Walli Cave Entrance Micro-organisms

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Abstract

The entrances of Piano Cave and Bone Cave at Walli (NSW) were examined to see whether the colours of the coatings were due to minerals or organisms. Both mineral and biological coatings were found. Some of the orange colours are due to the rapid precipitation of calcium carbonate (white) together with either reddish clays or red algae, or both. The dark greens and blacks are colonies of a green algae, which appears emerald green under the microscope. A pale green colour can occur where this algae co-exists with rapidly precipitated calcium carbonate such as moonmilk, and may persist as microscopic emerald-coloured speckles a few millimetres into the precipitate, presumably according to the ambient light levels and the substrate porosity. A purple colour resulted from a reddish mineral substrate (carbonate-cemented clays) with microscopic colonies of emerald-green algae. Bryophytes also develop in the shaded parts of cave entrances, usually away from the most direct sunlight. Some organisms form a blue-green colour, coating moist speleothems where there is sufficient filtered ambient light. Two types of filamental blue-green organisms were observed under the microscope, and may be a symbiosis of fungus and cyanobacteria.

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. The sample was 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, however fresh samples might be more useful.

When a trip to Walli Caves was organised by Sydney Speleological Society in July 2006, this was seen as an opportunity to further study the coatings near cave entrances. Piano Cave and Bone Cave were chosen due to their ease of access and having been previously described and studied (Wilkinson 1892, Frank 1974).

Aims

The aim was to visit Piano Cave and Bone cave which have interesting coatings near the entrance with a biological component rather than the purely mineralogical components previously examined by Rowling (2004). It was hoped to identify what was the cause of the colour seen.

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, and have a good representative collection of coloured coatings apparently due to micro-organism colonies. 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 teaspoonful). 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, describing, and scanning.

Piano Cave observations

Piano Cave was visited on Saturday 8th July 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 spent some time in the cave, mainly looking at historical signatures; Jill looked at some of the 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). She suspects a peculiar crystalline calcite coating on a bedrock pendant in one chamber to be an original crystal lining, possibly dating to a thermal period of the cave's development. This calcite was previously described in Rowling (2004) (map GR 045 086, WA12/5).

Sampling was done by Jill (with Steven Zanker) on the way out, just outside the old gate, where it is dim but dark enough to need a light (map, Figure 1). Samples are discussed below.

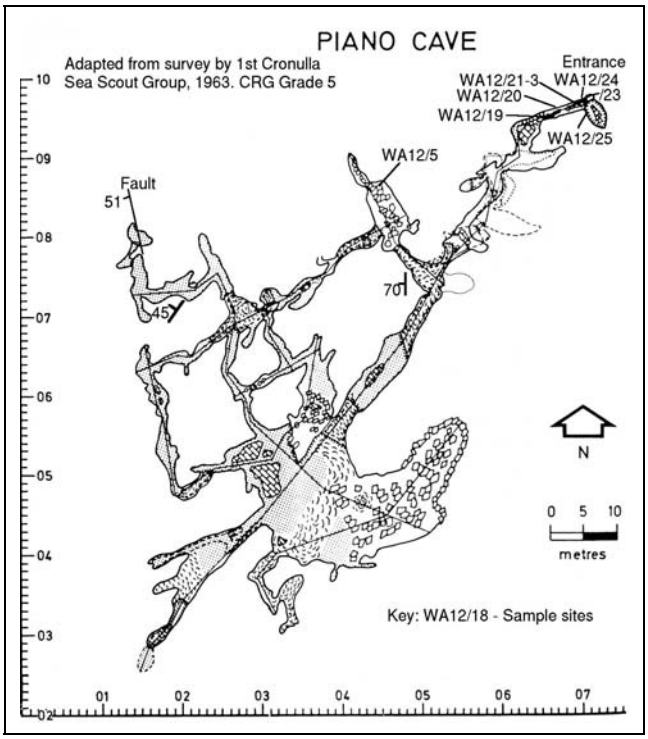

Figure 1: Piano Cave (plan): Sampled sites (Map based on Frank 1974).

