

# **Blue-green algae (Cyanobacteria): an introduction and description of their occurrence in cave entrances of South Island, New Zealand**

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## **An introduction to Cyanobacteria**

Cyanobacteria, commonly known as blue-green algae, are photosynthetic bacteria. They are found in the large majority of sunlit environments (Whitton and Potts 2000; Whitton 2012). Like plants, they use chlorophyll *a* to capture photons of light and channel this energy into making organic molecules from carbon dioxide and water. The waste product is oxygen. Most also contain phycocyanin, a bluish pigment which is responsible for their common name. The other resource they need for growth is a range of mineral nutrients, like nearly all plants and other algae. Their cell structure is prokaryotic (meaning before nucleus), a feature that they share with all other bacteria and archaea. The hereditary material is a simple twisted loop of DNA that lies in the centre of the cell. In contrast, all other organisms, including humans and plants, are eukaryotic (meaning true nucleus). Their cells each have a membrane-surrounded nucleus that contains the DNA in complex chromosomes.

Photosynthesis first evolved at least 3,500 million years ago (Schopf 2012). Some of the ancient microbes with this capacity resembled present-day cyanobacteria. Their major evolutionary innovation of oxygen release during photosynthesis eventually resulted in the accumulation of oxygen in the atmosphere from about 2,450 million years ago. This radically altered Earth's ecosystems and provided the oxygen required for the more efficient energy-generating process, called aerobic respiration that sustains the large majority of today's microbes, plants and animals.

Another outstanding evolutionary development was the transformation of a free-living single cell cyanobacterium into the structure called a chloroplast. Chloroplasts are found in cells of all so-called eukaryotic photosynthetic organisms, these being plants and eukaryotic algae. Evidence suggests that about 2,000 million years ago a single cell, eukaryotic protozoan retained an ingested cyanobacterium as a functioning unit rather than digesting it as food (Sánchez-Baracaldo et al. 2017). Over subsequent generations, many genes were lost from the cyanobacterium and others were transferred to the nucleus of the protozoan, which as a consequence gained control over most of its remaining functions. The cyanobacterium was now a chloroplast that divided when the cell divided resulting in daughter cells each with their own chloroplast. This photosynthetic

protozoan was the ancestor of all eukaryotic algae, for instance phytoplankton and seaweeds, and of all land plants. The origin of our verdant landscapes was a symbiotic merger of a cyanobacterium with a protozoan.

Another important function of their physiology is the ability of many to use molecular nitrogen ( $N_2$ ) as a nutrient. This is termed nitrogen fixation. No other types of algae, plants, fungi or animals can do this, although some have mutually beneficial relationships with cyanobacteria or other bacteria that can. Of huge importance are the nitrogen-fixing marine cyanobacteria that are largely responsible for maintenance of the nitrogen fertility of the oceans.

The Cyanobacteria is the most morphologically diverse group of bacteria. The wide range of structure they express in the modern world is also found in ancient fossils. The simplest forms comprise single cells, clusters of cells forming colonies, and cells remaining attached to one another to form unbranched chains or filaments. These have been found in rocks dating back to 2,600 million years before present. There are even older cyanobacteria-like fossil microbes from about 3,500 million years ago. However, it is impossible to unequivocally say that these could not be other types of bacteria. The most complex cyanobacteria have filaments up to several cells wide which produce side-branches. This morphology is found in fossils from about 400 million years ago.

Cyanobacteria are also remarkable amongst bacteria in general in that some filamentous forms differentiate two types of specialised cells. Heterocysts have thickened walls and lack the oxygen-producing stages of photosynthesis. In these ways they protect the key enzymes used in nitrogen-fixation from inhibition by oxygen. Without these adaptations, oxygen would pass into the cell from the external environment as well as being produced during photosynthesis. Species with heterocysts can therefore fix nitrogen during the day. Some other species that lack heterocysts restrict nitrogen fixation to night-time when there is no photosynthesis. Akinetes are cells that can survive adverse conditions following the death of undifferentiated vegetative cells. For instance, they can spend winter in lake sediments. They have much thickened walls and accumulate granules of chemicals that are energy and nutrient stores. On return of favourable conditions, the akinete undergoes cell division, the wall splits, and a new filament emerges.

The taxonomic system for cyanobacteria was originally largely based on their morphology as seen in specimens obtained from nature and viewed down a light microscope. Depending on how a species was defined there were two wildly divergent estimates of their total number. As increasing numbers were brought into culture and grown as pure strains another competing system was established based on morphology and patterns in cell division. In recent decades the development of molecular genetics has greatly modified concepts of evolutionary relationships amongst cyanobacteria. For instance, species with single cells and those with simple filaments were once each placed in their own order. Now it is clear that some single cell species are more closely related to

certain filamentous species than to other single cell species, which are more closely related to other simple filamentous species.

