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

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17 **Abstract**

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

32

33 **Keywords:** Extremophiles, Cyanidophytina, Phylogeny, Population structure, rbcL,  
34 Biodiversity

## 35 **Introduction**

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

48 A novel estimate of species richness of Cyanidiophyceae has recently come from the  
49 analysis of thermoacidophilic communities from aquatic and non-aquatic volcanic sites in  
50 Taiwan (Hsieh et al. 2015). The habitats so far explored, in search of polyextremophilic algae,  
51 have usually been characterized by strong acidity, as pH range is considered a greater  
52 constraint on the growth of Cyanidiophytina than temperature range. Thus, many explorations  
53 have focused in acidic geothermal areas (Brock 1978, Toplin et al. 2008, Hsieh et al. 2015).  
54 Currently, the genus *Cyanidium* encompasses two main species. These are *C. caldarium*  
55 (Tilden) Geitler, a polyextremophilic alga adapted to acidic and hot springs and fumaroles,  
56 usually rich in heavy metals, and *C. chilense*, a hypogean, neutrophilic (pH around 7.0) and  
57 mesophilic (20-25 °C) alga discovered in several caves worldwide (Schwabe, 1936;  
58 Friedmann, 1964; Skuja, 1970; LeClerc et al., 1983; Azua-Bustos et al., 2009; Darienko et al.,  
59 2010; Del Rosal et al., 2015; Cennamo et al., 2012; Ciniglia et al. 2017). The phylogenetically  
60 distinct thermoacidophilic *C. caldarium* and the neutrophilic and mesophilic *C. chilense* are  
61 clearly separated on the basis of both molecular and ecophysiological characters (Ciniglia et  
62 al., 2004). These findings suggest that other Cyanidiophytina could have a much wider  
63 distribution than those considered so far. This prompted us to search for alternative ecological  
64 niches, such as non-acidic environments.

65 In this study, we report on our new explorations of seven thermal baths located in  
66 Turkey and report the presence of Cyanidiophyceae populations on neutral/alkaline soils.  
67 Anatolian volcanism is a consequence of convergence occurring between Afro-Arabian and  
68 Eurasian plates and it can be considered as a bridge between the geothermal areas of Europe

69 and Asia. This zone is characterized by deposits of andesitic and rhyolitic lava, alternating  
70 with black and clastic sedimentary rocks, resulting from the solidification of mud mixed with  
71 water (Pearce et al., 1990). Although Turkey is still geologically active, intense volcanic  
72 activity has not been recorded for a number of years; Turkish volcanism varies from mildly  
73 alkaline volcanoes, such as Nemrut, to calc-alkaline/alkaline volcanoes, such as Ararat and  
74 Tendurek (Pearce et al. 1990).

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

## 82 MATERIALS AND METHODS

83

### 84 *X-ray diffraction (XRD)*

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

91

### 92 *Sample collection, isolation and cultivation*

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

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

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

115 ***DNA extraction, gene amplification and sequencing***

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

127

128 ***Phylogenetic analyses***

129 A total of 81 new *rbcL* sequences were obtained in this present study from our Turkish  
130 samples, and these were integrated with the 255 available *rbcL* sequences available at  
131 GenBank (Table S1). All sequences were aligned with published sequence data (Ciniglia et al.  
132 2004, Toplin et al. 2008, Skorupa et al. 2013, Ciniglia et al., 2014, Hsieh et al. 2015), using  
133 BioEdit Sequence Alignment Editor (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). No  
134 gaps or indels have been incorporated in the alignment. Newly determined sequences are all  
135 available on NCBI GenBank (Table S1). Maximum likelihood (ML) phylogenetic analysis of  
136 *rbcL* was performed using the GTR +  $\Gamma$  + I model implemented in RAxML software  
137 (Stamatakis 2008). Statistical support for each branch was obtained from 1,000 bootstrap  
138 replications using the same substitution model and RAxML program settings. Bayesian  
139 analyses (BA) were performed for combined and individual datasets with MrBayes v.3.1.1  
140 (Ronquist and Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo  
141 (MC3) with the GTR +  $\Gamma$  + I model. For each matrix, one million generations of two  
142 independent runs were performed with sampling trees generated every 100 generations. The  
143 burn-in period was identified graphically by tracking the likelihoods at each generation to  
144 determine whether they reached a plateau. Seven red algal taxa belonging to Bangiophyceae  
145 and Stylonematophyceae were chosen as outgroup taxa, being the closest relatives to  
146 Cyanidiophytina.

147 An estimate of genetic diversity was carried out using DNASP v.5.10.01 (Librado &  
148 Rozas 2009). For each population, the following statistics were computed: haplotype (h) (Nei  
149 1987) and nucleotide diversities ( $\pi$ ) (Nei 1987), with standard deviation. Population  
150 expansion, assessed by neutrality test (Tajima 1989, Fu&Li 1993) and mean number of  
151 pairwise differences (symbol) (Tajima, 1983).

