From Viterbo Hot Springs – More Aragonite Pearls (of wisdom), And Some Calcite Too – Do Nannos Rule?

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The following are a few of the most interesting pictures from continued work on the hot springs of Viterbo, mostly done since the original papers (Folk, 1993; 1994) with many partners in the field and at the SEM. They should reinforce the nannobacterial story on the precipitation of carbonate minerals.

Fig. 1 shows a hot spring orifice at Le Zitelle, Viterbo; type locality for the first discovery of nannobacteria in geology. Fig. 2 diagrams the chemistry of the emergent waters – over 500 ppm Ca, with a lot of HCO_3 and SO_4 (data of Malesani and Vanucci). In a nearby spring (fig. 3, Bagnaccio) a long traverse down an outflow trench shows the rapid change in water chemistry downstream. Temperature (red triangles) drops from 60° to 30° in the terminal pools; while pH (blue hexagons) rises from slightly acid 6.5 to over 7.6 as CO_2 degasses. For the first few meters, there is abundant outgassing of H_2S (stinks!) then it gradually oxidizes to sulfate. At first the water is too acid for $CaCO_3$ to precipitate; later, at such high temperatures (56-60°C) aragonite begins to form abundantly. Where filamentous S-bacteria especially flourish, their slimy mucus slows the precipitation rate and calcite forms instead of aragonite. In the carbonate sediment in the bottom of the trench the sulfur content shows mm-scale variation with depth, as shown by inserting a silver dollar (fig. 4).

Fig. 5 is a typical view of the fibrous mat formed by salmon-colored sulfur bacterial filaments, like streamers of hair. The carbonate is mainly aragonite with some calcite (fig. 6); the aragonite forms as radial needles encrusting bacterial filaments like a pipe-cleaner. Another common form is as fuzzy dumbbells (fig. 7; Pursell, 1985; Folk & Bendig, 1987); recently examined in detail by Corley (2009). My colleague at Mississippi State, Dr. Brenda Kirkland, prepared samples for TEM study (fig.8), in which acid removes all the aragonite mineral (thus white in the photo), and Osmium stains organic matter black. One can easily see that the central part of the dumbbells is very rich in organic material (I think nannobacterial cells), while the outer parts are relatively free. Her work at much higher magnification (Kirkland and Lynch, 2005; Folk & Kirkland, 2007; also the note on Viterbo Slime on this website), shows that nannobacteria have clearly defined cell walls and many have internal structures resembling ribosomes, in cells as small as 100nm. Dumbbell-shaped Ca oxalate precipitates from human urine (fig. 9) have also been found (Piccoli et al., 1984); would that these guys had looked at these objects at higher power – maybe they have nannobacteria as well?

Mud on the bottom of the cooler downstream bathing pools at Bagnaccio shows a mix of aragonite with calcite (fig. 10). Generally aragonite has formed first, as calcite crystals sometimes enclose them (fig. 11). But with precipitation rates of several mm/day near the surface (Folk, 1994) these two minerals must have formed within hours (or even minutes) of each other.

Now more on the nannobacterial story. Fig. 12 is a sample etched for 1 minute in 1% HCl; etching has exhumed a normal-size bacterial ellipsoid plus a cell of 0.2 microns (200 nm) that is at the lower limit of "life" as defined by microbiologists, plus a number of round cells down to 40 nm. A homunculus-like chain of about 8 nannobacterial cells arises from the calcite crystal. Inorganic minerals do not behave so strangely! This is indeed LIFE at the nm-scale, with a gradation up to normal cells.

Fig. 13 shows more nannobacterial cells evincing the "squashed-orange" effect; when two cells are adjacent, they deform plastically because of the flexible cell walls – again, not a property of minerals. The nannobacteria are less soluble (probably protected form solution by remnants of tough cell walls), so they float out on the duco mounting medium (fi. 14). And a glass slide placed in the water grew an algal/bacterial filament coated with nannobacterial cells (figure. 15). Fig 16 does not show a graduate student being drawn and quartered – instead it is a mucus strand with "big" bacteria attached.

Fig. 17 (SEM photo by Leo Lynch at Miss. State), shows the middle of a fuzzy dumbbell, rich in small balls, grading out into more IN-organic needles of aragonite – as seen in fig.8). By sampling the very tips of growing aragonite crystals, we can see the earliest stages of precipitation before IN-organic precipitation begins to obscure the scene. The "hook-em-horns" figure shows these growing tips, rich in nannocells. Fig. 19 is another end-on view of a nascent aragonite crystal that even shows the proper hexagonal symmetry for an aragonite twin. In fig. 20, the enlargement shows naked nannocells, not yet glued together by any inorganically precipitated aragonite.

Fig. 26 is a TEM slice taken by Kirkland and Lynch showing part of a fuzzy dumbbell. This specimen was NOT treated with acid, but it WAS stained with Osmium –

so aragonite crystals remain, appearing black because they are dense to transmitted electrons. The small round objects, about 40 nm are Os-stained nannobacterial cells, some showing cell walls and clearer centers. They are the same size as the tiny balls seen in SEM in fig. 17.

Turning now to calcite, similar features are observed. Nascent calcite crystals collected from the growing tips of bacterial mats are made of nm-size nannobacterial cells forming monolayers shingling over each other (fig. 22-24). Each layer is one-ball thick, as is the case in many clay minerals (see 'Italian clays" on this website). These samples are fresh, NOT etched in HCl. Fig. 25, 26 show a calcite crystal made of nannobacterial monolayers, but it has entangled filaments about 100 nm wide, also some sort of nanno-organism.

It thus seems that at Viterbo, nannobacteria are not just passive bystanders, but play THE major active role in precipitation of both aragonite and calcite crystals presumably because they have (-) charged cell walls that attract Ca++ ions. The crystals at first are made of aggregates of tiny nannocells. Later inorganic precipitation fills in the spaces between, so one ends up with "mature" crystals with smooth faces that would fool even the most dogged mineralogist into thinking they are IN-organic precipitate. T'aint so!

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Figure 1. Orifice at Le Zitelle Spring, Viterbo. Temperature=60°C, you can tolerate your finger in it for one second only. Water is precipitating white aragonite ($CaCO3$). Brownish stain caused by bacteria. For further details on Zitelle see Folk (1994).

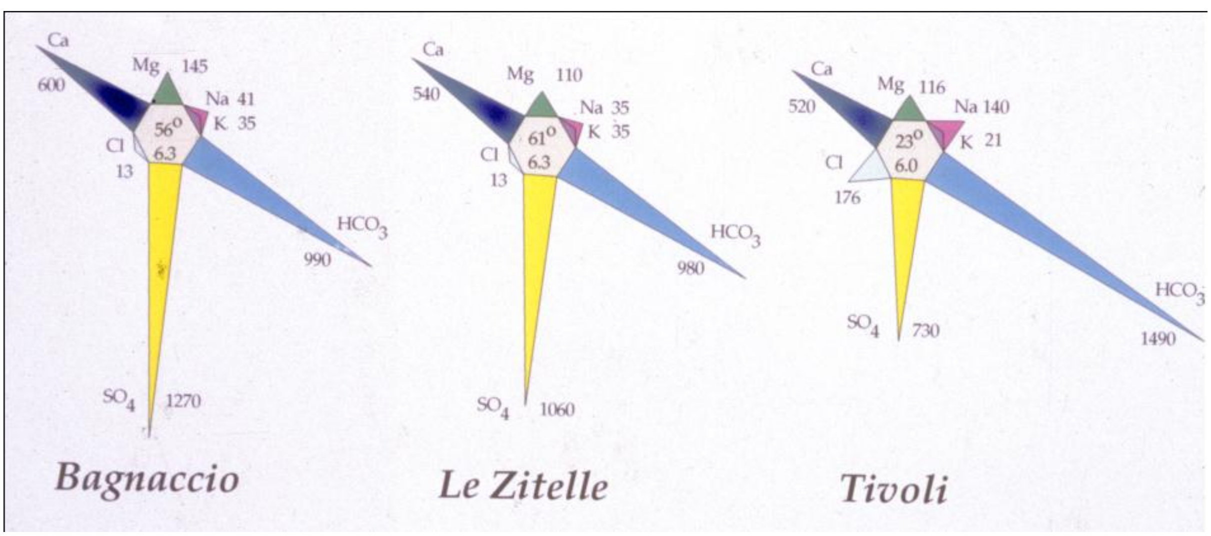

Figure 2. . Chemical properties of several spring waters. Because it is cooler at Tivoli, the precipitate is calcite.

