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Diversity and dynamics of picocyanobacteria and bloom-forming cyanobacteria in a large shallow eutrophic lake (lake Chaohu, China)

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ABSTRACT

The diversity and succession pattern of cyanobacteria, particularly picocyanobacteria and bloom-forming cyanobacteria, were examined monthly in a eutrophic lake (lake Chaohu) in China using a combination of light microscopy and 16S ribosomal RNA (rRNA) sequence analysis. The results showed that both picocyanobacteria and bloom-forming cyanobacteria have high levels of diversity. Microcystis *and* Anabaena *were the two predominant bloom-forming genera, and two obvious shifts occurred between them from spring to winter.* Anabaena *was dominant in spring, then it was rapidly replaced by* Microcystis *in summer and became dominant again in late autumn and early winter. Apart from water temperature, three forms of dissolved nitrogen (NO_3-N, NO_2-N, and NH_4-N) were important driving factors for their seasonal succession, as demonstrated by redundancy analysis. Clone libraries and sequence analysis revealed that picocyanobacteria (mainly* Synechococcus*-like) are also important cyanobacteria members in lake Chaohu. All 8 picocyanobacterial phylotypes belonged to the* Cyanobium *clade. The phylotypes could be further grouped into at least 7 distinct clusters, and 4 of these clusters do not belong to any of the previously described clusters. Picocyanobacteria accounted for more than 70% (percentage in the clone library) in March and April but only accounted for less than 10% from June to October during the* Microcystis *bloom. The relative abundance of picocyanobacteria was positively correlated with the mass ratio of dissolved inorganic nitrogen and phosphorus (*r*=0.965, P<0.01,* n*=10) and ammonium concentration (*r*=0.721, P<0.05,* n*=10).*

Key words: bloom-forming cyanobacteria, picocyanobacteria, Microcystis, Anabaena, *dynamics.*

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INTRODUCTION

Cyanobacteria are often dominant in autotrophic planktonic communities in eutrophic freshwater bodies worldwide (Dokulil and Teubner, 2000). Numerous cyanobacterial genera can form dense blooms, such as *Microcystis*, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Planktothrix* (Graham *et al*., 2010). Some of these cyanobacteria produce volatile compounds that produce odour and a wide range of toxins that are harmful to humans, domestic livestock, wild animals, and the aquatic environment (Stewart *et al*., 2008).

Studies on cyanobacteria in many eutrophic lakes usually focus on a few bloom-forming genera because of their large biomass and possible toxicity (Dokulil and Teubner, 2000). These bloom-forming genera persist for several months because of their physiological and metabolic advantages over other phytoplankton (Oliver and Ganf, 2000). During such a long period, cyanobacterial succession can occur among different genera or different morphological species of one dominant genus (Imai *et al*., 2009; Soares *et al*., 2009), which is possibly driven by different factors such as water temperature (Xu *et al*., 2010), ability to capture light (Kardinaal *et al*., 2007), and nutrient utilisation strategies (Rücker *et al*., 1997).

Information about the diversity and dynamics of picocyanobacteria in eutrophic and hypereutrophic freshwater environments is relatively sparse (Vörös *et al*., 1998; Hirose *et al*., 2003; Felföldi *et al*., 2009). Picocyanobacteria, a group of small autotrophic cyanobacteria with diameters ranging from approximately 0.2 to 2 μm (Raven, 1998), are ubiquitous in a variety of freshwater environments (Callieri and Stockner, 2002; Callieri, 2008) and contribute to a significant portion of total primary production (Stockner *et al*., 2000). Most former studies about freshwater picocyanobacteria focus on oligotrophic and mesoeutrophic habitats (Ivanikova *et al*., 2007; Becker *et al*., 2007; Wu *et al*., 2010; Callieri *et al*., 2012). The low biomass and small size of these habitats compared with other bloom-forming cyanobacteria in eutrophic water bodies have excluded picocyanobacteria in former studies based on light microscopy (Ouellette *et al*., 2006).

Molecular methods show that picocyanobacteria are abundant in eutrophic lakes (Kolmonen *et al*., 2004; Ouellette *et al*., 2006; Ye *et al*., 2011). Molecular methods are

useful for detecting and classifying cyanobacteria, especially picocyanobacteria, because they lack sufficient reliable morphologic features (Raven, 1998). One of the most appropriate targets for the analysis of environmental samples is the *16S ribosomal RNA (rRNA)* gene because of its preserved structure and function. Also, it has wildly been used in investigations on cyanobacterial diversity (Kirkwood *et al*., 2008; Foster *et al*., 2009; Kormas *et al.*, 2010; Lymperopoulou *et al*., 2011). However, polymerase chain reaction (PCR)-based molecular methods may have potential bias during DNA extraction, PCR, and cloning (von Wintzingerode *et al*., 1997). Therefore, the combination of molecular methods and traditional light microscopy can provide objective results in the study of cyanobacterial diversity.

In-depth information about the diversity and dynamics of cyanobacteria, particularly picocyanobacteria, in shallow eutrophic lakes with serious cyanobacterial blooms is still limited. The aim of this study was to obtain a comprehensive view of the diversity and dynamics of cyanobacteria, specifically the picocyanobacteria and the bloom-forming cyanobacteria in a large, shallow eutrophic lake, lake Chaohu. Their diversity and seasonal succession pattern were investigated using a combination of 16S rRNA clone library analysis and traditional light microscopy. Redundancy analysis (RDA) and correlation analysis were conducted to determine the possible factors that drive seasonal succession.

METHODS

Study site

Lake Chaohu (31° 25'-31° 43' N, 117° 17'-117° 52' E) is located in Southeast China and it has a total surface area of approximately 780 km^2 and an average depth of 2.7 m. It is the fifth largest freshwater lake in China. Apart from being a source of industrial and agricultural water, lake Chaohu is also a source of drinking water for nearby cities. The hypertrophic west region of lake Chaohu, which is near Hefei City, has suffered serious cyanobacterial blooms for several decades (Xie, 2009).

