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Diversity and biogeographical patterns of yeast communities in Antarctic, Patagonian and tropical lakes

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ABSTRACT

We investigated the distribution patterns of yeast communities in freshwater lakes along a latitudinal gradient in order to evaluate yeast biogeography at intercontinental (501–8000 km), regional (0–500 km) and local (0–1 km) geographical scales. We identified 285 yeast isolates belonging to 64 species based on sequence analysis of the ITS-5.8S region and the D1/D2 domains of the large subunit of rRNA genes. Distance decay analysis showed a significant negative slope curve at the intercontinental scale. At the intercontinental and regional scales, the dissimilarity of the yeast communities was correlated with geographical distance, with community similarity decreasing with increasing distance. The physiological profiles of the yeast communities from tropical and Patagonian lakes were similar but were different from those of Antarctic lakes. This is the first report of latitudinal patterns of lake yeast diversity along a gradient extending from Antarctic to tropical environments.

1. Introduction

Biogeography is the study of organisms' distribution patterns in geographical space, both in contemporary terms and in the context of their evolution and development over time, and the processes determining the distribution of biodiversity (Meadows, 2004; O'Malley et al., 2007). A central goal of biogeography is to understand the mechanisms that generate and maintain diversity, such as dispersal, speciation, extinction and species interactions (Martiny et al., 2006). Distance decay analysis assumes that the similarity of communities decreases as the distance between them increases (Nekola and White, 1999) and is used to demonstrate how selection, drift, dispersal and mutation shape biogeographical patterns (Hanson et al., 2012). The distance decay relationship can be influenced by environmental conditions and/or dispersal limitations (Anderson et al., 2006; Martiny et al., 2011).

Micro-organisms are diverse and abundant, and have been regarded by some as cosmopolitan because they exhibit short generation times, large population sizes and long distance dispersal (Fenchel and Finlay, 2004). Molecular taxonomy, which allows the accurate identification of microbial species, has revealed that micro-organisms exhibit unique biogeographical distribution patterns. Previous biogeographical studies have described the variability and composition of communities of bacteria and yeasts in soil and plants (Taylor et al., 2006; Vishniac, 2006; Maksimova et al., 2009; Kachalkin and Yurkov, 2012; Yurkov et al., 2015, 2016), bacterioplankton in lakes, and microfungi associated with palm, fungal endophytes and other organisms (Taylor et al., 2000; Vaz et al., 2014; Hyde et al., 2016). These studies show that the distribution of a microbial species can be influenced by geographical distance, historical factors (geology, evolution and island biogeography), time since niche colonization and/or environmental factors, depending on the scale analysed.

Some studies have attempted to determine yeast distribution patterns. Yeasts form part of the microbiota of most, if not all, natural ecosystems and can be cosmopolitan or endemic to specific

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habitats or regions (Starmer and Lachance, 2011; Lachance et al., 2016). Environmental conditions, such as temperature, pH, light and concentration of readily available nutrients are some of the ecological factors that determine the metabolic activity, growth, survival and biodiversity of yeasts (Libkind et al., 2009; Yurkov et al., 2015). Yurkov et al. (2015) reported that at the community level, geographical region and type of substrate (phylloplane *vs.* soil) determine yeast species distribution in birch forests in Russia. Nagahama (2006) reported yeast distribution in nine deep-sea environments in the Pacific Ocean and found that *Rhodotorula sphaerocarpa* (=*Rhodosporidium sphaerocarpum*), *Cyberlindnera saturnus* (=*Williopsis saturnus*) and *Candida pseudolambica* (*Pichia* clade), were isolated from all deep-sea sites, while other species, such as *Kluyveromyces nonfermentans*, occurred only at some sites. Several yeast species associated with plants and insects have geographical distributions that mirror their host's distribution (Starmer and Lachance, 2011; Lachance et al., 2016).

Surveys of the geographical distribution of aquatic yeast species are sparse, with most being focused on the influence of water pollution (Nagahama, 2006). Few species of yeast appear to be specifically associated with aquatic habitats. *Debaryomyces hansenii* is the most common ascomycetous yeast isolated from marine waters. This yeast is considered to be a ubiquitous species because it is found in different environments and regions. Other ubiquitous species frequently associated with aquatic habitats such as *Aureobasidium pullulans*, *Rhodotorula mucilaginosa* and *Vishniacozyma victoriae* (= *Cryptococcus victoriae*) are also found in different regions and environments (de García et al., 2007; Brandão et al., 2011; Vaz et al., 2011; Yurkov et al., 2015). Some species are endemic to specific regions: for example, *Metschnikowia australis* is associated with algae, marine invertebrates and seawater in Antarctica (Lachance, 2011; Godinho et al., 2013), suggesting that the ecological distribution of aquatic yeast communities could be influenced by geographical patterns and the local conditions of each environment.

In the present study, we investigated the spatial distribution of cultivable yeasts on a geographical transect from polar to tropical lakes. This gradient included lakes in Antarctica, southern Argentina and southeastern and northern Brazil. We explored whether environmental and/or geographical distances explained yeast community composition at three different geographical scales (intercontinental, regional and local) and between different lakes.

2. Material and methods

2.1. Characteristics of the lakes and sampling sites

2.1.1. Antarctic lakes

Water samples were collected from five different lakes in the Antarctic Specially Managed Area (ASMA) in Admiralty Bay, King George Island, South Shetland Islands (Table 1), during the austral summer season between December 2008 and January 2009. The five lakes sampled in the ASMA represent different environmental conditions: Agat Point and Wanda Lakes are influenced by marine water and Machu Picchu, Stanhouse and Refuge II Lakes are subjected to long periods of ice and snow-cover in the winter. Water temperature and pH were measured *in situ* using an YSI 650 multiparameter display system (YSI Environmental, USA). Three water samples (500 mL) were collected in sterile bottles from five sites spaced approximately 50 m apart and were transported on ice to the laboratory within 24 h of sampling for processing.

