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OBSERVATIONS FROM ACTIVE SULFIDIC KARST SYSTEMS: IS THE PRESENT THE KEY TO UNDERSTANDING GUADALUPE MOUNTAIN SPELEOGENESIS?

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Abstract.—Field investigations in active sulfide-rich cave systems in Mexico, Italy, and the United States have documented a three-step process of speleogenesis that includes an early sulfide-rich phreatic phase forming incipient karst conduits, a secondary sulfide-rich vadose-phreatic phase of chemical stoping, and a tertiary sulfide-depleted vadose phase of epigenic speleogenesis. The vadose-phreatic phase produces many geologic and biological characteristics unique to caves formed in sulfidic and hypogenic mixing environments. The tertiary vadose phase may destroy most of the evidence linking an ancient cave to a sulfidic origin. Commonly preserved evidence of previous sulfide-driven speleogenesis includes horizontal levels of large passages connected by vertical rifts, abruptly terminating passages, major passages formed parallel to axial planes, gypsum crusts on walls and ceiling, piles of gypsum sediments on the floor, subterranean rills, and speleosols. Caves in the Guadalupe Mountains prominently display all of these features. The robust microbial communities in vadose-phreatic phase sulfide-rich caves also impart characteristic isotopic, mineralogical, and lipid signatures that deserve further study in both active and ancient sulfidic cave environments.

INTRODUCTION

The discovery, exploration, and scientific investigations over the last twenty years into actively forming, sulfide-rich caves have advanced our understanding of important processes in the development of many karst systems. Multi-disciplinary teams investigating caves in Italy, Mexico, the United States, and elsewhere have recognized the aggressive, subaerial dissolution of conduits in sulfide-rich karst systems and the importance of chemoautotrophic bacteria in accelerating these processes. They also report common geologic, biological, and geomicrobiological characteristics in these caverns that distinguish them from epigenic caves. Some of these characteristics remain after the sulfidic waters abandon a cave system and preserve evidence of the origin of the spectacular Guadalupe Mountains caves in southeast New Mexico.

The sulfide bearing waters in all active examples discussed in this paper rise from depths as subterranean springs. The hypogenic waters are warmer than the ambient cave air and wall rock temperatures and have elevated CO_2 concentrations. Cooler meteoric water infiltrates from the surface and mixes with the hypogenic waters. These environmental influences of hydrochemical mixing, interactions between the phreatic and vadose zones, elevated CO_2, and convection/evaporation driven atmospheric circulation with vigorous condensation on walls and ceilings, strongly contribute to the morphology of these caves (Palmer, 1991). This paper presents a series of common observations from active sulfidic systems that may provide testable hypotheses for future exploration and study of both active and ancient sulfide-rich karst systems.

ACTIVE SULFIDIC KARST SYSTEMS

Cueva de Villa Luz, Tabasco, Mexico

The 2000-m long Cueva de Villa Luz (a.k.a. Cueva de las Sardinas) in Tabasco, Mexico (Fig. 1), contains a very aggressive, sulfidic environment and may display the most dynamic example of dissolution speleogenesis in the world. The cave passages form a ramiform and spongework maze with a generally horizontal, stream-bearing floor (Fig. 2 and 3) within the anticlinal flank of Cretaceous platform-margin limestone (INEGI, 1989) with patches of rudist-bearing bioherms. Most passages have irregular rooms with dead-end galleries and blind alcoves branching outward. Cupolas and more than 20 skylight-entrances punctuate the ceiling profile and result in ~25 m of vertical relief.

Hydrology and meteorology

More than two dozen subterranean springs rise through conduits in the cave floor ranging from ~2 cm wide to approximately 1 m x 2 m. The combined subterranean springs coalesce into a

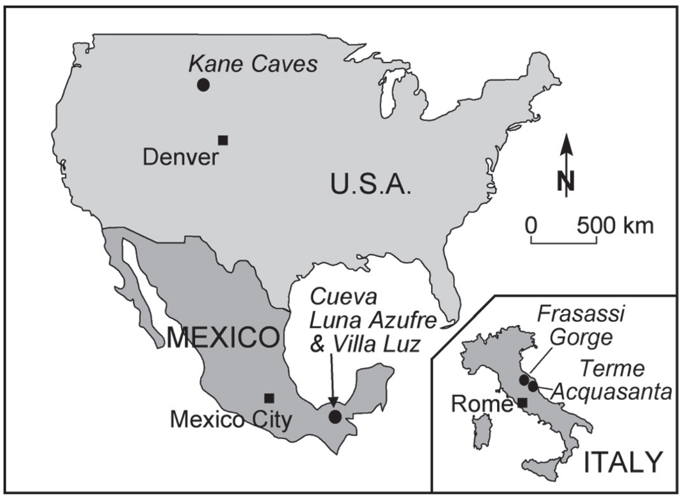

FIGURE 1. Site map of caves discussed in this article.

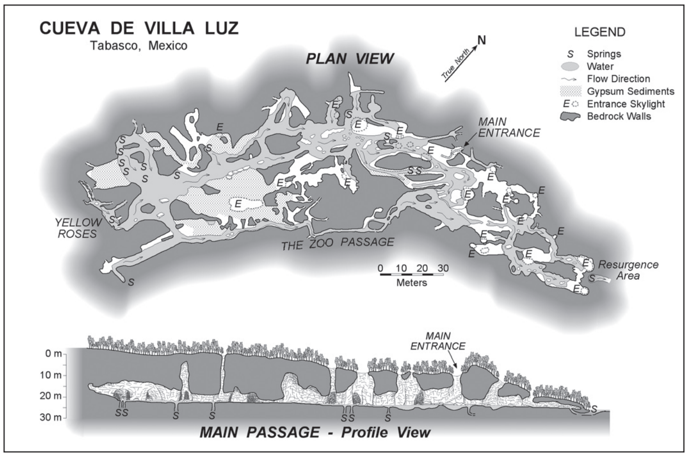

FIGURE 2. Map of Cueva de Villa Luz, Tabasco, Mexico (after Hose and Pisarowicz, 1999).

surface resurgence spring with a discharge of ~270-300 L/sec that is remarkably unaffected by the seasonal, tropical rainfall. No evidence of significant cave passages below the water table exists. The source of the sulfide is undetermined as there are several potential supplies. Recent investigations have supported derivation from the magmatic system associated with El Chichón volcano, 50 km to the west (Spilde *et al.*, 2004). However, brines from the rich petroleum basin or evaporite beds ~65 km north of the cave and a closer Tertiary magmatic system may also provide sulfide-rich waters (Hose and Pisarowicz, 1999).

