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Botryosphaeria, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* species associated with mango in Brazil

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Abstract Members of the Botryosphaeriaceae are well known fungi associated with dieback, canker and fruit rot on various hosts worldwide, including mango. The aim of this study was identify a large collection of Botryosphaeriaceae species associated with dieback and stem-end rot of mango in the semi-arid region of Northeastern Brazil, and compare the species in relation to mycelial growth, pathogenicity and virulence. A total of 115 isolates were sampled and based on morphology and DNA sequence data (ITS and EF1-α) seven taxa were identified, namely, *Botryosphaeria dothidea*, *B. mamane, Fusicoccum fabicercianum, Neofusicoccum parvum, N. brasiliense* sp. nov*, Neoscytalidium dimidiatum* and *Pseudofusicoccum stromaticum*. *B. dothidea* and *P. stromaticum* were the most commonly isolated species, which represented 37 % and 33 % of all isolates respectively. *B. mamane* is reported for the first time in association with mango diseases worldwide. There were significant differences among the species obtained in this study in relation to optimum temperature for mycelial growth and mycelial growth rates. All species were pathogenic on mango fruit. There were significant differences in virulence among the species, with *Ne. dimidiatum* and *N. parvum* being the most virulent species, while *P. stromaticum* was the least virulent.

Keywords Botryosphaeriaceae · Dieback · Stem-end rot · *Mangifera indica* · Pathogenicity · Virulence · Phylogeny

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Introduction

Brazil is one of the biggest producer and exporter of tropical fruits in the world. Mango (*Mangifera indica* L.) is one of the most exported products. Most of the mangoes grown for the international market comes from the São Francisco Valley region located in the Northeast part of the country. In 2010 the national production was 1 197 million t and the planted area reached 75 416 ha, generating about US$ 334 million. This ranks Brazil as third largest world producer after China and India (Agrianual 2012).

Mangoes are affected by various pests and pathogens. Among the wide range of destructive fungal pathogens that impact on mango production in Brazil are members of the Botryosphaeriaceae (Costa et al. 2010). The first report of species associated with mango in Brazil was in 1947 (Batista 1947) and they have become increasingly important (Tavares et al. 1991; Tavares 2002).

The species within Botryosphaeriaceae have a worldwide distribution and occur on a large variety of plant hosts including monocotyledons, dicotyledons, gymnosperms and angiosperms, on which they are found as saprophytes, parasites, and endophytes (von Arx 1987; Slippers and Wingfield 2007). These fungi are associated with different symptoms such as fruit rots, shoot blights, stem cankers, dieback and gummosis (von Arx 1987). In Brazil, stem-end rot is the main disease induced by Botryosphaeriaceae on mango, reducing fruit shelf-life and causing serious post-harvest losses (Junqueira and Junqueira 2007).

The taxonomy of species in the Botryosphaeriaceae is commonly based on the morphology of the anamorph states, which are most frequently encountered in nature. However, overlapping morphological characteristics has emphasized the utility of applying DNA sequence comparisons to resolve species (De Wet et al. 2008). Considerable changes have taken place recently in the taxonomy of the Botryosphaeriaceae (Liu et al. 2012). Historically, more than 18 anamorph genera have been associated with *Botryosphaeria* Ces. & De Not. In a phylogenetic study based on part of the 28S ribosomal DNA gene together with morphological characters revealed that *Botryosphaeria* is composed of several distinct lineages that correspond to individual genera (Crous et al. 2006). Only *B. dothidea* (Moug. : Fr.) Ces. & De Not. and *B. corticis* (Demaree & M.S. Wilcox) Arx & E. Müll. were retained in *Botryosphaeria*, while other species with *Fusicoccum* Corda like anamorphs were transferred to *Neofusicoccum* Crous, Slippers & A.J.L. Phillips. *Pseudofusicoccum* Mohali, Slippers & M.J. Wingf., or *Neoscytalidium* Crous & Slippers, which were introduced in that study.

The use of molecular tools has made a significant contribution towards the recognition of species in the Botryosphaeriaceae and numerous species have been described in recent years, both in native vegetation, and in diverse crops of economic importance (Phillips et al. 2002; Slippers et al. 2004a, b; Luque et al. 2005; Phillips et al. 2005; Liu et al. 2012). Pavlic et al. (2008) identified seven new species in Australian native vegetation. Based on DNA sequence data for five nuclear loci Pavlic et al. (2009a, b) identified three new species of *Neofusicoccum* within the *N. parvum/N. ribis* species complex in South Africa. In 2010, two new species, *N. batangarum* Begoude, Jol. Roux, Slippers and *Lasiodiplodia mahajangana* Begoude, Jol. Roux, Slippers were described from *Terminalia catappa* L. (Begoude et al. 2010). More recently *Fusicoccum ramosum* Pavlic, Burgess, M.J. Wingfield (Pavlic et al. 2008), *F. atrovirens* J.W.M. Mehl & B. Slippers (Mehl et al. 2011), *F. fabicercianum* S.F. Chen, D. Pavlic, M.J. Wingf. & X.D. Zhou (Chen et al. 2011), *B. fusispora* Boonmee, J.K. Liu & K.D. Hyde (Liu et al. 2012), and *B. schariffi* Abdollahzadeh, Zare, A.J.L. Phillips (Abdollahzadeh et al. 2013) were described in the *Botryosphaeria.* Regarding the genus *Lasiodiplodia*, 16 new species have been reported since 2004. (Pavlic et al. 2004, 2008; Burgess et al. 2006; Damm et al. 2007; Alves et al. 2008; Begoude et al. 2010; Abdollahzadeh et al. 2010; Ismail et al. 2012; Úrbez-Torres et al. 2012). The increase in the number of new species introduced is largely a result of the widespread use of DNA sequence data, but is also due to the exploration of new geographic regions and habitats.

Several species of Botryosphaeriaceae have been found associated with diseased mango trees and fruits worldwide: *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips, *B. dothidea* and *L. theobromae* (Pat.) Griffon & Maubl are some of the more common pathogens (Slippers et al. 2005; Javier-Alva et al. 2009; Costa et al. 2010). Recently, four new species of *Lasiodiplodia*, *B. schariffi* and *N. mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips were associated with this host in Iran (Abdollahzadeh et al. 2010, 2013). In Australia, species of Botryosphaeriaceae: *Neoscytalidium novaehollandiae* Pavlic, Burgess, M.J. Wingfield, *Ne. dimidiatum* (Penz.) Crous & Slippers, *Pseudofusicoccum adansoniae* Pavlic, Burgess, M.J. Wingfield, *P. ardesiacum* Pavlic, Burgess, M.J. Wingfield, *P. kimberleyense* Pavlic, Burgess, M.J. Wingfield, *L. iraniensis* Abdollahzadeh, Zare & A.J.L. Phillips and *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous were isolated from cankers and tip dieback of mango (Ray et al. 2010; Sakalidis et al. 2011), and in 2012, *L. egyptiacae* A.M. Ismail, L. Lombard & Crous was recorded in Egypt (Ismail et al. 2012).

In Brazil, dieback and stem end rot was reported in the early 1900s. Recently, diseases caused by members of the Botryosphaeriaceae have gained importance mainly because of changes in the cultivation management. Historically, this disease was attributed exclusively to *L. theobromae*. Recent studies using molecular methods revealed the presence of more species namely *B. dothidea, N. parvum* and *P. stromaticum* (Mohali, Slippers & M.J. Wingf.) Mohali, Slippers & M.J. Wingf. and six species of *Lasiodiplodia* causing diseases in mango in Northeast Brazil (Costa et al. 2010; Marques et al. 2012; Marques et al. 2013).

