

---

***Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan**

---

**Marelize van Wyk<sup>1\*</sup>, Ali O. Al Adawi<sup>2</sup>, Iqrar A. Khan<sup>3</sup>, Michael L. Deadman<sup>4</sup>, Abdullah. A. Al Jahwari<sup>2</sup>, Brenda D. Wingfield<sup>1</sup>, Randy Ploetz<sup>5</sup> and Michael J. Wingfield<sup>1</sup>**

<sup>1</sup>Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

<sup>2</sup>Ghadafan Agriculture Research Station, Ministry of Agriculture and Fisheries, P. O. Box 204 Sohar, 311, Sultanate of Oman

<sup>3</sup>Plant Protection Division, Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad, Pakistan

<sup>4</sup>Department of Crop Sciences, P.O. Box 34, Sultan Qaboos University, Al Khod 123, Sultanate of Oman

<sup>5</sup>Department of Plant Pathology, University of Florida, IFAS Tropical Research and Education, Homestead, FL 33031-3314 USA

Van Wyk, M., Al Adawi, A.O., Khan, I.A., Deadman, M.L., Al Jahwari, A.A., Wingfield, B.D., Ploetz, R. and Wingfield, M.J. (2007). *Ceratocystis manginecans* sp. nov., causal agent of a destructive mango wilt disease in Oman and Pakistan. *Fungal Diversity* 27: 213-230.

A serious wilt disease of mango trees (*Mangifera indica*) has recently appeared in Oman and Pakistan. Symptoms on affected trees resemble those of the mango disease known as “seca” in Brazil and include discolouration of the vascular tissue, gum exudation, galleries of the putative beetle vector of the fungal pathogen, wilting and rapid death. In both countries, the disease has been attributed to *Ceratocystis fimbriata*. This fungus is recognised as a complex of species and *C. fimbriata sensu stricto* is unlikely to be an appropriate name for the causal agent. We, therefore, considered the identity of Pakistan and Oman isolates using comparisons of combined DNA sequence data for partial ITS,  $\beta$ -tubulin and EF-1 $\alpha$  gene regions. These comparisons were supported with morphological characteristics. Results showed that isolates from mango in Pakistan and Oman represent a species, distinct from other species in the *C. fimbriata sensu lato* species complex. The name *Ceratocystis manginecans* sp. nov. is provided for the fungus, which is also shown to be closely related to but distinct from available isolates from mango in Brazil.

**Keywords:** Bark beetles, *Ceratocystis* spp., *Ceratocystis fimbriata*, *Ceratocystis omanensis*, mango diseases, Scolytidae

---

\*Corresponding author: Marelize van Wyk; e-mail: Marelize.vanwyk@fabi.up.ac.za

## Introduction

The genus *Ceratocystis* includes many plant and particularly tree pathogens (Kile, 1993; Upadhyay, 1993). Species of *Ceratocystis* are responsible for a wide range of disease symptoms including staining of the vascular tissue, cankers and wilting. *Ceratocystis fimbriata* Ell. & Halst. was the first species to be described in the genus, after it was found associated with black rot of sweet potato (Halsted and Fairchild, 1891). This fungus has subsequently been reported from six continents on a great number of hosts and with varying levels of pathogenicity.

The variability of isolates representing *C. fimbriata*, its wide host range and its extensive geographic distribution gave rise to the view that the fungus might represent a species complex (Webster and Butler, 1967a, b). More recently, DNA-based techniques have made it possible to recognise distinct taxa that might otherwise have been assigned to *C. fimbriata*. The first example emerged with the description of *C. albifundus* M.J. Wingf., De Beer & Morris, a fungus causing a serious wilt disease of *Acacia mearnsii* de Wildt. in South Africa, which had initially been identified as *C. fimbriata* (Morris *et al.*, 1993; Wingfield *et al.*, 1996). Likewise, *C. pirilliformis* Barnes & M.J. Wingf. from *Eucalyptus* in Australia (Barnes *et al.*, 2003) represents a cryptic species described in this complex. Recently, Engelbrecht and Harrington (2005) provided a re-description of *C. fimbriata sensu stricto* (*s.s.*) and defined this fungus as specifically representing the causal agent of sweet potato (*Ipomoea batatas* L.) black rot. Names have also been applied to isolates representing other cryptic species residing in various monophyletic lineages in the *C. fimbriata sensu lato* (*s.l.*) species complex (Van Wyk *et al.*, 2004; Engelbrecht and Harrington, 2005; Johnson *et al.*, 2005; Van Wyk *et al.*, 2007).

*Ceratocystis fimbriata* is known to be an important pathogen of mango in Brazil (Ploetz, 2003). The fungus was first recorded on these trees in the 1930s, where it caused a serious die-back disease known as “seca” or “murcha” disease (Ploetz, 2003). More recently, Silveira *et al.* (2007) associated *C. fimbriata* with mango branch blight in Rio de Janeiro State, where it also occurred on sugar apple (*Annona squamosa* L.), and suggested that the disease is endemic in the area. The disease of mango in Brazil is closely associated with infestations of the wood-boring scolytid *Hypocryphalus mangifera* Stebbing (Coleoptera: Scolytidae), which is also thought to spread the pathogen (Ribeiro, 1980; Yamashiro and Myazaki, 1985; Ploetz, 2003).

Severely diseased mango trees were observed in Oman early in 1998. Symptoms on these trees included dark staining of the affected wood and insect galleries, exudation of gum, leaf wilting and ultimately tree death (Al Adawi *et*

*al.*, 2003). Two *Ceratocystis* spp. were isolated from these trees. One of these is related to *C. moniliformis* Hedgec. and it was subsequently described as *C. omanensis* Al Subhi, M.J. Wingf. M. van Wyk & Deadman (Al Subhi *et al.*, 2006). The other fungus was morphologically similar to *C. fimbriata s.l.* and a detailed study incorporating morphological characteristics and DNA sequence comparisons, confirmed that it belonged to this species complex (Van Wyk *et al.*, 2005). Al Adawi *et al.* (2006) provided details of the disease in Oman including proof of pathogenicity of *C. fimbriata s.l.* and they also showed that *H. mangifera*, the same scolytid beetle associated with the fungus in Brazil, is closely linked to the disease in Oman.

