

Biodata of **Dr. Richard W. Castenholz**, author (with co-author **Timothy R. McDermott**) of “*The Cyanidiales: Ecology, Biodiversity, and Biogeography*”

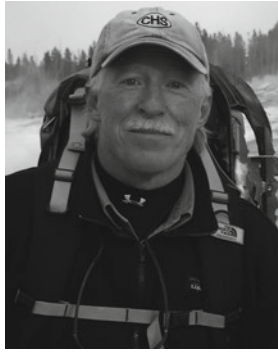
**Dr. Richard W. Castenholz** is Professor Emeritus in the Center for Ecology and Evolutionary Biology, University of Oregon. He received his B.S. in Botany at the University of Michigan (1952) and his Ph.D. in Botany at Washington State University (1957). He has been a faculty member at Oregon since 1957. His early research was on the ecology of freshwater and marine epilithic diatoms. In the 1960s he began the study of thermophilic cyanobacteria in hot spring mats. During the late 1960s and early 1970s *Chloroflexus* (the first known member of the phylum Chloroflexi) was described by Pierson and Castenholz. Later this developed into expansive studies of microbial mat phototrophs in other habitats, including hypersaline and Antarctic ponds and in endolithic habitats. As a result of these studies he and colleagues characterized the UV-screening pigment scytonemin in the sheaths of cyanobacteria and the motile UV escape strategy in several motile cyanobacteria in both thermal, temperate, and polar mats. Currently his main focus is on the geographical and phylogenetic diversity of the unicellular, thermo-acidophilic members of the Rhodophytan order Cyanidiales. His large culture collection of microbial phototrophs from extreme environments (CCMEE: <http://cultures.oregon.edu>) is housed in the Biology Department, University of Oregon.

E-mail: [rcasten@uoregon.edu](mailto:rcasten@uoregon.edu)



**Dr. Tim McDermott** is Professor of Soil & Environmental Microbiology at Montana State University. He received his PhD in Soil Microbiology in 1989 from the University of Minnesota and postdoctoral training at Washington State University. His laboratory employs ecophysiological and genomics approaches to study microbial communities and populations inhabiting the geothermal features in Yellowstone National Park. His focus is primarily on acidic features, which are also prime environments for thermoacidophilic eukaryotic algae belonging to the order Cyanidiales. Research projects include topics relevant to *in situ* gene expression and biogeochemical cycling in these environments.

E-mail: [timmcder@montana.edu](mailto:timmcder@montana.edu)



# THE CYANIDIALES: ECOLOGY, BIODIVERSITY, AND BIOGEOGRAPHY

**RICHARD W. CASTENHOLZ<sup>1</sup>**  
**AND TIMOTHY R. McDERMOTT<sup>2</sup>**

<sup>1</sup>*Center for Ecology and Evolutionary Biology, University of Oregon, 97403-5289, 5289 Eugene, Oregon, USA*

<sup>2</sup>*Thermal Biology Institute and Department of Land Resources, Environmental Sciences Montana State University, MT 59717, Bozeman, USA*

## 1. Introduction

The order Cyanidiales (or class Cyanidiophyceae) is comprised of asexual, unicellular red algae that are known to grow in low pH environments (0.2–3.5 or 4.0) and at moderately high temperatures (up to 56°C) and are typically found in acidic geothermal habitats throughout the Earth. No other photosynthetic microorganisms are known to inhabit this combination of conditions. The order Cyanidiales, since 1981, is thought to consist of three genera: *Cyanidium*, *Galdieria*, and *Cyanidioschyzon* (Ciniglia et al., 2004; Gross et al., 2001; Heilmann and Gross, 2001). This group of algae appears to be phylogenetically quite distinct from the main line of descent in the red algae and branches off quite early in geologic time (i.e. ~ 1.3–1.4 Ga), based on phylogenetic, molecular clock inference and fossil evidence for the first reputed macroalgae, which are presumed to be ancestors of the Rhodophyta (Yoon et al., 2002, 2004, 2006b). In this chapter, we comment on the ecology, biodiversity, and biogeography of these fascinating eukaryotic extremophiles, attempting to assimilate recent, important developments in our understanding of these algae.

## 2. Aspects of Cyanidiales Ecology

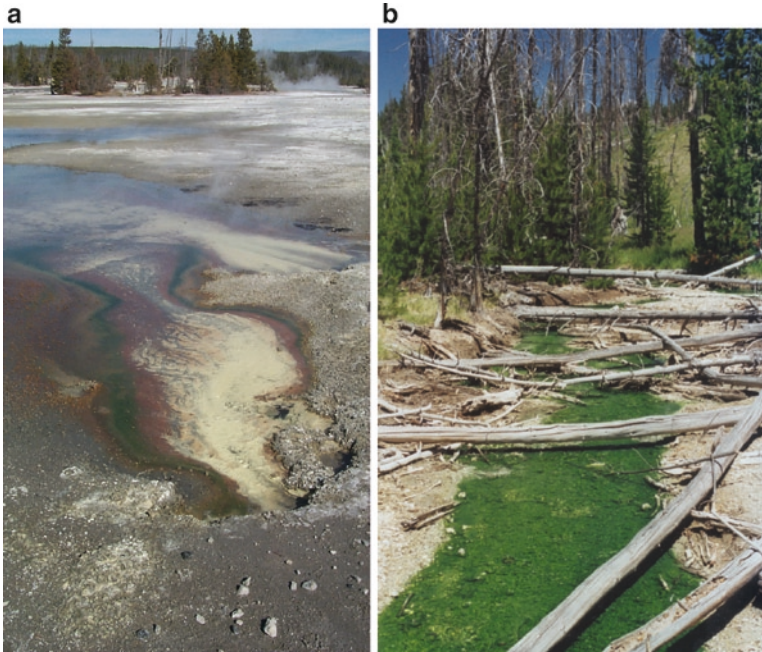
Prime habitats include warm-hot acidic springs and pools that emanate from geothermal sources, solid substrates bathed by steam from such pools, rock surfaces surrounding solfataras, and soils, gravel, and in crusts or as endoliths in such areas. Cyanidiales may even occur surrounding alkaline springs, bathed in steam where H<sub>2</sub>S is oxidized to sulfuric acid (R.W. Castenholz, 2002–2004, unpublished data). A few taxa of the Cyanidiales also occur in nonthermal acidic habitats (Gross et al., 2002), although at least one of these strains has retained its thermophilic properties (Gross and Gross, 2001). No other phototrophs are expected to be in this combination of low pH and elevated temperature, and thus such environments demarcate