Considering the number of species associated with mango elsewhere it seems likely that more species would be associated with this host in Brazil. Therefore, the objective of this study was to combine morphological characters with ITS, BT and EF1-α sequence data to characterize a large number of isolates and identify the species of Botryosphaeriaceae associated with *M. indica* in Brazil and to compare the species in relation to mycelial growth, pathogenicity and virulence.

Materials and methods

Sampling and fungal isolation

From April to June 2010, isolates were collected from plant tissue exhibiting dieback and stem end rot in commercial plantations of mango located in the São Francisco Valley, Northeastern Brazil. Plant tissues were surface disinfested with 70 % ethanol for 30 s and 1.5 % NaOCl for 1 min. Samples were then rinsed in sterile distilled water for 30 s and dried before small pieces (4–5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto potato dextrose agar (PDA, Acumedia, Lansing, USA) amended with 0.5 g l^{-1} streptomycin sulfate (PDAS).

Plates were incubated at 25 °C in the dark for 3 to 4 days. Fungal colonies emerging from plant tissue pieces that were morphologically similar to species of Botryosphaeriaceae (Sutton 1980; Phillips 2006) were transferred to fresh PDA plates and incubated at 25 °C in the dark, with observation at 3, 5 and 15 day. To obtain single-spore isolates, pycnidia were produced on 2 % water agar (WA) with autoclaved pine needles as a substrate after incubation for 3-week at 25 °C under a 12 h daily photoperiod with near-ultraviolet light (Slippers et al. 2004a,b). A single pycnidium was cut from each isolate under a stereo microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany) and placed in 250 μl of sterile water to produce a conidial suspension. A 20 μl aliquot was spread on PDAS and incubated at 28 °C in the dark for 24 h. A single germinating conidium was transferred to a new PDA plate. Stock cultures were stored on PDA slants at 5 °C in the dark.

DNA isolation, PCR amplification and sequencing

A portion of the translation elongation factor 1α (EF1-α) gene was sequenced for all 115 isolates collected from mango orchards. A total of 28 isolates were selected as representatives of the taxa found in our survey. For these isolates, the entire ITS rDNA cluster was sequenced and for six selected isolates of *Neofusicoccum* part of the β-tubulin genes was sequenced, in order to clarify the relationships among isolates obtained in this study (Table 1). Using a sterile 10 μl pipette tip, a small amount of aerial mycelium was scraped from the surface of a culture grown for 5 days on PDA at 25 °C and genomic DNA was extracted using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, USA) following the manufacturer's instructions. The ITS region was amplified using the primers ITS1 and ITS4 (White et al. 1990) as described by Slippers et al. (2004a), part of the β-tubulin gene was amplified using the primers BT2a and BT2b (Glass and Donaldson 1995) and part of the EF1-α gene was amplified using the primers EF1-688F and EF1-1251R (Alves et al. 2008) as described by Phillips et al. (2005). Each 50-μl polymerase chain reaction (PCR) mixture included 21 μl of PCR-grade water, 1 μl of DNA template, 1.5 μM of each primer, and 1 μl of PCR Master Mix (2X) (0.05 u μl^{-1} de *Taq* DNA polimerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP; Thermo Scientific, Waltham, USA). PCR reactions were carried out in a thermal cycler (Biocycler MJ 96; Applied Biosystems, Foster City, USA). The PCR amplification products were separated by electrophoresis in 1.5 % agarose gels in 1.0× Tris-acetate acid EDTA (TAE) buffer and were photographed under UV light after staining with ethidium bromide (0.5 μg ml^{-1}) for 1 min. PCR products were purified using the AxyPrep™ PCR Cleanup Kit (Axygen) following the manufacturer's instructions. ITS and EF1-α regions were sequenced in both directions using an ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems) at the Sequencing Platform LABCEN/CCB in the Universidade Federal de Pernambuco (Recife, Brazil).

Phylogenetic analyses

Sequences were edited with Chromas v. 2.32 (Technelysium Pty Lda, Brisbane, Australia). Sequences of both DNA regions of additional isolates were retrieved from GenBank. Sequences were aligned with ClustalX v. 1.83 (Thompson et al. 1997) and manually adjusted when necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young and Healey 2003). A partition homogeneity test was done to determine the possibility of combining the ITS and EF1-α datasets (Farris et al. 1995; Huelsenbeck et al. 1996). Sequences of other Botryosphaeriaceae species obtained from GenBank were included in the analyses (Table 1). *P. stromaticum* (CMW 13434 and CMW 13435) were used as outgroup in the phylogenetic analyses of *Botryosphaeria* and *Neoscytalidium* species and *B. dothidea* (CMW8000 and CBS110302) were used as outgroup to *Neofusicoccum* species and *Pseudofusicoccum* species.

Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swofford 2003) for Maximum-parsimony and MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003) for Bayesian analyses. Maximum-parsimony analyses were performed using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1,000 bootstrap replications (Hillis and Bull 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI).

Bayesian analyses employing a Markov Chain Monte Carlo method (MCMC) were performed. The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+Γ+G) was used. Four MCMC chains were run simultaneously, starting from random trees for 1,000,000 generations. Trees were sampled every 100th generation for a total of 10,000 trees. The first 1,000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 9,000 trees. This analysis was repeated three times starting from different random trees to ensure trees from the same tree space were sampled during