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



## 157 RESULTS

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

169 The identification of different genera and species in the Cyanidiophytina has  
170 previously been difficult, as there are few unequivocal morphological features to distinguish  
171 between them, and furthermore, there is homoplasy between some lineages. Thus, in order to  
172 identify the algal species been studied molecular tools were used. For this we first generated  
173 491 base pairs of *rbcL* sequence for the different isolates. These were aligned, including the  
174 81 new Turkish cyanidiophyceae isolates, (Table S1), and the existing 168 cyanidiophyceae  
175 *rbcL* sequences available from GenBank. These strains originated from Japan, Iceland, Italy,  
176 Kuril Islands, Kamchatka, USA, New Zealand, and seven outgroup taxa. *rbcL* phylogeny  
177 identified five cyanidiophyceae taxa from Turkey: *Galdieria sulphuraria*, *Galdieria maxima*,  
178 *Galdieria phlegrea*, *Cyanidium caldarium*, and *Cyanidioschyzon merolae* (Fig. 2). The  
179 inferred RAxML tree based on *rbcL* dataset showed several well-supported sublineages  
180 within *G. sulphuraria* and *G. maxima* clades. *G. sulphuraria* included at least five  
181 sublineages, including one defined by a New Zealand population (Fig. 2, subclade S1) and  
182 another with a USA population (Fig. 2, subclade S2). Accessions nested in an independent  
183 lineage, separable as two well-supported subclades (posterior probability/bootstrap: New  
184 Zealand subclade, 1/97; USA subclade, 1/98). We noted that 10 Turkish specimens grouped  
185 within *G. sulphuraria* and this was in two different subclades, 7 nesting with the Italian  
186 strains (Fig. 2, subclade S3; posterior probability/bootstrap 1/100), and 3 with the Icelandic  
187 strains along with the Russian *G. daedala* strain (Fig. 2, subclade S5; posterior  
188 probability/bootstrap 1/69). The sequences from Taiwan clustered with *G. partita* from  
189 Russia. Together the relations were clearly resolved with high statistical confidence.

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

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

201 Only two Turkish isolates were found to be closely related to *C. merolae*. The low  
202 level of intraspecific variation recorded in *C. merolae* did not generate any subclusterization  
203 associated to geographic populations. Our phylogenetic tree conformed to previously reported  
204 monophyly of Cyanidiophyceae (posterior probability, 1; ML LogDet bootstrap = 100%)  
205 (Fig. 2) (Ciniglia *et al.* 2004). However, by adding the *rbcL* sequences from the new Turkish  
206 isolates, at least six lineages within the class were indicated by the high bootstrap values,  
207 instead of the previously reported four lineages (Ciniglia *et al.* 2004; Ciniglia *et al.*, 2014).  
208 These six independent lineages were grouped in different monophyletic clades (Fig. 1),  
209 namely: 1) *C. merolae* (posterior probability 1/bootstrap, 99%); 2) *G. maxima* (1/99), sharing  
210 a common ancestor with *C. merolae*, but with strong evidence of molecular divergence  
211 between them; 3) the mesophilic lineage of *C. chilense* (1/100; Ciniglia *et al.*, 2017); 4)  
212 *C. caldarium* (1/100), clearly phylogenetically divergent from the mesophilic *C. chilense*  
213 (Yang *et al.*, 2016); 5) *G. sulphuraria* (1/100) and 6) *G. phlegrea* (0.94/97), as sister clades  
214 (1/ 100).

215

### 216 ***Genetic diversity and population differentiation***

217 Next an analysis of genetic diversity within and between populations of  
218 Cyanidiophyceae was performed by using DNAsp, which provides an estimate of the extent  
219 of genetic variation between individuals belonging to the same geographic population and  
220 between different populations. Results are listed in Table 2. We excluded *C. caldarium* from  
221 the analysis because of the low number of haplotypes and their restricted geographic  
222 distribution. A total of 159 haplotypes were recovered from 459 individuals analyzed and 149  
223 (95.5%) of the haplotypes were private, *i.e.* unique to a single locality. The highest values of

224 average sequence divergences were recorded for *G. sulphuraria* (K=19.47), and *G. maxima*  
225 (K=17.37), with a high level of haplotype diversity, as well (*G. sulphuraria*, hd, 0.83±0.028;  
226 *G. maxima*, hd, 0.956±0.006).