the environmental niche for the Cyanidiales. It is well known that cyanobacteria and other phototrophic prokaryotes do not occur in volcanic waters (warm or cold) below about pH 4, and only few species occur below pH 5 (Ward and Castenholz, 2000).

Results of recent, ecology-oriented studies have begun to shed light on what might be specific habitat preferences for the different genera. Environmental surveys, using PCR cloned *rbcL* sequences, have found that populations represented by *Galdieria*-A, *Galdieria*-B, and *Cyanidium* phylotypes appear to prefer, or at least are capable of withstanding, the rigors of an endolithic environment (Ciniglia et al., 2004; Yoon et al., 2006a). Interestingly, *Cyanidium* has not always been known to co-inhabit the endolithic and interlithic environments with *Galdieria* (Ciniglia et al., 2004; Yoon et al., 2006a). In these studies, *Cyanidioschyzon merolae* populations were encountered only in very humid or moist environments. Further detailed studies are necessary to determine if this was simply a sampling coincidence or if there are site-specific features that are important to various types of the Cyanidiales, although in extensive cultivation work in Yellowstone (>140 isolates from 20 disparate locations), we have observed similar trends and although *C. merolae* morphotypes were isolated from a soil crust at one site (Toplin et al., 2008). Usually, only *Galdieria*-*Cyanidium* morphotypes were isolated from these habitats. Surprisingly, thus far we have not yet isolated a bona fide genetically determined *Cyanidium* from any Yellowstone geothermal feature (Toplin et al., 2008). Our efforts in this regard continue.

The Cyanidiales are blue-green to green or even yellow-green in color due to the relative proportions of the predominant chloroplast pigments, c-phycoyanin, allophycocyanin, chlorophyll *a*, and a few carotenoids, all of which also occur in the cyanobacteria. However, phycoerythrin has not been found in these algae (Lin et al., 1990). The robust pigment composition of these algae and their very significant mat presence establish them visually as a dominant component of the microbial communities that inhabit the less extreme regions of thermal gradients in acidic geothermal springs (e.g. Fig. 1). Water temperatures in submerged mats can range between 38°C and 56°C, although in these particular environments the most robust mats typically are associated with water temperatures around 42°C (unpublished data) or 45°C (according to Brock, 1978).

The non-photosynthetic, acidophilic bacteria and archaea associated with these algae are poorly understood. Jackson et al. (2001) used PCR coupled to denaturing gradient gel electrophoresis to provide a qualitative assessment of the bacterial and archaeal diversity occurring in the outflow channel of a Yellowstone acidic geothermal spring referred to as Dragon Spring. Not unexpectedly, apparent diversity increased as temperature decreased, with some of the greatest diversity occurring in the green mat region dominated by the Cyanidiales. A more descriptive PCR-based study by Walker et al. (2005) followed, identifying many bacteria and archaea in Cyanidiales-inhabited endolithic samples taken immediately adjacent to Dragon Spring. *Mycobacterium*, an abundance of Proteobacteria groups, and *Sulfolobus* and *Leptospirillum* were detected in 16S rRNA gene PCR clone



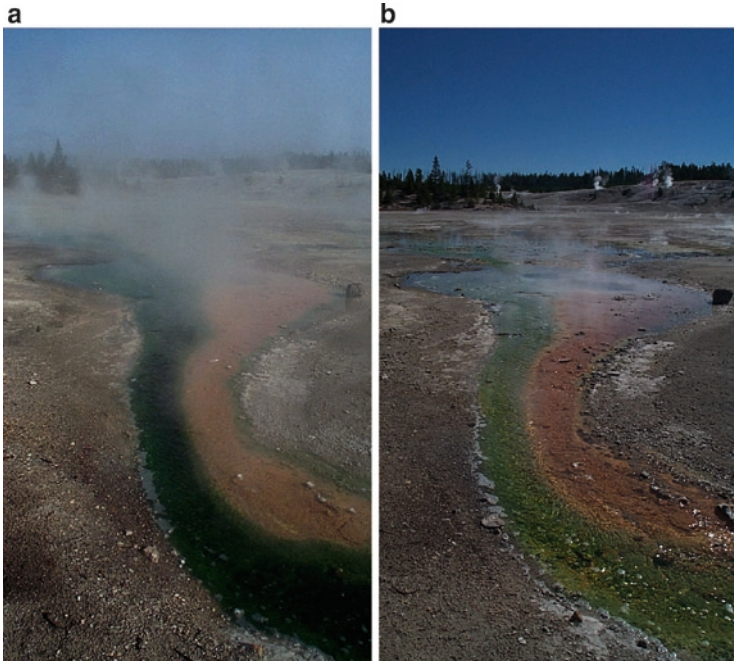
**Figure 1.** Photographs of Cyanidiales mats occurring in Yellowstone National Park. (a) Narrow green bands along the perimeter of Dragon Spring in Norris Geyser Basin. (b) Lemonade Creek located in the Amphitheater Springs area.