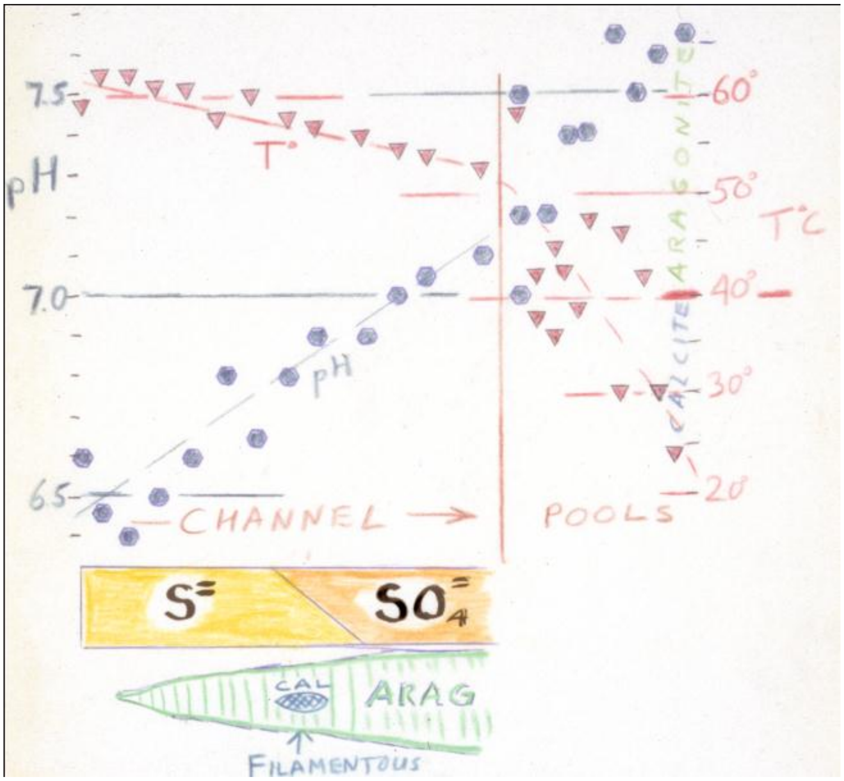

Figure 3. Downstream changes in pH, temperature and mineralogy. Jeff Springs (on the gravel road at Bagnaccio). Aragonite forms in channels at high temperatures, calcite in the terminal pools where water has cooled. Calcite forms at one spot in the hot channel because precipitation was slowed by thick slime.

Figure 4. Rapid changes in sulfur content of bottom mud shown in detail by inserting a silver dollar edgewise into the mud. Green color in mud is caused by (cyano) bacteria.

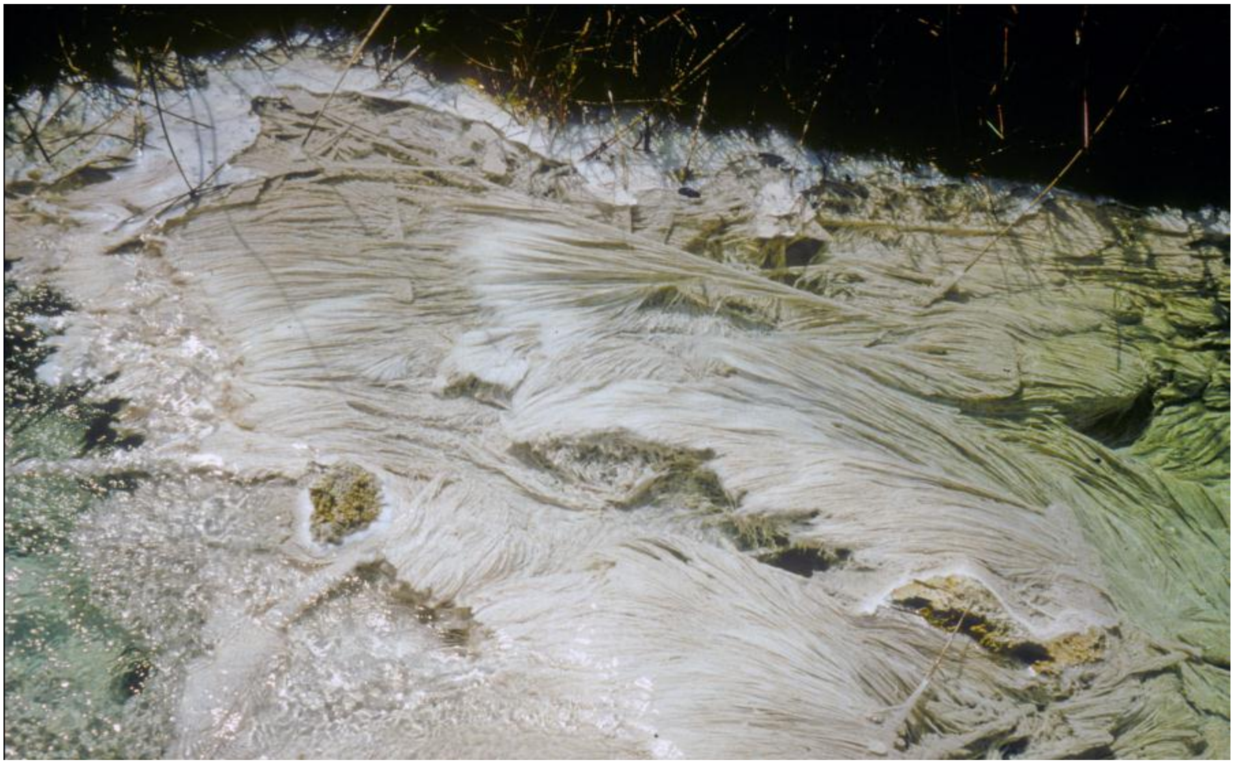

Figure 5. Sulfur-bacterial filaments (probably Chloroflexus) from Bagnaccio. Filaments many cm long are lithified and brittle due to precipitation of aragonite.

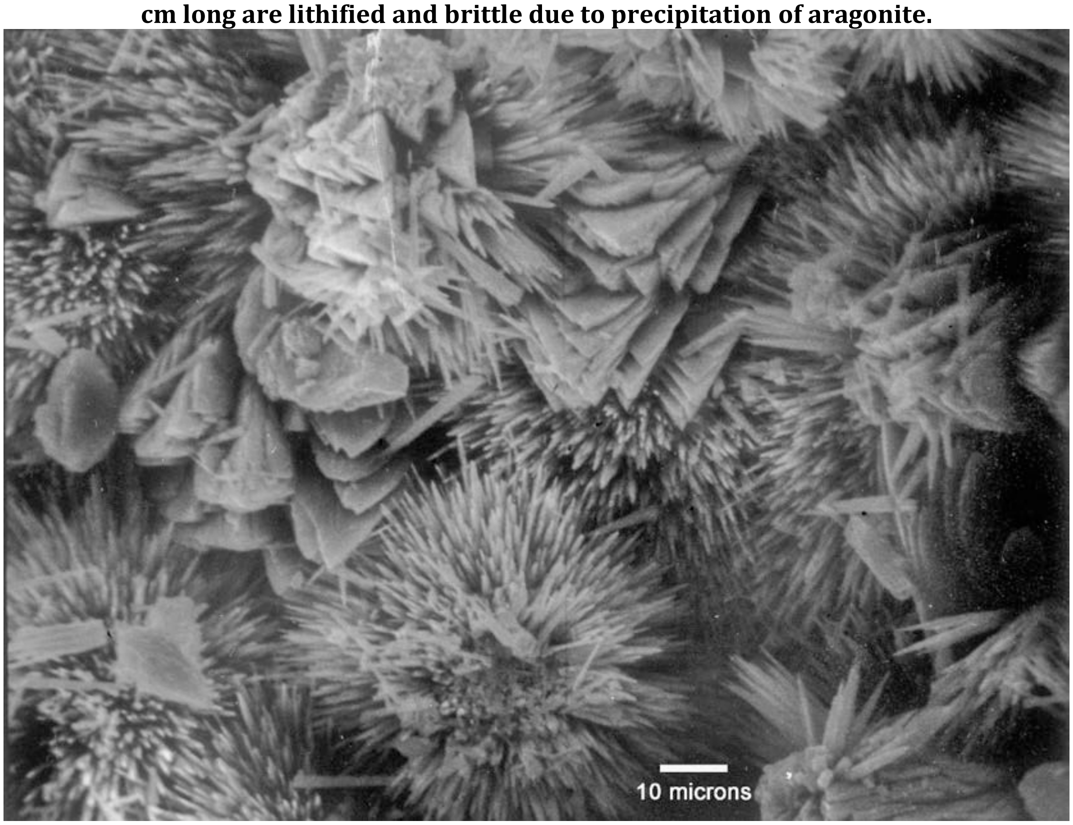

Figure 6. At Bagnaccio a mixture of “gothic arch” calcite with fibers of aragonite.