The springs share a consistent temperature (28^{o}C) with similar salinity and dissolved ion concentrations. Calcite is almost exactly at saturation and dolomite is slightly undersaturated. The waters are >60% saturated with gypsum (Palmer and Palmer, 1998). H_2S, CO_2, O_2, and formaldehyde vary temporally and from spring-to-spring (Hose, 2001), which is interpreted to represent different degrees of oxidation before the water enters the accessible cave. Springs with high sulfide concentrations (generally the larger volume conduits) release H_2S into the cave atmosphere. Atmospheric H_2S in the cave ranges from <1 ppm in downstream and some middle cave passages to >120 ppm in the isolated, upstream Yellow Roses passage. The percentage of atmospheric H_2S, CO_2 (0.03% to >3.2%), and O_2 (20.8% down to <9.5%) can fluctuate rapidly, seemingly the result of natural, episodic gas "bursts" from the springs and, in some cases, from anthropogenic disturbance of stream sediments. The multiple skylight entrances provide good air exchange with the surface. Hence, the H_2S readily oxidizes in the cave atmosphere. The ambient, mean annual temperature (and bedrock temperature) of ~25^{o}C, 3^{o} less than the sulfidic spring waters, sets up convective air flow in the cave passages. The relatively warm water vapor released at the springs readily condenses on walls and ceilings.

Geomicrobiology and speleosols

While physical processes will combine the O_2, H_2S, and condensation in the atmosphere to form sulfuric acid (H_2SO_4), the energy released by the reaction and the readily available CO_2 attracts chemoautotrophic bacteria that facilitate the reaction. These bacteria are nearly ubiquitous in the cave and form the primary base of a complex and robust ecosystem (Hose and Pisarowicz, 1999; Hose *et al.*, 2000). Dense bacterial mats line walls of several small springs and one large spring releases abundant masses of bacterial stringers, typically 1-2 cm long. Thin white and red microbial veils coat stream bottoms for several meters downstream from most sulfidic springs. However, subaerial microbial colonies represent the most distinctive features in the cave.

Rubbery, white, stalactite-like "snottites" are Villa Luz's most famous subaerial feature. Composed of colonies of sulfur-oxidiz-

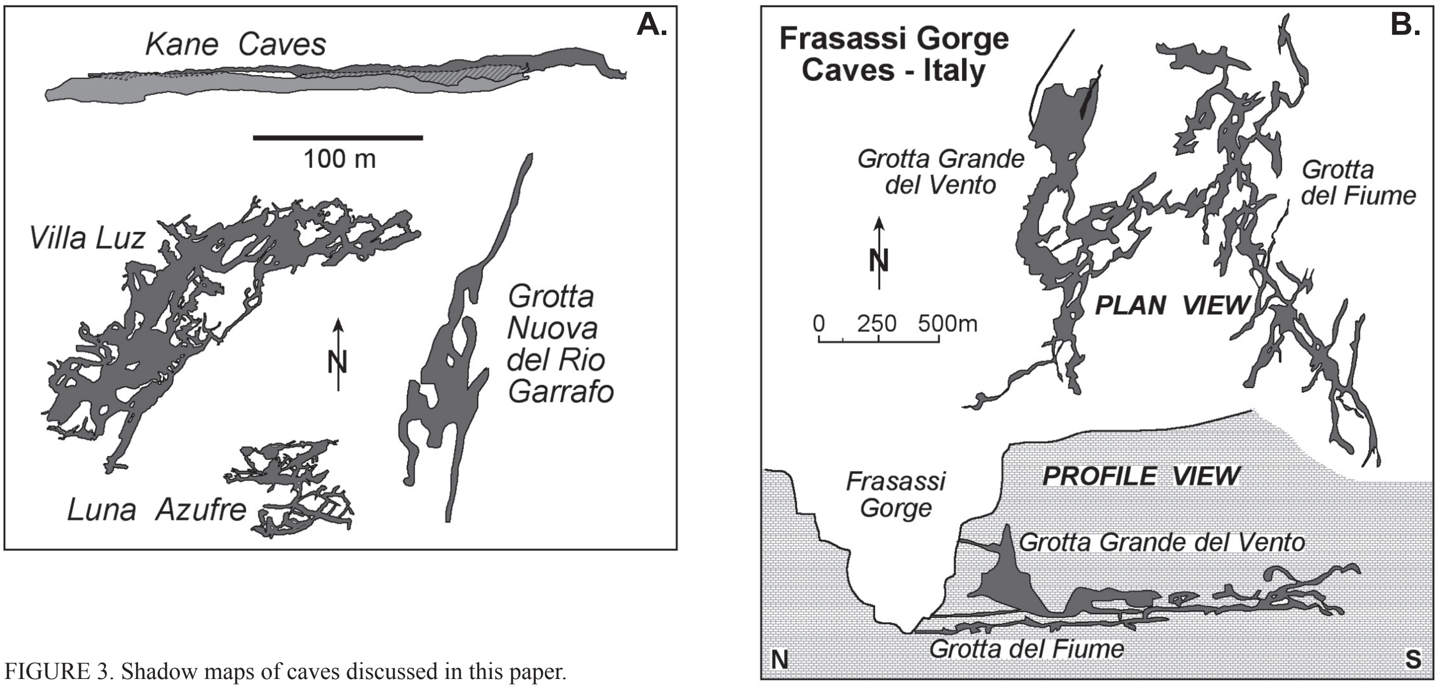

FIGURE 3. Shadow maps of caves discussed in this paper.

ing bacteria that produce sulfuric acid as a metabolic by-product (Hose *et al.*, 2000), individual snottites are ephemeral. They grow up to several centimeters per day, generally from the tips of coarse gypsum crystals (Fig. 4), in areas of relatively high atmospheric H_2S (typically ~3-25 ppm) and flourish during the rainy season (July – September). They do not grow in the passages with highest H_2S concentrations (typically >25 ppm). Snottites resemble soda straw stalactite, drapery, and "u-loop" speleothems, and drip sulfuric acid with measured pH values of 0.0–3.2 $\pm$0.1.

