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Effect of salinity on cyanobacterial community composition along a transect from Fuliya spring into the water of Lake Kinneret, Israel

Paper of the special Issue "Aquatic microbial ecology in the footsteps of Jürgen Overbeck"

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With 3 figures

Abstract: Cyanobacterial community composition was studied along a salinity gradient from the saline Spring Fuliya towards the water column of Lake Kinneret. The samples included a gradient of salinities ranging from 4270 mg Cl L^{-1} (Saline Spring) to 239 mg Cl L^{-1} (Lake Kinneret). Denaturing gradient gel electrophoresis (DGGE) and cloning of the 16 S rRNA gene, as well as cloning and sequencing of the *psbA* gene, were used to characterize cyanobacterial community composition. Despite the differences in salinity, similar cyanobacterial communities were observed in the lake and the saline spring, the only exception being the highest salinity sample (4270 mg Cl L^{-1}). Both, DGGE patterns and results of the clone libraries revealed the dominance of cyanobacteria with colonial *Gloeocapsa* and unicellular *Synechococcus* as the closest known cultured relatives, independently of the salinity. These results suggest that cyanobacterial populations inhabiting this freshwater lake and its saline sources can adapt to a wide range of salinities.

Key words: 16 S rRNA, *psbA*, cyanobacteria, Fuliya, Lake Kinneret.

Introduction

Lake Kinneret is a monomictic subtropical freshwater lake located in the northern part of Israel. Besides its importance as a freshwater resource for Israel, Lake Kinneret is a unique case of limnological and geochemical evolution. It originated from an inland water body with high salinity formed by a seawater arm that covered the subsiding rift valley during the Miocene (Hazan et al. 2005). As is typical of rift lakes, fast and large hydrological fluctuations in Lake Kinneret were accompanied by drastic variations of water salinity, involving a rapid transition from saline to freshwater (Hurwitz et al. 2000). At present, Lake Kinneret still possesses a relatively high salinity (~250 mg Cl^- L^{-1}), resulting from different saline springs (Rimmer & Gal 2003) that contribute less than 10 % of the lake-water, but provide almost 90 % of its salts (Klein-Bendavid et al. 2005).

Cyanobacteria are an essential component of phytoplankton communities in different aquatic environ-

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ments, and in the case of marine picocyanobacteria, they play a role in biogeochemical cycling at a global scale (Partensky et al. 1999, Scanlan & West 2002). In a previous study (Junier et al. 2007), we characterized the cyanobacterial communities in the water column of Lake Kinneret. Using sequence analysis of the 16 S rRNA gene, the gene coding for protein D1 of the photosystem II reaction centre (*psbA*), and the nitrogen regulator *ntcA*, we found similarities between cyanobacterial populations in Lake Kinneret and those described from marine environments (especially in the case of picocyanobacteria). This raised the question about the origin and dispersal of these populations, and in particular their connection with the variable levels of salinity that can be encountered in Lake Kinneret.

There has been always a long debate on the factors that determine the dispersion of bacterial communities. For example, the distribution of non-marine picocyanobacterial strains belonging to the branch of the picophytoplankton clade sensu Urbach et al. (Urbach et al. 1998) has resulted in two different theories explaining the global distribution of unicellular cyanobacteria. One theory (Ernst et al. 2003) suggests that picocyanobacteria have undergone ecosystem-dependent adaptive radiations within several brackish, freshwater and saline lake environments. This conclusion is an apparent contradiction of the second theory, commonly referred to as the "everything is everywhere" hypothesis (Crosbie et al. 2003), which postulates that free-living microbial species have a global distribution because their dispersal is rarely restricted by geographical boundaries.

Different studies support one or the other theory. For example, a study on thermophilic cyanobacteria in several hot springs at three different locations around the world suggested that their distribution patterns reflect geographical isolation, at both global and local scales (Papke et al. 2003). Since no correlation was observed between the biological patterns and 20 habitat-determining chemical characters, geographical isolation was considered as the determining factor for the speciation of microbial communities. Also, when freshwater and marine environments are considered simultaneously, the "everything is everywhere" hypothesis challenges the effect of salinity as a selective barrier. It has been shown that microbial communities in equivalent marine and freshwater habitats were generally very different (Glockner et al. 1999), even though they could perform similar ecological functions (Hobbie 1988).

In the present study we analyzed the cyanobacterial communities in the water column of Lake Kinneret and one of the main saline springs (Fuliya) that drain into the lake, in order to establish whether the differences in salinity have an effect on the composition of the cyanobacterial communities. Denaturing gradient gel electrophoresis (DGGE) of the 16 S rRNA gene and sequence analyses of the16 S rRNA gene and *psbA* were carried out at different depths of the water column of Lake Kinneret, and along a salinity gradient at the saline spring Fuliya (salinity ranging from 1247 to 4270 mg Cl L^{-1}). DGGE and cloning and sequencing were selected as complementary approaches to study the structure of cyanobacterial communities. The DGGE results showed that community composition was remarkably stable in spite of the marked differences in salinity. The main bands observed in the DGGE patterns could be related to dominant clones observed in the clone libraries. The sequence analysis confirmed the overlap between the communities of the water column of the lake and along the spring. In addition it provided information on the existence of a cluster of sequences specific to Lake Kinneret.