227 In *G. sulphuraria*, the analysis of genetic diversity was performed on 136 partial  
228 sequences of rbcL with 80 polymorphic sites and 33 different haplotypes (only two  
229 haplotypes were shared by Italy and Turkey and by Taiwan and Russia). The highest levels of  
230 haplotype diversity were found in the samples from New Zealand (hd = 0.867), Italy, and  
231 Taiwan (hd = 0.724 and 0.732). An average value of haplotype diversity was recorded in  
232 Turkey (hd = 0.600), despite the degree of nucleotide diversity higher than any other  
233 population ( $\pi = 0.0375$ ). Iceland exhibited comparatively lower values of these indices (hd =  
234 0.224;  $\pi = 0.0006$ ).

235 Genetic distance was represented as Fst for each pairwise combination of populations,  
236 based on rbcL marker. The value of inter-population pairwise genetic differentiation, Fst (5  
237 populations of *G. sulphuraria* analyzed: USA, Italy, Turkey, New Zealand, and Iceland) was  
238 significantly high (0.7788,  $P < 0.05$ ). Fst ranges from 0 to 1; Fst of 0 indicates panmixy with  
239 high interbreeding between populations, while a value of Fst of 1 means that the populations  
240 are fixed and do not interbreed. When considering the genetic differentiation between two  
241 populations, Fst values ranged from low (0.14) to high (0.97) (Table 3). The lowest level of  
242 genetic differentiation was recorded between Turkey and Italy, which were also the closest  
243 populations geographically (1950 km). However, high genetic divergences were found  
244 between the furthest and the closest *G. sulphuraria* populations, such as Taiwan and USA  
245 (0.97, 12254km), USA and Iceland (0.91, 5719km), Italy and USA (0.85, 8622km), New  
246 Zealand and Iceland (0.844, 17215km), Italy and Iceland (0.839, 3247 km), and New Zealand  
247 and Italy (0.71, 18559km). We next investigated the potential for isolation by distance (IBD)  
248 via statistical tests of correlations in order to weigh the contribution of geographic distance in  
249 the population structure. The correlation between genetic and geographic distances based on  
250 rbcL was weakly positive, but not statistically significant in *G. sulphuraria*, as shown in  
251 Fig. 3 ( $R = 0.264$ ,  $P = 0.333$ ). This thus rejected an isolation-by-distance model from these data.

252 In examinations of 245 *G. maxima* partial rbcL (434bp) sequences, these contained  
253 161 polymorphic sites and 100 haplotypes (Table 2). There was a high level of detected  
254 diversity (hd=0.956). Haplotype and genetic diversity of rbcL in Turkish populations,  
255 calculated from 40 sequences and 8 haplotypes were 0.652±0.069 (hd) and 0.02±0.0033 ( $\pi$ ) in  
256 43 polymorphic sites. The highest genetic diversity was found in the Taiwanese population,  
257 where among 149 individuals, 80 haplotypes and 108 parsimony informative sites showed

258 high haplotype diversity ( $0.957\pm 0.009$ ) with low genetic polymorphism ( $\pi=0.0373\pm 0.00067$ ).  
259 The Japanese population was the highest in both diversities ( $hd=0.861\pm 0.039$ ;  
260  $\pi=0.023\pm 0.00345$ ). This resulted from 23 sequences, 8 haplotypes, and 34 polymorphic sites.  
261 The level of haplotype and nucleotide diversity for the New Zealand population was  
262 calculated on the few sequences available (7 individuals, 4 haplotypes,  $hd=0.81\pm 0.13$ ,  
263  $\pi=0.028\pm 0.006$ ). The 24 Icelandic sequences showed a lower haplotype and nucleotide  
264 diversity ( $hd=0.163\pm 0.0098$ ;  $\pi=0.00073\pm 0.00051$ ). In the neutrality test of *G. maxima*, Tajima  
265 D and Fu and Li were both significantly negative for the Icelandic samples ( $D= -1.88381$ ;  $F=$   
266  $-2.796$  Table 2). However, all samples from the other regions showed negative values of  
267 Tajima D, but without statistical significance of Tajima and Fu and Li, except for Taiwan  
268 samples showing strong significantly negative values of F (Table 2).

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

278 Despite extensive sampling, current and previous molecular analysis has to date only  
279 identified 44 rbcL sequences from *C. merolae*. The majority belonged to individuals spread  
280 across the American territories, as few sequences were detected in the Turkish or Italian  
281 samples, and no sequences have yet been detected in Taiwanese samples. The analysis  
282 revealed the presence of 19 polymorphic sites, generating 19 haplotypes. The two most  
283 frequently represented were shared by the Turkish, Italian, and American samples. Genetic  
284 haplotype diversity was estimated using all of the isolates and gave results of  $0.918\pm 0.022$ ,  
285 with a very low degree of nucleotide diversity, namely  $\pi=0.00443\pm 0.00219$  (Tajima, -  
286  $1.73184$ ; Fu and Li,  $-3.456$ ). This indicates the absence of geographical population  
287 structuring. This was also shown by the low level of the overall genetic differentiation  
288 ( $F_{st}=0.05$ ). We could not perform correlation test for *C. merolae*, as well as for *G. phlegrea*  
289 and *C. caldarium*, because of the limited number of accessions and populations available for  
290 the analysis.

