Ceratocystis species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species

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Heath R.N., Wingfield M.J., Wingfield B.D., Meke G., Mbaga A. and Roux, J. (2009). *Ceratocystis* species on *Acacia mearnsii* and *Eucalyptus* spp. in eastern and southern Africa including six new species. Fungal Diversity 34: 41-67.

A survey of *Ceratocystis* species infecting wounds on non-native *Acacia mearnsii* and *Eucalyptus* spp. in Kenya, Malawi, South Africa and Tanzania resulted in the identification of eight species of which six were new to science. All identifications were based on morphology and DNA sequence data of the ribosomal RNA Internal Transcribed Spacer region, including the 5.8S operon as well as partial sequences of the β-tubulin and the Transcription Elongation Factor 1α genes. *Ceratocystis moniliformis* was found for the first time in Tanzania, while the wilt pathogen *C. albifundus* was commonly found on *A. mearnsii* in Kenya, South Africa and Tanzania. The undescribed *Ceratocystis* spp. are provided with the names *C. zombamontana* sp. nov., *C. polyconidia* sp. nov., *C. tanganyicensis* sp. nov., *C. obpyriformis* sp. nov., *C. oblonga* sp. nov. and *Thielaviopsis ceramica* sp. nov. All species introduced in this study are pathogenic on the hosts from which they were isolated.

Keywords: disease, plantation forestry, *Ophiostomatales*, wounds

Article Information
Received 4 February 2008
Accepted 13 June 2008
Published online 5 January 2009
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Introduction

Ceratocystis includes some well-known pathogens of trees, responsible for a wide range of disease symptoms including stem cankers, vascular wilts and root diseases (Kile, 1993). Most of the important pathogens are phylogenetically and morphologically very similar to *C. fimbriata* Ellis & Halst. *sensu lato* (*s.l.*). Several of the pathogenic species have been provided with new names, while others are recognized as unique based on phylogenetic inference (Wingfield *et al.*, 1996; Barnes *et al.*, 2003; Engelbrecht and Harrington, 2005; Johnson *et al.*, 2005; Van Wyk *et al.*, 2007; Rodas *et al.*, 2008) but have not been described formally. Well-known tree diseases caused by *Ceratocystis* spp. include oak wilt caused by *C. fagacearum* (Bretz) J Hunt (Bretz, 1952, Sinclair *et al.*, 1987), canker stain disease of plane trees (*Platanus* spp.) caused by *C. platani* (J.M. Walter) Engelbr. & T.C. Harr. (Engelbrecht and Harrington, 2005) and wattle wilt of *Acacia mearnsii* De Wild. caused by *C. albifundus* M.J. Wingf., De Beer & M.J. Morris (Morris *et al.*, 1993; Wingfield *et al.*, 1996). Many species, particularly those in the *C. coerulescens* (Münch) B.K. Bakshi species complex are agents of sap stain of timber (Münch, 1907) and various species, especially in the *C. moniliformis* (Hedgc.) C. Moreau *s.l.* species complex, appear to be saprobes (Davidson, 1935; Van Wyk *et al.*, 2006b).

Ceratocystis spp. residing in the *C. fimbriata s.l.* species complex require wounds for infection (DeVay *et al.*, 1963; Kile, 1993). These wounds can emerge from wind and hail damage, growth cracks, insect and other animal damage as well as human activities such

as grafting and pruning. Insects carry the *Ceratocystis* spp., which are ecologically adapted to this mode of dissemination, to the wounds, where infection can take place (DeVay *et al*., 1963; Kile, 1993). Stumps remaining from freshly harvested trees are also commonly infected by species of *Ceratocystis* (Roux *et al*., 2004). Recent studies have shown that artificially induced wounds are also commonly infected by *Ceratocystis* spp. and provide an opportunity to trap spe-cies infecting wounds from the environment (Barnes *et al*., 2003; Roux *et al*., 2004; Rodas *et al*., 2008).

Relatively little was known regarding *Ceratocystis* spp. occurring in Africa (Roux *et al*., 2005). During the course of the last two decades, there have been numerous studies treating these fungi on trees of the region. This is because of a serious wilt disease of *Acacia* spp. now known to be caused by *C. albifundus* (Morris *et al*., 1993; Wingfield *et al*., 1996; Roux *et al*., 2001a,b, 2005). More recently, *C. fimbriata s.l.* was reported to result in rapid wilting and death of *Eucalyptus* spp. in the Republic of Congo (Roux *et al*., 2000) and Uganda (Roux *et al*., 2001a). This fungus has also been isolated from wounds on *Eucalyptus* trees in South Africa (Roux *et al*., 2004) but although it was found to be pathogenic in inoculation tests, it has not been associated with disease under natural conditions. Most recently, two other species of *Ceratocystis*, *C. pirilliformis* I. Barnes & M.J. Wingf. and *C. moniliformis* have been recorded from *Eucalyptus* spp. in South Africa (Roux *et al*., 2004).

Population growth and globalisation is placing increasing pressure on native forests in Africa. For this reason, extensive forestry programmes, largely based on non-native species have been established. Diseases have already emerged as presenting serious constraints to the long term sustainability of forest plantations in Africa (Gibson, 1964; Roux *et al*., 2005) and this is likely to be an increasing trend in the future. Concern regarding diseases has prompted surveys for important groups of tree pathogens including species of *Ceratocystis*. The aim of this study was thus to expand current knowledge relating to *Ceratocystis* spp. in Africa, particularly those occurring on wounds on non-native *A. mearnsii* and *Eucalyptus* spp. in eastern and southern Africa.

Materials and methods

Collection of isolates

Isolates were collected from *A. mearnsii* and *Eucalyptus* spp. at four localities (Piet Retief, Tzaneen, Pietermaritzburg and Lothair) in South Africa and one each in Malawi (Zomba Mountain), Tanzania (Njombe) and Kenya (Thika). Collections were made from the stumps of freshly felled *Eucalyptus* spp. and *A. mearnsii* as well as from artificially induced wounds on the stems of *Eucalyptus* trees. In the case of the stumps, samples were collected between four days and four weeks after felling, by removing pieces of wood displaying stained vascular tissue and/or the presence of fungal growth.

Stem wounds were made on *Eucalyptus* trees using the technique previously described by Barnes *et al*. (2003). Twenty trees were selected randomly at each study site and wounds were made on the stems, approximately 1.5 meters from the ground. Approximately 100 cm^2 of bark was removed from the stems to expose the cambium. A horizontal slit was made into the xylem of the wound, approximately five mm deep. Samples were collected after six weeks by removing a piece of wood and bark from the top and bottom corners of the wound site and transferred to the laboratory in brown paper bags for further study.

