Viterbo Slime, TEM Revelations

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A real breakthrough in proving the biogenicity of the Viterbo nannobacteria was made by the Kirkland-Lynch team in working with the Transmission Electron Microscope (TEM) at Mississippi State.

The sample was a zucchini-green, slimy biofilm collected at Le Zitelle hot spring, Viterbo, Italy by Folk and Prof. Libby Stern in 2004. The sample was prepared by Dr. Amanda Lawrence using standard biological techniques at the Electron Microscope Center, Mississippi State. This involved microtoming to 80 nm, fixing and staining with osmium tetroxide, uranyl acetate and lead citrate; these acidic reactions dissolve away the aragonite to leave only organic material, and cell walls and internal "guts" stain black so they are visible in the TEM. They found round cells as small as 87 nm in diameter, with distinct cell walls and internal "dots" resembling ribosomes (Kirkland et al., 2005). Clearly these are not minerals.

Later, in 2006, Kirkland and Folk continued this work and found circular objects with cell walls as small as 50 nm (Folk and Kirkland, 2007). Cells of this volume are about 1/50 th size of the "lower limits of life" as proposed by the biological community—who consider the limit to be a sphere of 200-250 nm.

Quantitative measurement shows a continuous graduation in cell size from ca. 40 nm up to 300 nm or more—there is no "break" around 200 nm. Cell walls are only 10 nm thick in the tiniest cells, but are typically 20-30 nm in the larger ones, indicating that they are all of similar ilk. Ribosomes (black dots 10-30 nm), occur mainly in cells of 200-400 nm, but can be traced down to cells smaller than 90 nm. Circular cells with clear walls but no visible ribosomes measure mainly 50-100 nm., and there are many opaque circular objects of 40-60 nm that show no clear central region (perhaps an artifact of sectioning). But all these objects of whatever size take the same stains as "large" bacteria, thus are clearly organic entities of some kind and not just inorganic CaCO3.

These cell sizes are in the same diameter range as nannobacteria previously seen calcified at Viterbo and imaged by Scanning microscopy (Folk, 1993), and are similar to those cells quantitatively measured from the Martian meteorite ALH84001 (Folk and Taylor, 2002), and in the kaolin clays from east Texas (Folk, McBride and Yancey, 2012, in press GCAGS). Thus the argument that the "purported nannobacteria from Mars" are "too small to be life" is clearly without validity.

We may have at Viterbo a modern living studiable example of "primordial soup" from which earliest life emerges . . . a problem worth studying by professional microbiologists with lots of money and a modicum of courage!