Modern taxonomy of cyanobacteria takes into account all sources of evidence in an approach termed polyphasic (Komárek 2016). Importantly this includes molecular phylogenetics in which the sequences of nucleotides that make up the DNA of particular genes possessed by different species are compared and used to construct evolutionary trees. Additional data on morphology comes from light microscopy and on internal cell structure from transmission electron microscopy. Field specimens still provide valuable information on distribution and ecology whilst single strains grown in culture are indispensable for understanding biochemical and physiological characteristics. All this information is gradually producing a more stable taxonomy that reflects evolutionary relationships but there is recognition that this still needs to change, and sometimes radically, as new information comes to hand.

The largest compilation of species descriptions has been made for the Freshwater Flora of Central Europe. This massive work encompasses three volumes (Komárek and Anagnostidis 1999, Komárek and Anagnostidis 2005, Komárek 2013) and covers about 1,430 relatively well-established European species. It also contains descriptions of many species from outside Europe as well as of species that need clarification. In the short time since its publication, the classification at the level of orders and families has been reorganised. A recent, widely accepted taxonomy based on the polyphasic approach recognises eight orders containing 45 families and 281 genera (Komárek et al. 2014, Hauer and Komárek 2020). Difficulties and inconsistencies remain and much research is still needed.

Such a high level of diversity that has evolved over an immense period of time suggests that evolutionary adaptation to a wide range of habitats must have occurred. Indeed, this is the case, as there is barely a habitat that receives light and contains available water, even if only briefly, that does not support at least some cyanobacteria.

They are almost ubiquitous throughout illuminated habitats in marine and freshwater ecosystems. An example of marine phytoplankton is the unicell *Prochlorococcus marinus* (Biller et al. 2015). This is the smallest and most abundant photosynthetic organism on Earth. Its cells are less than one thousandth of a millimetre in diameter and number about  $10^{27}$ . It is predominantly found in tropical and subtropical oceans that are deficient in nutrients but its productivity is equivalent to that of all global croplands. In lakes, mass growths of cyanobacteria have become increasingly common due to eutrophication and climate warming (Paerl and Huisman 2008). Cyanobacteria generally grow better at higher temperatures than do other phytoplankton. At its extreme this is demonstrated by the thermophilic (heat-loving) cyanobacteria that flourish in hot springs at temperatures up to 74°C (Ward et al. 2012). At the opposite end of the temperature range, cyanobacteria usually dominate the

microbial communities that develop in meltwater ponds on glaciers (Quesada and Vincent 2012).

The surface layers of almost all soils support cyanobacteria, usually in association with diverse eukaryotic algae. Soil habitats range from the driest deserts, such as the Chilean Atacama Desert and the McMurdo Dry Valleys of Antarctica, to swamps and mires.

Rock surfaces at all altitudes, from those just above coastal high tide levels to those of high mountains, often have dark, thin crusts and mats of cyanobacteria, particularly where water seeps over them. Endolithic cyanobacteria occur below rock surfaces. Some species actively bore into limestone whilst others live in microscopic cavities between rock crystals in rock types that are translucent.

Symbioses are widespread. These are mostly mutualisms in which both partners benefit. A few lichens have cyanobacteria as the main photosynthetic partner whilst others with green algae photobionts have cyanobacteria restricted to specialised cephalodia. Within these structures the cyanobacteria, rather than being photosynthetic, are supplied with sugars that fuel their fixation of nitrogen. Several hornworts and liverworts have nitrogen-fixing cyanobacteria in specialised cavities as does the aquatic fern *Azolla*. Some cycads have these in intercellular spaces inside specialised roots that turn upwards and emerge at the soil surface, whilst species of just one angiosperm genus, *Gunnera*, take cyanobacteria into their cells in a specialised region of their rhizomes. In the marine ecosystem there are phytoplanktonic diatoms that have intracellular nitrogen-fixing filamentous cyanobacteria whilst intercellular single cell species are common within shallow-water sponges. One filamentous species is implicated in black ring disease of corals.

Cyanobacteria are of direct importance to human society in a wide variety of ways. Together with other types of algae, the occurrence and abundance of different species can be used as an indicator of water quality. An increase in nutrients, particularly nitrates and phosphates, in freshwater and marine ecosystems, often leads to visible mass growths, or blooms, whether of extensive mats in streams and rivers or densely turbid water in lakes and coastal waters. These blooms can be harmful or deadly for humans, our pets and stock animals due to their production of toxins (Metcalf and Codd 2012). Hepatotoxins damage the liver whilst neurotoxins affect function of the nervous system. One of the latter type has been implicated in neurodegenerative diseases. Long term exposure to poorly treated drinking water containing hepatotoxins has been linked to liver cancer. Another class of toxin causes an acute contact dermatitis. For instance, a species that forms mats on rocks in tropical seas may cause severe blistering if trapped under the bathing suits of swimmers.

