Got moonmilk? The characterization of moonmilk in Spider Cave, Carlsbad Caverns National Park, New Mexico

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INTRODUCTION

Location

Spider Cave is a part of the Guadalupe Mountains cave system in Carlsbad Caverns National Park of southeastern New Mexico (Fig. 1). The caves of the Guadalupe Mountains developed in the Permian Reef Complex, which surrounds the Delaware Basin (Hill, 2000). The formation of this cave system began during the late Paleozoic and continues through the present day (Hill, 2000). Most of the Guadalupe Mountains caves (including Carlsbad Cavern and Spider Cave) formed due to sulfuric acid speleogenesis (Hill, 2000; Polyak and Provencio, 2000). Spider Cave, along with the majority of the caves in the system, formed along the shelf crest of the backreef, which includes the Tansil, Yates and Seven Rivers formations. Spider Cave is within the Tansil (1295 m) and Yates (~1255 m) formations (Hill, 1992). A rough cave survey estimated the depth of Spider Cave to be ~40.75 m (Perrone, 2005).

The current temperature within Spider Cave is approximately 18 °C with some seasonal variation near the entrance, and the relative humidity within the cave is 100% (Plank, 2001). The entrance of the cave is within an arroyo; thus Spider Cave is subject to flooding. There are pools of water and dripping water within the cave and a flood line is present that indicates past flooding.

Time of formation

The age of Spider Cave was estimated utilizing the correlation between the age of caves within the Guadalupe Mountains determined by 40Ar/39Ar dating of alunite and the elevation of the caves (Polyak et al., 1998) (Fig. 2). This correlation does not include age dates for Spider Cave. Alunite is a sulfur-bearing clay (KAl₃(SO₄)₂(OH)₆) that formed when sulfuric acid etched out the caves in the Carlsbad Caverns National Park cave system. The entrance of Spider Cave is within the Tansil Formation (elevation 1295 m) and extends into the Yates Formation (elevation ~1255 m) (Hill, 1992). At this elevation, Spider Cave formed approxi-
approximately 6.0 million years ago (Fig. 2). This age places the formation of Spider Cave within the Miocene (5.3-7.1 Ma).

Moonmilk

Microorganisms have been implicated in the formation of secondary cave formations, known as speleothems, either passively by acting as nucleation sites onto which minerals can precipitate, or actively by producing substances that lead to a change in the microenvironment, which results in the precipitation of minerals (Northup et al., 2000). One such speleothem that has been the center of controversy is moonmilk (Fig. 3). Moonmilk has historically referred to a two-phase system of calcite (CaCO$_3$) crystals and water that is formed in caves (Bernasconi, 1981). The type of moonmilk found in Spider Cave of Carlsbad Caverns National Park is commonly referred to as “Crisco” and is composed of filamentous calcite that is associated with microorganisms, and has a slippery, greasy feel similar to clay (Boston et al., 2001).

Much of the moonmilk controversy centers on whether it is created biogenetically or abiogenetically. There have been studies in which bacteria were isolated from moonmilk and implicated in the formation of moonmilk (Hoeg, 1946; Caumartin and Renault, 1958; Mason-Williams, 1959; James et al., 1982; Danielli and Edington, 1983; Gradzinski et al., 1997); other studies have not found evidence of microorganisms in moonmilk (Shumenko and Olimpiev, 1977; Borsato et al., 2000).

Common morphological features found in SEM studies of cave moonmilk include stacked rhombohedral calcite (Boston et al., 2001; Gradzinski et al., 1997; Rogers and Moore, 1976), calcium carbonate coated filaments (also called needle fiber calcite crystals; Gradzinski et al., 1997), which take up nucleic acid stains at the ends of broken filaments (indicating the presence of DNA, and thus the presence of microorganisms) (Boston et al., 2001), as well as irregular grains of calcite, fine-grained siliciclastics (such as clay), interwoven calcified filaments, and ovoid bodies that are possibly bacterial (Boston et al., 2001; Gradzinski et al., 1997).

