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Spatial heterogeneity of cyanobacterial communities and genetic variation of *Microcystis* populations within large, shallow eutrophic lakes (Lake Taihu and Lake Chaohu, China)

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Abstract

Cyanobacteria, specifically *Microcystis*, usually form massive blooms in eutrophic freshwater lakes. Cyanobacterial samples were collected from eight sites of both Lake Taihu and Lake Chaohu in late summer to determine the diversity and distribution pattern of cyanobacteria and *Microcystis* in large, shallow, entropic lakes with significant spatial heterogeneity and long-term *Microcystis* bloom. Molecular methods based on denaturing gradient gel electrophoresis and clone library analysis were used. A similar heterogeneous distribution pattern of cyanobacteria in both lakes was observed. Most parts of these two lakes with high trophic level were dominated by *Microcystis*. However, in the regions with low trophic levels as well as low concentrations of chlorophyll *a*, *Synechococcus* occupied a considerable percentage. Different morphospecies and genotypes dominated the bloom-forming *Microcystis* populations in these two lakes. *Microcystis viridis* and *Microcystis novacekii* were dominant in Lake Chaohu, whereas *Microcystis flos-aquae* was dominant in Lake Taihu. Only 2 of the13 *Microcystis* operational taxonomic units were shared between these two lakes. Analysis of molecular variance based on 16S to 23S internal transcribed spacer sequences indicated the significant genetic differentiation of *Microcystis* between these two lakes (F_{st} = 0.19, $p < 0.001$). However, only 19.46% of the genetic variability was explained by the population variation between lakes, whereas most (80.54%) of the genetic variability occurred within the lakes. Phylogenetic analysis revealed no phylogeographic structure of *Microcystis* population in these two lakes, as illustrated by their cosmopolitan nature. Our results revealed that spatial heterogeneity within lakes has more impact on the cyanobacterial diversity than geographical isolation in a local scale.

Key words: cyanobacteria; *Microcystis*; *Synechococcus*; bloom; diversity
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Introduction

In many eutrophic temperate freshwater ecosystems, cyanobacteria form dense surface blooms annually and dominate phytoplankton communities. Several cyanobacteria genera such as *Microcystis*, *Anabaena*, *Planktothrix*, *Aphanizomenon*, and *Cylindrospermopsis*, have been documented extensively to form harmful algal blooms (Chorus and Bartram, 1999). Algal blooms cause problems such as foul odors, poor aesthetics, and threats to public health (Briand et al., 2003; Falconer and Humpage, 2005). High nutrient loading and water temperature are the main contributors to the massive proliferation of algal blooms (Paerl et al., 2001; Downing et al., 2001). These bloom-forming genera have different ecophysiological behaviors in the water column and their distribution is influenced strongly by physicochemical attributes of the aquatic environments (Dokulil and Teubner, 2000).

Many studies have reported the large genetic diversity of cyanobacteria within and across water bodies (Taton et al., 2006; Miller and McMahon, 2011). The dominant cyanobacteria vary in different lakes or in different areas of the same lake (Dokulil and Teubner, 2000). For example, the proportion of N-fixing species such as *Anabaena* sp., is higher at the outer part of the bay of Lake Victoria, whereas *Microcystis* sp. is more abundant at the inner part (Haande et al., 2011). *Microcystis* is one of the most studied genera of cyanobacteria because of its cosmopolitan distribution. *Microcystis* bloom populations generally comprise various morphospecies and genotypes with different functional traits including colony formation, growth rate and potential toxicity (Wilson et al., 2005, 2006; Kardinaal et al., 2007). Many researchers (Wilson et al., 2005; Humbert et al., 2005; Tanabe et al., 2009) have studied the diversity and spatial distribution pattern of *Microcystis*. However, studies on cyanobacteria distribution pattern in large, shallow, eutrophic freshwater lakes with significant spatial heterogeneity and long-term *Microcystis* bloom occurrence are still scarce.

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Many molecular methods have been widely used for microbial diversity studies, such as denaturing gradient gel electrophoresis (DGGE) which is a powerful tool for monitoring biodiversity of microbial populations in various environmental samples (Muyzer and Smalla, 1998). In recent years, this method has also been widely used in studies on the diversity and distribution of cyanobacteria in aquatic systems (Janse et al., 2003; Vareli et al., 2009; Ye et al., 2011). The 16S to 23S internal transcribed spacer (ITS) is a DNA region with a suitable degree of sequence heterogeneity, and this molecular marker was proved to be effective to distinguish closely related *Microcystis* species or subspecies (Janse et al., 2003). Molecular methods based on ITS, such as DGGE and sequences analysis, are used extensively in investigating the genotype diversity and dynamics of *Microcystis* in natural assemblages (Kardinaal et al., 2007; Humbert et al., 2005; Briand et al., 2009).

The aim of the current study is to investigate the distribution pattern and diversity of cyanobacteria in two large, shallow, eutrophic freshwater lakes (Lake Taihu and Lake Chaohu) with significant spatial heterogeneity and long-term *Microcystis* bloom occurrence. This study also aims to determine whether genetic differentiation and biogeographic structure existed between the *Microcystis* populations in these two lakes.

1 Materials and methods

1.1 Sampling and physicochemical parameters

Lake Taihu and Lake Chaohu are two of the largest shallow freshwater lakes in Southeast China. Lake Taihu (30°55′–31°33′N, 119°53′–120°36′E), with an area of about 2338 km^{2}and mean depth of 1.9 m, is the third largest freshwater lake in China. Lake Chaohu (31°25′–31°43′N, 117°17′–117°52′E), with a total surface area of about 780 km^2, is the fifth largest freshwater lake in China. *Microcystis* blooms have occurred annually in both lakes in recent decades. *Microcystis* blooms generally accumulate in the most eutrophic regions of these lakes, and are sometimes distributed by the wind across the lakes. Cyanobacteria samples were collected from Lake Taihu and Lake Chaohu in September 2010. Eight separate sites across both lakes with different eutrophic levels were set (Fig. 1). The environmental parameters including temperature, dissolved oxygen (DO), pH and conductivity, were measured *in situ* using Yellow Spring Instruments (YSI 6600, USA). After the samples were taken to the laboratory, 200 mL aliquots of each sample were filtered through a GF/C glass filter. The filtrate was used for the analysis of NO*x*-N (NO_2^--N plus NO_3^--N), NH_4^+-N, and PO_4^{3-}-P based on standard methods (Jin and Tu, 1990). The membranes were stored at –70°C and used in determining chlorophyll *a* (Chl-*a*) and phycocyanin (Zhang and Huang, 1991; Asai et al., 2001), as well as in the subsequent DNA extraction.

