Ceratocystis fimbriatomima, a new species in the *C. fimbriata sensu lato* complex isolated from *Eucalyptus* trees in Venezuela

Van Wyk, M.[1*], Wingfield, B.D.[1], Mohali, S.[2] and Wingfield, M.J.[1]

[1]Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), The University of Pretoria, Pretoria, South Africa, 0002
[2]Universidad de Los Andes, Facultad de Ciencias Forestales y Ambientales, Centro de Estudios Forestales y Ambientales de Postgrado (CEFAP), Merida, Venezuela

Van Wyk, M., Wingfield, B.D., Mohali, S. and Wingfield, M.J. (2009). *Ceratocystis fimbriatomima*, a new species in the *C. fimbriata sensu lato* complex isolated from *Eucalyptus* trees in Venezuela. Fungal Diversity 34: 175-185.

Species of *Ceratocystis* represent a group of important plant pathogens as well as saprobes that occur, primarily on woody substrates. The number of species in *Ceratocystis* has increased substantially in recent years, particularly as DNA-based methods have allowed for the recognition of cryptic taxa. The aim of this study was to identify isolates of a *Ceratocystis* sp. collected from freshly cut stumps of *Eucalyptus* trees in Venezuela. This was carried out using morphological comparisons with similar fungi as well as DNA sequence comparisons for the Internal Transcribed Spacer regions 1 and 2 including the 5.8S rDNA operon, part of the Beta-tubulin gene and part of the Transcription Elongation Factor 1-alpha gene region. Characteristics of the fungus in culture and its morphology resembled most species in the *C. fimbriata sensu lato* species complex. Microscopically, the fungus was most similar to *C. fimbriata sensu stricto*. Based on phylogenetic analyses, it was distinct from other species of *Ceratocystis sensu lato* having *C. manginecans* as its closest relative. The *Ceratocystis* sp. from *Eucalyptus* in Venezuela clearly represents a distinct taxon for which the name *C. fimbriatomima* sp. nov. is provided.

Key words: pathogen, phylogeny, species complex, tree wounds

Article Information
Received 2 February 2008
Accepted 20 May 2008
Published online 1 January 2009
*Corresponding author: Marelize van Wyk; email: marelize.vanwyk@fabi.up.ac.za

Introduction

Ceratocystis spp. in the *C. fimbriata sensu lato* (*s.l.*) species complex are mostly pathogens causing diseases of a large number of woody and some herbaceous plants (Kile, 1993). *Ceratocystis fimbriata* Ellis & Halst., the causal agent of black rot on sweet potato *Ipomoea batatas* (L.) Lam was the first species to be described and it typifies the genus (Halsted, 1890). Subsequent to its first disco-very, fungi identified as representing this species were isolated from a wide variety of hosts in many different parts of the world (Alexopoulos, 1962; Kile, 1993; Seifert *et al*., 1993).

Ceratocystis fimbriata has long been recognised to represent a complex of cryptic species (Webster and Butler, 1967a,b; Kile, 1993; Harrington, 2000). Studies based on DNA sequence data have confirmed this view and a recent trend has been to describe species that represent monophyletic lineages that occur in particular niches (Wingfield *et al*., 1996; Barnes *et al*., 2003; Marin *et al*., 2003; Van Wyk *et al*., 2004, 2007a,b; Engelbrecht and Harrington, 2005; Johnson *et al*., 2005). The first of these to be described was *C. albifundus* M.J. Wingf., De Beer & M.J. Morris which emerged as a pathogen of plantation-grown non-native *Acacia* spp. in South Africa in the early 1990's (Morris *et al*., 1993; Wingfield *et al*., 1996). Subsequently, many new species have been described in the *C. fimbriata s.l.* species complex including *C. pirilliformis* I. Barnes & M.J. Wingf. (Barnes *et al*., 2003), *C. polychroma* M. van Wyk, M.J. Wingf. & E.C.Y. Liew (Van Wyk *et al*., 2004), *C. cacaofunesta* Engelbrecht & T.C. Harr. (Engelbrecht and

Harrington, 2005), *C. platani* (J.M. Walter) Engelbrecht & T.C. Harr. (Engelbrecht and Harrington, 2005) and *C. atrox* M. van Wyk & M.J. Wingf. (Van Wyk *et al*., 2007b). *Ceratocystis fimbriata* is restricted to isolates from sweet potato and is appropriately referred to as *C. fimbriata sensu stricto* (*s.s.*) (Engelbrecht and Harrington, 2005).

Most *Ceratocystis* species in the *C. fimbriata s.l.* species complex cause or are associated with plant diseases (Kile, 1993). Symptoms associated with these fungi include root rot in tubular plants, vascular staining, cankers and vascular wilts in woody hosts. Some species threaten the propagation of woody crop plants such as coffee (Marin *et al*., 2003), cacao (Engelbrecht and Harrington, 2005), mango (Al Adawi *et al*., 2006; Van Wyk *et al*., 2007a) and timber crops such as *Eucalyptus* (Roux *et al*., 2004; Rodas *et al*., 2007) and *Acacia* (Morris *et al*., 1993; Wingfield *et al*., 1996; Roux *et al*., 2007). On *Eucalyptus*, *C. fimbriata s.l.* has been associated with serious canker and vascular wilt diseases in African and South American countries (Laia *et al*., 1999; Roux *et al*., 2000; Rodas *et al*., 2007).

