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Diversity of the endophytic fungi associated with the ancient and narrowly endemic neotropical plant *Vellozia gigantea* from the endangered Brazilian rupestrian grasslands

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ABSTRACT

This study focused on the taxonomy and diversity of the endophytic fungi associated with *Vellozia gigantea*, an endemic, ancient, and endangered plant species that occurs only in the rupestrian grasslands of Brazil. A total of 285 fungal isolates were recovered from leaves and roots of the *V. gigantea*, which were identified in 27 genera and 87 different taxa using molecular taxonomy methods. *Xylaria berteri*, *Diaporthe* sp. 1, *Nigrospora oryzae*, *Muscodor* sp. 1, *Colletotrichum aeschynomene*, and *Trichoderma viride* occurred in the highest frequency in both the leaf and root. *Diaporthe* was the most abundant genus, with 70 endophytic isolates recovered from the leaves and roots. Among all the taxa identified, 62 occurred as singlets, including those of the genera *Clonostachys*, *Coccomyces*, *Crucellisporiopsis*, *Daldinia*, *Myxotrichum*, *Pallidocercospora*, *Pezicula*, *Peyronellaea*, and *Pseudocercospora*. The diversity indices displayed high values, showing that *V. gigantea* shelters a diverse and rich mycobiota. Our results indicate that *V. gigantea* shelters in its tissues a highly diverse and cryptic mycobiota, including several rare species previously unreported as endophytes, but that are reported to have different ecological functions, which might be an important biological component contributing to the fitness of the plants living in the rupestrian grassland.

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1. Introduction

Different definitions of endophytes have been proposed since their discovery in the nineteenth century (Schulz and Boyle, 2005). Compant et al. (2016) describe endophytes as being any neutral, commensal, and/or beneficial microorganisms, as well as dormant saprobes and pathogens in the latent phase of their life cycle, which can be isolated from asymptomatic plant tissue. Additionally, endophytes seem to be closely associated with different parts of their host plants (Hardoim et al., 2015).

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Although many endophytic microbes have been regarded as symbionts that promote some benefit to their host, several endophytic species have been reported to be decomposers or parasites (Schulz and Boyle, 2005). According to Rosa et al. (2011), endophytic fungi include a high diversity of species, mainly in the phyla Ascomycota and Basidiomycota, which are associated with different plants around the world and represent an important group of the worldwide fungal diversity.

Among the typical biomes in Brazil, the Espinhaço Mountain Range has the rupestrian grasslands ('campo rupestre') which include valleys, canyons, ranges, and rock outcrops at high elevations that shelter a high richness of different endemic plant species (Lousada et al., 2011). The Espinhaço Mountain Range is located in eastern Brazil in the states of Minas Gerais and Bahia, and extends approximately 1000 km in a North-South direction. According to Silveira et al. (2015), the diversification of plant lineages present in the rupestrian grasslands occurred before the diversification of the Brazilian lowland savannas ('cerrado'), suggesting it is the most ancient open vegetation in eastern South America. In addition, the Brazilian rocky fields comprise more than 5000 plant species (about 15% of Brazilian plant diversity) in an area corresponding to 0.78% of its surface.

Brazil has a rich biodiversity of plant species, including those reported as being endemic. Among the endemic plants of the rupestrian grasslands, those of Velloziaceae occur at a high frequency and comprise about 240 predominately Neotropical and several other species (Menezes et al., 1994; Lousada et al., 2011). *Vellozia*, known locally as 'canela-de-ema', is the largest genus in the family and includes about 105 species (Menezes et al., 1994). *Vellozia gigantea* N. L. Menezes & Mello-Silva (Velloziaceae), a recently described species, is the tallest in the family, reaching more than 6 m, and is considered to be threatened by extinction (Lousada et al., 2011). However, there is few information about the biology of *V. gigantea*, including the age of its population. According to Alves (1994), the dracenoid species of *Vellozia*, like *V. gigantea*, may represent an ancient plant that could be hundreds of thousands of years old. Furthermore, *V. gigantea* has been classified as vulnerable owing to its small population size and very restricted distribution, being considered endemic to the rocky field vegetation in the rupestrian grasslands in eastern Brazil, specifically present in Serra do Cipó National Park (Mello-Silva and Menezes, 1999). In the present study, we focused on the taxonomy, diversity, community structure, ecology, and phylogenetic inferences of endophytic fungal communities associated with the ancient and narrowly endemic Neotropical plant species *V. gigantea*.

