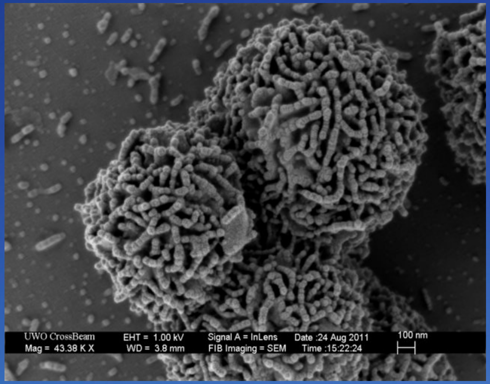
Future Metagenomic Applications for Space Missions



McGill U.

L.G. Whyte
Y.J. Chen
I. Altshuler
C. Maggoiri
E. Magnuson
B. O'Connor

Polar Microbiology & Astrobiology WHYTE LAB

NAS Planetary Protection Session
March 30, 2023 Washington DC

McGill

Objectives - Cryomicrobiology

- expand our fundamental knowledge of microbial diversity & ecology in unique polar ecosystems.

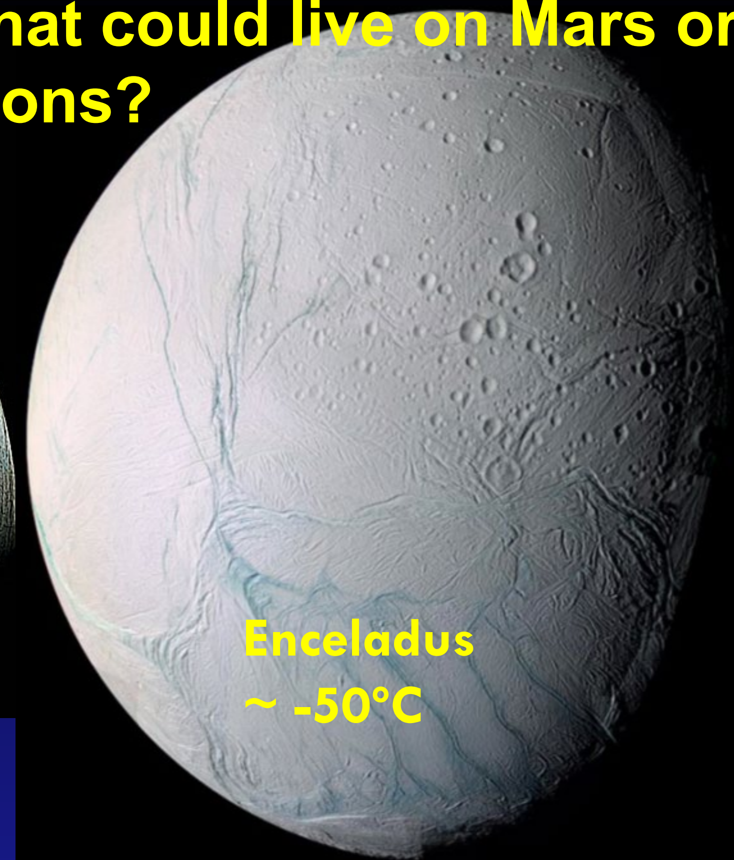
- What are the cold temperature limits of prokaryotic life?
- Are cryoenvironments biologically active in situ?
- Astrobiology relevance → life on Mars, Europa, Enceladus?
- Biotechnology / Health applications?

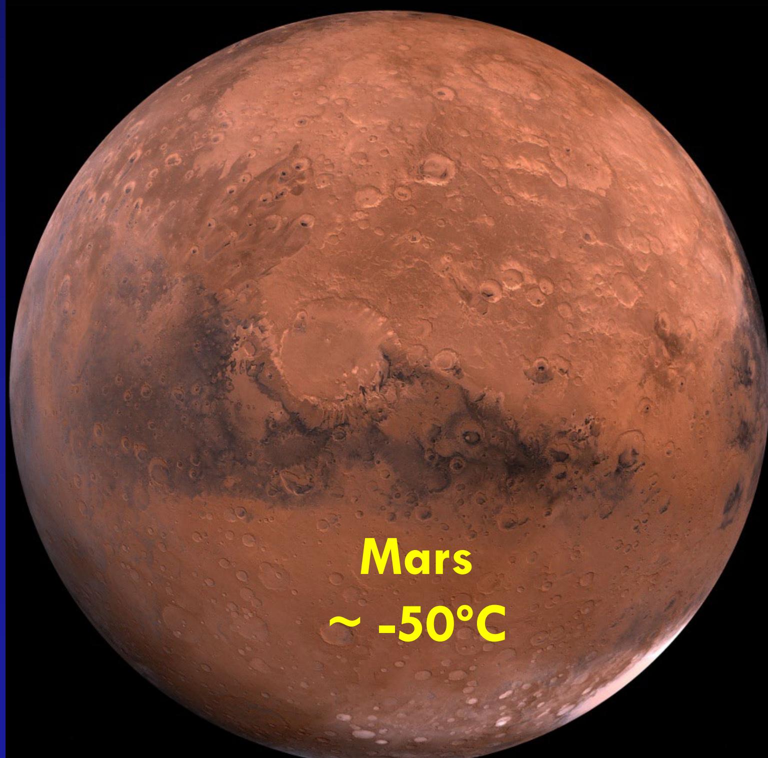
McGill

Gypsum Hill Cold Saline Spring, May, 2008


Eureka
Expedition Fjord
Montréal

Are there Earth Microbes that could live on Mars or the icy moons?

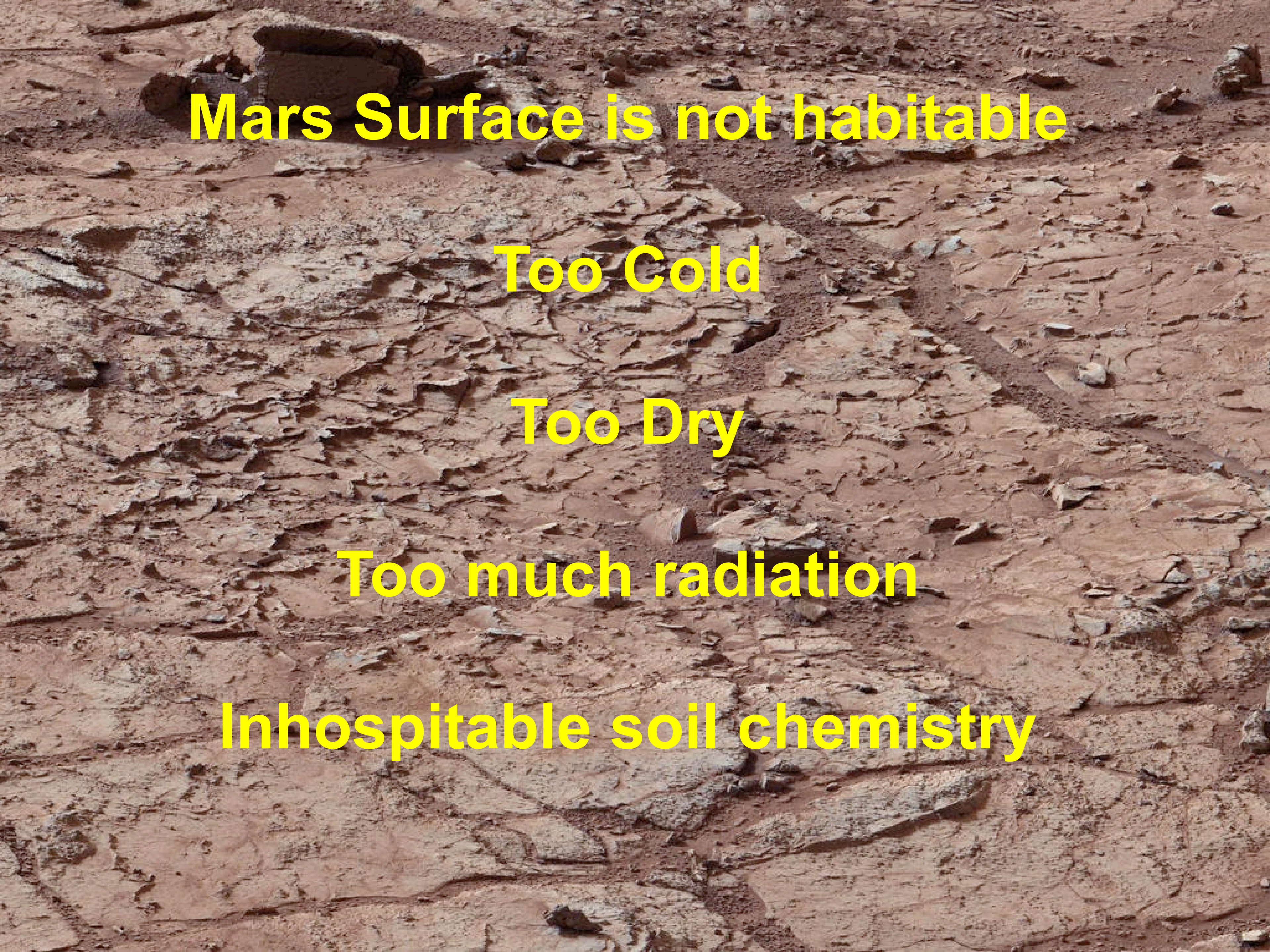




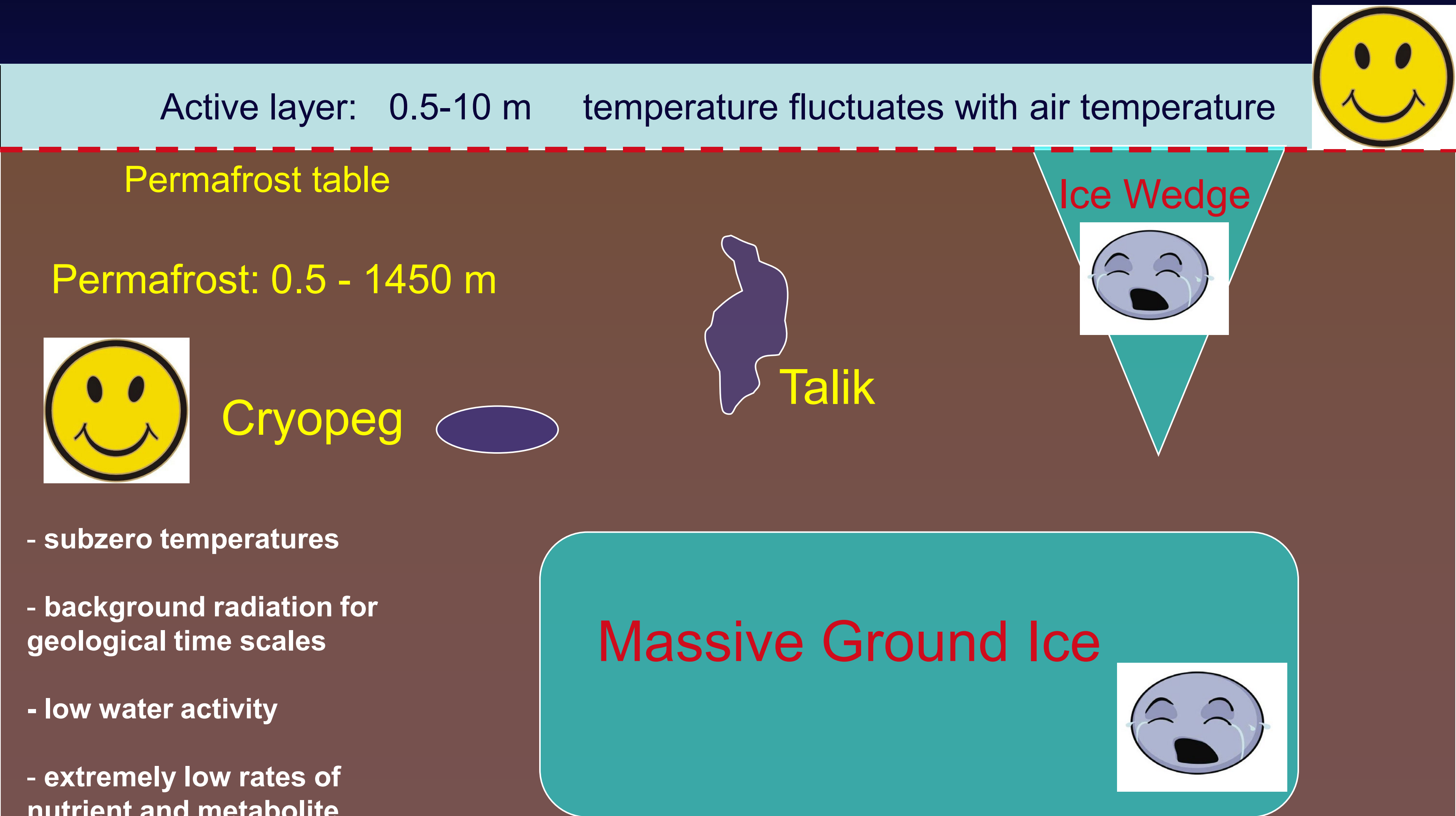
Searching for life in our solar system

- Very cold temperatures
- Liquid water? Salty brines?

Lyle Whyte McGill U.
Planetary Protection Session
Space Research Conf. May 3, 2022


Mars Surface is not habitable
Too Cold
Too Dry
Too much radiation
Inhospitable soil chemistry

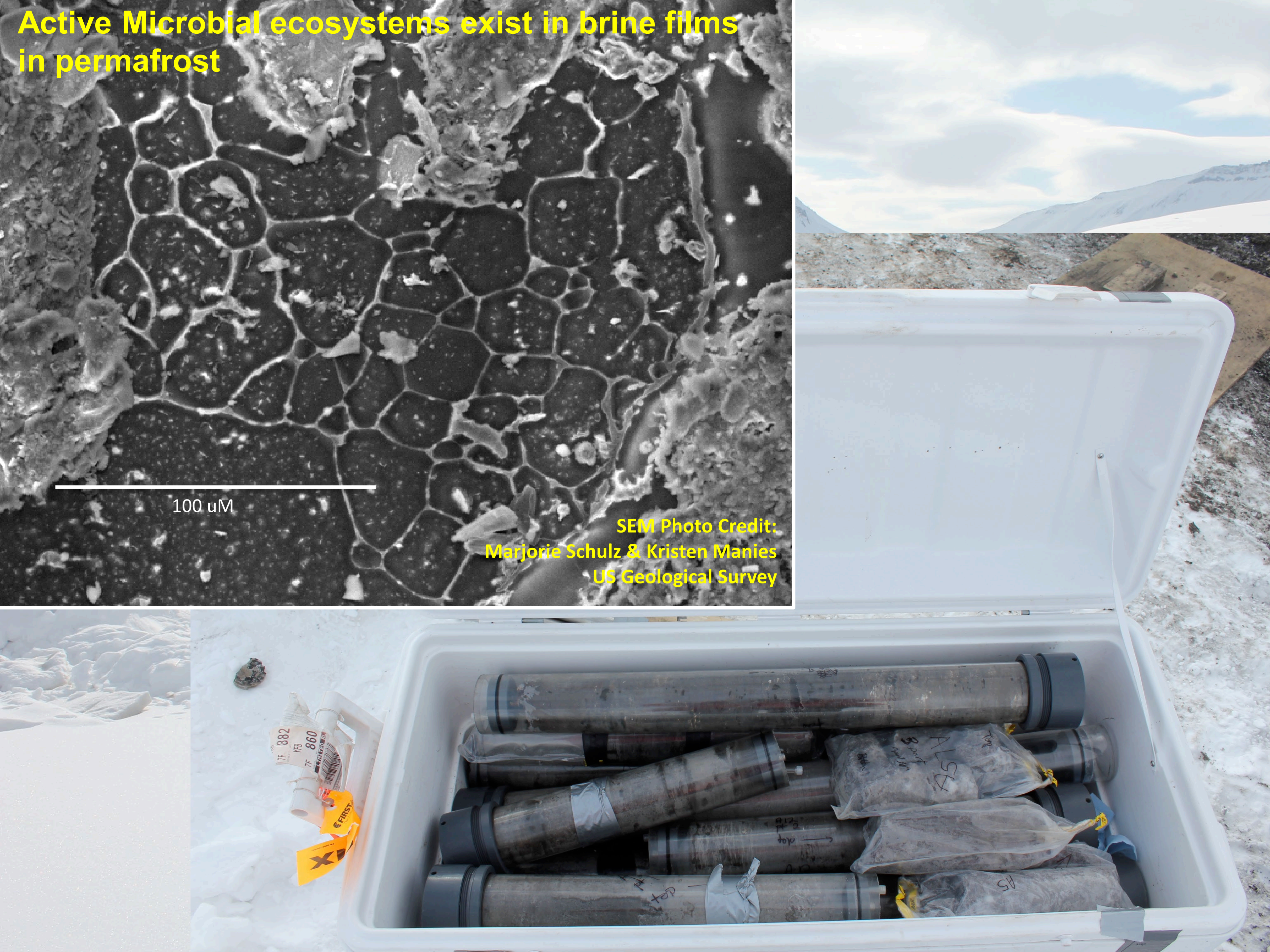
Permafrost: an extreme cryoenviroment



- subzero temperatures
- background radiation for geological time scales
- low water activity
- extremely low rates of nutrient and metabolite transfer