Bacteria and fungi make up black, grey, brown, beige, blue, and purple wall and ceiling coatings (Hose and Northup, 2004). Their complex geometric forms resemble clay vermiculations (Hill and Forti, 1997), but most of these deposits lack significant clay and, therefore, have been dubbed "biovermiculations" (Hose and Pisarowicz, 1999). The abundant, mushy colonies occur on bare limestone walls throughout most cave passages with very low (<1 ppm) to moderate (<25 ppm) H_2S concentrations. They exhibit average pH values of ~3.0. Some vermiculations trap sediments from clay seams higher on the wall and, perhaps, dust in the air. The biovermiculations nearly disappear near entrances during dry surface conditions, leaving thin shadows of mineral (gypsum and/or clay) deposit, and re-grow during the wet summer and fall seasons (Hose and Northup, 2004).

In addition to the notable and concentrated microbial colonies of snottites, biovermiculations, mat linings of springs, and stream floor veils, chemoautotrophic bacteria are nearly ubiquitous in sediments and weathered surfaces throughout the cave. Colorful, dense wall coatings ("speleosols") formed by microbial activity, fine-grained clastic sediments washed in from clay seams higher in the bedrock wall, and by-products (precipitates and residues) of the corrosion of limestone wall rock harbor abundant microbes (Northup *et al.*, 2005). Microcrystalline gypsum displays bacteria in SEM images (Hose *et al.*, 2000) and actinomycetes commonly colonize the surface of the gypsum pastes and speleosols.

Geologic features and speleogenesis

Two minerals typify Cueva de Villa Luz: Gypsum and elemental sulfur. Coarsely crystalline gypsum, including small "chandeliers" up to 4 cm long, and microcrystalline gypsum coat most walls throughout the cave. Gypsum deposits have a typical pH value of 3. The underlying limestone, which is extraordinarily pitted and etched, reveal where the gypsum has washed away (Fig. 5a and 5b). Subaerial deposits of sulfur cover the coarsely crystalline gypsum and are particularly prominent on the lower ~2 m of walls near the Yellow Roses spring, where the atmospheric H_2S concentration typically ranges from 30-100 ppm. The sulfur deposits are mushy (suggesting a large biological compo-

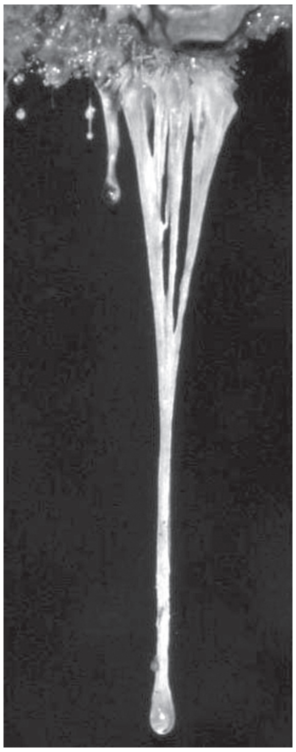

FIGURE 4. "Snottites" suspended from selenite crystals in Cueva de Villa Luz. Photo by L. Hose.

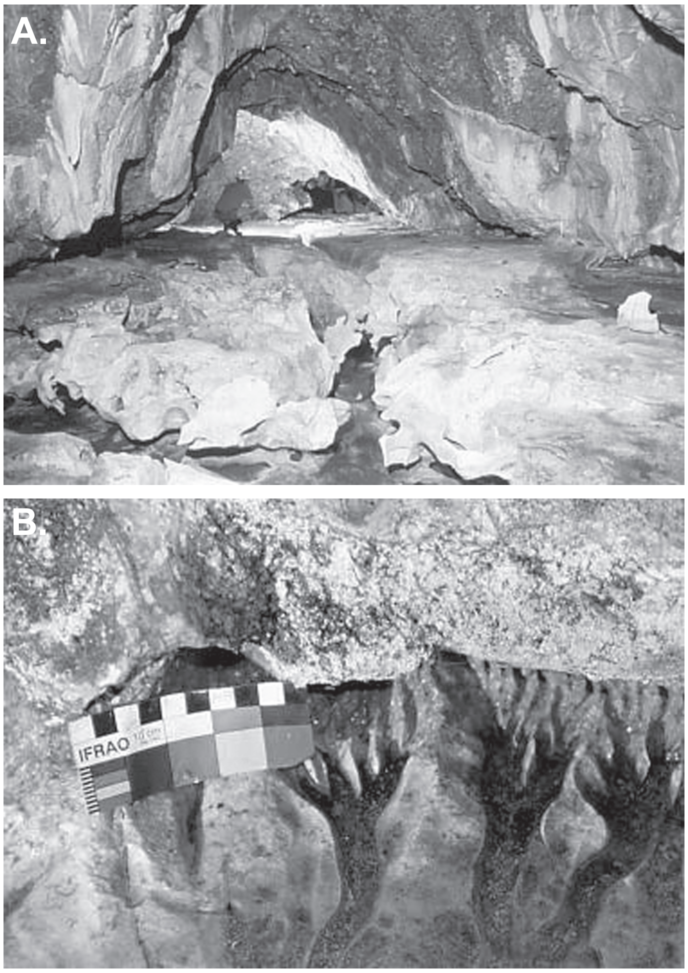

FIGURE 5. **A.** Downstream side passage in Cueva de Villa Luz. Note the exposed, highly corroded bedrock in the lower part of photo, where the stream has risen during floods and removed the overlying gypsum sediments. Photo by James Pisarowicz. **B.** Rills developed in limestone bedrock along the Cueva de Villa Luz stream shore in the middle part of the cave. Photo by L. Hose.

nent) and display "folia" morphologies. Calcite, mud, and halite folia have previously been considered subaqueous products of a declining, hypogenic water table (Davis, 1982; Hill, 1987; Hill and Forti, 1997). Villa Luz's folia are the only sulfur manifestation ever reported and they are actively growing in the subaerial environment.

