







Division - Soil Processes and Properties | Commission - Soil Biology

Yeast species and strains differing along an altitudinal gradient in the Brazilian forest domain

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ABSTRACT: Soil microbiota is an important component of the forest biomes, playing important roles in the soil aggregation and cycling of nutrients. Among the soil microorganisms stand out the yeasts, which are unicellular fungi involved in important soil ecological processes. The Brazilian Atlantic Forest is one of the main biodiversity hotspots in the world, and the effect of altitudinal gradient on the distribution patterns of yeast species across this ecosystem has not yet been addressed. Thus, this study aimed to investigate the occurrence and distribution of yeast species in soils along an altitude gradient (404; 1,016; 1,658; and 2,124 m above the sea level) of *Serra dos Órgãos* National Park located at Rio de Janeiro State, Brazil. Yeast species were described using a culture-based method. Species identification was performed using the fungal barcode locus, the D1/D2 region of 26S rRNA, and the gene genealogy was used to access the intraspecific distribution of strains along the altitudinal gradient. We isolated and identified a total of 76 yeasts including ten species belonging to eight genera. Basidiomycetes predominated over ascomycetes. *Saitozyma podzolica* and *Meyerozyma guilliermondii* were isolated at all altitudes. The principal component analysis showed that 88 % of sample distribution is explained by soil properties. For *S. podzolica*, the genetic genealogy suggested that intraspecific distribution is likely related to similar altitudes. Overall, the species composition and soil properties were modified as altitude was increasing, being more heterogeneous and richness in high altitudes.

Keywords: culturable yeast, soil ecology, forest, phylogeography, *Saitozyma*.

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INTRODUCTION

Fungal communities are an important component of the soil microbiota. Known to be unicellular fungi, yeasts are underexplored members of the soil fungi community in Brazilian soils. Yeast species play an important ecological role; they participate in processes such as decomposition of organic matter, soil aggregation, nutrient cycling in the soil (e.g., phosphate solubilization), biocontrol of soil pathogens, and plant growth promotion (Botha, 2011). Yeasts species occur in a wide range of different types of soil in the world (Stringini et al., 2008; Mestre et al., 2011; Yurkov et al., 2012a; Glushakova et al., 2015; França et al., 2016). Our knowledge about soil yeasts is biased towards temperate and boreal forests. Data from Africa, the Americas, and Asia are scarce, and the forest soils in the Southern hemisphere are strongly undersampled (Yurkov, 2018). Indeed, there are only a few studies which focused on soil yeast in Southern Hemisphere (e.g., Mestre et al., 2014). To the best of our knowledge, there is only one study in Brazil showing the soil yeast occurrence in both Atlantic forest and Neotropical savanna biomes (Moreira and Vale, 2018). The study demonstrates that the *Saitozyma podzolica* is a typical component in Brazilian soils, and its distribution is associated with soil properties, mainly with aluminum content.

Ecological factors are considered the main determinants of soil microbial community composition (Yurkov, 2017). The composition of the plant community, geographic distance, abiotic factors, soil physicochemical properties, and altitudinal variation are the main determinants of fungal distribution in the soil (Jain and Pandey, 2016; Chen et al., 2017). Habitats characterized by altitudinal gradients show dramatic changes in these ecological factors over short geographic distances (e.g., Siles and Margesin, 2016). Several studies have shown that fungal community composition in large altitudinal scale responds differently to climate conditions, plant, and soil properties (Meng et al., 2013; Jain and Pandey, 2016; Siles et al., 2017; Ren et al., 2018). It is important to point out that these authors focused only on soil filamentous fungi, whereas the distribution and dispersion abilities of yeast fungi can be different. With regard to the effect of altitude on soil yeast, França et al. (2016) observed that the yeast species composition altered along an altitude gradient in Alpine forest soils during the spring season. However, the Brazilian forests differ substantially from Subalpine forest community in terms of species diversity, climate, and seasonality. The altitude-specific effects on yeast species in soils of the Brazilian floristic domain are not yet known.

Representative of the humid tropical and subtropical rainforest biome, the Brazilian Atlantic forest is a hotspot and supports one of the highest numbers of richness and endemic species for flora and fauna on the planet (Myers et al., 2000; Ribeiro et al., 2009). Originally, it covered around 1,500,000 km² (Ribeiro et al., 2009); however, it is currently restricted to 98,800 km² of remnants forest, which is about 7 % of its original size (Morellato and Haddad, 2000). The state of Rio de Janeiro is entirely included in this Brazilian floristic domain and harbors some important remnants of the northern part of *Serra do Mar* mountain chain (Ayres et al., 2005). This region has an interesting altitudinal gradient that extends from 200 to 2,263 m of altitude. The heterogeneous environmental conditions and geographical characteristics favor a high diversity, flora and fauna endemism, and the occurrence of many species that require scientific description (Ribeiro et al., 2009), especially for the underexplored soil yeast species.

This study aimed to describe the yeast species over different altitudes in the Brazilian forest domain. Our hypothesis is that the altitudinal gradient affects the distribution of yeast species and strains. We addressed the following questions: (1) Which altitude exhibited the highest culturable yeast species richness? (2) Is there any relationship between the intraspecific distribution of polymorphisms, based on gene genealogy, and altitudinal gradient?