Many recent studies have recorded species of *Ceratocystis* in the *C. fimbriata s.l.* complex from countries in South America (Baker *et al*., 2003; Marin *et al*., 2003; Rodas *et al*., 2007). Other than reports of *C. fimbriata s.l.* from cacao and coffee in Venezuela in the 1950's (Pontis, 1951; Malaguti, 1952a,b; De Reyes, 1988), very little is known regarding these fungi in Venezuela. During the course of a recent survey of *Eucalyptus* diseases in Venezuela, a *Ceratocystis* sp. resembling *C. fimbriata s.l.* was commonly encountered on the freshly cut stumps of *Eucalyptus* trees.

Materials and methods

Isolates

Samples bearing ascomata typical of *Ceratocystis* spp. were collected from stumps of recently (three-week-old) felled *Eucalyptus grandis* x *E. urophylla* hybrid trees near Acarigua, Portuguesa State in Venezuela. The samples were wrapped in newspaper and placed in separate plastic bags and transported to the laboratory. Ascomata on the wood were inspected one week after collection and masses of ascospores were transferred to 2% Malt Extract Agar (MEA: 20% w/v; Biolab, Midrand, South Africa) supplemented with 100mg/L streptomycin sulphate (SIGMA). Pure cultures were obtained and these have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), The University of Pretoria, South Africa. Representative isolates were also lodged with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

PCR and sequencing reactions

Six isolates (Table 1) were grown for two weeks on 2% MEA, after which the mycelium was scrapped from the surface of cultures. DNA was extracted as described by Van Wyk *et al*., (2006) and PCR reactions were run for three gene regions as described by Van Wyk *et al*., (2006). The gene regions selected for sequencing were the Internal Transcribed Spacer region (ITS) one and four including the 5.8S rDNA operon, part of the Beta-tubulin (β-tubulin) gene and part of the Transcription Elongation Factor 1-alpha (EF1-α) gene region. The primers selected for the PCR and sequencing reactions were ITS1 and ITS4 developed by White *et al*., (1990), βt1a and βt1b developed by Glass and Donaldson (1995) and EF1F and EF1R developed by Jacobs *et al*., (2004).

For sequencing, two separate reactions were used for the forward and reverse primers, respectively. The reactions were run using the ABI PRISM^{TM} Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California) on an ABI PRISM^{TM} 3100 Autosequencer (Applied BioSystems, Foster City, California, USA). The resultant sequences were analyzed using the software programme Sequence Navigator (version 1.0.1) (Applied BioSystems, Foster City, California). These sequences, together with sequences for other *Ceratocystis* spp. from GenBank (Table 1) were aligned using the software programme MAFFT (http://timpani.genome.ad.jp/%7emafft/server/) (Katoh *et al*., 2002). A partition homogeneity test (PHT) was conducted in PAUP version

Table 1. Isolates of *Ceratocystis* spp. used in this study.

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	References (Sequence data)
C. albifundus	CMW4068	DQ520638 EF070429 EF070400	*Acacia mearnsii*	RSA	Van Wyk *et al*. (2007b)
C. albifundus	CMW5329	AF388947 DQ371649 EF070401	*Acacia mearnsii*	Uganda	Van Wyk *et al*. (2007b)
C. atrox	CMW19383 CBS120517	EF070414 EF070430 EF070402	*Eucalyptus grandis*	Australia	Van Wyk *et al*. (2007b)
C. atrox	CMW19385 CBS120518	EF070415 EF070431 EF070403	*Eucalyptus grandis*	Australia	Van Wyk *et al*. (2007b)
C. cacaofunesta	CMW15051 CBS152.62	DQ520636 EF070427 EF070398	*Theobroma cacao*	Costa Rica	Van Wyk *et al*. (2007b)
C. cacaofunesta	CMW14809 CBS115169	DQ520637 EF070428 EF070399	*Theobroma cacao*	Ecuador	Van Wyk *et al*. (2007b)
C. caryae	CMW14793 CBS114716	EF070424 EF070439 EF070412	*Carya cordiformis*	USA	Van Wyk *et al*. (2007b)
C. caryae	CMW14808 CBS115168	EF070423 EF070440 EF070411	*Carya ovata*	USA	Van Wyk *et al*. (2007b)
C. fimbriata s.s	CMW15049 CBS141.37	DQ520629 EF070442 EF070394	*Ipomoea batatas*	USA	Van Wyk *et al*. (2006) Van Wyk *et al*. (2007b)
C. fimbriata s.s.	CMW1547	AF264904 EF070443 EF070395	*Ipomoea batatas*	Papua New Guinea	Roux *et al*. (2000) Van Wyk *et al*. (2007b)
C. fimbriata s.l.	CMW8857	AY233868 AY233878 EU241483	*Annona muricata*	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l.	CMW8856 CBS121793	AY233867 AY233874 EU241484	*Citrus limon*	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l.	CMW10844	AY177238 AY177229 EU241481	*Coffea arabica*	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l.	CMW9565 CBS121790	AY233864 AY233870 EU241487	Soil	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l.	CMW5751 CBS121792	AY177233 AY177225 EU241493	*Coffea arabica*	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l.	CMW9572	AY233863 AY233871 EU241488	Mandarin	Colombia	Marin *et al*. (2003) Present study
C. fimbriata s.l	CMW14797 CBS114721	AY953382 EF433307 EF433316	*Mangifera indica*	Brazil	Van Wyk *et al*. (2007a)
C. fimbriata s.l	CMW15052 CBS600.70	EF433298 EF433306 EF433315	*Mangifera indica*	Brazil	Van Wyk *et al*. (2007a)

