

## **Project Report**

Cyanobacteria are key to understanding the evolution of the biosphere and the atmosphere. They are the only phylum that evolved the capability to carry out oxygenic photosynthesis, and the evolution of this pathway within Cyanobacteria is closely linked to the rise of oxygen on Earth. These organisms also have one of the oldest fossil records, with evidence for cyanobacteria in the form of stromatolites and body fossils of filamentous and coccoidal cells throughout the Proterozoic and possibly the Archean. However, the paucity of fossil evidence from Earth's early history makes it difficult to determine exactly when Cyanobacteria first evolved. For this reason, we use an approach that combines fossil evidence with modern molecular sequences to construct time calibrated cyanobacterial phylogenetic trees.

The goal of my research is to test and use molecular clock analyses to constrain the evolutionary timeline of Cyanobacteria, the timeline of the evolution of various cyanobacterial lineages, and the history of Earth's oxygenation. To do this, we need two key pieces of information: 1) ancient cyanobacterial fossils with which we can calibrate our models, and 2) the genetic sequences of modern analogs to those ancient fossils so that we can place fossil constraints on a specific node within our phylogenetic trees. One of the most ancient well-supported cyanobacterial fossils is the fossil *Eoentophysalis*, a ~2.0 Ga coccoidal fossil cyanobacterium found in the Belcher Supergroup of Canada<sup>1</sup>. Although this fossil is a prime candidate as a calibration point for molecular clock models, the genome sequences of the modern analogs (the second key piece of information in these models) are not available, preventing the

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<sup>1</sup> Hofmann, H., 1976, Precambrian microflora, Belcher Islands, Canada: significance and systematics: *Journal of Paleontology*, v. 50, n. 6, pp. 1040-1073.

use of this important fossil calibration. It is for this reason that we traveled to Shark Bay, Western Australia (Figure 1) to obtain modern analogs of *Eoentophysalis* and sequence their genomes.

The cyanobacterial fossil *Eoentophysalis* had unique patterns of division along three planes and formed characteristic pustular microbial mats that are analogous to modern pustular mats found in Shark Bay, Western Australia (Figure 2).formed by the cyanobacterium *Entophysalis*. However, whole genome sequencing has never been carried out on these mats, so the genomic information is still lacking. In July of this year, we carried out field work in Shark Bay to collect samples of pustular microbial mats to find and sequence *Entophysalis*. On July 9<sup>th</sup>, 2017, I left for Australia with Professor Tanja Bosak (associate professor of Geobiology in the Department of Earth, Atmospheric and Planetary Sciences at Massachusetts Institute of Technology), Professor Sara Pruss (associate professor of Geosciences at Smith College), and Dr. Lily Momper (William O. Crosby Fellow in the Department of Earth, Atmospheric and Planetary Sciences at Massachusetts Institute of Technology).

We spent seven days (July 12<sup>th</sup> to July 18<sup>th</sup>) at Carbla Point (26°14'05" S 114°12'05" E; Figure 1) collecting a variety of microbial samples from the peritidal and intertidal areas of Shark Bay. We collected samples of tufted microbial mats and pustular microbial mats (Figure 3) and placed them in sterile containers with three conditions: 1) collected without liquid so that mats would remain dry, 2) collected in sea water, and 3) collected in RNAlater, a solution that would preserved RNA for sequencing. We also used a Myron Company 6PIIFCE water probe to measure the salinity, oxidation/reduction potential, pH, and temperature of the seawater, and

collected sterile seawater for total organic carbon and dissolved organic carbon measurements. These measurements will provide important data that allow us to make artificial seawater medium in the lab and culture samples collected in the field. In addition to collecting samples at Carbla Point, we travelled to several other sites around Carbla and Hamelin pool to see and compare the microbial mats and stromatolites.

I will attempt to culture pustular microbial mats in the lab using the samples that we collected as inoculum. If culturing is successful, I will attempt to enrich or isolate *Entophysalis* from the microbial communities and sequence it. In parallel, we will use a metagenomic approach to sequence the entire microbial community and isolate the genomes of *Entophysalis* to be included in phylogenetic analyses and molecular clock models. We will use Illumina Sequencing to sequence whole genomes, which will be assembled and analyzed. From these genomes, I will identify 30 ribosomal proteins and add them to my existing dataset of cyanobacterial protein sequences. The inclusion of the newly sequenced taxa in our phylogenetic trees and molecular clocks will allow us to use the ancient *Eoentophysalis* as a deep fossil calibration that is currently lacking from models of cyanobacterial evolution. This work will provide important insights into the timing of cyanobacterial evolution and Earth's oxygenation, and will hold implications for the relationship between the biosphere and atmosphere on Earth, as well as the potential for life outside of our planet. Furthermore, it will enable us to better determine the timing of the evolution of different cyanobacterial groups, including those represented in the fossil record.

