environmental microbiology

Environmental Microbiology (2017) **19**(3), 1120–1133 doi:10.1111/1462-2920.13638

Phenotypic plasticity in freshwater picocyanobacteria

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Summary

Picocyanobacteria can occur as single-cell (Pcy) or as colonies (CPcy). Published evidence suggests that some Pcy strains have the capability to aggregate under certain culture conditions, however this has not been demonstrated to occur in natural environments. We investigated whether the Pcy and CPcy belong to the same species (i.e. phylotype), and the factors that determine their morphological and genetic variability in a hypertrophic shallow lake dominated by picocyanobacteria. Six main different morphologies and >30 phylotypes were observed. All sequences retrieved belonged to the '*Anathece* + *Cyanobium*' clade (Synechococcales) that are known to have the capability of aggregation/disaggregation. The temporal variation of picocyanobacteria morphotype composition was weakly correlated with the DGGE temporal pattern, and could be explained by the composition of the zooplankton assemblage. Laboratory experiments confirmed that the small cladoceran *Bosmina* favoured the dominance of CPcy, i.e. *Cyanodictyon* doubled the size of the colonies when present, most likely through the aggregation of single-cell picocyanobacteria into colonies. Flow cytometry cell sorting and 16S rRNA + ITS sequencing of the Pcy and CPcy cytometrically-defined populations revealed that some phylotypes could be found in both sorted populations, suggesting phenotypic plasticity in which various Synechococcales phylotypes could be found *in situ* either as single-cells or as colonies.

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Introduction

Picocyanobacteria are common components of plankton and are worldwide distributed in freshwater and marine systems (Stockner, 1991). These photoautotrophic organisms are usually present at densities of thousands of cells per mililitre and contribute importantly to global carbon cycling (Weisse, 1993). Picocyanobacteria occur as single-cells (Pcy) or embedded within a mucilaginous sheath (CPcy) as colonies and microcolonies (Callieri and Stockner, 2000; Passoni and Callieri, 2000; Crosbie *et al.*, 2003; Komárková and Šimek, 2003; Callieri *et al.*, 2012b).

Experiments with natural communities have suggested that some CPcy genera (e.g. *Aphanothece*, *Aphanocapsa*, *Cyanodictyon*) might have unicellular stages in their life cycle (Komárková and Šimek, 2003), while some solitary Pcy can assemble into aggregates of variable cell numbers. Callieri *et al.* (2012) suggested that microcolonies are in fact transitional forms from single-cells to large colonial morphotypes. In addition, Koblížek *et al.* (2000) and Callieri *et al.* (2011) showed that some strains of Pcy tend to form aggregates when exposed to certain light conditions, while Jezberová and Komárková (2007) demonstrated that the formation of colonies in single-cell picocyanobacterial species was induced by the presence of the predator *Ochromonas* sp. The opposite also seems to happen, as many genera within the order Synechococcales, originally described on the basis of the form of their colonies, quickly lose the mucilaginous envelope when in culture (Komárek *et al.*, 2014).

Thus, the colonial characteristic of many cyanobacteria is apparently tied to the environmental conditions (Komárek *et al.*, 2014, and references therein). This phenotypic plasticity, i.e. the potential ability of single-cell picocyanobacteria to aggregate or disaggregate, would have two major implications: (1) it challenges the use of colonial morphology as a valid taxonomic character to classify colonial cyanobacteria, and (2) the aggregation of single cells (<2 μm) into colonies (>2 μm) would directly affect microbial trophic web interactions, as these

cyanobacteria would increase their effective individual size, thus decreasing or even avoiding predation by small grazers.

The lack of morphological differences among cells of picocyanobacteria prompted the use of molecular techniques to discriminate among taxa (e.g. Wilmotte and Golubić, 1991; Komárek *et al.*, 2014). Although some studies have suggested that single-cells and some colonial strains are phylogenetically close (Crosbie *et al.*, 2003; Komárek *et al.*, 2011), the relationship between the Pcy and CPcy is still unclear. We aimed here at disentangling the diversity of lake picocyanobacteria by linking morphological, ecological and molecular information.

In some hypertrophic shallow lakes, the phytoplankton assemblage is permanently dominated by CPcy, e.g. lake Chascomús (Fermani *et al.*, 2013; Iachetti and Llames, 2015, Izaguirre *et al.*, 2015). In this particular system, the abundance of Pcy is also high and ranges among the highest values reported for aquatic systems. Given that CPcy coexist with Pcy, this lake constitutes an excellent study site to investigate whether the Pcy and CPcy belong to the same phylotypes (as defined by the 16S rRNA gene) or not, and what are the factors that determine the morphological and genetic composition of picocyanobacteria community. In order to answer these questions we performed: (1) a temporal analysis of the morphological and genetic composition of the picocyanobacterial community, and we related its dynamics with biotic (composition of potential grazers) and abiotic (physical and chemical) factors; (2) a laboratory experiment to test the effects of zooplankton on the composition of natural assemblages of the picocyanobacteria; and (3) a genetic characterization of the colonial and single-cell populations sorted by flow cytometry.

Results

Morphological composition of the picocyanobacterial community

Phytoplankton in lake Chascomús was permanently dominated by picocyanobacteria (Pcy + CPcy) with a biovolume that always surpassed the 50% contribution (average 74%) of the total phytoplankton biovolume (Supporting Information Fig. S1). These picocyanobacteria were accompanied by filamentous Cyanobacteria (e.g. *Phormidium* sp.), Chlorophytes (e.g. *Monoraphidium* spp., *Scenedesmus* spp.) and diatoms (e.g. *Synedra berolinensis*).

Four main different picocyanobacterial morphotypes were recognized: single-cell (Pcy), microcolonies, short trichomes and large colonies (Fig. 1, Table 1). The latter three morphotypes were grouped as CPcy. Cells of these morphotypes looked similar under the microscope: all of them were phycocyanin-rich, without aerotops (gas vesicles) and their cell-sizes did not differ significantly (average = 1.13 ± 0.08 μm, ANOVA, $P > 0.05$). Among the large colonies, three different morphospecies could be identified: *Cyanodictyon* sp., *Aphanocapsa*-like and *Eucapsis* sp. (Fig. 1).

The temporal dynamics of the four morphotypes was basically similar, with a main abundance peak during the coldest months of both years (Fig. 2a). However, some differences were observed among the large colonies (Fig. 2b): *Cyanodictyon* sp. showed two main peaks during both winters; the abundance of *Cyanodictyon* sp. was significantly correlated with the abundance of Pcy, microcolonies and short trichomes (Pearson correlation, $N = 39$, $r = 0.60$, $r = 0.56$, $r = 0.50$, $P < 0.01$); *Aphanocapsa*-like showed two peaks of abundance during the first winter and during the second spring season; while *Eucapsis* sp. showed the highest abundance during the first winter and then remained low until the end of the study period.

The number of cells per aggregate was estimated for each CPcy on each date, thus allowing comparison of the different morphotypes in terms of cells per mililitre. The total abundance of cells (including all morphotypes) showed a seasonality (Fig. 2c) with one main peak of similar abundance on each winter. However, the relative abundance of Pcy and CPcy was different in each winter. From the start of the study until mid-summer 2013 picocyanobacteria were similarly represented by Pcy and CPcy, while during the second winter peak Pcy accounted for about 75% of the relative abundance and CPcy were mostly represented by *Cyanodictyon* sp. (Fig. 2d). The NMDS analysis ordered the samples in two different groups (Fig. 3a). Samples corresponding to the first winter, in which the values of relative abundance between Pcy and CPcy were similar, grouped on the left side, while the remaining samples grouped on the right side of the figure. A comparison of these two groups by ANOSIM confirmed the significance of the separation (ANOSIM $R = 0.85$, $P < 0.01$).

The abiotic parameters (Supporting Information Table S1) explained little variation of the composition of picocyanobacteria (Mantel test, Pearson correlation, $r = 0.30$; $P < 0.05$). The variables that better explained the dynamics of the abundance of picocyanobacterial morphotypes were all indicative of seasonality. Pcy and CPcy abundance (in cells ml^{-1}) were significantly ($P < 0.01$) negatively correlated with temperature ($r = -0.62$; $r = -0.68$, respectively), mean irradiance in the water column (I_{mean}, $r = -0.49$; $r = -0.52$, respectively), and the ground level mean daily irradiance (I_0, $r = -0.53$; $r = -0.60$, respectively). Hence, higher abundances coincided with colder months.