Table 1 (continued). Isolates of *Ceratocystis* spp. used in this study.

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	References (Sequence data)
C. fimbriatomima	**CMW24174 CBS121786**	**EF190963 EF190951 EF190957**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. fimbriatomima	**CMW24176 CBS121787**	**EF190964 EF190952 EF190958**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. fimbriatomima	**CMW24376 CBS121788**	**EF190965 EF190953 EF190959**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. fimbriatomima	**CMW24377**	**EF190966 EF190954 EF190960**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. fimbriatomima	**CMW24378**	**EF190967 EF190955 EF190961**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. fimbriatomima	**CMW24379**	**EF190968 EF190956 EF190962**	***Eucalyptus* sp.**	**Venezuela**	**Present study**
C. manginecans	CMW13851 CBS121659	AY953383 EF433308 EF433317	*Mangifera indica*	Oman	Van Wyk *et al*. (2007a)
C. manginecans	CMW13852 CBS121660	AY953384 EF433309 EF433318	*Hypocryphalus mangifera*	Oman	Van Wyk *et al*. (2007a)
C. pirilliformis	CMW6569	AF427104 DQ371652 AY528982	*Eucalyptus nitens*	Australia	Barnes *et al*. (2003) Van Wyk *et al*. (2007b)
C. pirilliformis	CMW6579 CBS118128	AF427105 DQ371653 AY528983	*Eucalyptus nitens*	Australia	Barnes *et al*. (2003) Van Wyk *et al*. (2007b)
C. platani	CMW14802 CBS115162	DQ520630 EF070425 EF070396	*Platanus occidentalis*	USA	Van Wyk *et al*. (2007b)
C. platani	CMW23918	EF070426 EF070397 EU426554	*Platanus* sp.	Greece	Van Wyk *et al*. (2007b)
C. polychroma	CMW11424 CBS115778	AY528970 AY528966 AY528978	*Syzygium aromaticum*	Indonesia	Van Wyk *et al*. (2004)
C. polychroma	CMW11436 CBS115777	AY528971 AY528967 AY528979	*Syzygium aromaticum*	Indonesia	Van Wyk *et al*. (2004)
C. populicola	CMW14789 CBS119.78	EF070418 EF070434 EF070406	*Populus* sp.	Poland	Van Wyk *et al*. (2007b)
C. populicola	CMW14819 CBS114725	EF070419 EF070435 EF070407	*Populus* sp.	USA	Van Wyk *et al*. (2007b)
C. smalleyi	CMW14800 CBS114724	EF070420 EF070436 EF070408	*Carya cordiformis*	USA	Van Wyk *et al*. (2007b)

Table 1 (continued). Isolates of *Ceratocystis* spp. used in this study.

Species	Isolate no.	GenBank accession no.	Host	Geographical origin	References (Sequence data)
C. smalleyi	CMW26383 CBS114724	EU426553 EU426555 EU426556	*Carya cordiformis*	USA	Van Wyk *et al*. (2007b) This study
C. variospora	CMW20935 CBS114715	EF070421 EF070437 EF070409	*Quercus alba*	USA	Van Wyk *et al*. (2007b)
C. variospora	CMW20936 CBS114714	EF070422 EF070438 EF070410	*Quercus robur*	USA	Van Wyk *et al*. (2007b)
C. virescens	CMW11164	DQ520639 EF070441 EF070413	*Fagus americana*	USA	Van Wyk *et al*. (2007b)
C. virescens	CMW3276	AY528984 AY528990 AY529011	*Quercus robur*	USA	Van Wyk *et al*. (2004)