A chemical stoping process currently enlarges the subaerial passages as limestone (bedrock) ceilings and walls exposed to the acidic atmosphere convert to gypsum coatings (Palmer and Palmer, 1998; Hose and Pisarowicz, 1999; Hose *et al.*, 2000). This process is accompanied by a nearly two-fold volume increase in the rock (White and White, 1969; Palmer and Palmer, 2000). The pasty microcrystalline gypsum readily falls off overhanging surfaces (occasionally onto the head or shoulder of passing speleologists), forms sediment piles locally exceeding one meter thick, and slides down slopes. These small, subterranean "glaciers" ultimately either calve into the cave stream or the stream rises during an intense, localized storm. The undersaturated stream immediately dissolves the gypsum and removes it.

On the subaerial slopes, pits form in the gypsum deposits. The pits, some extending into the underlying limestone, are rill-lined and attributed to dissolution by dripping meteoric or condensate water. The rills on the underlying limestone surfaces are presumed to result from sulfuric acidity picked up by the dripping water as it flows along and through the gypsum. Less commonly, the coarsely crystalline crusts follow a similar fate of falling into the streams (due to crystal wedging), dissolving, and leaving the cave. The stoping process exposes limestone bedrock that biovermiculation colonies (apparent "pioneer" species) quickly claim and alter to new gypsum coatings.

Cueva Luna Azufre, Tabasco, Mexico

Another, active sulfidic cave, ~500 m southeast of Villa Luz and was discovered in 2005, partially explored, and 559 m of passage surveyed (Pisarowicz *et al.*, 2005). The four subterranean springs in Cueva Luna Azufre differ notably from the hypogenic waters in Villa Luz. Luna Azufre water temperatures vary from 29.0^{o} C to 30.3^{o} C, 1^{o} – 2^{o} warmer. All springs have very low flows (<1 L/sec), much higher salinity, generally higher dissolved oxygen, and lower hydrogen sulfide compared to Villa Luz springs. However, many of the same biological and geologic features mark Cueva Luna Azufre, including ramifying passages with irregular outlines, dead-end galleries, blind alcoves, and ceiling cupolas (Fig. 3a), skylight entrances, multiple springs, horizontal stream floors, widespread macro- and microcrystalline gypsum wall coatings, piles of stoped gypsum sediments on subaerial floors, biovermiculations, snottites, and fragments of bacterial mats ejected by spring waters.

Kane Caves, Wyoming, U.S.A.

The Kane Caves provide a compelling comparison of an active sulfidic cave and an abandoned sulfidic cave in the same setting (Fig. 6). An investigation of the 337-m long Lower Kane Cave in the Bighorn Basin of Wyoming (Fig. 1) by Egemeier (1973) provided the first English description of active sulfuric acid speleogenesis. Egemeier persuasively argued that the immediately overlying 310-m long Upper Kane Cave represented an older product of the same processes and setting. Egemeier (1971; 1987) further suggested that the processes observed in Lower Kane Cave may explain the speleogenesis of Carlsbad Cavern. Approximately 30 vertical meters separate the Kane caves, which have developed within the shallow-water, continental platform Mississippian Madison Limestone along a Little Sheep Mountain Anticline axial plane. Although no physical connection has been established between the two caves, the upper cave breathes out and the lower cave breathes in during very cold weather, suggesting air flow between them (Egemeier, 1973). Both caves have singular, linear "borehole" passages with nearly horizontal bedrock floors (Fig. 6 and 3a).

Lower Kane Cave

Most of Lower Kane Cave's walls and ceiling are covered with macro- and microcrystalline gypsum. Biovermiculations cover bare limestone on the wall, actinomycetes grow in several places, and a black speleosol covers one area (Hose and DuChene, 1999). The subaerial floors are mostly covered by stoped gypsum sediments. The lower cave has five low-flow (<1 L/sec) subterranean springs (some intermittent) rising from small, narrow rifts in the floor. The likely source of the sulfide is the adjacent Bighorn Basin petroleum reservoirs (Egemeier, 1971). Work by Engel *et al.* (2004b) demonstrated that sulfur-based chemoautotrophic bacteria in the cave facilitate the rapid, subaqueous dissolution of the limestone bedrock. Calcite speleothems are limited to a small area of flowstone and a few small stalactites. The Bighorn River back-floods into the cave, removes gypsum, and deposits mud near the mouth of the cave.

