

Relating Unique Cave Structures to Micro-Biomes. C. A. Lakrout¹, N. T. Jones², N. Tisato¹ ¹The University of Texas at Austin, Department of Geological Sciences, clakrout@utexas.edu, ²The University of Texas at Austin, College of Natural Sciences

Introduction: Caves have been considered a harsh environment for centuries because they are dark, have low nutrients, and sometimes have high concentrations of heavy metals and toxic gases. However, in recent decades, scientists have discovered a large variety of cave microbes [1]. Caves provide a refuge for life from harsh surface conditions, which is important when searching for life on other planets. If we expect to find life on other planets, it will likely be in caves [2].

Many cave formations originally thought to be abiotically deposited have been reconsidered as biologically mediated. One of these formations is called helictites. Helictites are oddly shaped cave deposits that develop sideways and upside down (against gravity). These formations are not very common, so their appearance is unique. Biologically mediated helictites have been found in a cave in France, called Asperge [3]. Another cave, Breezeway - Colorado, contains helictites in the Elkhorn room (figure 1). Here, we present analyses on Breezeway helictites showing that these deposits are associated with a specific microbiome, similar to that found in Asperge. Our findings reinforce the idea that life manifests in unusual ways and that biologically mediated deposits might be more frequent than previously thought.

Methods: Helictite samples were first collected in 2018. Five of these samples were analyzed in 2019 using micro-CT and Scanning Electron Microscopy (SEM) imaging. In 2021, another field trip was conducted with the purpose of collecting biological samples, which were collected from the Elkhorn room and two other control sites. Samples were collected from the walls of the caves and the helictites themselves in agar plates to be cultured and into sterile tubes for DNA extraction. This procedure was done at the helictite site and two control sites. Cultures were attempted and DNA sequenced to distinguish the microbiome of the helictite site.

Results: The micro-CT images revealed the presence of a low-density layer covering the calcite crystals of some helictites (figure 2). SEM images showed the presence of biological structures (figure 3). The cultures from the 2021 trip were slow growing, though showed the presence of bacterial life was strong at the helictite site (figure 3). The control sites had some, though much fewer indication of life. Bio cultures from the control sites developed less microbial diversity and different sets of microbes than those developed from the Elkhorn room.

Discussion: Samples were initially collected because of their interesting morphology, also recalling

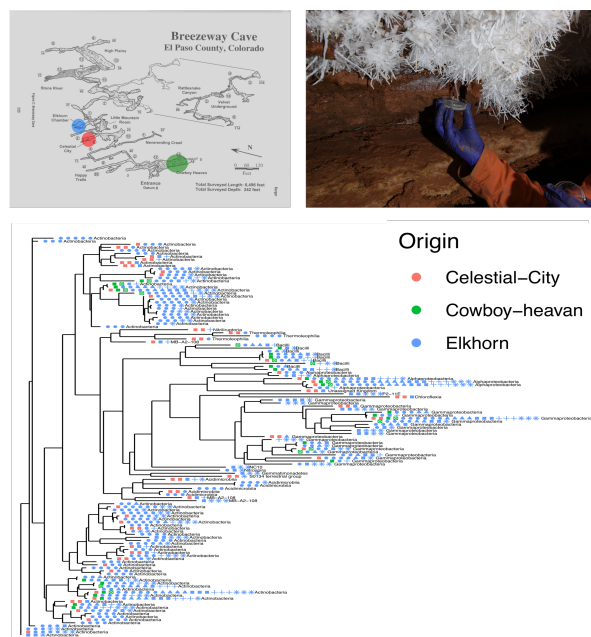


Figure 1: A map of the Breezeway cave showing the sample sites. Sampling a helictite with an agar plate. The phylogeny tree of the three sample sites with color-coded symbols to show which room the DNA was sequenced from.