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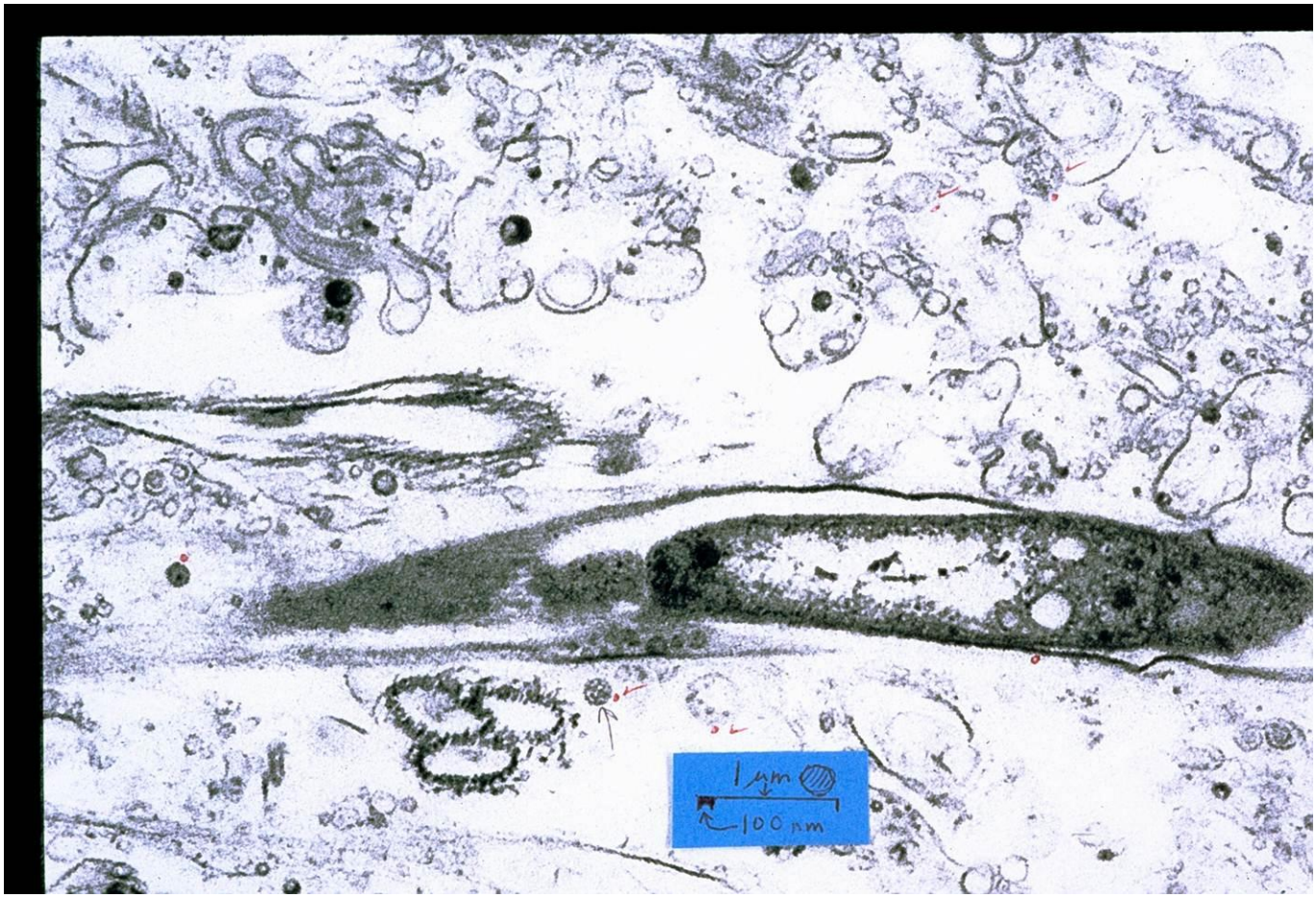
Figure 1. General view of TEM slice. A large organic body – bacterium? – shows cell walls and internal guts/vacuoles, and tiny dots, which are ribosomes. On the scale, the circle shows the "minimal size of life" – yet there are abundant circular objects in the photo that are a lot smaller but have similar cell walls and are some sort of biological object.

Figure 2. Enlarged view. Four cells, shown by check marks, have cell walls and internal "dots" that are probably ribosomes. Also there are many cells in the 50-100 nm range that show cell walls and lucent interiors.

Figure 3. From B. Kirkland 2005, a cell showing clear walls and internal dots. Gotta be "LIFE!"

Figure 4. A cell about 200 nm in diameter, at the "Lower Limit of Life," but with well-defined cell walls and internal ribosomes.

Figure 5. This specimen was TEM sectioned but NOT stained, so aragonite is preserved and shows up as dark objects. The aragonite forms dumbbell-shaped aggregates (top left) with growth zones (top right, curved band going from lower left to top right). A high-power view (middle photo) shows many round nannobacterial cells with lucent interiors about 50 nm in diameter. The bottom figure is an SEM photo of the same specimen. Balls of the same size are abundant along with rods of aragonite.