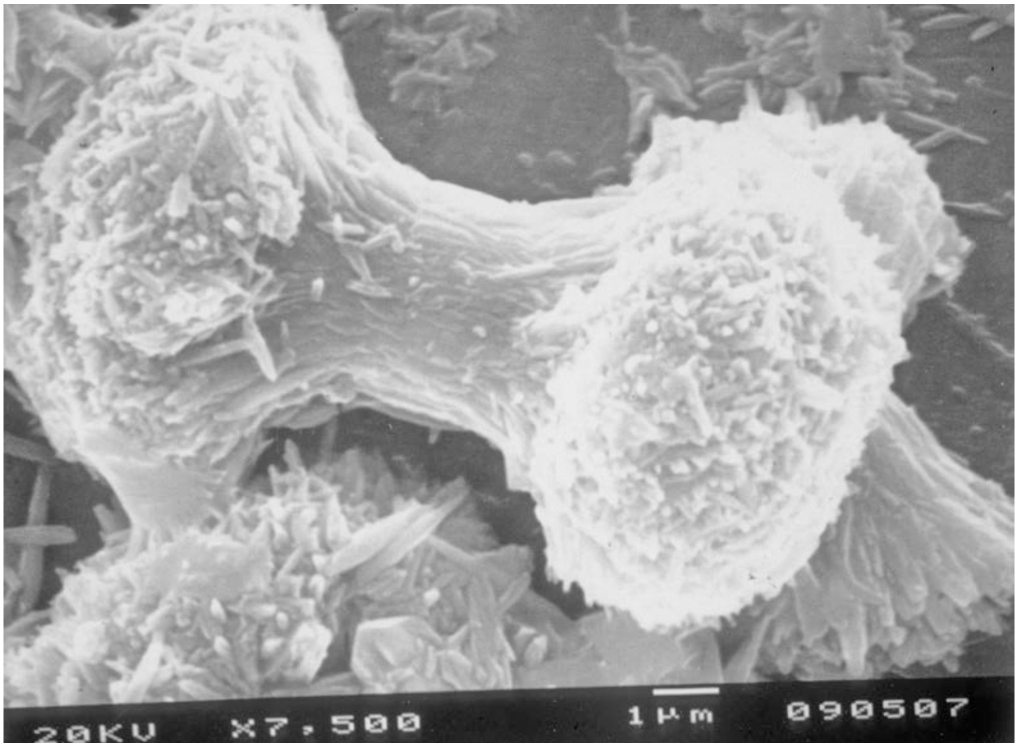

Figure 7. Fuzzy dumbbell of aragonite, bottom mud of a hot pool.

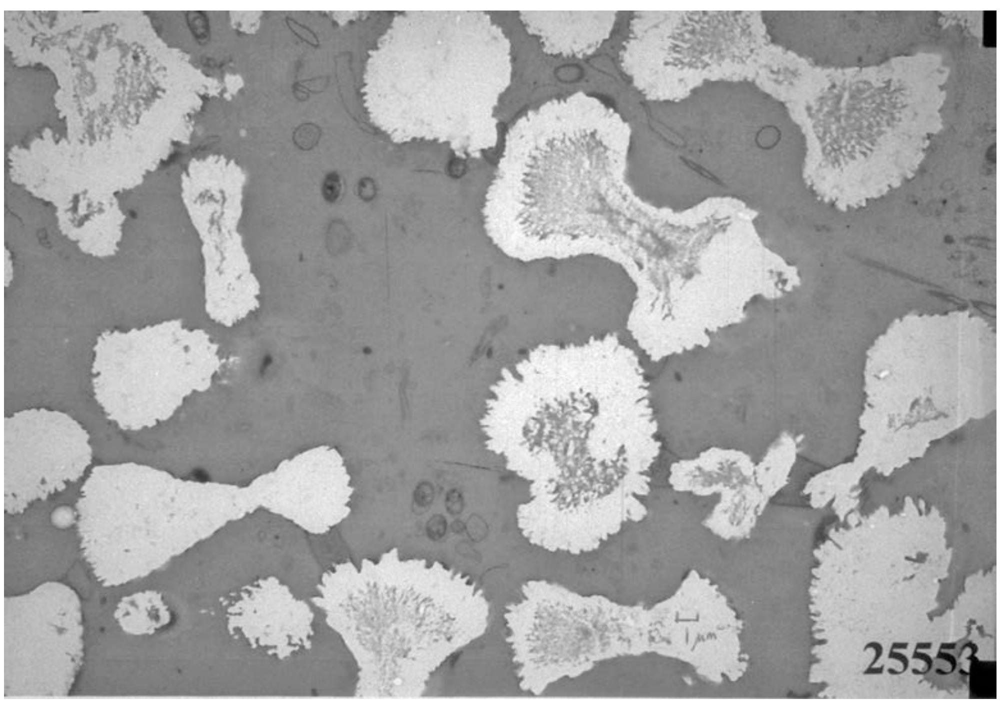

Figure 8. . TEM by Brenda Kirkland. Slice of fuzzy dumbbells averaging about 10 microns long from a patch of green slime, Le Zitelle. Organic rich cores stained dark by OsO_4. White in photo is where aragonite needles dissolved out because of acidic treatment. Dumbbells have organic cores and NON-organic outer parts.

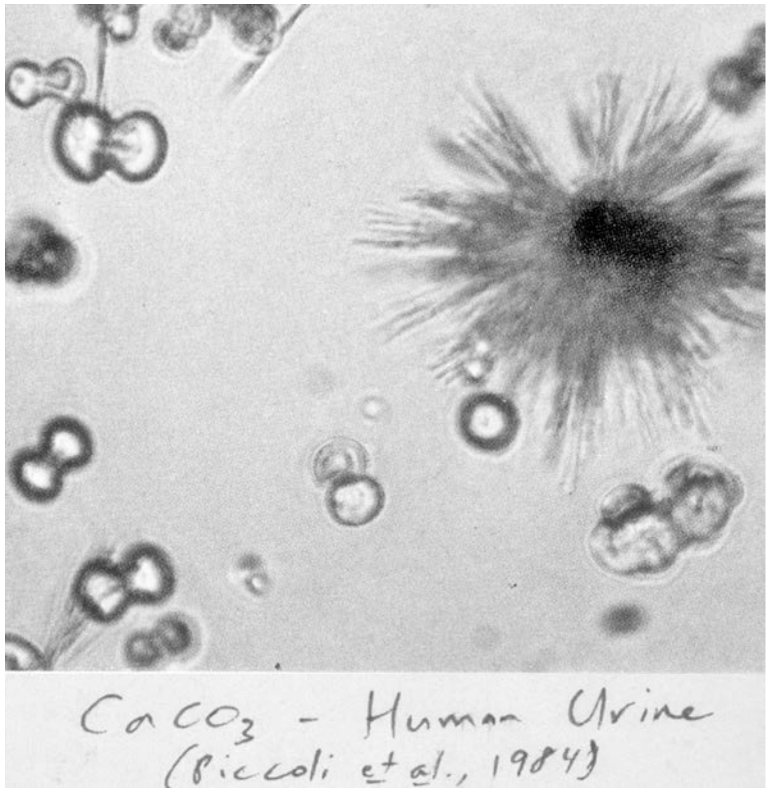

Figure 9. Dumbbells of Ca oxalate in human urine.

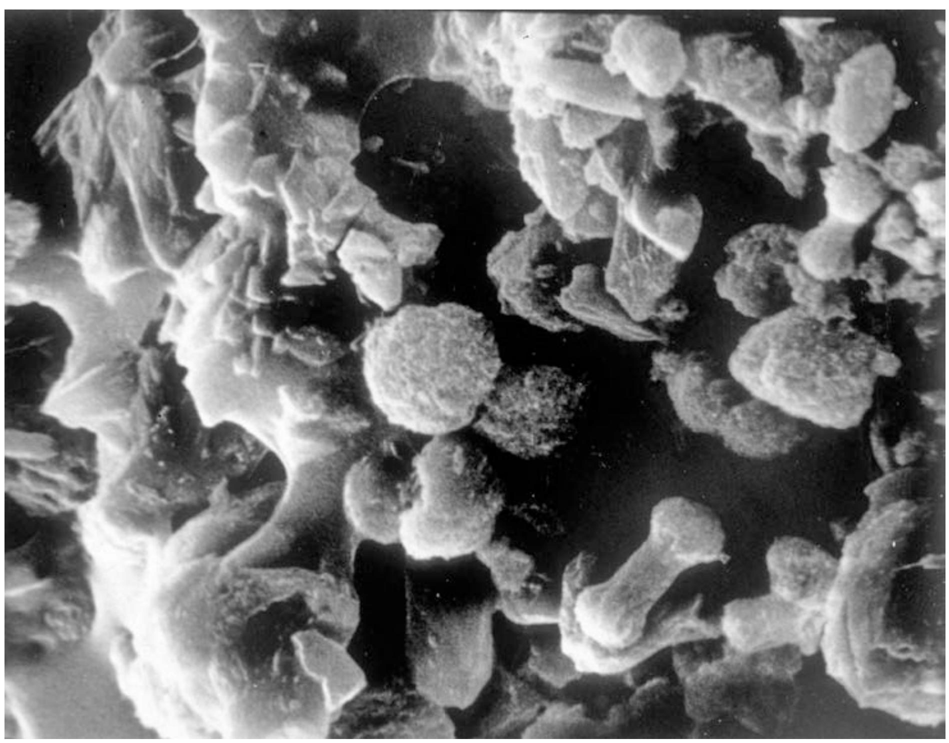

Figure 10. Mud from a cooler pool bottom, Bagnaccio. "George Washington" is calcite, and there are many aragonite dumbbells about 10 microns long.