Table 1 Isolates used in this study

Taxon	Culture accession no.[1]	Host	Location	Collector	GenBank accession no.[2]		
					ITS	EF-1α	BT
Botryosphaeria corticis	CBS 119047	*Vaccinium corymbosum*	USA	P.V. Oudemans	DQ299245	EU017539	
B. corticis	ATCC 22927	*Vaccinium* sp.	USA	R.D. Millholland	DQ299247	EU673291	
B. dothidea	CMW 8000	*Prunus* sp.	Switzerland	B. Slippers	AY236949	AY236898	
B. dothidea	CAP288	*Olive*	Italy	C. Lazzizera	EF638755	EF638732	
B. dothidea	CBS110302	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY259092	AY573218	
B. dothidea	CMM 3937	*Mangifera indica*	Brazil	M.W. Marques	**JX513643**	**JX513622**	
B. dothidea	CMM 3938	*M. indica*	Brazil	M.W. Marques	**JX513645**	**JX513624**	
B. dothidea	CMM 3940	*M. indica*	Brazil	M.W. Marques	**JX513644**	**JX513623**	
B. mamane	CMW 13429	*Eucalyptus hybrid*	Venezuela	S. Mohali	EF118048	GU134940	
B. mamane	CMW 13433	*E. hybrid*	Venezuela	S. Mohali	EF118049	GU134941	
B. mamane	CMM 1390	*M. indica*	Brazil	M.W. Marques	**KC184893**	**JX513627**	
B. mamane	CMM 3941	*M. indica*	Brazil	M.W. Marques	**KC184892**	**JX513626**	
B. scharifii	CBS 124703	*M. indica*	Iran	J. Abdollahzadeh	JQ772020	JQ772057	
B. scharifii	CBS 124702	*M. indica*	Iran	J. Abdollahzadeh/A. Javadi	JQ772019	JQ772056	
Fusicoccum atrovirens	CMW 22682	*Pterocarpus angolensis*	South Africa	J. Mehl/J. Roux	FJ888476	FJ888457	
F. atrovirens	CMW 22674	*P. angolensis*	South Africa	J. Mehl/J. Roux	FJ888473	FJ888456	
F. fabicercianum	CMW 24703	*E. grandis hybrid*	China	M.J. Wingfield/X.D. Zhou	HQ332195	HQ332211	
F. fabicercianum	CMW 27094	*Eucalyptus sp.*	China	M.J. Wingfield	HQ332197	HQ332213	
F. fabicercianum	CMM 3899	*M. indica*	Brazil	M.W. Marques	**JX513646**	**JX513625**	
F. fabicercianum	CMM 3905	*M. indica*	Brazil	M.W. Marques	**JX513642**	**JX513621**	
F. fabicercianum	CMM 3923	*M. indica*	Brazil	M.W. Marques	**JX513641**	**KC184894**	
F. fabicercianum	CMM 3925	*M. indica*	Brazil	M.W. Marques	**JX513640**	**JX513620**	
F. fabicercianum	CMM 3928	*M. indica*	Brazil	M.W. Marques	**JX513639**	**JX513619**	
F. ramosum	CMW 22674	*E. camaldulensis*	Australia	D. Pavlic	EU144055	EU144070	
Neofusicoccum andinum	CMW 13455	*Eucalyptus* sp.	Venezuela	S. Mohali	AY693976	AY693977	
N. andinum	CMW 13446	*Eucalyptus* sp.	Venezuela	S. Mohali	DQ306263	DQ306264	
N. arbuti	CBS 116131	*Arbutus menziesii*	USA	M. Elliott	GU251152	GU251284	
N. arbuti	CBS 117090	*A. menziesii*	USA	M. Elliott	GU251154	GU251286	
N. australe	CMW 6837	*Acacia* sp.	Australia	M.J. Wingfield	AY339262	AY339270	
N. australe	CMW 6853	*Sequoiadendron*	Australia	M.J. Wingfield	AY339263	AY339271	
N. batangarum	CMW 28363	*Terminalia catappa*	Africa	D. Begoude/J. Roux	FJ900607	FJ900653	FJ900634
N. batangarum	CMW 28315	*T. catappa*	Africa	D. Begoude/J. Roux	FJ900608	FJ800654	FJ900633
N. cordaticola	CMW 13992	*Syzigium cordatum*	South Africa	D. Pavlic	EU821898	EU821868	EU821838
N. cordaticola	CMW 14056	*S. cordatum*	South Africa	D. Pavlic	EU821903	EU821873	EU821843
N. eucalypticola	CBS 115766	*E. rossii*	Australia	M.J. Wingfield	AY 615143	AY615135	
N. eucalypticola	CMW 6539	*E. rossii*	Australia	M.J. Wingfield	AY 615141	AY615133	
N. eucalyptorum	CMW 10126	*E. grandis*	South Africa	H. Smith	AF283687	AY236892	
N. eucalyptorum	CBS 115791	*E. grandis*	South Africa	H. Smith	AF 283686	AY236891	
N. kwambonambiense	CMW 14023	*S. cordatum*	South Africa	D. Pavlic	EU821900	EU821870	EU821840
N. kwambonambiense	CMW 14140	*S. cordatum*	South Africa	D. Pavlic	EU821949	EU821889	EU821859
N. luteum	CMW 9076	*Malus* × *domestica*	New Zealand	S.R. Pennycook	AY 236946	AY236893	
N. luteum	CBS 110299	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY 259091	AY573217	
N. macroclavatum	WAC 12445	*E. globulus*	Australia	T.I. Burguess	DQ093197	DQ093218	DQ093207
N. macroclavatum	WAC 12446	*E. globulus*	Australia	T.I. Burguess	DQ093198	DQ093219	DQ093208

Table 1 (continued)

Taxon	Culture accession no.[1]	Host	Location	Collector	GenBank accession no.[2]		
					ITS	EF-1α	BT
N. mangiferum	CMW 7024	*M. indica*	Australia	G.I. Johnson	AY615185	DQ093221	
N. mangiferum	CMW 7797	*M. indica*	Australia	G.I. Johnson	AY 615186	DQ093220	
N. mediterraneum	PD 311	*Olea europea*	Italy	C. Lazzizera	GU251175	GU251307	
N. mediterraneum	PD 312	*Eucalyptus* sp.	Greece	P.W. Crous/M.J. Wingfield/A.J.L. Phillips	GU251176	GU251308	
N. nonquaesitum	PD 484	*Umbellularia californica*	USA	F. P. Trouillas	GU251163	GU251295	
N. nonquaesitum	PD 301	*Vaccinum corymbosum*	Chile	E.X. Briceño/J.G. Espinoza/B.A. Latorre	GU251185	GU251317	
N. occulatum	CBS 128008	*E. grandis* hybrid	Australia	T.I. Burgess	EU7301030	EU339509	EU339472
N. occulatum	MUCC 296	*E. pellita*	Australia	T.I. Burgess	EU301034	EU339512	EU339475
N. parvum	PD 106	*Prunus dulcis*	USA	T.J. Michailides	GU251139	GU251271	
N. parvum	ATCC58189	*Malus sylvestris*	New Zealand	G.J. Samuels	AF243395	AY236883	AY236912
N. parvum	CMM 1291	*M. indica*	Brazil	M.W. Marques	**JX513633**	**JX513613**	**KC794029**
N. parvum	CMM 1465	*M. indica*	Brazil	M.W. Marques	**JX513634**	**JX513614**	**KC794027**
N. parvum	CMM 3944	*M. indica*	Brazil	M.W. Marques	**JX513636**	**JX513616**	**KC794028**
N. ribis	CMW7054	*R. rubrum*	USA	B Slippers	AF241177	AY236879	AY236908
N. ribis	CMW 7772	*Ribis sp.*	USA	B. Slippers/G. Hudler	AY236925	AY236877	AY236906
N. umdonicola	CMW 14058	*S. cordatum*	South Africa	D. Pavlic	EU821934	EU821874	EU821844
N. umdonicola	CMW 14060	*S. cordatum*	South Africa	D. Pavlic	EU821935	EU821875	EU821845
N. viticlavatum	STE-U 5044	*V. vinifera*	South Africa	F. Halleen	AY343381	AY 343342	
N. viticlavatum	STE-U 5041	*V. vinifera*	South Africa	F. Halleen	AY343380	AY343341	
N. vitifusiforme	STE-U 5050	*V. vinifera*	South Africa	J.M. Van Niekerk	AY343382	AY343344	
N. vitifusiforme	STE-U 5252	*V. vinifera*	South Africa	J.M. Van Niekerk	AY343383	AY343343	
N. brasiliense	CMM 1269	*M. indica*	Brazil	M.W. Marques	**JX513629**	**JX513609**	**KC794032**
N. brasiliense	CMM 1285	*M. indica*	Brazil	M.W. Marques	**JX513628**	**JX513608**	**KC794030**
N. brasiliense	CMM 1338	*M. indica*	Brazil	M.W. Marques	**JX513630**	**JX513610**	**KC794031**
Ne. dimidiatum	PD 103	*Ficus carica*	USA	T.J. Michailides/P. Inderbitzin.	GU251106	GU251238	
Ne. dimidiatum	WAC 13284	*M. indica*	Australia	J. Ray	GU172382	GU172414	
Ne. dimidiatum	CMM 3979	*M. indica*	Brazil	M.W. Marques	JX513637	JX513618	
Ne. dimidiatum	CMM 3980	*M. indica*	Brazil	M.W. Marques	JX513638	JX513617	
Ne. novaehollandiae	WAC 13275	*M. indica*	Australia	J. Ray	GU172400	GU172432	
Ne. novaehollandiae	WAC 13303	*M. indica*	Australia	J. Ray	GU172398	GU172430	
Pseudofusicoccum adansoniae	CBS 122054	*Eucalyptus* sp.	Australia	D. Pavilic	EF585532	EF585570	
P. adansoniae	WAC 13299	*M. indica*	Australia	J. Ray	GU172404	GU172436	
P. ardesiacum	CBS 122062	*Ad. gibbosa*	Australia	D. Pavilic	EU144060	EU144075	
P. ardesiacum	WAC 13294	*M. indica*	Australia	J Ray	GU172405	GU172437	
P. kimberleyense	CBS 122061	*F. opposita*	Australia	D. Pavilic	EU144059	EU144074	
P. kimberleyense	WAC 13293	*M. indica*	Australia	J. Ray	GU172406	GU172438	
P. stromaticum	CMW 13435	*E. hybrid*	Venezuela	S. Mohali	DQ436935	DQ436936	
P. stromaticum	CMW 13434	*E. hybrid*	Venezuela	S. Mohali	AY693974	AY693975	
P. stromaticum	CMM 3953	*M. indica*	Brazil	M.W. Marques	**JX464102**	**JX464109**	
P. stromaticum	CMM 3961	*M. indica*	Brazil	M.W. Marques	**JX464103**	**JX464110**	
P. stromaticum	CMM 3964	*M. indica*	Brazil	M.W. Marques	**JX464104**	**JX464111**	
P. stromaticum	CMM 3965	*M. indica*	Brazil	M.W. Marques	**JX464105**	**JX464112**	

