

Bezerromycetales and Wiesneriomycetales ord. nov. (class Dothideomycetes), with two novel genera to accommodate endophytic fungi from Brazilian cactus

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Abstract

During a survey of endophytic fungi from the cactus *Tacinga inamoena* in a Brazilian tropical dry forest (Caatinga) some undescribed ascomycetous fungi were isolated. These fungi are characterized by superficial and immersed, globose to subglobose, smooth or hairy ascomata, bitunicate asci, and muriformly septate, ellipsoidal ascospores. Multigene phylogenetic analyses using sequences from partial ITS, SSU and LSU nrDNA and the translation elongation factor 1-alpha gene (*tef1*) demonstrated a monophyletic clade accommodating these endophytic fungi in the class Dothideomycetes, closely related to the order Tubeufiales. Based on morphological features and phylogenetic analyses, these fungi could not be placed in the order Tubeufiales, in the new order Wiesneriomycetales, or any other known genus in the class Dothideomycetes. Thus, two new genera (*Bezerromyces*, with *B. brasiliensis* and *B. pernambucoensis*, and *Xiliomyces* with *X. brasiliensis*), a new family (Bezerromycetaceae) and a new order (Bezerromycetales) are introduced to accommodate these novel taxa. Our phylogenetic analyses also demonstrated that the clade accommodating Wiesneriomycetaceae represents a new order, here introduced as Wiesneriomycetales.

Keywords: Endophytes, Fungal diversity, Multigene phylogeny, *Tacinga inamoena*, Taxonomy

Introduction

Endophytes are enigmatic microorganisms that live in tissues of all plants without appearing to cause damage. These microorganisms can help their hosts by protecting them against temperature, stresses caused by drought and humidity, UV light, herbivores, and plant pathogenic organisms (Redman et al. 2002; Hubbard et al. 2014; Jia et al. 2016). Endophytes can stimulate the growth of plants via the production of phytohormones, promoting the germination and dispersal of seeds, and the biocontrol of pathogens (Porrás-Alfaro and Bayman 2011; Vidal and Jaber 2015). These microorganisms can also be utilized for their biotechnological potential in the production of secondary metabolites, phytoremediation, and in the degradation of environmental pollution (Wang and Dai 2011; Chandra 2012; Santos et al. 2015). In addition to all environmental and biotechnological benefits, these microorganisms are extremely important for the estimation of fungal diversity. Several studies of endophytic fungi have contributed directly to the discovery of new fungi, their ecology, host–fungus relationships, and their distribution in different agricultural crops and natural environments. Studies on endophytic fungi have been conducted in a range of climatic and ecological zones, including temperate and tropical forests, polar regions, deserts, arid and semiarid environments (Arnold et al. 2000; Murali et al. 2007; Rosa et al. 2009; Sun et al. 2012; Bezerra et al. 2013, 2015; Nascimento et al. 2015; Massimo et al. 2015).

One of the most important Brazilian tropical dry forests is called “Caatinga”. This ecosystem is exclusive to Brazil, covers most states in the northeastern region of the country, and includes numerous plant species belonging to about 123 different families (Flora do Brasil 2020, <http://floradobrasil.jbrj.gov.br/>). Despite the fact that the Caatinga harbours several endemic species, including plants, birds, mammals and fishes, little attention has been paid to its conservation, considering the diverse biota and striking landscape, which results in an underestimation of Brazilian biodiversity (Bernard et al. 2014). According to information from the Caatinga Association (<http://www.acaatinga.org.br/>), only about 8% of the Caatinga ecosystem is presently protected. This ecosystem maintains 90 species of Cactaceae plants, with some being rare and endemic. These species require attention due to the fact that they appeared on the Convention on International Trade in Endangered Species of Wild Fauna and Flora list, underlining the importance of these cacti in the international wildlife trade (Cabral et al. 2013; Meiado et al. 2015; Taylor et al. 2015). Studies on fungal diversity in the Caatinga published to date have revealed many known and unknown taxa belonging to Ascomycota, Basidiomycota and Mucoromycotina (Maia et al. 2015). The study of the endophytic fungal community from plants of the Caatinga is still incipient, and very few reports have been published on fungal endophytes from cacti growing in this environment (Bezerra et al. 2012, 2013; Freire et al. 2015).

The first study of endophytic fungi from the cactus *Opuntia stricta* was published by Fisher et al. (1994) in Australia. They reported endophytic fungi belonging to 13 families and 8 orders. Similar results were obtained by Suryanarayanan et al. (2005), who studied 21 cacti species in the USA and reported the isolation of endophytes belonging to 8 families and 6 orders. Recently, Silva-Hughes et al. (2015) used a medicinal cactus *O. humifusa* in the USA to estimate the endophyte diversity and their antifungal activity. Using phylogenetic analyses they grouped the endophytes in 17 taxa representing 9 families and 6 orders. A

similar study on the diversity of the two native and sympatric cacti, *Myrtillocactus geometrizans* and *O. robusta* in Mexico, was recently published by Fonseca-García et al. (2016). Although they used molecular techniques to estimate the endophytic fungal diversity, several operational taxonomic units (OTUs) were identified only as Ascomycota and others were identified as fungi belonging to 14 different orders.

In Brazil, only three studies of endophytes from cacti growing in the Caatinga have thus far been conducted. The first, published by Bezerra et al. (2012), studied the cactus *O. ficus-indica* and the capacity of these endophytic fungi to produce hydrolytic enzymes. The authors identified 12 genera belonging to 8 families of 7 orders. Bezerra et al. (2013) studied the cactus *Cereus jamacaru* subsp. *jamacaru* and reported endophytic fungi belonging to 30 genera in 14 families of 10 orders in Ascomycota, 2 families of 3 orders in Basidiomycota, and 2 families of 1 order in Mucoromycotina. Freire et al. (2015) studied the influence of an insect (Hemiptera) on the endophytic fungal community of *O. ficus-indica*, and observed endophytes belonging to 9 families of 7 orders. These few reports on fungal endophyte diversity from cacti species revealed a great diversity of unknown fungi, which could greatly contribute to the global estimates of fungal microdiversity.

