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Cryptic dispersal in non-acidic environments from Turkey of Cyanidiophytina (Rhodophyta)

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Abstract

Cyanidiophytina are a group of polyextremophilic red algae with a worldwide, but discontinuous colonization. They are restricted to widely dispersed hot springs, geothermal habitats, and also some human-altered environments. Cyanidiophytina are predominant where pH is prohibitive for the majority of eukaryotes (pH 0.5-3). Turkey is characterized by areas rich in volcanic activity separated by non-volcanic areas. Here we show that Cyanidiophycean populations are present in thermal baths located around Turkey on neutral/alkaline soils. All known genera and species within Cyanidiophytina were detected in Turkey, including *Galdieria phlegrea*, recorded up to now only in Italian Phlegrean Fields. By phylogenetic analyses, Turkish *G. sulphuraria* strains are monophyletic with Italian and Icelandic strains, and with Russian *G. daedala* strain*s. G. maxima* from Turkey clustered with Icelandic, Kamchatka, and Japanese populations. The discovery of Cyanidiophytina in non-acidic Turkish soils raises new questions about the ecological boundaries of these extremophilic algae. This aids in the understanding of the dispersal abilities and distribution patterns of this ecologically and evolutionarily interesting group of algae.

Keywords: Extremophiles, Cyanidophytina, Phylogeny, Population structure, rbcL, Biodiversity

Introduction

Cyanidiophytina (Rhodophyta) are a group of red unicellular algae highly adapted to the environmental extremes offered by volcanic regions. These environments often support temperatures above 50 ºC and have high sulfuric acid concentrations that results in acidic pH levels prohibitive for most eukaryotes (Albertano et al. 2000; Brock 1978; Pinto et al. 2003; Pinto et al. 2007; Cennamo et al. 2017). The interest in global biodiversity and distribution patterns of thermoacidophilic Cyanidiophiceaen populations led to numerous explorations of volcanic regions both in and outside of Europe, such as Italy, Iceland, USA, New Zealand, and Japan. In this, molecular approaches were successfully used to assess the level of biodiversity in this group (Ciniglia et al. 2004; Yoon et al. 2004, 2006; Toplin et al. 2008, Ciniglia et al. 2014). This provided a hypothesis of the origin and dispersal routes of *Galdieria maxima* and *G. sulphuraria* in populations from Iceland and northeastern Asia. Cyanidiophytina mobility is still poorly understood.

A novel estimate of species richness of Cyanidiophyceae has recently come from the analysis of thermoacidophilic communities from aquatic and non-aquatic volcanic sites in Taiwan (Hsieh et al. 2015). The habitats so far explored, in search of polyextremophilic algae, have usually been characterized by strong acidity, as pH range is considered a greater constraint on the growth of Cyanidiophytina than temperature range. Thus, many explorations have focused in acidic geothermal areas (Brock 1978, Toplin et al. 2008, Hsieh et al. 2015). Currently, the genus *Cyanidium* encompasses two main species. These are *C. caldarium* (Tilden) Geitler, a polyextremophilic alga adapted to acidic and hot springs and fumaroles, usually rich in heavy metals, and *C. chilense*, a hypogean, neutrophilic (pH around 7.0) and mesophilic (20-25 °C) alga discovered in several caves worldwide (Schwabe, 1936; Friedmann, 1964; Skuja, 1970; LeClerc et al., 1983; Azua-Bustos et al., 2009; Darienko et al., 2010; Del Rosal et al., 2015; Cennamo et al., 2012; Ciniglia et al. 2017). The phylogenetically distinct thermoacidophilic *C. caldarium* and the neutrophilic and mesophilic *C. chilense* are clearly separated on the basis of both molecular and ecophysiological characters (Ciniglia et al., 2004). These findings suggest that other Cyanidiophytina could have a much wider distribution than those considered so far. This prompted us to search for alternative ecological niches, such as non-acidic environments.

In this study, we report on our new explorations of seven thermal baths located in Turkey and report the presence of Cyanidiophycean populations on neutral/alkaline soils. Anatolian volcanism is a consequence of convergence occurring between Afro-Arabian and Eurasian plates and it can be considered as a bridge between the geothermal areas of Europe and Asia. This zone is characterized by deposits of andesitic and rhyolitic lava, alternating with black and clastic sedimentary rocks, resulting from the solidification of mud mixed with water (Pearce et al., 1990). Although Turkey is still geologically active, intense volcanic activity has not been recorded for a number of years; Turkish volcanism varies from mildy alkaline volcanoes, such as Nemrut, to calc-alkaline/alkaline volcanoes, such as Ararat and Tendurek (Pearce et al. 1990).

The chemical composition of rocks collected in our sampling areas was determined by X-ray diffraction. Next a culture-dependent approach combined with rbcL gene sequencing was employed to characterize the phylogenetic positioning of algal diversity of the Cyanidiophycean populations we isolated from Turkey. We also added all of the available rbcL gene sequences from a wide geographic range, to refine the population structure and molecular variance. Then we explored the geographical distribution of global genetic variation in different species and genera of Cyanidia.

MATERIALS AND METHODS

X-ray diffraction (XRD)

XRD was performed on the mineralogical phases of substratum inorganic components occurring in the algal biofilms. XRD patterns were collected in the 3–90° 2θ range, according to the step scanning procedure with Co radiation on a Miniflex Diffractometer (Rigaku, Japan). The tube operated at 30 kV and 15 mA, and the counting time was 3600 s. The identification of mineralogical phases was performed with a search/match on the Joint Committee on Powder Diffraction Standards.

Sample collection, isolation and cultivation

Environmental samples were collected from seven Turkish thermal stations located in the south eastern, north eastern, and south western peninsula: 1) Cermik-Diyarbakir, 2) Biloris-Siirt; 3) Güçlükonak-Şirnak; 4) Nemrut crater lake-Bitlis; 5) Agri-Diyadin; 6) Kula-Manisa; 7) Germencik-Aydin (Fig. S1). For each station, samples were collected where algae were present either superficially or covered by crystals, crumbly soil, and mud layers, respectively (Fig. 1). The samples were collected from different microenvironments, such as the surface of the crystals, around the granules of crumbly soil and between the layers of mud (Fig. 1). Temperatures were measured with a digital thermometer (Field Environmental Instruments, Pittsburgh, Pennsylvania, USA). pH was measured with a portable pH meter (Hanna Instruments, Padova, Italy) and with pH strips (Macherey Nagel Bethelem, USA).

Sampling location, coordinates, pH, temperature, and habitat for each sampling site are summarized in Table 1. All samples were collected by scraping the mineral substratum and these were stored in sterile tubes. In order to obtain monoclonal cultures of each sample, serial dilutions were performed in a specific medium for Cyanidiophytina (Allen's medium, pH 1.5, Allen & Stanier 1968); multi-well plates were used for the isolations. Maximum dilution enrichments were also streak-plated onto Allen's medium supplemented with agar. Single colonies were chosen from each plate and suspended in liquid Allen's medium. Cultures in both tubes and plates were grown at 37°C under continuous fluorescent light. All isolates were numbered and stored in the Algal Culture Collection of University Federico II of Naples (ACUF, www.acuf.net). Cultures are available upon request to the authors.

Algal samples were inspected using a light microscope (Nikon Eclipse E800 equipped with Nomarski interference), in order to visualize strains grown in Allen's medium.

DNA extraction, gene amplification and sequencing

For DNA extraction, algal cells were suspended in a specific buffer (DNeasy Plant Mini Kit, Qiagen, Santa Clarita, California, USA) and ground with glass beads using a Mini-BeadBeater (BioSpec, Bartlesville, Oklahoma, USA) operated at 13,000 revolutions per min for 5 min. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Santa Clarita, California, USA). Four degenerate primers were used to amplify the rbcL gene from isolated samples (Ciniglia et al. 2004). The resultant products were purified with the QIAquick PCR purification kit (Qiagen) and used for direct sequencing using the BigDyeTM Terminator Cycle Sequencing Kit 3.1 (PE-Applied Biosystems, Norwalk, Connecticut, USA) and an ABI-3500 XL at the Microgem Laboratory (Naples, Italy). Forward and reverse electropherograms were assembled and edited using the program Chromas Lite v.2.1 (www.technelsium.com.au/chromas.html).

Phylogenetic analyses

A total of 81 new rbcL sequences were obtained in this present study from our Turkish samples, and these were integrated with the 255 available rbcL sequences available at GenBank (Table S1). All sequences were aligned with published sequence data (Ciniglia et al. 2004, Toplin et al. 2008, Skorupa et al. 2013, Ciniglia et al., 2014, Hsieh et al. 2015), using BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). No gaps or indels have been incorporated in the alignment. Newly determined sequences are all available on NCBI GenBank (Table S1). Maximum likelihood (ML) phylogenetic analysis of rbcL was performed using the GTR + Γ + I model implemented in RAxML software (Stamatakis 2008). Statistical support for each branch was obtained from 1,000 bootstrap replications using the same substitution model and RAxML program settings. Bayesian analyses (BA) were performed for combined and individual datasets with MrBayes v.3.1.1 (Ronquist and Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo (MC3) with the GTR + Γ + I model. For each matrix, one million generations of two independent runs were performed with sampling trees generated every 100 generations. The burn-in period was identified graphically by tracking the likelihoods at each generation to determine whether they reached a plateau. Seven red algal taxa belonging to Bangiophyceae and Stylonematophyceae were chosen as outgroup taxa, being the closest relatives to Cyanidiophytina.

An estimate of genetic diversity was carried out using DNASP v.5.10.01 (Librado & Rozas 2009). For each population, the following statistics were computed: haplotype (h) (Nei 1987) and nucleotide diversities (π) (Nei 1987), with standard deviation. Population expansion, assessed by neutrality test (Tajima 1989, Fu&Li 1993) and mean number of pairwise differences (symbol) (Tajima, 1983).

To assess population differentiation, pairwise F_{st} values were calculated as the pairwise genetic differentiation (pairwise F_{st} statistics) in ARLEQUIN version 3.5.2.2 (Excoffier and Lischer 2010) based on 50,000 permutations ($P < 0.05$). The isolation-by-distance was tested using a Pearson correlation in R, testing for a positive correlation between pairwise geographic distance (in km) and Fst average pairwise differences.

RESULTS

Soil and rock samples at the Anatolian volcanism region were surveyed in search of Cyanidiophytina species. Table 1 shows the location of the sampling sites, temperature and pH, along with the type of substratum for each sampling station. In all of the examined samples, quartz and potassium feldspars were the main minerals found, followed by calcyte (Kula Manisa and Germencik), pyroxenes and dolomites (Agri-Dyadin, Cermik-Dyiarbakir and Biloris-Sirt), and gypsum (Gucklukonak-Sirnak). These Turkish sites had mostly neutral pHs (Table 1). Despite this, all collected samples had Cyanidiophycean. We were successful in isolating cultures at all sites using Allen's medium at pH 1.5. Cyanidiophycean cultures grew abundantly, suggesting that although adapted to neutral soil, these microalgae were acid tolerant. The same medium was used to obtain single colonies, and axenic cultures were deposited at the Algal Collection of University Federico II (ACUF, www.acuf.net).

The identification of different genera and species in the Cyanidiophytina has previously been difficult, as there are few unequivocal morphological features to distinguish between them, and furthermore, there is homoplasy between some lineages. Thus, in order to identify the algal species been studied molecular tools were used. For this we first generated 491 base pairs of rbcL sequence for the different isolates. These were aligned, including the 81 new Turkish cyanidiophycean isolates, (Table S1), and the existing 168 cyanidiophycean rbcL sequences available from GenBank. These strains originated from Japan, Iceland, Italy, Kuril Islands, Kamchatka, USA, New Zealand, and seven outgroup taxa. rbcL phylogeny identified five cyanidiophycean taxa from Turkey: *Galdieria sulphuraria, Galdieria maxima, Galdieria phlegrea, Cyanidium caldarium*, and *Cyanidioschyzon merolae* (Fig. 2). The inferred RAxML tree based on rbcL dataset showed several well-supported sublineages within *G. sulphuraria* and *G. maxima* clades. *G. sulphuraria* included at least five sublineages, including on defined by a New Zealand population (Fig. 2, subclade S1) and another with a USA population (Fig. 2, subclade S2). Accessions nested in an independent lineage, separable as two well-supported subclades (posterior probability/bootstrap: New Zealand subclade, 1/97; USA subclade, 1/98). We noted that 10 Turkish specimens grouped within *G. sulphuraria* and this was in two different subclades, 7 nesting with the Italian strains (Fig. 2, subclade S3; posterior probability/bootstrap 1/100), and 3 with the Icelandic strains along with the Russian *G. daedala* strain (Fig. 2, subclade S5; posterior probability/bootstrap 1/69). The sequences from Taiwan clustered with *G. partita* from Russia. Together the relations were clearly resolved with high statistical confidence.

