environmental microbiology

Environmental Microbiology (2016) **00**(00), 00–00 doi:10.1111/1462-2920.13566

Microbial eukaryote communities exhibit robust biogeographical patterns along a gradient of Patagonian and Antarctic lakes

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Summary

Microbial eukaryotes play important roles in aquatic ecosystem functioning. Unravelling their distribution patterns and biogeography provides important baseline information to infer the underlying mechanisms that regulate the biodiversity and complexity of ecosystems. We studied the distribution patterns and factors driving diversity gradients in microeukaryote communities (total, abundant, uncommon and rare community composition) along a latitudinal gradient of lakes distributed from Argentinean Patagonia to Maritime Antarctica using both denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing (Illumina HiSeq). DGGE and abundant Illumina operational taxonomic units (OTUs) showed both decreasing richness with latitude and significant differences between Patagonian and Antarctic lakes communities. In contrast, total richness did not change significantly across the latitudinal gradient, although evenness and diversity indices were significantly higher in Patagonian lakes. Beta-diversity was characterized by a high species turnover, influenced by both environmental and geographical descriptors, although this pattern faded in the rare community. Our results suggest the co-existence of a 'core biosphere' containing reduced number of abundant/dominant OTUs on which classical ecological rules apply, together with a much larger seedbank of rare OTUs driven by stochastic and reduced dispersal processes. These findings shed new light on the biogeographical patterns and forces structuring inland microeukaryote composition across broad spatial scales.

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Introduction

Phototrophic and heterotrophic small eukaryotes of picoplanktonic, nanoplanktonic and microplanktonic size (i.e. microbial eukaryotes with a size ranging between 0.2 and $<$20 μm) are abundant in the upper photic zone of all lakes and oceans (Li *et al.*, 1994; Caron *et al.*, 1999; Foissner, 1999) and play important roles in the biogeochemical cycles (e.g. Pomeroy, 1974; Azam *et al.*, 1983; Stockner and Antia, 1986; Massana, 2011). The introduction of molecular techniques into microbial ecology has greatly increased our knowledge on microbial eukaryotes diversity (e.g. Epstein and López-García, 2008). Fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE), allow treating a relatively higher number of samples (van Hannen *et al.*, 1998), but provide limited information on the taxonomical composition of microbial communities and retrieve only the abundant or dominant

taxa, the so called 'core species' (Magurran and Henderson, 2003). In the last years, the introduction of novel high-throughput sequencing technologies permitted reaching saturation of microbial diversity, including the rarest taxa (the so-called 'rare biosphere', e.g. Sogin *et al*., 2006; Dawson and Hagen, 2009; Monchy *et al*., 2011; Lepère *et al*., 2013; Taib *et al*., 2013; Logares *et al*., 2014).

Diversity is considered a key property of ecosystems, necessary to understand the structure, functioning and dynamics of communities (e.g. MacArthur and Wilson, 1967; Magurran, 2004). It comprises two basic components, taxa richness (number of taxa in a community) and evenness (a measure of how similar taxa are in their abundances) (Magurran, 2004). Weak relationships between these two components in many aquatic datasets reflect independent components of diversity, suggesting that richness alone may be an incomplete surrogate for biodiversity (Soininen *et al*., 2012). Thus, richness and evenness should be treated separately (e.g. Hurlbert, 1971; Magurran, 1988; Legendre and Legendre, 1998) because both provide meaningful insights into community function (McNaughton, 1977) and may respond independently to different ecological processes (Ma, 2005). Beta-diversity (Whittaker, 1960) is usually applied in a broad sense to any measure of variation in taxa composition between sites (Anderson *et al*., 2011). As suggested by Baselga (2010; 2012), it can be partitioned into two components: turnover (the replacement of some taxa by others from site to site) and nestedness (the pattern characterized by the poorest site being a strict subset of the richest site, without replacement of taxa).

Ecological communities are also characterized by a few species exceptionally abundant, while the majority often remains remarkably rare (Fisher *et al*., 1943; Preston, 1948; Magurran, 2004). Therefore, communities can be separated into two components: 'core species', which are persistent and abundant, and 'rare or occasional species', which occur infrequently in the record and are typically low in abundance (Magurran and Henderson, 2003). This pattern is repeated across taxonomic groups (Magurran, 2004), including planktonic microorganisms (e.g. Pedrós-Alió, 2006; Mangot *et al*., 2013). These two components are not independent from each other, and rare taxa can be promoted from the 'rare biosphere' to the 'core biosphere' if conditions alter sufficiently and become adequate for the growth of a particular taxon. Likewise, a member of the 'core biosphere' can be grazed down or reduced by viral lysis below the threshold level to become a member of the 'rare biosphere' without disappearing from the ecosystem (Pedrós-Alió, 2006).

Recent studies have aimed to characterize the patterns of this 'rare biosphere' in ecosystems (Lepère *et al*., 2013; Logares *et al*., 2014). According to the 'seed bank theory' and as mentioned before, the 'rare biosphere' is supposed to play the role of a large inactive seed bank, dormant most of the time (e.g. as cysts or spores), and awaiting adequate conditions to develop (Lennon and Jones, 2011; Gibbons *et al*., 2013). Previous studies in freshwater seasonal variation (Lara *et al*., 2011; Simon *et al*., 2015) suggested that members of the eukaryotic community may enter some way of dormancy and constitute a 'seed bank' that participates to plankton community resilience over time. Furthermore, Simon *et al*. (2016) recently observed that the capacity to enter dormancy via the generation of resting forms (e.g., cysts, spores or other resistant metabolic stages) is the major explanation for the resilience of microbial communities observed in shallow freshwater ecosystems undergoing frequent droughts. Since a dormant microbial cell can survive for a long time in sub-optimal environments and can be transported passively over large distances in the meantime, some researchers argued that microbial species would have a cosmopolitan distribution (Finlay, 2002). The implications of these assumptions are twofold. First, it would mean that the large spatial diversity patterns observed in the 'rare biosphere' are independent of the forces that drive current diversity patterns in other life-forms (e.g. plants and animals), such as environmental factors and isolation by distance. Second, it would mean that beta-diversity patterns (i.e. variation in OTU composition among sites) will tend to be null. This globally more or less homogeneous rare and inactive biosphere would then bring only redundant information between different high-throughput sequencing surveys, and early molecular techniques such as DGGE would suffice to describe the biologically relevant part of diversity. Thus, if it were true that all microbes are easily dispersed globally, the long tail of the distribution of species abundances in any ecosystem would include all of the microorganisms on Earth, and the biodiversity of any ecosystem would be identical to the biodiversity on the planet (Pedrós-Alió, 2006). However, recent studies do not support these assumptions. It has been shown in freshwater (Debroas *et al*., 2015) and marine (Logares *et al*., 2014) systems that at least some of the rare microorganisms are active, and that microbial eukaryotes from high-mountain lakes are not globally distributed (e.g. Filker, *et al*., 2016). In addition, studies focusing on marine prokaryotes showed that rare OTUs followed similar distribution patterns to those of the most abundant members of the community and of the entire community (Galand *et al*., 2009; Logares *et al*., 2013). Similar patterns have been also observed for marine and freshwater microbial eukaryotes (Lepère *et al*., 2013; Logares *et al*., 2014).

The profound influence of microorganisms on human life and global biogeochemical cycles underlines the value of studying the biogeography of microorganisms (Lynch and Neufeld, 2015). The most common pattern in biogeographical studies is the decrease of richness with

Table 1. Comparisons between cell abundances and diversity properties of microbial eukaryotes from Patagonian and Antarctic water bodies.

