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Two new *Ceratocystis* species associated with mango disease in Brazil

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Abstract —*Mangifera indica*, a disease known as mango blight, murcha or seca da mangueira in Brazil, is caused by the canker wilt pathogen *Ceratocystis fimbriata* sensu lato. It is also closely associated with infestation by the non-native wood-boring beetle *Hypocryphalus mangiferae* (*Coleoptera: Scolytinae*). The aim of this study was to characterize *Ceratocystis* isolates obtained from diseased mango trees in Brazil. Identification was based on sequence data from ITS1+5.8S+ITS2 rDNA, part of the Beta-tubulin 1 gene, and part of the Transcription Elongation Factor 1-alpha gene. The Brazilian isolates grouped in two well defined and unique clades within *C. fimbriata* s.l. These were also distinct from *C. manginecans*, which causes a similar disease associated with *H. mangiferae* in Oman and Pakistan. Based on sequence comparisons and morphological characteristics, isolates representing the two phylogenetic clades are described as *C. mangicola* sp. nov. and *C. mangivora* sp. nov.

Key words — agricultural crop, bark beetles

Introduction

A disease typified by wilting of the leaves, flowers, and stems of mango trees (*Mangifera indica* L. (*Anacardiaceae*) mango) was first reported from Brazil in the 1930's (Viégas 1960, Ploetz 2003). The disease, commonly referred to as "mango blight", "seca" or "murcha da mangueira", represents one of the most important constraints to mango production in Brazil (Ploetz 2003). The causal agent of this disease was identified as *Ceratocystis fimbriata* Ellis & Halst. sensu lato (s.l.) (Viégas 1960, Piza 1966, Ribeiro 1980).

Ceratocystis fimbriata s.l. was first recognized as possibly encompassing more than one taxon by Webster & Butler (1967a, b), who showed host specificity amongst isolates of the fungus. Isolates of *C. fimbriata* s.l. are morphologically similar, but many can be differentiated through DNA sequence analyses. During the past decade, numerous new and cryptic species in the *C. fimbriata* complex have been described. Examples include the African fungus *C. albifundus* M.J. Wingf. et al. (Wingfield et al. 1996, Barnes et al. 2005), *C. larium* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2009a), *C. cacaofunesta* Engelbr. & T.C. Harr. (Engelbrecht & Harrington 2005), *C. fimbriatomima* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2009b), *C. curvata* M. van Wyk & M.J. Wingf., *C. ecuadoriana* M. van Wyk & M.J. Wingf., and *C. diversiconidia* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2011). In the strict sense, *C. fimbriata* is restricted to those isolates related to the sweet potato black-rot pathogen, first described by Halsted (1890) from diseased *Ipomoea batatas* (L.) Lam. (*Convolvulaceae*) (sweet potato) tubers in the USA (Engelbrecht & Harrington 2005). An alternative view is that phylogenetically different *C. fimbriata* s.l. isolates from various Brazilian hosts might represent populations of *C. fimbriata* s.s. rather than discrete taxa (Ferreira et al. 2010).

Ceratocystis species require wounds to infect trees (De Vay et al. 1963, Kile 1993). In Brazil, mango blight is closely associated with the wood-boring beetle *Hypocryphalus mangiferae* Stebbing (*Coleoptera: Scolytinae*) that is native to southern Asia (Wood 1982, Butani 1993, Atkinson & Peck 1994). It has been hypothesized that this insect aids in the dissemination of the fungus in Brazil (Ribeiro 1980, Yamashiro & Myazaki 1985, Ploetz 2003). Interestingly, the same beetle is associated with *Ceratocystis manginecans* M. van Wyk et al. that causes a serious disease of Mango trees in Oman and Pakistan (Al Adawi et al. 2006, Van Wyk et al. 2005; 2007a) and that has the same symptoms as mango blight in Brazil.

When Van Wyk et al. (2007a) described *C. manginecans,* only two *C. fimbriata* s.l. isolates from diseased mango in Brazil were included. These isolates differed phylogenetically from *C. manginecans* but were not treated as novel due to the small number of isolates available. Recently, a larger collection of *C. fimbriata* s.l. isolates associated with mango blight in Brazil has become available for study. The aim of this investigation was to compare these isolates with *C. manginecans* and thus to determine their identity.

Materials and methods

Isolates

A total of 15 isolates (Table 1) from diseased Mango trees obtained in Sao Paulo State in Brazil were transferred to 2% Malt Extract Agar (MEA) (20 g/L) (Biolab, Midrand, South Africa) and maintained at room temperature (~25°C). All cultures used are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI, University of Pretoria, South Africa). Representative isolates have also been deposited with the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). Cultures of representative isolates bearing fruiting structures of the fungi were dried on 30% glycerol and have been deposited with the National Collection of Fungi (PREM), South Africa.

Table 1. *Ceratocystis* spp. for which isolates or sequences were used in this study.

Species	Isolate .	GenBank acc. #	Host	Origin
C. acaciivora	CMW22563	EU588656, EU588636, EU588646	*Acacia mangium*	Indonesia
	CMW22564	EU588657, EU588637, EU588647	*A. mangium*	Indonesia
C. albifundus	CMW4068	DQ520638 EF070429 EF070400	*A. mearnsii*	RSA
	CMW5329	AF388947 DQ371649 EF070401	*A. mearnsii*	Uganda
C. atrox	CMW19383 CBS120517	EF070414 EF070430 EF070402	*Eucalyptus grandis*	Australia
	CMW19385 CBS120518	EF070415 EF070431 EF070403	*E. grandis*	Australia
C. cacaofunesta	CMW15051 CBS152.62	DQ520636 EF070427 EF070398	*Theobroma cacao*	Costa Rica
	CMW14809 CBS115169	DQ520637 EF070428 EF070399	*T. cacao*	Ecuador
C. colombiana	CMW9565 CBS121790	AY233864 AY233870 EU241487	soil	Colombia
	CMW5751 CBS121792	AY177233 AY177225 EU241493	*Coffea arabica*	Colombia
	CMW9572	AY233863 AY233871 EU241488	*Citrus reticulata*	Colombia
C. caryae	CMW14793 CBS114716	EF070424 EF070439 EF070412	*Carya cordiformis*	USA
	CMW14808 CBS115168	EF070423 EF070440 EF070411	*C. ovata*	USA
C. curvata	CMW22442 CBS122603	FJ151436 FJ151448 FJ151470	*Eucalyptus deglupta*	Colombia
	CMW22435 CBS122604	FJ151437 FJ151449 FJ151471	*E. deglupta*	Colombia
C. diversiconidia	CMW22445 CBS123013	FJ151440 FJ151452 FJ151474	*Terminalia ivorensis*	Colombia
	CMW22446	FJ151443 FJ151455 FJ151477	*T. ivorensis*	Colombia

TABLE 1, continued.

SPECIES	ISOLATE NO.	GENBANK ACC. #	HOST	ORIGIN
C. ecuadoriana	CMW22092 CBS124020	FJ151432 FJ151444 FJ151466	*E. deglupta*	Colombia
	CMW22093 CBS124021	FJ151433 FJ151445 FJ151467	*E. deglupta*	Colombia
C. fimbriata s.s.	CMW15049 CBS141.37	DQ520629 EF070442 EF070394	*Ipomaea batatas*	USA
	CMW1547	AF264904 EF070443 EF070395	*I. batatas*	Papua New Guinea
C. fimbriata s.l.	C1345	AY157966	*Eucalyptus* sp.	Brazil
	C1987	AY585344	*Eucalyptus* sp.	Brazil
	C2041	AY585345	*Acacia mearnsii*	Brazil
	CMW14811 CBS115171 C1905	AY526288	*Colocasia esculenta*	Brazil
	CMW14791 CBS114713 C1865	AY526286	*C. esculenta*	Brazil
	C1900	AY526287	*C. esculenta*	Brazil
	C2032	AY526289	*C. esculenta*	Brazil
	C925	AY157967	*Gmelina arborea*	Brazil
	CMW14806 CBS115166 C1782	AY526292	*Ficus carica*	Brazil
	CMW14796 CBS114720 C1715	AY526307	*Colocasia esculenta*	USA, Hawaii
	CMW14804 CBS115164 C1714	AY526306	*C. esculenta*	USA, Hawaii
	BPI596162	AY526305	*C. esculenta*	China
	C1558	AY157965	*Mangifera indica*	Brazil
C. fimbriatomima	CMW24174 CBS121786	EF190963 EF190951 EF190957	*Eucalyptus* sp.	Venezuela
	CMW24176 CBS121787	EF190964 EF190952 EF190958	*Eucalyptus* sp.	Venezuela
C. larium	CMW25434 CBS122512	EU881906 EU881894 EU881900	*Styrax benzoin*	Indonesia
	CMW25435 CBS122606	EU881907 EU881895 EU881901	*S. benzoin*	Indonesia
C. mangicola	**CMW14797** **CBS114721** **C1688**	**AY953382** **EF433307** **EF433316**	***Mangifera indica***	**Brazil**
	CMW27306	**FJ200256** **FJ200269** **FJ200282**	***M. indica***	**Brazil**
	CMW28907	**FJ200257** **FJ200270** **FJ200283**	***M. indica***	**Brazil**
	CMW28908	**FJ200258** **FJ200271** **FJ200284**	***M. indica***	**Brazil**

Table 1, continued.