The *G. maxima* assemblage included four subgroups reported in M1 to M4. Turkish specimens of *G. maxima* (n=40) clustered in two well-supported, different subclades, 13 of which clustered with Icelandic specimens (Fig. 2, subclade M1), 27 nesting with conspecific strains from Japan, Taiwan, and the Russian *G. maxima* authentic strain (Fig. 1, subclade M2). rbcL sequences from Taiwan and Japan both grouped into two subclades M2 and M3. *G. maxima* sequences from New Zealand did not group with any of conspecific strains collected from other locations, as in *G. sulphuraria* (Fig. 2, subclade M4).

The *G. phlegrea* clade was formed by Turkish (n=26) and Italian (n=8) isolates. This was strongly supported by high posterior probability/bootstrap values of 0.94/97%. *C. caldarium* from Turkey (n=3) were closely related to all other isolates with 100% bootstrap value.

Only two Turkish isolates were found to be closely related to *C. merolae*. The low level of intraspecific variation recorded in *C. merolae* did not generate any subclusterization associated to geographic populations. Our phylogenetic tree conformed to previously reported monophyly of Cyanidiophyceae (posterior probability, 1; ML LogDet bootstrap = 100%) (Fig. 2) (Ciniglia *et al.* 2004). However, by adding the rbcL sequences from the new Turkish isolates, at least six lineages within the class were indicated by the high bootstrap values, instead of the previously reported four lineages (Ciniglia *et al.* 2004; Ciniglia *et al.*, 2014). These six independent lineages were grouped in different monophyletic clades (Fig. 1), namely: 1) *C. merolae* (posterior probability 1/bootstrap, 99%); 2) *G. maxima* (1/99), sharing a common ancestor with *C. merolae,* but with strong evidence of molecular divergence between them; 3) the mesophilic lineage of *C. chilense* (1/100; Ciniglia *et al.*, 2017)*;* 4) *C. caldarium* (1/100), clearly phylogenetically divergent from the mesophilic *C. chilense* (Yang *et al.*, 2016); 5) *G. sulphuraria* (1/100) and 6) *G. phlegrea* (0.94/97), as sister clades (1/ 100).

Genetic diversity and population differentiation

Next an analysis of genetic diversity within and between populations of Cyanidiophyceae was performed by using DNAsp, which provides an estimate of the extent of genetic variation between individuals belonging to the same geographic population and between different populations. Results are listed in Table 2. We excluded *C. caldarium* from the analysis because of the low number of haplotypes and their restricted geographic distribution. A total of 159 haplotypes were recovered from 459 individuals analyzed and 149 (95.5%) of the haplotypes were private, *i.e.* unique to a single locality. The highest values of average sequence divergences were recorded for *G. sulphuraria* (K=19.47), and *G. maxi*ma (K=17.37), with a high level of haplotype diversity, as well (*G. sulphuraria*, hd, 0.83±0.028; *G. maxima*, hd, 0.956±0.006).

In *G. sulphuraria*, the analysis of genetic diversity was performed on 136 partial sequences of rbcL with 80 polymorphic sites and 33 different haplotypes (only two haplotypes were shared by Italy and Turkey and by Taiwan and Russia). The highest levels of haplotype diversity were found in the samples from New Zealand (hd = 0.867), Italy, and Taiwan (hd = 0.724 and 0.732). An average value of haplotype diversity was recorded in Turkey (hd = 0.600), despite the degree of nucleotide diversity higher than any other population (π = 0.0375). Iceland exhibited comparatively lower values of these indices (hd = 0.224; π= 0.0006).

Genetic distance was represented as Fst for each pairwise combination of populations, based on rbcL marker. The value of inter-populational pairwise genetic differentiation, Fst (5 populations of *G. sulphuraria* analyzed: USA, Italy, Turkey, New Zealand, and Iceland) was significantly high (0.7788, P<0.05). Fst ranges from 0 to 1; Fst of 0 indicates panmixy with high interbreeding between populations, while a value of Fst of 1 means that the populations are fixed and do not interbreed. When considering the genetic differentiation between two populations, Fst values ranged from low (0.14) to high (0.97) (Table 3). The lowest level of genetic differentiation was recorded between Turkey and Italy, which were also the closest populations geographically (1950 km). However, high genetic divergences were found between the furthest and the closest *G. sulphuraria* populations, such as Taiwan and USA (0.97, 12254km), USA and Iceland (0.91, 5719km), Italy and USA (0.85, 8622km), New Zealand and Iceland (0.844, 17215km), Italy and Iceland (0.839, 3247 km), and New Zealand and Italy (0.71, 18559km). We next investigated the potential for isolation by distance (IBD) via statistical tests of correlations in order to weigh the contribution of geographic distance in the population structure. The correlation between genetic and geographic distances based on rbcL was weakly positive, but not statistically significant in *G. sulphuraria*, as shown in Fig. 3 (R=0.264, P=0.333). This thus rejected an isolation-by-distance model from these data.

In examinations of 245 *G. maxima* partial rbcL (434bp) sequences, these contained 161 polymorphic sites and 100 haplotypes (Table 2). There was a high level of detected diversity (hd=0.956). Haplotype and genetic diversity of rbcL in Turkish populations, calculated from 40 sequences and 8 haplotypes were 0.652±0.069 (hd) and 0.02±0033 (π) in 43 polymorphic sites. The highest genetic diversity was found in the Taiwanese population, where among 149 individuals, 80 haplotypes and 108 parsimony informative sites showed high haplotype diversity (0.957±0.009) with low genetic polymorphism (π=0.0373±0.00067). The Japanese population was the highest in both diversities (hd=0.861±0.039; π=0.023±0.00345). This resulted from 23 sequences, 8 haplotypes, and 34 polymorphic sites. The level of haplotype and nucleotide diversity for the New Zealand population was calculated on the few sequences available (7 individuals, 4 haplotypes, hd=0.81±0.13, π=0.028±0.006). The 24 Icelandic sequences showed a lower haplotype and nucleotide diversity (hd=0.163±0.0098; π=0.00073±0.00051). In the neutrality test of *G. maxima*, Tajima D and Fu and Li were both significantly negative for the Icelandic samples (D= -1.88381; F= -2.796 Table 2). However, all samples from the other regions showed negative values of Tajima D, but without statistical significance of Tajima and Fu and Li, except for Taiwan samples showing strong significantly negative values of F (Table 2).

The inter-populational genetic differentiation, Fst calculated on 5 *G. maxima* populations (Turkey, Japan, Iceland, New Zealand, and Taiwan) was 0.55. However, the highest similarity in genetic structure calculated between two populations was accounted for the geographically closest populations Japan and Taiwan (Fst= 0.162). Low levels of genetic differentiation were also found between Turkey and Taiwan (Fst= 0.287) and Turkey and Japan (Fst= 0.257), despite the significant geographic distances between them. The highest Fst value was exhibited between Iceland and New Zealand, areas geographically far apart. A weakly positive correlation between genetic and geographic distances was detected for *G. maxima*, although it was not significant (R=0.145; P=0.763, Fig. 3).

Despite extensive sampling, current and previous molecular analysis has to date only identified 44 rbcL sequences from *C. merolae*. The majority belonged to individuals spread across the American territories, as few sequences were detected in the Turkish or Italian samples, and no sequences have yet been detected in Taiwanese samples. The analysis revealed the presence of 19 polymorphic sites, generating 19 haplotypes. The two most frequently represented were shared by the Turkish, Italian, and American samples. Genetic haplotype diversity was estimated using all of the isolates and gave results of 0.918±0.022, with a very low degree of nucleotide diversity, namely π=0.00443±0.00219 (Tajima, -1,73184; Fu and Li, -3,456). This indicates the absence of geographical population structuring. This was also shown by the low level of the overall genetic differentiation (Fst=0.05). We could not perform correlation test for *C. merolae*, as well as for *G. phlegrea* and *C. caldarium*, because of the limited number of accessions and populations available for the analysis.

DISCUSSION

Cyanidia are the most abundant photosynthetic protists found in extremely acidic, sulfur-rich environments that are close to active volcanoes (Brock *et al.*, 1978; Ciniglia *et al.*, 2004; Skorupa *et al.*, 2013; Toplin *et al.*, 2008). Until now Cyanidia have been isolated mainly in solfataras (Italy, Iceland, Japan, New Zealand, Yellowstone National Park, and Taiwan), where the condensation of sulfur dioxide and hydrogen sulfide produces crystals of sulfur subsequently oxidized to sulfuric acid resulting in acidification.

Turkey is characterized by collision volcanism, varying from mildly alkaline volcanoes, such as Nemrut, to calc-alkaline/alkaline ones, such as Mount Ararat (Pearce et al., 1990). Residual volcanic activity in Turkey explains the presence of many geothermal spots, with neutral and sub-neutral pH values, due to the limited presence of sulfuric acid. The main minerals detected in the areas explored were quartz, feldspars, calcite, and dolomites (Table 1). Narrow and thin biofilms of Cyanidia were detected in Turkish thermal baths, mostly in hypolithic and endolithic conditions.

Most of the species isolated from Anatolia were highly acidotolerant organisms, able to survive in a wide range of pH conditions (*Galdieria maxima, Galdieria phlegrea*, and *Cyanidium caldarium* between 1 and 7, *Galdieria sulphuraria* between 1 and 5.8). However, all species and strains, regardless of the ecological features of the sampling sites, remained well suited to acido-thermal or at least acidic growth conditions. One exception is represented by *Cyanidium chilense* (=cave *Cyanidium*, Schwabe, 1936, 1942; Hoffman, 1994; Ciniglia et al., 2017), which represents a separate monophyletic lineage within Cyanidiophytina, including several strains dispersed worldwide. It appears to be limited to cave habitats where pH and temperature are not extreme, and is unable to proliferate in laboratory conditions. Cyanidiophytina are thus abundant in mesophilic areas of Turkey, but are still adapted to thrive under acido-thermal environment.

According to Doemel and Brock (1971), the occurrence of *C. caldarium* in non-thermal habitat was frequent, being recorded in aquatic habitats between 20 °C and 55 °C and on soils at temperature between 10 °C and 55-57 °C. Pinto *et al.* (1993) similarly reported the presence of *C. caldarium*, *G. sulphuraria*, and *C. merolae* in more than 100 hydrothermal sites around Italy. These were not only in acidic hot springs, but also in acidic non-thermal ones, such as the sulfur mines. Recently, Hsieh et al. (2015) identified a novel mesophilic *Cyanidium* clade from non-thermal, but acidic sites in Taiwan, thus supporting the frequent occurrence of Cyanidiophytina in geothermal environments not necessarily in high temperature conditions (Gross et al., 2002).

Lowell & Castenholtz (2013) tested the ability of several *Cyanidium* to lower the external pH from 6 to more acidic values. They confirmed that many *Cyanidium* obtained from Yellowstone, Japan, Philippines, and New Zealand hot springs could acidify their growth environment. This suggested the importance of this process as survival strategy in confined environments, such as microbial mats, interstitial soil spaces, and endolithic niches. These algae appear to harbor adaptive responses to survive the non-ideal conditions during their dispersal, helped by wind flow, air particles, or birds. Despite the limited tolerance to desiccation and the absence of resting spores for Cyanidiophytina (Gross et al. 2002), the ability to lower the pH outside the cell would render them able to survive in non-acidic environments. This could potentially serve as a connection between the thermoacidic locations as a mechanism of long-distance migration (Brock, 1978; Gross, 1999).

