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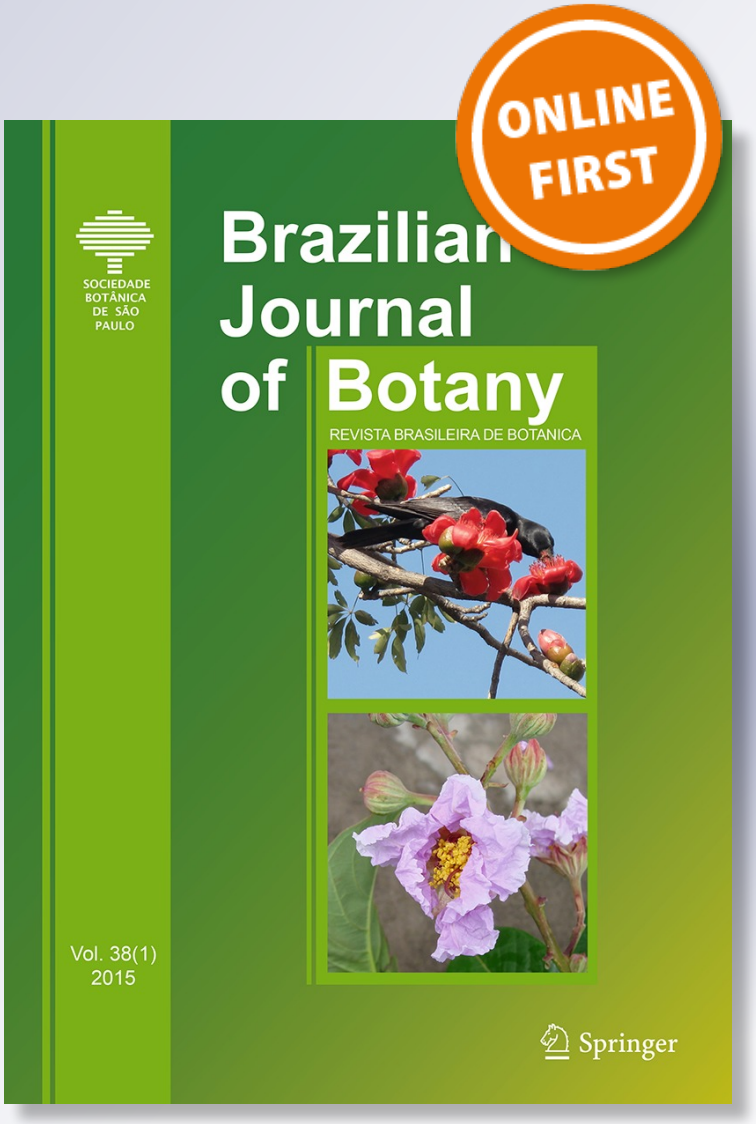

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Diversity of Mucorales in natural and degraded semi-arid soils

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Abstract Mucorales comprises fungi commonly isolated from soil, herbivore dung, and plant debris. Surveys in the semi-arid northeastern region of Brazil revealed 20 species of Mucorales, although this does not reflect the potential richness of these fungi in the Caatinga, the main domain of the region. Therefore, the aim of the present study was to compare the richness, diversity, frequency, and distribution of Mucorales from natural and degraded semi-arid soils of the Catimbau National Park, Pernambuco State, Brazil, and to provide a taxonomic key for the species found in the studied soils. Six samplings were performed in three natural areas with the following vegetation types: Carrasco, Sandy Caatinga, and Caatinga *s.s.,* as well as in an area of Caatinga *s.s.* under anthropic use. Thirteen taxa of *Absidia, Cunninghamella, Gongronella, Lichtheimia, Mucor, Rhizopus,* and *Syncephalastrum* were identified. Both richness and diversity were distinct among vegetation types. *Rhizopus microsporus* was the most common species. Soils from Carrasco exhibited a highest diversity and richness. The anthropic effects explain the low richness and diversity found on Caatinga *s.s*. Two species were reported for the first time in the Caatinga domain and a new species of *Absidia* was recorded.