Species	Isolate No.	GenBank Acc. #	Host	Origin
(***C. mangicola***)	**CMW28913**	**FJ200259** **FJ200272** **FJ200285**	***M. indica***	**Brazil**
	CMW28914	**FJ200260** **FJ200273** **FJ200286**	***M. indica***	**Brazil**
C. manginecans	CMW13851 CBS121659	AY953383 EF433308 EF433317	*M. indica*	Oman
	CMW13852 CBS121660	AY953384 EF433309 EF433318	*Hypocryphalus mangiferae*	Oman
	CMW23634	EF433302	*M. indica*	Pakistan
	CMW23628	EF433303	*M. indica*	Pakistan
C. mangivora	**CMW15052** **CBS600.70** **C74**	**EF433298** **EF433306** **EF433315**	***M. indica***	**Brazil**
	CMW27304 **CBS127204**	**FJ200261** **FJ200274** **FJ200287**	***M. indica***	**Brazil**
	CMW27305 **CBS128340**	**FJ200262** **FJ200275** **FJ200288**	***M. indica***	**Brazil**
	CMW27307	**FJ200263** **FJ200276** **FJ200289**	***M. indica***	**Brazil**
	CMW28909	**FJ200264** **FJ200277** **FJ200290**	***M. indica***	**Brazil**
	CMW28910	**FJ200265** **FJ200278** **FJ200291**	***M. indica***	**Brazil**
	CMW28911	**FJ200266** **FJ200279** **FJ200292**	***M. indica***	**Brazil**
	CMW28912	**FJ200267** **FJ200280** **FJ200293**	***M. indica***	**Brazil**
	CMW28916	**FJ200260** **FJ200281** **FJ200294**	***M. indica***	**Brazil**
C. neglecta	CMW17808 CBS121789	EF127990 EU881898 EU881904	*Eucalyptus* sp.	Colombia
	CMW18194 CBS121017	EF127991 EU881899 EU881905	*Eucalyptus* sp.	Colombia
C. obpyriformis	CMW23807 CBS122608	EU245004 EU244976 EU244936	*Acacia mearnsii*	South Africa
	CMW23808 CBS122511	EU245003 EU244975 EU244935	*A. mearnsii*	South Africa
C. papillata	CMW8857	AY233868 AY233878 EU241483	*Annona muricata*	Colombia
	CMW8856 CBS121793	AY233867 AY233874 EU241484	*Citrus limon*	Colombia

Table 1, continued.

Species	Isolate No.	GenBank Acc. #	Host	Origin
(*C. papillata*)	CMW10844	AY177238 AY177229 EU241481	*Coffea arabica*	Colombia
C. pirilliformis	CMW6569	AF427104 DQ371652 AY528982	*Eucalyptus nitens*	Australia
	CMW6579 CBS118128	AF427105 DQ371653 AY528983	*E. nitens*	Australia
C. platani	CMW14802 CBS115162	DQ520630 EF070425 EF070396	*Platanus occidentalis*	USA
	CMW23918	EF070426 EF070397 EU426554	*Platanus* sp.	Greece
C. polychroma	CMW11424 CBS115778	AY528970 AY528966 AY528978	*Syzygium aromaticum*	Indonesia
	CMW11436 CBS115777	AY528971 AY528967 AY528979	*S. aromaticum*	Indonesia
C. polyconidia	CMW23809 CBS122289	EU245006 EU244978 EU244938	*Acacia mearnsii*	South Africa
	CMW23818 CBS122290	EU245007 EU244979 EU244939	*A. mearnsii*	South Africa
C. populicola	CMW14789 CBS119.78	EF070418 EF070434 EF070406	*Populus* sp.	Poland
	CMW14819 CBS114725	EF070419 EF070435 EF070407	*Populus* sp.	USA
C. smalleyi	CMW14800 CBS114724	EF070420 EF070436 EF070408	*Carya cordiformis*	USA
	CMW26383 CBS114724	EU426553 EU426555 EU426556	*C. cordiformis*	USA
C. tanganyicensis	CMW15991 CBS122295	EU244997 EU244969 EU244929	*A. mearnsii*	Tanzania
	CMW15999 CBS122294	EU244998 EU244970 EU244939	*A. mearnsii*	Tanzania
C. tsitsikammensis	CMW14276 CBS121018	EF408555 EF408569 EF408576	*Rapanea melanophloeos*	South Africa
	CMW14278 CBS121019	EF408556 EF408570 EF408577	*R.. melanophloeos*	South Africa
C. variospora	CMW20935 CBS114715	EF070421 EF070437 EF070409	*Quercus alba*	USA
	CMW20936 CBS114714	EF070422 EF070438 EF070410	*Q. robur*	USA

Table 1, concluded.

Species	Isolate No.	GenBank Acc. #	Host	Origin
C. virescens	CMW11164	DQ520639 EF070441 EF070413	*Fagus americana*	USA
	CMW3276	AY528984 AY528990 AY529011	*Q. robur*	USA
C. zombamontana	CMW15235	EU245002 EU244974 EU244934	*Eucalyptus* sp.	Malawi
	CMW15236	EU245000 EU244972 EU244932	*Eucalyptus* sp.	Malawi

*CMW numbers are in the Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; CBS numbers are in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands; C numbers are in the T. Harrington collection Iowa State University, USA; BPI numbers are in the US National Fungus collection.

Phylogenetic comparisons

DNA was extracted from the isolates obtained from mango in Brazil according to Van Wyk et al. (2006). Three sets of analyses were run on DNA sequence data obtained from these isolates. The first dataset comprising the Internal Transcribed Spacer region 1 and 2 including the 5.8S rRNA operon (ITS) included sequences for all species in the *C. fimbriata* s.l. complex as well as most *C. fimbriata* sequences available in GenBank (http://ncbi.nlm.nih.gov) and from a variety of plants in Brazil. For the second dataset, three gene regions were targeted for PCR including the ITS, part of the Beta-tubulin 1 (βt) gene, and part of the Transcription Elongation Factor 1-alpha gene (EF1-α). Data for these three gene regions were combined. The third dataset consisted of only the isolates from Brazil and from mango with each gene region (ITS, βt, EF1-α) treated separately.

DNA amplification was achieved with the primer sets ITS1 and ITS4 (White et al. 1990), βt1a and βt1b (Glass & Donaldson 1995), and EF1F and EF1R (Jacobs et al. 2004), following the protocols described by Van Wyk et al. (2006). Amplification was assessed with the aid of gel electrophoresis in the presence of ethidium bromide. PCR amplicons were purified using 6% Sephadex G-50 columns (Steinheim, Germany) and sequenced in both directions using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California), with the same primers as those used for DNA amplification. Sequencing reactions were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California, USA).

Sequences were analysed using the software programme Chromas Lite 2.01 (http://www.technelysium.com.au). Sequence data obtained in this study for Brazilian isolates from mango were compared with those residing in the *C. fimbriata* s.l. clade for *Ceratocystis* obtained from GenBank or those previously published (Van Wyk et al. 2005, 2007a,b, 2009a,b, 2011). These sequences were aligned using MAFFT (http://timpani.genome.ad.jp/%7emafft/server/) (Katoh et al. 2002) and confirmed manually. Thereafter, the *C. fimbriata* s.l. dataset was analyzed using PAUP version 4.0b10* (Swofford 2002). Sequences for the three gene regions were analyzed separately and a partition homogeneity test (Swofford 2002) was used to determine whether the three datasets (ITS, βt and EF1-α) could be combined. The combined analyses were run as described in Van Wyk et al. (2009b). Sequences derived from this study were deposited in GenBank (Table 1) and the accompanying datasets and trees are deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S11630).

