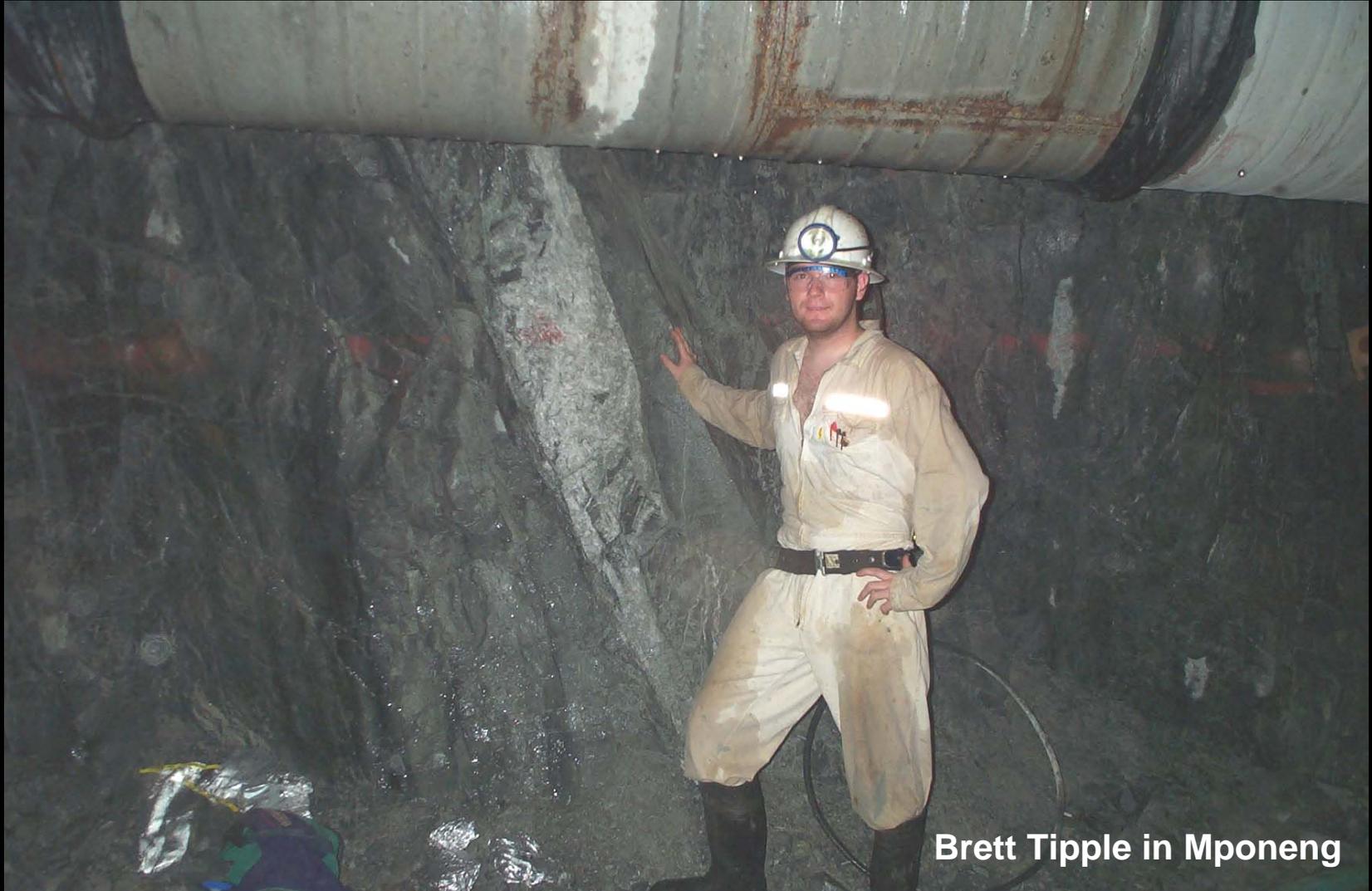


Kloof Mine , 3.3 kmbls, Photo by Arnand vanHeerden

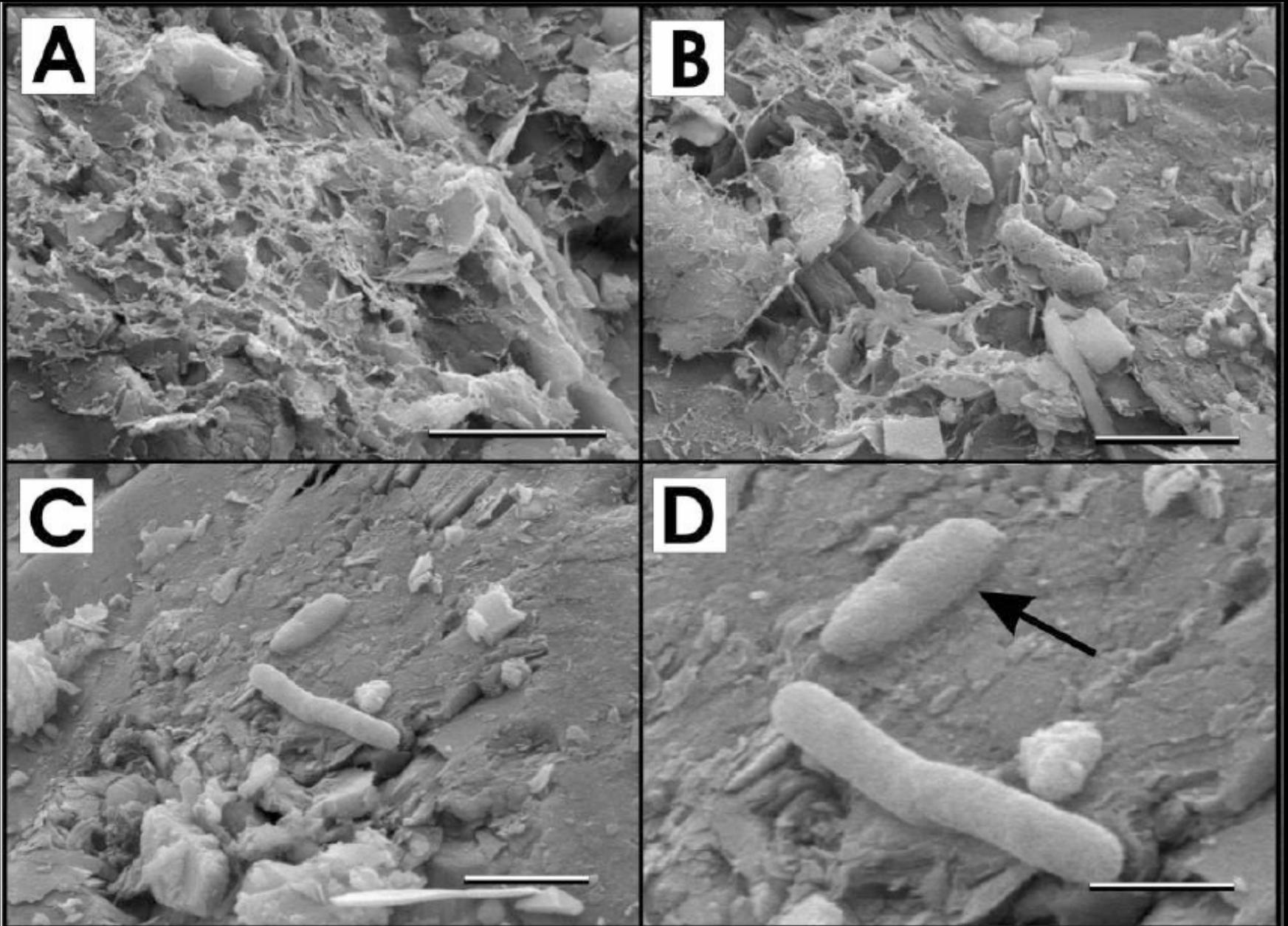


Evander Mine 8 Shaft 18 Level, 2.1 kmbls

MP104E46X fracture



Brett Tipple in Mponeng



Greg Wanger and Gordon Southam

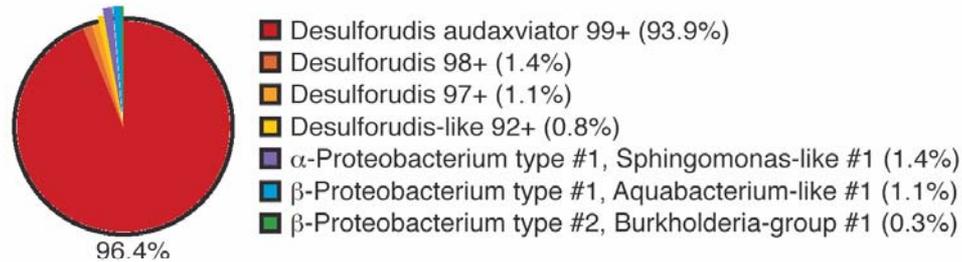
MP104E46X Summary

Depth	2.8 kmbls
Temp	>60 - 52°C
pH	9.3
H₂	1.9 - 3.7 mM
CH₄	8.6 - 16.6 mM
SO₄²⁻	0.5 - 1.9 mM
Cell density	3.3 x 10⁴ mL⁻¹
Volume filtered	5,600 L