Steven et al. 2006. Microbial Ecology and Biodiversity of Permafrost. *Extremophiles*

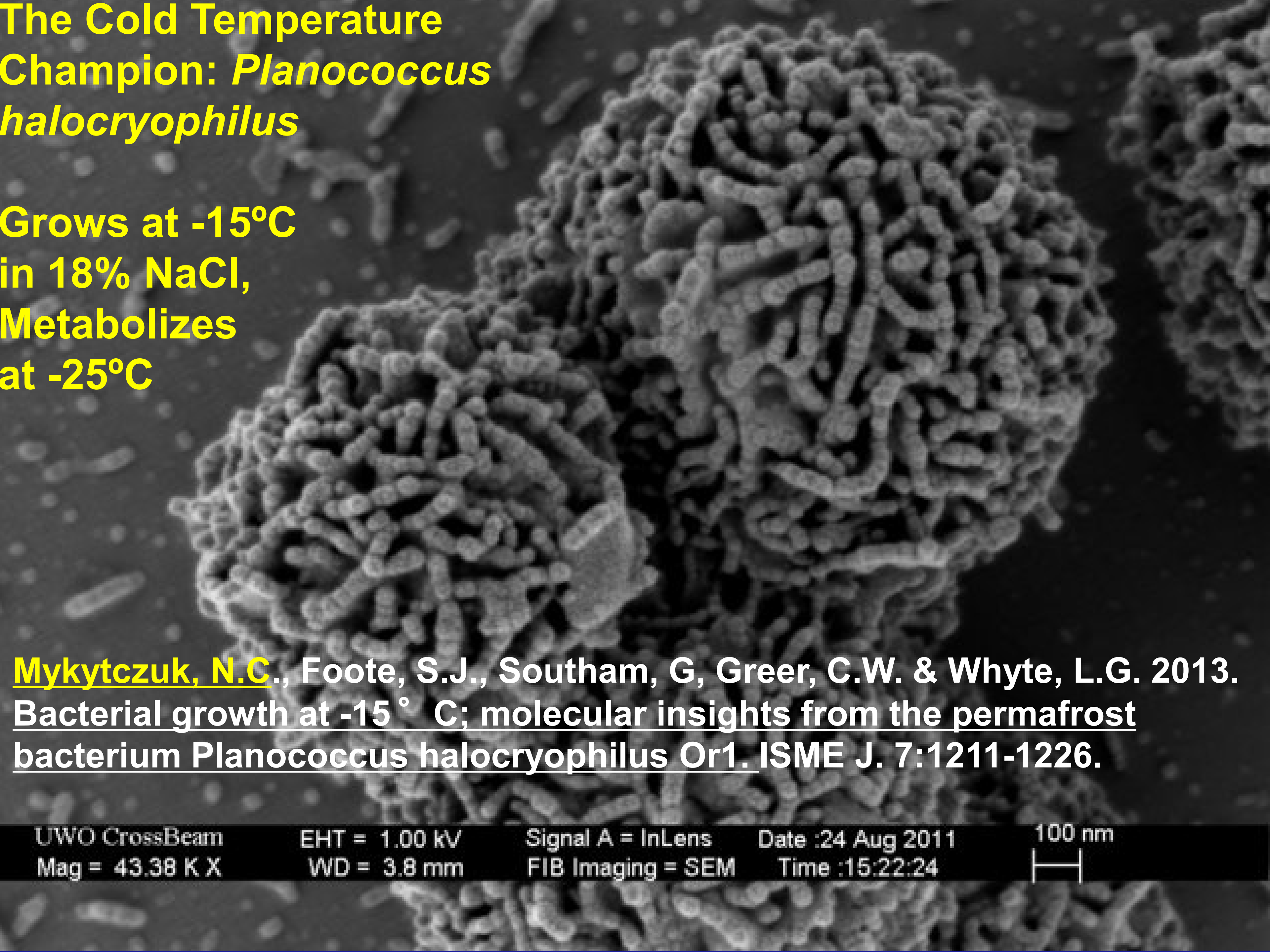
Active Microbial ecosystems exist in brine films in permafrost



SEM Photo Credit:
Marjorie Schulz & Kristen Manies
US Geological Survey

The Cold Temperature Champion: *Planococcus halocryophilus*

Grows at -15ºC in 18% NaCl, Metabolizes at -25ºC



<u>Mykytczuk, N.C</u>., Foote, S.J., Southam, G, Greer, C.W. & Whyte, L.G. 2013. <u>Bacterial growth at -15˚ C; molecular insights from the permafrost bacterium Planococcus halocryophilus Or1.</u> ISME J. 7:1211-1226.

Solute uptake: Genomic redundancy indicating osmotic/cold adaptation strategy in *Planococcus* Or1

Organism	Osmoregulatory genes: choline/betaine uptake and synthesis									
	Pro P	Pro V	Pro W	Pro X	Opu D	OpuA A	OpuA B	OpuA C	BetB *	Glyc ^
Planococcus sp. Or1		X(2)	X	X(2)	X(6)	X(3)	X(2)	X(2)	?	X
Psychrobacter cryohalolentis K5		X	X		X				X	
Psychrobacter arcticus 273-4	X								X	
Psychromonas ingrahamii		X(3)	X(3)						X	X
Colwellia psychrerythraea 34H		X	X	X					X(3)	
Desulfotalea psychrophila		X	X							
Exiguobacterium sibiricum 255-15	X	X		X	X		X	X		X

X, presence of gene; (#), number of copies in genome

Cryophilic microorganisms are also halotolerant / halophilic!

Mykytczuk et al., 2013. ISME J.

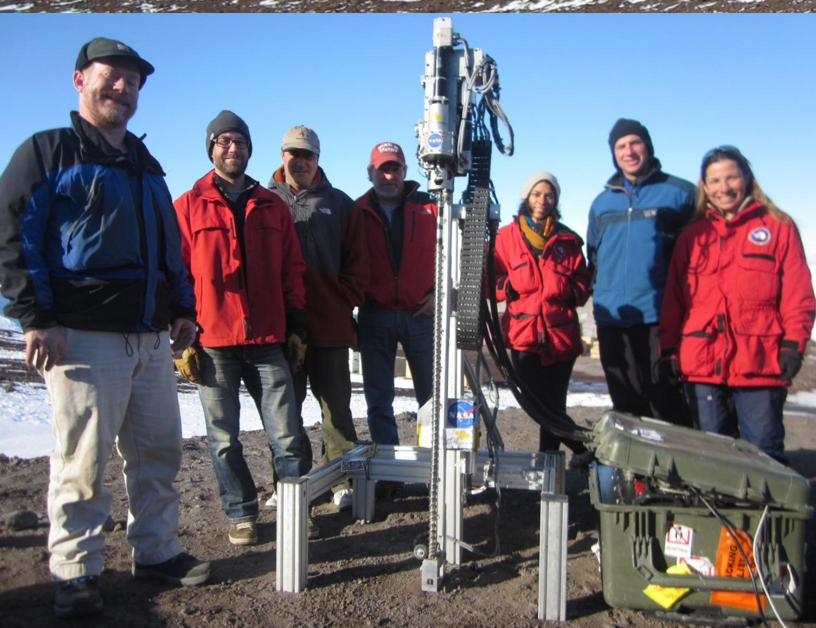
NASA ASTEP Project - IceBite: an auger and sampling system for ground ice on Mars

C. McKay (PI)

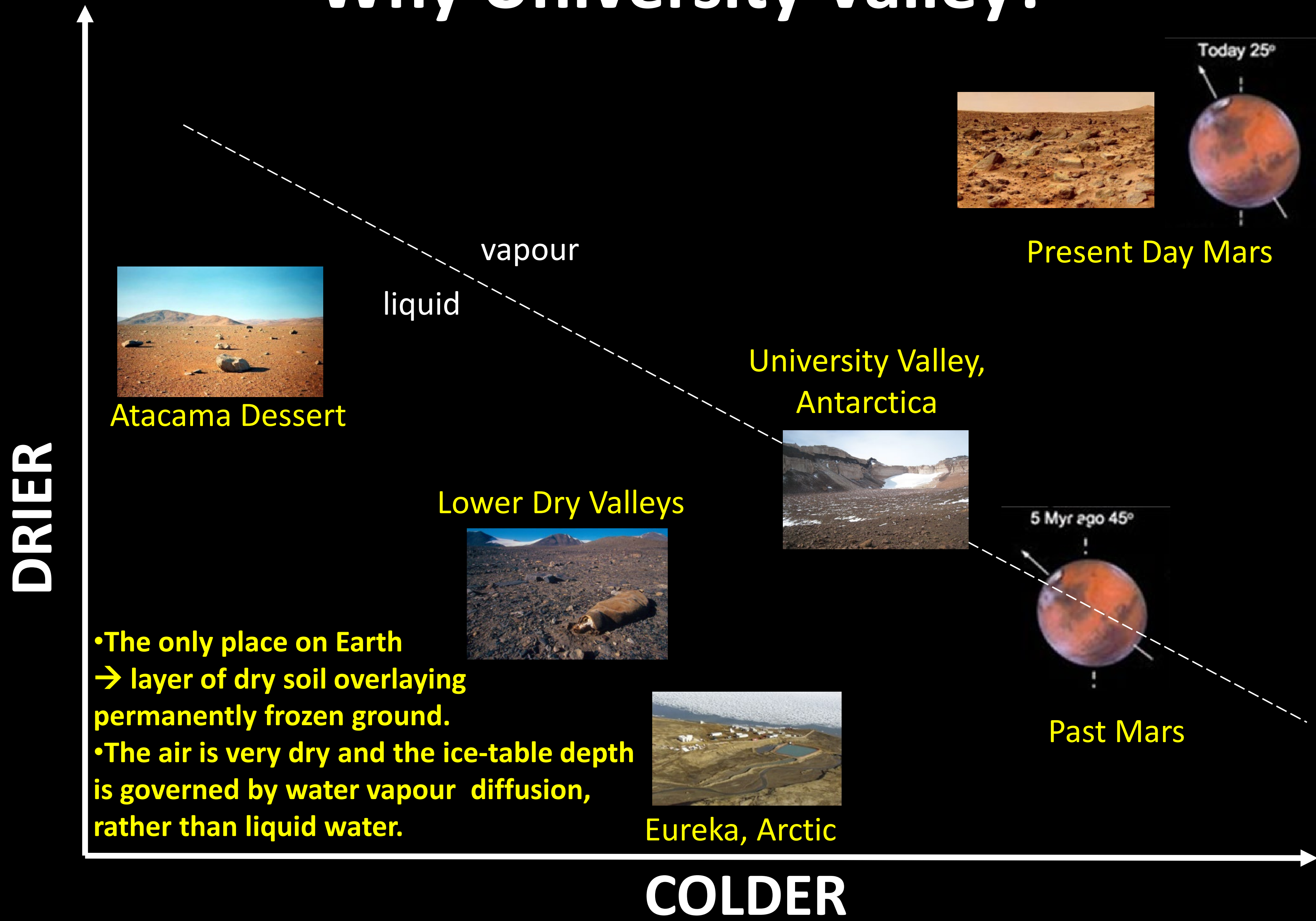
Alfonso Davila
Denis Lacelle
Wayne Pollard
Jackie Goordial
Jocelyne Diruggiero
Margarita Marinova

University Valley permafrost and cryptoendolith microbiology

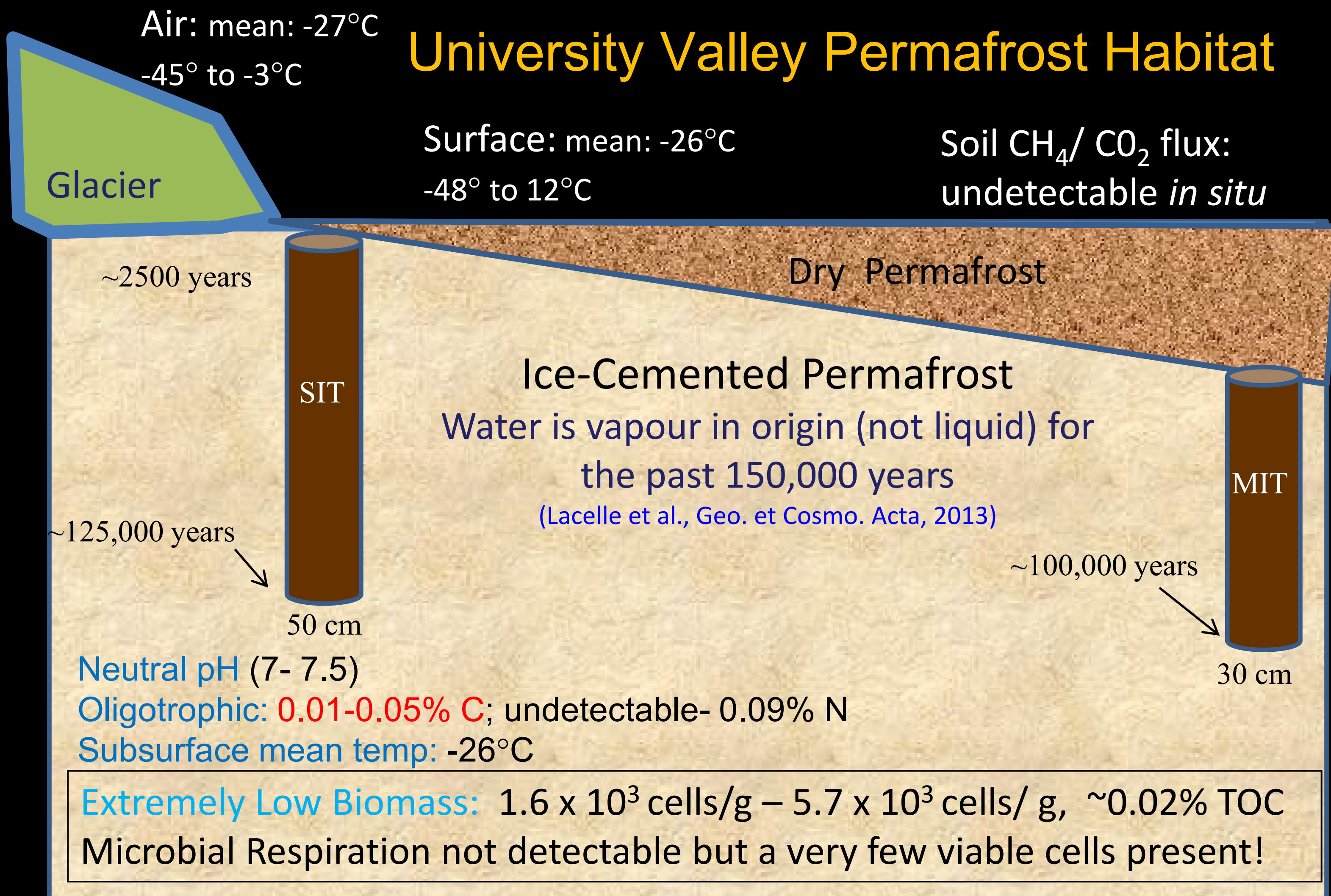
- Molecular Diversity
- Culturable microorganisms
- Microbial activity: *in situ*/ microcosms



Why University Valley?

