

Picocyanobacterial assemblages in ultraoligotrophic Andean lakes reveal high regional microdiversity

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Picocyanobacteria (Pcy) diversity was studied in a set of ultraoligotrophic North Patagonian Andean lakes. The glacial lake system includes a central lake (Lake Nahuel Huapi, with its side basin, Lake Moreno) and four satellite lakes (Lakes Espejo, Correntoso, Gutiérrez and Mascardi) derived from a larger paleolake. Automated ribosomal intergenic spacer analysis showed a Pcy community structure composed of 18 operational taxonomic units (OTUs), of which only one (OTU 1094) was widely distributed, being found at all depths in all of the lakes. Most of the others were observed in a few of the lakes. The principal component analysis revealed the habitat specificity of some Pcy OTUs: the satellite lakes have a highly irradiated epilimnion, while the central lake has a well-mixed euphotic zone that does not extend beyond the epilimnion. We also observed a distinctive vertical distribution of the OTUs and a significant correlation between Pcy chlorophyll-specific primary production and OTU 738, which was only found in the satellite lakes. Lastly, the high β -diversity of this lake district supports the hypothesis that microdiversity is higher in glacier-derived lake systems where habitat fragmentation due to geographic barriers results in rapid speciation.

INTRODUCTION

Picocyanobacteria (Pcy) play a fundamental role in oligotrophic water systems, contributing up to 80% of the total primary production (Bell and Kalff, 2001; Richardson and Jackson, 2007; Callieri, 2008). Pcy are the dominant picophytoplankters in ultraoligotrophic lakes, and are mainly represented by the genus *Synechococcus* (Stockner *et al.*, 2000; Callieri *et al.*, 2007b). The study of their genetic diversity has received much less attention than marine Pcy, and has been restricted to cultured strains (Ernst *et al.*, 1995; Robertson *et al.*, 2001; Crosbie *et al.*, 2003a; Ernst *et al.*, 2003). Nevertheless, studies on natural Pcy assemblages have been carried out in a limited number of lakes including

subalpine lakes (Becker *et al.*, 2002; Becker *et al.*, 2004; Becker *et al.*, 2007; Callieri *et al.*, 2007a), Laurentian Great lake (Ivanikova *et al.*, 2007) and more recently in English lakes (Sánchez-Baracaldo *et al.*, 2008).

Light intensity as well as light spectrum has been considered a major axis of niche differentiation in Pcy communities (Litchman, 2003; Stomp *et al.*, 2004; Stomp *et al.*, 2007). In oligotrophic oceans, Pcy ecotypes adapted to high- and low-light intensities have been observed at different depths in the water column (Rocap *et al.*, 2003; Johnson *et al.*, 2006) and a compartmentalization of different clades has been found under contrasting light environmental conditions (Fuller *et al.*, 2006).

The conservative 16S rRNA gene sequences show high pairwise similarity in freshwater Pcy (Crosbie *et al.*, 2003a) and do not mirror their physiological diversity (Rocap *et al.*, 2002). It has also been reported that community structure data can be also studied using the automated ribosomal intergenic spacer analyses (ARISAs; Fisher and Triplett, 1999). ARISA utilizes the length heterogeneity of the internal transcribed spacer (ITS) region between the 16S rRNA and 23S rRNA genes and is a promising way to examine changes in bacterial richness (Danovaro *et al.*, 2006; Fuhrman *et al.*, 2008; Ramette, 2009). ITS exhibits much length and sequence variation and has been considered in studies concerning the phylogenetic relationships among cultured and uncultured marine and freshwater Pcy (Ernst *et al.*, 2003; Chen *et al.*, 2006; Haverkamp *et al.*, 2008; Haverkamp *et al.*, 2009). ITS analyses have been already performed in many studies of picocyanobacterial diversity in order to single out closely related strains, where 16S rRNA gene sequence analysis lacks resolution (Rocap *et al.*, 2002; Crosbie *et al.*, 2003b), or to differentiate light-adapted ecotypes in the oceans (Rocap *et al.*, 2002; Ahlgren *et al.*, 2006). Using denaturing gradient gel electrophoresis, ITS was also helpful in the study of Pcy strain seasonal distribution in plankton (Becker *et al.*, 2002) and in periphyton (Becker *et al.*, 2004).

The North Patagonian ultraoligotrophic deep lakes (41°S and 71°W) (Fig. 1) spread over an area of ~1000 km² and appear as an intricate net of glacial valleys, which are generally interconnected. When the glaciers retreated from the Andean valleys during regional deglaciation, the water level in glacial paleolakes fell, producing several smaller, ice-free lakes (Tatur *et al.*, 2002). Consequently, lakes Espejo, Correntoso, Gutiérrez and Mascardi can be considered satellite lakes derived from the larger Lake Nahuel Huapi, while Lake Moreno is virtually a side basin of Lake Nahuel Huapi.

