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Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: a first study

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Abstract Endophytic fungi live within the healthy tissues of plants and can promote host species tolerance to different environmental stresses. However, most studies have been of plants in humid environments, and there are few reports of the benefits of such associations for plants of extreme environments. Our aims were to identify endophytic fungi using morphological taxonomy, to explore richness and estimate species frequency in a cactus, *C. jamacaru*, in Brazilian tropical dry forest (Caatinga). We thus identified 59 taxa, corresponding to 69.7 % of the total number of isolates; the other 30.3 % were sterile mycelia. *Cladosporium cladosporioides* and *Fusarium oxysporum* were the species most commonly isolated, followed by *Acremonium implicatum*, *Aureobasidium pullulans*, *Trichoderma viride*, *Chrysonilia sitophila*, and *Aspergillus flavus*. Forty-seven species are recorded for the first time as endophytic fungi of cacti, and 18 others as endophytes for Brazil; this suggests that *C. jamacaru* harbors a highly diverse fungal community as measured by a diversity index. However, species accumulation curves suggest that our study still underestimates endophyte diversity because it does not provide an exhaustive sample. To our knowledge, this is the first report of endophytic fungi from *C. jamacaru* in tropical dry forests.

Keywords Cactaceae · *Cladosporium cladosporioides* · Fungal diversity · *Fusarium oxysporum* · Semi-arid · Taxonomy

1 Introduction

Endophytic microorganisms are those that live within plant tissues without causing apparent damage to the host, and without producing external structures that emerge on the plant surfaces; they may or may not grow in culture medium (Azevedo and Araújo 2007). Many studies have reported that plants are colonized by dozens of endophytes. A tree in a rainforest or even a single leaf may harbor a wide diversity of fungal species, showing their importance in the estimation of fungal diversity (Arnold et al. 2000; Saikkonen et al. 2004). In addition to their key ecological relationships, endophytic fungi are considered important sources of bioactive metabolites (Tan and Zou 2001; Chandra 2012).

Although there have been advances in the study of endophytic fungi, there are few works verifying the associations of endophytic fungi in arid and semi-arid regions. Literature review shows Fisher et al. (1994a), Suryanarayanan et al. (2005), and Bezerra et al. (2012a) to have studied the

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association of endophytic fungi with cacti. Other studies exploring endophytes have focused on endophytic bacteria in cacti from desert areas in Mexico (Puente et al. 2004a, b, 2009a, b; Lopez et al. 2011, 2012). More recently, in the semiarid regions of the United States, Khidir et al. (2010) studied the community of endophytic fungi associated with the roots of grasses, and Loro et al. (2012) analyzed the diversity of endophytic fungi within plants in Venezuela. Sun et al. (2012) explored the endophytic fungi in leaves and stems of plants from the desert areas of China. Although few in number, these studies have helped us to understand plant-fungal interactions in environments considered extreme; providing information for new research into the benefits of these associations.

The literature shows only 14 % of studies relate to the biodiversity of tropical dry forest, while 86 % have been in humid regions (Sánchez-Azofeifa et al. 2005) and little research focuses on the endophytic fungi associated with plants in these regions. Tropical dry forests represent nearly half the tropical forests of the world, covering large areas in Africa, Australia, Central and South America, India, and South-East Asia (Murphy and Lugo 1986; Ceccon et al. 2006; Linares-Palomino 2006). In contrast to tropical humid forests, tropical dry forests occur in climates with highly seasonal rainfall (Ceccon et al. 2006).

In Brazil, tropical dry forest is named Caatinga because of the prevalent vegetation type, from caa (= forest) and tinga (= white) in the native Tupi language (Santos et al. 2012). The Caatinga is a ecosystem unique to Brazil, and in spite of its socioeconomic importance, it is submitted to intense and predatory exploration of its natural resources, and has been little protected or studied (Andrade et al. 2006). Brazil is the third center of diversity of the Cactaceae on the American continent (Taylor, in Oldfield 1997), with a total of 37 genera of native Cactaceae species, comprising about 30 % of the 120 species recorded in the New World (Zappi et al. 2010). Most cactus species are endemic to tropical dry forest (Taylor and Zappi 2004), and few studies have been conducted of their associations with endophytic microorganisms.

Cereus jamacaru DC. (Cactaceae) popularly known in Brazil as 'mandacaru', is a common columnar cactus, mainly found in the Caatinga, and with great importance for the sustainable development and biodiversity conservation of this ecosystem. It is especially important during the driest months because of its use as cattle food. It grows in soils with low nutrient levels, survives with minimal rainfall, and in addition, has medicinal properties (Andrade et al. 2006; Agra et al. 2007; Cavalcanti and Resende 2007; Rêgo et al. 2009).

It is important to preserve the Cactaceae in Brazil and to gain greater knowledge of their potential, including their relationships with endophytic microorganisms. There are few reports of fungal communities associated with cacti, and there are no studies of endophytic fungi from *C. jamacaru*. Our objectives were therefore, 1) to identify the endophytic fungi from *C. jamacaru* using morphological taxonomy, and 2) to explore richness and estimate species frequency of endophytic fungi in this cactus, growing in the tropical dry forest known as Caatinga; a unique and underestimated ecosystem in Northeastern Brazil. To our knowledge, this is the first report of endophytic fungi from *C. jamacaru* in tropical dry forests.