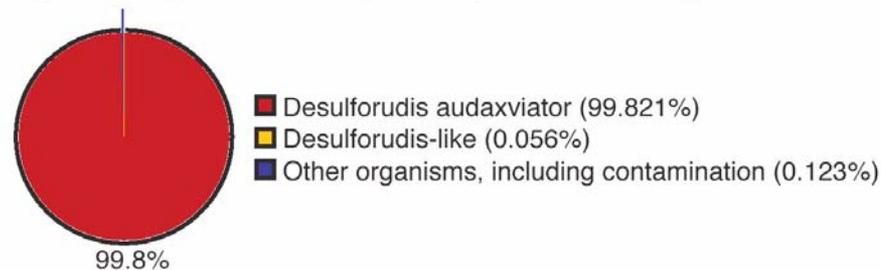
Lin, L.H., P.L. Wang, D. Rumble, et al. 2006. Long-term sustainability of a high-energy, low-diversity crustal biome. *Science* 314:479-482.

Population assessments of MP104E46X

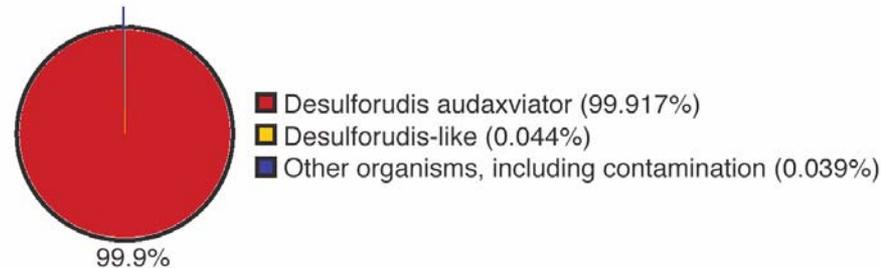
(A) SSU rRNA clone library (361 clones)



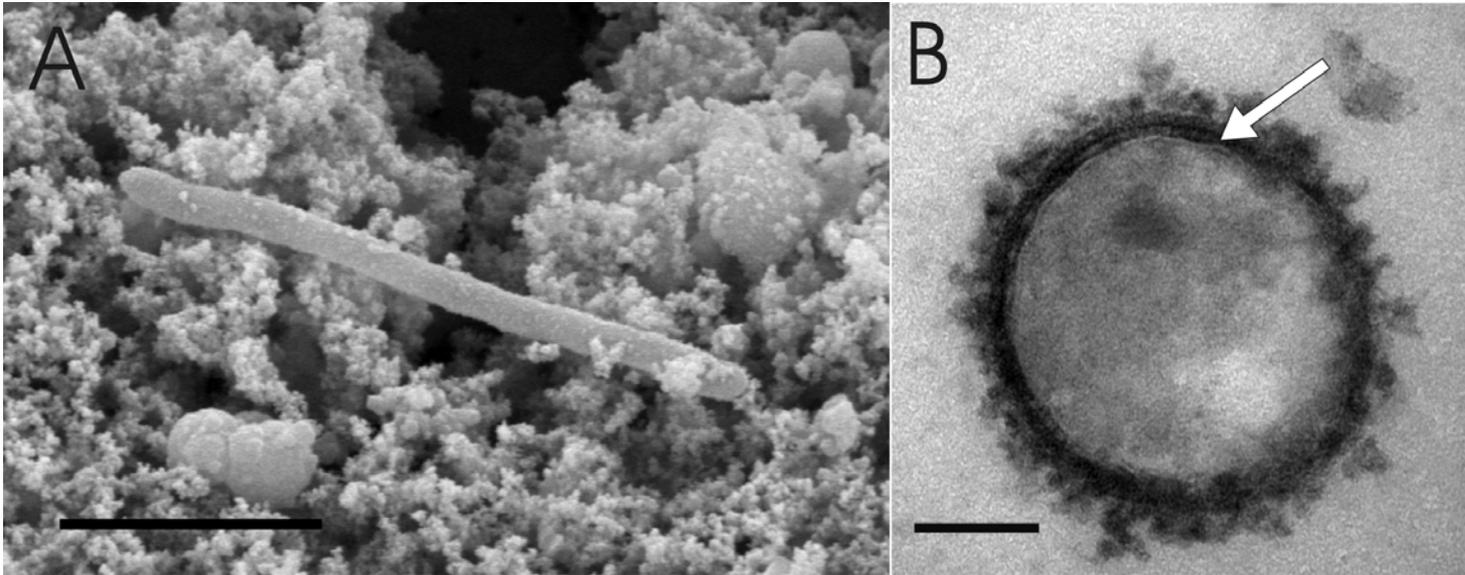
(B) Sanger metagenomic sequence (28,503 reads)



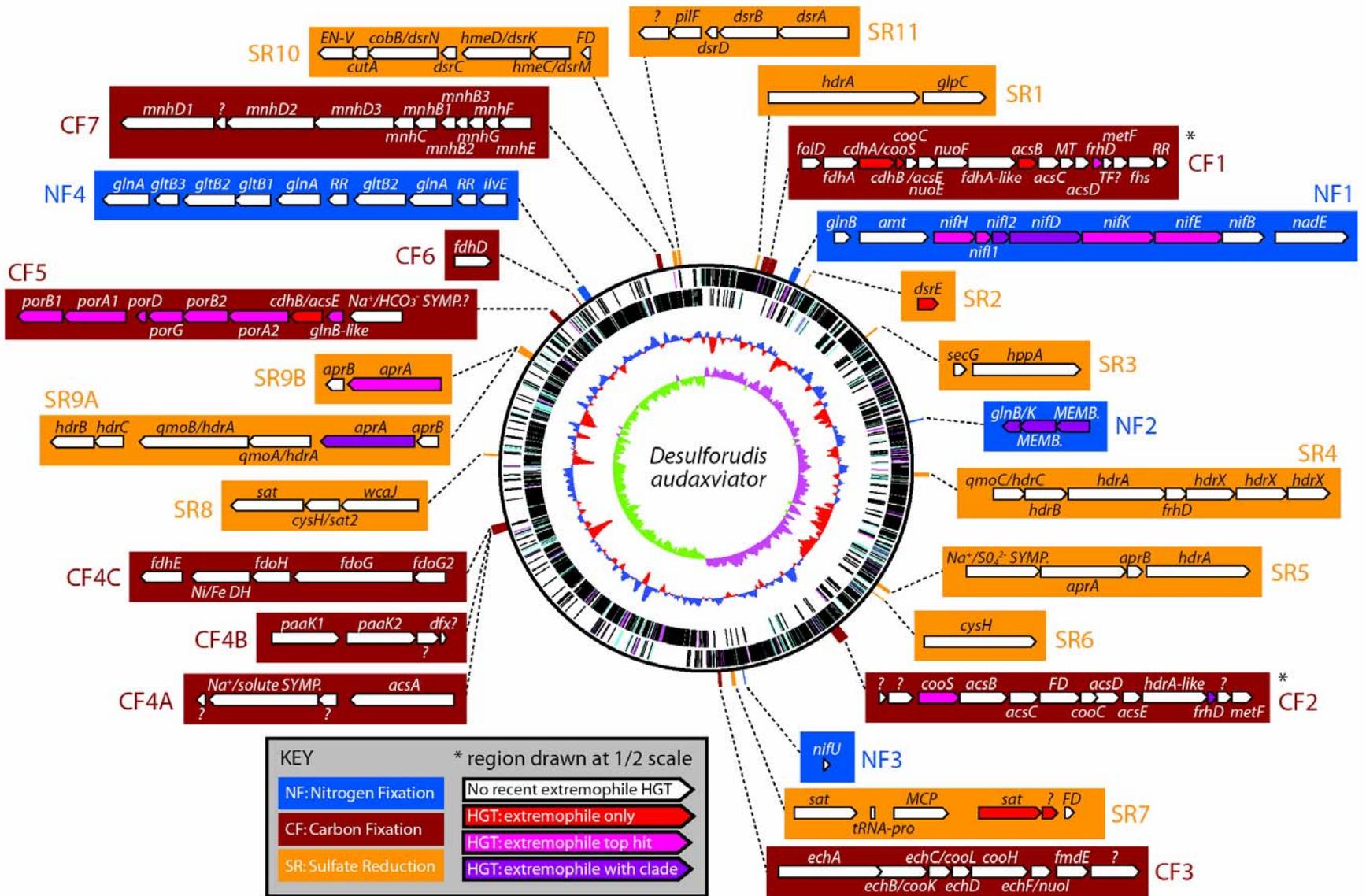
(C) 454 metagenomic sequence (478,807 reads)



Desulforudis audaxviator



Based on its rod-like morphology, its apparent use of the dissimilatory sulfate reduction pathway for energy production, and because of the journey this "audax viator" (bold traveler) undertook to live in the extreme depths of the Earth, we have named this organism "**Candidatus *Desulforudis audaxviator***".