These are all clear-water lakes with extinction coefficients of photosynthetically active radiation (PAR, K_d) as low as 0.09 m⁻¹ (Morris *et al.*, 1995; Callieri *et al.*, 2007b). Blue light dominates the deep euphotic layers of the lakes (Pérez *et al.*, 2002), and chlorophyll *a* shows a pronounced deep chlorophyll maximum (DCM) generally situated below the thermocline around 1% PAR level (Callieri *et al.*, 2007b; Pérez *et al.*, 2007). An average of 30% of total chlorophyll *a* can be attributed to Pcy, which are the main component of picophytoplankton in the lakes (Modenutti and Balseiro, 2002; Callieri *et al.*, 2007b).

The particular interconnected nature of North Patagonian lakes, their ultraoligotrophic state and the presence of a distinctive vertical distribution of irradiance

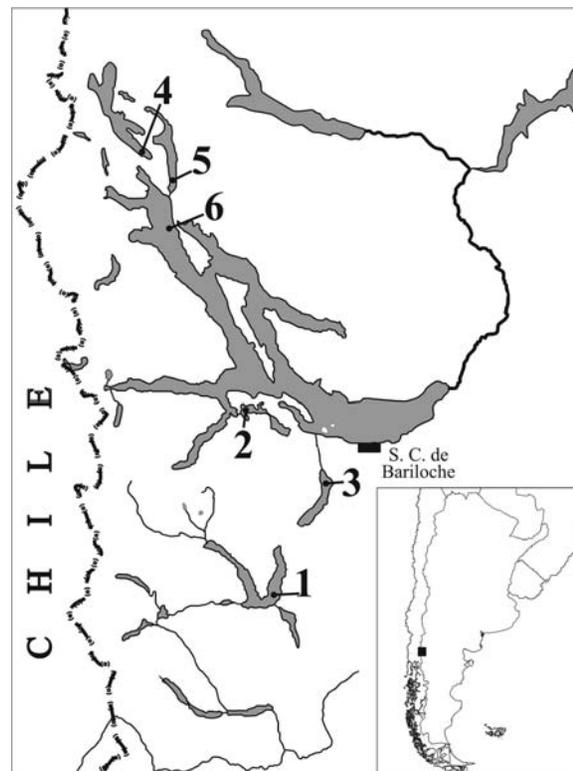


Fig. 1. Map of the Andean Patagonian lakes situated in North Patagonia, Argentina. The dots indicate sampling points. (1) Lake Mascardi, (2) Lake Moreno, (3) Lake Gutiérrez, (4) Lake Espejo, (5) Lake Correntoso, (6) Lake Nahuel Huapi.

(including ultraviolet wavelengths in the epilimnetic layers) (Modenutti *et al.*, 2008) makes them suitable environments for the study of Pcy diversity, habitat specificity and adaptation to various niches along a vertical gradient.

In this study, we aimed to characterize the diversity, composition and habitat specificity of the Pcy community in six deep Andean lakes. We estimated Pcy community structure using ARISA fingerprinting patterns at two different depths (25 and 1% of surface PAR). We also analysed the relationship between genetic diversity and primary production of Pcy assemblages.

METHOD

Site description

The six studied Andean North Patagonian lakes were Espejo, Correntoso, Nahuel Huapi, Moreno and Gutiérrez of the Atlantic watershed, and Mascardi of the Pacific watershed. Lakes are located at 41°S and 71°W, at an altitude of 760 m a.s.l. in the region of the Nahuel Huapi National Park, corresponding to the glacial lakes district of the Southern Andes, Patagonia,

Argentina (Fig. 1). The climate is cold-temperate (mean annual temperature: 8.7°C), and the area is characterized by a profuse hydrographic system including large and deep lakes ($Z_{\max} \geq 150$ m).

The lakes exhibit thermal stratification during late spring and summer. Penetration of PAR is high (up to 50 m) and dissolved organic carbon concentration is low ($< 50 \mu\text{M L}^{-1}$) (Corno *et al.*, 2009). The thermocline is always deeper than the 25% surface PAR depth. The trophic status is ultra-oligotrophic with very low nutrient concentrations (total dissolved phosphorus: $< 0.074 \mu\text{M L}^{-1}$), with a vertical heterogeneous distribution of chlorophyll *a* with a pronounced DCM at 1% of surface PAR (Callieri *et al.*, 2007b).

Sampling

The lakes were sampled during the austral summer (January 2005). Vertical profiles (0–60 m) of PAR (400–700 nm) were measured with a PUV 500B submersible radiometer (Biospherical Instruments). All sampling was carried out in duplicate, at mid-day, 1 h before solar noon. Samples for size-fractionated primary production, chlorophyll *a* concentration, Pcy abundance and molecular analyses were taken at 25 and 1% of surface PAR.