libraries. Additional studies have documented other eukaryotic microbes occurring with the Cyanidiales, but only in cooler regions of the geothermal outflows (ca.  $<39^{\circ}\text{C}$ ). Here the Cyanidiales habitat begins to overlap with that of other algae, such as *Chlorella* and *Paradoxia*-like algae (Ferris et al., 2005), as well as species of *Chlamydomonas*, *Dunaliella*, *Viridiella*, *Euglena*, diatoms (Gross, 2000), and the filamentous green alga, *Zygonium*. It is also well known that some species of fungi occur abundantly in cyanidial mats (Brock, 1978).

Heavy metals are common in the Cyanidiales habitats, although their concentrations can vary considerably. Waters in geothermal aquifers leach various minerals from aquifer wall rocks, resulting in high levels of silica, carbonates, and metals being brought to the surface. The acidic environment of Cyanidiales helps maintain the solubility of these metals at lower surface temperatures, although metal toxicity may actually be less at lower pHs, with the opposite true for toxic ions such as arsenite and selenite (Gross, 2000). *Cyanidium* has shown a high tolerance to Al (up to 200 mM) (Yoshimura et al., 1999, 2000), and *G. sulphuraria* can tolerate Hg concentrations up to  $\sim 10$  mM; roughly tenfold greater than *Cyanidium* (Pinto and Taddei, 1986). Other differences in heavy metal tolerance have been noted between *Galdieria* and *Cyanidium*. Albertano and Pinto (1986) reported that *Cyanidium* and *C. merolae* are more tolerant of arsenic, mercury,

and copper than *Galdieria*. Mercury has been documented to be present at extraordinarily high levels in Norris Geyser Basin in Yellowstone (Phelps, 1980), and indeed, we have documented total Hg levels in a Norris geothermal soil to be >200 µg/g-soil, and from which we have isolated *Galdieria sulphuraria* and another *Galdieria*-like type (Toplin et al., 2008). Recently, we have begun to examine the interactions of these algae with the toxic metalloids, arsenic and antimony. An isolate exhibiting 99% identity (both *rbcL* and 18S rDNA) to *Cyanidioschyzon merolae* (but with *Galdieria*-like morphology) obtained from Dragon Spring, is capable of oxidizing arsenite and antimonite (Lehr et al., 2007b). Further, ongoing studies concerning arsenic methylation (unpublished data) suggest that the Cyanidiales may be important contributors to the very significant arsenic methylation occurring in Yellowstone (Planer-Friedrich et al., 2006), and that process has been observed primarily in acidic habitats (Planer-Friedrich and Merkel, 2006). Thus, it would seem that these algae may contribute more to microbial community function in the geothermal setting than simply primary production.

UV radiation is well established as inhibitory to algae (e.g., Holm-Hanson et al., 1993), and two Yellowstone studies have examined the influence of UV irradiation on the Cyanidiales. Cockell and Rothschild (1999) did not find evidence of UV-based photosynthesis inhibition in the Cyanidiales that colonize Nymph Creek, a heavily shaded geothermal environment located in the Norris-Mammoth corridor. The use of mat cores in their study may have masked the actual effects of UV on cells exposed on the surface of the mat. In contrast, monthly measurements during a year-long study at Dragon Spring in the Norris Geyser Basin obtained several different lines of evidence that were all consistent with the suggestion that UV irradiance is likely a keystone environmental factor for these algae (Lehr et al., 2007a). Dragon Spring is located in an open, completely unshaded area, and thus is exposed to long photoperiods during the summer months, which coincides with maximum solar intensities and a major mat disturbance event referred to as “mat decline” (Lehr et al., 2007a) (Fig. 2). Various chemical and physical measurements showed that viable counts of Cyanidiales (assessed using the most probable number technique (MPN)) were significantly positively correlated with temperature and negatively correlated with UV/Visible irradiance (Lehr et al., 2007a). Additional measurements in that study also showed that photosynthesis is significantly inhibited by UV-A and UV-B exposure, and thus demonstrated evidence that the mat decline phenomenon is more closely related to UV exposure as opposed to visible light, although high intensities of visible radiation can also be inhibitory to members of the Cyanidiales (Brock, 1978). Furthermore, a comparison of PCR-generated 18S rRNA clone sequences and microsatellite sequences derived from DNA extracted during the mat decline period (July) versus those obtained when MPN counts were at a maximum (October) showed clear evidence that these major mat changes were accompanied by shifts in types dominating the population (Lehr et al., 2007a). Potentially, UV adaptation or sensitivity could contribute to diversification and/or to habitat preference (e.g., endolithic or soil habitats).



