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Bacterial community structure in a latitudinal gradient of lakes: the roles of spatial versus environmental factors

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SUMMARY

1. We analysed the latitudinal variation of bacterioplankton in 45 freshwater environments (lakes, shallow lakes and ponds) across a transect of more than 2100 km stretching from Argentinean Patagonia (45°S) to Maritime Antarctica (63°S), to determine the factors that mainly determine bacterioplankton community structure.
2. Bacterioplankton community composition (BCC) was assessed by a fingerprinting method (denaturing gradient gel electrophoresis) followed by band sequencing, whereas the abundances of total bacteria and picocyanobacteria were estimated by epifluorescence microscopy.
3. Bacterioplankton community composition was controlled by a combination of spatial (latitude and longitude) and environmental [e.g. phosphate, light diffuse attenuation coefficient (K_d) and dissolved organic carbon] factors. Total bacterioplankton abundance declined with latitude. A multiple regression analysis showed that phosphate, K_d and latitude had significant effects on total bacterioplankton abundance.
4. Of 76 operational taxonomic units identified in the studied lakes, 45 were shared between Patagonian and Antarctic water bodies, 28 were present only in Patagonian lakes and three were restricted to the Antarctic lakes. Significant differences were found in BCC between Patagonia and Antarctica. Among the sequences, 54% were similar (>97% sequence similarity) to others reported from cold habitats elsewhere on the planet (glaciers, high mountain lakes, Arctic).
5. Our results provide new evidence that supports the hypotheses of biogeographic patterns of bacterial assemblages and suggest that both spatial and environmental factors control bacterioplankton community structure.

Keywords: Antarctica, bacterioplankton, biogeography, lakes, Patagonia

Introduction

The incorporation of microbes as a subject in studies that explore the factors determining community composition was severely limited until recently. Recent studies have been able to investigate the relative influence of spatial (regional/geographic) and

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environmental factors on bacterial community composition (BCC) (see Dolan, 2005 and Martiny *et al*., 2006 for an overview; Van der Gucht *et al*., 2007; Barberán & Casamayor, 2010), but the importance of local environmental factors has been more intensively studied than the importance of regional factors. One reason for this might be that regional processes are more difficult to incorporate into ecological studies, since they act on a much larger scale (Logue & Lindström, 2008). Therefore, there is intense debate about the relative importance of environmental (e.g. lake trophic status, physicochemical variables) and spatial factors (e.g. latitude, longitude, geographic distance) in shaping microbial communities, and whether microbial organisms show a biogeographic signature in their distribution (Van der Gucht *et al*., 2007).

The historical view of microbial biogeography is that 'everything is everywhere, but the environment selects' (Baas-Becking, 1934; Brock, 1961). This idea presumes ubiquity of microbes based on the high abundance and high dispersal rates they are expected to have. This hypothesis was defended by Fenchel and Finlay in a series of publications (Finlay, 1998; Finlay & Clarke, 1999; Finlay, 2002; Fenchel & Finlay, 2004), in which they proposed that small organisms (<1 mm in length) tended to have a cosmopolitan distribution, a flat relationship between species and area, and at most a weak latitudinal gradient of diversity (Fenchel & Finlay, 2004). In support of the worldwide dispersion of microbes, Sommaruga & Casamayor (2009) and Schiaffino *et al.* (2009) reported that several bacterioplankton phylotypes found in high-altitude and low-latitude lakes, respectively, were similar to those reported from other extreme cold habitats elsewhere on the planet (polar habitats, glaciers, high mountain lakes), thus showing that taxon sorting by local environmental constraints (hydraulic retention time and nutrient status of the lakes, respectively) played a very important role in selecting similar phylotypes in all these types of environments.

In contrast to the hypothesis of bacterial cosmopolitanism, some studies have reported the existence of endemic species, restricted distributions and non-random patterns in bacterial diversity (e.g. Whitaker, Grogan & Taylor, 2003; Pommier, Pinhassi & Hagström, 2005; Pommier *et al*., 2007; Vyverman *et al*., 2010). Specifically for bacteria, Yergeau *et al.* (2007) provided evidence about factors shaping soil BCC along a 3200 km Antarctic transect using clone libraries of 16S rRNA genes and found that although bacterial richness declined with increasing latitude, habitat-specific patterns (presence or absence of vegetation cover) appeared to be also important and that geographical distance and vegetation cover (local environmental factors) influenced BCC. Pommier *et al.* (2007) studied marine bacterioplankton clone library data from nine locations distributed worldwide and found differences in the composition and richness of operational taxonomic units (OTUs) among locations, a high degree of endemism and decreasing OTU richness with increasing latitude. Similarly, Fuhrman *et al.* (2008) used amplified ribosomal intergenic spacer analysis (ARISA) fingerprinting to quantify marine bacterioplankton OTUs richness in 103 near-surface samples, taken from tropical to polar regions in both hemispheres, and found that OTU number declined significantly with increasing latitude and was positively correlated with water temperature.

The two studies above, which reported biogeographic patterns and influence of latitude in shaping bacterioplankton communities, were focussed on marine environments. In marine habitats (well-connected environments), waves and currents should facilitate dispersal and subsequent colonisation by marine bacteria (Collins, 2001). In contrast, inland water bodies can be considered to be islands within a sea of land (Reche *et al*., 2005) and disconnected and relatively heterogeneous habitats (Papke & Ward, 2004), so biogeographic patterns would be more likely. In addition, lakes also present a patchy distribution likely to generate dispersal constraints (Barberán & Casamayor, 2010). As a consequence, in their study of 30 lakes from Wisconsin with ARISA, Yannarell & Triplett (2005) found that differences in BCC were best explained by regional (i.e. northern versus southern Wisconsin lakes) and landscape (i.e. seepage lakes versus drainage lakes) factors. Crump *et al.* (2007) studied BCC from lakes and streams of an Arctic tundra catchment with denaturing gradient gel electrophoresis (DGGE) and suggested that at a restricted spatial scale (<10 km), BCC was controlled by a combination of geographic (elevation, lake hydrology) and environmental factors (conductivity, temperature, pH).

However, all these studies have focussed either on the spatial or on the local environmental components

influencing BCC, and few studies have investigated the relative influence of both types of factors (spatial and environmental) along an extensive range of spatial scales. As an exception, Van der Gucht *et al.* (2007) performed a metacommunity study based on DGGE fingerprinting of 16S rRNA genes that included 98 shallow meso- to eutrophic lakes in a north–south gradient in Europe (>2500 km) to study the impact of local and regional factors and showed a strong influence of local environmental factors (resources and grazing related factors) on BCC, with a marginal impact of spatial distance. Barberán & Casamayor (2010) carried out an empirical meta-analysis of different bacterial groups from a worldwide range of surface waters (18 inland water bodies of different sizes, altitudes and trophic status and 16 coastal marine sites) and found a strong component of environmental forces shaping aquatic microbial assemblages at a global scale and detected a geographic signal for some inland waters bacterial groups.

Despite the isolation of Antarctic and sub-Antarctic habitats, microbes can be transported aerially to the Antarctic Peninsula from South America or other Antarctic locations (Marshall, 1996; Hughes *et al.*, 2004). Hirano (1965) considered that when studying phytoplankton biogeography in Antarctica, it was necessary to also study the South American continent, probably because many Antarctic species could arrive from South America. In line with this general idea, a study of diatom diversity across a Patagonian–Antarctic transect revealed the presence of both endemic and cosmopolitan species in these two areas and an evident decrease in species richness with increasing latitude (Maidana *et al.*, 2005). The same pattern of decreasing algal richness was observed by Tell, Izaguirre & Allende (2011) along a Patagonian latitudinal transect.