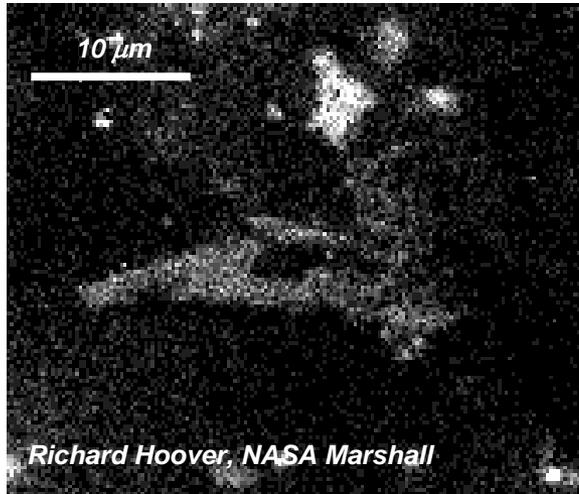
Some Features of Genome

Feature	Value
Genome size (bp)	2,349,476
%G+C	60.9
Predicted genes (ORFs)	2157
Percent of genome protein coding	86.8
Transposons	83
Transposon gene groups	30

Physiological Features

Feature	Notes
Endospores	Yes
Flagella	Yes
Sulfate reduction	SRB, Na ⁺ /SO ₄ ²⁻ symporter
Carbon fixation	
TCA - Reverse TCA	Incomplete
CODH - Wood Ljungdahl -	2 types, one archaeal
N fixation	Nitrogenase, archaeal
“hyperthermophile” genes	~50
Archaeal genes	85
Catalase, peroxidase, SOR	No

Radiation-driven Ecosystems?



Uranium X-ray map

Radiolysis splits water
(H_2 and H_2O_2 , O_2 , O^*)



Oxidants react with sulfide, FeS_2
($4\text{O}^* + \text{HS}^- \rightarrow \text{SO}_4^{2-} + \text{H}^+$)



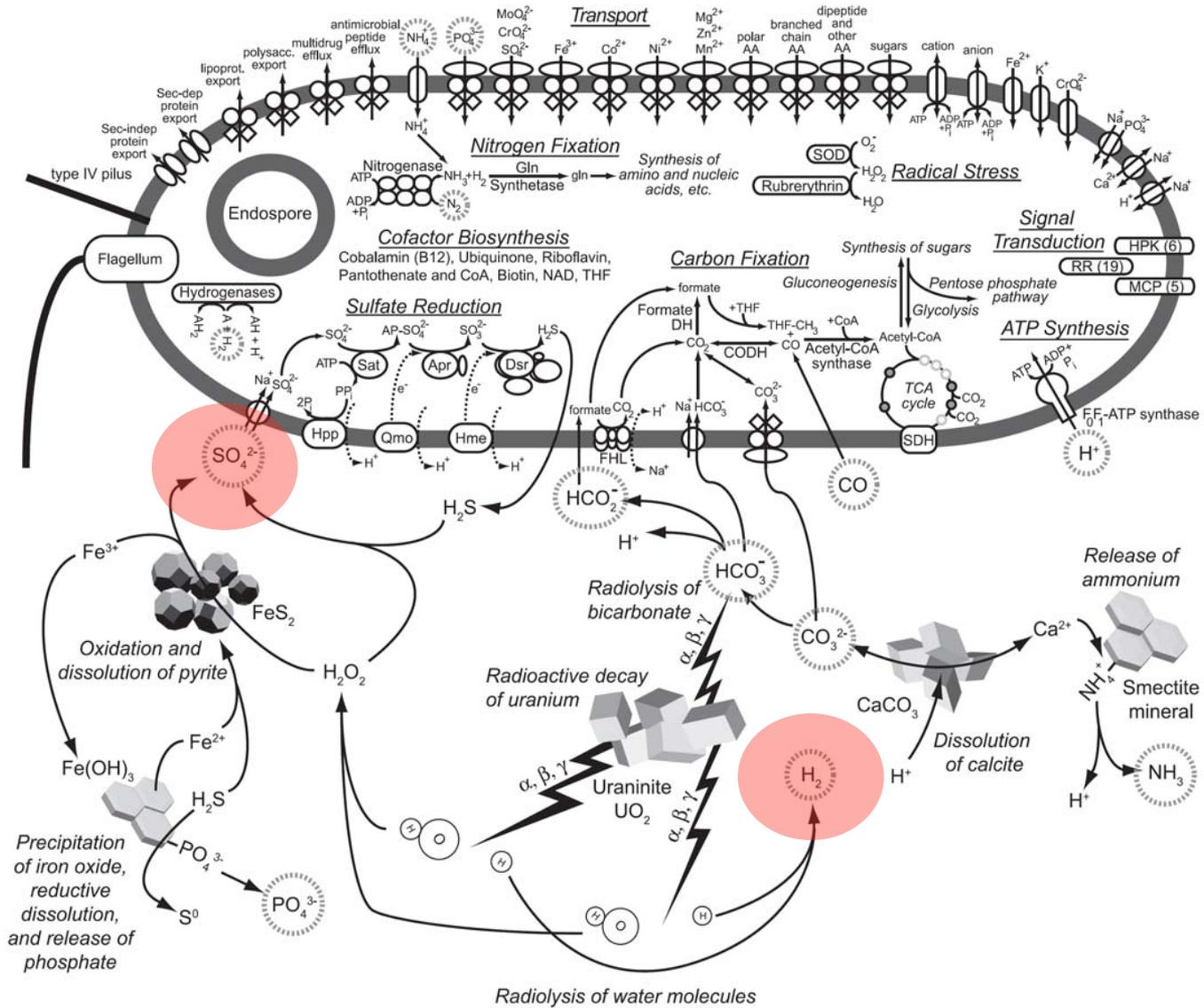
SO_4^{2-} (~11,000 ppm)