MATERIALS AND METHODS

Study area and sampling strategy

The study area belongs to the eastern coast of the Brazilian forest domain, in *Serra do Mar* mountain chain. The sampling was performed at the *Parque Nacional da Serra dos Órgãos* (PARNASO) in the state of Rio de Janeiro. The climate is super humid tropical (Cwb - subtropical highland climate, according to Köppen classification system), with average annual temperature ranging from 13-23 °C (reaching values of 38 to -5 °C in the highest parts). Rainfall ranges from 1,700 to 3,600 mm, with a concentration of rain in the summer (December to March) and a dry period in the winter (June to August). The soils in the sampled sites present great diversification, Ferrassols (*Latossolos*), and Lithosols (*Neossolos Litólicos*) in small areas of higher altitudes.

The predominant vegetation type is the montane rain forest, with submontane, ranging from 300 to 700 m; lower montane, from 700 to 1,100 m; upper montane, higher than 1,100 m above the sea level (Oliveira-Filho and Fontes, 2000); and high-altitude grasslands (*Campos de Altitude*), where altitude is higher than 1,800 m (Safford, 1999).

The samples were collected in June 2015. To obtain an altitudinal gradient, four different altitudes were established by vegetation type, namely: altitude 1 (404 m - submontane forest); altitude 2 (1,016 m - lower montane forest); altitude 3 (1,658 m - upper montane forest); and altitude 4 (2,124 m - high altitude grasslands) (Figure 1). Four sample points

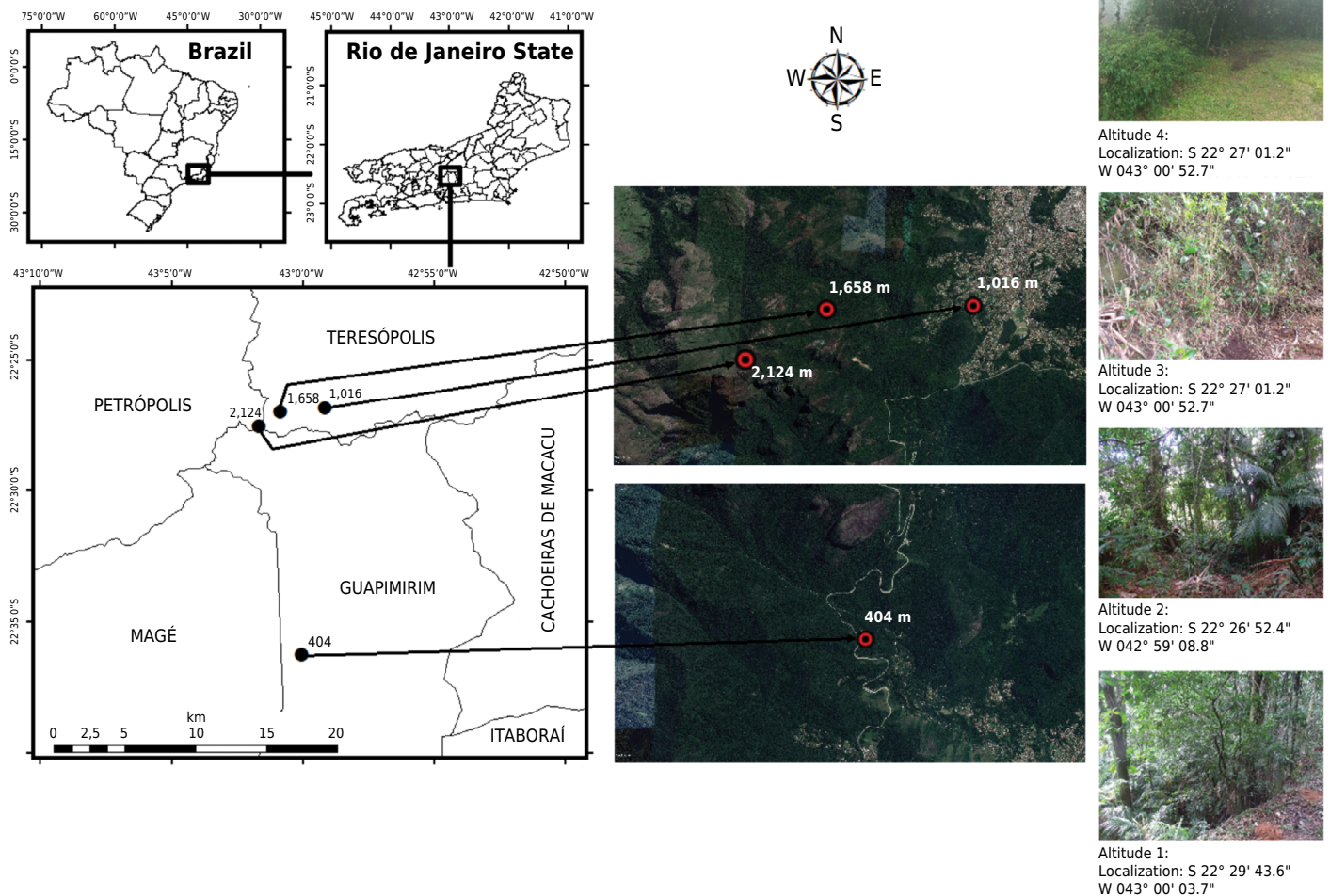


Figure 1. Geographical location of the PARNASO in the state of Rio de Janeiro, Brazil; and the four collecting points and their respective altitudes ranging from 404 to 2,124 m.

were marked by altitude, totaling 16 composite samples (4 sample points × 4 altitudes). At each sample point, three simple samples were collected in a layer of 0.00-0.20 m, and homogenized to obtain a representative composite sample. Soil samples were placed in sterile paper bags and transferred to the Mycology Laboratory at Universidade de Brasília (UnB, Brasília, Brazil) for further analyses.