			Patagonian lakes	Antarctic lakes
Epifluorescence	Autotrophic abundance	$n = 40$	$7.7 \times 10^{4***}$	$5.4 \times 10^{3***}$
	Heterotrophic abundance		$1.1 \times 10^{4**}$	$2.2 \times 10^{3**}$
DGGE	Dominant OTU richness	$n = 40$	21*	18*
	Dominant OTU richness	$n = 14$	20	18
Illumina HiSeq	Total OTU richness	$n = 14$	347	313
	Chao 1		539	493
	Abundant OTUs $\geq$1% richness		19**	10**
	Uncommon OTUs $>$0.1 to $<$1% richness		52	38
	Rare OTUs $\leq$0.1% richness		276	265
	Simpson Index (D)		13.4*	6.7*
	Shannon Index (H')		3.4*	2.6*
	Evenness (E_D)		0.041	0.022
	Evenness ($E_{H'}$)		0.584*	0.445*

Average per lake, $n = 40$ (28 Patagonian and 12 Antarctic water bodies) and $n = 14$ (8 Patagonian and 6 Antarctic water bodies).
Student's t-test $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

increasing latitude, although this pattern may depend on the spatial scale and the level of taxonomic resolution (e.g. Willig *et al.*, 2003; Hillebrand, 2004). However, there are no clear geographical patterns in relation to evenness and contrasting patterns have been reported (e.g. Willig *et al.*, 2003). South American and Antarctic lakes are propitious systems to study the diversity distribution patterns in microorganisms. Indeed, these freshwater systems can be found along a significant latitudinal gradient that exhibits relatively benign (low latitudes) to extremely harsh (higher latitudes) environmental conditions, as well as important barriers to dispersal (e.g. the Magellan Strait and the Drake Passage), that allow not only assessing their diversity patterns but also the importance of environmental and geographical factors in determining these patterns. Previous studies on microbial diversity patterns across Patagonian and Antarctic lakes have found a decreasing latitudinal diversity gradient for diatoms (Maidana *et al.*, 2005), Chlorococcales (Tell *et al.*, 2011), phytoplankton (Izaguirre *et al.*, 2016), and bacterioplankton (Schiaffino *et al.*, 2011).

Here, we have used both traditional fingerprinting (DGGE) and high-throughput sequencing technology (Illumina HiSeq) methods to study the patterns and drivers of latitudinal diversity gradients in total, abundant, uncommon and rare microbial eukaryote community along a gradient of freshwater environments (Fig. 1) stretching from Argentinean Patagonia (45° S) to Maritime Antarctica (63° S). We used DGGE, which focused on the dominant taxa, on the complete dataset (40 lakes), while the Illumina HiSeq approach that allowed reaching complete taxonomical reports was applied on a subset of samples (14 lakes). The use of both approaches provided a unique opportunity to compare their performance and conclusions. For both datasets, we hypothesised that OTU richness and diversity follow a decreasing pattern with increasing latitude, environmental harshness and geographical distance, just as it has been repeatedly shown for several other taxa (Brown and Lomolino, 1998; Gaston, 2000). In addition, given that environments from lower latitudes are less extreme and more productive than those from higher latitudes (Hawkins *et al.*, 2003; Currie *et al.*, 2004; Chase and Ryberg, 2004), we proposed that there are a higher proportion of dominant OTUs and evenness in lower latitudes than in higher latitudes. Furthermore, important changes in community compositions are assumed between Patagonian and Antarctic lakes due to environmental and geographical factors, which will be reflected by beta-diversity. Alternatively, we postulated that beta-diversity patterns of the rare biosphere are independent of the environmental and/or geographical distances, giving support to the 'seed bank theory'.

Results

Total abundances

In most water bodies, the abundance of autotrophic eukaryotes was greater than the abundance of heterotrophic ones (both $\leq$5 μm in diameter, Table 1), and both showed the same behaviour with respect to the trophic and latitudinal gradients (Table 2). Patagonian water bodies presented significantly higher autotrophic and heterotrophic eukaryotes abundances than Antarctic ones (Table 1). In addition, the abundance of autotrophic and heterotrophic eukaryotes decreased significantly towards higher latitudes and accordingly increased significantly with temperature and higher chl *a* (Table 2).

DGGE analysis

The DGGE gels performed for 40 lakes yielded a total of 819 bands located in 90 different positions (total dominant

Table 2. Spearman Rho correlations between microbial eukaryote properties (abundance, richness and diversity) and some environmental variables from all the studied water bodies.

		Lake area	Latitude	Temperature	Chlorophyll a
Epifluorescence ($n = 40$)	Autotrophic abundance	0.16	**−0.61****	**0.67****	**0.42***
	Heterotrophic abundance	−0.31	**−0.46***	**0.56****	**0.47***
DGGE	Dominant OTU richness ($n = 40$)	0.11	**−0.39**	0.14	**0.34**
	Dominant OTU richness ($n = 14$)	0.08	−0.32	0.15	**0.59**
Illumina HiSeq ($n = 14$)	Total OTU richness	−0.04	−0.01	0.10	−0.10
	Chao 1	−0.02	0.01	0.10	−0.29
	Abundant OTUs ≥1% richness	**0.74***	**−0.74***	**0.58**	−0.22
	Uncommon OTUs >0.1 to <1% richness	0.08	−0.18	0.22	0.19
	Rare OTUs ≤0.1% richness	−0.15	0.13	0.07	−0.20
	Simpson Index	0.48	−0.39	0.21	0.01
	Shannon Index	0.41	−0.39	0.30	0.05
	Evenness (E_D)	0.47	−0.29	0.10	−0.06
	Evenness ($E_{H'}$)	**0.54**	**−0.53**	0.40	0.02

$n = 40$ for DGGE and epifluorescence microscopy counts, and $n = 14$ for Illumina HiSeq reads and DGGE data.
Bold values represent $P < 0.05$, *$P < 0.01$, **$P < 0.0001$.

richness): 24 positions (27%) were exclusive in Patagonian lakes and 3 (3%) in Antarctic lakes, whereas 63 positions (70%) were shared between Patagonian and Antarctic water bodies. We successfully sequenced 26 different OTUs (representing 40% of the total intensity of the gels). Bands with the same position were sequenced from different lakes to confirm that they corresponded to the same sequence: e.g. bands 3 (a, b), 7 (a, b) and 17 (a, b) (Fig. 2). We found members of five taxonomic groups: 10 (38%) Stramenopiles (8 Chrysophyceae-Synurophyceae, 1 Bicosoecida, 1 Dictyochophyceae), 10 (38%) Alveolata (1 Alveolata, 8 Ciliophora and 1 Apicomplexa), 3 (12%) Archaeplastida (Chlorophyta), 2 (8%) Fungi and 1 (4%) Telonemida (Supporting Information Table S1). Some sequences showed their closest match and phylogenetic affiliation (between 98 and 100% of similarity) with well-described species of small phytoplankton (generally smaller than 5 μm) *Vitreochlamys incisa* (band 15), *Pyramimonas australis* str. (band 16), *Ostreococcus tauri* (band 26). Other sequences matched with OTUs belonging to taxonomic groups represented by larger cell sizes (e.g. Alveolata), possibly due to the inefficient sequential filtration (Supporting Information Table S1).

Illumina HiSeq analysis

From a total of 5 552 925 paired-end sequences, 3 670 418 passed the quality check. The total richness across all studied lakes was 1 505 different OTUs and ranged from 147 to 475 OTUs in each individual water body (332 ± 99 OTUs on average). Rarefaction curves performed for every water body showed that OTUs richness tended to reach a plateau, indicating that the sampling depth and sequencing coverage were good (Fig. 3). In general, Stramenopiles and Alveolata dominated diversity in the entire study (Fig. 4). Taxonomic composition showed clear differences between Patagonian and Antarctic water bodies: Cryptophyta were abundant in Patagonia but absent from Antarctic lakes, whereas Chrysophyta dominated in Antarctic lakes (Fig. 4). Ciliophora showed higher relative abundances and were more frequent in Patagonian than in Antarctic lakes, except in the Antarctic Pingüi Pond (Fig.

