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Community phylogenetic analysis of moderately thermophilic cyanobacterial mats from China, the Philippines and Thailand

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Abstract Most community molecular studies of thermophilic cyanobacterial mats to date have focused on *Synechococcus* occurring at temperatures of ~50–65°C. These reveal that molecular diversity exceeds that indicated by morphology, and that phylogeographic lineages exist. The moderately thermophilic and generally filamentous cyanobacterial mat communities occurring at lower temperatures have not previously been investigated at the community molecular level. Here we report community diversity in mats of 42–53°C recovered from previously unstudied geothermal locations. Separation of 16S rRNA gene-defined genotypes from community DNA was achieved by DGGE. Genotypic diversity was greater than morphotype diversity in all mats sampled, although genotypes generally corresponded to observed morphotypes. Thirty-six sequences were recovered from DGGE bands. Phylogenetic analyses revealed these to form novel thermophilic lineages distinct from their mesophilic counterparts, within *Calothrix*, *Cyanothece*, *Fischerella*, *Phormidium*, *Pleurocapsa*, *Oscillatoria* and *Synechococcus*. Where filamentous cyanobacterial sequences belonging to the same genus were recovered from the same site, these were generally closely affiliated. Location-specific sequences were observed for some genotypes recovered from geochemically similar yet spatially separated sites, thus providing evidence for phylogeographic lineages that evolve in isolation. Other genotypes were more closely affiliated to geographically remote counterparts from similar habitats suggesting that adaptation to certain niches is also important.

Keywords Cyanobacteria · Hot springs · Microbial mats · *Synechococcus* · Thermophiles

Introduction

Thermophilic mat communities occur in geothermal springs of neutral/alkaline pH and at temperatures of up to ~65°C (Brock 1978). Mat community composition is largely temperature-defined, and mats have been differentiated on the basis of the cyanobacterial taxa involved in primary production. At temperatures above 60°C *Synechococcus* is the only cyanobacterial genus encountered, and this has been the focus of most research on thermophilic mat diversity (Ferris et al. 1996a, 1996b; Ward et al. 1997, 1998). Studies have generally utilized 16S rRNA gene data from environmental samples to demonstrate that genotypic diversity far exceeds phenotypic diversity as estimated by observation and culture techniques (Ferris et al. 1996a). Although few locations worldwide have been studied, distinct phylogeographic groups have been shown to exist in the continental USA, Japan and New Zealand (Papke et al. 2003).

The moderately hot runoff channels and pools below ~60°C support a range of different cyanobacterial mats comprising filamentous and unicellular taxa and the occurrence of these is determined by temperature plus combined nitrogen and free sulphide levels. Nitrogen-poor waters support mats of diazotrophic cyanobacteria, usually *Fischerella* or *Calothrix* and/or *Pleurocapsa* at lower temperatures, whereas those rich in nitrogen support *Synechococcus* and *Phormidium* mats (Ward and Castenholz 2000). In sulphide-rich waters mats

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comprise *Oscillatoria* species that are sulphide tolerant/utilizing (Ward and Castenholz 2000). Filamentous mats have been extensively documented morphologically but have received little attention in terms of molecular diversity. Some *Oscillatoria* sequences are known from cultures (Miller and Castenholz 2001) and environmental DNA (Papke et al. 2003), and a low temperature *Synechococcus* mat (40–47°C) from Yellowstone National Park was found to support *Phormidium*, *Pseudanabaena* and *Spirulina*-like genotypes in addition to *Synechococcus* (Norris et al. 2002). It is reasonable to assume, however, that as with *Synechococcus* mats morphological diversity does not reflect genetic diversity. In addition, little is known about possible phylogeographic patterns among filamentous genotypes. A partial 16S rRNA gene phylogeny for *Oscillatoria* revealed genotypes from the continental USA, Japan and New Zealand that were similar although some New Zealand genotypes were unique (Papke et al. 2003). The deep branching between lineages that are observed for *Synechococcus* were, however, absent.

The paucity of molecular data for filamentous thermophilic cyanobacteria as compared to *Synechococcus* raises some important questions. Do moderately thermophilic cyanobacterial mats harbour greater genetic diversity than morphotypes suggest? Are filamentous thermophilic taxa phylogenetically distinct from their mesophilic counterparts? Are phylogeographic patterns apparent which may yield clues to the evolutionary forces that shape these thermophilic communities? In order to address these questions, at least in part, we set out to examine 16S rRNA gene-defined and morphological diversity in a range of filamentous cyanobacterial mat communities from moderately hot spring waters.

