Zoological Studies 34, Supplement I (1995)

Zoological Studies

Confocal Microscopy of Cyanobacteria in Calcite Speleothems

Guy Cox[1], Anya Salih[1], Julia James[2] and Bill Allaway[3]
[1]Electron Microscope Unit, University of Sydney, NSW 2006, Australia
[2]School of Chemistry, University of Sydney, NSW 2006, Australia
[3]School of Biological Sciences, University of Sydney, NSW 2006, Australia

Speleothems apparently deposited by the action of algae or bryophytes have long been known in caves where some light is present but the environment is protected from atmospheric weathering (Cubbon 1976). These include small tufa-like deposits and small stalactites containing cyanobacteria (Braithwaite and Whitton 1987), but are relatively insignificant in scale. We have recently described (Cox et al. 1989 a, b) an altogether different type of speleothem - massive, ridge-shaped stalagmites which can measure metres in length and height. Cyanobacteria are abundant in the superficial calcite layer and give the stalagmites a deep green colour when they are damp. Both in external appearance and cross section they resemble some stromatolites.

These stalagmites are found in large tunnel-like caves with high roofs and large entrances, open at both ends. Specimens from all known sites share certain distinctive morphological features. They are shaped like a humped ridge, and they have a very characteristic stepped or layered profile. In size they may be anywhere between 200mm long and 100mm high to 2m by 1m.

Scrapings from the surface of the stalagmites reveal predominantly coccoid cyanobacteria, with abundant sheaths, which have been assigned to the genus *Gloeocapsa* (Cox et al. 1989b). Massive stalagmites do not normally form in the relatively dry environment of large cave entrances. For this to happen requires fairly rapid removal of CO_2 from the drip water landing on the stalagmite. Photosynthetic CO_2 fixation by cyanobacteria would provide a mechanism for this, and seems likely to be the major source of calcite deposition in these stalagmites.

Under the microscope the flora of these stalagmites is revealed as an almost pure stand of one or possibly two species of *Gloeocapsa*. This is in striking contrast to nearby pebbles and bedrock surfaces, most of which support a very mixed community of cyanobacteria (filamentous and coccoid) and eukaryotic algae (*Chlorella*, diatoms, dinoflagellates and chrysophytes) often lichenized with fungal hyphae. It would appear that calcite deposition is sufficient to exclude all but those species adapted to it; this further supports our view that these stalagmites are the product of a specific association.

Further investigation requires an understanding of the organization of the cyanobacteria within the speleothem. The confocal microscope is the only tool capable of providing this information. Small (~500 μm thick) flakes were taken from the surfaces of some of these speleothems. These were imaged in well slides in the confocal microscope using the red and yellow autofluorescence of the chlorophyll and phycobilins of the cyanobacteria. It proved possible to obtain satisfactory images down to almost 250μm below the surface of these flakes, so that by scanning from both sides virtually the whole volume could be sampled. Clearly there will be some refractive index mismatch but in practice the water-filled porous calcite seems to be sufficiently close in mean refractive index to immersion oil for adequate imaging down to the point at which the lens touches the coverslip.

Fig. 1 shows a Voxel-View reconstruction of a confocal dataset from one of these flakes. A depth of 27μm is sampled from within the flake - cyanobacteria are 'cut' by the optical sections at both the top and bottom of the set. Cyanobacteria are organized in clusters joined by a common sheath, as is typical of *Gloeocapsa*. It is also clear