Soil characterization

The physicochemical analyses of soil samples were performed in the Laboratory of Soil Analysis, Vegetable Tissue and Fertilizer at Universidade Federal de Viçosa (UFV, Minas Gerais, Brazil), according to recommendations from Empresa Brasileira de Pesquisa Agropecuária (Donagema et al., 2011). The content of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), exchangeable acidity (H+Al), organic matter (OM), sand, silt, and clay was evaluated in those analyses. These results were used to calculate the following parameters: sum of exchangeable bases (SB), cation exchange capacity at pH 7.0 (T), effective cation exchange capacity (t), aluminum saturation index (m), and base saturation index (V) (Donagema et al., 2011).

The physical-chemical properties of the soils were submitted to analysis of variance and the means were compared by the Scott-Knott test at 5 % probability using the Assisat 7.7 statistical program (Silva and Azevedo, 2016).

Yeast isolation and molecular identification

From each soil sample, aliquots of 10 g were homogenized in 90 mL of 0.1 % peptone water (w/v). The suspension was vortexed using a shaker for one hour at 200 rpm. All soil samples were analyzed in three replicates and each of them was used to produce three 10-fold dilutions (10^{-1} , 10^{-2} , and 10^{-3}). Then, the dilutions were plated onto YM agar (0.3 % malt extract, 0.3 % yeast extract, 0.5 % peptone, 1 % glucose, and 2 % agar). Chloramphenicol ($100 \mu\text{g mL}^{-1}$) and 0.25 % sodium propionate (w/v) were added in the culture medium to inhibit bacterial and filamentous fungi growth, respectively. The plates were examined periodically and the grown colonies were purified and maintained in YM agar. Inoculation depletion isolation was used to obtain pure cultures from the colonies grown. The pure cultures were stored in 25 % glycerol in a freezer at $-80 \text{ }^{\circ}\text{C}$, and then deposited in Yeast Culture Collection of the Department of Plant Pathology at Universidade de Brasília (UnB, Brasília, Brazil).

Extraction of DNA was performed from the cell precipitate obtained by centrifugation of yeast culture grown in YM broth. Cells were lysed using extraction buffer (Tris-HCl 200 mmol L^{-1} , NaCl 250 mmol L^{-1} , EDTA 25 mmol L^{-1} pH 8, SDS 0.5 %, Triton X-100). Proteins were precipitated with chloroform: isoamyl alcohol (24:1) and subsequently centrifuged for 10 min at $16,000 \text{ g}$. The DNA was precipitated with isopropanol for one hour at $-20 \text{ }^{\circ}\text{C}$. The DNA samples were centrifuged and washed with 70 % ethanol (Kurtzman and Robnett, 1998). The DNA was then suspended in $30 \mu\text{L}$ of milli-Q water and stored at $-20 \text{ }^{\circ}\text{C}$.

The D1/D2 domain of the LSU rRNA gene was amplified by PCR using the following universal primers: NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') to identify ascomycetes and basidiomycetes (Kurtzman and Robnett, 1998; Fell et al., 2000). Amplification reactions were carried out with the PCR conditions: initial denaturation at $94 \text{ }^{\circ}\text{C}$ for 3 min; followed by 33 cycles of denaturation at $94 \text{ }^{\circ}\text{C}$ for 1 min, annealing at $56 \text{ }^{\circ}\text{C}$ for 45 s and extension at $72 \text{ }^{\circ}\text{C}$ for a 2 min; and a final extension at $72 \text{ }^{\circ}\text{C}$ for 7 min. The PCR products were purified using a USB[®] ExoSAP-IT[®] Kit (Affymetrix[®]) according to the manufacturer's instructions. The purified PCR products were sequenced at Universidade Católica de Brasília (UCB, Brasília, Brazil), using the sequencer ABI 3130xl Applied Bio systems, according to Sanger methodology (Sanger et al., 1997). We used the program Sequencher 4.8 to edit the sequences. For species identification, the nucleotide

sequences were compared with data deposited in the NCBI using the BLAST(n) algorithm (Altschul et al., 1990). The sequences were aligned and the molecular phylogenetic analysis was inferred by the maximum likelihood method in the MEGA 7 software (Kumar et al., 2016). The sequences of the isolates were deposited in GenBank (www.ncbi.nih.gov), with the access codes sequence: MK626569 to MK626644.