2. Materials and methods

2.1. Plant collection and isolation of fungal endophytes

Specimens of *V. gigantea* were collected in May 2013, at Serra do Cipó National Park (19°14′874″S; 043°30′574″W), a protected area in the state of Minas Gerais, Brazil. Identification of *V. gigantea* was based on comparisons with the voucher specimen deposited at the herbarium of the Institute of Biological Science (BHCB) of the Federal University of Minas Gerais, Brazil (http://sciweb.nybg.org/science2/IndexHerbariorum.asp) under the code BHCB 102620. The collection of the plant material was carried out according to the Brazilian biological diversity rules.

A total of 32 *V. gigantea* specimens were sampled, of which roots were obtained from 18 specimens and leaves from 32 specimens. Three leaves and 3 roots were obtained from each individual plant, placed in sterile plastic bags, and stored for less than 24 h at 10 °C until the isolation of endophytic fungi. Five fragments (approximately 0.5 cm long and 0.5 cm wide) of each leaf and root were cut using a flame-sterilised blade in a laminar flow hood. The tissue fragments were surface disinfected by immersing in 70% ethanol (1 min) and 2% sodium hypochlorite (3 min), followed by washing with sterile distilled water (2 min) (Carvalho et al., 2012). The fragments were plated onto Petri dishes containing potato dextrose agar (PDA; Difco, USA) supplemented with 200 mg L^{-1} chloramphenicol. The plates were incubated at 25 °C for 60 days. To test the effectiveness of the surface sterilisation, 100 μL of the final rinsing water was plated on PDA medium and incubated under the same conditions. Hyphal growth was monitored over an 8-week period. Using an aseptic technique, endophytes were transferred to PDA contained in 60-mm Petri plates and photographed after growth completion. The long-term preservation of filamentous fungal colonies was carried out in cryotubes containing 15% sterile glycerol at −80 °C, and in sterile distilled water at room temperature. All pure cultures of the endophytic fungal isolates were deposited in the Culture Collection of Microorganisms and Cells of the Federal University of Minas Gerais.

2.2. Fungal identification

The protocol for DNA extraction was described previously by Rosa et al. (2009). The internal transcribed spacer (ITS) region was amplified with the universal primers ITS1 and ITS4 (White et al., 1990). Amplification of the ITS region was performed as described by Rosa et al. (2009). Amplification of the β-tubulin (Glass and Donaldson, 1995) and ribosomal polymerase II genes (RPB2) (Houbraken et al., 2012) were performed with the Bt2a/Bt2b and RPB2-5F-Pc/RPB2-7CR-Pc 7CR primers, respectively, according to protocols established by Godinho et al. (2013). The obtained sequences were analyzed with SeqMan P with Lasergene software (DNASTAR Inc., Madison, WI, USA), and a consensus sequence was obtained using Bioedit v. 7.0.5.3 software (Carlsbad, ON, Canada). Representative consensus sequences of fungal taxa were deposited into GenBank (Table 1). To achieve species-rank identification based on ITS, β-tubulin data and ribosomal polymerase B2, the consensus sequence was aligned with all sequences from related species retrieved from the NCBI GenBank database using BLAST (Altschul et al., 1997). Taxa that displayed query coverage and identities ≤98% or an inconclusive taxonomic position were subjected to phylogenetic ITS, β-tubulin and ribosomal polymerase B2-based analysis in comparison with sequences of type species deposited in the

Table 1
Molecular identification of endophytic fungi associated with *Vellozia gigantea*. Identification conducted using BLASTn searches of the ITS, β-tubulin, polymerase II and D1-D2 domains.