We also compared the composition and abundance of micro- and mesozooplanktonic grazers (HF, rotifers, nauplii, copepods, cladocerans) with the picocyanobacterial morphological composition. The abundance of HF (avg. 1.3×10^4 flag. ml^{-1}) fluctuated without a seasonal pattern, although the highest abundances were

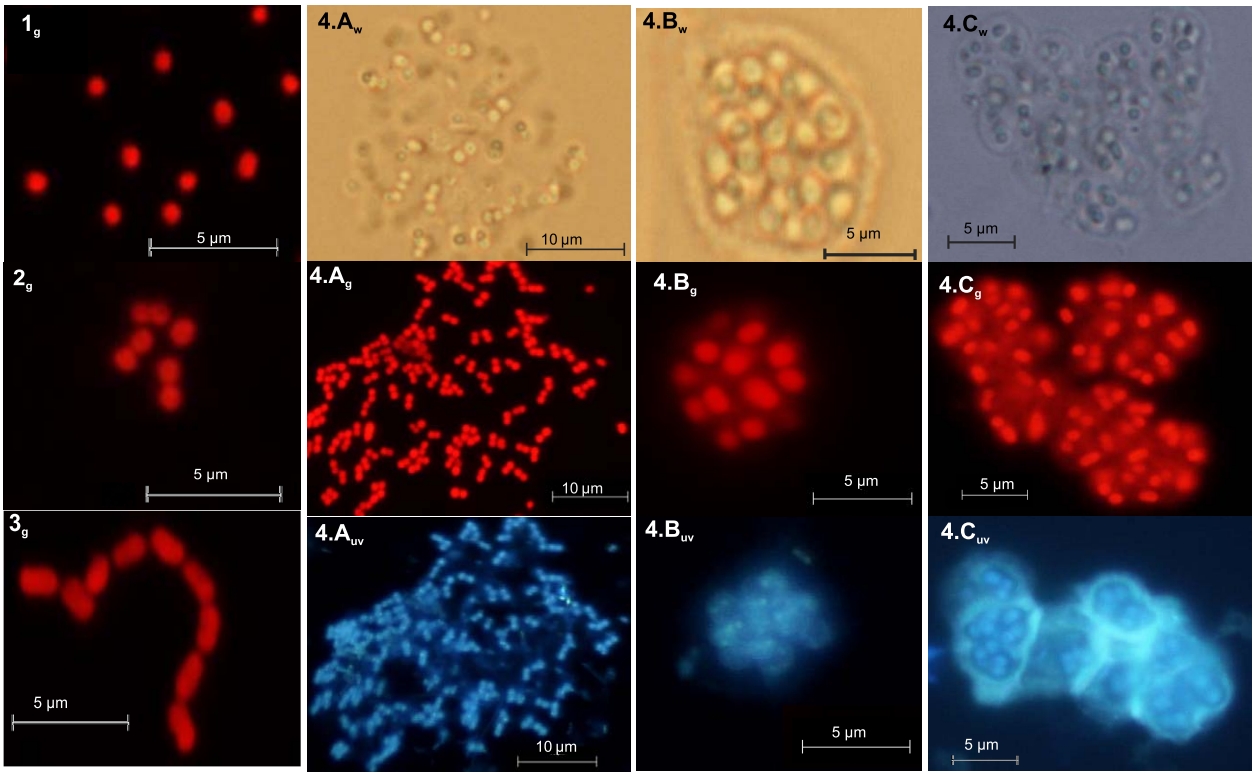


Fig. 1. Dominant picoyanobacterial morphotype in lake Chascomús: (1) single-cell; (2) microcolonies; (3) short trichomes; (4) large colonies: (4A) *Cyanodictyon* sp.; (4B) *Aphanocapsa*-like; (4C) *Eucapsis* sp. Microscope views: w = white light microscopy; g = epifluorescence under green light excitation; d = epifluorescence under UV excitation. [Colour figure can be viewed at wileyonlinelibrary.com]

observed at the beginning of the study (Fig. 4). Small cladocerans (*Bosmina* sp. and *Moina* sp.) were also particularly abundant until the first summer (300–1000 ind. l^{-1}), then the abundance declined and remained constantly low (avg. 33 ind. l^{-1}). Rotifers (mainly *Keratella americana*, *K. tropica*, *Brachionus havanensis*, and *B. caudatus*) showed seasonality with high abundance (> 2500 ind. ml^{-1}) during both warm seasons. Among the copepods, the cyclopoids (mainly *Acanthocyclops robustus*) showed a low and constant

Table 1. Morphological features of dominant picocyanobacterial morphotypes in lake Chascomús.

Morphotype	Features[a]	Cell shape	Cell dimensions (µm)
Single-cell (Pcy)		Spherical	1.09 ± 0.3 (n = 100 cells)
Microcolonies	Aggregates without a clear morphology, usually 3–12 cells	Spherical	1.03 ± 0.07 (n = 100 cells)
Short trichomes	solitary trichomes, short, usually 3–14 cells	Cylindrical	1.20 × 0.66 ± 0.4 (n = 100 cells)
Cyanodictyon sp.	Irregularly shaped colonies, 44.1 21.1 × 39.4 − 15.5 µm. Cells (4–460)[b] irregularly arranged in a diffuse, colorless mucilage	Spherical	1.17 ± 0.4 (n = 300 cells in 10 org.)
Aphanocapsa-like	spherical colonies, 11.8 − 4.9 µm diam. Cells (2–70)[b] regularly packed in a densely mucilage	Spherical	1.25 ± 0.4 (n = 300 cells in 10 org.)
Eucapsis sp.	Colonies more or less cubic in form 31.1 − 21.7 × 21 − 12.6 µm. Cells (8–310)[b] regularly arranged in groups of 4 cells, surrounded by densely and colorless mucilage.	Spherical or hemispherical after division	1.15 ± 0.2 (n= 300 cells in 10 org.)

a = colony dimensions measure on 100 organisms. b = Range of cells per colony registered during the study period.

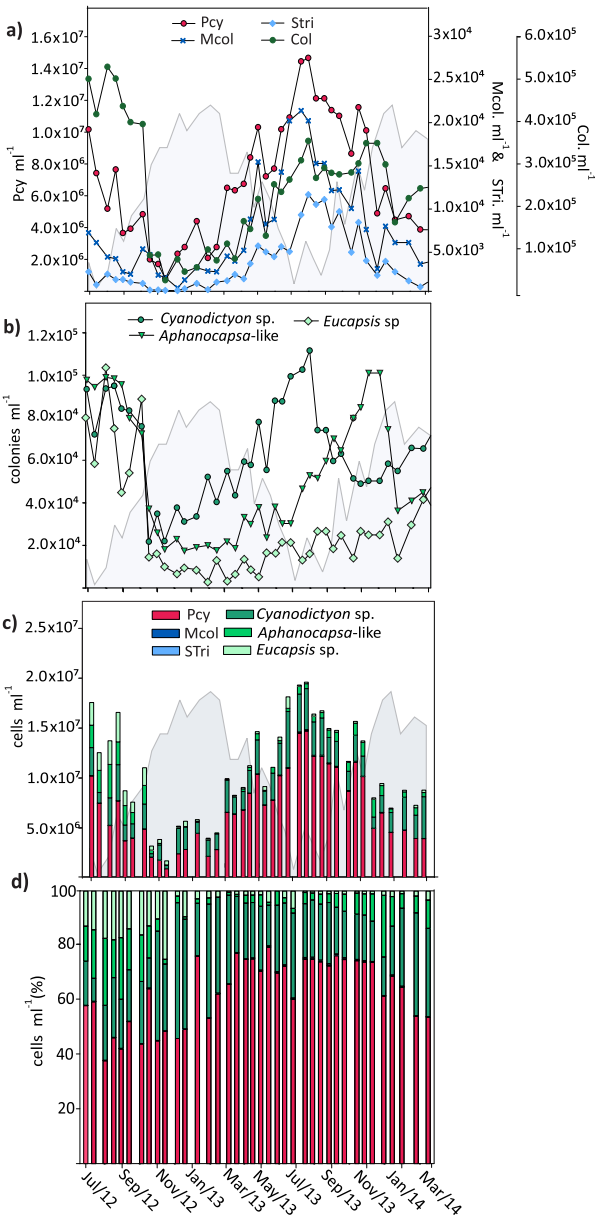


Fig. 2. Temporal changes in the abundance of dominant picocyanobacterial morphotypes in lake Chascomús from June 2012 to March 2014. (a) Single-cell (Pcy), microcolonies (Mcol), short trichomes (STri) and large colonies (Col) per millilitre. (b) Organisms per millilitre of the three dominant colonial morphospecies. c) Cells per millilitre of Pcy, Mcol, STri and the three dominant colonies morphospecies. d) Relative contribution in cells per millilitre. The shaded background area indicates water temperature which ranged between 5.5 and 27°C. [Colour figure can be viewed at wileyonlinelibrary.com]

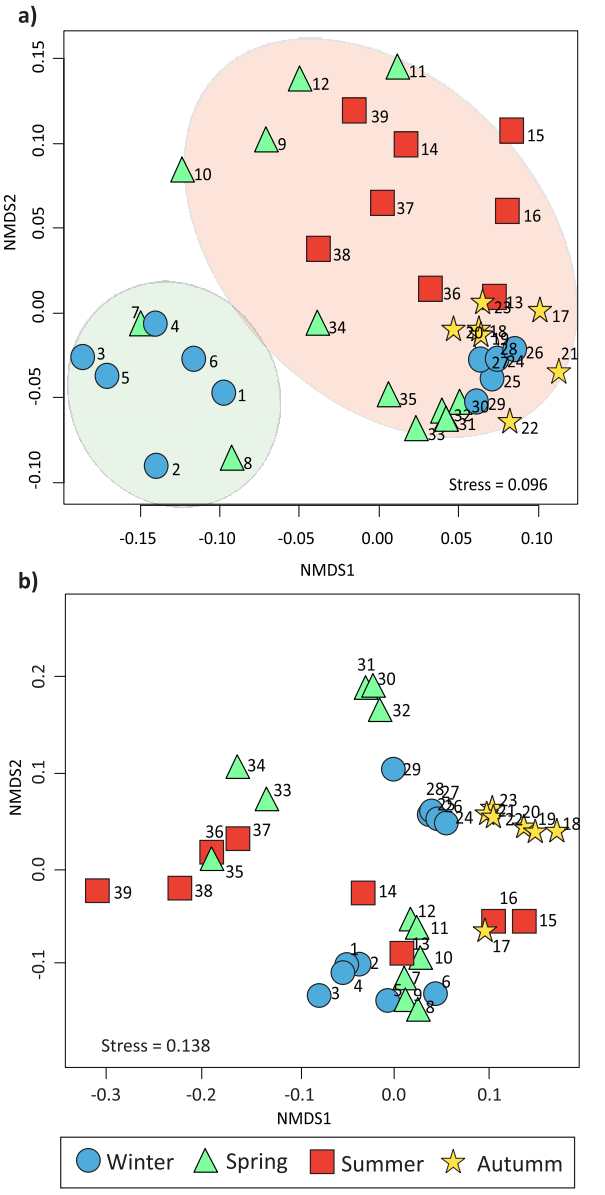


Fig. 3. Non-metric multidimensional scaling (NMDS) diagram based on (a) Bray–curtis similarity values of abundances in cells per millilitre of the dominant picocyanobacteria morphotypes and (b) DGGE relative band intensity, in lake Chascomús from June 2012 to March 2014. The numbers represent consecutive dates and the colours indicate the different seasons. [Colour figure can be viewed at wileyonlinelibrary.com]

abundance during the two years (Fig. 4) while the calanoids (*Notodiaptomus incompositus*) were the least abundant and evidenced a seasonal pattern.