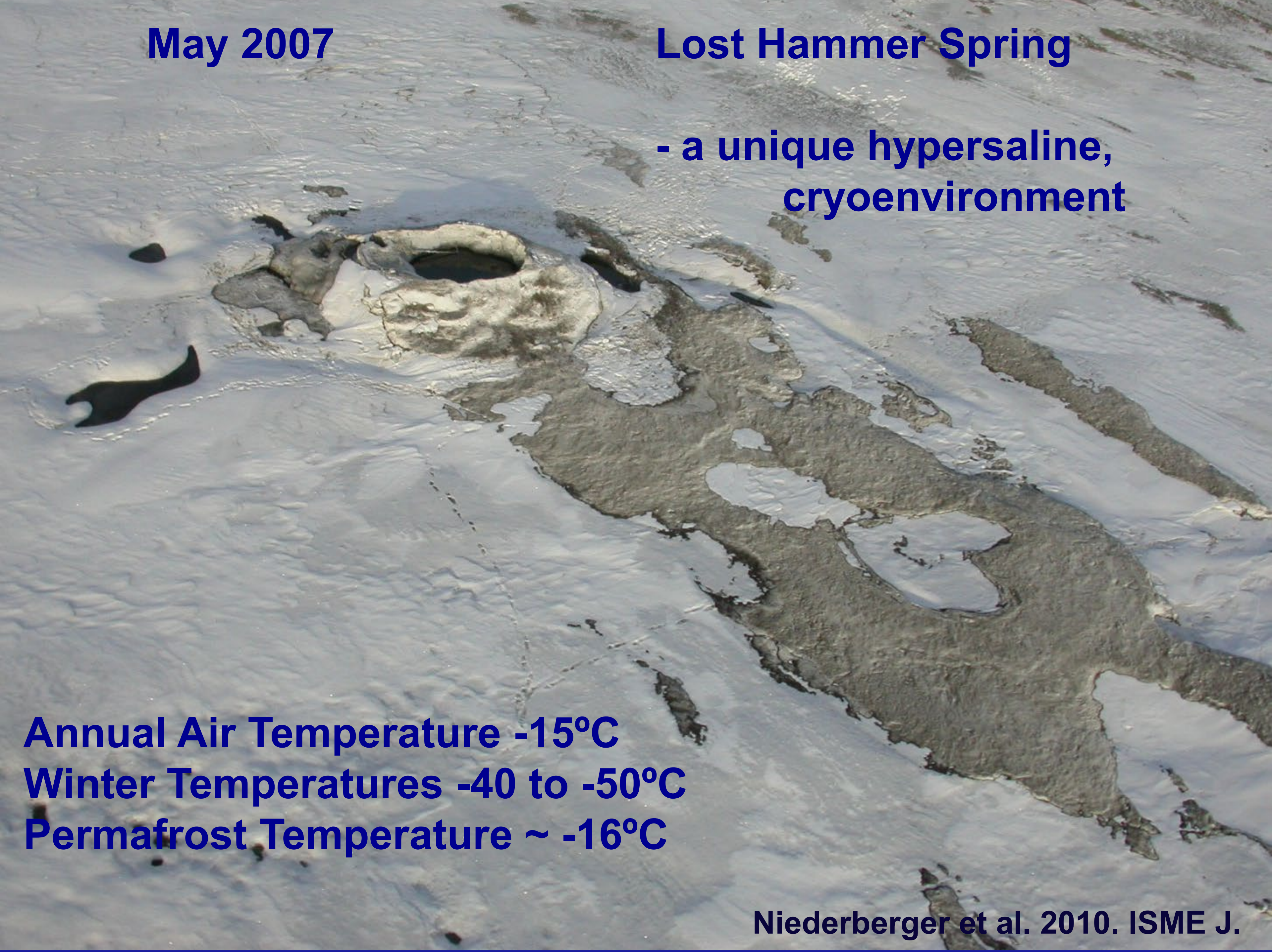


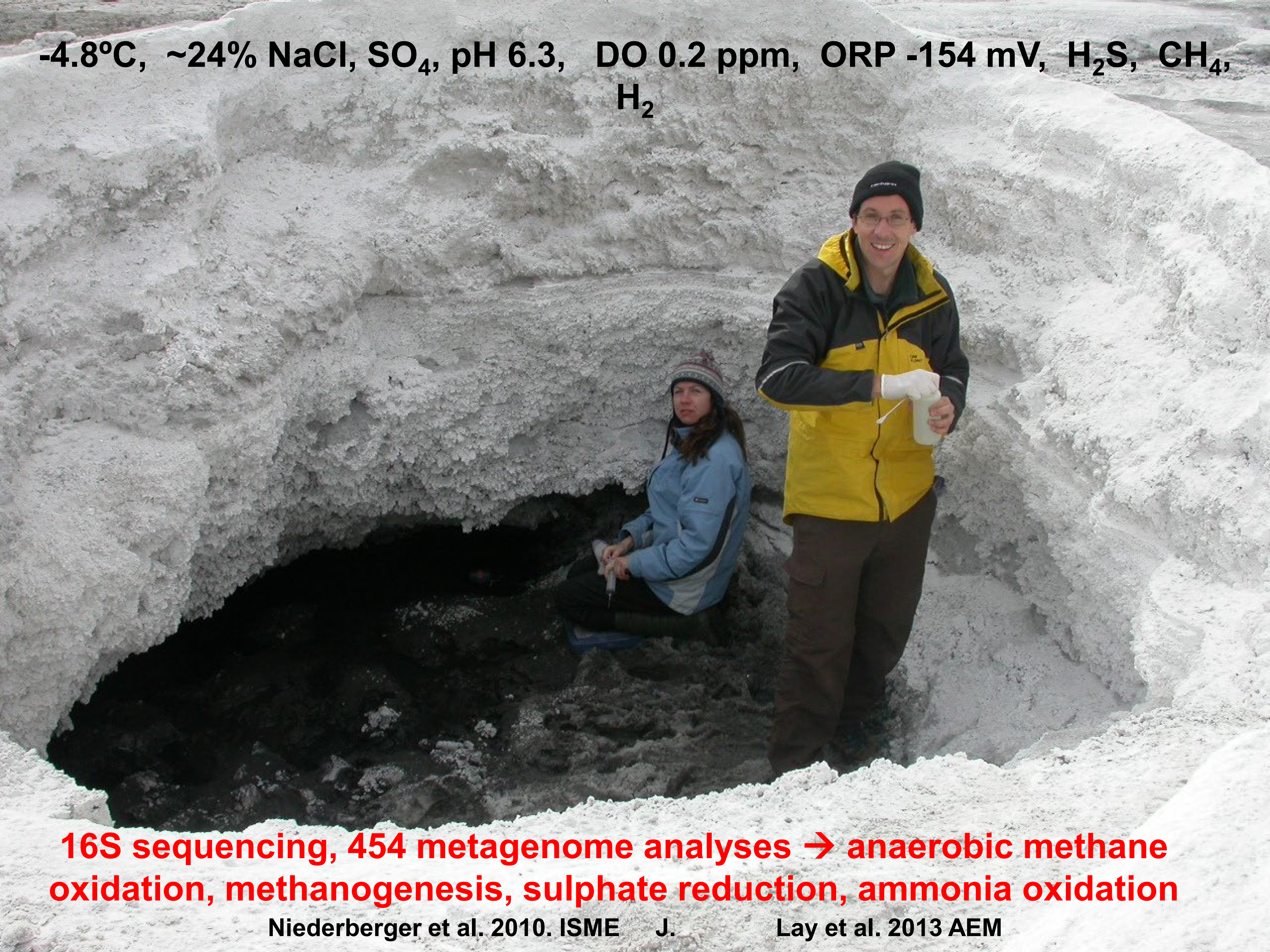
University Valley Permafrost Habitat

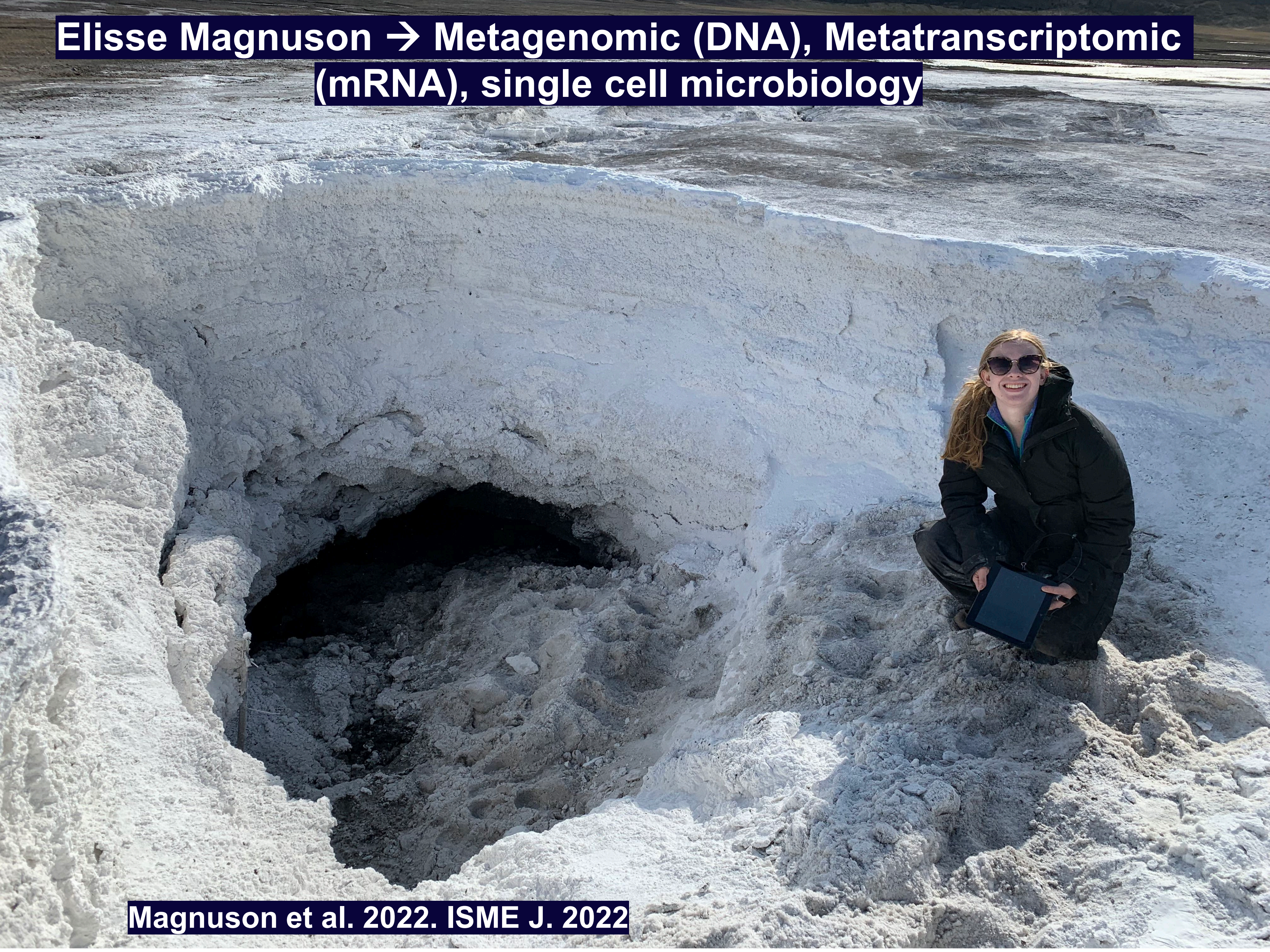


University Valley . . . . too cold, too dry, too old?

"It's (almost?) dead Jim"

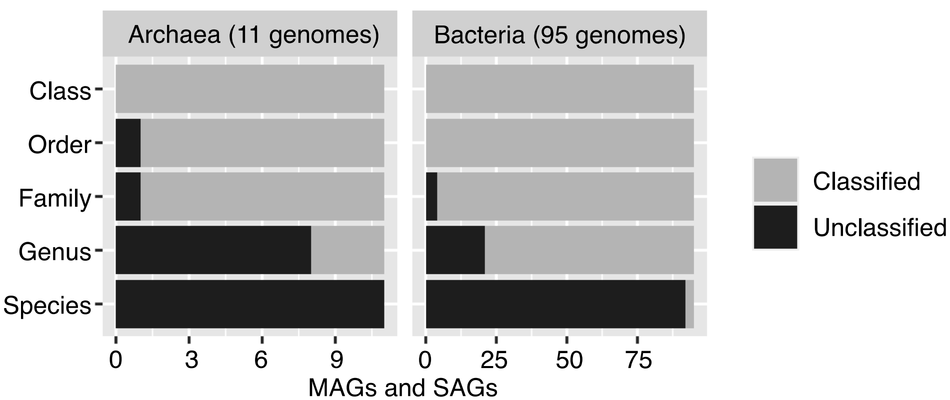

May 2007
Lost Hammer Spring
- a unique hypersaline, cryoenvironment
Annual Air Temperature -15ºC
Winter Temperatures -40 to -50ºC
Permafrost Temperature ~ -16ºC
Niederberger et al. 2010. ISME J.


-4.8ºC, ~24% NaCl, SO_4, pH 6.3, DO 0.2 ppm, ORP -154 mV, H_2S, CH_4, H_2
16S sequencing, 454 metagenome analyses → anaerobic methane oxidation, methanogenesis, sulphate reduction, ammonia oxidation
Niederberger et al. 2010. ISME J.
Lay et al. 2013 AEM

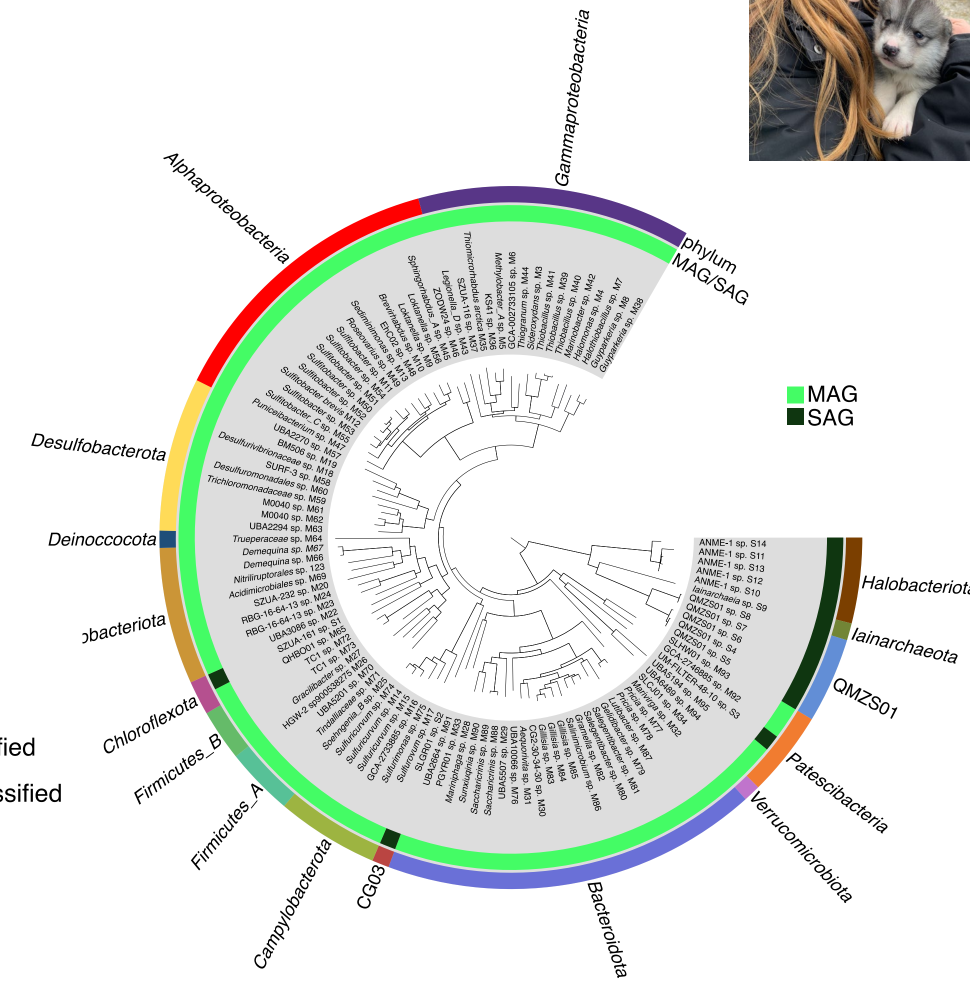

Elisse Magnuson → Metagenomic (DNA), Metatranscriptomic (mRNA), single cell microbiology
Magnuson et al. 2022. ISME J. 2022

Exploring Lost Hammer's taxonomic and metabolic diversity

- 106 genomes assembled through MAG and SAG sequencing
- Genomes from low-abundance ANME-1 and DPANN archaea recovered through SAG sequencing
- **High proportion of genomic novelty**



1a. Level of taxonomic novelty in the MAGs and SAGs by rank.