UFMGCB[a]	Tissue[b]	No of isolates	[c]Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	No of bp analyzed	Proposed taxa (GenBank acc. no)
9874	L, R	29	*Xylaria berteri* (GU324749)[d]	100	99	441	*Xylaria berteri* (KU7276850)[h]
			Xylaria berteri (FJ904911)[e]	100	99	337	(KU878163)[i]
9823	L, R	27	*Diaporthe nothofagi* (JX862530)[d]	99	93	459	*Diaporthe* sp. 1 (KU727686)[h]
			Diaporthe nothofagi (KF170922)[e]	99	88	439	(KU992319)[i]
9657	L,R	27	*Nigrospora oryzae* (KC771457)[d]	100	100	434	*Nigrospora oryzae* (KU727687)[h]
9886	L, R	20	*Muscodor equiseti* (JX089322)[d]	99	97	474	*Muscodor* sp. 1 (KU727688)[h]
9682	L, R	13	*Colletotrichum aeschynomenes* (NR120133)[d]	100	100	485	*Colletotrichum aeschynomenes*
			Colletotrichum aeschynomenes (JX010392)[e]	100	98	640	(KU727689)[h] (KX015126)[i]
9734	L, R	10	*Trichoderma viride* (DQ323428)[d]	99	100	481	*Trichoderma viride* (KU727690)[h]
9740	L, R	8	*Diaporthe schini* (NR111861)[d]	100	99	451	*Diaporthe* sp. 2 (KU727691)[h]
			Diaporthe masirevicii (KJ197257)[e]	96	95	390	(KX015134)[i]
9803	L, R	7	*Colletotrichum karstii* (HM585409)[d]	89	99	400	*Colletotrichum karstii* (KU727692)[h]
			Colletotrichum karstii (HM585428)[e]	100	100	463	(KU878164)[i]
9685	L, R	7	*Guignardia mangiferae* (JF261465)[d]	100	100	512	*Guignardia mangiferae*
			Guignardia mangiferae (AB704275)[e]	65	99	560	(KU727693)[h] (KX015127)[i]
9679	L, R	5	*Diaporthe arengae* (NR111843)[d]	99	93	455	*Diaporthe* sp. 3 (KU727694)[h]
			Diaporthe nothofagi (KF170922)[e]	100	90	493	(KU992320)[i]
9720	L, R	5	*Diaporthe miriciae* (KJ197282)[d]	99	99	466	*Diaporthe miriciae* (KU727695)[h]
			Diaporthe miriciae (KJ197262)[e]	100	99	399	(KU878162)[i]
9752	L, R	5	*Geotrichum vulgare* (NR077072)[d]	100	92	423	*Dipodascaceae* sp. (KU727696)[h]
9826	L, R	4	*Diaporthe foeniculina* (KC843295)[d]	100	98	491	*Diaporthe* sp. 4 (KU727697)[h]
			Diaporthe foeniculina (KC843209)[e]	99	96	450	(KU992321)[i]
9762	L, R	4	*Diaporthe sackstonii* (KJ197287)[d]	99	97	449	*Diaporthe* sp. 5 (KU727698)[h]
			Diaporthe acaciarum (KP004509)[e]	93	93	464	(KX015135)[i]
9678	L, R	4	*Fusarium circinatum* (NR120263)[d]	99	99	462	*Fusarium* sp. (KU727699)[h]
			Fusarium bulbicola (KF466437)[e]	72	99	378	(KX015136)[i]
9764	L, R	4	*Nemania abortive* (NR121350)[d]	99	89	460	*Xylariaceae* sp. 1 (KU727700)[h]
9807	L, R	3	*Bipolaris drechsleri* (KF500530)[d]	100	98	453	*Bipolaris drechsleri* (KU727701)[h]
9954	L	3	*Coccomyces proteae* (JX089385)[d]	100	93	438	*Coccomyces* sp. (KU727702)[h]
9959	L	3	*Diaporthe diospyricola* (KF777156)[d]	100	96	455	*Diaporthe* sp. 6 (KU727703)[h]