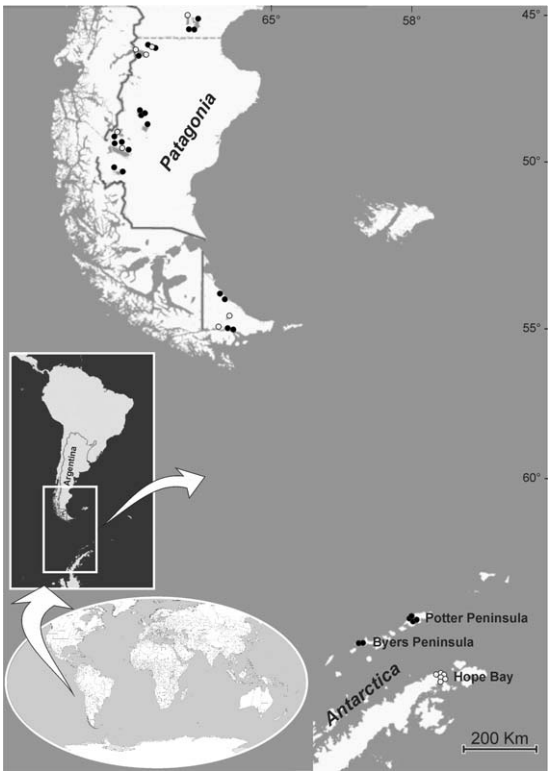

Fig. 1. Map showing the 40 locations of the studied water bodies. All sites were studied with DGGE, while those that also were studied with Illumina HiSeq (14 lakes) are shown with white circles.

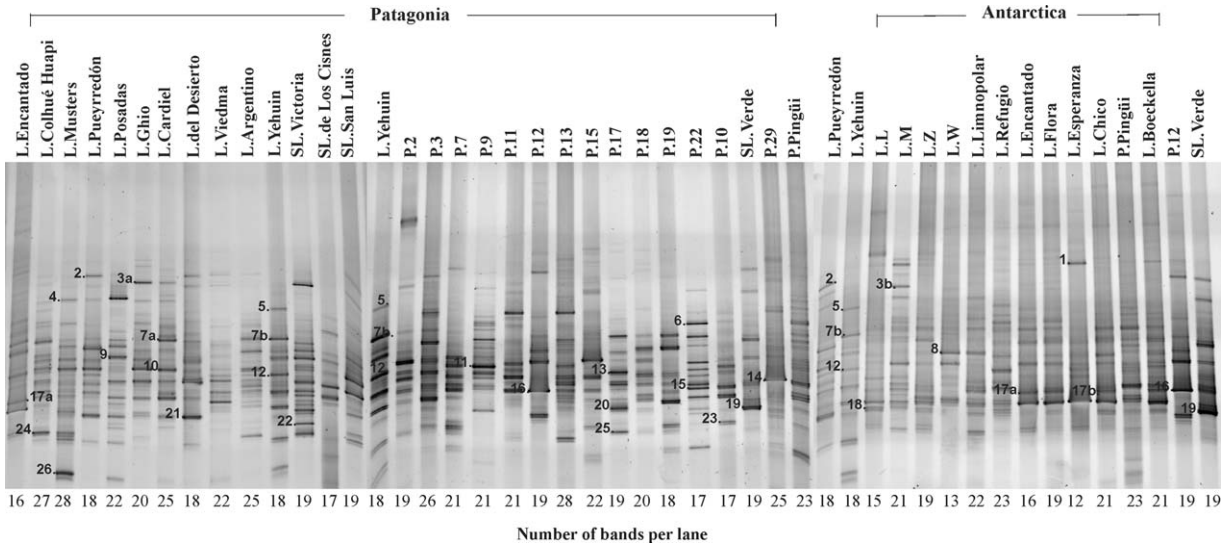

Fig. 2. Image of the DGGE gels run for 40 water bodies. Numbers in the gels indicate excised and sequenced bands. L.: lake, SL.: shallow lake, P.: pond.

4). This system has a large contribution of organic matter from penguins and represents an outlier in our dataset.

Globally, the 'uncommon and rare biosphere' presented a high percentage (92%) of exclusively uncommon or rare OTUs, whereas the 'core biosphere' showed a low percentage (4%) of exclusively dominant/abundant OTUs. Therefore, from the total 125 'core OTUs' found in 14 lakes, 120 (96%) were dominant in some lakes but also rare in other lakes, whereas only 5 (4%) were not found as rare in other lakes. In addition, 67% of these 125 OTUs were dominant just in one lake and 33% were dominant in two or more lakes (up to 6) lakes, and none of these OTUs was dominant in all studied lakes. On the other hand, from the total 1 500 OTUs found in the 'uncommon and rare biosphere' (OTUs $\leq$1%), 1 380 OTUS (92%) were always uncommon and/or rare (exclusive) while 120 of these OTUs (8%) were also found in the 'core biosphere' in other lakes. In all samples, we found a small number (around 8%) of abundant OTUs ($\geq$1%), while most OTUs (around 92%) were rare (OTUs $\leq$0.1%). Patagonian lakes showed significantly higher numbers of dominant OTUs ($\geq$1%) than Antarctic ones, whereas Antarctic lakes showed significantly higher numbers of rare OTUs ($\leq$0.1%) than Patagonian lakes (Fig. 5). Comparisons among each abundance category (total Illumina matrix, OTUs $\geq$1%, OTUs $>$0.1% to $<$1% and OTUs $\leq$0.1%) for all Patagonian and Antarctic water bodies are shown in Table 3. Patagonian lakes showed between 43 and 73% of exclusive OTUs, while Antarctic lakes showed between 24 and 33%. Patagonian and Antarctic lakes shared between 2 and

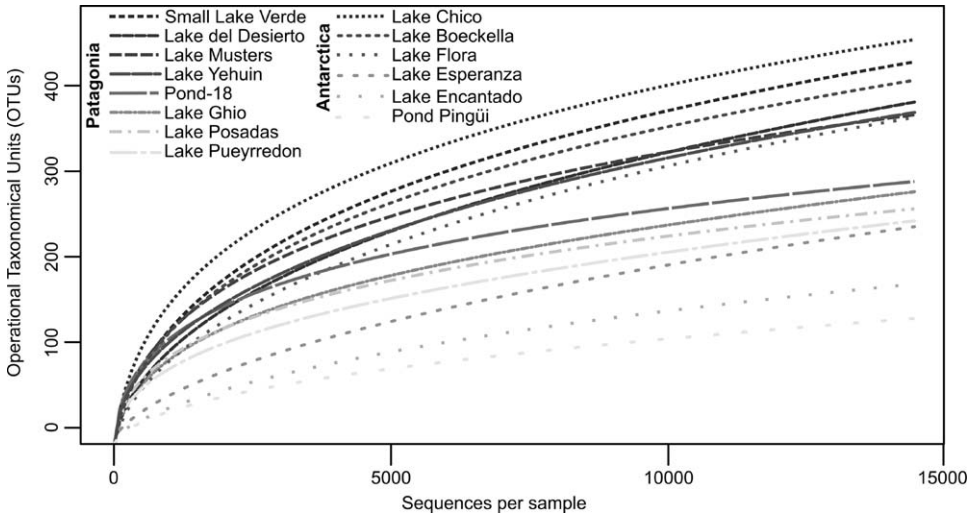

Fig. 3. Rarefaction curves for each water body using Illumina HiSeq dataset.

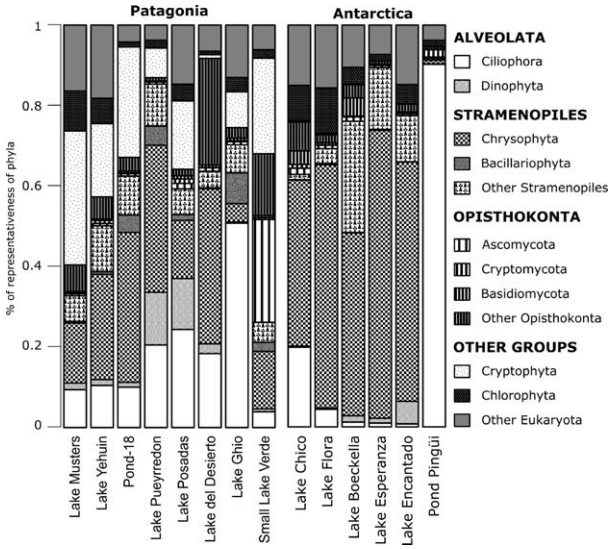

Fig. 4. Relative composition of phyla from the 14 water bodies studied with Illumina HiSeq.