Wood sections were examined for the presence of fruiting structures of *Ceratocystis* spp. In addition, wood pieces displaying vascular discoloration were baited for *Ceratocystis* spp. by placing these between two carrot slices (five mm thick) and incubating them at 25°C for 7-10 days (Moller and DeVay, 1968). Pieces of wood were also incubated in containers with moist tissue paper at 25°C for seven days to induce the formation of fruiting structures.

Once ascomata of *Ceratocystis* spp. were found, spore masses were lifted from their apices and transferred to 2% (w/v) malt extract agar (MEA) (Biolab, Midrand, South Africa) supplemented with streptomycin

sulphate (0.001 g vol^{-1}, SIGMA, Steinheim, Germany). Plates were then incubated at approximately 25ºC under natural day/night conditions. Isolates were purified on 2% MEA and are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative isolates were also deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. In order to prepare herbarium specimens, cultures bearing fruiting structures were dried on 30% glycerol and deposited with the National Fungal Herbarium of South Africa (PREM), Pretoria.

Morphology and growth in culture

All isolates collected in this study were grouped based on their culture morphology on 2% MEA after five days and were then studied microscopically for further differentiation. Representative isolates of each group were selected for further identification using DNA sequence comparisons.

For identification based on morphology, pure cultures were maintained on 2% MEA until fruiting structures formed. Fungal structures were mounted on glass slides in lactic acid and examined under a Zeiss Axioskop microscope. Images were captured using a HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd., Germany). Fifty measurements were made for each taxonomically relevant structure and averages and standard deviations (st. dev) were determined for each of these structures. Measurements are presented in this study as (minimum -) mean minus st. dev. - mean plus st. dev. (- maximum). Colours of cultures were defined based on the mycological colour charts of Rayner (1970).

Two test isolates of each species were selected to study growth in culture. These included one chosen to represent the holotype specimen and one of the paratypes. Growth rates of known species were not determined. Studies of growth in culture were performed by placing an agar disk (four mm diameter) overgrown with mycelium (mycelial side down) of selected five-day old isolates at the centres of 90mm Petri dishes containing 2% MEA. Petri dishes were incubated in the dark at temperatures ranging from 5°C to 35°C at 5°C intervals. Colony diameters were measured after seven days. Two measurements, perpendicular to each other, were made for each culture. Five replicates of each test strain were used at each temperature and averages of the ten measurements taken for each isolate were computed. The entire experiment was repeated once.

DNA isolation, PCR reactions and sequence analyses

DNA of representative *Ceratocystis* isolates (Table 1) was extracted using the method described by Van Wyk *et al*. (2006a). Three gene regions were amplified for sequencing and phylogenetic analyses. The ribosomal RNA Internal Transcribed Spacer regions (ITS) 1 and 2, and the 5.8S operon, were amplified using the primers ITS1 and ITS4 (White *et al*., 1990). Part of the beta-tubulin (*β*-tubulin) gene was amplified with primers Bt1a and Bt1b (Glass and Donaldson, 1995) and part of the Transcription Elongation Factor-1α (EF-1α) gene was amplified using the primers EF1F and EF1R (Jacobs *et al*., 2004).

Polymerase chain reaction (PCR) mixtures, for all three gene regions, consisted of 1 x Expand HF Buffer containing 1.5 mM $MgCl_2$ (Roche Diagnostics, Mannheim, Germany, supplied with the enzyme), 200 μM of each dNTP, FastStart *Taq* enzyme (2 U) (Roche Diagnostics, Mannheim, Germany), 200 ηM of the forward and reverse primers, and 2-10 ng DNA. Reactions were adjusted to a total volume of 25 μL with sterile water. The PCR programme was set for 4 min at 95°C for initial denaturation of the DNA. This was followed by 10 cycles consisting of a denaturation step at 95°C for 40s, an annealing step for 40s at 55°C and an elongation step for 45s at 70°C. Subsequently, 30 cycles consisting of 94ºC for 20s, 55ºC for 40s with a 5s extension step after each cycle, and 70ºC for 45s were performed. A final step of 10 min at 72ºC completed the programme. Amplification of the DNA for the three gene regions was confirmed under ultraviolet (UV) illumination using gel electrophoresis with 2% agarose in the presence of ethidium bromide. Amplicons were purified using 6%

Sephadex G-50 columns following the manufacturer's instructions (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, USA), 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) and sequences were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California, USA). Sequences were compared with those of closely related *Cera-tocystis* and *Thielaviopsis* spp. obtained from GenBank (http://www.ncbi.nlm.nih.gov), re-sulting in three datasets. The first set comprised three gene regions for isolates representing the *C. fimbriata s.l.* species complex, the second of the ITS gene region of *Thielaviopsis* spp. together with a small number of *Ceratocystis* spp., and the third set was made up of three gene regions for species in the *C. moniliformis s.l.* species complex. Sequences were aligned using the web interface (http://align.bmr.kyushu-u.ac.jp/mafft/software/) of MAFFT (Katoh *et al*., 2002) and confirmed manually.

Analyses were performed using Phylogenetic Analysis Using Parsimony (PAUP) version 4.0b10* (Swofford, 2002). To determine whether the sequences for the multiple gene regions could be combined into single datasets, partition homogeneity tests (Swofford, 2002) were conducted. 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 phylograms. Confidence intervals using 1000 bootstrap replicates were calculated. *Cerato-cystis virescens* (R.W. Davidson) C. Moreau was designated as the monophyletic sister outgroup taxon. 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 Mr Modeltest (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 100th 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.

Pathogenicity tests

The relative pathogenicity of the *Ceratocystis* spp. isolated from the various hosts was determined in inoculations performed in a greenhouse. An *E. grandis* W. Hill ex Maiden clone and *A. mearnsii* seedlings were maintained under greenhouse conditions for two weeks to acclimatise them to these conditions, prior to inoculation. The greenhouses were subjected to natural day/night conditions (~13 hours daylight/ ~11 hours darkness) and a temperature of approximately 25ºC was maintained. Tree diameters varied from 10 to 15 mm. The two fastest growing isolates for each of the six *Ceratocystis* spp. (CMW23809, CMW23818, CMW23807, CMW23808, CMW23802, CMW23803, CMW15992, CMW15999, CMW15235, CMW15236, CMW15242, CMW15248) were selected for the inoculation tests. Twenty trees were inoculated with each test strain. In addition, ten trees were inoculated with sterile MEA plugs, to serve as controls.