2 Materials and methods

2.1 Study site

Samples of *C. jamacaru* were collected from Tamanduá Farm in northeastern Brazil (07°1.524S, 037°23.518W) in areas of Caatinga, in dry season between September to November, 2011. The Tamanduá Farm includes 900 ha, of which 325 ha is a legal nature reserve (Private Natural Heritage Reserve). The Farm is located in the backlands of Espinharas, at an average altitude of 240 m with soils predominantly Entisols (Embrapa 1997). The climate is of type Bsh (semi-arid) according to the classification of Köppen (1948), with an average annual temperature of 32.8 °C and an average annual rainfall of 380–800 mm, concentrated within a short period of 2 to 4 months followed by a long dry period, lasting up to 8 months.

2.2 Plant material

Nine samples were randomly collected on each of three collecting trips, providing a total of 27 samples. Collected cladodes were 30–50 cm in length and were from different *C. jamacaru* individuals up to 3 m tall. Samples were packed in nylon bags, transported to the laboratory, and processed within 48 h.

2.3 Isolation of endophytic fungi from *Cereus jamacaru*

The material was processed as described by Bezerra et al. (2012a). Briefly, for asepsis of plant material were cut from each cladode three fragments of approximately 5 cm and were surface-sterilized by sequential washes in 70 % ethanol for 60 s, sodium hypochlorite 2–2.5 % for 180 s, 70 % ethanol for 30 s, and were then rinsed three times in distilled and sterilized water. After the processing were cut into segments of about 1 cm^2 totaling 45 fragments of each cladode. The fragments were then placed on potato-dextrose-agar medium (PDA) supplemented with chloramphenicol (100 mg/l) and tetracycline (50 mg/l) to restrict bacterial growth. The dishes were incubated at 28±2 °C for up to 30 days. Fungal growth was observed every day, and any fungal colony found was isolated, purified, and maintained on PDA for later identification. To check the efficacy of surface sterilization, 1 ml quantities of water from the last rinses were inoculated into Petri dishes containing the same medium, and the same incubation conditions.

2.4 Identification of endophytic fungi from *Cereus jamacaru*

For identification of endophytic filamentous fungi, microcultivations were performed, and the macro and micro morphological aspects of the somatic and reproductive structures were observed, using specific methodology and literature (Ellis 1971; Sutton 1980; Samson and Frisvad 2004; Leslie and Summerell 2006; Domsch et al. 2007). For identification of endophytic yeasts, we adopted the criteria of classical taxonomy observing the macro and microscopic characteristics, physiology and biochemical indicators (Lodder 1970; Kreger-Van Rij 1984; Kurtzman and Fell 1998; Barnett et al. 2000).

Scanning electron microscopy (SEM) was conducted at the Centro de Tecnologias Estratégicas do Nordeste (CETENE) in Recife, Pernambuco, Brazil.

Representative cultures of the endophytic fungi isolated from *C. jamacaru* are deposited in the URM Culture Collection (WDCM604) of the Federal University of Pernambuco, Recife, Brazil.

2.5 Data analysis

The absolute (f) and relative (fr) frequencies of isolated endophytic fungi were calculated. The absolute frequency was calculated as the total number of endophytic isolates, and the relative frequency was the number of isolates of each species divided by the total number of isolates. The colonization rate was calculated as the total number of segments from which fungi were isolated, in a sample divided by the total number of segments used for isolation (Larran et al. 2002).

Species diversity was calculated using Primer 6.0 software (Clarke and Gorley 2006). Analysis of species diversity was performed using Shannon's, Fisher's α, Simpson's, Margalef's, and Pielou's evenness indices. We also estimated species accumulation curves for these *C. jamacaru* endophytes (Jackknife 1; Jackknife 2; Chao 1; Chao 2; Bootstrap).

3 Results

In total, 27 cladodes (1,215 cladodes fragments) of *C. jamacaru* were used, from which 560 endophytic fungi were isolated. The colonization rate of the fragments by endophytic fungi was of 42.22 %.

The species most frequently isolated were members of *Cladosporium*, *Fusarium*, *Acremonium*, *Aureobasidium*, *Trichoderma*, *Chrysonilia*, and *Aspergillus*. Other genera showed a low frequency (one or two isolates) and were considered as isolation accidentals and/or rare. These species were of the genera *Boeremia*, *Candida*, *Cochliobolus*, *Guignardia*, *Pestalotiopsis*, *Phomopsis*, *Purpureocillium*, *Redaellia* (= *Aspergillus*), *Sarocladium*, *Sporobolomyces*, and *Syncephalastrum* (Table 1). We observed that the endophytic yeasts preferred to colonize the more internal tissues of *C. jamacaru*. Figure 1 illustrates the two species most frequently found and one less frequently isolated.