Upper Kane Cave

Limited remnants of gypsum remain in Upper Kane Cave and display rill-lined drip holes (pits) similar to those observed in Villa Luz's gypsum glaciers (Hose and DuChene, 1999). Most of the walls expose bedrock limestone and limestone breakdown

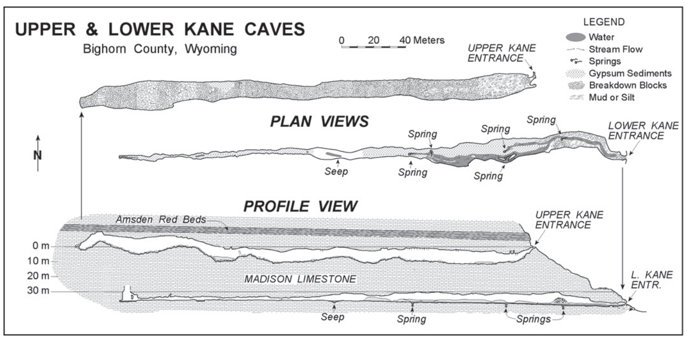

FIGURE 6. Map of Upper & Lower Kane Caves, Big Horn Basin, Wyoming. Upper Kane Cave immediately overlies Lower Kane Cave and has been offset on the plan view to show floor detail.

covers most of the floor. Calcite speleothems are more common than in the lower cave. Meteoric infiltration and mass wasting became the dominant processes after the sulfidic water table dropped. Gypsum wall and ceiling coatings detached and fell to the floor in most places. Limestone rockfalls from the ceiling covered the gypsum floor deposits ("glaciers") and, probably, rifts in the floor that previously served as subterranean springs. When the water table dropped and the relatively dense sulfidic gases stayed in the lower cave, chemoautotrophic bacteria in the upper cave lost their primary energy source. Today, no visible evidence of chemoautotrophic microbial colonies, such as biovermiculations, veils, actinomycetes, or stringers, has been reported.

Grotta del Fiume – Grotta Grande del Vento, Frasassi Gorge, Italy

The Frasassi Gorge cuts through a large anticline comprising a Lower Jurassic carbonate platform in the Apennines of east-central Italy (Fig. 1) (Coccioni *et al.*, 2002), exposing the entrances to numerous caves. All appear to have been formed by hypogenic, sulfidic processes (Galdenzi, 1990) but the Grotta del Fiume – Grotta Grande del Vento cave system provides a spectacular natural observatory of speleogenesis and modification in both active and abandoned passages. Grotta del Fiume is a resurgence cave with passages near and at the current sulfidic groundwater table. Higher passages now abandoned by the sulfidic waters comprise Grotta Grande del Vento, including two kilometers of spectacular commercialized passageways and chambers that rival the beauty and grandeur of Carlsbad Cavern. The landmark room, Ancona Abyss, has a volume of ~1 million m^3 (Galdenzi and Menichetti, 1995). A skylight entrance into the largest chamber is the only known natural entrance to the Grotta Grande part of the cave. The upper and lower cave, connected by a single passage, form a >20 km long system.

The entire system displays a ramifying morphology of irregular rooms, dead-end galleries, and blind alcoves branching outward with cupolas punctuating the ceilings and walls (Fig. 3b). Several other nearby caves with ~5 km of passages display the same general morphology. The gorge has multiple levels of mainly horizontal passages (1-10 m in diameter) in complex mazes and large rooms with flat, erosional rock floors and dome ceilings. The cave passage levels match alluvial gravel terraces in the adjacent surface valley and probably correlate to the same time of development (Bocchini and Coltorti, 1990). Tuccimei (2004) used uranium-thorium dates of calcite speleothems to demonstrate that the highest passages are older (>200 ka), based on the earliest date of vadose speleothem growth, and progressively lower passages are younger.

Hydrology and meteorology

Passages in Grotta del Fiume provide windows on the phreatic zone, although there is no evidence of cave passages large enough for humans to enter below the water table (Galdenzi, 2005). Two distinctive water types have been identified. Hypogenic water is sulfidic (up to 18 mg/L), saline (<2 g/L and rich in Cl^-, Na^+, and SO_4^-), and undersaturated with respect to gypsum (Cocchioni *et al.*, 2003). The source of the sulfide has been interpreted by Galdenzi and Menichetti (1995) as the product of sulfate reduction in underlying anhydrite and gypsum beds. Oxygen and hydrogen isotope data suggest that the sulfidic waters have a meteoric origin and a residence time in the aquifer of several decades (Tazioli *et al.*, 1990). Carbonate-rich, downward percolating water may make up 60% of the exposed phreatic waters during the spring but drops closer to 30% at the end of summer (Cocchioni *et al.*, 2003). This water infiltrates from the surface (mean rainfall is ~100 cm/a) with a low salinity, very low sulfate content, and high dissolved oxygen (Galdenzi and Maruoka, 2003). Stratification between the two waters occurs in some locations with the upper freshwater layer ranging from 0.2 to 5 m thick (Galdenzi, 2001).

The atmosphere in the active, lower passages contains low levels of H_2S (typically <8 ppm) and slightly elevated CO_2 (up to 0.06%) (Galdenzi, 2001). Highest concentrations of both gases are near the subterranean springs. Water temperature in the cave is ~13.5^{o}C, close to the air temperature of 13 °C, thus convection circulation is probably a minor process in the Frasassi Caves.

Geomicrobiology

Sulfur-oxidizing bacteria play an important role in the speleogenesis of the Frasassi Caves (Galdenzi *et al.*, 1999; Galdenzi and Sarbu, 2000), and can be studied in detail in the lower cave levels (Grotta del Fiume). Microbial mats on stream floors and subaerial walls, stringers just below the groundwater surface, snottites dangling from macrocrystalline gypsum clusters on walls, and dripping sulfuric acid with pH values <1 are common in the lower passages. Biofilm communities in sulfidic zones at Frasassi were surveyed using 16S rDNA cloning and fluorescence *in situ* hybridization (FISH) methods (Macalady *et al.* 2006; Macalady *et al.*, in prep.). Stream biofilms are white or grey, and dominated by relatives of known sulfur oxidizing and reducing groups in the γ-, β-, δ- and ε-Proteobacterial lineages. Several morphologically and phylogenetically distinct biofilm types typically co-occur in stream beds in a dynamic mosaic pattern influenced by changes in water flow rates and oxygen and sulfide concentrations. Sequences related to filamentous sulfate-reducing *Desulfonema* and sulfur-disproportionating *Desulfocapsa* species are particularly abundant in many of the biofilms, indicating that microorganisms mediate both sulfur oxidation and sulfur reduction within the streams. *In situ* measurements of S speciation in bulk stream water and within specific biofilm types using microelectrode voltammetry provide strong evidence for microbial acceleration of sulfur oxidation above abiotic rates, and confirm that phylogenetic differences between biofilm types result in differences in the pathways and extent of biological sulfur oxidation and reduction (Druschel *et al.*, 2005).