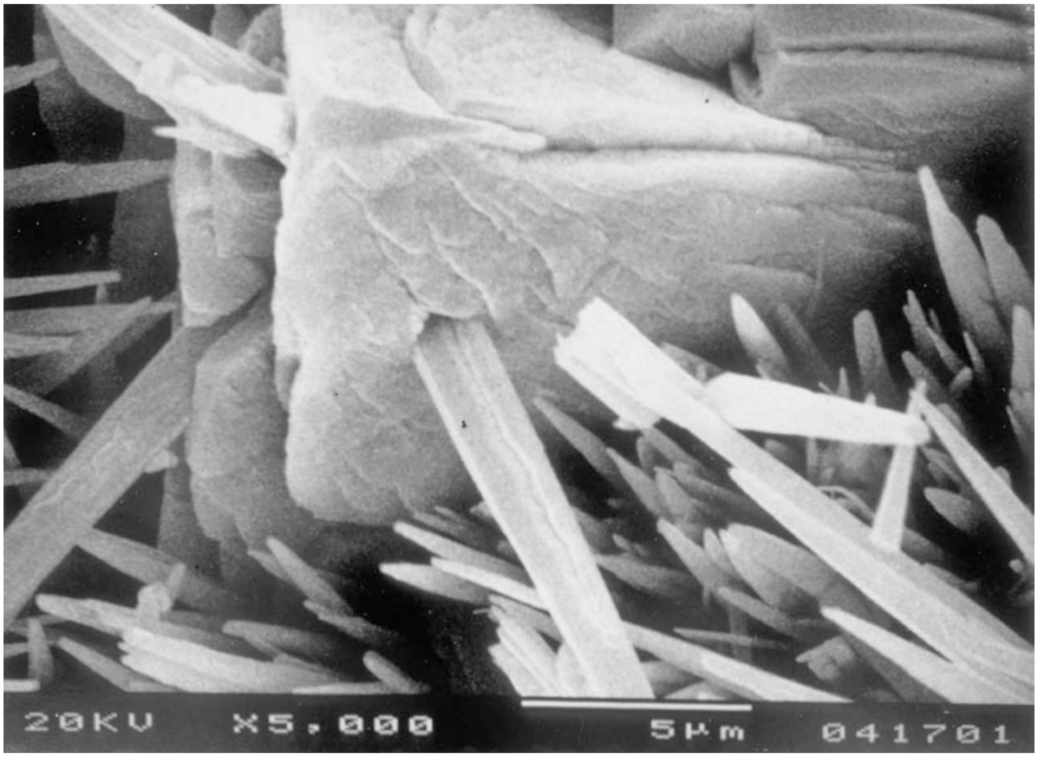

Figure 11. Needles of aragonite enclosed by later calcite—Bagnaccio.

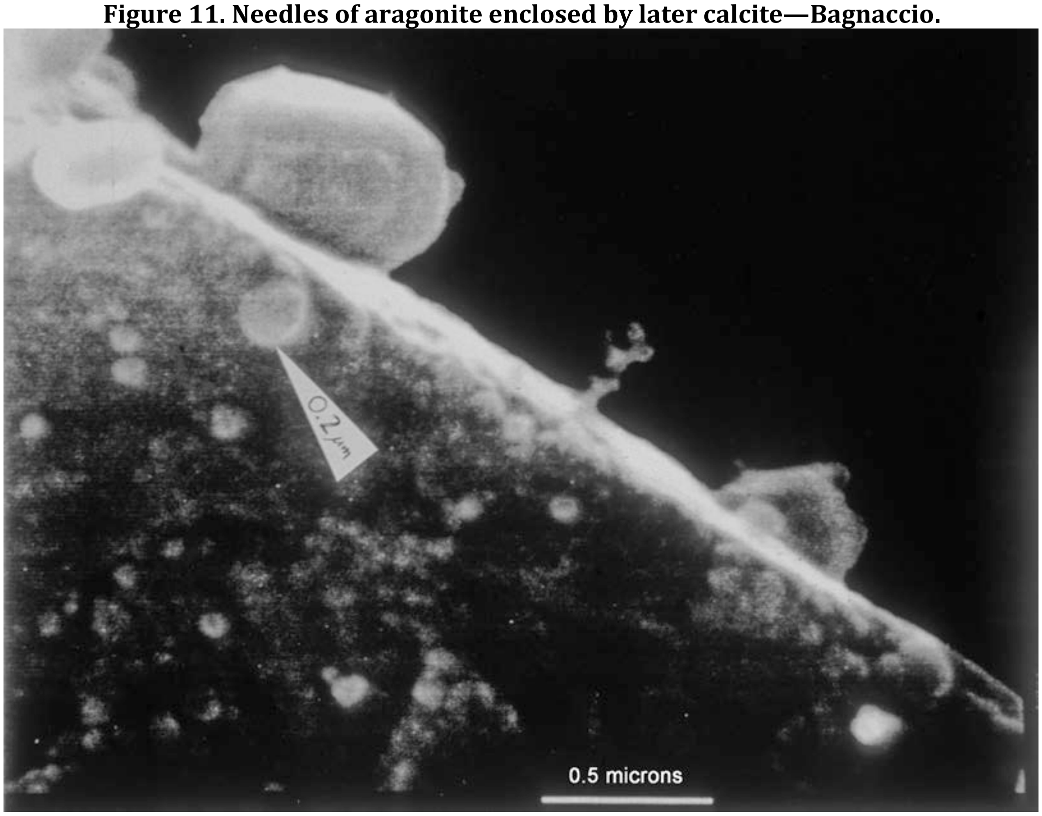

Figure 12. Older lithified travertine from a Roman/Etruscan age outflow channel atop an ancient artificial dike, Bullicame. Etched in 1% HCl for 1 minute. Cells of various sizes from "normal" bacteria to nannobacteria. "Lower limit of Life" at 0.2 microns is shown by the cell indicated by the arrow. A homunculus-- a chain of about 8 cells-- is clearly biological. Many round cells are shown, about 50-100 nm

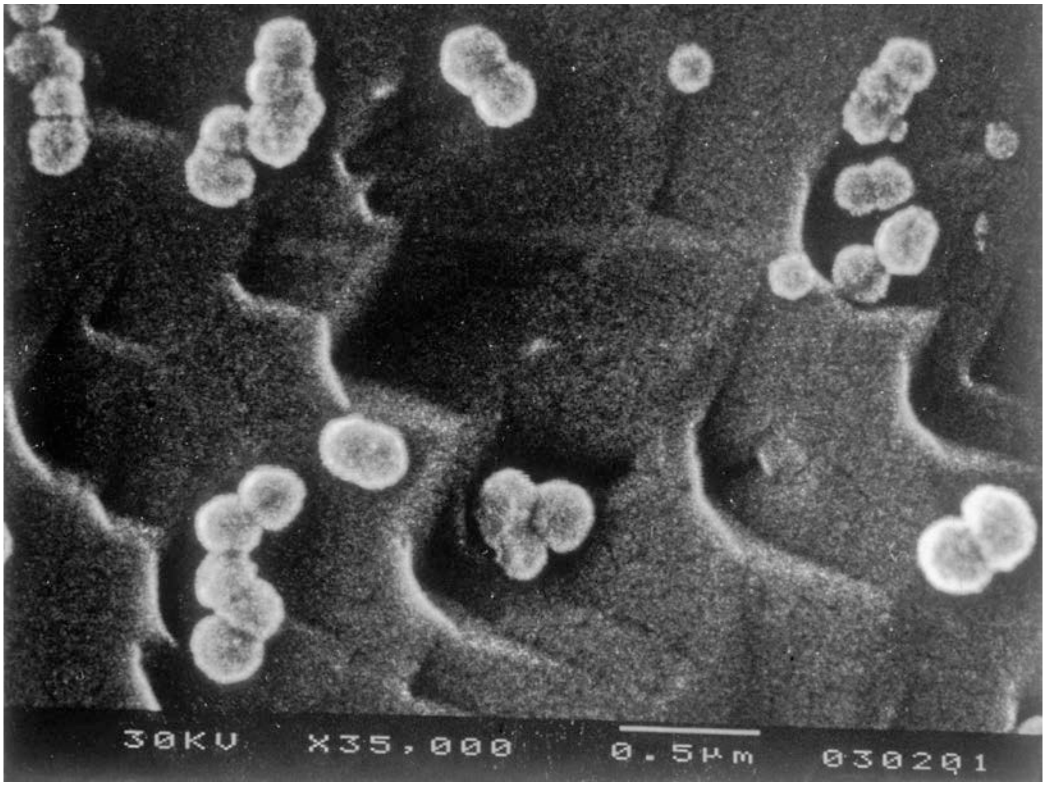

Figure 13. Fracture-filling vein of lithified aragonite, Bagnaccio, etched. Cells show the squashed-orange effect, denoting soft plastic deformation and favoring an organic origin.