During a recent survey of endophytic fungi associated with the cactus *Tacinga inamoena* from the Caatinga, four strains were obtained. These strains did not fit morphologically into any currently known genus. Therefore, we carried out DNA sequence analyses of four loci (ITS, SSU, LSU, and *tef1*) to determine the phylogenetic position of these strains and to resolve their taxonomic status.

Materials and methods

Isolation of endophytic fungi from cactus

Endophytic fungi were isolated as described by Bezerra et al. (2013) from the cactus *Tacinga inamoena* (K. Schum.) N.P. Taylor & Stuppy growing in Brazilian tropical dry forest (Caatinga), Catimbau National Park, Buíque municipality, Pernambuco state, Brazil (8°36'35"S, 37°14'40"W), and the Sustainable family farming plots, Itaíba municipality, Pernambuco state, Brazil (9°08.895S, 37°12.069W). The collections were authorized by the Ministério do Meio Ambiente (MMA)/Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio); permission number: 40331-1/authentication code 87451826 issued on 4 November, 2013.

Morphology

Endophytic fungi isolated from the cactus were cultured on malt extract agar (MEA), potato dextrose agar (PDA), water agar (WA), and synthetic nutrient deficient agar (SNA) (Crous et al. 2009), and incubated at 22 °C under a natural day–night cycle. Macro- and micro-morphological analyses (colony diameter, texture, pigmentation, margin appearance, exudates, and colours) were performed after 2 weeks, and the structures of reproduction were visualized after 2–3 months on WA and SNA culture media. Slide preparations were mounted in clear lactic acid. Endophytic strains are deposited in the culture collections of Micoteca URM Profa. Maria Auxiliadora Cavalcanti (Federal University of Pernambuco,

Recife, Brazil – www.ufpe.br/micoteca, WCDM 604) and the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (under Material Transfer Agreement – MTA No. 05/2015/Micoteca URM, issued on 14 April, 2015). Herbarium materials are deposited in Herbário URM Pe. Camilo Torrend (Federal University of Pernambuco, Recife, Brazil – <http://inct.florabrasil.net/participantes/herbarios-curadores/urm/>) and the CBS-KNAW Fungal Biodiversity Centre (CBS). Nomenclature and taxonomic information were deposited in MycoBank (www.mycobank.org) (Crous et al. 2004).

DNA extraction, amplification (PCR) and sequencing

Genomic DNA extraction was performed using the Wizard[®] Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. The primers NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), EF3Fd (Groenewald et al. 2013)/EF1-2218R (designed by S. Rehner - www.aftol.org/pdfs/EF1primer.pdf) and ITS5/ITS4 (White et al. 1990) were used to amplify part of the nuclear ribosomal small subunit (SSU) of the rDNA, part of the nuclear ribosomal large subunit (LSU) of the rDNA, part of the translation elongation factor 1-alpha (*tef1*), and the ITS region (first and second internal transcribed spacer regions and intervening 5.8S nrDNA), respectively. The ITS sequences were not included in the phylogenetic analyses.

Amplification reactions, with a total volume of 12.5 µL, were composed of 1× PCR buffer (Bioline, Luckenwalde, Germany), 5.6% dimethyl sulfoxide (DMSO) (v/v), 20 µM dNTPs, 0.2 µM of each forward and reverse primers, 0.25 U of *Taq* DNA polymerase (Bioline), and 10 ng of genomic DNA. The PCR conditions were: start step of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 50 s annealing (at 48 °C for SSU, LSU and ITS, and at 54 °C for *tef1*), and 1 min and 30 s extension at 72 °C, followed by a final extension step of 7 min at 72 °C and a cool-down step to 10 °C. The PCR amplicons were visualized with GelRed Nucleic Acid Gel Stain (Biotium, CA, USA) during 1% agarose gel electrophoresis.

Amplicons were sequenced using the same PCR primers sets with a BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (GE Healthcare Life Sciences, Eindhoven, The Netherlands) in MultiScreen HV plates (Merck Millipore, Darmstadt, Germany). Sequences were analysed on an ABI 3730xl DNA Analyzer (ThermoFisher Scientific, The Netherlands). The consensus sequences were computed and visually inspected using SeqMan v.7.0.0 (DNASTAR, Madison, WI, USA) and/or MEGA v.6 software (Tamura et al. 2013).

Phylogenetic analyses

To infer a preliminary phylogenetic relationship for the new sequences, an initial alignment of the newly generated sequences (SSU, LSU, and *tef1*) and 217 Dothideomycetes and Eurotiomycetes taxa, extracted from the dataset of Boehm et al. (2015, TreeBASE Study ID S16151) with two species from Lecanoromycetes as outgroup, was performed using the online MAFFT interface (Kato and Standley 2013; <http://mafft.cbrc.jp/alignment/server>). This alignment was used to infer a preliminary phylogenetic relationship for the new

sequences based on Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses (data not shown).

Based on the initial analysis, a second alignment was constructed on a subset from the original to which were added additional sequences from Boonmee et al. (2014), who introduced Tubeufiales as well as selected sequences from Patellariales (Boehm et al. 2015), Pleosporales (Zhang et al. 2009; Wanasinghe et al. 2014; Ariyawansa et al. 2015; Liu et al. 2015), Wiesneriomycetaceae (Suetrong et al. 2014; Pratibha et al. 2015; Santos 2015; Crous et al. 2016) and Valsariales (Zhang et al. 2009; Jaklitsch et al. 2015). In the second alignment, *Dendrographa decolorans* (DUKE 47570) was used as an outgroup taxon. The phylogenetic analyses were based on MP, ML and Bayesian Inference (BI). PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2003) was used to conduct the MP analyses with 1 000 bootstrap replicates. Alignment gaps were treated as missing data. For ML analyses, RAxML-HPC BlackBox (8.2.8) (Stamatakis 2008) was used to do a fast tree search using the GTRGAMMA substitution model and 500 fast bootstrap replicates. The BI analysis was performed using MrBayes v.3.2.1 (Ronquist et al. 2012). Based on MrModeltest v.2.2 (Nylander 2004) results, the three partitions were analysed using the GTR+I+G model. Support values (ML bootstrap – ML-BS; MP bootstrap – MP-BS; BI posterior probability – BPP) were calculated for all analyses and the tree length (TL), consistency index (CI), retention index (RI) and the rescaled consistency index (RC) for the MP analysis. The phylogenetic tree was visualized in Geneious v.5.5.7 (Kearse et al. 2012) and the layout of the tree was done in Adobe Illustrator v.CS5.1. The newly obtained sequences were deposited in the NCBI (www.ncbi.nlm.nih.gov; Table 1) and the alignment and phylogenetic tree in TreeBASE (Study ID S19537; www.treebase.org).

