

Multifaceted approach for the analysis of the phototrophic microbial community in a freshwater recreational area of Xochimilco, México

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Abstract The freshwater microbial community in a recreational area of Xochimilco, México was investigated and compared based on spatial (three different sites) and temporal (dry and rainy seasons) environmental variables. Many of the 16S- and 18S rRNA gene sequences recovered by DGGE fingerprinting analysis were related to phototrophic microbial phylotypes of known identity. Our genetic and morphological analysis indicated the ubiquitous presence of the microeukaryotic green algae *Desmodesmus-Scenedesmus* spp. and of the unicellular cyanobacteria *Cyanobium* spp. as the most representative populations in the samples. While 18S rRNA-DGGE fingerprinting analysis revealed a homogeneous community composition across sites and seasons, the 16S rRNA

showed significant differences between localities and seasons. None of the cyanobacteria species with potential to produce toxins were identified across the investigated samples. Correlations between biotic and abiotic variables evidenced an important difference between the dry and the rainy season, with a greater consistency in data from the rainy season. According to Principal Component Analysis (PCA), a strong relation between inorganic nitrogen, species richness, and subaquatic irradiance determines environmental variability in Xochimilco. Complementary and relevant data in results obtained from microscopy, fingerprinting, and statistical analysis applied in ecology indicate that a multifaceted approach to the study of microbial communities is necessary to accomplish a comprehensive scientific framework and to generate proper management strategies.

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Introduction

Planktonic organisms are good indicators of water quality and aquatic ecosystem health (Stevenson & Smol, 2003). However, the most accurate taxonomic identification is required to assess diversity and ecological role of specific species in communities, and there are still many species that have not been

described, yet abundant, particularly in the tropics. Molecular biology techniques have improved the taxonomic work required for recognizing such diversity and have found extensive applications in the areas of community structure and function (Yan et al., 2007). PCR-based methods are particularly valuable and effective as early warning tools for monitoring drinking or recreational freshwater systems to prevent acute and chronic exposure to toxic or problematic species present in the phytoplankton (Rengefors & Legrand, 2001; Kurmayer et al., 2003; Dittmann & Börner, 2005; Koskenniemi et al., 2007; Amer et al., 2009). In the monitoring of aquatic systems several methods have been successful, like for instance terminal-restriction fragment length polymorphism (T-RFLP/LH-PCR; Lepère et al., 2006; Nogales et al., 2007). However, denaturing gradient gel electrophoresis (DGGE) is perhaps the method that has increased most our understanding of community diversity in freshwater systems (Muyzer & Smalla, 1998; Casamayor et al., 2000; Demergasso et al., 2004; Unrein et al., 2005; Gucht et al., 2006; Hori et al., 2006; Oliveira & Goulder, 2006; among others).

In the present study, we analyzed the shallow hypereutrophic system of channels in Xochimilco (average of 3 m depth), located in Mexico City, using DGGE. The Xochimilco channels are all that is left of the large lake basin located in the Valley of Mexico, originally for the most part integrated by the Texcoco Lake. The migrant native tribes that arrived to the Xochimilco area of that basin developed a system of cultivation called the *chinampa*, which are built by accumulating lacustrine sediments at the bottom of the lake during low tides. This practice achieved its technological peak during the eleventh and fourteenth centuries and has continued to our days. Xochimilco (since 1988 designated by UNESCO as Cultural Human Patrimony) has been a sustainable wetland from its occupation time by local tribes. Finally, the severe periodical drought periods suffered by the Valley of Mexico have pressed the municipal authorities to maintain the water level by re-filling the net of channels with sewage water of secondary treatment. Environmental stress resulting from human activities thus enhanced the extra incoming of nutrients in water, resulting in the loss of ecological integrity of the aquatic system, which is evident in an increased turbidity, occasional low

dissolved oxygen levels and unpleasant odor problems. The system became heavily eutrophic and the phytoplankton is mainly composed of typical cyanobacteria and green chlorococcalean algae (Tavera et al., 2000), together with a massive growth of hydrophytes. Diversity of the whole pico- and microplankton communities (including both prokaryotic and eukaryotic organisms) was investigated in relation to environmental factors (PCA). The diversity was analyzed using morphological identification by light microscopy and SSU-rRNA-DGGE fingerprinting analysis. Our main objective was to explore how the photosynthetic planktonic community in the Xochimilco system of channels responds to the different land uses and catchment influences.

Materials and methods

Study area and sampling

Three localities in the Xochimilco channels distinguished by land use were chosen for collecting samples: La Virgen lagoon (19°16,622'N; 99°05,350'W), where agriculture is moderate; El Japón channel (19°16,804'N; 99°04,301'W), with intensive agricultural and livestock production; and El Bordo channel (19°17,175'N; 99°06,038'W), which is intensively used for touristic activities (Fig. 1).

Eighteen samples were collected with identical methods in 2007 during the dry (May) and the rainy season (September) to simultaneously evaluate spatial and temporal differences in the three chosen collection sites. 10 µm net samples were collected for microscopic identification. Simultaneously, water samples for DNA analysis were collected directly in bottles using a funnel with a 50 µm net to eliminate any group of organisms over that size. The water samples were immediately transported to the laboratory where fractions were obtained by serial filtration with polycarbonate filters of 10, 2.0, and 0.22 µm pore sizes, which were kept frozen (−80°C) until their analysis.

Morphological identification by light microscopy

Microscopy morphological analysis of the 10 µm net samples (Nikon E600 with DIC) was conducted to evaluate the nano- and microplankton species



Fig. 1 Map of the channel network area in México City: 1. El Bordo channel ($19^{\circ}17,175'N$; $99^{\circ}06,038'W$); 2. El Japón channel ($19^{\circ}16,804'N$; $99^{\circ}04,301'W$); 3. La Virgen lagoon ($19^{\circ}16,622'N$; $99^{\circ}05,350'W$). Photographs: **a** construction of a “chinampa” in Xochimilco; **b** panoramic of the “chinampa” network associated to the main channel system

richness, solely based on the presence of species in each sample (Table 1, Fig. 2). The morphological identification of eukaryotic algae and cyanobacteria was made according to appropriate relevant references (Komárek, 1975, 1989; Komárek & Fott, 1983; Comas & Komárek, 1984; Popovský & Pfiester, 1990; Komárek & Anagnostidis, 1999; Komárek et al., 1999; Hegewald, 2000; Comas et al., 2007).