Multivariate and haplotype network analyses

Principal Component Analysis (PCA) was performed using a correlation matrix in which the values of soil physical-chemical composition were used to identify similarities between the samples that were collected at different altitudes. The analyses were performed in the R software (R Core Team, 2017) using the 'FactoMineR' package (Lê et al., 2008).

Intraspecific diversity of a few common yeast species was assessed using a haplotype network analysis. Therefore, we used the sequences from the most frequent species *Saitozyma podzolica* that was isolated from all altitudes (Table 1). The alignment was performed using the Geneious Alignment tool available in the Geneious Prime 2019.0.4 program. The DnaSP 5.10 program (Librado and Rozas, 2009) determined the haplotypes in the alignment and inferred the diversity of the sequences. Based on coalescent theory, the NETWORK 4.5.1.6 program inferred the gene genealogy using the Median Joining haplotype network (Bandelt et al., 1999).

RESULTS

Yeast species inventory

Table 1. Inventory and occurrence frequency (%) of yeast species isolated at four altitudes in the PARNASO, Rio de Janeiro, Brazil

Yeast species	GenBank closest	Total isolates	Number of isolates			
			Altitude 1	Altitude 2	Altitude 3	Altitude 4
Basidiomycota						
Tremellales						
<i>Saitozyma podzolica</i>	KU316780	46	9 (64 %)	16 (73 %)	17 (85 %)	4 (20 %)
Trichosporonales						
<i>Apiotrichum porosum</i>	AB726863	11			1 (5 %)	10 (50 %)
<i>Apiotrichum</i> sp.	EF653947	1		1 (4.5 %)		
Leucosporidiales						
<i>Leucosporidium scottii</i>	AB726968	1			1 (5 %)	
<i>Leucosporidium golubevii</i>	AY212997	1				1 (5 %)
Cystofilobasidiales						
<i>Cystofilobasidium infirmominiatum</i>	DQ645523	1				1 (5 %)
Holtermanniales						
<i>Holtermanniella wattica</i>	FN428959	1				1 (5 %)
Ascomycota						
Saccharomycetales						
<i>Meyerozyma guilliermondii</i>	AB773379	11	5 (36 %)	4 (18 %)	1 (5 %)	1 (5 %)
<i>Candida natalensis</i>	AB436394	1		1 (4.5 %)		
<i>Nadsonia starkeyi-henricii</i>	FR716592	2				2 (10 %)
Total		76	14	22	20	20

A total of 76 strains were isolated in the present study. They represented ten yeast species (Table 1). We did not count the Colony Forming Units (CFUs) due to the low number of yeast colonies per plate seeded (<15). This low number of yeast colonies per plate was observed in samples from all collecting points.

The Basidiomycota phylum was predominant, with 62 isolates belonging to 7 species. The order Tremellales represented the largest number of strains with the *Saitozyma podzolica* species representing the most frequent (46 strains). The Ascomycota phylum had 14 isolates representing 3 species. All ascomycetous species belonged to the order Saccharomycetales, and *Meyerozyma guilliermondii* species representing the most frequent (11 strains) (Table 1).

The altitude 1 and altitude 2 showed similar species composition, sharing two species between them. The altitude 3 represented a transition among the altitudes, sharing species either from altitude 2 or from altitude 4. Altitude 4 presented the highest species richness among the altitudes, harboring seven species with four unique species for this altitude (Figure 2).

Saitozyma podzolica and *M. guilliermondii* were isolated at all altitudes indicating their wide distribution. *Apiotrichum porosum* was isolated only at high altitudes, i.e., altitudes 3 and 4. Some yeast species were isolated only at one of the altitudes. Specifically, four species (*L. golubevii*, *C. infirmominiatum*, *H. wattica* and *N. starkeyi-henricii*) were recovered solely at altitude 4, two species (*Apiotrichum* sp. and *C. natalensis*) at altitude 2, and one species (*L. scottii*) at altitude 3 (Figure 2).

Soil characterization

The sample soils of altitudes 1, 2, and 4 analyzed in this study were sandy clay, while the one from altitude 3 was sandy/sandy clay according to the textural classification of the Brazilian Soil Science Society (SBCS). In addition, all soil samples were slightly acid and had similar nutrient contents for K and Ca. On the other hand, Mg and Al contents were different among the samples at altitude 1 and altitude 4. The P contents had the highest values at altitude 4. The soil parameters OM, t, T, V, m, and H+Al had relative differences among the four altitudes (Table 2).