291 **DISCUSSION**

292 Cyanidia are the most abundant photosynthetic protists found in extremely acidic,  
293 sulfur-rich environments that are close to active volcanoes (Brock *et al.*, 1978; Ciniglia *et al.*,  
294 2004; Skorupa *et al.*, 2013; Toplin *et al.*, 2008). Until now Cyanidia have been isolated  
295 mainly in solfataras (Italy, Iceland, Japan, New Zealand, Yellowstone National Park, and  
296 Taiwan), where the condensation of sulfur dioxide and hydrogen sulfide produces crystals of  
297 sulfur subsequently oxidized to sulfuric acid resulting in acidification.

298 Turkey is characterized by collision volcanism, varying from mildly alkaline  
299 volcanoes, such as Nemrut, to calc-alkaline/alkaline ones, such as Mount Ararat (Pearce *et al.*,  
300 1990). Residual volcanic activity in Turkey explains the presence of many geothermal spots,  
301 with neutral and sub-neutral pH values, due to the limited presence of sulfuric acid. The main  
302 minerals detected in the areas explored were quartz, feldspars, calcite, and dolomites  
303 (Table 1). Narrow and thin biofilms of Cyanidia were detected in Turkish thermal baths,  
304 mostly in hypolithic and endolithic conditions.

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

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

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

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

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

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

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

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

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

387 We examined the population structure in *G. sulphuraria*, *G. maxima*, *G. phlegrea*, and  
388 *C. merolae*, measuring  $F_{st}$ , a parameter that provides a measure of population differentiation  
389 based on genetic variance between the populations. Pairwise comparisons between strains  
390 grouped by region have produced different results in the Cyanidiophyceae taxa. Large,  
391 significant  $F_{st}$  values across the hydrothermal locations were recorded in *G. sulphuraria* and  
392 *G. maxima* suggesting a high level of genetic differentiation, and a reduction in dispersal

393 ability of the individuals. However, in *G. maxima* low  $F_{st}$  values were recorded among the  
394 Asiatic populations, indicating that there is at least a small level of genetic differentiation  
395 between them, and a substantial level of gene flow. Perhaps this was due to the contiguity of  
396 the geothermal areas, being located on the Ring of Fire. Within *C. merolae* and *G. phlegrea*,  
397  $F_{st}$  values were not significantly different from zero ( $F_{st}= 0.05$  and  $0.013$ , respectively),  
398 indicating that populations from different geothermal springs were not genetically  
399 differentiated, suggesting a frequent gene flow among the geothermal springs. *G. phlegrea*  
400 populations to date have only been identified in Turkey and Italy, and it is intriguing that even  
401 in *G. sulphuraria*, the lowest level of genetic differentiation was recorded between the same  
402 populations. This supports the hypothesis of gene flow between Turkey and Italy. The level of  
403 genetic divergence of *G. phlegrea* was much lower than that observed in *G. sulphuraria* and  
404 in *G. maxima*. *G. phlegrea* has a restricted areal of dispersal, because of its peculiar  
405 adaptation to dry habitats, such as rock fissures, chasmoendolithic and cryptoendolithic  
406 environments. These habitats were very frequently encountered in Turkey, and are preferred  
407 by *G. phlegrea* in spite of fumaroles and hot springs.

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



427 and large subunit rDNA genes (SSU and LSU), concatenated with *rbcL*, should result in a  
428 substantial improvement in phylogenetic resolution.