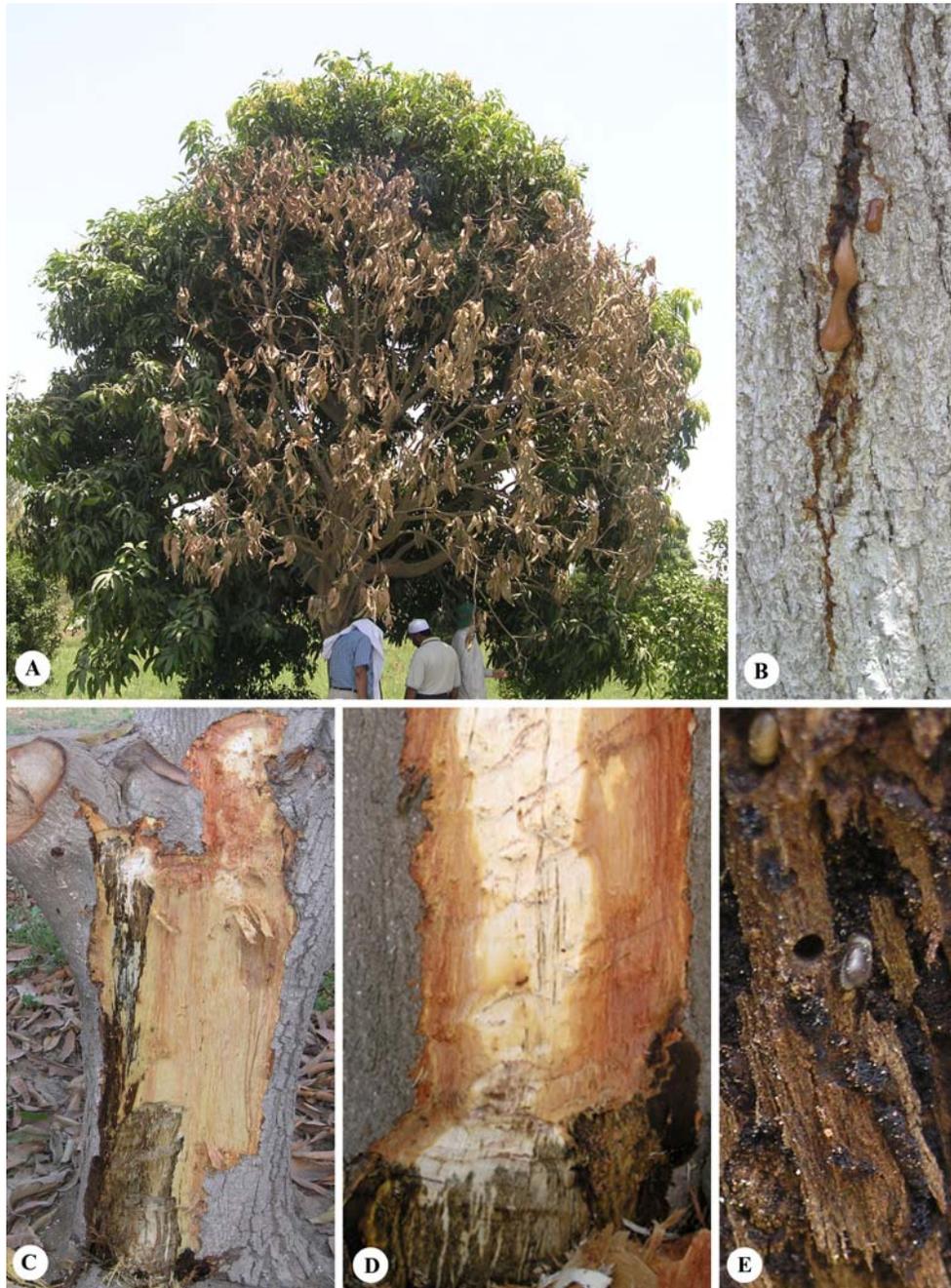
A wilt disease of mango similar to that observed in Oman was reported from Pakistan in 2005 (Malik *et al.*, 2005). Although little detailed evidence was provided to support this view, it was suggested that the disease is caused by *C. fimbriata*. Symptoms observed in Pakistan included gum exudation from the stems, vascular discolouration, branch death and holes in the bark as a result of insect infestation. The bark beetle *H. mangifera* (Florida Department of Agriculture, identification reference E2006-7581-701) was also found associated with this disease (Al Adawi, unpublished).

The pathogen responsible for mango wilt disease in Oman has been identified as *C. fimbriata s.l.*, which resides in the broad phylogenetic assemblage that includes *C. fimbriata s.s.*, various other cryptic species that have been provided with names, and a relatively large number that await taxonomic treatment (Van Wyk *et al.*, 2005). The aim of this study was to provide an appropriate taxonomic placement for the mango pathogen in Oman and Pakistan, based on isolates from mango trees and *H. mangifera* in both countries.

## Materials and methods

### *Isolates*

Isolates from mango in Oman used in this study were the same as those used in previous studies by Van Wyk *et al.* (2005). In the case of Pakistan, several mango farms in Faisalabad and Multan were visited in June 2005 and May 2006 to examine symptoms of mango decline disease. Symptoms on the trees (Fig. 1) were generally the same as those described from Oman (Van Wyk *et al.*, 2005), including rapid wilting of the leaves on infected parts of the trees, gum exuding from cracks in the bark, a streaked pattern of vascular discoloration and infestation by *H. mangifera*.



**Figure 1.** Wilting and internal symptoms observed on mango trees in Pakistan. **A.** Tree displaying rapid wilt in part of the crown. **B.** Gum exuding from a crack in the bark of an infected stem. **C, D.** Stem lesions showing typically streaked vascular discoloration. **E.** Entrance hole of *H. mangifera* and adult insect on infected stem.

