Informing models of life in Ocean World brines: lessons from active microbial life in briny waters of Lake Vida at -13C

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OVERVIEW:
Cold Adaptation in Antarctic microbes

In the last 25 years NASA Cassini and Galileo missions have discovered **Ocean Worlds** in the outer solar system, with cold, salty liquid oceans beneath ice crusts. Studying analogue environments on Earth and the life that persists there is crucial to our **search for life** in Ocean Worlds of the outer solar system, specifically Jupiter's moon, Europa and Saturn's moon, Enceladus, which are extremely promising for life outside Earth. Microorganisms in the subglacial Lake Vida are active, even at constant cold and high salt conditions. In this study we **mined the literature** to find specific protein families (PFAMs) linked to cold adaptation. Seven **metagenome assembled genomes (MAGs)** from Lake Vida were annotated with MetaERG and compared to the **cold associated PFAMs**. Our findings suggest that different microorganisms might employ different strategies to survive extreme cold. Further analysis will include metatranscriptome data.

INTRODUCTION:
Lake Vida as an Ocean World Analog
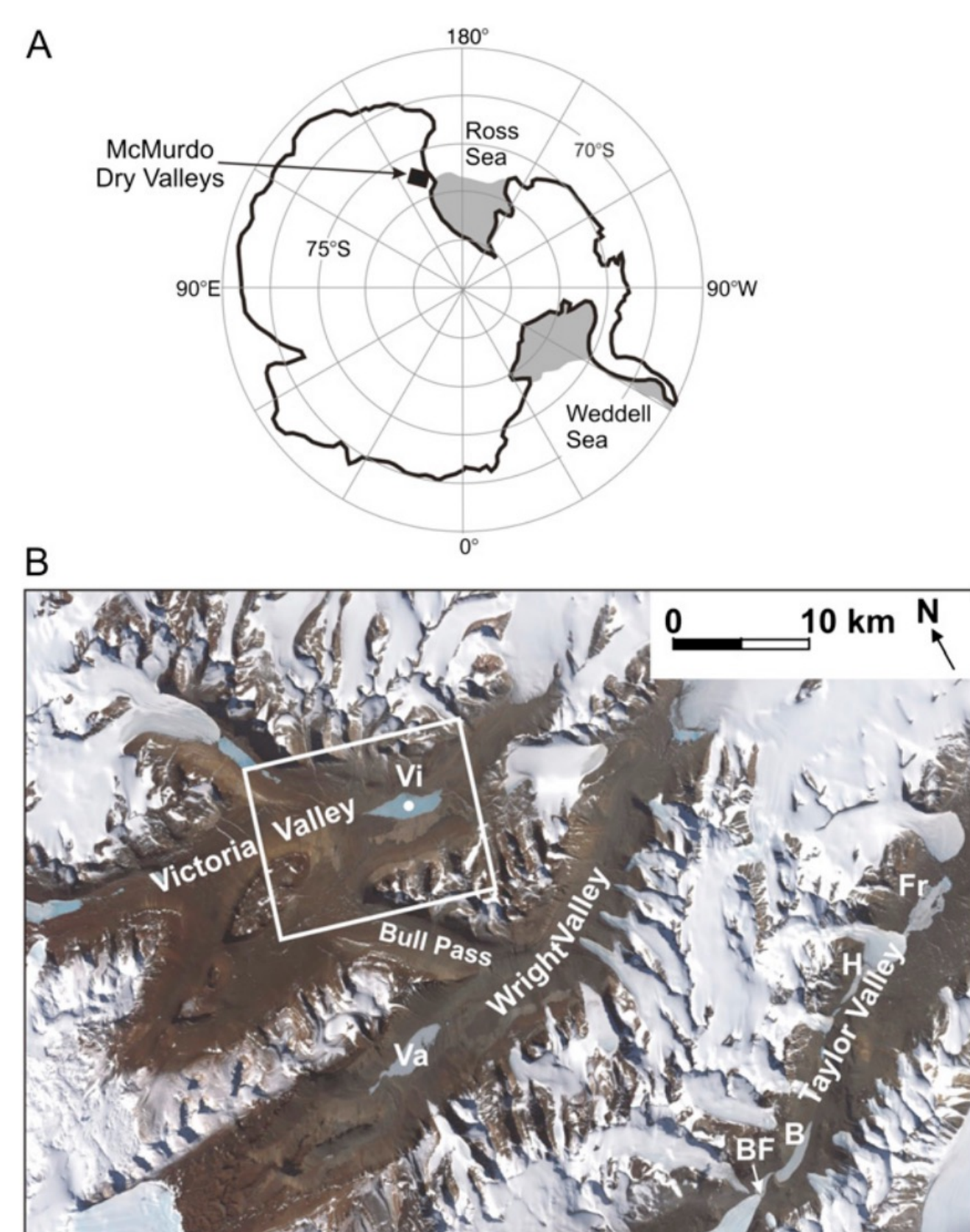
Figure 1. Map of Lake Vida, Antarctica (Murray et al., 2012)