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Introduction

Mucorales are characterized by the production of zygosporangia, a structure of sexual origin, bearing the zygospore, formed by the fusion of two gametangia of equal or unequal size and shape (Kendrick 2000). This is the largest order within the sub-phylum Mucoromycotina (Hibbett et al. 2007), with representatives usually isolated from substrates such as soil, dung, and plant material, decomposing organic matter and so performing nutrient cycling, or living as parasites of plants, animals and other fungi (Hill et al. 2000). Most Mucorales exhibit rapid growth, even in simple culture media, and are the first to colonize the substrate, degrading both simple and complex sugars (Richardson 2009), including pectins, lipids, proteins, and hemicelluloses (Domsch et al. 2007), being the latter the major constituent of lignocellulosic biomass, a fundamental part of plant cell walls (Scheller and Ulvskov 2010). Due to the ability of some Mucorales to produce several enzymes and organic acids, some species of this order have been used in different biotechnological process (Santiago and Souza-Motta 2006; Hoffmann et al. 2013).

The diversity of Mucorales is poorly studied in the soils of semi-arid ecosystems (Abdel-Hafez 1982; Abdullah and Al-Bader 1990; Grishkan and Nevo 2010), particularly in Brazil where only 20 species have been reported, which probably does not reflect the real richness of the group in this country (Santiago 2014). Caatinga is an exclusively Brazilian domain in the semi-arid region which includes several endemic species, with rich biodiversity and

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heterogeneous types of vegetation throughout the territory (Drumond et al. 2002). It is mainly composed of xerophytic vegetation, with diverse floristic composition (Andrade-Lima 1981), such as Caatinga *s.s.*, Sandy Caatinga and Carrasco (Rodal et al. 1998).

The Caatinga is being reduced due to agricultural expansion, which has caused an ecological imbalance, reducing the potential of soil and forest ecosystems (Alves et al. 2009). Environmental changes could be indicated by knowledge of the Mucorales in this community, estimated by variations of these organisms in impacted areas (Turco et al. 1994; Santiago et al. 2013). Considering the ecological importance of Mucorales in this community, the aim of the present study was to determine and compare the richness, diversity, frequency, and distribution of species of soils from natural and degraded ecosystems of the Caatinga in Pernambuco State. Additionally, a taxonomic key for the Mucorales present in these ecosystems was produced, thereby contributing to a better understanding of these fungi in the semi-arid northeastern region of Brazil and increasing their registers for the Neotropics.

Materials and methods

Study areas

The study areas are in the Catimbau National Park (8°31′55.8″S, 37°15′34.2″W), located between the hinterland and the wasteland of Pernambuco State, 285 km far from the capital Recife, covering the municipalities of Buíque, Ibimirim, Inajá and Tupanatinga. The mean annual temperature is 25 °C with rainfall of 1.060 mm per year. The rainy season lasts from April to July and the dry season is from August to March (Silva et al. 2011). The vegetation in the park varies from thorny bushes to non-thorny woods, with characteristic plants of Caatinga in areas of 'Carrasco', Caatinga *s.s.* and Sandy Caatinga (Andrade et al. 2004).

Carrasco is the term used to describe bushy vegetation without thorns of Caatinga in stony soils. It is found between 900 and 1000 m altitude and is composed by several species, such as *Banisteriopsis stellaris* Gates, *Buchenavia capitata* Eicheler, *Cnidosculus vitifolius* Pohl, *Eugenia tapacumensis* Berg, *Pyrostegia venusta* Miers, *Rollinia leptopetal* Safford, *Serjania lethalis* Hill, *Solanum baturitense* Huber, *Waltheria ferruginea* Hil and *Zanthoxylum stelligerum* Turez (Andrade et al. 2004).

Caatinga *s.s.* is characterized by thorny xerophytic vegetation on clay soils. It occurs up to 600 m altitude and the vegetation is composed of the following: *Alibertia rigida* K. Schum, *Arrabidaea corallina* Sandwith, *Guapira laxa* Furlan, *Coutarea hexandra* K. Schum and *Lantana camara* L. (Andrade et al. 2004).