In both regions, longitudinal cuts were made on 20 grafted mango trees affected by the disease, at the junction of the rootstock with the scion. This was done to determine whether vascular discolouration was present on the scion and/or rootstock. Forty samples including discoloured xylem were collected from diseased mango trees in Pakistan. Scolytid beetles found under the bark of affected trees were also collected and sent to the Division of Plant Industry, Florida Department of Agriculture & Consumer Services, Department of Agriculture (Gainesville, FL. USA) for identification.

Primary isolations from samples with vascular discolouration were made by incubating pieces of wood in moist chambers or, when fungal structures were not observed on the surface of the wood, by placing them between two slices of carrot (Moller and De Vay, 1968). Insects collected from under the bark of diseased trees were crushed and placed between carrot slices and incubated at room temperature for seven to 10 days. Ascospore masses from the apices of ascomata developing on infected wood or on carrot slices were transferred to 2% malt extract agar (MEA) (20 g/L) (Biolab, Midrand, South Africa) and maintained at room temperature (~25°C). These cultures were purified by means of single spores. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Representative cultures were dried on 30% glycerol and have been deposited with the National Herbarium of South Africa (PREM).

### *Phylogeny*

Four isolates (CMW 23628, CMW 23634, CMW 23641 & CMW 23643) from Pakistan and three isolates (CMW 13851, CMW 13852 & CMW 13854) from Oman were selected for phylogenetic studies. The isolates from Oman were those used in a previous study (Van Wyk *et al.*, 2005). DNA from the seven isolates was extracted as described by Van Wyk *et al.* (2007). Polymerase Chain Reactions for three gene regions, the Internal Transcription Spacer (ITS) region including the 5.8S rDNA operon (White *et al.*, 1990), Beta-tubulin ( $\beta$ t) (Glass and Donaldson, 1995) and Transcription Elongation Factor-1 alpha (EF-1 $\alpha$ ) (Jacobs *et al.*, 2004) were performed. The PCR conditions were as described by Van Wyk *et al.* (2007). The amplified products were purified using 6% Sephadex G-50 columns (Steinheim, Germany). PCR amplicons were 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 Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California). Sequence data for closely related *Ceratocystis* spp. were obtained from GenBank for comparative purposes. Sequences were aligned using MAFFT (<http://timpani.genome.ad.jp/%7emafft/server/>) (Katoh *et al.*, 2002) and confirmed manually. The dataset was analysed using PAUP version 4.0b10\* (Swofford, 2002). A partition homogeneity test (Swofford, 2002) was used to determine whether the three datasets could be combined. Gaps were treated as a fifth character and trees were obtained via stepwise addition of 1000 replicates with the Mulpar option in effect. The heuristic search option based on parsimony with stepwise addition was used to obtain the phylogram. Confidence intervals using 1000 bootstrap replicates were calculated. *Ceratocystis virescens* (Davidson) Moreau was designated as the out-group taxon due to its close relatedness, but with distinct differences to this group of fungi. All sequences derived from this study were deposited in GenBank (Table 1).

Phylogenetic trees based on Bayesian probabilities using a Markov Chain Monte Carlo (MCMC) algorithm were generated using MrBayes version 3.1.1 (Ronquist and Huelsenbeck, 2003). For each gene, a model of nucleotide substitution was determined using Mrmodeltest (Nylander, 2004) and these were included for each gene partition in MrBayes. One million random trees were generated using the MCMC procedure with four chains and sampled every 100<sup>th</sup> generation. To avoid including trees that were sampled before convergence, tree likelihood scores were assessed to determine the number of trees that were formed before the stabilization. Trees outside the point of convergence were discarded by means of the burnin procedure in MrBayes.

### ***Morphology***

Two isolates, one from Oman and the other from Pakistan, (CMW 23641 and CMW 13854 respectively) were selected for growth studies in culture. These isolates were grown for 14 d on 2% MEA, after which a 5mm plug was transferred to the centres of 90mm Petri dishes containing 2% MEA. Five replicates of each isolate were used for each of seven different incubation temperatures ranging from 5°C to 35°C at five degree intervals. The plates were incubated in the dark for seven days, after which two measurements of colony diameter were made at right angles to each other and averages were computed for each temperature. Colour designations were made for the cultures using the colour charts of Rayner (1970).

**Table 1.** *Ceratocystis* spp. for which isolates or sequences were used in this study<sup>a</sup>.

Species	Isolate no.	GenBank Accession no.	Host	Geographical origin
<i>C. albifundus</i>	CMW 4068	DQ520638 EF070429 EF070400	<i>Acacia mearnsii</i>	RSA
<i>C. albifundus</i>	CMW 5329	AF388947 DQ371649 EF070401	<i>A. mearnsii</i>	Uganda
<i>C. atrox</i>	CMW 19383 CBS 120517	EF070414 EF070430 EF070402	<i>Eucalyptus grandis</i>	Australia
<i>C. atrox</i>	CMW 19385 CBS 120518	EF070415 EF070431 EF070403	<i>E. grandis</i>	Australia
<i>C. cacaofunesta</i>	CMW 15051 CBS 152.62	DQ520636 EF070427 EF070398	<i>Theobroma cacao</i>	Costa Rica
<i>C. cacaofunesta</i>	CMW 14809 CBS 115169	DQ520637 EF070428 EF070399	<i>T. cacao</i>	Ecuador
<i>C. caraye</i>	CMW 14793 CBS 114716	EF070424 EF070439 EF070412	<i>Carya cordiformis</i>	USA
<i>C. caraye</i>	CMW 14808 CBS 115168	EF070423 EF070440 EF070411	<i>C. ovata</i>	USA
<i>C. fimbriata s.s</i>	CMW 15049 CBS 141.37	DQ520629 EF070442 EF070394	<i>Ipomoea batatas</i>	USA
<i>C. fimbriata s.s</i>	CMW 1547	AF264904 EF070443 EF070395	<i>I. batatas</i>	Papua New Guinea
<i>C. fimbriata s.l</i>	CMW 14797 CBS 114721	AY953382 EF433307 EF433316	<i>Mangifera indica</i>	Brazil
<i>C. fimbriata s.l</i>	CMW 15052 CBS 600.70	EF433298 EF433306 EF433315	<i>M. indica</i>	Brazil
<i>C. manginecans</i>	CMW 13851	AY953383 EF433308 EF433317	<i>M. indica</i>	Oman
<i>C. manginecans</i>	CMW 13852	AY953384 EF433309 EF433318	<i>Hypocryphalus mangifera</i>	Oman