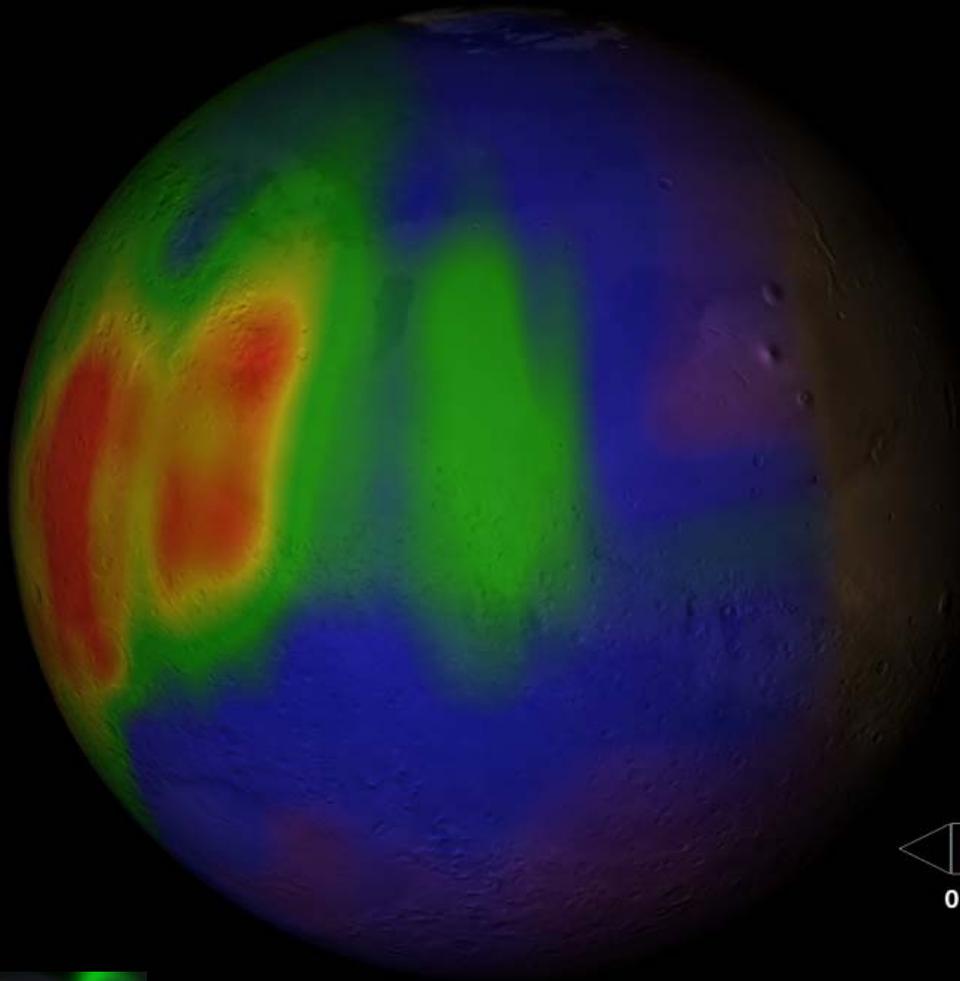
Estimated rates

H_2 production	~ 1 nM y^{-1}
SO_4^{2-} reduction	0.22 - 1.45 nM y^{-1}
Cell turnover	45 - 300 yrs

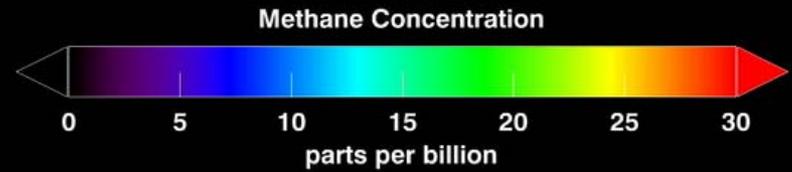
1) Lin, L.-H., J. A. Hall, J. Lippmann, et al. 2005. Radiolytic H_2 in the continental crust: Nuclear power for deep subsurface microbial communities. *Geochem. Geophys. Geosys.* 6:Q07003, doi:10.1029/2004GC000907.

2) Lin, L.H., P.L. Wang, D. Rumble, et al. 2006. Long-term sustainability of a high-energy, low-diversity crustal biome. *Science* 314:479-482.