Material and methods

Sites and sampling

Samples from three different depths (1, 15 and 20 m) were collected at station F (Longitude E 35.553 and Latitude N 32.849) with a maximum depth of 22 m representing the pelagic area of Lake Kinneret. These samples were obtained in April 2005, when the water column was not stratified. Samples were also collected from surface water at four sites of the saline spring Fuliya (Longitude E 35.518 and Latitude N 32.826) with salinities of 1247, 1510, 1650 and 4270 mg Cl L^{-1}. From each sample, water was filtered through 0.2 µm pore size filters (Supor-200, PALL Life Sciences) until the filter was clogged (between 300 and 400 ml of water). Filters were stored at –18 °C until DNA extraction.

DNA extraction and PCR amplification

DNA extraction was carried out with the UltraClean Soil DNA Kit (MoBio), following the manufacturer's guidelines. DNA concentrations and quality were checked by electrophoresis on 0.8 % agarose gels stained with ethidium bromide. For PCR between 1 and 2 µg/µl of DNA were used. For specific amplification of the cyanobacterial 16 S rRNA gene, PCR was carried out with the primers Cya106 F/Cya781 R (Nübel et al. 1997). For DGGE a nested PCR with the primers P3 (GC-clamped) and P2 (Muyzer et al. 1993) was performed using the cyanobacterial 16 S rRNA gene amplicons as template. Templates were purified using a multiscreen plate (Millipore) and diluted 50 times in water prior to the second amplification. A touchdown temperature program was used for nested PCR (Muyzer et al. 1993). For amplification of *psbA*, the primers psbA86 F/psbA980 R were used as described previously (Junier et al. 2007).

Denaturing gradient gel electrophoresis (DGGE)

A DCode System (BioRad) was used for DGGE of the 16 S rRNA PCR products. Separation was carried out in 7.5 % polyacrylamide gels with a gradient of 30 to 60 % of denaturants (100 % denaturants contained 420 g L^{-1} urea and 400 mL L^{-1} deionized formamide in 0.5× TAE) during 6 h at 200 V and 60 °C. Gels were silver stained (Sanguinetti et al. 1994) and scanned using a HP scanjet 5470c.

Cloning, sequencing and sequence analysis

For cloning the proof-reading *Pfu* DNA polymerase (Promega) was used. PCR products were cloned with the Zero Blunt PCR Cloning Kit (Invitrogen) according to the manufacturer's guidelines.16 S rRNA gene and *psbA* clone libraries were prepared with the samples from 15 and 20 m depth of Lake Kinneret, and from Fuliya with 1510 mg Cl L^{-1}. From each of these samples, 48 clones were picked randomly and checked for inserts of the expected size by PCR with the plasmid-specific primers M13 F/M13 R and agarose electrophoresis. For screening of these clones, one-shot sequencing with the M13 F primer was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit. Sequences were analyzed with an ABI 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommendations. The resulting nucleotide sequences were checked with BLAST (Altschul et al. 1997). Clones with identical sequences were grouped, and one clone per group was used for complete sequencing of both strands. In the case of *psbA* the nucleotide sequences were translated into protein for the phylogenetic analysis. Phylogenetic analyses were carried out using the software ARB (http://www.arb-home.de), as described previously (Junier et al. 2007). Sequences reported in this study have been deposited in GenBank under the accession numbers GU131229-GU131266.

Results and discussion

Composition of cyanobacterial populations analyzed using the 16 S rRNA gene

DGGE patterns of cyanobacterial 16 S rRNA gene products indicated that cyanobacteria community composition was very similar in the samples from the saline spring and from various depths of the lake (Fig. 1). With the only exception of the sample with the highest salinity (4270 mg Cl L^{-1}), banding patterns were similar in all samples. The most complex band pattern was obtained in the sample from 20 m depth of the water column, which contained 10 clearly differentiated bands. A very similar band pattern was observed at 15 m depth, while at 1 m depth two bands located in the medium part of the gradient were not observed. In the four samples from Fuliya DGGE band patterns were less complex. The samples Fuliya