Sampling and physicochemical measurements

Monthly sampling was conducted from September 2010 to August 2011 during cyanobacterial blooms (except January and February 2011 when the bloom disappeared) in three adjacent sites in the west region of lake Chaohu (Fig. 1). Water samples were collected from the surface layer (0 to 50 cm) using a 5 L Perspex sampler (Perspex, Weybridge, UK) and transferred to 5 L plastic containers. All of the containers were transported to the laboratory within 4 h and stored at 4°C until filtration. Water temperature, conductivity, dissolved oxygen (DO), and pH were measured on-site using a YSI meter (YSI 6600; Yellow Springs Instruments, Yellow Springs, OH, USA). The water samples were filtered through GF/C filters (Whatman, Maidstone, UK) and used for measuring dissolved nutrients [phosphate (PO_4-P), nitrate (NO_3-N), nitrite (NO_2-N), and ammonium (NH_4-N)] according to standard methods (Jin and Tu, 1990). The mass ratios of dissolved inorganic nitrogen (DIN; NH_4-N plus NO_3-N plus NO_2-N) and dissolved inorganic phosphorus (DIP; PO_4-P) (DIN:DIP) were also calculated based on their concentrations.

Light microscopic observations

Subsamples were fixed with 1% Lugol's iodine and used for enumeration and phytoplankton identification under a light microscope after concentration according to the Utermohl sedimentation method (Utermohl, 1958). The biovolume ($mm^3 L^{-1}$) of each species was calculated by multiplying its density by its estimated cell size (Hillebrand *et al*., 1999). These biovolumes were converted into biomass (mg L^{-1}) based on a phytoplankton cell density of 1 mg mm^{-3}.

16S ribosomal RNA sequence analysis

Ten samples collected at site CW1 from September 2010 to August 2011 were used for the genetic analysis of cyanobacteria. For DNA extraction, 200 mL of the water sample was filtered through a GF/C glass fiber filter. Although some picocyanobacteria may be loose when using the GF/C glass fiber filter, other filters with smaller pore size could not be used because of an extreme clogging by colonial and filamentous cyanobacteria after the first 10 mL of some water samples. Subsequently, the filter was cut into pieces. Genomic DNA extraction was conducted according to the method by Tillett and Neilan (2000). Primers CYA359F (5'-GGGGAATYTTCCG-

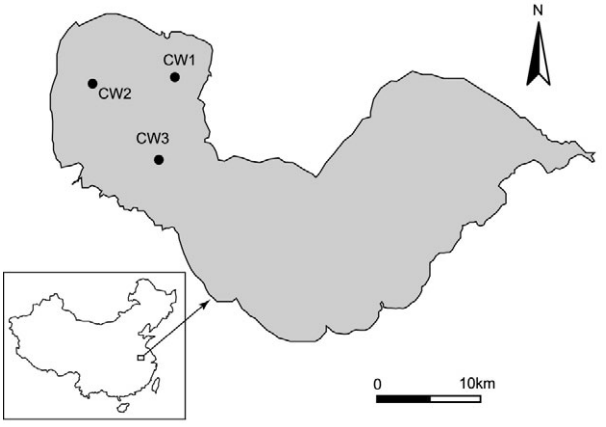

Fig. 1. Distribution of sampling sites in lake Chaohu in Central-East China (lower-left box).

CAATGGG-3') and CYA781R were used to specifically amplify the 16S rRNA sequences from cyanobacteria (although not exclusive). CYA781R is an equimolar mixture of CYA781a (5'-GACTACTGGGGTATCTAATCCCATT-3') and CYA781b (5'-GACTACAGGGGTATCTAATCCCTTT-3') (Nübel *et al*., 1997). All PCR reactions (final PCR volume of 25 μL) were performed using a Bio-Rad thermal cycler (Bio-Rad, Hercules, CA, USA). Polymerase chain reaction amplification was conducted as follows: 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and a final extension step at 72°C for 5 min.

For the clone library construction, three PCR products from each sample were mixed, purified, and cloned into the pGEM-T vector (Promega, Fitchburg, WI, USA) following the manufacturer's instructions. For each clone library, approximately 50 positive clones were randomly selected for sequencing using the T7 primer. Sequences with more than 97% similarity were treated as a phylotype. The coverage of the clone libraries based on Good's C estimator and the number of predicted phylotypes based on the S_{Chao1} index for each clone library were estimated according to the method by Kemp and Aller (2004). One representative sequence for each phylotype was selected, and its closest relative was obtained from the GenBank database after submitting the sequence to the BLAST search programme in the National Center for Biotechnology Information website. Afterwards, all partial 16S rRNA sequences and their relative sequences were imported into MEGA 4 to generate a phylogenetic tree using the neighbour-joining method with Jukes-Cantor distance correction. The partial 16S rRNA sequences of the cyanobacteria obtained in this study were deposited in GenBank under accession numbers JN944404 to JN944419.

Statistical analysis

Redundancy analysis was performed using CANOCO 4.5 to reveal the main factors that drive the seasonal succession of the dominant bloom-forming cyanobacteria. The tested environmental variables were temperature, conductivity, DO, pH, PO_4-P, NO_3-N, NO_2-N, NH_4-N, and DIN:DIP. All data were log(x+1)-transformed before RDA analyses. The forward selection of RDA was performed using Monte Carlo simulations with 499 unrestricted permutations. Explanatory factors (environmental variables) were considered significant when $P<0.05$. Pearson's correlation analysis was performed using the SPSS 17.0 software to analyse the relationship between picocyanobacteria and environmental variables.

RESULTS

Physicochemical characteristics of lake Chaohu

The water temperature of lake Chaohu peaked (nearly 30°C) in July and reached its nadir in December (approximately 5°C) during the sampling period. Water conductivity varied from 0.157 to 0.408 mS cm^{-1}, water pH was maintained at around 8 and reached a slight peak in July. The DO concentrations generally remained below 10 mg L^{-1} from April to November, but were considerably higher in December and March. The concentrations of NO_2-N, NO_3-N, and NH_4-N varied widely during the sampling period. The PO_4-P concentration sharply increased from March to August (except July), with a significant peak in August that gradually decreased until March. The DIN:DIP ratio showed considerable variation, with the smallest value of 1.5 in August and the highest value of 995.6 in March (Fig. 2).