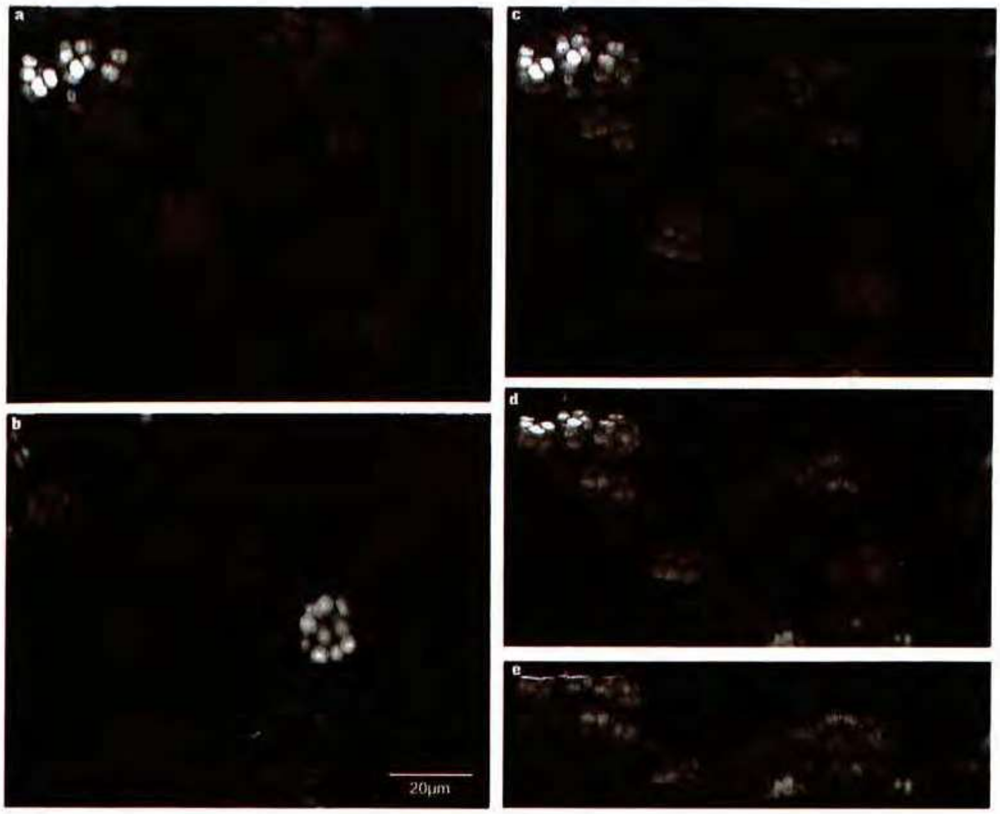

Fig. 1. Views of a confocal flyorescence dataset obtained on a Bio-Rad MRC600 confocal microscope. The original dataset sampled a volume 153×102×27 mm; this was been clipped slightly for volume rendering and pressntation. An oil immersion lens (x100, NA 1.3) was used. (a) Top view, looking straight down on the dataset. (b) bottom view, looking our from the inside of the calcite flake. (c), (d), (e) Tilt series, inclined 35°, 65° and 85° from the top view. In (e) we are looking almost along the plane of the flake.

that the cyanobacteria are overall quite uniformly distributed both laterally and vertically - there is no sign of vertical stratification at this scale, or indeed at the scale of the entire flake. Cell densities and arrangement are not visibly different when looking at either the top or the bottom of a flake. No point is more than 50μm from the nearest clump of cells, implying that the cyanobacteria are well positioned for effective removal of CO_2 from the water surrounding them. The whole pattern of their distribution strongly supports the view that this *Gloeocapsa* is adapted for life in this habitat, and that calcite is being deposited around them while they grow and divide - they are not an opportunistic species simply colonising new bare calcite surfaces.

The combination of confocal microscopy and volume rendering has provided a powerful tool for imaging this unusual bioherm in a way that would have been simply inconceivable a few years ago. Since all of the data is in digital form it is also simple to make it available for numerical analysis. These stalagmites are tens of thousands of years old, and still growing. Understanding the mechanism by which they form will provide the key to the information about past climates which lies buried in their lower layers.

We are very grateful to the management and staff of Jenolan Caves for allowing us to work in Nettle Cave, our study site. This work was supported by an Australian Research Council small grant.

REFERENCES

Braithwaite CJR, BA Whitton. 1987. Gypsum and halite associated with the cyanobacterium Entophysalis. Geomicrobiol. J. **5:** 43-55.

Cox GC, JM James, RAL Osborne, KA Legett. 1989a. Stromatolitic crayfish-like stalagmites. Proc. Univ. Bristol. Spelaeol. Soc. **18:** 339-358.

Cox GC, JM James, KA Legett, RAL Osborne. 1989b. Cyanobacterially deposited speleothems: subaerial stromatolites. Geomicrobiol. J. **7:** 245-252.

Cubbon BD. 1976. Cave Flora, in The Science of Speleology (ed. T.D. Ford and C.H.D. Cullingford). Academic Press, London, New York, San Francisco, pp 423-452.