Materials and methods

Sample recovery

Mat samples were collected from geothermal springs in various locations within Asia as indicated in Table 1. For each mat type three samples were taken. Sections (2 × 1 cm) were cut from mats using a scalpel and stored in sterile glass bottles in darkness on ice in the field (<8 h) and then at 4°C until processed (<2 weeks). Mats generally occurred in shallow (2–3 cm depth) water. Temperature and pH in the water channel for each location where mat growth occurred were recorded at several locations over a 5-min period to ensure sampling areas were representative of conditions generally experienced by the mat. The probes/thermometer were also inserted into holes left from biomass sampling, and values were not significantly different from the above. The pH meter (230-A, Orion) was calibrated on-site at sampling temperatures and used with an automatic temperature compensation (ATC) function when sampling. A thermometer was used to verify accuracy of the ATC digital thermometer. Hydrogen sulphide levels

Table 1 Moderately thermophilic mats used in this study

Mat	Location	GPS coordinates	Temp (°C)	pH	Dissolved H_2S	Observed morphotypes[a]	No. morphotypes	No. 16S rRNA gene-defined genotypes
Thp1	Pong Dued Thailand	N19° 14.641’ E98°41.358’	42	7.8	Nil	***Calothrix***	1	7
Phl	Los Banós Philippines	N14° 10.630’ E121° 12.333’	53	6.8	30 μM	***Fischerella***, *Oscillatoria*, *Synechococcus*	3	10
Thp2	Pong Dued Thailand	N19° 14.641’ E98°41.358’	51	7.7	Nil	***Phormidium***, *Cyanothece*, *Gloeothece*, *Synechococcus*	4	5
Ths	Sankhamphaeng Thailand	N18° 48.889’ E99° 13.604’	46	7.0	Nil	***Phormidium***, *Cyanothece*, *Synechococcus*	3	5
Tht	Teppanom Thailand	N18° 16.261’ E98° 23.787’	50	7.8	Nil	***Phormidium***, *Cyanothece*, *Gloeothece*, *Synechococcus*	4	7
Thp3	Pong Dued Thailand	N19° 14.641’ E98°41.358’	48	7.7	Nil	***Pleurocapsa***	1	3
Cht	Daggyai Tso Tibet, China	N29°35.413’ E85°44.486’	52	8.4	Nil	***Synechococcus***, *Pseudanabaena*	2	7

[a] Taxa in bold denote common/dominant morphotypes

were determined titrimetrically using methylene blue (HS-WR, Hach).

Microscopy

Identification of cyanobacterial morphotypes was carried out by microscopy using an Olympus BX50 compound microscope with and without Nomarski optics.

Community DNA recovery and PCR

DNA recovery from mat samples was achieved by lysis in CTAB with lysozyme, RNAse A and Proteinase K incubations, and phenol:chloroform extraction at 60°C. Genes of 16S rRNA were amplified by PCR using cyanobacteria-specific primers CYA359F and CYA781R (Nübel et al. 1997) with a $(GC)_{40}$ clamp added to the forward primer. The following PCR profile was used: 35 cycles of 1 min at 94°C, 50 s at 55°C, 1 min at 72°C; with an initial denaturation step of 3 min and a final extension step of 10 min. The PCR reaction mixture contained 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM of each primer, and 1.0 U of Taq DNA polymerase in 50 µl total volume. Since this is the first report of a molecular approach to biodiversity assessment of such thermophilic filamentous cyanobacterial mats, we first confirmed specificity of the PCR primers to twenty taxonomically diverse reference taxa from The University of Hong Kong Culture Collection.

DGGE and sequencing

Each PCR amplicon (2 µg amplified DNA) was separated by DGGE (Myers et al. 1988) in a urea/formamide denaturing gradient in 7% acrylamide gel, run at 150 V in 1 × TAE buffer (pH 8) at 60°C (DGGE-2001, CBS Scientific Co.). Bands were excised, soaked overnight in TE buffer (pH 8) at 4°C, re-amplified and purified (GFX, Amersham) prior to automated sequencing (ABI Prism 377, Applied Biosystems). Some bands could not be sequenced directly and were cloned (pDrive, Qiagen) before successful sequencing. All sequences have been deposited in GenBank, under accession numbers AY787591–AY787626.