A modeltest (MrModeltest2) was run on each gene region to determine nucleotide substitution rates (Nylander 2004) for incorporation into Bayesian analyses (MrBayes version 3.1.1) to determine whether nodes obtained with PAUP had statistical support (Ronquist & Huelsenbeck 2003). One million trees were generated using the Markov Chain Monte Carlo (MCMC) procedure. Four chains, two hot and two cold, were utilized to obtain the results. Trees were sampled every 100th generation and printed. Tree likelihood scores were assessed to determine the number of trees that had formed before stabilization to avoid including trees that had formed before convergence. Trees outside the point of convergence were discarded by means of the burn-in procedure (Ronquist & Huelsenbeck 2003).

Molecular Evolutionary Genetics Analysis (MEGA) 4 (Tamura et al. 2007) was used to determine the level of variation between the isolates from a wide range of hosts in Brazil for the ITS region only. In addition, this approach was applied for the ITS, βt, and EF1-α for the isolates obtained from mango trees in Brazil and including *C. manginecans* previously described from Oman and Pakistan. Sequences for each of the three gene regions were inspected to determine the number of fixed alleles between them.

An allele network was drawn using the software TCS (Clement et al. 2000). The dataset consisted of the combined gene regions (ITS, βt and EF1-α) of all the isolates obtained from mango in Brazil and including *C. manginecans* and *C. fimbriatomima*.

Culture characteristics and morphology

Based on the phylogenetic comparisons, two groups (B1 and B2) of isolates emerged. Three representatives from each of the two groups (CMW14797, CMW27306, CMW28907 and CMW15052, CMW27304, CMW27305) were randomly selected for growth studies in culture at different temperatures. The isolates were grown for 14 days on 2% MEA, after which 5mm diam. plugs were transferred to the centers of 90mm Petri plates containing 2% MEA. These plates were incubated at temperatures between 5 and 35°C at five degree intervals. Five plates were used for each isolate at each of the test temperatures and the entire experiment was repeated once. The colony colours for isolates were assigned using the colour charts of Rayner (1970).

For microscope studies, the same six isolates, representing the two groups (B1 and B2) that were used to compare culture characteristics were selected. These cultures were grown for 10 days on 2% MEA plates. Fungal structures were selected and mounted in lactic acid on glass slides. Photographic images were captured with a Carl Zeiss compound microscope and using a Zeiss Axio Vision camera system. For isolates CMW14797 and CMW28305, 50 measurements were made for taxonomically relevant morphological characteristics, while 10 measurements were taken for isolates CMW27306, CMW28907, CMW15052 and CMW27304. The averages and standard deviations (stdv) were computed for all the measurements that are presented in the descriptions as (minimum–) mean minus stdv – mean plus stdv (–maximum). Where the minimum value was the same as the mean minus the stdv, a parenthetical minimum was not included.

Results

Phylogenetic comparisons

ITS sequences for species in *C. fimbriata* s.l. —including unidentified isolates from *Colocasia* (*Araceae*) (taro), *Mangifera* (*Anacardiaceae*) (mango), *Gmelina* (*Lamiaceae*) (yemane) and *Ficus* (*Moraceae*) (fig) in Brazil— gave a 614 bp dataset for 83 isolates. This dataset consisted of 234 constant, 11 parsimony uninformative, and 369 parsimony informative characters. Of the five trees obtained in these analyses, one was selected for presentation (Fig. 1). The tree had the following characteristics; tree length = 1279, Consistency Index = 0.6, Rescaled Index = 0.5, Retention Index = 0.9.

MrModeltest2 selected the GTR+I+G model for the ITS gene region. These settings were included in the Bayesian analyses and four thousand trees were discarded because they were obtained outside the point of convergence. The Bayesian probabilities obtained in MrBayes were included in the phylogram (Fig. 1) obtained in PAUP. The probabilities obtained in the Bayesian analyses were similar to the support values obtained in PAUP.

The isolates from Brazil grouped into several polyphyletic clades (Fig. 1). These included a well-supported clade (Bootstrap 86%) represented by two isolates, one from *Acacia* (*Mimosaceae*) the other from *Eucalyptus* (*Myrtaceae*) A second clade included only isolates from taro (Bootstrap 85%). Isolates from mango and a *Eucalyptus* and *Gmelina* isolate resided in a discrete clade (Bayesian 91%), and *C. manginecans* isolates grouped in a clade sister to that clade (Bootstrap 87%). A group of isolates from mango resided in a clade with strong support (Bootstrap 99%, Bayesian 86%), and an isolate from fig was sister to that clade.

Amplicons of ~500 bp (ITS and β-tubulin) and ~800 bp (EF1-α) were obtained for the Brazilian isolates of *C. fimbriata* s.l. from mango (Table 1). The PHT resulted in a low P-value (P=0.01), possibly due to the small amount of variation in the βt gene region. Although the P-value was low, this value remained acceptable (Sullivan 1996, Cunningham 1997) to support combination of the data for the three gene regions. The combined dataset for the three gene regions consisted of a total of 1971 characters, 1066 of which were constant, 57 were parsimony-uninformative, and 848 were parsimony informative. Twenty-two most parsimonious trees were obtained, one of which (Fig. 2) was selected for presentation (Tree length = 2361, Consistency Index = 0.6, Rescaled Index = 0.5, Retention Index = 0.9).

MrModeltest2 selected the GTR+I+G model for the ITS gene region, the GTR+G model for the βt gene region and the HKY+I+G for the EF1-*α* gene region. These settings were included in the Bayesian analyses. Two thousand trees were discarded as they were outside of the point of convergence. The posterior probabilities for the branch nodes were included in the tree obtained with PAUP (Fig. 2). The posterior probabilities supported the bootstrap values obtained using PAUP.

The isolates from mango in Brazil grouped in two distinct clades (B1 and B2), with high bootstrap support (100% and 100%, respectively). These two phylogenetic groups were sister to *C. manginecans*, the species most closely related to them. All other species considered in this study, formed well-supported and distinct clades, confirming their unique nature.

The single ITS gene tree (Fig. 3) had a structure similar to the tree based on combined sequences for the three gene regions. The two groups of isolates from mango (B1 and B2) in Brazil grouped apart from *C. fimbriatomima* and *C. manginecans* with high bootstrap support. The single gene trees for the βt and EF1-α gene region did not distinguish between the two groups of isolates from mango in Brazil but they did distinguish *C. fimbriatomima* and *C. manginecans* from these two groups.

The number of fixed alleles between the four groups (three from mango and one from *Eucalyptus*), *C. manginecans,* the two groups of isolates obtained from Mango in Brazil (Group B1 and Group B2) and *C. fimbriatomima* varied within and between groups (Table 2). Analysis of the combined dataset for the three gene regions using TCS resulted in two allele trees (Fig. 4). *Ceratocystis fimbriatomima* was represented on its own while all three taxa from diseased mango including *C. manginecans* and the two groups identified in this study, resided in a single allele tree.

Table 2. Comparison of differing sequences and number of fixed alleles in *Ceratocystis* spp. from mango and the closely related species *C. fimbriatomima*. Shaded cells indicate variations within each species.

ITS	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**4**	16	6	10
C. mangivora	16	**2**	20	14
C. manginecans	6	20	**0**	14
C. fimbriatomima	10	14	14	**1**

βt	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**1**	0	5	8
C. mangivora	0	**3**	4	7
C. manginecans	5	4	**0**	3
C. fimbriatomima	8	7	3	**1**

EF-1α	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**1**	0	1	0
C. mangivora	0	**9**	1	0
C. manginecans	1	1	**0**	1
C. fimbriatomima	0	0	1	**0**