On a brighter note, we encourage the growth of some species for the valuable products that they produce (Grewe and Pulz 2012). Screening of many species has shown that some produce biochemicals active against viruses, bacteria,

algae, protozoa, fungi and insects as well as inhibitors of specific enzymes. These compounds could have importance in medicine and agriculture.

Cyanobacteria have been traditionally collected from nature for food in Central America, Africa and Asia. Modern approaches to cultivation in both open ponds and enclosed culture vessels produce thousands of tonnes dry weight each year. Western societies now consume large quantities of so-called "Spirulina". Really the label should state *Arthrospira* as the commercially grown species are distinct from true species of *Spirulina*. This is regarded as a rich source of vitamins, essential amino acids and fatty acids, minerals and antioxidant pigments but further clinical research is needed in order to confirm its efficacy. A small proportion of annual production is used as animal feed. There are reports of increases in egg production in poultry and improved colour of the yolk. In aquaculture, *Arthrospira* has been successfully fed to silver seabream, tilapia, prawns and shrimps. Extracts of *Arthrospira* are used in cosmetics and sunscreens. These include the blue and red phycobilin pigments that are also used as food colouring as well as in analytical procedures in laboratories.

Until the advent of the "Green Revolution" the nitrogen-fixing abilities of cyanobacteria in paddies contributed greatly to rice production. Recent studies have shown that they can replace chemical nitrogen fertiliser in modern rice production by fixing between 5 and >80 kg of nitrogen per hectare. There are indications that their production of plant hormones and growth regulators can stimulate seed germination and plant growth, for instance in wheat.

Cyanobacteria have been used to clean agricultural and industrial wastewaters, both by reducing the nutrient content and to absorb heavy metals. They have also found application in the removal of carbon dioxide from biogas produced from organic wastes by anaerobic digestors. The high concentration of carbon dioxide can be reduced by bubbling the biogas through cultures of *Arthrospira*. This increases the methane content and energy density of the biogas. Cyanobacteria themselves might directly contribute to bioenergy generation in the future. They have been investigated for ethanol and hydrogen production but sufficient yields would probably need genetically modified strains. This area of biotechnology is under development (Liang et al. 2018, Mishra et al. 2019).

### **Cyanobacteria in New Zealand**

Cyanobacteria were overviewed in the comprehensive New Zealand Inventory of Biodiversity (Broady and Merican 2012). At that time N.Z. was estimated to have about 413 species from 87 genera. It was thought likely that this was several hundred species short of a full inventory. This is still the case as there have been few new records since, but the checklist needs revision to add those and to reflect changes in classification and nomenclature.

The distribution of cyanobacteria in N.Z. reflects the broad range of habitats in which they occur globally, as summarised above. However, there is huge scope

for further work examining their diversity and distribution in all these habitats. Those in soils and associated with rock surfaces are particularly poorly known. The most thorough study of those in soils (Novis and Visnovsky 2011) used modern approaches to find five new species at one locality in the alpine zone of Arthur's Pass National Park. One of these was placed in a new genus, *Godleya*, named after Eric Godley, a former Director of Botany Division, DSIR. Extending such a study to the wide range of N.Z. natural and agricultural soils would surely be productive.

There has been surprisingly little focus on the nitrogen-fixing ability of cyanobacteria in N.Z. soils considering agriculture's current dependance on chemically synthesised fertiliser and its associated greenhouse gas emissions. How widespread and active are these in agricultural soils? Could their abundance and activity be stimulated? They have been known in indigenous grassland soils since a pioneering study by Elizabeth Flint (Flint 1958) and modern techniques have confirmed their presence in these soils (Chua et al. 2014).

New Zealand is rather special in having six native species of *Gunnera*, of which five are endemic. All form a symbiosis with the nitrogen-fixing cyanobacterium *Nostoc*. Researchers at University of Canterbury (Silvester and Smith 1969) were the first to demonstrate the significance of this with an estimate that it could contribute up to 72 grams of nitrogen per square metre per year. The encouragement of non-leguminous crop plants to form similar relationships with nitrogen-fixing bacteria is being actively researched (Dent and Cocking 2017).

Interestingly, a recent study of *Gunnera* species, including ones from N.Z., has shown that they have surprisingly high salt concentrations in their tissues even when these are in low concentration in the soils in which they grow (Green et al. 2017). As this capability was also found in the aquatic fern *Azolla*, also common in N.Z., then it seems to be linked to the possession of a cyanobacterial symbiosis.