The composition of the micro- and mesozooplanktonic grazers was correlated with the picocyanobacterial morphological composition (Mantel test, Pearson correlation, $r = 0.54$; $P < 0.01$). The effect of each group of grazers was also analysed separately. Even though correlations were significant in all cases, they were mostly weak and only the cladocerans had a strong correlation with the morphological composition of picocyanobacteria ($r = 0.53$; $P < 0.01$). Partial Mantel tests revealed that the predator community structure correlated with the picocyanobacterial composition when controlling for the abiotic variables (Pearson correlation, $r = 0.45$; $P < 0.01$). The Pcy abundance was

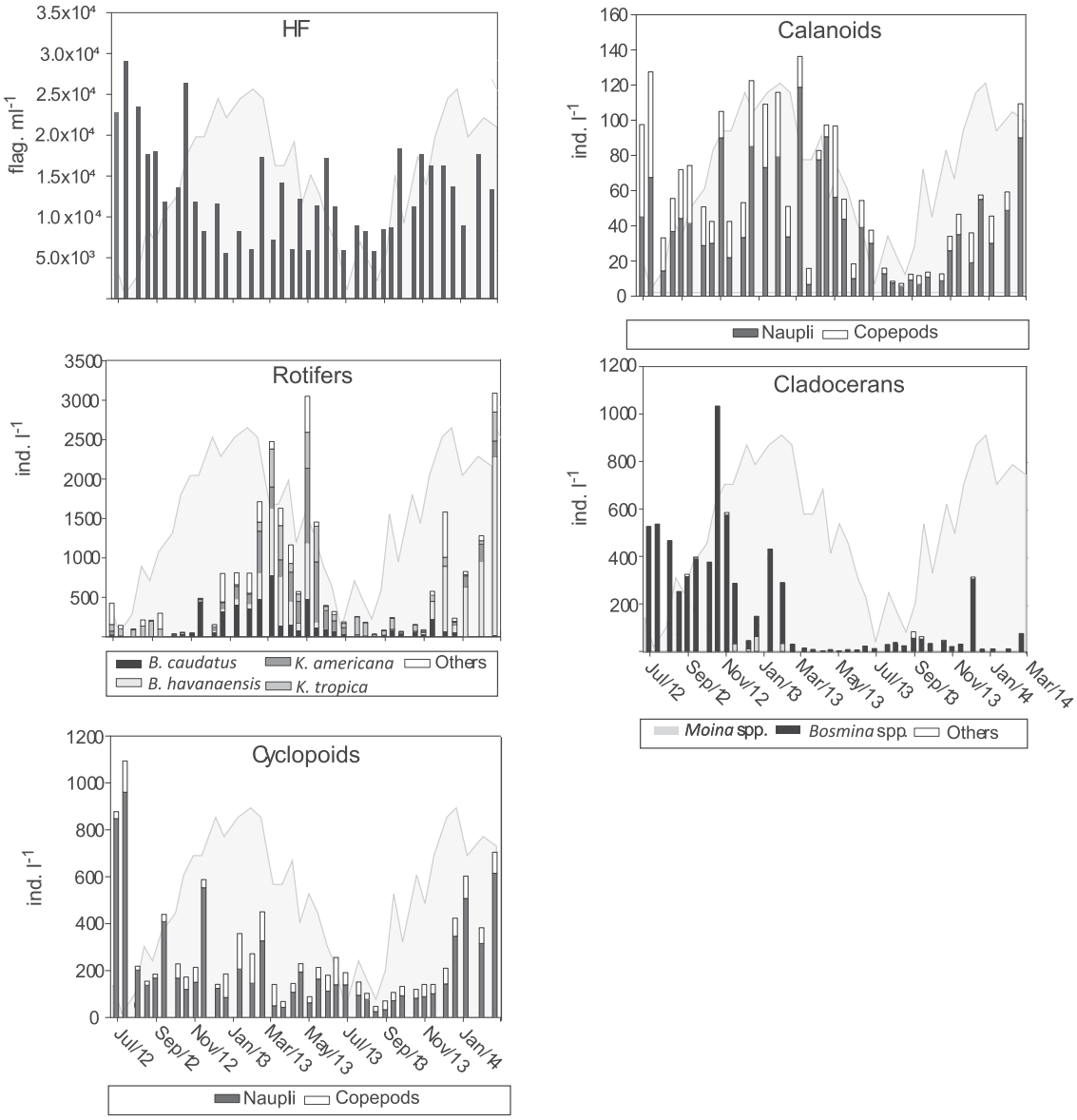


Fig. 4. Abundance of heterotrophic flagellates (HF) and zooplankton in lake Chascomús during the study period. The shaded background area indicates water temperature which ranged between 5.5 and 27°C.

negatively correlated with the abundance of small cladocerans ($N = 39$, $r = -0.51$; $P < 0.05$), while the percentage of cells in colonies was positively correlated with cladoceran and HF abundance ($N = 39$, $r = 0.51$ and 0.34 respectively; $P < 0.05$).

Effects of cladocerans on picocyanobacterial community composition

The effect of small cladocerans on the morphological composition of the picocyanobacteria was further assessed experimentally by comparing natural phytoplankton composition in the presence (Cla+) or absence of cladocerans (Cla−). Briefly, lake water containing all microorganisms was filtered to remove the zooplankton and then *Bosmina* sp. was added in the Cla+ treatment. Picocyanobacterial composition was compared against a control without cladocerans (Cla−) after six days of incubation.

At the beginning of the experiment (T0) Pcy comprised 56% of the total picocyanobacterial (Pcy + CPcy) abundance in the lake. At the end of the incubation, the total abundance of picocyanobacteria (Pcy + CPcy) as well as HF abundance did not significantly differ between treatments ($P > 0.05$). However, in terms of cells per millilitre, the relative and absolute abundance of Pcy was significantly lower in treatment Cla+ than in treatment Cla−,

whereas CPcy were significantly higher in treatment Cla+ (Fig. 5a and 5b). The final concentrations of Pcy and CPcy in the Cla− treatment did not differ significantly from T0.

Within the CPcy, the most evident change was observed for *Cyanodictyon* sp. This morphospecies increased significantly its total abundance (cells ml^{-1}) in treatment Cla+, by increasing the number of cells per colony ($P < 0.001$). In other words, this morphospecies doubled its effective size in the presence of small cladocerans (Fig. 5a).

Genetic characterization of the picocyanobacterial community

A total of 32 cyanobacterial clones were sequenced with ~ 2000 bp (16S rRNA gene plus the complete intergenic space region -ITS- fragment) from one environmental sample. Analysis of the 16S rRNA gene indicated that only 2 sequences were highly similar (>99%) to sequences of filamentous Cyanobacteria and the rest (30) were closely related to *Cyanobium* and *Synechococcus* (Supporting Information Table S3). The clustering analysis (99% of similarity of the 16S + ITS) revealed 23 different phylotypes, which belonged to the order Synechococcales and fell into the '*Anathece* + *Cyanobium*' clade proposed by Komárek *et al*. (2011), with highly supported bootstrap values (100%). Some clones were closely related to different strains of *Anathece, Cyanobium* and *Cyanodictyon*, whereas others were not phylogenetically related to any sequenced strain (Fig. 6) revealing new phylotypes within *Anathece* and *Cyanobium*. It is noteworthy that none of the retrieved sequences matched with the order Chroococcales, even though one morphotype was observed in our study that resembled *Aphanocapsa* (Fig. 1c).