Phylogenetic analyses

Approximate phylogenetic affiliations were determined by BLAST searches of the NCBI GenBank database. Multiple alignments were then created with reference to selected GenBank sequences using BioEdit version 5.0.9 (Hall 1999). Maximum likelihood analysis using PAUP* 4.0b8 (Swofford 2001) was used to illustrate the relationship of partial 16S rRNA gene sequences to representative cyanobacteria. Bayesian posterior probabilities (Rannala and Yang 1996) and bootstrap values (1000 replications) were calculated and were shown for branches supported by more than 50% of the trees.

Results and discussion

In this study we report 16S rRNA gene-defined community diversity in environmental samples of moderately thermophilic mats from seven different locations within Asia (Table 1). A total of 45 16S rRNA gene-defined genotypes were resolved by visual analysis of DGGE-banding patterns. For all samples studied genotypic diversity was greater than that observed by microscopy (Fig. 1), mirroring trends observed for *Synechococcus* mats from higher temperatures (Ferris et al. 1996a). Purely filamentous mats generally displayed fewer bands than those containing both filamentous and *Synechococcus*-like genotypes. Banding patterns were generally consistent between independent replicates from the same mat, although some within-mat variation occurred. This is to be expected when sampling mat communities where different microenvironments can exist within a small area.

All differentially migrating bands and co-migrating bands from different mat samples were sequenced wherever possible (36 out of 45 bands). Most were sequenced directly from excised bands, but some samples that generated ambiguous sequences were cloned prior to

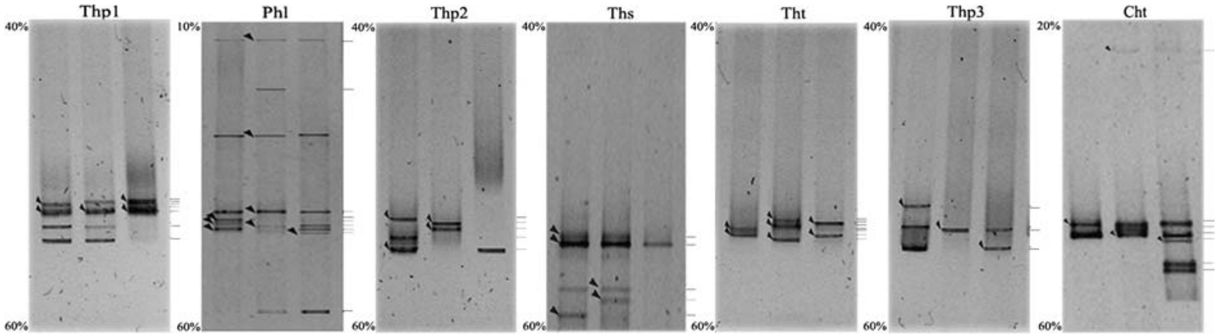

Fig. 1 DGGE banding patterns of 16S rRNA gene-defined diversity among moderately thermophilic cyanobacterial mats. *Thp1*, *Thp2*, *Thp3*, Pong Dued, Thailand; *Phl*, Los Banós, Philippines; *Ths*, Sankhamphaeng, Thailand; *Tht*, Teppanom, Thailand; *Cht*, Daggyai Tso, Tibet, China. *Percentages* refer to urea-formamide gradients employed for each gel. *Horizontal lines* indicate band migration positions, *arrows* denote those bands that were successfully sequenced

successful sequencing. It is likely that such bands contained mixed genotypes and this highlights a potential limitation to DGGE in community analysis. Nonetheless this can be favourably weighed in this case against the number of bands recovered per sample, which was greater than or similar to that observed for cyanobacteria in similar studies. The only morphotypes not represented in DGGE-derived sequences were the infrequently observed *Gloeothece* and *Pseudanabaena*.

All sequenced bands were provisionally identified on the basis of high similarity by BLAST search to cyanobacterial sequences in the NCBI GenBank database (Table 2). All but one *Oscillatoria*-like sequence (Thp2-1) corresponded to the observed morphotypes for each mat. Sequence divergence between similar genotypes in this study was greater than recorded for intraspecies 16S rRNA gene sequence variation in cyanobacteria (Tourova 2003) and so DGGE-derived genotypes are unlikely to represent multiple 16S rRNA gene copies from a single species. While *Fischerella*-, *Oscillatoria*- and *Synechococcus*-like sequences shared high similarity (95–99%) to published thermophilic cyanobacterial sequences, all other sequences recovered shared relatively low similarity (86–93%), reflecting the lack of previously published thermophilic sequences available for these genera and novel diversity reported here. Bearing in mind that the sequence data from this study is based upon a ~420 bp fragment of the rRNA gene, and that an overall 98% sequence similarity is generally considered to represent the same species, while <88% similarity suggests a new genus (Stackebrandt and Goebel 1994), we can assume that novel diversity at the species level or higher is identified for *Calothrix*-, *Cyanothece*-, *Phormidium*- and *Pleurocapsa*-like sequences.