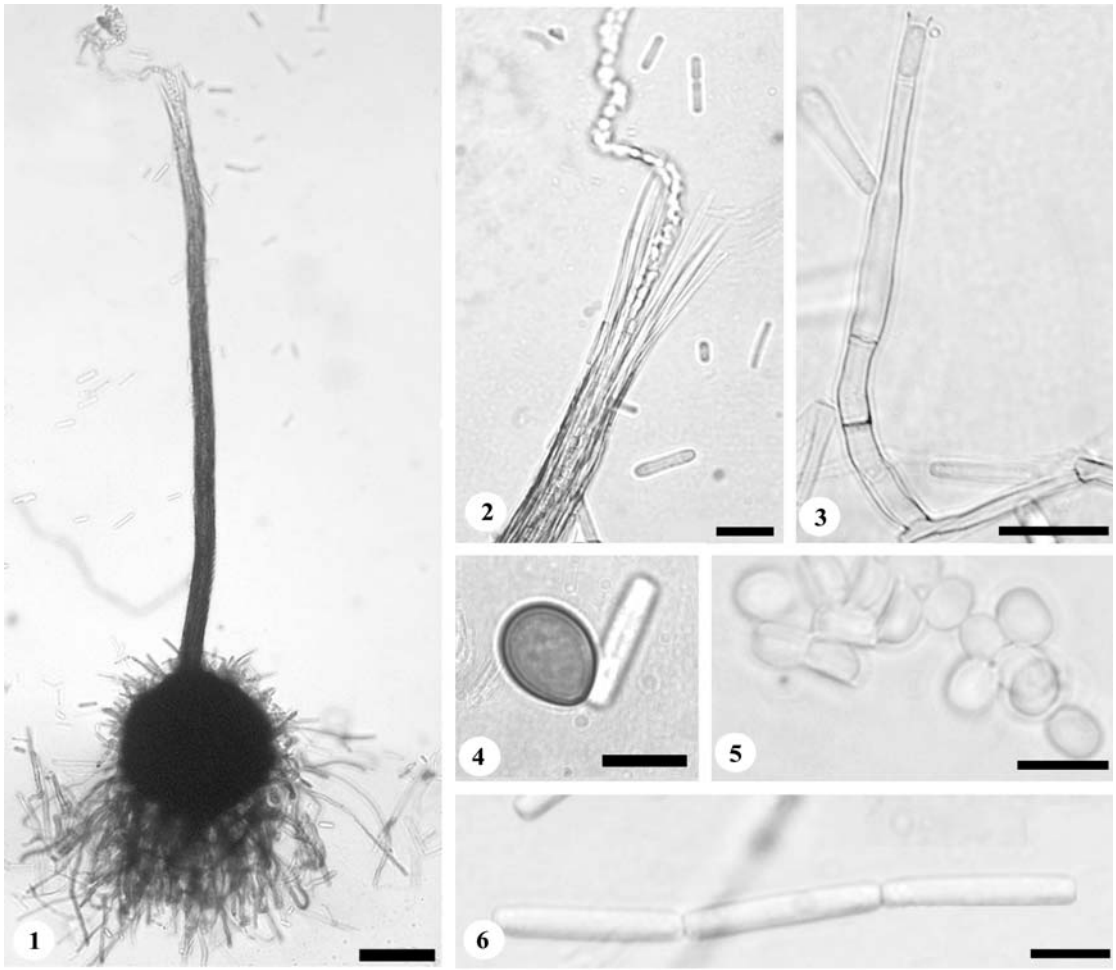
4.0b10* to determine whether the data sets could be combined (Swofford, 2002). In PAUP, all characters had equal weight, and gaps were treated as "fifth base". The heuristic search option based on parsimony was selected to search for optimal trees using heuristic algorithms (Swofford, 2002). The starting trees were obtained via stepwise addition, the sequences were randomly added and this was repeated 1000 times. To generate trees, the branch-swapping algorithm was set to tree-bisection-reconnection with the steepest decent not enforced. Polytomies were created by collapsing branches, if the maximum branch length was zero. The "Multrees" option was selected and topological constraints were not enforced. The tree was rooted with two isolates of *C. virescens* (R.W. Davidson) C. Moreau representing the outgroup taxon. Confidence intervals were obtained by calculating 1000 bootstrap replicates. All sequences derived from this study have been deposited in GenBank (Table 1).

The software program MrBayes (version 3.1.1) with the Markov Chain Monte Carlo (MCMC) algorithm was used to produce phylogenetic trees based on Bayesian probabilities (Ronquist and Huelsenbeck, 2003). A model of nucleotide substitution was determined for each gene region, using Mrmodeltest2 (Nylander, 2004). The nucleotide substitutions obtained were included for each gene partition in MrBayes. One million random trees were generated using the MCMC procedure with four chains, including hot and cold chains, and sampled every 100th generation. Tree likelihood scores were assessed to determine the number of trees that were formed before the stabilization, to prevent including trees that were formed before convergence. Trees outside the point of convergence were discarded by means of the burn-in procedure (Ronquist and Huelsenbeck, 2003).

Culture characteristics and morphology

Isolates (CMW24174, CMW24176, CMW24376 and CMW24377) morphologically resembling a species of *Ceratocystis* in the *C. fimbriata s.l.* species complex were grown for two weeks on 2% MEA. Subsequently, 4 mm plugs were transferred to the centres of five 90mm Petri dishes, containing 2% MEA, for seven different temperatures to be tested for growth for each of the isolates. These plates were incubated at 5°C to 35°C at 5°C intervals. Growth was assessed by taking two diameter measurements at right angles to each other for all plates after seven days of incubation. Averages of the ten diameter measurements for each isolate at each temperature were computed and the entire experiment was repeated once. The colour charts of Rayner (1970) were used to standardise the descriptions of colony colour.