Our study constitutes the first analysis of the structure of bacterioplankton communities in freshwater environments along a Patagonian–Antarctic transect, in which we attempt to disentangle the effects of spatial versus environment factors on bacterioplankton structure. In this survey, we analysed the bacterioplankton assemblages in 45 water bodies (including deep lakes, shallow lakes and ponds) from Argentinean Patagonia to Maritime Antarctica using the DGGE fingerprinting technique of PCR-amplified 16S rRNA genes and sequencing. We maximised the range of latitude as much as possible, but also maximised the variability in ecological regimes within the freshwater bodies, encompassing the variation (lake size, trophic status, altitude) existing in the region. The specific goals were (i) to describe dominant bacterioplankton composition (DGGE band pattern) and total bacterioplankton abundance along a Patagonian–Antarctic gradient with increasing climate severity, (ii) to statistically determine the degree of similarity of bacterioplankton composition between Antarctica and Patagonia and to study possible endemism in both regions and (iii) to statistically test whether spatial or environmental factors were more relevant in shaping bacterioplankton community structure.

Methods

Study sites

We studied 45 freshwater bodies located along a latitudinal transect from 45°22′ to 63°24′S of latitude (from Chubut Province, Argentinean Patagonia to Hope Bay, Antarctic Peninsula), spanning a gradient of increased climate severity along a distance of 2150 km and over 19° of latitude (Fig. 1). Different types of water bodies were sampled: deep lakes (L.), shallow lakes (S.L.) and ponds (P.), which differed in morphological and limnological characteristics (Table 1).

Following the classification given by Quirós & Drago (1999), the Patagonian lakes are included in two Geographical Lake Regions of Argentina: the 'Andean Patagonia Region' and the 'Patagonian Plateau Region'. The deepest lakes are situated in the Andean Patagonia and Tierra del Fuego. Lakes situated in Andean Patagonia were mainly formed by glacial and tectonic processes, which together with later strong fluvial erosion and hard rock composition accounts for their typically very dilute waters, thus being in general ultraoligotrophic or oligotrophic. This region hosts the largest and deepest glacial lakes in South America, which are usually elongated in an east–west orientation as determined by the shape of the glacial valleys that hold them (Díaz, Pedrozo & Baccala, 2000). The lakes located on the Patagonian Plateau are generally shallower than the Andean lakes and usually range from mesotrophic to eutrophic (Quirós & Drago, 1999), with higher total phosphorus

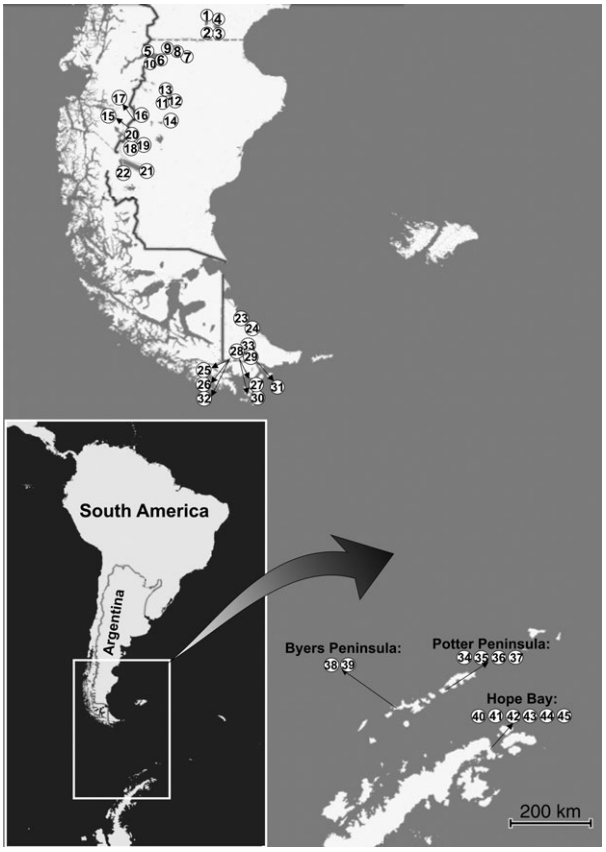

Fig. 1 Map showing locations of lakes along the Patagonian–Antarctic transect. Each number corresponds to a freshwater body (see Table 1).

and phytoplankton chlorophyll *a* (Quirós & Cuch, 1985). In Tierra del Fuego, some lakes are near the mountains and belong to the Andean Patagonian Region, while others are on the Patagonian Plateau of the island, among which most lakes in the SE are humic stained. Mean annual temperature in Patagonia ranges from 12 °C in the northeast to 3 °C towards the south. From the Andes and eastward, total annual precipitation decreases exponentially, from *c.* 2000 to <200 mm per year in the central portion of Patagonia (Paruelo *et al.*, 1998).

In Maritime Antarctica, we sampled lakes in three regions: Potter Peninsula, located on King George Island; Byers Peninsula on Livingston Island; and Hope Bay on the Antarctic Peninsula. Potter Peninsula (62°14′S, 58°38′W) is located in the southwestern extreme of the largest South Shetland Island, King George Island. A complete description of the area and the main characteristics of the water bodies were reported by Vinocur & Unrein (2000). Byers Peninsula lies at the western end of Livingston Island (62°37′S, 60°54′W), the second largest island of the South Shetland Islands. The main features of the lakes as well as the environmental characteristics of the region were reported by Toro *et al.* (2007). Hope Bay, situated at the northern end of the Antarctic Peninsula (63°24′S, 57°00′W), includes many shallow lakes, mostly of glacial origin. The geological and climatic characteristics of Hope Bay and the main features of the studied lakes have been previously described by Izaguirre *et al.* (1998) and Izaguirre, Allende & Marinone (2003).

Sampling regime, physical and chemical analyses

The samples were collected from the euphotic zone during the austral summer 2004 (Antarctica) and in late spring in Patagonia (during 2007 in Chubut and Santa Cruz and during 2008 in Tierra del Fuego). In deep lakes, integrated samples were collected within the epilimnetic region of the euphotic zone from the surface down to 5 m, whereas in shallow lakes, samples were obtained from about 30 cm below the surface. Temperature, pH and conductivity were measured *in situ* with a Horiba D-54 meter (Horiba, Kyoto, Japan) and dissolved oxygen (DO) with a HI 9146 Hanna portable meter (Hanna Instruments, Villafranca, PD, Italty). Samples for nutrient and chlorophyll *a* (Chl *a*) analyses were immediately filtered through Whatman® GF/F filters (Whatman, Maidstone, U.K.). Ammonia, nitrate and phosphate concentrations were determined using a Hach™DR/2800 spectrophotometer (Hach Company, Loveland, CO, U.S.A.) and their corresponding reagent kits (detection limit for all nutrients 0.01 mg L^{-1}) on the GF/F filtrates. Concentrations of Chl *a*, corrected for phaeopigments, were determined by spectrophotometry before and after acidification (HCl 0.1 N). Extractions of pigments from the GF/F filters were carried out in 90% acetone HPLC grade, with two 15-min sonication steps separated by overnight storage at 4 °C (Descy, Sarmento & Higgins, 2009). The equations published by Marker *et al.* (1980) were used for calculations. Dissolved organic carbon (DOC) was determined using a high-temperature Pt catalyst oxidation method (Shimadzu analyzer TOC-5000A, SM 5310B technique, detection limit: 0.1 mg L^{-1}) following the recommendations of Sharp *et al.* (1993). *In situ* underwater measurements of

Table 1 Main morphometric and physicochemical features of the 45 water bodies. The morphometric data for Hope Bay and Potter Peninsula were taken from Drago & Paira (1987), Izaguirre *et al.* (1998) and Vinocur & Unrein (2000). Byers Peninsula data were taken from Toro *et al.* (2007)