Table 1. GenBank accession numbers of type species and/or references strains used in this study. Sequences obtained in this study are shown in bold

Species	Collection accession number(s)	GenBank accession numbers			
		LSU	SSU	TEF1- α	ITS
<i>Acanthostigma chiangmaiense</i>	MFLUCC 10-0125	JN865197	JN865185	KF301560	JN865209
<i>Allophaeosphaeria muriformia</i>	MFLUCC 13-0349	KP765681	KP765682	–	KP765680
<i>Bambusaria bambusae</i>	CBS 139763	KP687813	KP687962	KP687983	KP687813
<i>Bezerromyces brasiliensis</i>	URM 7411 (CBS 141545)	KX518623	KX518627	KX518631	KX470390
<i>Bezerromyces pernambucoensis</i>	URM 7412 (CBS 141546)	KX518624	KX518628	KX518632	KX470391
<i>Botryobambusa fusicoccum</i>	MFLUCC 11-0143	JX646809	JX646826	–	NR_111793
<i>Botryosphaeria agaves</i>	MFLUCC 11-0125	JX646808	JX646825	–	NR_111792
<i>Botryosphaeria dothidea</i>	CBS 115476	DQ678051	DQ677998	DQ767637	KF766151
<i>Cophinforma atrovirens</i>	MFLUCC 11-0425	JX646817	JX646833	–	JX646800
<i>Dematiopleospora mariae</i>	MFLUCC 13-0612	KJ749653	KJ749652	KJ749655	KJ749654
<i>Dendrographa decolorans</i>	DUKE 47570	AY548815	AY548809	DQ883725	–
<i>Glyphium elatum</i>	BPI 892669	KM220934	KM220937	KM220932	KM220943
<i>Glyphium elatum</i>	BPI 892671	KM220936	KM220939	KM220933	KM220945
<i>Helicangiospora lignicola</i>	MFLUCC 11-0378	KF301531	KF301539	KF301552	KF301523
<i>Helicoma chiangraiense</i>	MFLUCC 10-0115	JN865188	JN865176	KF301551	JN865200
<i>Helicoma fagacearum</i>	MFLUCC 11-0379	KF301532	KF301540	KF301553	KF301524
<i>Hysteropatella clavispora</i>	CBS 247.34	AY541493	DQ678006	DQ677901	–

Species	Collection accession number(s)	GenBank accession numbers			
		LSU	SSU	TEF1- α	ITS
<i>Kellermania macrospora</i>	CBS 131716	JX444874	JX444902	–	KF766178
<i>Kellermania yuccigena</i>	CBS 131727	JX444883	JX444908	–	KF766185
<i>Murispora rubicunda</i>	IFRD 2017	FJ795507	GU456308	GU456289	–
<i>Myrmaecium fulvopruinatum</i>	CBS 139058	KP687861	KP687968	KP688030	KP687861
<i>Myrmaecium rubricosum</i>	CBS 139068	KP687885	KP687979	KP688053	KP687885
<i>Myrmaecium rubrum</i>	CBS 109505	GU456324	GU456303	GU456260	–
<i>Parawiesneriomyces syzygii</i>	CBS 141333	KX228339	–	–	KX228288
<i>Patellaria atrata</i>	CBS 958.97	GU301855	GU296181	GU349038	–
<i>Patellaria cf. atrata</i>	BCC 28876	GU371836	GU371828	–	–
<i>Patellaria cf. atrata</i>	BCC 28877	GU371837	GU371829	–	–
<i>Phaeotrichum benjaminii</i>	CBS 541.72	AY004340	AY016348	DQ677892	–
<i>Phyllosticta ampelicida</i>	CBS 237.48	DQ678085	DQ678034	–	–
<i>Phyllosticta citricarpa</i>	CBS 102374	GU301815	GU296151	GU349053	FJ538313
<i>Populocrescentia forlicesenensis</i>	MFLUCC 14-0651	KT306952	KT306955	–	KT306948
<i>Pseudogliophragma indicum</i>	MTCC 11985	KM052851	KM052852	–	KM052850
<i>Saccharata proteae</i>	CBS 115206	GU301869	GU296194	GU349030	KF766226
<i>Speiropsis pedatospora</i>	CBS 397.59	KR869797	–	–	KR822200
<i>Trichodelitschia bisporula</i>	CBS 262.69	GU348996	GU349000	GU349020	–
<i>Trichodelitschia munkii</i>	Kruys 201	DQ384096	DQ384070	–	–
<i>Tubeufia Chiangmaiensis</i>	MFLUCC 11-0514	KF301538	KF301543	KF301557	KF301530
<i>Tubeufia javanica</i>	MFLUCC 12-0545	KJ880036	KJ880035	KJ880037	KJ880034
<i>Valsaria insitiva</i>	CBS 127882	KP687886	KP687980	KP688054	KP687886
<i>Valsaria lopadostomoides</i>	CBS 139062	KP687868	KP687972	KP688037	KP687868
<i>Valsaria neotropica</i>	CBS 139064	KP687874	KP687974	KP688042	KP687874
<i>Valsaria robiniae</i>	CBS 139063	KP687870	KP687973	KP688039	KP687870
<i>Valsaria rudis</i>	CBS 139066	KP687879	KP687976	KP688047	KP687879
<i>Valsaria spartii</i>	CBS 139070	KP687843	KP687964	KP688013	KP687843
<i>Wiesneriomyces conjunctosporus</i>	BCC 4027	KJ425449	KJ425440	–	–
<i>Wiesneriomyces conjunctosporus</i>	BCC 18525	KJ425450	KJ425436	–	–
<i>Wiesneriomyces conjunctosporus</i>	BCC 20803	KJ425453	KJ425439	–	–
<i>Wiesneriomyces conjunctosporus</i>	BCC 40633	KJ425455	KJ425442	–	–
<i>Xiliomyces brasiliensis</i>	URM 7413 (CBS 141536)	KX518625	KX518629	KX518633	KX470392
<i>Xiliomyces brasiliensis</i>	URM 7414 (CBS 141535)	KX518626	KX518630	KX518634	KX470393