Among the isolates identified, 47 species were recorded for the first time as endophytic fungi of cacti, 18 others as endophytes in Brazil, and we consider about 58 % to be rare species (Table 1 and Fig. 2). These were distributed in 30 genera: Ascomycetes (24), Basidiomycetes (4), and Zygomycetes (2). Although 59 taxa were identified, corresponding to 69.7 % of the total number of isolates, 30.3 % of the fungi were sterile mycelia. The dominant endophytic fungi were members of the genera *Cladosporium* and *Fusarium* (Table 1 and Fig. 2).

The endophytic fungal richness from *C. jamacaru* was high (Table 2). The value on the Fisher-Alpha diversity index was 19.32, and on the Margalef index was 9.722; suggesting that *C. jamacaru* harbors a great diversity of endophytic fungi. In addition, it should be remembered that the accumulation curves of endophytic fungi did not reach stabilization point. The various richness indices we used estimated the number of species in *C. jamacaru* as 87 (using Jackknife 1), 106 (using Jackknife 2), 80 (using Chao 1), 106 (using Chao 2), and 72 (using a Bootstrap). We are certain that our methods have underestimated endophyte diversity and that our sampling was not exhaustive (Fig. 3).

4 Discussion

Very few investigations have been made of the endophytes in plants of dry environments (Puente et al. 2004a, b, 2009a, b; Murali et al. 2007; Khidir et al. 2010; Lopez et al. 2011, 2012; Suryanarayanan et al. 2011; Sun et al. 2012; Bezerra et al. 2012b; Loro et al. 2012), as most endophytic studies have focused on plants of tropical rainforests and temperate regions (Fisher et al. 1994b; Rodrigues and Petrini 1997; Arnold et al. 2000; Azevedo et al. 2000; Suryanaraynan and Kumaresan 2000; Kumaresan and Suryanaraynan 2001; Azevedo and Araújo 2007; Costa et al. 2012a, b; Lima et al. 2013). Plants in dry areas are exposed to many environmental stresses, including low water availability, high salinity, high diurnal temperature differences, high irradiance,

Table 1 Absolute (f) and relative (fr) frequency of endophytic fungi isolated from cactus *Cereus jamacaru* growing in tropical dry forest, Brazil

Endophytic fungi	Occurrence[a]	Collects			f	fr
		September	October	November		
Ascomycetes						
Acremonium charticola (Lindau) W. Gams	a, b		1		1	0.17
A. curvulum W. Gams	a		1		1	0.17
A. implicatum (J.C. Gilman & E.V. Abbott) W. Gams	–			18	18	3.2
A. pteridii W. Gams & J.C. Frankland	a	2			2	0.35
Aspergillus flavus Link	a		1	11	12	2.13
A. japonicus Saito	–		1		1	0.17
A. niger Tiegh.	a	3		1	4	0.71
A. ochraceus G. Wilh.	a	1		7	8	1.42
A. parasiticus Speare	a	2			2	0.35
A. sydowii (Bainier & Sartory) Thom & Church	a			1	1	0.17
A. terreus Thom	a			1	1	0.17
A. versicolor (Vuill.) Tirab.	a			3	3	0.53
Aureobasidium pullulans (de Bary) G. Arnaud	–	2	13	2	17	3.03
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	a			1	1	0.17
Candida etchellsii (Lodder & Kreger-van Rij) S.A. Mey. & Yarrow	a			1	1	0.17
C. magnoliae (Lodder & Kreger-van Rij) S.A. Mey. & Yarrow	a, b		1		1	0.17
Chrysonilia sitophila (Mont.) Arx	a, b			15	15	2.67
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–	44	32	28	104	18.53
C. sphaerospermum Penz.	–		1	3	4	0.71
Cochliobolus lunatus R.R. Nelson & F.A. Haasis	a	1	1		2	0.35
Curvularia brachyspora Boedijn	a, b		1		1	0.17
C. senegalensis (Speg.) Subram.	a	1			1	0.17
Cytospora Ehrenb.	a			14	14	2.49
Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij	a	4	2		6	1.06
Fusarium lateritium Nees	–	3			3	0.53
F. oxysporum Schltdl.	–	37	26	2	65	11.58
Gibberella fujikuroi var. *fujikuroi* (Sawada) Wollenw.	a	6	1		7	1.24
Guignardia bidwellii (Ellis) Viala & Ravaz	a	2			2	0.35
Nigrospora sphaerica (Sacc.) E.W. Mason	–		1	6	7	1.24
Penicillium aurantiogriseum Dierckx	–		2		2	0.35
P. brevicompactum Dierckx	a, b	2	2		4	0.71
P. chrysogenum Thom	a		2	1	3	0.53
P. commune Thom	a, b		2		2	0.35
P. funiculosum Thom	a			6	6	1.06
P. griseofulvum Dierckx	a	6			6	1.06
P. minioluteum Dierckx	a			3	3	0.53
P. restrictum J.C. Gilman & E.V. Abbott	a, b		1		1	0.17
P. waksmanii K.M. Zalessky	a, b		2		2	0.35
Pestalotiopsis guepinii (Desm.) Steyaert	–	2			2	0.35
Phoma leveillei Boerema & G.J. Bollen	a, b	1			1	0.17
P. pomorum Thüm.	a, b	2			2	0.35
P. putaminum Speg.	a		1		1	0.17
P. tropica R. Schneid. & Boerema	–	2	2	2	6	1.06
Phomopsis archeri B. Sutton	–			1	1	0.17
Pseudocochliobolus pallescens Tsuda & Ueyama	a		1		1	0.17
Purpureocillium lilacinum (Thom) Luangsa-ard, Hywel-Jones & Samson	a		1		1	0.17