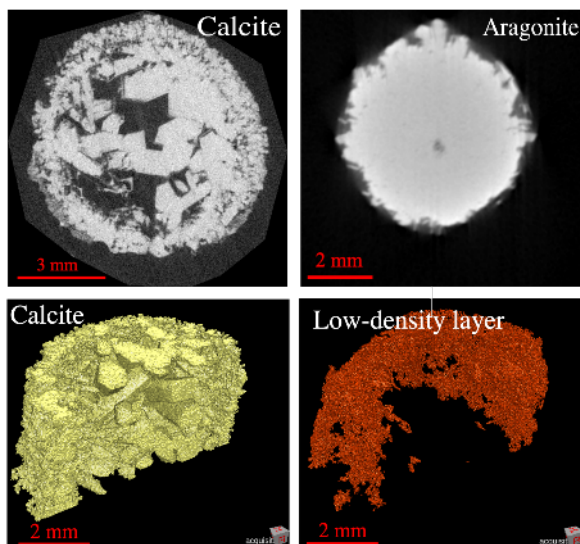


Figure 2: Micro-CT scans of helictites from Breezeway showing the morphology of calcite and aragonite found at the helictite site. 3D models of the calcite and accompanying low-density layer covering the calcite crystals.

Asperge helictites, which were found to be biologically mediated and suggesting that other caves with similar morphologies may host a vaster micro-biome than anticipated.

The low-density layer recognized at the micro-CT and SEM covers individual crystals of calcite like a porous crust. EDX analysis indicates that such a layer is made of calcium carbonate that seemed to be in the process of developing. Within the layer, connecting single crystals, we identified extracellular polymeric substance (EPS) remnants.

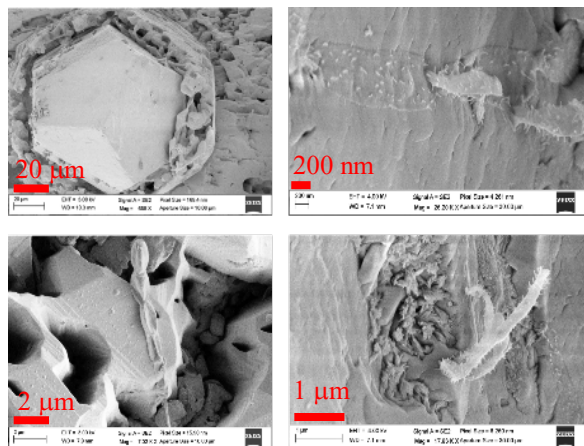


Figure 3: SEM images showing biological structures and calcium carbonate crystal disruption.

Microbial structures were also observed in dehydrated states from the freeze drying process to prepare samples for SEM. The presence of EPS and microbial structures suggest that there was life inhabiting these speleothems.

Analyses of the Elkhorn room's wall mud samples and millimetric white dots presented similar results to those found in Asperge, where the presence of microbes on the cave walls were found. In Breezeway, similar to Asperge, white dots and helictites were associated and found in one small area of the cave and sampled. The micro-biomes of Asperge and Breezeway show many similarities however, control rooms were not sampled in Asperge and therefore cannot be compared to the distribution of the micro biome in Breezeway.

The microbe samples for these analyses were sequenced and showed that Elkhorn had more quantity and diversity of life.

To verify the Elkhorn room's microbiome uniqueness, we performed DNA sequencing on the control group samples that were collected in the Cowboy Heaven and Celestial City rooms. Since these control rooms did not present helictites or white dots, only mud and regular speleothems were sampled. The microbes cultured by these analyses were sequenced, and species distribution showed that Elkhorn

microbiome is more diverse. By demonstrating the microbiome of Elkhorn as unique compared to the other rooms, we conclude that the unique helictites are somehow related to the unique microbiome. Such a conclusion is in line with the conclusion drawn for Asperge. More work needs to be conducted to understand the metabolic processes of the microbiome.

Conclusion: Understanding the interaction and potential of rock-microbe relationships is important in discovering life. Biologically mediated mineral deposits are evidence for life that can become part of the geological rock record for billions of years, far past the lifespan of an organism. This makes biologically mediated minerals a good source of evidence in searching for past life on other planets.

The next steps include metabolic studies to understand biological hierarchies and investigate possible new metabolic functions gathering energy from unusual sources, focusing on these and other caves with similar structures and replicating in-vitro mineral growth using Breezeway microbes.

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