1b. Phylogenetic tree of high- and medium-quality MAGs and SAGs.

Magnuson et al. 2022. ISME

Metatranscriptome sequencing revealed active microbial community is primarily lithoautotrophic (~60% of relative expression) ….. at -5ºC, 24% salinity, anoxic conditions

- **23% of relative expression attributed to sulfide-oxidizing Gammaproteobacteria**

- **Transcripts from active ANME-1 detected**

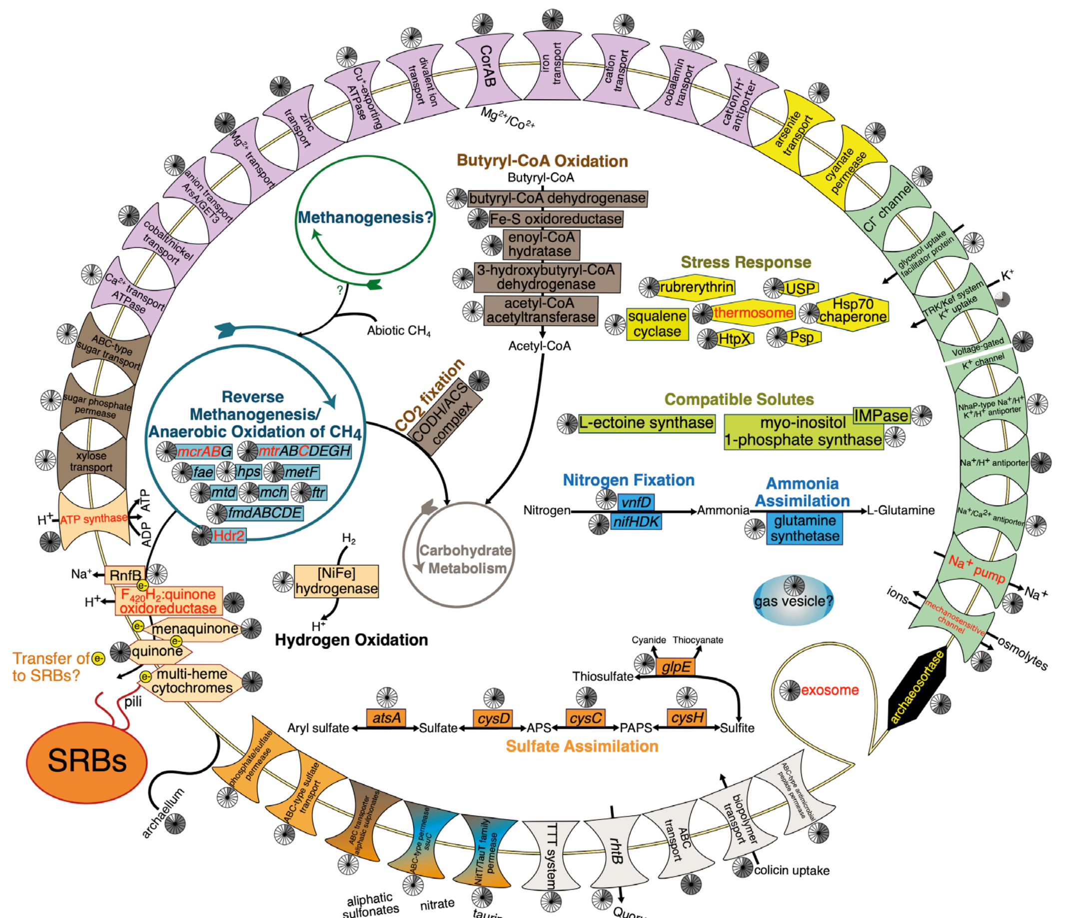


Figure 3. Metabolic reconstruction of ANME-1 SAGs. Genes with mapped transcripts are denoted by red text. Pie charts indicate the number of SAGs containing each gene (out of 17).

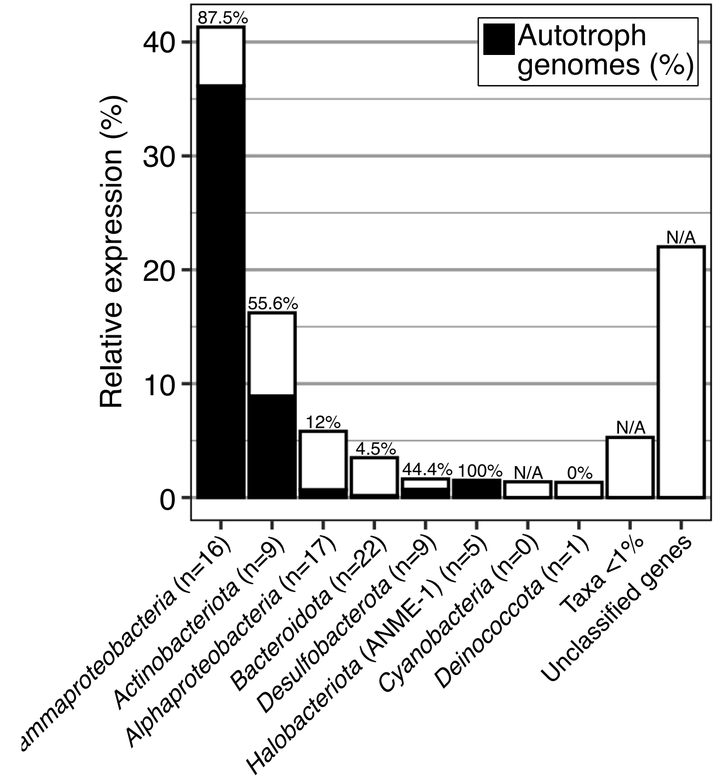


Figure 2. Percent relative expression by phylum, including both genes in MAGs and SAGs and unbinned genes classified by JGI. The percentage of autotrophic MAGs and SAGs (containing CO_2 fixation genes) in each phylum is indicated in black and noted above each column.

Magnuson et al. 2022. ISME

Figure 5. Model genome of LH Spring ANME-1 SAGs, an anaerobic methane oxidizing Archaea living at -5ºC in 25% salt!!!

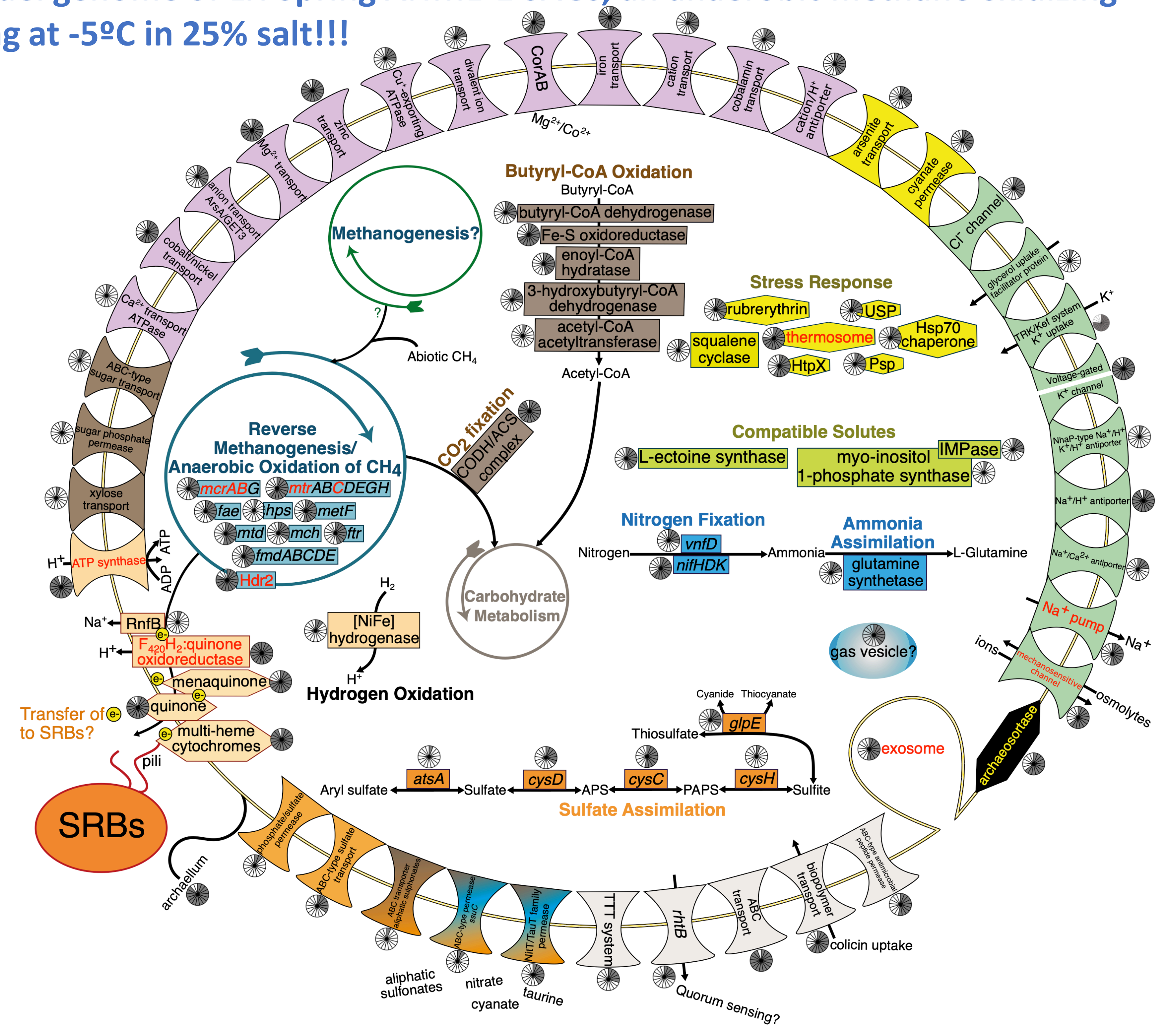
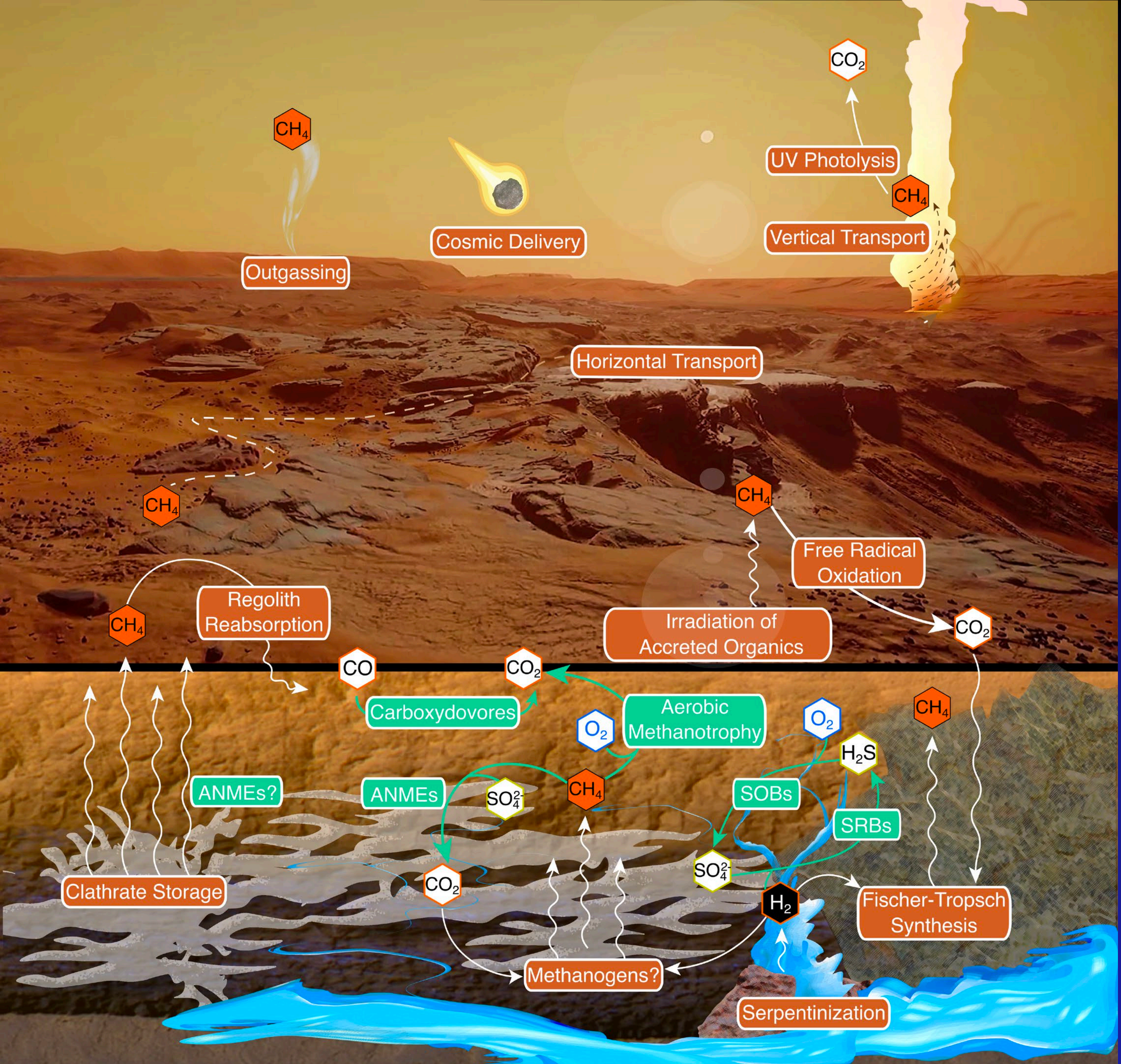


Figure 3. Metabolic reconstruction of ANME-1 SAGs. Genes with mapped transcripts are denoted by red text. Pie charts indicate the number of SAGs containing each gene (out of 17).



Magnuson et al. 2022. ISME J.

Lost Hammer Spring …… a Mars analogue Habitat on Earth

- Anaerobic CH_4-oxidizing ANME-1
- H_2-oxidizing, SO_4-reducing Desulfobacterota
- Mixotrophic CO-oxidizing Alphaproteobacteria
- H_2S-oxidizing Gammaproteobacteria scavenging trace O_2 …

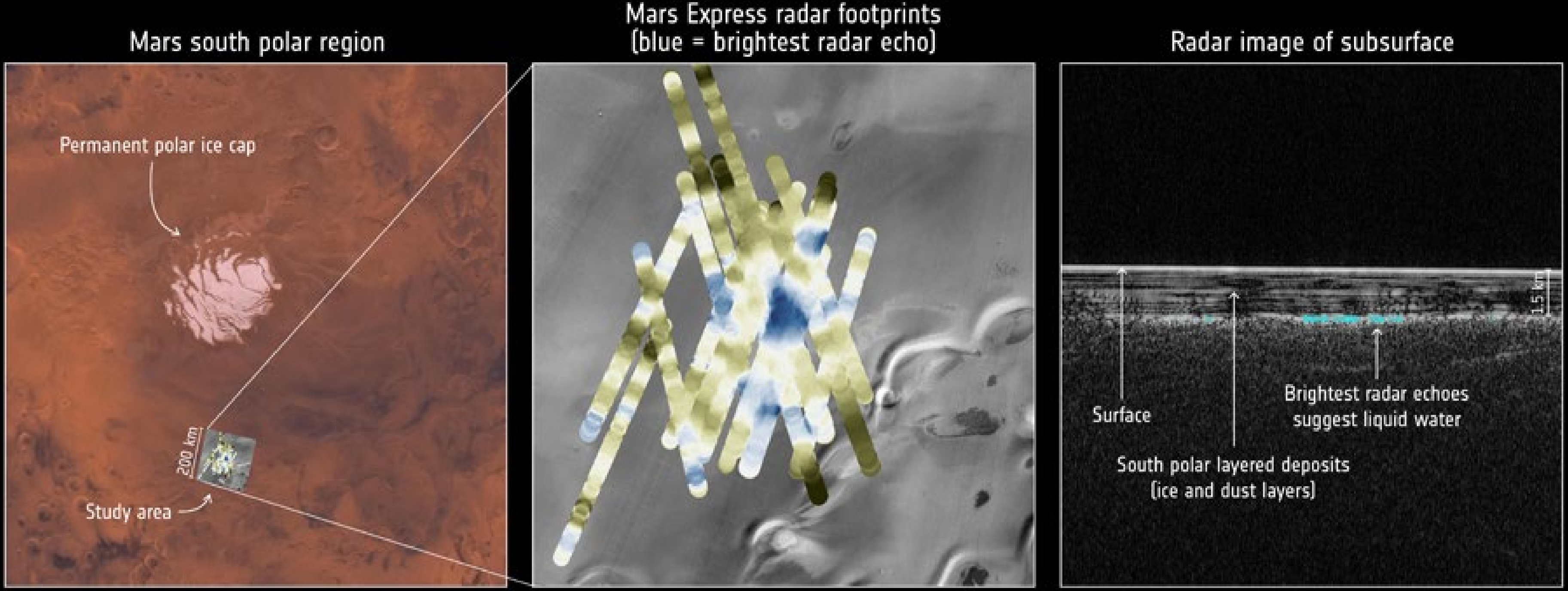
that potentially could exist in the Martian subsurface

Is there cold salty water on the surface of Mars?