Table 1 (continued)

Endophytic fungi	Occurrence[a]	Collects			f	fr
		September	October	November		
Redaellia Ciferri (= *Aspergillus*)	a, b	1			1	0.17
Sarocladium bacillisporum (Onions & G.L. Barron) Summerb.	a, b	1		1	2	0.35
Trichoderma viride Pers.	a	1	16		17	3.03
Basidiomycetes						
Rhodotorula foliorum (Ruinen) Rodr. Mir. & Weijman	a, b		1		1	0.17
R. minuta (Saito) F.C. Harrison	a		1		1	0.17
R. mucilaginosa (A. Jörg.) F.C. Harrison	a			1	1	0.17
R. pilatii (F.H. Jacob, Faure-Reayn. & Berton) Weijman	a, b	1	1		2	0.35
R. sonckii (Hopsu-Havu, Tunnela & Yarrow) Rodr. Mir. & Weijman	a	1			1	0.17
Sporobolomyces salmonicolor (B. Fisch. & Brebeck) Kluyver & C.B. Niel	a		2		2	0.35
Sterigmatomyces elviae Sonck & Yarrow	a,b		5		5	0.89
Tritirachium dependens Limber	a, b	4			4	0.71
Zygomycetes						
Cunninghamella echinulata var. *echinulata* (Thaxt.) Thaxt. ex Blakeslee	a, b	2		1	3	0.53
Syncephalastrum racemosum Cohn ex J. Schröt.	a, b			1	1	0.17
Sterile mycelia		58	85	27	170	30.3
Total		192	210	158	560	
Total taxa		26	30	25	59	

[a] Occurrence: (a) species reported for the first time as endophytes of members of the Cactaceae, and (b) species reported for the first time occurring as endophytes in Brazil

and nutrient deprivation. As endophytic fungi can aid against environmental stresses, it is somewhat surprising that the endophytes of dry area plants have received such little attention (Suryanarayanan et al. 1998; Sun et al. 2012).

Studies of the Cactaceae indicate similarities with our results of colonization rates. Fisher et al. (1994a), for example, studied 600 fragments of cactus from Australia and isolated 617 endophytic fungi across 23 taxa (Ascomycota). Suryanarayanan et al. (2005) used 1,050 fragments of cacti from Arizona (USA) to isolate 900 endophytes belonging to 22 fungal species (Ascomycota), and Bezerra et al. (2012a) used 45 fragments of forage cactus from Brazil to gain 44 isolates of endophytic fungi belonging to 13 species (Ascomycota). Studying the endophytic fungi of other plants, Khidir et al. (2010) analyzed the roots of grasses in the semiarid grassland of the United States, and found a colonization rate of 87.5 %. Sun et al. (2012) observed a colonization rate of 69 % of the fragments of stems and leaves of plants from the desert areas of China. Loro et al. (2012), studying the diversity of endophytic fungi of 18 plants of the semiarid regions of Venezuela, verified that all plant species contained endophytic fungi (incidence of 100 %), and obtained between 2 and 8 morphologically distinct isolates per plant. Most of the fungi that we found within *C. jamacaru* corresponded to genera already reported as endophytes (Larran et al. 2002; Azevedo and Araújo 2007; Vieira et al. 2011, 2012; Costa et al. 2012a).

In a previous study of *Opuntia ficus indica* cultivated in semiarid regions in Brazil, Bezerra et al. (2012a) observed that of the 44 endophytic fungi isolated, the genus *Cladosporium* was the most frequent. Fisher et al. (1994b) and Suryanarayanan et al. (2005) studying the endophytic fungi of cacti in Australia and Arizona, respectively, also isolated species of the genus *Cladosporium*. In studies of fungal endophytic in dry environments in New Mexico, Khidir et al. (2010) observed that the genera *Paraphaeosphaeria* and *Moniliophthora* were most commonly isolated, representing 58 % of the total. Studying the endophytic fungal community of the plants from desert areas in China, Sun et al. (2012) found only one isolate of *Cladosporium*, however they also identified some endophytic fungal genera generally found in the Cactaceae (*Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Phoma*, and *Ulocladium*). Loro et al. (2012) verified that the most common endophytic fungi in grasses and sedges from semiarid Northwest Venezuela are members of the genera *Cochliobolus* and *Phoma*.