			Diaporthe macintoshii (KJ197269)[e]	100	94	435	(KX015137)[i]
9824	L, R	3	*Diaporthe endophytica* (NR111847)[d]	99	97	486	*Diaporthe* sp.7 (KU727704)[h]
9893	L	3	*Medicopsis romeroi* (NR130697)[d]	97	90	438	*Trematosphaeriaceae* sp.
							(KU727705)[h]
9880	L	3	*Muscodor suthepensis* (JN558830)[d]	97	97	480	*Muscodor* sp. 2 (KU727706)[h]
9975	R	3	*Neofusicoccum parvum* (KF766204)[d]	62	86	473	*Botryosphaeriales* sp. 1
							(KU727707)[h]
9829	R	3	*Penicillium herquei* (NR103659)[d]	95	99	481	*Penicillium herquei* (KU727708)[h]
			Penicillium herquei (JN625969)[e]	100	99	444	(KU992317)[i]
9741	L, R	3	*Pestalotiopsis australasiae* (KM199297)[d]	100	100	508	*Pestalotiopsis australasiae*
			Pestalotiopsis australasiae (KM199410)[e]	100	100	400	(KU727709)[h] (KU840800)[i]
9960	L, R	3	*Phialocephala scopiformis* (NR119460)[d]	88	94	470	*Helotiales* sp. (KU727710) [h]
Vg1F5	L	2	*Candida michaelii* (AY520329)[g]	99	100	419	*Yamadazyma michaelii*
							(KU752546)[k]
Vg11F10	L	2	*Candida* sp. TT12 (HQ014450)[g]	100	100	480	*Yamadazyma riverae* (KU752547)[h]
			Candida sp. TT12 (HQ014447)[d]	100	100	480	(KU752549)[k]
9943	L, R	2	*Crucellisporiopsis marquesiae* (KP004443)[d]	98	89	407	*Hyaloscyphaceae* sp. (KU727711)[h]
9718	L	2	*Diaporthe inconspicua* (NR111849)[d]	100	99	485	*Diaporthe* sp. 8 (KU727712)[h]
9747	R	2	*Diaporthe maytenicola* (KF777157)[d]	99	96	455	*Diaporthe* sp. 9 (KU727713)[h]
9686	L, R	2	*Diaporthe terebinthifolii* (NR111862)[d]	100	98	461	*Diaporthe* sp. 10 (KU727714)[h]
			Diaporthe miriciae (KJ197262)[e]	100	90	399	(KX024588)[i]
9889	L, R	2	*Harpophora oryzae* (EU636699)[d]	97	86	450	*Magnaporthaceae* sp. 1
							(KU727715)[h]
9905	L	2	*Kabatiella lini* (FJ157873)[e]	65	84	401	*Dothideomycetes* sp. (KX024589)[i]
9891	L	2	*Mycoleptodiscus terrestris* (JN711860)[d]	99	84	473	*Magnaporthaceae* sp. 2
							(KU727716)[h]
9937	R	2	*Myxotrichum arcticum* (NR111037)[d]	97	98	480	*Myxotrichum* sp. (KU727717)[h]
9771	L, R	2	*Penicillium citrinum* (NR121224)[d]	98	99	460	*Penicillium citrinum* (KU727718)[h]
			Penicillium citrinum (KC345003)[e]	100	100	425	(KX015128)[i]
9903	R	2	*Pezicula sporulosa* (KF376107)[d]	100	98	429	*Pezicula sporulosa* (KU727719)[h]
9962	L	2	*Phaeophleospora stramenti* (KF901617)[d]	69	95	583	*Mycosphaerellaceae* sp.
							(KU727720)[h]
9949	R	2	*Quadricrura meridionalis* (NR119401)[d]	84	88	494	*Tetraplosphaeriaceae* sp.
							(KU727721)[h]
9736	R	2	*Trichoderma effusum* (NR111833)[d]	99	99	562	*Trichoderma effusum* (KU727722)[h]
9703	L	1	*Camarosporium aloes* (KF777142)[d]	100	90	470	*Botryosphaeriales* sp. 2
							(KU727723)[h]