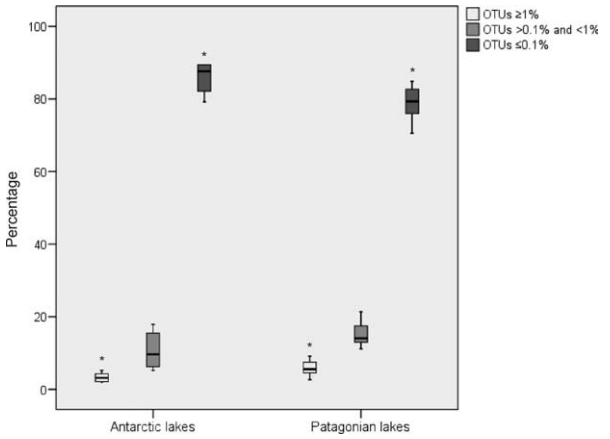

Fig. 5. Average percentage per lake of the abundant, uncommon and rare OTUs in Patagonian and Antarctic lakes. Illumina community matrix was studied considering the abundant OTUs ≥1% of the total number of sequences in a given sample and the less frequent OTUs with two different criteria: uncommon OTUs >0.1% to <1% and rare OTUs ≤0.1% of the total number of sequences in a given sample. *Student's *t*-test $P < 0.05$ for comparing each abundance category (OTUs ≥1%, OTUs >0.1% to <1% and OTUs ≤0.1%) between Patagonian and Antarctic lakes.

32% OTUs. For instance, the abundant OTUs community composition (≥1%) showed a total of 125 OTUs and only 2 OTUs belonging to Chrysophyceae were equally dominant in both Patagonian and Antarctic water bodies. All other abundant OTUs (123 OTUs) could dominate either in Antarctic (32) or in Patagonian (91) lakes, but never in both. Contrarily, the rare community composition (≤0.1%) showed a total of 1438 OTUs. From these OTUs, 461 were present in both Patagonian and Antarctic lakes, whereas 977 OTUs could only be present in Antarctic (359) or in Patagonian (618) lakes. This abundance category showed that only 3 OTUs (related to Fungi) were present in all water bodies.

To compare the responses of the individual diversity components (richness and evenness) we studied the relationship between them. No correlations between richness and evenness were found ($r = -0.07$ for E_D and $r = 0.39$ for E_H, both $P > 0.05$ and $n = 14$). Similar lack of relationship was found when studying the Patagonian lakes ($r = -0.41$ for E_D and $r = -0.27$ for E_H, both $P > 0.05$ and $n = 8$), or the Antarctic lakes separately ($r = 0.26$ for E_D and $r = 0.77$ for E_H, both $P > 0.05$ $n = 6$).

General patterns and comparisons

The abundant part of Illumina reads and the number of DGGE bands were significantly higher in Patagonian water bodies than in Antarctic ones (Table 1), and also decreased significantly with higher latitudes (Table 2). On the other hand, the total richness (as shown by Illumina

Table 3. Total, exclusive and shared number of sequence reads (Illumina HiSeq) for all Patagonian and Antarctic water bodies.

	Total OTUs	Shared OTUs	Exclusive OTUs	OTUs present in all water bodies	OTUs present in one water body
			Total matrix		
Patagonian lakes	1 505	486	663	24	445
Antarctic lakes			356		
			Abundant OTUs ≥1%		
Patagonian lakes	125	2	91	0	84
Antarctic lakes			32		
			Uncommon OTUs >0.1% to <1%		
Patagonian lakes	422	24	257	1	295
Antarctic lakes			141		
			Rare OTUs ≤0.1%		
Patagonian lakes	1 438	461	618	3	506
Antarctic lakes			359		

Total OTU richness from 14 water bodies: 8 Patagonian and 6 Antarctic ones.

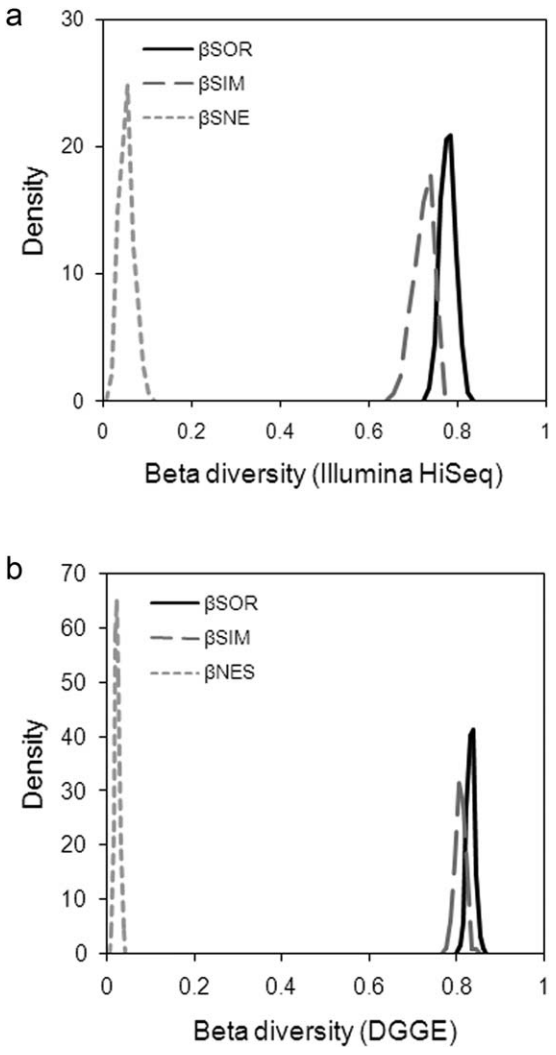

Fig. 6. Mechanisms driving variation in OTU composition in Patagonian-Antarctic lakes. The plots show the distribution of dissimilarity values due to overall beta-diversity (β_{SOR}), turnover (β_{SIM}) and nestedness (β_{NES}) based on (a) Illumina HiSeq and (b) DGGE data sets (a) Illumina HiSeq and (b) DGGE data sets, both n = 14 water bodies. Kernel density curves were constructed by re-sampling four lakes from each area 1000 times and computing the average β_{SOR}, β_{SIM} and β_{NES} respectively.

data) did not differ between Patagonian and Antarctic lakes (Table 1) and neither correlated with latitude (Table 2). Similar results were obtained with the richness estimator Chao 1 index (Tables 1 and 2). Both OTU diversity indices and evenness ($E_{H'}$) were significantly higher in Patagonian water bodies than in Antarctic ones, whereas neither diversity indices nor evenness changed with latitude, except Pielou evenness index ($E_{H'}$) that decreased with higher latitudes (Tables 1 and 2).

Microbial eukaryote communities exhibited high values of beta-diversity either based on DGGE ($\beta_{SOR} = 0.94$, $P < 0.001$) or on Illumina ($\beta_{SOR} = 0.84$, $P < 0.001$) datasets. In both cases, variation in community composition was mainly due to OTUs turnover (DGGE $\beta_{SIM} = 0.93$ and Illumina $\beta_{SIM} = 0.86$, both $P < 0.05$) and barely due to nestedness (DGGE $\beta_{NES} = 0.01$ and Illumina $\beta_{NES} = 0.02$, both $P < 0.05$) (Fig. 6a and b).