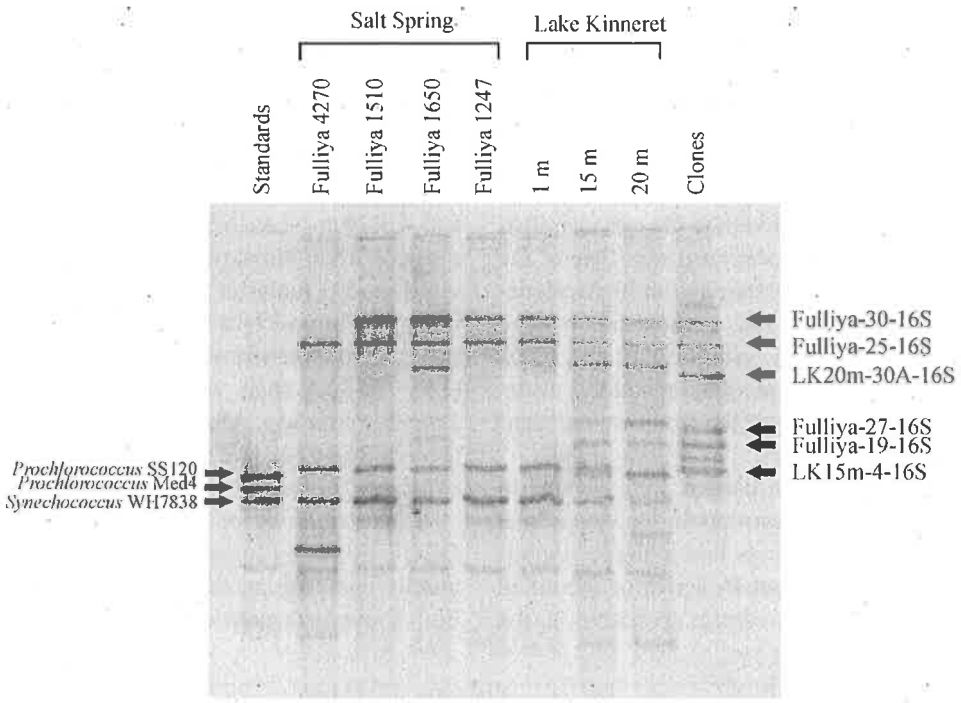


Fig. 1. DGGE analysis of 16 S rRNA gene PCR products amplified with cyanobacteria-specific primers from samples with different salinity in the spring Fuliya and at various depths of Lake Kinneret. Clones from the libraries prepared from the samples Fuliya 1650, Lake Kinneret 15 and 20 m were used as standards (line indicated as Clones). Strains from *Prochlorococcus* and marine *Synechococcus* were also included as standards (line 1).

1510, 1650 and 1247 contained four dominant bands (two each at high and low denaturant concentration) that were also observed in the sample from the water column of the lake. The most divergent pattern was observed in the Fuliya sample with 4270 mg Cl L^{-1}. Here, only three of the four common bands were observed (the band at the lowest denaturant concentration could not be observed), and one additional band that was not observed in any other sample appeared at a very high denaturant concentration. Considering the similarities in the DGGE profiles, the samples Fuliya 1510 and Lake Kinneret 15 and 20 m depth, which differ markedly in their salinity, were chosen for cloning and sequencing.

The short length of the PCR product used for the DGGE (less than 200 bp) makes this fragment unsuitable for phylogenetic studies. However, a comparison between the band patterns generated from the samples and the position of fragments produced by amplification on the identified clones in the clone libraries can contribute to the indirect identification of some of the bands in the DGGE gel. For this, a selection of clones (indicated as line "Clones" in Fig. 1) that were representative for the diversity of sequences in the libraries was used to identify the most relevant bands in the DGGE patterns. However, only three out of 11 bands in the samples corresponded to clones obtained in the libraries (Fig. 1). The band located at lower denaturant concentration, and observed in all the samples except from Fuliya 4270, corresponded to the clone Fuliya-30-16 S, for which the closest identified relative corresponded to *Cyanothece* spp. Another band, observed in all the samples, corresponded to the clone Fuliya-25-16 S, for which the closest identified relative corresponded to *Gloeocapsa* spp. One of the bands that migrated into the region with higher denaturant concentration corresponds to the band obtained for the clone LK15 m-4-16 S, which is part of a series of clones belonging to the picophytoplankton clade. This band was observed in all the samples, independently of the salinity or depth.

Five of the bands in the pattern were located at even higher denaturant concentration. However none of the clones from the libraries had a similar mobility in the denaturing gradient. A series of identified marine picocyanobacterial cultures available at the laboratory were included in the analysis (lane 1 Fig. 1) and for those also a band at a higher denaturant concentration was observed. In particular one of the bands observed in all the samples, independently of the salinity or depth, had a similar mobility as one band of *Synechococcus* WH7838.

