Kristin WOYCHEESE
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Project Title: Biogeochemistry and depositional facies of a serpentinizing fluid seep in the Zambales Range ophiolites, the Philippines

PROJECT REPORT

Abstract: The purpose of this field expedition was to collect samples to investigate the biogeochemical characteristics of a terrestrial serpentinizing seep located within the Zambales Range ophiolite. The results of this study demonstrated that depositional facies created by serpentinizing fluids preserve past records of microbial activity. Microbe-sized boreholes (2-5 um) and biologic material encrusted in carbonate minerals were observed via scanning electron microscopy (SEM). The presence of microbial communities within the carbonate minerals was verified via 16S rRNA amplicon sequencing and analysis. These data may be relevant to astrobiology studies, particularly detection of life in carbonates, by providing evidence for preferential preservation of microbial cells and traces in a serpentinizing seep.

Site Location: The field site is located at Manleluag Spring National Park in Mangatarem, Pangasinang province, Luzon, the Philippines (GPS Coordinates: N15°42.367', E120°16.935'). The climate is tropical, the average annual temperature is 27.4°C, and the average annual rainfall is 2223 mm. The serpentinizing seep is located on a densely vegetated slope in the eastern Zambales Mountains (Figure 2). The seep originates in a small pool and cascades into a larger, shallow pool (Figure 2B). At 10 meters from the source, precipitation of calcium carbonate creates meters of cascading terraces (Figure 2C and D). The precipitation of calcium carbonate appears to be dependent upon dissolved inorganic carbon (DIC). The carbonate buildups continue for many meters downstream, and coat fallen tree branches and roots (Figure 2D). Additional organic input enters the stream via leaf litter and insects, both of which are abundant in the tropical climate. This study increases the scope and breadth of serpentinizing seep ecosystems research as one of the few examples of microbial community analysis in a tropical serpentinizing seep ecosystem (see Schrenk et al., 2013 *Rev. Min. Geochem.*).

Preliminary Results: Samples were collected to test the hypothesis that serpentinizing fluid depositional facies preserve traces of microbial activity. *Fluid Geochemistry*: Fluid samples were collected at the source and at the base of the large apron pictured in Figure 2D. Sediment and carbonate samples were

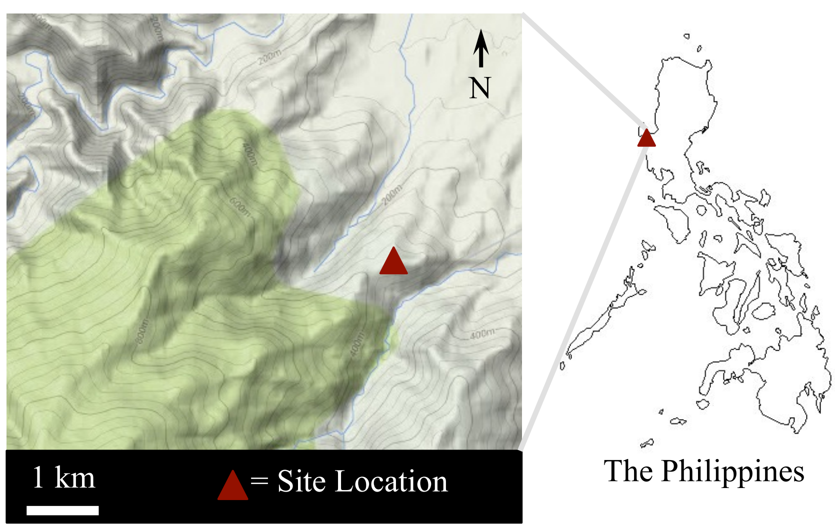

Figure 1: Site location and topographic map of Manleluag Spring.

collected at the source, 1.5 meters downstream, 10.6 meters downstream, and 19.5 meters downstream. At