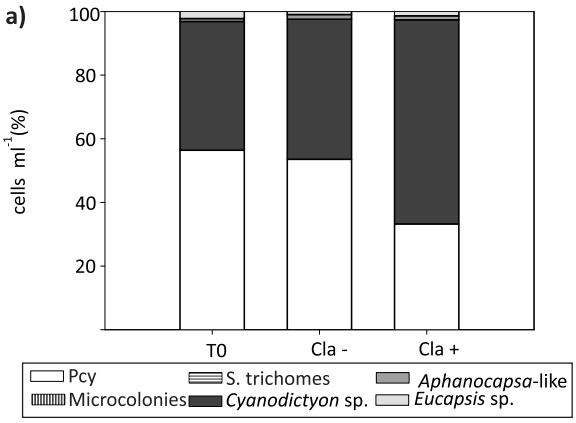
The DGGE (Denaturing Gradient Gel Electrophoresis) fingerprints of the 0.2 and 3 μm filters displayed identical patterns (Supporting Information Fig. S2). For simplicity, only the results obtained from the 0.2 μm pore-size filter are shown. The DGGE fingerprints evidenced a rather constant number of OTUs (i.e. band positions) throughout the sampled period. A total of twenty-three different OTUs were detected and sequenced (Supporting Information Fig. S3). All OTUs corresponded to Cyanobacteria, and 19 were highly similar (>98%) to cultures of *Cyanobium* and *Synechococcus* (Supporting Information Table S3). Five of the dominant phylotypes (based on band intensity) were common to all samples and two others were only present at the beginning of the study (Supporting Information Fig. S3). These seven sequenced bands were incorporated into the phylogenetic tree described above and fell within the clade '*Anathece* + *Cyanobium*'. Moreover, the dominant DGGE-based phylotypes presented a high phylogenetic similarity with the environmental clones (Fig. 6). The DGGE fingerprinting patterns varied non-randomly throughout the study. The ordination analysis (NMDS) showed an annual non-seasonal pattern (Fig. 3b). The DGGE pattern was weakly correlated with the morphological picocyanobacterial composition (Mantel test, Pearson correlation: $r = 0.15$; $P < 0.05$), and was not correlated with the abiotic variables nor was it correlated with the grazer community composition (Mantel test, $P > 0.05$).

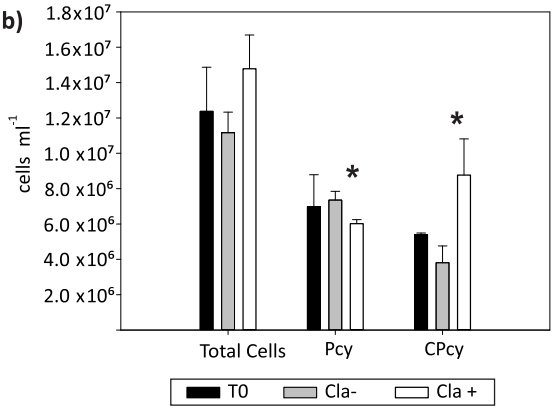
Sorting of single-cell and colonial picocyanobacteria

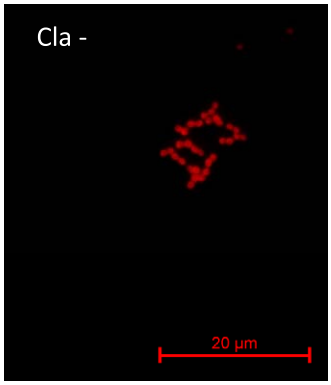
Two cytometric populations, corresponding to Pcy and CPcy, were identified in plots of red fluorescence after blue-light excitation (i.e. chlorophyll) vs. red fluorescence after red-light excitation (i.e. phycocyanin), and subsequently sorted (Supporting Information Fig. S4). CPcy were identified based on their relatively higher fluorescence and larger scatter signals than Pcy. The inspection of both sorted populations under epifluorescence microscopy corroborated the success of the sorting process, i.e. the Pcy sorted fraction was composed exclusively of single-cells and the CPcy by microcolonies, large colonies (mainly *Cyanodictyon* sp.) and short trichomes. From each sorted population partial 16S rRNA gene and complete ITS were PCR-amplified, cloned and sequenced. A total of 82 sequences (39 from the Pcy and 43 from the CPcy cytometrically-defined population) were retrieved. All the sequences were closely related to *Cyanobium* and *Synechococcus* (Supporting Information Table S3). A clustering analysis of the partial 16S rRNA gene fragment (700 bp) at 100% similarity revealed 43 different OTUs (Fig. 7a). Thirty-five OTUs were unique (singletons) and the rest (8 OTUs) were composed of multiple sequences (OTUs A to H in Fig. 7). Four OTUs (E, F, G and H) contained sequences from both sorted populations (Pcy and CPcy) comprising 44% of the retrieved sequences. Two OTUs (A and B) were represented by sequences exclusive from the CPcy cytometric population and two other OTUs (C and D) by sequences retrieved from the Pcy cytometric population.

We analysed the ITS within the 4 OTUs composed by sequences from both cytometrically-defined populations (E, F, G and H in Fig. 7). Small differences were observed in the ITS sequence within each OTU. However, there was not a common pattern among sequences retrieved from the Pcy and the CPcy cytometrically-defined population (Fig. 7b).

Phylogenetic analysis was used to incorporate the 16S rRNA gene fragment sequences (~700 bp) into the tree constructed with the complete 16S rRNA gene sequences (Fig. 6). As expected, all OTUs grouped within the '*Anathece* + *Cyanobium*' clade, and appeared associated to the environmental clones. Most OTUs that were retrieved from either Pcy, CPcy or both sorted populations clustered into four subclades (number 2, 3, 4, and 6 in Fig. 6) together with environmental clones, but without any closely related







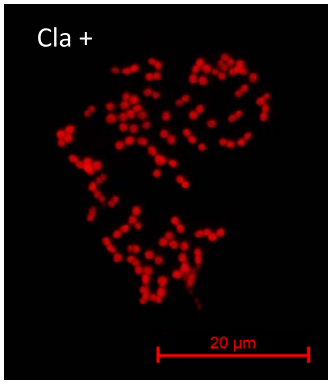


Fig. 5. Results of the experiment evaluating the structuring effect of Cladocerans on picocyanobacterial composition. Left panel: relative contribution (a) and absolute abundance (b) of Pcy and CPcy (in terms of cells per millilitre) at the beginning (T0) and the end of the experiment in the presence (Cla+) and absence (Cla−) of cladocerans. Asterisk indicates significant differences between the treatments (*t*-student, $P < 0.05$). Right panel: epifluorescence micrographs under green light excitation of *Cyanodictyon* sp. after six days of incubation in Cla− and Cla+ treatments. [Colour figure can be viewed at wileyonlinelibrary.com]

sequenced strain. Three OTUs found only in the CPcy population comprised a different subclade (number 5 in Fig. 6) together with strains of the colonial *Anathece*, *Cyanodictyon* and *Aphanothece*. Finally, subclade 1 was composed of sequences retrieved from the Pcy cytometrically sorted population and several environmental clones.

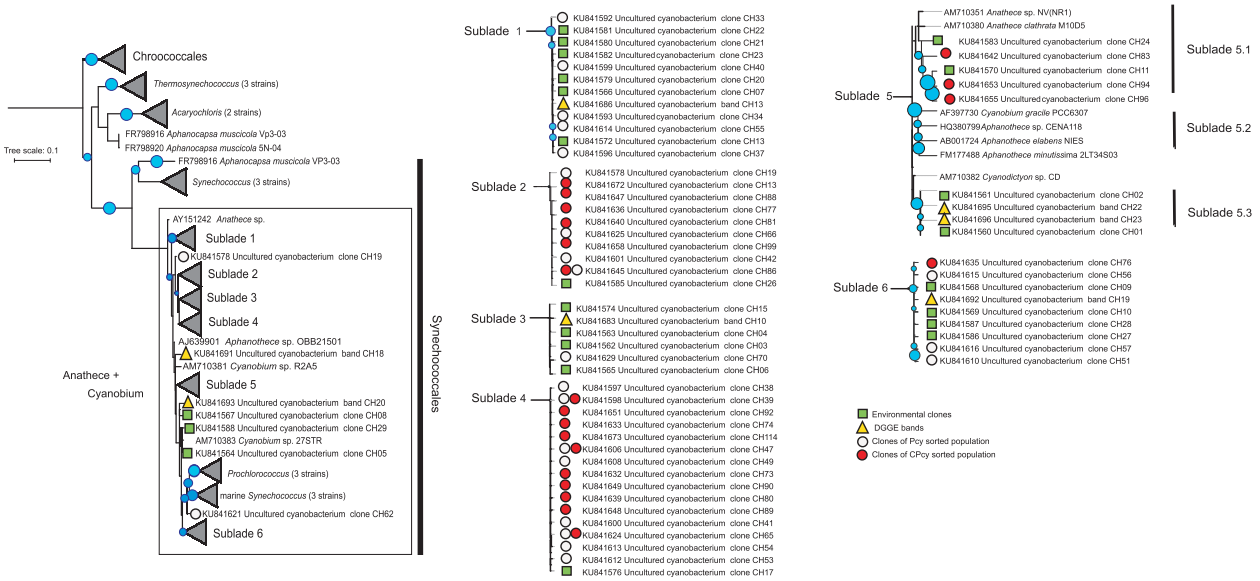


Fig. 6. Maximum-likelihood tree of the Synechococcales and Chroococcales clades *sensu* Komárek *et al.* (2011), inferred from 16S rRNA gene sequences. Terminal branches display the GenBank accession numbers and strain/isolate name. Circle size in the nodes indicate the bootstrap values (1000 replicates), values lower than 50 are not shown. [Colour figure can be viewed at wileyonlinelibrary.com]