Sequences obtained in this study are shown in bold

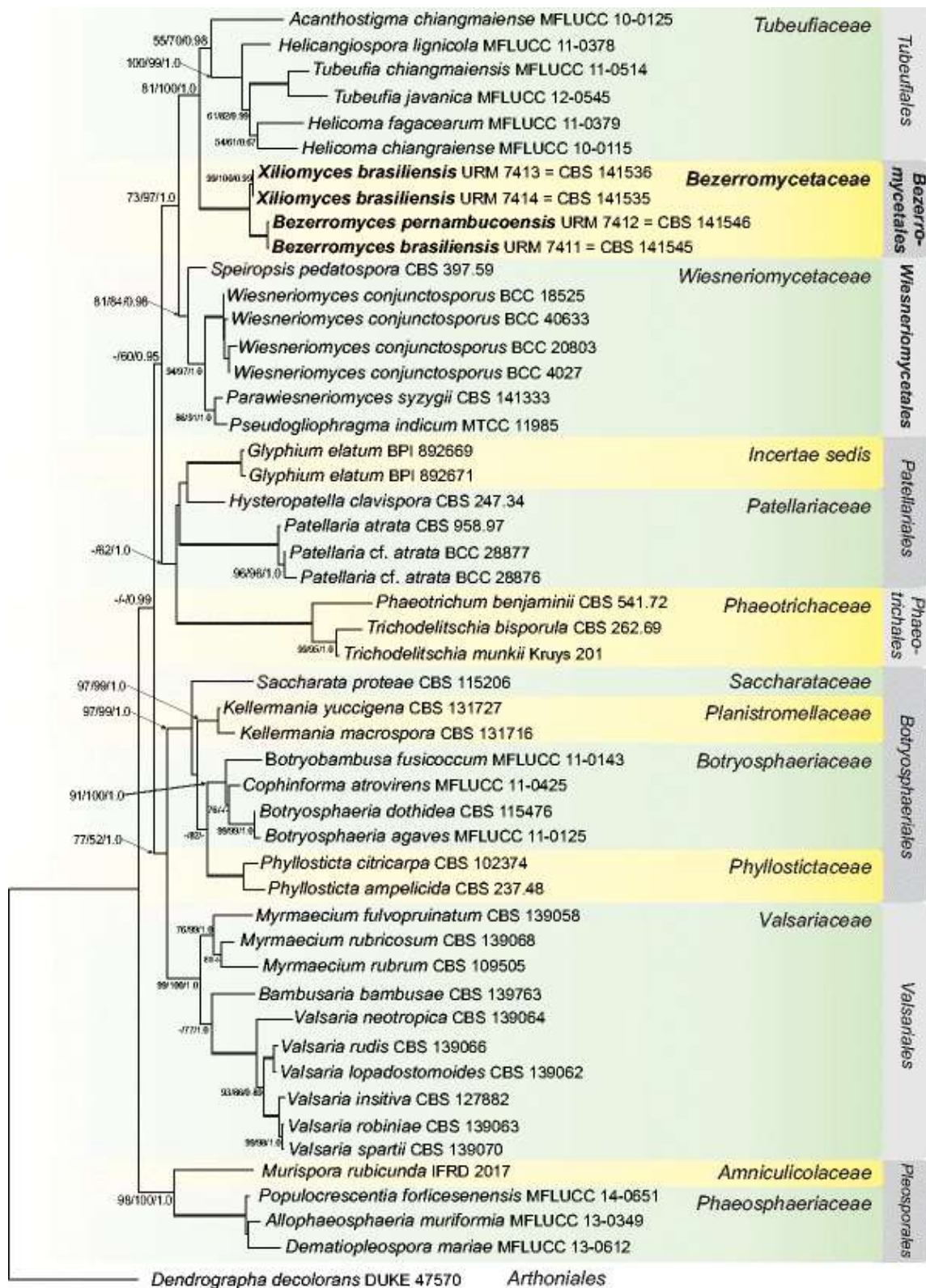


Fig. 1. Maximum likelihood (RAxML) tree obtained by phylogenetic analyses of the combined LSU nrDNA, SSU nrDNA, and *tef1* alignment of 50 taxa belonging to the nine orders shown to the right of the tree. Bootstrap support values from maximum parsimony (MP-BS) and maximum likelihood (ML-BS), and Bayesian posterior probabilities (BPP) are indicated at the nodes (MP-BS/ML-BS/BPP). Thickened branches represent clades with 100% MP-BS and ML-BS, and a BPP = 1.0. The scalebar represents the number of changes. *Dendrographa decolorans* (Arthoniales) was used as outgroup

Results

DNA sequence alignments and phylogenetic analyses

Following the results of preliminary phylogenetic analysis of the initial alignment (data not shown), the phylogenetic reconstruction of the second alignment was performed including sequences from 50 strains representing eight different orders in the Dothideomycetes and one order in the Arthoniomycetes, with the following distribution: Tubeufiales (6), Bezerromycetales ord. nov. (4), Wiesneriomycetales ord. nov. (7), Patellariales (6), Phaeotrichales (3), Botryosphaerales (9), Valsariales (10), Pleosporales (4) and Arthoniales (1) (Table 1). The three-gene dataset comprised of LSU nrDNA sequences for all 50 ingroup sequences, 48 SSU nrDNA sequences and 32 *tef1* sequences. After exclusion of ambiguous regions and introns, the combined dataset included 2628 characters (736 for LSU nrDNA, 1015 for SSU nrDNA, and 877 for *tef1*). In the MP analysis, 1891 characters were constant, 185 were variable and parsimony-uninformative and 558 were parsimony-informative. In the BI analysis, 296, 253, and 354 unique site patterns were present for LSU, SSU and *tef1*, respectively.