2.1.2. Patagonian lake

Steffen Lake is located in Argentinian Patagonia in the Nahuel Huapi National Park (Table 1). It has an area of 6.3 km^2 and an average depth of 76.8 m. The lake is of glacial origin, is oligotrophic, has a high transparency (Secchi disk: 13 m), and has limited human influence. A native Andean Patagonian forest composed of *Nothofagus* spp. surrounds the lake. Mean annual surface water temperature is *c.* 17 °C (Quirós, 1988). Three water samples (300–400 mL) were collected in sterile bottles from four sites located approximately 200 m apart on a transect along the lake. The samples were transported on ice to the laboratory within 24 h for processing. Water temperature was measured *in situ* and pH was measured in the laboratory with a 3310 Jenway pH meter (Staffordshire, UK).

2.1.3. Tropical Brazilian lakes

Samples were obtained from three tropical lakes in Brazil (Table 1). Dom Helvecio Lake is located in the Ecological State Park of Rio Doce, which comprises an area of 36,113 ha and constitutes the largest relict area of Atlantic rain forest in Minas Gerais state. The lake has a surface area of 6.87 km^2 and is considered to be the largest and deepest lake of the middle Rio Doce lake system. It is one of the deepest natural lakes in Brazil, with an average depth of 32.5 m. The lake is oligotrophic, warm, monomictic and has one circulation period, usually between May and August (Matsumura-Tundisi and Tundisi, 1995). Three water samples (100 mL) were collected in sterile bottles from six sites located approximately 100 m apart on a transect along the lake. Samples were transported to the laboratory on ice within 24 h for processing. Temperature was measured *in situ* and pH was measured in the laboratory with a HI 211 combined meter (Hanna instruments, Rhode Island, USA). Rico and de Dentro Lakes are located in Cantão State Park (9°10′S, 50°10′W), a protected area located in the west of the Tocantins state, which represents an ecotone area among the Cerrado, Amazon forest and Pantanal ecosystems (Santos and Lolis, 2007). A dense Amazonian forest surrounds the lakes and, in the rainy season (October–April), the whole plain is flooded as the water level rises by 7–10 m (Pinheiro and Dornas, 2009). The presence of

Table 1
Description of study sites and diversity indexes of yeasts included in the present study.

Environment	Lakes	Geographical coordinates	Total yeast counts (CFU L^{-1})[a]	Temperature (°C)[b]	Mean pH	Number of isolates	Number of yeast species	Number of singletons	Shannon index (H′)
Tropical	Lago Rico	9° 21′S, 50°00′ W	721.6 ± 918.0	23.5	6.9	50	19	6	2.60
Brazil	Lago de Dentro	9°21′ S, 49°58′ W	373.3 ± 426.2	23.0	7.1	56	19	6	2.38
	Dom Helvécio	19°29′ S, 19° 48′ W	774.1 ± 590.2	24.4	6.2	52	17	3	2.57
Patagonian, Argentina	Steffen	41°31′ S, 71°33′ W	52.2 ± 35.5	17.0	7.0	51	17	5	2.43
Antarctic	Stanhouse	62°04′S, 58°22′ W	53 ± 25.1	0.2	9.1	16	5	1	1.94
	Refúgio II	62°04′ S, 58°25′ W	5.6 ± 2.85	4.2	8.5	14		1	1.99
	Wanda	62°04′ S, 58°19′ W	37.71 ± 9.0	1.8	7.4	8	3	–	1.77
	Machu Picchu	62°05′S, 58°19′ W	20.8 ± 17.9	0.7	7.1	22	8	1	2.07
	Agat Point	62°11′ S, 58° 26′ W	73.5 ± 58.4	2.8	7.5	15	4	–	1.76

[a] Mean ± standard deviation.
[b] Temperature values are point measurements.

many lakes (about 800) and the flooding regime makes the region similar to the Pantanal biome. Three water samples (100 mL) were collected in sterile bottles from five sites approximately 30 m apart on a transect along the lakes. Samples were transported to the laboratory on ice within 24 h for processing. The temperature and pH of the water were measured *in situ* using a digital sampler (Horiba U-22, Fukuoka, Japan).

2.2. Yeast isolation

Samples of subsurface water (10–30 cm depth) from each lake were filtered through sterile nitrocellulose membranes (0.45 μm, 47 mm diameter) with a Nalgene filtration device (Nalgene™, Rochester, USA) and vacuum pump. The membranes were placed on the surface of yeast extract-malt extract agar medium (YMA, 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% dextrose, 2% agar, pH 4.0), containing chloramphenicol at 200 mg L^{-1}. Petri dishes were incubated at 15 °C (for samples from Antarctic and Steffen lakes) and at 20 °C (for those from tropical lakes) for 3–7 days. Yeast colony forming units (CFUs) were counted for quantitative analysis. Each morphotype was purified by repeatedly streaking on YMA medium. When possible, at least three randomly selected colonies of each morphotype were selected and stored at −80 °C prior to identification.

