# NASA Astrobiology Early Career Collaboration Award Report

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**Research description**

Explorations of historical records of methane on early Earth at the intersection of biogeochemistry and astrobiology are essential. This study is a comprehensive investigation into the timing, underlying mechanisms, and extent of methane accumulation in Earth's primordial atmosphere. The potential for methane production from both biotic and abiotic sources introduces an essential layer of complexity that lies at the heart of methane's utility as a potential biosignature elsewhere in the solar system and beyond. Among early microorganisms, methane-cycling microbes hold particular significance due to their potential role in planetary evolution. Microbial methanogenesis and methanotrophy (methane oxidation) processes appear to have played prominent roles in both the sources and sinks of methane during the Archaean eon. Nevertheless, it is equally crucial to consider plausible abiotic origins and the influence of photochemical reactions on the levels and temporal variations of atmospheric methane.

Remarkably, despite substantial research dedicated to unraveling the unknowns about Archean methane cycling, there has been a notable gap in studies that address potential scenarios of relatively small or highly variable biotic methane accumulation in the atmosphere. Various factors may contribute to such low levels of methane accumulation, including the nutritional prerequisites of methanogens and methanotrophs, notably the influence of trace metal deficiencies. Additionally, the timing and variability of oxygen input into the anaerobic biosphere and the emergence of aerobic and anaerobic methanotrophic pathways are critical factors that warrant exploration in this context.

While geological records offer valuable insights into the study of early Earth conditions and the evolution of life, their varying reliability and susceptibility to alteration over time introduce many uncertainties about early microbial evolution. For instance, studying the evolution of microbial methanogenesis and methanotrophy, especially within a planetary framework across extensive timescales, presents distinct challenges, primarily due to the conspicuous absence of discernible morphological characteristics or unequivocal organic and inorganic markers that can be attributed to these ancient microbial processes, although studies focused on sulfur cycling in the same oceans and carbon isotope fingerprints of methane oxidation give us some independent but indirect crosschecks.

In this research project, our primary objective has been to identify the probable ancestral pathway enzymes involved in methane cycling using available genomics data as a potentially powerful but underutilized way to study the evolution of these environmentally important microbes. Our ultimate goal is to reconstruct the evolutionary relationships, emergence timelines, and the natural history of the main groups of methanogens and methanotrophs in a planetary context and from that combination discern the origins and the full controls and consequences of their ecological proliferations.

**Visit to MIT**

The NASA Early Career Collaboration Award enabled me to advance my research in new directions through a collaborative visit to MIT. From July to August 2023, I had the opportunity to visit Prof. Greg Fournier's lab, which provided invaluable insights into deep-time phylogenomic studies within a planetary context. During this visit, I worked closely with Prof. Fournier and his group.

My work there initiated with some training sessions focused on their most recent phylogenetics pipeline. These skills in molecular phylogenetics are essential for the completion of my PhD thesis. Additionally, I gained access to their computing clusters, which allows me to conduct my genomics analysis and molecular clock calculations more efficiently. In sum, this highly successful visit facilitated a novel combined approach to studies of early methane cycling well suited to my background in biology and genomics. I feel very fortunate to be working in a unique research space made possible by the collaborative bridging of geological and biological expertise facilitated by this award.

**Figure 1:** One of our coding training sessions at Fournier Lab, MIT. Jack Payette, Chris Parsons, and I are working on the Python code that assists us in running the molecular clock based on Prof. Fournier's pipeline.

**Outcomes of this collaboration**

During my two months at MIT, in addition to the training and coding sessions, I initiated the generation of datasets for amino acid sequences in methanogens and methanotrophs, specifically targeting the alpha subunits of methyl-coenzyme M reductase (MCR) and particulate methane monooxygenase (pMMO) enzymes. The purpose of creating these datasets is to utilize available sequences to construct maximum likelihood and Bayesian phylogenetic trees, which illustrate the evolutionary relationships among various groups of methane-cycling microbes. These trees will serve as the foundation for our molecular clock analysis.

Throughout my time at MIT, I successfully reconstructed several gene and species trees for these microbes. I had the opportunity to discuss these results with fellow lab members and Prof. Fournier, contributing to the development of our research methodology and strengthening our collaboration. Moreover, I made preparations for the second phase of the project, building upon the insights gained from our initial analysis. Interestingly, we have identified the opportunity to calibrate four specific nodes in our pMMO tree, which are congruent with nodes found in previously published papers. These calibrations have led to the determination of four well-established age estimates for those groups of methanotrophs within our tree. This could be a game-changing result.