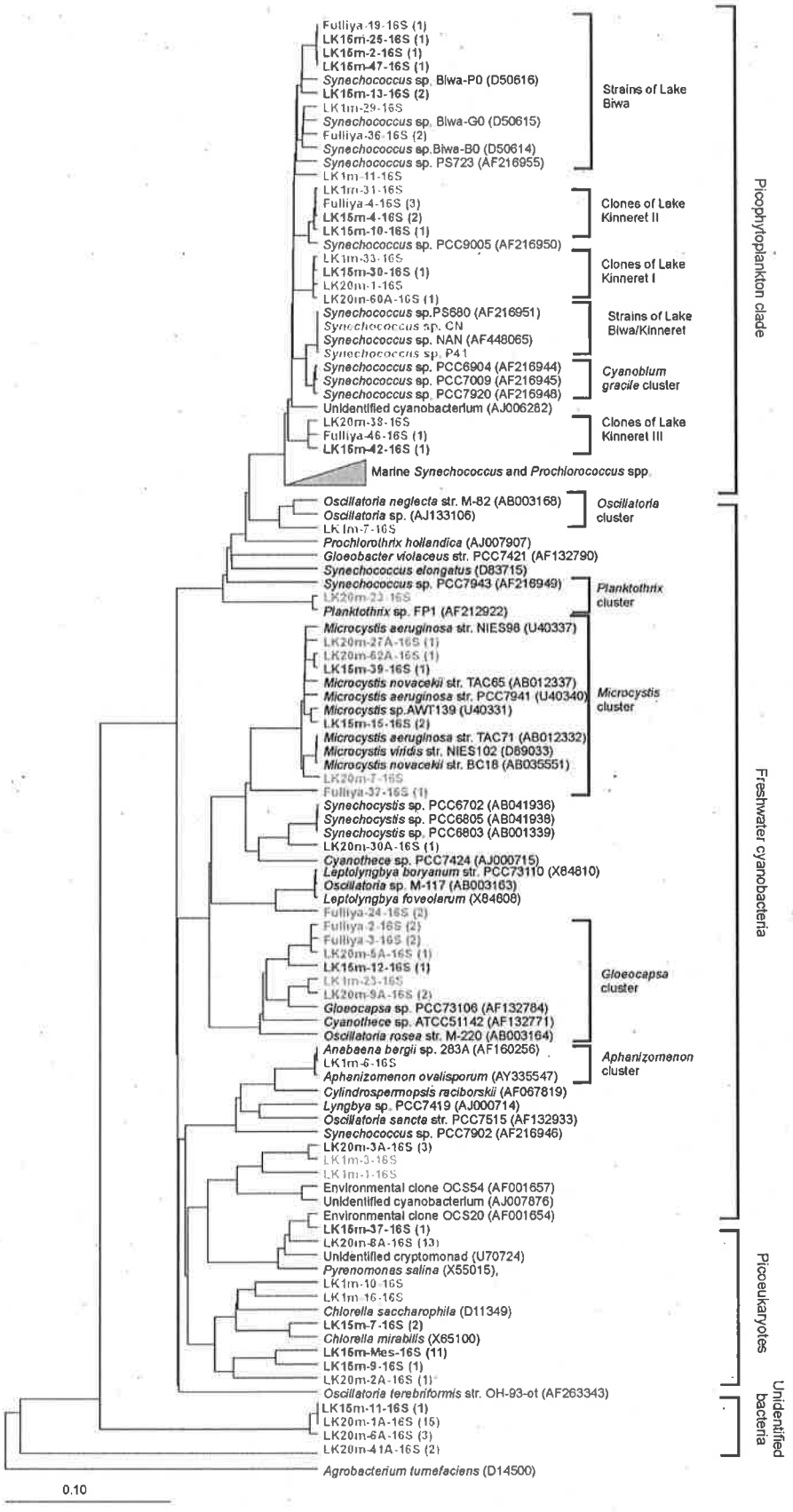
The bands obtained with the clones LK20 m-30 A-16 S (picophytoplankton clade), Fuliya-27-16 S (*Microcystis*-like), and Fuliya-19-16 S (picophytoplankton clade) did not appear in the patterns from the samples, and probably represent cyanobacteria in abundances below the detection limit of the DGGE.

In the phylogenetic analysis of the 16 S rRNA, the sequences from the clone libraries grouped with different groups of marine and freshwater cyanobacteria, picoeukaryotes and uncultured bacteria (Fig. 2). Although the fragment used here for phylogenetic inference corresponds only to a fraction of the 16 S rRNA gene, since the application of these group-specific product for cyanobacteria (Nübel et al. 1997), molecular approaches have contribute greatly to our understanding of the diversity and distribution of this group in nature and has lead to a revision of a traditional classification mainly based in morphological traits (Palinksa et al. 2006).

With the exception of the clusters *Oscillatoria, Planktothrix* and *Aphanizomenon*, all the groups of cyanobacteria that were detected during stable thermal stratification (Junier et al. 2007) were observed in the clone libraries from the present study. Five sequences from Lake Kinneret 15 m (LK15 m-15-16 S -with another identical clone- and LK15 m-39-16 S) and Lake Kinneret 20 m (LK20 m-27 A-16 S and LK20 m-62 A-16 S) grouped together with sequences from *Microcystis* and a clone from the previous study (Junier et al. 2007). One sequence from Fuliya (Fuliya-37-16 S) was distantly related to this group. One sequence from Lake Kinneret 20 m (LK20 m-30 A-16 S) was related to sequences from *Synechocystis* spp., while two sequences from Fuliya (Fuliya-24-16 S) were related to sequences from *Oscillatoria/Leptolyngbya*.

Clone libraries of 16 S rRNA gene support the DGGE results by suggesting that cyanobacterial community composition in the lake and the saline spring Fuliya overlaps considerably, as shown by the ex-

Fig. 2. Phylogenetic relationships of 16 S rRNA gene nucleotide sequences from cyanobacteria in Lake Kinneret 15 and 20 m and Fuliya saline spring. The tree represents the consensus between the trees obtained by neighbour-joining and maximum-likelihood. The number of identical clones that each complete sequence represents is shown in brackets. Sequences from the first clone library are shown in blue, while sequences from Fuliya, and Lake Kinneret 15 and 20 m are shown in green, red and orange, respectively (correct color code see online version).