No.	Ambient	Location	Latitude (°S)	Longitude (°W)	Altitude (m a.s.l.)	Area (km^2)	Temp. (°C)	pH	Conductivity ($\mu S\ cm^{-1}$)	DO (ppm)	N-NO_3^- (ppm)	N-NH_4^+ (ppm)	P-PO_4^{3-} (ppm)	K_d PAR (m^{-1})	Dissolved organic carbon (ppm)	Chl *a* ($\mu g\ L^{-1}$)
1	L. Musters	Chubut	45.55	69.14	277	438.506	12.6	7.8	310	9.3	0.01	0.22	0.38	1.15	10.8	2.62
2	P.2	Chubut	45.57	69.11	297	0.043	15.5	8.4	490	10.5	0.00	0.41	0.06	2.54	19.3	4.75
3	P.3	Chubut	45.60	69.00	269	0.002	14.1	8.1	110	10.9	0.01	0.41	0.20	2.27	13.5	9.68
4	L. Colhué Huapi	Chubut	45.37	68.95	280	762.559	10.0	8.7	1350	9.3	0.17	0.11	1.78	28.52	8.2	47.01
5	L. Pueyrredón	Santa Cruz	47.38	71.97	158	308.884	8.9	7.3	130	11.8	0.02	0.05	0.05	0.19	7.6	0.41
6	L. Posadas	Santa Cruz	47.45	71.81	160	39.877	10.0	7.8	210	9.8	0.04	0.01	0.07	0.09	6.1	0.38
7	P.7	Santa Cruz	47.34	70.99	447	0.001	27.0	8.3	1810	8.1	0.10	n.a.	14.10	1.17	n.a.	17.02
8	L. Ghio	Santa Cruz	47.27	71.51	400	59.464	12.8	8.2	4760	10.3	0.04	n.a.	0.84	0.34	42.0	0.11
9	P.9	Santa Cruz	47.20	71.60	580	0.024	20.7	8.2	4110	13.9	0.02	0.03	15.80	3.42	64.1	18.35
10	P.10	Santa Cruz	47.46	71.87	161	0.002	15.6	8.2	50	8.1	0.06	0.06	0.04	1.77	6.1	3.00
11	P.11	Santa Cruz	48.69	71.15	830	0.015	10.1	8.2	560	9.2	0.01	0.05	0.18	7.04	33.7	19.56
12	P.12	Santa Cruz	48.68	71.13	848	0.092	13.6	8.2	4600	9.0	0.03	0.00	0.10	1.70	43.5	5.43
13	P.13	Santa Cruz	48.63	71.14	880	0.005	17.3	8.3	230	9.3	0.18	0.00	0.10	n.a.	44.0	36.67
14	L. Cardiel	Santa Cruz	48.99	71.13	280	350.681	11.0	9.3	4360	10.3	0.10	0.00	1.39	0.23	11.8	0.76
15	P.15	Santa Cruz	49.26	72.89	425	0.005	7.3	7.3	80	11.9	0.15	0.01	0.96	0.49	7.0	1.78
16	L. del Desierto	Santa Cruz	49.08	72.89	506	9.273	8.2	6.9	20	11.9	0.12	0.02	0.15	0.12	5.1	0.20
17	P.17	Santa Cruz	49.13	72.93	459	0.004	14.8	7.0	50	9.5	0.14	0.03	0.27	0.61	11.0	0.29
18	P.18	Santa Cruz	49.59	72.30	253	0.185	12.3	8.2	380	10.3	0.14	0.20	0.26	0.42	5.4	3.87
19	P.19	Santa Cruz	49.59	72.31	253	0.076	13.6	8.3	560	11.3	0.02	0.15	0.15	0.54	11.7	3.17
20	L. Viedma	Santa Cruz	49.39	72.87	273	1219.190	8.3	6.7	40	11.4	0.03	0.01	0.25	1.62	5.5	1.02
21	L. Argentino	Santa Cruz	50.31	72.80	181	1419.485	10.0	6.9	40	9.3	0.17	0.00	0.13	1.31	4.0	0.17
22	P.22	Santa Cruz	50.32	72.79	184	0.995	12.3	8.1	610	10.5	0.05	0.02	0.24	3.06	n.a.	8.00
23	S.L. de Los Cisnes	Tierra del Fuego	53.79	67.78	8	3.116	12.7	9.0	25 800	12.0	0.01	0.12	0.40	2.73	50.2	0.41
24	S.L. San Luis	Tierra del Fuego	53.92	67.60	10	3.748	10.7	8.1	313	10.7	0.00	0.08	0.17	2.06	17.8	0.68
25	L. Acigami	Tierra del Fuego	54.83	68.56	20	19.197	4.5	7.6	78	11.2	0.01	0.07	0.10	1.50	5.1	0.12
26	S.L. Laguna Negra	Tierra del Fuego	54.84	68.59	29	0.138	6.1	6.9	66	10.1	0.00	0.13	0.07	0.80	12.0	0.41
27	L. Escondido	Tierra del Fuego	54.68	67.81	120	6.054	4.5	7.4	103	11.5	0.01	0.06	0.04	0.64	7.1	0.10
28	L. Fagnano	Tierra del Fuego	54.59	67.62	27	646.691	6.7	7.6	85	11.7	0.01	0.06	0.02	0.69	5.6	0.10
29	P.27	Tierra del Fuego	54.60	67.63	43	0.0003	8.3	6.5	205	7.7	0.00	0.08	0.08	3.30	24.8	0.34

Table 1 (Continued)

No.	Ambient	Location	Latitude (°S)	Longitude (°W)	Altitude (m a.s.l.)	Area (km^2)	Temp. (°C)	pH	Conductivity ($\mu S\ cm^{-1}$)	DO (ppm)	$N\text{-}NO_3^-$ (ppm)	$N\text{-}NH_4^+$ (ppm)	$P\text{-}PO_4^{3-}$ (ppm)	K_d PAR (m^{-1})	Dissolved organic carbon (ppm)	Chl *a* ($\mu g\ L^{-1}$)
30	S.L. Victoria	Tierra del Fuego	54.78	67.70	103	0.091	7.2	7.4	55	11.0	0.01	0.05	0.03	1.77	10.8	0.51
31	P.29	Tierra del Fuego	54.87	67.35	11	0.139	8.2	7.9	110	10.7	0.01	0.03	0.08	2.98	17.1	0.29
32	P.30	Tierra del Fuego	54.85	68.58	15	0.001	13.4	8.1	1284	10.5	0.02	0.06	0.03	2.16	6.9	0.31
33	L. Yehuin	Tierra del Fuego	54.36	67.78	50	42.482	8.0	8.0	197	11.5	0.01	0.13	0.04	0.41	9.3	0.10
34	L.L	Potter Peninsula	62.25	58.64	60	0.232	1.2	6.2	21	13.0	0.01	0.04	0.02	n.a.	n.a.	1.00
35	L.M	Potter Peninsula	62.25	58.65	30	0.003	4.4	6.7	93	12.6	0.01	0.03	0.02	n.a.	n.a.	1.20
36	L.W	Potter Peninsula	62.24	58.67	12	0.006	8.4	8.0	199	13.1	0.02	0.01	0.01	n.a.	n.a.	0.90
37	L.Z	Potter Peninsula	62.24	58.66	20	0.004	1.6	7.1	145	11.9	0.01	0.02	0.02	n.a.	n.a.	2.40
38	L. Refugio	Bayers Peninsula	62.67	61.00	5	0.120	3.5	7.6	247	10.8	0.74	0.09	0.17	n.a.	n.a.	28.75
39	L. Limnopolar	Bayers Peninsula	62.65	61.11	10	0.580	3.0	6.8	76	11.8	0.004	0.003	0.002	n.a.	n.a.	0.16
40	P. Pingüi	Hope Bay	63.40	57.01	20	0.002	0.8	7.1	1860	5.2	13.00	20.60	15.00	1.24	41.9	17.42
41	L. Boeckella	Hope Bay	63.40	57.00	49	0.070	0.2	6.5	19	17.3	0.14	0.47	0.21	1.08	0.7	3.70
42	L. Esperanza	Hope Bay	63.41	57.03	52	0.030	1.0	6.4	91	15.1	0.13	0.07	0.01	0.13	0.5	0.22
43	L. Encantado	Hope Bay	63.41	57.04	55	0.003	0.1	6.6	67	20.0	0.11	0.03	0.02	0.90	0.9	0.58
44	L. Flora	Hope Bay	63.41	57.04	52	0.006	3.0	6.3	32	26.3	0.09	0.01	0.01	0.13	0.4	0.65
45	L. Chico	Hope Bay	63.41	57.01	100	0.003	0.0	6.5	44	16.1	0.19	0.18	0.31	0.96	0.5	0.87

Temp., temperature; DO, dissolved oxygen; K_d PAR, diffuse attenuation coefficient; chl *a*., chlorophyll *a*; n.a., not available; P., pond; L., lake; S.L., shallow lake.

photosynthetically active radiation (PAR) were taken using a LI-COR radiometer equipped with a submersible spherical quantum sensor (Li-193 SA; Li-Cor PAR, Lincoln, NE, U.S.A.). The diffuse attenuation coefficient (K_d) was calculated as the slope of the relationship ln (irradiance) versus depth (Kirk, 1994). The geographic position of the water bodies was recorded using a geographic positioning system (Garmin, Olathe, KS, U.S.A.).