By far the greatest research effort in recent years has focused on cyanobacteria in our lakes, streams and rivers (e.g. McAllister et al. 2018, Puddick et al. 2019) including those in Canterbury (Harland et al. 2014). This has been stimulated by the increased nutrients and degraded water quality in so many of these due to human activities. The consequences have been turbid blooms in lakes and extensive mats covering sediments in flowing waters. The toxins produced by these noxious growths have had fatal consequences for farm stock and pets. More than 100 dogs have been killed at N.Z. rivers since 2005, and a dog death has been associated with neurotoxic growths in a farm pond (Wood et al. 2017). In Te Wairewa / Lake Forsyth a hepatotoxin has been found in the muscles and liver of shortfin eels at concentrations that can exceed the recommended levels for safe human consumption (Dolamore et al. 2017).

In contrast, although toxins accumulated in the tissues of mayfly larvae (*Deleatidium* spp.), no significant mortality occurred, even at doses far

exceeding those likely in the environment (Kelly et al. 2020). Further study is needed on whether the toxin might be transferred through food webs and affect animals at higher trophic levels. In another experimental study the N.Z. freshwater mussel *Echyridella menziesii* incorporated suspended material into sediments at similar rates whether or not exposed to a toxic strain of the cyanobacterium *Microcystis* (Collier et al. 2018).

The Cawthron Institute in Nelson now curates an internationally important culture collection that includes about 250 strains from 50 species of cyanobacteria, most of which were isolated from toxin-producing proliferations in lakes and rivers (Rhodes et al. 2016). This is a valuable resource for future research such as investigation of the factors that stimulate toxin production, and the comparison of toxic N.Z. strains with those from other regions (e.g. Ballot et al. 2018). The collection has been screened to test the potential of isolates for their use in microbial fuel cells (Luimstra et al. 2014).

Unwanted blooms of other algae might be assisted by the presence of cyanobacteria. Extensive mats of "didymo" (the introduced diatom *Didymosphenia geminata*) in South Island rivers of low nutrient status might receive nitrogen from nitrogen-fixing cyanobacteria associated with the mats (Novis et al. 2016).

Vigorous growths also have their advantages as shown by the entry of N.Z. into the commercial realm of "Spirulina" (*Arthrospira platensis*) production ([www.tahispirulina.co.nz](http://www.tahispirulina.co.nz)). This is harvested from purpose-built, covered, shallow ponds located in the small coastal community of Himatangi Beach in the North Island.

There is still much to be learned about N.Z. cyanobacteria ranging from their floristics and distribution patterns to the complexities of their impacts on other members of aquatic communities and their application in environmentally sustainable agriculture and aquaculture. An indication of their diversity is shown by a study of the lower Kaituna River and one of its tributary streams on Banks Peninsula (Merican 2013). This identified 56 species of which 29 were new N.Z. records. One can only speculate on the additional species that might occur in the numerous streams and rivers that have not been examined in detail. The following is a small contribution to knowledge of cyanobacterial diversity in one of the many N.Z. habitats in which they remain poorly known.

### **Cyanobacteria in New Zealand cave entrances**

Cyanobacteria and other algae become prominent in the landscape when growing conditions are challenging for plants. Many survive long periods of desiccation and intense light while others are able to grow at very low light intensities. The former conditions can occur on exposed rock surfaces at the outer limits of cave entrances, while the latter are prevalent in the increasingly dim but often humid environment with progression into cave entrances, in the

so-called twilight zone. In the more brightly lit outer zone there are often a few angiosperms, ferns and bryophytes (Giles 1984), including at risk species (e.g. Brownsey and de Lange 1997), but cyanobacteria are usually all that remain in the dimmest interior. Visible growths include mats and gelatinous colonies, especially where percolations of water are frequent, and thin crusts where rock surfaces are merely damp (Fig. 1). Colour varies from a bright blue-green to almost black as well as shades of brown, olive-green and even purple.



**Figure 1.** Cave entrances at Te Hapu, north-west Nelson. A. Hunters Cave with thin greenish crusts of algae on the walls. B-D, Hapu Bluffs caves with crusts of algae on various limestone formations.

Cave algae have been the focus of many studies overseas (e.g. Albertano 2012, Popović et al. 2019). In N.Z. there are very few published reports. The first focused on the control of algae, bryophytes and ferns that grow around artificial lamps, so-called lampenflora, in Waitomo Cave (Johnson 1979). This can be a serious problem in show caves worldwide and control methods are still being investigated (Estévez et al. 2019). Algae are involved in the formation of biokarst, a term used for landforms produced largely by the direct biological erosion and/or deposition of calcium carbonate. In Babylon Cave, Paparoa National Park, unidentified algae have a role in the formation of stalactites and small pinnacles that curve towards the light (Halliday 2015). Similar formations are found in Matainaka Cave on the Otago coast (Barth 2013). The widespread occurrence of algae encrusting cave entrances is confirmed by them being



apparent in least 11 photographs in the last ten years of issues of the New Zealand Speleological Bulletin.