The best tree (RAxML) obtained using the ML analysis is shown as Fig. 1, with the support values from the MP, ML and BI analyses plotted at the nodes. The sequences of the endophytic strains clustered together in a distinct clade, fully supported by all analyses (MP-BS = 100%, ML-BS = 100%, and BPP = 1), for which Bezerromycetales ord. nov. is proposed below. The order Tubeufiales is a sister lineage to Bezerromycetales ord. nov. and formed a distinct clade with high BPP support (MP-BS = 55%, ML-BS = 70%, and BPP = 0.98). We have observed that the family Wiesneriomycetaceae is positioned in a separate clade close to the new order and Tubeufiales with high support (MP-BS = 81%, ML-BS = 100%, and BPP = 1). Based on these results, we are introducing a new order, Wiesneriomycetales, below (MP-BS = 81%, ML-BS = 84%, and BPP = 0.98). The Tubeufiales–Bezerromycetales–Wiesneriomycetales group (MP-BS = 73%, ML-BS = 97%, and BPP = 1) is related to the orders Patellariales and Phaeotrichales, but with low support (ML-BS = 60%, and BPP = 0.95). In our analyses Tubeufiales is placed in a different phylogenetic position, compared to the phylogeny of Boonmee et al. (2014), where the order Tubeufiales was positioned as a separate lineage between Botryosphaerales and Patellariales. Phylogenetic analyses with MP, ML, and BI using the dataset of individual loci were also performed, but no topological phylogenetic conflict was observed (data not shown).

Taxonomy

Bezerromycetales J.D.P. Bezerra, C.M. Souza-Motta & Crous, ord. nov.

MycoBank MB817520

Ascomata superficial or immersed in culture media, pseudothecial, unilocular, globose to subglobose, gregarious or solitary, pale brown to brown at maturity, minutely papillate with ostiole, collapsing cupulate or laterally, smooth or hairy. *Peridium* comprising pale brown cells of *textura angularis*, and small cells of *textura prismatica*. *Hamathecium* comprising of numerous, filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate,

fissitunicate, cylindrical to cylindrical-clavate, short pedicellate, with or without a minute ocular chamber. *Ascospores* 1–2-seriate in ascus, hyaline when young, becoming pale brown to brown at maturity, ellipsoidal, muriformly septate, smooth or minutely verrucose. *Chlamydospores* sometimes linked to ascomata by hyphae, multiseptate, brown, dictyochlamydospore-like, globose to subglobose or ellipsoid to cylindrical. Asexual morph unknown. Known only as endophytic fungi associated with cactus species in tropical dry forests.

Type family: Bezerromycetaceae J.D.P. Bezerra, C.M. Souza-Motta & Crous.

Bezerromycetaceae J.D.P. Bezerra, C.M. Souza-Motta & Crous, fam. nov.

MycoBank MB817521

Morphological characters similar as for Bezerromycetales.

Type genus: *Bezerromyces* J.D.P. Bezerra, C.M. Souza-Motta & Crous.

Included genera: *Bezerromyces* J.D.P. Bezerra, C.M. Souza-Motta & Crous, *Xiliomyces* J.D.P. Bezerra, C.M. Souza-Motta & Crous.

Bezerromyces J.D.P. Bezerra, C.M. Souza-Motta & Crous, gen. nov.

MycoBank MB817522

Etymology: Named in honour of José Luiz Bezerra (J.L. Bezerra), an extraordinary mycologist from the previous Institute of Mycology at the University of Recife (IMUR), Pernambuco, Brazil.

Ascomata superficial or immersed in culture media, pseudothecial, unilocular, globose to subglobose, to 330 µm diam, gregarious or solitary, pale brown to brown at maturity, minutely papillate with ostiole, collapsing cupulate or laterally, hairy. *Peridium* comprising pale brown cells of *textura angularis*, and small cells of *textura prismatica*. *Hamathecium* comprising of numerous, filiform, septate, branched, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, short pedicellate, with or without a minute ocular chamber. *Ascospores* 1–2-seriate in ascus, hyaline when young, becoming pale brown to brown at maturity, ellipsoidal, muriformly septate, smooth. *Chlamydospores* sometimes linked to ascomata by hyphae, multiseptate, dictyochlamydospore-like, ellipsoid to cylindrical. Asexual morph unknown.

Type species. *Bezerromyces brasiliensis* J.D.P. Bezerra, C.M. Souza-Motta & Crous.

Bezerromyces brasiliensis J.D.P. Bezerra, C.M. Souza-Motta & Crous, sp. nov.

MycoBank MB 817523. Figure 2.



Fig. 2. *Bezerromyces brasiliensis* (URM 7411 = CBS 141545). **a** Ascomata superficial on WA medium. **b, c** Ascomata pseudothecial (in 85% lactic acid). **d–f** Asci and ascospores. **g** Pseudoparaphyses. **h** Ascospores germinating. **i, j** Chlamydospores. *Scale bars* (**b, c**) 250 μm , (**d**) 30 μm , (**e**) 20 μm , (**f–j**) 30 μm

Etymology: Name reflects the country (Brazil) where J.L. Bezerra was born.

Ascomata superficial or immersed in WA and SNA culture media, pseudothecial, unilocular, globose to subglobose, gregarious or solitary, pale brown to brown at maturity, minutely papillate with ostiole, collapsing cupulate or laterally, hairy, 86–207 μm high and 110–203 μm diam. *Peridium* comprising of pale brown cells of *textura angularis*, and small *textura prismatica*. *Hamathecium* comprising of numerous, filiform, septate, branched, hyaline pseudoparaphyses, sometimes inflated, 2–7 μm wide. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, short pedicellate, with or without a minute ocular chamber, 89–138 \times 19.5–28.5 μm . *Ascospores* 1–2-seriate in ascus, hyaline when young, becoming pale brown to brown at maturity, ellipsoidal, muriformly septate, smooth, 2–4 vertical septa and 3 transverse septa, 12–33 \times 8–15.5 μm . *Chlamydospores* sometimes linked to ascomata by hyphae, multiseptate, dictyochlamydospore-like, globose to subglobose or cylindrical, 13.5–27.5 \times 20.5–40 μm . Asexual morph unknown.

Culture characteristics: Colonies on MEA and PDA are similar, velvety, surface and reverse dark olive to brown, growing up to 5 cm at room temperature (22 °C) in one month.