Redundancy analyses (RDA) based on community composition versus environmental and geographical variables showed that in general Patagonian lakes ordinated apart from the Antarctic lakes (with some exceptions for the hypertrophic Antarctic Pingüi Pond and the Patagonian shallow lake Verde) when considering microbial communities from DGGE matrices ($n = 40$ and $n = 14$), total Illumina matrix and different Illumina abundance categories (abundant $\geq$1%, uncommon $>$0.1% to $<$1% and rare $\leq$0.1%) (Fig. 7). To study the main underlying driver of beta-diversity along the latitudinal gradient we applied variation partitioning and Mantel test (simple and partial) analyses (Table 4). RDA performed on each studied microbial eukaryote community matrix showed that forward selection retained the following variables as relevant: PCNM 1 and PCNM 3 (geographical variables), temperature, conductivity, DOC and lake area (environmental variables) (Table 4). Variation partitioning analyses showed that the amount of variation explained by each type of factor (geographical or environmental alone) varied with the molecular approach (DGGE or Illumina) and the community abundance category studied (Illumina data: abundant, uncommon or rare). Purely geographical and environmental factors explained similar and significant amount of variation on both DGGE ($n = 14$) and abundant Illumina microbial eukaryote communities. However, when moving to less abundant communities (i.e. uncommon or rare) the respective amount of explained variance and the significance faded; only purely geographical descriptors influenced significantly the rare communities (Table 4). In agreement with the results obtained with variance partitioning, Mantel tests showed that the similarity in total and abundant OTU community composition (both from Illumina reads) and in dominant OTU community composition (DGGE band pattern) decreased significantly when the distance among water bodies increased and grew up significantly when the water bodies showed more similar local environmental factors. However, the rare OTU community composition (OTUs $\leq$0.1%) only decreased significantly when the distance among water bodies increased and showed an independent effect of environmental parameters (Table 4). On the other hand, both tests did not show the same results with the total communities from Illumina. The Mantel approach showed a significant influence of geographical and environmental descriptors, whereas variation partitioning analysis showed only a higher and significant effect of geographical factors (Table 4).

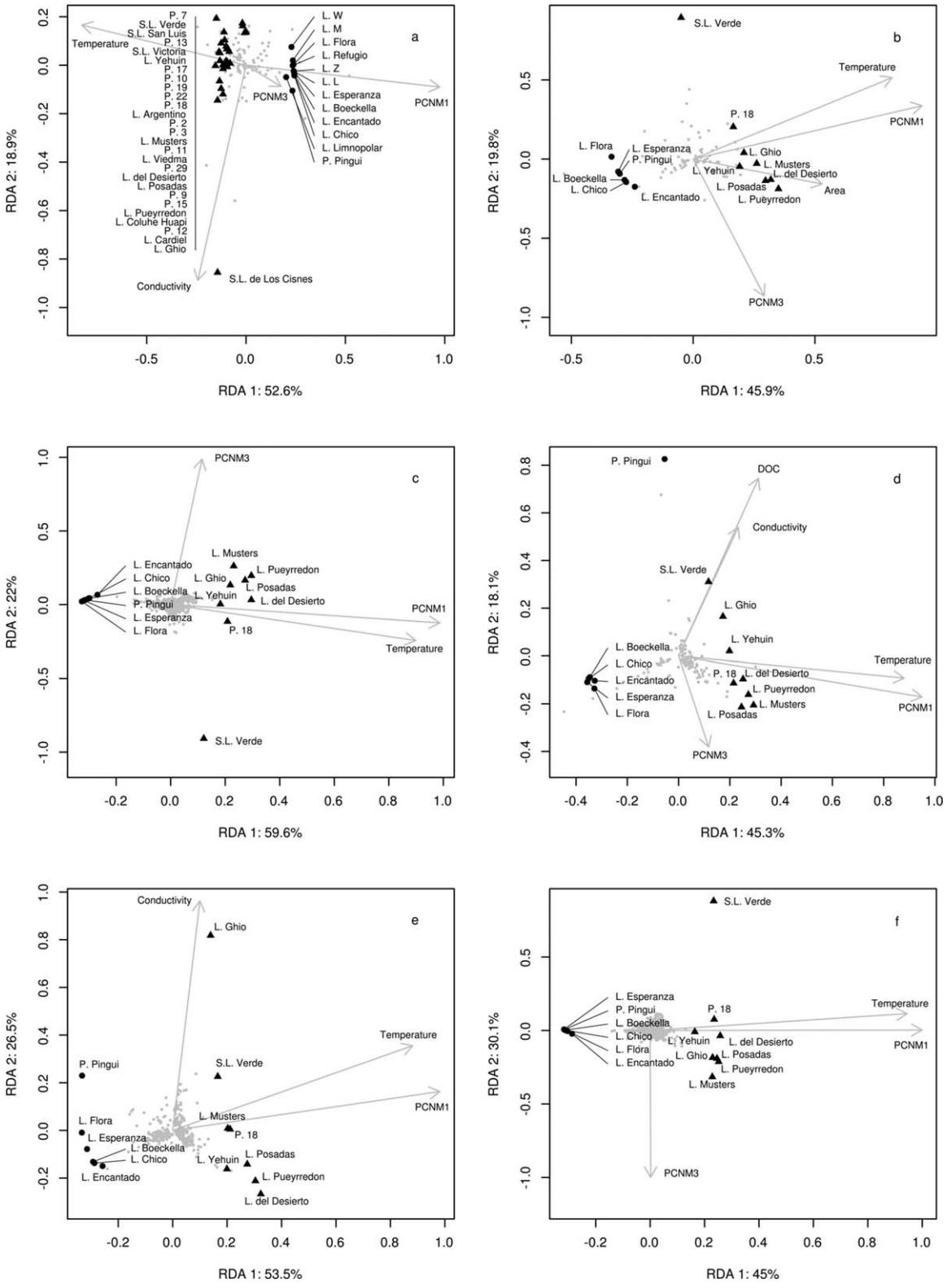

Fig. 7. Redundancy analyses using community composition versus environmental and geographical (PCNM) variables when considering microbial communities from DGGE matrices $n = 40$ (a), $n = 14$ (b), total Illumina matrix (c), abundant $\geq$1% OTUs (d), uncommon $>$0.1% to $<$1% OTUs (e) and rare $\leq$0.1% OTUs (f). Antarctic lakes are represented by circles and Patagonian lakes by triangles. DOC: dissolved organic carbon.

Table 4. Results of Mantel (simple and partial) and variation partitioning analyses to study the effect of geographical (PCNM) and environmental variables on microbial eukaryote community composition (based on DGGE band pattern and Illumina HiSeq reads).

		Mantel		Partial Mantel[a]		Variation partitioning			
Matrix type		*r*	*P*	*r*	*P*	Each factor (%)	Both factors (%)	Unexplained (%)	*Forward selection of variables*
DGGE ($n = 40$ lakes)	Geographical	**−0.26**	0.002	**−0.25**	0.002	**6**	5	82	PCNM1, PCNM3
	Environmental	**0.15**	0.020	**0.14**	0.020	**7**			Temperature, conductivity
DGGE ($n = 14$ lakes)	Geographical	**−0.56**	0.002	**−0.42**	0.002	**8**	8	78	PCNM1, PCNM3
	Environmental	**0.47**	0.002	**0.26**	0.020	**6**			Temperature, lake area
Illumina total OTUs	Geographical	**−0.67**	0.003	**−0.42**	0.003	**9**	17	72	PCNM1, PCNM3,
	Environmental	**0.76**	0.002	**0.60**	0.003	2			Temperature
Illumina abundant	Geographical	**−0.64**	0.002	**−0.61**	0.002	**6**	20	66	PCNM1, PCNM3, DOC,
OTUs ≥1%	Environmental	**0.57**	0.002	**0.54**	0.002	**8**			Temperature, conductivity
Illumina uncommon	Geographical	**−0.71**	0.002	**−0.61**	0.002	**5**	17	73	PCNM1, temperature,
OTUs >0.1 to <1%	Environmental	**0.60**	0.002	**0.43**	0.002	**5**			Conductivity
Illumina rare	Geographical	**−0.71**	0.002	**−0.63**	0.002	**9**	17	72	PCNM1, PCNM3
OTUs ≤0.1%	Environmental	**0.43**	0.004	0.16	0.106	2			Temperature

DOC: dissolved organic carbon.
a. The Partial Mantel test holds geographical or environmental matrix constant. Bold values are significant data ($P < 0.05$).

