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The Lewis and Clark Fund for Exploration and Field Research in Astrobiology

A Comparison of Subsurface Microbial Communities and Function (PORTUGAL)

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Abstract: In order to better understand the diversity and metabolisms of the terrestrial subsurface, seven thermal springs and two temperate springs arising along two major faults in Portugal were sampled for molecular and geochemical analysis.

Background:

Recent missions have revealed that extraterrestrial life within our solar system is most likely to be/have been present in the subsurface of Mars or moons of Jupiter and Saturn. Yet, despite it being an analogue for life on other planets, the Earth's subsurface biosphere remains one of the most poorly characterized biomes[1]. This is, in part, due to the difficultly in accessing and sampling subsurface waters—a task that often requires the use of drilling, submersibles, or deep mines. However, active terrestrial serpentinizing sites and surficial springs are becoming popular windows to view microbial life in the subsurface.[2] Of particular interest are thermal springs whose water has been warmed at depth before emerging at the surface. These waters contain organisms native to the subsurface and have been shown to exhibit a diverse range of metabolisms. To date, the majority of thermal spring research has focused on the ecosystems of the North American continent. Therefore, in order to address the Astrobiology Roadmap's Goal 4 (to show how life evolved and responded to environmental change on a planetary scale), the microbial diversity and metabolisms of geographically and geologically diverse sites need to be studied. In order to expand our understanding of the terrestrial subsurface, nine unique and biologically uncharacterized springs in Portugal were sampled as described below.

Sampling: The main objective during sampling was to collect enough biomass so that DNA extraction and sequencing could be performed. To do this, the thermal water of each site was filtered through a 0.2 μm Mediakap filters (Mfr # ME2M-10B-12S | Item# EW-29510-14) using either a peristaltic pump or via natural pressure. Water samples for aqueous chemistry (TOC/DOC, dissolved nutrients/ions, dissolved metals, etc.), physical properties (pH, ORP, temperature, salinity, conductivity, etc), and cell counts were also collected and brought back to Princeton University for further analysis.

Termas São Pedro do Sul (n=4): The thermal region of São Pedro do Sul is located approximately 100 km southwest of Porto, Portugal and was the first site visited during this field trip. Four thermal springs emitting water between 66°C and 67°C were sampled in the region. These springs arise within two distinct mineral producing areas around 1.2 km apart that are referred to as the "spa" and "vau" sectors. In the spa area, an artesian well dating back to the Roman empire and a well that samples water from 500-m depth were sampled. In the

vau sector, a small mineral spring and well naturally pumping water from a depth of 216-m were sampled. All of the thermal springs in São Pedro do Sul are found in Paleozoic granites and are associated with the Verin-Régua-Penacova fault.[3] As reported previously[4], the waters of the springs were found to be alkaline with high concentrations of sulfur.

Termas Caldas de Aregos (n=2): Two thermal wells were sampled in Caldas de Aeregos—a town located along the Duoro River. Like the thermal waters of São Pedro do Sul, the thermal waters at Caldas de Aregos also arise due to the activity of the Verin-Régua-Penacova fault. Thermal waters in Caldas de Aregos are slightly cooler (60˚C and 64˚C) with a reported pH of 9.2 (at 22˚C) and sulfate concentrations of 7.5 and 7.3 ppm, respectively.

Termas de Longroiva (n=3): The Longroiva thermal waters are located in northeast Portugal along the Vilariça fault. The region is characterized by a local mountain range (average altitude of 750-m) extending NNE-SSW from Trancoso, Portugal to Longroiva. Two streams (Massueime and Centieira) are fed by drainage from the mountains in the region that eventually feed the Côa River and Duoro River. Several springs arise in the region three of which are known to be rich in sulfur. The three sulfur rich water sources arising in granitoids were sampled: a thermal well (43˚C), a temperate well (21˚C), and a sulfurous mineral spring (18˚C). Although the temperatures of these waters are more moderate, it is believed that the waters are warmed at depths of up to 1200-m.[5]

Outreach: During this field trip, I also led a short course on astrobiology for 25 tenth graders at the local high school. Following the course, these students joined me at the two thermal springs located in Termas, São Pedro do Sul, to participate in a fieldwork activity. Their task was to analyze the water coming out of different springs in the thermal area using the same CheMet-kits, pH probe, and microscope I used in the field.

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[1] No work related to the grant was conducted June 20-24