Lake Vida is located in the Victoria Valley of the McMurdo Dry Valleys, Antarctica (Fig. 1). **The environment** of this dark, isolated subglacial lake is a highly anoxic, slightly acidic brine, containing C, H, N, Fe, and S, beneath 27+ m of ice, with temperatures around -13.4 C. A diverse **microbial community** exists and has been shown to be active, producing low levels of proteins in situ (Murray et al., 2012).

The ice-covered **Lake Vida is analogous to Ocean Worlds**, such as Europa and Enceladus (Fig.2). Specific adaptations to cold can be found in protein families linked to structural or metabolic changes. A single microbial genome may use one or more cold adaptation strategies. Understanding the cold adaptations of microorganisms in Lake Vida will **inform future NASA missions** to Ocean Worlds.
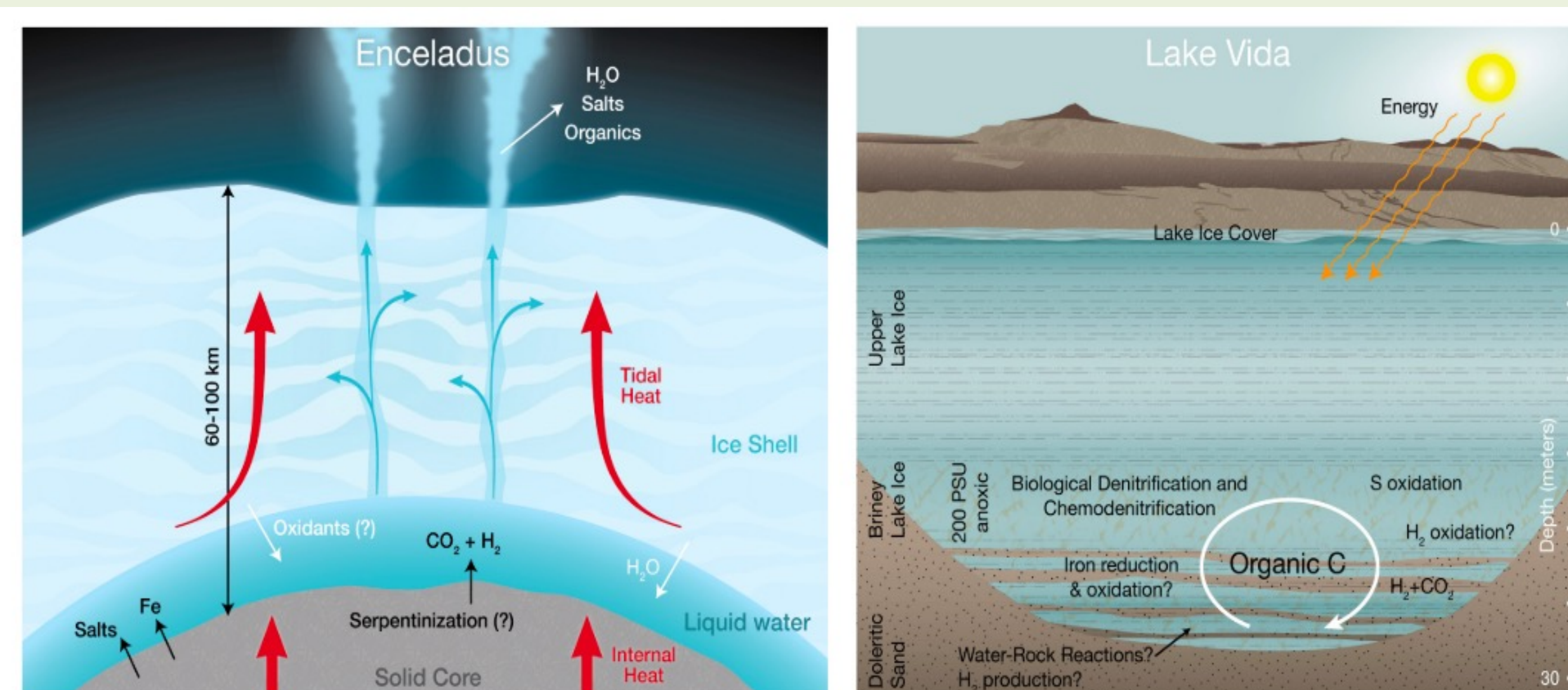
Figure 2. Schematic of Enceladus ice shell and ice capped Lake Vida