Sandy Caatinga is found between 600 and 800 m altitude. It consists of thorny xerophytic scrub vegetation on sandy sediments and includes the following species: *Arrabidaea corallina* Sandwith, *Bauhinia acuruana* Moric,

Table 1 Mucoralean species isolated from soil of the Catimbau National Park, Pernambuco State, Brazil, and its respective CFU/g of soil

Species	Study areas				
	Caatinga *s.s.*	Anthropized Caatinga	Carrasco	Sandy Caatinga	Total
Absidia cylindrospora var. *cylindrospora* Hagem	4.8×10^3	0.4×10^3	2.4×10^3	6×10^3	13.6×10^3
Absidia sp.	0.4×10^3	2×10^3	2×10^3	3×10^3	7.4×10^3
Cunninghamella elegans Lendn.	4.8×10^3	0	1×10^3	0	5.8×10^3
C. echinulata Thaxt.	0.4×10^3	2.2×10^3	0	0	2.6×10^3
Gongronella butleri (Lendn.) Peyronel and Dal Vesco	0	0	0	0.2×10^3	0.2×10^3
Lichtheimia brasiliensis A. L. Santiago, Lima and Oliveira	0.2×10^3	0	0.8×10^3	0	1×10^3
L. hyalospora (Saito) Kerst. Hoffm., Walther and K. Voigt	0	0	0.6×10^3	0	0.6×10^3
L. ramosa (Zopf) Vuill.	0	0	0.4×10^3	0.2×10^3	0.6×10^3
Mucor circinelloides Tiegh.	0.2×10^3	0	0.4×10^3	0.2×10^3	0.8×10^3
M. hiemalis Wehmer	0	0	0.6×10^3	0.2×10^3	0.8×10^3
Rhizopus arrhizus var. *arrhizus* A. Fisch.	1.6×10^3	1×10^3	1×10^3	0.8×10^3	4.4×10^3
R. microsporus Tiegh.	19.2×10^3	13.2×10^3	8.3×10^3	7.6×10^3	48.3×10^3
Syncephalastrum racemosum Cohn ex J. Schröt.	1.4×10^3	0.4×10^3	2.4×10^3	9.6×10^3	13.8×10^3
Total	33×10^3	19.2×10^3	19.9×10^3	27.8×10^3	99.9×10^3
Species richness	9b	6c	11a	9b	

Values followed by the same letter did not differ in Tukey's test ($p = 0.05$)

Table 2 Total values of colony forming units (CFU) of Mucorales from soils of Caatinga *s.s.*, anthropized Caatinga, Carrasco and Sandy Caatinga in the Catimbau National Park, Pernambuco State, Brazil

Areas	CFU g/soil
Caatinga *s.s.*	33.0×10^3 a
Anthropized Caatinga	19.2×10^3 a
Carrasco	19.9×10^3 a
Sandy Caatinga	27.8×10^3 a

Values followed by the same letter did not differ in Tukey's test ($p = 0.05$)

Table 3 Frequency (*F*i) and distribution (*D*i) of Mucorales isolated from soils of the Catimbau National Park, Pernambuco State, Brazil

Species	*F*i (%)	*D*i (%)
Absidia cylindrospora var. *cylindrospora*	5.90	0.160
Absidia sp.	4.51	0.080
Cunninghamella elegans	2.43	0.069
C. echinulata	1.73	0.031
Gongronella butleri	0.17	0.002
Lichtheimia brasiliensis	0.52	0.011
L. hyalospora	0.34	0.007
L. ramosa	0.34	0.007
Mucor circinelloides	0.34	0.009
M. hiemalis	0.34	0.009
Rhizopus arrhizus var. *arrhizus*	2.08	0.052
R. microsporus	21.70	0.570
Syncephalastrum racemosum	5.03	0.160

Banisteriopsis schizoptera B. Gates, *Caesalpinia microphylla* G. Don, *Chamaesyce thymifolia* Millsp., *Guapira laxa* Furlan, *Lantana camara* L., *Serjania lethalis* Hill, *Solanum baturitense* Huber, *Waltheria ferruginea* Hil, and *Zanthoxylum stelligerum* Turez (Andrade et al. 2004).