The molecular investigations on new cyanidiophycean isolates revealed the presence of all representatives of this class of microalgae, namely *G. sulphuraria*, *G. phlegrea*, *G. maxima, C. merolae*, and *C. caldarium* on hydrothermal soils around Turkey. The new rbcL sequences were mostly attributed to *G. phlegrea* and *G. maxima*, while *G. sulphuraria*, *C. merolae*, and *C. caldarium* sequences were rarely detected. Turkey is the first site in which all these species have been collected in one local. For example in Italy *G. maxima* has not yet been detected, while all other thermoacidophilic communities sampled to date have an incomplete number of species and strains (Toplin *et al.*, 2008; Skorupa *et al.*, 2013, Hsieh *et al.*, 2015).

Of remarkable interest is the detection of *G. phlegrea* in almost all of the sampling stations from Turkey, recorded to now only in one Italian area located within the Phlegrean Fields (Naples, Italy), adapted to relatively dry areas and to dim light (Ciniglia *et al.*, 2004; Pinto *et al.*, 2007). *G. phlegrea* possesses interesting ecophysiological traits, exhibiting maximal growth at 25 °C, which is lower than *G. sulphuraria* at 38 °C. It is known that amongst Rhodophyta, all Cyanidiophytina encountered an extensive reduction of their genome. It has been proposed that this is an adaptation strategy to stressful environmental conditions. *G. phlegrea* have regained genes through horizontal gene transfer, suggested as an ameliorative strategy for adaptation to specific environmental niches (Qiu *et al.*, 2013).

Genomic analyses revealed that *G. phlegrea* and *G. sulphuraria* belong to different taxa, since the protein divergences between them are comparable to the protein-divergence distances between humans and teleosts (Qiu *et al.*, 2013). The rbcL sequences of Turkish *G. sulphuraria* isolates showed the highest genetic variability both in terms of haplotype diversity and in nucleotide diversity, followed by Taiwanese conspecific specimens. *G.*

sulphuraria strains from Turkey clustered in two separate lineages, the former including Italian isolates, the latter including Icelandic strains. This finding suggests that there have been at least two separate introductions from Turkey in Western Europe; the levels of interpopulational genetic differentiation suggested a dispersal ability significantly higher between Turkey and Italy than between Turkey and Iceland, which would be consistent with a correlation between genetic and geographic distance.

Ciniglia *et al.* (2014) previously hypothesized that the northeastern Asian populations of *Galdieria* would be the potential donor of Icelandic *G. sulphuraria* populations, because of the occurrence of the Russian species *G. daedala* within the same clade, alongside some Turkish accessions. The strong monophyly among Turkey, Iceland, and Russian strains, along with the highly divergent haplotypes associated with Turkish accessions, would be consistent with Turkey in being a center of *G. sulphuraria* diversification and dispersal to Western European sites. A similar pattern was found in the *G. maxima* clade; Turkish isolates strictly grouped both with Icelandic and with Japanese and Taiwanese accessions, along with the Russian haplotype *G. maxima* IPPAS P507. In the present study, the combination of high haplotype and low nucleotide diversity is a signature of a rapid population expansion from a small effective population size (Avise 2000); Tajima's D test and Fu's Fs tests, applied to find out the population expansion, were both negative in all cases; this indicates excess of the rare mutations in populations, thus supporting the hypothesis of recent population expansions within Cyanidiophytina.

The discovery of Cyanidiophyceae in Turkey confirms the cosmopolitan distribution of these algae, despite the peculiar ecological requirements that are present in discontinuous and distant habitats. The worldwide distribution of extremophiles has been demonstrated also for *Sulfolobus,* an archea inhabiting the geothermal sulfuric springs at T > 70 °C and strongly acidic pH, isolated in several hot springs throughout Northern hemisphere (Brock et al., 1972; Zuo et al., 2015). It is intriguing for these extremophiles, such as the Cyanidiophytina, to understand how they can survive long-distance dispersal, through inhospitable environments, without tolerating desiccation and without producing resistance spores.

We examined the population structure in *G. sulphuraria*, *G. maxima*, *G. phlegrea*, and *C. merolae*, measuring Fst, a parameter that provides a measure of population differentiation based on genetic variance between the populations. Pairwise comparisons between strains grouped by region have produced different results in the Cyanidiophycean taxa. Large, significant Fst values across the hydrothermal locations were recorded in *G. sulphuraria* and *G. maxima* suggesting a high level of genetic differentiation, and a reduction in dispersal ability of the individuals. However, in *G. maxima* low Fst values were recorded among the Asiatic populations, indicating that there is at least a small level of genetic differentiation between them, and a substantial level of gene flow. Perhaps this was due to the contiguity of the geothermal areas, being located on the Ring of Fire. Within *C. merolae* and *G. phlegrea,* Fst values were not significantly different from zero (Fst= 0.05 and 0.013, respectively), indicating that populations from different geothermal springs were not genetically differentiated, suggesting a frequent gene flow among the geothermal springs. *G. phlegrea* populations to date have only been identified in Turkey and Italy, and it is intriguing that even in *G. sulphuraria*, the lowest level of genetic differentiation was recorded between the same populations. This supports the hypothesis of gene flow between Turkey and Italy. The level of genetic divergence of *G. phlegrea* was much lower than that observed in *G. sulphuraria* and in *G. maxima. G. phlegrea* has a restricted areal of dispersal, because of its peculiar adaptation to dry habitats, such as rock fissures, chasmoendolithic and cryptoendolithic environments. These habitats were very frequently encountered in Turkey, and are preferred by *G. phlegrea* in spite of fumaroles and hot springs.

Significant levels of genetic divergence were reported for other extremophilic microorganisms, such as in populations of *Sulfolobus solfataricus* where gene flow among different geothermal stations is limited (Whitaker et al., 2003). However, while in *S. solfataricus* the global population structure is mainly ascribed to isolation by distance, in Cyanidiophytina, namely in *G. sulphuraria* as well as in *G. maxima*, gene flow and species dispersal among populations was not found to increase with the geographic distance. This is notable as there was no significant positive correlation between genetic and geographic distance. For an extremophile, hot springs may be considered as island-like habitats occurring as clusters in globally distant regions. For an extremophilic organism to thrive in such conditions, they must adapt to drastically different conditions from the surrounding habitat through which they would have to disperse (Ramette and Tiedje, 2007). As such, it would be expected that geographical isolation might be an important component in the diversification of microextremophiles (Papke et al., 2003), as already observed in *S. solfataricus* (Whitaker et al., 2003). In stark contrast, our results suggest that for Cyanidiophyceae, their growth requirements limit dispersal, but do not prevent it. The discovery of such a high number of Cyanidiophycean species and strains from global explorations is helpful to better delineate ecological boundaries. Moreover, the phylogenetic analyses strongly support the reconstruction of the relationships between the 6 lineages recovered. For this purpose, sequencing of the whole rbcL gene as well as additional markers, such as the nuclear small and large subunit rDNA genes (SSU and LSU), concatenated with rbcL, should result in a substantial improvement in phylogenetic resolution.

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No potential conflict of interest was reported by the author(s).

AUTHOR CONTRIBUTIONS

A. Eren: conduction of experiments, analysis of results, contribution to draft writings; M. Iovinella: conduction of experiments, analysis of results, contribution to draft writings; S. J. Davis analysis of results, contribution to draft writings; D. Cioppa: isolation of strains, conduction of experiments; G. Pinto and A. Pollio: original concept, provision of resources; C. Ciniglia: original concept, provision of resources, draft editing.

Figures and Tables legends

Fig. 1. Pictures of some sampling points from the Turkish thermal areas for Cyanidiophytina. a, Cermik, Southeastern Turkey; b, c, endolithic growth of Cyanidiophytina in Germencik, Southwestern Turkey; d,e,f, Agri, Diyadin, Northeastern Turkey; g,h, Kula Manisa, Southwestern Turkey; i, Saart, Manisa, Southwestern Turkey; j, k,l, Salihli, Manisa, Southwestern Turkey.

Fig. 2. Consensus Bayesian tree of Cyanidiophytina based on rbcL sequences. The Bayesian posterior probability and maximum-likelihood (RAxML) bootstrap values (MLBT) are shown above the branches. Dashes indicate support values <50%.

Fig. 3. Correlation among genetic divergence and geographic distance. Each point represents a single pairwise comparison between seven isolated populations. Regression lines show relationships between genetic divergence and geographic distance (*G. sulphuraria*, R=0.245, P=0.333; *G. maxima*, R=0.145, P=0.763).

Table 1. Location, codes, habitat, pH, temperature and main minerals of sampling sites in Turkey.

Table 2. Statistics of rbcL haplotypes for the Turkish cyanidiophycean strains; n. sample size, v. variable sites, N. number of haplotypes, h. haplotype diversity, K. Average number of pairwise nucleotide differences, π nucleotide diversity. (significance *: $p < 0.05$; **; $p < 0.10$).

Table 3. Matrix of pairwise estimates of Fst between pairs of populations of *G. sulphuraria* and *G. maxima.*

Supplementary material

Fig. S1. Map of Turkey. Names indicate the sampling sites from where Cyanidiophytina were isolated.

Table S1. GenBank Accession numbers for taxa included in the phylogenetic analyses.

Location, region (coordinates)	**Sampling location code**	**Habitat**	**pH**	**T (°C)**	**Minerals**
Cermik, Diyarbakir Southeast Turkey (38°8'16"N, 39°28'3"E)	SET.CE	Thermal bath, on the wall inside and outside the hammam	7	24.6	Quartz, pyroxenes, dolomites
Biloris, Siirt Southeast Turkey (37°56'7"N, 41°56'12"E)	SET.BI	Thermal bath, on the wall, inside the hammam	7	25.8	Quartz, pyroxenes, dolomites
Gü.lükonak, Şirnak Southeast Turkey (37°28'10"N, 41°54'39"E)	SET.GU	Thermal bath, on the wall inside the hammam	1	54	Quartz, feldspars, gypsum
Nemrut crater lake East Turkey (38°37'33"N, 42°14'44"E)	CET.NE	Fumaroles	6.7	32-46	Quartz, feldspars, gypsum
Agri, Diyadin Northeast Turkey (39°32'26"N, 43°40'57"E)	NET.DI	Fumaroles, hot spring, hot pool, hot soil	6.5	45	Quartz, pyroxenes, dolomites
Kula, Manisa Southwest Turkey (38°32'45"N, 28°38'48"E)	SWT.KU	Hot soil-hot pool	5	41	Quartz, feldspars, miche, calcyte
Germencik, Aydin Southwest Turkey (37°52'15"N, 27°35'58"E)	SWT.GE	Hot spring	5.8	27	Quartz, feldspars, miche, calcyte