METHODS:
Literature mining, sample sequencing, bioinformatic analyses
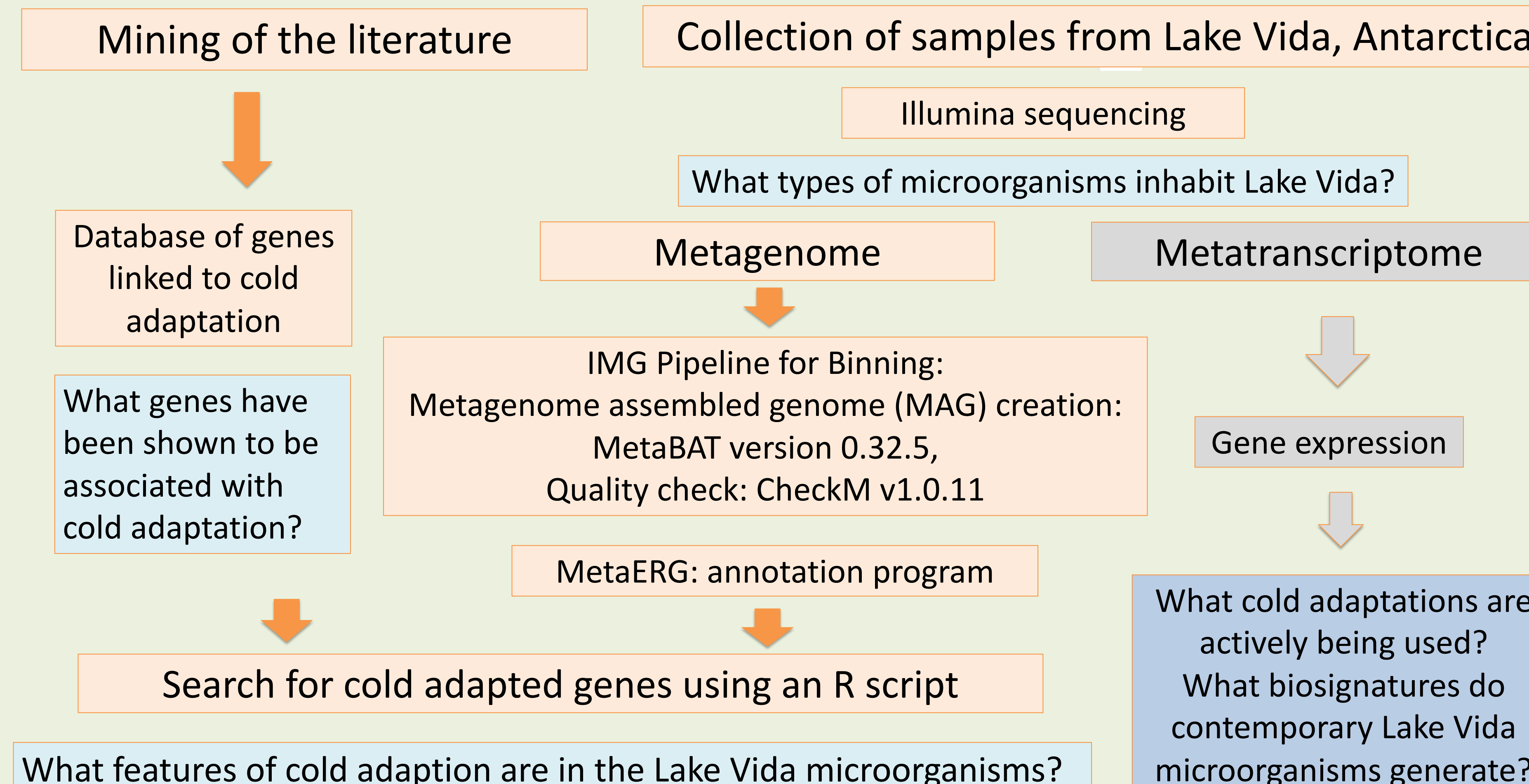
PRELIMINARY RESULTS:
A biodiverse community of organisms