Picocyanobacteria abundance

Samples were immediately preserved in formalin (2% final solution) cacodilate buffered (sol. 20%), stored in darkness at 4°C and processed within 2 weeks. Counting was performed on polycarbonate filters (0.2 μm pore size, OsmonicsTM) by autofluorescence of phycobiliproteins (Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar 100 \times objective, 1.25 \times additional magnification and filter sets for blue and green light excitation, Zeiss filter set 09: BP450-490, FT510, LP520, Zeiss filter set 14: LP510-KP560, FT580, LP590). A minimum of 200 cells were counted for each replicate.

Primary production and chlorophyll *a* determination

Primary production was measured using the ^{14}C technique (Steeman Nielsen, 1951). As dark bottle measurements, the “time 0” organic ^{14}C measurement was used by adding the isotope to the dark bottle and immediately filtering and analysing (Fahnenstiel *et al.*, 1994; Callieri *et al.*, 2007b). Lake water was sampled before starting the incubations and four replicate vials (25 mL each) were immediately filled with lake water. To each vial, 1.48 kBq $\text{NaH}^{14}\text{CO}_3 \text{ mL}^{-1}$ (Amersham) was added and then

incubated *in situ* for 4 h around noon. Incubations were carried out suspending the vials at 25% and 1% of surface PAR. At the end of the incubation, 500 μL aliquots were taken to check total activity. The samples were filtered using plastic disposable syringes and plastic filter holders (25 mm diameter). Polycarbonate filters (OsmonicsTM) of 2 μm were first used and the filtrate was concentrated on 0.22 μm pore size nitrocellulose membranes (MilliporeTM). Filters were acidified with 200 μL 1N HCl for 60 min in 20 mL scintillation vials. After adding 10 mL of scintillation liquid, the vials were counted in a Beckman LS3801 scintillation counter. Photosynthetic carbon assimilation was calculated based on the proportion between ^{14}C uptake and total inorganic carbon availability. The total inorganic carbon availability was determined from pH and alkalinity measurements.

For the determination of chlorophyll *a* content of Pcy, up to 250 mL of sampled water was filtered through a 2.0 μm pore size polycarbonate filter and the filtrate was subsequently filtered through a 0.2 μm pore size polycarbonate filter. Chlorophyll *a* was extracted with hot ethanol (Nusch, 1980) and measured with a 10-AU fluorometer (Turner Design). The primary productivity was normalized to $< 2 \mu\text{m}$ chlorophyll *a*.

Extraction of assemblage DNA

About 300 mL of lake water for each environmental sample was concentrated on 0.2 μm sterile hydrophilic polyethersulfone Supor[®] (Gelman Laboratory) and kept at -20°C in 2 mL of lysis buffer (50 mM Tris, 40 mM EDTA, 400 mM NaCl, 0.75 M Sucrose) for no longer than 2 months.

Nucleic acid extraction was performed directly from the filters using the Ultra Clean Bacterial Soil DNA Kit (Mbio Laboratories Inc., California) following the manufacturer’s protocol for the maximization of the extracted DNA amounts. Nucleic acid extracts were then checked on 0.8% agarose-TBE (Tris-Borate-EDTA, Promega) gels stained with ethidium bromide. Each extract was quantified spectrofluorometrically with a QuBit Fluorometer (Invitrogen), and stored at -80°C until further analysis.

Polymerase chain reaction (PCR)

To increase the amount of the amplified DNA signal, we used a semi-nested polymerase chain reaction (PCR) procedure. About 2 ng of total extracted DNA was used as a template for the first PCR using specific forward cyanobacterial primer 16S-359F (5'-GGGGAATYT TCCGCAATGGG-3') (Nübel *et al.*, 1997) and universal bacterial primer 23S-125R (5'-GGGTTBCCCCATT

CRG-3'). About 2 μL of each amplified samples was used as template for second PCR performed with the specific freshwater ITS cyanobacteria forward primer 16S-CSIF (5'-GYCACGCCCGAAGTCRTTAC-3') (Janse *et al.*, 2003) and with the universal bacterial 23S-125R (in this case TET labelled at 5'). The PCRs were executed in a volume of 50 μL containing forward and reverse primers (each at 0.3 μM) and 20 μL of PCR Master Mix (Promega). Thirty PCR cycles were used for both the DNA amplifications, consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, preceded by 3 min of denaturation at 94°C and followed by a final extension of 10 min at 72°C. All PCR amplifications were then purified with a PCR Purification Kit (Qiagen), resuspended in 50 μL of MilliQ water and checked on 1% agarose-TBE (Tris-Borate-EDTA, Promega) gels stained with ethidium bromide. Each amplification was then quantified using a QuBit Fluorometer (Invitrogen), and stored at -20°C until being processed.