The endophytes that we only observed as sterile mycelia (30.3 %) demonstrate that dry area plants harbor a diversity of fungi that may not sporulate in culture (Table 1 and Fig. 2). Studying cacti, Fisher et al. (1994a) found 1.78 % sterile mycelia in Australia; Suryanarayanan et al. (2005) found 6.44 % in Arizona, and Bezerra et al. (2012a) found 22.72 % in Brazil. Similarly, Sun et al. (2012) evaluating the

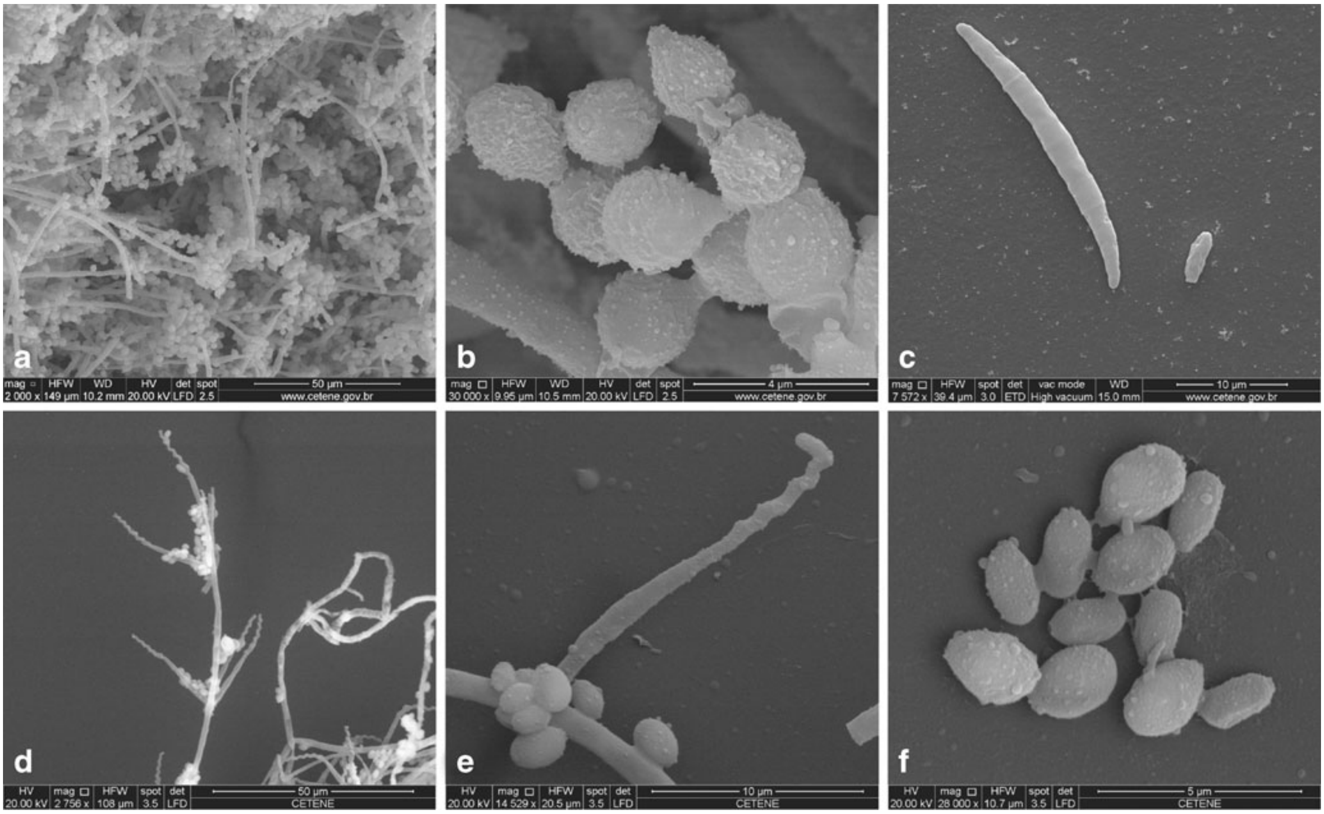

Fig. 1 Scanning electron microscope (SEM) photographs of three endophytes. *Cladosporium cladosporioides* (URM 6703): **a** Conidiophores and conidia. **b** Conidia. *Fusarium oxysporum* (URM 6704): **c** Macro and microconidia. *Tritirachium dependens* (URM 6705): **d** Conidiophores. **e** Conidiogenous cell geniculate. **f** Conidia

endophytic fungal community in stems and leaves of other plants in desert areas in China found 13.10 % sterile mycelia. In studies of endophytic fungi in plants of humid tropical and temperate regions, sterile mycelia also have been isolated. Guo et al. (2000), for example, found 16.45 % of isolates from palms distributed and cultivated in China were non-sporulating in culture. Arnold et al. (2000) grouped all the isolates obtained from two plant species in Panama into 418 endophytic morphospecies. Other studies in tropical and temperate regions have demonstrated that sterile mycelia may be isolated as endophytes (Arnold et al. 2001; Girlanda et al. 2002; Wang et al. 2005; Arnold and Lutzoni 2007; Unterseher et al. 2007; Linnakoski et al. 2012).