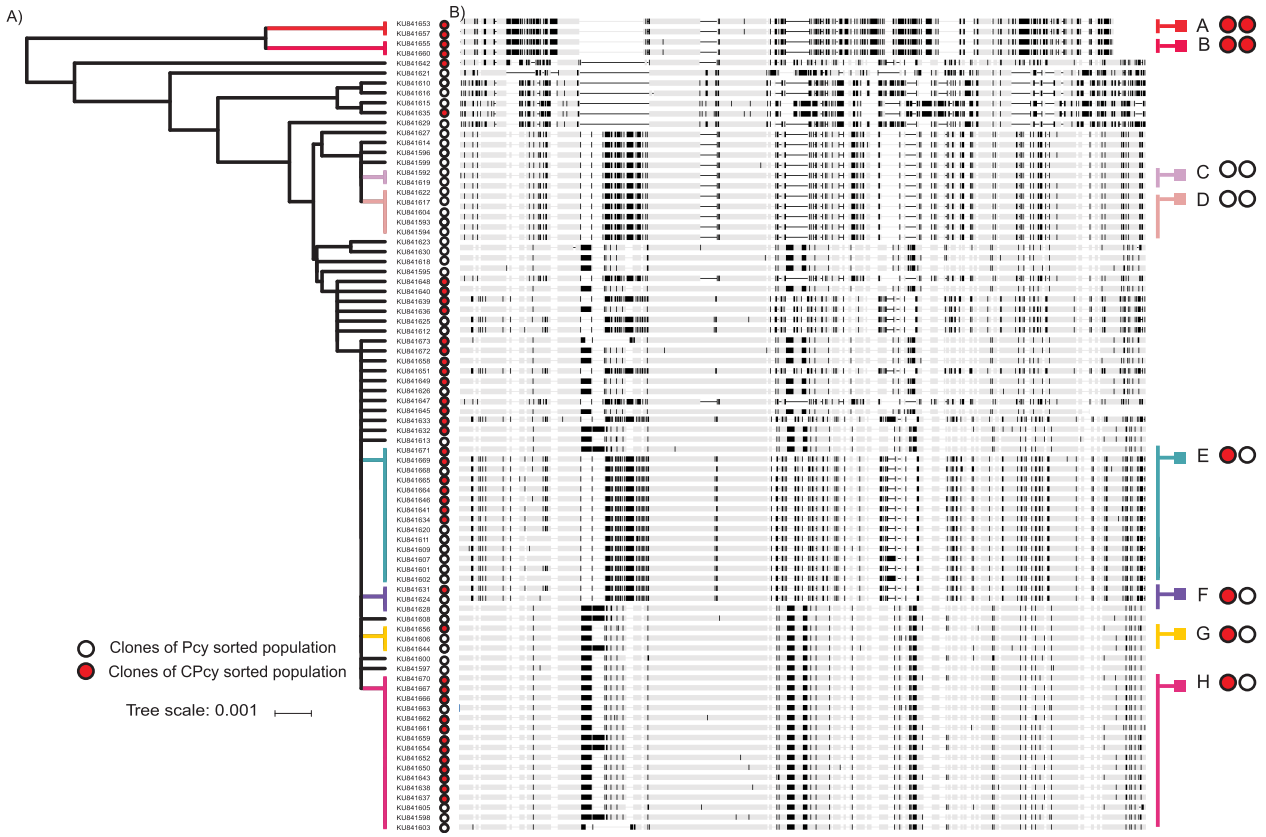


Fig. 7. (a) Results of cluster analysis of the partial 16S rRNA gene fragment (ca. 700 bp) at 100% similarity. (b) Graphic representation of a multiple alignment of 16S-23S rRNA intergenic space region (ITS) sequences of OTUs retrieved from Pcy and CPcy cytometrically defined populations. Boxes representing nucleotide sequences are connected by lines (gaps). Black boxes represent conserved region and grey boxes less conservative positions. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

We showed that some Synechococcales phylotypes were present in freshwater environments with either unicellular or colonial morphotypes in response to environmental conditions. Our findings also indicate that the zooplankton had a strong effect on the morphological composition of the picocyanobacterial assemblage. We experimentally demonstrated that the small cladoceran *Bosmina* induced changes by favouring the dominance of large colonies over unicellular forms. In particular, *Cyanodictyon* sp. doubled the number of cells per colony after six days of incubation. Basically the same trend was observed *in situ* in the lake (Fig. 8): the peak of >1000 ind. l^{-1} of *Bosmina* on 16th October 2012 coincided with a dramatic decrease in the abundance of colonies of *Cyanodictyon* sp., together with a significant increase of their colonial size from 20 to about 70 cells colony^{-1} (from ca. 10 to 35 μm in diameter). This increase in colony size was most likely due to the aggregation of single-cell picocyanobacteria.

Phenotypic plasticity has been demonstrated in many picoplanktonic organisms which develop grazing-resistant morphologies, such as trichomes or aggregates, in response to predation by nanoflagellates (heterotrophic bacteria: Jürgens and Matz, 2002; Pernthaler, 2005; Blom *et al.*, 2010; picocyanobacteria: Jezberová and Komárková 2007; Yang *et al.*, 2009). Zooplankton-induced morphological defences (e.g. the formation of spines, colonies and

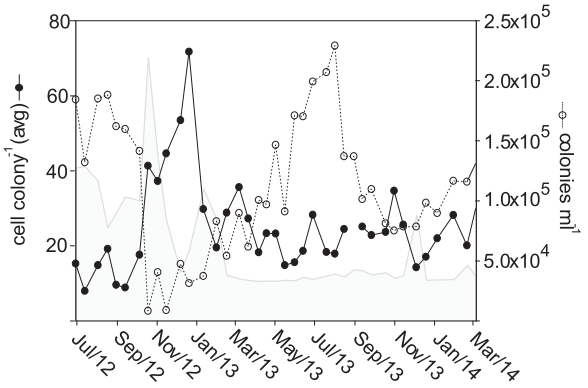


Fig. 8. Cells per colony and abundance of colonies of *Cyanodictyon* sp. from June 2012 to March 2014 in lake Chascomús. The shaded background area corresponds to the abundance of cladocerans community from figure 4.

thicker cell walls) have been also observed in many nano- and microphytoplanktonic species of Cyanobacteria, Chlorophyte and diatoms (van Donk *et al*., 2011). However, the effect of zooplankton on picoplankton phenotypic composition has usually been neglected, despite the fact that direct consumption of Pcy by zooplankton has been well documented (Callieri *et al*., 2012b, and cites therein; Motwani and Gorokhova, 2013; Miracle *et al*., 2014). Work and Havens (2003) observed that all zooplankton taxa from a eutrophic lake grazed on bacteria, eukaryotic algae and Cyanobacteria, yet colonial Cyanobacteria were negatively selected as compared with other food sources. Other studies suggested that CPcy were not grazed likely because of their mucilaginous envelopes (Blomqvist, 1996; Vrede, 1996). *Bosmina* is a selective grazer that consumes small particles by filtration and large particles by grasping (Demott and Kerfoot, 1982; Bleiwas and Stokes, 1985), so they could have either a direct effect on Pcy by consuming them (Motwani and Gorokhova, 2013), or an indirect effect through grazing on HFs (Demott and Kerfoot, 1982). In our experiment, the abundance of HF did not differ between treatments with or without the addition of *Bosmina*, suggesting a direct effect of *Bosmina* on the picocyanobacterial assemblage, inducing CPcy dominance. Thus, our results, together with previous published evidence, suggest that the colonial morphology would be an effective adaptation against grazing by these zooplankters.

The dynamics of the morphological composition of the picocyanobacterial assemblage was only weakly correlated with the DGGE fingerprinting. This would support that the changes in the morphotypes were not accompanied by changes in the composition of dominant phylotypes.

The sequences retrieved using different approaches (DGGE bands, cloning of environmental samples and flow cytometry-sorted populations) showed the co-existence of multiple picocyanobacterial phylotypes sharing a few morphotypes (i.e. only 6 morphologies were recognized in comparison to >30 different phylotypes), indicating possible niche partitioning exploited by the different phylotypes (Callieri, 2010). Although this temporal co-existence has previously been described for other systems, the number of phylotypes found in this work is the highest reported in the literature (Ernst *et al*., 1995; Postius and Ernst, 1999; Becker *et al*., 2004; Sánchez-Baracaldo *et al*., 2008; Callieri *et al*., 2012; Becker *et al*., 2012).

In addition, phylogenetic analysis demonstrated that all of the retrieved picocyanobacterial sequences belonged to the order Synechococcales and fell into the '*Anathece* + *Cyanobium*' clade (Komárek *et al*. 2011). This clade is comprised of strains of both typically single-cell genera (i.e. *Cyanobium* and *Synechococcus*) and typically colonial genera (e.g. *Anathece* and *Cyanodictyon*). Komárek *et al*. (2011; 2014) reported the observation that many of the colonies within this clade (e.g. *Cyanodictyon*) disintegrate easily after transfer into culture conditions, and their morphology is then similar to the Pcy *Cyanobium* and *Synechococcus*. It has also been shown that the single-cell strain *Cyanobium* 27-STR can change its ultrastructural morphology and aggregate rapidly when it is exposed to the presence of the predator *Ochromonas* sp. (Jezberová and Komárková, 2007). Interestingly, colonies of *Cyanodictyon* in lake Chascomús resemble the aggregates observed by Jezberová and Komárková (2007). Although these authors described the aggregates as *Aphanothece*-like colonies, the irregular arrangement of cells within a diffuse and colorless mucilage is very similar to our description of *Cyanodictyon* (Fig. 1). All these evidences together with the result of the experiment allow us to hypothesize that *Cyanodictyon* represents a flexible morphotype, being most likely the colonial phenotype of some unicellular species.