**Table 1 (continued).** *Ceratocystis* spp. for which isolates or sequences were used in this study<sup>a</sup>.

Species	Isolate no.	GenBank Accession no.	Host	Geographical origin
<i>C. manginecans</i>	CMW 13854	AY953385 EF433310 EF433319	<i>M. indica</i>	Oman
<i>C. manginecans</i>	CMW 23634	EF433302 EF433311 EF433320	<i>H. mangifera</i>	Pakistan
<i>C. manginecans</i>	CMW 23628	EF433303 EF433312 EF433321	<i>H. mangifera</i>	Pakistan
<i>C. manginecans</i>	CMW 23643	EF433304 EF433313 EF433322	<i>M. indica</i>	Pakistan
<i>C. manginecans</i>	CMW 23641	EF433305 EF433314 EF433323	<i>M. indica</i>	Pakistan
<i>C. pirilliformis</i>	CMW 6569	AF427104 DQ371652 AY528982	<i>E. nitens</i>	Australia
<i>C. pirilliformis</i>	CMW 6579	AF427105 DQ371653 AY528983	<i>E. nitens</i>	Australia
<i>C. platani</i>	CMW 14802 CBS 115162	DQ520630 EF070425 EF070396	<i>Platanus occidentalis</i>	USA
<i>C. platani</i>	CMW23918	EF070426 EF070397	<i>Platanus</i> sp.	Greece
<i>C. polychroma</i>	CMW 11424	AY528970 AY528966 AY528978	<i>Syzygium aromaticum</i>	Indonesia
<i>C. polychroma</i>	CMW 11436	AY528971 AY528967 AY528979	<i>S. aromaticum</i>	Indonesia
<i>C. populicola</i>	CMW 14789 CBS 119.78	EF070418 EF070434 EF070406	<i>Populus</i> sp.	Poland
<i>C. populicola</i>	CMW 14819 CBS 114725	EF070419 EF070435 EF070407	<i>Populus</i> sp.	USA
<i>C. smalleyi</i>	CMW 14800 CBS 114724	EF070420 EF070436 EF070408	<i>C. cordiformis</i>	USA

**Table 1 (continued).** *Ceratocystis* spp. for which isolates or sequences were used in this study<sup>a</sup>.

Species	Isolate no.	GenBank Accession no.	Host	Geographical origin
<i>C. variospora</i>	CMW 20935	EF070421	<i>Quercus alba</i>	USA
	CBS 114715	EF070437		
		EF070409		
<i>C. variospora</i>	CMW 20936	EF070422	<i>Q. robur</i>	USA
	CBS 114714	EF070438		
		EF070410		
<i>C. virescens</i>	CMW 11164	DQ520639	<i>Fagus americanum</i>	USA
<i>C. virescens</i>	CMW 3276	EF070441	<i>Quercus</i> sp.	USA
		EF070413		
		AY528984		
		AY528990		
		AY528991		

<sup>a</sup> / Isolates of *C. manginecans* were sequenced in this study. Other sequences for phylogenetic comparison were obtained from GenBank.

Morphological characteristics were assessed using 10-d-old cultures and structures were mounted in lactophenol on glass slides. Fifty measurements of each relevant taxonomic structure were made for a single isolate. Ten additional measurements of the relevant structures were made from two other randomly selected isolates. Averages (mean), standard deviation (stdv) and minimum (min) and maximum (max) measurements are presented for each structure as follows: (min-) mean minus stdv – mean plus stdv (-max).

## Results

### *Isolates*

Isolations from symptomatic xylem tissue from 40 mango trees, in Pakistan, yielded 43 isolates. Ten of these isolates were retrieved from adult *H. mangifera* beetles. Cultures generally resembled those of *Ceratocystis* spp. in the *C. fimbriata* s.l. species complex, having characteristic dark green colonies and the typical banana fruit odour.

### *Phylogeny*

A partition homogeneity test on the three DNA sequence data sets showed that they could be combined (P = 0.01) (Cunningham, 1997, Barker and Lutzoni, 2002). The combined dataset of sequences for the three gene regions

(1978 characters, 1176 were constant, 64 are parsimony uninformative) produced four most parsimonious trees of which one is presented (Fig. 2). The tree had a length of 1556 steps, a consistency index of 0.7333, a retention index of 0.8564 and a rescaled consistency of 0.6280. All the isolates from mango in Oman and Pakistan resided in a tightly nested clade (bootstrap = 88%), separate from other species in the *C. fimbriata s.l.* species complex. The isolates from mango in Pakistan and Oman were phylogenetically most closely related to each other. The isolates from Brazil formed a group (bootstrap = 66%) separate from the other mango isolates. The two clades incorporating the mango isolates were also separate from other species in the *C. fimbriata s.l.* species complex. Other than the isolates from Brazil, the species most closely related to those from Pakistan and Oman and Brazil were *C. fimbriata s.s.*, *C. cacaofunesta* Engelbrecht and Harrington and *C. platani* Engelbrecht and Harrington. The posterior probability of the branch nodes of the combined tree generated using Bayesian inference supported the bootstrap values obtained with PAUP. Eight thousand trees were discarded due to the fact that they were outside of the point of convergence.