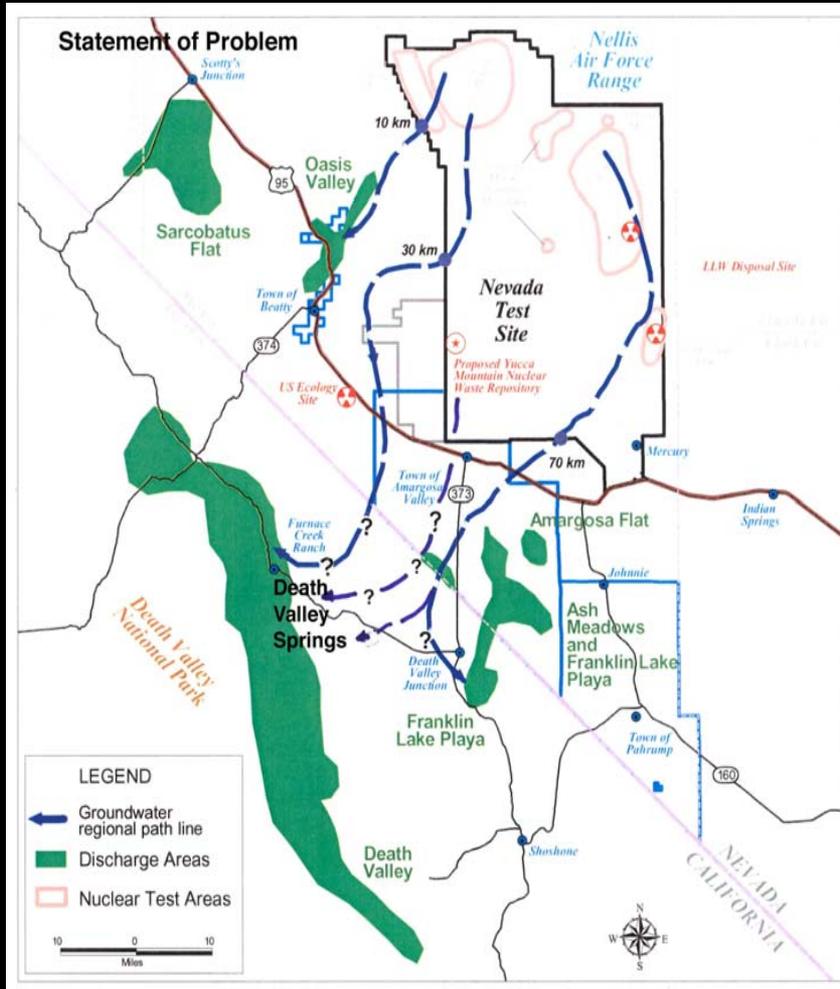


Methane release:
Northern summer



NASA's Infrared Telescope Facility (IRTF) atop Mauna Kea

Part II: NTS/Death Valley Flow System

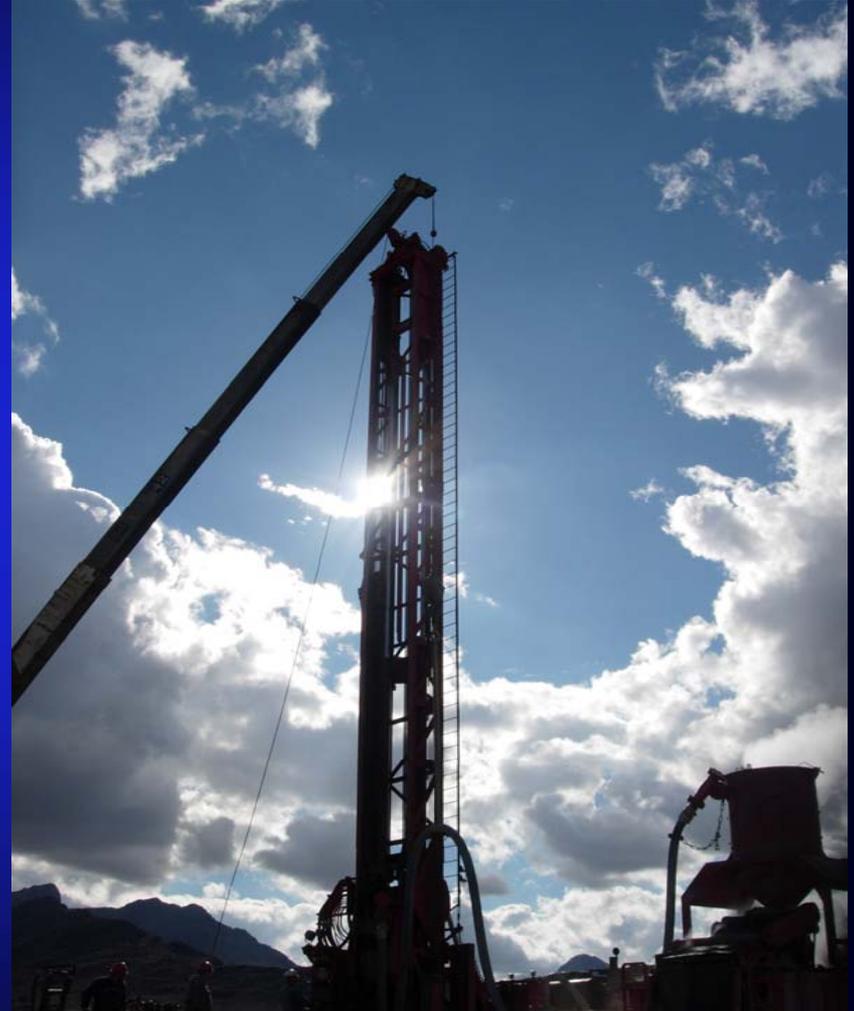


From Mike King, Devils Hole workshop, 2007

Windows into the Death Valley Flow System

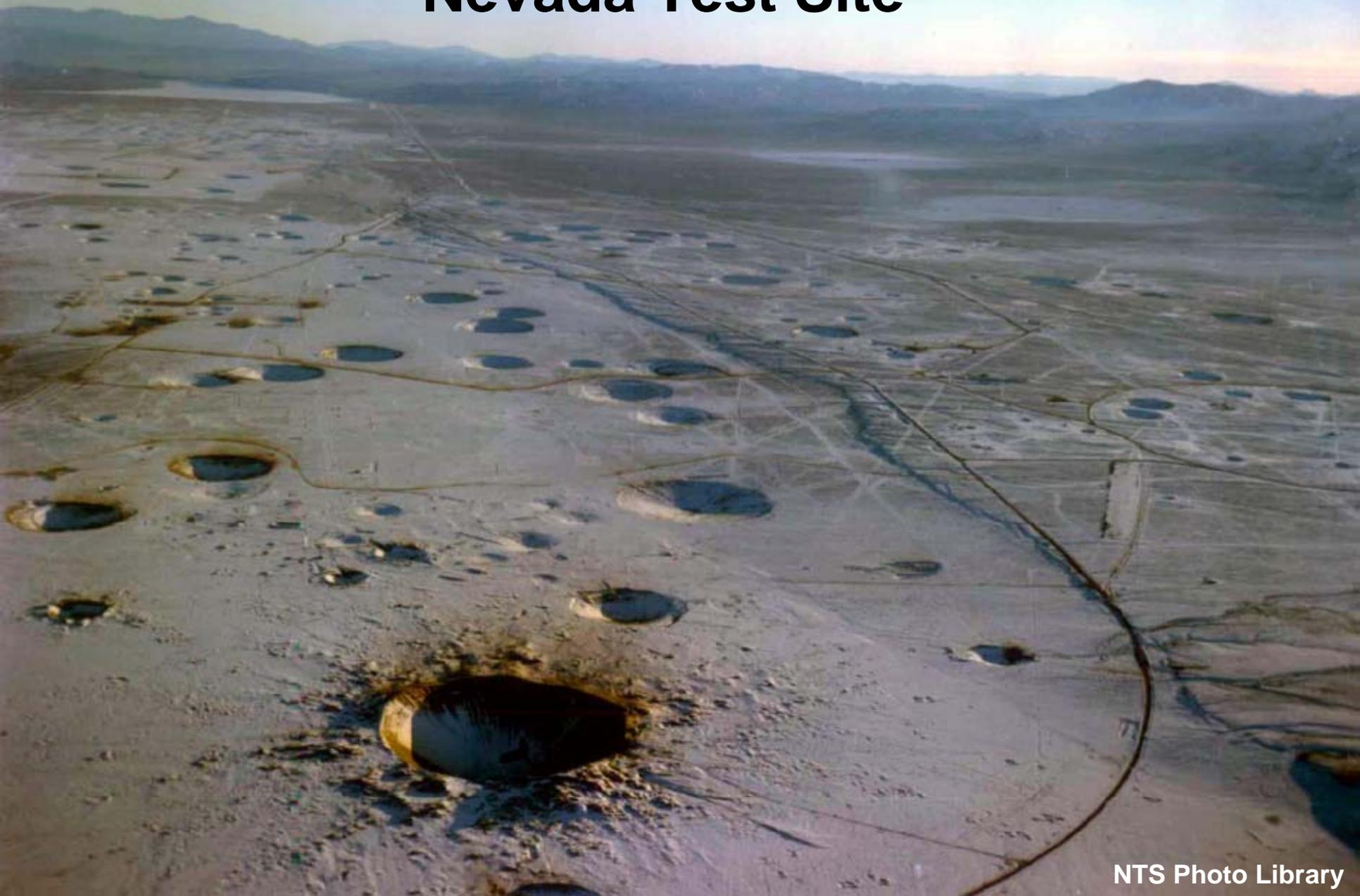


Devils Hole



Well BLM1, Nye CO, NV

Nevada Test Site

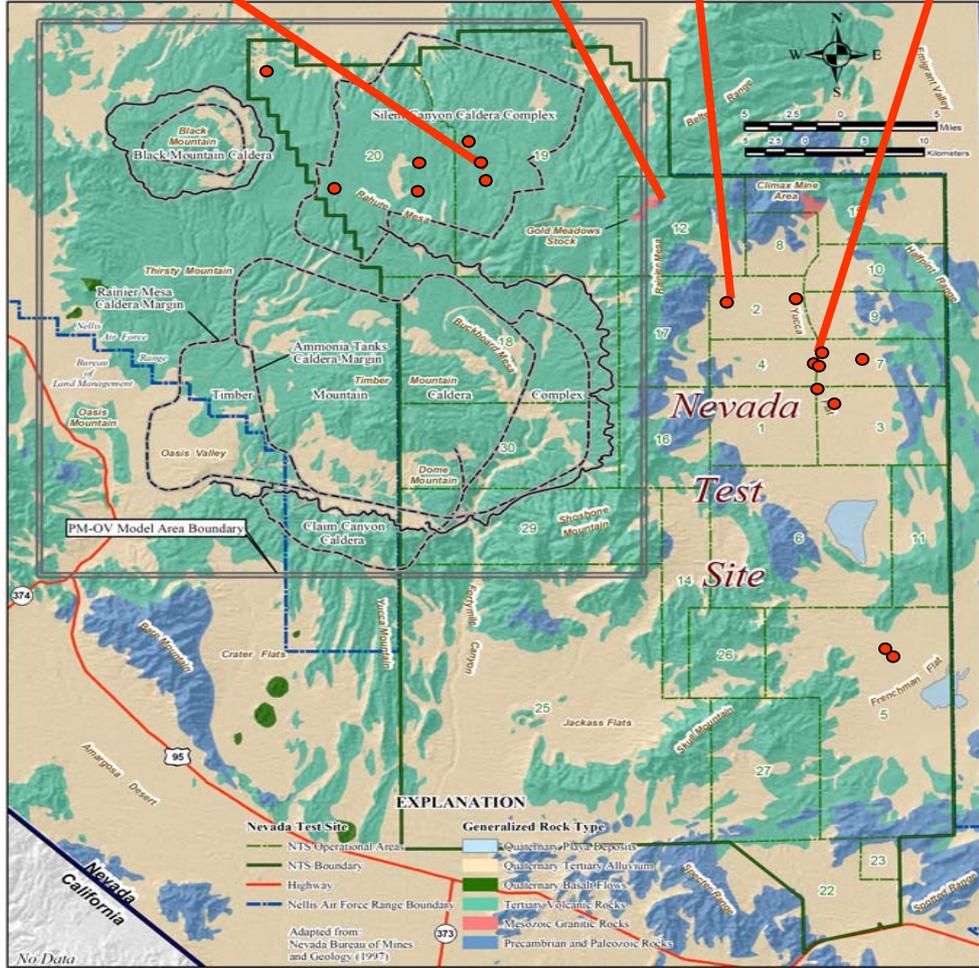


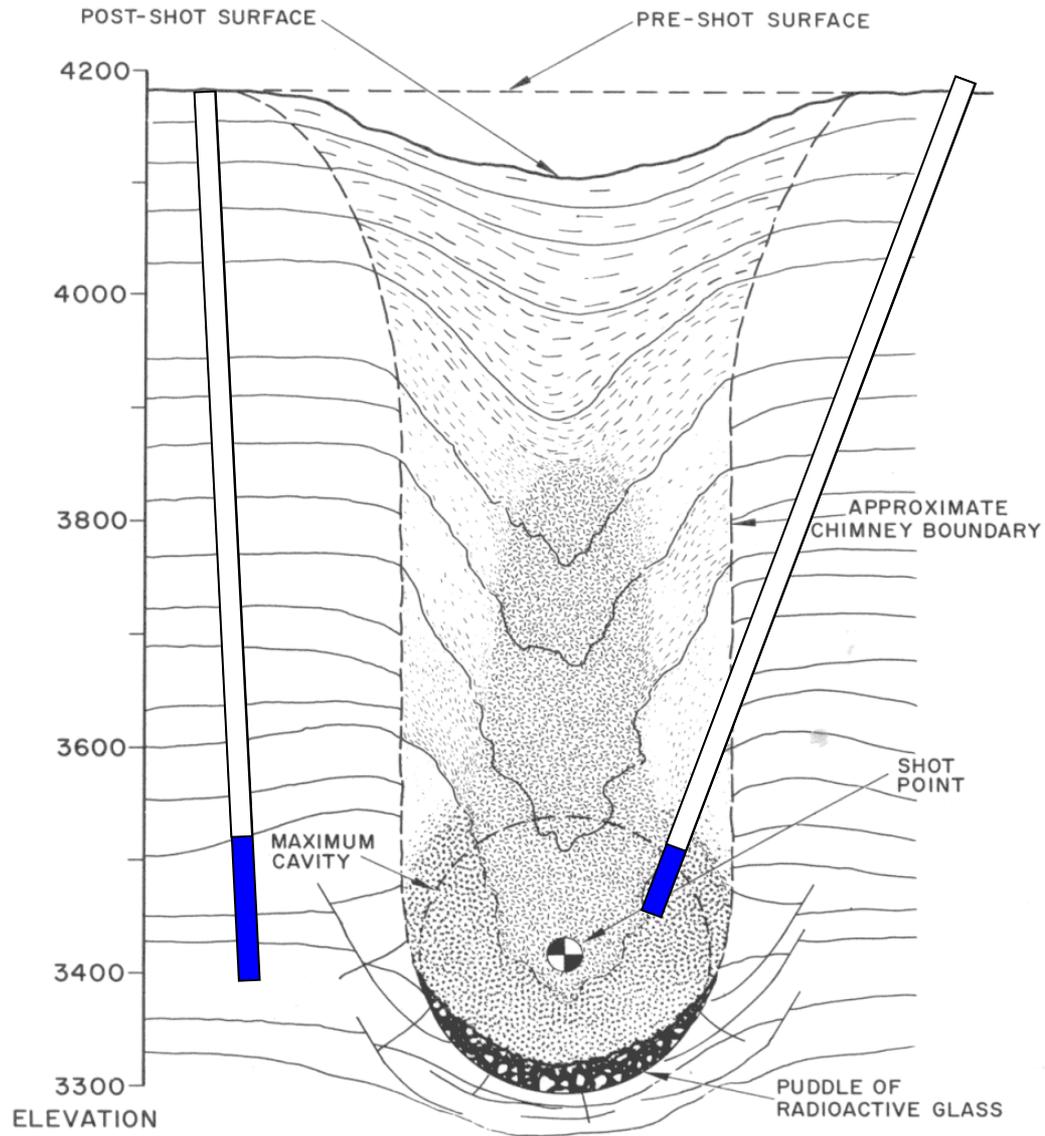
**CHANCELLOR
U-19ad PS#1A**

**U12N
Tunnel
Complex**

**NASH
UE-2ce**

**GASCON
UE-4t,
U-4t PS#3A**





Hot well sampling at NTS



(DOE-cleared photo, J.C. Bruckner)

Aquatic chemistry 2008 NTS hot well survey (from M. Zavarin)^a

Test	Well	pH	T (°C)	Cond. mS/cm	DIC	Cl ⁻	NO ₃ ⁻	SO ₄ ⁻	Na ⁺	Ca ²⁺