Automated rRNA intergenic spacer analysis and operational taxonomic unit sequences

To study the Pcy diversity, we used the ARISA fingerprinting technique, which amplifies the ITS region in the rRNA operon plus small parts of the 16S rRNA and 23S rRNA genes. The purified TET-labelled PCR products were denatured (3 μL of PCR product, 2.5 μL of deionised formamide and 0.5 μL of internal size standard; ROX 2500, Applied Biosystems) at 94°C for 2 min, then chilled on ice and carried for the analysis. ARISA fragments were separated and detected by electrophoresis at 2800 V for 7 h in a denaturing 5% acrylamide gel with an ABI Prism 377 automated sequencer. The sizes of ARISA fragments, which represent different operational taxonomic units (OTUs), were determined by comparison with the internal size standard ROX 2500 (Applied Biosystems) by using the local southern size-calling method of the software GeneScan 3.7 (Applied Biosystems). To align ARISA profiles of different runs, we binned the peaks in different fixed windows (Hewson and Fuhrman, 2006) depending on fragment length (300/700–3 bp, 700/1000–5 bp, >1000–10 bp). The peak heights of all the ARISA profiles collected from the same lake were standardized following the procedures described by Dunbar *et al.* (Dunbar *et al.*, 2001). All the peaks smaller than 350 bp and those longer than 1300 bp size were not considered because the amplified fragments are composed by the ITS region (for Pcy even more than 1000 bp) (Ernst *et al.*, 2003) plus 300–350 bp belonging to the next genes (about 200 bp from 16S rRNA gene and 125 bp from 23S rRNA gene). All peaks within

1.9 bp from a higher peak (commonly called “shoulder peaks”) were eliminated. The detection threshold applied to ARISA profiles was calculated according to the approach suggested by Luna *et al.* (Luna *et al.*, 2006) and in our case was 0.24% of the total fluorescence.

Amplified ITS fragments, generated for ARISA analysis, were cloned using pGEM-T Easy Vector (Promega) according to the manufacturer’s instructions as described in Michaud *et al.* (Michaud *et al.*, 2009). For the PCR amplification, the vector-specific primers, M13F (5'-GTAAAACGACGGCCAG-3') and M13R (5'-CAGGAAACAGCTATGAC-3'), were used. Sequence reaction was carried out by Macrogen Inc. (South Korea). The ITS sequences retrieved from this research are published in GenBank (GQ401166, GQ401167, GQ401168, GQ401169).

Calculations and statistical analysis

The epilimnetic mean irradiances were calculated for each lake as follows:

$$I_m = I_0 \bullet \frac{1 - e^{(-K_d \bullet \zeta)}}{K_d \bullet \zeta}$$

where I_0 is the irradiance at the surface, K_d the PAR diffuse attenuation coefficient and ζ the depth of the mixed layer.

Richness, Shannon–Weaver diversity index and evenness were calculated according to Pielou (Pielou, 1984), on the ARISA OTU relative fluorescence. Pcy community composition belonging to different lakes and depths were analysed through principal component analysis (PCA) using CANOCO 4.5.

The chlorophyll *a*-specific primary production of Pcy at depths of 25% and 1% surface PAR as dependent variable, and the corresponding relative proportion of OTU fluorescence as independent variables, were analysed through forward stepwise regression with SigmaStat 3.5 software.

Statistical differences were tested by paired *t*-test and two-way ANOVA. Normality and homoscedasticity were checked and transformations were applied when necessary to fulfil these conditions. All statistics were carried out using SigmaStat 3.1.

RESULTS

Lake physical characteristics

The six lakes were thermally stratified, although thermocline depth varied between lakes. In the lakes

Table I: Physical, chemical and biological characteristics of the six Andean North Patagonian lakes

	Nahuel Huapi	Moreno	Gutiérrez	Mascardi	Espejo	Correntoso
Area	557	5.2	16.4	39	30	19.5
Z_{\max}	464	90	111	218	245	>100
Z_{therm}	>50	19.3	22	11.3	15	13.3
I_m	279	559	583	751	698	835
$Z_{1\%}$	48.8	32.8	37.4	27.9	41.4	43.4
K_d	0.094	0.140	0.127	0.165	0.111	0.106
Temp 25%	13	16	15	17	18	18
Temp 1%	10	8.0	8.0	7.4	7.2	7.2
TDP 25%	2.55	2.55	1.23	1.89	2.38	2.05
TDP 1%	2.22	2.87	2.05	2.05	1.07	1.40

Area: lake surface (km²); Z_{\max} , maximum lake depth (m); Z_{therm} , depth of the thermocline (m); I_m , mean epilimnetic irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$); $Z_{1\%}$, depth of the 1% of surface irradiance; K_d , extinction coefficient of photosynthetically active radiation; Temp 25%, temperature (°C) at the depth of 25% of surface irradiance; Temp 1%, temperature (°C) at the depth of 1% of surface irradiance; TDP 25%, total dissolved phosphorus ($\mu\text{g L}^{-1}$) at the depth of 25% of surface irradiance; TDP 1%, total dissolved phosphorus ($\mu\text{g L}^{-1}$) at the depth of 1% of surface irradiance.