The cyanobacteria in this preliminary survey were found in samples from 14 caves in the vicinity of Karamea, Charleston and Punakaiki in Buller District, West Coast, South Island in 1999 and 2001 (Table 1, p. 39). Small samples of scrapings were made from the range of visible growths at each location. These were subsequently air-dried and stored until viewed in a rehydrated condition using a light microscope at magnifications up to 1000x. Illustrations were made of representative specimens using a drawing tube attachment and photomicrographs were taken. Identifications were based on morphology and used recent floras (Komárek and Anagnostidis 1999, Komárek and Anagnostidis 2005, Komárek 2013).

Nineteen species were distinguished (Table 1). Identifications to species are tentative and compare specimens ("cf.") with the morphologically closest species (morphospecies) in the literature. Confirmation requires use of the polyphasic approach with the application of cultures and molecular phylogenetics. The flora was dominated by unicellular, colony-forming species from the orders Chroococcales and Chroococciopsidales (Figs. 2 and 3, p. 40). Filamentous species (Figs. 4 and 5, p. 41) were less frequently observed. The single example of simple unbranched filaments from the Oscillatoriales, *Lyngbya* sp. (Fig. 4A, B; Fig. 5A) was notable for its mucilaginous sheath, being heavily encrusted with calcium carbonate. The remaining morphospecies were from the Nostocales and possessed the characteristic nitrogen-fixing heterocyst cells (e.g. Fig. 4D). Of these, *Scytonema* cf. *myochrous* also had a calcium carbonate encrusted sheath (Fig. 5B) while *Stigonema* sp. had the most complex morphology with filaments up to several cells wide that formed true branches (Fig. 4F; Fig. 5H).

The number of species is somewhat less than that recorded elsewhere. For instance, 42 species were found in a single cave in Israel (Vinogradova et al. 1998), 37 in just two caves in Serbia (Popović et al. 2019), 32 from 10 Polish caves (Czerwik-Marcinkowska et al. 2015) and 22 and 17 from two caves in Spain (Urzi et al. 2010, Martínez and Asencio 2010). Undoubtedly more taxa would be found with more detailed examination of samples from the caves included here, and with extension of the study to the many other caves in limestone, marble and volcanic rocks at varied altitudes and in different climatic zones of both main islands.

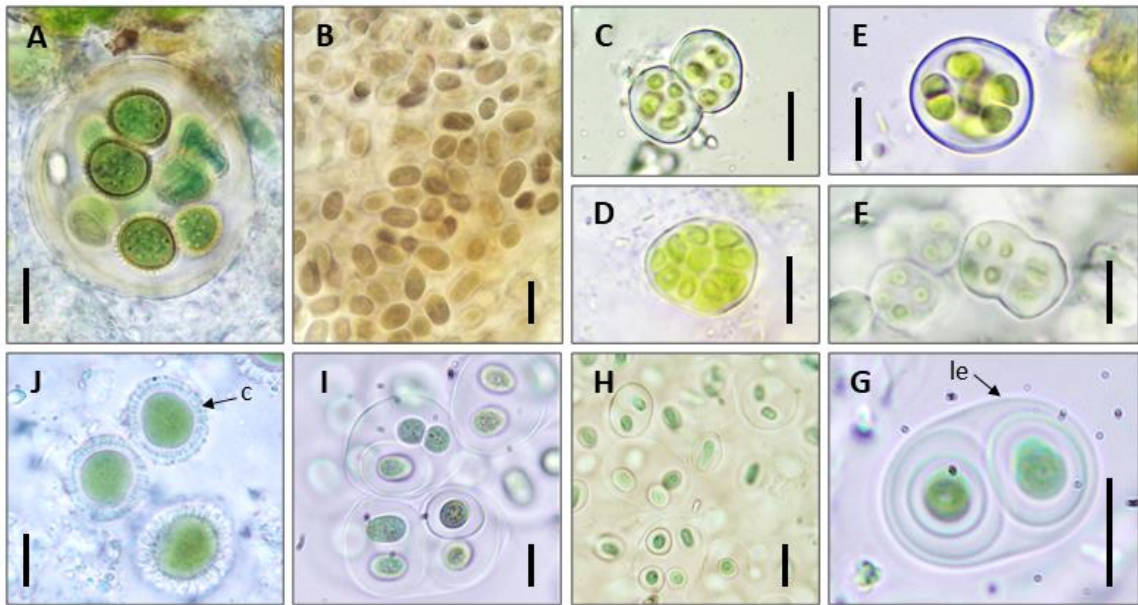
The morphospecies found are similar to those in other studies. Unicells in colonies are often the dominant forms. However, there is almost certainly cryptic diversity that light microscopy alone is unable to distinguish. The use of cultures and the application of other microscopical technologies and of molecular phylogenetics would likely be productive. The techniques used in the discovery of a new genus and species of Chroococcales, *Chalicogloea cavernicola*, in a Spanish cave (Roldán et al. 2013) would be a good model to follow.