For microscopic measurements, fungal



Figs 1-6. Morphological characteristics of *Ceratocystis fimbriatomima*. **1.** Ascomata with globose base and long neck. **2.** Divergent ostiolar hyphae. **3.** Primary conidiophore, flask-shaped phialides producing cylindrical conidia. **4.** Dark, sub-globose chlamydospore and cylindrical conidia. **5.** Hat-shaped ascospores. **6.** Chain of cylindrical conidia. Bars; **1.** = 100 μm, **2.** = 10 μm, **3.** = 20 μm, **4.** = 10 μm, **5.** = 10 μm, **6.** = 10 μm.

structures, taken from 10 d-old cultures on 2% MEA were mounted in lactic acid. Fifty measurements were made of each taxonomically relevant structure from the culture CMW24174 and 10 measurements for these structures were made for isolates CMW-24176, CMW24376 and CMW24377. The minimum, maximum, average and standard deviation (stdv) was calculated for the measurements of each structure. The measurements are thus presented as (minimum-) stdv minus the mean – stdv plus the mean (-maximum). A Carl Zeiss microscope with a Zeiss Axio Vision camera system was used to asses the measurements and to capture photographic images of all relevant taxonomic structures.

Results

Isolates

Fresh fungal structures were commonly found on the specimens collected from *Eucalyptus* stumps in Venezuela. The structures were characteristic of *Ceratocystis* spp. having a *Thielaviopsis* anamorph (Figs 1-6). Seventeen isolates of the *Ceratocystis* sp. were made from the samples taken from five *Eucalyptus* trees. One isolate (CMW24174) was chosen to represent the fungus and three additional isolates (CMW24176, CMW24376 and CMW24377) were chosen as additional specimens for description. These cultures, grown on 2% MEA, were dried down and have been deposited with the National Collection of Fungi (PREM), Pretoria, South Africa (Table 1.)