GASCON	U-4t PS#3a	8.5	20.3	558	---	73.6	<0.4	0.44	75.3	10.9
CHANCELLOR	U-19 PS#1A	8.9	95-65	862	110	26.9	2.11	63.4	96.0	1.1
NASH	UE-2ce	6.8	34.2	736	308	14.0	5.7	18.1	38	71.8
Tunnel Vent	U12N.10	9.4	22.7	1110	178	35.5	1.9	288.6	221	14

^aData are preliminary

Preliminary radiological data from NTS hot wells

Test	Well	Sample Date	³ H (pCi/L)	¹⁴ C (pCi/L)	⁹⁹ Tc (pCi/L)	¹²⁹ I (pCi/L)	^{239,240} Pu (pCi/L)
GASCON	U-4t PS#3a	03/19/08	3.0E3	nd	3.49E-3	2.52E-4	ip
CHANCELLOR	U19ad PS#1A	05/01/08	1.3E7	4.0E2	2.54E1	1.32E-2	2.7E1
NASH	UE-2ce	07/02/08	2.7E5	8.0E1	2.28E-3	1.06E-2	<0.04
U12N Tunnel	U12N.10	08/20/08	6.26E6	ip	ip	9.89E-1	ip

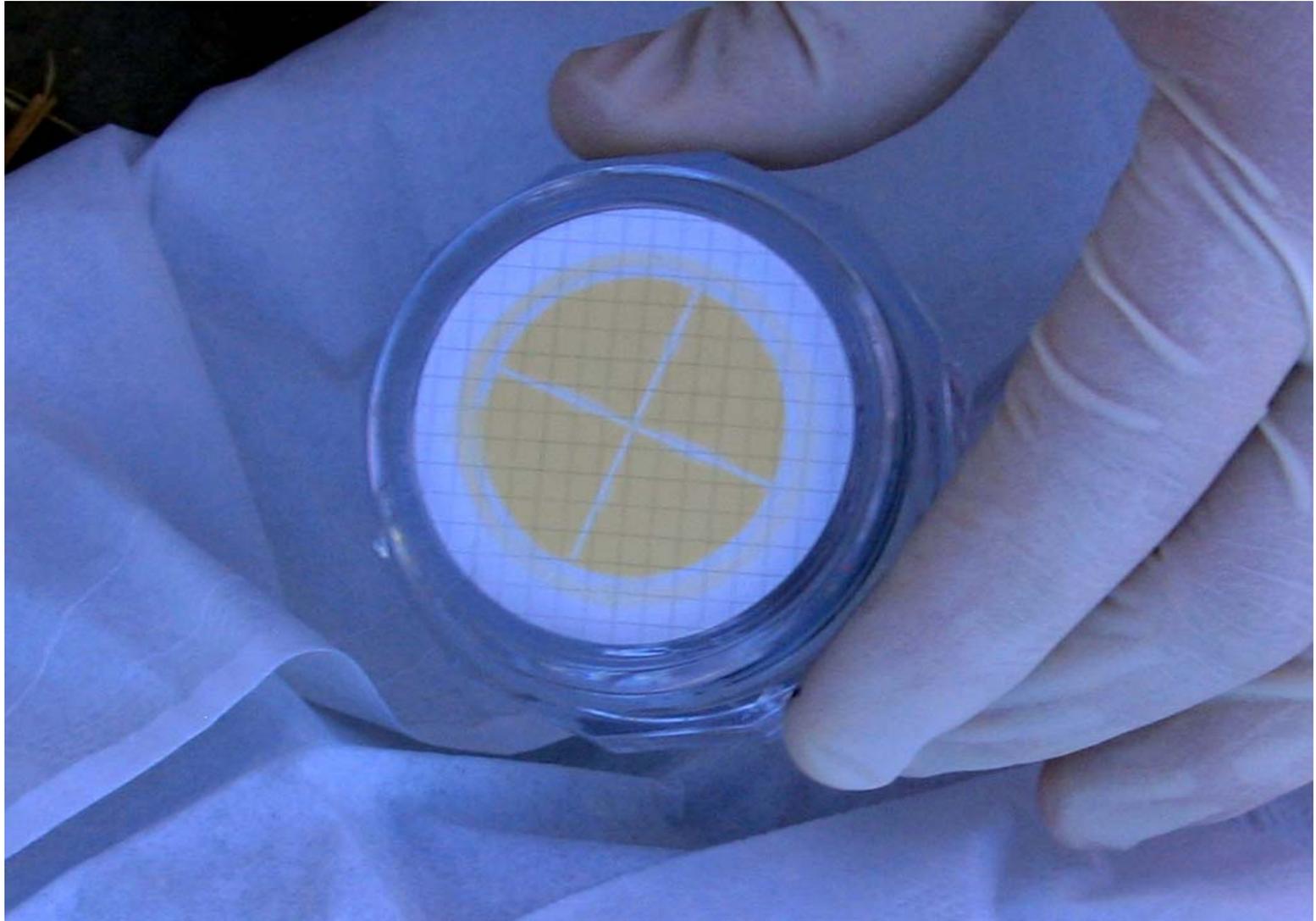
ip = analysis in progress

Abundance of major NTS microbial types.