Energy Dispersive X-ray Spectrometry (EDS) studies of Spider Cave moonmilk show that it consists mainly of carbon, oxygen and calcium, a composition consistent with calcium carbonate, though there is a small signature of excess carbon that indicates the presence of organic material (Boston, 2001).

Short-term culture studies demonstrated that there is an active biological community within Spider Cave moonmilk consisting of Actinomyces spp., Bacillus spp., Micromonas spp., and Streptomyces spp., which are types of soil bacteria, as well as algae and unidentified isolates (Boston et al., 2001).

METHODS

Cave Survey

A rough survey of the cave was performed to locate a moonmilk line and to map the sampled areas (Perrone, 2005). There are two main areas within the cave that contain moonmilk; the area used for this paper is referred to as “the study area” and is located at the lowest elevation within the cave (Fig. 4). A more accurate survey of the cave was performed by Art and Peggy Palmer (Perrone, 2005), and these data were incorporated into the first survey to achieve more accurate depth measurements.

Thin Sections

Petrographic thin sections were made from moonmilk collected from sample sites 1-7 and A1-A3. A block of moonmilk was taken from the ground between A1 and A2, and a large piece of popcorn-like moonmilk was collected from the wall in an area between A1 and A2 (Fig.5) (see Perrone, 2005).

Microprobe Analysis

Microprobe analysis of thin section samples was performed on a Cameca SX50 instrument and run at 15 kV and 20 mA at the University of Texas at El Paso. Thin section samples from sample sites 1 and 6, A1 and A2, and a block of moonmilk found on the
ground between A1 and A2 were polished and carbon-coated for microprobe analysis (Fig. 5). Images of the samples were collected, as well as some EDS spectra. For the EDS analyses, all of the spectra were collected for the beam shooting area, and images were collected using X-Ray maps. Images are in black and white, and white indicates that there is more of that element.

**Scanning Electron Microscopy**

Scanning Electron Microscopy (SEM) was performed using a JEOL 5800LV SEM operating at 15 kV-10 kV, and Energy Dispersive X-ray Spectrometry (EDS) was performed using an Oxford EDS analyzer system. Both instruments are at the University of New Mexico. Three different procedures were used to prepare cave moonmilk samples for SEM and EDS. Three sets of samples were taken from three different locations within the study area (Fig. 5; A1, A2, and A3). One set of samples was neither fixed nor dehydrated; they were placed on an SEM stub in the cave. A second set of samples from the same locations was fixed and dehydrated using a procedure from New Mexico State University’s EM laboratory (Perrone, 2005). The third set of samples from the same locations were fixed using a procedure from the University of New Mexico’s SEM laboratory (Perrone, 2005). The NMSU lab fixation technique used a cacodylate buffer, while the UNM lab fixation technique utilized a bicarbonate buffer.

**TABLE 1. Summary of thin section morphology.**

<table>
<thead>
<tr>
<th></th>
<th>Type 1: Crystalline</th>
<th>Type 2: Diffuse</th>
<th>Type 3: Recrystallized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td>Calcite</td>
<td>Calcite</td>
<td>Calcite</td>
</tr>
<tr>
<td><strong>Layers</strong></td>
<td>Continuous</td>
<td>Discontinuous</td>
<td>None</td>
</tr>
<tr>
<td><strong>Thickness</strong></td>
<td>Regular</td>
<td>Irregular</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Crystal size</strong></td>
<td>Microspar (&lt;5μm)</td>
<td>Micrite (&lt;1μm)</td>
<td>Micrite (&lt;1μm)</td>
</tr>
<tr>
<td><strong>Staining</strong></td>
<td>No stain</td>
<td>Dark stain</td>
<td>Light stain</td>
</tr>
</tbody>
</table>
RESULTS

Thin Sections

Three microscopic morphological types were observed in thin section (Table 1). Type 1 has sharp, planar crystalline boundaries on regular sized crystals, Type 2 has diffuse, irregular crystalline boundaries, and Type 3 has a recrystallized appearance. The majority of sample sites have all three morphological types present (Fig. 5). Two sites (A1 and P) have only Type 1 and Type 2 present (Fig. 5), and two sites (A3 and B) have only Type 3 present (Fig. 5).