Gutiérrez, Mascardi, Moreno, Correntoso and Espejo, the depth of the thermocline ranged from 11 to 22 m, while in the largest Lake Nahuel Huapi it was deeper than 50 m (Table I). Epilimnetic temperatures at 25% surface PAR ranged between 13 and 18°C and the hypolimnetic ones at 1% surface PAR were around 7–8°C (Table I). Euphotic zones ($Z_{1\%}$ in Table I) were deep in all lakes, ranging from 30 m (Lake Mascardi) to 50 m (Lake Nahuel Huapi). With the exception of the latter, the euphotic zone extended beyond the mixed layer (see $Z_{1\%}$ and Z_{therm} in Table I), and thus included the upper part of the hypolimnion. As a consequence of the location of $Z_{1\%}$ and Z_{therm} , Lake Nahuel Huapi exhibited the lowest mean epilimnetic irradiance (I_m) followed by Lake Moreno; and lakes Gutiérrez, Mascardi, Espejo and Correntoso have a comparatively more illuminated epilimnion (Table I).

Picocyanobacteria abundance, chlorophyll *a* and chlorophyll-specific production

The direct observation of the natural assemblages indicated that picophytoplankton, in lakes Moreno, Gutiérrez, Mascardi, Correntoso, and Espejo, was entirely composed of small prokaryotic phycoerythrin-rich cells (mean biovolume: $0.31 \pm 0.1 \mu\text{m}^3$ per cell).

Pcy abundances ranged from $18.9 \pm 1.1 \times 10^3$ to $132.2 \pm 3.2 \times 10^3 \text{ cell mL}^{-1}$. The abundances at 1% of surface PAR were significantly higher than those at 25% of surface PAR (paired *t*-test: $n = 6$, $P = 0.006$) (Fig. 2A). Chlorophyll *a* concentration in the size $< 2 \mu\text{m}$ ranged from 0.07 ± 0.007 to $1.88 \pm 0.4 \mu\text{g L}^{-1}$ and in general was very low at 25% surface PAR while at 1% was higher forming a DCM (Fig. 2B). We found significant difference among lakes and the two different depths (two-way ANOVA between lakes and between depths, $P < 0.0001$). The

chlorophyll-specific production was higher at 25% surface PAR than at 1% surface PAR except in Lake Mascardi (Fig. 2C) and ranged from 0.05 ± 0.01 to $2.68 \pm 0.13 \text{ mg C (mg Chl)}^{-1} \text{ h}^{-1}$. In particular, the values obtained are comparatively lower in lakes Nahuel Huapi and Moreno than in the other lakes (Fig. 2C).

Pcy community composition and OTU richness

A total of 18 OTUs were found, some of them appeared to be site-specific, while others displayed a wide distribution among lakes (Fig. 3). The OTU 1094 occurred in all samples and comprised 21–95% of the total amplicons, and was dominant in lakes Nahuel Huapi and Moreno. OTU 683 was present in most of the lakes and depths, whereas OTU 528 appeared only in Lake Nahuel Huapi at the depth of 1% PAR. OTU 738 occurred only in four lakes: Gutiérrez, Mascardi, Espejo and Correntoso. Remarkably, one fragment (OTU 656) was present only in samples from Lake Espejo. The following environmental ITS sequences were recognized as cyanobacteria: OTU 1094 (AL-Clone01, GQ401166), 683 (AL-Clone02, GQ401167), 656 (AL-Clone03, GQ401168), 528 (AL-Clone04, GQ401169), representing up to 95% of the total ARISA signal. These sequences exhibited a low similarity ($< 98\%$) with the already published Pcy ITS sequences.

Considering the whole data set, no differences were observed in OTU richness, diversity and evenness of ARISA fragments (two-way ANOVA $P > 0.05$) (Table II).

The PCA analysis (total variance explained by the first three axes = 93%) showed two clear groups of samples, and two isolated samples (Fig. 4A). The first group comprised samples of lakes Nahuel Huapi and