Discussion

Our results show that microbial eukaryote total richness did not change with latitude (Table 2) and did not differ significantly between Patagonian and Antarctic lakes (Table 1). This contrasts with the prevailing general view of decreasing richness with latitude (Pianka, 1966; Gaston and Blackburn, 2000; Hillebrand, 2004) also observed in soil protists (Lara *et al.*, 2016), although there are a number of exceptions for aquatic (e.g. Crow, 1993; Passy, 2010) and soil (Fernández *et al.*, 2016) eukaryotic microorganisms. Therefore, the decreasing latitudinal gradient in taxa richness often reported for larger organisms (Rosenzweig, 1995) is not straightforward for microbial freshwater eukaryotes taken as a whole. Our data are consistent with the report of Hillebrand and Azovsky (2001), which showed that the effect of latitudinal diversity gradient decreases with decreasing body size of the taxa under study and almost disappears for protists (though this paper was based on an incomplete taxonomic sampling for protists). However, when only the abundant fraction of OTUs was considered (from Illumina and DGGE), we observed both a decrease in richness with latitude (Table 2) and significant differences between Patagonian and Antarctic lakes (Table 1). Accordingly, cell abundances (both auto- and heterotrophic) decreased also with latitude and were significantly higher in Patagonia than in Antarctica, probably corresponding to the abundant part of diversity (Tables 1 and 2). Similar decreases with latitude of cell abundances and richness of the dominant taxa were previously observed for bacterioplankton (Schiaffino *et al.*, 2011) and phytoplankton (Izaguirre *et al.*, 2016) in the studied lakes, and for autotrophic picoplankton in the Atlantic Southern Ocean (Doolittle *et al.*, 2008).

The diversity and evenness indices of the whole community did not correlate with latitude (except evenness Pielou index $E_{H'}$ that correlated negatively with latitude, Table 2), whereas both were significantly higher in Patagonian lakes than in Antarctic ones (except E_D, Table 1). As mentioned before, diversity has two basic components, richness (number of OTUs in a community) and evenness (how similar are OTUs in their abundances) (Magurran, 2004), which are combined in diversity indices (e.g. Shannon or Simpson indices). We found that total richness did not change in different latitudes, but as abundance was influenced by latitude (Tables 1 and 2), diversity changed significantly between Patagonian and Antarctic lakes. This pattern of diversity would be due to an increase in harsh climatic conditions with latitude, limiting the development of taxa having lower tolerance to climatic adversity. Protists in high-latitude lakes are constrained by cold temperatures, low inorganic nutrient supply and low light availability for much of the year due to ice cover and polar darkness (Rengefors *et al.*, 2012; Charvet *et al.*, 2014). Basically, many of the OTUs found in Antarctic systems could not reach the threshold to be abundant, likely because these ecophysiological constrains. These could also explain why Patagonian lakes showed more dominant OTUs, whereas Antarctic lakes showed more rare OTUs.

Many authors suggest that richness and evenness should be treated separately (e.g. Hurlbert, 1971; Magurran, 1988; Legendre and Legendre, 1998) because both provide meaningful insights into community function (McNaughton, 1977) and may respond independently to different ecological processes (Ma, 2005). Wilsey and Stirling (2007) proposed that the richness component is more sensitive to migration rates, while evenness is more

sensitive to biotic and environmental interactions. We found that evenness and richness were not correlated along the latitudinal gradient of lakes, suggesting that microbial eukaryote communities responded independently to different ecological factors. As no changes in total OTU richness were found in our dataset, but changes in evenness Pielou index ($E_{H'}$) along the latitudinal gradient of lakes emerged (Tables 1 and 2), we can suppose that microbial eukaryotes would be more regulated by biotic interactions and environmental stressors, than dispersal and migration. Accordingly, Soininen *et al*. (2012) stated that species richness and evenness may respond to different environmental factors or in a different way to a given factor, thus reflecting independent components of biodiversity.

We found a small number (around 8%) of highly abundant OTUs ($\geq$1%), representing the 'core biosphere', while most OTUs (around 92%) were less frequent (Fig. 5), the so called 'rare biosphere'. In line with our results, Nolte *et al*. (2010) showed that a small number of highly abundant protistan OTUs are present throughout the entire year, while most OTUs are rare and highly restricted. Similarly, Debroas *et al*. (2015) found that freshwater protists were represented by a high number (77%) of rare OTUs.

Interestingly, we observed differences in taxonomic composition between Patagonian and Antarctic water bodies, being Cryptophyta frequent in Patagonia and absent from Antarctic lakes, whereas Chrysophyta dominated in Antarctic lakes (Fig. 4). This result was also supported by microscopic observations (Izaguirre *et al*., 2016). Accordingly, Charvet *et al*. (2012; 2014) found that Arctic lakes often contain many OTUs related to Chrysophyta, suggesting they are well adapted to cope with the low nutrient supply and strong seasonality that characterize Polar Regions. Indeed, it has been shown that at least some heterotrophic strains are specifically adapted to the Antarctica (Boenigk *et al*., 2007).

Multiple-sites dissimilarity measures of variation in OTU composition conducted on Illumina and DGGE dataset revealed that there is a high and significant dissimilarity in the OTU composition between any pair of lakes taken at random from our study site. These analyses also revealed for both datasets that variation in OTU composition is characterized by turnover and to a much lower extent to nestedness (Fig. 6). Most OTUs are replaced among lakes while very few OTUs co-occur among lakes. These results give further support to those shown by the Mantel test and variation partitioning analyses and suggest that each lake may have a unique and little explored microbial eukaryote community. It is worth mentioning that, although a percentage of OTUs is shared between Patagonian and Antarctic lakes (i.e. around 32% of the total Illumina matrix and 70% from DGGE data), turnover remains as an important phenomenon in driving variation in OTUs composition between any pair of lakes (i.e. OTUs are replaced between lakes). Therefore, these values of shared OTUs between Patagonian and Antarctic lakes should not be confused with the amount of dissimilarity that exists between any pair of lakes in terms of their OTU composition. Indeed, an OTU only needs to be present in one Patagonian lake and in one Antarctic lake to be considered a shared OTU. Moreover, the number of shared OTUs is a descriptive approach that does not say anything about the phenomenon characterising beta-diversity patterns (turnover or nestedness) along the studied gradient.

Mantel test and variation partitioning analyses showed that beta-diversity changed significantly along geographical and environmental gradients (Fig. 4 and Table 4). The distance-based approach Mantel test (that provides an assessment of the significant interactions) and canonical variation partitioning (that allows quantification of the major sources of variation in the dependent variables using transformed raw data) are complementary approaches (Tuomisto and Ruokolainen, 2006). Particularly, these two approaches showed that environmental and geographical factors were important in shaping abundant/dominant microbial eukaryote communities, whereas only the geographical factors were important driving rare microeukaryote communities ($\leq$0.1%). These results point towards the existence of an abundant fraction of diversity ('core biosphere') ruled by standard macroecological rules, such as the latitudinal gradient of diversity and environmental filters. It coexists with a 'rare biosphere' that seems to be only weakly influenced by geographical position and not at all by environmental filters. Besides, variation partitioning showed that the percentage of variance explained in the communities decreased in the rarest fractions of diversity, suggesting higher randomness. The combined effect of environmental and geographical factors on the microbial community structure was also found by Cottenie (2005) and Lovejoy and Potvin (2011). Soininen *et al*. (2011) found that freshwater planktonic communities may be controlled by both dispersal-driven assembly and local ecological determinism, with a scale-dependant balance between these two forces. In addition, Lepère *et al*. (2013) and Logares *et al*. (2014) also found a highly significant effect of geographical distance on both rare and abundant/dominant freshwater and marine microbial eukaryotes respectively.

Direct observation of microbial eukaryotes confirmed that autotrophic and heterotrophic eukaryote abundances ($\leq$5 μm in diameter) not only showed a latitudinal gradient (Tables 1 and 2), but also their abundances increased with increasing chl *a* (Table 2). Similarly, Rochera *et al*. (2013) found that the abundance of autotrophic picoplankton from 12 lakes of Byers Peninsula increased along a trophic gradient.