Wounds were made on the tree stems using a cork borer (5 mm diam) in such a way that a disc of bark was removed to expose the cambium. Mycelial plugs of a similar size were taken from the edges of seven-day-old actively growing cultures and placed in the wounds with the mycelium facing the cambium. Wounds were sealed with labora-tory film (Parafilm "M", American National Can™ Chicago, Illinois, USA) to protect the inoculated fungus and the cambium from desiccation. Lesion lengths were measured six weeks after the trees were inoculated. The experiment was repeated once and the data were pooled for analyses. The differences between

Table 1. *Ceratocystis* isolates obtained and used in this study.

Species	Culture no.	Host	Geographical origin	Collector(s)	GenBank Accession no. ITS, BT, EF1α
C. albifundus	[b] CMW4068	*Acacia mearnsii*	South Africa	J. Roux	DQ520638, EF070429, EF070400
"	[b] CMW5329	"	Uganda	"	AF388947, DQ371649, EF070401
"	[a] CMW23823, CMW23824	"	South Africa	R.N. Heath	
"	[a b] CMW23825	"	"	"	EU245010, EU244982, EU244942
"	[a] CMW23826-CMW23837	"	"	"	
"	[a b] CMW23838	"	"	"	EU245009, EU244981, EU244941
"	[a b] CMW24860	"	Tanzania	R.N. Heath & J. Roux	EU245011, EU244983, EU244943
"	[a b] CMW24861	"	"	"	EU245012, EU244984, EU244944
	[a] CMW24862-CMW24887	"	"	"	
"	[a b] CMW24685	"	Kenya	"	EU245013, EU244985, EU244945
"	[a b] CMW24686	"	"	"	EU245014, EU244986, EU244946
"	[a] CMW27876, CMW27877	"	"	"	
C. atrox	[b] CMW19383	*Eucalyptus grandis*	Australia	M.J. Wingfield	EF070414, EF070430, EF070402
"	[b] CMW19385	"	"	"	EF070415, EF070431, EF070403
C. bhutanensis	[b] CMW8217, CBS114289	*Ips schmutzenhoferi*	Bhutan	M.J. Wingfield, T. Kirisits, D.B. Chhetri	AY528957, AY528962, AY528952
"	[b] CMW8242, CBS112907	"	"	"	AY528956, AY528961, AY528951
C. cacaofunesta	[b] CMW15051, CBS152.62	*Theobroma cacao*	Costa Rica	A.J. Hansen	DQ520636, EF070427, EF070398
C. cacaofunesta	[b] CMW14809, CBS115169	"	Ecuador	C. Suarez	DQ520637, EF070428, EF070399
C. caryae	[b] CMW14793, CBS114716	*Carya cordiformis*	USA	J. Johnson	EF070424, EF070439, EF070412
"	[b] CMW14808, CBS115168	*C. ovata*	"	"	EF070423, EF070440, EF070411
C. fimbriata	[b] CMW15049, CBS141.37	*Ipomoea batatas*	"	C.F. Andrus	DQ520629, EF070442, EF070394
"	[b] CMW1547	*I. batatas*	Papua New Guinea	E.C.H. McKenzie	AF264904, EF070443, EF070395
C. moniliformis	[a b] CMW22284	*E. grandis*	Tanzania	J. Roux & R.N. Heath	EU245015, EU244987, EU244947
"	[a b] CMW22289	*E. grandis*	"	"	EU245016, EU244988, EU244948
"	[a] CMW27864-CMW27875	"	"	"	
"	[a] CMW17584	*Eucalyptus* sp.	South Africa	G. Kamgan Nkuekam	
"	[a b] CMW17587	"	"	"	EU245017, EU244989, EU244949
"	[a b] CMW17690	"	"	"	EU245018, EU244990, EU244950
"	[a] CMW27863	"	"	"	
"	[b] CMW9590	"	"	J. Roux	AY528985, AY528996, AY529006
"	[b] CMW4114	"	Ecuador	M. J. Wingfield	AY528986, AY528997, AY529007
C. moniliformopsis	[b] CMW10215, CBS115793	*E. obliqua*	Australia	Z.Q. Yuan	AY528999, AY528988, AY529009

Table 1 (continued). *Ceratocystis* isolates obtained and used in this study.

Species	Culture no.	Host	Geographical origin	Collector(s)	GenBank Accession no. ITS. BT, EF1α
"	[b] CMW9986, CBS109441	*E. obliqua*	Australia	Z.Q. Yuan	AY528987, AY528998, AY529008
C. oblonga	[a b] CMW23802	*A. mearnsii*	South Africa	R.N. Heath	EU245020, EU244992, EU244952
"	[a b] CMW23803	"	"	"	EU245019, EU244991, EU244951
"	[a b] CMW23804	"	"	"	EU245021, EU244993, EU244953
"	[a] CMW23805	"	"	"	
C. obpyriformis	[a b] CMW23806	*A. mearnsii*	South Africa	"	EU245005, EU244977, EU244937
"	[a b] CMW23807	"	"	"	EU245004, EU244976, EU244936
"	[a b] CMW23808	"	"	"	EU245003, EU244975, EU244935
"	[a] CMW27862	"	"	"	
C. omanensis	[b] CMW11046, CBS118112	*Mangifera indica*	Oman	A.O.Al-Adawi	DQ074739, DQ074729, DQ074734
"	[b] CMW11048, CBS115780	"	"	"	DQ074742, DQ074732, DQ074737
C. pirilliformis	[b] CMW6569	*Eucalyptus nitens*	Australia	M.J. Wingfield	AF427104, DQ371652, AY528982
"	[b] CMW6579	"	"	"	AF427105, DQ371653, AY528983
C. platani	[b] CMW14802, CBS115162	*Platanus occidentalis*	USA	T.C. Harrington	DQ520630, EF070425, EF070396
"	[b] CMW23918	"	Greece	M.J. Wingfield	EU426554, EU426555, EU426556
C. polonica	[b] CMW5026	n/a	n/a	n/a	AY233907
"	[b] CMW1165	n/a	n/a	n/a	AY233906
C. polychroma	[b] CMW11424, CBS115778	*Syzygium aromaticum*	Indonesia	M.J. Wingfield	AY528970, AY528966, AY528978
"	[b] CMW11436, CBS115777	"	"	"	AY528971, AY528967, AY528979
C. polyconidia	[a b] CMW23809	*A. mearnsii*	South Africa	R.N. Heath	EU245006, EU244978, EU244938
"	[a] CMW23810-CMW23817	"	"	"	
"	[a b] CMW23818	"	"	"	EU245007, EU244979, EU244939
"	[a b] CMW23819	"	"	"	EU245008, EU244980, EU244940
"	[a] CMW23820-CMW23822	"	"	"	
C. populicola	[b] CMW14789, CBS119.78	*Populus* sp.	Poland	J. Gremmen	EF070418, EF070434, EF070406
"	[b] CMW14819, CBS114725	"	USA	T. Hinds	EF070419, EF070435, EF070407
C. resinifera	[b] CMW20931, CBS100202	*Picea* sp.	Norway	H. Solheim	U75616
"	[b] CMW26371, CBS100204	"	USA	T. Hinds	U75618
C. savannae	[b] CMW17300	*Acacia nigrescens*	South Africa	G. Kamgan Nkuekam	EF408551, EF408565, EF408572
"	[b] CMW17279	*Combretum zeyheri*	"	"	EF408552, EF408566, EF408573
C. smalleyi	[b] CMW14800, CBS114724	*Carya cordiformis*	USA	G. Smalley	EF070420, EF070436, EF070408
C. tanganyicensis	[a] CMW15988-CMW15990	*A. mearnsii*	Tanzania	R.N. Heath & J. Roux	
"	[a b] CMW15991	"	"	"	EU244997, EU244969, EU244929
"	[a b] CMW15992	"	"	"	EU244999, EU244971, EU244931