Morphological results

During the sampling period, the cyanobacterial biomass ranged from 2.6 to 44.3 mg L^{-1}. Cyanobacteria often accounted for more than 60% of the total phytoplankton biomass (Fig. 2). Nineteen cyanobacteria species belonging to 13 genera were identified under a microscope in the 30 samples. *Microcystis* (4 morphological species, namely *M. viridis*, *M. novacekii*, *M. aeruginosa,* and *M. wesenbergii* were detected; the former 2 were dominant), *Anabaena* (*A. circinalis*), and *Aphanizomenon* (*A. flos-aquae*, *A. gracile*, and *A. issatschenkoi*) were the three dominant genera. The other ten genera, namely, *Pseudanabaena*, *Dactylococcopsis* (*D. irreguloris* and *D. acicularis*), *Planktothrix*, *Lyngbya*, *Spirulina*, *Raphidiopsis*, *Merismopedia*, *Chroococcus*, *Aphanothece*, and *Aphanocapsa* were fewer or were only detected occasionally. Two clear shifts were observed between *Microcystis* and *Anabaena* during the sampling period (Fig. 3). *Microcystis* was exclusively dominant in warmer months, accounting for more than 99% in July and August. By contrast, *Anabaena* was exclusively dominant in cooler months, accounting for more than 94% in December, March, April, and May. *Aphanizomenon* was the subdominant genus, appearing in early summer and late autumn during the shift between *Microcystis* and *Anabaena*.

Redundancy analysis

Four significant environmental variables, namely temperature ($P=0.002$, $F=15.34$), NO_3-N ($P=0.002$, $F=9.26$), NO_2-N ($P=0.004$, $F=5.51$), and NH_4-N ($P=0.022$, $F=3.62$) were obtained after the forward selection with Monte Carlo permutation test. The RDA ordination of the dominant cyanobacteria genera (*Microcystis*, *Anabaena*, and *Aphanizomenon*) and the samples from the 3 sites in relation to these variables are shown in Fig. 4. The first RDA axis accounted for 85.8% of the relationship between species and environmental variables. This axis was mainly positively correlated with water temperature and

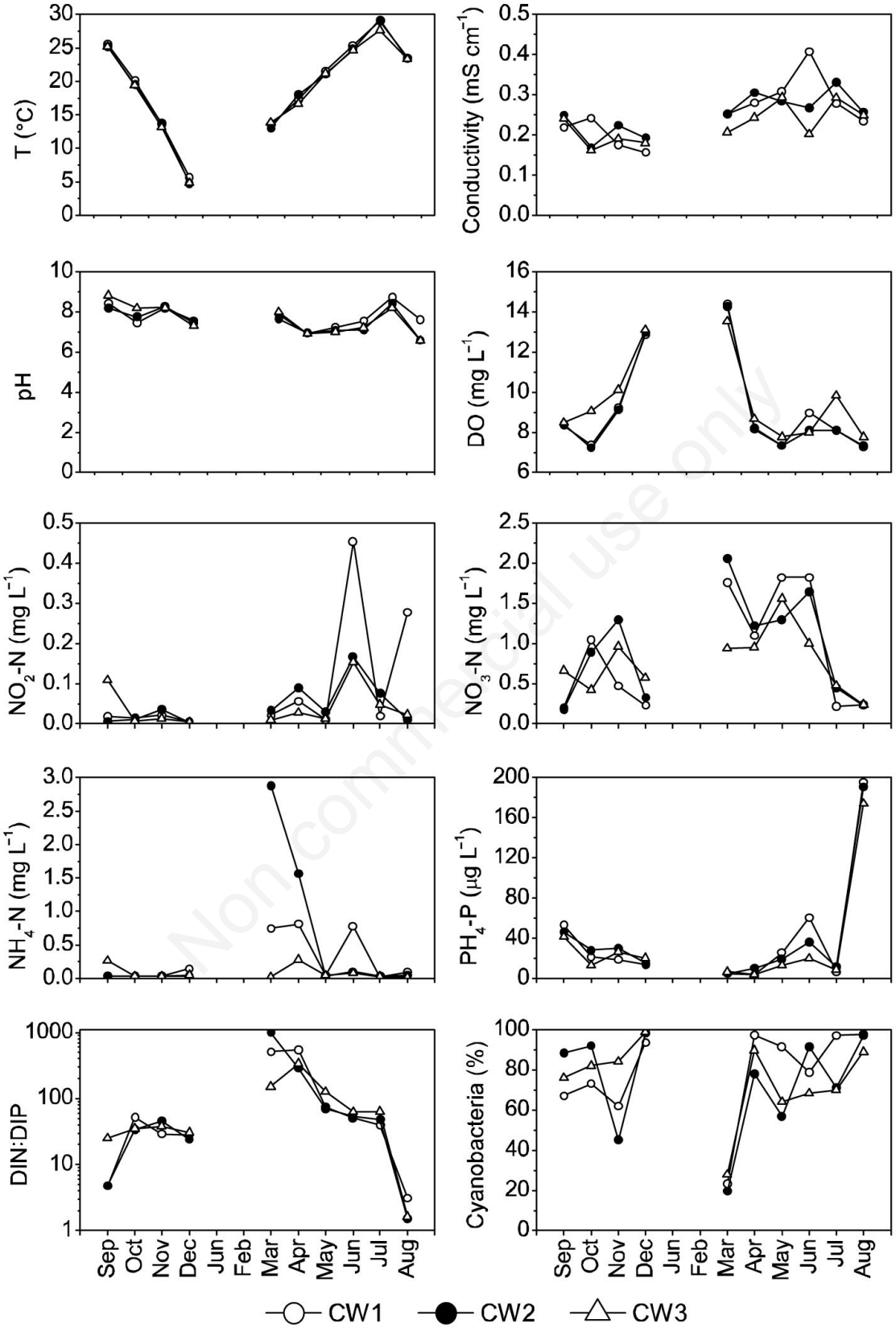

Fig. 2. Annual variations in physicochemical parameters, nutrients, and relative abundance of cyanobacteria in total phytoplankton (based on biomass) in three sampling sites (CW1, CW2, and CW3) in lake Chaohu from September 2010 to August 2011.

negatively correlated with NO_3-N and NH_4-N. Axis 2 was mainly positively correlated with NO_2-N. These 4 significant variables explained 65.3% of the total variance in the cyanobacteria community (Tab. 1). The most discriminant variable was temperature, which explained 35% of the total variance, followed by NO_3-N (17%), NO_2-N (8%), and NH_4-N (5%).

