LONG-TERM PERSISTENCE OF MICROORGANISMS IN GEOLOGICAL MATERIALS: LAZARUS, RIP VAN WINKLE, AND THE WALKING DEAD. P.J. Boston[1,2], M.N. Spilde[3], D.E.Northup[4], & C. McMillan[5] [1]EES Dept. New Mexico Institute of Mining & Technology, 801 Leroy Place, Socorro, New Mexico 87801; pboston@nmt.edu, [2]National Cave and Karst Research Institute, 1400 Commerce Dr., Carlsbad, NM 88220; [3]Institute of Meteoritics, Univ. New Mexico, Albuquerque, NM 87131; [4]Biology Dept. Univ. New Mexico, Albuquerque, NM 87131; [5]Northern Arizona University, Flagstaff, AZ 86011.

Introduction: Many have speculated about the potential for very long term survival of microorganisms [1] and some investigators have reported such finds in halite even as early as the 1960's [2,3]. Besides the curiosity value of such reports, if they are true such very long-term viability of microbes has significant implications for Earth evolution, for life on other planets, and perhaps for the plausibility of microbial swapping from one planet to another, or even panspermia.

Lazarus Taxa in Microorganisms: Concepts in macroscopic evolution that pertain to very long lived "survivor" taxa that seem to disappear from the fossil record only to reappear in far distant future geological epochs [e.g. 4] may be simply a preservation issue with these organisms, but in the microbial world, we suggest that such "Lazarus Taxa" may actually be disappearing from the surface biota for geologically significant periods of time, surviving in the subsurface, only to be reintroduced to the aboveground biosphere by crustal geological processes (uplift, orogeny, cavern formation, faulting, canyon incision, etc.). If such genomic storage occurs in the subsurface, it may provide an additional means of evolutionary adaptation to changing environments aboveground, and similar mechanisms might enable quiescent survival of organisms during super-annual climatic cycles on planets like Mars.

Rip Van Winkle Microorganisms: Selenite (crystalline gypsum) crystals of extraordinary size have been discovered in cave chambers intersected by mining activity at the Naica Mine (Industries Peñoles) in Chihuahua, Mexico [5]. Within the crystals, inclusions are numerous. They often contain solid materials, fluids, and sometimes gas. In addition, highly colored red, orange, and black deposits on chamber walls appear to have surface morphologies suggestive of microbial biofabrics (Figure 1). The cave chambers are associated with an active hydrothermal base metal ore deposit and exhibit temperatures presently ranging from ~ 40-60°C. The chambers have been drained of hydrothermal water during mining of zinc, lead, silver and minor copper, thus allowing access to the chambers about a decade ago. During Feb. 2008 and Dec. 2009, we collected fluid and solid samples for direct microscopic inspection (Figure 2), bulk chemistry, live culture, and DNA analysis. Based on our results, we estimate that organisms that we have recovered from inclusions may have been trapped within their crystalline time capsules from between 10,000 to 100,000 years. The range of time depends upon the exact depth in the crystal that was sampled and which age estimation we accept for the crystals of the three studies that have been published to date. U/Th dates published are subject to uncertainties in the location of broken crystal sampled for the analysis. However, crystal growth experiments in the mine produced calculated ages of ~400 ky for the large crystals [6]. The deepest fluid inclusion that we sampled was at a crystal depth of approximately 3-5 cm which yields approximate ages of 30-50 ky since entombment. This range is based on growth rates ranging from 0.5 - 1.45 mm per 1 ky [7].

Molecular data from the fluid inclusions, and a variety of wall microbial/mineral deposits shows a plethora of strains that appear to be entirely novel, but whose closest relatives (at the 90% similarty range) include organisms from tantalizing environments around the world including volcanic soils, other caves in different hemispheres, and many that have been identified from a variety of unusual and extreme chemical circumstances.

Walking Dead Microorganisms: The experience of entombment in fluid inclusions, sediments, or other geological materials cannot be an easy one to survive. Extreme toughness in the face of environmental insults appears to be common in extremophiles, and quite noticeable in many subsurface strains that we have studied. Other recent experiments within our group that help to illustrate this amazing survivability have involved organisms that are making their living metabolizing copper sulfides, an interesting story in itself, but what we recount here of interest is the regrowth of organisms after being subjected to preparation and repeated examination with scanning electron microscopy (SEM) and electron microprobe techniques. Samples are air dried, dried in a vacuum oven at 100^{o}C, vacuum-coated with Au/Pd coatings to render them electron dense, and then repeatedly exposed to high voltage electron beams for imaging, and stored between analyses in a dessicator. The copper sulfide microbial communities have regrown on the sample stubs, punching up through the Au/Pd coatings to produce the same brown fuzz that originally attracted us to study the copper sulfides in the first place! These organisms are tough little buckaroos, and perhaps a model of good candidates for surviving geological entombment, long term burial in sediments, or even carried along as bioburden in spacecraft.