Phylogenetic analysis was used to further resolve identities and establish relationships between sequences obtained in this study and representative GenBank sequences. Branching patterns within trees generally had high levels of support, with lower bootstrap and Bayesian posterior probability values for a few branches probably reflecting current ambiguities in cyanobacterial taxonomy and sequence length limitations to the analysis. The *Fisherella*-like sequences grouped closely with unpublished sequences (AY236467–AY236480) from a nearby geothermal pool at the same location. This lineage included thermophilic *Fischerella* and *Mastigocladus* (*Fischerella*) sequences from as far apart as Iceland and the Philippines, and these probably belong to *F. muscicola* (Fig. 2). The temperature from which *Fischerella*-like sequences were recovered is within the known range for this genus and *Fischerella* is known to occur in springs of pH 5 and above (Ward and Castenholz 2000).

In contrast the *Calothrix*- and *Pleurocapsa*-like sequences likely represent novel diversity at the species level or higher, forming distinct and possibly thermophilic lineages within each genus (Fig. 2). Since GenBank sequences available for comparison were all mesophiles from other continents, it is also possible that phylogeo-

Table 2 Identity of sequences obtained from community DNA of moderately thermophilic cyanobacterial mats

Code[a]	Genbank accession number	Closest GenBank match			Code[a]	Genbank accession number	Closest GenBank match		
		Identity	Accession number	Percent similarity			Identity	Accession number	Percent similarity
Thp-1	AY787591	*Calothirix brevissima*	AB074504	93	Ths-2	AY787609	*Cyanothece* sp.	AY429141	92
Thp-2	AY787592	*Calothirix brevissima*	AB074504	91	Ths-3	AY787610	*Cyanothece* sp.	AY429141	91
Thp-3	AY787593	*Calothrix* sp.	AJ133164	87	Ths-4	AY787611	Cyanothece *sp.*	AY429141	91
Thp-4	AY787594	*Calothirix brevissima*	AB074504	92	Ths-5	AY787612	*Pleurocapsa minor*	Z82810	87
Thp-5	AY787595	Calothirix brevissima	AB074504	90	Tht-1	AY787613	*Cyanothece* sp.	AY429141	91
Phl-1	AY787596	*Fischerella* sp.	AB093487	99	Tht-2	AY787614	*Cyanothece aeruginosa*	Z82775	88
Phl-2	AY787597	*Oscillatoria* sp.	AF317508	98	Tht-3	AY787615	*Cyanothece* sp.	AY429141	92
Phl-3	AY787598	*Oscillatoria* sp.	AF317508	99	Tht-4	AY787616	Synechococcus *sp.*	AF285243	95
Phl-4	AY787599	*Oscillatoria* sp.	AF317508	98	Tht-5	AY787617	Unknown Oscillatoriales	AF401743	88
Phl-5	AY787600	*Fischerella* sp.	AB093487	99	Tht-6	AY787618	*Cyanothece* sp.	AB067581	88
Phl-6	AY787601	*Fischerella* sp.	AB093487	98	Tht-7	AY787619	Unknown Oscillatoriales	AF401748	89
Phl-7	AY787602	*Thermosynechococcus elongatus*	AP005376	99	Thp3-1	AY787620	*Pleurocapsa minor*	Z82810	88
Phl-8	AY787603	*Oscillatoria* sp.	AF317508	98	Thp3-2	AY787621	*Pleurocapsa minor*	Z82810	87
Thp2-1	AY787604	*Oscillatoria* sp.	AF317508	99	Thp3-3	AY787622	*Pleurocapsa minor*	Z82810	87
Thp2-2	AY787605	*Cyanothece* sp.	AY429141	92	Cht-1	AY787623	*Synechococcus* sp.	AF285243	89
Thp2-3	AY787606	*Cyanothece* sp.	AB067581	90	Cht-2	AY787624	*Thermosynechococcus elongatus*	AP005376	98
Thp2-4	AY787607	*Synechococcus* sp.	AF132774	95	Cht-3	AY787625	*Thermosynechococcus elongatus*	AP005376	95
Ths-1	AY787608	*Cyanothece* sp.	AY429141	92	Cht-4	AY787626	*Thermosynechococcus elongatus*	AP005376	95