Our ongoing research will focus primarily on running the molecular clocks, a time-consuming aspect of the project due to the demanding computational requirements. Subsequent efforts will center on correlating these results to independent geochemical/environmental constraints, which are a specialty of the Lyons group, thus allowing a comprehensive view of methane's early relationships to climate evolution, habitability, and biosignature potential.

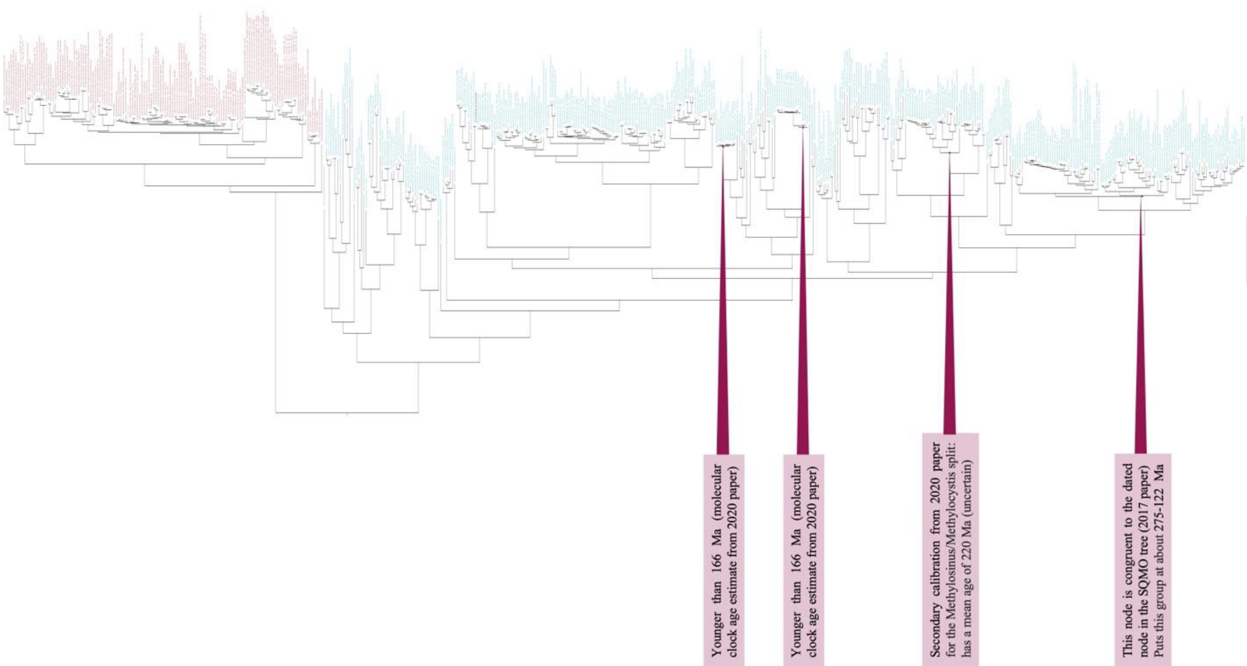


**Figure 2:** Reconstructed phylogenetic tree for the alpha subunit of the pMMO enzyme. Phylogenetic relationships were inferred using Maximum Likelihood (ML) with iqtree2. Bootstrap analysis for the ML tree was conducted with 1000 bootstrap replicates. Four calibration points for specific bacterial groups in the pMMO tree are identified and highlighted in purple.