- Dark lines are observed appearing seasonally on the Martian surface – *recurring slope lineae (RSLs)*
- Recent Spectral evidence suggests they contain hydrated salts and water

Martian RSLs in Newton Crater

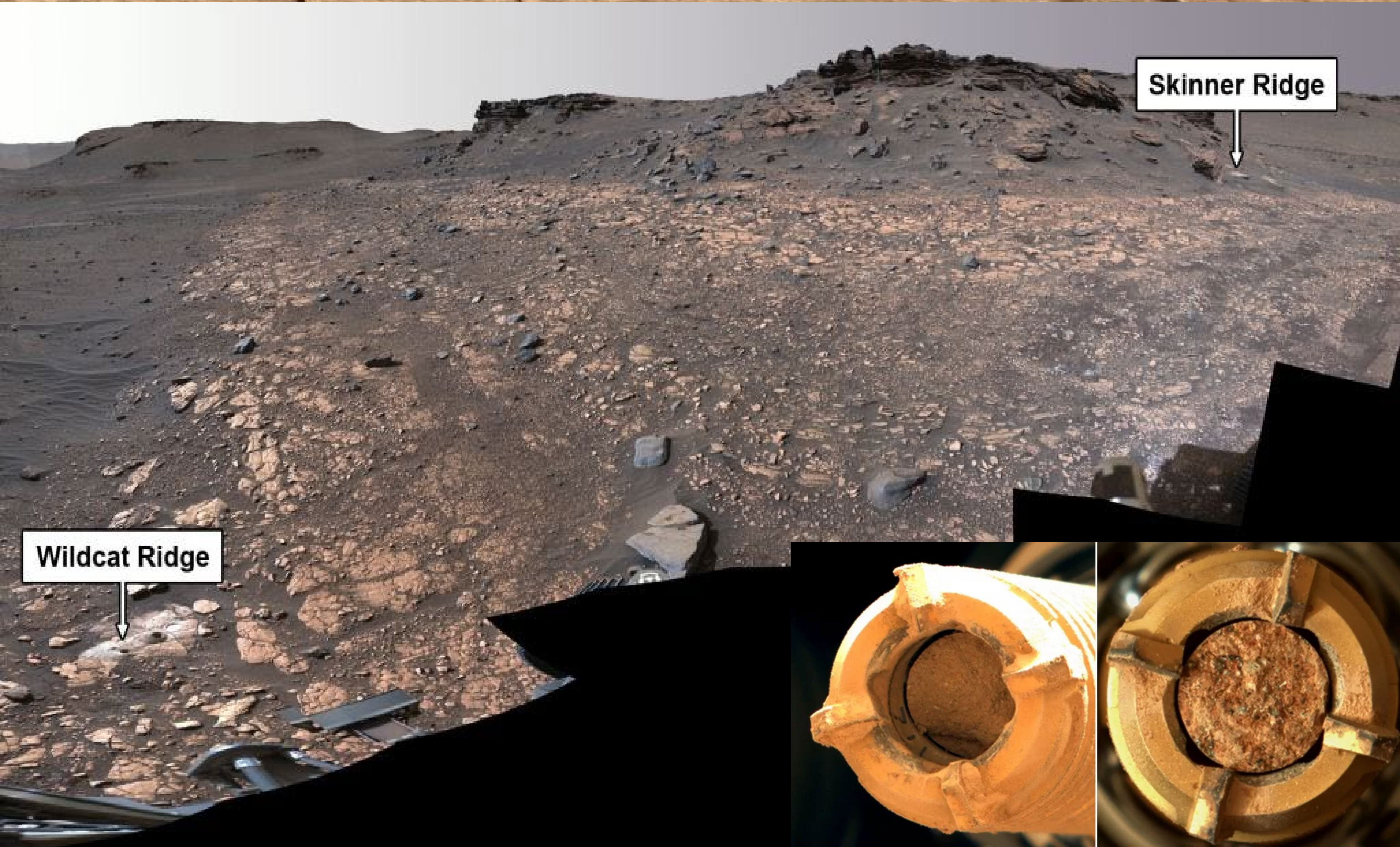
Discovery of hypersaline subsurface lake on Mars!



Science June 2018

Jezero Crater "WildCat Ridge" May 2022

Organic C rich / sulphate rich, sedimentary rock core sample retrieved from an ancient lake bed formed from an evaporating saltwater lake ~ 3.5 bya ….



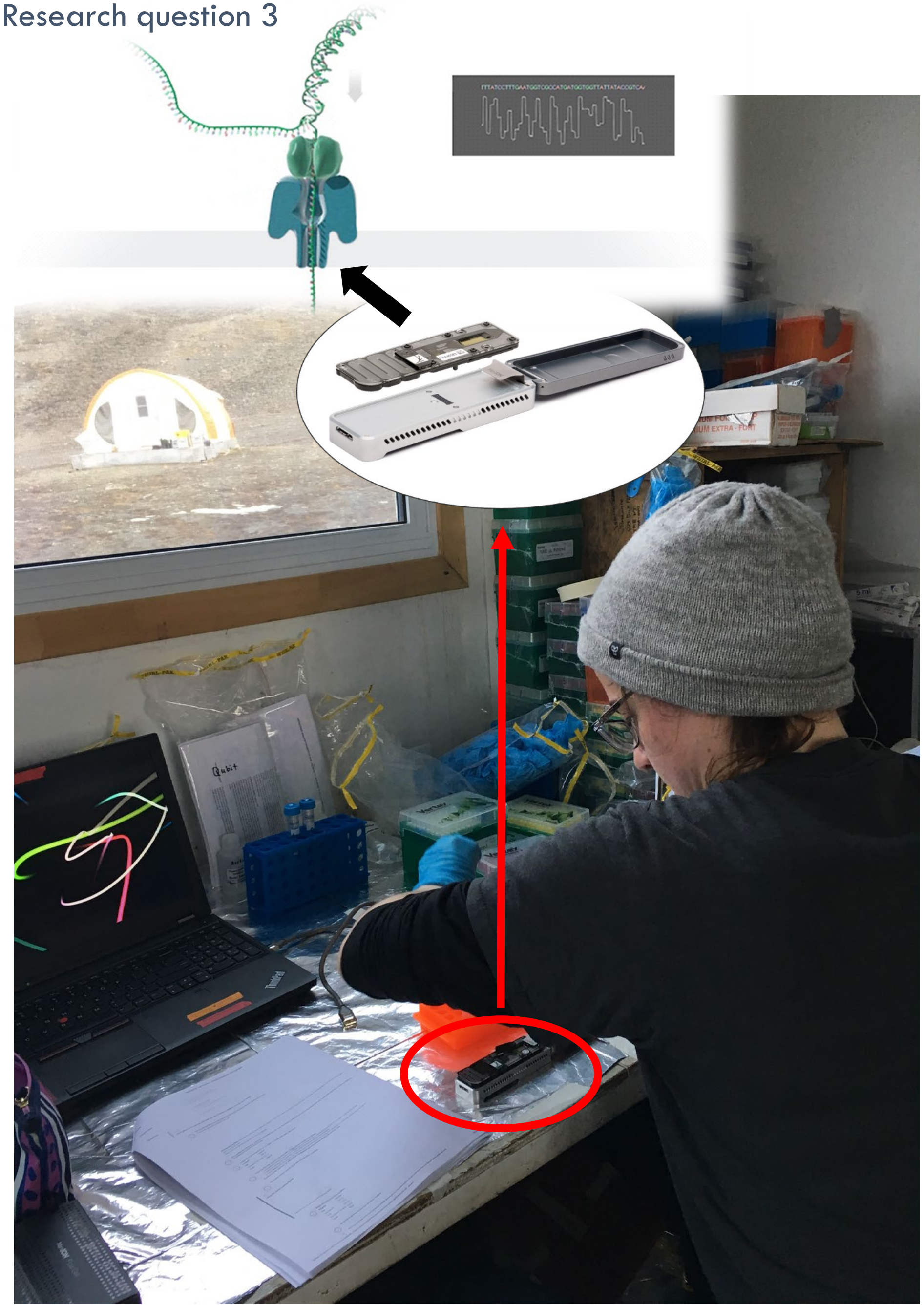
MinION sequencing: life detection and metagenomics

The **Oxford Nanopore Technologies (ONT) MinION** is:

- **Small** (90 g), **low cost**, **sensitive**, can **detect unambiguous biosignatures** (DNA, RNA), generates **long reads** (e.g. genome-length contigs)

Can its advantages (e.g. long reads) overcome its disadvantages (e.g. high error rate) to make it a useful tool for **life detection** and **metagenomics**?

Goordial et al. 2017. Front. Microbiol.; Maggiori et al. 2020. Astrobiol.; Maggiori et al. 2021 Sci. Reports



MinION sequencing

Illumina sequencing

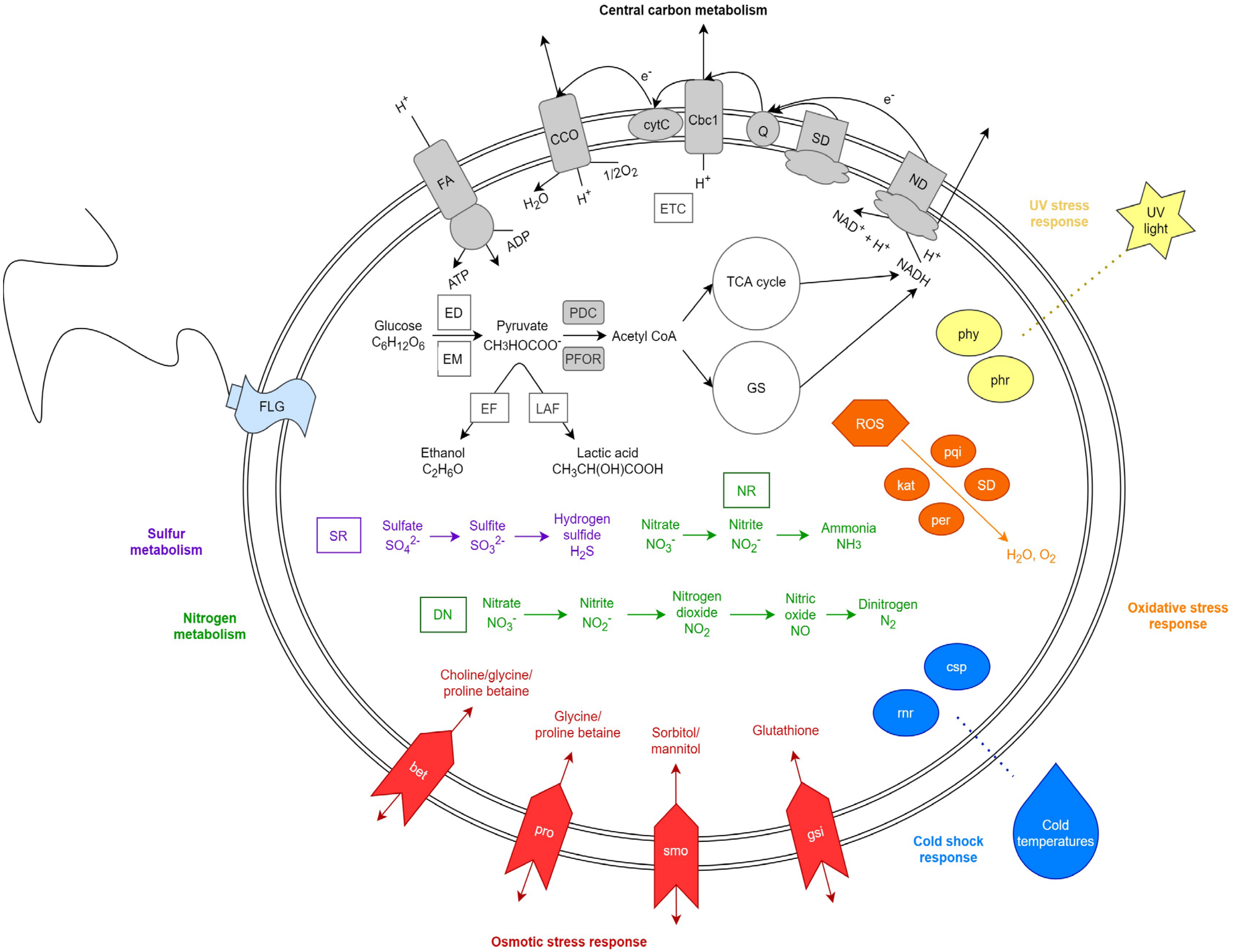
Long error-prone MinION reads (from the field)

Short accurate HiSeq reads (from the lab)

Long and accurate hybrid contiguous sequences

Metagenome-assembled genomes (MAGs)

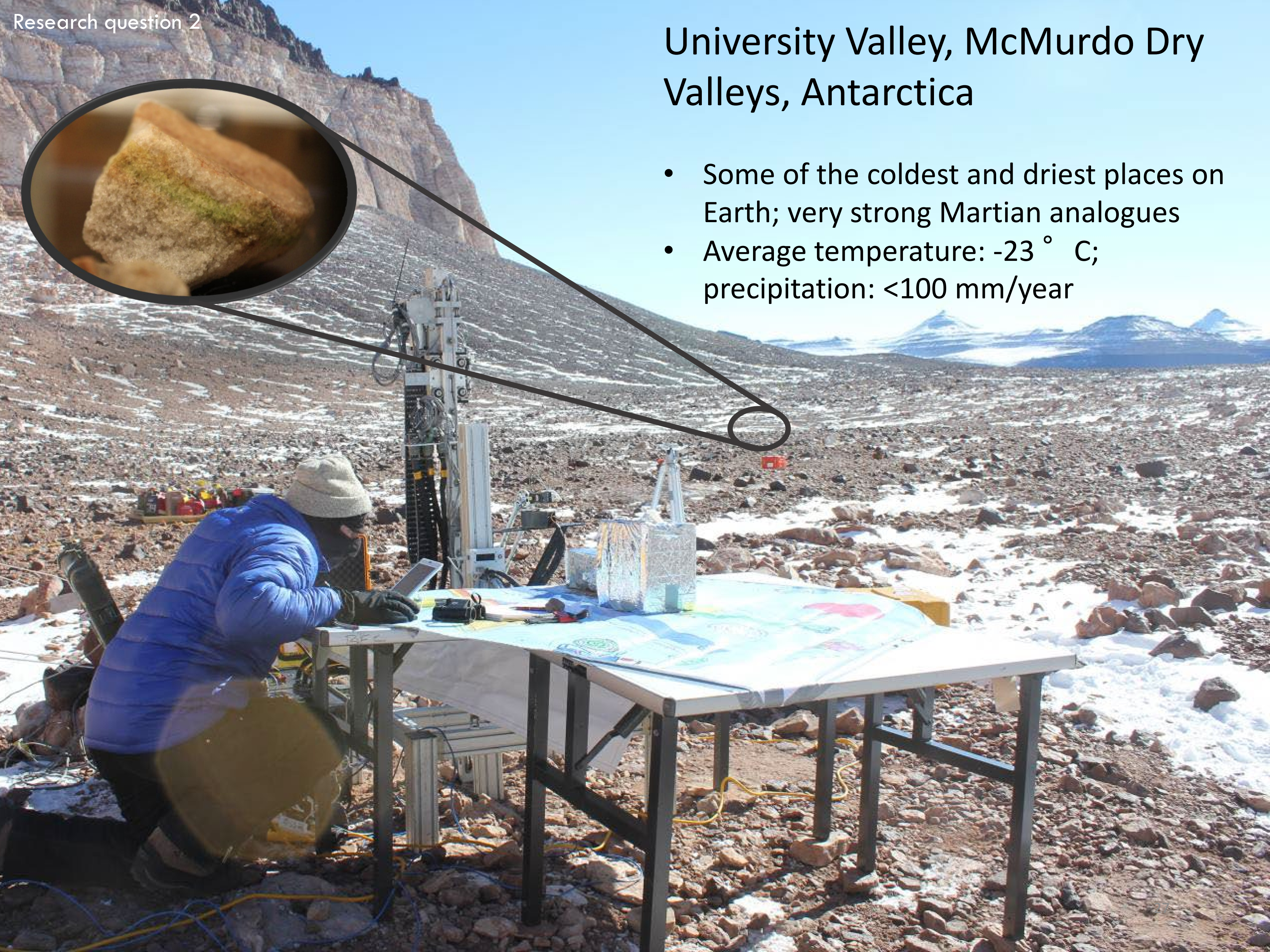
Hybrid MAG from a novel Arctic sea ice cryoconite metagenome