Table 1 (continued). *Ceratocystis* isolates obtained and used in this study.

Species	Culture no.	Host	Geographical origin	Collector(s)	GenBank Accession no. ITS. BT, EF1α
C. tanganyicensis	[a] CMW15993-CMW15998	*A. mearnsii*	Tanzania	R.N. Heath & J. Roux	
"	[a b] CMW15999	"	"	"	EU244998, EU244970, EU244939
"	[a] CMW16000-CMW16007	"	"	"	
"	[a] CMW27878-CMW27892	"	"	"	
C. tribiliformis	[b] CMW13013	*Pinus merkusii*	Indonesia	M.J. Wingfield	AY528993, AY529003, AY529014
"	[b] CMW13015	*Pinus merkusii*	Indonesia	M.J. Wingfield	AY528994, AY529004, AY529015
C. tsitsikammensis	[b] CMW14276	*Rapanea melanophloeos*	South Africa	G. Kamgan Nkuekam	EF408555, EF408569, EF408576
"	[b] CMW14278	"	"	"	EF408556, EF408570, EF408577
C. variospora	[b] CMW20935, CBS 114715	*Quercus alba*	USA	J. Johnson	EF070421, EF070437, EF070409
"	[b] CMW20936, CBS 114714	*Q. robur*	"	"	EF070422, EF070438, EF070410
C. virescens	[b] CMW11164	*Fagus americana*	"	D. Houston	DQ520639, EF070441, EF070413
"	[b] CMW3276	*Quercus* sp.	USA	T. Hinds	AY528984, AY528990, AY5289991
C. zombamontana	[a b] CMW15251	*Eucalyptus* spp.	Malawi	R.N. Heath & J. Roux	EU245001, EU244973, EU244933
"	[a b] CMW15235	"	"	"	EU245002, EU244974, EU244934
"	[a b] CMW15236	"	"	"	EU245000, EU244972, EU244932
"	[a] CMW15242	"	"	"	
T. basicola	[b] C1602	n/a	n/a	n/a	AF275490
"	[b] C1373	n/a	n/a	n/a	AF275482
T. ceramica	[a b] CMW15245	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux	EU245022, EU244994, EU244926
	[a] CMW15237	"	"	"	
"	[a] CMW15238	"	"	"	
	[a] CMW15240, CMW15241	"	"	"	
"	[a b] CMW15246	"	"	"	EU245023, EU244995, EU244927
"	[a b] CMW15248	"	"	"	EU2450024, EU244996, EU244928
	[a] CMW15249	"	"	"	
T. ceramica	[a] CMW17139-CMW17141	*Eucalyptus* sp.	Malawi	R.N. Heath & J. Roux	
"	[a] CMW24169-CMW24172	"	"	"	
T. ovoidea	[b] C1375	n/a	n/a	n/a	AF275483
"	[b] C1376	n/a	n/a	n/a	AF275484
T. populi	[b] CBS484.71	*Populus* sp.	n/a	n/a	AF275479
"	[b] CBS486.71	"	n/a	n/a	AF275480

[a] Isolates obtained in this study
[b] Isolates used in Phylogenetic analysis in this study

variables were log10 transformed to obtain a normalized distribution. The data were then subjected to a univariate procedure using SAS (SAS Version 8.2, 2001). To determine whether the inoculated fungi were responsible for the lesion development, re-isolations were made from the lesions and the fungi identified based on morphology.

Results

Isolates

Isolations from ascomata and mycelium on wounds or carrot baits yielded cultures that, based on morphology, clearly represented a number of different *Ceratocystis* spp. A total of 136 isolates were obtained in this study. Of these, 106 were isolated from *A. mearnsii*, 23 cultures from *Eucalyptus* spp. and seven isolates were of a *Thielaviopsis* sp. from *Eucalyptus* spp. for which no sexual fruiting bodies were found (Table 1). Symptoms associated with fungal infection of the wounds and stumps were a discoloration of the vascular tissue in a streaked pattern, developing above and below the wounds. Vascular streaking of the wood associated with the cut stumps spread downwards into the roots.

Morphology and growth in culture

The *Ceratocystis* spp. obtained in this study could be separated into eight distinct groups based on culture morphology. Selected isolates representing these groups (designated Group A to Group H) were chosen for further study and others have been preserved (Table 1). Group A isolates were from *A. mearnsii* in Tanzania (CMW15773, CMW15781), South Africa (CMW23825, CMW23838) and Kenya (CMW24685, CMW24686) and produced light-coloured mycelium bearing ascomata with light coloured bases, similar to those of *C. albifundus*. Group B isolates (CMW15235, CMW15236, CMW15251) from *E. grandis* in Malawi, produced light brown to greyish-white colonies (17""i) and ascomata with black ascomatal necks. Group C isolates (CMW23806, CMW23807, CMW23808) were obtained from *A. mearnsii* from South Africa and had greyish-white colonies (21"k) and ascomata with black ascomatal necks similar to those of *C. pirilliformis*. Group D isolates (CMW15991, CMW15992, CMW15999) were from *A. mearnsii* in Tanzania and produced dark grey to greenish colonies (25"m) similar to those of *C. fimbriata s.l.* This fungus produced abundant chlamydospores not typically found in *C. fimbriata s.s.* Isolates (CMW23809, CMW23818, CMW23819) residing in Group E were from *A. mearnsii* in South Africa and produced dark brown to greenish-brown colonies (19"i) and ascomata with black ascomatal necks and had an extremely strong alkaloid odour. Group F isolates (CMW23802, CMW23803, CMW23804) were from *A. mearnsii* in South Africa and produced white-coloured colonies that turned light brown with age and were similar to those of *C. savannae* Kamgan & Jol. Roux. These isolates, however, produced nodules on the hyphae, a characteristic not found in *C. savannae* (Kamgan *et al*., 2008). Group G isolates were from *Eucalyptus* spp. in Tanzania (CMW22284, CMW22289) and South Africa (CMW17587, CMW17960). These isolates were characterized by hyaline to grey to black mycelium, similar to that found in *C. moniliformis*. Group H isolates (CMW15245, CMW15246, CMW15248) represented a *Thielaviopsis* sp., from a *Eucalyptus* sp. in Malawi without any production of sexual structures and were characterized by light to dark brown mycelium (21"b).