Samples and Discussion - Piano Cave

Sample WA12/19

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.

Sample WA12/20

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

Sample WA12/21

The dark green stalactitic coating in a sheltered ceiling corner near the cave entrance (Figure 2) appears to be a mixture of green algae and bryophytes on a substrate of calcite moonmilk. The algae colonies occur throughout the moonmilk, indicating some light penetrates the porous material. The moonmilk holds water, which in turn supports the algae. Moonmilk is a particularly fine form of mainly calcium carbonate, often with a fibrous "thatch" structure due to biological activity. It can be made of needle-form calcite ("lublinite"), sometimes with small quantities of aragonite and vaterite, and is usually fairly quickly precipitated leading to a small crystal size.

Figure 2: Piano Cave: Sample site WA12/21, 22, 23 with various coloured coatings.

Sample WA12/22

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 anomalous colour with UV (purple and green-white with afterglow), with underlying brown to reddish sediment (possibly illite cemented with calcite). A small amount of green material appears to be green algae (Figure 2).

Sample WA12/23

The blue-grey-green coatings near the North wall of the cave entrance is 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.

Sample WA12/24

This sample appears purplish from a distance, comprising reddish coloured flakes and chips which are layered pink and green coatings on speleothems on the North wall of the cave entrance. The purple-colouring appears to be an optical effect caused by green algal colonies developing on porous reddish layered sediments, mainly calcite with clays. The algal colonies have typically developed 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.

Sample WA12/25

The sheltered area near the cave entrance exposed intermittently to daylight is colonised by several organisms including bryophytes, green algae and two types of light grey-green filamentous bacteria forming coralloid shapes on the wall. The substrate is moonmilk and cave sediment comprising calcite and clays. Under the microscope, the green algae forms emerald-green spherical colonies. The filamentous material takes two forms. One form is easily visible under a microscope at 50x, and forms grey-green filaments 1 to 2 mm long and about 0.03 mm diameter, comprising emerald green interior filaments coated with a silvery exterior (possibly calcite), and glow weakly green under UV light. It is thought that these are calcite-coated bacterial threads (Figures 3 and 6). The other filamentous form is about an order of magnitude finer, hard to see under 70x magnification, and comprises a network of short filaments which can re-join forming green-grey threads a little like fungal hyphae. It is assumed that the second form is a different species to the first form. Possibly the colony is a symbiosis of cyanobacteria and a fungus. A purple fluorescence seen under UV light was thought to be caused by moonmilk.

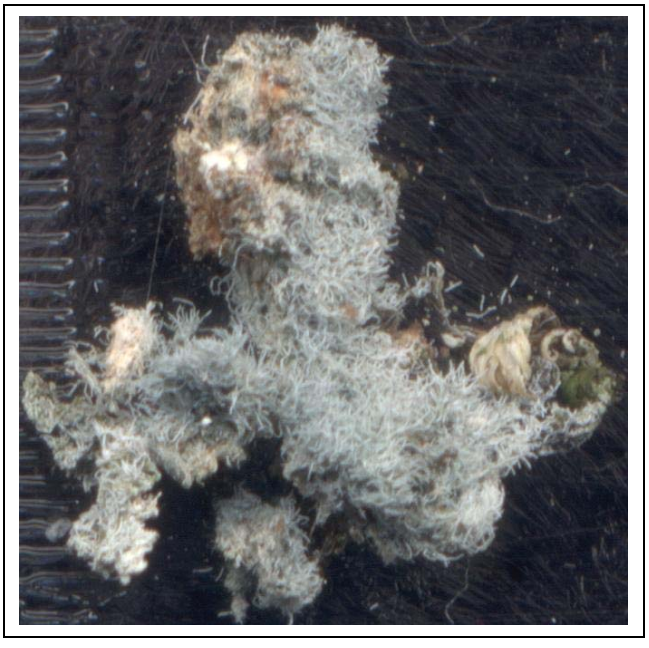

Figure 3: Filamentous micro-organisms and bryophyte, Piano Cave entrance, scanned.