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546

547 **DISCLOSURE STATEMENT**

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

549

550 **AUTHOR CONTRIBUTIONS**

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

556 **Figures and Tables legends**

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

562

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

566

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

571

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

574

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

578

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

581

582 **Supplementary material**

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

585

586 **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

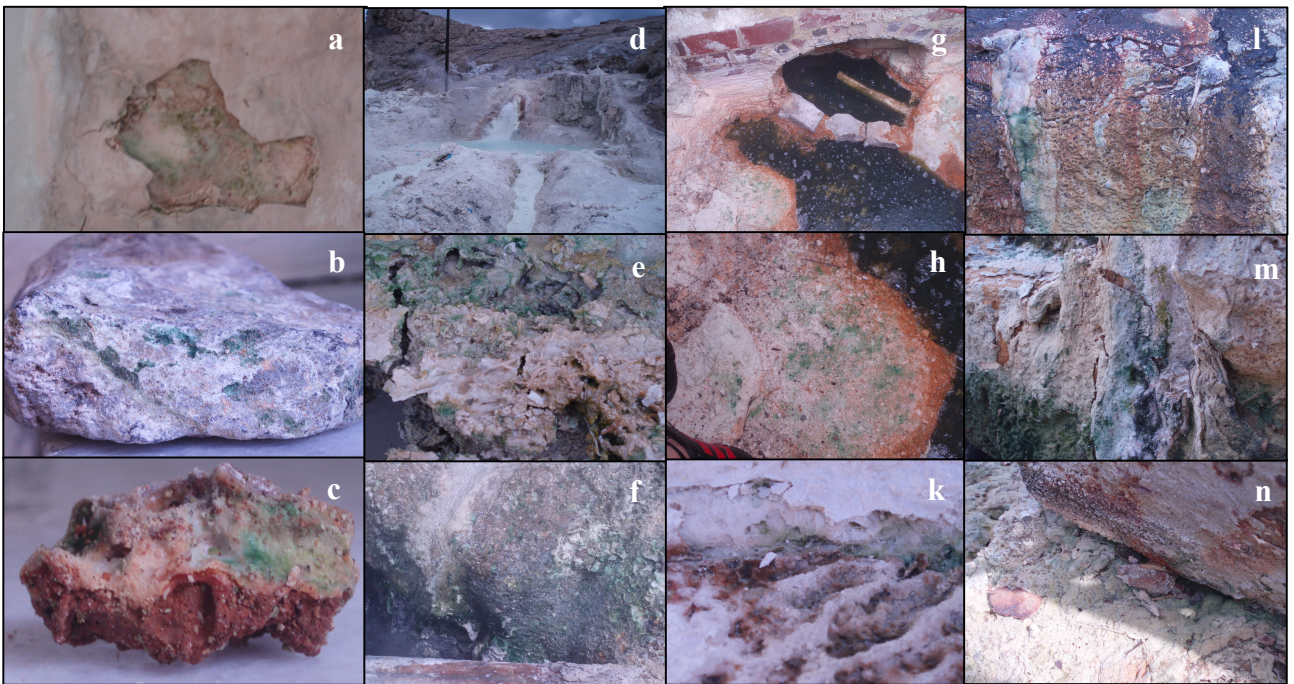


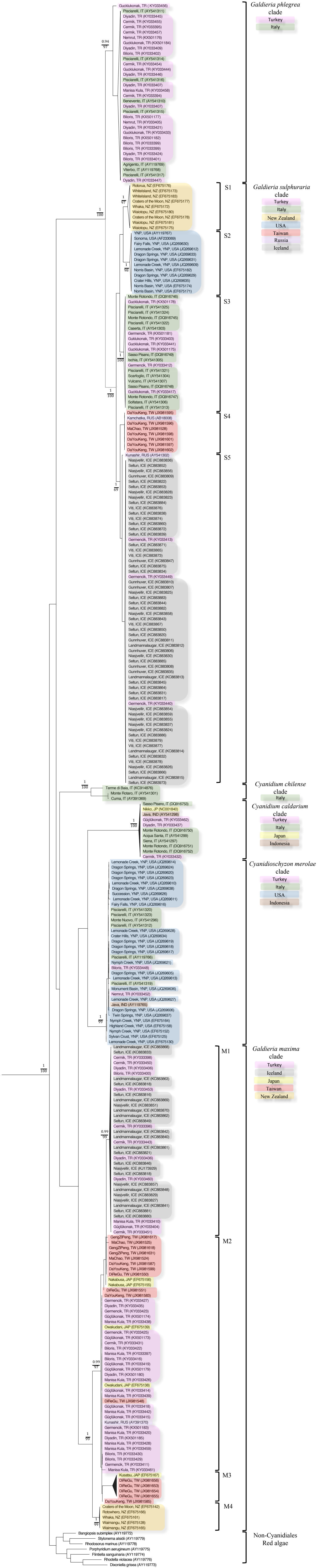
Phylotype	n	v	N	K	h	$\pi$	Tajima	Fu and Li F*	
<i>G.sulphuraria</i>	ALL	136	80	33	19,47	0,83±0,028	0,0426±0,00356	-0,35987	-0,42141
	Italy	15	8	7	1,29	0,724±0,121	0,00283±0,0019	-1,744	-1,992
	USA	13	5	4	1,2	0,6±0,131	0,00269±0,00157	-0,84	-1
	Turkey	10	39	3	17,13	0,6±0,131	0,0375±0,00482	-1,17	1,3426
	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
<i>G. maxima</i>	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
<i>C. merolae</i>	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
<i>G.phlegrea</i>	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 cyanodiophycean strains; n . sample size, v . variable sites, N . number of haplotypes, h. haplotype diversity, K. Average number of pairwise nucleotide differences,  $\pi$  nucleotide diversity. (significance \*:  $p < 0.05$ ; \*\*:  $p < 0.10$ ).