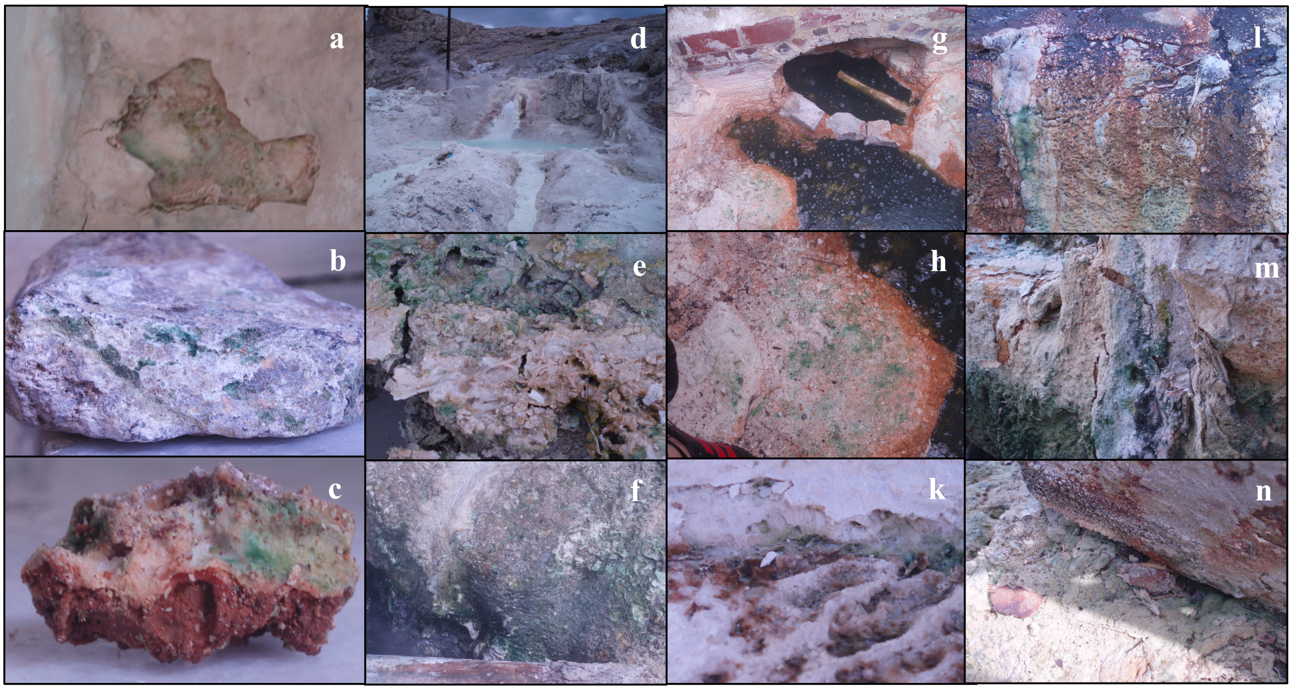
Table 1. Location, codes, habitat, pH, temperature and main minerals of sampling sites in Turkey

Phylotype		**n**	**v**	**N**	**K**	**h**	**π**	**Tajima**	**Fu and Li F***
G.sulphuraria	ALL	136	80	33	19,47	0,83±0,028	0,0426±0,00356	-0,35987	-0,42141
	Italy	15	8	7	1,29	0,724±0,121	0,00283±0,0019	-1,744	-1,992
	USA	13	5	4	1,2	0,6±0,131	0,00269±0,00157	-0,84	-1
	Turkey	10	39	3	17,13	0,6±0,131	0,0375±0,00482	-1,17	1,3426
	Taiwan	27	39	6	6,83	0,732±0,054	0,015±0,0035	-1,405	0,637
	Iceland	59	6	7	0,267	0,224±0,072	0,00058±0,000115	-1,95362 *	-3,337 * *
	New Zealand	10	8	6	2,022	0,867±0,085	0,0044±0,002	-1,23	-1,43
G. maxima	ALL	245	161	100	17,3721	0,956±0,006	0,038±0,0012	-1,38	-5,416 **
	Turkey	40	43	8	8,8	0,652±0,069	0,02±0,0033	-0,52	-2,2
	Japan	23	34	8	10,52	0,861±0,039	0,023±0,00345	-0,302	-0,815
	Iceland	24	4	3	0,3333	0,163±0,0098	0,00073±0,00051	-1,88381 *	-2,796 *
	Taiwan	149	108	80	17,08	0,957±0,009	0,0373±0,00067	-0,6142	-5,3 **
	New Zealand	7	44	4	13,05	0,81±0,13	0,028±0,0059	-1,58	-1,836
C. merolae	ALL	44	19	19	2,0296	0,918 ±0,022	0,00443±0,00219	-1,73184	-3,456 **
	Turkey	2	1	2	1	1±0,5	0,0028±0,00109	-	-
	USA	35	17	17	2,2454	0,934±0,021	0,0049±0,00053	-1,515	-2,89 *
	Italy	5	2	3	0,8	0,7±0,2	0,00175±0,00066	-0,97	-0,95
G.phlegrea	ALL	34	26	7	2,3244	0,458±0,104	0,0051±0,00272	-2,3221 **	-3,4274 **
	Italy	8	4	2	1	0,250±0,180	0,0022±0,0016	-1,5347	-1,7974
	Turkey	26	24	6	2,72	0,517±0,113	0,006±0,0028	-2,20 **	-3,267 * *

Table 2. Summary statistics of rbcL haplotypes for the Turkish cyanidiophycean strains; n . sample size, v . variable sites, N . number of haplotypes, h. haplotype diversity, K. Average number of pairwise nucleotide differences, π nucleotide diversity. (significance *: p< 0.05; **; p< 0.10).

G. sulphuraria	ICE	ITA	NZE	TWN	TUR	USA
ICE	***	0,839	0,844	0,87	0,55	0,91
ITA		***	0,71	0,88	0,14	0,85
NZE			***	0,91	0,511	0,831
TWN				***	0,67	0,97
TUR					***	0,68
USA						***