Snottites at Frasassi typically have pH values between 0 and 1.5 and drip from macroscopic (1-2 cm long) $CaSO_4 \cdot 2H_2O$ crystals or from microcrystalline gypsum crusts associated with elemental sulfur. The snottites are among the lowest diversity microbial communities known (Macalady *et al.*, 2006a). Prominent microorganisms in the snottites are relatives of the extreme acidophiles *Acidithiobacillus thiooxidans*, *Acidimicrobium ferrooxidans*, the archaeon *Ferroplasma acidophilum*, and filamentous fungi. Protists, *Sulfobacillus* species, and members of uncultured bacterial lineage TM6 are present in much lower cell densities. Cells that hybridize with *Acidithiobacillus*-specific oligonucleotide probes and oxidize thiosulfate and elemental S can be readily cultured from the snottites (Vlasceanu *et al.*, 2000; Jones *et al.* 2005). Microorganisms are ubiquitous in slightly less acidic (pH 1.5 - 4.5) microcrystalline gypsum crusts that cover much larger areas of limestone walls than the snottites. Microorganisms in the gypsum crusts have an average density of 10^6-10^7 cells per cm^3 (Lyon *et al.*, 2004).

Abundant biovermiculations grow on exposed limestone walls lacking gypsum crusts throughout the lower (Grotta del Fiume) cave, but are extremely rare in the upper cave (Grotta Grande del Vento). Unlike stream biofilms and snottite communities, the biovermiculations surveyed to date do not contain relatives of any known sulfur oxidizing bacteria, raising the question of what chemical species power their growth. The biovermiculation bacteria are extremely diverse, representing largely uncultured groups in 17 different major evolutionary lineages (Macalady *et al.*, in prep.).

Geologic features and speleogenesis

Abundant macro- and microcrystalline gypsum and some elemental sulfur occur on subaerial surfaces in the lower cave passages. Galdenzi (1990) attributes all gypsum in the cave to subaerial processes. Chemical stoping, as described in the Cueva de Villa Luz section, actively enlarges the Grotta del Fiume passages (Galdenzi and Maruoka, 2003). The underlying limestone is riddled with corrosion pockets, which become evident following removal of the gypsum coating.

Galdenzi *et al.* (1997) reported on a 5-year experiment in which limestone tablets were suspended in the air immediately above and in the water immediately below the sulfidic water/air interface. Microcrystalline gypsum formed on the subaerial tablets but the undersaturated groundwater prevented gypsum growth on the subaqueous tablets. The average weight loss, measured after gypsum removal, was an aggressive ~15-20 mg/cm^2/a for both settings. The dissolution rates accelerated with time. A biofilm at the limestone-gypsum interface provided direct evidence of the important H_2S oxidizing role of Grotta del Fiume's bacterial communities.

The upper passages (Grotta Grande del Vento) contain massive gypsum floor deposits (Fig. 7) as well as huge calcite speleothems, reminiscent of Big Room in Carlsbad Cavern. The gypsum "glaciers", products of wall and ceiling coatings falling to the floor after the water table retreated from the upper level, have a maximum thickness of 5 m and volume of >1000 m^3. However, infiltration of freshwater from the surface has removed most of the gypsum that once covered the walls, ceiling, and floor. Rockfall and speleothem growth have provided extensive vertical relief on the previously nearly horizontal floor.

Galdenzi (1990) summarizes speleogenesis in the Frasassi Caves as a three-step process at each level:

1. Phreatic phase – Many small tunnels start to form by corrosion in the uppermost phreatic zone.
2. Vadose-phreatic phase – Long stability of the water table accompanies a gradual and progressive enlarging of the cave passage. Hydrologic circulation facilitates gas exchange, releases H_2S and water vapor into the cave atmosphere, and forms a strongly corrosive environment.
3. Vadose phase – Phreatic circulation drops and develops a new, deeper cave network. Percolating vadose water removes gypsum deposits and precipitates calcite speleothems.

GROTTA NUOVA DEL RIO GARRAFO, ACQUASANTA, ITALY

Italy boasts many other interesting active and paleo-sulfidic caves but Grotta Nuova del Rio Garrafo near the town of Terme Acquasanta, Italy (Fig. 1), must rank near the top. The cave has formed in pre-Miocene limestone in the core of an eastern Apennine anticline. The entrance is ~10 m directly above a perennial, freshwater, surface stream yet the cave passages form a ramifying pattern of irregular rooms with dead-end galleries and blind alcoves branching outward that ultimately drop ~50 m (40 m below the streambed) to a sulfidic aquifer.

A reconnaissance investigation of the cave revealed that the upper and middle levels of passages provide little evidence of a