**Table 1.** Cyanobacteria observed in samples from entrances of 14 limestone caves in Buller District, West Coast, South Island, New Zealand.

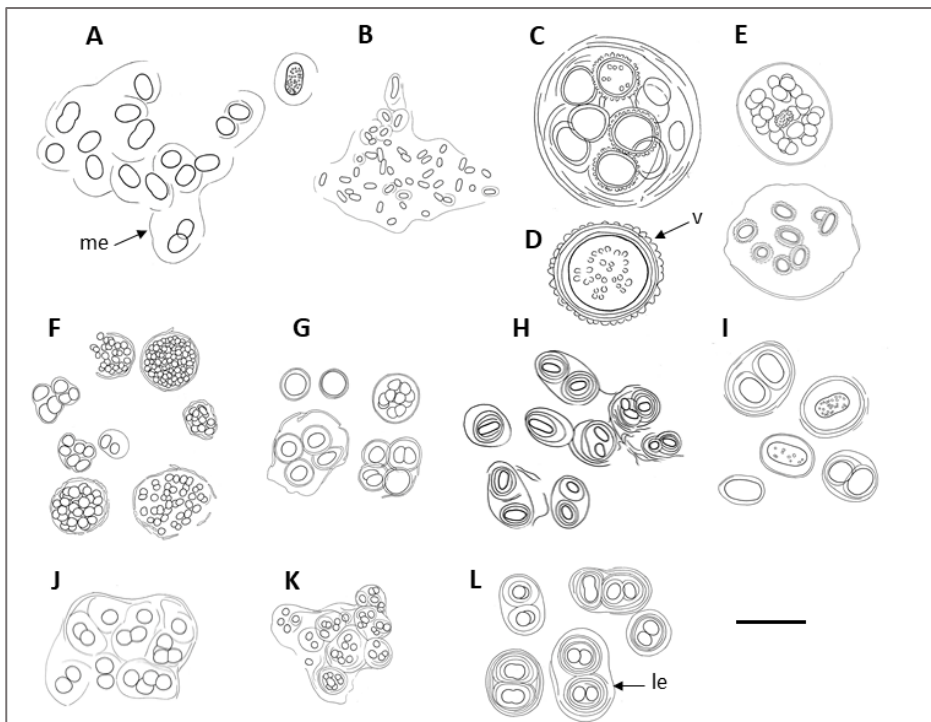
Taxa <sup>1</sup>	Caves <sup>2</sup>													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<b>Chroococcales</b>														
<i>Asterocapsa</i> cf. <i>divina</i>	+			+										
<i>Asterocapsa</i> sp.			+			+						+		
<i>Aphanothece</i> cf. <i>pallida</i>		+	+											
<i>Aphanothece</i> cf. <i>saxicola</i>	+		+			+								
<i>Gloeocapsa</i> cf. <i>aeruginosa</i>	+		+			+	+		+					
<i>Gloeocapsa</i> cf. <i>bituminosa</i>				+							+			
<i>Gloeocapsa</i> cf. <i>caldariorum</i>			+	+				+						
<i>Gloeothece</i> cf. <i>pallida</i>	+			+										
<i>Gloeothece</i> cf. <i>tepidariorum</i>											+		+	
<b>Chroococciopsidales</b>														
<i>Chroococciopsis</i> cf. <i>kashaii</i>	+	+	+		+		+							
<i>Chroococciopsis</i> sp.														+
Unidentified calcified unicell			+											
<b>Oscillatoriales</b>														
<i>Lyngbya</i> sp.												+		
<b>Nostocales</b>														
<i>Calothrix</i> sp.	+													
<i>Nostoc</i> sp.	+	+												
<i>Scytonema</i> cf. <i>myochrous</i>	+		+	+	+	+								+
<i>Tolypothrix</i> sp.	+					+								
<i>Rivularia</i> sp.						+								
<i>Stigonema</i> sp.						+						+		

<sup>1</sup> Arranged alphabetically within orders according to Komárek et al. (2014).