G. maxima	ICE	JAP	TWN	TUR
ICE	***	0,4	0,64	0,67
JAP		***	0,21	0,06
TWN			***	0,56
TUR				***


a
d
g
l
b
e
h
m
c
f
k
n


Galdieria phlegrea
clade
Turkey
Italy
Gucklukonak, TR (KY033456)
Pisciarelli, IT (AY541311)
Diyadin, TR (KY033445)
Cermik, TR (KY033455)
Cermik, TR (KY033395)
Cermik, TR (KY033457)
Nemrut, TR (KX501176)
Gucklukonak, TR (KX501184)
Diyadin, TR (KY033409)
Biloris, TR (KY033402)
Pisciarelli, IT (AY541314)
Cermik, TR (KY033454)
Gucklukonak, TR (KY033444)
Diyadin, TR (KY033446)
Pisciarelli, IT (AY541316)
Diyadin, TR (KY033407)
Manisa Kula, TR (KY033458)
Cermik, TR (KY033394)
Benevento, IT (AY541310)
Diyadin, TR (KY033407)
Pisciarelli, IT (AY541315)
Biloris, TR (KX501177)
Nemrut, TR (KY033405)
Diyadin, TR (KY033421)
Gucklukonak, TR (KY033433)
Biloris, TR (KX501182)
Biloris, TR (KY033399)
Biloris, TR (KY033399)
Diyadin, TR (KY033424)
Biloris, TR (KY033401)
Agrigento, IT (AY119769)
Viterbo, IT (AY119768)
Pisciarelli, IT (AY541317)
Dyadin, TR (KY033447)
0.94
97
1
100
Galdieria sulphuraria
clade
Turkey
Italy
New Zealand
USA
Taiwan
Russia
Iceland
S1
1
97
Rotorua, NZ (EF675176)
WhiteIsland, NZ (EF675173)
WhiteIsland, NZ (EF675183)
Craters of the Moon, NZ (EF675177)
Whaka, NZ (EF675172)
Waiotopu, NZ (EF675180)
Craters of the Moon, NZ (EF675178)
Waiotopu, NZ (EF675181)
Waiotopu, NZ (EF675175)
S2
1
98
YNP, USA (AY119767)
Sonoma, USA (AF233069)
Fairy Falls, YNP, USA (JQ269630)
Lemonade Creek, YNP, USA (JQ269612)
Dragon Springs, YNP, USA (JQ269633)
Dragon Springs, YNP, USA (JQ269631)
Lemonade Creek, YNP, USA (JQ269609)
Norris Basin, YNP, USA (EF675182)
Dragon Springs, YNP, USA (JQ269629)
Crater Hills, YNP, USA (JQ269635)
Norris Basin, YNP, USA (EF675174)
Norris Basin, YNP, USA (EF675171)
S3
1
100
Monte Rotondo, IT (DQ916746)
Gucklukonak, TR (KX501178)
Pisciarelli, IT (AY541325)
Pisciarelli, IT (AY541324)
Monte Rotondo, IT (DQ916745)
Pisciarelli, IT (AY541322)
Caserta, IT (AY541303)
Germencik, TR (KX501181)
Guklukonak, TR (KY033403)
Gucklukonak, TR (KY033441)
Gucklukonak, TR (KX501175)
Sasso Pisano, IT (DQ916749)
Ischia, IT (AY541305)
Germencik, TR (KY033412)
Pisciarelli, IT (AY541321)
Scarfoglio, IT (AY541304)
Vulcano, IT (AY541307)
Sasso Pisano, IT (DQ916748)
Gucklukonak, TR (KY033417)
Monte Rotondo, IT (DQ916747)
Solfatara, IT (AY541306)
Pisciarelli, IT (AY541313)
1
100
S4
DaYouKeng, TW (JX981595)
Kamchatka, RUS (AB18008)
DaYouKeng, TW (JX981596)
MaChao, TW (JX981528)
DaYouKeng, TW (JX981598)
DaYouKeng, TW (JX981601)
DaYouKeng, TW (JX981597)
DaYouKeng, TW (JX981602)
S5
1
69
Kunashir, RUS (AY541302)
Niasjvellir, ICE (KC883836)
Seltun, ICE (KC883852)
Niasjvellir, ICE (KC883856)
Gunnhuver, ICE (KC883809)
Seltun, ICE (KC883822)
Seltun, ICE (KC883853)
Niasjvellir, ICE (KC883828)
Niasjvellir, ICE (KC883823)
Seltun, ICE (KC883884)
Viti, ICE (KC883876)
Seltun, ICE (KC883838)
Viti, ICE (KC883874)
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Seltun, ICE (KC883839)
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Viti, ICE (KC883873)
Gunnhuver, ICE (KC883847)
Seltun, ICE (KC883875)
Seltun, ICE (KC883834)
Germencik, TR (KY033449)
Gunnhuver, ICE (KC883810)
Gunnhuver, ICE (KC883807)
Niasjvellir, ICE (KC883825)
Seltun, ICE (KC883883)
Seltun, ICE (KC883844)
Seltun, ICE (KC883882)
Niasjvellir, ICE (KC883858)
Seltun, ICE (KC883843)
Viti, ICE (KC883867)
Seltun, ICE (KC883850)
Seltun, ICE (KC883820)
Gunnhuver, ICE (KC883811)
Landmannalaugar, ICE (KC883812)
Gunnhuver, ICE (KC883806)
Niasjvellir, ICE (KC883830)
Seltun, ICE (KC883885)
Gunnhuver, ICE (KC883808)
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Landmannalaugar, ICE (KC883813)
Seltun, ICE (KC883845)
Seltun, ICE (KC883864)
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Niasjvellir, ICE (KC883859)
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Niasjvellir, ICE (KC883837)
Niasjvellir, ICE (KC883824)
Seltun, ICE (KC883886)
Viti, ICE (KC883879)
Viti, ICE (KC883877)
Landmannalaugar, ICE (KC883814)
Seltun, ICE (KC883832)
Viti, ICE (KC883878)
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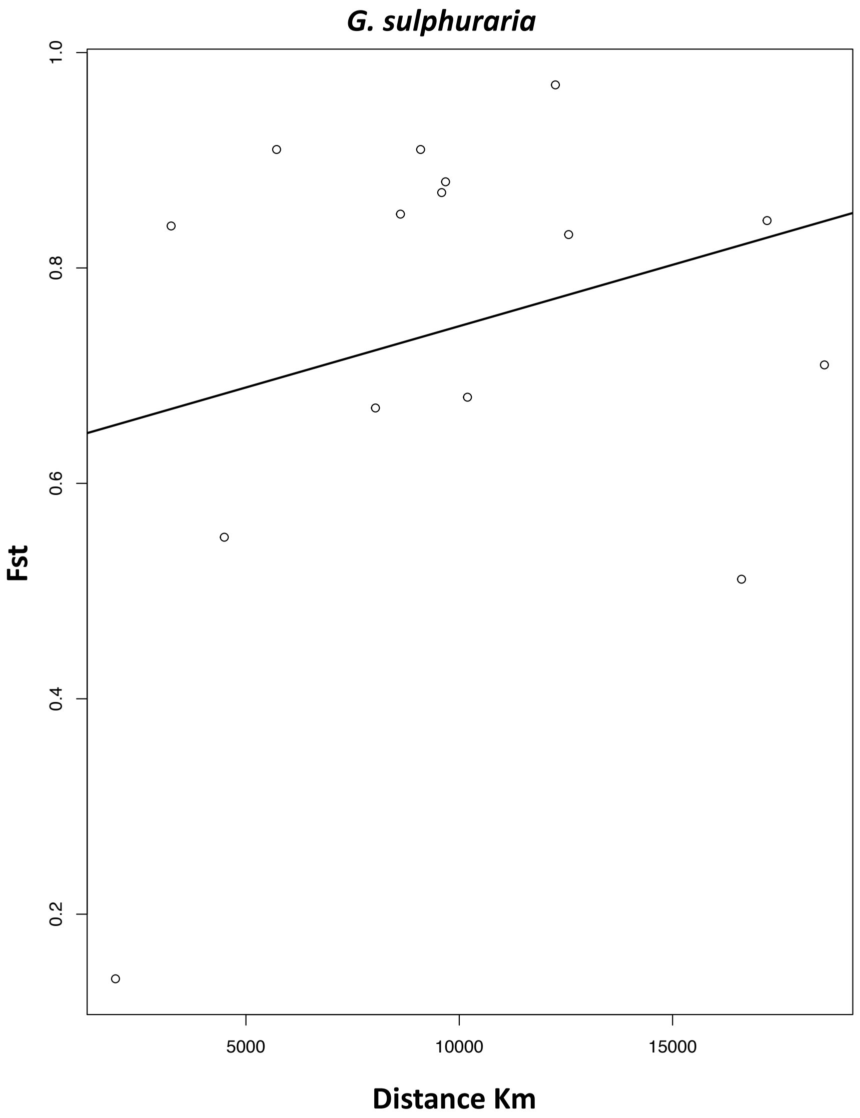
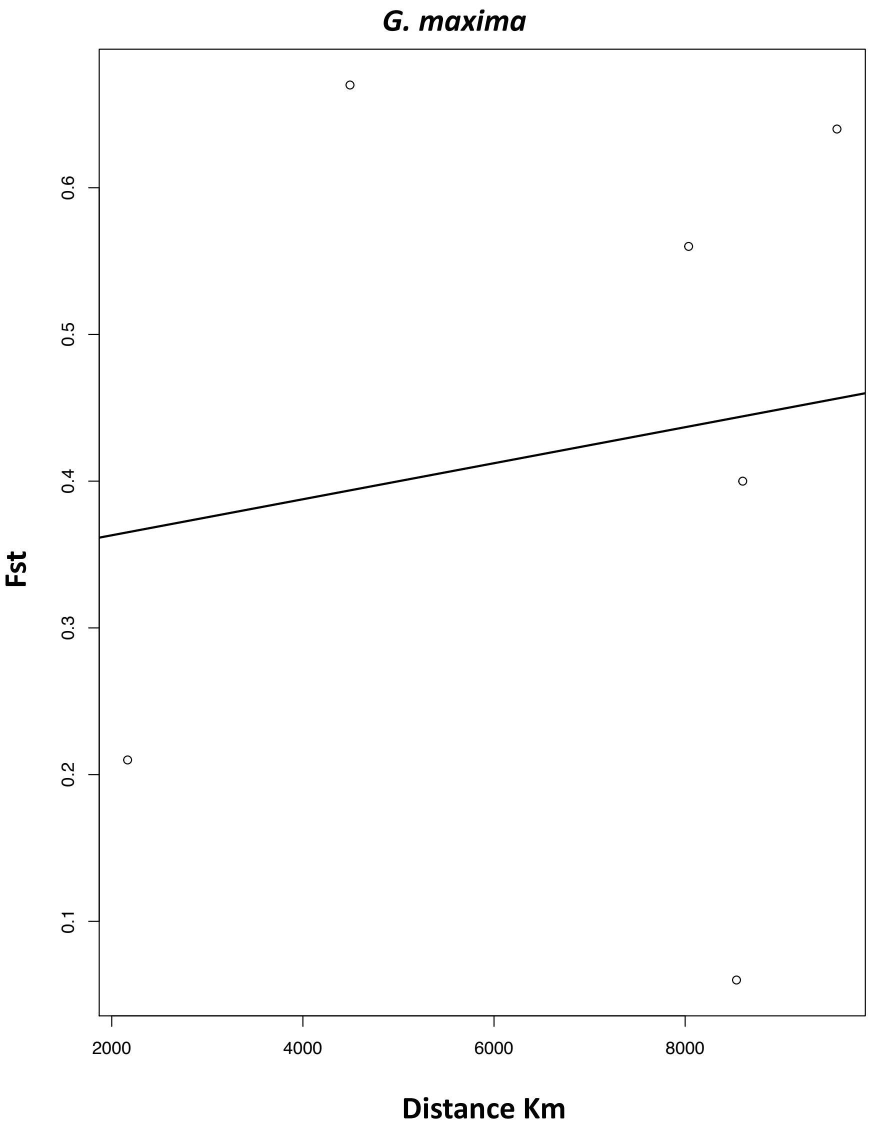
Seltun, ICE (KC883866)
Landmannalaugar, ICE (KC883815)
Seltun, ICE (KC883973)
Cyanidium chilense
clade
Italy
1
100
Terme di Baia, IT (KC914876)
Monte Rotaro, IT (AY541301)
Cuma, IT (AY391369)
Cyanidium caldarium
clade
Turkey
Italy
Japan
Indonesia
1
100
Sasso Pisano, IT (DQ916753)
Nikko, JP (NC001840)
Java, IND (AY541298)
Güçlükonak, TR (KY033462)
Diyadin, TR (KY033437)
Monte Rotondo, IT (DQ916750)
Acqua Santa, IT (AY541299)
Siena, IT (AY541297)
Monte Rotondo, IT (DQ916751)
Monte Rotondo, IT (DQ916752)
Cermik, TR (KY033432)
Cyanidioschyzon merolae
clade
Turkey
Italy
USA
Indonesia
1
99
Lemonade Creek, YNP, USA (JQ269614)
Dragon Springs, YNP, USA (JQ269624)
Dragon Springs, YNP, USA (JQ269620)
Dragon Springs, YNP, USA (JQ269623)
Lemonade Creek, YNP, USA (JQ269610)
Dragon Springs, YNP, USA (JQ269638)
Succession, YNP, USA (JQ269626)
Lemonade Creek, YNP, USA (JQ269611)
Fairy Falls, YNP, USA (JQ269616)
Pisciarelli, IT (AY541320)
Pisciarelli, IT (AY541323)
Monte Nuovo, IT (AY541296)
Pisciarelli, IT (AY541312)
Lemonade Creek, YNP, USA (JQ269628)
Crater Hills, YNP, USA (JQ269634)
Dragon Springs, YNP, USA (JQ269619)
Dragon Springs, YNP, USA (JQ269618)
Dragon Springs, YNP, USA (JQ269617)
Pisciarelli, IT (AY119766)
Nymph Creek, YNP, USA (JQ269621)
Biloris, TR (KY033448)
Dragon Springs, YNP, USA (JQ269605)
Lemonade Creek, YNP, USA (JQ269613)
Pisciarelli, IT (AY541319)
Monument Basin, YNP, USA (JQ269636)
Nemrut, TR (KY033452)
Lemonade Creek, YNP, USA (JQ269627)
Java, IND (AY119765)
Dragon Springs, YNP, USA (JQ269606)
Twin Springs, YNP, USA (JQ269637)
Nymph Creek, YNP, USA (EF675164)
Highland Creek, YNP, USA (EF675158)
Nymph Creek, YNP, USA (EF675152)
Sylvan Crust, YNP, USA (EF675125)
Lemonade Creek, YNP, USA (EF675130)
1
100
Galdieria maxima
clade
Turkey
Iceland
Japan
Taiwan
New Zealand
M1
0.99
99
Landmannalaugar, ICE (KC883868)
Seltun, ICE (KC883833)
Cermik, TR (KY033398)
Cermik, TR (KY033450)
Diyadin, TR (KY033406)
Biloris, TR (KY033400)
Landmannalaugar, ICE (KC883863)
Seltun, ICE (KC883818)
Diyadin, TR (KY033453)
Seltun, ICE (KC883816)
Landmannalaugar, ICE (KC883869)
Niasjvellir, ICE (KC883851)
Landmannalaugar, ICE (KC883870)
Landmannalaugar, ICE (KC883862)
Seltun, ICE (KC883849)
Cermik, TR (KY033396)
Landmannalaugar, ICE (KC883842)
Landmannalaugar, ICE (KC883840)
Cermik, TR (KY033443)
Landmannalaugar, ICE (KC883861)
Seltun, ICE (KC883821)
Diyadin, TR (KY033436)
Seltun, ICE (KC883846)
Niasjvellir, ICE (KJ173929)
Seltun, ICE (KC883818)
Diyadin, TR (KY033460)
Niasjvellir, ICE (KC883857)
Landmannalaugar, ICE (KC883848)
Niasjvellir, ICE (KC883829)
Niasjvellir, ICE (KC883827)
Landmannalaugar, ICE (KC883841)
Seltun, ICE (KC883881)
Seltun, ICE (KC883880)
Manisa Kula, TR (KY033410)
Güçlükonak, TR (KY033404)
Cermik, TR (KY033451)
M2
0.99
97
GengZiPeng, TW (JX981617)
MaChao, TW (JX981525)
GengZiPeng, TW (JX981618)
GengZiPeng, TW (JX981631)
MaChao, TW (JX981524)
DaYouKeng, TW (JX981587)
DaYouKeng, TW (JX981599)
DiReGu, TW (JX981550)
Nakabusa, JAP (EF675156)
Nakabusa, JAP (EF675155)
DiReGu, TW (JX981551)
DaYouKeng, TW (JX981583)
Germencik, TR (KY033427)
Diyadin, TR (KY033435)
Germencik, TR (KY033423)
Güçlükonak, TR (KX501174)
Manisa Kula, TR (KY033438)
Owakudani, JAP (EF675139)
Germencik, TR (KY033425)
Güçlükonak, TR (KX501173)
Cermik, TR (KY033431)
Biloris, TR (KY033422)
Manisa Kula, TR (KY033397)
Biloris, TR (KY033416)
Güçlükonak, TR (KY033419)
Güçlükonak, TR (KX501179)
Diyadin, TR (KX501180)
Manisa Kula, TR (KY033426)
Owakudani, JAP (EF675138)
Güçlükonak, TR (KY033414)
Manisa Kula, TR (KY033439)
DiReGu, TW (JX981548)
Güçlükonak, TR (KY033418)
Manisa Kula, TR (KY033442)
Güçlükonak, TR (KY033415)
Kunashir, RUS (AY391370)
1
99
Germencik, TR (KX501183)
Manisa Kula, TR (KY033420)
Diyadin, TR (KX501185)
Manisa Kula, TR (KY033428)
Manisa Kula, TR (KY033459)
Biloris, TR (KY033430)
Biloris, TR (KY033429)
Germencik, TR (KY033411)
Manisa Kula, TR (KY033461)
M3
Kusatsu, JAP (EF675167)
DiReGu, TW (JX981656)
DiReGu, TW (JX981653)
DiReGu, TW (JX981654)
DiReGu, TW (JX981655)
DaYouKeng, TW (JX981585)
M4
1
99
Craters of the Moon, NZ (EF675142)
Rotowhero, NZ (EF675166)
Whaka, NZ (EF675161)
Waimangu, NZ (EF675128)
Waimangu, NZ (EF675165)
Non-Cyanidiales
Red algae
Bangiopsis subimplex (AY119772)
Stylonema alsidii (AY119779)
Rhodosorus marinus (AY119778)
Porphyridium aerugineum (AY119775)
Flintiella sanguinaria (AY119774)
Rhodella violacea (AY119776)
Dixoniella grisea (AY119773)