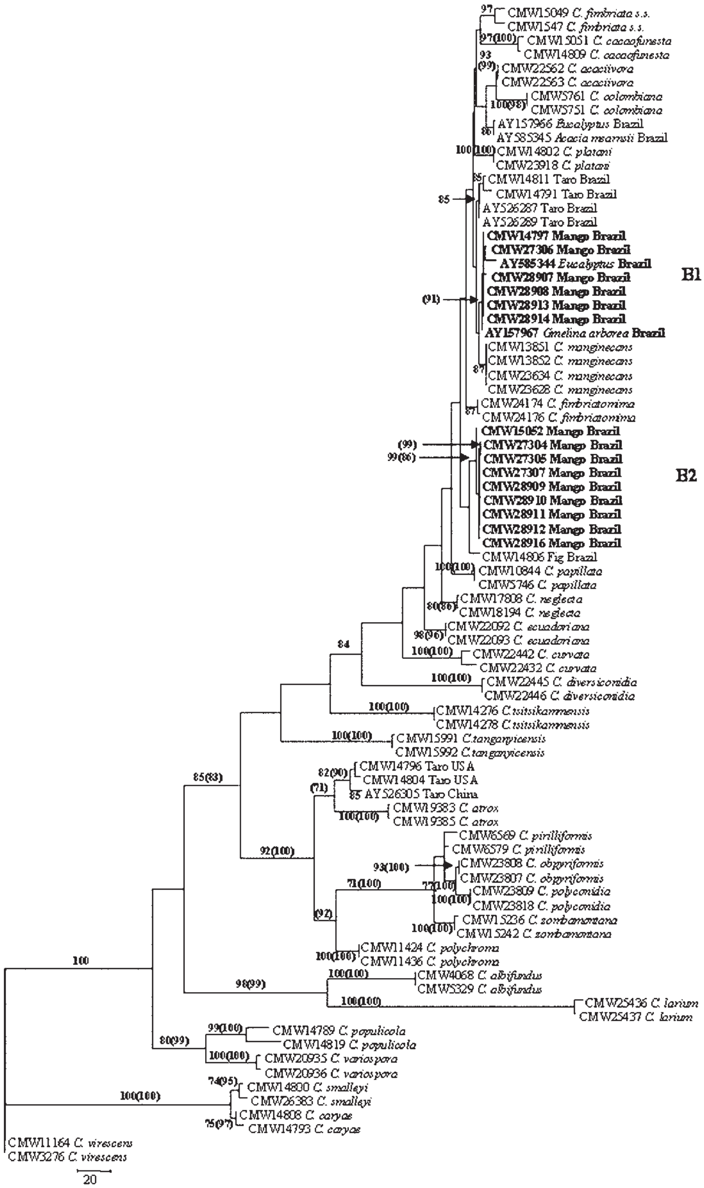


Figure 1. Phylogenetic tree based of the ITS gene region for *Ceratocystis mangicola* (B1), *C. mangivora* (B2), and other species in the *C. fimbriata* s.l. complex including isolates from Brazil obtained from various hosts. Bootstrap values are indicated at the branches, with Bayesian support in brackets.

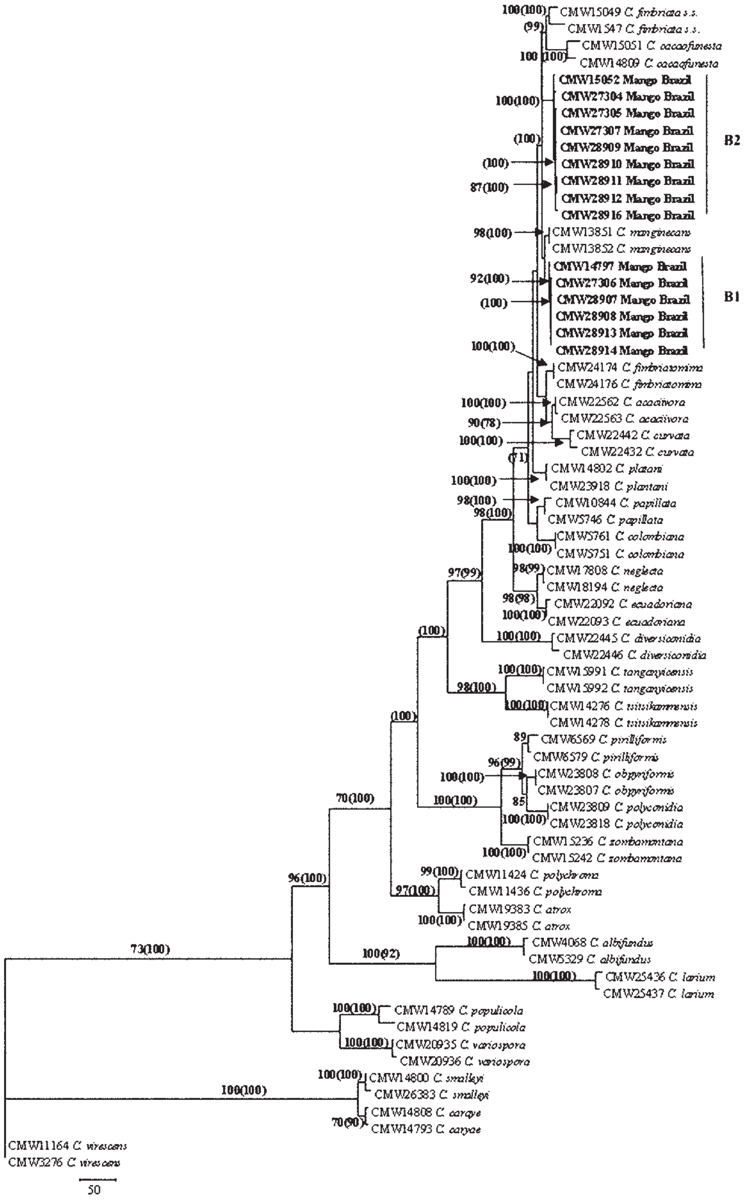


Figure 2. Phylogenetic tree based on the combined regions of the ITS, β-tubulin and EF1-α for *Ceratocystis mangicola* (B1), *C. mangivora* (B2), and other species in the *C. fimbriata* s.l. species complex. Bootstrap values are indicated at the branches, with Bayesian support in brackets.

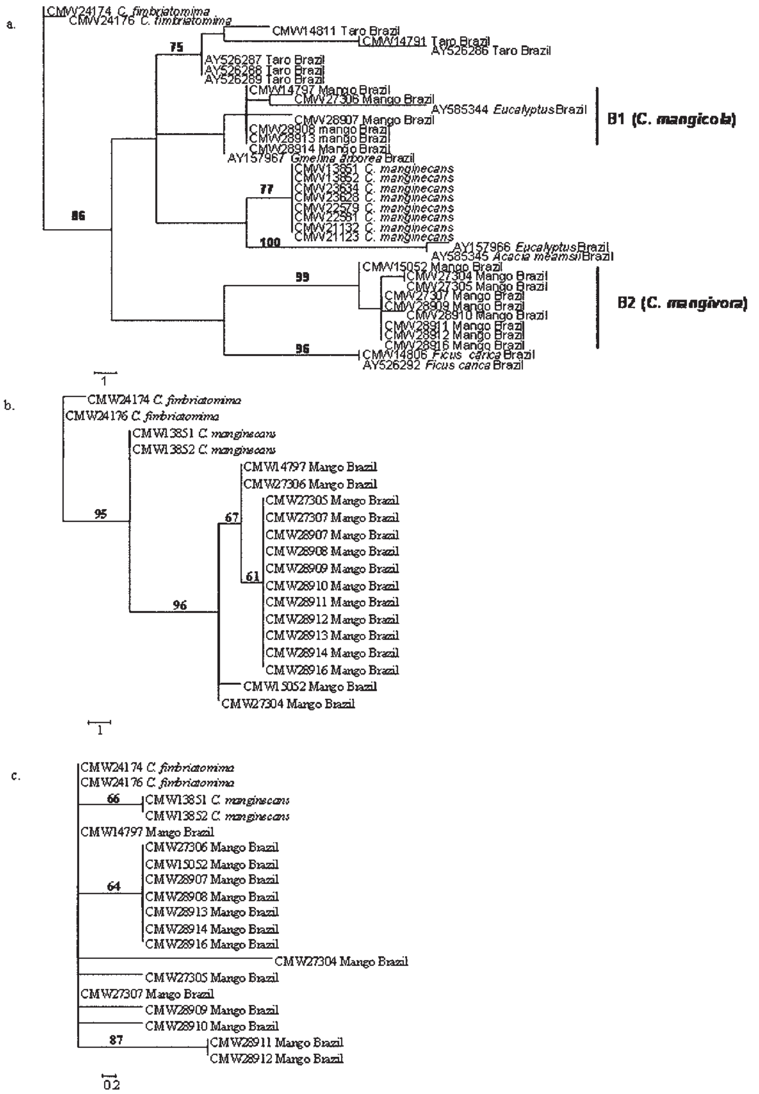


FIGURE 3. Three separate unrooted phylogenetic trees representing three gene regions. Isolates representing the two groups from Brazil mango as well as *Ceratocystis manginecans* and *C. fimbriatomima* were included. a. ITS. b. βt. c. EF1-α.