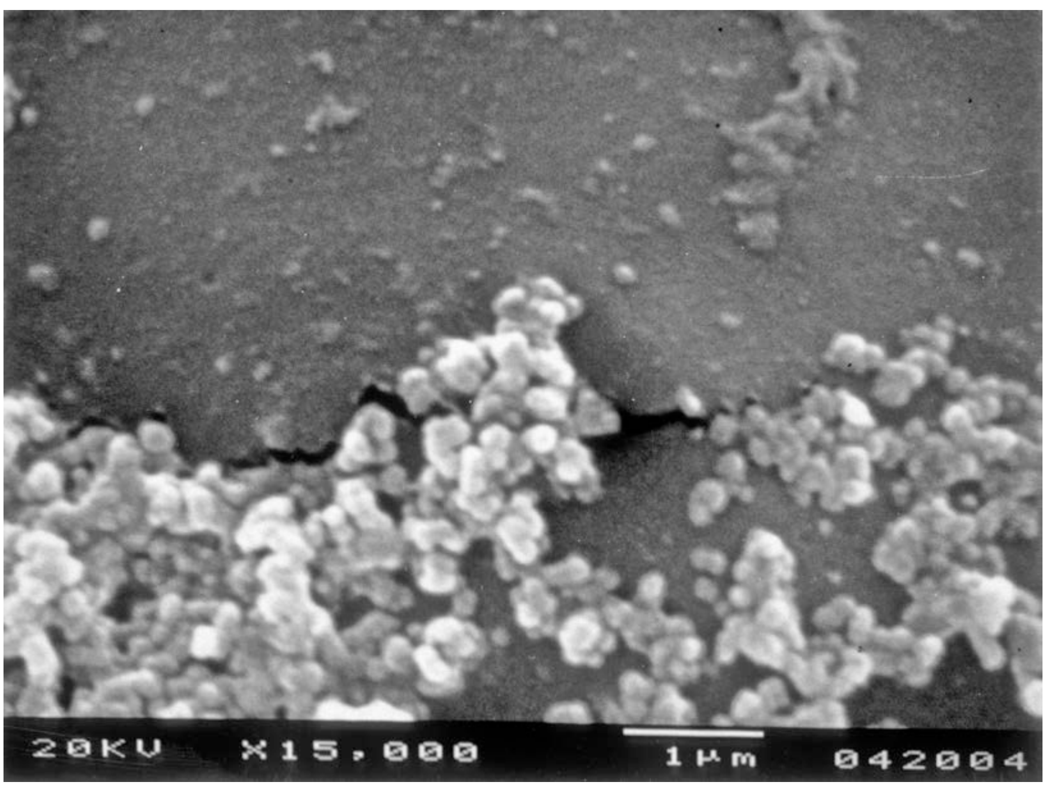

Figure 14. Sulfur-bacterial mat, Bagnaccio. A prolonged etch in HCl dissolved the host aragonite and liberated nannobacterial cells that floated off onto the Duco mounting medium.

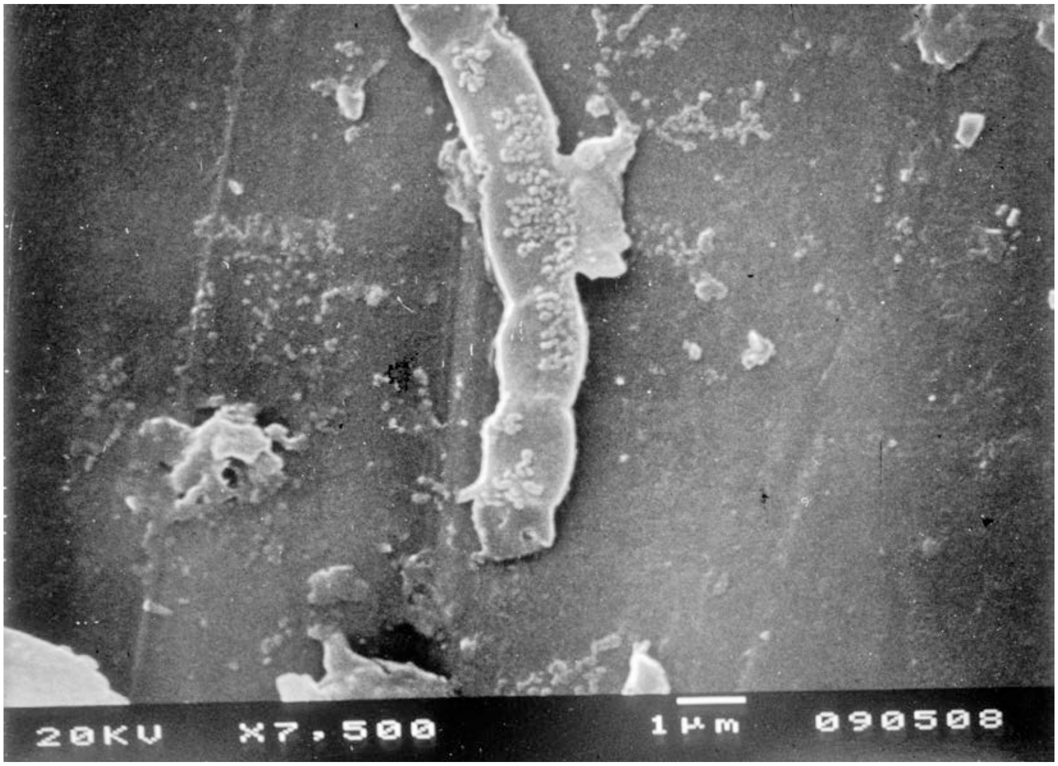

Figure 15. At Bagnaccio we inserted a glass slide into an underwater green slime. This (?cyano) bacterial filament grew, and is decorated with tiny nanobacterial cells of about 50-100 nm.

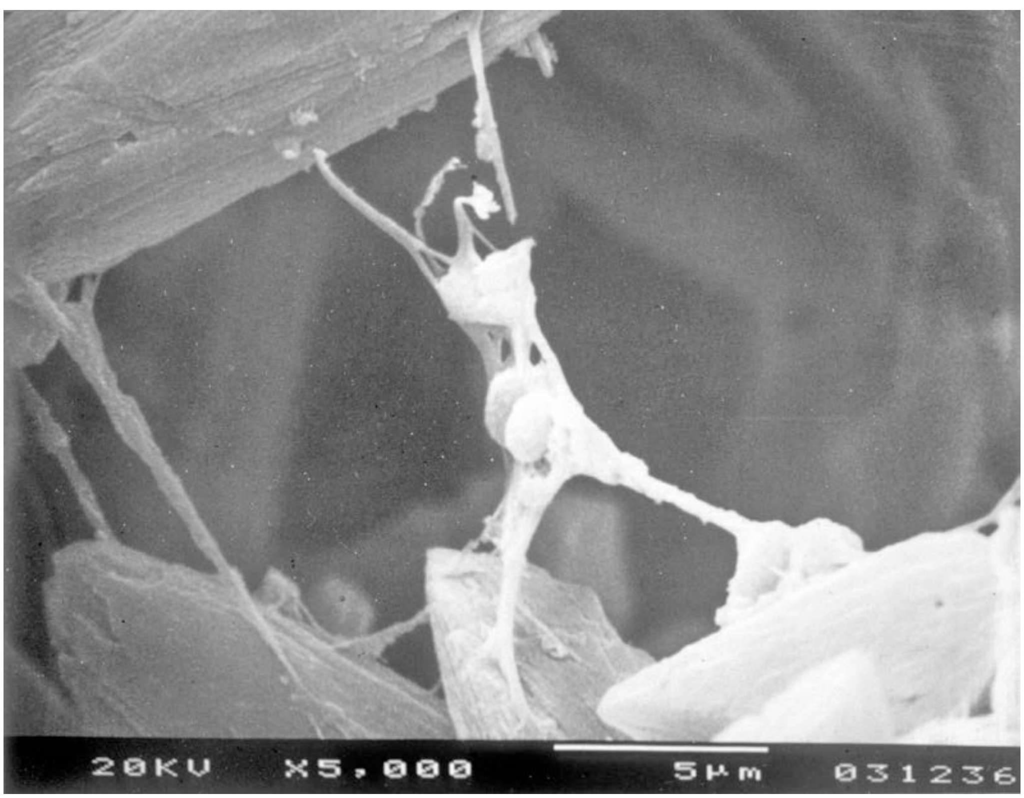

Figure 16. Bullicame. Not a student being drawn and quartered, but two “normal” bacterial cells attached by mucus strands to calcite crystals.