2.3. Yeast identification

All yeast isolates were grouped based on colony morphology, physiological tests and sequence analyses. The physiological characteristics of all isolates were tested according to assimilation patterns of carbon and nitrogen sources. Fifty-one physiological tests were performed, *viz*. glucose fermentation, assimilation of carbon compounds (acetate, cellobiose citrate, D-arabinose, D-glucitol, D-gluconate, D-glucosamine, D-mannitol, D-ribose, D-xylose, DL-actate, ethanol, erythritol, ethyl acetate, galactitol, galactose, glucitol, glucose, glycerol, hexadecane, inulin, isopropanol, lactose, L-arabinose, L-rhamnose, L-sorbose, maltose, melibiose, melezitose, methanol, myo-inositol, ribitol, *N*-acetyl-D-glucosamine, raffinose, salicin, soluble starch, succinate, sucrose, trehalose and xylitol), utilization of nitrogen compounds (lysine, nitrite and nitrate), growth in amino-acid-free medium, growth at 30 °C, growth on YMA with 10% sodium chloride and 50% glucose, resistance to acetic acid, acid production (solubilisation of $CaCO_3$) and the formation of starch-like compounds (Kurtzman et al., 2011). The response for each test was assigned a value of 0 (no growth), 0.5 (weak or variable) or 1 (positive).

Isolates with identical morphological and physiological characteristics were grouped together and subjected to PCR fingerprinting with the core sequences of the primer $(GTG)_5$ (Libkind et al., 2003). Yeast strains with identical PCR fingerprint patterns were grouped together and putatively considered to belong to the same species (Sampaio et al., 2001; Brandão et al., 2011). At least 50% of the yeast isolates of each molecular group were identified by sequencing. Species identification was performed by sequence analysis of the ITS-5.8S region and the D1/D2 variable domains of the large subunit of rRNA genes as described previously (Kurtzman and Robnett, 1998). DNA extraction was performed according to Brandão et al. (2011). The amplified DNA was concentrated, cleaned and sequenced in an ABI 3130 Genetic Analyzer automated sequencing system using BigDye v3.1 and POP-7 polymer. The sequences obtained were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST; https://www.ncbi.nlm.nih.gov/pubmed/2231712) (Altschul et al., 1990).

2.4. Analysis of ecological data

Species accumulation curves were used to determine whether a sufficient number of samples had been obtained from each environment (Colwell et al., 2004). The extrapolated richness was estimated using the nonparametric estimator Chao2 (Colwell and Coddington, 1994) and the richness was compared using a heat map, in which the similarities were computed based on a dendrogram. Yeast diversity in each lake was measured using the Shannon (H′) index ($H = -\Sigma ni/n\ ln\ (n_i/n)$, where n_i is the number of individuals of the taxon i and n is the total number of individuals (Ryan et al., 1995). The Kruskal-Wallis test was used to test for differences in the numbers of CFUs between lakes.

β-diversity was evaluated using the rate of distance decay, which assumes that community similarity decreases with increasing geographical distance (Nekola and White, 1999). The yeast community similarity based on the Jaccard index was regressed against geographical distance (ln-transformed) and the distance decay relationship was calculated as the slope of least-squares linear regression (Nekola and White, 1999). The geographical distance was logarithmically transformed owing to the large distances between the lakes (Martiny et al., 2011; Vaz et al., 2014). Geographical distance between the sampling sites was classified as either intercontinental (501–8000 km), regional (0–500 km) or local (0–1 km). In addition, we tested whether the slope of the distance decay curve of each sampling site was significantly different from zero using a randomisation procedure with 1000 iterations. The yeast richness was compared between lakes using the heatmap.2 function in R, using 'complete' (complete-linkage) as the clustering method.

To investigate the relationship between yeast community similarity and the geographical distance and environmental characteristics, we applied the ranked partial Mantel test (Goslee and Urban, 2007; Martiny et al., 2011; Vaz et al., 2014). Principal component analysis (PCA) of the environmental variables (pH and temperature) was performed and the dissimilarities were computed for the first component. Correlations were examined with the Spearman correction, with the P values being based on 10,000 permutations.

Multiple regression matrices (MRM) were used to determine the relative importances of geographical distance and environmental variables to the similarities of the yeast communities (Goslee and Urban, 2007). To reduce the effect of spurious relationships between variables, we performed the MRM test, removed the non-significant variables and then repeated the test (Martiny et al., 2011). Temperature and pH were identical at the collection points in each Antarctic lake and were hence not included in the tests at the local scale. We tested the significance of each model by performing 10,000 permutations.

The yeasts were grouped according to their physiological characteristics and agglomerative hierarchical clustering using the function hclust (Oksanen, 2009). All analyses were performed using the R package (R Development Core Team, 2012).

3. Results and discussion

3.1. Diversity analysis

The heatmap analysis confirmed that the lakes were colonized by distinct sets of yeast species according to the environment studied. *Sporobolomyces japonicus*, *Pichia kudriavzevii*, *Papiliotrema laurentii* and *A. pullulans* occurred mainly in de Dentro and Rico lakes in tropical Brazil. *M. australis* was prevalent in Agat point and Wanda lakes in Antarctica, while *Leucosporidium muscorum*, *V. victoriae* and *R. mucilaginosa* dominated in Stanhouse, Machu

Picchu and Refuge II lakes (Fig. 1). The distance decay analysis showed a significant negative slope curve when compared at the intercontinental scale (slope = −0.004, $P < 0.001$) (Fig. 2). At the local scale, the distance decay was also significant for polar