### ***Morphology***

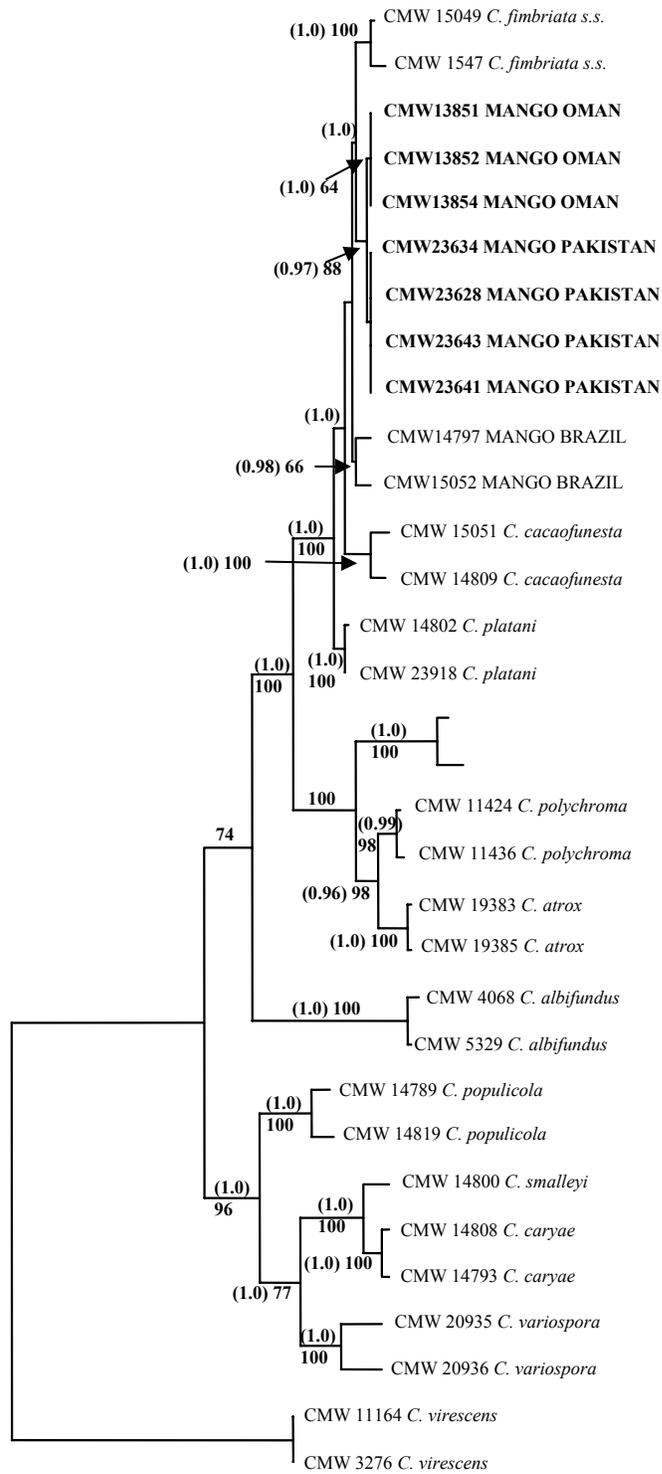
Isolates CMW 13854 and CMW 23634 from Oman and Pakistan, respectively, displayed similar growth patterns in culture and grew optimally between 20-25°C. No growth was observed at 5°C, 10°C and 35°C. After 7 days, both cultures reached an average of 27mm and 29 mm at 15°C and 30°C, respectively. At 20°C both isolates had reached an average of 43 mm and at 25°C an average diameter of 45 mm was reached.

Isolates of the *Ceratocystis* sp. from Pakistan and Oman were morphologically indistinguishable. The fungus could be easily distinguished from *C. fimbriata s.s.* by the production of both secondary and primary conidiophores and both cylindrical and barrel-shaped conidia, respectively. *Ceratocystis fimbriata s.s.* does not produce secondary conidiophores that produce barrel-shaped conidia. The *Ceratocystis* sp. from Pakistan and Oman is morphologically similar to *C. platani*. In this respect, both fungi produce primary and secondary conidiophores and the two conidial forms. They could,

---

**Figure 2.** A phylogenetic tree based on analysis of sequence data for the combined regions of the ITS,  $\beta$ -tubulin and EF1- $\alpha$  for *C. manginecans* and other species in the *C. fimbriata s.l.* species complex. The phylogram was obtained using the heuristic search option based on parsimony with *C. virescens* as the out-group. Bootstrap values are indicated in bold while Bayesian support is indicated in brackets.

# Fungal Diversity



— 10 changes

however, be distinguished from each other by the fact that *C. platani* has longer ascomatal necks (535-835  $\mu\text{m}$  vs 557-635  $\mu\text{m}$ ), longer ostiolar hyphae (20-90  $\mu\text{m}$  vs 45-59  $\mu\text{m}$ ) and both the conidiophore forms are shorter than those in isolates from mango in Pakistan and Oman (24-90  $\mu\text{m}$  vs 81-109  $\mu\text{m}$  and 35-50  $\mu\text{m}$  vs 65-77  $\mu\text{m}$ ).

### ***Taxonomy***

The *Ceratocystis* sp. from mango in Oman and Pakistan resides in a phylogenetic group, distinct from other species in the *C. fimbriata* species complex. Although all of these fungi are morphologically very similar, the mango taxon from Oman and Pakistan can be distinguished from its closest phylogenetic relatives based on morphological characteristics. The fungus is consequently described as a new taxon as follows.