1.2 Microscopic observations

Subsamples were fixed with 1% Lugol's iodine for microscopic observation. Morphological studies of the samples were carried out according to the morphological descriptions of the species conducted by Komárek and Anagnostidis (1999).

1.3 DNA isolation and PCR-DGGE

The GF/C glass filters containing the collected phytoplankton were cut into pieces. Subsequently, community DNA extraction was conducted using the potassium xanthogenate sodium dodecyl sulfate method, as described by Tillett and Neilan (2000). DGGE was used to investigate the distribution pattern of cyanobacteria. Primers CYA359F (5′-GGGGAATYTTCCGCAATGGG-3′) with a 40 bp GC-clamp (5′-CGCCCGCCGCGCCCCG CGCCGGTCCCGCCGCCCCCGCCCG-3′) attached to its 5′ end and CYA781R were used to amplify the

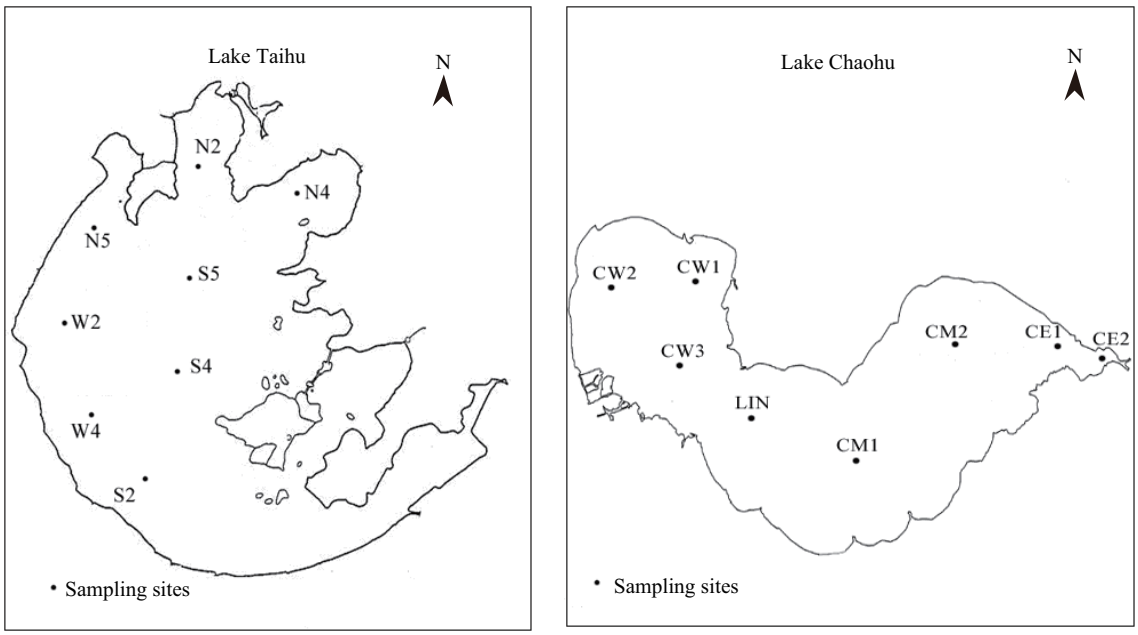

Fig. 1 Distribution of sampling sites in Lake Taihu and Lake Chaohu.

16S rRNA sequences of the cyanobacteria. CYA781R was an equimolar mixture of CYA781a (5′-GACTACTG GGGTATCTAATCCCATT-3′) and CYA781b (5′-GACT ACAGGGGTATCTAATCCCTTT-3′) (Nübel et al., 1997). Polymerase Chain Reaction (PCR) was performed in the Bio-Rad thermal cycler. PCR amplification was conducted as follows: 5 min at 94°C, 35 cycles consisting of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and a final extension step at 72°C for 5 min. All PCR products were verified on 1.2% (*W*/*V*) agarose gels stained with Goldview.

DGGE was performed using the DGGE-2001 system (CBS Scientific Company, USA). The PCR products were applied directly onto 6% (*W*/*V*) polyacrylamide gels with a denaturing gradient of 40%–70% (100% denaturant corresponding to 7 mol/L urea and 40% (*V*/*V*) formamide). Electrophoresis was performed in 1× TAE (40 mmol/L Tris-acetate (pH 7.4), 20 mmol/L acetate, and 1 mmol/L disodium EDTA) at 60°C for 16 hr at 75 V. After electrophoresis, the gels were stained in SYBR Green I (1:10,000 dilution; Molecular Probes) for 30 min, and then photographed using the Bio-Rad gel explorer. The DGGE band position and intensity were determined and modified manually using Quantity One software version 4.6 (Bio-Rad, USA). Cluster analysis of DGGE fingerprints, considering the band intensities, was conducted by the weighted pair group with mathematical averages (WPGMA) using Multivariate Statistical Package (MVSP) version 3.1 (Kovach Computing Services).

1.4 Cloning and sequencing

According to the result of the cluster analysis of the DGGE profile, two representative samples of each lake were selected to estimate the genetic diversity of cyanobacteria through the construction of 16S rRNA clone libraries. PCR was performed using primers CYA359F (without GC-clamp) and CYA781R according to the procedure described in Section 1.3.

