

Life in the Dark: the Blue Dragon Flow as an analog for the Martian subsurface

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Introduction: Although previous exploration of Mars has focused on the surface, astrobiologists are beginning to shift their attention underground. Subsurface environments can shield organisms from radiation and temperature fluctuations, allowing them to evolve and persist in otherwise harsh conditions [1]. Furthermore, undisturbed and relatively constant conditions also enhance the preservation of evidence of past biological activity. For these reasons, lava tubes and basaltic caves are appealing targets in the search for life on Mars [2]. Volcanic environments on Earth with relevant similarities, such as the iron-rich basalts found at Craters of the Moon (CotM) National Monument in Idaho, can serve as useful analogs for these subsurface locales on Mars [3]. However, little is known about the habitability of and biological community inhabiting these basaltic lava tubes. Additionally, linking extant biological communities to biosignature detection via synergistic investigations can be a powerful tool for refining and interpreting data from the search for life on Mars.

Study Site: The ~2ka Blue Dragon Flow at CotM is one of the closest terrestrial analogs to Martian basalts due to its iron-rich composition. To better understand the biological community and detectable biosignatures associated with the Blue Dragon Flow, we investigated five lava tubes using petrographic analyses, DNA and cDNA sequencing, and mass spectrometry for biosignature detection.

Methods: Samples were collected in August 2016, August 2017, and January 2018 from publicly accessible as well as publicly barred lava tubes. Samples were crushed and analyzed on a Bruker D8 Discover X-Ray Diffractometer (XRD) to determine sample mineralogy.

To determine the microbes associated with the samples collected, we isolated DNA and sequenced across the 16S taxonomic marker gene. We also extracted RNA and reverse transcribed and sequenced cDNA in order to identify metabolically active community members. Nucleic acids were extracted for sequencing using a ZymoBIOMICS DNA extraction kit. The DNA and cDNA were then used to build sequencing libraries and were sequenced on the Illumina MiSeq platform. We analyzed community abundance and composition with DADA2 and R and generated a non-metric multidimensional scaling

(NMDS) ordination to understand differences among locations and sampling timepoints. Finally, we used metagenomic sequencing to illuminate the metabolic potential of the communities.

The nature and distribution of organic molecules associated with the samples were characterized using pyrolysis and derivatization gas chromatography mass spectrometry (GCMS) experiments. Ten milligrams of each sample was analyzed using a Frontier Lab pyrolyzer coupled to a ThermoFisher GCMS mounted with a MXT-5 column. A cold-trap was used to trap the volatile compounds during the pyrolysis experiments (similar to the analyses done by the Sample Analysis at Mars (SAM) instrument on the *Curiosity* rover).

Results: *Lava Tube Petrography and Mineralogy:* Petrographic and mineralogical XRD analyses uncovered few differences between basalt on the surface of the lava flow and in the lava tube interiors. Samples of coatings and alteration features inside the tubes often contained salts such as sulfates and carbonates.

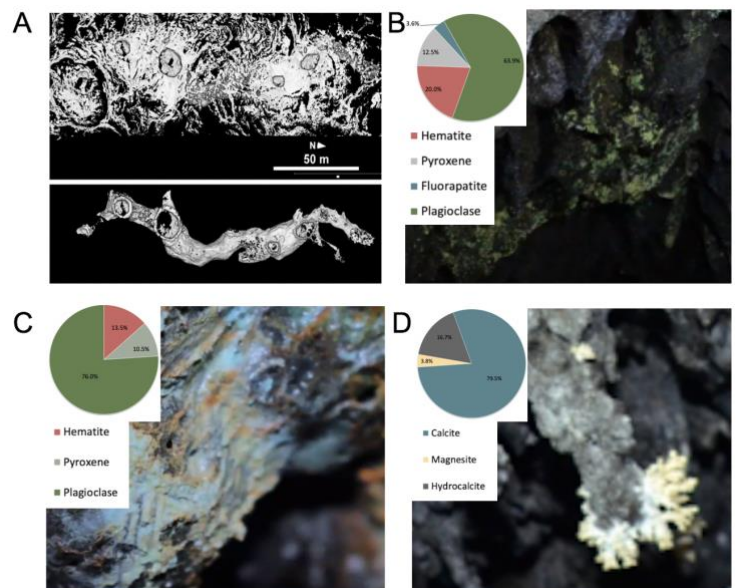


Figure 1. LIDAR imaging of Indian Tunnel from B. Garry [4] (A) and crystalline mineralogy of samples 221 (Arco Tunnel yellow deposit, (B), 209 (Arco Tunnel green deposit, (C) and Last Chance coral deposit (D) from

XRD analysis, all samples contained x-ray amorphous material as well.