**Figure 2.** Photographs documenting mat decline during the long photoperiods of summer. Geothermal spring flow merging with that derived from Pinwheel Geyser (during a quiescent period) located along the boardwalk at Norris Geyser Basin. (a) January, 2005 and (b) early July, 2005. (Reprinted with permission from the *Journal of Phycology*).

### 3. Biodiversity

Confirmation of the relatedness of the cyanidia to the red algae was produced without the use of genetics with the identification of floridosides in all three genera in addition to other red algal components (De Luca and Moretti, 1983). However, the cell wall (when present) is largely (50–55%) proteinaceous, unlike most of the red algae (Bailey and Staehelin, 1968). With only a single membrane surrounding the plastid, it was determined to have been the result of a primary endosymbiosis (De Luca and Moretti, 1983). There is no doubt now that the Cyanidiales form a distinct phylogenetic clade. However, for many years “*Cyanidium*” was classified as a cyanophyte or a green alga with anomalous pigmentation, and so it was when M.B. Allen isolated the first culture (Allen, 1959). A more complete history of these sequential events may be found in Brock (1978), Pinto et al. (1994), and Pinto (2007). Important recent developments in the study of these algae include the sequencing of the complete genome of *Cyanidioschyzon merolae* (Matsuzaki et al., 2004) and a nearly complete genome of *Galdieria sulphuraria* (Barbier et al., 2005).

The complete genomes of the chloroplast (Ohta et al., 2003) and mitochondrion (Ohta et al., 1998) of *C. merolae* have also been published.

The type genus and species *Cyanidium caldarium* was first described in 1933 as a cyanophyte (cyanobacterium) by Lothar Geitler (Geitler, 1933) and unknowingly later by Joseph Copeland in 1936, again as an unusual cyanophyte and named *Pluto caldarius* (Copeland, 1936). An organism referred to as *Cyanidium caldarium* was studied extensively by Doemel and Brock (1971, 1978), but at that time there had been no division into three genera and all members of this group were referred to as *Cyanidium caldarium*. The Doemel and Brock studies were probably using *Galdieria sulphuraria*, if indeed only one “type” was involved. Since then and until molecular methods had been applied, the three recognized genera and several species have been distinguished mainly by morphology and reproductive patterns. Taxa of the genus *Cyanidioschyzon* are smaller (usually 1–2  $\mu\text{m}$  in breadth) with an oval, club- or pear-like shape (see Matsuzaki et al., 2004). They reproduce by binary cytokinesis and lack a rigid cell wall. *Galdieria* and *Cyanidium* have a more spherical shape, a rigid cell wall, and reproduce through the formation of 4–32 small daughter cells within a mother cell. Some types have also been distinguished by physiological characteristics. For example, isolates of *G. sulphuraria* have the ability to grow heterotrophically in the dark on at least 50 different carbon sources, including several sugars, sugar alcohols, amino acids, and TCA cycle intermediates (Barbier et al., 2005; Gross and Schnarrenberger, 1995; Oesterhelt and Gross, 2002). This characteristic of *G. sulphuraria* has been viewed as a probable explanation for its ability to grow in darkness or semi-darkness in soil, gravel, and as endolithic populations, using organic carbon released from other microorganisms in these habitats (Ferris et al., 2005; Gross and Schnarrenberger, 1995; Oesterhelt and Gross, 2002). *G. maxima* may also grow heterotrophically, although poorly according to Gross et al. (2001). *G. sulphuraria* lacks the ability to use nitrate as the sole nitrogen source, but a few other *Galdieria*-like isolates also lack this ability (Toplin et al., 2008).

Thermo-acidic environments are scattered disparately like small islands throughout the Earth. Since members of the Cyanidiales tested thus far (>30) do not tolerate desiccation (Toplin et al., 2008), the geographical separation and isolation of thermo-acidic sites may have led to allopatric speciation events over time (Gross et al., 2001). One large geothermal “island” with many acidic habitats is the Yellowstone geothermal region, an approximately 9,000  $\text{km}^2$  area with numerous hydrothermal features that vary in pH, temperature, exposure to high solar radiation, water availability, and concentrations of soluble metals and metalloids (Nordstrom et al., 2005). Any of these physico-chemical factors could exert selection pressures, potentially providing opportunities for distinct species or ecotypes to arise even within an area as small as YNP. Indeed, evidence of habitat specialization has been found for the thermophilic cyanobacterium *Mastigocladus (Fischerella) laminosus* within YNP (Miller et al., 2006), *Synechococcus* (Ward et al., 2006, Bhaya et al., 2007), and *Sulfolobus* (Whitaker et al., 2003).

Cultivation-independent work conducted in Italy has identified novel *rbcL* phylotypes representing a novel lineage referred to as *Galdieria*-B (Ciniglia et al., 2004). Our studies have used both genetic and morphological criteria to examine