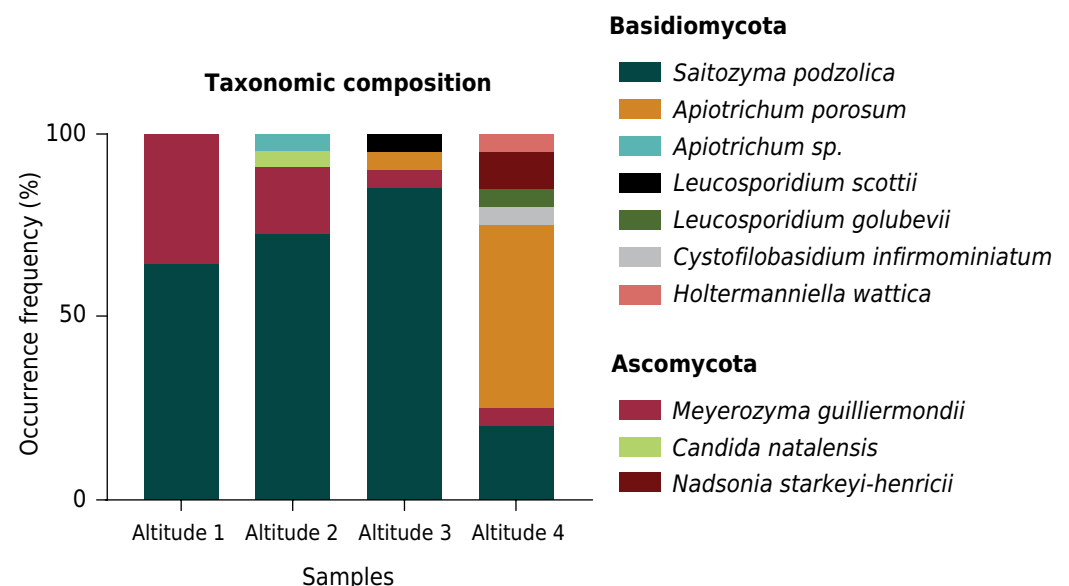


Figure 2. Taxonomic composition of soil yeast species obtained at four altitudes (404; 1,016; 1,658; and 2,124 m) in the PARNASO.

Table 2. Mean values of the chemical and physical properties of the soils at four altitudes in the PARNASO, Rio de Janeiro, Brazil

Property ⁽¹⁾	Altitude 1	Altitude 2	Altitude 3	Altitude 4
pH(H ₂ O)	4.54	4.61	4.58	4.67
P (mg dm ⁻³)	2.02 b	4.07 ab	8.07 ab	31.80 a
K (mg dm ⁻³)	48.75 a	39.50 a	51.25 a	42.75 a
Ca ²⁺ (cmol _c dm ⁻³)	0.45 a	0.23 a	0.45 a	0.35 a
Mg ²⁺ (cmol _c dm ⁻³)	0.16 a	0.08 b	0.15 a	0.10 ab
Al ³⁺ (cmol _c dm ⁻³)	1.30 b	1.70 ab	1.70 ab	2.05 a
H+Al (cmol _c dm ⁻³)	5.30 c	7.40 b	13.02 a	11.47 a
SB (cmol _c dm ⁻³)	0.73 a	0.41 a	0.73 a	0.57 a
t (cmol _c dm ⁻³)	2.03 b	2.11 b	2.43 ab	2.62 a
T (cmol _c dm ⁻³)	6.03 b	7.81 b	13.76 a	12.04 a
V (%)	12.10 a	5.35 b	5.27 b	4.90 b
m (%)	64.12 b	80.12 a	70.10 ab	78.07 a
OM (kg dag ⁻¹)	1.88 b	3.19 b	8.33 a	8.48 a
Rem-P (mg L ⁻¹)	25.87 a	21.92 ab	10.02 c	16.37 bc
Coarse sand (kg kg ⁻¹)	430	470	450	450
Thin sand (kg kg ⁻¹)	150	140	170	150
Silt (kg kg ⁻¹)	130	140	140	150
Clay (kg kg ⁻¹)	290	250	240	250

⁽¹⁾ pH in water at a soil:solution ratio of 1:2.5; Ca²⁺, Mg²⁺, and Al³⁺: extractor KCl 1 mol L⁻¹; H+Al: extractor SMP; SB: sum of bases; T: cation exchange capacity at pH 7.0; t: effective cation exchange capacity; V: bases saturation; m: aluminum saturation; Rem-P: remaining phosphorus; OM: organic matter, oxidation Na₂Cr₂O₇ 2 mol L⁻¹ + H₂SO₄ 5 mol L⁻¹; P, K, Fe, Zn, Mn, Cu: Mehlich-1 extractor; S: extractor Monocalcium Phosphate Acetic Acid; B: hot water extractor. Means followed by the same letter in the columns do not differ from each other. The Scott-Knott test was applied at 5 % probability.

Principal component analysis showed the association of environmental characteristics and distribution of soil samples at each site, with axes 1 and 2 explaining 88.6 % of the effects of these variables (Figure 3). The soil samples were distributed into four groups. We verified a pattern of sample distribution and the influence of each variable. Altitude 2 was more similar to altitude 1 and 3 and was positively correlated with the properties m, V, and P-rem. Altitude 4 was similar only to altitude 3 and was positively correlated with the properties t, T, OM, H+Al, and Al.