A sample from each lake was collected in the site with dense *Microcystis* bloom to estimate the genetic diversity of *Microcystis* spp. The samples were used in the construction of clone libraries based on the ITS sequences. The primers, CSIF (5′-G(T/C)CACGCCCGAAGTC(G/A)TTAC-3′) and ULR (5′-CCTCTGTGTGCCTAGGTATC-3′), were used referred to a previous study (Janse et al., 2003). The amplification program comprised the following steps: an initial denaturation step at 94°C for 5 min, followed by 30 cycles for 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. Finally, the tubes were incubated for 10 min at 72°C. The PCR products were verified on 1.2% (*W*/*V*) agarose gels.

For each clone library, three PCR products were pooled, purified, and ligated into the pGEM-T vector (Promega, Germany), and then transformed into competent DH5α cells following the manufacturer's instruction. Approximately 50 positive clones in each clone library were selected for the sequencing, which was performed using the T7 primer by the Shanghai Sangon Biological Engineering Technology, Ltd., China. Sequences with more than 97% similarity were grouped in one operational taxonomic unit (OTU). Library clone coverage, Shannon-Wiener index and Pielou evenness index of these six clone libraries were calculated based on OTU distribution as described previously (Good, 1953; Shannon and Weaver, 1949; Pielou, 1966).

1.5 Phylogenetic trees and statistical analysis

One representative sequence from each OTU was selected for further phylogenetic analysis. First, these sequences were compared with those available in the National Center for Biotechnology Information database using Basic Local Alignment Search Tool to obtain their related sequences. Afterward, all partial 16S rRNA sequences and ITS sequences were imported into MEGA4 to generate two phylogenetic trees using the neighbor-joining method with the Jukes-Cantor distance correction (Jukes and Cantor, 1969). Patterns of the genetic variation based on the ITS sequence within and between lakes were estimated via analysis of molecular variance (AMOVA) using Arlequin software 3.1 (Excoffier et al., 2005). Comparison of the physiochemical factors between Lake Taihu and Lake Chaohu was performed using SPSS 17.0 software.

1.6 Nucleotide sequence accession numbers

The partial 16S rRNA sequences of cyanobacteria and ITS sequences of *Microcystis* obtained in the current study were deposited in GenBank under accession numbers JN646782 to JN646813.

2 Results

2.1 Physicochemical characteristics of Lake Taihu and Lake Chaohu

The spatial variation range of the environmental parameters in these two lakes is listed in Table 1. Samples were collected in September 2010, at which the water temperature in Lake Taihu and Lake Chaohu was above 25°C. The concentration of PO_4^{3-}-P in Lake Taihu was significantly lower than that in Lake Chaohu (ANOVA, $p < 0.05$), whereas the concentration of NO*x*-N (mg/L) and conductivity (mS/cm) were higher in Lake Taihu than in Lake Chaohu (ANOVA, $p < 0.05$). A high range in the concentrations of nutrients, PC and Chl-*a* was found in both lakes. The environmental parameters of the two samples of each lake used for clone library construction are also listed in Table 1. One site (N5 for Lake Taihu and CW2 for Lake Chaohu) had higher concentrations of phycocyanin, Chl-*a*, and PO_4^{3-}-P, and the other one had lower concentration of these parameters.

2.2 Morphological diversity

Five genera of cyanobacteria, including *Microcystis*, *Aphanizomenon*, *Anabaena*, *Pseudanabaena* and *Planktothricoides*, were observed in Lake Chaohu under a microscope. Within the *Microcystis* genus, *Microcystis aeruginosa*, *Microcystis viridis*, *Microcystis wesenbergii*,

Table 1 Environmental parameters of the four selected sites and the spatial variation range in Lake Taihu and Lake Chaohu

Variable	N5[a]	W4[a]	Lake Taihu	CW2[b]	CE2[b]	Lake Chaohu
Temperature (°C)	26.8	27.1	26.6–27.5	25.2	25.7	25.2–25.7
pH	9.55	9.51	9.51–9.66	8.18	8.50	8.18–9.19
DO (mg/L)	6.54	6.98	6.54–7.78	8.40	7.74	7.74–9.71
Conductivity (mS/cm)	0.63	0.67	0.57–0.68	0.25	0.24	0.19–0.25
NO*x*-N (mg/L)	0.40	0.96	0.12–1.23	0.19	0.10	0.01–0.77
NH_4^+-N (mg/L)	0.15	0.04	0.03–0.15	0.03	0.06	0.03–0.26
PO_4^{3-}-P (μg/L)	18.55	2.47	2.35–18.55	45.97	21.85	1.64–52.96
Chl-*a* (μg/L)	88.49	10.03	10.02–88.49	19.55	3.62	3.62–26.18
Phycocyanin (μg/L)	69.84	10.18	4.45–69.84	44.57	9.85	8.67–58.91

[a] Selected site of Lake Taihu used for clone library construction; [b] selected site of Lake Chaohu used for clone library construction.

and *Microcystis novacekii* were detected, with *M. viridis* and *M. novacekii* as the dominant species. The presence of cyanobacterial genera, such as *Microcystis, Anabaena*, and *Planktothricoides*, was observed in Lake Taihu. *Microcystis flos-aquae*, *M. aeruginosa*, *M. wesenbergii*, and *Microcystis ichthyoblabe* were detected, with *M. flos-aquae* as the dominant *Microcystis* species.

2.3 DGGE and cluster analysis

The spatial distribution pattern of cyanobacterial communities in Lake Taihu and Lake Chaohu was estimated via DGGE analysis based on the 16S rRNA gene sequence (Fig. 2a). Most of the samples from these two lakes showed a similar band pattern, and were consistent with the *Microcystis*-dominant sites. However, the band patterns of CE1, CE2, and CM2 samples at the east of Lake Chaohu were significantly different from those in other samples. The cluster analysis showed that all samples were classified into four clusters based on their DGGE fingerprints similarity: two for Lake Taihu and two for Lake Chaohu (Fig. 2b). Based on this result, two samples of Lake Chaohu (CE2 and CW2) and two samples of Lake Taihu (N5 and W4) were selected to construct four 16S rRNA clone libraries for the cyanobacterial diversity analysis. Sites CW2 and N5, representing dense *Microcystis* blooms, were selected in the analysis of the genetic diversity of *Microcystis* based on ITS.