Physical and chemical measurements

Total phosphorus was analyzed by the acidic digestion method following the Hach procedure (1997), as well as total inorganic nitrogen; NO_3-N through the cadmium reduction method; NO_2-N by the diazotization method; and NH_4-N by the salicylate method. Vertical light attenuation coefficient, measured with data collected with Hobo loggers, was calculated as K_d according to Kirk (1986). Secchi depth and common hydrology parameters such as temperature, pH, and conductivity were measured in situ using portable Conductronic (México) equipment. Chlorophyll *a* was determined only during the rainy season sampling using the spectrophotometer method according to Lorenzen (1967), with acidic correction for pheopigments (Table 2).

DNA extraction, SSU-PCR-DGGE fingerprinting, and genetic identification

A half section of each filter was used for DNA extraction by the method previously described in Tillet & Neilan (2000). Extracts were kept frozen at $-20^{\circ}C$ until analysis. Quantification of DNA was made via spectrophotometry (NanoDrop 1000 Technologies [Saveen Werner]) and integrity confirmed in 1% agarose gels.

All PCR were performed using 0.2 U of Hotstar Taq DNA polymerase (Qiagen) in a 25 μ l reaction. The PCR mixture contained 1 \times Hotstar Taq polymerase buffer, 0.5 pmol of forward and reverse primers, 0.2 mM dNTPs, and 10 ng of template DNA.

The cyanobacterial-specific 16S rRNA and eukaryote-specific 18S rRNA genes were analyzed by DGGE via PCR amplification with the oligonucleotide primers CYA106F (with 40 nucleotide GC

Table 1 Species richness in each sample based on the number of species present

	X ₀ 16	X ₀ 17	X ₀ 18	X ₀ 5	X ₀ 19	X ₀ 20	X ₀ 21	X ₀ 8	X ₀ 22	X ₀ 23	X ₀ 24	X ₀ 11
<i>Closteriopsis</i> sp	□	□	□	□	■	□	□	□	□	□	□	□
<i>Coelastrum pseudomicroporum</i>	■	■	□	□	□	□	□	□	□	□	□	□
<i>Desmodesmus armatus</i>	□	□	□	■	□	□	□	□	□	□	□	□
<i>D. armatus</i> (morphospecies <i>helveticus</i>)	□	□	□	□	□	□	□	■	■	■	■	□
<i>D. armatus</i> (m. <i>pseudohelveticus</i>)	□	□	□	□	□	□	□	■	■	■	■	□
<i>D. armatus</i> var. <i>spinosus</i>	□	■	□	□	□	□	□	□	□	□	□	■
<i>D. communis</i>	■	■	■	■	■	■	■	■	■	■	■	■
<i>D. intermedius</i>	■	■	■	□	□	□	□	□	■	■	■	■
<i>D. opoliensis</i> var. <i>opoliensis</i>	■	■	□	□	□	□	■	■	■	■	■	□
<i>D. opoliensis</i> var. <i>carinatus</i>	■	■	■	■	■	□	■	■	■	■	■	■
<i>D. opoliensis</i> var. <i>mononensis</i>	■	■	■	■	□	□	■	■	■	■	■	□
<i>D. pannonicus</i>	■	■	□	□	□	□	□	■	□	□	□	□
<i>D. subspicatus</i>	□	□	□	□	■	■	□	■	■	■	■	□
<i>Dyctiosphaerium pulchellum</i>	■	■	□	□	□	□	□	□	□	□	□	□
<i>Golenkinia radiata</i>	□	□	■	■	■	■	■	□	■	■	■	■
<i>Micractinium crassisetum</i>	■	■	■	□	□	□	□	□	■	■	■	□
<i>Monoraphidium contortum</i>	□	■	■	□	□	□	□	■	□	□	□	□
<i>M. irregulare</i>	■	■	■	□	□	■	□	□	□	□	■	■
<i>Oocystis borgei</i>	□	□	□	□	■	□	□	□	■	□	■	□
<i>O. lacustris</i>	□	□	□	□	□	□	□	□	■	■	■	□
<i>Pediastrum boryanum</i> var. <i>boryanum</i>	■	■	□	□	□	□	□	□	■	■	■	■
<i>P. boryanum</i> var. <i>longicorne</i>	■	■	■	□	■	□	■	■	□	■	■	□
<i>P. duplex</i>	■	□	□	□	■	□	□	□	□	□	□	□
<i>P. tetras</i>	■	■	■	□	□	□	□	□	□	■	■	□
<i>Raisiella hemisphaerica</i>	□	■	□	□	□	□	□	□	□	□	□	□
<i>Scenedesmus acuminatus</i>	□	□	□	■	□	□	□	□	□	□	□	■
<i>S. dimorphus</i>	■	■	□	□	□	□	□	□	□	□	□	□
<i>Tetraedron</i> sp	■	■	□	□	□	□	□	□	□	□	□	□
<i>Tetrastrum heteracanthum</i>	□	□	□	□	□	□	□	□	■	□	□	□
<i>Chlamydomonas</i> sp	□	□	□	□	□	□	■	□	□	□	□	□
<i>Chlorogonium elongatum</i>	□	□	□	□	□	□	□	□	■	■	□	□
<i>Coccomonas</i> sp	□	■	■	□	■	□	■	□	□	□	□	□
<i>Pteromonas angulosa</i>	□	■	■	■	□	□	■	■	□	□	□	□
<i>Euglena acus</i>	■	■	■	□	□	□	■	□	□	□	■	□
<i>Euglena</i> ca. <i>proxima</i>	□	■	□	□	□	□	□	□	□	□	□	□
<i>Euglena</i> sp	□	□	□	□	□	■	□	□	□	□	□	□
<i>Phacus indicus</i>	■	■	□	□	□	□	□	□	□	□	□	□
<i>Phacus</i> sp	□	□	□	■	□	□	□	□	□	□	□	□
<i>Phacus tortus</i>	■	■	□	□	■	□	■	□	□	□	□	□
<i>Trachelomonas</i> sp	■	■	□	□	□	□	□	□	□	□	□	□
<i>Peridiniopsis oculatum</i>	■	■	■	■	■	□	■	■	■	■	■	■
<i>Goniochloris contorta</i>	□	■	□	□	□	□	□	□	□	□	□	□
<i>G. mutica</i>	□	□	□	□	□	□	□	□	□	■	□	□
<i>Amphora copulata</i>	□	■	□	□	□	□	□	□	□	□	□	□
<i>Aulacoseira granulata</i> var. <i>granulata</i>	■	□	■	□	■	□	□	□	□	□	□	□