It is well known that in all aquatic ecosystems, the sequential filtration process allows the passage of cells

larger than their nominal pore sizes and can lead to the retention of smaller cells if the filters are clogged (Díez *et al.*, 2001). Sørensen *et al.* (2013) found that the abundance of large protist (>10 μm as ciliate and dinoflagellate), often found among picoeukaryotes studies, correlated negatively with the pore size chosen for the end filter in the sequential filtration, suggesting that extracellular DNA adhering to small particles may be the source of larger protist OTUs in picoplanktonic size fractions. We observed also large ciliates by microscopy (such as *Stentor* sp.), which may have contracted and passed through the filters (Gabriela Küppers, personal communication). On the other hand, temporal samplings are important for adequate diversity and species richness estimates (Nolte *et al.*, 2010). It is likely that a broader seasonal sampling would have increased the taxonomic diversity even further and possibly defined better the 'core and rare biosphere'. However, given the considerable amount of sampled lakes and the relatively broad scale studied (2100 km) in our work, we decided to take an integrated water sample in each lake during the same season to make them comparable. Lepère *et al.* (2013) found that temporal variations in the composition of the small protist community do not affect the importance of geographical distance in explaining community composition, and they suggested that the use of a single sample per lake should be enough to analyse the spatial distribution of lacustrine protists.

Although the high-throughput sequencing approach brought obviously more information than DGGE on community composition, comparing both approaches has certain limitations. Indeed, the first PCR step used different primers sets amplifying, respectively, the V9 and V1-3 regions of the SSU rRNA. It has been shown that primers amplifying these regions in eukaryotes are not entirely universal, and therefore, biases may be observed in the respective community compositions (Hadziavdic *et al.*, 2014). Moreover, the taxonomic resolution of the different variable regions of the gene is not equivalent and varies from taxon to taxon (Hu *et al.*, 2015). Nevertheless, in spite of these important methodological differences, a similar amount of variance could be explained with both Illumina abundant OTUs (≥1%) and DGGE (Table 4), even though patterns observed in the RDA analyses showed some differences (Fig. 7b and d). In that sense, DGGE gave a picture of diversity comparable with the abundant fraction of the communities, and therefore does not lose its relevance as a fingerprinting technique (Pedrós-Alió, 2006). Our data is consistent with that of Pommier *et al.* (2010), who found that fingerprinting approaches such as DGGE clustered samples in a similar way than a high-throughput sequencing approach (in that case, pyrosequencing).

In summary, we found more abundant OTUs in Patagonian lakes (higher E_H evenness indices and higher dominant/abundant richness) than in Antarctic lakes, while total OTU richness did not change. Accordingly, the 'core biosphere' was significantly larger in Patagonian lakes than in Antarctic ones, while the 'rare biosphere' was larger in Antarctic lakes. In addition, the microbial eukaryote community composition (beta-diversity) changed significantly between Patagonian and Antarctic lakes and was mainly characterized by OTUs turnover. Both geographical position of the water bodies and the local environmental descriptors influenced beta-diversity (mainly abundant/dominant microbial eukaryote community composition), whereas only the geographical factors were important in shaping the rare OTU community composition. Thus, our results suggest that the latitudinal gradient becomes evident depending on the diversity component under evaluation (i.e. total richness, dominant richness, diversity indices and composition). These findings definitely shed new light on the biogeographical patterns and forces that structure inland microbial eukaryote composition across broad spatial scales. Growing information about microbial eukaryote diversity and distribution patterns makes protist the subject of active arguments over their ecology (Caron *et al.*, 2009). However, microbial eukaryotes comprise an immense array of different sizes and lifestyles, and, even though some general conclusions can be drawn, the study of individual taxa may lead to diverging conclusions. Altogether, patterns in the spatial distribution of organisms provide important baseline information about mechanisms that regulate the diversity of life and the complexity of ecosystems (Levin, 1992; Green *et al.*, 2004).

Experimental procedures

Studied lakes and samplings

Samples were collected once in 40 freshwater bodies (ranging from oligotrophic to eutrophic) from Chubut Province, Argentinean Patagonia, to Hope Bay, Antarctic Peninsula (45°22′ to 63°24′ S of latitude) (Fig. 1). In Antarctic lakes, samples were taken during the austral summer 2004, whereas in Patagonian lakes, samples were collected in late spring 2007 and 2008. Integrated samples were collected within the euphotic zone from the surface down to 5 m in deep lakes and from about 30 cm below the surface in shallow lakes. Temperature, pH, conductivity and dissolved oxygen (DO) were measured *in situ* with portable meters (Horiba D-54 meter, Kyoto, Japan and Hanna HI 9146, Villafranca, Italy). Nutrients (Ammonium N-NH_4, nitrate N-NO_3, nitrite N-NO_2 and phosphate P-PO_4), phytoplanktonic chlorophyll *a* (chl *a*), dissolved organic carbon (DOC) and light diffuse attenuation coefficient (K_d) were analysed following the methods described in Schiaffino *et al.* (2011).

A complete description of the studied area and the main characteristics of the water bodies were previously reported in different papers: Quirós and Cuch (1985) and Quirós and Drago (1999) for Patagonia, Vinocur and Unrein (2000) for Potter Peninsula, Toro *et al.* (2007) for Byers Peninsula and Izaguirre *et al.* (1998) for the Antarctic Peninsula (Hope Bay).

The geographical positions of the studied water bodies, as well as their main limnological characteristics were detailed in Schiaffino *et al*. (2011). The classification of the trophic status of the water bodies was based mainly on chl *a* taking into account the ranges presented in the Wetzel (2001) compilation.

Quantification of total abundances

Abundances of autotrophic and heterotrophic eukaryotes of picoplanktonic and nanoplanktonic size (0.2–5 μm) from each water body were obtained by epifluorescence, using an Olympus microscope (Olympus BX40F4, Tokyo, Japan) at 1000× magnification. Samples for epifluorescence microscopy were preserved with filtered cold glutaraldehyde 10% (1% final concentration) and filtered through a 0.2 and 0.6 μm pore-size polycarbonate filters. Autotrophic eukaryotes (≤3 μm in diameter) counts were performed using both blue and green-wavelength excitation and DAPI stained heterotrophic eukaryotes (flagellates of ≤5 μm in diameter) counts were performed using blue and UV-wavelength excitation.

Molecular methods

DNA extraction. Around 1000 ml of water samples were prefiltered *in situ* through a 50 μm net to remove zooplankton, then filtered with a vacuum pump first through a 20 μm pore-size polycarbonate filter and then through a 3 μm 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) and stored at −80°C until DNA extraction. Fractionation with 3 μm filters was chosen to be consistent with previous work (e.g. Massana *et al*., 2004; Vaulot *et al*., 2008) focusing on microbial eukaryotes. The 0.2–3 μm size fraction was used for this study. The procedures followed for DNA extraction (phenol/chloroform extraction) and touchdown polymerase chain reaction (PCR) amplifications were previously described in detail (Unrein *et al*., 2005).

DGGE analysis. This approach was performed for a total of 40 Patagonian and Antarctic lakes. Results from this fingerprinting method were presented showing the analysis of the entire dataset ($n = 40$) and the analysis of the 14 ($n = 14$) lakes. To perform DGGE amplifications we used the primers designed by Díez *et al*. (2001) targeting the V1-V3 variable regions of 18S rRNA gene: Euk1F (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and Euk516r-GC (5′-ACC AGA CTT GCC CTC C-3′) with a 40 bp GC-clamp. DGGE analyses were carried out with a DGGE-2000 system (CBS Scientific Company, Del Mar, CA). The 0.75 mm-thick gels of polyacrylamide (acrylamide:bisacrylamide ratio of 37:5:1) were run at 100 V and 60 °C for 16 h in a linear 35 to 55% 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, Grand Island, NY) and visualized with UV radiation using a Chemidoc system and the Quantity One software (Bio-Rad, Hercules, CA). Samples were run in 3 DGGE gels and about 800 ng of PCR product from environmental samples were applied to individual lanes in the gels. Between 2 and 4 samples were run in all the DGGEs to allow comparison among the gels; thus, respective positions of individual bands in different gels could be determined. Digitized DGGE images were analysed using the Gel-Pro 4.0 software (National Institutes of Health, Bethesda, MD). Changes in band intensities are likely due to relative changes in the abundances of the corresponding populations. With DGGE approach we obtained the dominant OTUs.