[a] Codes refer to mat type and location from which sequences were obtained, see Table 1

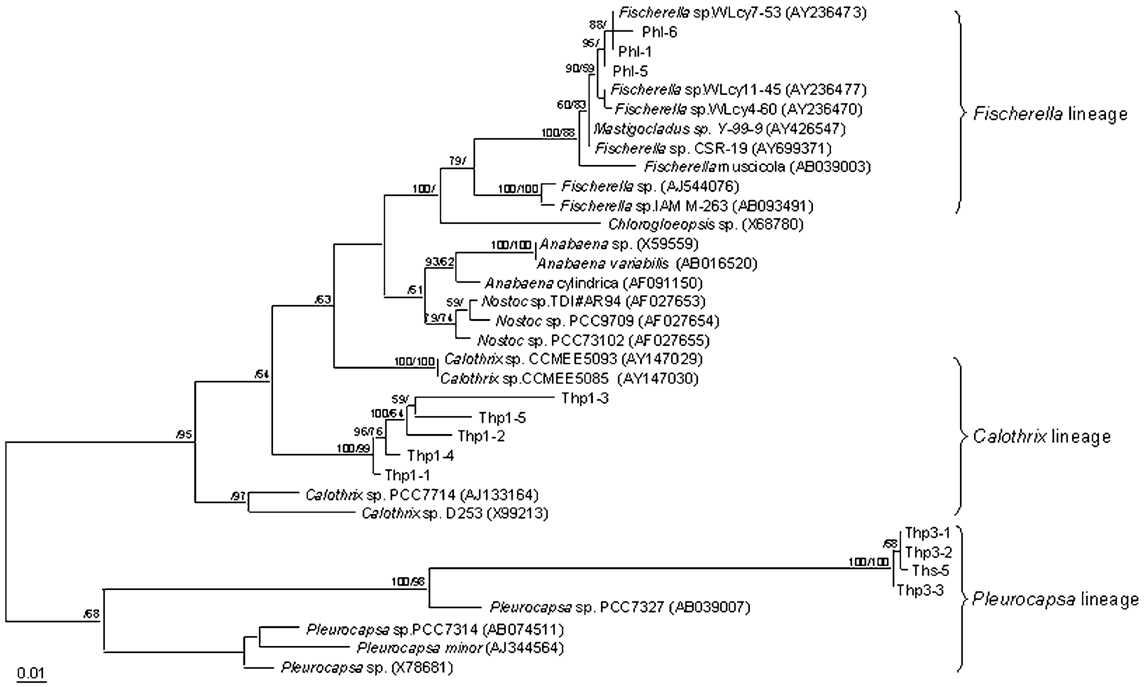

Fig. 2 Phylogenetic relationships among moderately thermophilic diazotrophic cyanobacteria based upon Maximum Likelihood analysis of partial 16S rRNA gene sequence data. Sequence codes refer to those given in Table 2, where *Thp1*, *Thp2*, *Thp3*, Pong Dued, Thailand; *Phl*, Los Banós, Philippines; *Ths*, Sankhamphaeng, Thailand; *Tht*, Teppanom, Thailand; *Cht*, Daggyai Tso, Tibet, China. Unrooted trees are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1000 replications (second number), shown for branches supported by more than 50% of the trees. *Scale bar* represents 0.01 nucleotide changes per position

graphic factors alone may be responsible. The *Calothrix*- and *Pleurocapsa*-like sequences were recovered from three locations in Thailand of 42–48°C, which are close to the 35–47°C range recorded for these taxa in other studies (Ward and Castenholz 2000). The range of pH for growth of thermophilic *Calothrix* and *Pleurocapsa* is unknown. *Calothrix* was recorded from only one site and this may reflect the availability of sinter pebbles for colonization, which were absent at all other sites except mat Cht, where temperatures were above the limit for growth of *Calothrix*. Since *Pleurocapsa* occurred in mat Ths but not the morphologically similar mats Thp2 (51°C) and Tht (50°C) that were separated by ~100 km but with similar aqueous geochemistry, we may conclude that the upper temperature limit for *Pleurocapsa* growth probably lies between 48–49°C. This is the first report of thermophilic *Calothrix* sequence data, and only one *Pleurocapsa* sequence is previously recorded from a geothermal environment in Japan (Papke et al. 2003). The sequence data for the latter taxon spans a fragment of the 3′ end for the 16S rRNA gene and ITS region and so is not comparable with our data.

