Walli Cave Entrance Micro-organisms

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Introduction

Walli Caves (NSW) are located on private grazing property about 215 km west of Sydney. During a cave aragonite study, samples were obtained of the Cliefden Caves Limestone at the surface at Walli (Rowling 2004), some of which had surface coatings. It was apparent that some of the colours seen were a thin coating of calcium carbonate containing a biological colouring agent which was initially thought to be a red algae. A few years after collection, the surface samples were scraped to obtain some of the mainly white and pale orange coatings, and sent to Dr Brett Neilan who heads the University of New South Wales School of Biotechnology and Biomolecular Sciences, where it was examined by students and staff at the School.

Dr Neilan later commented that the material was "indeed a cyanobacterium ... most closely related to nitrogen-fixing filamentous species" and that one match of the material's DNA was to a bacterium found in deep sea sediment. No fungus was detected in the sample, as it was old.

A trip to Walli Caves organised by Sydney Speleological Society in July 2006 was an opportunity to further examine the coatings near cave entrances.

Materials and methods

There are no particular restrictions on sampling imposed by the land owner. Permission to sample was obtained by personal communication to Peter Wellings of the Sydney Speleological Society and the trip leader.

Samples were to be taken of the different types present, based on appearance. The caves chosen are relatively close to the camp site, have a good representative collection of coloured coatings apparently due to micro-organism colonies, and have been previously studied and described (Wilkinson 1892, Frank 1974).

A steel blade was used to scrape the samples into new ziplock polyethylene bags, aiming to obtain about 1 g of material (about a heaped teaspoonfull). Samples were to be taken from areas while preserving the natural appearance of the site, and photos were taken of the sampled areas. Part of each sample was transferred to a microscope slide for viewing and scanning.

Piano Cave observations

Piano Cave was visited on 8/7/2006 with members of Sydney Speleological Society including Ernie Byrnes, Larry Zanker and Steven Zanker and Jill Rowling. The weather was rather cold but the cave was warm and humid, typical for Walli. The group mainly looked at historical signatures; Jill looked at geomorphological features such as ceiling canyons and some evidence that warm water had risen into the cave from sumps and springs (Figure 1 map, GR 029 038). A peculiar calcite coating in one chamber may be a hydrothermally deposited crystal lining (map GR 045 086, WA12/5) Rowling (2004). Jill obtained samples of coloured coatings just outside the old gate, where it is dim but dark enough to need a light (map, Figure 1).



Samples and discussion - Piano Cave

A reddish coating on stalactites and other speleothems just outside and south of the old cave gate and in the twilight zone is mainly mineral, comprising calcite and clays. The red may be either humic acids or iron oxides (or both).



Figure 2: Sample WA12/19.

A white coating on the otherwise black limestone bedrock of the entrance tunnel just outside and North East of the old cave gate, at about head height and in the twilight zone comprises minerals



Figure 3: Sample WA12/20.

resembling gypsum and barite, characteristic of Walli (Rowling 2004).

A dark green stalactitic coating in a sheltered ceiling corner near the cave entrance appears to be a mixture of green algae and bryophytes (mosses and liverworts) on a moonmilk substrate (often needle-form calcite, trace aragonite and vaterite). Algal colonies occur



Figure 4: Looking towards Piano Cave entrance.

throughout the moonmilk, indicating some light penetrates the porous material. The moonmilk holds water, which in turn supports the algae.



Figure 5: Piano Cave: Samples WA12/21, 22, 23 with coloured coatings.

The pinkish band between the green and grey areas appears to be mainly mineral, comprising a creamy white crystalline coating (probably calcite) over moonmilk. This is possibly a mixture of calcite, small quantities of aragonite and vaterite as it has an anomolous colour with UV (purple and green-white with afterglow),



Figure 6: Coloured coatings, Piano Cave.

with underlying brown to reddish sediment (possibly clays cemented with calcite). A small amount of green material appears to be green algae.

The blue-grey-green coatings near the North wall of the cave entrance comprise a fine grained mineral (probably calcite) coated with green algae, filamentous organisms and bryophytes. The filamentous organisms favour the finer grained material and appear to develop around the grains, presumably as they are sheltered. Some of the clear mineral grains have a high refractive index; at Walli this is commonly gypsum or barite.

This sample appears purplish from a distance, comprising reddish coloured flakes and chips which are pink and green layers on speleothems on the North wall of the cave entrance. The purple-colouring appears to be an optical effect caused by tiny green algal colonies developing on porous reddish layered sediments, mainly calcite with clays. The algae typically grows between the mineral grains. The reddish outer layer appears to be calcite; below this is a layer of emerald green algal colonies. Below that is clear columnar calcite, then porous reddish material (possibly clays), a white layer (moonmilk) and more clays.