Gene genealogy

A total of 45 sequences of *S. podzolica* were included in the analysis. The alignment of the D1/D2 domain of the LSU rRNA gene contained 540 positions. One sequence from altitude 1 was removed from the analysis due to its shorter size (513 bp). For the analysis, we did not consider missing data and neither sites with gaps. We observed five substitutions, three transitions, and two transversions in the alignment. Among 45 isolates, four haplotypes were observed and they were subdivided into two genealogical lineages separated by four mutation steps (Figure 4a). One lineage, depicted in green, occurred in the first three altitudes (1, 2, and 3), but especially in the lower altitudes 1 and 2 (Figure 4b). The second lineage, depicted in red, appeared in altitudes 2, 3, and 4, and contrarily to the first lineage, its frequency was greater in the higher altitudes (3 and 4). Interestingly, this admixture takes place in the middle altitudes. The haplotype H1 was the most frequent (n = 24) and it occurred in altitudes 1, 2, and 3, while H2 (n = 1) only in altitude 2. The haplotype H3 was the second more frequent (n = 19) and appeared in altitudes 3 and

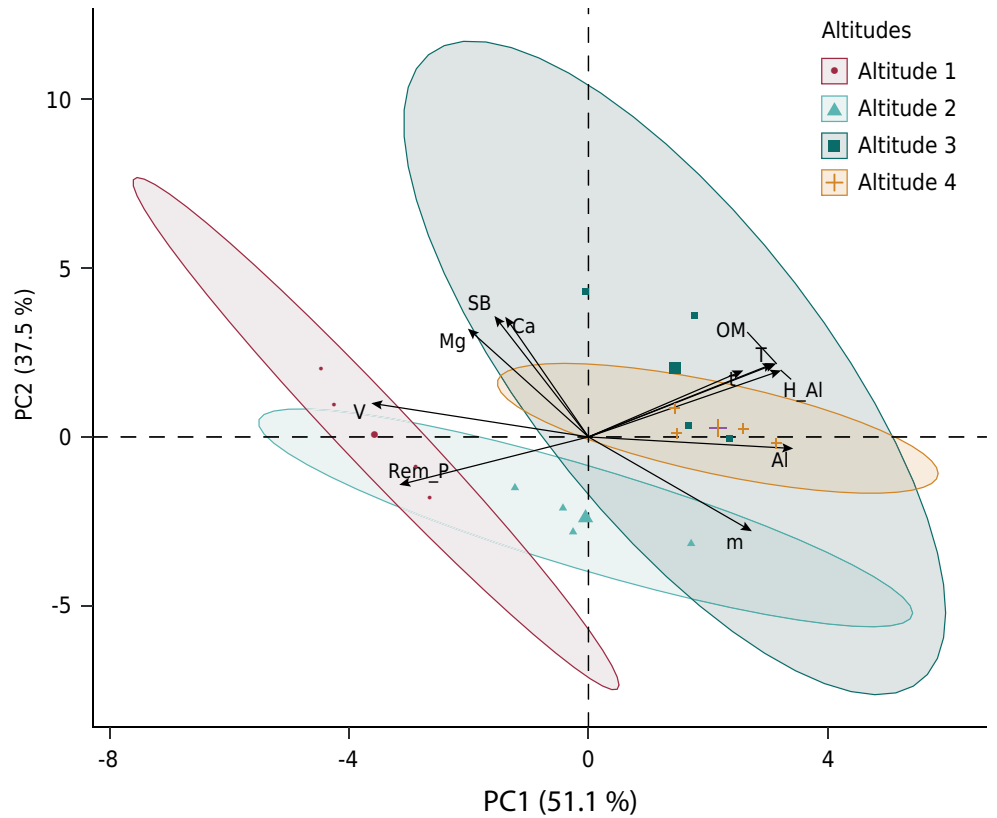


Figure 3. Principal component analysis using data for physicochemical soil properties in four different altitudes of the PARNASO. Soil parameters: Mg: magnesium; Ca: calcium; Al: aluminum; m: aluminum saturation index; H+Al: exchangeable acidity; Rem_P: remaining phosphorus; OM: organic matter; SB: sum of exchangeable bases; T: cation exchange capacity at pH 7.