Specimen examined: Brazil, Pernambuco, Catimbau National Park (8°36'35"S, 37°14'40"W), as endophytic fungus from cactus *Tacinga inamoena*, Sep. 2013, J.D.P. Bezerra (holotype URM 89943; isotype CBS H-22686; culture ex-type URM 7411 = CBS 141545).

Bezerromyces pernambucoensis J.D.P. Bezerra, C.M. Souza-Motta & Crous, **sp. nov.**

Mycobank MB817524. Figure 3.



Fig. 3. *Bezerromyces pernambucoensis* (URM 7412 = CBS 141546). **a** Ascomata superficial on WA medium. **b** Ascomata pseudothecial and asci. **c** Asci, ascospores and pseudoparaphyses. **d–f** Asci and ascospores. **g** Ascospores. **h** Pseudoparaphyses. **i, j** Ascospores germinating. Scale bars (**b**) 220 μ m, (**c**) 100 μ m, (**d–h**) 30 μ m, (**i**) 25 μ m, (**j**) 15 μ m

Etymology: Name reflects the Brazilian state (Pernambuco), where J.L. Bezerra was born.

Ascomata superficial or immersed in WA and SNA culture media, pseudothecial, unilocular, globose to subglobose, gregarious or solitary, liked by hyphae, pale brown to brown at maturity, minutely papillate with ostiole, collapsing cupulate or laterally, hairy, 130–380 μ m high and 135–330 μ m diam. *Peridium* comprising of pale brown cells of *textura angularis*,

and small *textura prismatica*. *Hamathecium* comprising of numerous, filiform, septate, branched, hyaline pseudoparaphyses, 2.3–3.2 µm wide. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, short pedicellate, with or without a minute ocular chamber, 60.5–114 × 20.5–31.5 µm. *Ascospores* 1–2-seriate in ascus, hyaline when young, becoming pale brown to brown at maturity, ellipsoidal, muriformly septate, smooth, 2–4 vertical septa and 3 transverse septa, 10.5–36 × 7.5–14.8 µm. *Chlamydospores* sometimes linked to ascomata by hyphae, multiseptate, dictyochlamydospore-like, ellipsoid to cylindrical, 13.5–38 × 20–40.5 µm. Asexual morph unknown.

Culture characteristics: Colonies on MEA and PDA are velvety, surface and reverse dark olive to brown, growing up to 5 cm at room temperature (22 °C) in 1 month.

Specimen examined: Brazil, Pernambuco, Catimbau National Park (8°36'35"S, 37°14'40"W), as endophytic fungus from cactus *Tacinga inamoena*, Sep 2013, J.D.P. Bezerra (holotype URM 89944; isotype CBS H-22687; culture ex-type URM 7412 = CBS 141546).

Notes: *Bezerromyces brasiliensis* and *B. pernambucoensis* differ in ascomata, asci, and ascospore size. *Bezerromyces* differs from *Populocrescentia* (Ariyawansa et al. 2015), *Dematiopleospora* and *Allophaeosphaeria* (Wanasinghe et al. 2014; Liu et al. 2015) in peridium structure, papillate ostiole, ascomata shape, muriform and fusiform ascospores. Also, it can be differentiated from *Murispora* (Zhang et al. 2009) forming muriform and fusiform ascospores, and having a phoma-like asexual morph (Wanasinghe et al. 2015). All of these genera are affiliated to Pleosporales, which is phylogenetically distant from *Bezerromyces*.

Xiliomyces J.D.P. Bezerra, C.M. Souza-Motta & Crous, gen. nov.

MycoBank MB817525.

Etymology: Named in honour of the Maria Auxiliadora de Queiroz Cavalcanti (Xilia), a mycologist from the previous Institute of Mycology at the University of Recife (IMUR), Pernambuco, Brazil.

Ascomatal structures superficial or immersed in culture media, pseudothecial-like, unilocular, globose to subglobose, up to 188 µm diam, gregarious or solitary, pale brown to brown at maturity, minutely papillate with ostiole, smooth. *Chlamydospores* multiseptate, dictyochlamydospore-like, globose to subglobose, up to 26 µm diam.

Type species: *Xiliomyces brasiliensis* J.D.P. Bezerra, C.M. Souza-Motta & Crous.

Xiliomyces brasiliensis J.D.P. Bezerra, C.M. Souza-Motta & Crous, **sp. nov.**

MycoBank MB817526. Figure 4.