(*continued on next page*)

Table 1 (*continued*)

UFMGCB[a]	Tissue[b]	No of isolates	[c]Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	No of bp analyzed	Proposed taxa (GenBank acc. no)
9918	L	1	*Camarosporium aloes* (KF777142)[d]	100	90	468	*Pleosporineae* sp. (KU727724)[h]
Vg27R5	R	1	*Candida parapsilosis* (JN091166)[g]	100	100	481	*Lodderomyces* (*Candida*) *parapsilosis* (KU752548)[k]
9972	R	1	*Cladophialophora boppii* (EU103997)[d]	99	91	519	*Herpotrichiellaceae* sp. (KU727725)[h]
9708	R	1	*Clonostachys rosea* (AF358231)[d]	97	99	487	*Clonostachys* cf. *rosea* (KU727726)[h]
			Clonostachys rosea (KJ413352)[e]	75	99	394	(KX015129)[i]
9859	L	1	*Colletotrichum cymbidiicola* (NR111694)[d]	98	100	491	*Colletotrichum* sp. 1 (KU727727)[h]
9802	L	1	*Colletotrichum gigasporum* (KF687715)[d]	98	92	454	*Colletotrichum* sp. 2 (KU727728)[h]
			Colletotrichum gigasporum (KF687866)[e]	100	98	623	(KX060161)[i]
9939	R	1	*Crucellisporiopsis marquesiae* (KP004443)[d]	100	99	475	*Crucellisporiopsis marquesiae* (KU727729)[h]
9697	R	1	*Daldinia loculata* (AF176959)[d]	100	99	395	*Daldinia loculata* (KU727730)[h]
			Daldinia loculata (AY951698)[e]	100	98	352	(KX015130)[i]
9690	L	1	*Diaporthe acaciarum* (KP004460)[d]	99	99	456	*Diaporthe acaciarum* (KU727731)[h]
			Diaporthe acaciarum (KP004509)[e]	100	97	426	(KX015131)[i]
9770	L	1	*Diaporthe acaciarum* (KP004509)[e]	100	94	382	*Diaporthe* sp. 11 (KX060162)[i]
9664	L	1	*Diaporthe acaciigena* (KC343005)[d]	99	94	476	*Diaporthe* sp. 12 (KU727732)[h]
			Diaporthe acaciigena (KC343973)[e]	90	93	462	(KX060163)[i]
9663	L	1	*Diaporthe anacardii* (NR111841)[d]	99	95	486	*Diaporthe* sp. 13 (KU727733)[h]
			Diaporthe foeniculina (KC843209)[e]	99	94	454	(KX060164)[i]
9765	R	1	*Diaporthe anacardii* (NR111841)[d]	99	95	483	*Diaporthe* sp. 14 (KU727734)[h]
			Diaporthe foeniculina (KC843209)[e]	100	94	401	(KX060165)[i]
9779	L	1	*Diaporthe citri* (KC843311)[d]	100	96	455	*Diaporthe* sp. 15 (KU727735)[h]
			Diaporthe foeniculina (KC843209)[e]	100	96	405	(KX060166)[i]
9801	R	1	*Diaporthe citri* (KC843311)[d]	100	92	486	*Diaporthe* sp. 16 (KU727736)[h]
			Diaporthe citri (KC843187)[e]	98	86	417	(KX060167)[i]
9693	R	1	*Diaporthe eucalyptorum* (NR120157)[d]	99	99	480	*Diaporthe eucalyptorum* (KU727737)[h]
9674	R	1	*Diaporthe hickoriae* (NR103699)[d]	99	92	455	*Diaporthe* sp. 17 (KU727738)[h]
			Diaporthe sclerotioides (KC344161)[e]	100	91	491	(KX060168)[i]
9672	L	1	*Diaporthe kongii* (NR111616)[d]	98	99	450	*Diaporthe kongii* (KU727739)[h]
			Diaporthe kongii (KJ197272)[e]	100	99	448	(KX015132)[i]
9659	R	1	*Diaporthe masirevicii* (KJ197257)[d]	100	98	455	*Diaporthe masirevicii* (KU727740)[h]
			Diaporthe masirevicii (KJ197257)[e]	100	98	493	(KU992318)[i]