PCR and sequencing reactions

Amplicons of ~500 bp (ITS and β-tubulin) and ~800 bp (EF1-α) were obtained

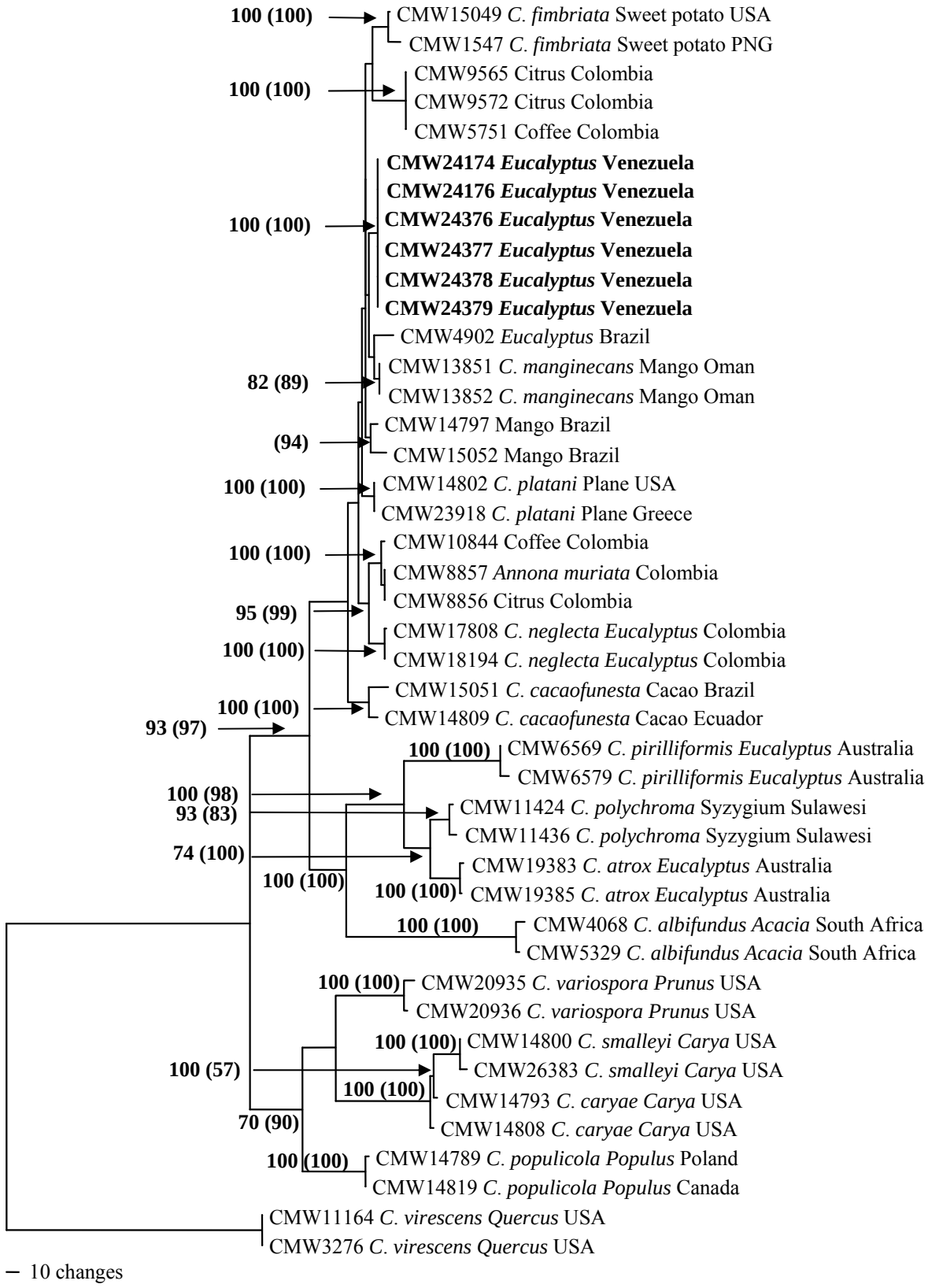


Fig. 7. Phylogenetic tree based on the combined regions of the ITS, β-tubulin and Ef1-α for *C. fimbriatomima* and other species in the *C. fimbriata s.l.* species complex. *Ceratocystis virescens* represents the out-group taxon. Bootstrap values are indicated at the branch nodes while Bayesian values are indicated in brackets.

tubulin) and ~800 bp (EF1-α) were obtained from the six isolates chosen for DNA sequence analysis (Table 1). The PHT resulted in a low P-value (P=0.01), possibly attributed to the minimal variation in the β-tubulin gene region. Although the P-value was low, studies (Sullivan, 1996; Cunningham, 1997) suggest that the data could still be combined. The combined dataset for the three gene regions had a total of 1944 characters. Of these 1944 characters, 1164 were constant, 45 were parsimony-uninformative and 735 were parsimony informative. Thirty-six most parsimonious trees were obtained, one of which was selected for presentation (Fig 7). This tree had a length of 1501 steps and is described as follows: Consistency Index = 0.7382, Retention Index = 0.8805 and Rescaled Consistency Index = 0.6500.

Based on the phylogenetic analysis, the *Ceratocystis* sp. from *Eucalyptus* in Venezuela grouped separately from all the described *Ceratocystis* spp. in the *C. fimbriata s.l.* species complex. The closest phylogenetic relative of this fungus was *C. manginecans* (Fig 7). The posterior probabilities for the tree emerging from the phylogenetic analysis were high with the *Ceratocystis* sp. from Venezuela supported 100%. All other species used in this study for comparison resided in groups with high bootstrap support and represented distinct phylogenetic taxa (Fig 7).

For both the ITS and the β-tubulin gene regions, MrModeltest2 selected the GTR+G model to support the datasets best. The HKY+G model was selected for the EF1-α gene region. These model settings were included in the Bayesian analysis and 3000 trees were discarded due to the fact that they were outside of the point of convergence (burn-in) when analysing the Bayesian inference. The posterior probability of the branch nodes of the combined tree obtained with the Bayesian inference supported the bootstrap values obtained with PAUP (Fig 7).

Culture characteristics and morphology

The cultures of the *Ceratocystis* sp. from *Eucalyptus* in Venezuela had a greenish olivaceous (33'"f) colour (Rayner, 1970). No growth was observed at 5°C, 10°C and 35°C, and limited growth was observed after seven days at 15°C (26mm) and 20°C (39mm). At 25°C and 30°C the cultures grew rapidly, reaching 50mm and 45mm, respectively in seven days. The cultures had a banana odour similar to that of many *Ceratocystis* spp.

Taxonomy

The *Ceratocystis* sp. from Venezuela isolated from freshly cut *Eucalyptus* stumps is phylogenetically distinct from all other *Ceratocystis* spp. residing in the *C. fimbriata s.l.* clade. It is also morphologically different to all of these species and is, therefore, described as a new species as follows:

Ceratocystis fimbriatomima M. van Wyk & M.J. Wingf. **sp. nov.**

(Figs 1-6)

MycoBank: 511432

Etymology: From the Latin *fimbriato* + Greek *mimos* (= '*fimbriata*-mimicking'), referring to the morphlogical similarity to *C. fimbriata s.s.*

Ascosporae lateraliter visae cucullato-pileiformes, non septatae, hyalinae, in vagina inclusa; vagina exclusa 2-4 x 4-6 µm. *Conidiophora secondaria* (phialide infundibuliformi) et *conidia secondaria* (doliiformia) desunt. *Chlamydosporae* umbrinae (6-) 10-14 (-15) µm longae, (6-) 7-11 (-12) µm latae.

Ascomatal bases dark, globose, unornamented (142-) 173-215 (-234) µm in diam. *Ascomatal necks* dark at bases becoming lighter towards the apices, (446-) 660-890 (-1070) µm long, apices (16-) 18-24 (-28) µm wide, bases (28-) 32-42 (-47) µm wide. *Ostiolar hyphae* divergent, (40-) 49-61 (-68) µm long. *Ascospores* hyaline, hat-shaped in side view, invested in sheath, 2-4 µm long, 4-6 µm wide, accumulating in buff-yellow masses at tips of ascomatal necks.