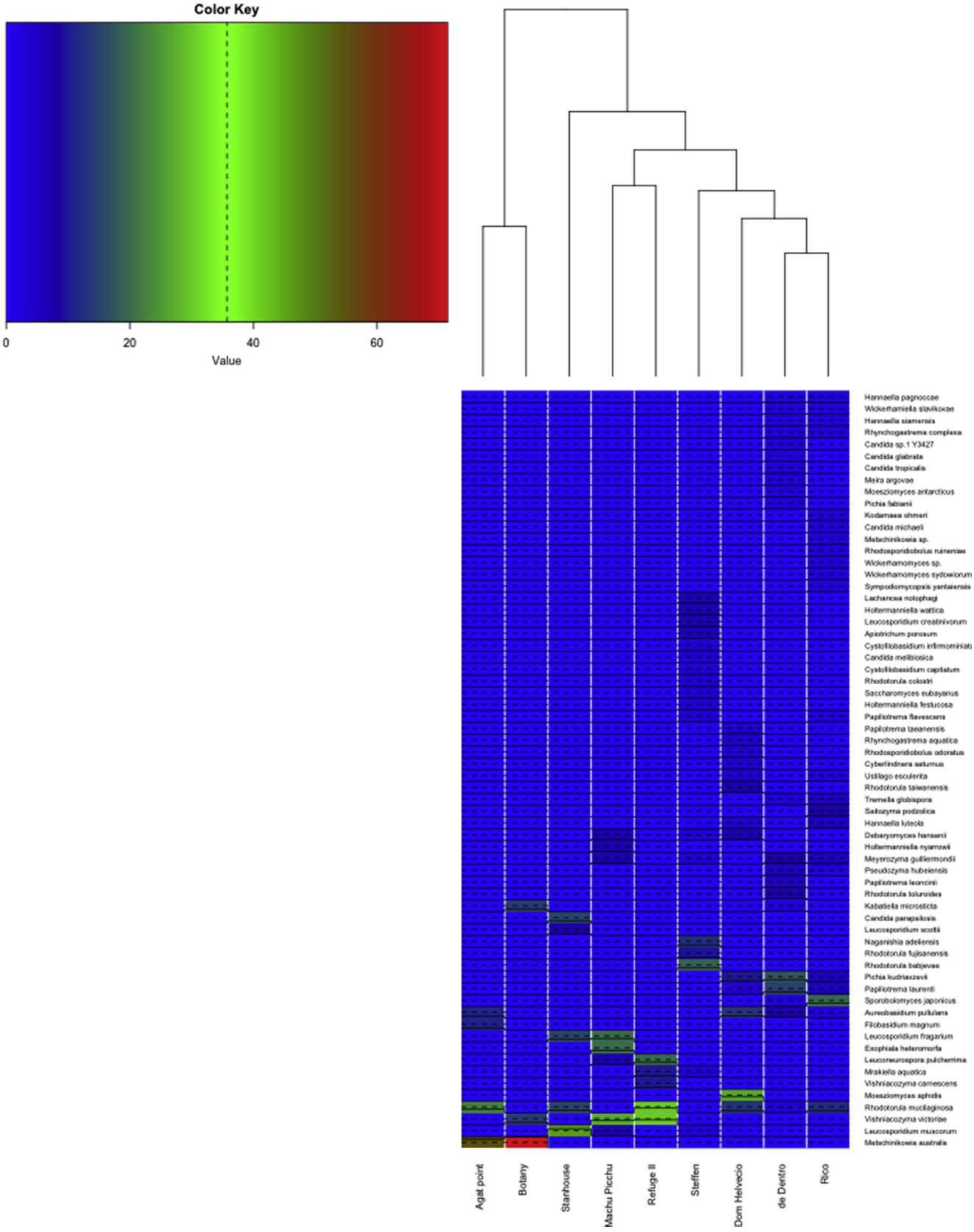

Fig. 1. Heatmap of yeast richness in temperate (Argentinian Patagonian), tropical (Brazilian) and polar (Antarctic) lakes.

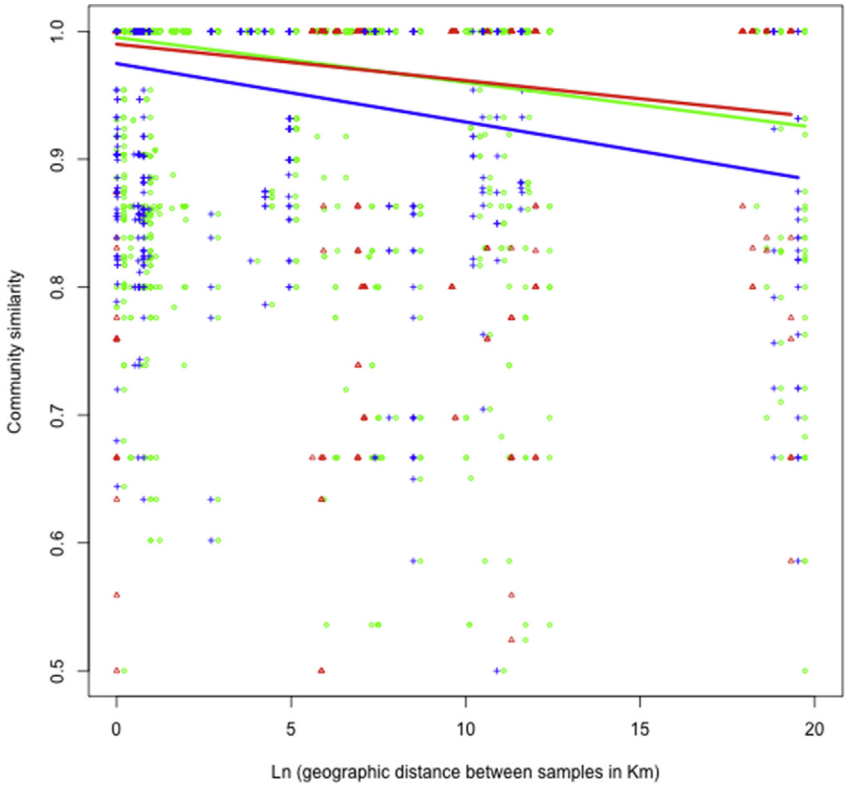

Fig. 2. Distance decay relationship for the yeast communities. Pairwise community similarities were calculated using the Jaccard index and plotted against the natural logarithms of the distances among study sites. The lines denote significant ($P < 0.001$) linear regressions at the intercontinental scale (green) and at local scales in tropical (blue, Brazil) and polar (red, Antarctic) environments.

