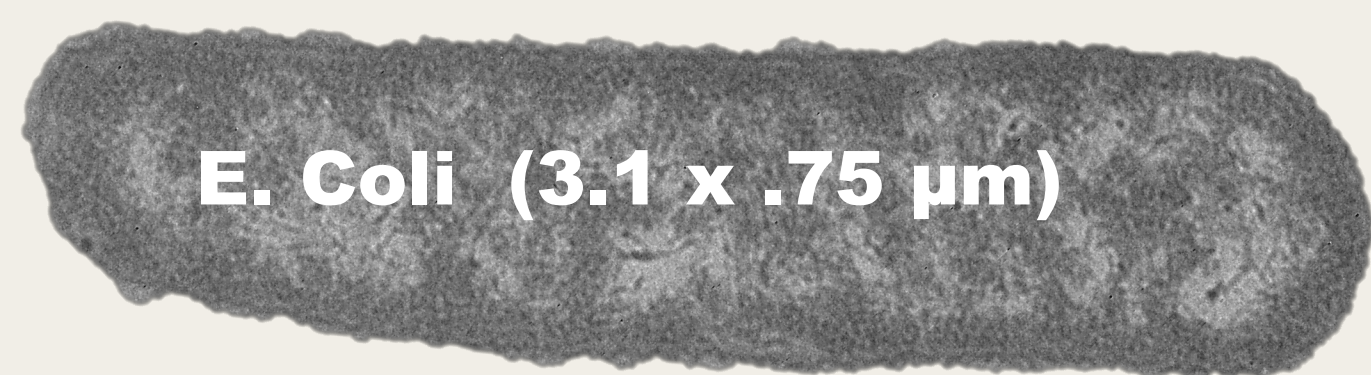
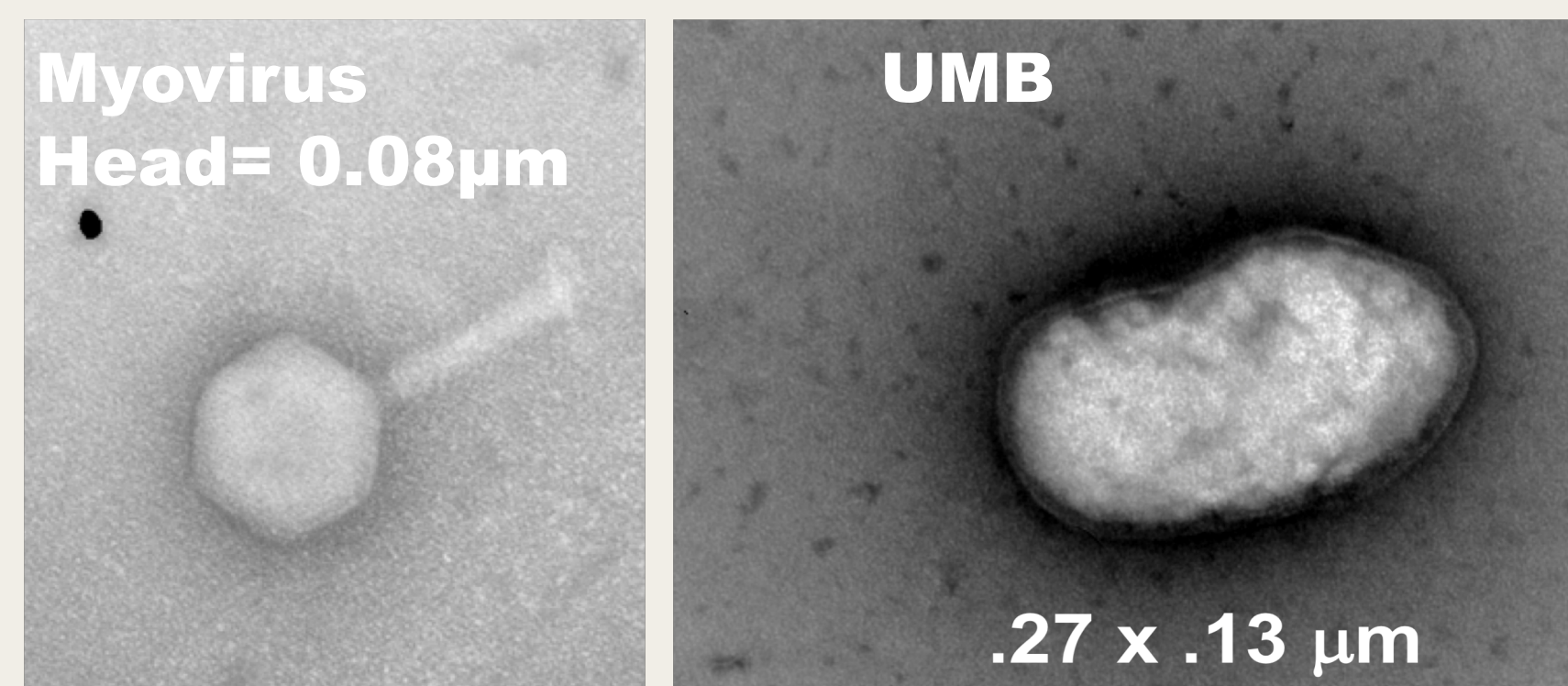


