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Evaluating the Biogenicity of Biovermiculations in Mammoth Cave, KY, USA

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Caves have been observed on Mars and Earth's moon; therefore, terrestrial caves can be considered astrobiological analogue environments. Microbe-rich dendritic rock-coatings found on cave walls, referred to as biovermiculations, are conspicuous potential biosignatures. Most recent studies describing biovermiculations are from sulfidic caves and relate biogenic mechanisms to sulfur cycling reactions [1][2]. Here, we characterize the biogeochemistry of biovermiculations in a carbonate karst cave, Mammoth Cave (MC), Kentucky, USA to draw comparisons to sulfidic acid biovermiculations and evaluate their biogenicity. We hypothesize that MC biovermiculations are formed by a distinct mode of microbiological life that moves rock-surface material into vermiculated patterns. We integrate morphological classification, spatial mapping, live and epifluorescence microscopy, 16S rRNA gene sequencing, and metagenomics to evaluate biogenicity and formation mechanisms. MC biovermiculations occur in five morphological classes: simple, globule, intermediate, and complex A/B (Fig. 1). Observed vermiculated material includes soot, fine-grain mud, and cricket guano. Spatial analyses indicate that morphology corresponds more strongly with material availability and substrate rheology than adjacency to other morphologies. Light microscopy reveals motile microorganisms potentially capable of active material redistribution. Biovermiculation growth extends into 19th–20th century candle soot but minimally alters post-1970s deposits, constraining the timescale of growth. Preliminary analyses suggest that microbial communities are conserved among biovermiculations and unique from other locations. After Proteobacteria, Desulfobacterota is the second most abundant phylum in biovermiculation samples, which is 10x more abundant than most other locations. After organic carbon oxidation, the most common metabolism genes are for sulfur and sulfite oxidation, iron and sulfate reduction, and methanotrophy. Ongoing DNA and RNA comparisons between morphologies and non-vermiculated material will help illuminate the role of microbial activity in material redistribution. We will contextualize these observations with C/N isotopic fingerprinting and analysis of biovermiculations from other mid-latitude karst sites. Overall, this study develops a multiparameter framework for assessing the biogenicity of biovermiculations in caves, a terrestrial analogue environment.

[1] Hose, Palmer, Palmer, Northup, Boston & DuChene (2000), *Chemical Geology* 169, 399-423.

[2] Jones, Lyon & Macalady (2008), *Journal of Cave and Karst Studies* 70, 78-93.