Samples with Type 1 and Type 2 morphologies have layering (<0.25 mm). Type 1 layers have a regular, continuous thickness, microspar-sized crystals, and do not take on the Alizarin Red S (Fig. 6a and b; white color). Type 2 layers have an irregular, discontinuous thickness, micrite-sized particles, and take on the stain (Fig. 6a and b; dark gray color). The samples with completely recrystallized morphologies of Type 3 exhibit no features; they are homogenous calcite micrite (Fig. 6c). The calcite stain is a light gray, though in some places it is not present. This can be interpreted as the sample varying in thickness when cut. These samples show no evidence of past layering and appear to be completely recrystallized.

Samples that appear to have all three morphological types have an overall recrystallized appearance (Fig. 6d). The crystals are micrite-sized, and they display a diffuse, light gray stain for calcite utilizing Alizarin Red S. However, evidence of past layering can be seen. The stain is not regular; rather some areas within the samples take on a darker stain, and other areas have no stain.

Figures 6a-b show samples that were found at a higher elevation (A2 and popcorn-like moonmilk from the wall between A1

![Image](image_url)

FIGURE 6. Thin section pictures of moonmilk. White represents those areas that did not take the Alizaren Red S stain; dark gray represents those areas that did take the stain; and light gray represents those areas that partially took the stain. (a) Moonmilk from A1 showing Type 1 and Type 2 morphologies. (b) Moonmilk from A1 showing Type 1 and Type 2 morphologies. (c) Moonmilk from sample site 1 showing Type 3 morphology. (d) Moonmilk from sample site 3 showing a combination of Type 1, Type 2, and Type 3 morphologies.
and A2), while those samples that appear to show remnants of Type 1 and Type 2, but were mostly recrystallized (Fig. 6d) are found at a lower elevation (sample sites 1-7, and A1). Samples that were completely recrystallized (Fig. 6c) are found at the lowest elevations (A3 and the block of moonmilk found on the ground between A1 and A2).

Composition

The thin sections from Figure 6 were used for microprobe and EDS analysis. Only the maps for calcium and magnesium from site A1 (Type 1 crystalline and Type 2 diffuse) and sample site 1 (combination of the three morphologies) are shown. All elemental data are summarized in Table 2.

X-ray maps of the Type 3 recrystallized samples indicate that the samples are high in carbon and oxygen. EDS analysis confirms these results and indicates that levels of carbon and oxygen are highest in recrystallized samples. All other elements are at low levels, except for chlorine. Chlorine is found in the epoxy, and the recrystallized samples are very porous, which traps the epoxy in the pores. Thin sections showing Type 1 crystalline morphology can be seen in Figures 7a and b. There is more elemental diversity in the crystalline layer than in the recrystallized samples. Calcium (Fig. 7a), magnesium (Fig. 7b), sulfur, and silica are present in a greater abundance, while the carbon and oxygen levels are much lower than in the recrystallized samples. Thin sections showing Type 2 diffuse morphology can be seen in Figures 7a and b. There are more elements indicated in the diffuse layer than in both the recrystallized and crystalline samples, indicating higher levels of aluminum, calcium (Fig. 7a), magnesium (Fig. 7b), silica and sulfur. The levels of carbon and oxygen are lower in the diffuse samples than in the recrystallized samples, but similar to the crystalline samples.

Thin sections that have characteristics of Type 1, 2, and 3 can be observed in Figures 7c and d. These samples contain a greater variety of elements than do the recrystallized samples. There are higher amounts of aluminum, calcium (Fig. 7c), magnesium (Fig. 7d) and sulfur. The levels of carbon and oxygen are not as high and while there is some chlorine in the samples it is not as abundant as the more porous recrystallized samples.

