



## **Biomineralization and biosignatures of coralloid-type speleothems from lava tubes of Galapagos Islands: evidences on the fossil record of prokaryotes**

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Lava tubes have traditionally been considered of little interest from a mineralogical point of view. Recently, this type of volcanic caves has received particular attention because lava tubes have been described on Mars. Speleothems, or secondary mineral deposits in lava tubes are mainly composed of siliceous minerals. Coralloid-type speleothems are found either on basaltic cave walls or on the surface of other speleothems. Several authors attribute a microbially mediated origin to their formation. This type of speleothems was recorded within Royal Palm Cave of Santa Cruz Island in Galapagos Archipelago (Ecuador), a lava tube 600 m long, 5 to 15 m height and 2 to 10 m width. The Galapagos Islands are an archipelago of 19 volcanic islands located some 1500 km west of Ecuador, in the Pacific Ocean. These islands host one of the most biodiverse settings on Earth, studied by Charles Darwin.

Beige and greyish small coralloids were collected in Royal Palm Cave and analysed by field emission scanning electron microscopy with energy dispersive X-ray spectroscopy (FESEM-EDS), X-ray micro-computed tomography (micro-CT) and mineralogical analyses for morphological, 3D microstructural and compositional characterization, as well as for assessing microbe-mineral interactions and biogenicity. In addition, 16S rRNA gene analyses were performed to identify microbial communities associated with the coralloid-type speleothems. The coralloids showed internal compositional zonation along the growth direction of the speleothems, according to micro-CT data. Internal layering was clearly discernable by the differences in opacity of the distinct mineralogical phases to X-rays, being dominated by alteration products of siliceous composition, whereas more opaque phases, usually Ca-rich minerals, were dominant in the outermost part of the speleothems. X-ray diffraction and infrared spectroscopy reinforced that the first stage of deposition is mainly composed of opal A and clay minerals, whereas the final stage mainly consists of low crystalline calcite. FESEM-EDS analysis revealed mineralized bacterial filaments rich in Si on the coralloid samples, as well as minerals precipitation associated with extracellular polymeric substances (EPS), which serve as nuclei for preferential precipitation on the extracellular sheaths. This suggests that biological activity played a major role in the development of these speleothems. In addition, imprints of filamentous cells and microboring readily preserved on siliceous minerals were observed on the coralloid speleothems. These features are recognized as biosignatures valuable for astrobiology and may represent modern analogs of the fossil record of prokaryotes.

DNA-based analyses showed that bacteria belonging to Actinobacteria (31%) Gemmatimonadetes (25%) and Proteobacteria (24%) phyla dominated in this cave ecosystem, followed by Acidobacteria, Firmicutes and Nitrospirae. Most of the identified phylotypes were affiliated to chemoautotrophs, including thermophilic bacteria such as *Ferrithrix thermotolerans*, and other mineral utilizing microorganisms like *Aciditerrimonas ferrireducens*, *Desulfuromonas* sp. and *Desulfovibrio* sp., indicating that Galapagos lava tubes host highly specialized subsurface biosphere dominated by microorganisms able to interact with minerals and promote biomineralization.

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