Anamorph: *Thielaviopsis*

Primary conidiophores phialidic, flask-shaped, (49-) 60-94 (-122) µm long, 3-5 µm wide at the apices, 5-9 µm wide at broadest points and 4-7 µm wide at bases. *Secondary conidiophores* flaring or wide mouthed absent. *Primary conidia* cylindrical in shape (14-) 20-28 (-31) µm long, 3-5 µm wide. *Secondary conidia*, barrel-shaped conidia, absent. *Chlamydospores* hair brown (17""i), subglobose (6-) 10-14 (-15) µm long, (6-) 7-11 (-12) µm wide.

Habitat: On cut stumps of recently (three-week-old) felled *Eucalyptus grandis* x *E. urophylla* hybrids.

Known distribution: Venezuela.

Material examined: VENEZUELA**,** Acarigua, Portuguesa State, isolated from bases of felled *Eucalyptus* trees, *M.J. Wingfield*, **holotype** Herb. *PREM59439*; *culture ex-type CMW24174 = CBS 121786*, July 2006. VENEZUELA**,** Acarigua, Portuguesa State, isolated from *Eucalyptus* trees, *M.J. Wingfield*, **paratype** Herb. *PREM59437*; *culture ex-paratype CMW24176 = CBS121787*, July 2006. VENEZUELA**,** Acarigua, Portuguesa State, isolated from *Eucalyptus* trees, *M.J. Wingfield*, **paratype** Herb. *PREM59615*; *culture paratype CMW24376 = CBS 121788*, July 2006. VENEZUELA**,** Acarigua, Portuguesa State, isolated from *Eucalyptus* trees, *M.J. Wingfield*, **paratype** *culture ex-paratype CMW24177*, July 2006.

Discussion

A new species of *Ceratocystis* from the stumps of freshly-cut *Eucalyptus* trees in Venezuela has emerged from this study. Primary recognition of this fungus as distinct from other species in the genus is based on phylogenetic analyses of sequence data for the ITS, β-tubulin and EF1-α gene regions. In this respect, the fungus clearly resides in the *C. fimbriata s.l.* species complex. Its morphology, with hat-shaped ascospores produced from ascomata without spines on their bases, which would reside in the *C. moniliformis s.l.* group, is also consistent with this taxonomic placement.

Phylogenetic data for the three gene regions combined, produced a high level of confidence that *C. fimbriatomima* from Venezuela is distinct from all described species. Phylogenetically, the species closest to *C. fimbriatomima* is *C. manginecans* but *C. fimbriata s.s.* is also relatively closely related to it. All other species included in this study for comparative purposes were confirmed as distinct from each other and from *C. fimbriatomima* with high levels of confidences. Other species in the *C. fimbriata s.l.* species complex that have been isolated from *Eucalyptus* are *C. atrox* (Van Wyk *et al.*, 2007b), *C. pirilliformis* (Barnes *et al.*, 2003) and *C. neglecta* (Rodas *et al.*, 2007) are clearly different to *C. fimbriatomima*.

Morphological characteristics of *C. fimbriatomima* are most similar to those of *C. fimbriata s.s.*, and its name has been chosen to reflect this fact. Both these fungi lack flaring secondary phialides as well as the barrel-shaped conidia that are produced from such phialides. These structures are found in all other species in the *C. fimbriata s.l.* complex. *Ceratocystis fimbriatomima* can be distinguished from its closest relative *C. fimbriata s.s.* based on the ostiolar hyphae and primary conidiophores that are both shorter in *C. fimbriata s.s.* than in *C. fimbriatomima*. Furthermore, the ascospores of *C. fimbriata s.s.* are much longer than those of *C. fimbriatomima*.

Various *Ceratocystis* spp. have been found on *Eucalyptus* spp. but only those in the *C. fimbriata s.l.* species complex might be confused with *C. fimbriatomima*. *Ceratocystis atrox* is known only from Australia and it has a very distinct association with the wood boring insect *Phoracantha acanthocera* (Macleay) (Cerambicydae: Coleptera) (Van Wyk *et al.*, 2007b). *Ceratocystis pirilliformis* was first found on *Eucalyptus* in Australia (Barnes *et al.*, 2003) but it is also known from South Africa, where it is thought to be introduced (Roux *et al.*, 2004). *Ceratocystis fimbriatomima* is very different to *C. pirilliformis* in having globose as opposed to pear-shared ascomatal bases (Barnes *et al.*, 2003; Roux *et al.*, 2004). *Ceratocystis neglecta*, recently found on *Eucalyptus* in Colombia (Rodas *et al.*, 2007) differs from *C. fimbriatomima* in that it has secondary conidiophores and secondary, barrel-shaped, conidia. The ascomatal necks of *C. fimbriatomima* are also longer and the primary conidiophores shorter than those of *C. neglecta*. It is thus unlikely that *C. fimbriatomima* could be confused with other *Ceratocystis* spp. in the *C. fimbriata s.l.* species complex that occurs on *Eucalyptus* spp.