Table 1 continued

	X ₀ 16	X ₀ 17	X ₀ 18	X ₀ 5	X ₀ 19	X ₀ 20	X ₀ 21	X ₀ 8	X ₀ 22	X ₀ 23	X ₀ 24	X ₀ 11
<i>A. granulata</i> var. <i>angustissima</i>	■	□	□	■	□	□	□	■	□	□	■	□
<i>Cocconeis placentula</i>	□	□	□	□	□	□	□	□	■	□	■	□
<i>Cyclotella meneghiniana</i>	■	■	■	■	□	□	■	□	□	■	■	□
<i>Cymbella mexicana</i>	□	□	■	□	□	□	□	□	□	□	□	□
<i>Denticula kuetzingii</i>	■	■	□	□	□	□	□	□	□	□	□	□
<i>Gomphonema affine</i>	□	□	□	□	□	□	□	□	■	□	■	□
<i>Sellaphora pupula</i>	□	■	□	□	□	□	□	□	□	□	□	□
<i>Staurosira construens</i> var. <i>venter</i>	□	■	□	□	□	□	□	□	□	■	■	□
<i>Staurosirella leptostauron</i>	□	□	□	□	□	□	□	□	■	□	□	□
<i>Thalassiosira visurgis</i>	□	■	□	□	□	■	□	□	□	□	□	□
<i>Anabaenopsis</i> sp	□	□	□	□	□	□	■	□	□	■	■	■
<i>Cyanobium</i> sp	□	□	■	■	■	■	■	□	□	■	■	□
<i>Merismopedia</i> sp. 1	□	□	■	□	□	□	□	□	□	□	■	□
<i>Mensmopedia</i> sp. 1	■	■	□	□	□	□	□	□	□	□	□	□
<i>Synechococcus</i> cfr. <i>lividus</i>	□	□	□	■	□	□	□	□	□	□	□	□
<i>Synechococcus nidulans</i>	□	□	□	□	□	□	□	□	■	■	■	□
<i>Synechococcus</i> sp	■	■	■	■	□	□	■	□	■	□	■	■
Cryptophyta	□	□	□	□	□	□	□	□	□	■	□	□
Richness values	27	35	20	14	13	7	16	13	21	23	28	11

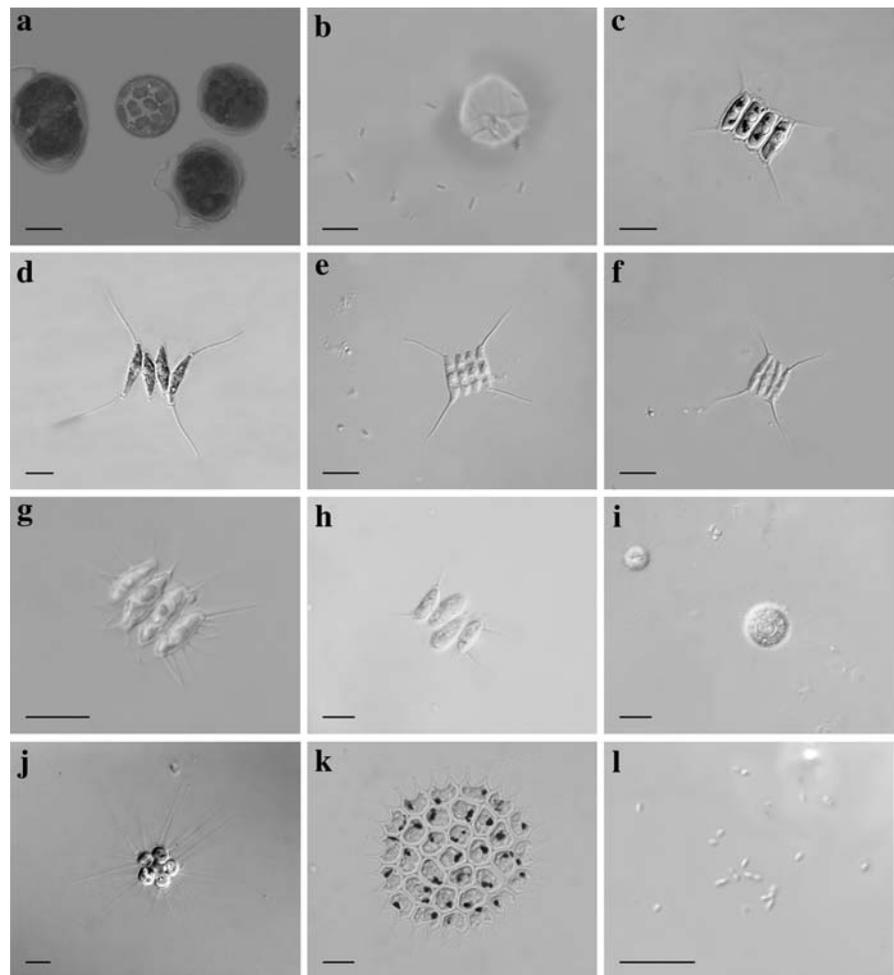
clamp at the 5' end) and CYA781R (Nübel et al., 1997); and Euk1A and Euk516r (with 40 nucleotide GC clamp at the 5' end) (Díez et al., 2001a), respectively. Amplified products were 675 and 560 bp, respectively. The PCR program for the cyanobacterial-specific 16S rRNA and eukaryote-specific 18S rRNA genes were as previously described by Nübel et al. (1997) and Díez et al. (2001a), respectively. PCR products were analyzed on 1% agarose gels stained with ethidium bromide.