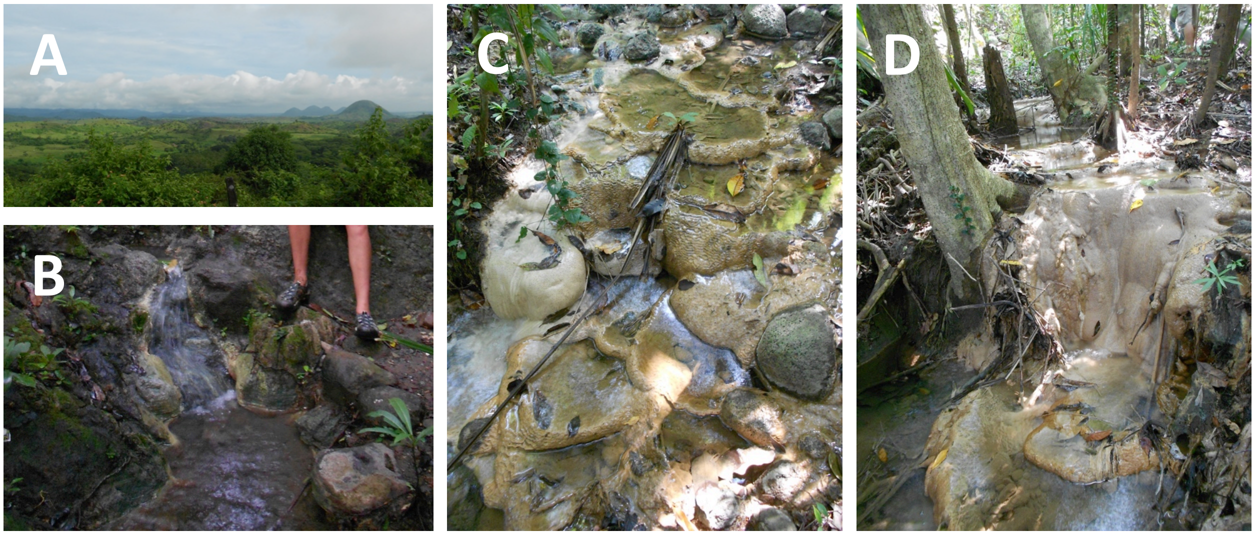

Figure 2: Site location. A) The hilly terrain of the Zambales ophiolite range in Luzon, Philippines. B) The source spring and outflow channel/lower pool at Manleulag Spring National Park. C) Terraces and rimstone dams, 10.6 meters from source. D) A large accretion of terraces on tree roots and fallen branches, 19.5 meters from source.

the source, DIC was approximately 0.4 ppm, while DOC was 0.12 ppm. Near the base of the large carbonate terrace (approximately 19.5 meters downstream), DIC was 4.4 ppm and DOC was 0.73 ppm. The pH shifted slightly from 10.83 to 10.23 from the source to the terrace, and the oxidative-reductive potential (ORP) fell by half, from -424.7 to -213.2. Temperature decreased insignificantly, from 34.44˚C at the source, to 32.55˚C at the base of the terrace. Dissolved oxygen increased from 0.86% to 4.69%. *SEM-EDX*: Samples were examined via scanning electron microscopy (SEM) equipped with X-ray electron dispersive spectroscopy (EDX). Results suggest that microbial activity leaves both chemical and physical traces in the precipitated carbonates forming downstream of the seep's surface expression (Table 1, Figure 3).

Table 1: EDX-derived elemental weight % of solids at Manleluag.

Element Wt.%	source sed.	light band	dark band	calcite rhomb.	root
C	24.4	1.72	36.2	4.86	52.4
O	32.2	40.1	41.0	46.5	30.1
Mg	0	2.85	4.4	2.16	1.2
Al	1.5	3.6	2.85	3.64	0.93
Si	2.53	8.88	7.11	6.65	2.22
S	-	-	0.25	-	-
Ti	18.6	-	-	-	-
Mn	0.84	-	-	-	-

Ca	0.37	38.2	7.38	32.7	12.1
Fe	19.6	3.92	0.89	3.54	1.09

Elemental analysis of samples indicates that the weight percent of carbon varies between light (calcite; 1.72 wt.% C) and dark (biofilm; 36.16 wt.% C) layers in a section of encrusted root (Table 1; Figure 3C). All samples are relatively low in magnesium, typical for serpentinizing systems (Table 1). In the solids, calcium varies from 38.18 wt.% in mineral-rich layers to 7.38 wt.% in the dark biofilm layers (Table 1, Figure 3C). The dark layers also contain 0.25 wt.% S, which was not detected elsewhere in the sample (Table 1). Elements such as Fe, Al, and Si are also present, and may reflect composite parent material (Table 1).

SEM analysis of terrace samples indicate that roots and microbes are present in the terrace structure, and serve as nucleation points for carbonate crystallization (Figure 3A, B; Table 1). Chemical traces of microbial activity are recorded in meso-structures (Table 1). Physical traces are also recorded, as endolithic burrows or pit marks and by entombment in calcite crystals (Figure 3D).