(slope = −0.003, $P = < 0.001$) and tropical (slope = −0.005, $P = < 0.001$) lakes (Fig. 2). Using ranked partial Mantel tests, yeast community dissimilarity was highly significantly correlated with geographical distance at the intercontinental scale ($\rho = 0.26$, $P < 0.001$). At the regional scale, there was a marginally significant correlation between yeast communities in tropical Brazil ($\rho = 0.096$, $P < 0.058$) (Table 2). These findings are consistent with the strong effect of geographical distance on yeast community structure indicated by the MRM analysis (Table 3). Two mechanisms can explain the decline in similarity between yeast communities and increasing geographical distance. Firstly, environmental conditions become increasingly different as distance between sampling sites increases. If the yeast species within a community are adapted to specific lake conditions, then communities would be expected to become increasingly different with distance since species are sorted according to their niche requirements. Secondly, several yeast species have restricted dispersal and tend to colonize sites with similar ecological conditions (Vaz et al., 2014). Therefore, for these species, a distance decay relationship would emerge even without differences in lake conditions or yeast nutritional requirements.

MRM analysis showed the factors that best explain the correlation. Distance, pH and temperature were statistically significant terms in the model at an intercontinental scale. The ß value shows that the similarity increased as the distance and pH decreased and temperature increased. Temperature did not play a significant role ($P > 0.05$) at the local level and was removed from the final model (Table 3). At the local scale, when each lake was analysed separately, the proportion of the variability explained by the MRM models was statistically significant when compared with the samples from the transects across Rico Lake ($R^2 = 21\%$, $P < 0.005$) and de Dentro Lake ($R^2 = 17\%$, $P < 0.05$) (Table 3). The geographical distance influenced the yeast community similarity for both lakes, while pH value was significant only for de Dentro Lake. Water pH varied from 6.80 to 7.22 in de Dentro Lake and the negative ß value in the MRM analysis suggests that the yeast community similarity increases as pH decreases (Table 3). However, this influence of pH on yeast community similarity was not observed for the other lakes. Further studies on organic matter fractionation in these lakes may be important to understand acidification processes in freshwater environments. However, our results suggest that pH value could be an important factor in selecting the yeast communities across de Dentro Lake.

We compared the yeast communities based on the physiological profiles of the species and the nutritional profile of the yeast community found in each lake. We found two distinct groups based on similar physiological profiles (Fig. 3). One group consisted of yeast communities from tropical and Patagonian lakes and another group contained yeast communities from Antarctic lakes. Most species were able to assimilate more than 25 carbon compounds, presenting a wide nutritional profile. These species could compete for nutrients more efficiently in environments with limited available nutritional sources, such as in the Antarctic lakes and Steffen Lake. Cosmopolitan species such as *A. pullulans*, *D. hansenii* and *R. mucilaginosa* had broad nutritional profiles and were found in all lakes. *Saccharomyces eubayanus, Lachancea nothofagi, Rhodotorula colostri, Rhodotorula fujisanensis* and *M. australis* had limited nutritional profiles and were restricted to lakes of cold regions (Patagonia and Antarctica).

We demonstrated that yeast communities in Antarctic, Patagonian and Brazilian tropical lakes are not randomly distributed. These lakes constitute unique ecosystems that select yeast

Table 2
Partial Mantel test results, where Spearman ρ represents the correlation between the yeast community dissimilarity and either geographic distance or environmental distance.

Correlation between yeast communities	Controlling for:	Intercontinental		Regional scales					
				Tropical Brazil		Argentinian Patagonia		Antarctica	
		ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
Geographic distance	Environmental distance	0.26	<0.001	0.096	0.058	–	–	−0.022	0.64
Environmental distance	Geographic distance	0.00071	0.49	−0.031	0.67	–	–	0.059	0.14

The environmental variables were first examined using a principal components analysis that considered temperature and pH of each lake. The *P* values are one-taled and based on 10,000 permutations.

Table 3
Results of the multiple regressions on matrices (MRM) analysis for the yeast communities by spatial scale.

	Intercontinental	Local		Tropical Brazil			Argentinian Patagonia	Antarctica[b]				
		Tropical Brazil	Antarctica	DH[a] $R^2 = 0.05$	LR $R^2 = 0.2$[*b]	LD $R^2 = 0.17$[**c]	STF $R^2 = 0.09$	ST $R^2 = 0.002$	MP $R^2 = 0.002$	RF $R^2 = 0.012$	WA $R^2 = 0.001$	AP $R^2 = 0.002$
Ln (geographic distance)	−0.26	−0.09	0.02	0.03	0.44*	0.44**	−0.09	−0.04	−0.02	0.11	0.03	0.04
pH[d]	−0.18	−0.01	0.06	−0.23	0.02	−0.56**	0.17	–	–	–	–	–
Temperature	0.1	−0.04	0.008	–	–	–	–	–	–	–	–	–

Levels of significance denoted by: * $P < 0.01$, ** $P < 0.001$.

[a] Lakes: DH: Dom Helvécio, LR: Lago Rico, LD: Lago de Dentro, STF: Steffen, AP: Agat Point, WA: Wanda, MP: Machu Picchu, RF: Refuge II, ST: Stanhouse.

[b] $P \leq 0.01$.

[c] $P \leq 0.001$: The variation (R2) of the community similarity that is explained by the remaining variables and the partial regression coefficients (β) of the final model are reported. If the partial regression is reported, then its significance level (one-way tests) is $P < 0.05$.