Determination of the abundance of autotrophic and heterotrophic prokaryotes

Total bacterioplankton and picocyanobacteria abundances were determined by epifluorescence using an Olympus microscope (Olympus BX40F4, Tokyo, Japan) at ×1000 magnification. Water samples from each lake were filtered through a 0.2-μm-pore size polycarbonate black filter, and cells were stained with 4, 6 diamidino-2-phenyl-indole (10 μg mL^{-1} final concentration), following the procedure outlined by Porter & Feig (1980). Bacterioplankton counting was performed by epifluorescence microscopy using UV excitation (freshwater archaea would be included in these counts), and picocyanobacteria counting was carried out using both blue- and green-wavelength excitation.

DNA extraction and PCR amplification

Water samples were pre-filtered *in situ* through a sterile 50-μm net to remove zooplankton and collected in a plastic bottle. Depending on the trophic status of the lake, between 500 and 2000 mL of sample were filtered with a vacuum pump first through a 20-μm-pore size polycarbonate filter and then through a 3- and 0.2-μm-pore size polycarbonate filters (diameter 47 mm; Millipore, Cork, Ireland). The filters were placed in cryovials with 1.8 mL of lysis buffer (40 mM EDTA, 50 mM Tris–HCl, 0.75 M sucrose), stored first in liquid nitrogen and then at −80 °C until nucleic acid extraction. For this study, we used the 0.2-μm-pore size filters (0.2–3 μm fraction) to study free-living bacterial communities.

The procedure followed for nucleic acid extractions (phenol/chloroform extraction) was fully described by Unrein *et al*. (2005). Amplifications of 16S rRNA gene fragments were performed using the touchdown polymerase chain reaction (PCR) procedure using a Bio-Rad thermocycler (Bio-Rad, Hercules, CA, U.S.A.). To perform DGGE amplifications, we used the primers designed by Muyzer, De Waal & Uitterlinden (1993) and Muyzer *et al*. (1995): 358f-GC 5′-CCT ACG GGA GGC AGC AG-3′ (target group: bacteria; with a 40-bp GC-clamp sequence attached to the 5′ end) and 907RM 5′-CCG TCA ATT CMT TTG AGT TT-3′ (target group: universal). The primers produced fragments of about 540 bp, equivalent to positions 358–907 (in *Escherichia coli*) in the variable V3–V5 region of the 16S rRNA gene. The PCR mixtures (50 μL) contained 1 μL of extracted DNA as a template, 1 μL of deoxynucleoside triphosphate mix at a concentration of 10 mM each one, 1.5 μL of $MgCl_2$ (solution 25 mM), 2.5 μL of BSA (3 mg mL^{-1}), 5 μL of PCR buffer (Invitrogen, Grand Island, NY, U.S.A.), 2.5 μL of each primer at a concentration of 10 μM, 0.25 μL of Taq DNA polymerase (Invitrogen) and 33.75 μL of 0.2-μm-filtered sterilised water. The PCR programme included an initial denaturation at 94 °C for 5 min, followed by 10 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min (decreased by 1 °C every cycle until 55 °C, at which an additional 20 cycles were carried out), and extension at 72 °C for 3 min and a final extension cycle at 72 °C for 10 min. An aliquot of the PCR product was analysed by electrophoresis in a 1% agarose gel, stained with SYBR SAFE (0.5 × final concentration; Invitrogen) and quantified by comparison with a standard (Low DNA Mass Ladder; Invitrogen).

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis was performed with a DGGE-2000 system (CBS Scientific Company, Del Mar, CA, U.S.A.). Electrophoresis was performed with 0.75-mm-thick 6% polyacrylamide gels (acrylamide/bisacrylamide ratio of 37.5 : 1) submerged in 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA, pH 7.4) at 60 °C. Approximately 800 ng of PCR product from environmental samples was applied to individual lanes in the gel. The gels were run at 100 V for 16 h in a linear 40–80% denaturant agent gradient (100% denaturant agent was defined as 7 M urea and 40% deionised formamide). The gels were stained for 45 min in 1× TAE buffer with Sybr-Gold nucleic acid stain (Invitrogen) and visualised with UV radiation using a Chemidoc system and the Quantity One software (Bio-Rad). Forty-one samples (four samples

were lost accidentally when performing DNA extraction: L. Acigami, S.L. Laguna Negra, L. Escondido, and P.27) were run in three DGGE gels. Between three and four samples were run in all the DGGEs to allow comparison among the gels; thus, positions of the bands in different gels could be determined. Digitised DGGE images were analysed using the GEL-PRO 4.0 software (National Institutes of Health, Bethesda, MD, U.S.A.). A band was defined as a stain signal when its intensity was above 0.2% of the total intensity for each lane (e.g. Reche *et al*., 2005) and was then considered as an OTU. Bands migrating at the same position in the gels in different samples were considered to represent the same OTU. We applied the term 'dominant OTUs' because DGGE fingerprints yield only the dominant members of the bacterioplankton community. Hereinafter, we use 'number of dominant OTUs' and 'DGGE band number' interchangeably for the total number of DGGE bands per lake.

Band sequencing

Denaturing gradient gel electrophoresis bands were excised with scalpel blades from the gel, resuspended in 20 μL of Milli-Q water, stored at 4 °C overnight and then kept at −20 °C until PCR reamplification. Five microlitres of supernatant was used for PCR reamplification with the original primer set, and the PCR products were purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Germany). Between 30 and 50 ng of the reamplified PCR product were used for a sequencing reaction with the corresponding forward primer in Macrogen Sequencing Service (Macrogen, Republic of Korea). The sequences obtained (around 500 bp) were screened for chimeras using the Ribosomal Database Project (Maidak *et al*., 2001) and then compared with public DNA databases using BLAST (Altschul *et al*., 1997). Sequence data generated in this study were deposited in GenBank under the Accession numbers HM358514-HM358596.

Statistical analyses

The similarity in BCC among water bodies was first assessed by cluster analysis on a qualitative matrix (BCC presence/absence), using the Jaccard index and the unweighted pair-group mean average (UPGMA) algorithm with software SPSS 15.0.1 (StatSoft, Tulsa, OK, U.S.A.).

Another matrix was constructed with the BCC obtained by DGGE taking into account the relative intensity of each band compared with the total band intensity in each lane (100%). This 'quantitative community matrix' was tested against a second matrix obtained from both environmental and spatial data corresponding to the same samples using the Canonical Correspondence Analysis (CCA), with the purpose of identifying the controlling factors of BCC in the lakes. Given that this 'community matrix' based on DGGE band intensities showed a strong unimodal response, CCA was performed. The following variables were included in the analysis: latitude, lake area, phosphate, diffuse attenuation coefficient (K_d) and DOC. Forward selection was used for adding environmental variables to the model. Variables strongly correlated were eliminated from the analysis as they provided redundant information. Significance of the canonical axes was assessed using Monte Carlo permutation tests. To further explore the patterns in BCC revealed by DGGE, the canonical variation partitioning procedure was employed (Borcard, Lengendre & Drapeau, 1992). This type of analysis allows the discrimination of patterns related to one set of explanatory variables while controlling a second set of explanatory variables, called the covariables (Legendre & Legendre, 1998). In particular, this analysis is useful to measure the relative importance of non-spatial environmental factors (physics, chemistry, morphometry) and spatial factors (geographic coordinates) in biogeographic studies (Cottenie, 2005; Yannarell & Triplett, 2005; Langenheder & Ragnarsson, 2007), and it is appropriate to determine how much variation can be related to each factor. The whole variation of the BCC matrix was partitioned as follows: non-spatial environmental variation (environment alone), spatial variation that is not shared by the environmental variables (space alone), spatially structured environmental variation (environment + space) and unexplained variation and stochastic fluctuations (Borcard *et al*., 1992). The significance of these components was evaluated with a Monte Carlo permutation test. The canonical variation partitioning approach was carried out with BCC matrix (based on DGGE band intensities and DGGE band presence/absence) and spatial matrix (geographic coordinates) while controlling for the effect of the non-spatial environmental descriptors (lake area, phosphate, K_d, DOC) and with the BCC matrix

and non-spatial environmental data set while controlling for space (spatial matrix). We used partial CCA for the BCC intensity matrix and partial redundancy analysis (RDA) for the BCC presence/absence matrix, since responses were unimodal and linear respectively. Calculations were performed with software CANOCO (ter Braak, 1991).