Sequence and phylogenetic analysis

Ten samples collected from CW1 were selected to construct clone libraries (1 library per month) for further genetic analysis. The coverage of the clone libraries was satisfactory according to Good's C estimator, and more than 61.5% of the predicted phylotypes were obtained for most of the clone libraries based on the S_{Chao1} index (Fig. 5). A total of 421 clones were analysed, corresponding to 16 cyanobacteria (383 clones), 1 *Cryptomonas* chloroplast (13 clones), 1 *Teleaulax* chloroplast (8 clones), 1 *Thalassiosira* chloroplast (15 clones), 1 uncultured phototrophic eukaryote chloroplast (1 clone), and 1 uncultured bacterial (1 clone) phylotype.

All the representative sequences of the 16 cyanobacterial phylotypes and their closest relatives are shown in Tab. 2. Phylotypes M1 and A1 were related to *Microcystis* and *Anabaena*, respectively. Some of the other phylotypes belonged to *Pseudanabaena*, *Woronichinia naegeliana*, and uncultured cyanobacteria. However, 8 phylotypes (S1 to S8) were related to picocyanobacteria: 3 (S1 to S3) to *Cyanobium,* and 5 (S4 to S8) to *Synechococcus*. These picocyanobacterial sequences had considerable diversity, with a maximum of 5% in pairwise comparisons (423 bp of 16S rRNA). *Synechococcus elongatus* PCC7942 was used as the outgroup in the phylogenetic analysis of picocyanobacteria. All the picocyanobacterial sequences derived in this study clustered into the *Cyanobium* clade (*sensu* Ernst *et al*., 2003), which contains nearly all freshwater-derived picocyanobacterial sequences and was distant from the marine *Synechococcus* clade. However, these picocyanobacterial sequences were grouped into at least 7 distinct clusters in the phylogenetic tree (Fig. 6). Four clusters, namely phylotypes S2, S6, S7, and S8, did not belong to any previously described lineages within the

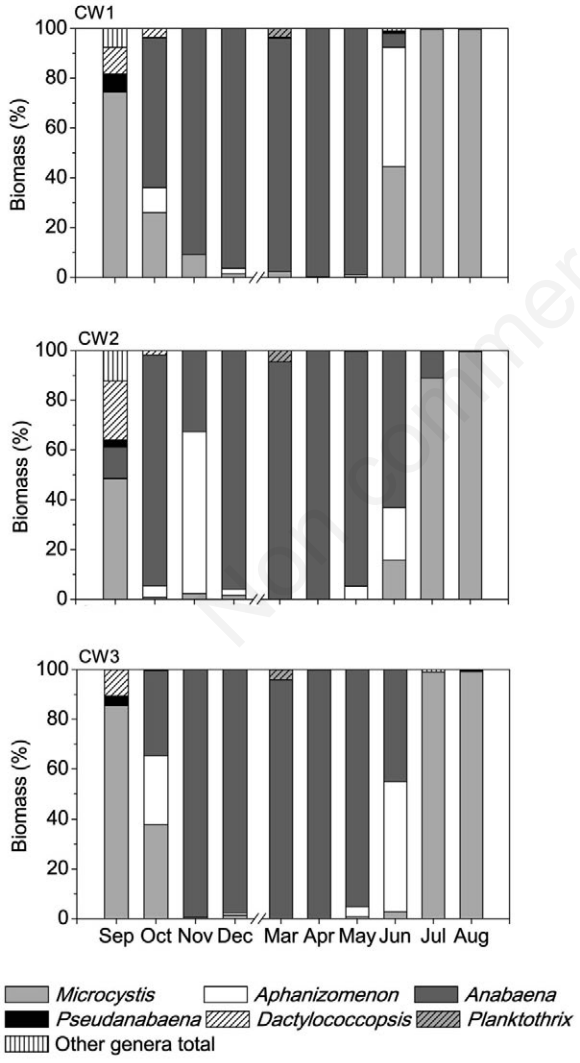

Fig. 3. Temporal variations (September 2010 to August 2011) in cyanobacterial community composition in the three sampling sites of lake Chaohu.

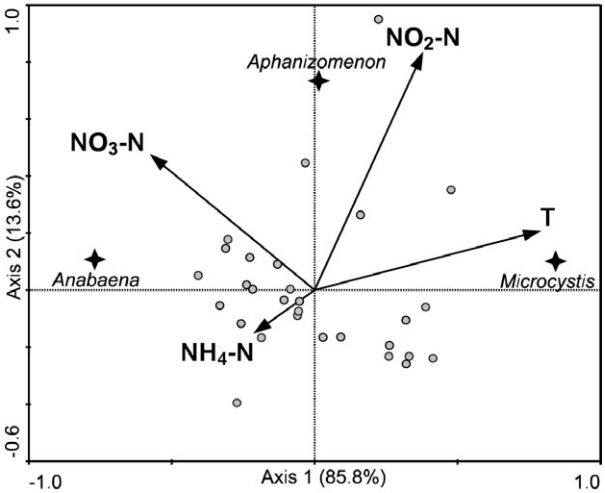

Fig. 4. Redundancy analysis ordination diagram including 30 samples (gray circles) from all three sites in lake Chaohu (September 2010 to August 2011), with abiotic variables as vectors and the dominant cyanobacterial genera (*Microcystis*, *Anabaena*, and *Aphanizomenon* – indicated by stars) on the two-dimensional space of the first and second axes. T=water temperature.

Cyanobium clade, and their related sequences were derived from different habitats worldwide (Tab. 2).