No growth rate studies were performed for Groups A and G as these represent known species. The optimal growth for isolates (CMW15236, CMW15235) representing Group B was 20-25°C. No growth was observed at 5°C and 35°C. At 20 and 25°C an average of 41mm of growth was observed after 14 days. The optimal growth for Group C (CMW23808, CMW23807) isolates was 25°C and they did not grow at 5°C or at 35°C. At 25°C, an average of 52mm growth was observed after seven days. Group D isolates (CMW15992, CMW15999) grew optimally at 20°C but did not grow at 5°C or at 35°C and reached an average of 27mm of growth after seven days. Group E isolates (CMW23809, CMW23818) grew optimally at 20-25°C with minimal growth at 5°C and 35°C and produced an average growth of 81mm in seven

days. Isolates in Group E produced an average growth of 72mm at 20°C and 81mm at 25°C after seven days. Group F isolates (CMW23803, CMW23802) grew optimally at 25°C. Growth was also observed at 5°C and at 35°C. Cultures reached an average of 83mm of growth after seven days. Group H isolates (CMW15245, CMW15248) produced optimal growth at 25°C reaching an average of 90mm of growth after four days. No growth was observed at 5°C, 10°C or at 35°C.

Phylogenetic analyses

DNA sequencing yielded amplicons of ~500bp for both the ITS and *β*-tubulin gene regions and amplicons of ~750bp for the EF1-α gene region. Partition homogeneity tests showed that data from the three gene regions could be combined for both the *C. fimbriata s.l.* and *C. moniliformis s.l.* datasets. The *C. fimbriata s.l.* data set had a P value of 0.001 and the *C. moniliformis s.l.* data set a P value of 0.16 for the partition homogeneity tests.

One most parsimonious tree was obtained for the combined data set of the *C. fimbriata s.l.* group (Fig. 1). This tree had a length of 1597 base pairs. There were 1915 characters, with 1135 of these characters being constant, 37 characters being parsimony-uninformative and 743 characters being parsimony-informative, with Consistency Index (CI) = 0.7276, Retention Index (RI) = 0.9003 and Rescaled Consistency (RC) = 0.6551. In the phylogenetic tree, *C. fimbriata s.s.*, *C. platani* Engelbrecht and Harrington, *C. cacaofunesta* Engelbr. & T.C. Harr., *C. pirilliformis*, *C. polychroma* M. van Wyk, M.J. Wingf. & E.C.Y. Liew, *C. albifundus*, *C. caryae* J.A. Johnson & T.C. Harr., *C. smalleyi* J.A. Johnson & T.C. Harr. *C. variospora* (R.W. Davidson) C. Moreau, *C. populicola* J.A. Johnson & T.C. Harr., *C. tsitsikammensis* Kamgan & Jol. Roux and *C. atrox* M. van Wyk & M.J. Wingf. all represented distinct clades, supported by high bootstrap values. The isolates collected in this study formed four separate and distinct clades. Groups emerging from the phylogenetic analyses were consistent with the groups defined based on their morphology.

The isolates obtained from *A. mearnsii* from Tanzania (Group A, Group D) grouped in two clades. Group D isolates (CMW15991, CMW15992, CMW15999) resided in a distinct clade (Clade 1), close to *C. tsitsikammensis* with strong bootstrap support (100%). Group A isolates (CMW24806, CMW24861) resided in the *C. albifundus* clade (Clade 2), with 100% bootstrap support. Isolates (CMW-24685, CMW24686) from *A. mearnsii* from Kenya, treated as Group A (CMW24685, CMW24686), also grouped in the *C. albifundus* clade (Clade 2). Isolates from *A. mearnsii* in South Africa (Group A, Group C, Group E) resided in three clades. The first group of isolates, Group A (CMW23825, CMW23838) grouped within the *C. albifundus* clade (Clade 2). The other two groups of isolates (CMW23806, CMW23807, CMW23808) designated Group C (Clade 3) and Group E (CMW23809, CMW23818, CMW23819) (Clade 4) resided in two distinct clades with strong (100%) bootstrap support, close to the *C. pirilliformis* clade. Isolates (CMW15235, CMW15236, CMW15251) obtained from *E. grandis* from Malawi, designated Group B, also formed a distinct clade, separate from any other isolates and with strong (100%) bootstrap support, close to *C. pirilliformis* (Clade 5). Posterior probability values calculated for the branch nodes sup-ported the bootstrap values for all these clades and suggested that four previously undescribed species in the *C. fimbriata s.l.* group were collected during this study (Fig. 1).

Three most parsimonious trees were obtained for the data set including the *Thielaviopsis* spp. (Fig. 2). In the phylogenetic tree, *C. bhutanensis* M. van Wyk, M.J. Wingf. & Kirisits, *C. omanensis* Al-Subhi, M.J. Wingf, M. van Wyk & Deadman, *T. ovoidea* (Nag Raj & W.B. Kendr.) A.E. Paulin, T.C. Harr. & McNew, *T. populi* (Veldeman ex Kiffer & Delon) A.E. Paulin, T.C. Harr. & McNew, *T. basicola* (Berk. & Broome) Ferraris, *C. polonica* (Siem.) (Siemaszko) C. Moreau and *C. resenifera* T.C. Harr. & M.J. Wingf. all represented distinct clades, supported by high bootstrap values. *Thielaviopsis* isolates collected from *Eucalyptus* in

Table 2. Morphological differences between species described in this study and closely related species in the *C. fimbriata sensu lato* group.