Fulliya-19-16S (1)
LK15m-25-16S (1)
LK15m-2-16S (1)
LK15m-47-16S (1)
Synechococcus sp. Biwa-P0 (D50616)
LK15m-13-16S (2)
LK1m-29-16S
Synechococcus sp. Biwa-G0 (D50615)
Fulliya-36-16S (2)
Synechococcus sp.Biwa-B0 (D50614)
Synechococcus sp. PS723 (AF216955)
LK1m-11-16S
LK1m-31-16S
Fulliya-4-16S (3)
LK15m-4-16S (2)
LK15m-10-16S (1)
Synechococcus sp. PCC9005 (AF216950)
LK1m-33-16S
LK15m-30-16S (1)
LK20m-1-16S
LK20m-60A-16S (1)
Synechococcus sp.PS680 (AF216951)
Synechococcus sp. CN
Synechococcus sp. NAN (AF448065)
Synechococcus sp. P41
Synechococcus sp. PCC6904 (AF216944)
Synechococcus sp. PCC7009 (AF216945)
Synechococcus sp. PCC7920 (AF216946)
Unidentified cyanobacterium (AJ006282)
LK20m-38-16S
Fulliya-46-16S (1)
LK15m-42-16S (1)
Marine Synechococcus and Prochlorococcus spp.
Oscillatoria neglecta str. M-82 (AB003168)
Oscillatoria sp. (AJ133106)
LK1m-7-16S
Prochlorothrix hollandica (AJ007907)
Gloeobacter violaceus str. PCC7421 (AF132790)
Synechococcus elongatus (D83715)
Synechococcus sp. PCC7943 (AF216949)
LK20m-22-16S
Planktothrix sp. FP1 (AF212922)
Microcystis aeruginosa str. NIES98 (U40337)
LK20m-27A-16S (1)
LK20m-62A-16S (1)
LK15m-39-16S (1)
Microcystis novacekii str. TAC65 (AB012337)
Microcystis aeruginosa str. PCC7941 (U40340)
Microcystis sp.AWT139 (U40331)
LK15m-15-16S (2)
Microcystis aeruginosa str. TAC71 (AB012332)
Microcystis viridis str. NIES102 (D89033)
Microcystis novacekii str. BC18 (AB035551)
LK20m-7-16S
Fulliya-37-16S (1)
Synechocystis sp. PCC6702 (AB041936)
Synechocystis sp. PCC6805 (AB041938)
Synechocystis sp. PCC6803 (AB001339)
LK20m-30A-16S (1)
Cyanothece sp. PCC7424 (AJ000715)
Leptolyngbya boryanum str. PCC73110 (X84810)
Oscillatoria sp. M-117 (AB003163)
Leptolyngbya foveolarum (X84808)
Fulliya-24-16S (2)
Fulliya-2-16S (2)
Fulliya-3-16S (2)
LK20m-5A-16S (1)
LK15m-12-16S (1)
LK1m-23-16S
LK20m-9A-16S (2)
Gloeocapsa sp. PCC73106 (AF132784)
Cyanothece sp. ATCC51142 (AF132771)
Oscillatoria rosea str. M-220 (AB003164)
Anebaena bergii sp. 283A (AF160256)
LK1m-6-16S
Aphanizomenon ovalisporum (AY335547)
Cylindrospermopsis raciborskii (AF067819)
Lyngbya sp. PCC7419 (AJ000714)
Oscillatoria sancta str. PCC7515 (AF132933)
Synechococcus sp. PCC7902 (AF216946)
LK20m-3A-16S (3)
LK1m-3-16S
LK1m-1-16S
Environmental clone OCS54 (AF001657)
Unidentified cyanobacterium (AJ007876)
Environmental clone OCS20 (AF001654)
LK15m-37-16S (1)
LK20m-8A-16S (13)
Unidentified cryptomonad (U70724)
Pyrenomonas salina (X55015),
LK1m-10-16S
LK1m-16-16S
Chlorella saccharophila (D11349)
LK15m-7-16S (2)
Chlorella mirabilis (X65100)
LK15m-Mes-16S (11)
LK15m-9-16S (1)
LK20m-2A-16S (1)
Oscillatoria terebriformis str. OH-93-ot (AF263343)
LK15m-11-16S (1)
LK20m-1A-16S (15)
LK20m-6A-16S (3)
LK20m-41A-16S (2)
Agrobacterium tumefaciens (D14500)
0.10
Strains of Lake Biwa
Clones of Lake Kinneret II
Clones of Lake Kinneret I
Strains of Lake Biwa/Kinneret
Cyanobium gracile cluster
Clones of Lake Kinneret III
Oscillatoria cluster
Planktothrix cluster
Microcystis cluster
Gloeocapsa cluster
Aphanizomenon cluster
Picophytoplankton clade
Freshwater cyanobacteria
Picoeukaryotes
Unidentified bacteria

istence of related sequences in overlapping clades. This is the case of several sequences from Fuliya (Fuliya-24-16 S, 2, and 3), as well as sequences from Lake Kinneret 15 m (LK15 m-12 A-16 S) and 20 m (LK20 m-5 A-16 S, and 16), which together with sequences from the previous study (represented by the clone LK1 m-23-16 S) (Junier et al. 2007), were grouped in a cluster shown as *Gloeocapsa* cluster in Fig. 2. This group of sequences, which might represent a new, yet uncultured group of cyanobacteria, was previously found to be the most common group in the stratified water column of Lake Kinneret (Junier et al. 2007). Interestingly, the analysis of the Kinneret samples from April and the saline spring Fuliya indicates that representatives from this group can tolerate different salinity conditions, and that it may occur year-round in the water column of Lake Kinneret. These results complement the regular microscopic observations made in samples from the lake during stable summer stratification indicating the dominance of Chroococcales in the epilimnion (Zohary 2004), but it also suggests that this group persists over the mixing period in the whole water column.