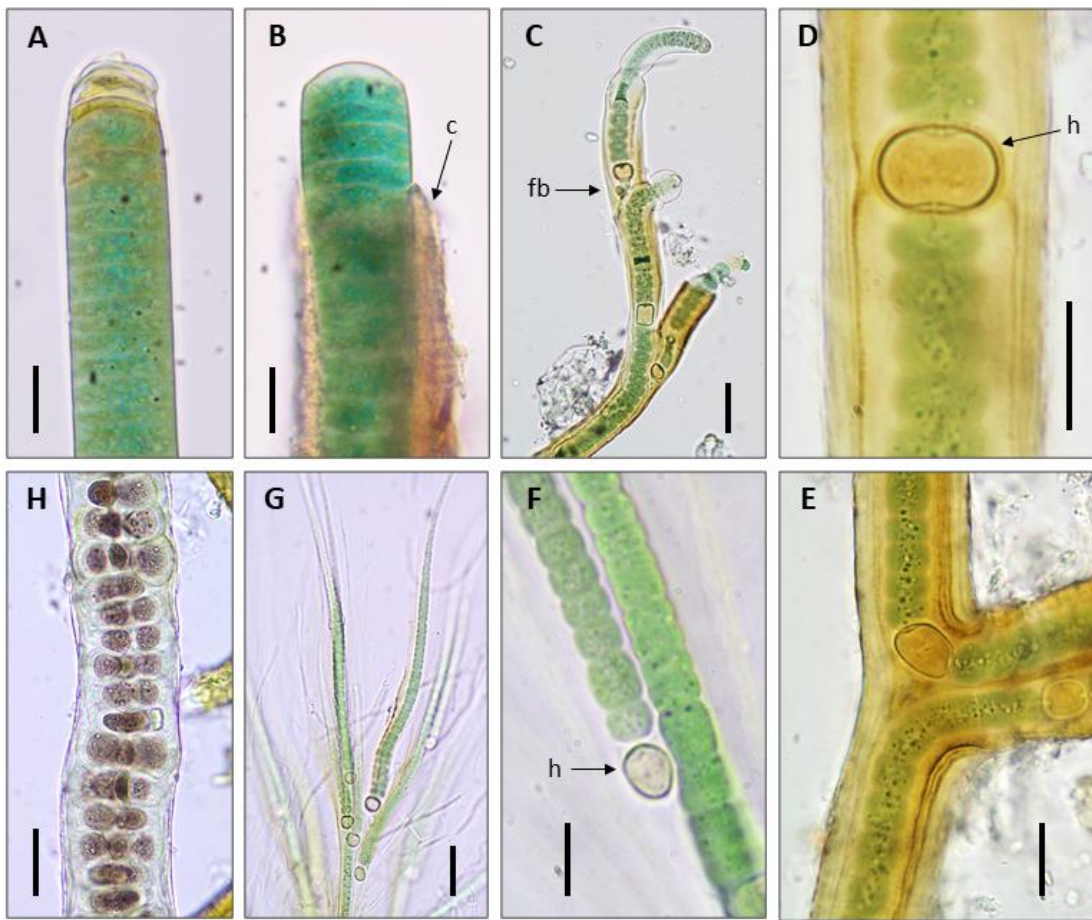
<sup>2</sup> Karamea: 1 - Oparara Arch, 2 - Moria Gate, 3 - Honeycomb Hill Cave, 4 - Honeycomb Hill Cave T entrance. Charleston, Waitakere/Nile River and Tiropahi River: 5 - Winchhead Cave. 6 - Te Ananui/Metro Cave, 7 - Venturer Cave, 8 - Cave "A", 9 - Cave "B", 10 - Cave "C", 11 - Hole in the Cliff. Potikohua/Fox River: 12 - A Fox River cave, 13 - Fox River Cave. Punakaiki: 14 - Punakaiki Cavern.



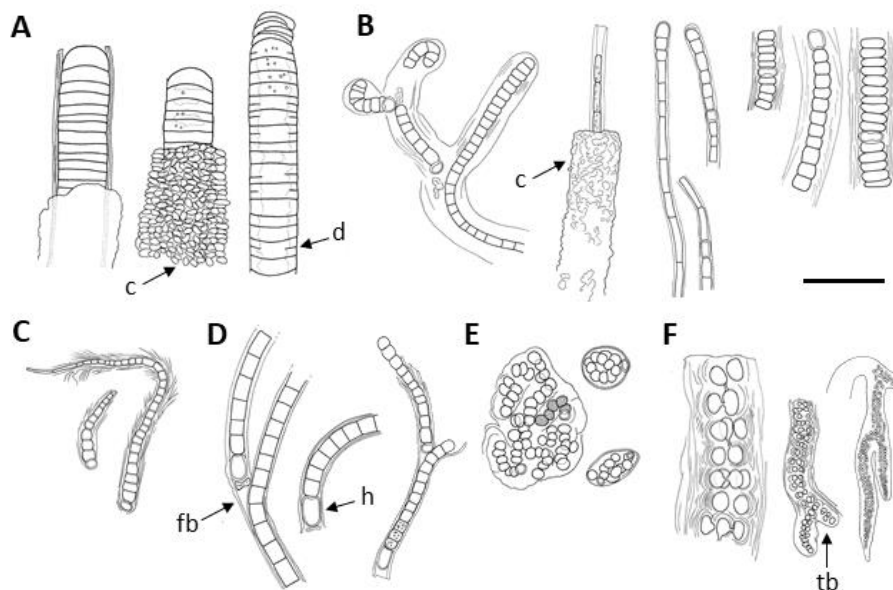
**Figure 2.** Single cell and colonial cyanobacteria. A: *Asterocapsa* cf. *divina*. B: *Aphanothece* cf. *pallida*. C, D: *Chroococciopsis* cf. *kashaii*. E: *Chroococciopsis* sp. F: *Gloeocapsa* cf. *aeruginosa*. G: *Gloeocapsa* cf. *caldariorum*. H: *Gloeothece* cf. *palea*. I: *Gloeothece* cf. *tepidariorum*. J: unidentified unicells in thin, calcified mucilaginous coatings. Scale-lines are equivalent to 1/100 mm = 10  $\mu$ m. c - calcified mucilaginous envelope; le - lamellated mucilaginous envelope.