2.4 Clone library analysis

Six clone libraries were constructed. CE2-359, CW2-359, N5-359, and W4-359 were the16S rRNA clone libraries used for cyanobacteria diversity analysis. CW2-ITS and N5-ITS were the ITS clone libraries used for *Microcystis* diversity analysis. The diversity analysis of these clone libraries is shown in Table 2. The coverage values were greater than 93%, suggesting that most of the cyanobacteria or *Microcystis* diversity were identified in all the clone libraries. For the four 16S rRNA clone libraries, W4-359

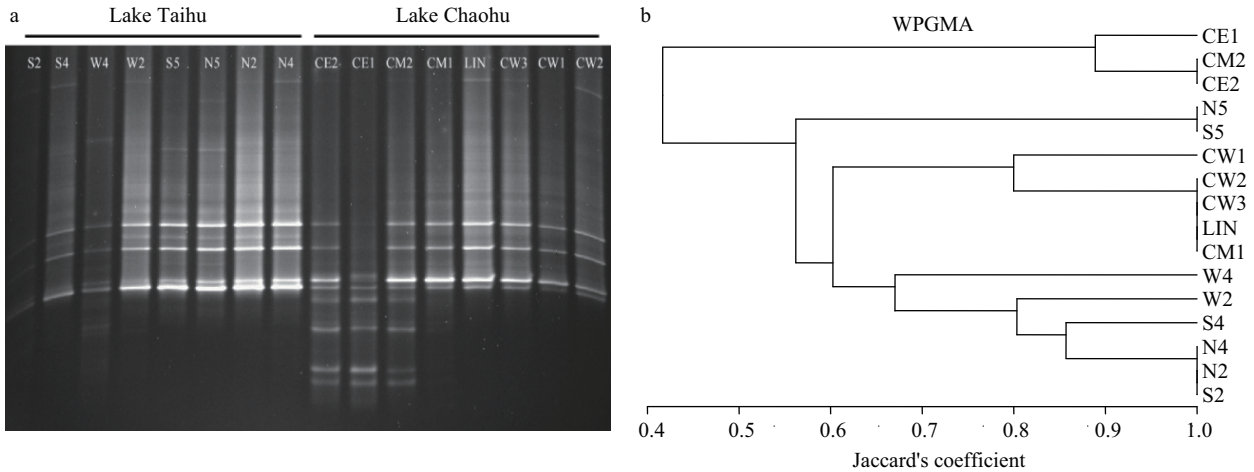

Fig. 2 (a) Denaturing gradient gel electrophoresis (DGGE) banding profile based on cyanobacterial 16S rRNA gene fragments from samples of Lake Taihu and Lake Chaohu; (b) weighted pair group with mathematical averages (WPGMA) dendrogram based on the similarities of DGGE profiles.

Table 2 Number of sequences and corresponding OTUs, coverage value, Shannon-Wiener index, and Pielou evenness index of the six clone libraries

Clone	Number of sequences	Number of OTUs	Coverage value (%)	Shannon-Wiener index	Pielou evenness index
CE2-359	45	4	98	0.861	0.591
CW2-359	35	4	94	0.474	0.402
W4-359	46	8	93	1.378	0.496
N5-359	37	1	100	0	1
CW2-ITS	45	7	98	1.568	0.685
N5-ITS	39	8	95	1.848	0.793

OTUs: operational taxonomic units; ITS: 16S to 23S internal transcribed spacer.

had the highest Shannon-Wiener index and N5-359 had the highest Pielou evenness index. For the two ITS clone libraries, N5-ITS had both higher Shannon-Wiener index and Pielou evenness index.

2.5 Genetic diversity of cyanobacteria

According to the closest relatives of all the OTUs (Table 3) in four cyanobacteria clone libraries, six OTUs related to four cyanobacteria genera were detected in Lake Chaohu, and eight different OTUs related to five cyanobacteria genera (one OTU W4-359-13 was related to the diatom) were detected in Lake Taihu. Three genera, namely, *Microcystis*, *Synechococcus*, and *Planktothricoides*, were detected in both lakes. *Pseudanabaena* was only detected in Lake Chaohu, whereas *Anabaena* and *Limnothrix* were only detected in Lake Taihu. The neighbor-joining tree shows the phylogenetic positions of all the representative cyanobacterial partial 16S rRNA gene sequences obtained from these four cyanobacterial clone libraries (Fig. 3).

The distribution of every OTU (indicated by their closest relatives) in these four clone libraries is shown in Fig. 4. *Microcystis* related OTU was dominant in the clone library CW2-359 (88.6%) of Lake Chaohu and the clone library N5-359 (100%) of Lake Taihu. However, *Synechococcus* related OTUs were dominant in the clone library CE2-359 (82.2%) of Lake Chaohu and the clone library W4-359 (60.9%) of Lake Taihu. All four *Microcystis* related OTUs in these four clone libraries belonged to the same OTU.