Ultramicrobacteria Genome Database Project

Abdullah Salim, Tien Tran, Andrew Putt and Terry Hazen

Introduction

Ultramicrobacteria are a largely uncultured and highly metabolically active group of globally abundant bacteria.



Transmission electron micrograph of a myovirus and candidate UMB from the Y-12 Complex, Oak Ridge, TENN. Image of E. Coli from Jon Marles-Wright for size

Importance:

- UMB have been confirmed to be an active and functioning group of microorganisms rather than just stressed cells [1].
- Recent research indicates that UMB aid in bioremediation and nutrient cycling, but future and current investigations of UMB are limited by:
 - a wide variety of nomenclature
 - a lack of databases containing functional, genomic, or spatial data specific to candidate UMB

Nomenclature commonly used for small bacteria		
Common Names	Definition	Size
Ultramicrobacteria UMB [1]	Bacteria that maintain a volume of $\leq 0.1 \mu\text{m}^3$, full genomes measuring 1 to 3.2 megabases.	$< 0.3 \mu\text{m}$ $\geq 0.1 \mu\text{m}$
Ultrasmall Bacteria USB [2]	Bacteria with an average volume of $0.009 \mu\text{m}^3$, tightly spiraled DNA, and incomplete genomes ~ 0.9 megabases.	$< 0.2 \mu\text{m}$ $\geq 0.1 \mu\text{m}$
Nanobacteria [1]	Uncultured and often unsubstantiated ranging from 0.01 to $0.1 \mu\text{m}$.	$< 0.1 \mu\text{m}$
Mycoplasma [3]	Cell-wall lacking pathogenic bacteria	$< 0.1 \mu\text{m}$

Questions:

A.) Can we create a database of environmental UMB using current UMB genomic, spatial, and geochemical data?

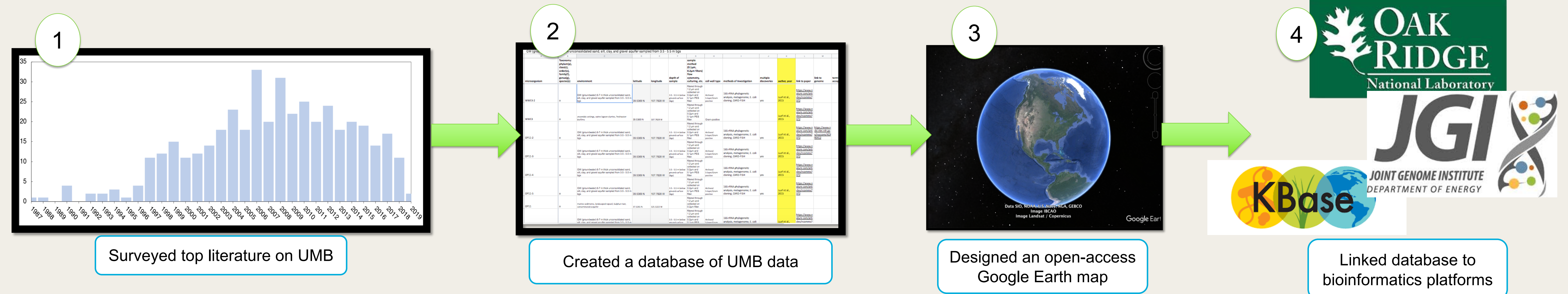
B.) Can we use this database to distinguish differences among environmental and parasitic UMB given current UMB data?