DGGE of the PCR products was carried out using a Dcode system (BioRad). DGGE was run at 75 volts for 16 h in 0.75 mm, 6% polyacrylamide gels (37.5:1 acrylamide bisacrylamide) submerged in 1× TAE buffer (40 mM Tris, 40 mM acetic acid, and 1 mM EDTA, pH 7.4) at 60°C, as previously described (Díez et al., 2001b). A linear gradient of denaturing agents from 45 to 65% was used to resolve both the 16S rRNA and 18S rRNA genes. After electrophoresis, the gel was stained in 1× TAE buffer containing SYBR-Gold Nucleic Acid Stain (1:10,000 dilution, Molecular Probes, Invitrogen AB, Sweden) and the results were recorded using a molecular imager (ChemiDoc

XRS system, BioRad). The DGGE banding pattern revealed from each sample was compared by image analysis using the QuantityOne software (BioRad), as previously described (Schauer et al., 2000). The number of DGGE bands present was considered to be the number of phylotypes in each sample. A dendrogram was constructed by cluster analysis using the software Statistica 7.0 by a similarity matrix (City Block distance and Ward's method) obtained taking into account the presence or absence of individual DGGE bands in all lanes (communities).

The most dominant bands were excised from the gels and submerged in 20 µl DNAase RNAase-free H₂O (UltraPure, Gibco) and stored at 4°C overnight. An aliquot of the eluted DNA was subjected to an additional round of PCR using the same primers. The reamplified PCR products were cleaned using a PCR clean-up kit (GFX, AmershamTM) and sequenced (with the corresponding forward primer) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), on an ABI PRISM model 377 (v.3.3) automated sequencer (DNA Technology, Denmark). Partial 16S rRNA and 18S rRNA sequences

Fig. 2 Most common algae present in the phytoplankton of Xochimilco: **a** *Cyclotella meneghiniana* (center) and *Peridiniopsis oculatum*; **b** empty theca of *P. oculatum* and *Synechococcus* sp.; **c** *Desmodesmus communis*; **d** *D. opoliensis* var. *opoliensis*; **e** *D. opoliensis* var. *mononensis*; **f** *D. opoliensis* var. *carinatus*; **g** *D. subspicatus*; **h** *D. intermedius*; **i** *Golenkinia radiata*; **j** *Micractinium crassisetum*; **k** *Pediastrum boryanum*; **l** *Cyanobium* sp. and *Synechococcus nidulans*. Bar = 10 μ m



were aligned in Bioedit, version 7.0.4.1 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>), using ClustalW. All sequences were subjected to Blast searches (Altschul et al., 1997; www.ncbi.nlm.nih.gov/blast) and the closest relatives from GeneBank were included for phylogenetic analysis. Only sequences from published studies or culture collections were included, and the reference taxa were used for phylogenetic inference from distance approximations by the neighbor-joining method and Kimura two-parameter (K2P) in PAUP (version 4.0b10, Sinauer Associates Inc., Sunderland, MA). One thousand bootstrap replicates were performed for both data sets. The 16S rRNA gene sequence of *Agrobacterium* sp. (EF550174) and the 18S rRNA gene sequence of *Euglena gracilis* (M12677) were used as outgroups. The sequences generated in this study have been deposited in the

GenBank database under accession numbers (16S rRNA-DGGE bands): FJ919674–FJ919692, (18S rRNA-DGGE bands): FJ919693–FJ919703.

Statistical analysis

We carried out a PCA (Statistica 7.0) for searching of those variables that could better explain differences between localities and physical–chemical as well as biological factors. Variables were organized as active and supplementary. The active-variable group included total phosphorus, ammonia, nitrate, nitrite, irradiance (Kd), and species richness; the supplementary-variable group included temperature, pH, and conductivity. Because chlorophyll *a* was not measured in the dry season, the corresponding data were not included in the PCA.

Table 2 Values of parameters registered in each sampling site in the rainy and the dry seasons

Locality	Season	Sample	Richness	Total Phosphorus	Ammonium	Nitrate	Nitrite	Kd	Temperature	pH	Conduct.
Virgen	Dry	Xo5	14	8.70	2.49	5.3	0.0	29	19.1	8.1	822
Virgen	Rainy	Xo 16	27	0.23	0.56	0.6	0.0	27	20.6	8.8	760
Virgen	Rainy	Xo 17	35	0.33	0.61	0.6	0.0	26	19.1	8.5	705
Virgen	Rainy	Xo 18	20	1.04	0.58	0.6	0.0	25	24.5	8.8	786
Japon	Dry	Xo8	13	3.06	2.40	3.8	0.0	29	18.7	8.2	843
Japon	Rainy	Xo 19	13	0.14	0.79	1.2	0.1	26	20.6	8.5	896
Japon	Rainy	Xo 20	7	0.33	0.81	1.5	0.1	25	20.0	8.4	846
Japon	Rainy	Xo21	16	0.52	0.77	1.4	0.1	26	20.5	9.4	871
Bordo	Dry	Xo 11	11	7.10	3.65	5.6	0.0	26	27.7	9.1	810
Bordo	Rainy	Xo 22	21	0.17	0.55	0.5	0.0	29	19.8	10.6	619
Bordo	Rainy	Xo 23	23	0.11	0.50	0.4	0.0	26	20.6	11.2	621
Bordo	Rainy	Xo 24	28	0.15	0.54	0.8	0.0	27	19.2	10.4	445

Xo5, Xo8 and Xo11 samples collected in the dry season. Nitrogen inorganic forms as $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$; concentrations of nitrogen forms and Ptot in mg l^{-1} ; Kd values calculated according to Kirk (1986); temperature in Celsius degrees; pH in pH-units and conductivity in $\mu\text{S cm}^{-1}$ (K_{25}). Species richness values are equal to number of species present in each sample

Results

Statistical analysis: PCA

Xochimilco is a shallow, warm, tropical body of water with low subaquatic irradiance, high conductivity, and high pH (Table 2). Apparently, from the hard data presented here, there were small differences when comparing the rainy and the dry seasons; however, PCA and DGGE fingerprinting analyses demonstrated some important distinctions in diversity connected with climate and hydrochemistry. According to PCA, variables measured in Xochimilco showed statistically significant correlations (Table 3a). Judging from eigenvalues, there were no redundancies for the active variables (Table 3b). The PCA reduced the complete environmental variability in Xochimilco into two dimensions (principal components with eigenvalue >1) and communalities produced by PCA (based on cosines of correlations) pointed out to the contribution of active and supplementary variables to explain environmental variability (Table 3c).

Projection of the variables on the two-factor plane synthesized the PCA analysis (Fig. 3a), and exhibited an important control of nitrogen over the species richness as well as a very strong positive correlation between irradiance and species richness (Table 3a). This projection also denoted that those variables

considered in this study as ‘active’ were in fact the most important to explain spatial and temporal differences in phytoplankton.