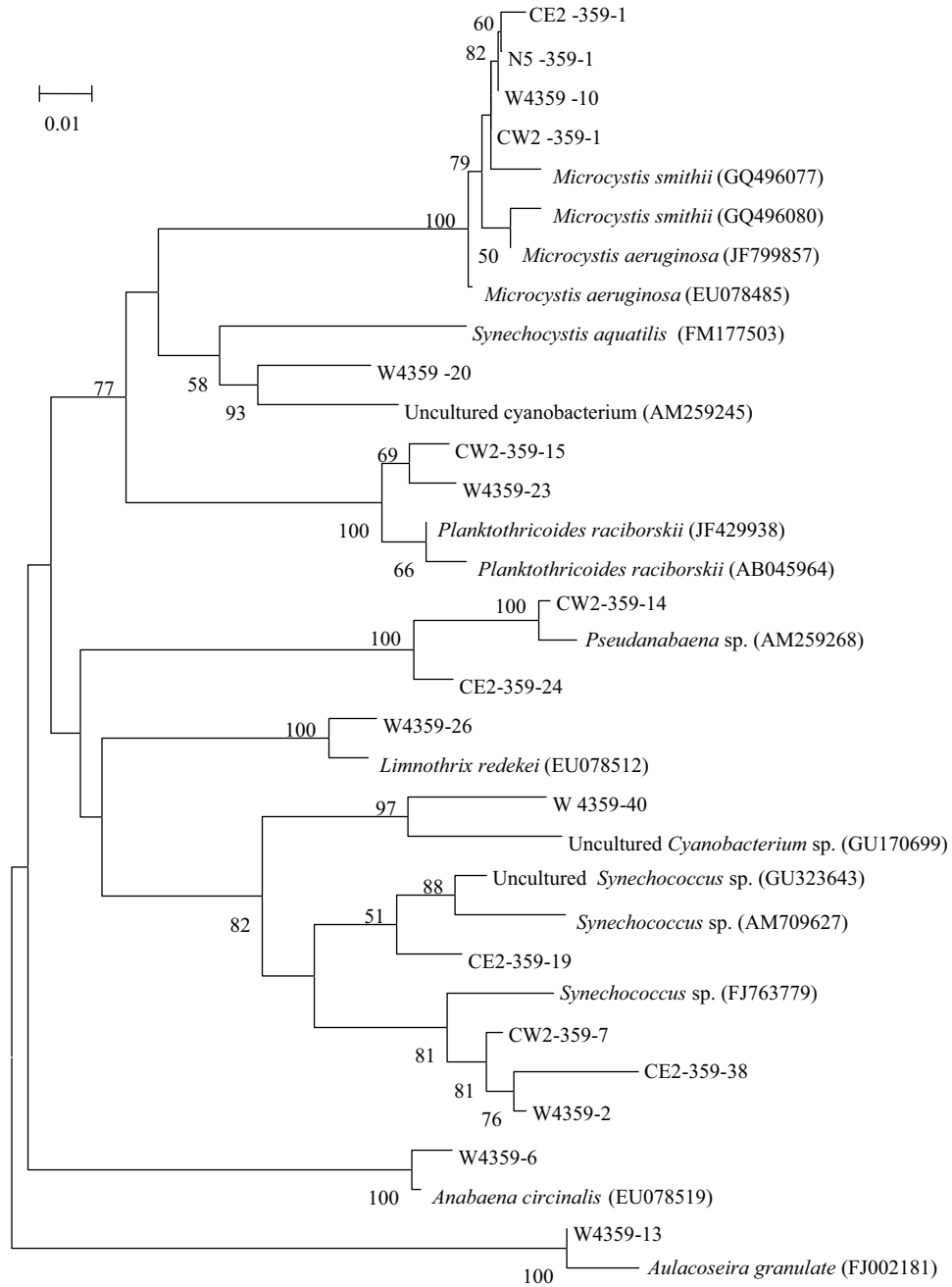

Fig. 3 Neighbor-joining tree showing phylogenetic relationships of representative partial 16S rRNA sequences obtained from four cyanobacterial clone libraries and the closest relative sequences obtained from GenBank (with their accession numbers in parentheses). Only bootstrap values above 50% (from 1000 bootstrap replicates) are shown at the nodes. Scale bar represents 0.01 substitutions per site.

Table 3 The closest relatives of OTUs in four cyanobacteria clone libraries in Lake Taihu and Lake Chaohu

Lake	Clone library	OTU[a]	Accession number	Closest relatives (Accession number)	Similarity (%)
Lake Chaohu	CE2-359	CE2-359-1	JN646782	*Microcystis* sp. (GQ496077)	100
		CE2-359-19	JN646783	Uncultured *Synechococcus* sp. clone (GU323643)	99
		CE2-359-24	JN646784	*Pseudanabaena* sp. (AM259268)	97
		CE2-359-38	JN646785	*Synechococcus* sp. (FJ763779)	99
	CW2-359	Cw2-359-1	JN646786	*Microcystis aeruginosa* G-01 (JF799857)	100
		Cw2-359-7	JN646787	*Synechococcus* sp. (FJ763779)	99
		Cw2-359-14	JN646788	*Pseudanabaena* sp. (AM259268)	100
		Cw2-359-15	JN646789	*Planktothricoides raciborskii* (JF429938)	99
Lake Taihu	N5-359	N5-359-1	JN646790	*Microcystis* sp. (GQ496077)	100
	W4-359	W4-359-2	JN646791	*Synechococcus* sp. (FJ763779)	99
		W4-359-6	JN646792	*Anabaena circinalis* (EU078519)	99
		W4-359-10	JN646793	*Microcystis* sp. (GQ496080)	100
		W4-359-13	JN646794	*Aulacoseira granulata* (FJ002181)	100
		W4-359-20	JN646795	Uncultured *cyanobacterium* (AM259245)	96
		W4-359-23	JN646796	*Planktothricoides raciborskii* (AB045964)	99
		W4-359-26	JN646797	*Limnothrix redekei* (EU078512)	100
		W4-359-40	JN646798	Uncultured *Cyanobacterium* sp. clone (GU170699)	95

[a] Operational taxonomic units.

However, the dominant *Synechococcus* related OTU in the clone libraries CE2-359 and W4-359 was different. The subdominant *Synechococcus* related OTU in the clone library CE2-359 was the same to the dominant one in the clone library W4-359. Two different *Pseudanabaena* related OTUs with small relative abundance (< 6%) were detected in clone libraries CE2-359 and CW2-359, and one *Planktothricoides* related OTU with a relative abundance of less than 9% was detected in clone libraries CW2-359 and W4-359. The relative abundances of other OTUs in the clone library W4-359 were all less than 6.5%. Without considering the minority in these OTUs, the distribution patterns of cyanobacteria in Lake Taihu and Lake Chaohu were very similar, with *Synechococcus* dominating the regions with relatively low concentration of Chl-*a* (also phycocyanin). This pattern was affected by the biomass of *Microcystis* in the bloom-forming period of Lake Taihu and Lake Chaohu.