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 4). 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 5).

Figure 4: Entrance, Bone Cave, with Kurrajong tree which drops leaves and nuts into the cave.

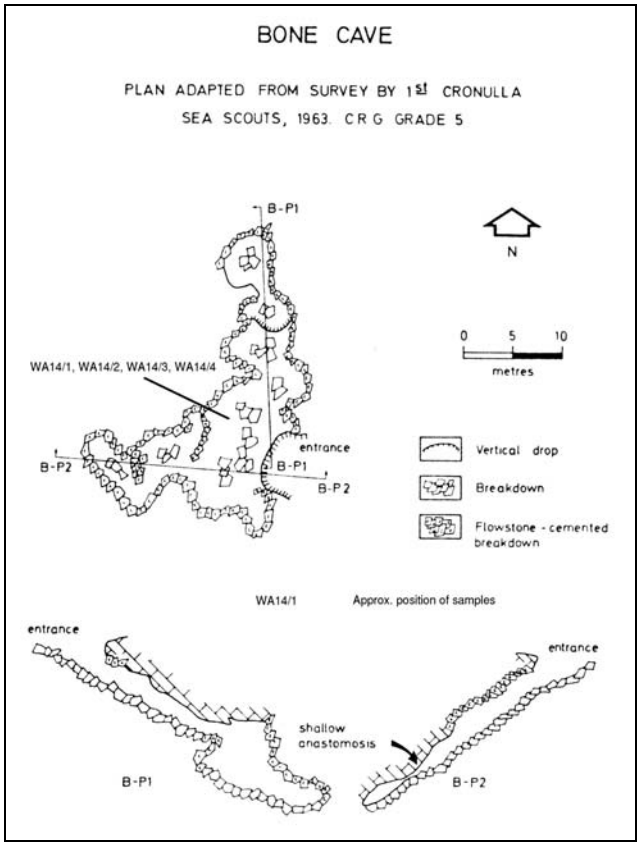

Figure 5: Bone Cave: Sampled sites (Map based on Frank 1974).

The white concrete-like coralloids are mainly mineral (for example, calcite, huntite, aragonite and gypsum) with occasional spherical colonies of green algae buried in between the grains. Anomalous colours under long-wave UV light (yellow-white and purple with greenish afterglow) suggest mineral components are mainly calcite with some moonmilk (needle-form calcite possibly with trace aragonite and vaterite) and possibly some huntite. The presence of algae was surprising since the material did not look obviously green.

Sample WA14/2

The sample comprised blue-green “fluff” developed over a tan-coloured soft sediment. The soft crumbly sediment substrate is composed of calcite, clays and some organic material. The blue-grey-green fluffy material comprises two

Samples and Discussion - Bone Cave

Sample WA14/1

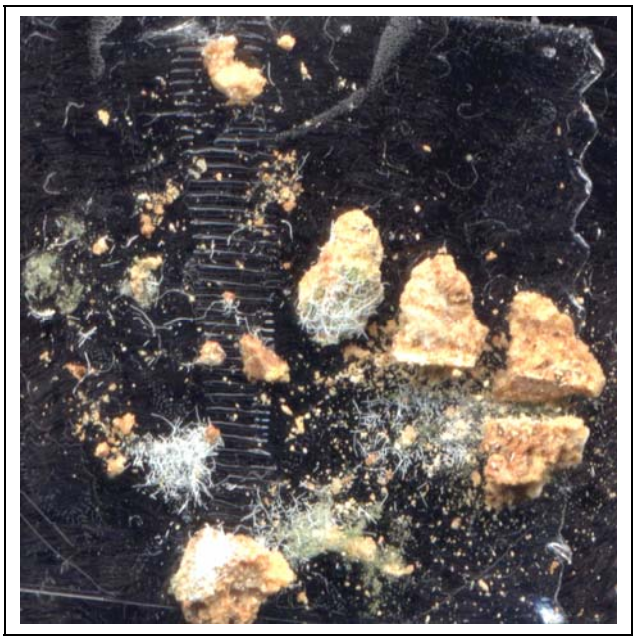

Figure 6: Filamentous micro-organisms, two types, on sediment from Bone Cave (scanned). Grains are about 1 mm diameter.