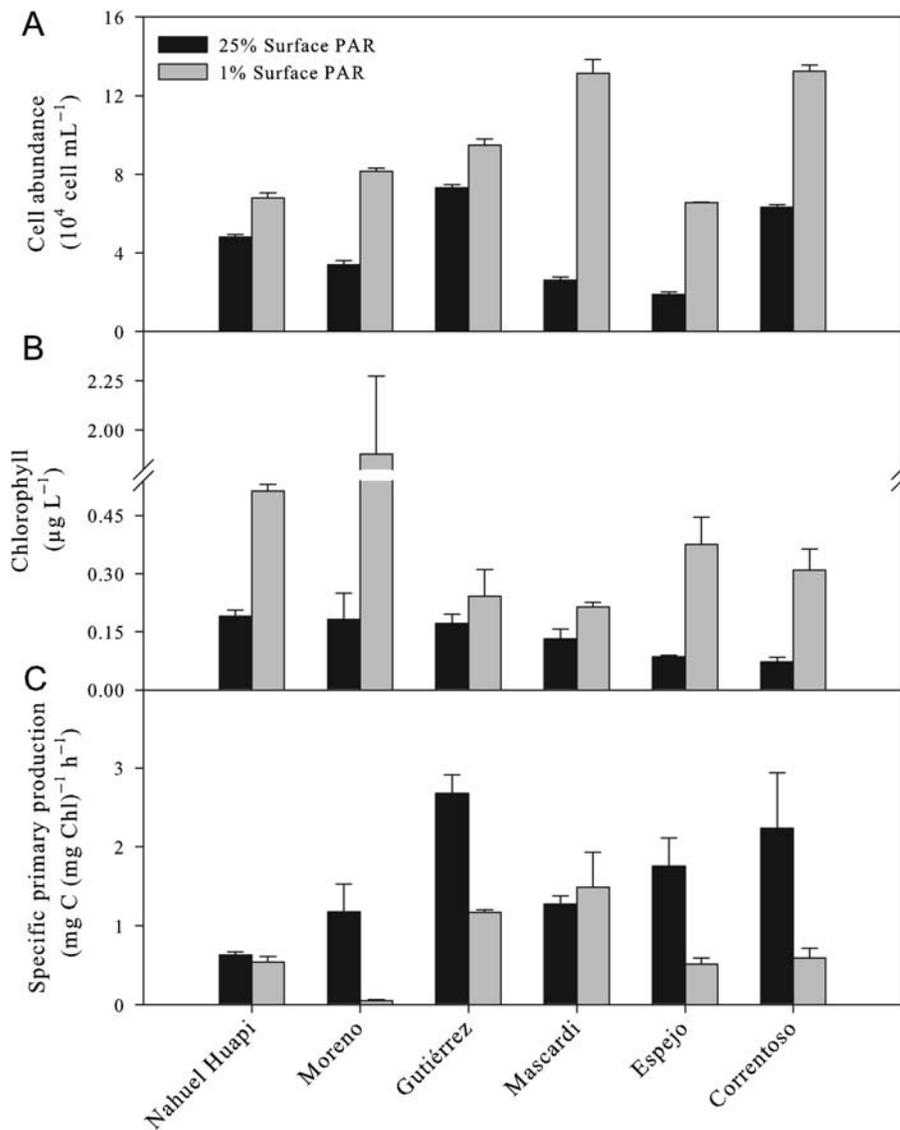


Fig. 2. Picocyanobacteria cell abundance (A), chlorophyll *a* (B) and chlorophyll-specific production (C) in the size range <2 μm of the different lakes at 25% and 1% surface PAR depths.

Moreno at both depths. The second one, included lakes Mascardi, Correntoso and epilimnetic samples of Gutiérrez and Espejo; while deep samples of these two lakes appear as isolated samples. The most important OTUs that determined this configuration were OTU 1094, and 738, both with high correlation with axis 1 (Fig. 4B). OTU 1094 has a widespread distribution, while 738 was absent in the first group but very important, almost dominating, in the second one, and much more scarce in the two isolated samples (hypolimnetic samples from Gutiérrez and Espejo). In a third place, and correlated to axis 2 (Fig. 4B), appear OTUs 656, 443 and 489 present only in Lake Espejo, the former dominating the deep sample of this lake and the other

two only present in the deep sample of this lake. The analysis showed that all other OTUs had less contribution to the observed ordination.

OTU richness and chlorophyll-specific primary production relationship

We analysed the dependence of the chlorophyll-specific primary production on the different OTUs and we observed that the only variable included in the model was the OTU 738 (forward stepwise regression, for OTU 738: $R^2 = 0.54$, $P = 0.007$). In all cases, the other OTUs were not statistically significant. The exclusive presence of OTU 738 in Lakes Gutiérrez,