Table 1 (continued)

Taxon	Culture accession no.[1]	Host	Location	Collector	GenBank accession no.[2]		
					ITS	EF-1α	BT
P. stromaticum	CMM 3967	*M. indica*	Brazil	M.W. Marques	**JX464106**	**JX464113**	
P. stromaticum	CMM 3971	*M. indica*	Brazil	M.W. Marques	**JX464107**	**JX464114**	
P. stromaticum	CMM 3978	*M. indica*	Brazil	M.W. Marques	**JX464108**	**JX464115**	

Newly deposited sequences are shown in bold

[1] CBS = Centraalbureau voor Schimmelcultures Utrecht, Netherlands; CMW = Forestry and Agricultural Biotechnology Institute, University of Pretoria South Africa; MUCC = Murdoch University Culture Collection, Perth, Australia; ATCC = American Type Culture Collection, Manassas, Virginia; WAC = Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia; CAP = culture collection of A.J.L. Phillips, Lisbon, Portugal; CMM = culture collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; STE-U = Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa PD = Culture Collection of Plant Pathogenic Bacteria, Plant Protection Service, The Netherlands

[2] Sequence numbers in bold were obtained in the present study

each analysis. Phylogenetic trees were visualized using Treeview (Page 1996). Sequences derived in this study were deposited in GenBank. Representative isolates obtained in this study were deposited in the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Brazil). Phylogenetic trees were deposited in TreeBASE (S14389).

Morphological characterization

Representatives of the different groups identified in the phylogenetic analysis were used to study colony morphology and conidial characteristics. The color and aerial hyphal growth from isolates were recorded during 15 days of growth on 2 % PDA at 25 °C in the dark. Conidial characteristics were determined from cultures grown on 2 % WA containing autoclaved pine needles and incubated under near-ultraviolet light, as described above. Conidia and other structures were mounted in 100 % lactic acid and digital images recorded with a Leica DFC320 camera on a Leica DMR HC microscope fitted with Nomarski differential interference contrast optics (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The length and width of 50 conidia per isolate were measured with the Leica IM500 measurement module. Mean and standard errors of the conidial measurements, including mean length to width (L/W) of the ratio conidial measurements were calculated.

These isolates were also used to determine the effect of temperature on colony growth of different species. A 3-mm-diameter mycelial plug from the growing margin of a 3-day-old colony was placed in the center of a 90-mm-diameter 2 % PDA plate, and four replicates of each isolate were incubated at temperatures ranging from 5 °C to 35 °C in 5 °C intervals in the dark. After a 2-day incubation period, the colony diameter (mm) was measured in two perpendicular directions. The experiment was done twice. Colony diameters were plotted against temperature and a curve was fitted by a cubic polynomial regression ($y = a + bx + cx^2 + dx^3$). Optimal temperature was estimated from the regression equation and numeric summary with TableCurve™ 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA). Optimum temperature was defined as the temperature that produced the maximum mycelial growth rate. The colony diameter data at 30 °C were used to calculate the mycelial growth rate (mm/day). One-way analyses of variance (ANOVA) were conducted with data obtained from optimum temperature and mycelial growth rate experiments, and means were compared by Fisher's least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Pathogenicity and virulence in fruits

The isolates used in the morphological characterization were selected for this test. Mango fruits (cv. Tommy Atkins) at stage three of maturation (Assis 2004), which had not been treated with fungicides, were washed in running water, surface disinfested in 70 % ethanol for 1 min and 1 % NaOCl for 5 min, then rinsed in sterile distilled water. After drying, the fruits were placed on plastic trays, on the base of each were four layers of paper towels wetted with distilled water to increase humidity. Each fruit was put on a sterilized Petri plate to avoid direct contact with water and was wounded at the median region by pushing the tip of four sterile pins through the surface of the skin to a depth of 3 mm. A mycelial plug (5 mm in diameter) removed from the margin of a 5-day-old PDA culture grown at 28 °C in the dark of each isolate was immediately placed on the wound. A non-colonized agar plug was used for the control. The trays were enclosed in plastic bags and incubated at 25 °C in the dark. The plastic bags and paper towels were removed after 48 h, and the fruits were kept at the same temperature. Isolates

were considered pathogenic when the lesioned area advanced beyond the 5-mm diameter inoculum point. The virulence of the isolates was evaluated from measurement of the lesion length at 5 days after inoculation in two perpendicular directions on each fruit. The experiment was arranged in a completely randomized design with six replicates per treatment (isolate) and one fruit per replicate. The experiment was conducted twice. Differences in virulence caused by Botryosphaeriaceae species were determined by one-way ANOVA and means were compared by LSD test at the 5 % significance level using STATISTIX.

Results

DNA sequencing and phylogenetic analyses

A total of 115 isolates were obtained from mango stems and fruits collected from Northeast regions of Brazil. From this total, 7 species of Botryosphaeriaceae were identified based on phylogenetic analysis of the partial translation elongation factor 1α (EF1-α) gene: *Botryosphaeria dothidea*, *B. mamane*, *N. parvum*, *Fusicoccum fabicercianum, Neofusioccum* sp., *Ne. dimidiatum* and *Pseudofusicoccum stromaticum*. To confirm the identity of the isolates, the internal transcribed spacer (ITS) sequence was obtained for 28 isolates representing each putative species and part of the β-tubulin gene was sequenced for six isolates of *Neofusicoccum*.

PCR products for the ITS were approximately 580 bp in size, while those for β-tubulin and EF1-α were approximately 450 bp. The ITS and EF1-α sequences were combined in three different data sets corresponding to 1) *Botryosphaeria* and *Neoscytalidium* species, 2) *Neofusicoccum* species, 3) *Pseudofusicoccum* species. Each data set was analyzed separately to produce three phylogenetic trees, one for each genus.

The combined ITS and EF dataset of the *Botryosphaeria* and *Neoscytalidium* consisted of 29 ingroup and 2 outgroup sequences. The alignment contained 827 characters including coded alignment gaps. Of these characters 543 were constant, 11 were variable and parsimony uninformative and 273 were parsimony informative. A heuristic search of the 273 parsimony informative characters generated 16 most parsimonious trees (TL=387; CI=0.902; RI=0.965; HI=0.098) each with similar clade topologies, and one is presented in Fig. 1. The phylogenetic analyses of Maximum-parsimony (MP) and Bayesian methods (BM) produced nearly identical topologies (Bayesian tree not shown). Isolates from mango clustered in three different clades corresponding to known species. The isolates CMM3928, CMM3925, CMM3923, CMM3899 and CMM3905 clustered together with *F. fabicercianum* (CMW27094=CBS127193, culture ex-type) in a strongly supported clade (MP bootstrap=95 %, BM probability=0.83). Isolates CMM3937, CMM3938, CMM3940 clustered within *B. dothidea*. Isolates CMM3941 and CMM 1390 clustered within *B. mamane* D.E. Gardner in a well-supported clade (MP/BM: 100/1.0), while CMM3979 and CMM3980 resided in a clade together with *Ne. dimidiatum*.