***Ceratocystis manginecans* M. van Wyk, A Al Adawi & M.J. Wingf. sp. nov.**  
(Fig. 3a-g)

MycoBank: 510851

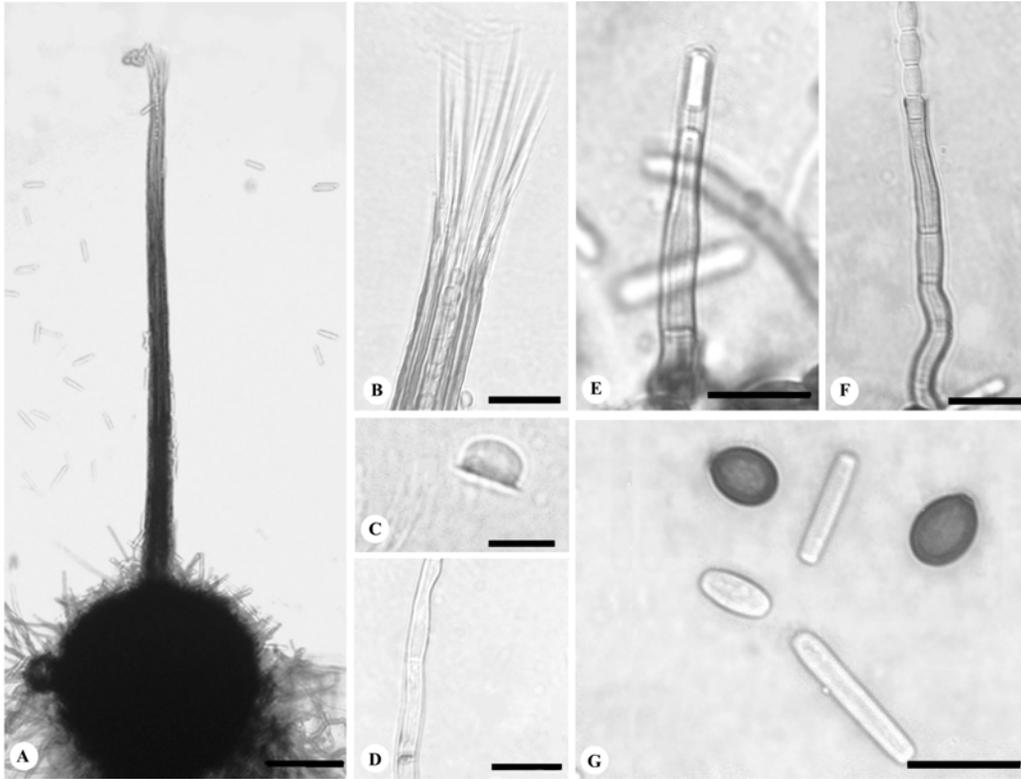
*Etymology*: The name of this fungus is derived from the Latin word *neco* “to kill, slay or put to death”, which refers to the fact the fungus is responsible for a serious disease of mango trees.

*Hyphae* laeves segmentatae. *Bases* ascomatum nigrae, globosae. *Colla* ascomatum basi atro-brunnea, apicem versus pallescentia. *Hyphae ostiolaris* divergentes, hyalinae. *Ascospores* in massa globosa alba vel fulva in apicibus collorum ascomatum crescunt; lateraliter visa cucullatae, non septatae, hyalinae.

*Anamorph* *Thielaviopsis*: conidiophorae bifformes, primariae hyalinae, secundariae hyalinae. Evolutio *conidiorum* phialidicorum per faciendum parietum annularium; conidia biformia singula vel catenata. *Conidia primaria*; *secundaria*. *Chlamydo-spores* coffeinae vel umbrinae, globosae vel subglobosae.

*Colonies* grayish olive (21"") in colour on 2% MEA. *Odour* banana. *Hyphae* smooth and segmented. *Ascomatal bases* globose, black, (153-)192-254(-281)  $\mu\text{m}$  in diam. *Ascomatal necks* dark brown becoming lighter towards apices (514-)557-635(-673)  $\mu\text{m}$  long, (25-)32-42(-48)  $\mu\text{m}$  wide at base, (14-)16-22(-26)  $\mu\text{m}$  wide at tip. *Ostiolar hyphae* hyaline, divergent, (42-)45-59(-69)  $\mu\text{m}$  long. *Asci* evanescent, not seen. *Ascospores* hyaline, hat-shaped, 3-4  $\mu\text{m}$  in length, 4-5  $\mu\text{m}$  wide excluding sheath, 7-8  $\mu\text{m}$  wide including sheath.

*Thielaviopsis anamorph*: *Conidiophores* of two morphological forms. *Primary conidiophores* phialidic, lageniform, hyaline, (72-)81-109(-144)  $\mu\text{m}$  long, 5-7(-9)  $\mu\text{m}$  wide at bases, 6-8(-9)  $\mu\text{m}$  wide at broadest point, 3-6  $\mu\text{m}$  wide at tips. *Secondary conidiophores*, tube-like, flaring at mouths, short, hyaline, (59-)65-77(-84)  $\mu\text{m}$  long, 5-8  $\mu\text{m}$  wide at bases and (5-)6-8  $\mu\text{m}$  wide at tips. *Conidia* of two types. *Primary conidia*, hyaline, cylindrical, (15-)23-29(-33)  $\mu\text{m}$  in length, 3-6  $\mu\text{m}$  wide. *Secondary conidia*, hyaline, barrel-shaped, (8-)9-



**Figure 3.** Morphological characteristics of *Ceratocystis manginecans* (from holotype). **A.** Globose ascoma. **B.** Divergent ostiolar hyphae. **C.** Hat-shaped ascospores. **D.** Segmented hypha. **E.** Primary phialidic conidiophore with emerging cylindrical conidia. **F.** Secondary conidiophore with emerging chain of barrel-shaped conidia. **G.** Dematiaceous chlamydospores, cylindrical- and barrel shaped conidia. Scale bars: A = 100  $\mu\text{m}$ , B = 20  $\mu\text{m}$ , C = 5  $\mu\text{m}$ , D = 20  $\mu\text{m}$ , E = 20  $\mu\text{m}$ , F = 20  $\mu\text{m}$ , G = 20  $\mu\text{m}$ .

11(-12)  $\mu\text{m}$  in length, 5-7(-8)  $\mu\text{m}$  wide. *Chlamydospores* brown, thick-walled, globose to sub-globose, (11-)12-14  $\mu\text{m}$  in length by 9-11(-12)  $\mu\text{m}$  wide.

*Specimens examined:* OMAN, from diseased *Mangifera indica* trees, isolated *A. O. Al Adawi*, **holotype** Herb. PREM 59612; culture ex-type CMW 13851 = CBS 121659, 2002. OMAN, from diseased *Mangifera indica* trees, isolated *A. O. Al Adawi*, **paratype** PREM 59613; culture ex-paratype CMW 13852 = CBS 121660, 2002. PAKISTAN, from bark beetle, isolated *A. O. Al Adawi*, **paratype** PREM 59614; culture ex-paratype CMW 23634 = CBS 121661, 2002.