Nothing is known regarding the pathogenicity of *C. fimbriatomima* or whether it might cause a disease on *Eucalyptus* in Venezuela. It was found on freshly cut stumps where infections were typically on green tissue. This ecological niche might indicate that the fungus is a pathogen although

inoculation tests will be required to resolve this question.

Acknowledgements

We thank the National Research Foundation (NRF), members of the Tree Protection Co-operative Programme (TPCP), the THRIP initiative of the Department of Trade and Industry and the Department of Science and Technology (DST)/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) for funding. We also thank Dr. Hugh Glen for providing the Latin description and for suggesting a name for the new species.

References

Al Adawi, A.O., Deadman, M.L., Al Rawahi, 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.

Alexopoulos, C.J. (1962). *Introductory Mycology*. 2nd edn. John Wiley & Sons, Inc., United States of America.

Baker, C.J., Harrington, T.C., Kraus, U. and Alfenas, A.C. (2003). Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. Phytopathology 93: 1274-1284.

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.

Cunningham, C.W. (1997). Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14: 733-740.

De Reyes, L.C. (1988). Necrosis del tronco y ramas del cacao en Venezuela. 10a Conferencia Internacional de Investigacion en Cacao, Venezuela, 485-489.

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. (1890). Some fungous disease of the sweet potato. Agricultural College Experiment Station Bulletin 76: 1-32.

Harrington, T.C. (2000). Host specialization and speciation in the American wilt pathogen *Ceratocystis fimbriata*. Fitopatologia Brasileira 25: 262-263.

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.A. Webber). APS Press, St. Paul, Minnesota, USA: 173-183.

Laia, M.L., Alfenas, A.C. and Harrington, T.C. (1999). Isolation, detection in soil, and inocultion of *Ceratocystis fimbriata*, causal agent of wilting, dieback and canker in *Eucalyptus*. In : *Proceedings of the 12th Biennial Conference of the Australasian Plant Pathology Society*, *Canberra*, *Australia*, *27-30 September*. 77. (ed. L. Morin).

Malaguti, G. (1952a). *Ceratostomella fimbriata* en el cacao de Venezuela. Acta Cientifica deVenezuela 3: 94-97.

Malaguti, G. (1952b). Una podredumbre del tallo de *Crotalaria juncea*, causada por *Ceratostomella fimbriata*. Agronomica Tropical, Maracay 4: 287-292.

Marin, M., Castro, B., Gaitan, A., Preisig, O., Wingfield, B.D. and Wingfield, M.J. (2003). Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. Journal of Phytopathology 151: 395-405.

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.

Nylander, J.A.A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pontis, R.E. (1951). A canker disease of the coffee tree in Colombia and Venezuela. Phytopathology 41: 178-184.

Rayner, R.W. (1970). A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey.

Rodas, C., Roux, J., Van Wyk, M., Wingfield, B.D. and Wingfield, M.J. (2007). *Ceratocystis neglecta* sp. nov., infecting *Eucalyptus* trees in Colombia. Fungal Diversity 28: 73-84.

Ronquist, F. and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Roux, J., Heath, R.N., Labuschagne, L., Nkuekam, G.K. and Wingfield, M.J. (2007). Occurrence of the wattle wilt pathogen, *Ceratocystis*

albifundus on native South African trees. Forest Pathology 37: 1-11.

Roux, J., Van Wyk, M., Hatting, H. & Wingfield, M.J. (2004). *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. Plant Pathology 53: 414-421.

Roux, J., Wingfield, M.J., Wingfield, B.D., Bouillett, J.P. and Alfenas, A.C. (2000). A serious new disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. Forest Pathology 30: 175-184.

Seifert, K.A., Wingfield, M.J. and Kendrick, W.B. (1993). A nomenclature for described species of *Ceratocystis*, *Ophiostoma*, *Ceratocystiopsis*, *Ceratostomella* and *Sphaeronaemella*. In: *Ceratocystis and Ophiostoma*: *Taxonomy*, *Ecology*, *and Pathogenicity* (eds. M.J. Wingfield, K.A. Seifert and J.A. Webber). APS Press, St. Paul, Minnesota, USA: 269-287.

Sullivan, J. (1996). Combining data with different distributions of among-site variation. Systematic Biology 45: 375-380.

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

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

Van Wyk, M., Pegg, G., Lawson, S. and Wingfield, M.J. (2007b). *Ceratocystis atrox* sp. nov associated with *Phoracantha acanthocera* infestations on *Eucalyptus* in Australia. Australian Journal of 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., Van der Merwe, N.A., Roux, J., Wingfield, B.D., G.N. Kamgan and Wingfield, M.J. (2006). Population genetic analyses suggest that the *Eucalyptus* fungal pathogen *Ceratocystis fimbriata* has been introduced into South Africa. South African Journal of Science 102: 259-263.

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, USA: 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.