Character	*C. pirilliformis*	*C. zombamontana*	*C. polyconidia*	*C. tanganyicensis*	*C. obpyriformis*	*C. tsitsikammensis*
ASCOMATA						
Base						
Shape	Pyriform to Globose	Obpyriform	Globose	Subglobose	Obpyriform	Globose
Colour	Black	Dark brown to black	Dark brown to black	Dark brown to black	Dark brown to black	Black
Diameter	115-187(-206) µm	(106-)132-181(-208) µm	(133-)153-223(-277) µm	(119-)138-177(-205) µm	(149-)166-206(-228) µm	(124-)143-175(-186) µm
Ornamentation	None	None	None	None	None	None
Neck						
Colour	Black	Dark brown to black	Dark brown to black	Dark brown to black	Dark brown to black	Black
Collar	None	None	None	None	Collar	None
Length	372-683(-778)µm	(264-)315-443(-535) µm	(326-)429-589(694) µm	(302-)365-484(-558) µm	(477)569-675(-708) µm	(217-)321-425(-465) µm
Width (tip)	12-21 (-25) µm	(13-)16-21(-23) µm	(11-)13-19(-23) µm	(13-)14-18(-21) µm	(13-)16-21(-26) µm	n/a
Width (base)	19-33(-40)µm	(24-)30-38(-42) µm	(20-)26-34(-39) µm	(19-)24-32(-37) µm	(26-)28-36(-45) µm	(31-)32-47(62) µm
Ostiolar hyphae						
Orientation	Convergent	Divergent	Divergent	Divergent	Divergent	Divergent
Length	n/a	(16-)20-25(-27) µm	(36-)39-47(-57) µm	(17-)39-47(-47) µm	(34.-)39-47(-54) µm	(23-)28-38(-42) µm
Ascospores						
Colour	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
Shape (side view)	Hat-shaped	Hat-shaped	Hat-shaped	Hat-shaped	Hat-shaped	Hat-shaped
Length	3-5(-7)µm	(4-)5-6(-6) µm	(3-)3-4(-5) µm	(3-)3-5(-6) µm	(3-)3-4(-6) µm	(4-)5-(-7) µm
Width	2-4(-5)µm	(3-)3-4(-5) µm	(4-)4-5(-6) µm	(4-)4-6(-8) µm	(3-)4-5 (-7) µm	(2-)3-4(-5) µm
Aggregation	n/a	Yellow-buff	Yellow-buff	Yellow-buff	Yellow-buff	n/a

Table 3. Morphological differences between species described in this study and closely related species in the *C. moniliformis sensu lato* group.

Character	*C. oblonga*	*C. savannae*	*C. moniliformis*[a]
ASCOMATA			
Base			
Shape	Obpyriform	Globose	Globose
Colour	Dark brown to black	Dark brown	Brown / black
Diameter	(13-)180-254(-315) µm	(155-)178-217(-248) µm	90-180
Ornamentation	(8-)11-16(-19) µm	(1-)3-8(-13) µm	12-16 x 6 µm
Neck			
Colour	Dark brown to black	Dark brown	Na
Collar	Disc shaped	Disc shaped	Na
Length	(405-)502-721(-881) µm	(359-)455-703(-775) µm	Na
Width (tip)	(12-)13-18(-23) µm	(13-)12-21(-24) µm	Na
Width (base)	(30-)46-69(-76) µm	(37-)48-59(-62) µm	Na
Ostiolar hyphae			
Orientation	Divergent	Divergent	Divergent
Length	(22-)23-27(31) µm	(17-)25-40(-46) µm	12-18 µm
Ascospores			
Colour	Hyaline	Hyaline	Hyaline
Shape (side view)	Hat-shaped	Hat-shaped	Oval, one side flat
Length	(3)-3-4(-4) µm	(5-)5-5 (-6) µm	4-5 µm
Width	(6-)7-8(-8) µm	(2-)3-3(-4) µm	3-4 µm
Aggregation	Yellow-buff	Straw yellow	Na

[a]Hedgcock, 1906

Moreau and *C. resenifera* T.C. Harr. & M.J. Wingf. all represented distinct clades, supported by high bootstrap values. *Thielaviopsis* isolates collected from *Eucalyptus* in Tanzania (Group H) formed a separate, well supported clade (Fig. 2). This tree had a length of 265 steps, the total number of characters were 468, with 303 of these being constant and 165 parsimony-informative, with CI = 0.8868, RI = 0.9690 and RC = 0.8593. In the phylogenetic tree, the *Thielaviopsis* isola-tes obtained in this study grouped most close-ly to *C. bhutanensis* and *C. omanensis*, sepa-rate from other known *Thielaviopsis* spp. and with strong bootstrap support (Fig. 2). These isolates were then further compared to all *Ceratocystis* spp. in the *C. moniliformis s.l.* group using all three gene regions, confirming their unique nature, as well as their affinity to *C. bhutanensis* and *C. omanensis* (Fig. 3).

Two most parsimonious trees were obtained for the combined data set of the *C. moniliformis s.l.* group, of which one is presented (Fig. 3). This tree had a length of 578 steps, the total number of characters were 1273, with 844 constant, three characters parsimony-uninformative and 426 parsimony-informative characters, with CI = 0.9135, RI = 0.9595 and RC = 0.8765. In the phyloge-netic tree, *C. savannae*, *C. omanensis*, *C. bhutanensis*, *C. moniliformis*, *C. tribiliformis* M. van Wyk & M.J. Wingf. and *C. monili-formopsis* Z.Q. Yuan & C. Mohammed all resided in distinct clades, supported by high bootstrap values. Clades emerging from the phylogenetic analyses were consistent with those (Group G, Group F, Group H) that emerged based on morphology for the African isolates. Isolates (CMW23802, CMW23803, CMW23804) obtained from *A. mearnsii* from South Africa (Group G) grouped in a distinct clade (Clade 6) with strong (100%) bootstrap support, closest to *C. savannae*. Isolates in Group F obtained from *Eucalyptus* in Malawi (CMW15242, CMW15245, CMW15248) grouped in a distinct clade (Clade 7) with strong (100%) bootstrap support, most closely related to the isolates obtained from *A. mearnsii* from South Africa (Clade 6) and *C. savannae*. Isolates in Group H obtained from *Eucalyptus* spp. in Tanzania (CMW22284, CMW22289) and South Africa (CMW17587, CMW17960) grouped to together within the *C. monilifor-*