Soil samples

Soil samples were collected inside the park in four areas. Three of them were natural areas of Carrasco, Sandy Caatinga, and Caatinga *s.s.* The fourth area contained Caatinga *s.s.* under rural anthropogenic stress, with family farming systems and ranching animals. Collections were performed monthly between August 2012 and January 2013 in the four areas. In each area, eight quadrants of 25 m^2 were randomly distributed, with a minimum distance of 10 m between them. Six soil sub-samples were collected in each quadrant (20 cm deep) using sterilized spatulas, totaling 48 replicates for each area and 192 from the four areas. The samples were placed in plastic bags, kept in polystyrene boxes with ice and taken to the laboratory, where volumes of the six replicates of each quadrant were homogenized, resulting in a composite sample per quadrant, totaling eight composite samples per collection area. In total, 32 composite soil samples were considered for analysis.

Isolation, purification and identification

For each compound soil sample, 5 mg were sprinkled on the surface of three Petri dishes containing wheat germ

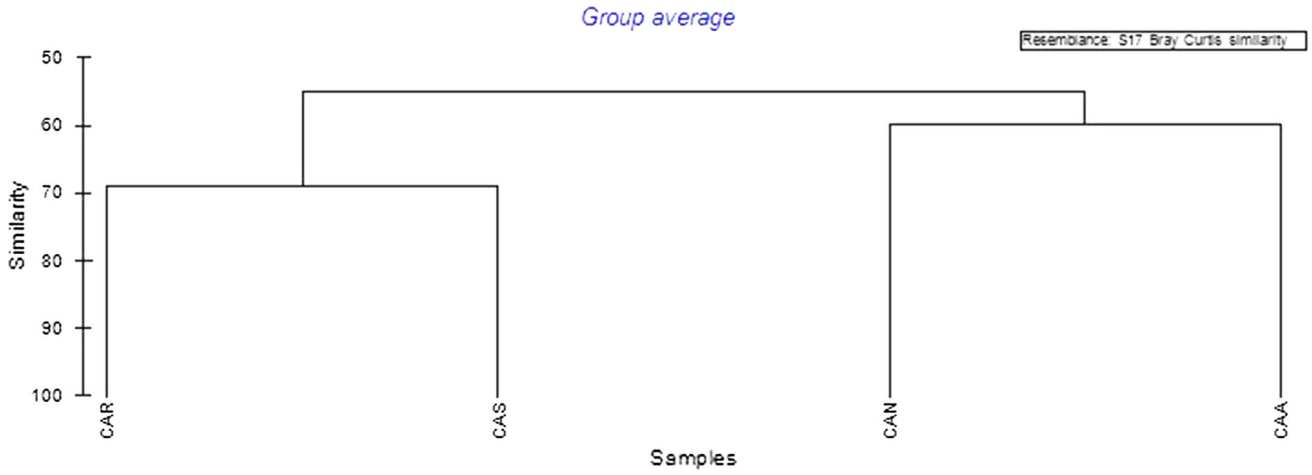

Fig. 1 Dendrogram of Bray–Curtis similarity analysis between Mucorales in soils of Caatinga *s.s.* (CAN), anthropized Caatinga (CAA), 'Carrasco' (CAR), and Sandy Caatinga (CAS) in the Catimbau National Park, Pernambuco State, Brazil

agar culture medium (Benny 2008) plus chloramphenicol (80 mg/L). The plates were left on a bench for 72 h (28 ± 1 °C) under alternating light and dark periods of 12 h. For purification, fragments of colonies were transferred separately to Petri dishes with malt extract agar (O'Donnell 1979) plus chloramphenicol (80 mg/L). Pure cultures were observed in a stereomicroscope (Carl Zeiss Axioscope 40) and a light microscope (Leika EZ4). The Mucorales were identified by analyzing the color, appearance and diameter of colony, and microstructures as described by Benny (1982), Schipper (1978, 1984, 1990), Domsch et al. (2007), Hesseltine and Ellis (1964), Hoffmann et al. (2007), Zheng and Chen (2001) and Zheng et al. (2007).

Ecological analyses

The species frequencies were calculated using the following formula: $Fi = Ji/k$, where Fi = the frequency of species i; Ji = the number of samples in which the species i occurred; k = the total number of soil samples. The species distribution was calculated using the following formula: $Di = (Ni/N) \times 100$, where Di = the distribution of species i; Ni = the number of colony forming units (CFU) of species i; N = the total number of CFU. The species distribution was classified as follows: <0.5 % = rare; >0.5 < 1.5 % = occasional; >1.5 < 3.0 % = common; >3.0 % = abundant (Schnittler and Stephenson 2000). The species diversity was estimated by the Shannon Wiener Index [log2: $H0' = \sum(pi) \times (log2pi)$] using Primer software (Clarke and Gorley 2006).