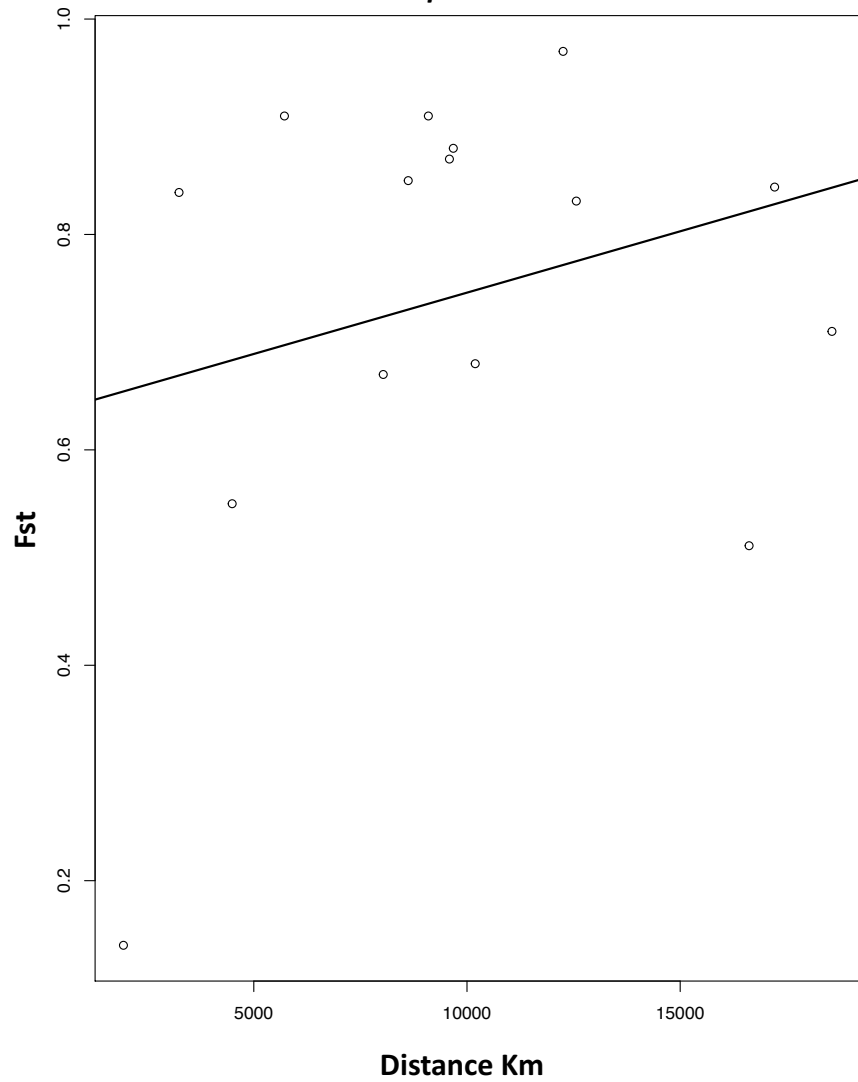
<b><i>G. sulphuraria</i></b>	<b>ICE</b>	<b>ITA</b>	<b>NZE</b>	<b>TWN</b>	<b>TUR</b>	<b>USA</b>
ICE	***	0,839	0,844	0,87	0,55	0,91
ITA		***	0,71	0,88	0,14	0,85
NZE			***	0,91	0,511	0,831
TWN				***	0,67	0,97
TUR					***	0,68
USA						***

<b><i>G. maxima</i></b>	<b>ICE</b>	<b>JAP</b>	<b>TWN</b>	<b>TUR</b>
ICE	***	0,4	0,64	0,67
JAP		***	0,21	0,06
TWN			***	0,56
TUR				***