Hence, several Cyanobacteria belonging to the '*Anathece* + *Cyanobium*' clade seem to switch between Pcy and CPcy morphologies depending on the environmental conditions. Sorting and sequencing of Pcy and CPcy from lake Chascomús support this assertion, as some phylotypes appeared in both cytometrically defined populations. The DGGE patterns of the 0.2-3 μm and the 3-20 μm fractions were also identical (Supporting Information Fig. S2) reinforcing the idea that the dominant phylotypes were present in both size fractions. Thus, our study demonstrates the *in situ* coexistence of single-cell and colonial picocyanobacterial morphologies within the same phylotype (as defined by the 16S rRNA gene), which is consistent with phenotypic plasticity among picocyanobacteria. Additionally, the small within-OTU variation observed in the ITS (Fig. 7) can potentially be related to intraspecific genotypic variation. We do not dismiss the possible existence of some genomic differences outside the 16S rRNA gene that can control morphotype variation.

Phylogenetic classification of the phylotypes retrieved revealed new sub-clades within the '*Anathece* + *Cyanobium*'. Most of them were represented by sequences recovered from both sorted populations and from environmental clones. Furthermore, a sub-clade was conformed by OTUs found only in the CPcy fraction and some colonial strains, and another sub-clade by OTUs found in the Pcy fraction and several environmental clones. In addition, one phylotype from the Pcy sorted population (clone CH62) was closely related to the marine *Synechococcus*. Therefore, we cannot dismiss the existence in the lake of purely unicellular or colonial phylotypes within this clade.

In summary, the multifaceted approach we used provides firm evidence that CPcy phylotypes might have unicellular stages while some solitary Pcy can gather into groups, and that the relative composition of Pcy and CPcy is determined by the structure of the zooplankton assemblage. Among the different morphospecies identified in our

study, *Cyanodictyon* sp. appears to be the most phenotypically flexible. Our results also point to the the likely direct effect of zooplankton (i.e. *Bosmina*) on picocyanobacteria promoting by changes in their morphology, and ultimately modifying the predator-prey relationship within the microbial food web.

Experimental procedures

Study site and sampling

Chascomús (35°36′S 58°02′W) is a large (area = 30.1 km^2), shallow (mean depth 1.9 m) lake located in the Pampa region of Argentina within the temperate region (Iriondo and Drago, 2004). This lake is highly turbid, hypertrophic and typically alkaline (Lagomarsino *et al*., 2011). Vertical and horizontal homogeneity of the water column is commonly observed for most parameters due to constant mixing by the persistent wind shear (Torremorell *et al*., 2007; Diovisalvi *et al*., 2010).

The lake was sampled fortnightly from April 2012 to March 2014 at a central point. Measurements of temperature, pH, conductivity, turbidity, dissolved oxygen concentration and Secchi disk readings were made *in situ*. In addition, subsurface water samples were collected and transported in 10 l polypropylene containers to the laboratory for analyses of chemical and physical parameters and biological communities. Technical details of the abiotic variables and chlorophyll-*a* measured during this period were already described in Torremorell *et al*. (2015) and Lagomarsino *et al*. (2015), and are summarized in Supporting Information Table S1.

Microscopical determination and counting

Phototrophic community. In order to analyse the different size fractions of phytoplankton, two sets of samples were obtained: (i) samples for epifluorescence, preserved with 10% glutaraldehyde (final concentration 1%), and (ii) for inverted microscopy fixed with 2% acidified Lugol's iodine solution.

Samples for epifluorescence were filtered on a 0.22 μm pore-size black polycarbonate filter following the standard DAPI staining procedure (Porter and Feig, 1980). All samples were diluted (1:30) with 0.22 μm prefiltered lake water due to the high amount of organisms and suspended particulate matter. Details of sample processing for epifluorescence are described in Fermani *et al*. (2013). Different cyanobacterial morphotypes were defined based on the number, shape and size of the cells, as well as on the mucilage features (Table 1). In addition, the taxonomic assignment of each CPcy morphotype was performed following Komárek and Anagnostidis (1998) and Komárek *et al*. (1999; 2014; 2015). Epifluorescence counting was performed to determine the abundance of Pcy, microcolonies and short trichomes as well as the number of cells per unit in all the CPcy morphotypes described in Table 1 (i.e. microcolonies, short trichomes and the three large colonial morphospecies). Due to the low abundance and high error when counting picoeukaryotic algae (<2 μm), these were estimated with a FACSCalibur flow cytometer (Becton Dickinson) following Gasol and Moran (2015).

The number of colonies per mililitre (i.e. *Aphanocapsa*-like, *Cyanodictyon* sp. and *Eucapsis* sp.), together with other less abundant nano- and microphytoplankters (mainly chlorophytes and diatoms) were estimated by inverted microscopy following the protocol as described in Iachetti and Llames (2015).

The abundance of all picocyanobacterial morphotypes was estimated in terms of cells per millilitre by multiplying the number of cells per colony for each morphotype on each date (obtained by epifluorescence) by the number of colonies/microcolonies per mililitre.

The biovolume of the different morphospecies was estimated using geometric models (Hillebrand *et al*., 1999). For all picocyanobacterial morphotypes, the sizes of cells and colonies were estimated by image analysis following Massana *et al*. (1997). The images were analysed with the Image-Pro Plus 4.5 software (Media Cybernetics). For other phytoplankters, we used the cell dimensions measured previously in Pampean shallow lakes (Allende *et al*., 2009; Iachetti and Llames, 2015).

Heterotrophic community. To enumerate heterotrophic flagellates (HF), samples fixed with glutaraldehyde were diluted (1:30), stained with DAPI and filtered onto a 0.8 μm pore-size black polycarbonate filter (MSI), following the same procedure described above for autotrophic picoplankton. The zooplankton was collected by pouring 50 l of lake water through a 50 μm plankton net and the filtered water was preserved in 4% formalin. Rotifers and crustaceans were enumerated on Sedgwick-Rafter and Bogorov counting chambers. For more details see Fermani *et al*. (2013).

Experimental set-up

Based on the results obtained from our temporal analysis, we investigated the response of the picocyanobacterial community to the presence of small cladocerans. The experiment consisted of two treatments: presence of cladocerans (Cla+) and absence of cladocerans (Cla−). The experiment was carried out on July 2015 when cladoceran abundance was 68 ind. l^{-1}. Six glass containers were filled with 50 ml of lake water pre-filtered through a 50 μm mesh to remove zooplankton. In the Cla+ treatment, 50 *Bosmina* sp. were added to reach a concentration of 1000 ind. l^{-1}, which corresponds to the maximum abundance observed *in situ* (Fig. 4). These cladocerans were isolated from lake Chascomús the day before the experiment, and starved for 24 h on lake water filtered through 0.22 μm. Each treatment was run in triplicate. The glass containers were incubated on a rotating plankton wheel (0.5 rpm) placed within a growth chamber under simulated *in situ* conditions (temperature: 11°C; photoperiod: 8/16 h light/dark; illumination: 8.40 W m^{-2}). In addition, samples to study cyanobacterial community composition and HF abundance were taken at the beginning of the experiment and after six days of incubation. The identification and counting of the different organisms were performed as described above.

DNA extraction

On each sampling date, 50 ml of lake water pre-filtered through a 50 μm net to remove large particles were sequentially filtered through a 3 μm and a 0.22 μm pore-size polycarbonate filters (Millipore). The filters were frozen (−80°C) until DNA extraction. Genomic DNA from filters was

extracted using a CTAB protocol (Fernandez Zenoff *et al*., 2006), as described in Llames *et al*. (2013).

DGGE analysis

The 16S rRNA gene fragments were amplified with specific cyanobacterial primers CYA359F (CG) and CYA781R$_{(a)}$ (Supporting Information Table S2) using the PCR protocol described in Llames *et al*. (2013). The PCR products obtained (~500 pb) were analysed on a 40-65% denaturant DGGE gel (see details in Supporting Information material). Bands form each position were excised from the DGGE gel, reamplified and purified using a QiaQuick PCR purification kit (QiaGen) and sequenced with CYA359F primer (Supporting Information Table S2**)** (Macrogen, South Korea). Digitized DGGE images were analysed using the TotalLab 100 software (Nonlinear Dynamics), as previously described Llames *et al*. (2013).

Cloning of the environmental sample

One environmental clone library was constructed using the DNA extracted from the 0.22 μm filter collected on 5th March 2014. This sample was selected because it presented the highest number of bands in the DGGE analysis. The 16S rRNA gene plus the 16S-23S Internal Transcribed Spacer (ITS) PCR products (~2200 pb) were cloned and sequenced. Genomic DNA was amplified with primers 27F and PITSEND (R) (Supporting Information Table S2). The amplification was performed using the PCR protocol described in Llames *et al*. (2013). The StrataClone PCR Cloning Kit (Agilent Technologies) was used for cloning, following the manufacturer's instructions. Clones were screened by a direct PCR. Positive clones were processed for restriction fragment length polymorphisms (RFLP) with HaeIII (Sigma Aldrich). The resulting restriction enzyme fragment patterns were compared and sorted visually on 1% agarose gels. When possible, two clones of the same pattern were selected. A total of 42 clones were sequenced using the primer listed in Supporting Information Table S2 (Macrogen, South Korea). The sequences were edited using Pregap4 and assembled using gap4 from the Staden package (Staden, 1996). The presence of chimeras was checked using UCHIME software (Edgar *et al*., 2011). The 16S rRNA genes were edited manually using ARB (http://www.arb-home.de).