The dynamics of *Microcystis*, *Anabaena*, and picocyanobacteria based on their percentage in the 16S rRNA clone libraries during different months is shown in Fig. 7. *Microcystis* and *Anabaena* dominated in different months. *Anabaena* was dominant in May, and then *Microcystis* replaced it from June to October, until *Anabaena* dominated again in December. This succession pattern was consistent with the results obtained under light microscopy, as illustrated by the scatter plot of the percentages of these two bloom-forming cyanobacteria obtained through light microscopic observation and the 16S rRNA clone library (Fig. 8). Picocyanobacteria, which are difficult to detect under light microscopy, were also an important component of cyanobacteria in lake Chaohu, especially in April (82%) and March (70%). Afterwards, their relative abundance gradually decreased, accounting for a very small proportion (<10%) from June to October when *Microcystis* was dominant, and then a small peak appeared again in November. A seasonal variation in these picocyanobacterial phylotypes was also observed. The predominant phylotype S3 was found only in April, whereas S1 and S2 prevailed before and after April. The correlations between the relative abundance of picocyanobacteria and the physicochemical variables in CW1 were analysed using a Pearson's correlation analysis. Significantly positive relationships were found among the relative abundance of picocyanobacteria (percentage in the clone library), DIN:DIP (r=0.965, P<0.01, n=10), and NH_4-N (r=0.721, P<0.05, n=10).

DISCUSSION

In this study, the cyanobacterial bloom lasted from April to December in the sampling region in lake Chaohu. Nineteen cyanobacteria species, belonging to 13 genera and 16 different phylotypes (8 of them belonged to picocyanobacteria), were detected under light microscopy and 16S rRNA clone library analysis, respectively. These two approaches

Tab. 1. Summary of redundancy analysis between the dominant cyanobacterial genera (*Microcystis*, *Anabaena*, and *Aphanizomenon*) and the four significant environmental variables (water temperature, NO_3-N, NO_2-N, and NH_4-N).

	Axis 1	Axis 2
Eigenvalues	0.560	0.089
Species-environment correlations	0.879	0.709
Cumulative percentage variance		
of species data	56.0	64.9
of species-environment relation	85.8	99.4
Sum of all eigenvalues	1.000	-
Sum of all canonical eigenvalues	0.653	-

Tab. 2. Sixteen phylotypes obtained in lake Chaohu: closest relatives of their represented sequences.

Phylotype	Accession number	Closest relatives (accession number)	Isolation source	Similarity (%)
M1	JN944404	*Microcystis aeruginosa* (JF799857)	Pond water, India	100
C1	JN944405	Uncultured cyanobacterium (EU642172)	Lake Michigan, USA	99
C2	JN944406	Uncultured cyanobacterium (FR648036)	Seawater	100
C3	JN944407	Uncultured cyanobacterium (GQ848173)	Lake Taihu, China	97
C4	JN944408	Uncultured cyanobacterium (FJ774047)	Lake Marathon, Greece	99
P1	JN944409	*Pseudanabaena* sp. (AM259268)	Lake Tuusulanjarvi, Finland	100
A1	JN944410	*Anabaena circinalis* LMECYA 123 (EU078519)	Freshwater reservoir, Portugal	100
W1	JN944411	*Woronichinia naegeliana* (AJ781043)	Reservoir Letovice, Czech Republic	98
S1	JN944412	*Cyanobium* sp. LB03 (AY183115)	Lake Biwa, Japan	100
S2	JN944413	*Cyanobium* sp. JJ2-3 (AM710363)	Freshwater reservoir, Czech Republic	99
S3	JN944414	*Cyanobium* sp. JJ9-A3 (AM710378)	Freshwater reservoir, Czech Republic	99
S4	JN944415	Uncultured *Synechococcus* sp. clone Kanui-2 (EF638720)	Lake Kanui Newsland	99
S5	JN944416	*Synechococcus* sp. Suigetsu-CG2 (AB610891)	Lake Suigetsu, Japan	99
S6	JN944417	Uncultured *Synechococcus* sp. clone BS.L4.3 (DQ275607)	Baltic sea	99
S7	JN944418	*Synechococcus* sp. Suigetsu-CG2 (AB610891)	Lake Suigetsu, Japan	98
S8	JN944419	*Synechococcus* sp. BS2 (HM346183)	Lake Taihu, China	99