[d] The pH and temperature did not vary in the five Antarctic lakes and were hence not analysed for in the MRM analysis. There was only one lake on Patagonian (Argentina) ecosystem and it was not possible to do the MRM analysis for this local scale.

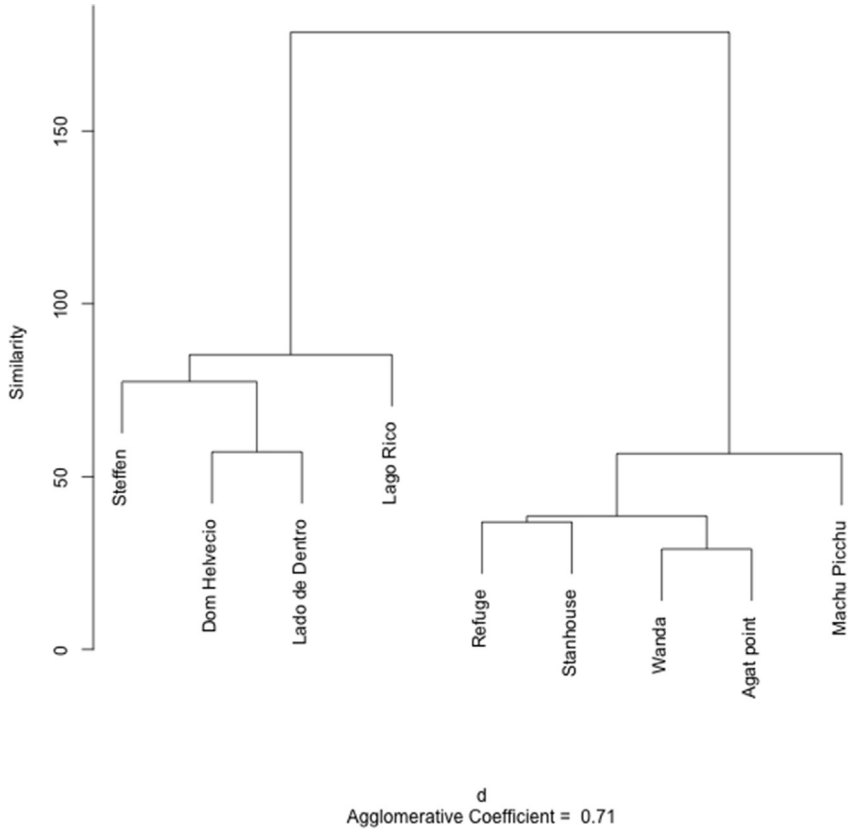

Fig. 3. Dendrogram representing the similarity of the physiological profiles of the yeast communities from tropical Brazilian (Dom Helvécio, Lago de Dentro and Lago Rico), Argentinian Patagonian (Steffen) and Antarctic (Refuge II, Stanhouse, Wanda, Agat Point and Machu Picchu) lakes.

communities that are able to tolerate stressful conditions and nutrient availabilities. We observed a distance decay relationship at all intercontinental scales that could be explained by dispersion limitations and/or environmental selection. The influence of the environment or geographical distance in this relationship varied between the distance scales. Furthermore, yeast communities in lakes, mainly those of Antarctica and Patagonia, showed species with restricted nutritional profiles, suggesting habitat specificity for these yeasts.

3.2. Yeast occurrence and diversity

We obtained and identified 285 yeast isolates belonging to 36 genera and 64 species (Table 4). The yeast species most commonly isolated were *A. pullulans, Pa. laurentii, D. hansenii, L. muscorum, M. australis, P. kudriavzevii, Moesziomyces aphidis, Rhodotorula babjevae, Rh. mucilaginosa, S. japonicus* and *V. victoriae*. Basidiomycetous yeasts were predominant in all lakes, representing 65.6% of the total isolates. These yeasts are often found associated with the phyllosphere of terrestrial plants and their occurrence in tropical and temperate aquatic environments might be the result of run-off from surrounding plant substrates (Brandão et al., 2011; Starmer and Lachance, 2011). Basidiomycetous yeasts are more nutritionally versatile and tolerant to extreme environmental conditions compared with ascomycetous yeasts (Brandão et al., 2011), which could explain their frequent occurrence in Antarctic lakes. *M. australis* was isolated only from Agat Point and Wanda Lakes. This yeast is indigenous to Antarctic marine habitats (Lachance, 2011) and has been isolated from marine sediment, seawater and thalli of the algal species *Adenocystis utricularis*, *Desmarestia anceps* and *Palmaria decipiens* (Vaz et al., 2011; Godinho et al., 2013). The occurrence of this yeast species only in Agat Point and Wanda Lakes further supports the view that *M. australis* is of marine origin, since these lakes are fed by seawater.

V. victoriae has been previously isolated from lichens, mosses and soil (Thomas Hall et al., 2002). This species was the most frequent species obtained from different substrates in Antarctica (Vaz et al., 2011; Santiago et al., 2016) and is found in other cold environments, such as the Calderone Glacier in Italy (Branda et al., 2010), ice from subglacial environments in Arctic habitats (Butinar et al., 2007) and a Patagonian lake in Argentina (Brandão et al., 2011). However, this yeast could be more widespread, since its occurrence has been reported in other regions, for instance in rice leaves in Thailand (Tantirungkij et al., 2015), or in soil in Mediterranean forests (Yurkov et al., 2015). Two *C. parapsilosis* isolates were obtained from Stanhouse Lake. This species is an opportunistic pathogen and is common in aquatic environments polluted by sewage (Nagahama, 2006). The occurrence of this yeast species in Stanhouse Lake could be associated with guano from seabirds, the effect of the oceanic tide or the presence of humans due to tourism in the Antarctic Peninsula (Chryssanthou et al., 2011).