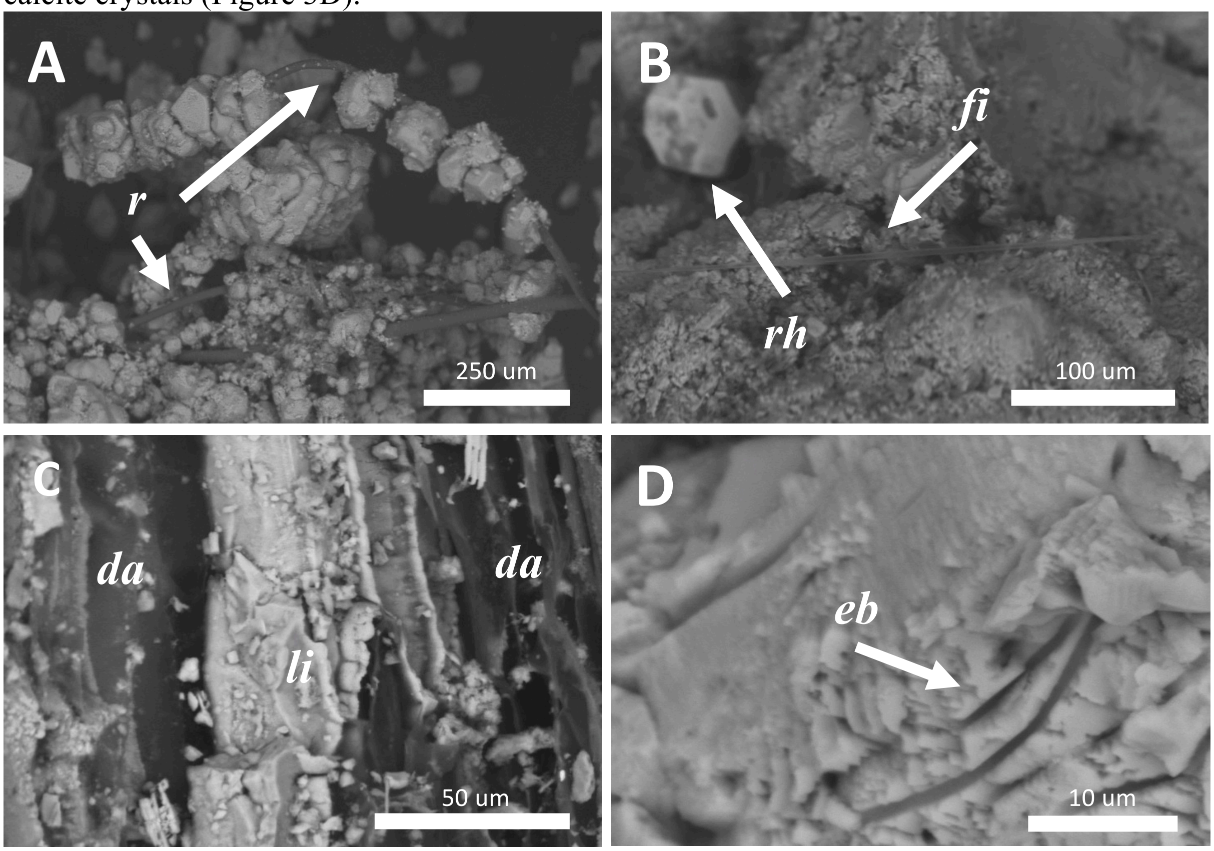

Figure 3: Scanning electron microscopy (SEM) of samples collected at Manleluag Spring National Park. A) Microterracette samples consist of rhomboid calcite crystals, shown here precipitating on organic matter (likely a plant root, *r*). B) Putative microbial filament (*fi*) interbedded with calicte crystals (note rhombohedron, *rh*, in background). C) Alternating layers of organic-rich biofilm and calcium carbonate crystals growing around a tree root. D) Possible evidence of endolithic borings (*eb*) in calcite from a rimstone dam.

Microbial Community: 16S rRNA amplicon sequencing analysis identified taxa capable of methanogenesis, hydrogen oxidation, fermentation, sulfur oxidation, and iron oxidation. Based on taxa abundances, heterotrophy dominates over photosynthesis-driven autotrophy for tens of meters downstream from the seep. The results of 16S rRNA amplicon sequencing are presented in Woycheese, et al. (*in prep.* for *Frontiers in Extreme Microbiology*).

Discussion: The results of this field expedition suggest that microbial filaments have the ability to be trapped and bound by abiotic calcium carbonate precipitation triggered by high pH, low DIC serpentinizing fluid exposure to surface conditions. Furthermore, SEM-EDX analysis indicates that chemical activity can also be recorded in the mineral matrix, at least over short time scales (further work is necessary to determine the fidelity of geochemical signals in the fossil record). In general, terrace facies preserve microbial traces more readily than lithified pool bottom facies, which demonstrated crystal degradation and secondary precipitation of aragonitic needles growing on calcite rhomboids. The dissolution and re-precipitation occurring in pool bottoms appears to erase many physical traces of past microbial activity. These results suggest that microbes are preferentially preserved in air-water interface depositional facies, such as terraces. Therefore, terraces, and not pool bottoms, may serve as more appropriate targets for astrobiology studies, in the event that similar structures are located on Mars or elsewhere.

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