- 90.9% complete, 0.6% contaminated
- Identified as *Octadecabacter* by metaerg, MiGA, and GTDB-tk
- Contains 1 16S gene aligning to *Octadecabacter arcticus* with 98% identity
- The average nucleotide identity (ANI) of Hybrid_5 was calculated at 93.51% with *O. arcticus*, below the threshold of 95% similarity for identical species
- Other factors that indicate novelty are present
  - this MAG contains the genus's only known instances of genomic potential for nitrate reduction, denitrification, sulfate reduction, and fermentation
- Hybrid MAG contains genomic features not present in HiSeq counterpart (e.g. some stress response genes, 16S)

University Valley, McMurdo Dry Valleys, Antarctica

- Some of the coldest and driest places on Earth; very strong Martian analogues
- Average temperature: -23 ° C; precipitation: <100 mm/year



Mars Custom UVC light chamber

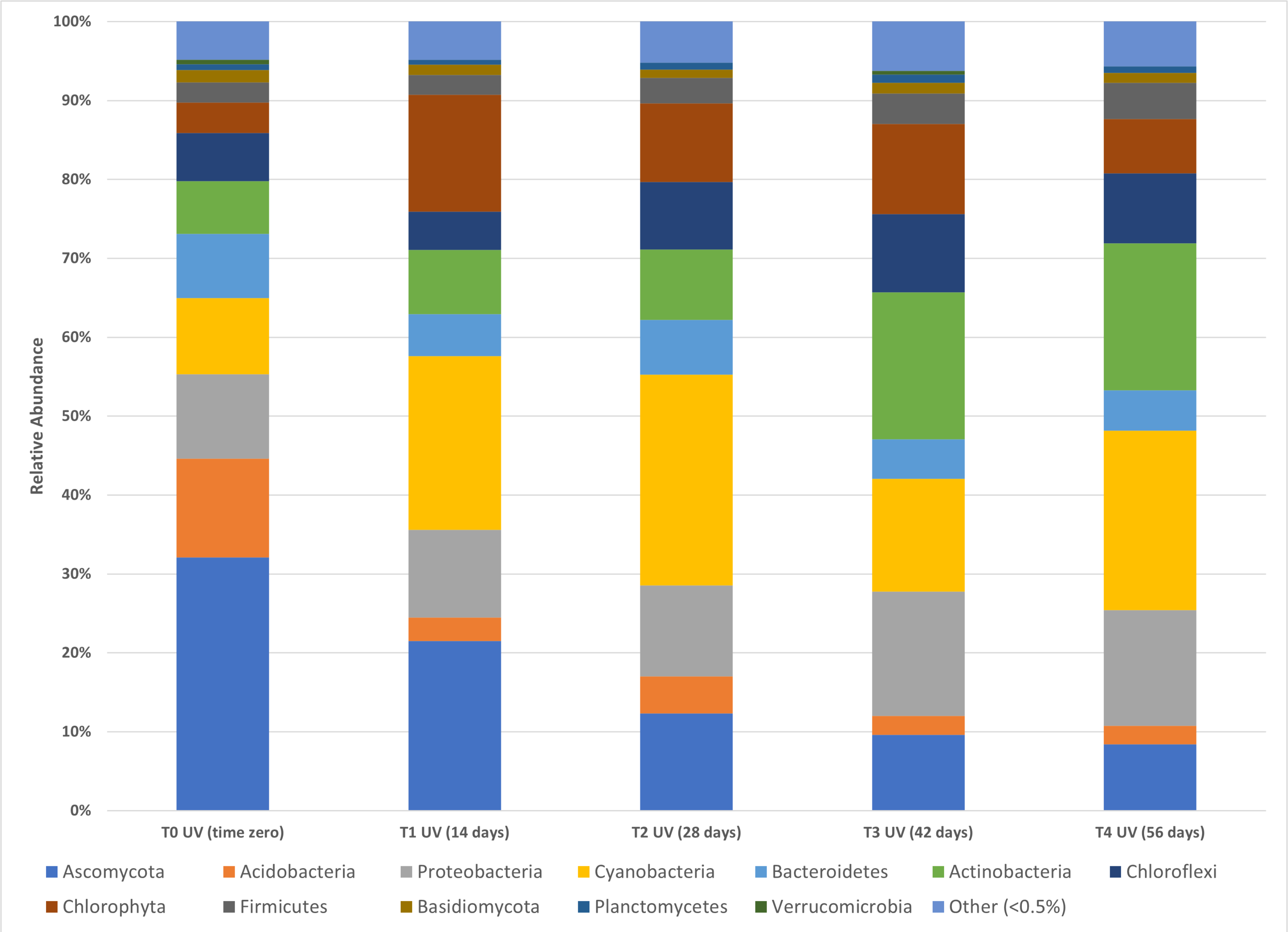
	Average UVC flux (W/m²)
Custom UVC chamber	~4 – 70
Mars	~0.9 – 1.2

Custom UVC chamber containing University Valley cryptoendoliths (left) incubated at -25ºC.

Received a UV exposure equivalent to ~ 278 Martian years (~ 522 Earth Years)

Unsterilized, covered negative control is circled in red. Sterilized and uncovered negative control is circled in blue.

MinION successfully detected and sequenced DNA after UVC radiation exposure ~ 278 Martian years (~ 522 Earth Years)



Future Metagenomic Applications for Space Missions

Biosignature / Extant Life Detection

Nanopore sequencing should be a viable detection tool for unambiguously detecting life …. In future robotic and human missions ….

Detecting NA polymers in extraterrestrial samples ?

Identifying and characterizing microbes in Earth extreme environments analogous to Mars and Icy Moons

Planetary Protection Applications

Detecting and characterizing forward contamination

Detecting and characterizing terrestrial and extraterrestrial NA polymers (??) in MSR samples ….

Future Metagenomic Applications for Space Missions

Future Challenges to obtain high quality metagenomes and metagenome assembled genomes…..

Nanopore sequencing accuracy needs improvement

Requires robust NA Polymer extraction methods …..

Requires very low level detection limits ...... 10 - 100 cells / g

Automation and TRL for robotic missions will …. ?

Very large sequence data sets will require high level computing and data transfer on robotic missions ……


Merci beaucoup!
Canada Research Chair Program &
Canadian Foundation for Innovation
Polar Continental Shelf Project,
National Resources Canada
Northern Scientific Training Program,
FQRNT, NSERC
Polar Microbiology & Astrobiology
WHYTE LAB
CSA ASC
NASA
NSERC
CRSNG
McGill
McGill
TSI
Trottier
Space Institute
at McGill
Institut spatial
Trottier
de McGill

ExoMars 2028 . . . . . . ??

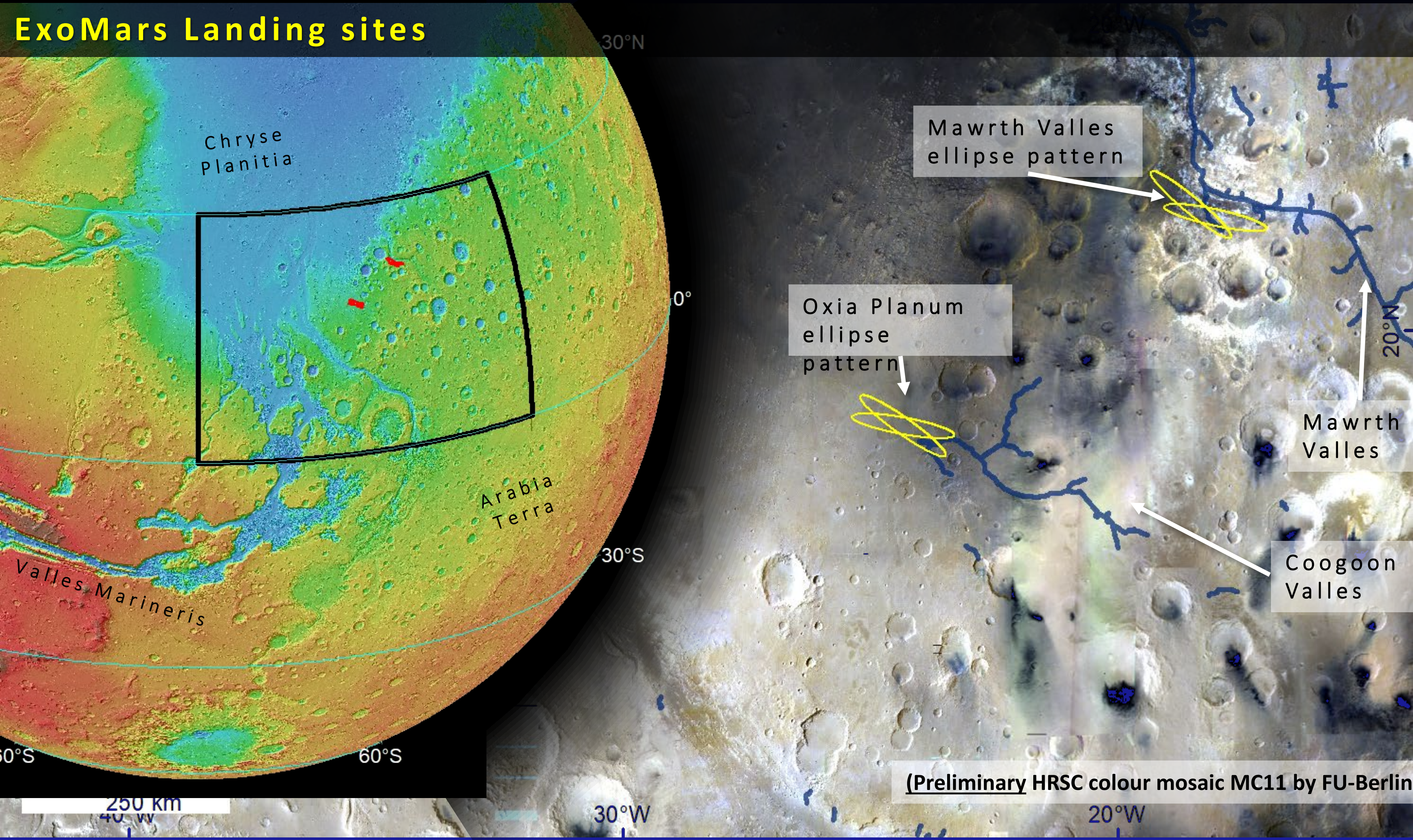
Will travel across the Mars surface to search for biosignatures in ~3.5 -4 BYA sediments / outcrops from Mars distant and warm past ….

→ collect 1-2 m subsurface samples with a drill.

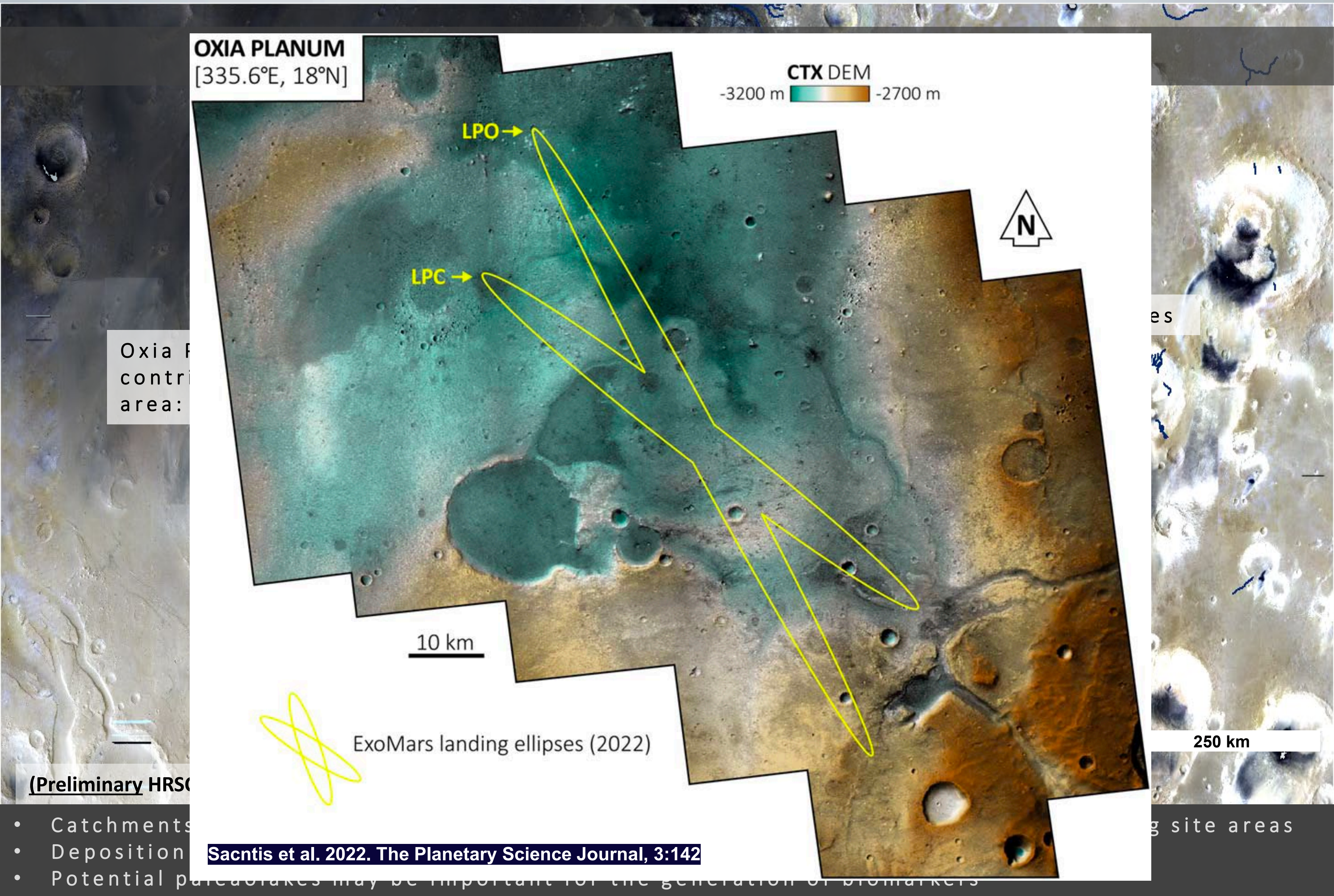
→ Strong Target for ….
Extant Microbial Life ?????
Past Microbial Life ?

→ analyse with next-generation life detection instruments

ExoMars 2028 landing site …. Oxia Planum … a 4 bya shallow sea basin?