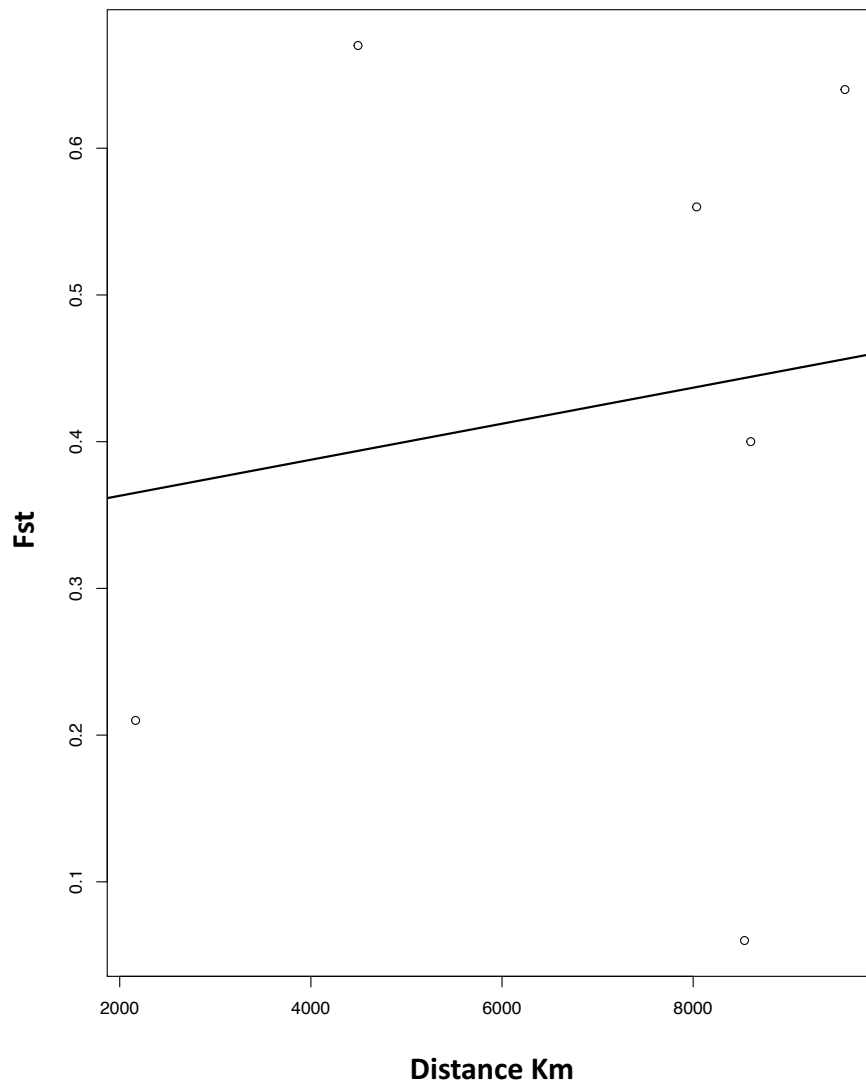




*G. sulphuraria*



*G. maxima*



Taxa	Strain	Sampling Site	GenBank number
<i>Cyanidiales sp.</i>	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	
<i>Cyanidioschyzon merolae</i>	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	
<i>Cyanidium caldarium</i>	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
<i>Galdieria daedala</i>	RK1	Japan, Nikko	NC_001840
	IPPAS P508	Russia, Kunashir	AY541302

<i>Galdieria maxima</i>	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	
IPPAS P500	Russia, Kamchatka	AB18008	
<i>Galdieria partita</i>			
<i>Galdieria phlegrea</i>	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
<i>Galdieria phlegrea</i>	ACUF009	Italy, Viterbo	AY119768
<i>Galdieria sulphuraria</i>	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	