Statistical analysis

A comparison of species richness between the communities in the studied areas was performed by Analysis of Similarity (ANOSIM Primer v6) in which the matrix of Bray–Curtis similarity was plotted (Clarke and Gorley 2006). Species accumulation curves were also calculated for each area, which enabled an estimate of the total richness in each area using the Chao 1 and Jacknife 1 estimators (Clarke and Gorley 2006). Differences in species richness and CFU/g of soil among areas were evaluated by the Analysis of Variance (ANOVA) and means were compared using Tukey's test ($p = 0.05$) using Statistica for Windows 5.1 (Statsoft 1997).

Results

Thirteen taxa of Mucorales belonging to *Absidia, Cunninghamella, Gongronella, Lichtheimia, Mucor, Rhizopus,* and *Syncephalastrum* were isolated from the collected soils, totalizing 99.9 × 10^3 CFU/g of soil (Table 1). The highest number of CFU/g of soil (33 × 10^3) was found in the Caatinga *s.s.* However, according to the ANOVA and the Tukey's test ($p = 0.05$), no significant differences were found among the soils for CFU (Table 2). Nine taxa were isolated from Caatinga *s.s.*, six from Caatinga *s.s.* under anthropic stress, 12 from Carrasco and 9 from Sandy Caatinga. *Rhizopus microsporus* produced the highest number of CFU/g of soil (48.3 × 10^3), followed by *S. racemosum* (13.8 × 10^3) and *A. cylindrospora* (13.6 × 10^3). *Rhizopus microsporus* was the most common species (Fi = 21.7 %) and was classified as occasional (Di = 0.57 %) in the studied areas, followed by *A. cylindrospora* var. *cylindrospora* (Fi = 5.90 %) and *S. racemosum* (Fi = 5.03 %). *Absidia cylindrospora* var. *cylindrospora*, *C. elegans*, *C. echinulata*, *G. butleri, L. brasiliensis, L. hyalospora, M. circinelloides, M. hiemalis, R. arrhizus* var. *arrhizus* and *S. racemosum* exhibited a low distribution (Di < 0.5 %) and were considered rare in the Catimbau National Park (Table 3).

The similarity between communities of Mucorales was higher in soils of Carrasco and Sandy Caatinga (69.13 %) (Fig. 1). The other areas exhibited less similarity of species: Caatinga *s.s.* and Carrasco = 56 % and Caatinga *s.s.* and Sandy Caatinga = 51.54 %. The similarity of Mucorales between the native and anthropized Caatinga was 60.18 %. The diversity of Mucorales was higher in soils of Carrasco ($H' = 1.89$), followed by Sandy Caatinga ($H' = 1.56$) and native Caatinga *s.s.* ($H' = 1.31$). Soils of the anthropized Caatinga *s.s.* were less diverse ($H' = 1.03$). According to the results of Chao 1 indicators, the expected richness was reached within all areas, while the Jackknife 1 estimated greater richness in areas of Caatinga *s.s.* (11 species) and Sandy Caatinga (14 species).

Identification key for species of Mucorales from the Catimbau National Park

1. Sporangiophores bearing sporangia……...4
1. Sporophores bearing merosporangia or sporangiola over the surface of a fertile vesicle……2
2. Cylindrical merosporangia produced ……*Syncephalastrum racemosum*
2. Pedicellate unispored sporangiola produced……..3
3. Cream to brownish colonies, dark giant sporangiola present in cultures after 7 days of incubation……...*Cunninghamella echinulata*
3. Gray colonies, dark giant sporangiola absent……*C. elegans*
4. Sporangia without apophyses; rhizoids and stolons absent or rare……...5
4. Sporangia with apophyses; rhizoids and stolons present……6
5. Sporangiophores repeatedly branched, with circinate branches and columellae obovoid……..*M. circinelloides*