Slides courtesy of P. Fawdon, M. Balmes, Open University, UK



Slides courtesy of P. Fawdon, M. Balmes, Open University, UK

Exomars 2022 Science Flow ….. Biosignature Targeting / Detection

PANCAM "BioCam" + ISEM
Step 1

- Geology, Geomorphology, Biosignatures, Mineralogy, geochemistry
- DRILL SITE SELECTION

CLUPI
Step 2.1

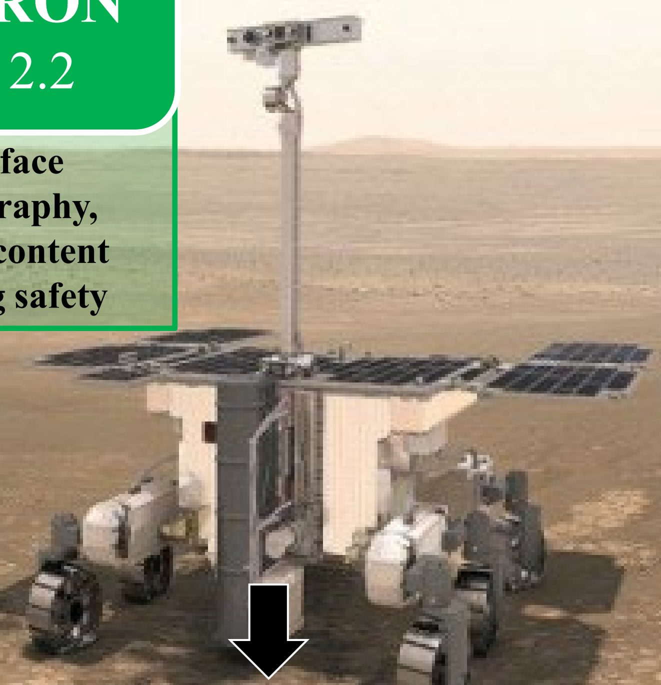
- Microimaging
  - Morphological biosignatures, mineral-replaced structures…

WISDOM + ADRON
Step 2.2

- subsurface stratigraphy, water content drilling safety

+

DRILL SITE SELECTION

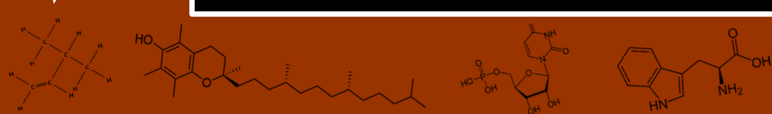


MaMISS
Step3

Imaging of subsurface
- Stratigraphy, water-related minerals…

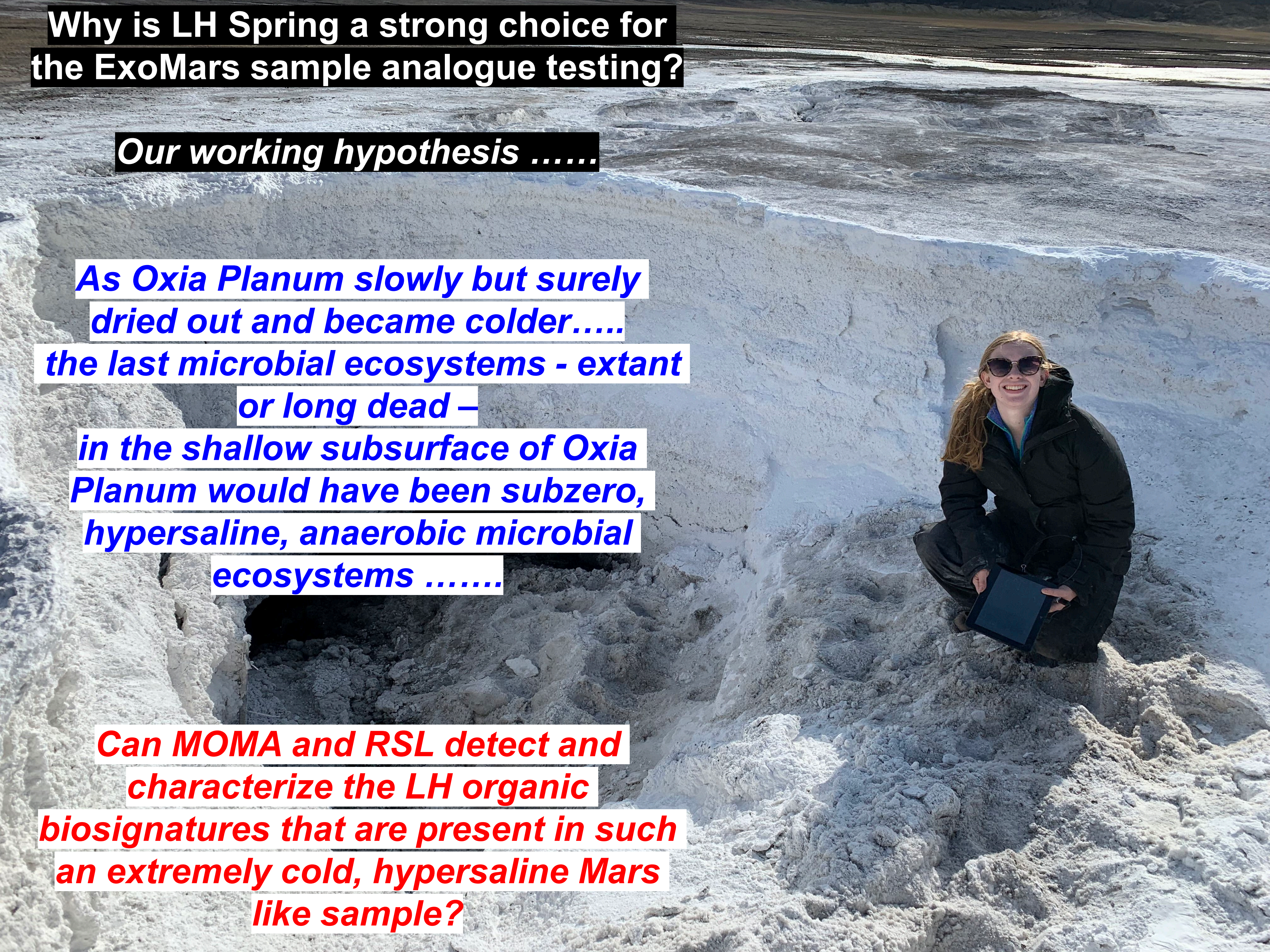
Samples

MOMA (org. C characterization)
RLS (org. C detection, mineralogy)
uOmega (mineralogy, geology)



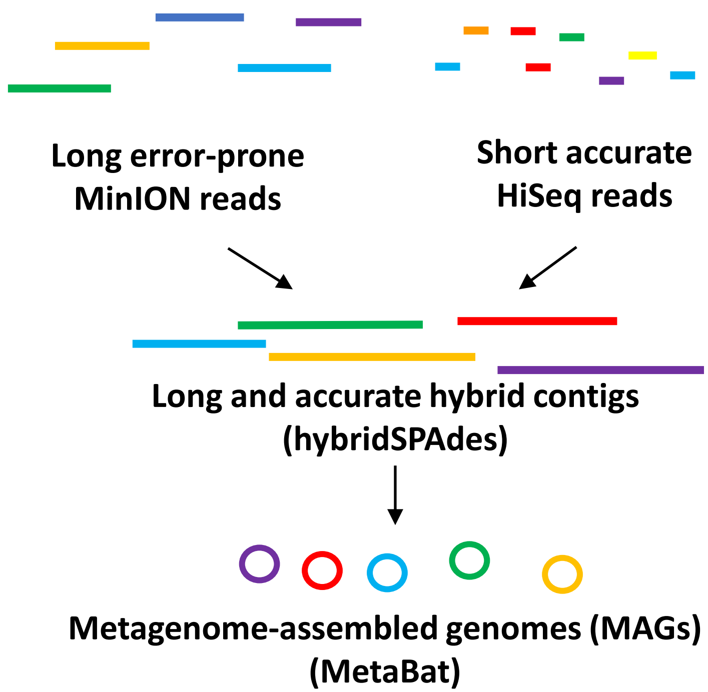
L. Whyte
A. Fairen
F. Westall
L. Preston
C. Freissinet

(Fernandez and Whyte unpublished)


Why is LH Spring a strong choice for the ExoMars sample analogue testing?
Our working hypothesis ……
As Oxia Planum slowly but surely dried out and became colder….. the last microbial ecosystems - extant or long dead – in the shallow subsurface of Oxia Planum would have been subzero, hypersaline, anaerobic microbial ecosystems …….
Can MOMA and RSL detect and characterize the LH organic biosignatures that are present in such an extremely cold, hypersaline Mars like sample?

Hybrid Illumina + MinION metagenome hybrid assemblies overcomes high error rates, produce longer (>1000 bp)contigs, more ultra-long (>50 000 bp) contigs, and more classified coding sequences.

Hybrid MAGs --> more complete, less contaminated, and have single contigs covering a higher proportion of the MAG.



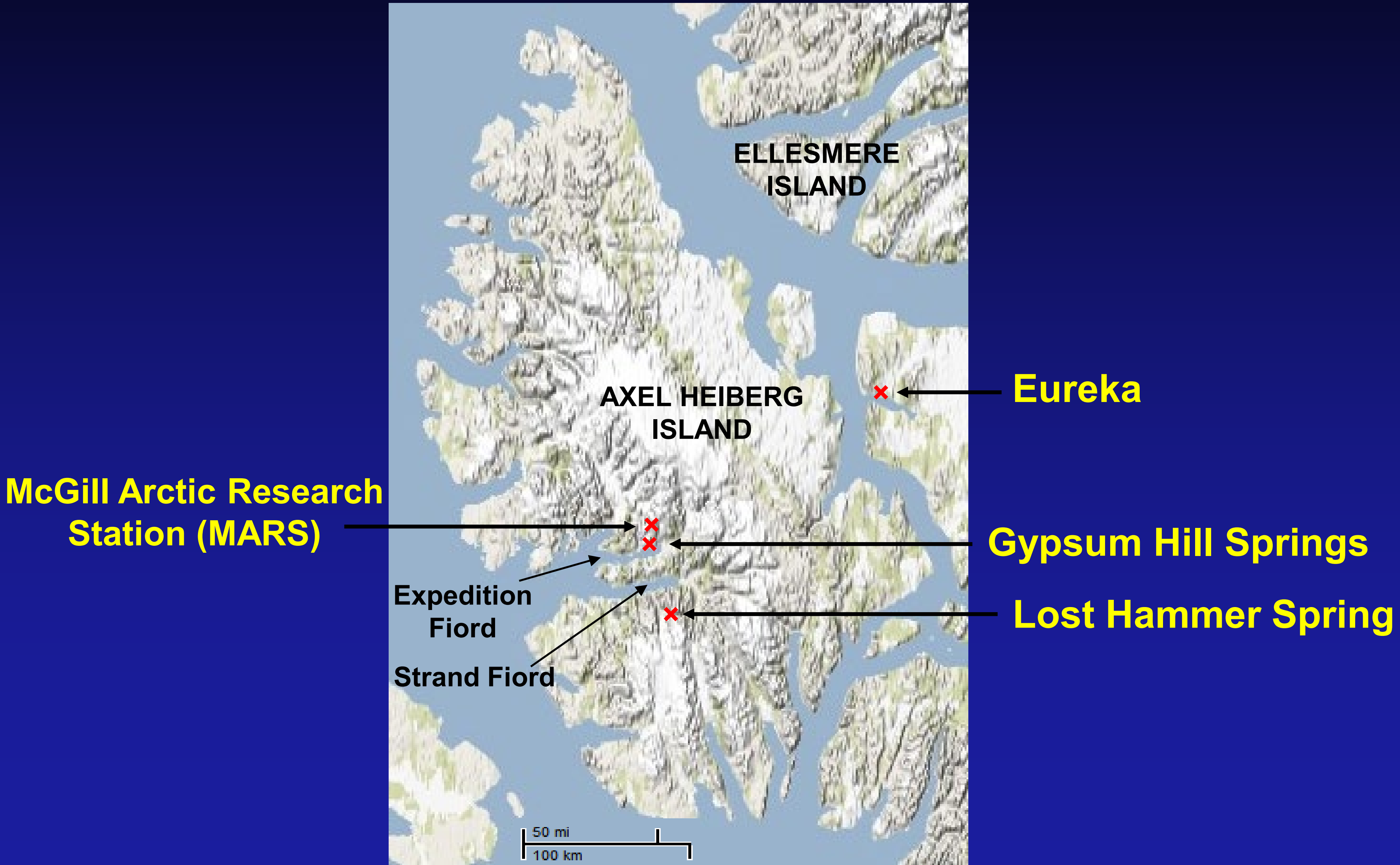
Hybrid assembly schematic.

Assembly type	Bin ID	Genome size (bp)	Longest contig (bp)	Mean contig length (bp)	N50	Completeness (%)	Contamination (%)	Taxonomy
HiSeq	**HiSeq_31**	**3 218 255**	**51 014**	**7926**	**9977**	**90.6**	**1.7**	**Octadecabacter sp.**
	HiSeq_14	3 193 410	34 424	5592	7860	80.2	3.4	Polaromonas sp.
	HiSeq_32	2 854 964	29 037	6781	3796	77.3	0.6	Pseudomonas sp.
	HiSeq_30	1 577 574	14 969	4612	5723	52.1	1.7	Nonlabens sp.
	HiSeq_8	1 930 594	28 420	5274	4775	51.7	3.4	Flavobacterium sp.
	HiSeq_19	1 677 201	32 650	5392	5599	51.3	1.7	Nonlabens sp.
Hybrid	**Hybrid_5**	**3 275 525**	**122 374**	**14 557**	**19 997**	**90.9**	**0.6**	**Octadecabacter sp.**
	Hybrid_20	3 024 995	47 365	9166	11 848	84.2	1.0	Pseudomonas sp.
	Hybrid_35	3 189 002	54 478	8032	10 334	79.5	1.4	Polaromonas sp.
	Hybrid_12	1 885 346	36 215	7141	11 449	64.0	1.6	Nonlabens sp.
	Hybrid_27	1 821 856	32 650	6093	19 324	56.3	0.6	Nonlabens sp.
	Hybrid_21	2 613 398	43 486	8799	6309	50.8	0.8	Flavobacterium sp.

Comparison of HiSeq-only vs. Hybrid MAG features.

Maggiori et al. 2021. Submitted.

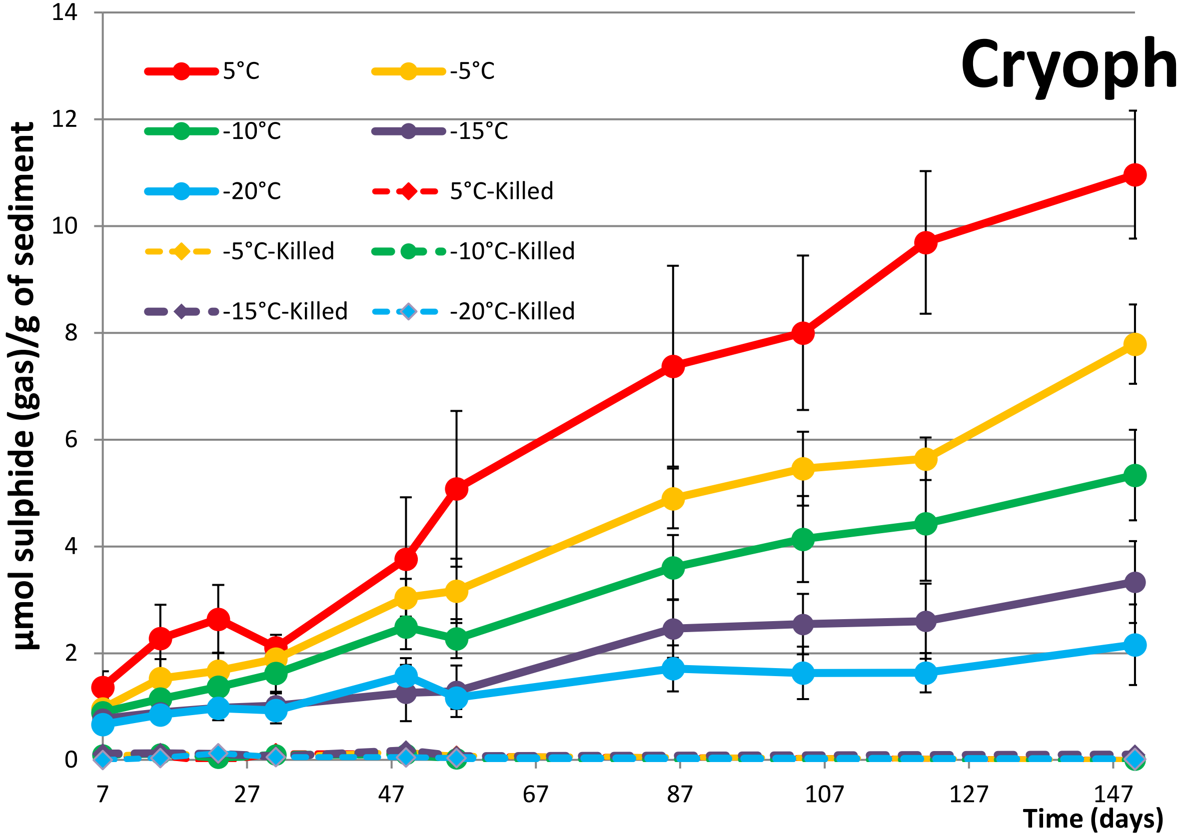
Axel Heiberg Island, Nunavut



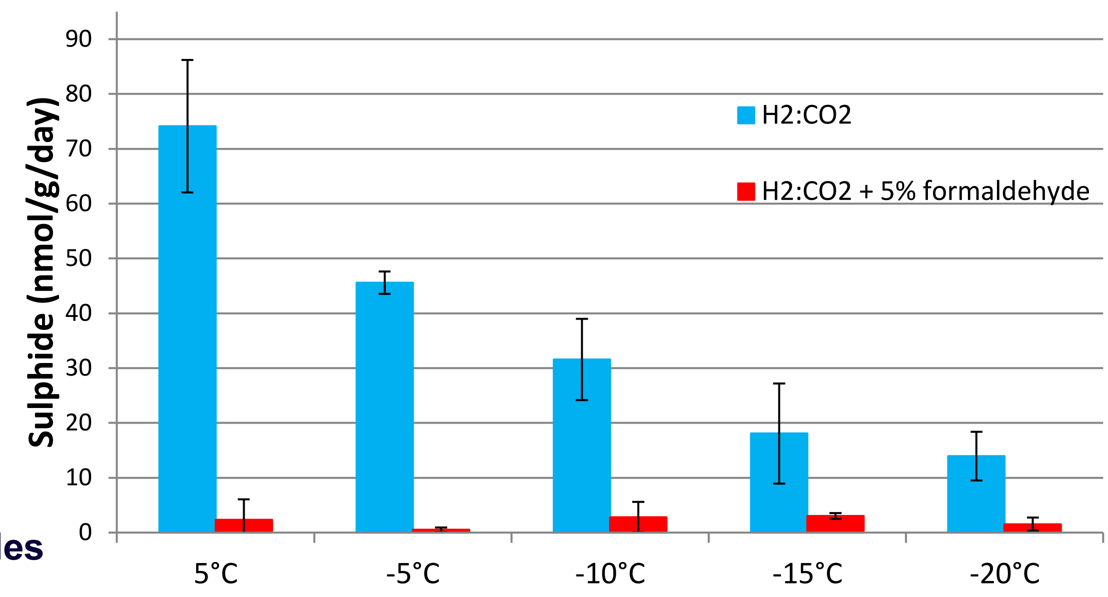
Cryophilic, hydrogenotrophic, sulphate reduction?