Most sequences from the *Phormidium* mats (Thp2, Ths, Tht) resolved into a phylogenetically uncertain *Cyanothece*-like group that spanned clades within the Oscillatoriales. The molecular systematics of the Oscillatoriaceae is, however, polyphyletic and not satisfactorily resolved at present (Wilmotte 1994; Litvaitis 2002). Curiously no *Phormidium*-like sequences were obtained for mats Thp2 and Ths, although sequences from mat Tht resolved unambiguously into a *Phormidium* lineage (Fig. 3). Further work is required to resolve this particular ambiguity, and sequence analysis of the entire 16S rRNA gene or additional loci may help in this regard. It should be noted, however, that even where near-complete 16S rRNA gene sequences have been used, conflicts between morphological and molecular identification of *Phormidium*-like sequences have still arisen (Nadeau et al. 2001; de la Torre et al. 2003). On a relatively small spatial scale (~100 km), distinct populations of *Cyanothece*-like sequences existed within Thailand at geochemically similar sites, indicating that small-scale phylogeographic effects also occur for at least some moderately thermophilic cyanobacteria. Such small-scale variations have been recorded for *Synechococcus* within north America (Ward et al. 1998).

Oscillatoria-like sequences all shared high similarity to known thermophilic oscillatorians, but formed a distinct phylogenetic group within the thermophilic *O. amphigranulata* lineage, sharing highest affinity with cultivated thermophilic oscillatorians (Miller and Castenholz 2001). All but one of our *Oscillatoria*-like sequences were recovered in association with *Fischerella*-like sequences in free-floating Philippine mats growing in moderately sulphidic conditions. These cyanobacteria are known to be moderately/highly sulphide-tolerant, occur at up to 62°C and at pH 6.5 and above (Ward and Castenholz 2000). A single *Oscillatoria*-like sequence was also recovered from Thailand (Thp2) in non-sulphidic conditions, but was absent from morphologically