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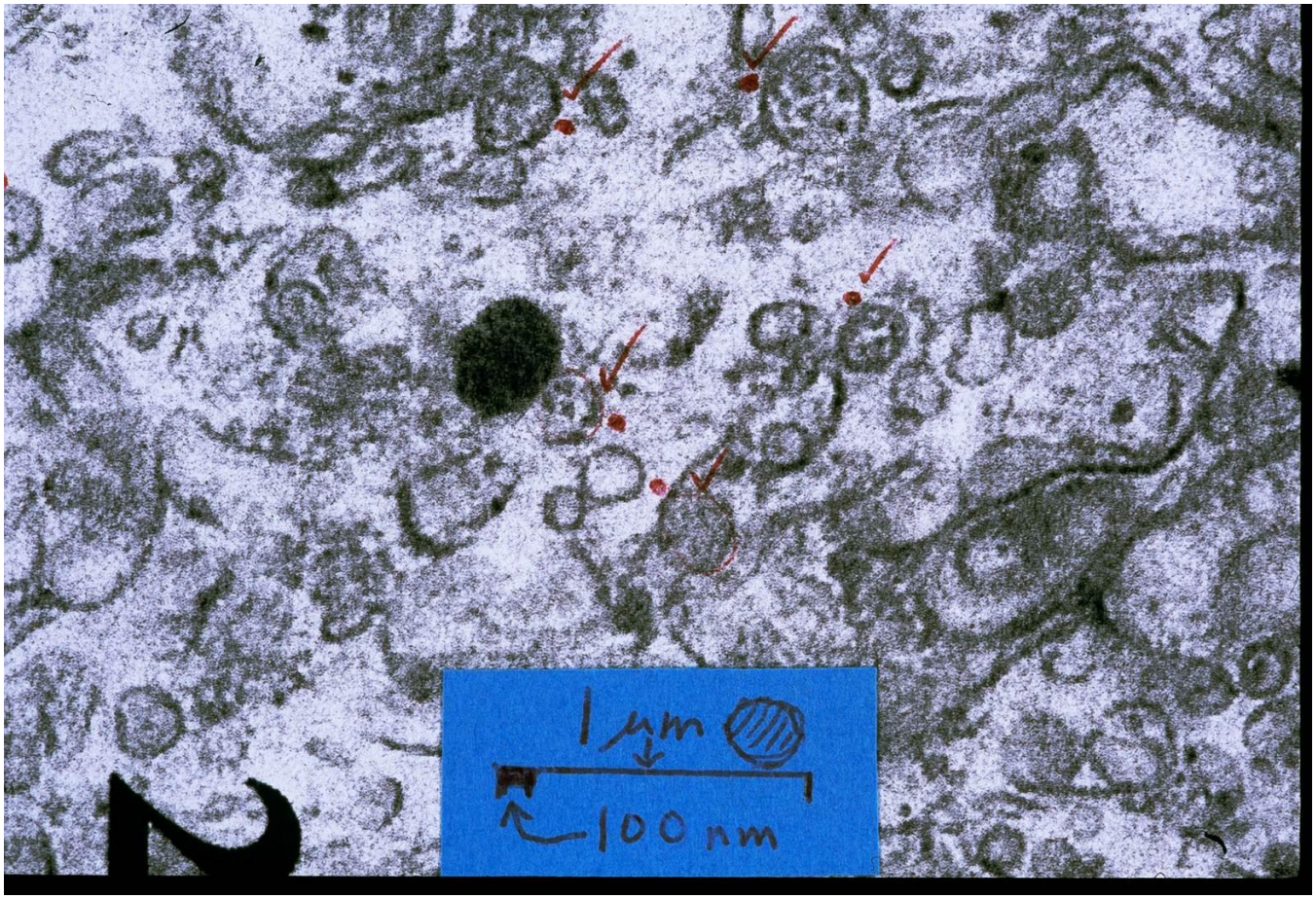

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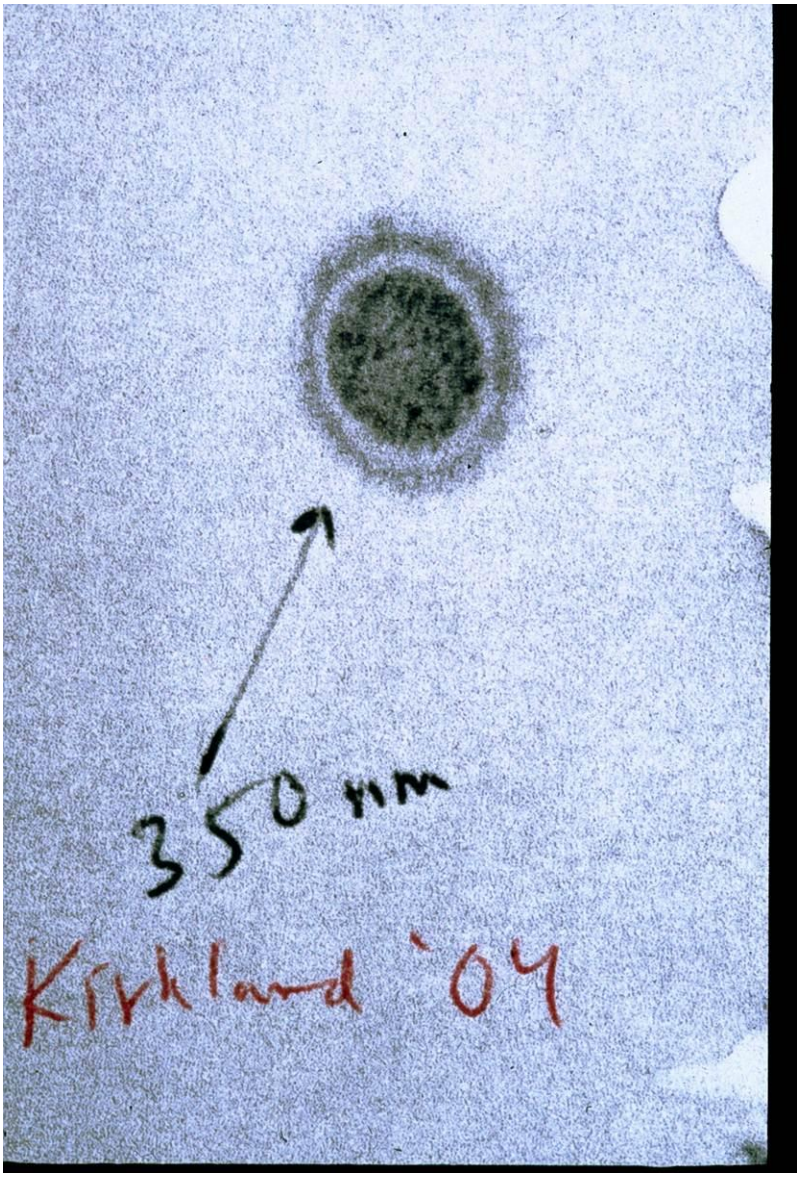