Physiotype	GASCON	NASH	U12N
Nitrate reducers	10^2	10^4	nd
Sulfate reducers	nd	10^2	10^2
Iron reducers	nd	nd	10^3
Fermentative	10^2	nd	10^1
Methanogens	--	--	10^2

nd = none detected

Hot Well Bacteria on Filter



(DOE-cleared photo, J.C. Bruckner)

NTS Diversity

Chloroflexi

Nitrospirae

Firmicutes

Actinobacteria

Acidobacteria

B-C group

δ

β

γ

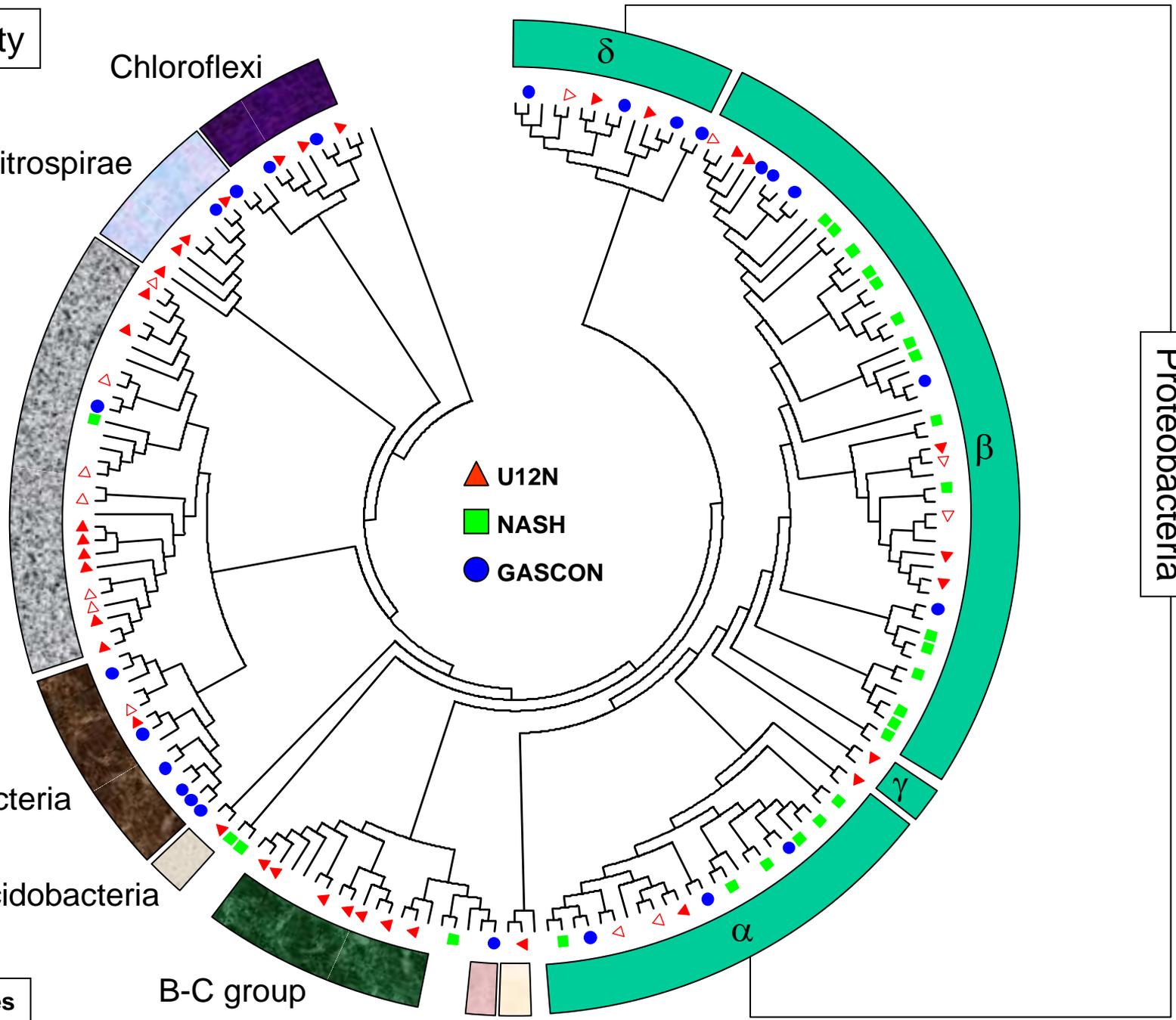
α

Proteobacteria

- ▲ U12N
- NASH
- GASCON

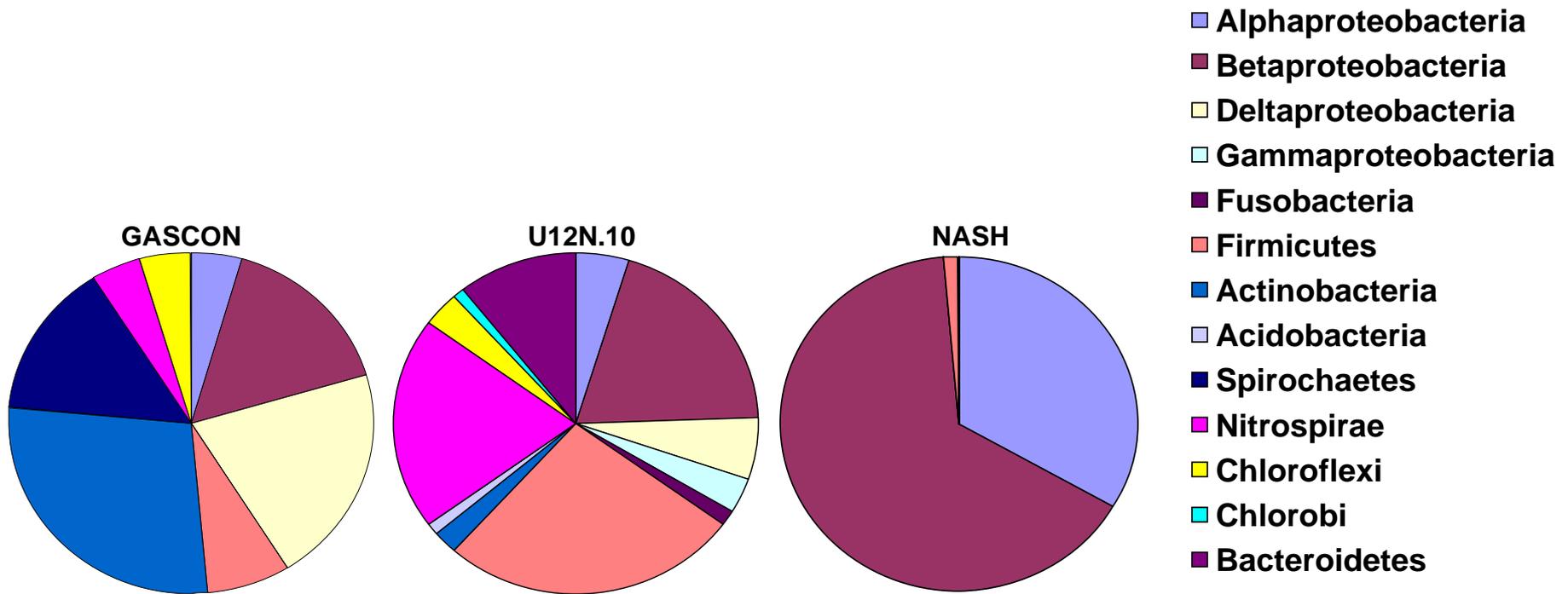
S = Spirochaetes
F = Fusobacteria

S F



Nearest described microorganisms to clones (>97% based on BLAST pairwise alignment)

Phylum	Species	Similarity	Location (# clones)	Isolate description
a-proteobacteria	<i>Phenylobacterium lituiforme</i>	98%	GASCON (1)	Thermophile, subsurface aquifer
a-proteobacteria	<i>Phenylobacterium falsum</i>	98%	NASH (7)	Alkaline, subsurface aquifer
b-proteobacteria	<i>Aquabacterium</i> sp. GPTSA100-18	99%	NASH (1)	Isolated from Indian warm springs
b-proteobacteria	<i>Azospira oryzae</i> strain N1	98%	NASH (7)	Selenate/selenite reducer
b-proteobacteria	<i>Ralstonia</i> sp. 22	99%	NASH (3)	Arsenite oxidizer
b-proteobacteria	<i>Delftia acidovorans</i> str. SPH1	99%	U12N (1)	Degrader of organics
b-proteobacteria	<i>Siderooxidans ghiorsii</i> str. LD-1	95% ^a	GASCON (3)	Neutrophilic Fe(II) oxidizer
d-proteobacteria	<i>Desulfovibrio</i> sp. zt31	97%	GASCON (7)	Sulfate reducer
d-proteobacteria	<i>Pelobacter venetianus</i>	95% ^a	U12N (1)	Dissimilatory Fe(III) reducer
g-proteobacteria	<i>Thiovirga sulfuroxydans</i>	99%	U12N (3)	Microaerophilic sulfur oxidizer
Firmicutes	<i>Desulforudis audaxviator</i> MP104C	98%	U12N (1)	Deep biosphere,