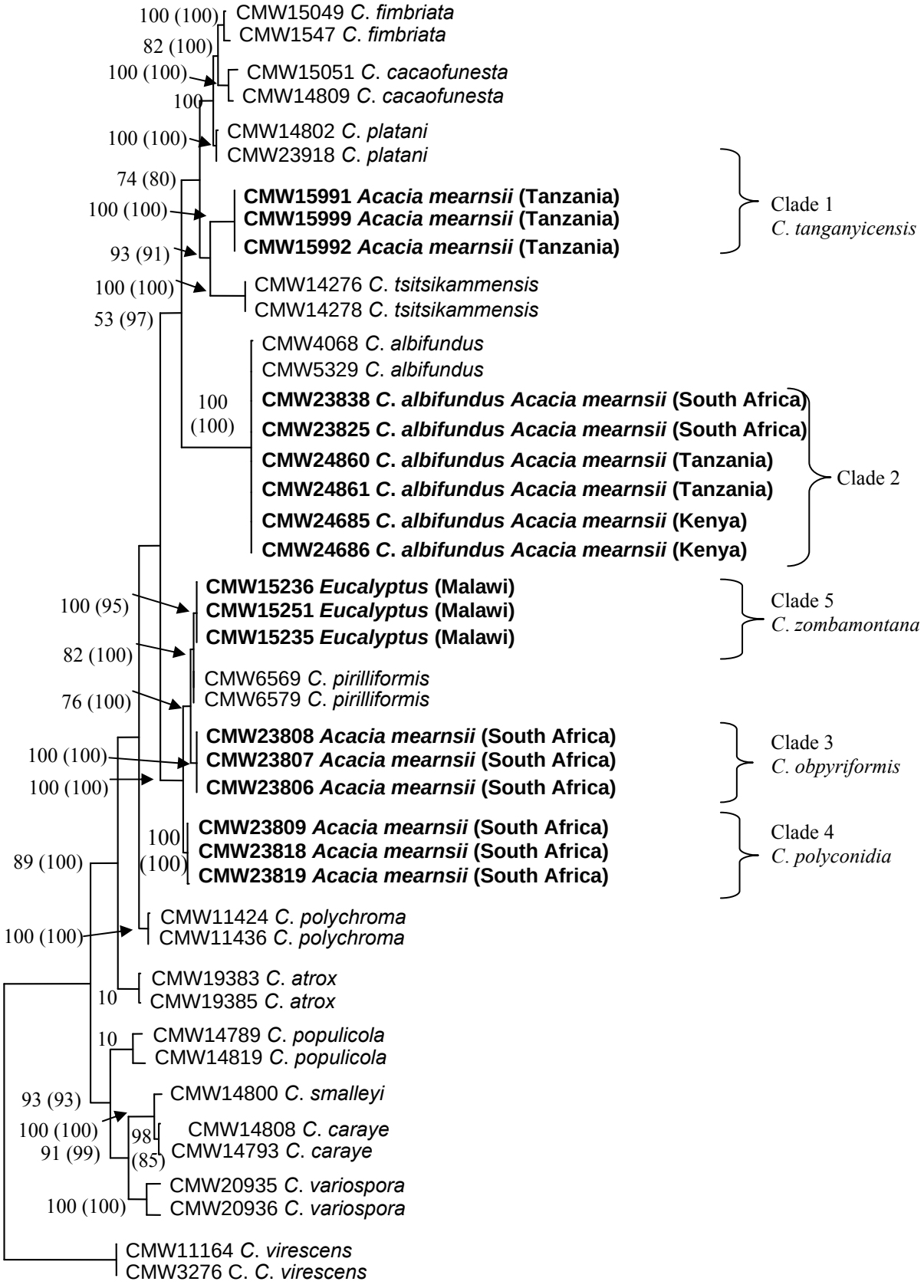

Fig. 1. Phylogenetic tree based on the combined ITS, *β*-tubulin and EF1-α gene regions of the *Ceratocystis fimbriata sensu lato* group of isolates. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated above the branches with posterior probability values in brackets. *Ceratocystis virescens* represents the out-group taxon. Clades 1-5 (Bold) indicate isolates identified in this study.

mis clade (Clade 8). Posterior proba-bility values calculated for the branch nodes supported the bootstrap values (Fig. 3).

Taxonomy

Comparison of DNA sequence data for the African isolates resulted in distinct phylogenetic lineages, consistent with the groups that emerged from morphological comparisons. These thus showed that five previously undescribed *Ceratocystis* spp. and one new *Thielaviopsis* sp. were amongst the isolates collected in this study (Table 2, Table 3). These fungi are consequently described as new taxa.

Ceratocystis zombamontana R.N. Heath & Jol. Roux, **sp. nov.** (Fig. 4)

MycoBank: 511245

Etymology: The name refers to the Zomba mountain in Malawi where this fungus was first isolated.

Coloniae badiae. *Colla ascomatum* atrobrunnea vel nigra, apicem versus pallescentia (264-) 315–442 (-535) µm longa, apicibus (13-) 156–21 (-23) µm latis. *Ascosporae* lateraliter visae cucullatae vel pileiformes (4-) 5–6 (-6) × (3-) 3–4 (-5) µm, e supra visae doliiformes (5-) 6–7 (-8) × (5-) 6–7 (-8) µm. *Anamorpha Thielaviopsis* cum conidiophoris phialidicis in mycelio singulis, hyalinis. Conidiophorae primariae ampulliformes. Conidia primaria cylindrica baciliformia; secondaria doliiformia. *Chlamydosporae* absunt.

Colonies hair brown (17""i), reverse hair brown (17""i). *Mycelium* mostly superfi-cial and smooth, sparse tawny olive (19"i) aerial mycelium. *Optimal temperature* for growth 20-25°C, minimal growth at 5°C and no growth at 35°C. Slow growing, reaching 41 mm in 14 days at 20 and at 25°C. *Ascomatal bases* dark brown to black, subglobose to ovoid, (130-) 152–196 (-222) µm long, (106-) 132–181 (-208) µm wide. Ornamentation absent. *Ascomatal necks* dark brown to black at base becoming lighter brown to hyaline towards apices, (264-) 315–443 (-535) µm long, (24-) 30–38 (-42) µm wide at bases of necks, (13-) 16–21 (-23) µm wide at tips of necks. *Ostiolar hyphae* divergent, hyaline, (16-) 20–25 (-27) µm long. *Asci* not observed. *Ascospores* accumulating in round, white to yellow (yellow-buff 19d) masses at the apices of the ascomatal necks, embedded in sheath tissue, aseptate, hyaline, cucullate (hat-shaped) in side view, (4-) 5–6 (-6) µm long, (3-) 3–4 (-5) µm wide without sheath, doliiform in top view, (5-) 6–7 (-8) µm long, (5-) 6–7 (-8) µm wide including sheath.