the diversity of a large number of isolates of the Cyanidiales obtained from 13 distant and chemically disparate sites within Yellowstone, and from larger spatial scales that include Japan, New Zealand, and Iceland. Our most common Yellowstone isolate appears to be a *Galdieria* or *Cyanidium* morphotype, since it possesses a cell wall and divides internally to produce an even number of small daughter cells that eventually are liberated from the mother cell. Based on the 18S rDNA and *rbcL* markers, however, these cells show 99–100% identity to the morphologically very different *Cyanidioschyzon merolae* (Toplin et al., 2008). Although morphology alone can differentiate the genus *Cyanidioschyzon* from *Galdieria* and *Cyanidium* (see above), at this point only genetic means can distinguish taxa of this order that, in some cases, may be so distantly related that they may constitute new, previously undescribed species. The situation with the aforementioned Cyanidiales isolates is similar in many ways to the problem with unicellular microorganisms in general. Comparisons of the sequences of one or two genes may be inadequate. Other genes or their products or partial or complete genomes are needed to further establish the genetic relatedness of this novel member of the Cyanidiales. Microorganisms that are identical, based on 18S rRNA gene sequence criteria, but which are distinctly different species is not without precedence (e.g., in dinoflagellates, Logares et al., 2007). Short sequence repeats (i.e., microsatellites) have been useful for identifying genetic diversity among these isolates (Lehr et al., 2007a; Toplin et al., 2008), but at this point there is no meaningful correlation between these variants and specific habitat characteristics (Toplin et al., 2008). It is important to note that indisputable *C. merolae* morpho-phylotypes also occur in Yellowstone and have also been cultured (Toplin et al., 2008).

#### 4. Biogeographical Considerations

Apart from the quandary of how to characterize and name this provisional new species or variety that is incorrectly identified as *Cyanidioschyzon merolae* (using only the sequences of two genes), there are also major subgroups of the Cyanidiales in other distant provinces (i.e., Japan, New Zealand, and Iceland) that do show genetic distinctiveness, particularly using chloroplast *rbcL* gene sequences, and none of which show closest identity to *C. merolae*. For the isolates from these spatially disparate locations, at this time the use of only 18S rDNA and *rbcL* sequences seems adequate to point out several taxa quite distinct from the two major groups in Yellowstone (see Table 1). As indicated by phylogenetic analysis (one based on *rbcL*, the other on 18S rDNA sequences), there are three distinct groups (clades) of isolates from Japan that show closest identity to an isolate from the Kurile Islands (off the northeast coast of Hokkaido, Japan) named *Galdieria maxima* (Table 1) (Toplin et al., 2008). Similarly, three phylogenetic clades from New Zealand also show genetic separation from the Yellowstone isolates and from the Japanese strains (the latter at least at the *rbcL* locus) (Toplin et al., 2008). In contrast, eight isolates from several sites in southwest Iceland were identical to each other at both the *rbcL* and 18S rDNA loci, and like one

**Table 1.** Nearest BLAST relative for each type of isolate from YNP, Japan, New Zealand, and Iceland for *rbcL*. The number in the parentheses is the % identity to the nearest GenBank relative. The percent variation for each group is also listed for each gene, followed by the number of isolates compared (n). (Modified from Toplin et al., 2008.)

	Nearest Blast Relative <i>rbcL</i>	% Variation Within Group + number of strains analyzed
<b>YNP</b>		
Type IA/IB	<i>C. merolae</i> 10D (99)	0.38, n = 16
Type II	<i>G. sulphuraria</i> UTEX 2393 (99)	0.38, n = 5
<b>Japan</b>		
Type IIIA	<i>G. maxima</i> IPPAS507 (99)	1, n = 9
Type IIIB	<i>G. maxima</i> IPPAS507 (96)	1, n = 14
Type IIIC	<i>G. maxima</i> IPPAS507 (93)	0, n = 1
<b>New Zealand</b>		
Type IV	<i>G. sulphuraria</i> SAG 108.79 (96)	0.8, n = 10
Type V	<i>G. maxima</i> IPPAS P507 (93)	1, n = 8
Type VI	<i>G. maxima</i> IPPAS P507 (91)	0, n = 1
<b>Iceland</b>		
Type VII	<i>G. maxima</i> IPPAS507 (96)	0, n = 8

particular clade from Japan, showed the closest identity to *Galdieria maxima* (E. Perry et al., 2008, unpublished data).

The most cosmopolitan of all cyanidial “species”, *G. sulphuraria* has also been isolated (3 strains) from endolithic habitats in southwestern Iceland (Gross and Oesterheld, 1999). How did cyanidia find their way to Japan, New Zealand, and Iceland from the Yellowstone or Italian (or other) geothermal areas or vice versa? Indeed, it may be difficult to identify the ancestral source of the Cyanidiales evolutionary clade. If the Cyanidiales with its present general characteristics is as ancient as indicated by molecular clock inference, dissemination difficulties may not be so relevant, since the disparate geothermal “islands” may represent remnants of an origin that occurred long before present continental positions existed. Still, however, issues of dispersal still exist, since many volcanically active island sites that support populations of Cyanidiales came into existence in more recent geologic times (e.g., Azores, Iceland). However, members of the Cyanidiales appear to be absent in their appropriate temperature zone in volcanic areas of Hawaii (Brock, 1978). Identifying the origin of the Cyanidiales may prove difficult. However, the most likely method of dissemination may be better understood through experimentation. We (and other authors) have been unable to show desiccation tolerance (at less than ~90% relative humidity) that would permit aerial transport of these algae over great distances (>2,000 km) with no suitable habitat intervening. However, testing pure cultures may not be a suitable method. Certainly, to successfully colonize a newly available volcanic site, enough cells to constitute a viable founder population would be necessary. It is possible that small amounts of moist acidic mud containing viable

cells could be transported by birds, or even more likely, cells could be carried in the alimentary track of water birds. It was shown many years ago that this is possible even in the case of algae that do not produce resting cells or spores (Proctor, 1959).