In our survey, 69.7 % of isolates were identified by their morphological characteristics as belonging to 59 taxa; and we consider 58 % to be rare species. Although morphological taxonomy contributes greatly to the elucidation of the richness of endophytic fungi, the addition of molecular methods in the identification of these organisms has helped to distinguish morphospecies, and genera with species complexes such as *Cladosporium* (Bensch et al. 2012). It has also increased our knowledge of endophytic diversity, including, in particular, those fungi that do not sporulate in culture (Suryanarayanan et al. 2011). Therefore, the exclusive use of morphological taxonomy may to some extent misrepresent the presence of some common species in the cactus *C. jamacaru*, previously found in other habitats. We expect to continue exploration the diversity of fungal endophytes from *C. jamacaru*, adding molecular methods to assist in the identification of these microorganisms. Guo et al. (2000), Wang et al. (2005), and Suryanarayanan et al. (2011) have highlighted the importance of molecular techniques to aid identification of non-sporulating fungi.

Interestingly, the plant pathogen *Fusarium oxysporum* was isolated as an endophyte from *C. jamacaru*. This species is reported as causing diseases in plants of agricultural interest and cacti of the genus *Opuntia* in which it causes wilting of the cladodes (Postma and Rattink 1992; Souza et al. 2010); however, it has also been predominantly isolated as an endophyte (Xing et al. 2010; Wang et al. 2011; Vieira et al. 2012). Also isolated, were species of *Aspergillus* and *Penicillium*, which may be thermophilic opportunistic pathogens in humans and other mammals, and produce mycotoxins (Hoog et al. 2000; Summerbell 2005; Magan 2007; Posada et al. 2007; Kharwar et al. 2008; Houbraken et al. 2011; Loro et al. 2012; Bezerra et al. 2012a). Although Zygomycetes and Basidiomycetes are rarely isolated as

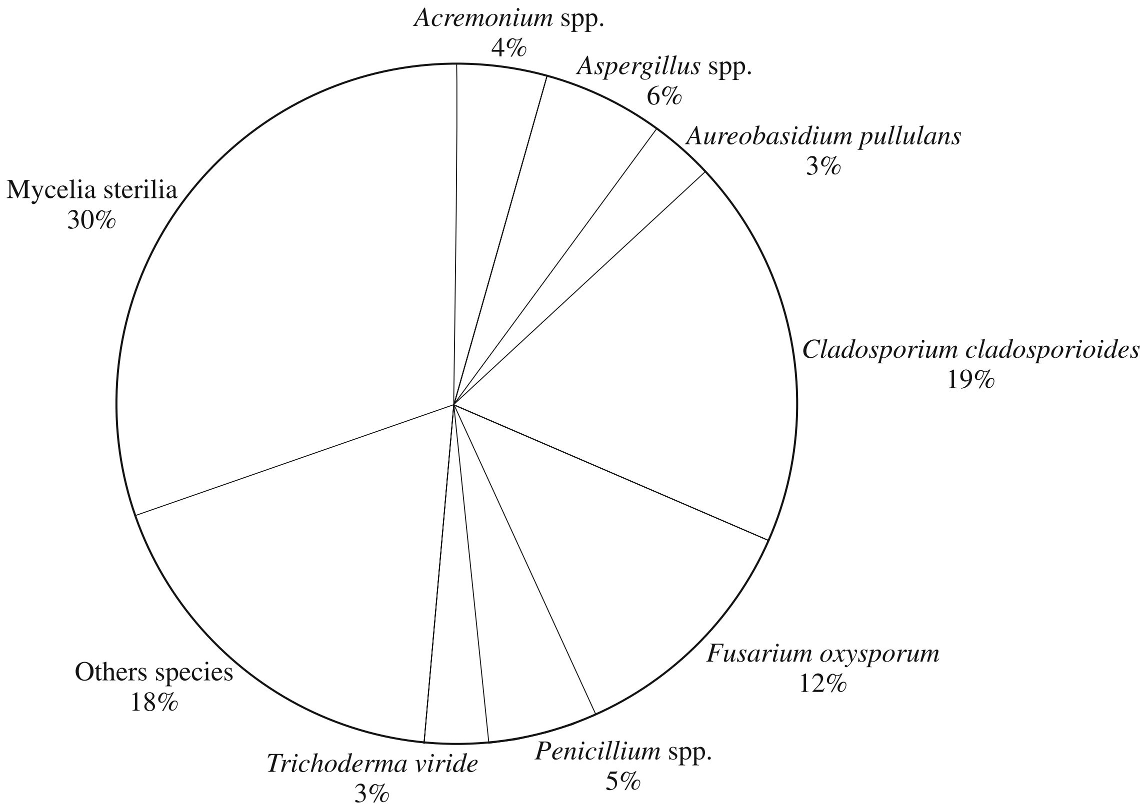

Fig. 2 Relative frequency of the endophytic fungi isolated from the cactus *Cereus jamacaru*, growing in tropical dry forest in Brazil

endophytes (Stone et al. 2004; Wang et al. 2005), we isolated two species of Zygomycetes and eight species of Basidiomycetes from *C. jamacaru*.