Cell sorting and clone library

Samples for flow cytometry samples were cryopreserved with glyTE (Rinke *et al*., 2014). For cell sorting we selected one sample (28th August 2013) in which the abundance of the different morphologies was high. Electrostatic sorting was performed with a FACSAria II SORP flow cytometer (Becton-Dickinson) equipped with a 100 mW blue argon-ion (488 nm) laser and a 70 mW red helium-neon (633 nm) laser. The instrument was prepared for sorting following the Aseptic Sort procedure (McIntyre *et al*., 2010). Sterilized PBS served as the sheath fluid. The sorter was set in 4-way purify sort mode and with a flow sorting rate of ca. 150-200 events/s. Single-cell (Pcy) and colonial (CPcy) picocyanobacterial populations were discriminated based on their red fluorescence after blue and red light excitation (Supporting Information Fig. S4). A minimum of 100 000 events of each population were simultaneously sorted into two sterilized 1.5 ml polypropylene tubes containing 0.5 ml of TE buffer. After sorting, 0.2 ml of the contents of each tube was processed for observation under epifluorescence microscopy. Five μl of TE- sorted cells content was used for PCR amplification. To lyse picocyanobacterial cells we added a 5 min. heating step at 94°C to the PCR cycle described above. The picocyanobacterial 16S rRNA gene (partial) and the ITS (complete) were PCR-amplified using the specific cyanobacterial primers Cya-771-F and PITSEND-R (Supporting Information Table S2). The StrataClone PCR Cloning Kit (Agilent Technologies) was used for cloning, following the manufacturer's instructions. Clones were sequenced using the primers listed in Supporting Information Table S2 (Macrogen, South Korea). The sequences were edited as described above. Sequences shorter than 1500 pb were excluded from the analyses.

The remaining sequences were analysed by an agglomerative clustering at a 100% identity threshold of the 16S rRNA gene in order to define OTUs retrieved from sorted populations (USEARCH software; Edgar, 2010). After this clustering, we further performed a comparative analysis of the ITS sequences (GENEIOUS 9.1.5 software; Kearse *et al*., 2012) looking for patterns among the groups defined based on the 16S rRNA sequences. We considered that Identical 16S rRNA gene sequences and no differences within the ITS between single-cell (Pcy) and colonial (CPcy) picocyanobacteria populations would provide evidence that both phylotypes belong to the same species.

Phylogenetic analysis

The partial and complete 16S rRNA gene sequences retrieved in this study (DGGE bands, environmental and sorted clones) were used in further phylogenetic analyses. When identical sequences (>99% similarity of 16S + ITS sequence) were obtained from the same sample, only one representative was selected. Sequences closely related with filamentous cyanobacteria identified by BLASTN comparison to GenBank (http://blast.ncbi.nlm.nih.gov) were excluded. The sequences were aligned and incorporated into the alignment of complete sequences of 16S rRNA gene published by Komárek *et al*. (2011) (http://dx/doi.org/10.1080/09670262.2011.606373) using MAFFT v.7 (Katoh and Standley, 2013). The phylogenetic tree was constructed using the Maximum likelihood method with the GTRGAMMA model and 1000 bootstraps using the program RaxML v.7.0.4 (Stamatakis, 2006). *Synechococcus* PCC 6301 was used as outgroup. The tree was drawn using iTOL (http://itol.embl.de; Letunic and Bork, 2006).

Nucleotide sequence accession numbers

The sequences reported in this paper have been deposited in GenBank under the following accession numbers: KU841560-KU841591 for environmental clones; KU841592-KU841630 for clones of the sorted Pcy population; KU841631-KU841673 for the clones of the sorted CPcy population; and KU841674 - KU841696 for the DGGE bands.

Data analysis

All statistical analyses were performed within the R environment (R Development Core Team, 2014). Pearson correlation coefficients were computed to investigate the relationship between the picocyanobacterial community, environment variables, and the heterotrophic community (HF and zooplankton). One-way ANOVA test (Kruskal-Wallis method for multiple comparisons) was carried out to analyse differences in the cell-size of dominant cyanobacterial morphotypes. Furthermore, the significance of the differences between treatments in the six-day experiment was assessed with a Student's Test using the Vegan R-package (Oksanen *et al.*, 2013).

Abundance of Pcy and CPcy in cells per millilitre and DGGE relative band intensities (the relative contribution of each band to the total band signal in the lane) were ordered by nonmetric multidimensional scaling (NMDS) with Hellinger transformation and Bray–Curtis index by the APE (Paradis *et al.*, 2004) and Vegan R-packages. To test the statistical significance of the groups obtained by NMDS, analyses of similarity (ANOSIM) using 999 permutations were conducted with the Vegan R-package. Mantel tests, using Pearson's correlation, were performed in order to investigate the relationship between cyanobacterial community composition and the abiotic/biotic variables. A matrix based on DGGE relative band intensities was compiled to compare (with a Mantel test) the morphological and genetic information. Simple and partial Mantel tests were carried out using Bray–Curtis similarity matrices for both community data picocyanobacteria (Hellinger transformation) and zooplankton, and Euclidean distance dissimilarity matrices for standardized environmental data, all using the Vegan R-package.

Acknowledgements

This study was supported by the Argentinean Network for the Assessment and Monitoring of Pampean shallow-lakes (PAMPA^2–CONICET), 3PA and the ANPCyT (PICT-2011-1029 and PICT-2014-1290). We thank to Dr. F. M. Cornejo and Dr. I. Ferrera for their help in molecular analyses and the rest of the team at the Institut de Ciències del Mar, CSIC, Barcelona. Dr. G. Salerno, M. Kolman and M. Perez-Cenci for their laboratory assistance with the clone library. Agustina Ganuza (CIC-INTECH) for technical assistance with the flow cytometry. We thank Dr. M. Devercelli and V. Quiroga for their advice on an early draft and for critical revision of the manuscript, and two anonymous reviewers for valuable comments on the manuscript.

References

Allende, L., Tell, G., Zagarese, H., Torremorell, A., Pérez, G., Bustingorry, J., *et al.* (2009) Phytoplankton and primary production in clear-vegetated, inorganic-turbid, and algal-turbid shallow lakes from the pampa plain (Argentina). *Hydrobiologia* **624:** 45–60.

Becker, S., Singh, A.K., Postius, C., Böger, P., and Ernst, A. (2004) Genetic diversity and distribution of periphytic Synechococcus spp. in biofilms and picoplankton of Lake Constance. *FEMS Microbiol Ecol* **49:** 181–190.

Becker, S., Sánchez-Baracaldo, P., Singh, A.K., and Hayes, P.K. (2012) Spatio-temporal niche partitioning of closely related picocyanobacteria clades and phycocyanin pigment types in Lake Constance (Germany). *FEMS Microbiol Ecol* **80:** 488–500.

Bleiwas, A., and Stokes, P. (1985) Collection of large and small food particles by *Bosmina*. *Limnol Oceanogr* **30:** 1090–1092.

Blom, J.F., Horn, K., Šimek, K., and Pernthaler, J. (2010) Aggregate formation in a freshwater bacterial strain induced by growth state and conspecific chemical cues. *Environ Microbiol* **12:** 2486–2495.

Blomqvist, P. (1996) Late summer phytoplankton responses to experimental manipulations of nutrients and grazing in unlimed and limed Lake Njupfatet, central Sweden. *Arch Für Hydrobiol* **137:** 425–455.

Callieri, C. (2010) Single cells and microcolonies of freshwater picocyanobacteria: a common ecology. *J Limnol* **69:** 257–277.

Callieri, C., and Stockner, J. (2000) Picocyanobacteria success in oligotrophic lakes: fact or fiction? *Analysis* **59:** 72–76.

Callieri, C., Lami, A., and Bertoni, R. (2011) Microcolony formation by single-cell *Synechococcus* strains as a fast response to UV radiation. *Appl Environ Microbiol* **77:** 7533–7540.

Callieri, C., Caravati, E., Corno, G., and Bertoni, R. (2012a) Picocyanobacterial community structure and space-time dynamics in the subalpine Lake Maggiore (N. Italy). *J Limnol* **71:** 95–103.

Callieri, C., Cronberg, G., and Stockner, J.G. (2012b). Freshwater Picocyanobacteria: single cells, microcolonies and colonial forms. In *Ecology of Cyanobacteria II: Their Diversity in Space and Time*, Whitton B.A. (ed.). Springer Science+Business Media B.V, London, pp. 229–269.

Crosbie, N.D., Pöckl, M., and Weisse, T. (2003) Rapid establishment of clonal isolates of freshwater autotrophic picoplankton by single-cell and single-colony sorting. *J Microbiol Methods* **55:** 361–370.

Demott, W.R., and Kerfoot, W.C. (1982) Competition among cladocerans: nature of the interaction between Bosmina and Daphnia. *Ecology* **63:** 1949–1966.

Diovisalvi, N., Berasain, G., Unrein, F., Colautti, D., Fermani, P., Llames, M.E., *et al.* (2010) Chascomús: estructura y funcionamiento de una laguna pampeana turbia. *Ecol Austral* **20:** 115–127.

van Donk, E., Ianora, A., and Vos, M. (2011) Induced defences in marine and freshwater phytoplankton: A review. *Hydrobiologia* **668:** 3–19.

Edgar, R.C. (2010) Supplementary Material - Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460–2461.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194–2200.

Ernst, A., Marschall, P., and Postius, C. (1995) Genetic diversity among *Synechococcus* spp. (cyanobacteria) isolated from the pelagial of Lake Constance. *FEMS Microbiol Ecol* **17:** 197–203.