9854	L	1	*Diaporthe maytenicola* (KF777157)[d]	99	94	483	*Diaporthe* sp. 18 (KU727741)[h]
			Diaporthe foeniculina (KC843209)[e]	99	94	497	(KX060169)[i]
9695	R	1	*Diaporthe nothofagi* (KF170922)[e]	97	88	460	*Diaporthe* sp. 19 (KX060170)[i]
9754	L	1	*Diaporthe saccarata* (NR120260)[d]	100	97	449	*Diaporthe* sp. 20 (KU727742)[h]
			Diaporthe macintoshii (KJ197269)[e]	98	95	431	(KX060171)[i]
9665	R	1	*Diaporthe sojae* (KJ590719)[d]	99	97	437	*Diaporthe* sp. 21 (KU727743)[h]
			Diaporthe masirevicii (KJ197257)[e]	99	95	475	(KX060172)[i]
9970	R	1	Fungal sp. (AY699698)[d]	86	93	449	Fungal sp. (KU727744)[h]
9794	L	1	*Fusarium inflexum* (U34577)[d]	99	99	427	*Fusarium* sp. (KU727745)[h]
			Fusarium oxysporum (KR072629)[e]	91	100	301	(KX060173)[i]
9729	R	1	*Hypoxylon liviae* (KC968922)[d]	100	91	401	*Xylariaceae* sp. 2 (KU727746)[h]
9950	R	1	*Lecythophora decumbens* (HE610337)[d]	98	99	478	*Lecythophora decumbens* (KU727747)[h]
9866	L	1	*Muscodor equiseti* (JX089322)[d]	99	100	475	*Muscodor equiseti* (KU727748)[h]
9790	L	1	*Muscodor oryzae* (JX089321)[d]	99	99	501	*Muscodor oryzae* (KU727749)[h]
9810	L	1	*Muscodor suthepensis* (JN558830)[d]	97	100	502	*Muscodor suthepensis* (KU727750)[h]
9967	L	1	*Mycosphaerella stramenti* (DQ303042)[d]	99	99	485	*Mycosphaerella stramenti* (KU727751)[h]
9675	R	1	*Neopestalotiopsis surinamensis* (KM199351)[d]	99	100	464	*Neopestalotiopsis surinamensis*
			Neopestalotiopsis surinamensis (KM199465)[e]	99	99	432	(KU727752)[h] (KX060174)[i]
9694	L	1	*Ochrocladosporium adansoniae* (KF777176)[d]	100	90	510	*Pleosporales* sp. (KU727753)[h]
9931	L	1	*Pallidocercospora ventilago* (KF777177)[d]	98	100	413	*Pallidocercospora* sp. (KU727754)[h]
9772	R	1	*Paraconiothyrium estuarinum* (AY642530)[d]	98	98	494	*Paraconiothyrium* sp. (KU727755)[h]
			Paraconiothyrium estuarinum (JX496355)[e]	85	94	396	(KX060175)[i]
9793	R	1	*Paraphaeosphaeria parmeliae* (KP170654)[d]	100	99	491	*Paraphaeosphaeria parmeliae* (KU727756)[h]
9928	L	1	*Penicillium quebecense* (NR121507)[d]	100	99	472	*Penicillium quebecense*
			Penicillium quebecense (JN606622)[f]	100	98	718	(KU727757)[h] (KU992316)[j]
9864	L	1	*Peyronellaea prosopidis* (KF777180)[d]	100	96	419	*Peyronellaea* sp. (KU727758)[h]
9979	L	1	*Pseudobotrytis bisbyi* (KF733464)[d]	97	85	437	*Coniochaetales* sp. (KU727759)[h]
9945	L	1	*Pseudocercospora norchiensis* (GU269753)[d]	100	100	439	*Pseudocercospora norchiensis* (KU727760)[h]
9946	R	1	*Pseudolachnea fraxini* (JQ889287)[d]	100	84	457	*Sordariomycetes* sp. (KU727761)[h]
9915	L	1	*Pseudophialophora schizachyrii* (KF689650)[d]	99	89	401	*Magnaporthaceae* sp. 3 (KU727762)[h]
9953	L	1	*Stagonospora perfecta* (KF251258)[d]	100	87	395	*Massarinaceae* sp. 1 (KU727763)[h]