What organisms are present? A diverse community of microorganisms was identified through varying SSU rRNA gene datasets. Although slight differences in the community composition exist, Pseudomonodales and Flavobacteriales are abundant in all, with many other overlaps (Fig. 3).
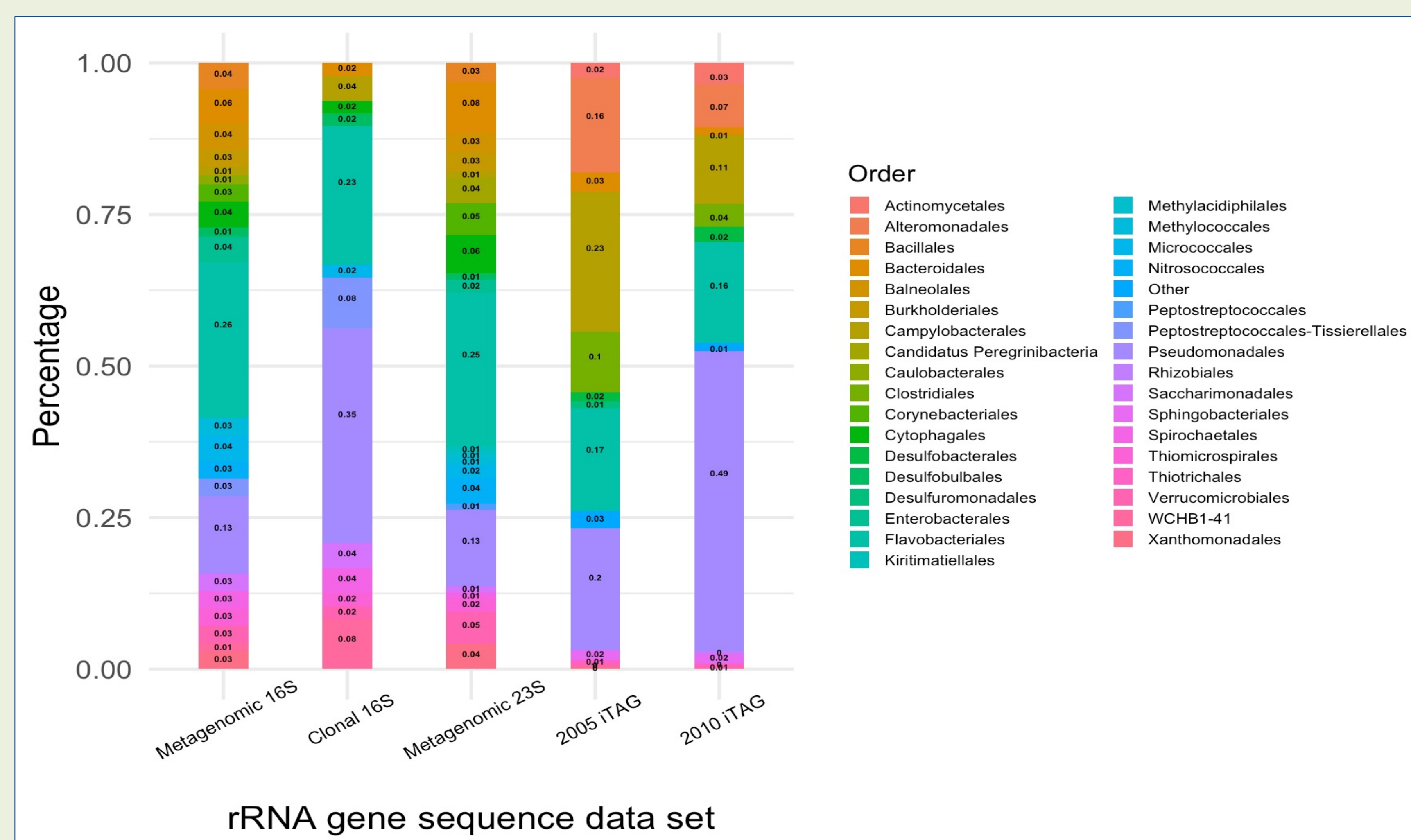
Figure 3. Stacked Bar Graph of the organisms at the Order level found in the Lake Vida Brine, as distinguished by 16S, Clonal 16S, 23S and 2005 and 2006 iTAG data.