As previously mentioned, the closest known cultured relatives to this group of sequences belong to the genus *Gloeocapsa* and therefore additional ecological information could be obtained from the knowledge of this genus. However, little information is available on the ecological role of *Gloeocapsa*. This genus has been found in the Markham Ice Shelf (Vincent et al. 2004), in hypersaline microbial mats (Fourcans et al. 2004), and is listed as a common cyanobacterial genus associated to endo or epilithic habitats (Pentecost & Whitton 2012). In the last habitat types, important environmental adaptations (i.e. water stress, various light intensities, pH, temperature, among others) have been reported suggesting an important phenotypic plasticity that is revealed for example in the pigment composition of this group (Pentecost & Whitton 2012). The results from the present study suggest that in addition to the previously known adaptations, relatives to this genus might be adapted to a wide range of salinities. However, in order to better understand the physiological implications of salinity, cultured representatives from the clades identified here need to be obtained, which was unfortunately beyond the scope of the present study.

In the group corresponding to the picophytoplankton clade, the clones Fuliya-19-16 S and Fuliya-36-16 S (together with another identical clone), as well as four clones from Lake Kinneret 15 m (LK15 m-25 A-16 S, 2, 47 and 13), were grouped together with sequences from *Synechococcus* sp. isolated from Lake Kinneret (Israel) and Lake Biwa (Japan) (Kane et al. 1997), forming the cluster designated "Strains LK". Two clones from Fuliya (Fuliya-4-16 S and 46), four clones from Lake Kinneret 15 m (LK15 m-4 A-16 S, 10, 30, and 42) and one clone from Lake Kinneret 20 m (LK20 m-60 A-16 S), grouped with clones previously detected in the stratified water column (Junier et al. 2007). These three clusters containing exclusively sequences from Lake Kinneret were designated LK clusters I, II and III. The presence of different groups of picocyanobacteria in the samples from April corresponded with previous reports based on microscopic observations (Malinsky-Rushansky et al. 2002, Malinsky-Rushansky et al. 1995), suggesting that these groups appear during the whole year at different depths of the water column. The best-known representatives of the picophytoplankton clade, marine *Synechococcus* and *Prochlorococcus* dominate primary production in vast areas of the ocean (Partensky et al. 1999, Scanlan & West 2002), and are so far restricted to marine habitats, which may suggest a clear differentiation between freshwater and marine clades. However, the results from Lake Kinneret and the saline spring Fuliya suggest the existence of groups within the picophytoplankton clade, which are not necessarily restricted to marine or freshwater habitats, but rather can tolerate a wide range of salinities.

If the geological origin of the Lake Kinneret is taken into consideration, it is surprising that none of the sequences from the present study clustered with the typically marine cyanobacterial clusters. However this has also been observed for strains of *Synechococcus* (Haverkamp et al., 2009) and *Pseudoanabaena* (Acinas et al. 2009) isolated from the brackish Baltic Sea. In these two studies, as well as the present study, sequences from the isolates or the clones tend to group together into environment-specific clusters suggesting common speciation within similar environments as postulated previously for Lake Kinneret (Junier et al. 2007).

Composition of cyanobacterial populations analyzed using *psbA*

The gene coding for protein D1 of the photosystem II reaction centre (*psbA*) has been used to study picophytoplankton diversity in marine environments (Zeidner et al. 2003). In the present study, the *psbA* gene was used to analyze freshwater cyanobacterial communities. In agreement with the results obtained with the 16 S rRNA gene, the clone libraries of *psbA* also indicate overlap in the picocyanobacteria communities

in Lake Kinneret and those in the saline spring Fuliya. Two clone libraries from the samples of Lake Kinneret 15 m and Fuliya 1510 revealed the dominance of one of the groups of *Synechococcus*-like sequences (Fig. 3) that were not represented in the previous study carried out during stable thermal stratification (Junier et al. 2007). The detection of this new cluster of *psbA* sequences coincided with the detection of several new *Synechococcus*-like 16 S rRNA sequences belonging to the picophytoplankton clade. Additionally, some clonal sequences from the lake and Fuliya were grouped with the cluster of sequences from 1 m, which have been detected previously (Junier et al. 2007).

Surprisingly, although *psbA* sequences related to different freshwater cyanobacteria were found during stable thermal stratification (Junier et al. 2007), sequences related to these groups were not found in samples from April, which contained only picocyanobacteria-like *psbA*. However, it is important to consider that although this appears to be in contradiction with the results obtained for the 16 S rRNA gene, which suggested the clear dominance of freshwater cyanobacteria, in the case of *psbA*, an overwhelming majority of studies dealing with picocyanobacteria have been restricted to marine habitats, therefore biasing the reference dataset towards marine sequences. In addition, the importance of having culture representatives for dominant freshwater clades, such as for example the *Gloeocapsa* clade is once more evident, since the data gathered so far does not allow the evaluation of amplification biases in the case of *psbA* for freshwater groups.