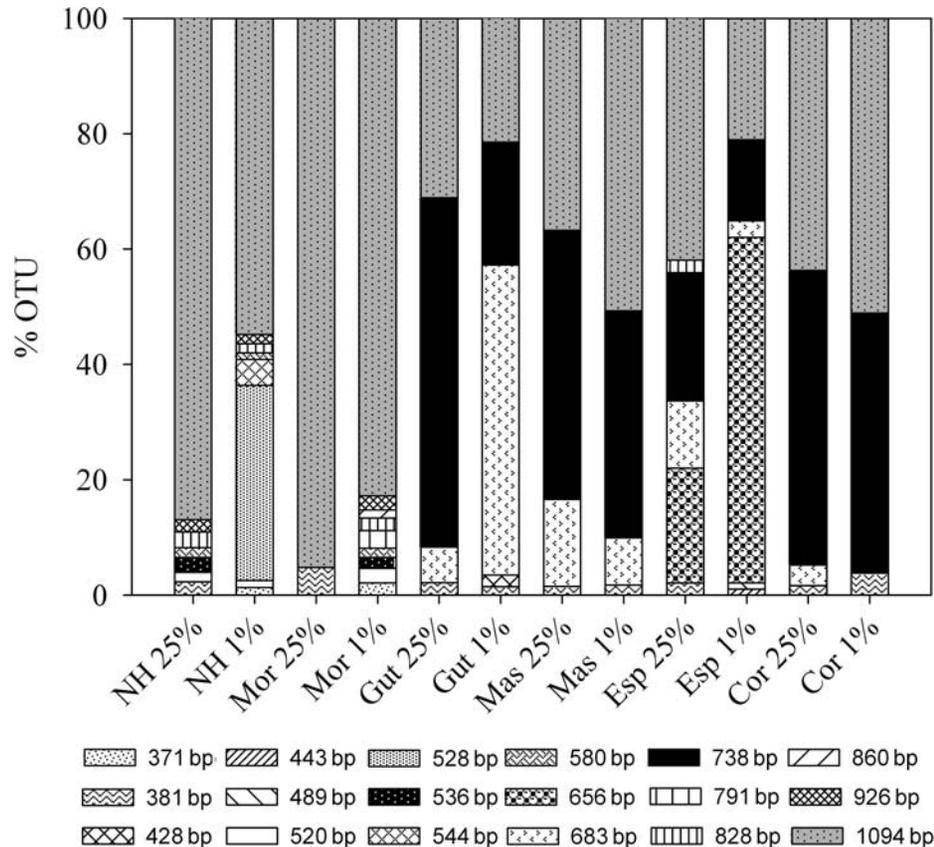


Fig. 3. Proportion of single OTUs obtained by ARISA on the ITS fragment at different depths (25% and 1% of surface PAR) in Andean lakes. NH, Lake Nahuel Huapi; Mor, Lake Moreno; Gut, Lake Gutiérrez; Mas, Lake Mascardi; Esp, Lake Espejo; Cor, Lake Correntoso.

Table II: Richness, Shannon–Weaver diversity index and evenness calculated on ARISA OTU relative fluorescence of the six Andean North Patagonian lakes

Lakes	Richness	Diversity (Shannon index)	Evenness (Pielou)
Nahuel Huapi 25%	7	0.619	0.318
Nahuel Huapi 1%	8	1.128	0.542
Moreno 25%	2	0.192	0.277
Moreno 1%	9	0.816	0.371
Gutiérrez 25%	4	0.921	0.665
Gutiérrez 1%	5	1.134	0.705
Mascardi 25%	4	1.073	0.774
Mascardi 1%	4	0.987	0.712
Espejo 25%	6	1.436	0.801
Espejo 1%	6	1.109	0.619
Correntoso 25%	4	0.891	0.643
Correntoso 1%	3	0.826	0.752

Mascardi, Correntoso and Espejo (Fig. 3) matched with the ordination plot (PCA, Fig. 4). The relative importance of this OTU comprised 14–61% of the total

amplified DNA in these lakes. Pcy communities which included OTU 738 showed a relatively higher specific primary production.

DISCUSSION

Our data showed that 50% of significant ARISA fragments were unique to one lake or one lake group. The existence of dominant genotypes was also reported from lakes in the North hemisphere in particular in the pelagic waters of Lake Superior (Ivanikova *et al.*, 2007). Nevertheless, our study also showed habitat specificity of some Pcy OTU, since PCA analysis showed an ordination of two lake groups, one corresponding to Nahuel Huapi-Moreno and the other to Gutiérrez, Mascardi, Espejo and Correntoso. During lake evolution, some Pcy OTUs could result from specific adaptations, favoured by even minor differences in irradiance conditions. In this sense, the satellite lakes have a highly irradiated epilimnion, while the central lake has an epilimnion deeper than the photic layer. Since the situation