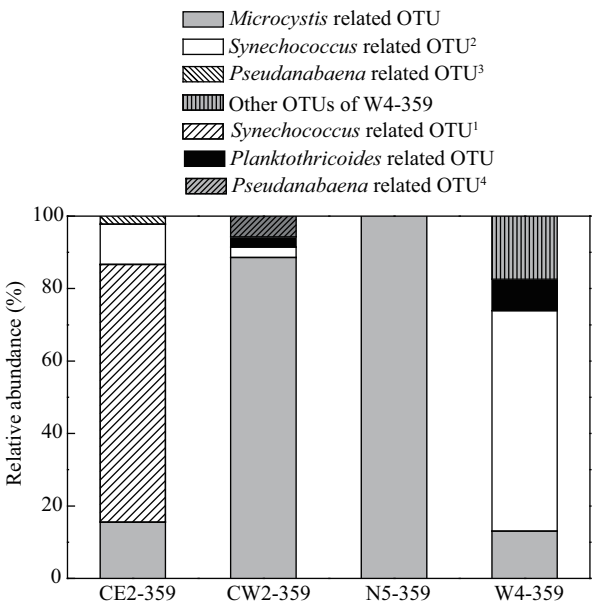

Fig. 4 Relative abundance of OUT (operational taxonomic unit) distribution in four 16S rRNA clone libraries of Lake Taihu and Lake Chaohu. OTU[1] and OTU[2] belonged to different *Synechococcus* OTUs. OTU[3] and OTU[4] belonged to different *Pseudanabaena* OTUs. Other OTUs of W4-359 included OTU related to *Anabaena* (6.5%), *Aulacoseira* (4.3%), *Limnothrix* (2.2%), and two OTUs related to uncultured cyanobacterium (4.3%).

2.6 Genetic diversity of *Microcystis*

Two clone libraries, N5-ITS from Lake Taihu and CW2-ITS from Lake Chaohu, were constructed for the genetic diversity analysis of *Microcystis* based on the ITS sequences. The 45 total sequences obtained from the clone library N5-ITS were divided into 8 OTUs, and 39 sequences obtained from the clone library CW2-ITS were divided into 7 OTUs. OTU CW2-ITS-4 dominated in Lake Chaohu (46.7%), whereas OTUs N5-ITS-6 and N5-ITS-1 dominated in Lake Taihu (25.6% and 23.1%, respectively). Aside from the specific OTUs in each lake, two OTUs were shared in these two lakes, as shown in Fig. 5, and were the dominant *Microcystis* OTUs in Lake Taihu. The percentage of each OTU in these two clone libraries is shown in Fig. 5.

AMOVA analysis showed a distinct genetic differenti-

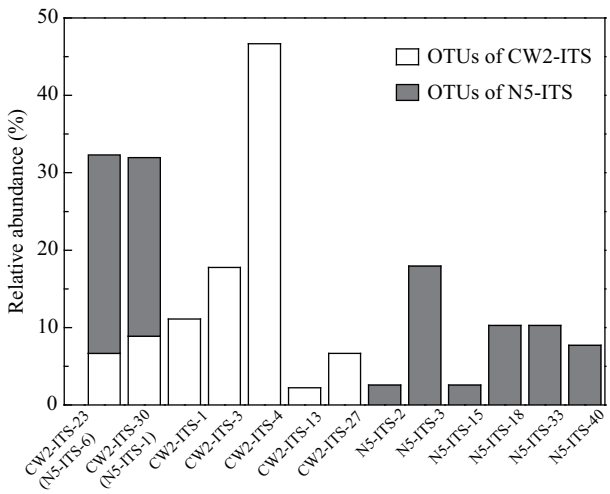

Fig. 5 Relative abundance of every *Microcystis* OTU (operational taxonomic unit) in two ITS (16S to 23S internal transcribed spacer) clone libraries from Lake Taihu and Lake Chaohu. CW2-ITS-30 and N5-ITS-1 were the same, CW2-ITS-23 and N5-ITS-6 were the same.

ation of *Microcystis* populations between these two lakes (F_{st} = 0.19, p < 0.001), and 80.54% of the variability occurred within the lakes instead of between lakes (19.46%). Most of the OTUs could not be classified into certain *Microcystis* morphospecies, but their phylogenetic relationship could be seen clearly in the phylogenetic tree (Fig. 6). According to Fig. 6, all the OTUs were divided mainly into two separate clusters. The dominant