To determine the correlation between similarity and distance matrices, standard and partial Mantel tests were also performed using zt software version 1.1 (Bonnet & Van de Peer, 2002). The BCC similarity matrices (based on DGGE band intensities and DGGE band presence/absence) were obtained using Bray Curtis and Jaccard index, respectively, the spatial matrix was obtained using simple Euclidean distances and the standardised environmental similarity matrix using 1 minus a standardised Euclidean distance in which raw values of environmental variables were first transformed to their standard normal deviate equivalents [(x − mean) divided by the SD] to accommodate the different units of the different variables (Martiny *et al.*, 2006). To further explore the effect of each type of environmental component on BCC similarity matrix, the standardised environmental similarity matrix (which included physics, chemistry and morphometry data) was separated in two different similarity matrices: physicochemical (phosphate, K_d, DOC) and morpho-topographical (lake area and altitude).

Stepwise multiple regressions were performed using DGGE band number per lake (dependent variable) and the following environmental variables (independent variables): latitude, lake area, phosphate, DOC, K_d, pH, DO and dissolved inorganic nitrogen (DIN) = nitrate + nitrite + ammonia. Other stepwise multiple regressions were assessed using total bacterioplankton abundance per lake (dependent variable) and the same environmental variables utilised in the first multiple regression analysis. Variables for statistical tests that were not normally distributed were $\log_{10}$-transformed or $\log_{10}$ + 1-transformed to fit normality assumptions, which were checked with Kolmogorov–Smirnov tests. Correlations between all variables were carried out using Spearman's rho test. Correlations and multiple regressions were carried out with SPSS 15.0.1 (StatSoft).

To statistically test the degree of resemblance of BCC (based on DGGE band intensities using Bray Curtis distance and DGGE band presence/absence using Jaccard index) between Antarctica and Patagonia, an analysis of similarity (ANOSIM) was performed using PAST software version 2.0 (Hammer, Harper & Ryan, 2001).

Results

The studied water bodies covered a large latitudinal gradient (Fig. 1) and ranged from oligotrophic to eutrophic (chl *a* levels varied from 0.1 to 47.01 μg L^{-1}, Table 1). The abundances of prokaryotes increased with increasing trophic status of the lake: in oligotrophic lakes, bacterioplankton abundances ranged from 9.6×10^4 to 1.3×10^7 and picocyanobacteria from 0 to 3.6×10^5 cells mL^{-1}; in mesotrophic lakes, bacterioplankton varied from 5.3×10^5 to 1.3×10^7 and picocyanobacteria from 0 to 1.4×10^6 cells mL^{-1}; and in eutrophic lakes, bacterioplankton ranged from 3.0×10^6 to 1.0×10^8 and picocyanobacteria from 1.1×10^2 to 5.7×10^6 cells mL^{-1}. Correlations between picoplankton abundances (bacterioplankton and picocyanobacteria) and environmental variables are shown in Table 2.

We ran samples from 41 water bodies in three DGGE gels (Fig. 2). Along the latitudinal transect, the number of DGGE bands per sample ranged from 10 to 35. Patagonian lakes presented between 12 and 35 bands per lake, whereas Antarctic lakes showed between 10 and 21. Analysis of the gels gave a total of 863 bands located in 76 different DGGE positions, which represent the total dominant bacterioplankton richness along the Patagonian–Antarctic transect. Each DGGE position was considered to represent a single bacterial OTU. Among these 76 OTUs, 45 were shared between Patagonian and Antarctic water bodies, 28 were present only in Patagonian lakes and three were exclusive to Antarctic lakes.

Fifty different positions from a total of 76 were successfully sequenced (these 50 positions represented 80% of the total intensity of the three DGGE gels). Bands with the same position were sequenced from different lakes to confirm that they corresponded to the same sequence: e.g. bands 4 (a, b) or 45 (a, b, c, d, e, f, g) (Fig. 2). Sequence similarities among these ranged from 99.5 to 100%, indicating that they indeed are most likely the same OTU. Among the 50 sequenced positions, we found three Cyanobacteria (*Synechococcus* sp.), 17 Bacteroidetes, 11 Betaproteobacteria, nine Actinobacteria, one Alphaproteobacte-

Table 2 Spearman's rho correlation coefficients among denaturing gradient gel electrophoresis (DGGE) band number (n = 41) and picoplankton abundances (Bacterioplankton and Picocyanobacteria, n = 45) versus environmental variables

	DGGE band number	Bacterioplankton abundance	Picocyanobacteria abundance	Lake area	Latitude	Temperature	DOC	K_d	Phosphate
DGGE band number	1								
Bacterioplankton abundance	**0.44***	1							
Picocyanobacteria abundance	**0.39**	**0.71*****	1						
Lake area	**0.54*****	0.06	**0.30**	1					
Latitude	**−0.44***	**−0.65*****	**−0.77*****	−0.26	1				
Temperature	**0.33**	**0.67*****	**0.71*****	−0.02	**−0.83*****	1			
DOC	0.22	**0.73*****	**0.57*****	−0.02	**−0.57*****	**0.65*****	1		
K_d	0.05	**0.64*****	0.29	−0.22	−0.20	**0.34**	**0.51*****	1	
Phosphate	0.17	**0.63*****	**0.57*****	0.10	**−0.50****	**0.41***	**0.54*****	0.26	1
Chl *a*	−0.08	**0.55*****	0.27	**−0.35**	−0.29	**0.30**	**0.31**	**0.44***	**0.43***

Bold data represent $P < 0.05$; *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.
DOC, dissolved organic carbon; K_d, diffuse attenuation coefficient; Chl *a*, chlorophyll *a*.

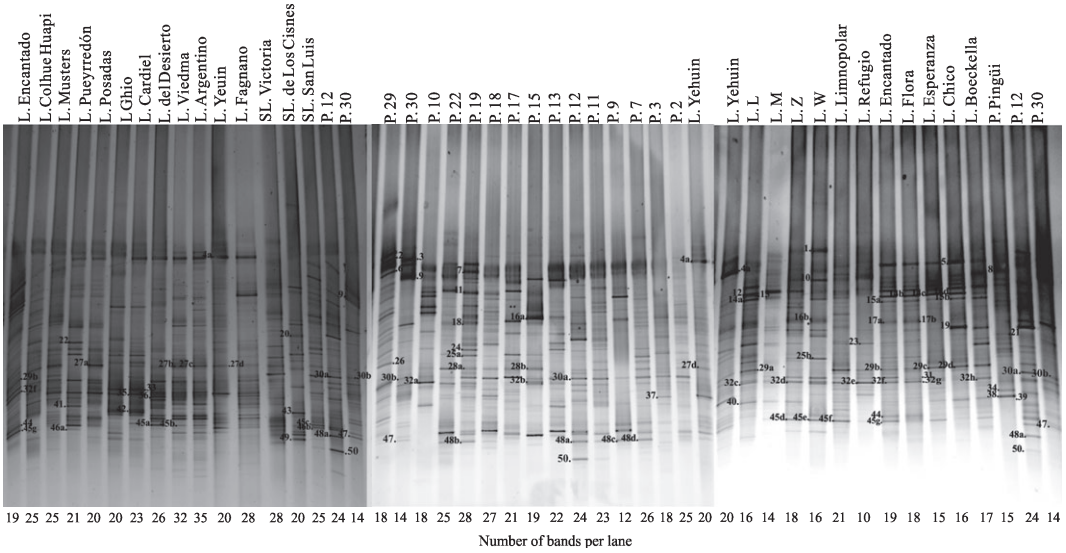

Fig. 2 Picture of denaturing gradient gel electrophoresis gels run for the 41 sampled lakes. Numbers in gel indicate excised and sequenced bands.

ria, one Firmicutes, one Acidobacteria, four unknown bacteria, one mitochondrial and two chloroplast genes. The complete information (closest match, GenBank accession number, fragment length, sequence similarity, source of the closest match, closest phylogenetic affiliation and phylogenetic group) of each sequenced DGGE band is presented in the Table S1. For 54% of the sequenced OTUs, the closest matches corresponded to environmental sequences found in polar regions (Arctic and Antarctic) or in cold habitats elsewhere in the world (glaciers and high mountain lakes).