Hypotheses:

A. We can create the first publicly accessible database to identify UMB from several environmental locations.

B.) We can identify differences in UMB community composition across environments and perform genomic comparisons.

Methods:

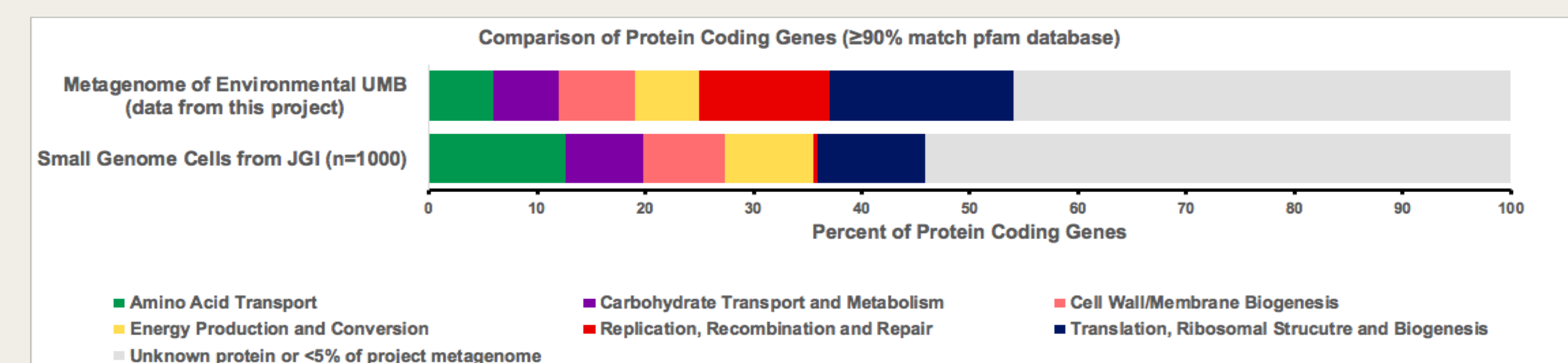


Results:

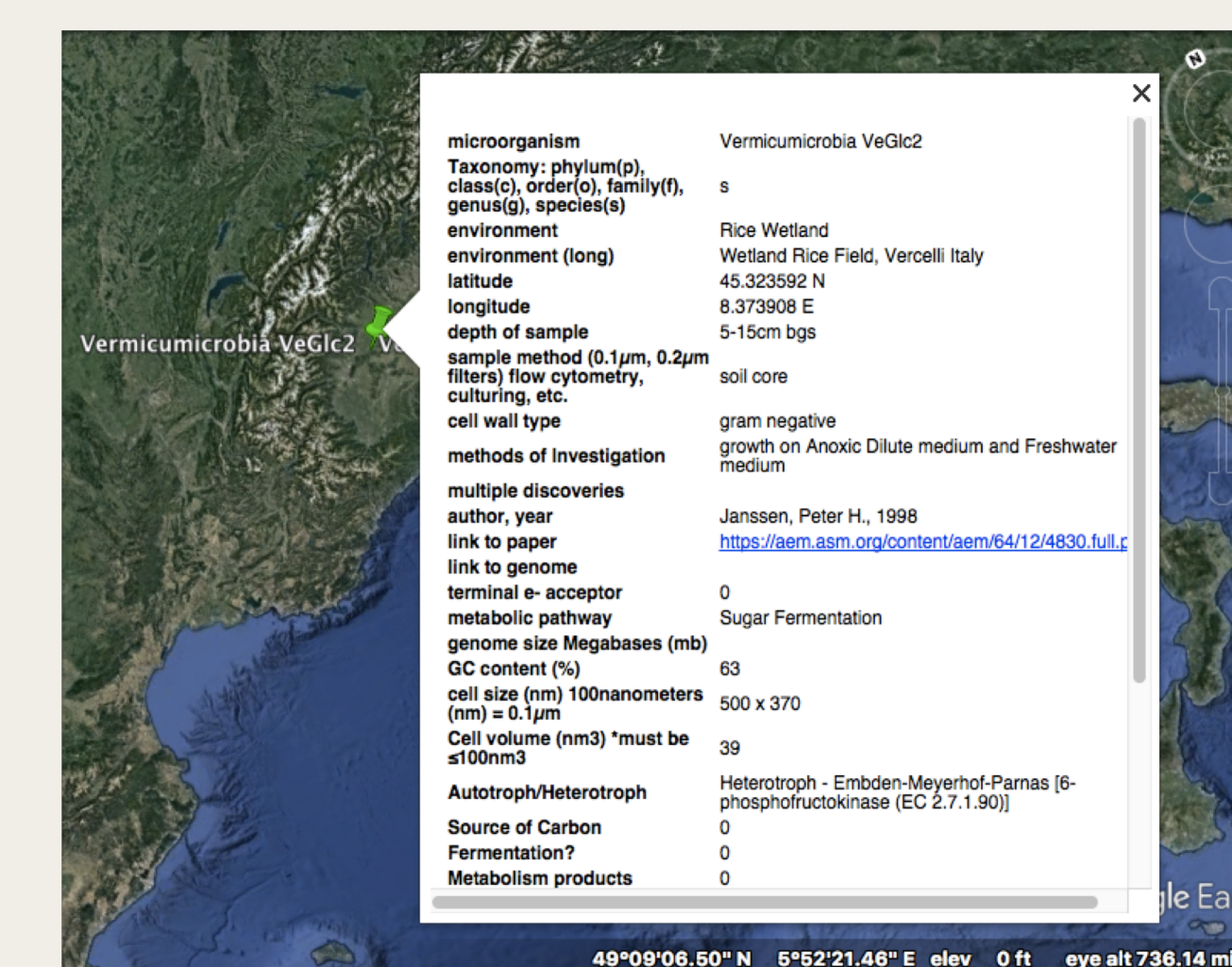


Image of the open-access Google Earth map linked to this database to perform rapid investigations of environments and organisms of interest (screen shot grabbed on 10 April 2019).

Three Prominent Orders of UMB		
Order	Environments	Notable Characteristics
OD1	anoxic groundwater samples, marine sediments, landscaped topsoil, Sulphur river, cave sediments	OD1 are microorganisms that possess both fermentative and respiratory energy pathways, can metabolize nitrogen and fatty acids.
WWE3	anaerobic settings, swine lagoon slurries, freshwater biofilms	WWE3 are oval-shaped and are likely related to nutrient cycling in wastewater sludge. WWE3 form a distinct monophyletic group deeply branching apart from all known bacterial divisions.
OP11	marine sediments, landscaped topsoil, Sulphur river, and contaminated aquifer	Common in reduced environments containing sulfur compounds such as sulfates and sulfides in marine environments, and within contaminated aquifers.



Comparison of Protein Coding Genes compared between metagenome of environmental UMB vs small genome bacteria identified on JGI using pfam library (>90% protein match cutoff).



This is the organism information populated from this database when a researcher clicks on a Google Earth map marker (screen shot grabbed on 10 April 2019).

Conclusions

- OD1, WWE3, and OP11 were commonly identified among the UMB orders we collected data for and analyzed in our database.
- Previously identified UMB candidates like RB2256 were nullified as being UMB in this investigation based on criteria for UMB classification.
- Genomic data in the UMB database was compared to small genome bacterial data (most of these cells being classified as parasitic) in JGI from which we identified more proteins for replication and translation from environmental UMB than small genome JGI entries.
- This database was integrated into a Kbase narrative for future genome investigations.

Future Work

- This database will serve as a master resource that can be built on and used to address the driving factors for small genomes, similarities in microbial community structure, and variations of UMB across environments.

REFERENCES

[1] Luef B, Frischkorn KR, Wrighton KC, Holman HYN, Birarda G, Thomas BC, Singh A, Williams KH, Siegerist CE, Tringe SG, et al.: Diverse uncultivated ultra-small bacterial cells in groundwater. *Nature Communications* 2015, 6. [2] Cavicchioli R, Ostrowski M: Ultramicrobacteria. *eLS* 2003. [3] Duda VI, Suzina NE, Polivtseva VN, Boronin AM: Ultramicrobacteria: Formation of the concept and contribution of ultramicrobacteria to biology. *Microbiology* 2012, 81:379-390. [4] Morowitz HJ, Wallace DC: Genome Size and Life-Cycle of Mycoplasma. *Annals of the New York Academy of Sciences* 1973, 225:62-73.

ACKNOWLEDGEMENTS

- ❖ Oak Ridge National Lab
- ❖ KBASE (<https://kbase.us/>)
- ❖ Lawrence Berkely National Lab
- ❖ JGI (<https://jgi.doe.gov/>)