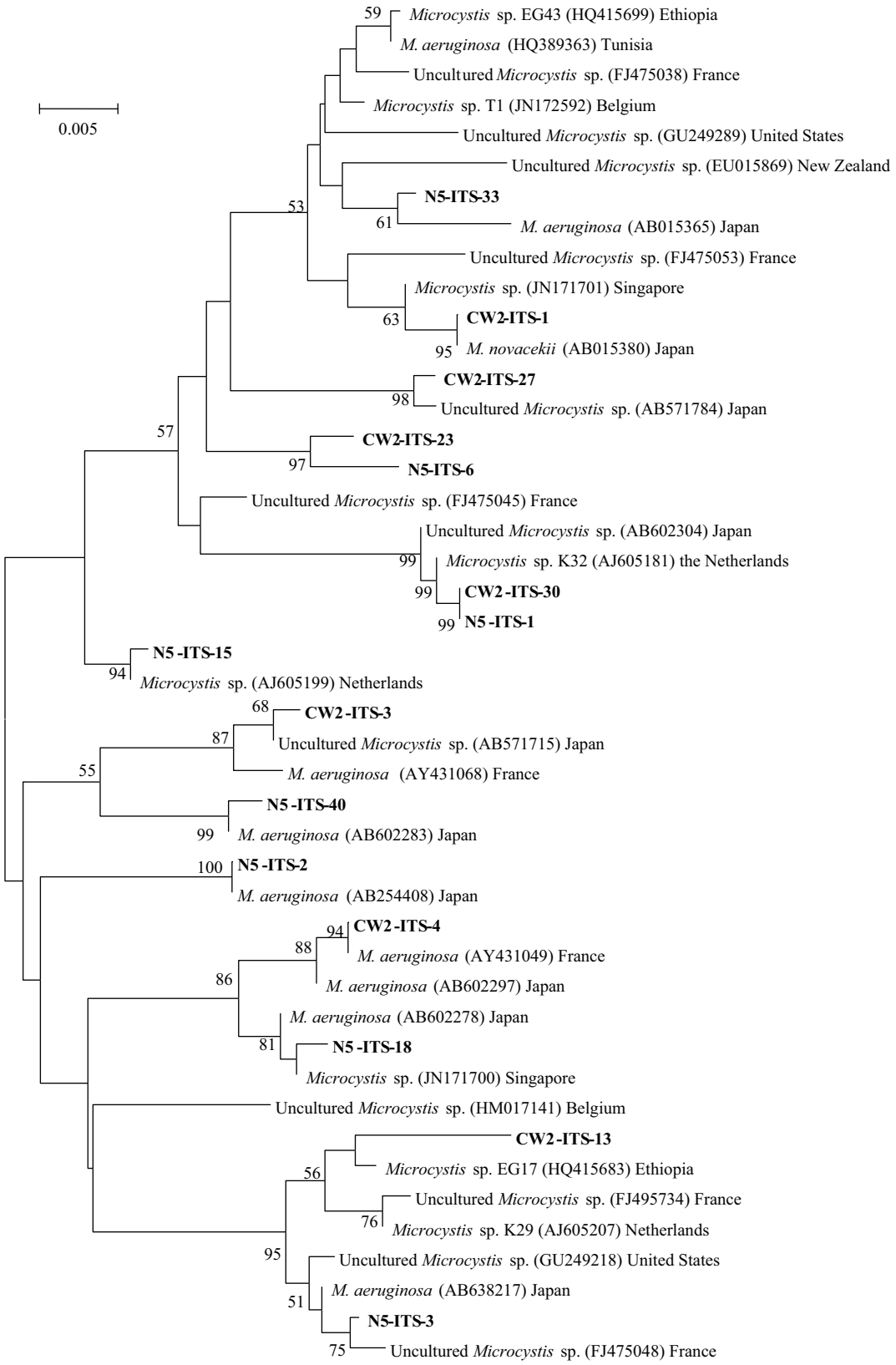

Fig. 6 Neighbor-joining tree showing phylogenetic relationships of *Microcystis* ITS (16S to 23S internal transcribed spacer) sequences obtained in this study and the related sequences from the GenBank database (with their accession numbers in parentheses). Sequences obtained in this study are marked in bold. Bootstrap values above 50% (from 1000 bootstrap replicates) are shown at the nodes. Scale bar represents 0.005 substitutions per site.

OTUs from Lake Taihu (N5-ITS-1 and N5-ITS-6) and Lake Chaohu (CW2-ITS-4) were in different clusters. However, other OTUs from these two lakes and many sequences obtained from several other countries around the world (Japan, Singapore, New Zealand, Ethiopia, Tunisia, France, Belgium, Netherlands, and United States) were intermixed in the two clusters and were not grouped based on their origin.

3 Discussion

3.1 Distribution pattern and diversity of cyanobacteria

Lake Taihu and Lake Chaohu are two of the most famous large, shallow, eutrophic lakes in China, which have suffered from cyanobacterial bloom for several decades. Combining the results of microscopic observation and molecular method, the overall cyanobacterial diversity of these two lakes was discovered to be similar in the genus level, in spite of some rare species. *Microcystis* was dominant in most of the sample sites in these two lakes. This similarity in cyanobacterial diversity may be due to the similar physical and chemical environment conditions of these two lakes. They are both large, shallow, eutrophic lakes with violent disturbances by wind-driven waves, and are located in Southeast China under similar latitude. Similar cyanobacterial communities were also found in two shallow, eutrophic Mediterranean lakes in Greece, as documented by Kormas et al. (2010). Through comparison of the genetic structure of cyanobacteria in four neighboring eutrophic lakes, Miller and McMahon (2011) indicated that the differences of cyanobacterial community between these lakes are small and the diversity of cyanobacteria could be explained by the heterogeneity within lakes.

Lake Taihu and Lake Chaohu have large surface areas, and the distribution of pollution sources in the surrounding areas was uneven. These factors led to the different nutrient levels in different regions of these two lakes (Deng et al., 2007; Qin, 2008), as demonstrated by the large range of PO_4^{3-}-P concentration within both lakes (Table 1). Sites CW2 and N5 represented the hypereutrophic regions of these two lakes. CW2 is adjacent to Hefei City and three seriously contaminated rivers, namely, Nanfei River, Pai River and Shiwuli River. Site N5 is near Zhushan Bay, situated in the northwest part of Lake Taihu. Caoqiao River is one of the main inflow river channels of Zhusan Bay. Hundreds of industrial facilities are distributed densely along the Caoqiao River (Yao and Xue 2010). The dense accumulation of *Microcystis* blooms in these two sample sites can be ascribed to better growth conditions for the bloom-forming *Microcystis* in these areas. *Microcystis* demonstrated a preference to eutrophic conditions (Paerl et al., 2001). The Currents or winds might also leading to the accumulation of *Microcystis* blooms in these areas (Briand et al., 2009). This heterogeneous distribution of *Microcystis* blooms could enhance the spatial heterogeneity within these two lakes.