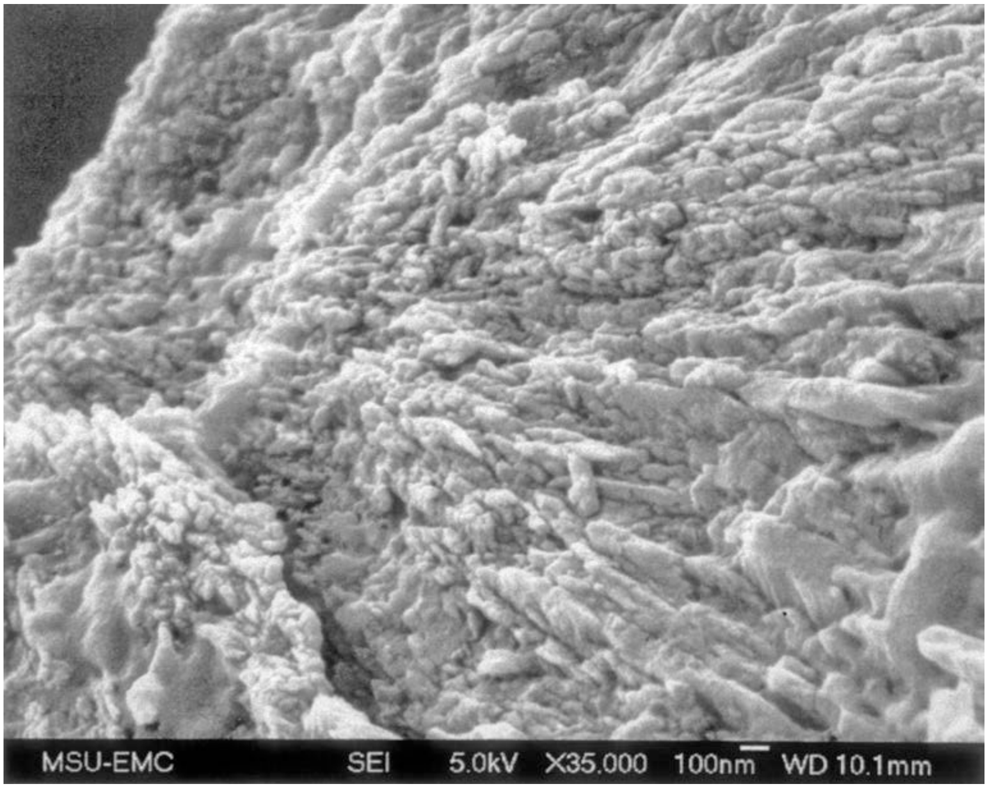

Figure 17. Center of aragonite spherulite, etched. Nannobacterial cells more abundant at left (near center of spherulite itself), and inorganic aragonite crystals further out (at right). Compare with fig.8. SEM photo by Leo Lynch.

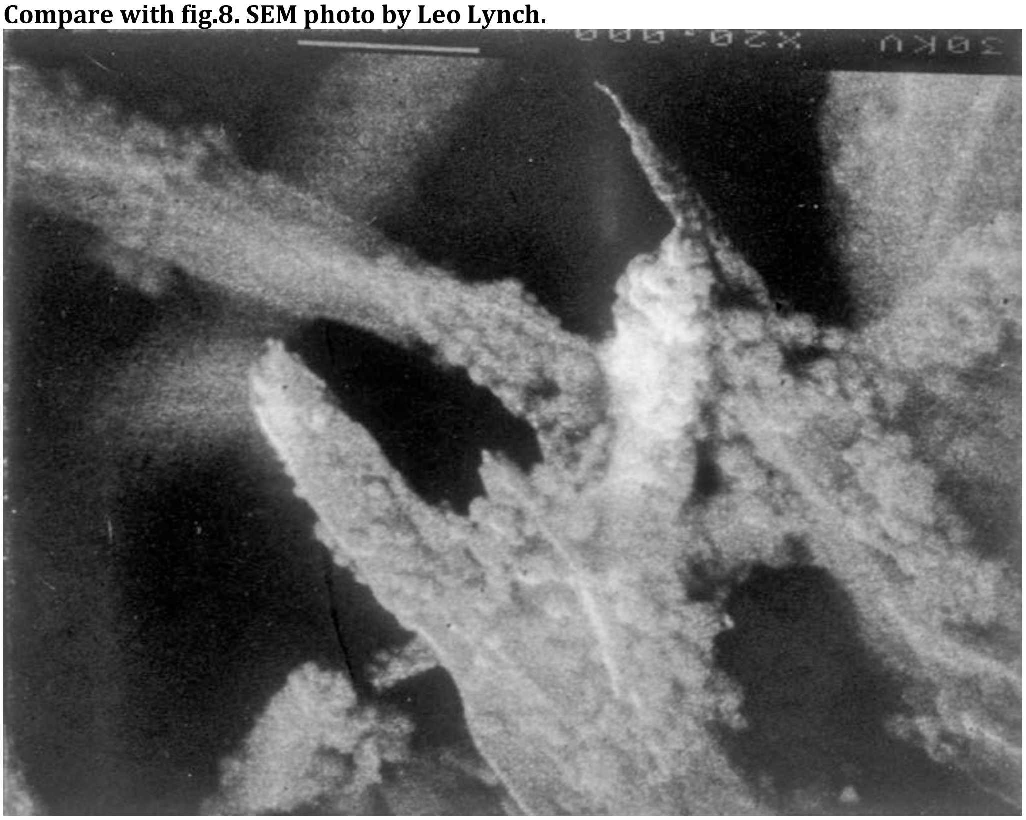

Figure 18. Nascent, growing tip of aragonite spherulite, Bagnaccio;etched. Nannobacterial cells swarm on this “hook-em-horns” feature.

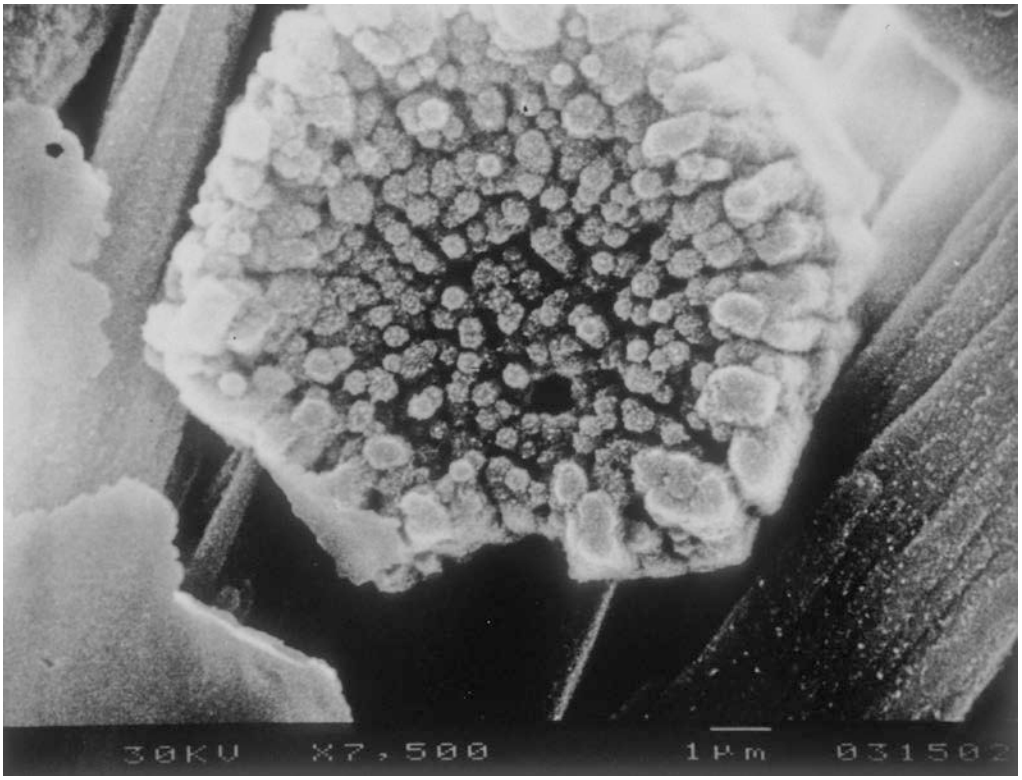

Figure 19.From a fuzzy dumbbell, end of an aragonite crystal, not etched. Pseudo-hexagonal form is assumed by the grouping of nannobacterial cells.