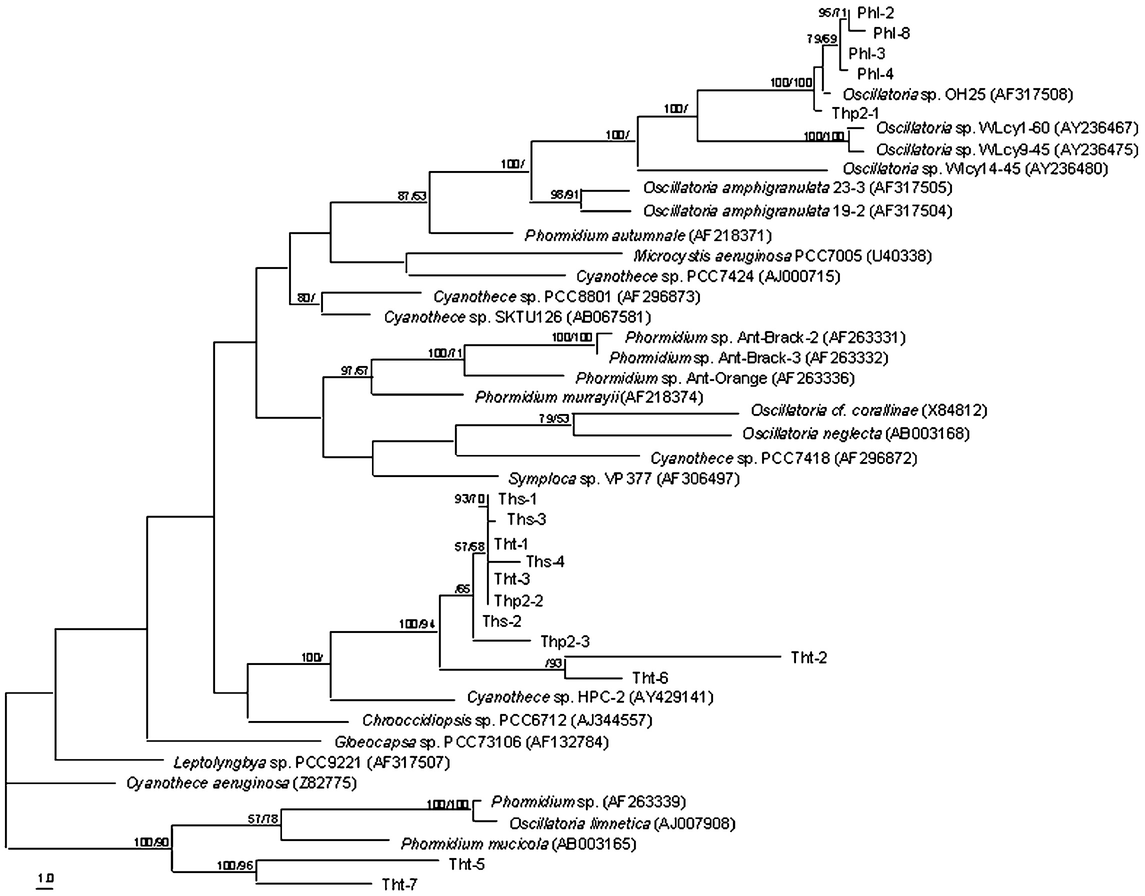

Fig. 3 Phylogenetic relationships among moderately thermophilic Oscillatoriales and *Cyanothece*-like cyanobacteria based upon Maximum Likelihood analysis of partial 16S rRNA gene sequence data. Sequence codes refer to those given in Table 2, where *Thp1*, *Thp2*, *Thp3*, Pong Dued, Thailand; *Phl*, Los Banós, Philippines; *Ths*, Sankhamphaeng, Thailand; *Tht*, Teppanom, Thailand; *Cht*, Daggyai Tso, Tibet, China. Unrooted trees are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1000 replications (second number), shown for branches supported by more than 50% of the trees. *Scale bar* represents 1 nucleotide change per position

similar mats (Ths and Tht). Since all DGGE bands were sequenced for these samples, and assuming the resolution between mats bearing similar morphotypes is comparable, this is probably an accurate reflection of occurrence for this genotype. *Oscillatoria*-like sequences from Philippines, Thailand and the USA largely grouped independently within the thermophilic *Oscillatoria* lineage suggesting novel phylogeographic lineages may have arisen in isolation at each location. This view is also supported by analysis of *Oscillatoria*-like sequences from New Zealand and Japan (Papke et al. 2003). This greater resolution among thermophilic *Oscillatoria* sequences as compared to other filamentous cyanobacteria in this study may reflect, at least in part, the greater number of known genotypes for this taxon. All sequences recovered in our study were nonetheless phylogenetically distinct and so expand the known limits of this thermophilic group.

The *Synechococcus* sequences obtained from moderately thermophilic mats were all unique, yet, generally shared high sequence similarity with known *Synechococcus* from other geothermal environments, although most of these were recovered from hotter habitats than those in our study. *Synechococcus* is known to occur within the temperature and pH range of mats used in this study. Philippine and Tibetan sequences were most closely affiliated with the C1 lineage, which largely comprises *Synechococcus* from relatively lower temperatures (as in this study) and a number of sequences from Japanese springs. That the Philippine sequence resolved in this way is understandable bearing in mind the geographic proximity and geochemical similarities between locations, namely that both are sulphidic and of neutral pH, arising from the same fault system. All but one of the Tibetan sequences grouped loosely with C1 lineage sequences, expanding this grouping with at least three novel lineages. Prevailing atmospheric conditions and winds dictate that the most likely dispersal direction would be West–East, and Tibetan geothermal springs have been in continuous existence for longer than most Japanese and Philippine springs, which are associated with recent volcanic activity. We can therefore speculate that the shallow branching Japanese and Philippine sequences may have evolved from Tibetan ancestors. The phylogenetic placement of *Synechococcus*-like sequences from Thailand is curious since they form a separate