<i>Galdieria sulphuraria</i>	ACUF018	Italy, Scarfoglio	AY541304
	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	
<i>Cyanidium chilense</i>	Sybil cave	Italy, Cuma	AY391369
	sp.19	Italy, Monte Rotaro	AY541300
	sp.20	Italy, Monte Rotaro	AY541301
		Italy, Terme di baia	KC914876
<i>Galdieria sp.</i>	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
	clone 12.ENVS.DYK.ditch45.2.2	Taiwan, DaYouKeng	JX981569
	clone 12.ENVS.DYK.ditch45.2.3	Taiwan, DaYouKeng	JX981570
	clone 12.ENVS.DYK.ditch45.2.5	Taiwan, DaYouKeng	JX981571
	clone 12.ENVS.DYK.ditch45.2.6	Taiwan, DaYouKeng	JX981572
	clone 12.ENVS.DYK.ditch45.2.7	Taiwan, DaYouKeng	JX981573
	clone 12.ENVS.DYK.ditch45.2.8	Taiwan, DaYouKeng	JX981574
	clone 12.ENVS.DYK.ditch45.2.9	Taiwan, DaYouKeng	JX981575
	clone 12.ENVS.DYK.ditch45.2.10	Taiwan, DaYouKeng	JX981576
	clone 12.ENVS.DYK.ditch45.2.12	Taiwan, DaYouKeng	JX981577
	clone 12.ENVS.DYK.ditch45.2.13	Taiwan, DaYouKeng	JX981578
	clone 12.ENVS.DYK.ditch45.2.14	Taiwan, DaYouKeng	JX981579
	clone 12.ENVS.DYK.ditch45.2.15	Taiwan, DaYouKeng	JX981580
	clone 12.ENVS.DYK.ditch45.4.1	Taiwan, DaYouKeng	JX981581
	clone 12.ENVS.DYK.ditch45.4.6	Taiwan, DaYouKeng	JX981583
	clone 12.ENVS.DYK.ditch45.4.8	Taiwan, DaYouKeng	JX981585
	clone 12.ENVS.DYK.endolithic.2	Taiwan, DaYouKeng	JX981586
	clone 12.ENVS.DYK.endolithic.4	Taiwan, DaYouKeng	JX981587
	clone 12.ENVS.DYK.endolithic.6	Taiwan, DaYouKeng	JX981588
	clone 12.ENVS.DYK.endolithic.7	Taiwan, DaYouKeng	JX981589
	clone 12.ENVS.DYK.endolithic.8	Taiwan, DaYouKeng	JX981590
	clone 12.ENVS.DYK.endolithic.10	Taiwan, DaYouKeng	JX981591
	clone 12.ENVS.DYK.endolithic.11	Taiwan, DaYouKeng	JX981592
	clone 12.ENVS.DYK.endolithic.12	Taiwan, DaYouKeng	JX981593
	clone 12.ENVS.DYK.endolithic.13	Taiwan, DaYouKeng	JX981594
	clone 12.ENVS.DYK.endolithic.14	Taiwan, DaYouKeng	JX981595
	clone 12.ENVS.DYK.endolithic.15	Taiwan, DaYouKeng	JX981596
	clone 12.ENVS.DYK.endolithic.16	Taiwan, DaYouKeng	JX981597
	clone 12.ENVS.DYK.endolithic.17	Taiwan, DaYouKeng	JX981598
	clone 12.ENVS.DYK.endolithic.18	Taiwan, DaYouKeng	JX981599
	clone 12.ENVS.DYK.endolithic.21	Taiwan, DaYouKeng	JX981600
	clone 12.ENVS.DYK.endolithic.22	Taiwan, DaYouKeng	JX981601
	clone 12.ENVS.DYK.endolithic.23	Taiwan, DaYouKeng	JX981602
	clone 12.ENVS.DYK.endolithic.24	Taiwan, DaYouKeng	JX981603
	clone 12.ENVS.DYK.endolithic.25	Taiwan, DaYouKeng	JX981604
clone 12.ENVS.DYK.endolithic.26	Taiwan, DaYouKeng	JX981605	
clone 12.ENVS.DYK.endolithic.28	Taiwan, DaYouKeng	JX981606	

<i>Galdieria</i> 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
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clone 05.ENVS.DRG.stream42.shaded.18	Taiwan, DiReGu	JX981675	
THAL001.DRG01.Gp	Taiwan, DiReGu	KJ125464	
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	

clone12.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
clone12.ENVS.GZP.epilithic.low7	Taiwan, GengZiPeng	KC313268
clone12.ENVS.GZP.epilithic.low8	Taiwan, GengZiPeng	KC313269
clone12.ENVS.GZP.epilithic.low9	Taiwan, GengZiPeng	KC313270
clone12.ENVS.GZP.epilithic.low10	Taiwan, GengZiPeng	KC313271
clone12.ENVS.GZP.epilithic.low11	Taiwan, GengZiPeng	KC313272
clone12.ENVS.GZP.epilithic.low12	Taiwan, GengZiPeng	KC313273
clone12.ENVS.GZP.epilithic.low13	Taiwan, GengZiPeng	KC313274
clone12.ENVS.GZP.epilithic.low14	Taiwan, GengZiPeng	KC313275
clone12.ENVS.GZP.epilithic.low15	Taiwan, GengZiPeng	KC313276
clone12.ENVS.GZP.epilithic.low16	Taiwan, GengZiPeng	KC313277
clone12.ENVS.GZP.epilithic.low18	Taiwan, GengZiPeng	KC313278
clone12.ENVS.GZP.epilithic.low19	Taiwan, GengZiPeng	KC313279
clone12.ENVS.GZP.epilithic.low20	Taiwan, GengZiPeng	KC313280
clone12.ENVS.GZP.epilithic.low21	Taiwan, GengZiPeng	KC313281
clone12.ENVS.GZP.epilithic.low22	Taiwan, GengZiPeng	KC313282
clone12.ENVS.GZP.epilithic.low23	Taiwan, GengZiPeng	KC313283
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	
<i>Cyanidium caldarium</i>	ACUF767	Turkey, Cermik	KY033432	
	ACUF775	Turkey, Diyadin	KY033437	
	CloneT17	Turkey, Güçlükonak	KY033462	
<i>Cyanidioschyzon merolae</i>	CloneT01	Turkey, Biloris	KY033448	
	CloneT05	Turkey, Nemrut	KY033452	
<i>Galdieria maxima</i>	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	
	<i>Galdieria phlegrea</i>	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,Gucklukonak	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
<i>Galdieria sulphuraria</i>	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.



**Kula-Manisa**

**Germencik-Aydin**

**Agri-Diyadin**

**Nemrut-Bitlis**

**Cermik-Diyarbakir**

**Biloris-Siirt**

**Sirnak**