During thermal stratification (Junier et al. 2007) a differential distribution according to depth was observed for the clonal sequences of *psbA* from Lake Kinneret. This suggested a separation of ecotypes similar to the distribution observed for *Prochlorococcus* in stratified water bodies (Moore et al. 1998, Rocap et al. 2003). The results from the sampling during mixis did not show a clear separation of the clones at 15 m

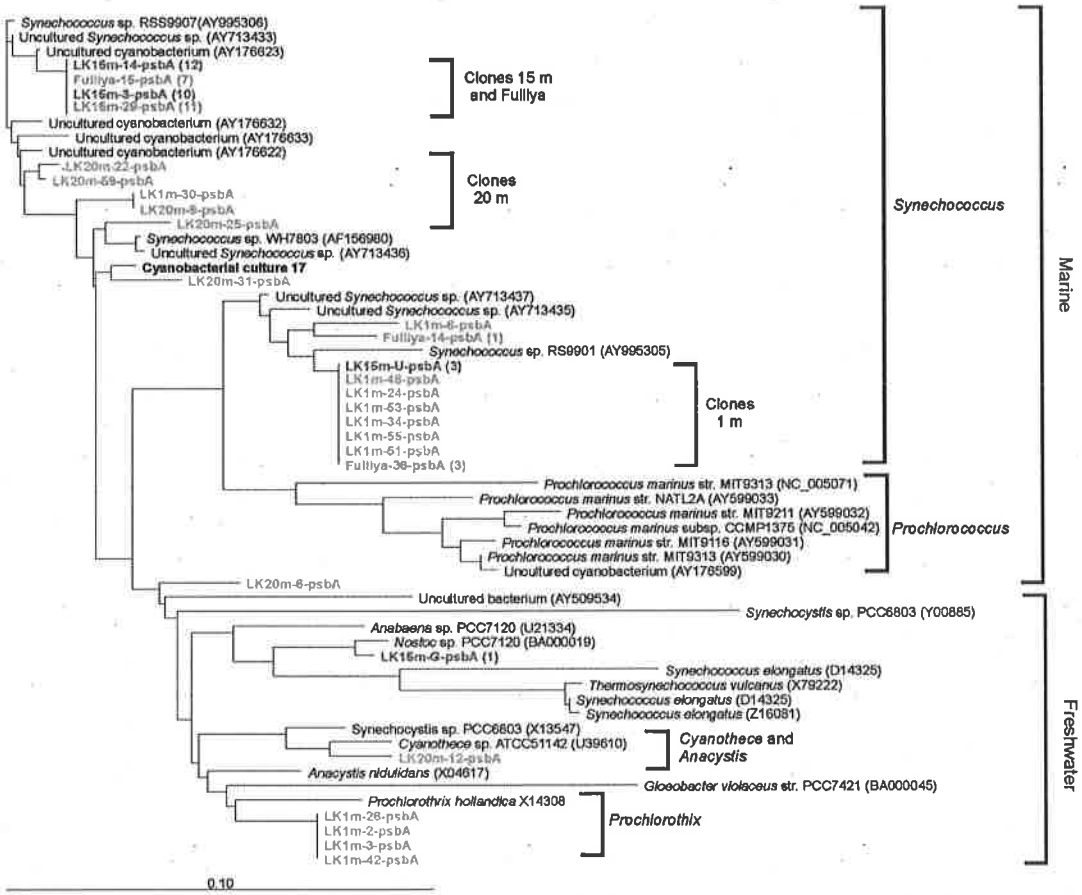


Fig. 3. Phylogenetic relationships of PsbA proteins. Evolutionary distances were determined using the neighbour-joining algorithm in ARB. Clones from this study are shown in color according to Fig. 2. The number of identical clones that each complete sequence represents is shown in brackets.

from those at surface water, and might suggest that changes in the water column triggered by stratification might be responsible for the segregation of different populations of picocyanobacteria in Lake Kinneret.

Everything is everywhere: cyanobacterial communities in a saline gradient in Lake Kinneret

While certain studies have indicated ecosystem-specific adaptive radiation (Ernst et al. 2003) and niche speciation among ecotypes (Johnson et al. 2006, Rocap et al. 2003), others indicate a global distribution of unicellular cyanobacteria (Crosbie et al. 2003). In Lake Kinneret we have found specific populations of cyanobacteria that seem to be able to adapt to a wide range of environmental conditions, specifically salinity, supporting the fact that these populations can easily migrate and colonize new habitats, which is an essential requirement for the everything is everywhere theory. Furthermore, the characterization of the cyanobacterial communities in Lake Kinneret and Fuliya suggest that the distinction between freshwater and marine cyanobacteria can be less clear than previously thought, particularly in the case of the new *Gloeocapsa*-like and the picocyanobacteria populations. However, it is important to mention that recent studies characterizing the microdiversity of populations of isolates of *Synechococcus* (Haverkamp et al. 2009) and *Pseudoanabaena* (Acinas et al. 2012) from the brackish Baltic Sea have shown than a multilocus approach is needed to reveal genetic diversification within cyanobacteria. Therefore, specifically adapted genotypes for various salinities can be hidden underneath the single-gene analysis carried out for the 16 S rRNA and *psbA* genes used here.

Acknowledgments

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References

Acinas, S. G., Haverkamp, T. H. A., Huisman, J. & Stal, L. J., 2009: Phenotypic and genetic diversification of *Pseudoanabaena* spp. (cyanobacteria). – ISME J. **3**: 31–46.

Altschul, S. F. and others, 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. – Nucleic Acids Res. **25**: 3389–3402.

Crosbie, N. D., Pockl, M. & Weisse, T., 2003: Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16 S rRNA gene and *cpcBA*-intergenic spacer sequence analyses. – Appl. Environ. Microbiol. **69**: 5716–5721.