Scanning Electron Microscopy

Some of the thin sections were viewed using SEM, which showed that differential staining appears to be due to the surface topography of the samples (Fig. 8a). Crystalline areas that do not stain are lower in topography, whereas diffuse areas that do stain are higher in topography. Alizaren Red S stains only the top most layer of any sample, which would explain why the crystalline layers do not stain, despite all layers being made of calcite.

SEM images of Spider Cave moonmilk from all areas show an overall texture that has a biofilm-like appearance. The biofilm-like texture is smooth and curd-like and appears like crumpled tissue paper. Interwoven within the biofilm-like texture are filaments that give a mat-like appearance. High magnification examination of the samples shows that there are two main forms present, both of which are composed of calcium carbonate. There are filamentous forms composed of long and flexible filaments that take up nucleic acid stains, indicating they contain organic material (Fig. 8b) (Boston et al., 2001). Also present are stacked rhombohedrons of calcite referred to as lublinite (Fig. 8c). Lublinite is a common form found in moonmilk from many different caves. A feature found less commonly in the Spider Cave samples is clay particles. These can be recognized by their round, tissue-paper like appearance and the presence of aluminum and silica in EDS analyses (Fig. 8c).

Preparation technique played a critical role in the textures observed under SEM. Those samples that were not fixed, or fixed using the procedure from the University of New Mexico’s EM laboratory were similar in appearance, while the samples that were fixed using New Mexico State University’s EM laboratory procedure had a very different appearance. The UNM procedure called for a bicarbonate buffer during the fixation process, while the NMSU procedure did not. The samples that were fixed without the bicarbonate buffer appeared to be decalcified and took on a wool-like appearance that is an artifact of the preparation technique (Fig. 8d).

DISCUSSION

Temperature

Because the temperature range between glacial and interglacial periods varies significantly and dating of the moonmilk is imprecise, it is not possible to determine temperature during moonmilk formation. Different temperatures would have had a different affect on the formation of moonmilk, as well as changing the chemistry of the cave water. Current temperature of the cave (18 °C) is high for the Guadalupe Mountains cave system, and per-

**TABLE 2.** Summary of elemental data from EDS of thin sections (see Perrone, 2005).

<table>
<thead>
<tr>
<th>Content</th>
<th>Type 1: Crystalline</th>
<th>Type 2: Diffuse</th>
<th>Type 1, 2 and 3</th>
<th>Type 3: Recrystallized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>350</td>
<td>325</td>
<td>50-125</td>
<td>25</td>
</tr>
<tr>
<td>Mg</td>
<td>75</td>
<td>40</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Al</td>
<td>None</td>
<td>40</td>
<td>0-30</td>
<td>0-25</td>
</tr>
<tr>
<td>Si</td>
<td>50</td>
<td>50</td>
<td>25-75</td>
<td>50</td>
</tr>
<tr>
<td>S</td>
<td>None</td>
<td>25</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Cl</td>
<td>None</td>
<td>None</td>
<td>25-50</td>
<td>25-75</td>
</tr>
<tr>
<td>C</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>150-200</td>
<td>150-225</td>
</tr>
<tr>
<td>O</td>
<td>75</td>
<td>75</td>
<td>200-250</td>
<td>200-225</td>
</tr>
<tr>
<td>Fe</td>
<td>None</td>
<td>75</td>
<td>None</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Na</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>25-50</td>
</tr>
<tr>
<td>K</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>0-25</td>
</tr>
<tr>
<td>P</td>
<td>None</td>
<td>&lt;50</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Values in counts per second. Crystalline and diffuse data are based on Figures 6a and b, recrystallized data are based on Figure 6c, and Type 1, 2 and 3 data are based on Figure 6d.
haps this was the case in the past as well. However, moonmilk could have formed during overall lower temperatures (i.e. during an ice age), which would have caused slower precipitation rates as well as changed the types of elements available in the water that entered the cave and isotopic ratios. Warmer temperatures would have caused faster precipitation rates and different elemental and isotopic ratios. Changes in water chemistry would also affect the types of minerals that could precipitate, as well as the trace elements present in the minerals and the elements available for biological processes. It is possible that lower temperatures could have caused the small crystal size of the Spider Cave moonmilk (as well as moonmilk found in other caves).