Nevertheless, little is known of the migratory or accidental movement of water birds in the distant past. Since Yellowstone, Japan, and New Zealand have had continuous volcanic, geothermal activity for several millions of years, a very rare transport of cells of the Cyanidiales could, nevertheless, have occurred with subsequent speciation events, particularly if somewhat different chemical environments might have selected for new varieties. Preliminary evidence indicates that a great overlap of similar acidic habitats occurs in all of these major volcanic locations. If dispersal of this type of organism is very easy and frequent for reasons we do not understand, we might expect a greater similarity among isolates from distant sites. Since it is agreed that light-exposed sites in Iceland have been exposed for colonization for less than 10,000 years after complete glaciation, it is not unusual that there is little apparent variation among the Cyanidiales of Iceland, suggesting that the time for proliferating founder populations to evolve new species was insufficient (Gross and Oesterhelt, 1999; E. Perry et al., 2008, unpublished data). There is a considerable danger currently that thermobiologists could be the most likely transport vectors. Thus, it is very important that these biologists sterilize their equipment and boots before moving from one major thermal geographic area to another. In our case, a new pair of boots has been purchased in all cases.

## 5. Evolution of the Cyanidiales

The Order Cyanidiales or the class Cyanidiophyceae (depending on the hierarchy chosen by different authors) constitutes a very early and distinct branch of the Rhodopyta (red algae), presumably with chloroplasts similar to the primordial chloroplast for all photosynthetic eukaryotes (Yoon et al., 2002, 2006b). Evidence indicates that it was a primary endosymbiosis. A somewhat novel hypothesis, however, is that the eukaryotic “host” of the cyanidial lineage may indeed have been quite ancient (acidophilic or not), and that this group was originally achlorotic (plastids absent), and that a thermophilic cyanobacterial endosymbiont was incorporated, and that by its presence (and genes) imparted thermophily to ancestors of the Cyanidiales. Of course, what we mean by later, is an endosymbiosis possibly during the late Proterozoic ( $>1 \times 10^9$  years ago). From what we know of present day thermophilic cyanobacteria, they do not tolerate acidic conditions (Kallas and Castenholz, 1982a, b). Thus, the harboring of a thermophilic cyanobacterial symbiont in a host that maintains a pH close to neutrality creates a pH climate that is acceptable for essentially all cyanobacteria. Since it is agreed that the ancestor or ancestors of all red algae presumably acquired a cyanobacterium as an endosymbiont that later evolved into a plastid, this hypothesis, in general terms, should not be very controversial. What is controversial is that the Cyanidiales

lineage may have acquired a thermophilic cyanobacterium as an endosymbiont that was different from the supposed primordial cyano-chloroplast that evolved eventually into all chloroplasts of red algae, green algae, and plants. If the Cyanidiales are as ancient and as separate from the main line of the red algae as proposed by Yoon et al. (2006b), there may be no overwhelming reason to reject this hypothesis.

## 6. Future Directions

Extensive cultivation work coupled with the application of molecular tools has begun to shed considerable light on the Cyanidiales. From only a handful of recent studies we have learned a great deal about their biodiversity at the population level, and future work that integrates genomics-level studies will no doubt greatly expand our foundational knowledge and uncover yet additional pieces of the puzzle, but it is important not to ignore phenotypic attributes, such as morphology, life history, physiology, and ecology. Current efforts that have primarily focused on populations of Cyanidiales in the geothermal features located in Italy and Yellowstone need to be expanded to consider additional geothermal islands distributed around the globe. Indeed, recent explorations of the Cyanidiales in Japan, New Zealand, and Iceland (e.g., Toplin et al., 2008) certainly indicate there is much to learn. Because of their island-like distribution, clear restrictions on transport, and relatively simple genetic structure (with no apparent sexual cycle), the Cyanidiales have become an exceptional model for addressing fundamental questions in evolution.

## 7. Acknowledgments

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## 8. References

- Albertano, P. and Pinto, G. (1986) The action of heavy metals on the growth of three acidophilic algae. *Boll. Soc. Natur. Napoli* **95**: 319–328.
- Albertano, P., Ciniglia, C., Pinto, G. and Pollio, A. (2000) The taxonomic position of *Cyanidium*, *Cyanidioschyzon* and *Galdieria*: an update. *Hydrobiologia* **433**: 137–143.
- Allen, M.B. (1959) Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. *Arch. Mikrobiol.* **32**: 270–277.
- Bailey, R.W. and Staehelin, L.A. (1968) The chemical composition of isolated cell walls of *Cyanidium caldarium*. *J. Gen. Microbiol.* **54**: 269–276.
- Barbier, G., Oesterhelt, C., Larson, M.D., Halgren, R.G., Wilkerson, C., Garavito, C., Benning, R.M. and Weber, A.P. (2005) Comparative genomics of two closely related unicellular thermo-

- acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. *Plant Physiol.* **137**: 460–474.
- Bhaya, D., Grossman, A.R., Steunou, A.-S., Khuri, N., Cohan, F.M., Hamamura, N. et al. (2007) Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses. *ISME J.* **1**: 703–713.
- Brock, T.D. (1978) *Thermophilic Microorganisms and Life at High Temperatures*. Springer, New York, USA.
- Ciniglia, C., Yoon, H.S., Pollio, A., Pinto, G., and Bhattacharya, D. (2004) Hidden biodiversity of the extremophilic Cyanidiales red algae. *Mol. Ecol.* **13**: 1827–1838.
- Cockell, C.S. and Rothschild, L.J. (1999) The effects of UV radiation A and B in diurnal variation in photosynthesis in three taxonomically and ecologically diverse microbial mats. *Photochem. Photobiol.* **69**: 203–210.
- Copeland, J.J. (1936) Yellowstone thermal myxophyceae. *Annal. New York Acad. Sci.* **36**: 1–232.
- De Luca, P. and Moretti, A. (1983) Floridosides in *Cyanidium caldarium*, *Cyanidioschyzon merolae* and *Galdieria sulphuraria* (Rhodophyta, Cyanidiophyceae). *J. Phycol.* **19**: 368–369.
- Doemel, T.D. and Brock, T.D. (1971) The physiological ecology of *Cyanidium caldarium*. *J. Gen. Microbiol.* **67**: 17–32.
- Ferris, M.J., Magnuson, T.S., Fagg, J.A., Thar, R., Kuhl, M., Sheehan, K.B. and Henson, J.M. (2003) Microbially mediated sulphide production in a thermal, acidic algal mat community in Yellowstone National Park. *Environ. Microbiol.* **5**: 954–960.
- Ferris, M.J., Sheehan, K.B., Köhl, M., Cooksey, K., Wigglesworth-Cooksey, B., Harvey, R. and Henson, J.M. (2005) Algal species and light microenvironment in a low-pH, geothermal microbial mat community. *Appl. Environ. Microbiol.* **71**: 64–71.
- Geitler, L. (1933) Diagnoses neuer Blaualgen von den Sunda-Insela. *Arch. Hydrobiol. Suppl.* **12**: 622–634.
- Gross, W. (2000) Ecophysiology of algae living in highly acidic environments. *Hydrobiologia* **33**: 31–37.
- Gross, W. and Gross, S. (2001) Physiological characterization of the acidophilic red alga *Galdieria sulphuraria* isolated from a mining area. *Nova Hedwigia, Beiheft* **123**: 523–530.
- Gross, W. and Oesterhelt, C. (1999) Ecophysiological studies of the red alga *Galdieria sulphuraria* isolated from southwest Iceland. *Plant Biol.* **1**: 694–700.
- Gross, W. and Schnarrenberger, C. (1995) Heterotrophic growth of two strains of the acidothermophilic red alga *Galdieria sulphuraria*. *Plant Cell Physiol.* **36**: 633–638.
- Gross, W., Heilmann, I., Lenze, D. and Schnarrenberger, C. (2001) Biogeography of the Cyanidiales (Rhodophyta) based on 18S ribosomal RNA sequence data. *Eur. J. Phycol.* **36**: 275–280.
- Gross, W., Oesterhelt, C., Tischendorf, G. and Lederer, F. (2002) Characterization of a non-thermophilic strain of the red algal genus *Galdieria* isolated from Soos (Czech Republic). *Eur. J. Phycol.* **37**: 477–482.
- Heilmann, I. and Gross, W. (2001) Genetic diversity of thermo-acidophilic red algae according to random amplified polymorphic DNA (RAPD) analysis. *Nova Hedwigia Beiheft* **123**: 531–539.
- Holm-Hanson, O., Lubin, D., and Helbling, E.W. (1993) Ultraviolet radiation and its effects on organisms in aquatic environments, In A.R. Young, L. Bjorn, J. Mohan, and W. Nultsch (eds.) *Environmental UV Photobiology*. Plenum Press, New York.
- Jackson, C.R., Langner, H.W., Donahoe-Christiansen, J., Inskip, W.P. and McDermott, T.R. (2001) Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. *Environ. Microbiol.* **3**: 532–542.
- Kallas, T. and Castenholz, R.W. (1982a) Internal pH and ATP-ADP pools in the cyanobacterium, *Synechococcus* sp. during exposure to growth-inhibiting low pH. *J. Bacteriol.* **149**: 229–236.
- Kallas, T. and Castenholz, R.W. (1982b) Rapid transient growth at low pH in the cyanobacterium *Synechococcus* sp. *J. Bacteriol.* **149**: 237–246.
- Lehr, C.R., Frank, S.D., Norris, T.B., D'Imperio, S., Kalinin, A.V., Toplin, J.A., Castenholz, R.W. and McDermott, T.R. (2007a) Cyanidia (Cyanidiales) population diversity and dynamics in an acid-sulfate chloride spring in Yellowstone National Park. *J. Phycol.* **43**: 3–14.