(sediment microcosms with $H_2:CO_2$ headspace)

→ Subzero anaerobic lithoautotrophy in a hypersaline environment?



Amendments/controls with $N_2:CO_2$ and acetate didn't yield any sulphide production.



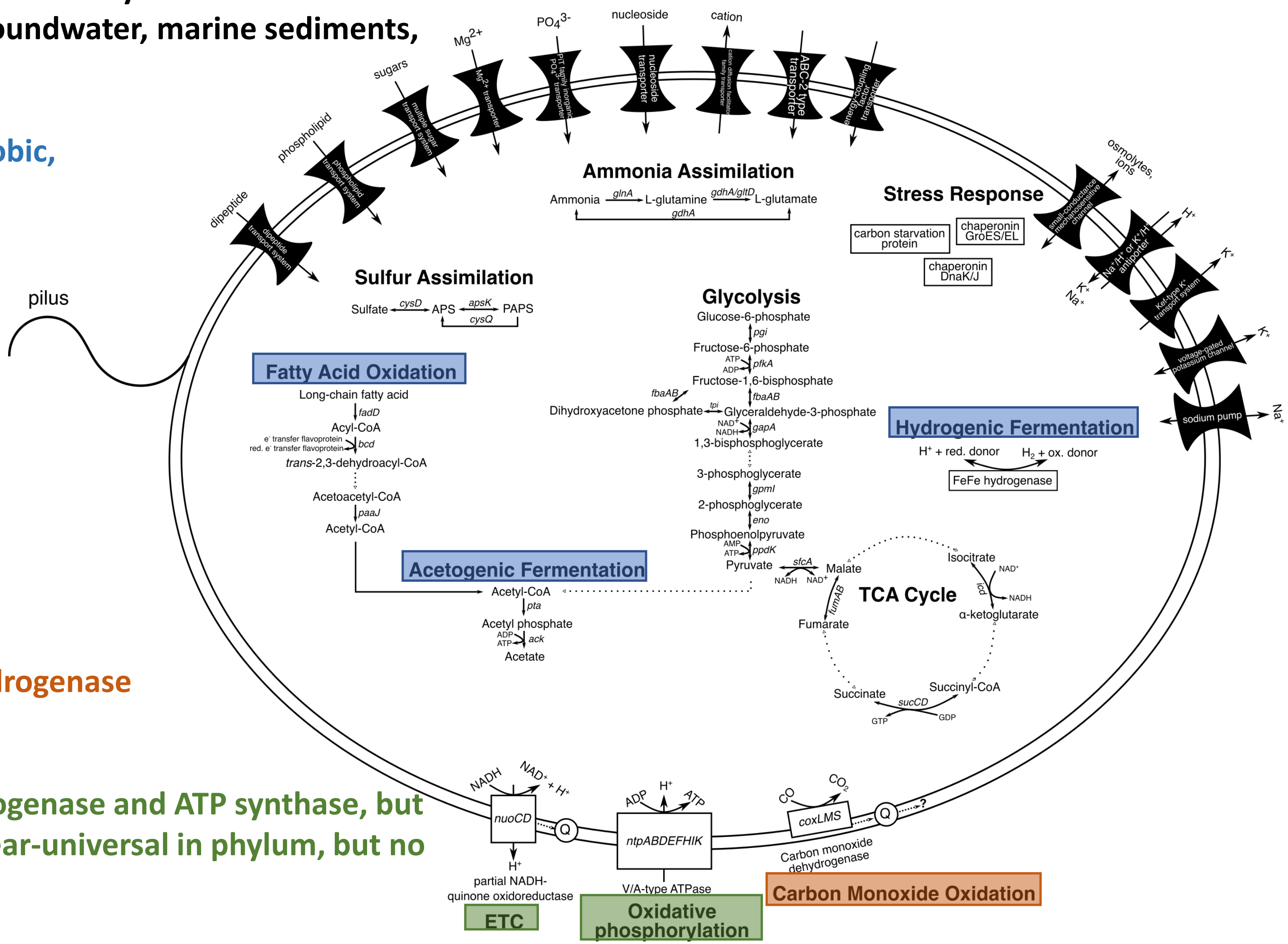
Unable to show methanogenesis or anaerobic methane oxidation under ambient conditions in LH sediments.

Lamarche Gagnon et al. 2015. Extremophiles

Newly-defined candidate phylum CG03 SAG

- 20 genomes (GTDB), previously classified as Elusimicrobia, from groundwater, marine sediments, hypersaline soda lakes

- heterotrophic, anaerobic, producing acetate and hydrogen as fermentation products

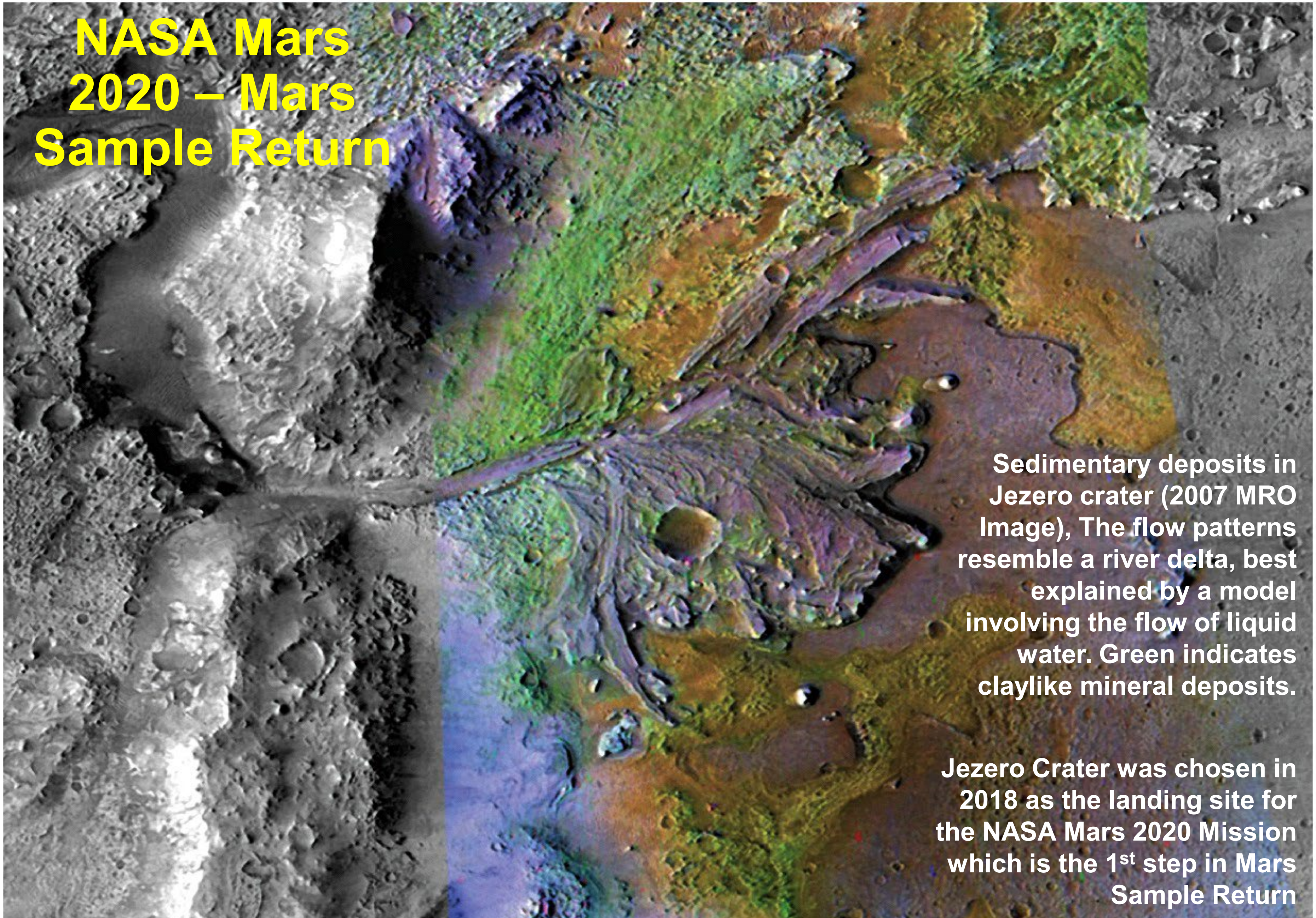


- homolog to CO dehydrogenase
  -> mixotrophy?

- partial NADH dehydrogenase and ATP synthase, but no other ETC genes (near-universal in phylum, but no clear functionality)

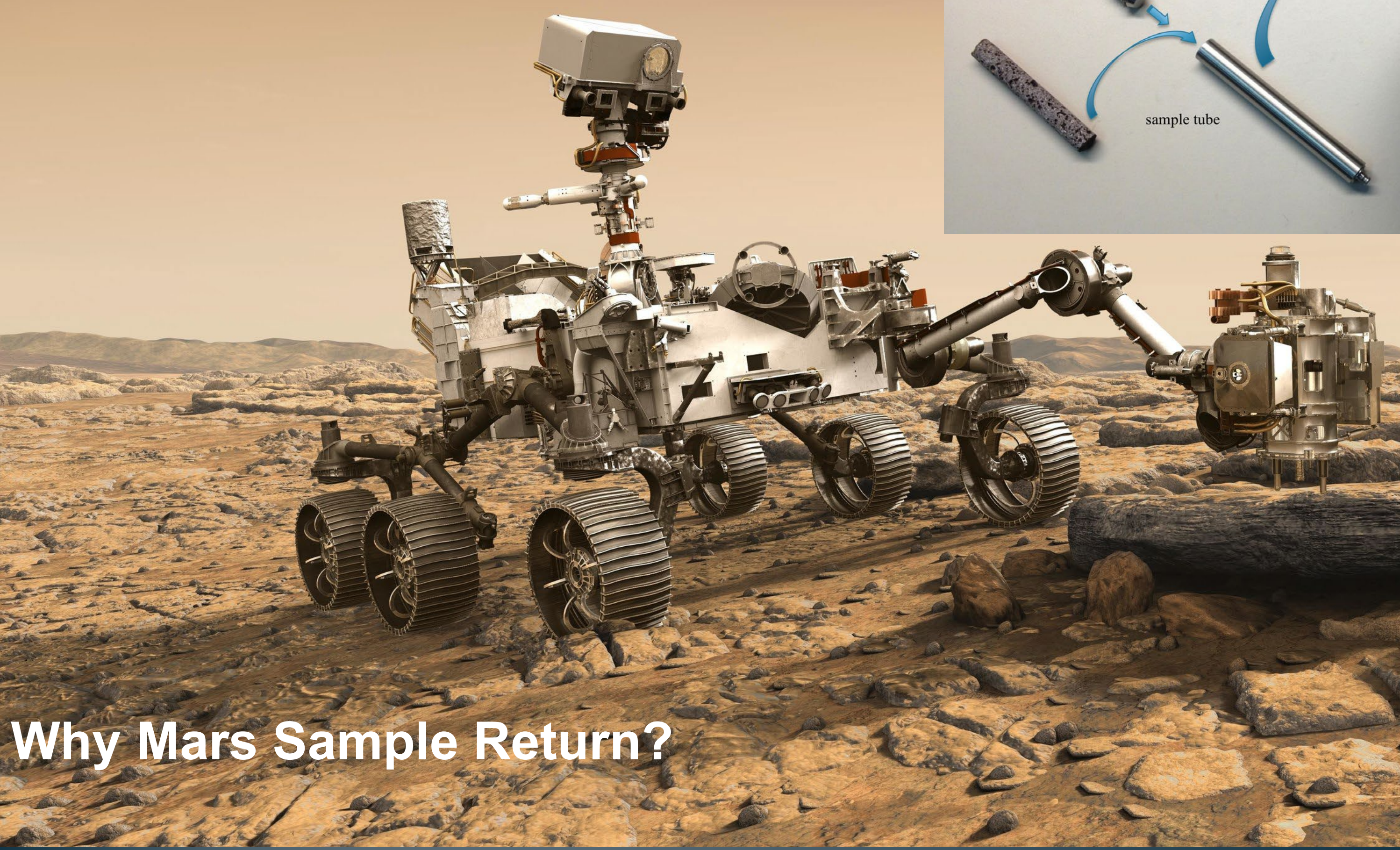
- also present in nearby Gypsum Hill spring (~7C, 8% salinity), overrepresented in metatranscriptome compared to metagenome (10x rel. abundance)

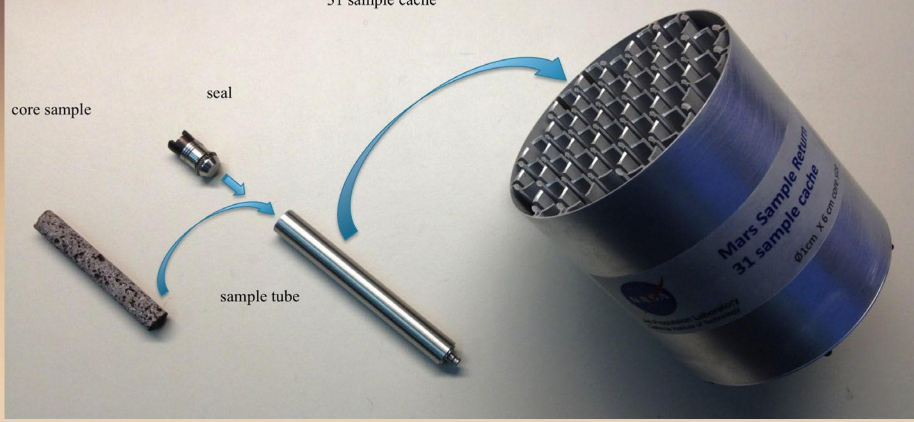
Evidence of past water on Mars



Microbiology: An Evolving Science, Third Edition Figure 22.23

Mars 2020 – 1st Step Towards Sample Return





Why Mars Sample Return?

- So many more (and more powerful) laboratories and instruments on Earth.
- Future instruments will be better – returned samples are the *gift that keeps on giving.*
- Some important analyses not possible (e.g., radiometric age dating)
- Signs of ancient microbial life may only be discovered by humans using a combination of instruments and techniques here on Earth. - The proof that life exists on Mars needs to be extraordinary.