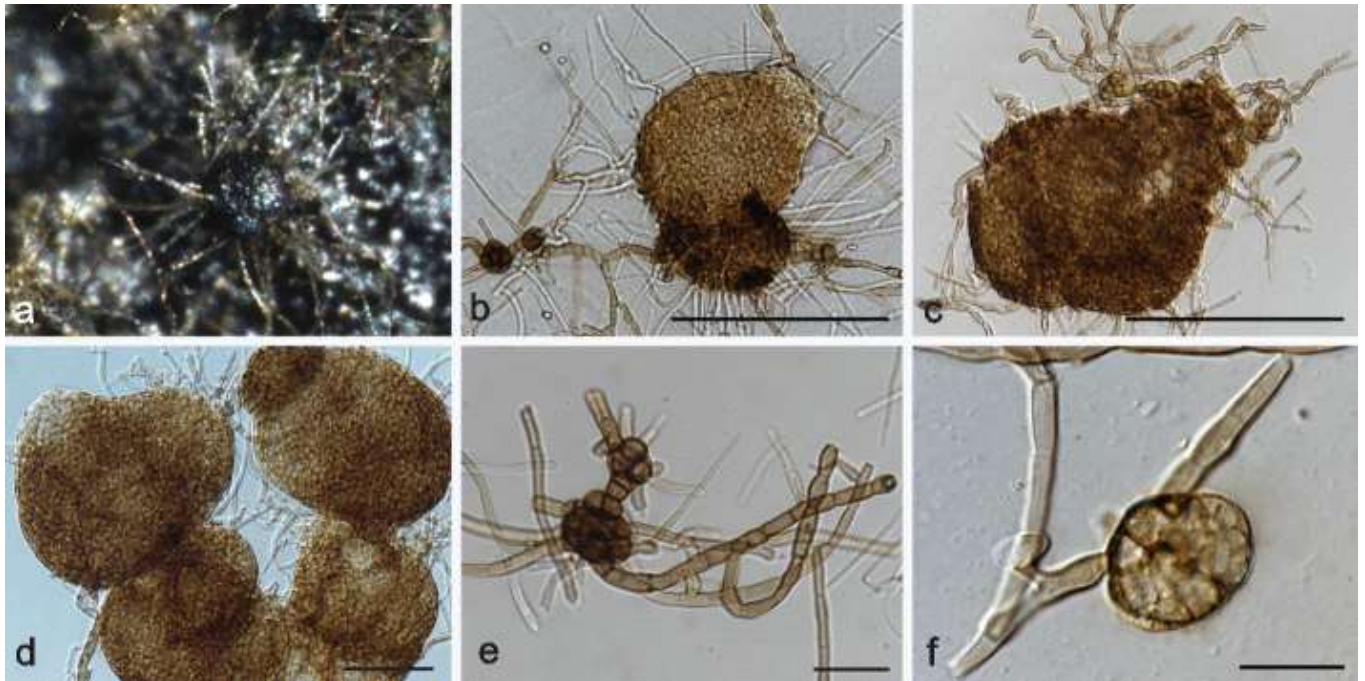


Fig. 4. *Xiliomyces brasiliensis* (URM 7413 = CBS 141536). **a** Ascomatal structures superficial on WA medium. **b–d** Ascomatal structures pseudothecial-like. **e, f** Chlamydospores. Scale bars (**b**) 250 μm , (**c**) 175 μm , (**d**) 100 μm , (**e**) 25 μm , (**f**) 15 μm

Etymology: Name reflects the country (Brazil) where M.A.Q. Cavalcanti was born.

Xiliomyces brasiliensis differs from *B. brasiliensis* and *B. pernambucoensis* by unique fixed alleles based on alignment of the combined LSU nrDNA, SSU nrDNA and *tef1* loci deposited in TreeBASE (S19537) and ITS nrDNA sequences deposited in GenBank: LSU nrDNA positions 164 (C), 456 (G), 661 (gap), and 663 (C); SSU nrDNA position 740 (C), 741 (C), 742 (G), 743 (T), 745 (A), and 746 (A); *tef1* positions 1824 (C), 1830 (C), 1869 (G), 1899 (C), 2034 (T), 2088 (C), 2103 (C), 2110 (G), 2128 (G), 2136 (C), 2212 (G), 2300 (A), 2341 (G), 2344 (C), 2380 (C), 2406 (A), 2416 (G), 2424 (A), 2527 (C), 2590 (C), 2605 (G), and 2629 (C); ITS nrDNA positions 73 (C), 79 (C), and 185 (C).

Culture characteristics: Colonies on MEA and PDA are velvety, surface and reverse dark olive to brown, growing up to 5 cm at room temperature (22 °C) in 1 month.

Specimens examined: Brazil, Pernambuco state, Itaíba municipality, Curral Velho farm (9°08.895S, 37°12.069W), as endophytic fungi from cactus *Tacinga inamoena*, Sep. 2013, J.D.P. Bezerra (holotype URM 89945; isotype CBS H-22680; culture ex-type URM 7413 = CBS 141536; *ibid.* URM 7414 = CBS 141535).

Notes: *Xiliomyces brasiliensis* is described here without well-defined sexual or asexual structures. After more than 5 months of incubation under different conditions and culture media, with and without sterilized cactus tissues, we observed only ascomatal-like structures and chlamydospores. A blast search of GenBank using the SSU and LSU nrDNA sequences demonstrated the close relationship with genera in Tubeufiales (SSU nrDNA: identities = 98–99%; LSU nrDNA: identities = 94–95%); *tef1* sequences demonstrated low identities with genera belonging to Pleosporales. The ITS nrDNA sequences have distant similarity to uncultured fungi [e.g. GenBank JX136098; Identities 482/531 (91%), Gaps

17/531 (3%)], fungi from rock formations [e.g. GenBank AY843120; Identities 451/491 (92%), Gaps 17/491 (3%)], and fungal endophyte from *Juniperus deppeana* [e.g. GenBank KP991001; Identities 428/454 (94%), Gaps 11/454(2%)]. In the phylogenetic tree (Fig. 1), the *Xiliomyces* sequences cluster as a sister lineage to *Bezerromyces* in the Bezerromycetales.

Wiesneriomycetales J.D.P. Bezerra, R.J.V. Oliveira, C.M. Souza-Motta, J.Z. Groenewald & Crous, ord. nov.

Mycobank MB818886

Conidiomata sporodochial, synnematal, solitary to gregarious, with or without setae. Setae subulate, septate, erect, flexuous, pigmented, thick-walled. Conidiophores macronematous, mononematous, penicillate, septate, branched, straight or flexuous, solitary or gregarious. Conidiogenous cells holoblastic, monoblastic, polyblastic, discrete, determinate, terminate, clavate, cylindrical, slightly ampulliform. Conidia solitary to gregarious, hyaline, pale, aseptate, catenate, connected by narrow isthmi, fusiform to falcate, cylindrical, subcylindrical, cuneiform, branched. Sexual morph unknown.

Type family: Wiesneriomycetaceae Suetrong, Rungjindamai, Somrithipol. & E.B.G. Jones

Wiesneriomycetaceae Suetrong, Rungjindamai, Somrithipol. & E.B.G. Jones, Phytotaxa 176: 288. 2014.

Description: see Suetrong et al. (2014) and similar as for Wiesneriomycetales.

Type genus: *Wiesneriomyces* Koord.

Included genera: *Wiesneriomyces* Koord., Verh. K. Akad. Wet., tweede sect. 13: 246. 1907, *Pseudogliophragma* Phadke & V.G. Rao, Norw. JI Bot. 27: 127. 1980, *Speiropsis* Tubaki, J. Hattori bot. Lab. 20: 171. 1958, *Parawiesneriomyces* Crous & M.J. Wingf., Persoonia 36: 389. 2016.

Notes: Based on our phylogenetic analyses, the order Wiesneriomycetales is introduced to accommodate the family Wiesneriomycetaceae. Fungi in this order are characterized by sporodochial or synnematal conidiomata with or without setae; conidiophores are macronematous, mononematous or penicillate; conidia hyaline to slightly pigmented in chains connected by narrow isthmi. To date no sexual morph is known for any of these genera. These fungi have a wide distribution in tropical and subtropical regions, growing on different substrates (Suetrong et al. 2014; Pratibha et al. 2015; Santos 2015; Crous et al. 2016). In addition, some authors have circumscribed *Speiropsis* as an aquatic hyphomycete (Barbosa and Gusmão 2005; Santos 2015).