Table 1 (*continued*)

UFMGCB[a]	Tissue[b]	No of isolates	[c]Top BLAST search results (GenBank accession number)	Query cover (%)	Identity (%)	No of bp analyzed	Proposed taxa (GenBank acc. no)
9901	L	1	*Stagonospora pseudocaricis* (KF251260)[d]	100	86	459	*Massarinaceae* sp. 2 (KU727764)[h]
9667	R	1	*Xylaria arbuscula* (JN601145)[e]	100	99	500	*Xylaria arbuscula* (KU727765)[h]
			Xylaria arbuscula (GQ478226)[d]	100	96	360	(KX015133)[i]

[a]UFMGCB = Culture of Microorganisms and Cells from the Federal University of Minas Gerais. [b]Tissue: L = leaf and R = root. [c]Top BLAST search results represent the first sequences matched with the endophytic fungi sequences of *Vellozia gigantea*. Taxa subjected to phylogenetic analysis based on the [d]ITS1-5.8S-ITS2, [e]β-tubulin, [f]Polymerase II and [g]D1/D2 regions for elucidation of taxonomic positions. [h]ITS1-5.8S-ITS2, [i]β-tubulin, [j]Polymerase II and [k]D1/D2 sequences deposited in the GenBank.

GenBank database, with estimations conducted using MEGA Version 6.0 (Tamura et al., 2013). The maximum composite likelihood method was employed to estimate evolutionary distances with bootstrap values calculated from 1000 replicate runs. The information about fungal classification generally follows Kirk et al. (2008), MycoBank (http://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org) databases.

2.3. Ecological analysis

To quantify species diversity, richness and dominance, we used Fisher's α, Margalef's and Simpson's indices, respectively. All diversity and similarity indices were performed using the computer program PAST, version 1.90 (Hammer et al., 2001).

3. Results

3.1. Taxonomic and diversity analyses

Two hundred eighty-five fungal isolates were recovered as endophytes of *V. gigantea*, and were identified into 27 genera and 87 taxa (Table 1). *Xylaria berteri*, *Diaporthe* sp. 1, *Nigrospora oryzae*, *Muscodor* sp. 1, *Colletotrichum aeschynomene*, and *Trichoderma viride* occurred in the highest frequency in both the leaf and root. *Diaporthe* was the most abundant genus with 70 endophytic isolates recovered from the leaves and roots. The taxa distribution in *V. gigantea* tissue was as follows: 24 taxa occurred in both leaf and root tissues, 36 were obtained only in the leaves, and 27 were only in the roots (Table 1).

Among the total taxa identified, 62 (70%) occurred as singlets (occurrence $\leq$ 2 isolates), including those of the genera *Clonostachys*, *Coccomyces*, *Crucellisporiopsis*, *Daldinia*, *Myxotrichum*, *Pallidocercospora*, *Pezicula*, *Peyronellaea*, and *Pseudocercospora*, which displayed a phylogenetic relationship with fungi obtained from different environments of the world.

Additionally, the diversity indices displayed high values: Fisher $\alpha = 42.68$ (diversity), Margalef = 15.21 (richness), and Simpson = 0.96 (dominance). Fifty-four taxa displayed low molecular taxonomic identities with sequences of fungi deposited in GenBank, and were identified on the genera, family, class, or order levels and may represent new species.

4. Discussion

4.1. Taxonomic and diversity analyses

Our results showed that the diversity of the fungal species associated with *V. gigantea* was high when compared with other studies of endophyte communities from other plant hosts. The abundant cosmopolitan fungal genera (*Xylaria*, *Diaporthe*, *Nigrospora*, *Muscodor*, *Colletotrichum*, and *Trichoderma*) obtained as endophytes of *V. gigantea* represent widely seen common taxa reported in different plants from tropical environments (Carvalho et al., 2012; Ferreira et al., 2015).