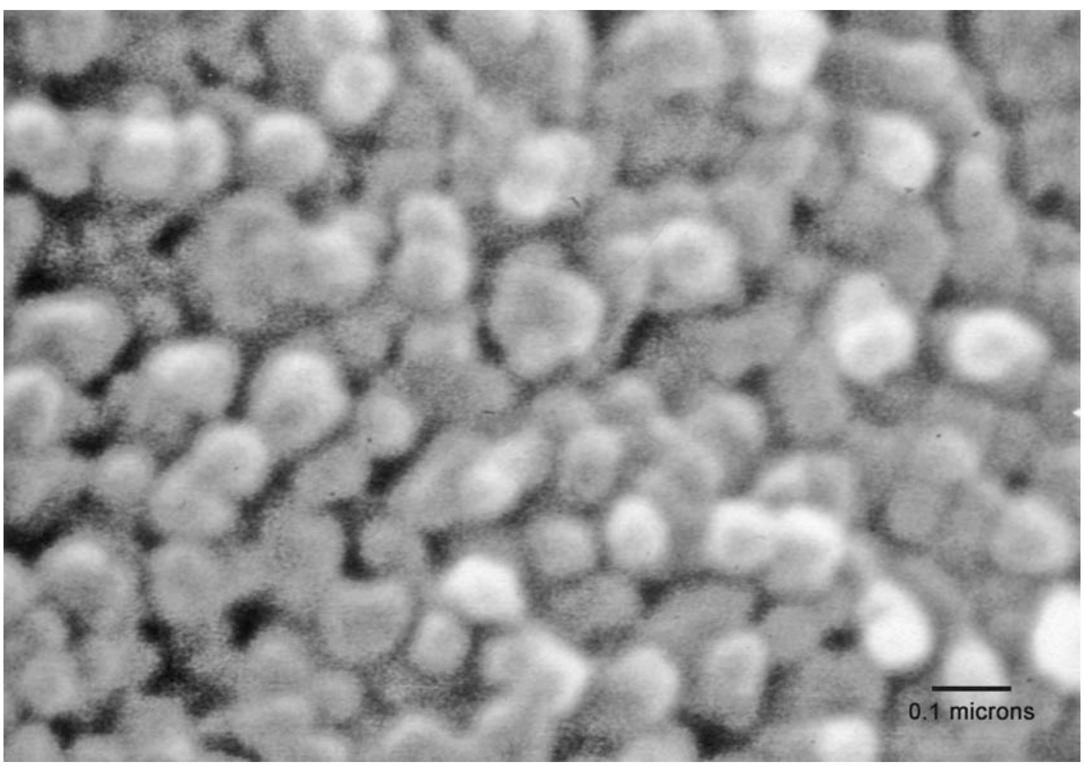

Figure 20.Enlargement of fig.19.Entire "crystal" seems to be made up of 50 nm balls with no binding cement between them.

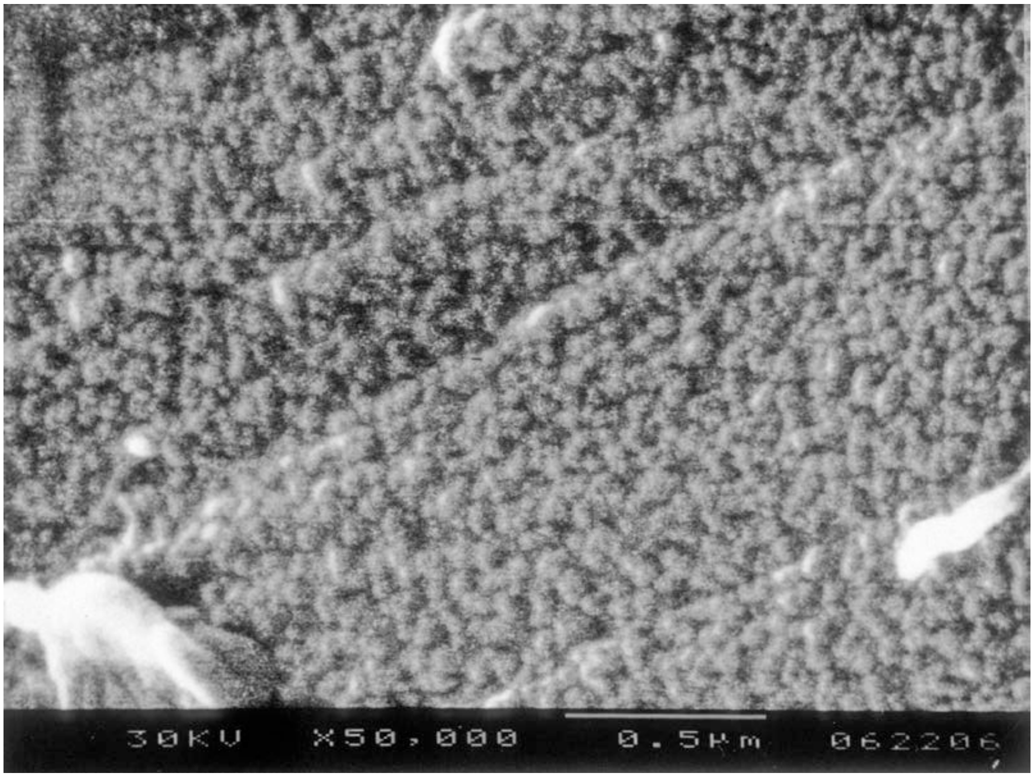

Figure 21.Nascent calcite crystal; successive monolayer sheets of 40 nm nannobacterial cells shingling over one another.

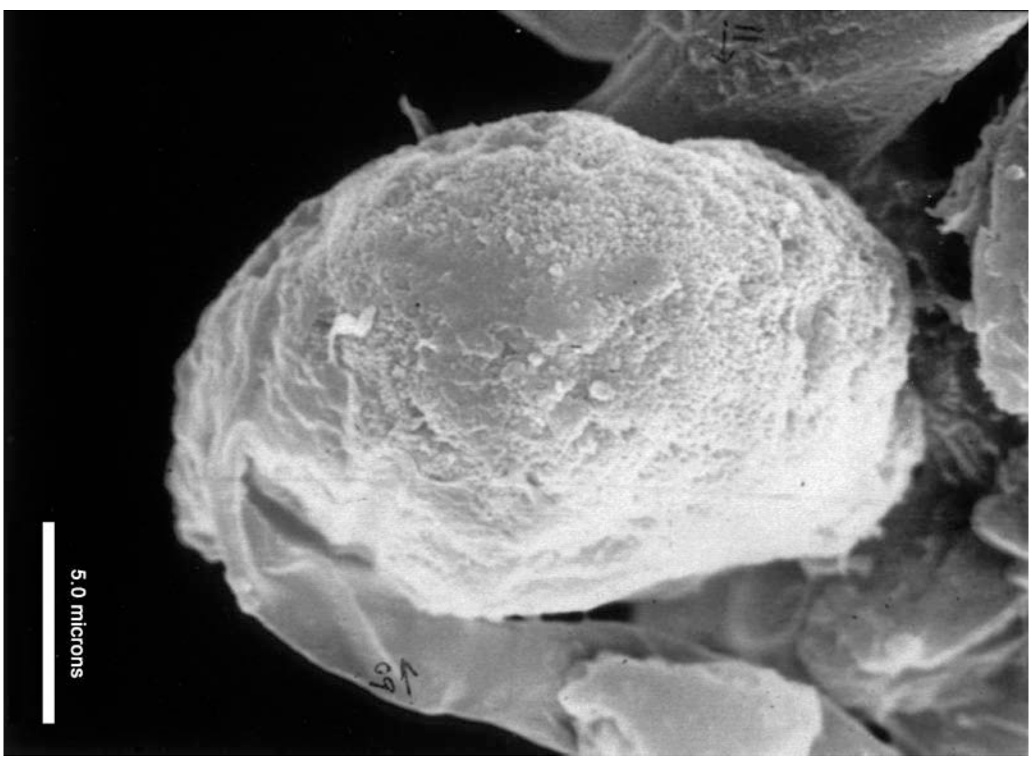

Figure 22.Bullicame calcite, not etched, showing shingling sheets.

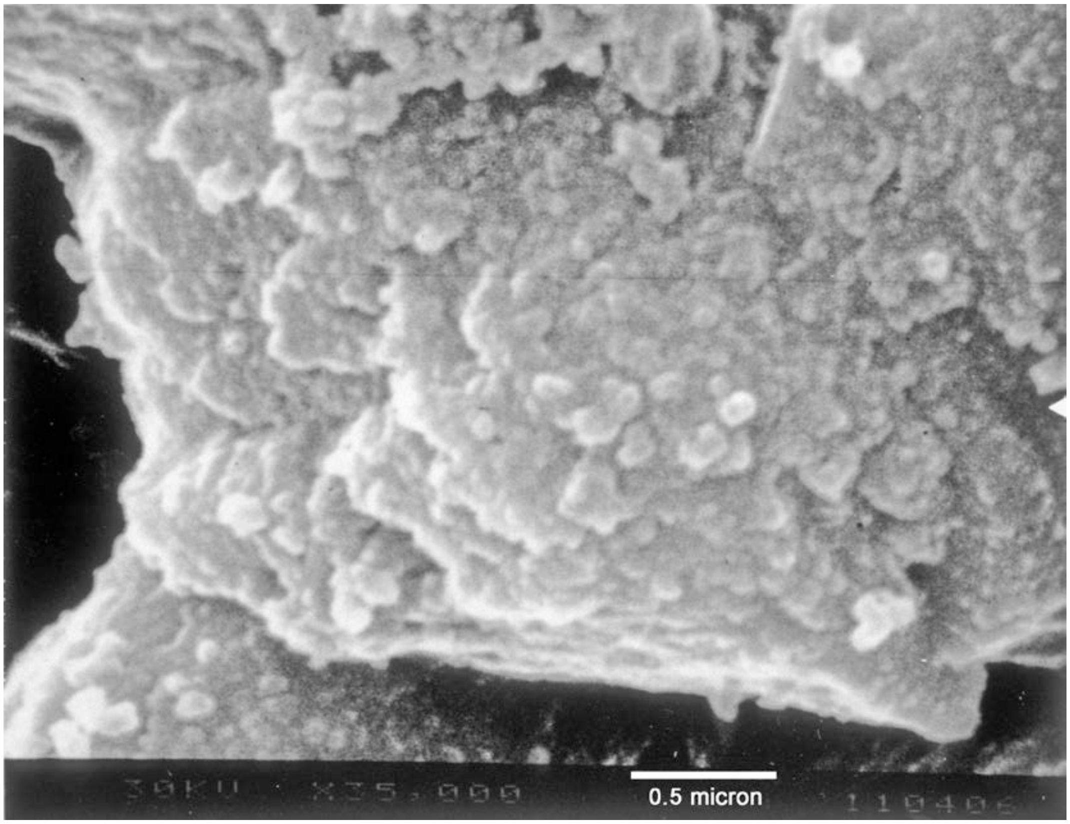

Figure23. As above, enlarged.