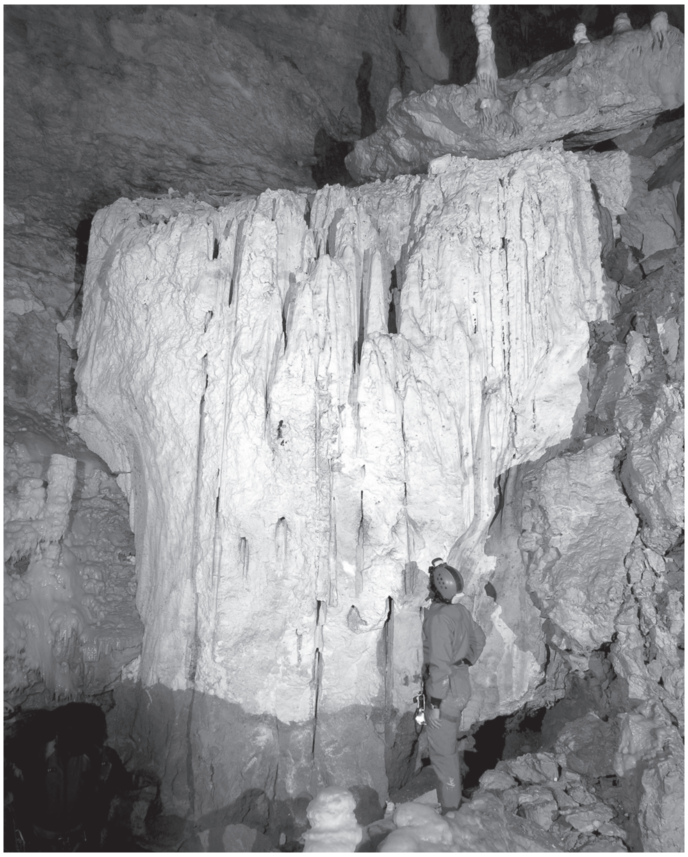

Figure 7. Massive gypsum sediment pile ("glacier") in Grotta Grande del Vento with rill-lined pits formed by dripping meteoric or condensate water. Photo by Art and Peg Palmer.

sulfidic origin, although the pattern of passages suggests hypogenic and/or mixing processes. Fine-grained, clastic sediments (probably from flooding by the surface stream) cover most of the floor but there are no permanent streams. The air temperature is 10^{o}C in these parts of the cave. The walls are bedrock, although one room in the middle of the cave displays prominent microbial clusters on the walls, assumed to be actinomycetes colonies.

Deeper in the cave, the air temperature is higher and a fog rises from the pits and passages below. Coarsely crystalline gypsum grows on the walls. Low levels of H_2S become detectible. The air temperature, fog, gypsum deposits, and H_2S progressively increase with decreasing elevation in the cave. Hundreds of tiny (<1 cm) snottites hang from coarse gypsum crystals in the last chamber above the sulfidic stream passage. The snottites are composed of bacteria, archaea related to *Ferroplasma* species, and filamentous fungi, similar to snottites from other sulfidic caves (Jones *et al.*, 2005).

Walls in the narrow stream passage are covered with gypsum, elemental sulfur, and apparent speleosols. A small, 42^{o}C stream hosting thick white microbial biofilms enters the chamber from a thin crack at the base of a wall. Galdenzi (2005) reports that the water is highly saline (measured as >6 g/L) with significant amounts of chloride, sulfate, and carbonate, and the atmosphere has ~2.7% CO_2, >250 ppm H_2S, "high" SO_2, and 16.5% O_2. There has not been a full accounting of what gases displace the oxygen in this passage. The source of the sulfidic, thermal water is also not well understood.

COMMON FEATURES IN ACTIVE SULFIDIC CAVES

All of the caves in this study share the following traits: 1) Major passages formed parallel to axial cleavage within the core of anticlines. These anticlines may represent shallow drapes over buried faults, providing relatively permeable pathways to facilitate the flow of hypogenic waters; 2) Phreatic conduits much smaller (too small to allow human entry) than most air-filled passages, which tend to be many meters wide and high; 3) Multiple springs rising from the floor through narrow rifts, holes, or sediment into large, subaerial, nearly horizontal stream passages; 4) Extensive macro- (including selenite and chandelier forms) and microcrystalline gypsum weathering products on walls and ceilings; 5) Large piles of gypsum sediments on floors resulting from chemical stoping in subaerial passages; 6) Abundant chemoautotrophic microbial biofilms in both the subaqueous and subaerial environments, including abundant biovermiculations on exposed limestone bedrock where sulfidic gases are very low to moderate and snottites suspended from macrocrystalline gypsum in areas with low to moderate levels of H_2S; 7) Rills within and under gypsum sediments on vertical and sub-vertical walls; 8) Speleosols on subaerial walls; 9) Abruptly terminated passages and cupolas; 10) Sulfuric acid drips with pH values below 3; 11) Dead-end galleries, blind alcoves, and, with the exception of the Kane Caves, ramifying mazes of irregular rooms with passages branching outward (Fig. 3); 12) With the exception of the Kane Caves, elemental sulfur on non-carbonate (generally gypsum), subaerial walls near highly sulfidic, subterranean springs; and 13) Thermal gradients between the sulfidic water and wall temperatures that facilitates mild to vigorous convective air exchange and condensation processes.

PROPOSED SPELEOGENESIS MODEL

All caves in this study support the three-step model proposed by Galdenzi (1990). The early phreatic phase develops incipient conduits near the top of the sulfidic aquifer (Fig. 8: Phase 1). The corrosive strength of these hypogenic waters is restricted until they mix with infiltrating, meteoric water or air. Thus, phreatic conduits remain small below the mixing boundary. Once the sulfidic water table drops, the vadose-phreatic phase accelerates conduit enlargement, mostly through another multi-step process of sulfidic subterranean spring waters degassing, convection driving the relatively dense H_2S gas upward through the cave atmosphere, microbial oxidation of H_2S to H_2SO_4, which alters limestone walls and ceilings to gypsum, chemical stoping of the limestone walls and ceilings, and removal of the resulting spoils (gypsum sediments) as solute in cave streams (Fig. 8: Early Phase 2). Stream passages and paleo-phreatic rifts rapidly enlarge during this phase. As the water table drops, surface downcutting continues, sulfidic waters and gases abandon the original passage, and lower passages may form (Fig. 8: Late Phase 3). Epigenic processes also impact the cave development, but the aggressive speleogenesis overwhelms their impact.