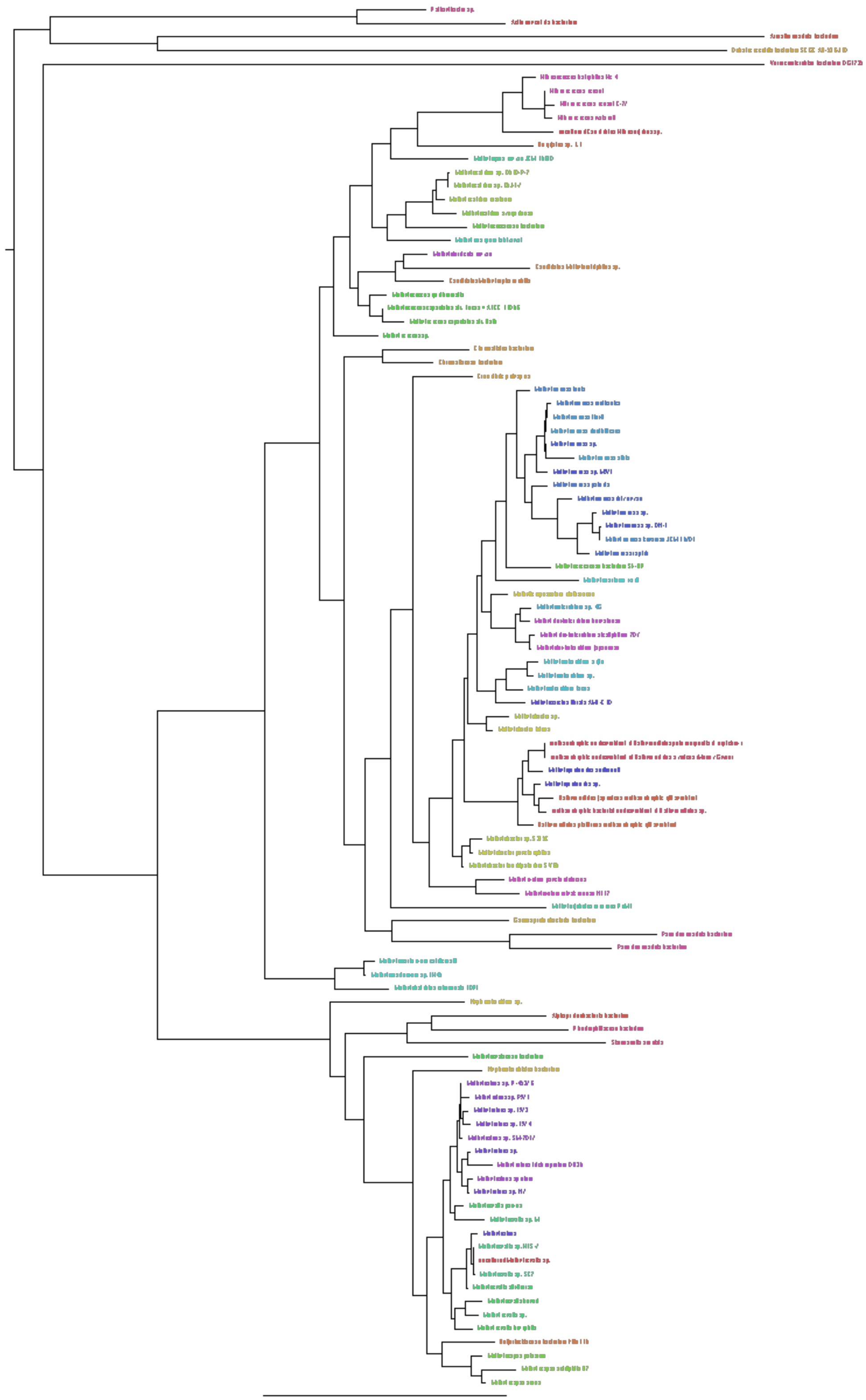

**Figure 3:** Reconstructed phylogenetic tree based on the 16S ribosomal RNA for the bacterial group on the pMMO tree. Phylogenetic relationships were inferred using Maximum Likelihood (ML) with iqtree2. Bootstrap analysis for the ML tree was performed with 1000 bootstrap replicates.

**Thanks to the NASA Astrobiology Program**

I am deeply grateful for the funding provided by the NASA Astrobiology Early Career Collaboration Award, which has supported this project and provided the opportunity for the collaborative research described above. This award facilitated a valuable learning experience that enabled me to initiate the computational aspect of this project, which might not have been possible otherwise.

Prof. Fournier's expertise in phylogenomic, molecular clock analysis, and identifying Horizontal Gene Transfer (HGT) events in early microbial communities made it easy to explore genomic databases and conduct phylogenomic analyses with high accuracy. This collaboration has also opened the door to future work, such as investigating the deep history of early oxygen-producing bacteria, which had a significant impact on shaping the early Earth's conditions and could have implications in astrobiology and biomarker detection on potentially oxic worlds beyond Earth. In other words, doors have opened with potential for career-long impact.

Importantly, the outcomes may also challenge long-held views on early methane on Earth, which were developed without our wider scope of data, approaches, and interdisciplinarity. We are eagerly looking forward to sharing the final results with the astrobiology community in the next few months through presentations and publications. Once again, I would like to express my sincere thanks to the NASA Astrobiology Program for providing this exceptional opportunity.