Total phosphorus and ammonium were privileged in the 2-dimension scatter plot (Fig. 3b) based on correlations between variables, and clearly evidenced differences between the dry and the rainy seasons, with a greater consistency in data from the rainy season.

Microscopic analysis

Our microscopy analysis evidenced low diversity of morphotypes (Table 2), mainly in the El Japón channel where a bloom of *Peridiniopsis oculatum* (Stein) Bourrelly dominated in both seasons (Fig. 2a, b); this species was also present, but less abundantly, in La Virgen lagoon and El Bordo channel.

In general, Chlorophyta phototrophic algae mostly dominated the eukaryotic fraction of the phytoplankton, Chlorococcales (*sensu lato*) being the best-represented group (Fig. 2; Table 3) including the most abundant species *Desmodesmus opoliensis* (Chod.) E. Hegew. et. A. Schmidt and varieties, *D. subspicatus* (Chod.) E. Hegew. et. A. Schmidt, *D. communis* (E. Hegew.) E. Hegew. et. A. Schmidt and *D. intermedius* (Chod.) E. Hegew. et. A. Schmidt, as identified according to Hegewald’s (2000) proposal and based

Table 3 According to PCA, active, and supplementary variables explain well environmental variability in Xochimilco

(a) Correlations for active and supplementary (*) variables

	Ptot	Ammonium	Nitrate	Nitrite	Kd	Richness	*Temperature	*pH	*Conductivity
Ptot	1.000000	0.088250	0.051254	-0.061424	-0.660669	-0.134761	0.833500	-0.385334	0.363824
Ammonium	0.088250	1.000000	0.943908	0.914092	-0.562115	-0.756537	-0.061444	-0.637652	0.790238
Nitrate	0.051254	0.943908	1.000000	0.969891	-0.443471	-0.762953	-0.132359	-0.490683	0.617217
Nitrite	-0.061424	0.914092	0.969891	1.000000	-0.315547	-0.762941	-0.168003	-0.352379	0.565312
Kd	-0.660669	-0.562115	-0.443471	-0.315547	1.000000	0.547100	-0.465040	0.572236	-0.583786
Richness	-0.134761	-0.756537	-0.762953	-0.762941	0.547100	1.000000	-0.235636	0.231561	-0.642075
*Temperature	0.833500	-0.061444	-0.132359	-0.168003	-0.465004	-0.235636	1.000000	-0.202717	0.352797
*pH	-0.385334	-0.637652	-0.490683	-0.352379	0.572236	0.231561	-0.202717	1.000000	-0.731983
*Conductivity	0.363824	0.790238	0.617217	0.565312	-0.583786	-0.642075	0.352797	-0.731983	1.000000

(b) Eigenvalues of correlation matrix and related statistics variables. Active variables only

	Eigenvalue	Total (%)	Cumulative	Cumulative
1	3.898163	64.96938	3.898163	64.9694
2	1.487207	24.78679	5.385370	89.7562
3	0.320846	5.34744	5.706216	95.1036
4	0.236475	3.94126	5.942692	99.0449
5	0.039619	0.66032	5.982311	99.7052
6	0.017689	0.29482	6.000000	100.0000

(c) Communalities based on correlations (cosines) active and supplementary variables (*)

	From 1	From 2
Ptot	0.046015	0.8993814
Ammonium	0.921038	0.934567
Nitrate	0.907226	0.951344
Nitrite	0.841765	0.956575
Kd	0.417056	0.883669
Richness	0.765062	0.765401
*Temperature	0.007880	0.629606
*pH	0.278553	0.368003
*Conductivity	0.546450	0.5895579

All variables considered as active in the analysis have important contribution to this explanation, as eigenvalues do not show redundancy between variables. Factor 1 enhances status of species richness related to nitrogen and light. These variables contribute to the highest fraction (64.9%) of environmental variability in Xochimilco

on the revision of material in the UTEX collection (UTEX, 2009).

The cyanobacterial fraction, also represented by few morphotypes, was composed primarily by the picocyanobacteria *Synechococcus nidulans* (Pringsheim) Komárek, *Synechococcus* cf. *lividus*, *Synechococcus* sp., *Cyanobium* sp., *Merismopedia* sp. 1 and, *Merismopedia* sp. 2 (Fig. 2; Table 1). The most abundant and one of the most frequent of these species was *Cyanobium* sp.

Genetic analysis by SSU-rRNA-DGGE fingerprinting and sequence identification

While phototrophic picoplanktonic species were very homogeneous according to observations in light microscopy (Fig. 2; Table 3), our 16S rRNA-DGGE fingerprinting results (Fig. 4a) showed significant differences between size fractions, localities and seasons. Figure 4b shows the clustering analysis of 16S rRNA-DGGE fingerprints based upon the

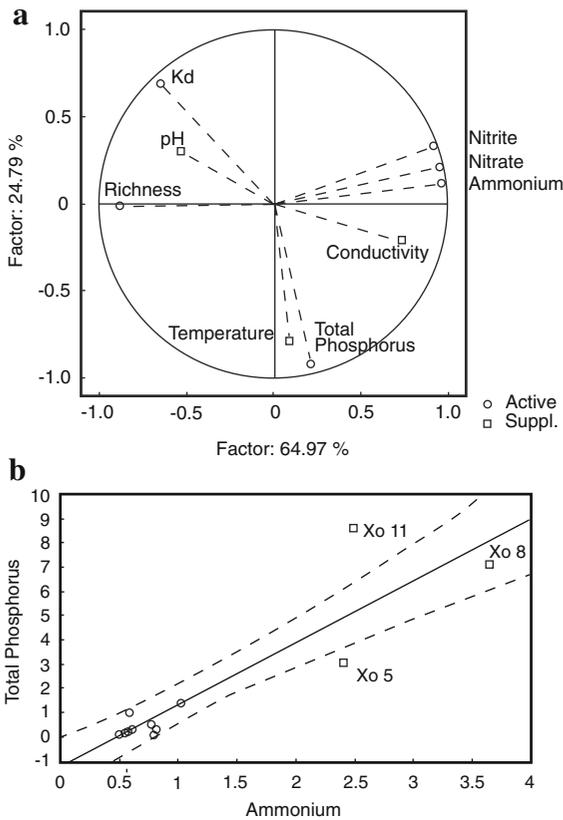


Fig. 3 **a** Projection of variables on the factor-plane 1x2. Vectors nearest the circle correspond to the active variables. The species richness vector, strongly negative correlates to inorganic nitrogen forms ($r = -0.756$, $r = -0.762$, $r = -0.762$), but not to total phosphorus (orthogonal), $r = -0.134$. **b** 2-dimension scatter plot based on correlations between variables. Sites studied in the rainy season (*open circle*) show more involved relationship of nutrients to general environment conditions than those sampled during the dry season (*open square*—outlier cases). Total phosphorus = $-1.2073 + 2.5521$ (0.95 confidence)