The *Neofusicoccum* combined ITS and EF dataset (9 isolates from this study and 40 sequences retrieved from GenBank) was composed of 783 characters including gaps, of which 557 were constant, 14 were variable and parsimony uninformative and 212 were parsimony informative. A heuristic search generated 70 most parsimonious trees (TL=361; CI=0.770; RI=0.919; HI=0.230) with similar clade topologies, and one is presented in Fig. 2a. In this dataset, the isolates from Brazil clustered in two clades. The majority of isolates (CMM3945, CMM3944, CMM1271, CMM3943, CMM1291, CMM1465) clustered together with *N. parvum* supported by an MP bootstrap of 96 % and BM probability of 1.00. While the isolates CMM1285, CMM1338 and CMM1269 were not clearly resolved and bootstrap support for the branches was generally low, they grouped next to *N. cordaticola* and *N. kwambonambiense*. The *Neofusicoccum* combined ITS, β-tubulin and EF1-α dataset included 20 ingroup and 2 outgroup taxa and was composed of 1,183 characters including gaps, of which 1,090 were constant, 22 were variable and parsimony uninformative and 71 were parsimony informative. Maximum parsimony analysis of the remaining 71 parsimony informative characters produced a single most parsimonious tree with TL=104; CI=0.913; RI=0.940; HI=0.087. Isolates CMM1285, CMM1338 and CMM1269 formed a well-supported clade (MP/BM=99/1.00) representing a new phylogenetic species. And the other isolates clustered together with *N. parvum* (MP/BM=99/1.0) (Fig. 2b).

The *Neofusicoccum* spp. combined ITS and EF dataset included 17 taxa including the outgroup comprised 864 characters including gaps, of which 521 were constant, 308 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 35 parsimony informative characters resulted in a single tree with TL=364 steps CI=0.975; RI=0.920; HI=0.025 (Fig. 3). In this dataset, the mango isolates grouped together with *P. stromaticum* with MP bootstrap=51 % and BM probability of 0.94.

Results of this study showed that *B. dothidea* (37 %) and *P. stromaticum* (33 %) were the most prevalent fungi isolated from *Mangifera indica* in São Francisco Valley, followed by *N. parvum* (9 %), *F. fabicercianum* (7 %), *Ne. brasiliense* (10 %), and *Ne. dimidiatum* (2 %) (Fig. 4).

Taxonomy

Neofusicoccum brasiliense M.W. Marques, A.J.L. Phillips & M.P.S. Câmara **sp. nov**. MycoBank MB804730.

Etymology: The name refers to Brazil, the country where this fungus was first found.

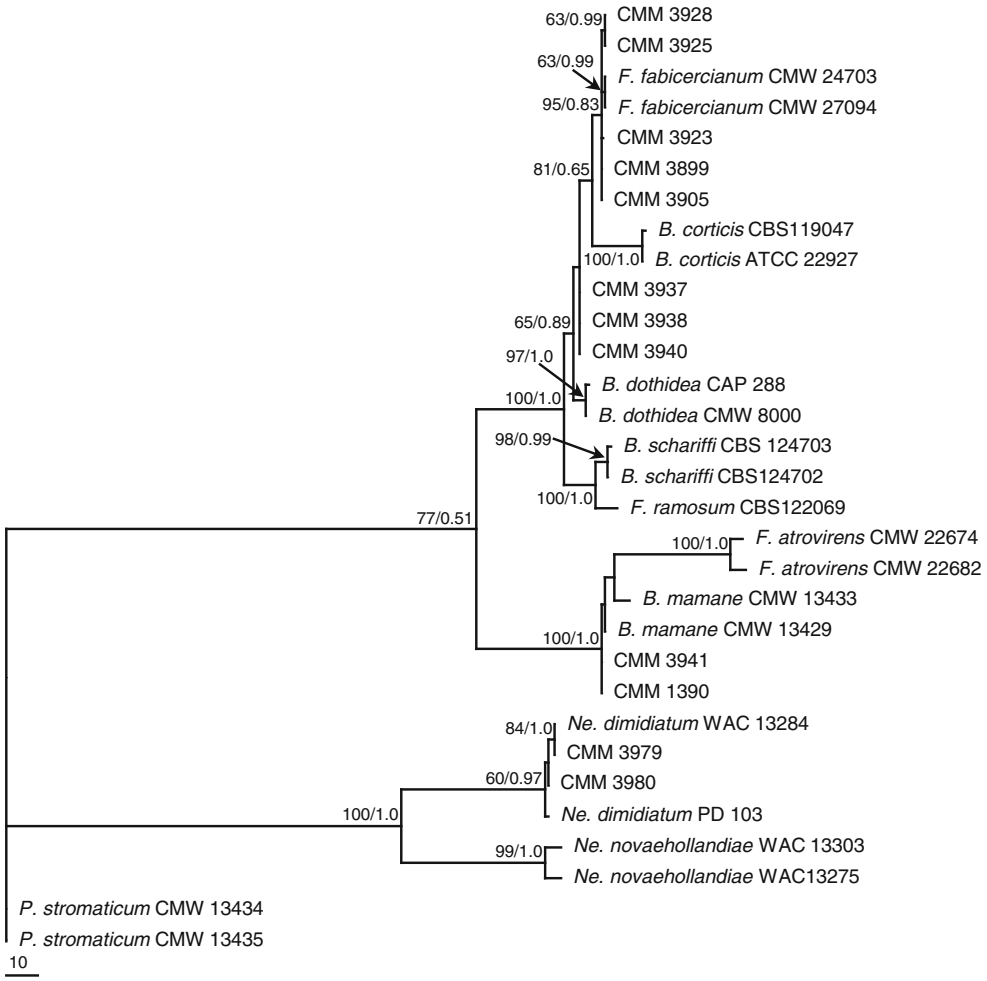

Fig. 1 One of 16 most parsimonious trees (TL=387; CI=0.902; RI=0.965; HI=0.098) obtained from combined ITS and EF1-α sequence data. Maximum parsimony bootstrap support values from 1,000 replications and Bayesian posterior probability scores are shown at the nodes. The tree was rooted to *P. stromaticum* (CMW 13434 and CMW 13435). The *bar* represents 10 changes

Cultures sterile. *Neofusicoccum brasiliense* differs from its closest phylogenetic neighbor, *N. kwambonambiense* and *N. umdonicola,* by unique fixed alleles in three loci based on alignments of the separate loci deposited in TreeBase as study S14389: ITS positions 132 (C), 137 (T), 154 (C), 164 (A), 363 (A), 407 (T); EF-1α positions 56(T) and 206 (GAP); BT positions 32(C), 61 (T), 96 (C), 115(A), 175 (C), 235 (A), 251 (A), 301 (C) 316(C), 391(T);

Culture characteristics: aerial mycelia white, becoming dark greenish-grey or greyish with the reverse side of the colonies greenish black after 4–5 days at 25 °C. Optimum temperature for mycelial growth: 27.7±0.6. Mycelial growth rate: 29.5±1.89 mm/day.

Holotype: Brazil, Pernambuco, Petrolina, Lote 1195 - DISNC (40° 31′ 00″, 09° 20′ 14.8″), on *Mangifera indica* stems, 2010, coll. M.W. Marques, holotype living culture CMM 1338; isotype living culture in URM 7005.