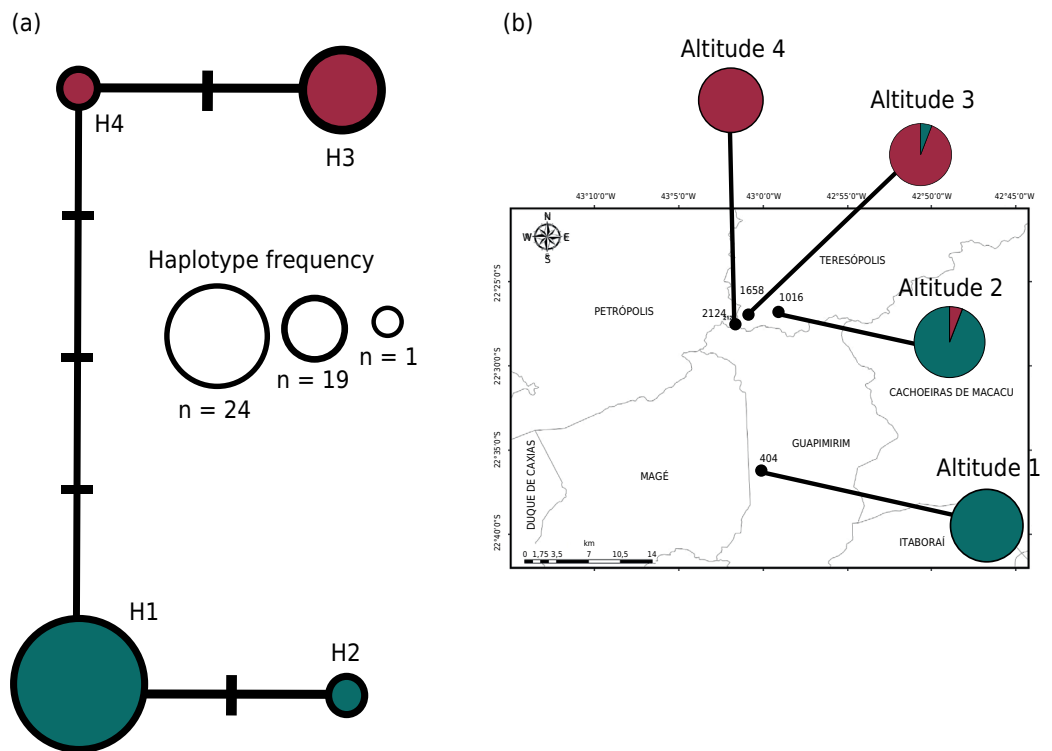


Figure 4. Median-joining network of the four haplotypes of *Saitozyma podzolica*. (a) The four haplotypes (H1, H2, H3, and H4) belong to two genealogical lineages, one depicted in green, and another one depicted in red. The circle sizes are proportional to the relative frequencies, major circle (n = 24), medium circle (n = 19), and small circles (n = 1). The number of mutation steps is indicated with small bars when more than one step occurs. (b) The distribution of the two genealogical lineages along the altitudinal gradient (altitudes 1, 2, 3, and 4).

4, while the H4 (n = 1) only in altitude 4. The D1/D2 domain gene genealogy indicated that strain diversity is likely related to similar altitudes.

DISCUSSION

The soil yeast species composition differed along the altitude gradient in the Brazilian forest domain. Basidiomycetous yeasts were found more often than ascomycetes in the studied soils, which is in agreement with previous reports on yeast species from forest soils (Sláviková and Vadkertiová, 2000; Stringini et al., 2008; Pimenta et al., 2009; Yurkov et al., 2012a; Mestre et al., 2014; Moreira and Vale, 2018). It has been suggested that basidiomycetous yeasts may be dominant in forest soils due to strategies and adaptations such as the production of polysaccharide capsules and stronger ability to use a wide range of carbon sources, including complex compounds (Vishniac, 2006; Connell et al., 2008; Yurkov 2018).

A total of ten yeast species were identified in 16 soil samples collected along an altitudinal gradient in the Brazilian forest domain (Table 1). Although the Brazilian Atlantic Forest is a recognized biodiversity hotspot (Myers et al., 2000) and is characterized by a high proportion of endemic vascular plant species, we did not detect endemic or new yeast species in the studied soil samples. A greater sampling effort and application of independent cultivation techniques are necessary to access more deeply the diversity in this ecosystem since this study already reports the presence of yeast species.

Yeast species that were found in the Brazilian forest domain are commonly reported in soil samples worldwide. The most frequent species in this study, *Saitozyma podzolica*, is a typical inhabitant of acid soils with high organic matter content, moisture, and sometimes the presence of aluminum and iron oxides (Buzzini and Yurkov, 2017; Yurkov, 2018). The occurrence of *S. podzolica* in soils has been well positively correlated with high rainfall values, low soil pH, and dissolved carbon compounds (Vishniac, 2006; Yurkov et al., 2012b). In our study, the aluminum content, aluminum saturation index (m), and base saturation index (V) likely favor the occurrence of *S. podzolica* in all samples, according to the PCA, mainly at altitudes 1 and 2 (Figure 3).

The second most frequent species in this study, *Meyerozyma guilliermondii* has been reported previously in soil substrates (Mokhtarnejad et al., 2015; Yurkov et al., 2016; Glushakova et al., 2017; Tepeeva et al., 2018). This yeast has also been isolated from food and other environmental niches, indicating its ubiquitous distribution (Corte et al., 2015), especially in phylloplane (Limtong and Koowadjanakul, 2012; Limtong and Kaewwichian, 2014; Nasanit and Krataithong, 2015; Sperandio et al., 2015). Some *M. guilliermondii* strains are notably able to grow under rocks due to their ability as phosphate-solubilizing soil yeast (Nakayan et al., 2013; Sarabia et al., 2018), contributing to weathering processes, soil formation, and solubilization of insoluble nutrients to the soil microbial community (Botha, 2006). Indeed, the rocks are one of the main reservoirs of phosphorus (Bini and Lopez, 2016). Notably, *M. guilliermondii* was more frequent at altitudes 1 and 2 (Figure 2), where soil samples were mainly influenced by the remaining phosphorus content (P-rem) (Figure 3).

Our results show that altitude 4 presented the highest species richness. This altitude has a phytophysiology known as high-altitude grasslands (*Campos de Altitude*), whose vegetation is characterized as a mosaic of shrubs and small copses of short trees that often develops on rocky outcrops with intense solar radiation, and low temperatures (Safford, 1999; Cronenberger and Viveiros-de-Castro, 2007). It is completely different from the vegetation types found at other altitudes. This set of conditions may favor the emergence of yeast species adapted to this environment (Figure 2). Remarkably, four species (*Leucosporidium golubevii*, *Cystofilobasidium infirmominiatum*, *Holtermanniella wattica*, and *Nadsonia starkeyi-henricii*) were isolated only at this altitude. Interestingly,

S. podzolica and *M. guilliermondii*, the most frequent species at other altitudes, displayed low frequency at altitude 4 (Table 1).