Ernst, A., Becker, S., Wollenzien, U. I. & Postius, C., 2003: Ecosystem-dependent adaptive radiations of picocyanobacteria inferred from 16 S rRNA and ITS-1 sequence analysis. – Microbiology **149**: 217–228.

Fourcans, A. et al., 2004: Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). – FEMS Microbiol. Ecol. **51**: 55–70.

Glöckner, F. O., Fuchs, B. M. & Amann, R., 1999: Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. – Appl. Environ. Microbiol. **65**: 3721–3726.

Haverkamp, T. H. A., Schouten, D., Doeleman, M., Wollenzien, U., Huisman, J. & Stal, L. J., 2009: Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. – ISME J. **3**: 397–408.

Hazan, N. et al., 2005: The late quaternary limnological history of Lake Kinneret (Sea of Galilee), Israel. – Quatern. Res. **63**: 60–77.

Hobbie, J. E., 1988: A comparison of the ecology of planktonic bacteria in fresh and salt water. – Limnol. Oceanogr. **33**: 750–764.

Hurwitz, S., Stanislavsky, E., Lyakhovsky, V. & Gvirtzman, H., 2000: Transient groundwater-lake interactions in a continental rift: Sea of Galilee, Israel. – GSA Bulletin **112**: 1694–1702.

Johnson, Z. I., Zinser, E. R., Coe, A., McNulty, N. P., Woodward, E. M. & Chisholm, S. W., 2006: Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. – Science **311**: 1737–1740.

Junier, P., Witzel, K.-P. & Hadas, O., 2007: Genetic diversity of cyanobacterial communities in Lake Kinneret (Israel) using 16 S rRNA gene, *psbA* and *ntcA* sequence analyses. – Aquat. Microb. Ecol. **49**: 233–241.

Kane, M., Maeda, H., Fukunaga, T. & Nishi, K., 1997: Molecular phylogenetic relationship between strains of cyanobacterial picoplankton in Lake Biwa, Japan. – J. Mar. Biotechnol. **5**: 41–45.

Klein-Bendavid, O., Gvirtzman, H. & Katz, A., 2005: Geochemical identification of fresh water sources in brackish groundwater mixtures; the example of Lake Kinneret (Sea of Galilee), Israel. – Chem. Geol. **214**: 45–59.

Malinsky-Rushansky, N., Berman, T., Berner, T., Yacobi, Y. Z. & Dubinsky, Z., 2002: Physiological characteristics of picophytoplankton, isolated from Lake Kinneret: responses to light and temperature. – J. Plankton Res. **4**: 1173–1183.

Malinsky-Rushansky, N., Berman, T. & Dubinsky, Z., 1995: Seasonal dynamics of picophytoplankton in Lake Kinneret, Israel. – Freshw. Biol. **34**: 241–254.

Moore, L. R., Rocap, G. & Chisholm, S. W., 1998: Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. – Nature **393**: 464–467.

Muyzer, G., De Waal, E. C. & Uitterlinden, A. G., 1993: Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16 S rRNA. – Appl. Environ. Microbiol. **59**: 695–700.

Nübel, U., Garcia-Pichel, F. & Muyzer, G., 1997: PCR primers to amplify 16 S rRNA genes from cyanobacteria. – Appl. Environ. Microbiol. **63**: 3327–3332.

Palinska, K. A., Thomasius, C. F., Marquardt, J. & Golubic, S., 2006: Phylogenetic evaluation of cyanobacteria preserved as historic herbarium exsiccata. – Int. J. Syst. Evol. Microbiol. **56**: 2253–2263.

Papke, R. T., Ramsing, N. B., Bateson, M. M. & Ward, D. M., 2003: Geographical isolation in hot spring cyanobacteria. Environ. – Microbiol. **5**: 650–659.

Partensky, F., Hess, W. R. & Vaulot, D., 1999: *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. – Microbiol. Mol. Biol. Rev. **63**: 106–127.

Pentecost, A. & Whitton, B. A., 2012: Subaerial cyanobacteria. – In: Whitton, B. A. (ed.): Ecology of Cyanobacteria II: Their Diversity in Space and Time. – Springer.

Rimmer, A. & Gal, G., 2003: Estimating the saline springs component in the solute and water balance of Lake Kinneret, Israel. – J. Hydrol. **284**: 228–243.

Rocap, G. et al., 2003: Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. – Nature **424**: 1042–1047.

Sanguinetti, C. J., Neto, E. D. & Simpson, A. J. G., 1994: Rapid silver staining and recovery of PCR products separated by polyacrylamide gels. – Biotechniques **17**: 915–918.

Scanlan, D. J. & West, N. J., 2002: Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. – FEMS Microbiol. Ecol. **40**: 1–12.

Urbach, E., Scanlan, D. J., Distel, D. L.,Waterbury, J. B. & Chisholm, S. W., 1998: Rapid diversification of marine picophytoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). – J. Mol. Evol. **46**: 188–201.

Vincent, W. F., Mueller, D. R. & Bonilla, S., 2004: Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. – Cryobiology **2**: 103–112.

Zeidner, G. et al., 2003: Molecular diversity among marine picophytoplankton as revealed by *psbA* analyses. – Environ. Microbiol. **5**: 212–216.

Zohary, T., 2004: Changes to the phytoplankton assemblage of Lake Kinneret after decades of a predictable, repetitive pattern. – Freshw. Biol. **49**:1355–1371.