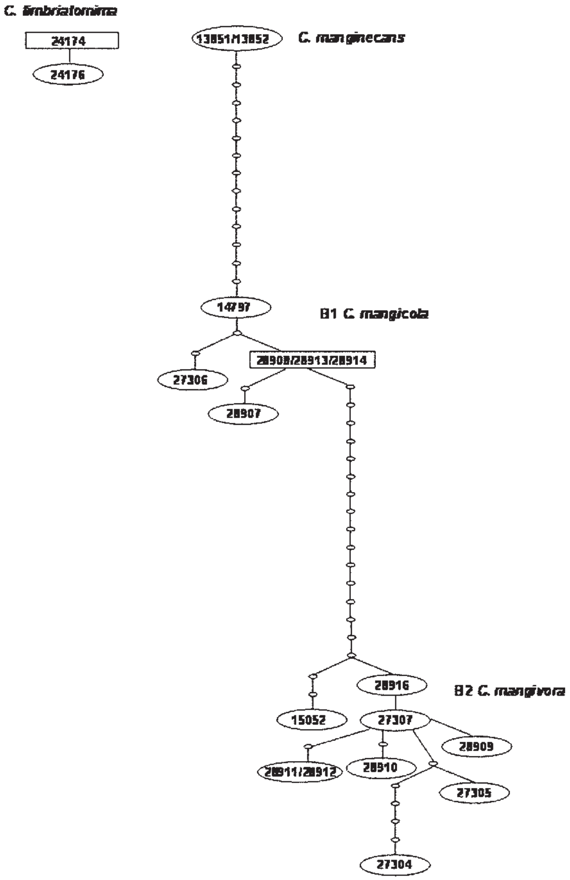


Figure 4. An allele network of the two groups of isolates from Brazil as well as a closely related species *C. manginecans* also isolated from mango trees and *Ceratocystis fimbriatomima*. The numbers represent CMW numbers (Table 1).

Culture characteristics and morphology

Isolates representing Group B1 were morphologically similar to other species in *C. fimbriata* s.l. They produced a banana odour, typical of fungi in this group. After 2 weeks on 2% MEA, the colonies had a dark brown (snuff brown, 15"k) colour (Rayner 1970) with large numbers of perithecia visible on the surface of the cultures. At 5°C and 35°C, no growth was observed after 7 days. At 10°C (8 mm), 15°C (22 mm), 20°C (36 mm) and 30°C (20 mm) diminished growth was observed after 7 days while the optimum temperature for growth of these isolates was 25°C (44 mm).

Isolates residing in Group B2 were similar to those in Group B1 producing a banana odour and they had a similar morphology. After 2 weeks on 2% MEA, the colonies also had a dark brown (snuff brown, 15"k) colour (Rayner 1970) with many perithecia produced on the culture surface. As with the isolates in Group B1, after seven days, there was no growth at either 5 or 35°C. Some growth was observed at the other temperatures tested 10°C (7 mm) and 15°C (22 mm), 20°C (41 mm), 30°C (36 mm) and 25°C (45 mm) represented the optimum temperature for growth.

Taxonomy

Based on DNA sequence comparisons and (to a lesser extent) morphology, isolates from mango in Brazil could be separated into two distinct groups. These groups represent previously unknown species that are described as follows:

Ceratocystis mangicola M. van Wyk & M.J. Wingf., **sp. nov.** FIGURE 5

MYCOBANK MB511886

Hyphae ostiolares hyalinae divergentes convergentesque, (47–)57–73(–79) µm longa. Conidiophorae biformes; primariae phialidicae, lageniformes, hyalinae; secondariae copiosae, tubiformes, apicibus expansis, hyalinae.

TYPE: Brazil, São Paulo State, from diseased *Mangifera indica* trees, isolated C.J. Baker C1688, 2000. (**holotype** PREM60182 (culture dried on 30% glycerol); culture ex-type CMW14797 = CBS114721).

ETYMOLOGY: The epithet refers to the fact that the fungus occurs on mango.

Colonies brown (15"k) on 2% MEA. Odour banana. Hyphae smooth and segmented. Ascomatal bases globose to sub-globose, dark-brown to black, (125–)139–199(–230) µm wide, (115–)136–192(–236) µm long. Ascomatal necks brown becoming lighter towards apices (541–)766–980(–1103) µm long, (21–)26–36(–46) µm wide at base, (15–)19–27(–33) µm wide at tip. Ostiolar hyphae of two types; hyaline, divergent and convergent, (47–)57–73(–79) µm long. Asci evanescent, not seen. Ascospores hyaline, hat-shaped, 3–4 µm long, 3–4 µm wide excluding sheath, 5–6 µm wide including sheath.

Thielaviopsis ANAMORPH: Conidiophores of two morphological forms. Primary conidiophores phialidic, lageniform, hyaline, (59–)71–119(–140) µm long, (3–)4–6(–7) µm wide at base, 5–7(–8) µm wide at broadest point, 3–5(–8) µm wide at tips. Secondary conidiophores, abundant, tube-like, flaring at apices, hyaline, (53–)72–114(–148) µm long, 4–6(–7) µm wide at bases and 6–8(–9) µm wide at tips. Conidia of two types. Primary conidia, hyaline, cylindrical, (15–)18–24(–29) µm long, (3–)4–6 µm wide. Secondary conidia, abundant, hyaline, barrel to sub-globose shaped, (6–)7–9(–11) µm long, 6–8 µm wide. Chlamydospores rare, brown, thick-walled, globose to sub-globose, (12–)14–16(–17) µm long by (9–)11–13(–14) µm wide.

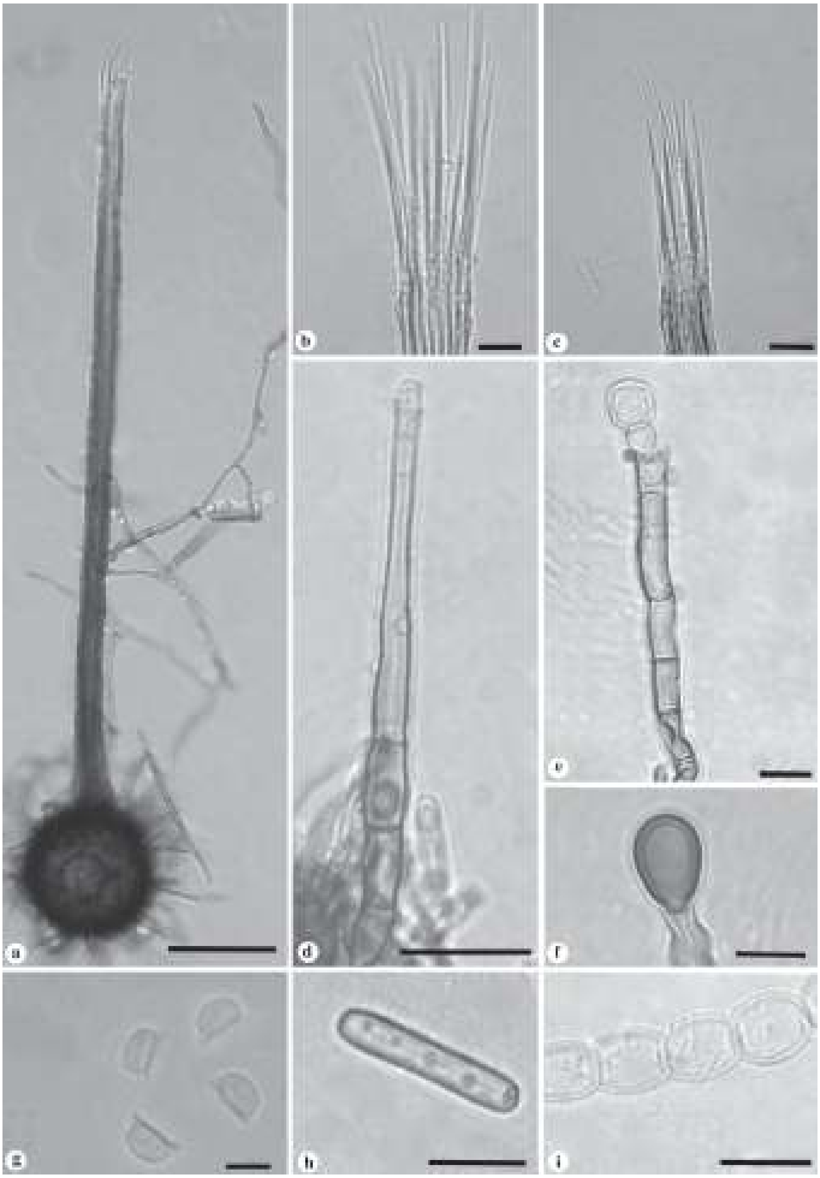


Figure 5. Morphological characteristics of *Ceratocystis mangicola* (from holotype): a. Globose ascomata. b. Divergent ostiolar hyphae. c. Convergent ostiolar hyphae. d. Primary phialidic conidiophore. e. Secondary conidiophore with emerging chain of barrel-shaped conidia. f. Dark colored chlamydospores. g. Hat-shaped ascospores. h. Cylindrical conidia. i. Chain of barrel-shaped conidia. Scale bars: a = 100 µm, b–c, e–f, h–i = 10 µm, d = 20 µm, g = 5 µm.