presence or absence of all observed bands and confirmed local and seasonal variability. For instance, El Bordo samples, including both dry and rainy seasons that were separately subclustering, form a cluster that is clearly distinct from the other two localities. Moreover, pattern similarities for 0.2 μm filter size samples were revealed. For La Virgen and El Japón localities, and depending on the season, 0.2 μm samples always clustered together, which indicates certain homogeneity between both locations (Fig. 4b). In El Bordo, 0.2 μm samples from both

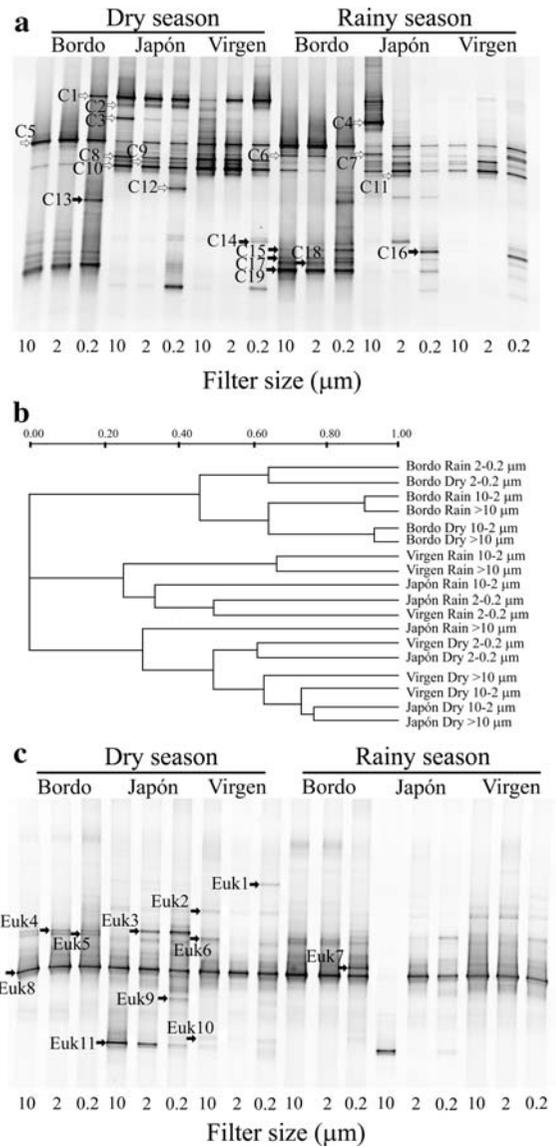


Fig. 4 **a** DGGE fingerprint of the specific cyanobacteria 16S rRNA gene fragments (including chloroplast of phototrophic eukaryotes). **b** Dendrogram showing the degree of similarity (by presence/absence of each DGGE band) between the cyanobacterial community present in **a**. Scale bar indicates linkage distances. **c** DGGE fingerprint of the Eukaryotic 18S rRNA gene fragments collected from the Xochimilco channels at the El Bordo, El Japón, and La Virgen sampling sites in 2007 during the dry and rainy seasons. The 16S and 18S rRNA sequences obtained from the DGGE bands that were excised from the gels are numbered and denoted by *black arrows*. Designations correspond to those shown in the phylogenetic reconstructions in Figs. 5 and 6

seasons clustered together and separated from the rest of the same filter size samples.

The 16S rRNA-DGGE profiles (Fig. 4a) indicate the presence of three to six bands (DGGE bands 16–21) that are phylogenetically affiliated to freshwater unicellular *Synechococcus* and/or *Cyanobium* phylotypes (Fig. 5). These phylotypes were well represented in El Bordo site during the rainy season; while in the two other sites these same bands were present in the rainy season in a much lower relative abundance and only in the picoplanktonic fraction between 0.2 and 2 μm . One additional band (DGGE band 14) was present in El Bordo, both during the dry and the rainy seasons, which was affiliated to cyanobacteria closely related to the Pseudanabaenaceae (Fig. 5); this band was only present in the small fraction size (0.2–2 μm ; Fig. 4a), which demonstrates its low relative abundance within the community.

The presence of cyanobacteria in El Bordo clearly defines a particular and different local pattern with respect to La Virgen and El Japón locations. In the latter two localities, DGGE bands were mainly affiliated to some chloroplasts of eukaryotes that were amplified using the set of primers chosen in this study for cyanobacteria 16S rRNA gene amplification (see below). For instance: DGGE bands 7 and 12 were affiliated with two chloroplasts of cryptophytes; DGGE bands 1–4, to diatom chloroplasts; and DGGE bands 5, 6, 8–10, and 11 formed a separate cluster of unknown affiliation.

Furthermore, the analysis of the 18S rRNA-DGGE profiles (Fig. 4c) revealed a more homogeneous community all over the sites and seasons. The most representative phylotypes recovered (DGGE bands 2, 4, 5, and 8) were those closely related to the colonial green algae *Scenedesmus* spp. (Chlorophyta; Fig. 6). However, species in this genus actually belong to subgenus *Desmodesmus* or *Scenedesmus* (Hegewald, 2000). These species of Chlorophyta were totally dominating the samples and specially important and ubiquitous was the one related to DGGE band 8 (Fig. 4c). The rest of the DGGE bands were tentatively affiliated to fungi (DGGE band 7) or heterotrophic organisms such as ciliates (DGGE band 1), dinoflagellates (DGGE band 10, and 11), uncultured alveolates (DGGE band 9) and stramenopiles (DGGE band 3), all of them with a much lower relative abundance in the samples investigated through DGGE.