## Discussion

*Ceratocystis manginecans* described in this study is one of a growing number of cryptic species, which in the past would have been assigned to the

well-known plant pathogen, *C. fimbriata*. These cryptic species have only become obvious in recent years and subsequent to the ability to distinguish them based on phylogenetic inference. Results of this study have shown that the newly described *C. manginecans* from mango in Oman and Pakistan, can easily be distinguished from *C. fimbriata s.s.* and other species in the *C. fimbriata s.l.* species complex, using DNA sequence comparisons. Although most of these species are morphologically very similar, accounting for the fact that they were treated as a single taxon in the past, *C. manginecans* can also be distinguished from its closest phylogenetic relatives based on morphological characteristics.

Phylogenetic comparisons using sequence data for three gene regions were used in this study, to distinguish between *C. manginecans* and other species in the *C. fimbriata s.l.* species complex. In this respect, it is possible to distinguish the fungus based on sequences of the ITS region of the rDNA operon, and various other species in the group have been recognised at this level (Wingfield *et al.*, 1996; Barnes *et al.*, 2003; Engelbrecht and Harrington, 2005). However, there are many situations where single gene regions provide insufficient data to distinguish species, as is for example evident for most species in the *C. moniliformis s.l.* species complex (Van Wyk *et al.*, 2006; Al Subhi *et al.*, 2006). The fact that sequence comparisons for three gene regions distinguish *C. manginecans* from its closest relatives provides confidence that this is a unique taxon.

*Ceratocystis manginecans* is the cause of a disease that seriously threatens the mango industry of Oman (Al Adawi *et al.*, 2006). The disease has resulted in the death of a great number of trees in that country and it has recently been recognised as a serious constraint to mango cultivation in Pakistan (Malik *et al.*, 2005). Accurate recognition of the pathogen responsible for this disease will hopefully contribute to measures that will reduce its impact. Certainly, recognizing that *C. manginecans* differs from *C. fimbriata s.s.*, will promote and improve understanding of the disease and its possible origin.

The serious mango disease caused by the fungus that has been referred to as *C. fimbriata s.l.* in Brazil, has symptoms very similar to those observed in Oman and Pakistan (Viegas, 1960; Ribeiro, 1980; Ploetz, 2003; Al Adawi *et al.*, 2006). Our analyses suggest that the disease in Brazil is not caused by *C. fimbriata s.s.* nor by *C. manginecans*, but rather by another cryptic species in this complex. The morphology of the Brazilian isolates used in this study is distinct from *C. manginecans* (authors, unpublished data) but since we examined only two isolates, we have chosen not to provide a name.”

Inoculation tests with *C. manginecans* in Oman have shown that the fungus is a virulent pathogen, able to kill mango trees rapidly (Al Adawi *et al.*, 2006). Although we are not aware of similar tests in Pakistan, it is likely that

the same will be true in that country. The fungus represents a serious threat to the mango growing communities in these countries, many of which are dependant on this crop for sustainability.

The origin of *C. manginecans* in Oman and Pakistan is unknown. It has, however, been suggested that it might have been introduced into Oman, originally from South America (Van Wyk *et al.*, 2005). *Ceratocystis manginecans* could easily have been introduced on soil associated with this material as this fungus is well-adapted to a soil-borne habitat due to the presence of the soil surviving structures the chlamydospores that are present in this fungus. There have been anecdotal suggestions that a producer from Pakistan imported planting stock from Brazil and that this was also established at a production site in Oman. This could explain the original outbreak in Oman and then only later in Pakistan.

An intriguing similarity between the mango disease caused by *C. manginecans* in Oman and Pakistan and the disease in Brazil, is that the scolytid beetle, *H. mangifera*, is associated with the disease in all three areas (Al Adawi *et al.*, 2006; Van Wyk *et al.*, 2006; Ploetz, 2003). *Ceratocystis manginecans* can easily be isolated from this insect in both Oman and Pakistan as has been found in the present and previous studies (Al Adawi *et al.*, 2006; Van Wyk *et al.*, 2006). In Oman and Pakistan, trees are commonly found with insect probing damage, before the onset of infection. In this respect, the insect appears to play an important role in disease spread and development. This has also been suggested for the disease in Brazil (Ribeiro, 1980), although our study suggests that the fungus in Brazil represents a species different to *C. manginecans*.

*Hypocryphalus mangifera* is thought to be native to southern Asia, in areas such as India (Wood, 1982; Butani, 1993; Atkinson and Peck, 1994) and Pakistan where mango is also native (Wood, 1982; Mukherjee, 1997). If this is true, the insect would have been introduced into Brazil. It is intriguing to speculate that *C. manginecans* could have been introduced into Oman and Pakistan, possibly from Brazil, after which it would have established a relationship with an insect, native to the area. In Brazil, the situation would then be opposite, with the insect vector being introduced and having become associated with a native fungus, similar to but distinct from *C. manginecans*. Studies to determine the origin of *C. manginecans* are planned and should help to resolve these questions.

The mango disease caused by *C. manginecans* seriously threatens the future of mango cultivation in Oman and Pakistan. It could also threaten mango cultivation in other parts of that region such as India, which is the world's most important producer. Important sources of mango germplasm (monoembryonic

*M. indica* evolved in India and polyembryonic *M. indica* in Southeast Asia), and other species of *Mangifera*, which are most diverse in Southeast Asia could also be threatened. Every effort must thus be made to prevent the introduction of *C. manginecans* and its *H. mangifera* vector into new environments. Understanding its mode of spread and promoting quarantine procedures to limit its movement should be a high priority.

### Acknowledgements

We are grateful to the Sultan Qaboos University, and Ministry of Agriculture and Fisheries, Sultanate of Oman for providing funds to support this study. We also acknowledge the University of Pretoria and the National Research Foundation (NRF) South Africa for financial support. The Pakistan government is acknowledged for providing support to undertake sampling of trees in that country. We further thank Dr. Hugh Glen who suggested a name for *C. manginecans* and provided the Latin diagnosis and Dr. Martin Coetzee for advice regarding analysis of DNA sequence data.