HABITAT & DISTRIBUTION: Isolated from *Mangifera indica* trees and associated with the wood-boring scolytine *Hypocryphalus mangiferae*. Known from São Paulo State, North West Brazil.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL. SÃO PAULO STATE, VOTUPORANGA,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13750-1, 2007, PREM60183, living culture CMW27306; isolated C.J. Rossetto 13959, 2008, PREM60184, living culture CMW28907; isolated C.J. Rossetto 13911, 2008, PREM60186, living culture CMW28913; **SÃO PAULO STATE, SANTA BÁRBARA D'OESTE**, from diseased *Mangifera indica* trees isolated C.J. Rossetto 13977, 2008, PREM60185, living culture CMW28908; **SÃO PAULO STATE, PINDORAMA**, from diseased *Mangifera indica* trees isolated C.J. Rossetto 13966, 2008, PREM60187, living culture CMW28914.

NOTES: *Ceratocystis mangicola* (B1; CMW14797) is distinguished from all other species in the *C. fimbriata* s.l. complex based primarily on phylogenetic inference. However, it also has ostiolar hyphae that are both divergent and convergent as opposed to being only divergent in most species of this genus.

Ceratocystis mangivora M. van Wyk & M.J. Wingf., **sp. nov.** FIGURE 6

MYCOBANK MB512368

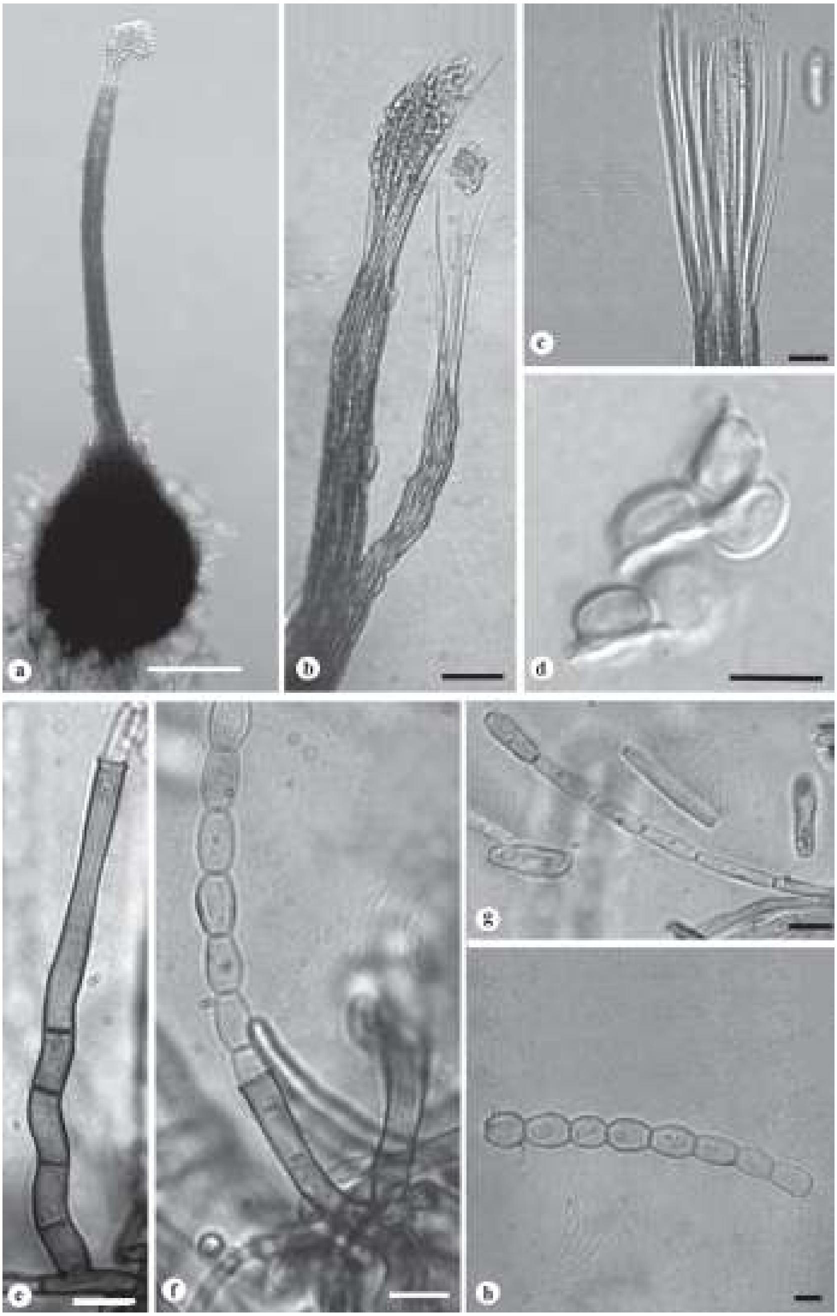
Bases ascomatum globosae vel obpyriformes. Colla ascomatum brunnea, apicem versus pallescentia, apice in duo vel plura ramose. Conidiophorae biformes; primariae phialidicae lageniformes hyalinae; secondariae abundantes tubiformes apice expansae hyalinae. Chlamydosporae absunt.

TYPE: Brazil. São Paulo State, Campinas, from diseased *Mangifera indica* trees, isolated C.J. Rossetto 12132, 2001. (**holotype** PREM60570 (culture dried on 30% glycerol); culture ex-type CMW27305 = CBS128340).

ETYMOLOGY: The epithet refers to the fact that the fungus causes a disease on mango.

Colonies brown (15"k) on 2% MEA. Odour banana. Hyphae smooth and segmented. Ascomatal bases globose to obpyriform, dark-brown to black, (171–)188–244(–295) µm wide, (174–)192–256(–310) µm long. Ascomatal necks brown becoming lighter towards apices, branching at apices into two or more necks, (394–)437–575(–654) µm long, (21–)26–34(–40) µm wide at base, (16–)19–29(–35) µm wide at tip. Ostiolar hyphae hyaline, divergent and convergent, (60–)75–91(–96) µm long. Asci evanescent, not seen. Ascospores hyaline, hat-shaped, 3–5 µm in length, 4–6 µm wide excluding sheath, 5–8 µm wide including sheath.

Thielaviopsis ANAMORPH: Conidiophores of two morphological forms. Primary conidiophores phialidic, lageniform, hyaline, (70–)78–106(–124) µm long, (3–)5–7 µm wide at base, 5–7(–8) µm wide at broadest point, 3–5 µm wide at tips. Secondary conidiophores, abundant, tube-like, flaring at apices, hyaline, (42–)62–100(–118) µm long, (3–)4–6 µm wide at bases and (4–)6–8(–9) µm wide at tips. Conidia of two types: Primary conidia, hyaline, cylindrical, (12–)16–24(–31) µm long, 2–5 µm wide. Secondary conidia, abundant, hyaline,


a
b
c
d
e
f
g
h

barrel-shaped, (8–)9–13(–15) µm long, (5–)6–8(–9) µm wide. Chlamydospores absent.

HABITAT & DISTRIBUTION: Isolated from *Mangifera indica* trees. Associated with the wood-boring scolytid *Hypocryphalus mangiferae*. Known from São Paulo State, Central East Brazil.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL, SÃO PAULO STATE,** from diseased *Mangifera indica* trees, isolated M. Barreto Figueiredo, 1970, PREM60188, living culture CMW15052 = CBS600.70; **BRAZIL, SÃO PAULO STATE, CAMPINAS,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 12093, 2001, PREM60189, living culture CMW27304 = CBS127204; isolated C.J. Rossetto 12036, 2001, PREM60190, living culture CMW27307; **BRAZIL, SÃO PAULO STATE, SANTA BÁRBARA D'OESTE,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13988, 2008, PREM60191, living culture CMW28909; isolated C.J. Rossetto 13987, 2001, PREM60192, living culture CMW28910. **BRAZIL, SÃO PAULO STATE, TUPI (NEAR PIRACICABA),** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13989, 2008, living culture CMW28911; isolated C.J. Rossetto 13989-1, 2008, living culture CMW28912; **BRAZIL, SÃO PAULO STATE, VALINHOS,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13986, 2008, living culture CMW28916.