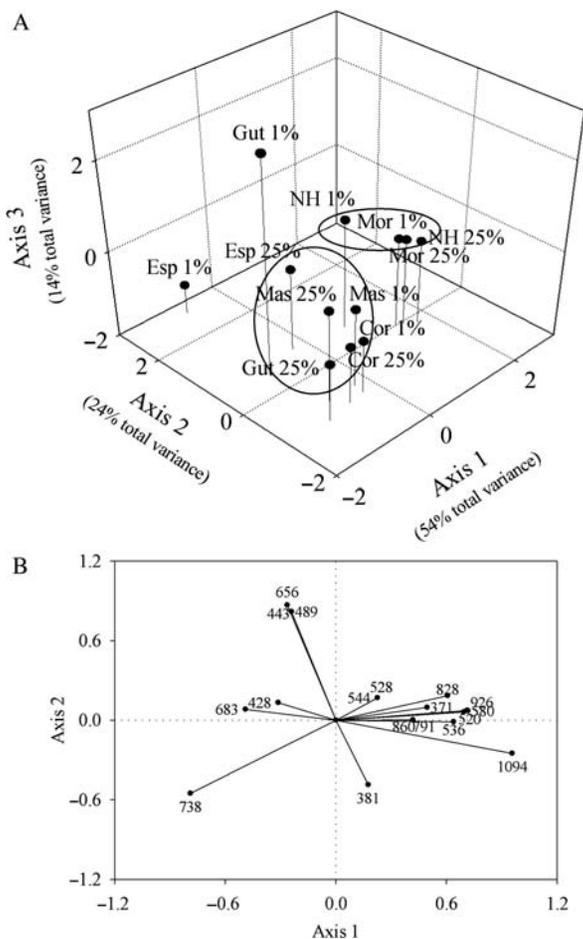


Fig. 4. (A) First three axes of the principal component analysis (PCA) on the Pcy community composition (ARISA bands) belonging to the different lakes and depths. (B) Vectors resulted from the PCA analysis. References: NH, Lake Nahuel Huapi; Mor, Lake Moreno; Gut, Lake Gutiérrez; Mas, Lake Mascaridi; Esp, Lake Espejo; Cor, Lake Correntoso.

of satellite lakes was established early in geological times, late Pleistocene (13.2 ky B.P.) (Tatur *et al.*, 2002), it is likely that this condition allowed the colonization of distinctive OTUs. In addition, we obtained statistical evidence that Pcy chlorophyll-specific primary production was positively correlated to OTU 738 only found in the four satellite lakes, and with high importance in axis 1 of the PCA. The correlation above suggests the habitat specificity of this OTU which was present only in one lake group and seems to drive the photosynthetic production of the Pcy community in those lakes.

In this sense, microdiversity is emphasized in glacier derived lake systems where the fragmentation of the habitat due to geographic barriers allows more rapid speciation than marine waters (Sánchez-Baracaldo *et al.*, 2008) giving a high β -diversity to the area. Thus, the

mean number of OTU per lake depth is 5.1, whereas the total number of OTU in the studied lakes rises to 18. This means that each single lake increases significantly the total diversity of the region. The limited diversity of each lake assemblage and the extreme oligotrophy of the lakes (low nutrient concentrations, low organism abundances, low non-target DNA, low humic acids and others PCR inhibitors) decreased the risk of PCR biases as well as the risk of competition-overlap of the DNA fragments belonging to different OTUs. The occurrence of multiple ribosomal operons is limited in oligotrophic environments by the dominance of slow growing organisms (Klappenbach *et al.*, 2000), in particular Pcy belonging to genus *Synechococcus* or *Prochlorococcus* (Stewart and Cavanaugh, 2007).

The coexistence of multiple ecotypes requires adaptation to diverse niches, determined by the habitat heterogeneity throughout the water column (Ahlgren and Rocap, 2006). In the stratified ocean, *Prochlorococcus* ecotypes clearly segregate in the water column according to light. However, in the same environment, *Synechococcus* ecotypes have been observed to coexist (Ahlgren and Rocap, 2006). Our PCA analysis showed that the deep samples from lakes Espejo and Gutiérrez are isolated samples, suggesting a distinctive vertical distribution of the OTUs. A similar situation was observed in Lake Kinneret, where a distinct library composition has been found in samples above and below the thermocline, resulting in a distinctive vertical distribution (Junier *et al.*, 2007). In Andean lakes, the dissimilarity in the relative composition of the Pcy community along the water column is probably enhanced by the temporary isolation due to summer stratification and suggests a great acclimation ability of certain OTUs. Wind exposure and wind speed variations cause considerable changes in thermocline depth and in the mean epilimnetic irradiance of these lakes, affecting the coexistence of photosynthetic species (Modenutti *et al.*, 2008).

The low similarity (<98%) between the already published Pcy ITS sequences and those retrieved in the North Patagonian Andean lakes suggests the presence of genetically distinct Pcy assemblages.

In conclusion, we successfully applied ITS analysis recognizing a complex Pcy assemblage of Andean Patagonian ultraoligotrophic lakes. We also showed that the distribution of Pcy diversity varied between lakes suggesting biogeographical traits of Pcy communities in Patagonia. The observed community structure (also in vertical gradient) results probably from physical constraints, such as light resulting in emerging communities with particular physiological adaptations. Finally, future research in remote areas such as the Patagonian region, should aim to enlarge the knowledge on Pcy sequences

in order to clarify the unanswered phylogenetic relationships among freshwater Pcy diversity, often confined to boreal systems.

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