G. sulphuraria
Fst
1.0
0.8
0.6
0.4
0.2
5000
10000
15000
Distance Km


G. maxima
Fst
0.6
0.5
0.4
0.3
0.2
0.1
2000
4000
6000
8000
Distance Km

Taxa	**Strain**	**Sampling Site**	**GenBank number**
Cyanidiales sp.	CHJ-4	USA, Crater Hills, YNP	JQ269635
	CHJ-5	USA, Crater Hills, YNP	JQ269634
	DS1-6	USA, Dragon Springs, YNP	JQ269623
	DS1-9	USA, Dragon Springs, YNP	JQ269631
	DS2-2	USA, Dragon Springs, YNP	JQ269638
	DS2-5	USA, Dragon Springs, YNP	JQ269629
	DS3-1	USA, Dragon Springs, YNP	JQ269633
	DS3-3	USA, Dragon Springs, YNP	JQ269624
	DSB-9	USA, Dragon Springs, YNP	JQ269617
	DSC-8	USA, Dragon Springs, YNP	JQ269618
	DSD-7	USA, Dragon Springs, YNP	JQ269605
	DSE-8	USA, Dragon Springs, YNP	JQ269619
	DSF-12	USA, Dragon Springs, YNP	JQ269620
	DSH-4	USA, Dragon Springs, YNP	JQ269606
	SFFL-8	USA, Fairy Falls, YNP	JQ269630
	SFFR-7	USA, Fairy Falls, YNP	JQ269616
	SFFL-5	USA, Fairy Falls, YNP	JQ269630
	LCBCEL-7	USA, Lemonade Creek, YNP	JQ269610
	LCBTERR-12	USA, Lemonade Creek, YNP	JQ269611
	LCCYEGR-4	USA, Lemonade Creek, YNP	JQ269614
	LCASUB-11	USA, Lemonade Creek, YNP	JQ269627
	LCBSUB-5	USA, Lemonade Creek, YNP	JQ269628
	LCCBLGR-8	USA, Lemonade Creek, YNP	JQ269613
	LCBTERR-6	USA, Lemonade Creek, YNP	JQ269612
	LCBCEL-5	USA, Lemonade Creek, YNP	JQ269609
	RIVER1B-5	USA, Monument Basin, YNP	JQ269636
	NCB-4	USA, Nymph Creek, YNP	JQ269621
	SSI-6	USA,Succession, YNP	JQ269622
	SSII-1	USA,Succession, YNP	JQ269626
	TS-4	USA, Twin, YNP	JQ269637
Cyanidioschyzon merolae	ACUF201	Indonesia, Java	AY119765
	ACUF202	Italy, Monte Nuovo	AY541296
	ACUF001	Italy, Pisciarelli	AY119766
	Clone-C16	Italy, Pisciarelli	AY541319
	CloneA1	Italy, Pisciarelli	AY541312
	CloneD1	Italy, Pisciarelli	AY541320
	CloneE10	Italy, Pisciarelli	AY541323
	10D	Italy, Sardinia	D63675
	10D	Italy, Sardinia	NC_004799
	CCMEE5625	USA, Highland Creek, YNP	EF675158
	CCMEE5576	USA, Lemonade Creek, YNP	EF675130
	CCMEE5506	USA, Norris Basin, YNP	EF675146
	CCMEE5507	USA, Norris Basin, YNP	EF675160
	CCMEE5631	USA, Norris Basin, YNP	EF675140
	CCMEE5639	USA, Norris Basin, YNP	EF675127
	CCMEE5640	USA, Norris Basin, YNP	EF675137
	CCMEE5584	USA, Nymph Creek, YNP	EF675164
	CCMEE5585	USA, Nymph Creek, YNP	EF675152
	CCMEE5593	USA, Obsidian Creek, YNP	EF675124
	CCMEE5610	USA, Sylvan Crust, YNP	EF675125
	CCMEE5609	USA, Sylvan Springs, YNP	EF675144
Cyanidium caldarium	ACUF182	Indonesia, Java	AY541298
	ACUF 020	Italy, Acqua Santa	AY541299
	isolate MR4-22	Italy, Monte Rotondo	DQ916750
	isolate MR5-5	Italy, Monte Rotondo	DQ916751
	isolate MR6-C35	Italy, Monte Rotondo	DQ916752
	Clone C2	Italy, Pisciarelli	AY541318
	isolate SP1-10	Italy, Sasso Pisano	DQ916753
	ACUF019	Italy, Siena	AY541297
	RK1	Japan, Nikko	NC_001840
Galdieria daedala	IPPAS P508	Russia, Kunashir	AY541302

Galdieria maxima	ACUF419	Iceland, Landmannalaugar	KC883840
	ACUF420	Iceland, Landmannalaugar	KC883841
	ACUF421	Iceland, Landmannalaugar	KC883842
	ACUF428	Iceland, Landmannalaugar	KC883848
	ACUF449	Iceland, Landmannalaugar	KC883861
	ACUF450	Iceland, Landmannalaugar	KC883862
	ACUF451	Iceland, Landmannalaugar	KC883863
	ACUF456	Iceland, Landmannalaugar	KC883868
	ACUF457	Iceland, Landmannalaugar	KC883869
	ACUF458	Iceland, Landmannalaugar	KC883870
	ACUF404	Iceland, Niasjvellir	KC883827
	ACUF406	Iceland, Niasjvellir	KJ173929
	ACUF407	Iceland, Niasjvellir	KC883829
	ACUF438	Iceland, Niasjvellir	KC883851
	ACUF445	Iceland, Niasjvellir	KC883857
	ACUF389	Iceland, Seltun	KC883816
	ACUF392	Iceland, Seltun	KC883818
	ACUF393	Iceland, Seltun	KC883819
	ACUF396	Iceland, Seltun	KC883821
	ACUF411	Iceland, Seltun	KC883833
	ACUF425	Iceland, Seltun	KC883846
	ACUF436	Iceland, Seltun	KC883849
	ACUF468	Iceland, Seltun	KC883880
	ACUF469	Iceland, Seltun	KC883881
	CCMEE5664	Japan, Kusatsu	EF675145
	CCMEE5665	Japan, Kusatsu	EF675129
	CCMEE5667	Japan, Kusatsu	EF675151
	CCMEE5676	Japan, Kusatsu	EF675143
	CCMEE5677	Japan, Kusatsu	EF675132
	CCMEE5678	Japan, Kusatsu	EF675168
	CCMEE5679	Japan, Kusatsu	EF675163
	CCMEE5680	Japan, Kusatsu	EF675167
	CCMEE5681	Japan, Kusatsu	EF675157
	CCMEE5660	Japan, Nakabusa	EF675156
	CCMEE5661	Japan, Nakabusa	EF675150
	CCMEE5662	Japan, Nakabusa	EF675154
	CCMEE5663	Japan, Nakabusa	EF675159
	CCMEE5674	Japan, Nakabusa	EF675153
	CCMEE5675	Japan, Nakabusa	EF675155
	CCMEE5657	Japan, Owakudani	EF675139
	CCMEE5658	Japan, Owakudani	EF675162
	CCMEE5659	Japan, Owakudani	EF675138
	CCMEE5669	Japan, Owakudani	EF675126
	CCMEE5670	Japan, Owakudani	EF675148
	CCMEE5672	Japan, Owakudani	EF675131
	CCMEE5673	Japan, Owakudani	EF675141
	CCMEE5705	New Zealand, Rotowhero	EF675166
	CCMEE5703	New Zealand, Waimangu	EF675165
	CCMEE5704	New Zealand, Waimangu	EF675128
	CCMEE5713	New Zealand, Waiotopu	EF675147
	CCMEE5709	New Zealand, Whaka	EF675161
	CCMEE5715	New Zealand, Whaka	EF675149
	CCMEE5720	New Zealand, White Island	EF675134
	CCMEE5716	New Zealand, Craters of the	EF675142
	IPPAS P507	Russia, Kunashir	AY391370
Galdieria partita	IPPAS P500	Russia, Kamchatka	AB18008
Galdieria phlegrea	ACUF063	Italy, Agrigento	AY119769
	ACUF012	Italy, Benevento	AY541310
	ACUF002	Italy, Pisciarelli	AY541311
	CloneB15	Italy, Pisciarelli	AY541314
	CloneB19	Italy, Pisciarelli	AY541315
	CloneB20	Italy, Pisciarelli	AY541316

	CloneC1	Italy, Pisciarelli	AY541317
Galdieria phlegrea	ACUF009	Italy, Viterbo	AY119768
Galdieria sulphuraria	ACUF376	Iceland, Gunnhuver	KC883806
	ACUF380	Iceland, Gunnhuver	KC883807
	ACUF381	Iceland, Gunnhuver	KC883808
	ACUF382	Iceland, Gunnhuver	KC883809
	ACUF383	Iceland, Gunnhuver	KC883810
	ACUF384	Iceland, Gunnhuver	KC883811
	ACUF413	Iceland, Gunnhuver	KC883835
	ACUF427	Iceland, Gunnhuver	KC883847
	ACUF385	Iceland, Landmannalaugar	KC883812
	ACUF386	Iceland, Landmannalaugar	KC883813
	ACUF387	Iceland, Landmannalaugar	KC883814
	ACUF388	Iceland, Landmannalaugar	KC883815
	ACUF399	Iceland, Niasjvellir	KC883823
	ACUF400	Iceland, Niasjvellir	KC883824
	ACUF402	Iceland, Niasjvellir	KC883825
	ACUF403	Iceland, Niasjvellir	KC883826
	ACUF405	Iceland, Niasjvellir	KC883828
	ACUF408	Iceland, Niasjvellir	KC883830
	ACUF414	Iceland, Niasjvellir	KC883836
	ACUF415	Iceland, Niasjvellir	KC883837
	ACUF442	Iceland, Niasjvellir	KC883854
	ACUF443	Iceland, Niasjvellir	KC883855
	ACUF444	Iceland, Niasjvellir	KC883856
	ACUF446	Iceland, Niasjvellir	KC883858
	ACUF447	Iceland, Niasjvellir	KC883859
	ACUF390	Iceland, Seltun	KC883817
	ACUF395	Iceland, Seltun	KC883820
	ACUF397	Iceland, Seltun	KC883822
	ACUF398	Iceland, Seltun	KC883973
	ACUF409	Iceland, Seltun	KC883831
	ACUF410	Iceland, Seltun	KC883832
	ACUF412	Iceland, Seltun	KC883834
	ACUF416	Iceland, Seltun	KC883838
	ACUF417	Iceland, Seltun	KC883839
	ACUF422	Iceland, Seltun	KC883843
	ACUF423	Iceland, Seltun	KC883844
	ACUF424	Iceland, Seltun	KC883845
	ACUF437	Iceland, Seltun	KC883850
	ACUF439	Iceland, Seltun	KC883852
	ACUF440	Iceland, Seltun	KC883853
	ACUF448	Iceland, Seltun	KC883860
	ACUF452	Iceland, Seltun	KC883864
	ACUF454	Iceland, Seltun	KC883866
	ACUF459	Iceland, Seltun	KC883871
	ACUF460	Iceland, Seltun	KC883872
	ACUF463	Iceland, Seltun	KC883875
	ACUF470	Iceland, Seltun	KC883882
	ACUF472	Iceland, Seltun	KC883883
	ACUF473	Iceland, Seltun	KC883884
	ACUF474	Iceland, Seltun	KC883885
	ACUF475	Iceland, Seltun	KC883886
	ACUF453	Iceland, Viti	KC883865
	ACUF455	Iceland, Viti	KC883867
	ACUF461	Iceland, Viti	KC883873
	ACUF462	Iceland, Viti	KC883874
	ACUF464	Iceland, Viti	KC883876
	ACUF465	Iceland, Viti	KC883877
	ACUF466	Iceland, Viti	KC883878
	ACUF467	Iceland, Viti	KC883879
	ACUF011	Italy, Caserta	AY541303
	ACUF015	Italy, Ischia	AY541305
	isolate MR4-21	Italy, Monte Rotondo	DQ916745
	isolate MR5- C17	Italy, Monte Rotondo	DQ916746
	isolate MR6- C36	Italy, Monte Rotondo	DQ916747
	CloneA12	Italy, Pisciarelli	AY541313
	CloneD15	Italy, Pisciarelli	AY541322
	CloneD5	Italy, Pisciarelli	AY541321
	CloneE11	Italy, Pisciarelli	AY541324
	CloneE12	Italy, Pisciarelli	AY541325
	isolate SP1-10	Italy, Sasso Pisano	DQ916748
	isolate SP3-C2	Italy, Sasso Pisano	DQ916749