Discussion

Studies of endophytic fungi in different countries have revealed a huge fungal diversity associated with plants growing in distinct biomes (Bezerra et al. 2015; Massimo et al. 2015; Nascimento et al. 2015; Fonseca-García et al. 2016; Siddique and Unterseher 2016). Several taxa have been described based on endophytic isolates, and have contributed to the

discussion of fungal–host relationships, taxonomic novelties and ecological distribution (Siqueira et al. 2008; Glienke et al. 2011; Oliveira et al. 2014, 2016; Knapp et al. 2015). These molecular data have greatly improved our understanding of phylogenetic relationships, clarifying fungal classification including new fungi from different environments, with the aim of establishing their global distribution and diversity (Kirk et al. 2013; Crous et al. 2014a, b, 2015a, b).

Studies on endophytic fungi have directly contributed to fungal diversity estimates with new taxa associated with distinct biomes around the world (Fisher et al. 1993; Dreyfuss and Chapela 1994; Strobel and Daisy 2003; Kemler et al. 2013). These morphological characters and molecular data are crucial to describing these new taxa, and for clarifying obstacles in understanding dothideomycete systematics (Schoch et al. 2009). Our study again underlined the importance of these microorganisms for fungal taxonomy, and the need for preservation of these cactus species and environments.

The new order Bezerromycetales is proposed here to accommodate a new family, Bezerromycetaceae. A preliminary phylogenetic analysis using 219 representative taxa of Dothideomycetes, Eurotiomycetes and Lecanoromycetes based on Boehm et al. (2015) revealed Bezerromycetales as a unique clade related to Tubeufiales (data not shown). Different phylogenetic analyses using three loci (SSU nrDNA, LSU nrDNA and *tef1*) and morphological characters, such as the muriformly septate ascospores, supported this clade as new order in the class Dothideomycetes. Tubeufiales was proposed by Boonmee et al. (2014) based on morphological characteristics and DNA sequences to accommodate the unique family Tubeufiaceae, which now has 19 accepted genera, including asexual and sexual morphs. This order is characterised by having superficial, oval and bright ascomata, bitunicate asci, mostly with long fusiform to filiform, transeptate ascospores, and having hyphomycetous asexual morphs with helicosporeous conidia (Boonmee et al. 2014).

In addition, representative sequences from the family Wiesneriomycetaceae, proposed by Suetrong et al. (2014) as order *incertae sedis*, represented a strongly supported clade closely related to the new order Bezerromycetales and Tubeufiales. In their study, the phylogenetic analyses of Suetrong et al. (2014) showed Wiesneriomycetaceae as a monophyletic clade between Patellariales and Tubeufiales, with Tubeufiales as closest sister group. This family is morphologically characterized by sporodochial conidiomata with setae, macronematous and branched conidiophores, and hyaline to slightly pigmented conidia in uniseriate chains connected by narrow isthmi, lacking a sexual morph (Suetrong et al. 2014). Pratibha et al. (2015) included the genus *Pseudogliophragma* in this family and placed Wiesneriomycetaceae in the order Tubeufiales. Another study by Santos (2015) included the genus *Speiropsis* in this family and proposed *Pseudogliophragma* as synonym of *Wiesneriomyces*. Recently, Crous et al. (2016) included the novel monotypic genus *Parawiesneriomyces* in the Wiesneriomycetaceae. Based on these morphological characteristics and on the results obtained in our phylogenetic analyses, we are introducing the order Wiesneriomycetales to accommodate the family Wiesneriomycetaceae.

The class Dothideomycetes is one of the most important and diverse classes in the phylum Ascomycota. It comprises phytopathogenic fungi, endophytes, fungi with different habits and habitats, and also fungi with biotechnological potential (Wijayawardene et al. 2014;

Santos et al. 2015; Woudenberg et al. 2015). In recent years, this class has received significant attention and several papers have highlighted its importance to fungal taxonomy, based on its fungal diversity and on new studies performed to improve the classification of dothideomycetous fungi (Schoch et al. 2009; Hyde et al. 2013; Wijayawardene et al. 2014). Studies on fungal endophytes have contributed directly with new taxa added to the Dothideomycetes and contributed to the discussion on the origin of the association between plants and fungi (Slippers and Wingfield 2007; Glienke et al. 2011; Bezerra et al. 2012, 2013; Gomes et al. 2013; Phillips et al. 2013; Knapp et al. 2015).

Several new genera were recently described with phenotypic characteristics, such as muriformly septate ascospores, similar to *Bezerromyces*, but these were placed in other families and orders. The genus *Populocrescentia* was described by Ariyawansa et al. (2015) as saprobic on dead and hanging branches of *Populus nigra* collected in Italy. This genus belongs to the family Phaeosphaeriaceae (Pleosporales) and is also distinct from *Dematiopleospora* and *Allophaeosphaeria* (both Pleosporales) in its peridium structure, papillate ostioles, ascomata shape, muriform and fusiform ascospores (Wanasinghe et al. 2014; Liu et al. 2015). Another genus, *Murispora* (Zhang et al. 2009), has a freshwater habitat and is saprobic, characterised by muriform and fusiform ascospores, and is placed in the family Amniculicolaceae (Pleosporales); recently, additional novel species, including some with phoma-like asexual morphs, have been included in this genus by Wanasinghe et al. (2015). The orders phylogenetically closely related to Bezerromycetales, such as Botryosphaeriales (Schoch et al. 2006; Slippers et al. 2013), Tubeufiales (Boonmee et al. 2014), and Wiesneriomycetales ord. nov. have different sexual and asexual morphological characters (ascomata and asci shape, ascospores shape, septation, and asexual morphs), and are phylogenetically distinct from Bezerromycetales. The description of Bezerromycetales as a new order in the present study highlights the need to collect fungal biodiversity from a range of diverse environments and substrates, as these diverse niches frequently harbour fungal lineages that are still missing in current phylogenetic studies.

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