Discussion

Previously recorded (Tavera et al., 2000) and our own data show that, according to the average values of total phosphorus (1.82 mg l^{-1}) and total inorganic nitrogen (3.05 mg l^{-1}), Xochimilco is a hypereutrophic body of water. Also chlorophyll *a* may be generally high according to our data from the rainy season, ranging from 0.051 to 1.69 mg l^{-1} . In a hypereutrophic environment and without a physiology-experimental approach to phytoplankton species, it is not possible to talk about nutrient-limitation of phytoplankton, simply because of nutrient abundance (Florida LAKEWATCH, 2000; Bruger et al., 2007), however, in Xochimilco disproportion of N and P is rather high and some quality impact can be expected over phytoplankton in connection to such disproportion. As our results have shown, inorganic nitrogen is significantly affecting species richness (generally poor).

Results of the PCA analysis suggest that the photosynthetic planktonic community responds to differences between localities, therefore to the catchment influence in the system of channels. Phytoplankton was evaluated as species richness and, according to PCA, it is sensitive both to nitrogen level and to irradiance. In Xochimilco, considering that La Virgen and El Japón are influenced by agriculture in contrast to El Bordo that is used for touristic activities, incoming nitrogen from the catchment area must be different and this may be also the situation with phosphorus. However, our values of total phosphorus did not correlate to species richness and it seems interesting that total phosphorus correlates negative-significantly to *K_d* ($r = -0.66$, Fig. 3, Table 3a), which indicates that the organic phosphorus linked to biomass also reflects the effect of penetration of light in the water column; regardless that biomass may not be the only cause for values of *K_d*, it should be important (Cristofor et al., 1994). Nitrogen influences the species diversity and we suspect that phosphorus could control the phytoplankton biomass, as has been observed in tropical eutrophic environments (Bruger et al., 2007). In addition, the correlation denoted by the total phosphorus and ammonia show dispersion of cases in samples from the dry season, which means that the nutrient-influence over phytoplankton occurs with different emphasis along the year (Fig. 3b), better



Fig. 5 Phylogenetic affiliations of cyanobacterial (and eukaryotic chloroplast) 16S rDNA—DGGE-band sequences from Xochimilco planktonic samples collected from three different locations during the dry and rainy seasons. The tree was

inferred using the neighbor-joining distance algorithm with the Kimura 2P model correction. Sequences from this study are in *bold*. Bootstrap values >50 are shown

correlating in the rainy season. Our PCA analysis showed in summary that species richness of the phytoplankton is a correct attribute for assessing ecological integrity in Xochimilco because algae translate the ecological status of the environment in terms of nitrogen and subaquatic irradiance, two of the most important variables in eutrophic environments in the tropics (Dávalos-Lind & Lind, 1993; Lewis, 1996, 2002). It is also clear that behavior of these variables changes during the dry and the rainy seasons.

Our genetic analyses were targeted to resolve dominant groups of pico- and nanoplankton, with size classes 0.2–2 and 2–20 μm , respectively. The DGGE profiles for the 16S rRNA gene showed important differences in phototrophic prokaryotic communities between fraction size, localities, and seasons. This result was in accordance with the interpretation derived from our PCA analysis in regards to the control of phytoplankton and it is relevant because we were not able to distinguish by microscopic methods changes in species richness that were supported by our DGGE analysis. The DGGE fingerprinting differences observed between localities may show that the small size fraction of phytoplankton is responding to environmental conditions more than the organisms of the larger size fraction (Drakare et al., 2003). Regarding the cyanobacteria, *Synechococcus* cf. *lividus*, *S. nidulans*, and *Merismopedia* spp. observed by microscopy were rather infrequent and scarce in samples, but *Synechococcus* sp. and above all *Cyanobium* sp. truly represented the picoplanktonic fraction, which has been previously observed in other freshwater environments (Postius et al., 1996; Ivanikova et al., 2007). Our 16S rRNA profiles showed a wider diversity spectrum being integrated by at least six different phylotypes without morphological distinction, and no cyanobacteria species with potential to produce toxins were identified across the samples investigated.

In the contrary, phylogenetic analysis of the 18S rRNA gene partial sequences recovered from the DGGE gel revealed a very homogeneous community and was coincident with the observation of species by light microscopy across the sampled sites and seasons (Table 2).

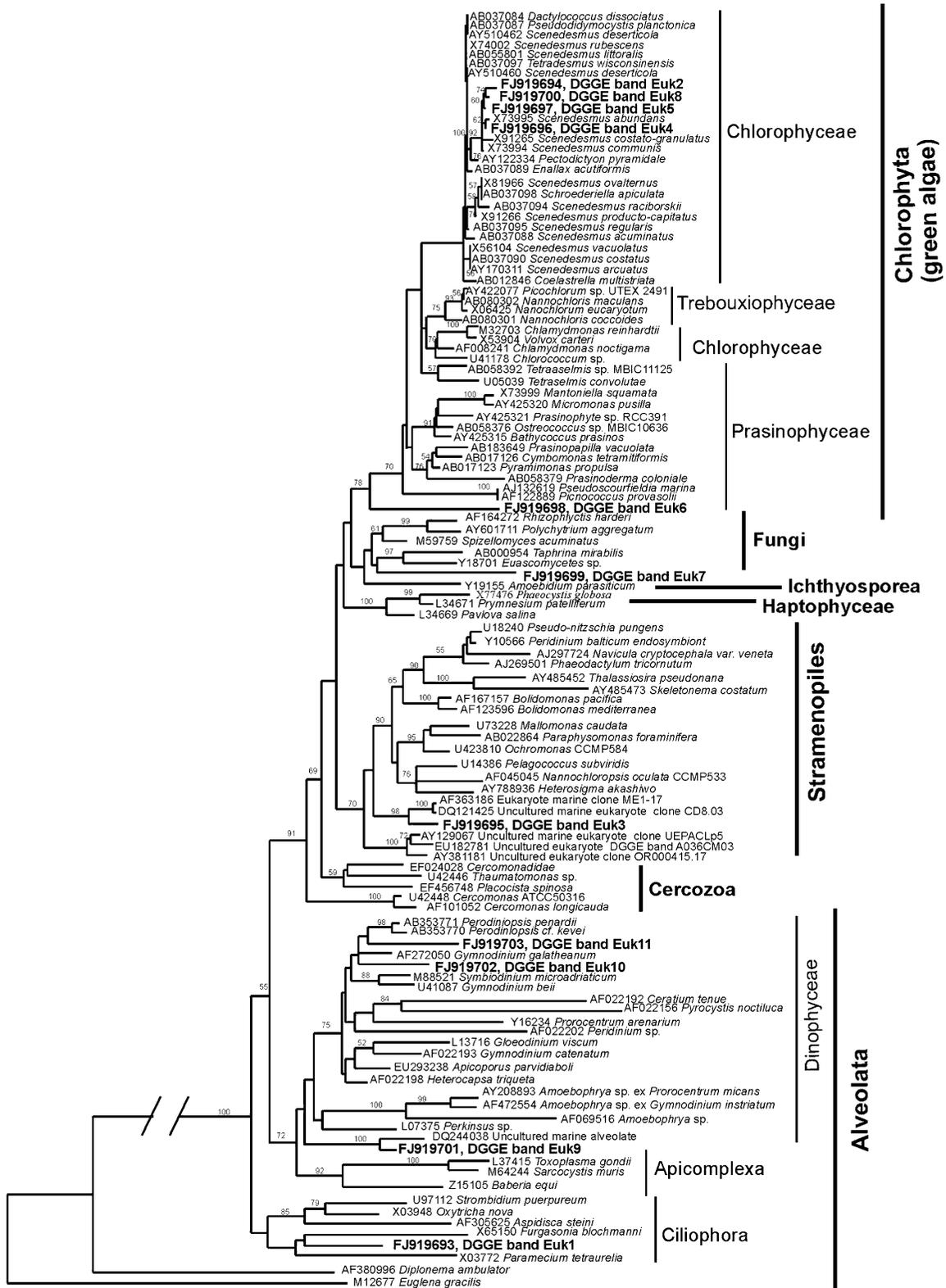
One of the most variable and abundant species in our samples, determined by microscopy as *Desmodesmus subspicatus*, is referred in literature as