We also isolated some pigmented fungi; *Aureobasidium, Cladosporium, Curvularia*, and *Phoma*. Other works about endophytes suggest that pigmented fungi can confer host plant tolerance to drought, aridity, and/or extreme temperatures (Redman et al. 2002; Mandyam and Jumpponen 2005; Khidir et al. 2010), and these fungi associated with roots can be involved in the absorption of water (Barrow 2003; Mandyam and Jumpponen 2005; Barrow et al. 2008). Furthermore, some studies have raised the hypothesis that endophytic fungi can alter hormone levels that control stomatal behavior and osmotic adjustment (Malinowski and Belesky 2000; Mandyam and Jumpponen 2005). Morsy et al. (2010) suggested that these relationships may be influenced by the involvement of osmoprotectants, the pigment melanin, and thermophilic proteins, as observed in cultures of *Curvularia protuberata*. Research on fungi from extreme environments, although sometimes challenging, is clearly justified and important to understanding the factors that shape microbial communities in these areas, giving a perspective on the benefits for the environment (Magan 2007; Newsham 2012).

Our results suggest a high endophytic fungal diversity in *C. jamacaru*. This is in contrast to some studies which have indicated that the endophytic diversity in dry areas and/or desert is low because of factors such as low rainfall and low

Table 2 Endophytic fungal diversity indices from *Cereus jamacaru* growing in tropical dry forest, Brazil. Isolates identified as sterile mycelia are not included

Indices	Collects			Total
	September	October	November	
Richness of species	26	30	25	59
Number of isolates	134	125	131	390
Margalef's	5.104	6.006	4.923	9.722
Pielou's	0.6976	0.7317	0.8068	0.7335
Fisher's α	9.617	12.52	9.167	19.32
Shannon's (H')	2.273	2.489	2.597	2.991
Simpson's	0.8127	0.8662	0.9008	0.8904

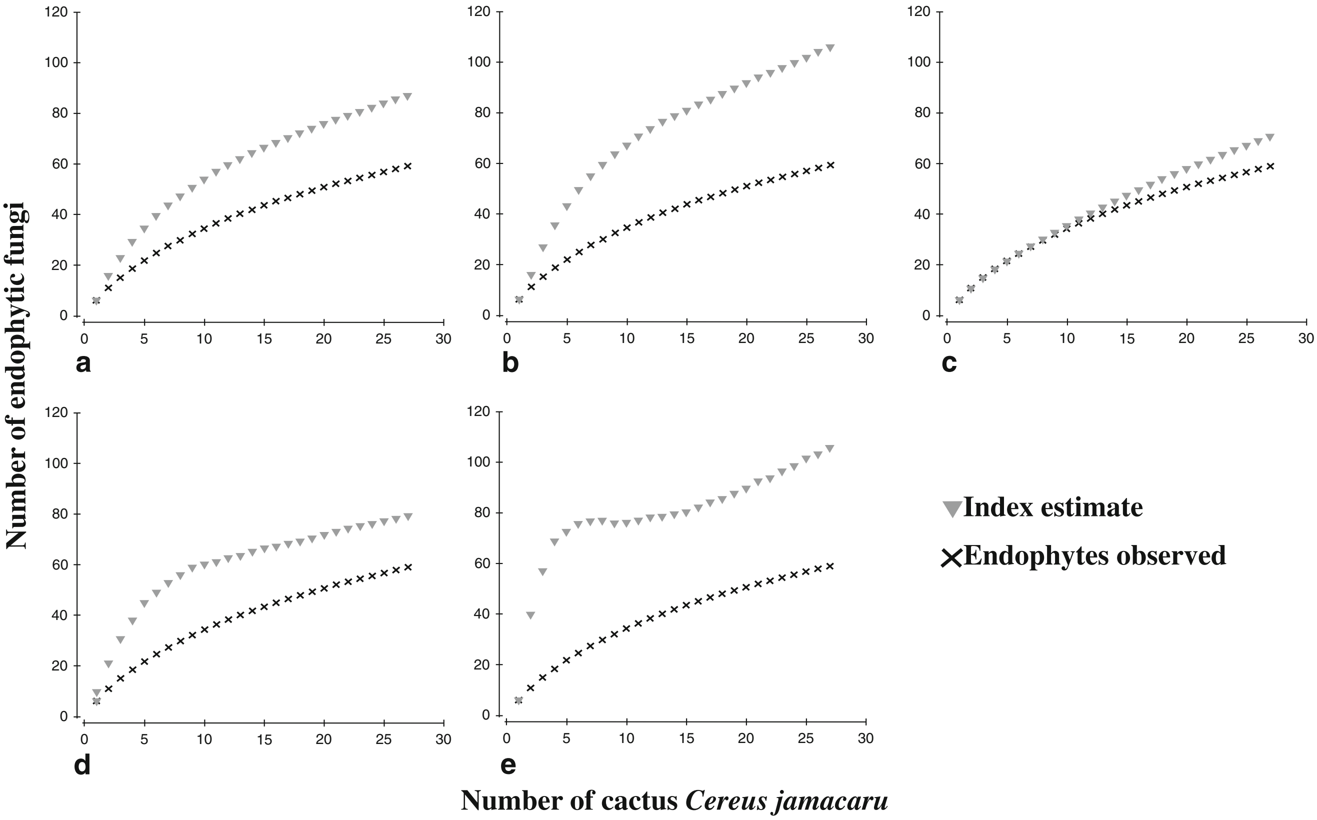

Fig. 3 Accumulation curves of endophytic fungi from *C. jamacaru* growing in tropical dry forest in Brazil. (▼) indicates the accumulation curves of endophytic fungal estimates for each index, and (×) indicates the accumulation curves of the endophytic fungi. (**a** Jackknife 1; **b** Jackknife 2; **c** Bootstrap; **d** Chao 1; **e** Chao 2)