**Figure 3.** Single cell and colonial cyanobacteria. A: *Aphanothece* cf. *pallida*. B: *Aphanothece* cf. *saxicola*. C, D: *Asterocapsa* cf. *divina*. E: *Asterocapsa* sp. F: *Chroococciopsis* cf. *kashaii*. G: *Chroococciopsis* sp. H: *Gloeothece* cf. *palea*. I: *Gloeothece* cf. *tepidariorum*. J: *Gloeocapsa* cf. *bituminosa*. K: *Gloeocapsa* cf. *aeruginosa*. L: *Gloeocapsa* cf. *caldariorum*. Scale-line is equivalent to 1/50 mm = 20  $\mu$ m for all except D for which it is 1/100 mm = 10  $\mu$ m. me - mucilaginous envelope; v - verrucose ("warty") envelope surrounding cell; le - lamellated mucilaginous sheath.



**Figure 4.** Filamentous cyanobacteria. A, B: *Lyngbya* sp. C, D, E: *Scytonema* cf. *myochrous*. F, G: *Rivularia* sp. H: *Stigonema* sp. Scale-lines are equivalent to 1/100 mm = 10  $\mu$ m for A, B, D, E, F and 1/50 mm = 20  $\mu$ m for C, G, H. c - calcified mucilaginous sheath; fb - false-branch; h - heterocyst.



**Figure 5.** Filamentous cyanobacteria. A: *Lyngbya* sp. B: *Scytonema* cf. *myochrous*. C: *Calothrix* sp. D: *Tolypothrix* sp. E: *Nostoc* sp. F: *Stigonema* sp. Scale-line is equivalent to 1/50 mm = 20  $\mu$ m for A and 1/25 mm = 40  $\mu$ m for B-F. c - calcified sheath; d - dividing cell; fb - false-branch; h - heterocyst; tb - true branch.

Although many taxa found in caves have wide distributions outside this habitat, there are some for which records are all or mostly from within caves. At least four of these have distinct morphologies with filaments having characteristic branching patterns. These include the magnificently named *Symphonema cavernicola* (Asencio et al. 1996). None of these was found in this preliminary survey. If they are truly absent from N.Z. caves then this would suggest limitations in their global distribution either due to dispersal barriers or to environmental factors.

Undoubtedly there are patterns in distribution of cyanobacteria within individual N.Z. caves in response to gradients in light intensity and water availability but these have not been investigated. A few descriptive studies have been made elsewhere. For instance, in three caves in Spain, colonial unicellular morphospecies dominated at greater light intensities and were gradually replaced by filamentous forms with decreasing light (Roldán et al. 2004). This was also the pattern in a cave in Israel (Vinogradova et al. 1998). In contrast, in an Australian cave, it was a unicellular form that occurred in the dimmest conditions, remarkably to less than 1/50,000th the intensity of full sunlight (Cox et al. 1981). There is great potential for detailed studies to clarify these patterns in N.Z. caves.

Cave entrances in N.Z. have been classified as a critically endangered naturally uncommon ecosystem (Holdaway et al. 2012) as they occupy less than a total of 100 hectares and have relatively high levels of disturbance. Their flora can easily be damaged by visitors and animals such as goats that access them for shelter. Manaaki Whenua / Landcare Research lists five threatened plants (<https://www.landcareresearch.co.nz/publications/factsheets/rare-ecosystems/subterranean-or-semi-subterranean/cave-entrances>). The algae including cyanobacteria are equally prone to disturbance and their visual contribution to the aesthetic qualities of cave entrances can easily be compromised. The values of the twilight zone flora are recognised in a recent plan for the management of West Coast caves and karst landscapes (Silverwood and Millar 2018) and management policies include measures for protection, for instance by route marking around sensitive areas.

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