A cold adaptation database was created from mining the literature. **346** putative cold adapted protein families were identified, linked to a variety of processes, from protein-folding chaperones to lipid biosynthesis to compatible solute synthesis. An R script was written to search for these cold adapted PFAMs in the annotated MAGs.

PRELIMINARY RESULTS:
Cold adapted PFAMs in 7 MAGs

The seven MAGs, with a variety of taxonomic identities (Table 1), all contain cold adapted PFAMs. The number of total hits, ratios of those that are most prevalent, and percentage of cold adapted PFAMs found in the MAGs out of all identified cold adapted PFAMS in the database vary between organisms (Table 1).

Table 1. Cold adapted PFAMs within seven MAGs annotated by MetaERG from Lake Vida, Antarctica

MAG Number	Class	total PFAM hits	% cold adapted PFAMs found
3	Kiritimatiellae	422	0.38
5	Gammaproteobacteria	488	0.51
6	Actinobacteria	624	0.52
7	Spriochaetia	646	0.55
8	Campylobacteria	24	0.06
10	Actinobacteria	322	0.42
15	Saccharimonadia	115	0.19

The presence of cold adapted PFAMs in all seven MAGs show the potential for cold adaptation in the Lake Vida brine microorganisms. The differences in total hits and PFAM ratios found in each MAG indicate that the organisms are likely utilizing different cold adaptive strategies to survive the extremes of Lake Vida.

CONCLUSION:
Future Work

SSU rRNA gene surveys using different sequencing strategies show **minor differences in community composition** perhaps due to amplification biases. All seven MAGs analyzed showed putative cold adaptation PFAMs, with variation in depth of the hits, suggesting that **microorganisms can employ a variety of cold adaptation strategies**. Future work will include analyses of cold adaptation genes using COG and EC numbers (enzymes), as well as investigating the **metatranscriptome** for expression of these genes mapped to the rRNA gene and MAG sequences. Attention will also be given to the types of biosignatures that contemporary Lake Vida microorganisms generate.

REFERENCES:

Murray, A. E., Kenig, F., Fritsen, C. H., McKay, C. P., Cawley, K. M., Edwards, R., et al. (2012). Microbial life at -13 C in the brine of an ice-sealed Antarctic lake. *Proc. Natl. Acad. Sci.* 109, 20626–20631. doi:10.1073/pnas.1208607109.