Fig. 6 Phylogenetic affiliations of Eukaryotic 18S rRNA—DGGE-band sequences present in samples collected in Xochimilco channels in three different locations during the dry and rainy seasons. The tree was inferred using neighbor-joining distance algorithm with the Kimura 2P model correction. Sequences from this study are in *bold*. Bootstrap values >50 are shown

Scenedesmus subspicatus Chodat or *Scenedesmus abundans* (Kirchner) Chodat (Kuhn & Pattard, 1990; Trainor & Egan, 1990; Terry & Stone, 2002); these names being actually synonyms (Hegewald, 2000).

In DNA surveys, many phylotypes appear under the name *Desmodesmus subspicatus*. However, the only 18S rRNA sequences included in NCBI databases of the *Desmodesmus* species in particular *D. subspicatus* or *Scenedesmus subspicatus* are still unpublished or from a different region of the SSU. For that reason, the match sequences of our DGGE bands (2, 4, 5, and 8) coincident with sites and size fractions where *Desmodesmus-Scenedesmus* morphotypes were observed, were indeed closer to other *Scenedesmus* species and clustered separately from the rest of the Chlorophyceae organisms (Fig. 6). We presume that the ubiquitous DGGE band 8 affiliated to *Scenedesmus* phylotype recovered by our 18S rRNA gene analysis could represent the unknown chloroplast of band 5 in our 16S rRNA-DGGE profile. The great majority of species in *Scenedesmus* are considered environmental-induced forms (Trainor, 1998; John & Tsarenko, 2003) and despite of acceptance of subgenus *Desmodesmus* (Hegewald, 2000), modern authors still assign species only to *Scenedesmus* in the conviction that molecular analysis is far to be satisfactory for this genus (John & Tsarenko, op. cit.). In addition, it has been previously reported that within the genus *Scenedesmus* only a few well-supported clades are found, indicating that the 18S rRNA data lack sufficient resolution to distinguish among most of the *Scenedesmus* species (Lewis & Flechtner, 2004). For the time being, it is quite difficult to resolve whether our 18S rRNA-DGGE bands tentatively affiliated to *Scenedesmus* phylotypes, are in reality *Desmodesmus subspicatus*.

A similar situation occurred in relation to the dinoflagellate *Peridiniopsis oculatum*. In disagreement with our microscope observation, it was not possible to relate this species with any phylotype present in the public database. However, our sequences recovered from DGGE Euk 10 and 11



are coincident to our microscopic evaluation in the dominance-abundance of this species in samples from El Japón and La Virgen and, according to our 18S rRNA phylogeny (Fig. 6), both phylotypes certainly belong to Dinophyceae. It is important to remark that with PCR-dependent techniques, the identity of freshwater *Peridinium* species has remained elusive, owing to the relatively small number of *Peridinium* SSU-rRNA sequences in public sources. In addition, most of the studies related to such species have been only performed on evolutionary relationships of their plastids (Ki & Han, 2005).

From the different approaches undertaken in this study, we have found that microscopy, ecology, and molecular tools produced sound results and are complementary to each other for integrating the survey of aquatic ecosystems. Particularly, the use of the DGGE genetic fingerprinting analysis may not only enhance recognition of genetic differences but, as multiple samples can be analyzed simultaneously, it might also support the monitoring of microbial community and environmental conditions fast enough to follow the rapid changes experimented by the urban aquatic systems and those influenced by industrial or agronomical activities (Casamayor et al., 2000; Demergasso et al., 2004; Gucht et al., 2006; Lepère et al., 2006; Nogales et al., 2007; Amer et al., 2009), in particular in those areas where the microbial communities are poorly investigated, such as in Xochimilco.

Based in utilization of these tools within a multifaceted approach, we conclude that abiotic factors in the channels studied in Xochimilco are likely to drive the genetic diversity and thereby the differences in community patterns. The photosynthetic planktonic community respond to the different use and catchment influence in the channel system, permeated by the climatic conditions during the dry and the rainy seasons.

Finally, results assessed in this study points to an aspect still not considered in the majority of studies on microbial diversity, that is, embracing biodiversity and function of ecosystem one may reveal paraphyletic relations between groups of algae (Medlin et al., 2007). In Xochimilco, even dealing with partial sequences, this may be the situation with diatoms and dinoflagellates, groups with common paraphyly and unclear phylogenetic relationships. Also the controversial identity of microscopy and

DGGE phylotypes that we observed in some results is a positive sign because it indicates the limitations of using only one of the two approaches (Pedrós-Alió, 2005). We agree that future studies of microbial communities will follow multifaceted approaches as well as the scrutiny of several different gene markers to structure concatenated analyses with the aim to clarify the phylogeny of microorganisms.

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