Factors determining bacterial community composition

The results of the cluster analysis (Fig. 3) using the complete DGGE band pattern (presence/absence dataset) suggest a geographical and morphometric lake effect on BCC. The lakes from Antarctica clustered together (group I), except the eutrophic Pingüi

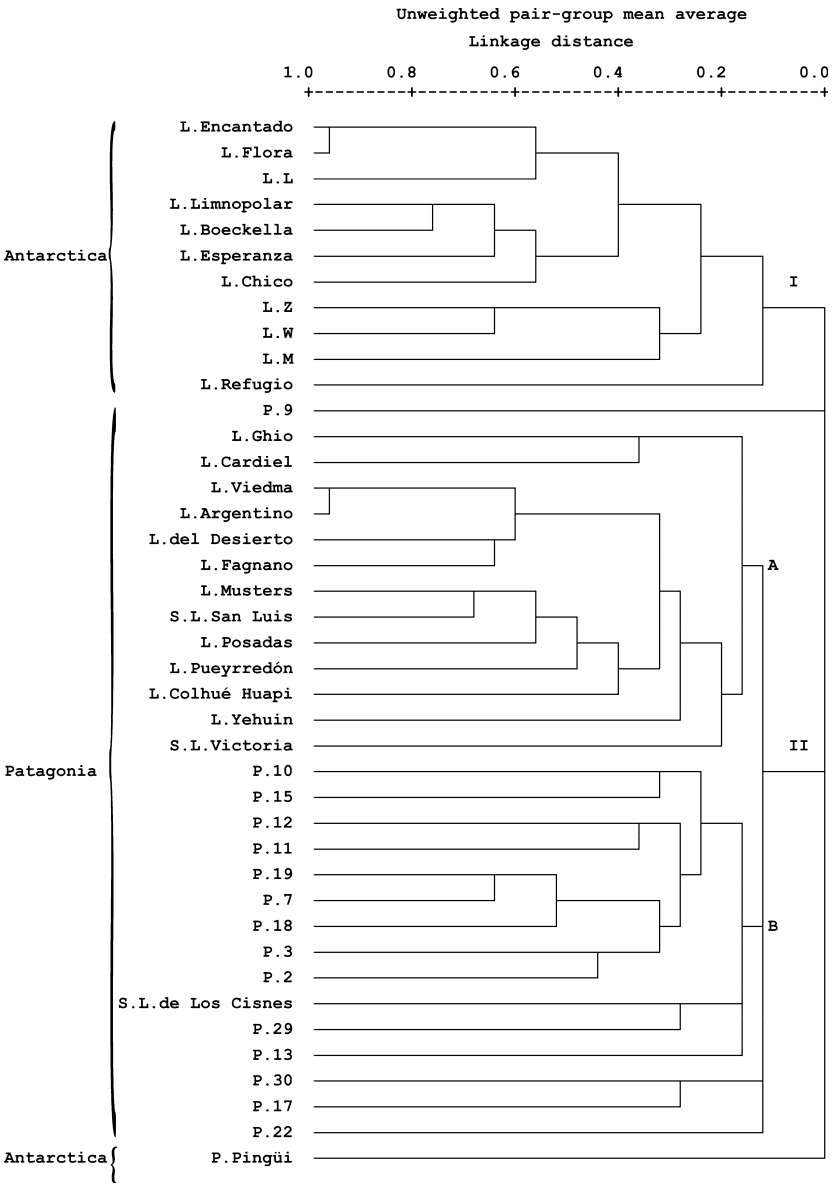

Fig. 3 Unweighted pair-group mean average dendrogram resulting from the cluster analysis performed among samples based on the denaturing gradient gel electrophoresis band positions (bacterioplankton community composition presence/absence) presented in Fig. 2.

Pond. The water bodies from Patagonia also grouped together (group II), and the subgroups within it, in general, reflect differences in morphometric and limnological characteristics: group II-A consists mainly of large, deep and oligotrophic lakes, whereas group II-B consists mainly of small, shallow and mesotrophic–eutrophic water bodies. In addition, significant differences were found in lake BCC (based on DGGE band intensities and DGGE band presence/absence) between Antarctic and Patagonian region (ANOSIM $r = 0.46$ (Bray Curtis) and $r = 0.69$ (Jaccard), both $P < 0.0001$).

The result of the CCA using the bacterial DGGE band intensity profiles and both environmental and spatial variables together from the 41 water bodies is shown in Fig. 4. The first two axes accounted for 59.0% of the variance (axis 1: 38.5%, axis 2: 20.5%). The environmental variables were significantly correlated with the first axis ($P = 0.022$), and the test of significance of all canonical axes was also significant

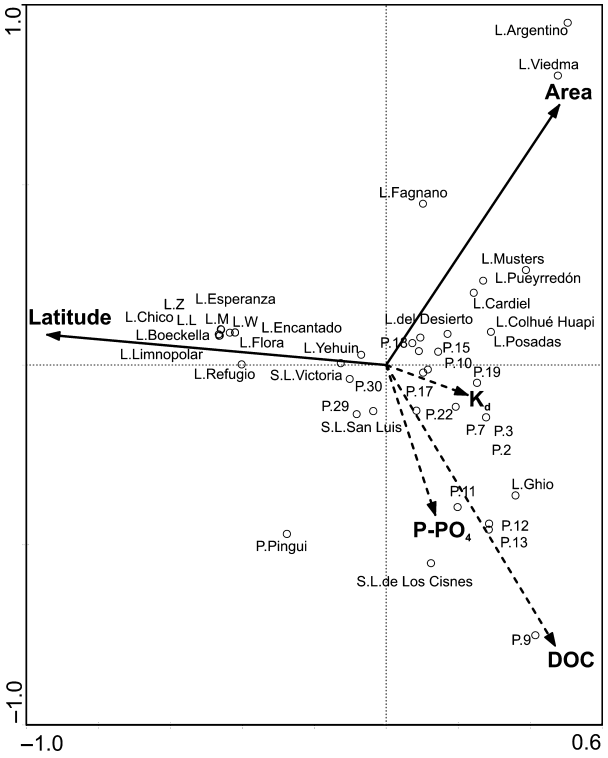

Fig. 4 Band biplot corresponding to the Canonical Correspondence Analysis (CCA) based on the band intensity patterns of the denaturing gradient gel electrophoresis gels presented in Fig. 2 and environmental data. Significant environmental variables ($P < 0.05$) are indicated with solid arrows, while dotted arrows are not significant. P-PO_4, phosphate concentration; DOC, dissolved organic carbon; K_d, diffuse attenuation coefficient.

($P = 0.012$). The first axis is mainly defined by latitude (intraset correlation coefficient: −0.86); the second axis is mainly correlated with lake area and DOC (intraset correlation coefficient: 0.63 and −0.68, respectively). This analysis clearly shows the separation of the water bodies by latitude: Antarctic lakes are ordinated in the middle-left side of the figure. Lakes with higher surface area, as L. Argentino, L. Viedma, L. Fagnano, L. Musters, L. Pueyrredón, L. Cardiel, L. Colhué Huapi, are plotted together in the upper-right side of the graph. In addition, the majority of the ponds and shallow lakes are situated together with higher levels of DOC and phosphate. The most important and significant variable was latitude ($P = 0.002$); lake area showed a lower significance in the ordination of the lakes ($P = 0.078$); the other variables included in the analysis were not significant (Fig. 4).