NOTES: Isolates of *C. mangivora* (B2; CMW27305) can have ascomatal necks that branch dichotomously at the apices with ostiolar hyphae being either divergent or convergent. Isolates of this species also did not produce chlamydospores in culture.

Discussion

Results of this study showed that a relatively large collection of *Ceratocystis* isolates from mango trees suffering from Mango blight in Brazil reside in two distinct phylogenetic clades. These groups are, furthermore, distinct from *C. manginecans,* which causes a similar disease of mango in Oman and Pakistan (Al Adawi et al. 2006, Van Wyk et al. 2005, 2007a). The isolates residing in these two groups are consequently treated as distinct taxa and the names *C. mangicola* and *C. mangivora* have been provided for them.

The mango tree blight in Brazil, known for almost a century, was previously ascribed to *C. fimbriata* s.l., which we now recognize represents a relatively large number of cryptic taxa. These species are morphologically very similar, and although individual species can be distinguished from their closest relatives, recognition based solely on morphological characteristics would

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FIGURE 6. Morphological characteristics of *Ceratocystis mangivora* (from holotype): a. Globose to obpyriform ascomata. b. Ascomatal neck branching into two apices with both convergent and divergent ostiolar hyphae. c. Convergent ostiolar hyphae. d. Hat-shaped ascospores. e. Primary conidiophore. f. Secondary conidiophore with emerging chain of barrel-shaped conidia. g. Chain of cylindrical conidia. h. Chain of barrel-shaped conidia. Scale bars: a = 100 µm, b–c, e–g = 10 µm, d, h = 5 µm.

be very difficult. This situation exists for many groups of fungi, for example *Fusarium* species in the *Gibberella fujikuroi* species complex (Leslie et al. 1992, O'Donnell et al. 2000). *Ceratocystis mangicola* is phylogenetically most closely related to *C. manginecans,* a known pathogen of mango and other crops in Oman and Pakistan. *Ceratocystis mangivora* (also described in this study) has no well-defined sister group but is also closely related to the other two mango pathogens. Isolates of these species were not only distinct from each other but also phylogenetically distinct from the mango pathogen, *C. manginecans*. The species phylogenetically most closely related to these mango pathogens from Brazil is *C. fimbriatomima*, which was first isolated from *Eucalyptus* trees in Venezuela (Van Wyk et al. 2009b).

Phylogenetic analyses of sequences for the ITS gene region strongly supported separation of *C. mangicola* and *C. mangivora*. In contrast, the βt and EF1-α gene regions for the single gene trees showed little or no variation between *C. mangicola* and *C. mangivora*. This is not uncommon for species in the *C. fimbriata* complex (Van Wyk et al. 2010). Similarly the allele trees for four species; *C. fimbriatomima* and the three mango pathogens, *C. manginecans*, *C. mangicola,* and *C. mangivora* showed that the three species from mango were most closely related to each other. This suggests a common ancestor for the three mango pathogens and the fact that they have probably undergone speciation relatively recently.

Ceratocystis mangicola and *C. mangivora* are morphologically very similar, both producing dark brown cultures with a banana odour that is characteristic of many species of *Ceratocystis.* However, isolates representing the two species could be distinguished from each other based on various micro-morphological characteristics. Thus, *C. mangicola* isolates have both divergent and convergent ostiolar hyphae, a characteristic not noted for other *C. fimbriata* s.l. species except *C. mangivora* described here. Isolates of *C. mangivora* consistently display branched ascomatal necks that give rise to either convergent or divergent ostiolar hyphae. Furthermore, similar to some species in *C. fimbriata* s.l., *C. mangivora* did not produce chlamydospores in culture while these structures are very obvious in cultures of *C. mangicola*. *Ceratocystis mangicola* isolates also have globose to sub-globose ascomatal bases compared to the globose to obpyriform bases in *C. mangivora.*

A previous study on *C. fimbriata* s.l. from different hosts (including mango in Brazil) treated the isolates as a genetically diverse population representing a single taxon (Ferreira et al. 2010). It would have been interesting to include data from that study's isolates in our own research, which might have provided a more robust species delimitation for *C. mangicola* and *C. mangivora*. The unavailability of the isolates for study and the absence of their sequence data from GenBank preclude comparisons at that level.

An interesting aspect of the mango blight disease in Brazil and in Oman is the fact that the pathogens are associated with the same wood-boring insect (*H. mangiferae*) in both areas of the world. *Hypocryphalus mangiferae* is a monophagous bark beetle found only on *Mangifera* species (Schedl 1961). Its source area is likely the same as mango trees in tropical Asia (Wood 1982, Butani 1993, Kostermans & Bompard 1993). Both tree and beetle have been introduced into Brazil (Wood 1982, Butani 1993, Kostermans & Bompard 1993).

Both *C. mangicola* and *C. mangivora* are suspected to be native to Brazil. As with most *Ceratocystis* species, a wound is required for *C. mangicola* and *C. mangivora* to infect mango trees (Silva et al. 1959). Intensive studies of diseased mango trees in Brazil have shown that *H. mangiferae* is the only insect present during the early disease stages. *Xyleborus* species (*Coleoptera: Curculionidae*) typically appear when the disease spreads down towards the larger branches and *Cerambycidae* only appear when the disease reaches the trunk regions (Silva et al. 1959, Castro 1960, Medeiros & Rossetto 1966, Rossetto et al. 1980). Studies have also shown that *H. mangiferae* is the primary vector of *C. mangicola* and *C. mangivora* in Brazil (Ribeiro & Rossetto 1971). A similar vector relationship has also been shown for *C. manginecans* in Oman (Al Adawi et al. 2006, Van Wyk et al. 2007).

The fact that *H. mangiferae* has become associated with three cryptic species of *Ceratocystis* is not surprising. Species in this group of fungi easily establish relationships with insects (Kile 1993, Roux & Wingfield 2009), probably facilitated by the fruity aromas that they produce. The association between *C. mangicola*, *C. mangivora,* and *C. manginecans* and *H. mangiferae* is very similar to emerging new associations between ambrosia beetles and tree pathogens such as those found in Laurel Wilt Disease in the USA (Mayfield et al. 2008

In this study we chose to provide names to species reflected by phylogenetic lineages (*C. mangicola, C. mangivora,* and *C. manginecans*) rather than to treat them as population components of the single species *C. fimbriata*. In doing so we could easily define distinctly different phylogenetic groupings and provide a mechanism to distinguish their differences. Defined clades showing such differences are undoubtedly valuable in studying important diseases, including aspects of host pathogen interaction and resistance. Furthermore, providing separate names for *C. mangicola, C. mangivora,* and *C. manginecans* facilitates quarantine procedures and efforts to curb the global movement of tree pathogens (Wingfield et al. 2001, Slippers et al. 2005).

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Literature cited

Al Adawi AO, Deadman ML, Al Rawahi AK, Al Maqbali YM, Al Jahwari AA, Al Saadi BA, Al Amri IS, Wingfield MJ. (2006) Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman. European Journal of Plant Pathology116: 247–254. http://dx.doi.org/10.1007/s10658-006-9056-x

Atkinson TH, Peck SB. 1993. Annotated checklist of the bark and ambrosia beetles (*Coleoptera: Platypodidae* and *Scolytidae*) of tropical southern Florida. Florida Entomologist 77: 313–329. http://dx.doi.org/10.2307/3496101

Barnes I, Nakabonge G, Roux J, Wingfield BD, Wingfield MJ. (2005) Comparisons of populations of the wilt pathogen *Ceratocystis albifundus* in South Africa and Uganda. Plant Pathology 54: 189–195. http://dx.doi.org/10.1111/j.1365-3059.2005.01144.x

Butani DK. 1993. Mango: pest problems. Periodical Expert Book. Agency D-42 Vivek Vihar: Delhi (India).

Castro R. da Silva 1960. Contribuição ao estudo de *Hypocryphalus mangiferae* (Stebbing, 1914) (*Coleoptera, Scolytidae*). Ciclo biológico e etiologia. Recife. Tese para concurso de professor livre-docente da 9ª cadeira- entomologia e parasitologia- da Escola Superior de Agricultura da Universidade Rural de Pernambuco, 54.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659. http://dx.doi.org/10.1046/j.1365-294x.2000.01020.x

Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14: 733–740.