	ACUF018	Italy, Scarfoglio	AY541304
Galdieria sulphuraria	ACUF017	Italy, Solfatara	AY541306
	ACUF021	Italy, Vulcano	AY541307
	CCMEE5706	New Zealand, Craters of the Moon	EF675177
	CCMEE5712	New Zealand, Craters of the Moon	EF675178
	CCMEE5717	New Zealand, Rotorua	EF675176
	CCMEE5707	New Zealand, Waiotopu	EF675181
	CCMEE5714	New Zealand, Waiotopu	EF675180
	CCMEE5719	New Zealand, Waiotopu	EF675175
	CCMEE5718	New Zealand, Whaka	EF675179
	CCMEE5708	New Zealand, Whaka	EF675172
	CCMEE5710	New Zealand, WhiteIsland	EF675183
	CCMEE5711	New Zealand, WhiteIsland	EF675173
	LCATERR-7	USA, Lemonade Creek, YNP	JQ269608
	CCMEE5511	USA, Norris Basin, YNP	EF675174
	CCMEE5572	USA, Norris Basin, YNP	EF675182
	CCMEE5573	USA, Norris Basin, YNP	EF675171
	UTEX2393	USA, Sonoma, California	AF233069
	SAG 108.79	USA, Yellowstone	AY119767
Cyanidium chilense	Sybil cave	Italy, Cuma	AY391369
	sp.19	Italy, Monte Rotaro	AY541300
	sp.20	Italy, Monte Rotaro	AY541301
		Italy, Terme di baia	KC914876
Galdieria sp.	clone 12.ENVS.DYK.ditch60.1.1.1	Taiwan, DaYouKeng	JX981552
	clone 12.ENVS.DYK.ditch60.1.1.2	Taiwan, DaYouKeng	JX981553
	clone 12.ENVS.DYK.ditch60.1.1.3	Taiwan, DaYouKeng	JX981554
	clone 12.ENVS.DYK.ditch60.1.1.5	Taiwan, DaYouKeng	JX981555
	clone 12.ENVS.DYK.ditch60.1.1.6	Taiwan, DaYouKeng	JX981556
	clone 12.ENVS.DYK.ditch60.1.1.7	Taiwan, DaYouKeng	JX981557
	clone 12.ENVS.DYK.ditch60.1.1.9	Taiwan, DaYouKeng	JX981559
	clone 12.ENVS.DYK.ditch60.1.1.11	Taiwan, DaYouKeng	JX981561
	clone 12.ENVS.DYK.ditch60.1.1.12	Taiwan, DaYouKeng	JX981562
	clone 12.ENVS.DYK.ditch60.1.1.15	Taiwan, DaYouKeng	JX981563
	clone 12.ENVS.DYK.ditch60.1.2.3	Taiwan, DaYouKeng	JX981564
	clone 12.ENVS.DYK.ditch60.1.2.6	Taiwan, DaYouKeng	JX981565
	clone 12.ENVS.DYK.ditch60.1.2.8	Taiwan, DaYouKeng	JX981566
	clone 12.ENVS.DYK.ditch60.1.3.5	Taiwan, DaYouKeng	JX981568
	clone12.ENVS.DYK.ditch45.2.2	Taiwan, DaYouKeng	JX981569
	clone12.ENVS.DYK.ditch45.2.3	Taiwan, DaYouKeng	JX981570
	clone12.ENVS.DYK.ditch45.2.5	Taiwan, DaYouKeng	JX981571
	clone12.ENVS.DYK.ditch45.2.6	Taiwan, DaYouKeng	JX981572
	clone12.ENVS.DYK.ditch45.2.7	Taiwan, DaYouKeng	JX981573
	clone12.ENVS.DYK.ditch45.2.8	Taiwan, DaYouKeng	JX981574
	clone12.ENVS.DYK.ditch45.2.9	Taiwan, DaYouKeng	JX981575
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	clone12.ENVS.DYK.ditch45.2.12	Taiwan, DaYouKeng	JX981577
	clone12.ENVS.DYK.ditch45.2.13	Taiwan, DaYouKeng	JX981578
	clone12.ENVS.DYK.ditch45.2.14	Taiwan, DaYouKeng	JX981579
	clone12.ENVS.DYK.ditch45.2.15	Taiwan, DaYouKeng	JX981580
	clone12.ENVS.DYK.ditch45.4.1	Taiwan, DaYouKeng	JX981581
	clone12.ENVS.DYK.ditch45.4.6	Taiwan, DaYouKeng	JX981583
	clone12.ENVS.DYK.ditch45.4.8	Taiwan, DaYouKeng	JX981585
	clone12.ENVS.DYK.endolithic.2	Taiwan, DaYouKeng	JX981586
	clone12.ENVS.DYK.endolithic.4	Taiwan, DaYouKeng	JX981587
	clone12.ENVS.DYK.endolithic.6	Taiwan, DaYouKeng	JX981588
	clone12.ENVS.DYK.endolithic.7	Taiwan, DaYouKeng	JX981589
	clone12.ENVS.DYK.endolithic.8	Taiwan, DaYouKeng	JX981590
	clone12.ENVS.DYK.endolithic.10	Taiwan, DaYouKeng	JX981591
	clone12.ENVS.DYK.endolithic.11	Taiwan, DaYouKeng	JX981592
	clone12.ENVS.DYK.endolithic.12	Taiwan, DaYouKeng	JX981593
	clone12.ENVS.DYK.endolithic.13	Taiwan, DaYouKeng	JX981594
	clone12.ENVS.DYK.endolithic.14	Taiwan, DaYouKeng	JX981595
	clone12.ENVS.DYK.endolithic.15	Taiwan, DaYouKeng	JX981596
	clone12.ENVS.DYK.endolithic.16	Taiwan, DaYouKeng	JX981597
	clone12.ENVS.DYK.endolithic.17	Taiwan, DaYouKeng	JX981598
	clone12.ENVS.DYK.endolithic.18	Taiwan, DaYouKeng	JX981599
	clone12.ENVS.DYK.endolithic.21	Taiwan, DaYouKeng	JX981600
	clone12.ENVS.DYK.endolithic.22	Taiwan, DaYouKeng	JX981601
	clone12.ENVS.DYK.endolithic.23	Taiwan, DaYouKeng	JX981602
	clone12.ENVS.DYK.endolithic.24	Taiwan, DaYouKeng	JX981603
	clone12.ENVS.DYK.endolithic.25	Taiwan, DaYouKeng	JX981604
	clone12.ENVS.DYK.endolithic.26	Taiwan, DaYouKeng	JX981605
	clone12.ENVS.DYK.endolithic.28	Taiwan, DaYouKeng	JX981606

Galdieria sp.	clone12.ENVS.DYK.endolithic.29	Taiwan, DaYouKeng	JX981607
	clone12.ENVS.DYK.endolithic.30	Taiwan, DaYouKeng	JX981608
	THAL006.DYK01.Gp	Taiwan, DaYouKeng	KJ125469
	THAL007.DYK02.Gp	Taiwan, DaYouKeng	KJ125470
	clone12.ENVS.DRG.stream40.sun.1.3	Taiwan, DiReGu	JX981533
	clone12.ENVS.DRG.stream40.sun.2.2	Taiwan, DiReGu	JX981534
	clone12.ENVS.DRG.stream40.sun.2.5	Taiwan, DiReGu	JX981536
	clone12.ENVS.DRG.stream40.sun.3.1	Taiwan, DiReGu	JX981537
	clone12.ENVS.DRG.stream40.sun.3.2	Taiwan, DiReGu	JX981538
	clone12.ENVS.DRG.stream40.sun.3.3	Taiwan, DiReGu	JX981539
	clone12.ENVS.DRG.stream40.sun.3.7	Taiwan, DiReGu	JX981540
	clone12.ENVS.DRG.stream40.sun.3.13	Taiwan, DiReGu	JX981541
	clone12.ENVS.DRG.stream40.sun.3.14	Taiwan, DiReGu	JX981542
	clone12.ENVS.DRG.stream40.sun.3.15	Taiwan, DiReGu	JX981543
	clone12.ENVS.DRG.stream40.sun.3.20	Taiwan, DiReGu	JX981546
	clone12.ENVS.DRG.stream40.sun.4.6	Taiwan, DiReGu	JX981548
	clone12.ENVS.DRG.stream40.sun.4.9	Taiwan, DiReGu	JX981549
	clone12.ENVS.DRG.stream40.sun.4.10	Taiwan, DiReGu	JX981550
	clone12.ENVS.DRG.stream40.sun.4.15	Taiwan, DiReGu	JX981551
	clone 05.ENVS.DRG.stream42.sun.2	Taiwan, DiReGu	JX981643
	clone 05.ENVS.DRG.stream42.sun.3	Taiwan, DiReGu	JX981644
	clone 05.ENVS.DRG.stream42.sun.4	Taiwan, DiReGu	JX981645
	clone 05.ENVS.DRG.stream42.sun.5	Taiwan, DiReGu	JX981646
	clone 05.ENVS.DRG.stream42.sun.6	Taiwan, DiReGu	JX981647
	clone 05.ENVS.DRG.stream42.sun.7	Taiwan, DiReGu	JX981648
	clone 05.ENVS.DRG.stream42.sun.8	Taiwan, DiReGu	JX981649
	clone 05.ENVS.DRG.stream42.sun.9	Taiwan, DiReGu	JX981650
	clone 05.ENVS.DRG.stream42.sun.10	Taiwan, DiReGu	JX981651
	clone 05.ENVS.DRG.stream42.sun.11	Taiwan, DiReGu	JX981652
	clone 05.ENVS.DRG.stream42.sun.12	Taiwan, DiReGu	JX981653
	clone 05.ENVS.DRG.stream42.sun.13	Taiwan, DiReGu	JX981654
	clone 05.ENVS.DRG.stream42.sun.14	Taiwan, DiReGu	JX981655
	clone 05.ENVS.DRG.stream42.sun.15	Taiwan, DiReGu	JX981656
	clone 05.ENVS.DRG.stream42.sun.16	Taiwan, DiReGu	JX981657
	clone 05.ENVS.DRG.stream42.shaded.1	Taiwan, DiReGu	JX981658
	clone 05.ENVS.DRG.stream42.shaded.2	Taiwan, DiReGu	JX981659
	clone 05.ENVS.DRG.stream42.shaded.3	Taiwan, DiReGu	JX981660
	clone 05.ENVS.DRG.stream42.shaded.4	Taiwan, DiReGu	JX981661
	clone 05.ENVS.DRG.stream42.shaded.6	Taiwan, DiReGu	JX981663
	clone 05.ENVS.DRG.stream42.shaded.7	Taiwan, DiReGu	JX981664
	clone 05.ENVS.DRG.stream42.shaded.8	Taiwan, DiReGu	JX981665
	clone 05.ENVS.DRG.stream42.shaded.9	Taiwan, DiReGu	JX981666
	clone 05.ENVS.DRG.stream42.shaded.10	Taiwan, DiReGu	JX981667
	clone 05.ENVS.DRG.stream42.shaded.11	Taiwan, DiReGu	JX981668
	clone 05.ENVS.DRG.stream42.shaded.12	Taiwan, DiReGu	JX981669
	clone 05.ENVS.DRG.stream42.shaded.13	Taiwan, DiReGu	JX981670
	clone 05.ENVS.DRG.stream42.shaded.14	Taiwan, DiReGu	JX981671
	clone 05.ENVS.DRG.stream42.shaded.15	Taiwan, DiReGu	JX981672
	clone 05.ENVS.DRG.stream42.shaded.16	Taiwan, DiReGu	JX981673
	clone 05.ENVS.DRG.stream42.shaded.17	Taiwan, DiReGu	JX981674
	clone 05.ENVS.DRG.stream42.shaded.18	Taiwan, DiReGu	JX981675
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	THAL002.DRG02.Gp	Taiwan, DiReGu	KJ125465
	THAL003.DRG03.Gp	Taiwan, DiReGu	KJ125466
	THAL004.DRG04.Gp	Taiwan, DiReGu	KJ125467
	THAL008.DRG05.Gp	Taiwan, DiReGu	KJ125471
	THAL005.GZP01.Gp	Taiwan, GengZiPeng	KJ125468
	clone 12.ENVS.GZP.epilithic.1	Taiwan, GengZiPeng	JX981624
	clone 12.ENVS.GZP.epilithic.2	Taiwan, GengZiPeng	JX981625
	clone 12.ENVS.GZP.epilithic.3	Taiwan, GengZiPeng	JX981626
	clone 12.ENVS.GZP.epilithic.4	Taiwan, GengZiPeng	JX981627
	clone 12.ENVS.GZP.epilithic.5	Taiwan, GengZiPeng	JX981628
	clone 12.ENVS.GZP.epilithic.6	Taiwan, GengZiPeng	JX981629
	clone 12.ENVS.GZP.epilithic.7	Taiwan, GengZiPeng	JX981630
	clone 12.ENVS.GZP.epilithic.8	Taiwan, GengZiPeng	JX981631
	clone 12.ENVS.GZP.epilithic.9	Taiwan, GengZiPeng	JX981632
	clone 12.ENVS.GZP.epilithic.10	Taiwan, GengZiPeng	JX981633
	clone 12.ENVS.GZP.epilithic.12	Taiwan, GengZiPeng	JX981634
	clone 12.ENVS.GZP.epilithic.13	Taiwan, GengZiPeng	JX981635
	clone 12.ENVS.GZP.epilithic.14	Taiwan, GengZiPeng	JX981636
	clone 12.ENVS.GZP.epilithic.16	Taiwan, GengZiPeng	JX981638
	clone 12.ENVS.GZP.epilithic.17	Taiwan, GengZiPeng	JX981639
	clone 12.ENVS.GZP.epilithic.18	Taiwan, GengZiPeng	JX981640