The canonical variation partitioning performed to discriminate the relative importance of non-spatial environmental and spatial factors showed that both exerted influence on BCC. For the BCC intensity matrix, the relative importance of the spatial components was 11.0%, while for non-spatial environmental factors, it was 11.8% (Monte Carlo permutation test $P < 0.05$ for all canonical axes), and for spatial component of environmental influences, it was 2.2% (environment + space). For the BCC presence/absence matrix, the relative importance of environment alone was 15.1%, while for space alone, it was 12.0% (Monte Carlo permutation test $P < 0.002$ for all canonical axes), and for environment plus space, it was 3.7% (Fig. 5a,b). The Mantel and partial Mantel tests were significant and also indicated that both spatial and environmental factors influenced BCC (Fig. 5c,d). To further disentangle the major factors controlling BCC, the similarity matrix obtained from BCC presence–absence profile was compared to matrices of spatial distribution, physicochemical

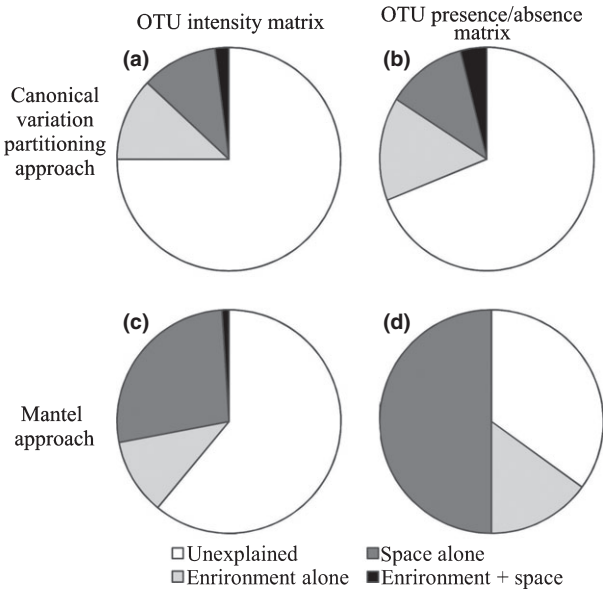

Fig. 5 Results of canonical variation partitioning of the Bacterioplankton community composition (BCC) matrices into different components (a) for the BCC intensity matrix (unimodal response) using partial CCA and (b) for the BCC presence/absence matrix (linear response) using partial redundancy analysis. Results of the partial Mantel test into different components are shown in (c) for the BCC intensity matrix and (d) for the BCC presence/absence matrix. Four different components are shown: pure environmental variation that is not shared by spatial variables (environment alone), pure spatial variation that is independent of any environmental factors (space alone), spatial structure in BCC that is shared by the environmental data (environment + space) and unexplained variation.

constraints and morpho-topographical features (lake area and altitude) using Mantel test and partial Mantel test (Table 3). These analyses indicated that the spatial distribution of the lakes had a significant effect on bacterial community similarity and that the physicochemical constraints matrix but not the morpho-topographical matrix had an effect on bacterial community similarity (Table 3).

Factors determining DGGE band number

The stepwise multiple regressions procedure performed with DGGE band number per lake and the environmental variables (latitude, lake area, phosphate, DIN, DOC, K_d, pH and DO) showed that only latitude and lake area had a significant effect on the dependant variable and were selected for the regression model, being the most important variables explaining the number of dominant OTUs. The selected model (1) had statistically significant partial regression coefficients (adjusted multiple coefficient of determination $R^2 = 0.49$, $P = 0.0001$, $n = 41$):

(1) Denaturing gradient gel electrophoresis band number = $36.90 - 0.32 \times \text{latitude} + 8.50 \times 10^{-9} \times \text{area}$, with latitude in degrees and area in m^2. The standardised partial regression coefficients (beta weights) were 0.51 (area), −0.39 (latitude).

Denaturing gradient gel electrophoresis band number declined with increasing latitude, with a significant negative correlation ($r = -0.44$, $P = 0.004$, $n = 41$). DGGE band number had a weaker relationship with temperature ($r = 0.33$, $P = 0.036$, $n = 41$) than with latitude and was not correlated with Chl *a* concentration ($r = -0.08$, $P = 0.63$, $n = 41$). In addition, DGGE band number was observed to increase with lake area, resulting in a significant positive correlation ($r = 0.54$, $P = 0.0001$, $n = 41$) (Table 2).

Table 3 Results of Mantel test and partial Mantel test for the comparison of bacterioplankton community composition similarity matrix (based on denaturing gradient gel electrophoresis band presence–absence) with spatial distribution matrix (distance between pairs of lakes calculated from latitude and longitude), the physicochemical constraints matrix (phosphate, dissolved organic carbon, diffuse attenuation coefficient) and the morpho-topographical matrix (lake area and altitude)

	Mantel		Partial Mantel†	
Matrix type	*r*	*P*	*r*	*P*
Spatial distribution	−0.503*	0.0001	–	–
Physicochemical constraints	0.296*	0.0001	0.305*	0.0001
Morpho-topographical constraints	0.082	0.1020	0.036	0.3066

*$P < 0.05$.

†The partial Mantel test holds spatial distribution constant.

Factors determining bacterioplankton abundance

The stepwise multiple regressions procedure performed with bacterial abundance and the environmental variables (latitude, lake area, phosphate, DIN, DOC, K_d, pH and DO) showed that only latitude, phosphate and K_d had a significant effect on the dependant variable (total bacterial abundance per lake) and were selected for the regression model, being the most important variables explaining bacterial abundances. The selected model (2) showed statistically significant partial regression coefficients (adjusted multiple coefficient of determination $R^2 = 0.65$, $P = 0.0001$, $n = 45$):

(2) Bacterioplankton abundance = $10.05 - 0.07 \times \text{latitude} + 0.08 \times \text{phosphate} + 0.05 \times K_d$, with latitude in degrees, phosphate in mg L^{-1} and K_d in m^{-1}. The standardised partial regression coefficients (beta weights) were −0.57 (latitude), 0.38 (phosphate), 0.28 (K_d).

Bacterioplankton abundance declined with increasing latitude, with a significant negative correlation ($r = -0.65$, $P = 0.0001$, $n = 45$), whereas it increased significantly with water temperature, DOC, K_d and phosphate concentration ($r = 0.67$, $P = 0.0001$, $n = 45$; $r = 0.73$, $P = 0.0001$, $n = 45$; $r = 0.64$, $P = 0.0001$, $n = 45$; $r = 0.63$, $P = 0.0001$, $n = 45$, respectively) (Table 2).

Discussion

Our results show that bacterioplankton community structure was influenced by both spatial and environmental factors. These findings are in line with the results obtained by other authors. In particular, Langenheder & Ragnarsson (2007) studied a set of coastal rock pools at small spatial scales (<500 m) and found that bacterioplankton composition was influenced by local environmental conditions and spatial factors; Yannarell & Triplett (2005) reported that environmental, regional, temporal and landscape level features interact to determine the makeup of bacterial assemblages in northern temperate lakes; and Cottenie

(2005), based on a large published data set with information on community structure of a broad range of taxa (included bacteria), found that *c.* 50% of the variation in community composition was explained by both local and spatial variables. On the other hand, in a bacterial metacommunity study carried out in 98 shallow meso- to eutrophic lakes covering a north–south gradient in Europe (>2500 km), a strong influence of local environmental factors (resources and grazing) was found, whereas spatial distance showed only a marginal effect (Van der Gucht *et al.*, 2007). Teasing apart both types of factors in biogeographic analysis is essential because the spatial dimension may have a spurious correlation with crucial local environment factors that affect BCC (Sommaruga & Casamayor, 2009) and the difficulty of co-varying space and habitat characters can only, to some extent, be disentangled by multivariate analysis (e.g. Beisner *et al.*, 2006; Langenheder & Ragnarsson, 2007; Van der Gucht *et al.*, 2007). In our study, the canonical partitioning approach was useful to discriminate the influence of each type of factor. We observed that both geographic position of the lakes and environmental conditions influenced bacterioplankton communities. Similar results were obtained from Mantel and partial Mantel tests. However, for the Mantel approach, the relative importance of space alone and the total explained variation were higher (in BCC intensity and BCC presence–absence matrices) than in canonical partitioning analyses. Although canonical analysis was found to have much higher power than the Mantel approach, the latter analysis is appropriate for testing the variation in beta diversity among groups of sites (Legengre, Borcard & Peres-Neto, 2005).