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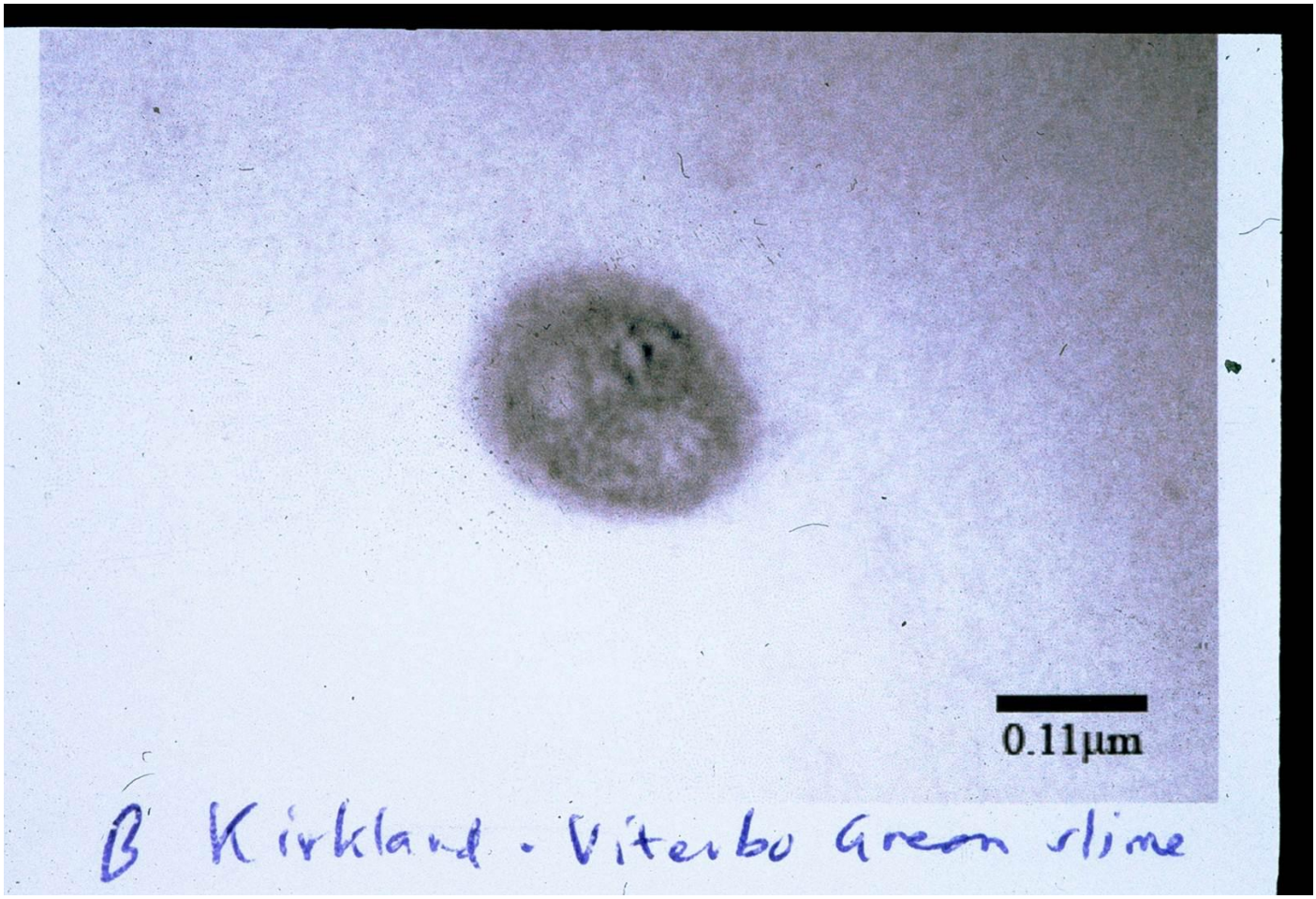