Fermani, P., Diovisalvi, N., Lagomarsino, L., Zagarese, H.E., and Unrein, F. (2013) The microbial food web structure of a

hypertrophic warm-temperate shallow lake, as affected by contrasting zooplankton assemblages. *Hydrobiologia* **714:** 115–130.

Fernandez Zenoff, V., Sineriz, F., and Farias, M.E. (2006) Diverse responses to UV-B radiation and repair mechanisms of bacteria isolated from high-altitude aquatic environments. *Appl Environ Microbiol* **72:** 7857–7863.

Gasol, J.M., and Moran, X.A. (2015) Flow cytometric determination of microbial abundances and its use to obtain indices of community structure and relative activity. In *Hydrocarbon and Lipid Microbiology Protocols*, MacGenity, T.J., *et al.* (ed.). Berlin: Springer Protocols Handbooks. Springer-Verlag, pp. 1–29.

Hillebrand, H., Dürselen, C.D., Kirschtel, D., Pollingher, U., and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J Phycol* **35:** 403–424.

Iachetti, C.M., and Llames, M.E. (2015) Light limitation helps stabilize the phytoplankton assemblage steady-state in a temperate and highly turbid, hypertrophic shallow lake (Laguna Chascomús, Argentina). *Hydrobiologia* **752:** 33–46.

Iriondo, M.H., and Drago, E.C. (2004) The headwater hydrographic characteristics of large plains: the Pampa case. *Ecohydrol Hydrobiol* **4:** 7–16.

Izaguirre, I., Sánchez, M.L., Schiaffino, M.R., O'farrell, I., Huber, P., Ferrer, N., *et al.* (2015) Which environmental factors trigger the dominance of phytoplankton species across a moisture gradient of shallow lakes? *Hydrobiologia* **752:** 47–64.

Jezberová, J., and Komárková, J. (2007) Morphological transformation in a freshwater Cyanobium sp. induced by grazers. *Environ Microbiol* **9:** 1858–1862.

Jürgens, K., and Matz, C. (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie Van Leeuwenhoek* **81:** 413–434.

Katoh, K., and Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30:** 772–780.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., *et al.* (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28:** 1647–1649.

Koblížek, M., Josef, K., and Masojídek, J. (2000) Cell aggregation of the cyanobacterium *Synechococcus elongatus*: role of the electron transport chain. *J Phycol* **36:** 662–668.

Komárek, J., and Anagnostidis, K. (1998). Cyanoprokaryota 1. Chroococcales. Gustav Fischer, Jena-Stuttgart- Lübeck-Ulm.

Komárek, J., Kopecky, J., and Cepak, V. (1999) Generic characters of the simplest cyanoprokaryotes and *Cryptogam Algol* **20:** 209–222.

Komárek, J., Kaštovský, J., and Jezberová, J. (2011) Phylogenetic and taxonomic delimitation of the cyanobacterial genus Aphanothece and description of Anathece gen. nov. *Eur J Phycol* **46:** 315–326.

Komárek, J., Kaštovský, J., Mareš, J., and Johansen, J.R. (2014) Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* **86:** 295–335.

Komárková, J., and Šimek, K. (2003) Unicellular and colonial formations of picoplanktonic cyanobacteria under variable environmental conditions and predation pressure. *Arch Hydrobiol Suppl Algol Stud* **109:** 327–400.

Lagomarsino, L., Pérez, G.L., Escaray, R., Bustingorry, J., and Zagarese, H.E. (2011) Weather variables as drivers of seasonal phosphorus dynamics in a shallow hypertrophic lake (Laguna Chascomús, Argentina). *Fundam Appl Limnol/Arch Für Hydrobiol* **178:** 191–201.

Lagomarsino, L., Diovisalvi, N., Bustingorry, J., Escaray, R., and Zagarese, H.E. (2015) Diel patterns of total suspended solids, turbidity, and water transparency in a highly turbid, shallow lake (Laguna Chascomús, Argentina). *Hydrobiologia* **752:** 21–31.

Letunic, I., and Bork, P. (2007). Interactive Tree Of Life (iTOL): An online tool for phylogenetic tree display and annotation. *Bioinformatics* **23(1):**127–128.

Llames, M.E., Giorgio, P.A., Zagarese, H., Ferraro, M., and Izaguirre, I. (2013) Alternative states drive the patterns in the bacterioplankton composition in shallow Pampean lakes (Argentina). *Environ Microbiol Rep* **5:** 310–321.

Massana, R., Gasol, J.M., Bjørnsen, P.K., Black, N., Hagström, ÅK.E., Hietanen, S., *et al.* (1997) Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. *Sci Mar* **61:** 397–407.

McIntyre, C., Flyg, B., and Fong, T. (2010) Fluorescence-activated cell sorting for CGMP processing of therapeutic cells. *BioProcess Int* **8:** 44–53.

Miracle, M.R., Vicente, E., Sarma, S.S.S., and Nandini, S. (2014) Planktonic rotifer feeding in hypertrophic conditions. *Int Rev Hydrobiol* **99:** 141–150.

Motwani, N.H., and Gorokhova, E. (2013) Mesozooplankton grazing on Picocyanobacteria in the Baltic Sea as inferred from molecular diet analysis. *PLoS One* **8:** 1–8.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., *et al.* (2013). Vegan: community ecology package. R package version 2.0-7. http://CRAN.R-project.org/package=vegan.

Paradis, E., Claude, J., and Strimmer, K. (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20:** 289–290.

Passoni, S., and Callieri, C. (2000) Picocyanobacteria single forms, aggregates and microcolonies: survival strategy or species succession? *Verh Internat Verein Limnol* **27:** 1879–1883.

Pernthaler, J. (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* **3:** 1–11.

Porter, K.G., and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25: 943–948. *Limnol Oceanogr* **25:** 943–948.

Postius, C., and Ernst, A. (1999) Mechanisms of dominance: Coexistence of picocyanobacterial genotypes in a freshwater ecosystem. *Arch Microbiol* **172:** 69–75.

R Development Core Team (2014). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rinke, C., Lee, J., Nath, N., Goudeau, D., Thompson, B., Poulton, N., *et al.* (2014) Obtaining genomes from uncultivated environmental microorganisms using FACS-based single-cell genomics. *Nat Protoc* **9:** 1038–1048.

Sánchez-Baracaldo, P., Handley, B.A., and Hayes, P.K. (2008) Picocyanobacterial community structure of freshwater lakes and the Baltic Sea revealed by phylogenetic analyses and clade-specific quantitative PCR. *Microbiology* **154:** 3347–3357.

Staden, R. (1996) The Staden sequence analysis package. *Mol Biotechnol* **5:** 233–241.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stockner, J.G. (1991) Autotrophic picoplankton in freshwater ecosystems: The view from the summit. *Int Rev Der Gesamten Hydriobiologie* **76:** 483–492.

Torremorell, A., Bustigorry, J., Escaray, R., and Zagarese, H.E. (2007) Seasonal dynamics of a large, shallow lake, laguna Chascomús: The role of light limitation and other physical variables. *Limnol - Ecol Manag Inl Waters* **37:** 100–108.

Vrede, K. (1996) Regulation of bacterioplankton production and biomass in an oligotrophic clearwater lake - The importance of the phytoplankton community. *J Plankton Res* **18:** 1009–1032.

Weisse, T. (1993) Dynamics of autotrophic picoplanckton in marine and freshwater systems. *Adv Microb Ecol* **13:** 327–370.

Wilmotte, A., and Golubić, S. (1991) Morphological and genetic criteria in the taxonomy of Cyanophyta/Cyanobacteria. *Arch Für Hydrobiol Algol Stud* **64:** 1–24.

Work, K.A., and Havens, K.E. (2003) Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. *J Plankton Res* **25:** 1301–1306.

Yang, Z., Kong, F., Yang, Z., Zhang, M., Yu, Y., and Qian, S. (2009) Benefits and costs of the grazer-induced colony formation in Microcystis aeruginosa. *Ann Limnol Int J Limnol* **45:** 203–208.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Average (AVG), standard deviation (SD) and range of the main physical, chemical and biological parameters, measured during the study period. In all cases n= 39.

Table S2. Primer sets used.

Table S3. Phylogenetic affiliation of the sequences obtained in lake Chascomús from June 2012 to March 2014.

Fig. S1. Temporal fluctuation of phytoplankton biovolume in lake Chascomús from June 2012 to March 2014. Colonial coccoids which cells features were no similar to Pcy were analysed separately.

Fig. S2. DGGE profiles of 0.2 and 3 μm filters obtained with cyanobacteria-specific PCR amplification of DNA from samples collected from December 2012 to March 2013 in lake Chascomús.

Fig. S3. DGGE profiles obtained with cyanobacteria-specific PCR amplification of DNA from samples collected from June 2012 to March 2014 in lake Chascomús. (a) Images of the original DGGE gels. (b) Heatmap of DGGE profiles constructed using gplots and RColorBrewer packages in the R environment (R Development Core Team, 2014). Sampling dates are indicated at the bottom side of the figure. The numbers in the digitalized picture indicate the bands that were sequenced (Table S3). The arrows indicate the seven dominant genotypes referred by in the text.

Fig. S4. Flow cytometry plots showing picocyanobacterial populations. The dotted lines indicate the gates used for single-cell (Pcy) and colony (CPcy) sorting. As internal standard, 1 μm-diameter yellowgreen fluorescent latex beads (Fluoresbrite, Polysciences) was used.