**Spider Cave vs. Chocolate High**

One of Spider Cave’s lowest points in elevation (approximately 1260 m) is equivalent to one of the highest points of elevation within Carlsbad Cavern—Chocolate High (between 1210-1265 m).
Both Chocolate High and areas containing moonmilk within Spider Cave show similar structural trends and are within jointed limestone and dolomite facies of the Yates Formation, which lies between 1210 m to 1265 m in elevation (Hill, 1992). Moonmilk areas of Spider Cave are all within limestone and dolomite; specific rock layers occupied by moonmilk in Chocolate High have not been identified, but Chocolate High is within dolomite and limestone layers that typify the Yates Formation. Despite similarities in the formation of the areas containing moonmilk and the geology of the parent rock, moonmilk in Spider Cave is fundamentally different in distribution and character to that of moonmilk in Chocolate High. Spider Cave has rooms completely coated with calcite-rich moonmilk, displays evidence of flooding by meteoric water, and the entrance is located within an arroyo. Chocolate High has only small patches of moonmilk (1-10 cm in length and less than 1 cm thick), no evidence of flooding by surface water, and no source of surface water (Perrone, 2005). Chemical analyses of moonmilk in other areas of Carlsbad Cavern indicate occurrences of aragonite, hydromagnesite, huntite and dolomite-rich moonmilk; there are no recorded occurrences of calcite-rich moonmilk such as the Spider Cave moonmilk (Hill, 1992). Although moonmilk in Chocolate High has not been analyzed, it is similar enough to moonmilk in the rest of Carlsbad Cavern to hypothesize that it is not calcite-rich (Table 3).

There are two main differences between Spider Cave and Chocolate High: (1) the entrance of Spider Cave is in an arroyo, providing direct access to surface water, while there is no water source leading into Chocolate High; and (2) the location of Spider Cave moonmilk is at the lowest point of elevation for the cave system, which would allow for the pooling of water, while Chocolate High is at one of the highest points of elevation, and therefore water is unlikely to pool in that area for long periods of time.

Thus, while the limestone and dolomite contribute to the composition of the moonmilk by providing ions, the location of the areas that contain moonmilk within Spider Cave allow for the formation of moonmilk in great quantities due to the pooling of water. Only patches of moonmilk are possible in Chocolate High as water cannot pool in this area. Also, the entrance to Spider
Three main morphological and compositional types appear to dominate Spider Cave moonmilk: Type 1 crystalline, Type 2 diffuse, and Type 3 recrystallized. These types are not always distinct; often there appears to be a combination of these three types in a given sample.

Compositional analyses show that there is a predominance of carbon and oxygen in all three types. However, Type 1 and Type 2 morphologies have a lower amount of carbon and oxygen and a higher amount of silica, aluminum, sulfur, magnesium and calcium than Type 3 or the combination of types.

The abundance of elements (calcium, magnesium, aluminum and silica) present in a given sample (Table 2) roughly corresponds to the elevation at which the sample was taken. Samples with low concentrations of these elements (and much higher concentrations of carbon and oxygen) and completely recrystallized calcite are at the lowest elevations where they would have remained in the phreatic environment the longest and experienced the most recrystallization. Those samples that have higher amounts of elements and have both Type 1 and Type 2 morphologies are at the highest elevations where they would not have been underwater as long and were thus subject to less recrystallization. Those with a combination of morphologies and an intermediate elemental concentration are found in the middle elevations.