Caveats and Concerns: The primary caveats with claims of live microbial antiquity (or claims of extractable DNA) center around two issues. The first issue is potetial contamination of specimens with live environmental organisms or biomolecules that would be expected in a given environment. The second concern is geological and geochemical processes that may have compromised the original materials over geological time and overprinted them with organisms that could have been introduced into a system long after it's claimed age. Although one can never completely rule out contamination, the taxonomic affinities and variety of organisms identified by molecular and culturing techniques can provide some confidence that at least contamination is unlikely when large numbers of extremophiles are found. Addressing the second issue of geological alteration can be much trickier, requiring a plausibility argument based on the apparent pristine nature of the mineralogical and petrographic setting and a thorough discussion of all possible confounding geochemical processes that may have occurred in that setting over time. We now believe that our hot crystal communities, and the communities living in wall material in the Naica caverns both show that we are looking at Naica indigenes, some of which have apparently been off the social circle for quite some time! If we are correct, then this adds another geological storage possibility (crystalline gypsum) to the small list of materials in which long term microbial survival has been reported (ice and halite).

References: [1] Fendrihan S., et al. (2006) *Revs Environ Sci Biotech* 5:203–218. [2] Reiser, R. and Tasch, P. (1960) Trans Kansas Acad Sci 63:31–34. [3] Dombrowski H. (1963) *Annals NY Acad Sci* 108:453–460. [4] Mamay S.H. and Bateman R.M. (1991) *Am J Botany* 78(4):489-496. [5] Garcia-Ruiz J.M. et al (2007) *Geol* 35(4):327–330. [6] Sanna L. et al. (2010) *Int J Speleo* 39(1):35-46. [7] Lauritzen, S-E. et al. (2008) *Proc. Int'l Cong Geol.* Oslo, Norway.

Figure 1: Biotextural appearance of red wall material in Cueva de Los Cristales, Naica, MX.

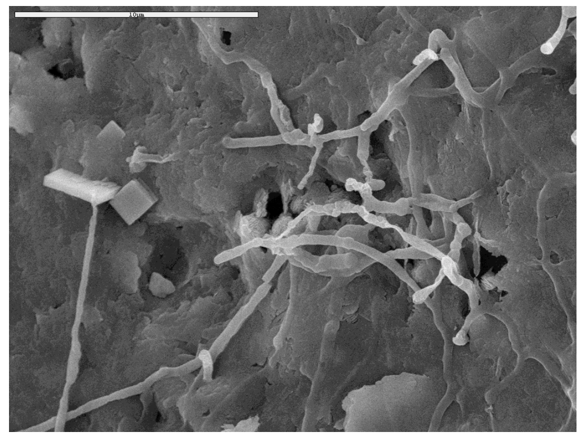

Figure 2: Filamentous microbial forms protruding from iron oxide and clay coated cave chamber walls. These organisms are still very much alive in the walls (although presumably NOT on this SEM specimen stub…but you never know….see below).

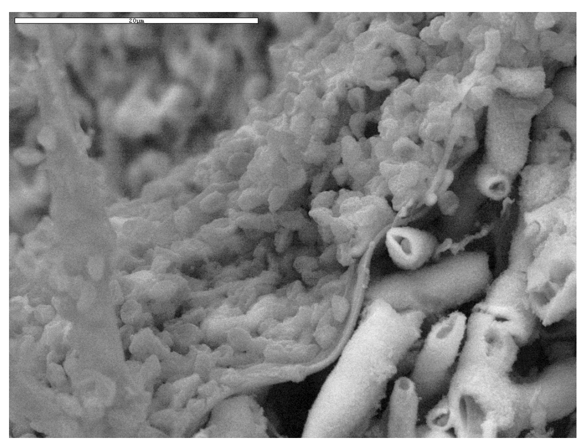

Figure 3: Survivors of SEM preparation, small cells on left are bacteria-sized, broken hyphae on right are in the size range of fungi.