Biological Community: 16S rRNA gene and cDNA sequencing, as well as metagenomic analysis of secondary sulfate deposits in the lava tubes—hypothesized to be microbially mediated [5]—identified aerobic, microaerophilic and anaerobic organisms with potential roles in metal oxidation as well as S, C and N cycling (Figure 2). Despite extreme fluctuations in ambient temperatures at the surface, seasonal differences in the subsurface microbial community structure were minimal, as were differences year to year (Figure 3).

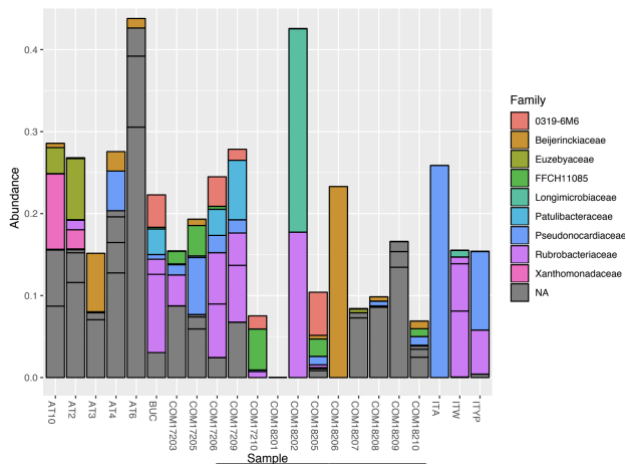


Figure 2. 16S rRNA gene sequencing demonstrates the diversity of lava tube microbial communities, shown here on the family level.

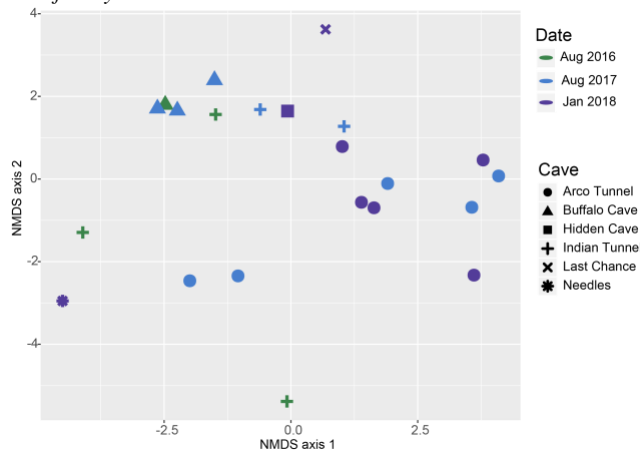


Figure 3. Cave communities remained stable over time despite seasonal climatic shifts.

Detection of Organic Molecules: A high diversity and abundance of organic molecules were recovered in secondary mineral deposits and biofilms using pyrolysis-GCMS methods similar to those aboard the *Curiosity* Rover. Organic molecules include nitrogen

and oxygen-bearing molecules as well as hydrocarbons. The addition of wet chemistry reagents such as N-(tert-Butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) and Tetramethyl ammonium hydroxide (TMAH) significantly enhanced our detections and highlighted the presence of carboxylic and fatty acids (Figure 4) and other potential organic biomarkers.

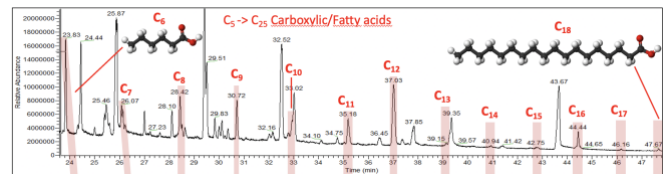


Figure 4. Example of organic molecules that were detected with pyrolysis and derivatization-GCMS including C₆-C₂₁ carboxylic and fatty acids in sample 204 (Hidden Cave, 2018).

Discussion: These results reveal a diverse and active biological community within the darkness of the Blue Dragon Flow lava tubes. The community remained consistent over a three-year sampling period despite seasonal temperature shifts, thus demonstrating the power of lava tubes as environmental stabilizers. The constancy of this environment also bodes well for preservation of organic markers. We detected a range of organic molecules on cave biofilm surfaces which, when combined with our mineralogy and metagenomic results, are likely related to the microbial communities present in the lava tube. These results open a window into the biotic potential of the Martian subsurface, and the rich trove of possibility for future exploration.

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References: [1] Boston, P. et al. (2001). *Astrobiology* 1, 25–55. [2] Northup, D.E. et al. (2011). *Astrobiology* 11, 601–618. [3] Hughes, S.S. et al. (2019). *Astrobiology*, 19(3), 260-283. [4] Garry, W.B. et al., (2017) LPSC 48, Abs. 1207. [5] Richardson, C. D. et al. (2012). *Planet. Space Sci.* 65, 93–103.