Several organisms form green cave coral shapes on the wall near the entrance, intermittently exposed to daylight: bryophytes, green algae and two types of microscopic, light grey-green filamentous organisms. The

Sample WA12/25



Figure 7: Sample WA12/25.

substrate is moonmilk and cave sediment (calcite and clays).

Under the microscope, green algae forms emerald-green spherical colonies.

The filamentous material takes two forms.

Grey-green filaments are visible under the microscope at 50x. They are 1 to 2 mm long and about 0.03 mm diameter, comprising emerald green inner filaments with a silvery outer coating (possibly calcite), and glow weakly green under UV light. They may be calcite-coated bacterial threads (Figure 8).



Figure 8: Sample WA12/25.

The other filamentous form is about an order of magnitude finer, hard to see under 70x magnification, and comprises a network of short, grey-green filaments which re-join, resembling fungal hyphae.

Possibly the whole colony is a symbiosis of cyanobacteria and a fungus.

A purple fluorescence seen under UV light was thought to be caused by moonmilk.

Bone Cave observations

Jill visited Bone Cave accompanied by Larry Zanker on Sunday 9th July 2006, a sunny, cold day and much warmer inside the small cave (Figure 9). The entrance area was littered with Kurrajong leaves and nuts.



Figure 9: Entrance, Bone Cave, with Kurrajong tree.

The lower area of the cave had mildly elevated CO_2 . Part of the ceiling has an interesting inverted canyon shape, reminiscent of past warm spring activity. The twilight area has coloured deposits (plant and mineral) on the walls. Four samples of coatings were taken, including some like white concrete, also pale green, blue-green and black coatings. The samples were all taken from the hanging wall inside the cave entrance, where the light is dimmer and a torch is needed (Figure 10).



Figure 10: Bone Cave sampled sites (Map based on Frank 1974).

Samples and discussion - Bone Cave



Figure 11: Coloured deposits on the hanging wall, Bone Cave.

Concrete-like white coralloids are mainly mineral with occasional spherical colonies of green algae between the grains. Anomalous colours under long-wave UV light (yellow-white and purple with greenish afterglow) suggest mainly



Figure 12: Sample WA14/1.

calcite with moonmilk (mostly needle-form calcite) & possibly huntite.

Blue-grey-green "fluff" has developed over a tan-coloured sediment of calcite, clays and organic material. Two different types of organisms are present, similar to WA12/25, namely a larger filamentous form (cyanobacteria) which can be seen on low power, and a very fine mesh form (possibly fungus), visible only under high power (Figure 13). Under long wave UV light, the fluff fluoresced slightly purple so it may have a carbonate coating such as calcite or vaterite.



Figure 13: WA14/2.

This dark green sample comprises mainly dark emerald green (to black) colonies of green algae, coating tan-coloured cave sediments. The layered sediment comprises reddish clays, clear columnar calcite crystals and possibly some aragonite (or a paramorph of calcite after aragonite). The green algal colonies coat the sediment grains and lie between the crystals.

The sample comprises scrapings of the light green material from coralloids. The mineral component appears to be calcite grains, moonmilk and possibly some huntite based on the long wave UV response (glows purplish and white with a short, green afterglow). The biological component is made of



Figure 14: Sample WA14/4.

small emerald-green colonies of algae amongst the grains.

Conclusions

Some reddish-brown colours

seen near cave entrances at Walli are due to a thin covering of clear and white calcite over cave sediments including calcite and reddish clays. Purplish colours result from an optical effect of emerald-green algae colonies on reddish-brown sediment. White coatings and soft deposits may be moonmilk, mainly calcite with possibly small quantities of aragonite or vaterite, often with an organic component. The light green coatings on the white deposits are small emerald green colonies of green algae developed on clear calcite and white moonmilk.



Figure 15: Coloured coatings, Bone Cave.

The shade of green is determined by the density of colonies.

Dark green coatings are usually dense colonies of green algae (emerald green under the microscope).

Black coatings near the cave entrance are usually dense colonies of green algae, black to emerald green under the microscope, suggesting possibly two different species.



Figure 16: Coloured coatings, Bone Cave.

The blue-green and grey-green coatings are dense colonies of at least two different species, possibly cyanobacteria and fungus. One (bacterium) type forms long filaments which are apparently coated with calcite, and the other (fungal) type forms a fine mesh of thinner green filaments, also possibly with a calcite coating.

Bryophytes colonise the lighter areas, where they compete with other organisms, depending on available light, soil and water.



Figure 17: Coloured coatings, Bone Cave.

References

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