5. Sporangiophores unbranched or weakly branched sympodially and columellae ellipsoidal……..*M. hiemalis*

6. Apophyses globose; rhizoids and poorly-developed stolons present……..*Gongronella butleri*

6. Apophyses not globose; stolons and well-developed rhizoids present……...7

7. Apophyses slightly evident, sporangiophores arising from aerial hyphae, stolons, or growing opposite to rhizoids……8

7. Apophyses present or absent, sporangiophores arising from stolons, never opposed to rhizoids……..9

8. Simple or branched rhizoids; sporangiophores often reaching 1.5 mm in length; sporangia larger than 100 µm in diameter……*Rhizopus arrhizus* var. *arrhizus*

8. Poorly-developed rhizoids; sporangiophores never exceeding 1 mm in length; sporangia smaller than 100 µm in diameter……*R. microsporus*

9. Subsporangial septa present; cylindrical sporangiospores and giant cells absent ……..10

9. Subsporangial septa rare or absent; globose or ovoid sporangiospores; giant cells present or absent……..11

10. Sporangiophores usually single or 2 in whorls; sporangiospores up to 7.5 µm in length; columellae projection up to 5.75 µm; produces brown pigment on MEA at 25°C……*Absidia* sp.

10. Sporangiophores usually 4 in whorls; sporangiospores up to 5.5 µm in length; columellae projection up to 4.5 µm; brown pigment absent on MEA at 25°C……*A. cylindrospora* var. *cylindrospora*

11. Rough, globose or subglobose sporangiospores exceeding 5.5 µm in length……*Lichtheimia hyalospora*

11. Smooth or slightly rough, sub-globose or ellipsoidal sporangiospores, not exceeding 5.5 µm in length……12

12. Giant cells present, globose; columellae sub-globose or spatulate, some with one or more projections……..……..*L. ramosa*

12. Giant cells absent; short hemispherical, globose, and sub-globose columellae without projections……*L. brasiliensis*

Discussion

The results of this manuscript increase the knowledge of the Mucorales in soils of Caatinga from 20 to 23 taxa. However, the richness of only 13 species appears to be lower when compared with the results of Santiago et al. (2013) that isolated 19 taxa of Mucorales from soils of Caatinga, and Schoenlein-Crusius et al. (2006) and Trufem (1981a, b, c) that reported more than 40 taxa from Atlantic Forest soils in southeastern Brazil. However, the richness of Mucorales from Caatinga was higher when compared to studies from other semi-arid regions. Abdel-Hafez (1982) reported seven species of Mucorales in Saudi Arabia soils, including *C. echinulata*, *M. circinelloides*, *R. arrhizus* var. *arrhizus* (as *R. oryzae*) and *S. racemosum*. Studies regarding soil fungi from Southern Desert of Iraq (Abdullah et al. 1986) reported only five species of *Absidia*, *Actinomucor*, *Mucor,* and *Rhizopus*, including *R. arrhizus*. However, the author suggests that Mucorales have been common in that soil. Grishkan et al. (2009) reported species of Mucorales in soil from Israel, such as *A. cylindrospora* var. *cylindrospora*, *C. elegans*, *C. echinulata*, *G. butleri, L. hyalospora* (as *Mycocladus blakesleeanus*), *M. circinelloides* and *M. hiemalis*, corroborating the results of this manuscript.

Most species isolated from soils of the Catimbau National Park had previously been observed in the semi-arid region of Brazil. Oliveira et al. (2013) isolated *A. cylindrospora, G. butleri*, *R. microsporus, R. arrhizus* var. *arrhizus* (as *R. oryzae*), and *S. racemosum* from a similar Sandy Caatinga (same soil type) in Chapada São José, an area of the same National Park, although the number of CFU/g of soil and species richness were lower than those observed in the present study. The use of different isolation techniques and culture media by the aforementioned authors may explain the differences found. Furthermore *A. cylindrospora*, *C. elegans*, *R. arrhizus* var. *arrhizus* (como *R. oryzae*), *R. microsporus* and *S. racemosum* were cited in soils of Canindé de São Francisco and Jaguararí in Bahia (Cavalcanti et al. 2006; Santiago and Souza-Motta 2006). *Cunninghamella echinulata* was observed in soils of Triunfo and Serra Talhada, while *A. cylindrospora*, *R. arrhizus* var. *arrhizus*, *R. microsporus,* and *S. racemosum* where isolated from soils of Cabrobó and Belém de São Francisco in Pernambuco (Santiago et al. 2013; de Souza et al. 2013), corroborating the results of the present study.