On the other hand, in our study, we found that the number of OTUs per lake decreased with latitude. Nevertheless, as the molecular technique employed (DGGE) is not useful to assess the total bacterioplankton richness in one lake, our results should not be interpreted in the framework of the latitudinal gradients of biodiversity. However, some authors have observed latitudinal gradients in bacterial diversity; in particular, Fuhrman *et al.* (2008) found a decreasing pattern of diversity in planktonic marine bacteria analysing samples taken from tropical to polar in both hemispheres, and Pommier *et al.* (2007) also observed a latitudinal gradient of OTU richness studying coastal waters. Contrarily, Chu *et al.* (2010) did not find the same pattern in soil bacteria communities studied with barcoded pyrosequencing technique and argued that the controls on the bacterial communities would be different from those observed for macroorganisms. In light of these discrepancies, further studies on bacterial diversity using other techniques will be necessary.

In agreement with the results obtained by Pommier *et al.* (2007) and Fuhrman *et al.* (2008) for marine bacterioplankton, in our study, DGGE band number correlated with temperature but not with chl *a* concentration, suggesting that temperature was more influential than productivity in determining the latitudinal pattern.

We also found that OTU number was directly correlated with lake area, in agreement with other reports (Horner-Devine *et al.*, 2004; Bell *et al.*, 2005; van der Gast *et al.*, 2005; Reche *et al.*, 2005). Nevertheless, other studies did not find this relationship (Lindström & Leskinen, 2002; Zwart *et al.*, 2002). Thus, even though some authors have linked this positive relationship with Island-Biogeography Theory (i.e. that larger islands support more species than smaller islands; MacArthur & Wilson, 1967), such assumptions should be treated with caution because of the limitations of the molecular technique used (Lindström *et al.*, 2007). However, even if DGGE band numbers do not reflect the real richness of a community, a different number of DGGE bands could reflect a difference in rank-abundance of populations (i.e. in the number of populations above the detection threshold) and a significant correlation between DGGE band numbers and lake surface area might still have some ecological meaning.

We observed significant differences in bacterioplankton composition between Antarctic and Patagonian lakes, even though 45 OTUs were shared between these two continents. It has been postulated that despite the isolation of Antarctic and sub-Antarctic habitats, microbes can be transported aerially to the Antarctic Peninsula probably from South America or other Antarctic locations (Marshall, 1996; Hughes *et al.*, 2004). Nevertheless, significant differences in BCC were still found, probably due to the low temperatures and climatic severity of the Antarctic continent.

The previously discussed patterns were found using a fingerprinting method that allows comparison of lakes on the basis of the more abundant populations, since this method does not detect genotypes

that individually might constitute <1% of the individuals in a given sample (Muyzer *et al.*, 1993; Murray, Hollibaugh & Orrego, 1996). Nevertheless, this methodological limitation will not affect the general observed patterns because we focussed on conservative criteria and we dealt with the most abundant bacterial groups detected. In line with this idea, in a study of the spatial patterns of bacterial communities in the NW Mediterranean Sea, Pommier *et al.* (2010) observed the same clustering of samples using the 300 most abundant OTUs than with a complete dataset retrieved by pyrosequencing, suggesting that fingerprinting approaches were sufficient to cluster the samples appropriately despite the fact that they could not retrieve most of the richness in the samples. With DGGE technique, we expected to retrieve the dominant organisms that are developing in a lake at a given moment and we did not expect to obtain bacterial ribotypes that might have arrived to that place by stochastic events. Some species are present over time with high abundances and are likely responsible for most ecosystem function (Pedrós-Alió, 2006). Magurran & Henderson (2003) called these 'core' species. Those species below the abundance detection thresholds of molecular techniques can be considered 'occasional' species that form a seed bank of rare species that grow slowly or not at all or may have sporadic appearance. Nevertheless, although the molecular technique performed is adequate to retrieve the dominant bacterial taxa in each lake, we should be awarded of possible temporal changes in the bacterial assemblages, since our study was based on a large regional scale survey where samples were obtained in one occasion. In spite of this, a previous study carried out in some Antarctic lakes that were included in this work showed that the temporal variations (monthly and interannual) of BBC in a given lake were less important than the differences among lakes because of their trophic conditions (Schiaffino *et al.*, 2009).

The lakes we studied exhibit contrasting features in relation to their trophic status, which account for differences in bacterioplankton and picocyanobacteria abundances. These microbial groups increased from oligotrophic to eutrophic water bodies, being consistent with generally accepted principles of microbial ecology (Gasol & Duarte, 2000). In this work, we maximised the range of latitude as much as possible, but also maximised the variability in ecological regimes within the freshwater bodies, including as much variation (e.g. lake size, trophic status, altitude) as existing in the area. Whether the signal of environmental constraints on community composition can be detected may depend on the heterogeneity of ecosystems selected and the magnitude of change in environmental factors included in the analysis (Sommaruga & Casamayor, 2009). With pooling ecosystems of very similar characteristics in an analysis, there is a risk of asserting only the significance of the spatial or regional signal (Dolan, 2006).

Another interesting finding of our work is that approximately half (54%) of the obtained sequences had their closest matches with bacteria detected in other similar cold habitats, not only from Antarctica, but also from elsewhere in the world. This finding is consistent with those observed in previous surveys of bacterioplankton from Antarctic (Schiaffino *et al.*, 2009) and alpine lakes (Sommaruga & Casamayor, 2009), in which 63 and 81%, respectively, of the sequenced OTUs matched sequences and clones found in polar regions or in cold habitats elsewhere. Hughes *et al.* (2004) found similar results when investigating sequences obtained from Antarctic Peninsula air samples. We only remark here a similarity among these sequences, but to be completely certain that phylotypes in different cold regions are exactly the same organisms, it will be necessary to compare the entire genome, because even identical 16S rRNA gene sequences may not necessarily correspond to identical genotypes and even less so to ecotypes (Hahn & Pöckl, 2005). As Sommaruga & Casamayor (2009) have discussed, one may wonder whether this is just a coincidence resulting from a biased comparison of genetical identity or the result of bacterial dispersal and successful colonisation of a similar suitable environment in different parts of the world.

Our results show the existence of phylotypes that are found in similar environment elsewhere on earth but also show the presence of apparently endemic phylotypes. However, it is important to remember that a species only recorded in one place may inhabit another part of the world but below a threshold of detection or may inhabit a place that has not been sampled yet. Among the sequenced OTUs, some bacteria have only been retrieved, so far, from Antarctic lakes (e.g. *Polaromonas vacuolata*, *Flavobacterium weaverense*, *Sphingobacterium antarcticum*), suggesting that they are endemic to this continent and one of the sequences (sequence number 2) from a Patagonian pond showed

a very low similarity percentage (93%) with other sequences from GenBank, suggesting endemism. It seems that it is possible to find not only ubiquitous ribotypes but also endemic ones, and that this will depend on the dispersal ability and on the biological characteristics of each species. Pommier *et al.* (2007) found that the global marine bacterioplankton community showed a high degree of endemism and also included some cosmopolitan OTUs. Whereas some microbial taxa might disperse globally, others will only disperse over very short distances (the propagules must survive the conditions encountered during dispersion and colonisation), creating non-random distributions of microbial assemblages (Martiny *et al.*, 2006). Some recent studies suggest that dispersal ability is probably not high for all bacteria (Papke & Ward, 2004; Hervàs *et al.*, 2009). The long-standing hypothesis 'everything is everywhere, but the environment selects' (Baas-Becking, 1934) is limited in the literal sense because severe environments can eliminate even bacterial resting stages, and recent studies also challenge this hypothesis by identifying geographic isolation in some microbial taxa (Crump *et al.*, 2007). Moreover, not all microorganisms produce spores, cysts and resting propagules that can withstand long range dispersal (Martiny *et al.*, 2006), and different bacterioplankton taxa might differ in their functional plasticity and the ease of their live dispersal (Logue & Lindström, 2008).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Phylogenetic affiliation, following BLAST searches, of the sequences retrieved from the denaturing gradient gel electrophoresis bands in Fig. 2.

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