Once the water table drops sufficiently to remove the influence of H_2S in the atmosphere, epigenic processes dominate and may eliminate direct evidence of the earlier sulfidic environment (Fig. 8: Phase 4). Infiltrating freshwater removes gypsum and sulfur. The water's carbonic acid component may also continue dissolving the bedrock walls and floors, removing the morphologies left by sulfuric acid speleogenesis. Organic and detrital material from the surface may be introduced and cover floors and shelves. Rock fall within the cave also covers the floor, potentially hiding the previous upwelling rifts, rills, and gypsum deposits. Calcite speleothems also cover the original morphology of the ceilings, walls, and floors. Chemoautotrophic microbes mostly or entirely

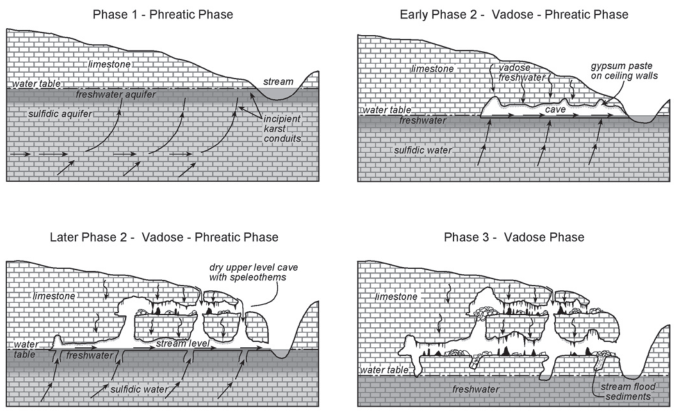

FIGURE 8. Cartoon representing the proposed three-step model for the development of each level of the sulfidic caves in this study. Modified after Galdenzi (1990).

abandon the cave. The only remaining imprints of the first two phases of sulfidic speleogenesis may be a ramifying pattern of irregular rooms and mazes with passages branching outward, dead-end galleries, blind alcoves, and cupolas. However, these features may also result from hypogenic processes by a carbon dioxide rich aquifer and mixing processes along coastal margins (Mylroie *et al.*, 1995).

POTENTIAL BIOSIGNATURES IN ANCIENT SULFIDIC CAVES

Biological communities in active sulfur caves have the potential to impart isotopic, mineralogic and lipid signatures that may be of use in identifying fossil caves formed by sulfuric acid speleogenesis. A few patterns have emerged in light of several published reports and numerous preliminary investigations. Sulfidic subterranean springs and streams host dense biofilms containing organisms related to sulfur oxidizers with a wide variety of biochemical pathways and metabolic characteristics. Relatives of sulfur-oxidizing ε-Proteobacteria are common and diverse (Engel *et al.*, 2003; Northup *et al.*, 2005; Macalady *et al.*, 2006), especially under high sulfide and low oxygen conditions. Other filamentous and non-filamentous sulfur oxidizers such as *Beggiatoa*, *Thiobacillus,* and *Thiothrix* relatives may play an equally important role depending on geochemical conditions (Hose *et al.*, 2000; Engel *et al.*, 2004a; Lyon *et al.*, 2004; Macalady *et al.*, 2006). This observation is significant because carbon isotopic fractionation associated with sulfide-driven microbial CO_2 fixation/primary productivity may be smaller for ε-Proteobacteria using the rTCA cycle than for organisms using the Calvin Cycle/RUBISCO (Campbell *et al.*, 2003; Macalady and Banfield, 2003). Sulfur reducing bacteria are ubiquitous, abundant and diverse in stream biofilms and underlying sediments (Lavoie *et al.*, 1998; Hose *et al.*, 2000; Engel *et al.*, 2004a; Lyon *et al.*, 2004; Macalady *et al.* 2006). Sulfate reduction is associated with a strong isotopic fractionation (Canfield, 2001), and therefore has the potential to influence the isotopic composition of sulfide degassing from streams and subsequently trapped as sulfur and sulfate minerals on cave walls.

Snottites in caves on different continents appear to have strikingly similar population structures and are among the lowest diversity natural communities known. Both Frasassi and Villa Luz snottites are dominated by sulfur-oxidizing *Acidithiobacillus* species, with minor populations representing *Acidimicrobium* species, acidophilic archeaea, fungi, and protists (Hose *et al.*, 2000; Northup *et al.*, 2002; Jones *et al.*, 2005). Interestingly, the low pH of snottites and some biovermiculations may provide a unique biosignature of sulfuric acid speleogenesis in the form of N isotopes. Acidic wall biofilms in Lower Kane Cave (Stern *et al.*, 2003) and Frasassi (Galdenzi and Sarbu, 2000) contain some of the most depleted N signatures found in any organic matter. Stern and colleagues (2003) proposed that NH_3 originating in neutral, sulfidic water is trapped as NH_4^+ by acidic wall solutions, providing an abundant source of isotopically depleted N that is incorporated into organic matter by acidophilic microbes. Future research will test the utility of this potential biosignature in older, non-active levels of the Frasassi cave system and in the ancient caves of the Guadalupe Mountains.

EVIDENCE OF SULFIDE-DRIVEN SPELEOGENESIS IN THE GUADALUPE MOUNTAINS

The caves of the Guadalupe Mountains provide ample evidence of their sulfidic as well as hypogenic origins. Most, if not all, of the major caves are formed near the crest of the Reef, Guadalupe Ridge, and McKittrick Hill Anticlines and the Huapache Monocline (Hill, 2000), and their major passages trend parallel to axial cleavage. These structures have been interpreted as drape folds over buried, syndepositional and uplift-related faults and fractures (Koša and Hunt, 2006). General characteristics of Guadalupe Mountain caves are ramifying patterns consisting of large rooms with narrow rifts extending downward, successive passages arranged in crude levels, deep rills in carbonate rocks, and spongework and network mazes (Palmer and Palmer, 2000; Palmer, 2006). Northup *et al.* (2000) described evidence of robust chemoautotrophic processes in the past, including possible lithified snottites, and abundant speleosols. Other "extraordinary features" in the Guadalupe Mountains caves include rills, gypsum "glaciers", sulfur masses, (calcite) folia, and mega-crystalline gypsum "chandeliers" (Davis, 2000). All of these characteristics are consistent with observations and data from actively developing sulfidic caves and provide compelling evidence of the hypogenic, sulfidic speleogenesis of the major caves in the Guadalupe Mountains.

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