### References

- Al Adawi, A.O., Deadman, M.L., Al Rawahi, A.K., Khan, A.J. and Al Maqbali, Y.M. (2003). *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. *Plant Pathology* 52: 419.
- Al Adawi, A.O., Deadman, M.L., Al Rawahi, A.K., Al Maqbali, Y.M., Al Jahwari, A.A., Al Saadi, B.A., Al Amri, I.S. and Wingfield, M.J. (2006). Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman. *European Journal of Plant Pathology* 116: 247-254.
- Al Subhi, A.M., Al Adawi, A.O., Van Wyk, M., Deadman, M.L. and Wingfield, M.J. (2006) *Ceratocystis omanensis*, a new species from diseased mango trees in Oman. *Mycological Research* 110: 237-245.
- Atkinson, T.H. and Peck, S.B. (1994). Annotated checklist of the bark and ambrosia beetles (Coleoptera: Platypodidae and Scolytidae) of tropical Southern Florida. *Florida Entomologist Online* 77: 313-329.
- Barker, F.K. and Lutzoni, F.M. (2002). The utility of the incongruence length difference test. *Systematic Biology* 51: 625-637.
- Barnes, I., Roux, J., Wingfield, M.J., Old, K.M. and Dudzinski, M. (2003). *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. *Mycologia* 95: 865-871.
- Butani, D.K. (1993). *Mango: Pest Problems*. Periodical Expert Book. Agency D-42, Vivek Vihar, Delhi, India.
- Cunningham, C.W. (1997). Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733-740.
- Engelbrecht, C.J. and Harrington, T.C. (2005). Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* on sweet potato, cacao and sycamore. *Mycologia* 97: 57-69.
- Glass, N.L. and Donaldson, G.C. (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, B.D. and Fairchild, D.G. (1891). Sweet-potato black rot. *Journal of Mycology* 7: 1-11.

- Jacobs, K., Bergdahl, D.R., Wingfield, M.J., Halik, S., Seifert, K.A., Bright, D.E. and Wingfield, B.D. (2004). *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research* 108: 411-418.
- Johnson J.A., Harrington T.C. and Engelbrecht C.J.B. (2005). Phylogeny and taxonomy of the North American clade of the *Ceratocystis fimbriata* complex. *Mycologia* 97: 1067-1092.
- Katoh, K., Misawa, K., Kuma, K. and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066.
- Kile, G.A. (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.
- Malik, M.T., Khan, S.M., Dasti, A.A. and Kazmi, M.R. (2005). First record of *Ceratocystis fimbriata*, causal organism of mango sudden death in Pakistan. *Pakistan Journal of Phytopathology* 17: 187-191.
- Moller, W. and DeVay, J. (1968). Insect transmission of *Ceratocystis fimbriata* in deciduous fruit orchards. *Phytopathology* 58: 1499-1508.
- Morris, M.J., Wingfield, M.J. and De Beer, C. (1993). Gummosis and wilt of *Acacia mearnsii* in South Africa caused *Ceratocystis fimbriata*. *Plant Pathology* 42: 814-817.
- Mukherjee, S.K. (1997). Introduction: Botany and Importance. In: *The Mango: Botany, Production and Uses* (ed. R.E. Litz). CAB International, Wallingford, UK: 1-19.
- Nylander, J.A.A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Ploetz, R.C. (2003). Diseases of mango. In: *Diseases of Tropical Fruit Crops* (ed. R.C. Ploetz). CABI Publishing, Wallingford, Oxford: 327-363.
- Rayner, R.W. (1970). A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.
- Ribeiro, I.J.A. (1980). Seca de mangueira. Agentes causais e studio da molesta. In: *Anais do I Simposio Brasileiro Sobre a Cultura de Manguera*. Sociedade Brasileira de Fruticultura, Jacoticobal, November 24-28, 1980, 123-130.
- Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.
- Silveira, S.F., Harrington, T.C., Mussi-Dias, V., Engelbrecht, C.J.B., Alfenas, A.C. and Silva, C.R. (2006). *Annona squamosa*, a new host of *Ceratocystis fimbriata*. *Fitopatologia Brasileira* 31: 394-397.
- Swofford, D.L. (2002). PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Upadhyay, H.P. (1993). Classification of the ophiostomatoid fungi. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity* (eds. M.J. Wingfield, K.A. Seifert and J.F. Webber). APS Press, St. Paul, Minnesota: 269-287.
- Van Wyk, M., Al Adawi, A.O., Wingfield, B.D., Al Subhi, A.M., Deadman, M.L. and Wingfield, M.J. (2005). DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman. *Australasian Plant Pathology* 34: 587-590.
- Van Wyk, M., Pegg, G., Lawson, S. and Wingfield, M.J. (2007). *Ceratocystis atrox* sp. nov. associated with *Phoracantha acanthocera* infestations on *Eucalyptus* in Australia. *Australasian Plant Pathology* 36: 407-414.
- Van Wyk, M., Roux, J., Barnes, I., Wingfield, B.D., Liew, E.C.Y., Assa, B., Summerell, B.A. and Wingfield, M.J. (2004). *Ceratocystis polychroma* sp. nov., a new species from *Syzygium aromaticum* in Sulawesi. *Studies in Mycology* 50: 273-282.

- Van Wyk, M., Roux, J., Barnes, I., Wingfield, B.D. and Wingfield, M.J. (2006). Molecular phylogeny of the *Ceratocystis moniliformis* complex and description of *C. tribiliformis* sp. nov. *Fungal Diversity* 21: 181-201.
- Viegas, A.P. (1960). Mango blight. *Bragantia* 19: 163-182 (abstracted in *Review of Applied Mycology* 42: 696, 1963)
- Webster, R.K. and Butler, E.E. (1967a). A morphological and biological concept of the species *Ceratocystis fimbriata*. *Canadian Journal of Botany* 45: 1457-1468.
- Webster, R.K. and Butler, E.E. (1967b). The origin of self-sterile, cross-fertile strains and culture sterility in *Ceratocystis fimbriata*. *Mycologia* 59: 212-221.
- White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A sequencing guide to methods and applications*. (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White) Academic Press, San Diego: 315-322.
- Wingfield, M.J., De Beer, C., Visser, C. and Wingfield, B.D. (1996). A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. *Systematic and Applied Microbiology* 19: 191-202.
- Wood, S.L. (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. and 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.

(Received 28 May 2007; accepted 19 July 2007)