Additionally, several endophytes of *V. gigantea* displayed low molecular taxonomic identities with sequences of fungi deposited in GenBank, and were identified on the genera, family, class, or order levels and may represent new species. As an example, the endophytic yeast *Yamadazyma riverae* UFMGCB Vg11F10 recovered from the leaves of *V. gigantea* was recently described as a new species (Lopes et al., 2015). However, more detailed taxonomic (polyphasic taxonomy) studies will be necessary to describe these endophytic taxa.

We detected several minority taxa (singlets) within the fungal community, including those of the genera *Clonostachys*, *Coccomyces*, *Crucellisporiopsis*, *Daldinia*, *Myxotrichum*, *Pallidocercospora*, *Pezicula*, *Peyronellaea*, and *Pseudocercospora*, which seem to be rare or unreported as endophytic species. *Clonostachys rosea*, described as *Gliocladium roseum*, is the anamorph stage of *Bionectria ochroleuca* (Bionectriaceae) (Schroers et al., 1999), which is a common soil saprobic fungus that occurs in different habitats (Sutton et al., 1997). Vega et al. (2008) reported the endophytic *Clonostachys rosea*, recovered from coffee leaves in Colombia, as an entomopathogenic species with pathogenic activity against the Coleoptera *Hypothenemus hampei*, the most devastating pest of coffee worldwide. Additionally, *Clonostachys rosea* was described as an endophyte of cucumber (Chatterton et al., 2008) and able to induce expression of defence genes in wheat and canola (Lahlali and Peng, 2013). This same fungal species was found to be a mycoparasite that produces chitanase and glucanase enzymes and peptides against known phytopathogenic fungi (Rodríguez et al., 2011). The genus *Coccomyces* (Rhytismataceae) comprises about 116 species

of ligninolytic fungi (Kirk et al., 2008). Hirose et al. (2013) recovered *Coccomyces sinensis* as a decomposer endophyte of *Camellia japonica*. The genus *Crucellisporiopsis* includes only three recognised species (Nag Raj, 1993). *Crucellisporiopsis marquesiae* was recently described as a new species that occurs on the twigs of *Marquesia acuminata* (Dipterocarpaceae) in Zambia, Africa (Crous et al., 2014). *Daldinia* (Xylariaceae) comprises about 20 species of wood-inhabiting fungi (Ju et al., 1997), including taxa reported as endophytes (Whalley, 1996). We recovered *Daldinia loculata* as an endophyte of *V. gigantea*, and since the plant is an endemic species adapted to arid conditions, it suggests that *Daldinia* species are adapted ecologically and physiologically to xerophytic conditions (Ingold, 1965). Additionally, *Daldinia loculata* is able to colonise burned substrates (Ju et al., 1997). *Myxotrichum* sp. was recovered from the root tissue of *V. gigantea*. The genus *Myxotrichum* (Myxotrichaceae) includes many cellulolytic and mycorrhizal species associated with members of the Ericaceae and Epacridaceae (Cannon and Kirk, 2007). The cosmopolitan genus *Pseudocercospora* (teleomorph *Mycosphaerella*) is a recognised plant pathogen fungal group which is commonly associated with leaves and fruits of different host plants of arid, cool temperate, sub-tropical, and tropical regions (Crous et al., 2013). In addition, *Pseudocercospora* includes endophytic and saprobic species, and is used as a biological control agent of weeds (Den Breeÿen et al., 2006). *Pezicula* (Dermateaceae) includes species growing endophytically, such as *Pezicula sporulosa*, which is associated with shrubs and trees, whereas other species are parasitic (Abeln et al., 2000).

In this study, the endophytic fungal community of *V. gigantea* was found to be rich, diverse, and complex, and included taxa that are mutualistic, saprobic, and parasitic. Some of these fungi have the ability to control phytopathogens (mycoparasitic) and insects (entomopathogenic), whereas others are xerophytic (fire-adapted), or involved in endophyte-mycorrhizal fungi interactions. We suggest that the natural endophytic fungal microbiota associated with *V. gigantea* might contribute in part to the plant's fitness and its millennial age in the natural environment; however, further detailed studies will be necessary to prove these hypothesis.

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