Lichtheimia was represented by three species, but exhibited a low distribution. The species of this genus are able to grow well at high temperatures (Hoffmann et al. 2013) and those isolated in the present study were also mentioned in other soils of Caatinga and are commonly found in semi-arid Brazilian regions (Cavalcanti et al. 2006; Santiago and Souza-Motta 2006; Santiago et al. 2013; Santiago et al. 2014). Thermophilic and thermotolerant fungi are detected over areas receiving highest incidence of solar radiation because they present greater spore production and germination in soil heated by the sun (Abdullah and Al-Bader 1990).

Mucor circinelloides and *M. hiemalis* are among the most common taxa of Mucorales isolated from soil (Domsch et al. 2007) and have been reported in Brazilian Atlantic Forest ecosystems (Schoenlein-Crusius and Milanez 1997; Schoenlein-Crusius et al. 2006; de Souza et al.

2008). These species are now reported for the first time in the Brazilian Caatinga domain, including Caatinga *s.s.*, Sandy Caatinga and Carrasco ecosystems. *Absidia* sp. was observed in all of the ecosystems studied. This species has morphological and genetic characteristics that differ from other taxa of the genus and will be published in a subsequent paper.

The statistical analyses among the communities of Mucorales in the studied soils did not exhibit significant differences in terms of CFU. However, the diversity and richness of Mucorales isolated from the soils of natural areas were higher in Carrasco than in the other areas, which could be due to the higher water availability in this type of vegetation, where rainfall is retained (Andrade et al. 2004). According to the species richness estimators, the soils of Sandy Caatinga and Caatinga *s.s.* may still contain a greater number of species than that actually observed.

The results of the present study show that the species richness and diversity of Mucorales are higher in soils of preserved Caatinga *s.s.* than in the soil of the anthropized area, although without significant differences in the numbers of CFU/g of soil. The activity of grazing goats, sheep, and cattle has caused irreversible changes in the ecosystem, degrading the vegetation and reducing plant diversity (Alves et al. 2009). A reduction of the plant community can change certain abiotic factors, such as the temperature and moisture content in the soil, while also reducing the diversity of fungi (Raymundo and Tauk-Tornisielo 1997). Moreover, the suppression of herbaceous plants caused by indiscriminate grazing leads to soil erosion, commits the hydro capacity and makes the soil more compacted, favoring superficial drainage, with serious consequences for geotopes and diversity in this ecosystem (Alves et al. 2009).

With regard to the distribution of Mucorales, all species were rare except *R. microsporus*, which is occasional in soils of tropical countries (Jennessen et al. 2005). In a community, there are usually few taxa with a high frequency, while most have a low frequency and distribution (Richardson 2001; Santiago et al. 2013). In general, the occurrence of Mucorales in semi-arid regions is much lower than that of other filamentous fungi (Abdel-Hafez 1982; Abdullah et al. 1986; Grishkan and Nevo 2010; Oliveira et al. 2013).

The species composition was similar between soils of Carrasco and Sandy Caatinga (69.13 %), and between the soil of preserved and anthropized Caatinga *s.s.* (60.18 %). This result was expected, since the species richness and diversity could be estimated directly by the plant community (Schmit and Mueller 2006). In this case, Carrasco and Caatinga *s.s.* have very distinct flora, while the Sandy Caatinga shares floristic composition with both vegetation types, but it is closer to the high-altitude Carrasco (Andrade et al. 2004). In addition to soil factors and climatic conditions, altitude variations also influence changes in the rhizosphere and in microbial communities (Pandey and Palni 2007).

The results of the present study indicated that the diversity and richness of Mucorales in soils of Carrasco was higher than in Sandy Caatinga and Caatinga *s.s.*, whereas the anthropized area exhibited low richness and diversity. Two species were recorded for the first time in the Caatinga domain and a new species of *Absidia* was also recorded. Further studies in areas of Caatinga will possibly enable the isolation of other species, thereby expanding knowledge about the Mucorales in semi-arid regions.

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