Here we point out some ecological factors that may have contributed to the presence of species detected at altitude 4. Due to the low vegetation in altitude 4, soil microorganisms are more exposed to UV radiation. Notably, *Cystofilobasidium infirmominiatum*, a species identified herein, is part of the group of yeast species known as pigmented (reddish-pink yeasts). The presence of pigment has been associated with tolerance to UV radiation in habitats, like phylloplane (Fonseca and Inácio, 2006), which are exposed to high UV radiation (Libkind et al., 2009). This could be a strategy for survival of these species in adverse environments, exposed to UV radiation due to high altitudes and low vegetation, such as found at altitude 4.

Low temperature in high-altitude grasslands is another environmental factor that may have influenced the presence of some yeast species at altitude 4. The average annual temperature in the park varies from 13 to 23 °C and may reach -5 °C in the highest sites (Oliveira-Filho and Fontes, 2000). Thus, the occurrence of the psychrotolerant species (as *L. scottii*, *L. golubevii*, *C. infirmominiatum*, and *N. starkeyi-henricii*) only in higher altitudes seems to be related to those conditions (Deak, 2006). Species of the genus *Leucosporidium* have been frequently isolated from cold environments such as Antarctic soils and lakes (Vishniac, 2006; Connell et al., 2008; Vaz et al., 2011), and are common inhabitants of low-temperature environments, such as the polar regions, or temperate regions during cold seasons (Vaz et al., 2011). Beyond that, *Leucosporidium* genus harbor species that are potential sources of cold-active enzymes and have the ability to degrade phenol and phenolic compounds (Buzzini et al., 2012; García et al., 2015), pointing to an ecological role of species in the soil. Moreover, *H. wattica* (García et al., 2007; Branda et al., 2010), *C. infirmominiatum*, and *A. porosum* (Mestre et al., 2014), which have often been related to low-temperature environments, were isolated only at altitude 4.

It is noteworthy that *A. porosum* was the species with the highest frequency at altitude 4. *Apiotrichum porosum* also was one of the species with the highest relative frequency in samples of Patagonian forest soils, whose annual average temperature is 10 °C, reaching values up to -20 °C (Mestre et al., 2014). This yeast is a typical soil species able to degrade hemicellulose and phenolic compounds. This ability enables the assimilation of alternative sources of nutrients, which may be beneficial for survival in soils with adverse conditions, as low temperatures and low availability of nutrients and water (Mestre et al., 2014). In addition, *S. podzolica* presents the ability to assimilate typical plant compounds by actively participating in the mineralization of plant material on soils (Middelhoven et al., 2001; Yurkov et al., 2012b). Furthermore, *S. podzolica* and *A. porosum* are known as oleaginous yeasts because of their ability to produce lipids suitable for various industrial applications (Schulze et al., 2014).

The analysis of polymorphisms of the D1/D2 region in the strains of the most frequent species demonstrates the effect of altitude on the intraspecific distribution of *S. podzolica*. The haplotype network showed that several strains (haplotypes) of *S. podzolica* inhabit soils at different altitudes (Figure 4). Genetically closer haplotypes were found at the same altitudes. Here, strains at altitudes 1 and 2 were genetically closer to each other (despite the spatial separation of sampling sites, see Figure 1) than to haplotypes from higher altitudes. Likewise, the similarity of haplotypes at altitudes 3 and 4 was higher compared with two other sampling sites. This separation of haplotypes mirrored the separation of soil samples observed in the PCA (Figure 3). We suggest that the genetic differences found in *S. podzolica* strains may be associated with the altitudinal gradient, changes in vegetation type and soil properties. Indeed, the soil properties from altitude 3 and 4 were different from those at altitudes 1 and 2 (Table 2). Overall, habitats with similar properties harbor a similar yeast species (Yurkov, 2017). Additionally, altitude





3 exhibited a genetic admixture in the *S. podzolica* sequences, which is consistent with the transition feature of the yeast species composition observed in figure 2.



Although the amount of species detected in this study is not large enough to discuss the effects of altitude and vegetation on the soil yeast, our observations indicate that there is a relationship between yeast species and altitudinal change. This is more evident when we look toward the intraspecific distribution detected in *S. podzolica* strains. To the best of our knowledge, this is the first study that evaluated the influence of an altitudinal gradient on the distribution of yeast species in the Brazilian Atlantic forest soils. Further studies are needed with a greater sampling effort to understand the distribution of these species in Brazilian soils. Taken together, our work highlights the importance of studies to describe yeast species, as well as to elucidate their distribution in areas of extreme biological importance. Herein, PARNASO contains one of the largest remnants of the Atlantic Rainforest.




CONCLUSIONS

Soil yeast from the higher altitude exhibited greater richness, with an increased diversification from lowland to highland. This richness is likely related to changes in the type of vegetation (lower montane rain forest to high-altitude grasslands). Furthermore, the gene genealogy of *Saitozyma podzolica* suggests genetic proximity among closer altitudes, which may be related to soil physicochemical properties.



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