In regions with lower eutrophic level (CE2 and W4, also with lower concentration of phycocyanin and Chl-*a*), picocyanobacteria *Synechococcus* was dominant in the cyanobacterial community. These results showed a similar heterogeneous distribution of cyanobacteria in these two bloom-forming lakes, and were consistent with results of previous investigations in other 32 lakes. The percentage contribution of picocyanobacteria (mainly represented by *Synechococcus* spp.) could exceed 70% when Chl-*a* concentration was below 10 μg/L, and no more than 10% when Chl-*a* concentration was above 100 μg/L (Vörös et al., 1998). *Synechococcus* are ubiquitous autotrophic picoplankton in marine and freshwater systems. They contribute significantly to the primary production of ecosystems (Postius and Ernst, 1999). Hu et al. (2004) found that the growth of *Synechococcus* could be inhibited by cyanotoxin microcystin-RR, which is produced by freshwater cyanobacteria, such as *Microcystis*. *Microcystis* can change buoyancy provided by gas vesicles in response to irradiance and nutrients, unlike *Synechococcus* (Visser et al., 2005). However, *Synechococcus* has the advantage of nutrient acquisition because its small size enables it to acquire and use resources more effectively than larger phytoplankton cells (Raven, 1998). Ye et al. (2011) found that the quantity of *Synechococcus* was about 10 times more than that of *Microcystis* during the bloom seasons, as estimated by real-time PCR techniques based on the samples collected in a eutrophic region of Lake Taihu in 2006. The relationship between *Synechococcus* and *Microcystis* may be very complex, and their interaction in the bloom-forming eutrophic lakes require further study.

3.2 *Microcystis* diversity and differentiation in Lake Taihu and Lake Chaohu

Microcystis was detectable in all sampling sites. AMOVA analysis also showed a distinct genetic differentiation of *Microcystis* between these two lakes (F_{st} = 0.19, p < 0.001), but only 19.46% of the genetic variability was explained by population variation between lakes. Most (80.54%) of the genetic variability occurred within lakes. Similarly, a study of *M. aeruginosa* diversity in a number of geographically closely situated lakes in the southern peninsula of Michigan showed that *M. aeruginosa* was genetically diverse within and among lakes, and 59% of the genetic variation was explained by within population variation (Wilson et al., 2005). Other studies of the spatial genetic diversity of *Microcystis* in 15 lakes of Europe and Morocco and 4 reservoirs of Brazil also confirmed a considerable genetic variation within and among lakes (Bittencourt-Oliveira et al., 2001; Janse et al., 2004). All these studies conducted in different lakes revealed that most of the genetic variation of *Microcystis* could be explained by within-habitat rather than among-habitat differences. Tanabe et al. (2009) indicated that local genetic drift or selection might have far more impact on the fine-scale spatial genetic diversity of *M. aeruginosa* than geographic factors.

Both classical microscopy and ITS sequence analysis revealed the differentiation of *Microcystis* populations in these two lakes. The difference of environmental condi-

tions might responsible for this differentiation between these two lakes. Sabart et al. (2009) pointed out that the community structure of *Microcystis* was determined by very local selection pressures in each aquatic ecosystem. Several biotic or abiotic factors have been reported to shape the composition of *Microcystis* populations. Xu et al. (2011) found that pH was the primary factor for the marked changes of composition and proportion of the *Microcystis* ITS genotype from the upper to the lower reaches of Qinhuai River in China. The nutrient availability (nitrogenous compounds and phosphorus) also showed a significant impact on the proportion of *Microcystis* with or without the microcystin synthetase gene (Davis et al., 2010). In addition to the influences of local conditions, van Gremberghe et al., (2009a) pointed out that priority effects (i.e. the arrival order of colonists) between different *Microcystis* strains might have significant effects on the final composition of *Microcystis*. The influence of grazing impact from different grazers on *Microcystis* community dynamics has also been reported (van Gremberghe et al., 2009b). Although several environmental parameters (e.g., temperature, conductivity, and PO_4^{3-}-P) and the dominant level of *Microcystis* vary between Lake Taihu and Lake Chaohu, more data from different lakes in this region are needed to assess the real impact of these factors on *Microcystis* population structure.

3.3 Phylogenetic analysis of *Microcystis* ITS sequences

According to the result of the phylogenetic analysis, the two generated clusters in the phylogenetic tree were not related to the geographical origin of the ITS sequences except for the dominant OTU of each lake. This means a high phylogenetic relatedness existed between these sequences derived from Lake Taihu and Lake Chaohu. High genetic relatedness of *Microcystis* between strains from geographically distant regions of the northern hemisphere was also observed by Wu et al. (2007) based on the PC-IGS sequence analysis. The ITS sequences in this study was compared with those available in GenBank, and the result showed that most *Microcystis* strains were also observed in other freshwater systems like in Japan, France, Ethiopia, New Zealand, and United States. The lack of biogeographic structure of these sequences suggested that most of the *Microcystis* genotypes in these two lakes are likely to be ubiquitous, as concluded by Sabart et al. (2009). The study compared the *M. aeruginosa* ITS sequences derived from the regions near Loire River in France with those available in GenBank. Most recently, van Gremberghe et al. (2011) pointed out that *M. aeruginosa* might have a true cosmopolitan distribution based on the phylogeography study of 311 ITS sequences derived from six continents.

4 Conclusions

Our data present an image of the diversity and distribution pattern of cyanobacteria within and between two large eutrophic lakes suffering from serious *Microcystis* blooms. *Microcystis* dominated in regions with high trophic level, whereas *Synechococcus* dominated in regions with lower trophic level and low Chl-*a* concentration. Spatial heterogeneity could be the main reason explaining the cyanobacterial diversity and heterogeneous distribution in these two lakes rather than geographical isolation. Evident differentiation of *Microcystis* populations between these two lakes was found, although they were not phylogeographically structured according to the result of the phylogenetic analysis. The ecological implications of different dominant *Microcystis* morphospecies or genotypes and the factors determining their diversity and distribution require further study.

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