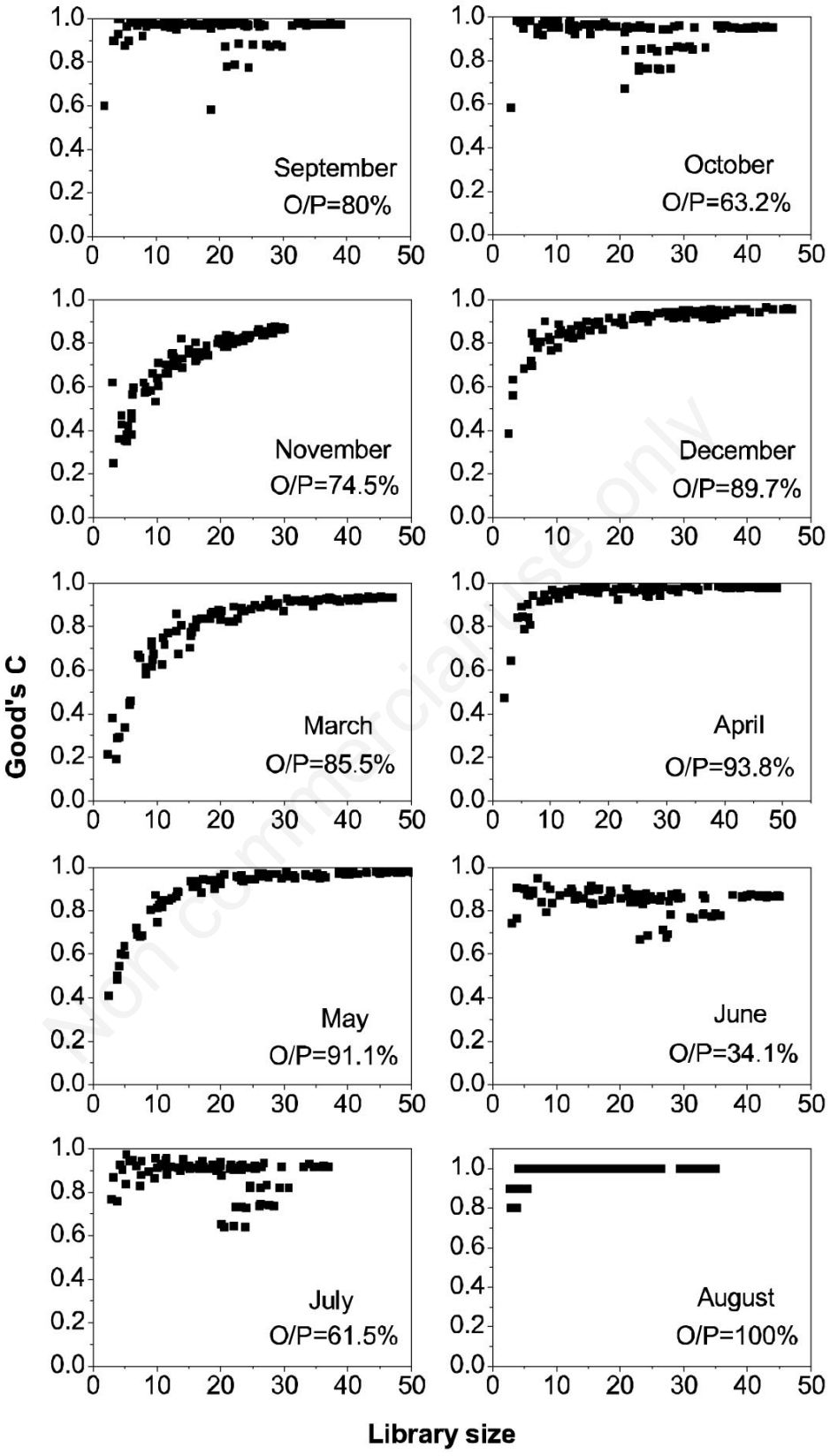

Fig. 5. Clone library coverage based on Good's C estimator of the ten cyanobacterial clone libraries across different months in lake Chaohu. O/P refers to the ratio of observed and predicted number of phylotypes based on the S_{Chao1} index.

produced consistent results regarding the dominant bloom-forming genera (*Microcystis* and *Anabaena*) and their succession pattern. *W. naegeliana* was only detected through the 16S rRNA clone library analysis, and this species has not been previously reported in lake Chaohu probably because of its low abundance and its morphological characteristics similar to *Microcystis* (Yu *et al*., 2011). Some genera with lower abundance under light microscopy (*Aphanizomenon*, *Dactylococcopsis*, *Planktothrix*, *Lyngbya*, *Spirulina*, and *Aphanocapsa*) were not detected using