vegetation density (Arnold et al. 2000; Suryanarayanan et al. 2002a, b, 2003, 2005). Murali et al. (2007), for example, analyzing the endophytic communities in India, identified 60 and 51 species in tissues of 15 different trees in dry thorn and dry deciduous forests, respectively. These authors suggest that the low diversity of endophytes in their study may be due to a group of fungi dominated by *Colletotrichum gloeosporioides*, *Phomopsis* spp., and *Phyllosticta capitalensis*, and shared by trees belonging to different families and in these two different forests. This would not be the case in our study, since of these taxa we have only isolated *Phomopsis archeri*. Similarly, studying endophytes associated with other cacti, Fisher et al. (1994a) isolated 23 taxa, Suryanarayanan et al. (2005) isolated 22 species of fungi, and Bezerra et al. (2012a) isolated 15 taxa in *Opuntia ficus-indica*. The high richness of endophytes from *C. jamacaru* in Brazilian tropical dry forest could be related to plant diversity in the Caatinga (Siqueira Filho et al. 2012).

Overall, the community of endophytic fungi in cacti in the Brazilian tropical dry forest is very diverse, as is indicated by our estimated Fisher-Alpha value (19.32). Studying 21 species of cacti in Arizona, Suryanarayanan et al. (2005) obtained a Fisher-Alpha value of between 2.3 and 0.6. The Fisher-Alpha values we found are low in comparison with those obtained by Arnold and Lutzoni (2007). These authors described the increasing endophytic community diversity recovered from angiosperms in southern boreal forest (Fisher-Alpha value of 9.2), to the temperate zone (Fisher-Alpha value of 25.7) to the tropics (Fisher-Alpha value of 30.9). In contrast, the Fisher-Alpha estimates we found were greater than those found by Murali et al. (2007) and Suryanarayanan et al. (2011) in plants of dry thornland, dry deciduous forests, and montane evergreen forest. These comparisons should be interpreted cautiously because saturation curves in some these studies indicated the fungal community diversities were underestimated (Loro et al. 2012).

Studying leaves and stems in desert areas in China, Sun et al. (2012) obtained values for Shannon's index varying from 0.29 to 4.78, and for Simpson's index from 1.00 to 6.60. Although there is some variation, these estimates are relatively similar to ours. Similar fungal diversity estimates were also obtained from grasses and sedges in the neotropical semiarid region of northwestern Venezuela (H'=2.27–3.06) (Loro et al. 2012). Our values for Shannon's index (H'=2.991) and Pielou's evenness index (J=0.7335) were similar to those of Tadych et al. (2012) in their study of endophytic and pathogenic fungi in developing cranberry ovaries (flower to mature fruit) of *Vaccinium macrocarpon* collected in New Jersey, USA. Our Margalef's richness index value for fungi from *C. jamacaru* (D_{mg}=9.722) was greater than that for the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in China (Yuan et al. 2010), and was higher than that for the endophytic community from *Cannabis sativa* in the Netherlands (Kusari et al. 2012). This indicates that the diversity of fungal endophytes in cactus in Brazilian tropical dry forest is very high.

Accumulation curves for the endophytic fungi we observed indicate that the endophytic community was not completely sampled. Similar results were obtained in other surveys of plants in dry environments (see Murali et al. 2007; Khidir et al. 2010; Suryanarayanan et al. 2011; Loro et al. 2012; Sun et al. 2012) and other tropical environments (Arnold et al. 2000; Arnold and Lutzoni 2007). The results we obtained suggested that further sampling would add new species of endophytes to those known from this cactus.

Detailed investigations of the composition of endophytic fungal communities in tropical and/or temperate plants frequently find new taxa and reveal new distributions of already known species. Because endophytes are not easily observable, species diversity can be relatively high and as only a small proportion of the potential hosts have been examined, endophytes represent a substantial number and diversity of undiscovered fungi (Arnold et al. 2000). Studies of endophytic fungi are needed to provide fundamental information for the assessment of global diversity and the distribution of these important microbes (Tan and Zou 2001; Strobel and Daisy 2003; Stone et al. 2004; Siqueira et al. 2011).

5 Conclusion

Based on our observations, we suggest that the cactus *C. jamacaru* harbours a high diversity of endophytic fungi, and that further sampling will add further new species to its known endophyte community. *Cladosporium cladosporioides* and *Fusarium oxysporum* were the most frequently isolated endophytic species, suggesting that they may have ecological importance for the Cactaceae. Further studies of endophytic fungi from the Cactaceae are needed to understand the relationships between plants and fungi from arid and semi-arid regions, and the benefits such fungi confer on their hosts, as well as the mechanisms evolved to adapt these plants to extreme living conditions. It may even be possible to transfer the appropriate genes from endophytic fungi to plants, to obtain plants of economic importance that are resistant to water shortage and potentially buffered against climate change.

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