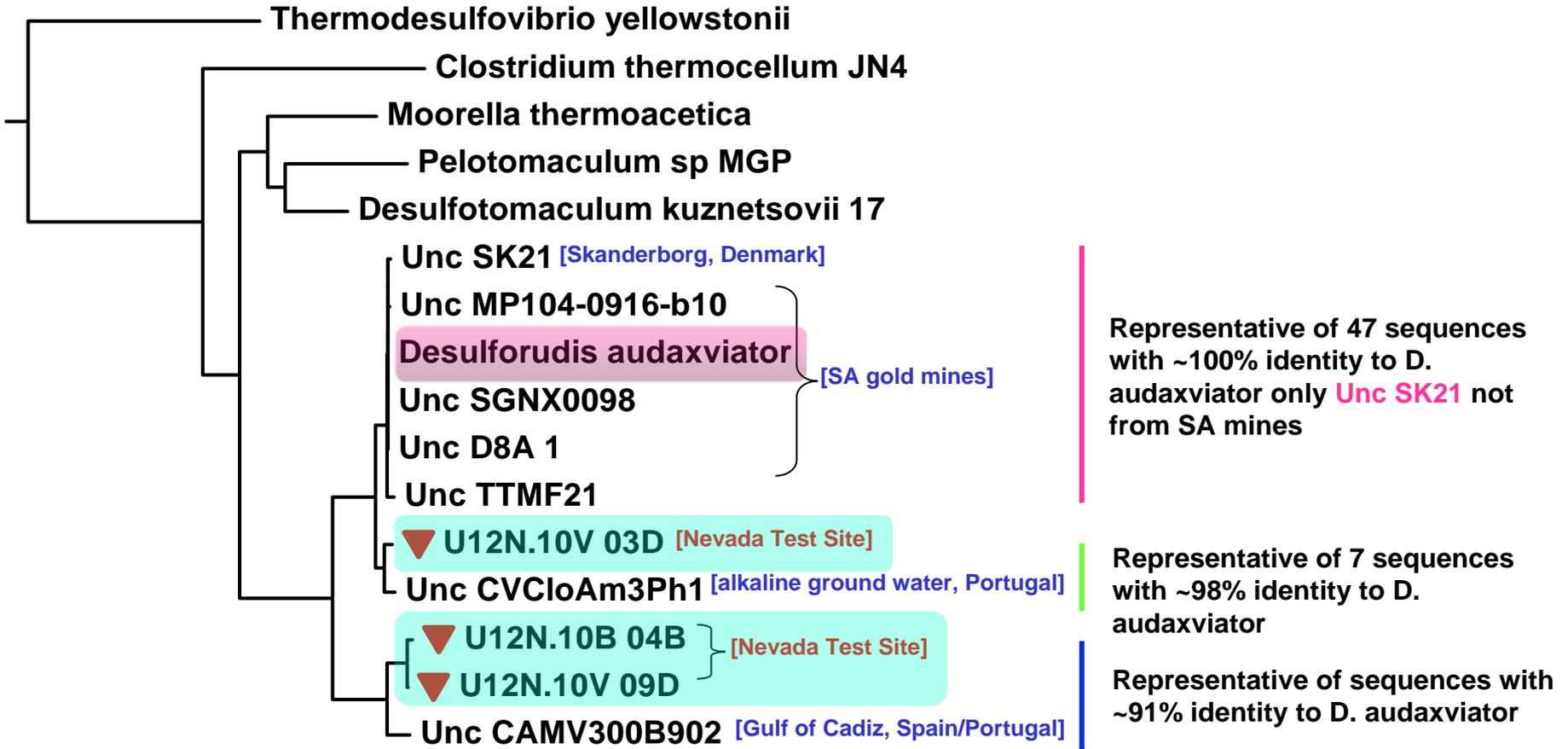


Distribution of NTS clones by phyla. Total numbers of clones per site: Nash (73), GASCON (68), U12N (86).

Hot well sampling at NTS



(DOE-cleared photo, J.C. Bruckner)



Note: 836,814 SSU rRNAs in RDP Release 10

Conclusions

- Earth has an isolated microbial ecosystem deep underground
- Deep biosphere appears to be fueled by radiochemistry and completely independent of solar inputs
- *Million-to-one* discovery of *Desulforudis audaxviator* in deep radioactive habitats on two continents suggests role for radiation in sustaining microbial populations at the NTS

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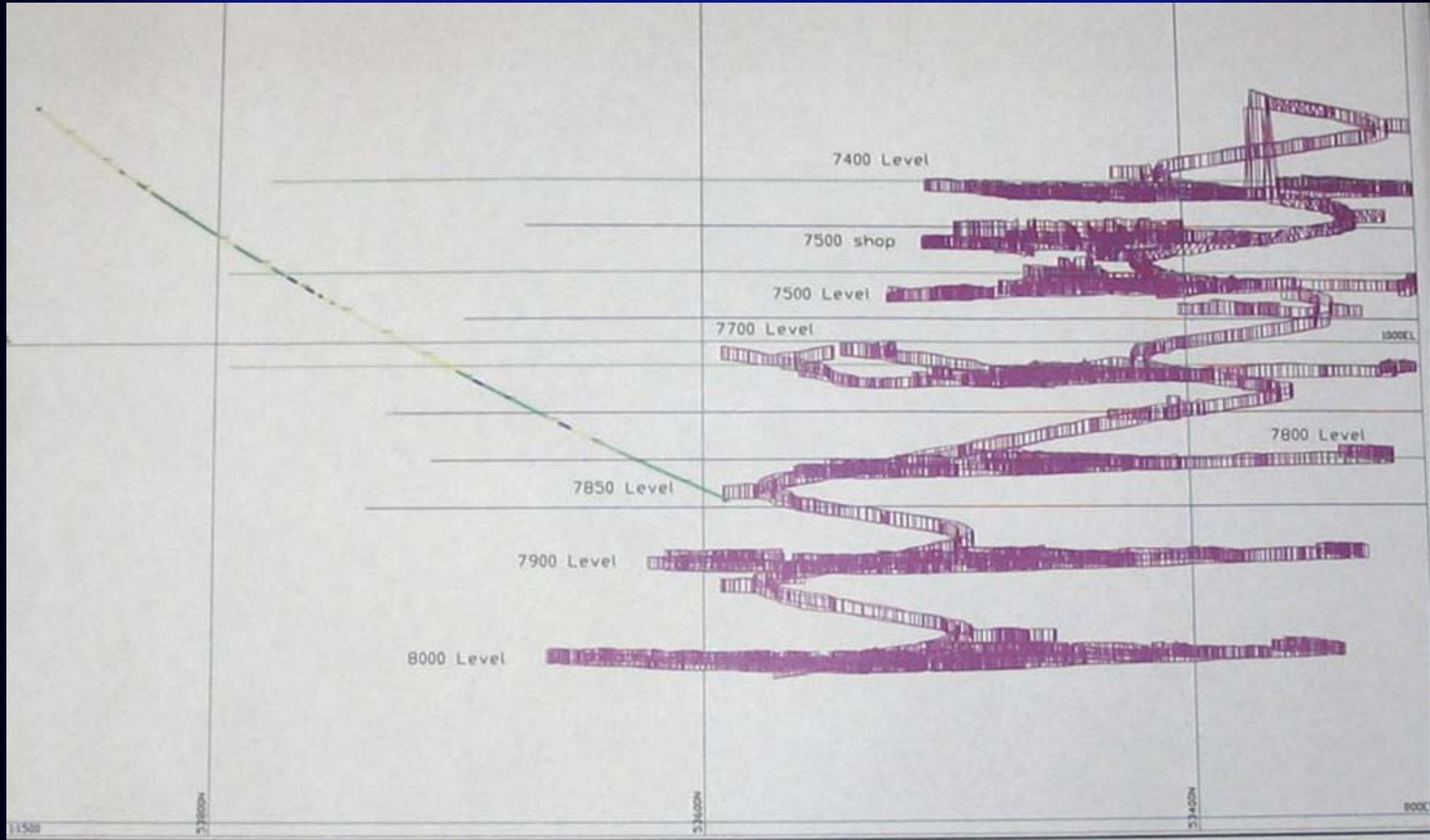
Future Directions

Kidd Creek Mine

Timmins, Ontario



Kidd Creek Mine 7850 Borehole



Kidd Creek Mine

7850 Borehole



DUSEL

Schematic view of DUSEL facilities. Actual implementation will depend on site.

Source: DUSEL S1 Study