R. babjevae was the most frequent species obtained from the temperate Steffen Lake in Argentina. This species has a wide geographical distribution, since it has been isolated from several different substrates in Europe, America and Asia (Sampaio, 2011) and from freshwater (Libkind et al., 2003) and marine environments (Gadanho et al., 2003). *L. muscorum,* the second most frequently isolated species in this environment, has been previously isolated from lakes and glaciers in Patagonian Argentina (de García et al., 2007; Libkind et al., 2009; Brandão et al., 2011). Most yeasts isolated from Steffen Lake were allochthonous species and may be associated with plants and plant-related substrates

Table 4
Identification, number of isolates and origin of the yeast species isolated from the lakes studied.

Yeast species	No. of isolates	Tropical Brazil			Patagonian Argentina	Antarctic				
		DH[a]	LR	LD	STF	SH	MP	AP	WA	RF
Apiotrichum porosum	2				2					
Aureobasidium pullulans	11	4	1	5				1		
Candida glabrata (*Nakaseomyces* clade)	1			1						
Candida melibiosica (*Metschnikowiaceae* clade)	1				1					
Candida michaeli (*Yamadazyma* clade)	1		1							
Candida parapsilosis (*Lodderomyces*/*C. albicans* clade)	2					2				
Candida tropicalis (*Lodderomyes*/*C. albicans* clade)	1			1						
Candida sp.A UFMG-CM-Y3404	1		1							
Candida sp.B UFMG-CM-Y3427 (*Diutina* clade)	1			1						
Cyberlindnera saturnus	1	1								
Cystofilobasidium capitatum	1				1					
Cystofilobasidium infirmominiatum	1				1					
Debaryomyces hansenii	11	8			2		1			
Exophiala heteromorpha	4						4			
Filobasidium magnum	2							2		
Hannaella luteola	3	1	2							
Hannaella pagnoccae	5		2	3						
Hannaella siamensis	3	2	1							
Holtermanniella festucosa	2				2					
Holtermanniella nyarrowii	1						1			
Holtermanniella wattica	3				3					
Kodamaea ohmeri	1		1							
Kabatiella microsticta	2			1					1	
Lachancea nothofagi	2				2					
Leuconeurospora pulcherrima	3						1			2
Leucosporidium creatinivorum	2				2					
Leucosporidium fragarium	7					2	5			
Leucosporidium muscorum	11				1	9	1			
Leucosporidium scottii	1					1				
Meira argovae	1			1						
Metschnikowia australis	16							10	6	
Meyerozyma guilliermondii	7		2	3	1		1			
Moesziomyces antarcticus	1			1						
Moesziomyces aphidis	12	12								
Mrakiella aquatica	4				3					1
Naganishia adeliensis	9				9					
Papiliotrema flavescens	3		1		2					
Papiliotrema laurenti	11	3	2	6						
Papiliotrema leoncinii	2	2								
Papilotrema taeanensis	2	2								
Pichia fabianii	1			1						
Pichia kudriavzevii	23	5	2	16						
Pseudozyma hubeiensis	5		1	4						
Rhodotorula babjevae	11				11					
Rhodotorula colostri	1				1					
Rhodotorula fujisanensis	5				5					
Rhodotorula mucilaginosa	22	7	6	2		2		2		3
Rhodotorula taiwanensis	2	2								
Rhodotorula toruloides	3		3							
Rhodosporidiobolus odoratus	1	1								
Rhodosporidiobolus ruineniae	1		1							
Rhynchogastrema aquatica	1	1								
Rhynchogastrema complexa	3		1	2						
Saccharomyces eubayanus	1				1					
Saitozyma podzolica	3		3							
Sporobolomyces japonicus	19		18	1						
Sympodiomycopsis yantaiensis	1		1							
Tremella globispora	3		2	1						
Ustilago esculenta	2	1	1							
Vishniacozyma carnescens	1									1
Vishniacozyma victoriae	16						8		1	7
Wickerhamiella slavikovae	2		1	1						
Wickerhamomyces sp. UFMG-CM-Y3346	2		2							
Wickerhamomyces sydowiorum	1		1							
Total	285	52	54	54	50	16	22	15	8	14

[a] DH: Dom Helvécio, LR: Lago Rico, LD: Lago de Dentro, STF: Steffen, SH: Stanhouse, MP: Machu Picchu AP: Agat Point, WA:Wanda, RF: Refuge II.

surrounding this environment.

Tropical lakes, which were located in protected areas and have limited human influence had the highest number of yeast isolates, with *P. kudriavzevii* (23 isolates) being the most frequent species, followed by *S. japonicus*, *R. mucilaginosa* and *Pa. laurentii*. Most yeast species obtained from Brazilian tropical lakes have been previously isolated from other tropical freshwater environments. *P. kudriavzevii* is often found in aquatic environments with high levels

Table 5
P values from Kruskal-Wallis analyses showing statistical differences between yeast counts ($CFU^{-1}L$) in the lakes studied.