If elements such as silica, aluminum, sulfur, magnesium, and calcium were once abundant (or at least present) in the original fabrics (i.e. Type 1 and Type 2) they appear to have been leached out by the meteoric flood waters during the recrystallization process (Type 3, and mixed fabric comprising Types 1, 2 and 3). Type 1 crystalline morphologies have continuous, regular layers that do not vary in thickness. The calcite crystals in these layers are distinguishable. Due to the regular nature of the Type 1 layers, these are interpreted as abiotic crystals (Grotzinger and Rothman, 1996; Tucker and Wright, 1990; Bathurst, 1975). Type 2 diffuse morphologies have discontinuous, irregular layers that vary in thickness. The calcite crystals in these layers are too small to be distinguished. Due to the diffuse, irregular nature of the Type 2 layers, these are interpreted as biotic layers (Grotzinger and Rothman, 1996; Tucker and Wright, 1990; Bathurst, 1975).

Type 1 and Type 2 morphologies may be the original morphologies present in the samples. These types are found at the highest elevations, which have remained underwater for the shortest amount of time. Type 3 recrystallized morphologies lack both the Type 1 and Type 2 characteristics; rather they are homogenous calcite. Type 3 morphologies occur at the lowest elevations where they would have remained underwater the longest. Type 3 is interpreted as being recrystallized Type 1 and Type 2 forms. Type 2 diffuse layers show the highest concentrations of aluminum and sulfur, which possibly indicates the use of sulfur in microbial processes and the possible trapping of silt and mud by a microbial mat.

Alternating biotic and abiotic layers (Type 1 and Type 2) could be the result of a variety of factors. Stromatolites can result from an influx of storm water covering microbial mats with sediment, thus producing layers of biotic mats and abiotic sediment (Reid et al., 2000; Riding, 2000). In Spider Cave, flood waters entering the cave could have mechanically altered the biotic layers by depositing sediments on top of them. Chemical analyses of the moonmilk samples indicate that aluminum and silica are present, and they appear to be most abundant in the transition between biotic and abiotic layers. Influx of meteoric water also could have changed the water chemistry, allowing for increased or decreased microbial activity. Finally, flood waters would have caused a release of CO₂ into the cave environment, which could have caused the precipitation of abiotic crystals. Micropor-sized crystals indicate that crystallization may have happened relatively quickly, resulting in the formation of smaller crystals. Once the flooding and the churning of the water stopped, a standing pool of water would have been left, allowing for microbial activity to commence.

**Future Studies**

A new classification system for moonmilk is necessary; the term moonmilk is too general. Each moonmilk cave needs an in-depth study such as this to determine the depositional environment. Only then can a new classification system be implemented.

**CONCLUSIONS**

1. A comparison between moonmilk found in Spider Cave to moonmilk found in Chocolate High of Carlsbad Cavern suggests that standing water is necessary for Crisco moonmilk to form.
2. Spider Cave moonmilk shows three main morphology types: Type 1—crystalline (continuous, regular layers with microspar-sized crystals that do not take the Alizarin Red S stain), Type 2—diffuse (discontinuous, irregular layers with micrite-sized crystals that stain dark) and Type 3—recrystallized (no layers with micrite-sized crystals that stain light).
CHARACTERIZATION OF MOONMILK AT SPIDER CAVE

Also present are morphologies that show a combination of these types.

3. Type 1 and Type 2 have higher abundances of calcium, magnesium, aluminum, silica and sulfur, and Type 3 has higher abundances of carbon and oxygen.

4. Elemental abundance corresponds to elevation; lower elevation sites (i.e. Type 3 samples) would allow water to pool longer, thus allowing recrystallization to take place for a longer period of time than in those areas that are at higher elevations (Type 1 and 2 samples).

5. The Type 2 diffuse layers typify biotic processes, while the Type 1 crystalline layers typify abiotic processes. This suggests that the original fabric was formed similarly to stromatolitic processes wherein microbial activity in a mat is followed by abiotic processes.

6. A new classification system for moonmilk must be generated to differentiate among different forms of moonmilk.

REFERENCES


Scanning electron micrograph of calcite moonmilk in Cueva de Las Barrancas, New Mexico. Calcite rods and draped biofilm and microorganisms dot the miniature landscape. Pasty white moonmilk may be a product of biological interaction with the rock surfaces of caves. It occurs in caves all over the world and its origin is an active topic of investigation. Image by M. Spilde and P. Boston.