different types of organisms which have developed on the sediment. The organisms may be two types of cyanobacteria or a bacterium and a fungus, and are similar to the types observed in sample WA12/25, comprising a larger filamentous form (cyanobacteria) which can be seen on low power, and a very fine mesh networking form (possibly a fungus) which is seen under high power. The second type forms a three dimensional network (Figure 6). Under long wave UV light, the fluffy material fluoresced slightly purple.

Sample WA14/3

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 small quantities of aragonite (or a paramorph of calcite after aragonite). The green algal colonies coat the sediment grains and lie between the crystals.

Sample WA14/4

The sample comprises scrapings of the light green material from coralloids. The mineral component appears to be calcite with some moonmilk (needle-form calcite, possibly trace aragonite and vaterite) 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 small emerald-green colonies of algae amongst the grains.

Conclusions

Many of the reddish brown colours seen near cave entrances are due to a thin covering of clear and white calcite over cave sediments including calcite and reddish clays. White coatings and soft deposits may be moonmilk, mainly calcite and 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" (mixture of possibly calcite and small quantities of aragonite). 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 algae. These are usually black to emerald green under the microscope, suggesting possibly two different species. 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 cyanobacteria, fungus and algae, depending on available light, soil and water.

Acknowledgements

Particular thanks are given to Dr. Brett Neilan and students at the University of New South Wales School of Biological and Biomolecular Sciences for their insight into the types of micro-organisms to be encountered near cave entrances. Thanks are given to members of the Sydney Speleological Society for enthusiastically supporting the trip.

References

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Appendix

Summary of Samples

Table 1 lists the sampled material.

Table 2 suggests the composition of the sampled material.

Sample No.	Cave	Location	General Appearance
WA12/19	Piano	Dark/twilight zone, map GR 067 095	Reddish coating on speleothems
WA12/20	Piano	twilight zone, map GR 068 096	White coating on bedrock
WA12/21	Piano	sheltered zone, ceiling, map GR 070 096	Green coating on white powder
WA12/22	Piano	sheltered zone, ceiling, map GR 070 096	Pinkish powder
WA12/23	Piano	sheltered zone, ceiling, map GR 070 096 & sheltered zone, map GR 072 097	Blue-green powder
WA12/24	Piano	sheltered zone, ceiling, map GR 071 097	Reddish flakes
WA12/25	Piano	entrance wall, map GR 071 095	Green and grey fluff
WA14/1	Bone	Hanging wall, base of rubble pile	White powder
WA14/2	Bone	Hanging wall, base of rubble pile	Blue-green fluff
WA14/3	Bone	Hanging wall, base of rubble pile	Dark green material
WA14/4	Bone	Hanging wall, base of rubble pile	Light green material

Table 1: Samples from the entrances of Piano Cave and Bone Cave, Walli Caves.

Sample No.	Suggested mineral components	Suggested biological components
WA12/19	calcite, clays	-
WA12/20	gypsum, barite	-
WA12/21	"lublinite" calcite, trace aragonite, vaterite	Green algae and actinomycetes (in moonmilk)
WA12/22	"lublinite" calcite, clays	actinomycetes (in moonmilk)
WA12/23	"lublinite" calcite, trace gypsum or barite	filamentous cyanobacteria
WA12/24	calcite, clays	green algae
WA12/25	"lublinite" calcite	filamentous cyanobacteria, fungus, bryophytes, green algae
WA14/1	"lublinite" calcite, trace aragonite, vaterite, huntite	green algae
WA14/2	"lublinite" calcite, clays	filamentous cyanobacteria, fungus
WA14/3	calcite, clays, trace aragonite	green algae
WA14/4	"lublinite" calcite, trace aragonite, vaterite, huntite	green algae

Table 2: Estimated composition of samples.