Piza CdT. (ed.) 1966. Anais do Simposio Sobre a Seca da Mangueira. (Abstract. Review of Applied Mycology 46: 378, 1967

DeVay JE, Lukezic FL, English WH, Trujillo EE. 1963. *Ceratocystis* canker of stone fruit trees. Phytopathology 53: 873.

Engelbrecht CJB, Harrington TC. 2005. Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. Mycologia 97: 57–69. http://dx.doi.org/10.3852/mycologia.97.1.57

Ferreira EM, Harrington TC, Thorpe DJ, Alfenas AC. 2010. Genetic diversity and interfertility among highly differentiated populations of *Ceratocystis frimbriata* in Brazil. Plant Pathology 59: 721–735. http://dx.doi.org/10.1111/j.1365-3059.2010.02275.x

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Halsted BD. 1890. Some fungous diseases of the sweet potato. New Jersey Agricultural College Experiment Station. 76: 1–32.

Jacobs K, Bergdahl DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD. 2004. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycological Research 108: 411–418. http://dx.doi.org/10.1017/S0953756204009748

Kamata N, Esaki K, Kato K, Igeta Y, Wada K. 2002. Potential impact of global warming on deciduous oak dieback caused by ambrosia fungus *Raffaelea* sp. carried by ambrosia beetle *Platypus quercivorus* (*Coleoptera: Platypodidae*) in Japan. Bulletin of Entomological Research 92: 119–126. http://dx.doi.org/10.1079/BER2002158

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. http://dx.doi.org/10.1093/nar/gkf436

Kile GA. 1993. Plant diseases caused by species of *Ceratocystis* sensu stricto and *Chalara*. In: *Ceratocystis* and *Ophiostoma:* taxonomy, ecology and pathogenicity (eds. M.J. Wingfield, K.A. Seifert and J.F. Webber). APS Press: St. Paul (Minnesota). 173–183 pp.

Kostermans AJGH, Bompard JM. 1993. The mangoes. Their botany, nomenclature, horticulture and utilization. Academic Press, New York. 233 pp.

Leslie JF, Plattner RD, Desjardins AE, Klittich CJR. 1992. Fumonisin B1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 82: 341–345. http://dx.doi.org/10.1094/Phyto-82-341

Mayfield AE, Smith JA, Hughes M, Dreaden TJ. 2008. First report of Laurel wilt disease caused by a *Raffaelea* sp. on avocado in Florida. Plant Disease 92: 976. http://dx.doi.org/10.1094/PDIS-92-6-0976A

Medeiros JWA de, Rossetto CJ.1966. Seca-da-mangueira. I Observações preliminares. O Agronomico, Campinas, SP, Brasil. 18:1–11.

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik I. 2000. A multigene phylogeny of the *Giberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience 41: 61–78. http://dx.doi.org/10.1007/BF02464387

Ploetz RC. 2003. Diseases of mango. 327–363, in: RC Ploetz (ed.). Diseases of tropical fruit. CABI Publishing: Wallingford Oxford (UK).

Rayner RW. 1970. A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.

Ribeiro IJA. 1980 Seca da mangueira. Agentes causais e estudo da molestia. 123–130, in: Anais do I Simposio Brasiliero Sobre a Cultura de Mangueira. Sociedade Brasileira de Fruticultura, Jaboticabal, Novembro 24–28, 1980.

Ribeiro IJA, Rossetto CJ. 1971. Seca-da-mangueira. V Isolamento de *Ceratocystis fimbriata* de *Hypocryphalus mangiferae* e freqüência de sintomas iniciais no campo. 607-616, in: Anais do I Cogresso Brasileiro de Fruticultura. Campinas, 12–16 de julho 1971. Vol. 2.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3. Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Rossetto CJ, Ribeiro IJA, Igue T. 1980. Seca-da-mangueira. III. Comportamento de variedades de mangueira, espécies de coleobrocas e comportamento de *Hypocryphalus mangiferae*. Circular 106. Instituto Agronômico de Campinas. 44 p.

Roux J, Wingfield MJ. 2009. *Ceratocystis* species: Emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. Southern Forests 71: 115–120. http://dx.doi.org/10.2989/SF.2009.71.2.5.820

Schedl KE. 1961. *Hypocraphalus mangifera* Stebbing. In: *Scolytidae* und *Platypodidae* Afrikas I. Revista de Entomologia Mocambique 4: 543–544.

Silva JN, Gayão TC, Castro RS. 1959. A morte das mangueiras do Recife (Resultados preliminares do estudo dessa doença). Pernambuco, Instituto Agronômico do Nordeste, Boletim Técnico, 7: 38.

Slippers B, Stenlid J, Wingfield MJ. 2005. Emerging pathogens: fungal host jumps following anthropogenic introduction. Trends in Ecology and Evolution 20: 420–421. http://dx.doi.org/10.1016/j.tree.2005.05.002

Sullivan J. 1996. Combining data with different distributions of among-site variation. Systematic Biology 45: 375–380. http://dx.doi.org/10.1093/sysbio/45.3.375

Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analyses (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599. http://dx.doi.org/10.1093/molbev/msm092

Van Wyk M, Al Adawi AO, Wingfield BD, Al Subhi AM, Deadman ML, Wingfield MJ. 2005. DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman. Australasian Plant Pathology 34: 587–590. http://dx.doi.org/10.1071/AP05080

Van Wyk M, Roux J, Barnes I, Wingfield BD, Wingfield MJ. 2006. Molecular phylogeny of the *Ceratocystis moniliformis* complex and description of *C. tribiliformis* sp. nov. Fungal Diversity 21: 181–201.

Van Wyk M, Al Adawi AO, Khan A, Deadman ML, Al Jahwari AA, Wingfield BD, Ploetz RC, Wingfield MJ. 2007a. *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. Fungal Diversity 27: 213–230. http://dx.doi.org/10.1071/AP07042

Van Wyk M, Pegg G, Lawson S, Wingfield MJ. 2007b. *Ceratocystis atrox* sp. nov associated with *Phoracanthta acanthocera* infestations on *Eucalyptus* in Australia. Australian Journal of Plant Pathology 36: 407–414.

Van Wyk M, Wingfield BD, Clegg PA, Wingfield MJ. 2009a. *Ceratocystis larium* sp. nov., a new species from *Styrax benzoin* wounds associated with incense harvesting in Indonesia. Persoonia 22: 75–82. :10.3767/003158509X439076

Van Wyk M, Wingfield BD, Mohali S, Wingfield MJ. 2009b. *Ceratocystis fimbriatomima*, a new species in the *C. fimbriata sensu lato* complex isolated from *Eucalyptus* trees in Venezuela. Fungal Diversity 34: 173–183.

Van Wyk M, Wingfield BD, Wingfield MJ. 2010. *Ceratocystis* **species** in the *Ceratocystis fimbriata* complex. In: *Ceratophiostoma***.** North Stadbroke Island, Brisbane, Australia, August **2006.** (In press)

Van Wyk M, Wingfield BD, Wingfield MJ. 2011. Four new *Ceratocystis* spp. associated with wounds on *Eucalyptus*, *Schizolobium* and *Terminalia* trees in Ecuador. Fungal Diversity 46:111–131. http://dx.doi.org/10.1007/s13225-010-0051-3

Viegas AP. 1960. Mango blight. Bragantia 19: 163–182 (abstracted in Review of Applied Mycology 42: 696, 1963)

Webster RK, Butler EE. 1967a A morphological and biological concept of the species *Ceratocystis fimbriata*. Canadian Journal of Botany 45: 1457–1468. http://dx.doi.org/10.1139/b67-149

Webster RK, Butler EE. 1967b. The origin of self-sterile, cross-fertile strains and culture sterility in *Ceratocystis fimbriata*. Mycologia 59: 212–221. http://dx.doi.org/10.2307/3756794

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols: A sequencing guide to methods and applications. Academic Press: San Diego (USA).

Wingfield MJ, De Beer C, Visser C, Wingfield BD. 1996. A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. Systematic and Applied Microbiology 19: 191–202.

Wingfield MJ, Slippers B, Roux J, Wingfield BD. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the southern hemisphere. BioScience 51: 134–140. http://dx.doi.org/10.1641/0006-3568(2001)051[0134:WMOEFF]2.0.CO;2

Wood SL. 1982. The bark and ambrosia beetles of North and Central America (*Coleoptera: Scolytidae*), a taxonomic monograph. Great Basin Naturalist, Memoirs 6: 1–1356.

Yamashiro T, Myazaki I. 1985. Principal pests and diseases of mango - *Mangifera indica* L. – in the State of Sao Paulo and updated control methods. Biológico 51:41–50.