Environments		Tropical Brazil			Patagonian Argentina	Antarctica				
		DH	LR	LD	STF	AP	WA	MP	RF	ST
Tropical Brazil	DH[a]		n.s.	n.s.	0.022	0.006	0.001	<0.001	<0.001	0.011
	LR			n.s.	n.s.	n.s.		0.005	<0.001	n.s.
	LD				n.s.	n.s.	0.026	<0.001	<0.001	n.s.
Patagonian Argentina	STF					n.s.	n.s.	n.s.	0.0006	n.s.
Antarctica	AP						n.s.	n.s.	0.022	n.s.
	WA							n.s.	n.s.	n.s.
	MP								n.s.	n.s.
	RF									0.015
	ST									

[a] Lakes: DH: Dom Helvécio, LR: Lago Rico, LD: Lago de Dentro, STF: Steffen, AP: Agat Point, WA: Wanda, MP: Machu Picchu, RF: Refuge II, ST: Stanhouse. n.s.: not significant ($P > 0.05$).

of organic matter from industrial and domestic waste, but this yeast is often found in soil, on fruits and various natural environments (Nagahama, 2006; Kurtzman, 2011), and is also considered to be an opportunistic pathogen (Kurtzman, 2011). The high frequency of this species in de Dentro and Dom Helvecio Lakes could be also attributed to faecal contamination by animals (Brandão et al., 2010). The opportunistic pathogenic species *Candida tropicalis* was also isolated from de Dentro lake, but this species is frequently isolated from rotting wood (Cadete et al., 2012), and so this isolate was possibly not of human or animal origin.

A. pullulans, *Pa. laurentii*, *D. hansenii, Meyerozyma guilliermondii* and *R. mucilaginosa* are widely distributed in nature and were isolated from more than three lakes, suggesting cosmopolitan distributions. These species have been previously isolated from worldwide aquatic environments (Libkind et al., 2003; Nagahama, 2006; Butinar et al., 2007; Vaz et al., 2011) and could have been introduced with allochthonous organic matter.

Based on analyses of the sequences of the D1/D2 domains of rRNA, we found four isolates that could represent three new species. Two isolates identified as *Wickerhamomyces* sp. UFMG-CM-Y3346 (GenBank accession number KJ608555) showed one to two nucleotide differences from several yeast strains deposited in GenBank. These strains could represent a new *Wickerhamomyces* species lacking a formal description. The strain identified as *Candida* sp.A UFMG-CM-Y3404 (GenBank accession number KR815866) belongs to the Metschnikowiaceae clade and had 85% identity with *Candida pimensis* and *Candida picachoensis* and 89% identity with *Candida picinguabensis*. One isolate, identified as *Candida* sp.B UFMG-CM-Y3427 (GenBank accession number KR815865), differed by two nucleotide substitutions from *Candida* sp. BG02-7-16-015A-2-1 and likely represents the same species. This new species is phylogenetically related to the clade *Diutina* (Khunnamwong et al., 2015). The species *Wickerhamiella slavikovae*, *Hannaella pagnoccae* and *Papiliotrema leoncinii* were recently described by Hagler et al. (2013), Landell et al. (2014) and Pagani et al. (2016), respectively, using isolates from this study. All new species that we isolated originated from tropical lakes.

Mean total yeast counts in each lake are shown in Table 1. Water samples collected in Antarctica yielded lower numbers of yeast colonies than the Brazilian and Patagonian lakes (Table 5), with the highest yeast counts being obtained from tropical Brazilian lakes. Yeast diversity and density in aquatic environments may thus have been influenced by biotic factors, including allochthonous sources like soil and plant debris (Nagahama, 2006; Brandão et al., 2010, 2011) and abiotic factors such as pH, temperature and UV radiation (Brunati et al., 2009).

The highest yeast densities obtained from tropical lakes could be related to the occurrence of a dense and diverse plant community surrounding the lakes. The yeast density of Antarctic lakes was similar to those found in studies of Patagonian glacial meltwaters from the Frías, Castaño Overo and Río Manso glaciers, located on Mount Tronador in the Nahuel Huapi National Park in Northwestern Patagonia, Argentina (de García et al., 2007). Antarctic lakes are also subjected to long periods of ice and snow-cover and dramatic changes of photosynthetic activity between the winter and summer seasons (Brunati et al., 2009). The Antarctic lakes in our study do not have surrounding vegetation, which could explain the correspondingly low yeast counts and Shannon values (Table 1). The low yeast densities observed in the Argentinian lake studied here may be related to the meltwater rivers draining from the lake that are oligotrophic and characterized by low nutrient concentrations (de García et al., 2007; Libkind et al., 2009). In addition, the community of plants surrounding this lake is composed mainly of *Nothofagus* species and is of low diversity. The low diversity of plant species around the lake could also explain the low yeast densities, since the yeast occurrence in water bodies reflects inputs from terrestrial sources such as soil and plant debris. High UV radiation exposure might also play a role in reducing the frequencies of yeasts in Antarctic and Patagonian lakes, in particular in the subsurface waters studied here (Libkind et al., 2009; Brandão et al., 2011).

The Shannon diversity values of the lakes studied are shown in Table 1. The highest diversity values were for tropical Brazilian and temperate Argentinian lakes, and were similar to those found for the Nahuel Huapi Lake in Patagonia (Brandão et al., 2011) and a tropical Brazilian lake surrounded by Atlantic rain forest in Brazil (Medeiros et al., 2008). These results support the hypothesis that aquatic environments surrounded by dense and diverse vegetation support diverse yeast communities (Brandão et al., 2011).

The sampling did not capture the richness of yeasts in the temperate and tropical lakes studied, demonstrated by the lack of asymptote for the corresponding species accumulation curves (Supplementary Fig. 1). Moreover, the extrapolated richness using the Chao2 estimator indicated that our sampling effort was enough to account for 67%, 73% and 79% of the yeast species in tropical, polar and temperate lakes, respectively. More samples are thus needed to estimate the actual diversity of yeasts in these lakes (Colwell et al., 2004). However, in Antarctic lakes the curve approximated an asymptote (Supplementary Fig. 1), indicating that increasing sampling will not significantly increase the number of species found. This finding might be owing to the geographical isolation and inhospitality of Antarctica.

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Supplementary data

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