	clone 12.ENVS.GZP.epilithic.19	Taiwan, GengZiPeng	JX981641
	clone12.ENVS.GZP.epilithic.low1	Taiwan, GengZiPeng	KC313262
	clone12.ENVS.GZP.epilithic.low2	Taiwan, GengZiPeng	KC313263
	clone12.ENVS.GZP.epilithic.low3	Taiwan, GengZiPeng	KC313264
	clone12.ENVS.GZP.epilithic.low4	Taiwan, GengZiPeng	KC313265
	clone12.ENVS.GZP.epilithic.low5	Taiwan, GengZiPeng	KC313266
	clone12.ENVS.GZP.epilithic.low6	Taiwan, GengZiPeng	KC313267
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	clone12.ENVS.GZP.epilithic.low10	Taiwan, GengZiPeng	KC313271
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	clone12.ENVS.GZP.epilithic.low24	Taiwan, GengZiPeng	KC313284
	clone12.ENVS.GZP.epilithic.low25	Taiwan, GengZiPeng	KC313285
	clone12.ENVS.GZP.epilithic.low26	Taiwan, GengZiPeng	KC313286
	clone12.ENVS.GZP.epilithic.low27	Taiwan, GengZiPeng	KC313287
	clone12.ENVS.GZP.epilithic.low28	Taiwan, GengZiPeng	KC313288
	clone12.ENVS.GZP.epilithic.low29	Taiwan, GengZiPeng	KC313289
	clone12.ENVS.GZP.epilithic.low30	Taiwan, GengZiPeng	KC313290
	clone12.ENVS.GZP.soil.low1	Taiwan, GengZiPeng	KC313291
	clone12.ENVS.GZP.soil.low3	Taiwan, GengZiPeng	KC313292
	clone12.ENVS.GZP.soil.low4	Taiwan, GengZiPeng	KC313293
	clone12.ENVS.GZP.soil.low5	Taiwan, GengZiPeng	KC313294
	clone12.ENVS.GZP.soil.low9	Taiwan, GengZiPeng	KC313295
	clone12.ENVS.GZP.soil.low11	Taiwan, GengZiPeng	KC313296
	clone12.ENVS.GZP.soil.low12	Taiwan, GengZiPeng	KC313297
	clone12.ENVS.GZP.soil.low13	Taiwan, GengZiPeng	KC313298
	clone12.ENVS.GZP.soil.low17	Taiwan, GengZiPeng	KC313299
	clone12.ENVS.GZP.stream45.1.1	Taiwan, GengZiPeng	JX981609
	clone12.ENVS.GZP.stream45.1.4	Taiwan, GengZiPeng	JX981611
	clone12.ENVS.GZP.stream45.1.6	Taiwan, GengZiPeng	JX981612
	clone12.ENVS.GZP.stream45.1.7	Taiwan, GengZiPeng	JX981613
	clone12.ENVS.GZP.stream45.1.8	Taiwan, GengZiPeng	JX981614
	clone12.ENVS.GZP.stream45.1.9	Taiwan, GengZiPeng	JX981615
	clone12.ENVS.GZP.stream45.1.10	Taiwan, GengZiPeng	JX981616
	clone12.ENVS.GZP.stream45.1.11	Taiwan, GengZiPeng	JX981617
	clone12.ENVS.GZP.stream45.1.12	Taiwan, GengZiPeng	JX981618
	clone12.ENVS.GZP.stream45.1.13	Taiwan, GengZiPeng	JX981619
	clone12.ENVS.GZP.stream45.1.16	Taiwan, GengZiPeng	JX981620
	clone12.ENVS.GZP.stream45.1.20	Taiwan, GengZiPeng	JX981621
	clone12.ENVS.GZP.stream45.1.21	Taiwan, GengZiPeng	JX981622
	clone12.ENVS.GZP.stream45.1.22	Taiwan, GengZiPeng	JX981623
	clone05.ENVS.DRG.stream42.sun.1	Taiwan, GengZiPeng	JX981642
	clone12.ENVS.MC.sulfurFume1.1.2	Taiwan, MaChao	JX981516
	clone12.ENVS.MC.sulfurFume1.1.6	Taiwan, MaChao	JX981517
	clone12.ENVS.MC.sulfurFume1.1.7	Taiwan, MaChao	JX981518
	clone12.ENVS.MC.sulfurFume1.2.5	Taiwan, MaChao	JX981520
	clone12.ENVS.MC.sulfurFume1.2.3	Taiwan, MaChao	JX981519
	clone12.ENVS.MC.sulfurFume1.1.3	Taiwan, MaChao	JX981521
	clone12.ENVS.MC.sulfurFume1.3.5	Taiwan, MaChao	JX981523
	clone12.ENVS.MC.sulfurFume1.3.7	Taiwan, MaChao	JX981524
	clone12.ENVS.MC.sulfurFume1.3.8	Taiwan, MaChao	JX981525
	clone12.ENVS.MC.sulfurFume1.3.16	Taiwan, MaChao	JX981528

Taxa	Strain	Sampling Site	GenBank number
Cyanidium caldarium	ACUF767	Turkey, Cermik	KY033432
	ACUF775	Turkey, Diyadin	KY033437
	CloneT17	Turkey, Güçlükonak	KY033462
Cyanidioschyzon merolae	CloneT01	Turkey, Biloris	KY033448
	CloneT05	Turkey, Nemrut	KY033452
Galdieria maxima	ACUF653	Turkey,Biloris	KY033400
	ACUF764	Turkey,Biloris	KY033430
	ACUF763	Turkey,Biloris	KY033429
	ACUF735	Turkey,Biloris	KY033422
	ACUF698	Turkey,Biloris	KY033416
	ACUF650	Turkey, Cermik	KY033398
	CloneT03	Turkey, Cermik	KY033450
	ACUF647	Turkey, Cermik	KY033396
	cloneT04	Turkey, Cermik	KY033451
	ACUF766	Turkey, Cermik	KY033431
	ACUF783	Turkey, Cermik	KY033443
	ACUF774	Turkey,Diyadin	KY033436
	CloneT06	Turkey,Diyadin	KY033453
	ACUF665	Turkey,Diyadin	KY033406
	CloneT13	Turkey,Diyadin	KY033460
	ACUF772	Turkey,Diyadin	KY033435
	cloneT18	Turkey,Diyadin	KX501185
	ACUF773	Turkey,Diyadin	KX501180
	ACUF671	Turkey,Manisa Kula	KY033410
	ACUF648	Turkey,Manisa Kula	KY033397
	ACUF731	Turkey,Manisa Kula	KY033420
	cloneT12	Turkey,Manisa Kula	KY033459
	ACUF776	Turkey,Manisa Kula	KY033438
	ACUF777	Turkey,Manisa Kula	KY033439
	CloneT14	Turkey,Manisa Kula	KY033461
	ACUF743	Turkey,Manisa Kula	KY033428
	ACUF741	Turkey,Manisa Kula	KY033426
	ACUF782	Turkey,Manisa Kula	KY033442
	ACUF673	Turkey,Germencik	KY033411
	ACUF739	Turkey,Germencik	KY033425
	ACUF736	Turkey,Germencik	KY033423
	CloneT15	Turkey,Germencik	KX501183
	ACUF742	Turkey,Germencik	KY033427
	ACUF660	Turkey, Güçlükonak	KY033404
	ACUF697	Turkey, Güçlükonak	KY033415
	ACUF722	Turkey, Güçlükonak	KX501174
	ACUF769	Turkey, Güçlükonak	KX501179
	ACUF724	Turkey, Güçlükonak	KY033419
	ACUF714	Turkey, Güçlükonak	KY033418
	ACUF695	Turkey, Güçlükonak	KY033414
	ACUF710	Turkey, Güçlükonak	KX501173
Galdieria phlegrea	ACUF657	Turkey,Biloris	KY033402
	ACUF656	Turkey,Biloris	KY033401
	ACUF652	Turkey,Biloris	KY033399
	ACUF780	Turkey,Biloris	KX501182
	ACUF765	Turkey,Biloris	KX501177
	ACUF625	Turkey, Cermik	KY033394
	ACUF668	Turkey, Cermik	KY033408
	CloneT07	Turkey, Cermik	KY033454
	ACUF642	Turkey, Cermik	KY033395
	CloneT08	Turkey, Cermik	KY033455
	CloneT10	Turkey, Cermik	KY033457
	ACUF667	Turkey,Diyadin	KY033407
	ACUF669	Turkey,Diyadin	KY033409
	ACUF 771	Turkey,Diyadin	KY033434
	ACUF 658	Turkey,Guklukonak	KY033403
	ACUF737	Turkey,Diyadin	KY033424
	ACUF 734	Turkey,Diyadin	KY033421
	ACUF 787	Turkey,Diyadin	KY033446
	ACUF785	Turkey,Diyadin	KY033445
	cloneT09	Turkey,Gucklukonak	KY033456
	ACUF784	Turkey,Gucklukonak	KY033444
	ACUF770	Turkey,Gucklukonak	KY033433
	cloneT16	Turkey,Gucklukonak	KX501184
	cloneT11	Turkey,Manisa Kula	KY033458
	ACUF664	Turkey,Nemrut	KY033405

	ACUF738	Turkey,Nemrut	KX501176
	ACUF788	Turkey,Dyadin	KY033447
Galdieria sulphuraria	ACUF779	Turkey,Germencik	KY033440
	ACUF676	Turkey,Germencik	KY033413
	cloneT02	Turkey,Germencik	KY033449
	ACUF674	Turkey,Germencik	KY033412
	ACUF778	Turkey,Germencik	KX501181
	ACUF781	Turkey,Gucklukonak	KY033441
	ACUF725	Turkey,Gucklukonak	KX501175
	ACUF768	Turkey,Gucklukonak	KX501178
	ACUF700	Turkey,Gucklukonak	KY033417

Table S1. GenBank Accession numbers for taxa included in the phylogenetic analyses.


Agri-Diyadin
Kula-Manisa
Nemrut-Bitlis
Germencik-Aydin
Cermik-Dyiarbakir
Biloris-Siirt
Sirnak