- Lehr, C.R., Kashyap, D.R. and McDermott, T.R. (2007) New insights into microbial oxidation of arsenic and antimony oxidation. *Appl. Environ. Microbiol.* **73**: 2386–2389.
- Lin, S., Offner, G.D. and Troxler, R.F. (1990) Studies on *Cyanidium caldarium* phycobiliprotein pigment mutants. *Plant Physiol.* **93**: 772–777.
- Logares, R., Rengefors, K., Kremp, A., Shalchian-Tabrizi, K., Boltovskoy, A., Tengs, T., Shurtleff, A. and Klaveness, D. (2007) Phenotypically different microalgal morphospecies with identical ribosomal DNA: a case of rapid adaptive evolution? *Microb. Ecol.* **53**: 549–561.
- Matsuzaki, M., Misumi, O., Shin-I, T., Maruyama, S., Takahara, M., Miyagishima, S.Y. and Mori, T. (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. *Nature* **428**: 653–657.
- Miller, S.R., Purugganan, M.D. and Curtis, S.E. (2006) Molecular population genetics and phenotypic diversification of two populations of the thermophilic cyanobacterium *Mastigocladus laminosus*. *Appl. Environ. Microbiol.* **72**: 2793–2800.
- Nordstrom, D.K., Ball, J.W. and McClesley, R.B. (2005) Ground water to surface water: chemistry of thermal outflows in Yellowstone National Park, In: W.P. Inskeep (ed.) *Geothermal Biology and Geochemistry in Yellowstone National Park*. Thermal Biology Institute, Montana, pp. 73–94.
- Oesterheld, C. and Gross, W. (2002) Different sugar kinases are involved in the sugar sensing of *Galdieria sulphuraria*. *Plant Physiol.* **128**: 291–299.
- Ohta, N., Sato, N. and Kuroiwa, T. (1998) Structure and organization of the mitochondrial genome of the unicellular red algae *Cyanidioschyzon merolae* deduced from the complete nucleotide sequence. *Nucleic Acids Res.* **26**: 5190–5198.
- Ohta, N. et al. (2003) Complete sequence analysis of the plastid genome of the unicellular red alga *Cyanidioschyzon merolae*. *DNA Res.* **10**: 67–77.
- Phelps, D. (1980) Distribution of soil mercury and the development of soil mercury anomalies in the Yellowstone geothermal area, Wyoming. *Econ. Geol.* **75**: 730–741.
- Pinto, G. and Taddei, R. (1986) Evaluation of toxic effects of heavy metals on unicellular algae. V – analysis of the inhibition manifesting itself with an increased lag phase. *Boll. Soc. Natur. Napoli* **95**: 303–316.
- Pinto, G. (2007) Cyanidiophyceae: looking back – looking forward, In: J. Seckbach (ed.) *Algae and Cyanobacteria in Extreme Environments*. Springer, Dordrecht, The Netherlands, pp. 389–397.
- Pinto, G., Albertano, P. and Pollio, A. (1994) Italy's contribution to the systematics of *Cyanidium caldarium* 'sensu lato', In: J. Seckbach (ed.) *Evolutionary Pathways and Enigmatic Algae: Cyanidium caldarium (Rhodophyta) and Related Cells*. Kluwer, Dordrecht, The Netherlands, pp. 157–166.
- Planer-Friedrich, B. and Merkel, B.J. (2006) Volatile metals and metalloids in hydrothermal gases. *Environ. Sci. Technol.* **40**: 3181–3187.
- Planer-Friedrich, B., Lehr, C., Matschullat, J., Merkel, B.J., Nordstrom, D.K. and Sandstrom, M.W. (2006) Speciation of volatile arsenic at geothermal features in Yellowstone National Park. *Geochimica* **70**: 2480–2491.
- Proctor, V.W. (1959) Dispersal of fresh-water algae by migratory water birds. *Science* **130**: 623–624.
- Toplin, J.A., Norris, T.B., Lehr, C.R., McDermott, T.R. and Castenholz, R.W. (2008) The thermoacidophilic Cyanidiales: biogeographic and phylogenetic diversity in Yellowstone National Park, Japan, and New Zealand. *Appl. Environ. Microbiol.* **74**: 2822–2833.
- Walker, J.J., Spear, J.R. and Pace, N. (2005) Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature* **434**: 1011–1014.
- Ward, D.M. and Castenholz, R.W. (2000) Cyanobacteria in geothermal habitats, In: B.A. Whitton and M. Potts (eds.) *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer, Dordrecht, The Netherlands, pp. 37–59.
- Ward, D.M., Bateson, M.M., Ferris, M.J., Köhl, M., Wieland, A., Koepfel, A. and Cohan, F.M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. *Philos. Trans. R. Society Lond. B. Biol. Sci.* **361**: 1997–2008.

- Whitaker, R.J., Grogan, D.W. and Taylor, J.W. (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* **301**: 976–978.
- Yoon, S.Y., Hackett, J.D., Pinto, G. and Bhattacharya, D. (2002) The single, ancient origin of chromist plastids. *Proc. Natl. Acad. Sci. USA* **99**: 15507–15512.
- Yoon, S.Y., Hackett, J.D., Ciniglia, C., Pinto, G. and Bhattacharya, D. (2004) A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* **21**: 809–818.
- Yoon, H.S., Ciniglia, C., Wu, M., Comeron, J.M., Pinto, G., Pollio, A. and Bhattacharya, D. (2006a) Establishment of endolithic populations of extremeophilic Cyanidiales (Rhodophyta). *BMC Evol. Biol.* **6**: 78 (12 pp) (online).
- Yoon, H.S., Muller, K.M., Sheath, R.G., Ott, F.D. and Bhattacharya, D. (2006b) Defining the major lineages of red algae (Rhodophyta). *J. Phycol.* **42**: 482–492.
- Yoshimura, E., Nagasaka, S., Sato, Y., Satake, K. and Mori, S. (1999) Extraordinary high aluminum tolerance of the acidophilic thermophilic alga, *Cyanidium caldarium*. *Soil Sci. Plant Nutr.* **45**: 721–724.
- Yoshimura, E., Nagasaka, S., Satake, K. and Mori, S. (2000) Mechanism of aluminum tolerance in *Cyanidium caldarium*. *Hydrobiologia* **433**: 57–60.