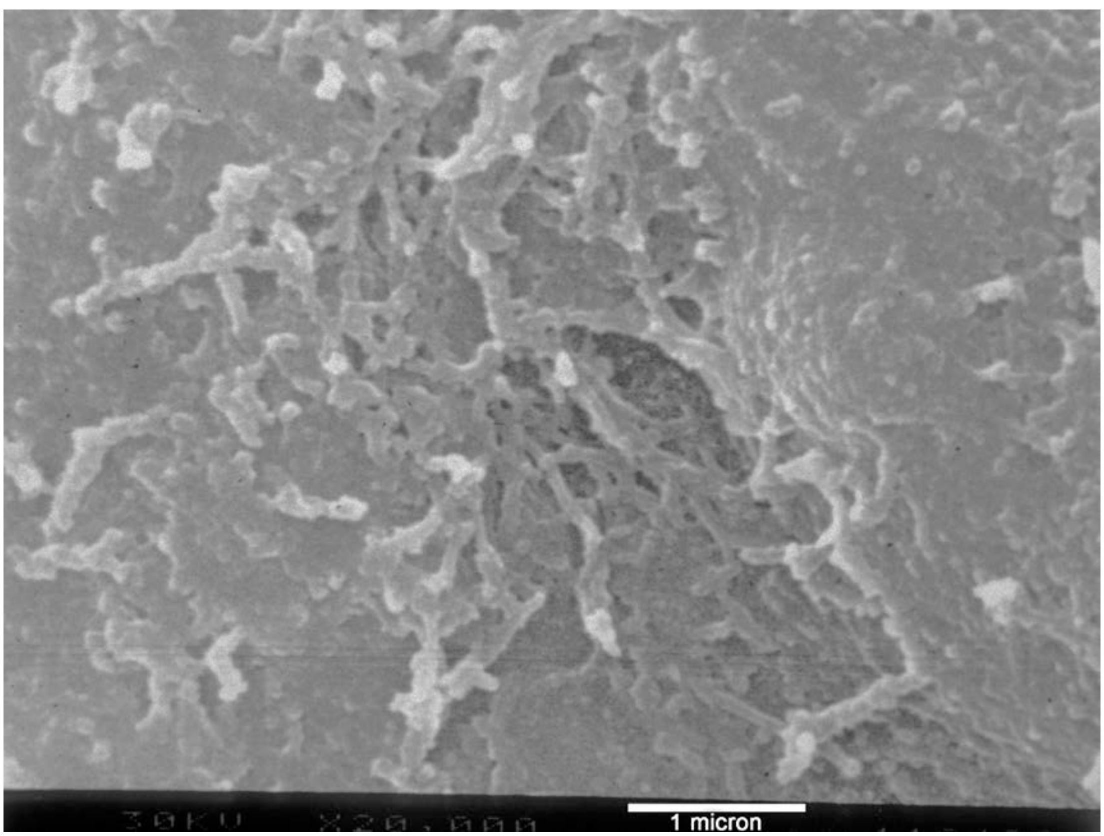

Figure24. Calcite, not etched, showing organic filaments 100 nm wide.

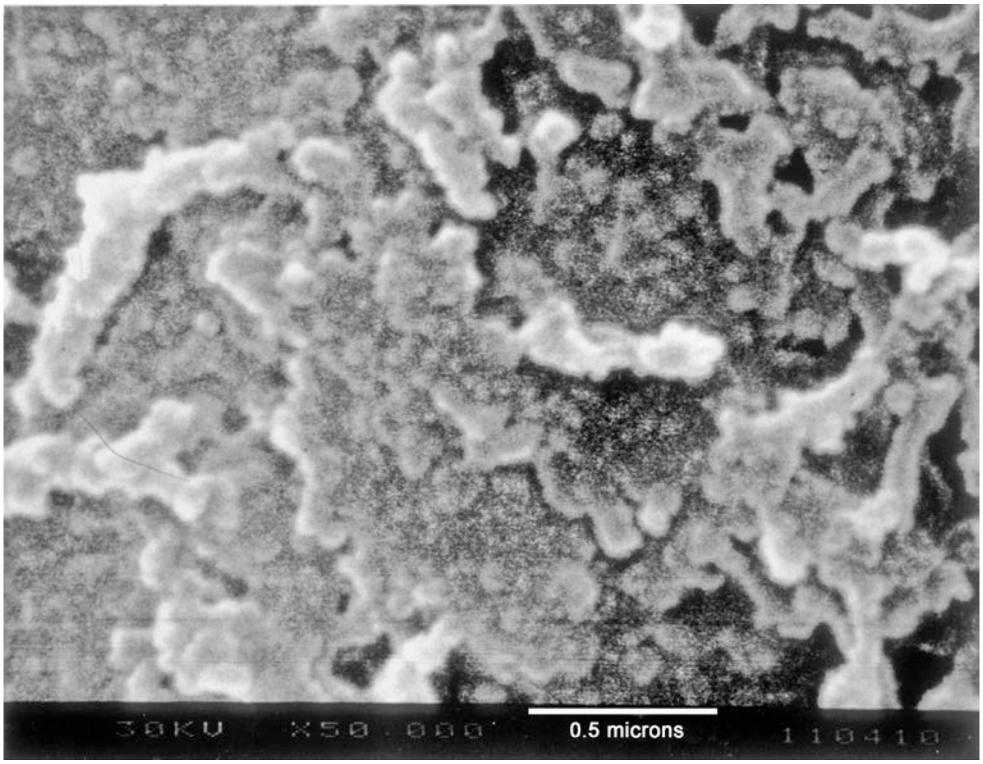

Figure25. Same as fig.24 also with lots of nannobacterial cells.

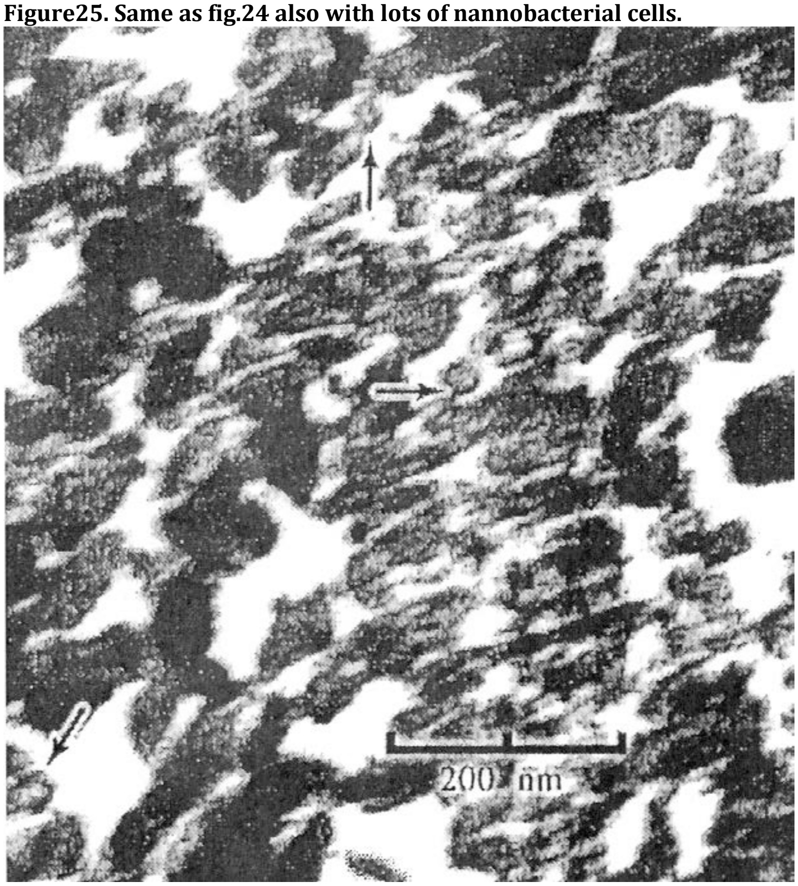

Figure26. TEM by Brenda Kirkland of part of an aragonite fuzzy dumbell analogous to fig.8 and 17. Balls show apparent electron-dense cell walls and luscent center, indicating that they are organic and not minerals.