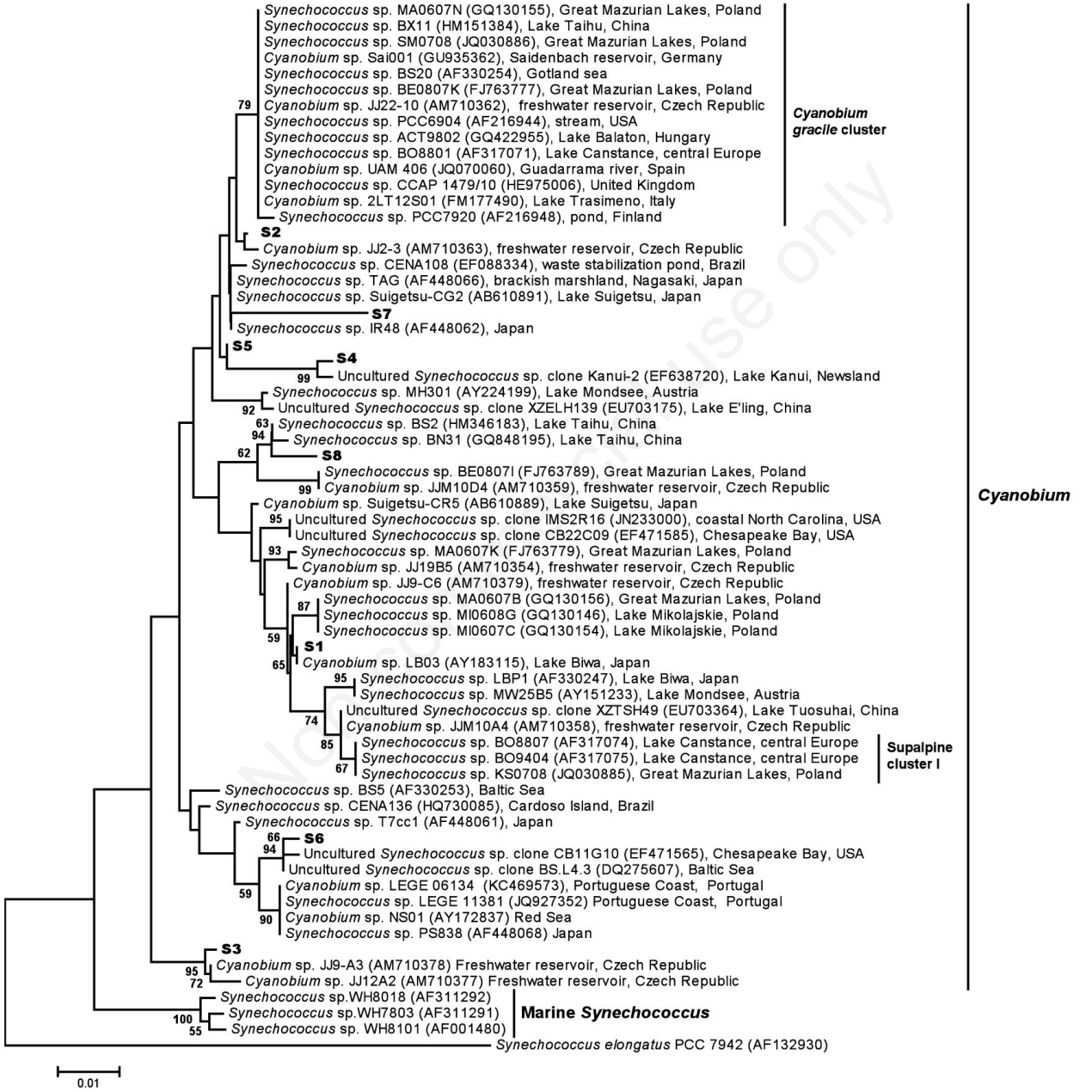

Fig. 6. Neighbour-joining tree of the representative partial 16S rRNA sequences of picocyanobacterial phylotypes obtained in this study (in bold) and their relative sequences obtained from GenBank (given with strain names, their accession numbers, and isolation details of habitats and locations). Bootstrap values above 50% (from 1000 bootstrap replicates) are shown at the nodes. Two major phylogenetic clades, *Cyanobium* and marine *Synechococcus*, are indicated on the right. Two lineages within the *Cyanobium* clade (*Cyanobium gracile* cluster and subalpine cluster I) defined by Ernst *et al.* (2003) are also indicated. Scale bar represents 0.01 substitutions per site. The outgroup is *Synechococcus elongatus* PCC 7942.

a molecular approach, which may be ascribed to the limited sequences analysed or the potential bias in DNA extraction, PCR, and cloning (von Wintzingerode *et al*., 1997). These results suggest the need for a combination of morphologic and molecular approaches in cyanobacterial community analysis (Kormas *et al*., 2011). More powerful next-generation sequencing techniques such as 454 pyrosequencing can be used in the future to assess cyanobacterial diversity (Eiler *et al.*, 2013).

Microcystis and *Anabaena* were the predominant bloom-forming genera as shown by light microscopy and 16S rRNA clone library analysis. *Microcystis* is the most dominant bloom-forming genus in lake Chaohu according to historical investigations (Xie, 2009). *Anabaena* too is a major bloom-forming genus (Deng *et al*., 2007). *Microcystis* was exclusively dominant in July and August and had 4 morphological species. However, all of them were grouped into 1 phylotype according to the similarities of their 16S rRNA sequences because *Microcystis* phenotypes do not necessarily reflect their phylogeny (Otsuka *et al*., 1998). *Anabaena* comprised more than 94% of the cyanobacterial bloom in March, April, May, and December, as revealed by the morphologic method. However, only one *Anabaena* sp. (*A. circinalis*) was detected using both light microscopy and genetic analysis.

The similar succession pattern of bloom-forming cyanobacteria was also found in other shallow eutrophic water bodies. In lake Qingshan, *Microcystis* dominates from June to August, but it is rapidly replaced by *Anabaena* and *Oscillatoria* from September to November (Ni *et al.*, 2012). In another shallow eutrophic lake – lake Tuusulanjärvi (Finland) – the dominant cyanobacteria shift from *Microcystis* in mid-summer to nitrogen-fixing genera (*Anabaena* and *Aphanizomenon*) in late summer, and the driving factors are temperature, radiation, and inorganic nitrogen and phosphorus concentrations (Rajaniemi-Wacklin *et al.*, 2008). In the present study, RDA analysis shows that the main driving force for the shifting of *Anabaena* and *Microcystis* in lake Chaohu was water temperature. The different responses of *Anabaena* and *Microcystis* to high temperature have been reported in several studies. *Microcystis* prefers temperatures above 25°C (Chu *et al*., 2007). *A. circinalis* is dominant below 25°C, and collapsed when the average daily temperature suddenly increased from 24.5 to 26.6°C in an Australian reservoir (Bormans *et al*., 2005). Nalewajko and Murphy (2001) demonstrated that the growth rates of *Microcystis* sp. are higher than that of *Anabaena* sp. when grown at

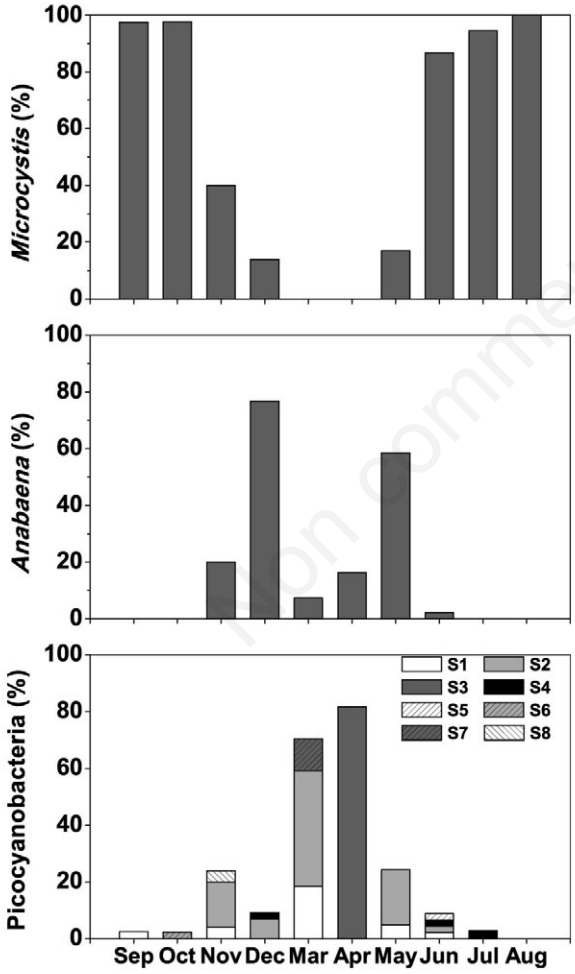

Fig. 7. Temporal variations in the relative abundance of *Microcystis, Anabaena*, and picocyanobacteria in total cyanobacteria (based on their percentage in the 16S rRNA clone libraries) sampled at site CW1 in lake Chaohu from September 2010 to August 2011. Temporal variations in the eight picocyanobacterial phylotypes are also indicated.