Specimen examined: Brazil, Pernambuco, Petrolina, Farm Boa Esperança (40°27′30.8″, 09°20′03.2″), on *Mangifera indica* stem, 2006, coll. V.S.O. Costa (paratype living culture CMM 1269, ex-paratype living culture URM 7006). Brazil, Bahia, Casa Nova, Farm Fortaleza (40°52′ 46.2″, 09°17′ 07.2″), on *Mangifera indica* stem, 2006, coll. V.S.O. Costa (paratype living culture CMM 1285, ex-paratype living culture URM 7004).

Notes: A light yellowish pigment was observed in the media of three isolates examined. Isolates could not be induced to sporulate on any of the media defined in this study, nor on sterilized plant host tissue placed on WA.

Morphology and cultural characteristics

The isolates that were identified in the phylogenetic analysis using the combined data set were used to study colony morphology and conidial characteristics. Isolates of *N. brasiliense* did not sporulate and for this reason the description was based on molecular data. The conidia of *Ne. dimidiatum* were ellipsoid to ovoid, hyaline, with an acutely rounded apex, truncate base, initially aseptate, becoming brown and 2-septate at maturity, with the central cell darker than the end cells. The mycelium was composed of branched, septate, brown hyphae which disarticulated into 0-1-septate phragmospores. All other isolates produced anamorph structures on the pine needles on WA within 2–

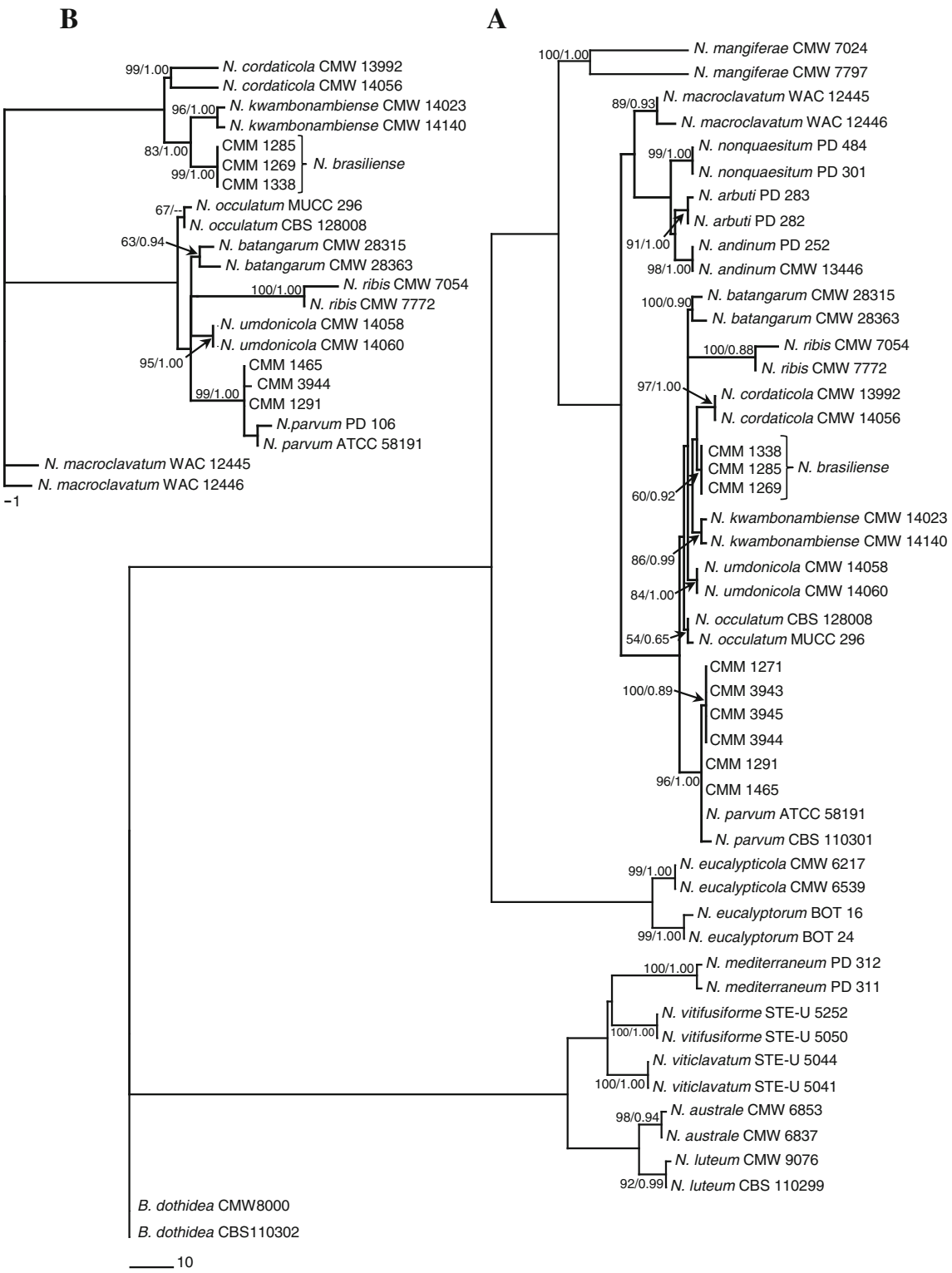

Fig. 2 **a**. One of the most 70 parsimonious trees (TL=361; CI=0.770; RI=0.919; HI=0.230) obtained from combined ITS and EF1-α. The bar represents 1 change. **b**. One of the two most parsimonious trees (TL=104 CI=0.913; RI=0.940; HI=0.087) resulting from maximum parsimony analysis of combined ITS, EF1-α and BT sequence data for *Neofusicoccum* species. Maximum parsimony bootstrap support values from 1,000 replications and Bayesian posterior probability scores are shown at the nodes. The bar represents 10 changes

4 week. No sexual (teleomorph) structures were observed during this study and the species produced hyaline, elongate and thin-walled, fusoid conidia. All isolates on PDA grew rapidly, covering the entire surface of the Petri dishes within 4 days. The aerial mycelium was initially white, turning dark greenish-grey or greyish after 4–5 days at 25 °C under near