DGGE bands were removed from the gel and resuspended in 20 μl of Milli-Q water. The PCR reamplifications were performed with the original primer set and the PCR products were purified with the QIAquick PCR Purification kit (QIAGEN, Hilden, Germany). The reamplified PCR products 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 with KeyDNATools and then compared with the curated ribosomal eukaryotic database PR^2 (Guillou *et al*., 2013). All 18S rRNA gene sequences obtained in this study were deposited in GenBank under accession numbers KC923040-KC923068.

Illumina HiSeq. To further explore the diversity distribution patterns, a subset of 14 water bodies (8 Patagonian and 6 Antarctic lakes) from the entire dataset (40 lakes) were analysed with this technology. We amplified extracted DNA using primers specific to the V9 variable region of the 18S rRNA gene using the protocol as in Amaral-Zettler *et al*. (2009), and adapted after Lara *et al*. (2015). Sequencing was performed by the company Fasteris (Geneva, Switzerland) using Illumina HiSeq 2500 technology; paired end reads were around 200 bp in length.

Quality check (Phred score filtering, trimming of the primers and chimera removal) of the sequences was performed following the pipeline developed by de Vargas *et al*. (2015); Briefly, we kept sequences that passed the filtering based on quality values from the paired fastq file, by evaluating the expected error in a 50 bp sliding window and discarding sequences with more than 1% of error in the worst quality window. Sequences were clustered using SWARM (Mahé *et al*., 2014). The obtained sequences were then taxonomically classified with the curated ribosomal eukaryotic database PR^2 (Guillou *et al*., 2013) using the software GGSearch (McWilliam *et al*., 2013). Sequences affiliated with Metazoa and Embryophyta were removed from the analysis. Sequences were also aligned against the SILVA bacterial and archaeal database to remove possible prokaryotic OTUs.

Data analyses

The Illumina community matrix was studied considering the total OTUs, the abundant OTUs ≥1% of the total number of sequences in a given sample (Pedrós-Alió, 2006; Fuhrman, 2009) and the less frequent OTUs with two different criteria: uncommon >0.1% to <1% and rare ≤0.1% (Fuhrman, 2009; Vergin *et al*., 2013). The justification for the use of these thresholds is arbitrary, and lies in studies that indicate that organisms that make ≥1% of the total cell number can be detected with PCR-dependent techniques, while those that comprise ≤0.1% are difficult to retrieve (Muyzer *et al*., 1993; Casamayor *et al*., 2000). In order to compare the different

samples, we randomly selected the same number of Illumina sequences from each lake (matching the sample with the lowest number of reads, i.e. 14 437 sequences after primers trimming and quality filtering).

To study the diversity patterns, we calculated two different diversity indices for each sample using the total OTUs from the Illumina data: Shannon's index ($H' = -\sum pi \ln(pi)$) and Simpsoń 's reciprocal index ($D = 1/\sum pi^2$), were *pi* is the relative abundance of each OTU (*n/N*). In addition, two different evenness indices were also calculated based on the Simpsoń s reciprocal index ($E_D = D/S$) and the Shannoń s index ($E_{H'} = H'/\ln S$, Pielou index), where *S* is the OTU richness. To estimate total richness, we computed the Chao 1 index (Chao 1 $= S_{obs} + (f1^2/2f2)$, where S_{obs} is the observed number of OTUs, *f*1 is the number of singleton OTUs (represented by a single Illumina read), and *f*2 is the number of doubleton OTUs (Chao, 1984). Furthermore, to determine whether sampling was deep enough to get a reasonable estimate of OTU richness, we performed rarefaction curves with the rarefaction function R package vegan v. 2-3, 2. The latitudinal diversity pattern was assessed by correlating (Spearman Rho correlations) the diversity components (richness and evenness) and indices recorded in each lake with latitude. In addition, to compare the responses of the individual diversity components, we performed Spearman Rho correlations between them. Student's *t*-tests were used to compare the differences between Patagonian and Antarctic datasets (abundances, richness, evenness and diversity indices). Some variables were LN-transformed to meet normality and homoscedasticity assumptions.

We computed multiple-sites dissimilarity measures of variation in OTUs composition (Baselga, 2012) based on Illumina and DGGE community matrices to explicitly assess the spatial patterns of beta-diversity and their underlying phenomena (pure turnover, pure nestedness, or a combination of both with one of them prevailing over the other) in the model groups under study. In this approach, beta-diversity (β_{SOR}) is first calculated and then partitioned into its spatial turnover (β_{SIM}) and nestedness-resultant dissimilarity (β_{NES}) components. β_{SOR} and β_{SIM} are represented by the dissimilarity values retrieved through the calculation of Sørensen and Simpson dissimilarity indices respectively; while β_{NES} is computed as the difference between these metrics (*op. cit.*). These metrics range from 0 to 1, where higher values are consistent with higher values of dissimilarity among sites. Differences in the number of lakes among the areas studied were controlled for by re-sampling four lakes from each area 1000 times and computing the average β_{SOR}, β_{SIM} and β_{NES} respectively. Multiple-site dissimilarity measures of beta-diversity were computed using the R package betapart (Baselga and Orme, 2012).

We finally determined if environmental parameters and/or geographical distance influenced microbial eukaryote community composition (beta-diversity), and if so, which one had the strongest influence. We also wanted to know if the three abundance categories (abundant $\geq$1%, uncommon $>$0.1% to $<$1% and rare $\leq$0.1%) were influenced by the same parameters and if these parameters explained the same amount of variance. We also treated DGGE data in order to evaluate its performance with Illumina data, comparing the patterns using DGGE and the abundant part of the Illumina reads. We first transformed OTU abundance data using a Hellinger transformation prior to applying redundancy analysis (RDA) (Legendre and Gallagher, 2001; Ramette, 2007). Geographical coordinates were transformed into Principle Coordinates of Neighbourhood Matrix (PCNM; Borcard and Legendre, 2002). Raw environmental variables were transformed to their standard normal deviate equivalents [(x—mean) divided by the SD] to accommodate the different units of the different variables (Legendre and Birks, 2012). Relevant variables were selected for each abundance category and DGGE data by forward selection based on RDA (ordistep function, R package vegan v. 2-3, 2). In order to assess the relative contribution of each factor (environmental vs. geographical descriptors), we performed a variation partitioning based on partial RDA (varpart R package vegan v. 2-3, 2) on each community matrix (DGGE data and each abundance category from Illumina), also based on the variables that were kept after forward selection. We also performed standard and partial Mantel tests to examine the respective effect of geographical distance versus environmental factors on community composition; this distance-based approach is efficient in analyzing spatial patterns in community similarity and this is an alternative way to assess beta-diversity (Legendre *et al*., 2005; Tuomisto and Ruokolainen, 2006; Soininen *et al*., 2007; 2011). We calculated Bray Curtis distance matrices for community matrices (each abundance category from Illumina and DGGE datasets) and Euclidean distance matrices for the standardised environmental parameters (Martiny *et al*., 2006) and the PCNM variables that were selected previously.

Acknowledgements

This work was supported by a grant from the Argentinean Funds for Technical and Scientific Investigation (FONCYT, PICT 32732 and PICT 2013-0794), the Spanish Project MIX-ANTAR (REN 2002-11396-E/ANT), the Swiss NSF project 310003A_143960 and the Program 'Luis Santaló' of the National Research Council of Spain and the National Council of Scientific and Technical Research of Argentina (CSIC-CONICET, PROBA 2007AR0018). The authors also thank the members of the Antarctic Esperanza Station for logistic support and Dr A. Quesada and his team for providing the samples from the Byers Peninsula. L.D.F. is supported by CONICYT (doctoral scholarships N° 21110037 and 78130011) and the Wildlife Conservation Society (Karukinka grant N° 2012).

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