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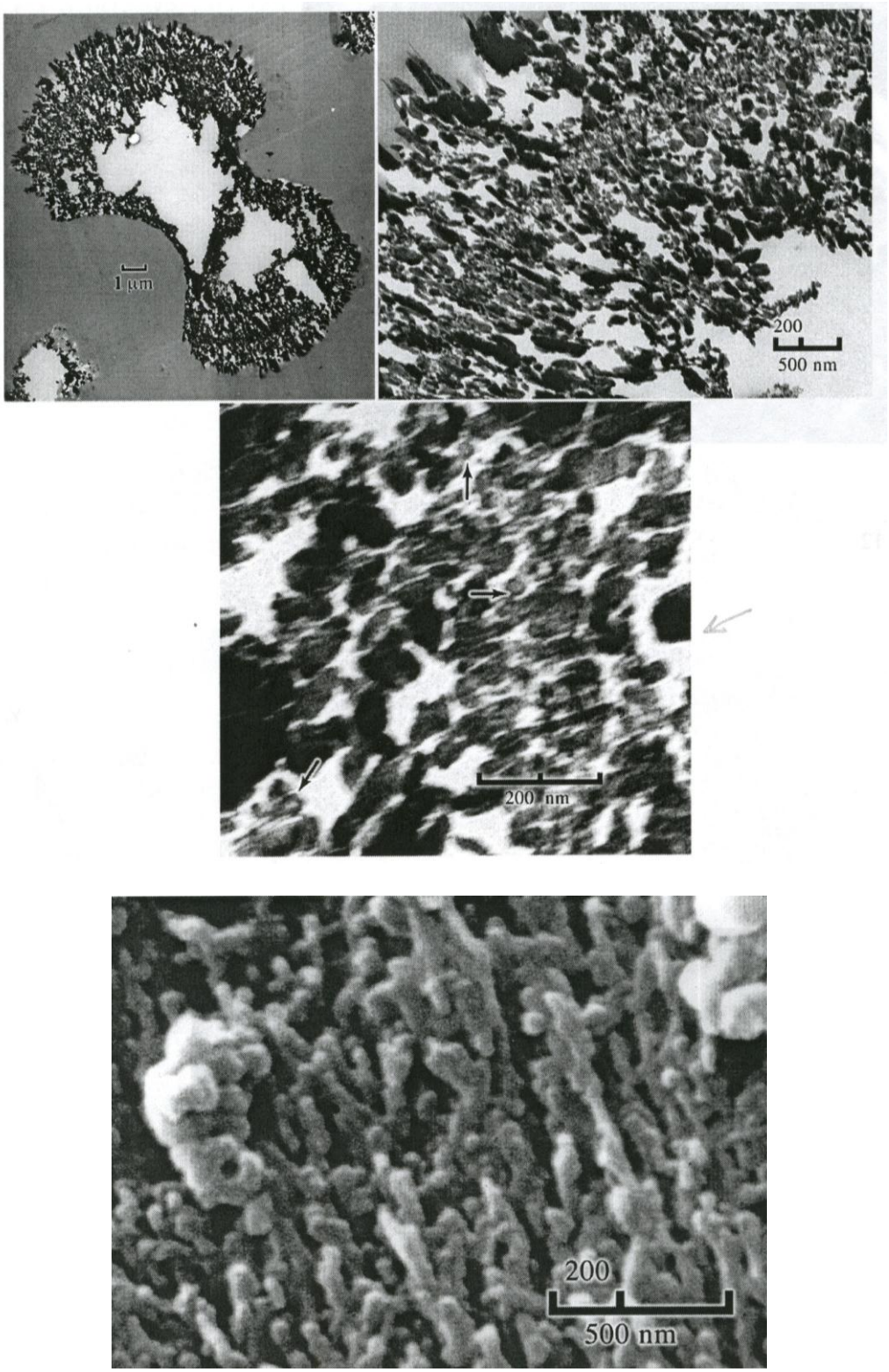

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