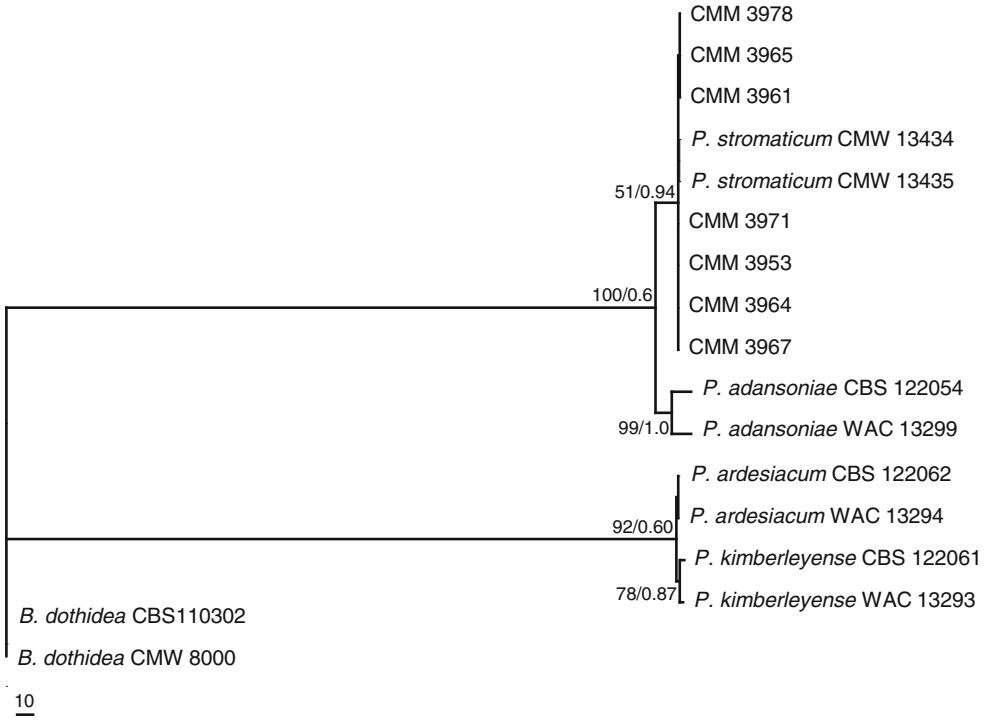

Fig. 3 Single most parsimonious tree (TL=364; CI=0.975; RI=0.920; HI=0.025) obtained from combined ITS and EF1-α sequence data for *Pseudofusicoccum* species. Maximum parsimony bootstrap support values from 1,000 replications and Bayesian posterior probability scores are shown at the nodes. The tree was rooted to *B. dothidea* (CAP 288 and CMW 8000). The *bar* represents 10 changes

UV-light. Conidial dimensions of the species obtained in this study were similar to those previously described in the literature (Table 2).

There were significant differences ($P \leq 0.05$) among the species obtained in this study in relation to optimum temperature for mycelial growth and mycelial growth rates. The optimum temperature varied from 25.4 °C to 30.8 °C. Optimum temperature for growth differed significantly between the species. *Ne. dimidiatum* (30.8 °C) and *P. stromaticum* (30.4 °C) had the highest optimum temperature for growth, while *N. parvum* had the lowest. The mycelial growth rate of *Ne. dimidiatum* (41.2 mm/day) was significantly higher than all other species. Growth rates differed significantly among the other species, which varied from 19.7 to 29.5 mm/day. *B. dothidea* and *F. fabicercianum* had the lowest mycelial growth rates with 21.9 and 19.7 respectively (Table 3).

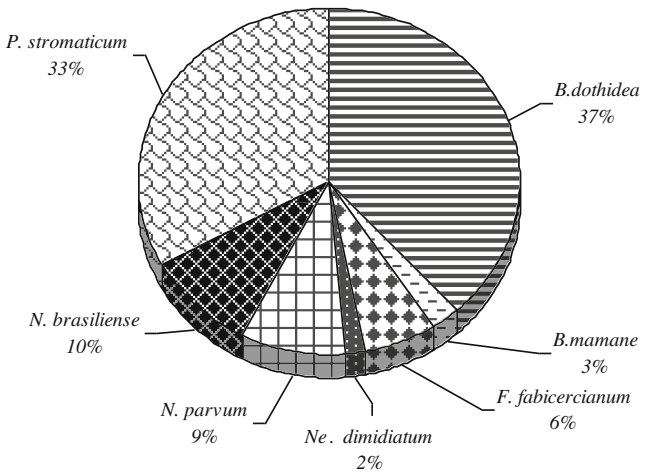

Fig. 4 Frequency (%) of Botryosphaeriaceae species associated with dieback and stem-end rot of mango in São Francisco Valley ($n=115$), Northeastern Brazil

Pathogenicity and virulence on fruits

All species of *Botryosphaeria*, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* collected in this study were pathogenic in fruit. Inoculations resulted in irregularly shaped, roughly circular, black to brown lesions on the surface of the fruit. Analysis of variance showed that there were significant differences ($P \leq 0.05$) in the virulence among species. *Ne. dimidiatum, N. parvum* and *B. dothidea* were the most virulent species. *P. stromaticum* produced the smallest lesions on mango fruits. The lesion length induced by *B. mamane*, *F. fabicercianum* and *N. brasiliense* had intermediate levels of virulence (Fig. 5).

Discussion

In this study, seven different species of Botryosphaeriaceae distributed in four genera were identified and characterized from *M. indica* in Northeastern Brazil. These identifications were supported by morphology and DNA sequence data (ITS and EF1-α).

In Northeastern Brazil, the mango cultivation has increased considerably lately and the use of new cultivation technology,

Table 2 Comparison of conidial size of species examined in this study and previous studies

Species	Conidial size (μm)	L/W ratio	References
Botryosphaeria dothidea	19.5–22.8×4–4.9	4.8	This study
	18–20×4–5	4.1	Slippers et al. 2004a
B. mamane	29.4–31.4×7.3–8.7	4.0	This study
	28–43×5–7	5.8	Mohali et al 2007
Fusicoccum fabicercianum	18.1–21.2×4. 7–6.9	3.7	This study
	19.6–24.4×5.2–6.4	3.8	Chen et al. 2011
Neofusicoccum parvum	14.6–16.4×5.8–6.4	2.7	This study
	15–19×4–6	3.1	Slippers et al. 2004a
Neoscytalidium dimitiatum	9.1–12.3×3–5.3	2.8	This study
Pseudofusicoccum stromaticum	22.2–24.3×6.3–7.0	4.0	This study
	20–23×5–6	4.0	Mohali et al. 2006

such as floral induction by application of potassium nitrate and paclobutrazol together with long periods of exposure of plants to water stress (Junqueira and Junqueira 2007), has likely contributed to the resurgence of diseases such as stem-end rot and dieback. These diseases were associated exclusively with *L. theobromae* and, later *N. parvum, B. dothidea* and *P. stromaticum* were reported (Costa et al. 2010; Marques et al. 2012). In this study, four more species were found to be associated with these diseases. These include: *B. mamane, Fusicoccum fabicercianum, N. brasiliense* and *Ne. dimidiatum.*

The species *B. dothidea* and *N. parvum* are commonly associated with mango diseases worldwide (Slippers et al. 2005) and are generally found in temperate regions (Burgess et al. 2006; Pavlic et al. 2007; Sakalidis et al. 2011). Recent work reported *B. dothidea* had a broad distribution on *M. indica* in Iran, and was found in a variety of climates ranging from temperate and humid to the semi-arid regions and the humid tropical regions (Abdollahzadeh et al. 2013). In the present study, *B. dothidea* was the most prevalent species found in the semi-arid regions of Brazil and represented 37 % of all the isolates examined. *B. dothidea* is common on both cultivated and indigenous hosts in the Northern Hemisphere (Zhou and Stanoxz 2001; Slippers et al. 2004a; Piskur et al. 2011). This suggests a Northern Hemisphere origin for this fungus and implies that it was introduced into the Southern Hemisphere together with plant material. Worldwide, this species is associated with a wide variety of hosts. In Brazil it has been reported on pear, apple, *Vitis* sp., *Eucalyptus* and mango (Mendes et al. 1998; Becker and Ieki 2002; Costa et al. 2010).

Neofusicoccum parvum is one of the most common pathogens of mango causing fruit stem-end rot, dieback and blossom blight (Slippers et al. 2005). It was reported in Brazil for the first time in 2009 (Costa et al. 2010). This species is closely related to *N. ribis*, often showing overlapping morphological characters, which has led to confusion and misidentification, requiring, therefore the use of molecular methods. However, the use of only ITS may underestimate the real diversity, mainly among closely related species or cryptic species (Taylor et al. 2000). Therefore, the sequence data of ITS is normally combined with the other genes such as EF1-α, β -tubulin, which have been applied successfully to discriminate cryptic species and elucidate phylogenetic relations (Mohali et al. 2007; De Wet et al. 2008; Phillips et al. 2008; Sakalidis et al. 2011). Recently, four species, *N. batangarum, N. cordaticola* Pavlic, Slippers, M.J. Wingfield, *N. kwambonambiense* Pavlic, Slippers, M.J. Wingfield and *N. umdonicola* Pavlic, Slippers, M.J. Wingfield were identified in this complex based on congruence between genealogies of multiple genes (Pavlic et al. 2009a, b; Begoude et al. 2010).