Appendix:

Figure 1: Locality map showing the location of Carbla Point in Shark Bay, Western Australia.



Figure 2: Modern pustular microbial mats (left) in Shark Bay from our field work compared to fossil *Eoentophysalis* (right; Hofmann, 1976).

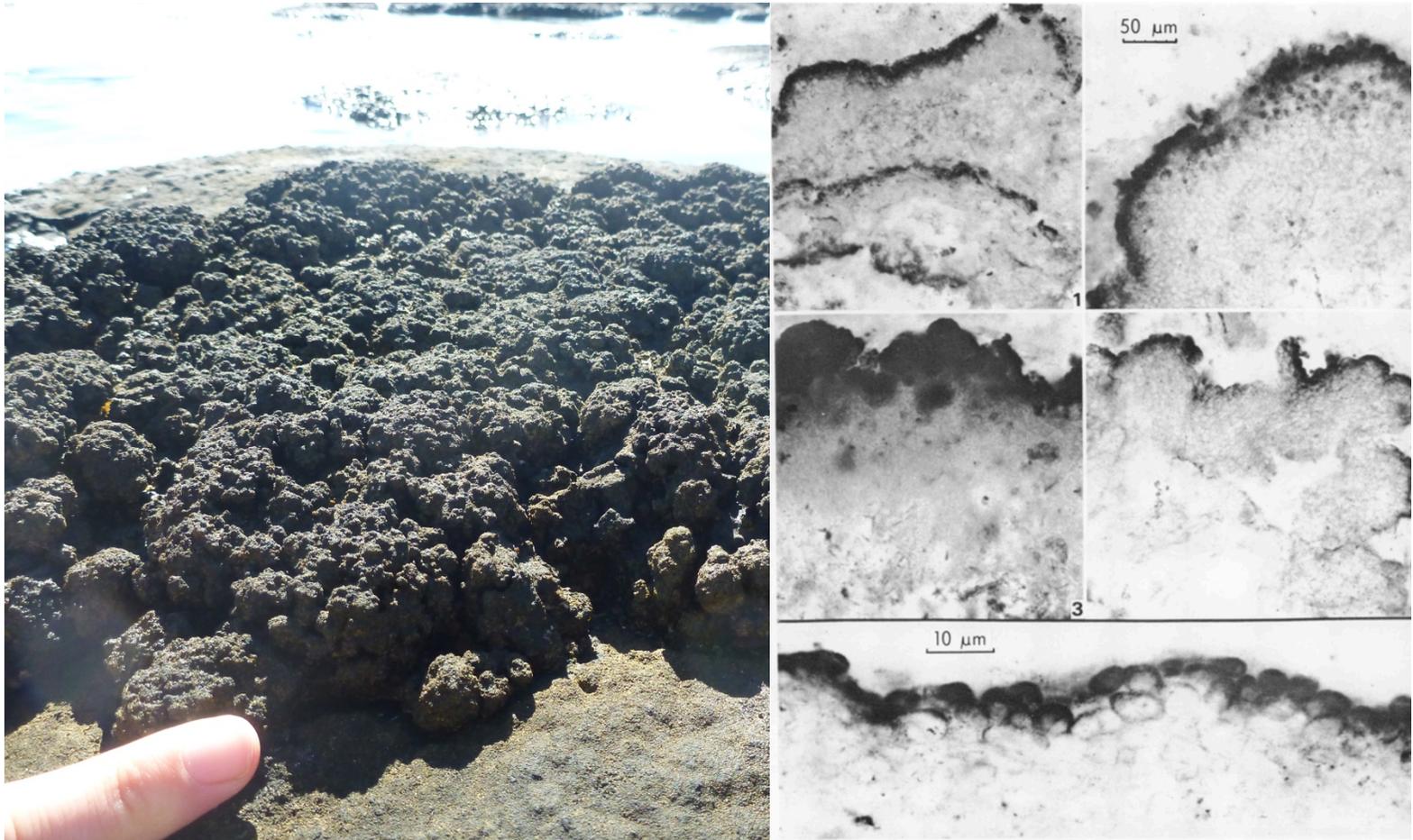


Figure 3: Field photo of me (Kelsey Moore) at Carbla Point.