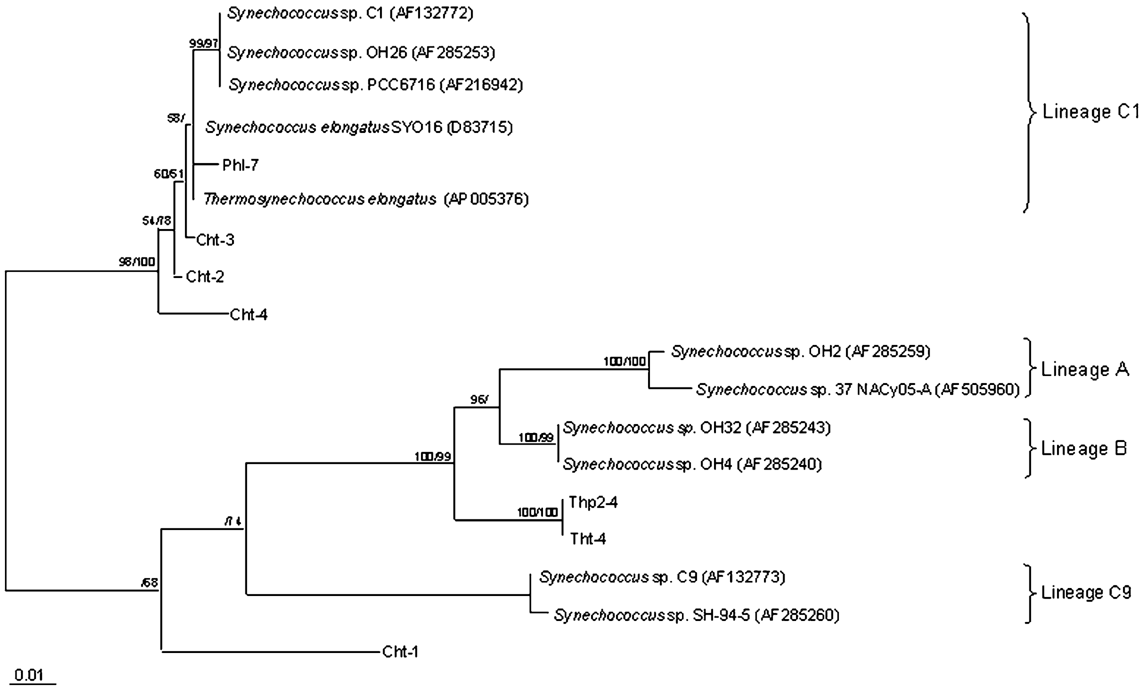

Fig. 4 Phylogenetic relationships among moderately thermophilic *Synechococcus* based upon Maximum Likelihood analysis of partial 16S rRNA gene sequence data. Sequence codes refer to those given in Table 2, where *Thp1*, *Thp2*, *Thp3*, Pong Dued, Thailand; *Phl*, Los Banós, Philippines; *Ths*, Sankhamphaeng, Thailand; *Tht*, Teppanom, Thailand; *Cht*, Daggyai Tso, Tibet, China. Unrooted trees are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1000 replications (second number), shown for branches supported by more than 50% of the trees. *Scale bar* represents 0.01 nucleotide changes per position

group that is most closely affiliated with sequences only previously recorded for geothermal springs in the continental USA, rather than geographically more proximal Asian sites. In addition, a single Tibetan sequence forms a distinct lineage separate from all other A/B and C9 sequences. Although more locations worldwide would need to be studied in order to make robust conclusions, it would appear that distinct phylogeographic groupings independent of temperature and pH do exist, as observed for north American and Japanese springs (Papke et al. 2003). There was no evidence for grouping of *Synechococcus*-like sequences from four different locations in our study on the basis of pH, although low pH-adapted genotypes distinct from those occurring at pH 6.1 and above (and most similar to our sequences) may exist in north American springs of pH 5 (Ruff-Roberts et al. 1994). The reason why some geochemically similar locations appear to support narrowly defined thermophilic genotypes, while others such as Daggyai Tso have far greater diversity is at present unclear. It may simply relate to greater diversity in predominantly *Synechococcus* mats compared to mats dominated by other cyanobacterial taxa, but factors such as age of springs and disturbance events may also be a factor. Whether the composition of moderately thermophilic mats is constant over time is currently unknown, although *Synechococcus* populations are known to be stable within mats over time and recover after disturbance (Ferris and Ward 1997; Ferris et al. 1997; Norris et al. 2002).

In conclusion, we have characterized community molecular diversity of moderately thermophilic cyanobacterial mats from a range of geothermal springs. The 16S rRNA gene-defined diversity of all mats exceeded that observed by microscopy. Genotypes resolved into distinct thermophilic phylogenetic lineages separate from their mesophilic counterparts. Furthermore, some evidence for phylogeographic patterns emerged among moderately thermophilic cyanobacteria, across relatively short regional and also inter-continental distances. These features are in general agreement with observations of more thermophilic *Synechococcus* mats, which suggests that cyanobacterial communities in moderately thermophilic habitats are affected by similar evolutionary pressures.

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