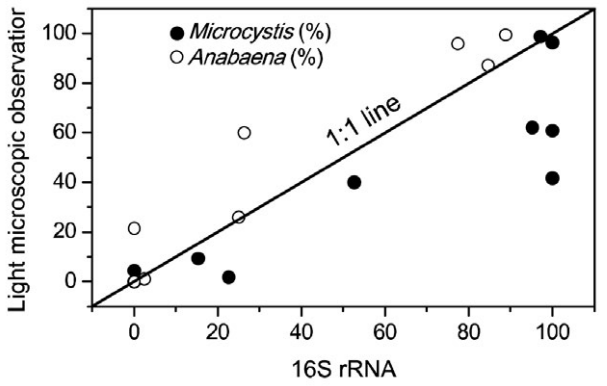

Fig. 8. Scatter plot of the percentages of the two dominant bloom-forming cyanobacteria (*Microcystis* and *Anabaena*) obtained under light microscopic observation and 16S rRNA clone library. The solid line is the 1:1 line.

temperatures above 26°C in laboratory experiments. Dissolved nitrogen (mainly NO_3-N) was also an important driving factor for the seasonal cyanobacterial succession in lake Chaohu. *Anabaena* is a superior species in nitrogen-limited or low N:P ratio water bodies because of its nitrogen-fixing ability (Tezuka and Nakano, 1993; Havens *et al*., 2003). However, the positive relationship between the biomass of *Anabaena* and NO_3-N concentration, which is the main component of dissolved nitrogen in lake Chaohu, demonstrates that the presence of numerous *Anabaena* was not due to nitrogen deficiency. *Anabaena* heterocysts, which are responsible for nitrogen fixation, were not found in all 10 water samples. In addition, no relationship was found between the *Anabaena* biomass and the DIN:DIP ratio. These results indicate that the *A. circinalis* in lake Chaohu is most likely a non-nitrogen-fixing strain that mainly depends on ambient NO_3-N for survival and proliferation. Therefore, the succession of bloom-forming cyanobacteria in lake Chaohu was mainly driven by water temperature and dissolved nitrogen (mainly NO_3-N).

Picocyanobacteria were also dominant in lake Chaohu, as shown by molecular methods. These picocyanobacteria exhibit high genetic diversity and are polyphyletic because they were located in several distinct branches of the 16S rRNA phylogenetic tree. This result is similar to that of previous reports showing that individual freshwater bodies can support a diverse group of picocyanobacteria with polyphyletic origins (Robertson *et al*., 2001; Sánchez-Baracaldo *et al*., 2008). The high richness of freshwater picocyanobacteria compared with their marine counterparts has been demonstrated by many studies (Crosbie *et al*. 2003; Ivanikova *et al*., 2007; Sánchez-Baracaldo *et al*., 2008; Felföldi *et al*., 2009). This finding may be explained by the long evolutionary history of cyanobacteria. Phylogenetic analysis based on multiple genes, morphologic characters and relaxed molecular clock analysis demonstrates that the earliest cyanobacteria were probably unicellular, had a small cell diameter, and lived in freshwater environments before dispersing into coastal brackish and marine habitats (Sánchez-Baracaldo *et al*., 2005; Blank and Sánchez-Baracaldo, 2009). Picocyanobacteria may have also speciated more rapidly in freshwater environments because of geographical separation than in relatively homogeneous marine environments (Felföldi *et al*., 2009). All 8 phylotypes from lake Chaohu were grouped into the *Cyanobium* clade (*sensu* Ernst *et al*., 2003). This clade contains nearly all of the previously isolated small, coccoid, and rod-shaped picocyanobacteria species, which have been identified provisionally as *Synechococcus*-like strains (Callieri and Stockner, 2002; Padisák *et al*., 2003). However, at least 4 novel groups that do not belong to previously defined subclusters were found within the *Cyanobium* clade (Ernst *et al*., 2003; Crosbie *et al*., 2003; Sánchez-Baracaldo *et al*., 2008). These groups may have formed because of an ecosystem-dependent adaptive radiation (Ernst *et al*., 2003).

Picocyanobacteria showed a remarkable seasonal change referred to the number of clones retrieved. Picocyanobacteria showed a large peak in early spring when they accounted for more than 70% of the total cyanobacteria and a considerably smaller peak in autumn. This result is consistent with previous findings that indicated that the population peaks of picocyanobacteria in temperate lakes usually exhibit a typical bimodal pattern, one in spring or early summer and one in autumn (Stockner *et al*., 2000). However, picocyanobacteria accounted for <10% during the *Microcystis* bloom in summer. Pearson's correlation analysis showed that DIN:DIP and NH_4-N were positively related to the relative abundance of picocyanobacteria. Likewise, Takamura and Nojiri (1994) found a positive correlation between the picophytoplankton contribution to phytoplankton biomass and N:P ratio. Buskey *et al*. (2003) also found that NH_4-N addition stimulates the growth of *Synechococcus* in mixed populations. Other abiotic and biotic factors such as competitive exclusion, size-selective protozoan grazing, and allelopathy (Callieri and Stockner, 2000) may have important effects on the annual dynamics of picocyanobacteria. The possible suppression effect of *Microcystis* on picocyanobacteria could not be excluded either, considering the gradual decrease in *Synechococcus* percentage during the development of *Microcystis* bloom.

CONCLUSIONS

In conclusion, this study reveals the dominant status of picocyanobacteria and two bloom-forming cyanobacteria (*Anabaena* and *Microcystis*), and their succession during a long bloom period in lake Chaohu, a large, shallow, and eutrophic subtropical lake. Picocyanobacteria and *Anabaena* are dominant during the cold months, and their dominance is rapidly replaced by *Microcystis* with the increase in temperature. Apart from temperature, nutrient statuses such as dissolved nitrogen concentration (NO_3-N and NH_4-N) and DIN:DIP were also very important for the temporal variations in these algae. However, the interactions between picocyanobacteria and these bloom-forming cyanobacteria are still unclear. Further studies on the ecological traits of these genera and their interactions may help elucidate the mechanisms underlying the seasonal succession of these genera in shallow eutrophic lakes.

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