Neofusicoccum brasiliense is recognized as another species in the complex *N. parvum*/*N. ribis*, closely related

Table 3 Optimum temperature for mycelial growth and mycelial growth rate at 30 °C of species associated with dieback and stem-end rot of mango in Northeastern Brazil

Species	n	Optimum temperature (°C) ± SE	Mycelial growth rate (mm/day) ± SE
Botryosphaeria dothidea	6	27.9±0.36 b	21.9±1.09 d
Botryosphaeria mamane	1	28.5±0.89 b	22.3±2.68 cd
Fusicoccum fabicercianum	6	27.1±0.36 b	19.7±1.09 d
Neofusicoccum parvum	10	25.4±0.28 c	25.3±0.83 c
Neofusicoccum brasiliense	2	27.7±0.63 b	29.5±1.89 b
Neoscytalidium dimidiatum	2	30.8±0.63 a	41.2±1.89 a
Pseudofusicoccum stromaticum	8	30.4±0.31 a	24.9±0.99 c

Mean ± standard error. Values within columns followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$)

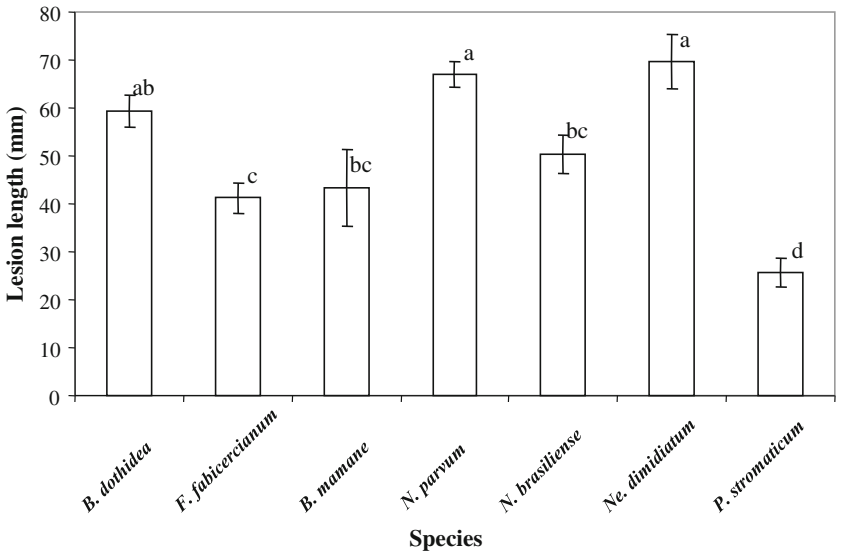

Fig. 5 Mean lesion lengths (mm) caused by species associated with dieback and stem-end rot of mango in Northeastern Brazil, 72 h after inoculation with mycelium colonized agar plugs onto wounded fruits of Tommy Atkins cultivar. *Bars* above columns are the standard error of the mean. Columns with same *letter* do not differ significantly according to Fisher's LSD test ($P \leq 0.05$)

to *N. kwambonambiense* and *N. cordaticola*. Since it failed to sporulation in culture, our description was based in molecular data. In this study, this species was the third most prevalent species found on mango.

Fusicoccum fabicercianum was recently described from *Eucalyptus* sp. in southern China (Chen et al. 2011). Phylogenetically, *F. fabicercianum* is closely related to *B. corticis*, *B. dothidea*, *B schariffi* and *F. ramosum*. This species is a new record for Brazil and is reported here for the first time on mango.

An interesting finding in this study was the identification of *B. mamane* associated with mango. This species has previously only been reported in Hawaiian (Gardner 1997) and Venezuelan native vegetation (Mohali et al. 2006). Only the ITS sequence is available for the ex-type isolate (isolate 97-59). For this reason sequences of regions other than ITS come from isolates that are not associated with the type, its host or locality. Unfortunately, these isolates might not be true *B. mamane* since there are 4 bp differences in ITS from the ex-type.

In this study *P. stromaticum* was the second most abundant species comprising 38 % of the isolates, indicating this genus is more widely distributed than it believed earlier. This species has been reported only on non-native *Eucalyptus* and *Acacia* spp. in Venezuela (Mohali et al. 2006, 2007). Later, it was reported causing a disease of mango in Brazil (Marques et al. 2012). According to Pavlic et al. (2008), the fact that all *Pseudofusicoccum* spp. occurred on native hosts in a relatively undisturbed area of Australia or in the case of *P. stromaticum* on Australian plants suggests that the species are most likely native to that country. Based in our survey this is clearly not the case, since this species was found in non-native plant species (mango) and intensively cultivated areas.

The species *Ne. dimidiatum* is characterized by conidia formed in arthric chains in the aerial mycelium, powdery to the touch. In addition to arthroconidia the cultures produce *Fusicoccum*-like conidia in pycnidia. *Ne. dimidiatum* has been isolated from different substrates including plant tissues, soil, human skin and nails, and is known as a plant pathogen (Punithalingam and Waterston 1970; Crous et al. 2006). In Australia this species was collected as an endophyte in tissues of plants (Pavlic et al. 2008). Later, these fungus were isolated from mango showing dieback and canker symptoms and produced lesions during pathogenicity trials on mango fruit and excised stems (Ray et al. 2010; Sakalidis et al. 2011). The same was found in the present study, this species was isolated only from mango branches with dieback or canker. However, it was able to cause lesions on mango fruit, and was the most virulent species in the pathogenicity test. In Brazil, this species was reported for the first time causing collar and root rot in the biofuel plant *Jatropha curcas* L. (Machado et al. 2012). To our knowledge this is the first report related to mango trees in Brazil. Studies about these species resurging in the country and their roles in the disease epidemiology must be undertaken.

In Brazil, *B. dothidea, N. parvum* and *L. theobromae* were found in the main mango production regions, but their relative prevalence differed in each region. In the São Francisco Valley, *B. dothidea* and *N. parvum* were more prevalent than *L. theobromae*, whereas *L. theobromae* was the predominant species in the Assú Valley (Costa et al. 2010). In this study, all species were reported only in São Francisco Valley. In Western Australia, eight taxa of Botryosphaeriaceae were identified in mango, the most commonly encountered species included *Ne. novaehollandiae, Ne. dimidiatum* and *P. adansoniae* (Sakalidis et al. 2011). In eastern Australia the species identified were *N. parvum, N. mangiferum, B. dothidea* and *P. kimberleyense* (Slippers et al. 2005). The species distribution may be due to a number of factors including the predominant cultivars, differences in climate and soil type and cultivation practices (Lazzizera et al. 2008).

Inoculation of mango fruits was made and all the species showed the potential to cause damage. *Ne. dimidiatum* and *N. parvum* caused the largest lesions on the fruit. Similar data were found by Sakalidis et al. (2011), when pathogenicity of Botryosphaeriaceae species was tested in fruits and branches of mango. Studies about its epidemiology and its impact on mango culture should be done. In this study, the origins of the Botryosphaeriaceae species collected from *M. indica* are unknown. Maybe, the semi-arid climatic conditions and the use of practices that lead the plant to longer periods of stress (floral induction and water restriction) have led to these diseases acquiring more importance lately. The incidence and pathogenicity indicate that all the species are economically important for mango cultivation, because they have the potential to reduce fruit quality especially in postharvest.

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