Thielaviopsis anamorph: *Conidiophores* occurring singly on mycelium, conidiophores phialidic, hyaline, primary conidiophores flask shaped (55-) 69–101 (-119) µm long, (4-) 5–7 (-8) µm wide at bases, (3-) 3–5 (-5) µm wide at tips. Secondary conidiophores flaring, (72-) 74–117 (-128) µm long, (4-) 4–6 (-7) µm wide at bases, (4-) 4–4 (-6) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, baciliform, (15-) 16–21 (-26) µm long, (3-) 3–4 (-6) µm wide, secondary conidia barrel-shaped, (5-) 6–7 (-9) µm long, (3-) 3–4 (-4) µm wide. *Chlamydospores* absent.

Material examined: Malawi, Zomba Mountain (S 15° 21.269, E 035° 18.163). Isolated from wounds on *Eucalyptus grandis*. Collected: R.N. Heath and J. Roux, 2004. PREM59804 (h**olotype)**, CMW15236 = CBS122296 (**culture ex-type).** *Additional specimens*: Malawi, Zomba Mountain (S 15° 21.269, E 035° 18.163). Isolated from wounds on *Eucalyptus grandis*. Colected: R.N. Heath and J. Roux, 2004. PREM59805 (paratype), CMW15235 = CBS122297; CMW15251 = CBS122298 = PREM59806; CMW15242 = PREM59807 (**culture ex-type**).

Ceratocystis polyconidia R.N. Heath & Jol. Roux, **sp. nov.** (Fig. 5)

MycoBank: 511246.

Etymology: The name refers to the abundance of primary conidia produced.

Coloniae isabellinae. *Colla ascomatum* atro-brunnea vel nigra, apicem versus pallescentia vel hyalina (326-) 589–429 (-694) µm longa, apicibus (11-) 13–19 (-23) µm latis. *Ascosporae* lateraliter visae cucullatae vel pileiformes (3-) 3.1–4.1 (-5) x (3.5-) 4.3–5.3 (-5.8) µm, e supra visae doliiformes. *Anamorpha Thielaviopsis* cum conidiophoris phialidicis in mycelio singulis, hyalinis. Conidiophorae primariae ampulliformes. Conidiophorae secondariae expansae. Conidia primaria cylindrica baciliformia; secondaria doliiformia. Chlamydosporae terminales, singulae, parietibus crassis, subglobosae, brunneae.

Colonies isabella (19"i), reverse isabella (19"i). *Mycelium* mostly superficial, smooth, segmented, sparse hazel (17"i) aerial mycelium. *Optimal temperature* for growth

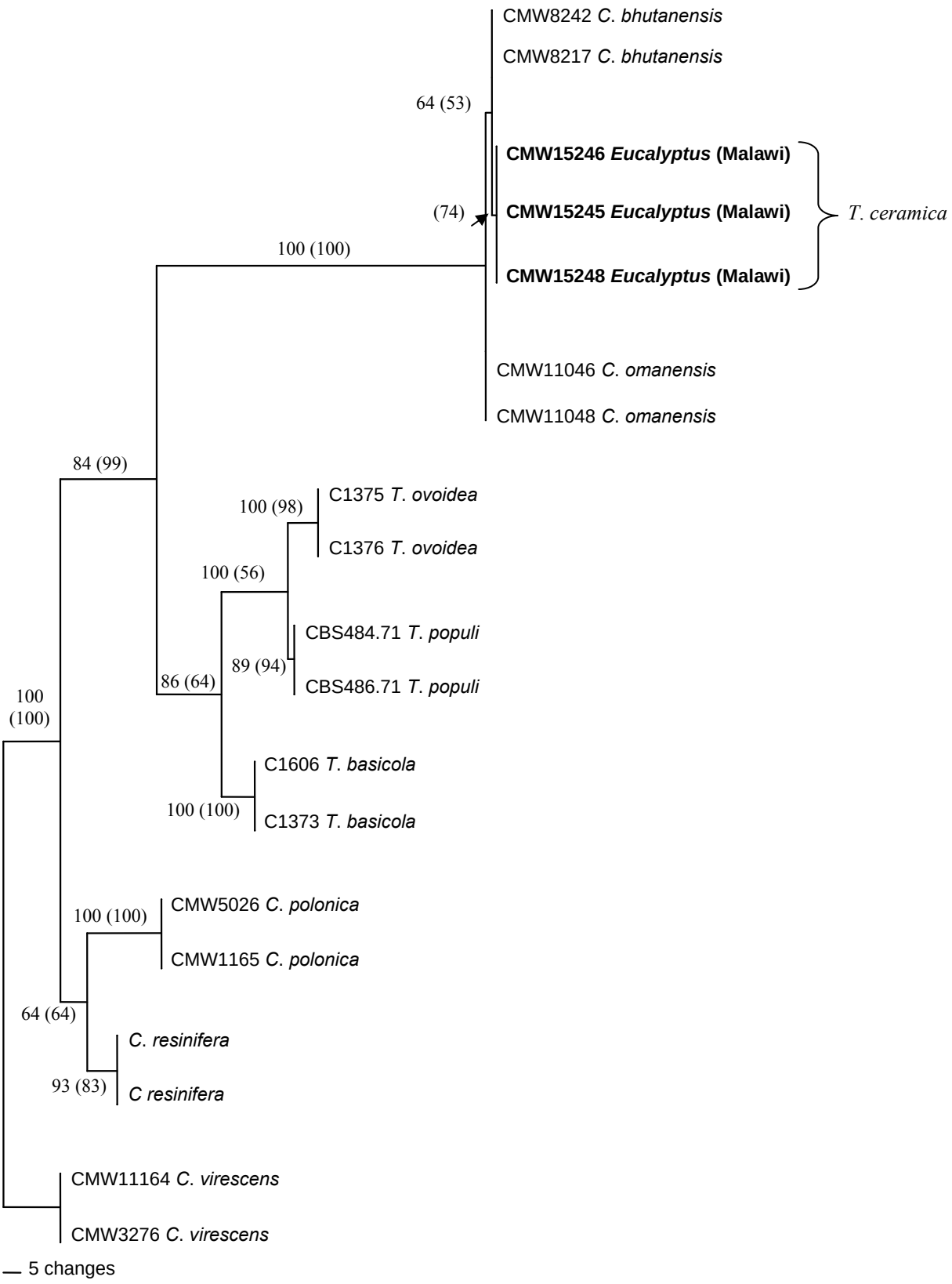

Fig. 2. Phylogenetic tree based on the ITS gene region of *Thielaviopsis* spp. and *Ceratocystis* spp. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated above the branches with posterior probability values in brackets. *Ceratocystis virescens* represents the out-group taxon

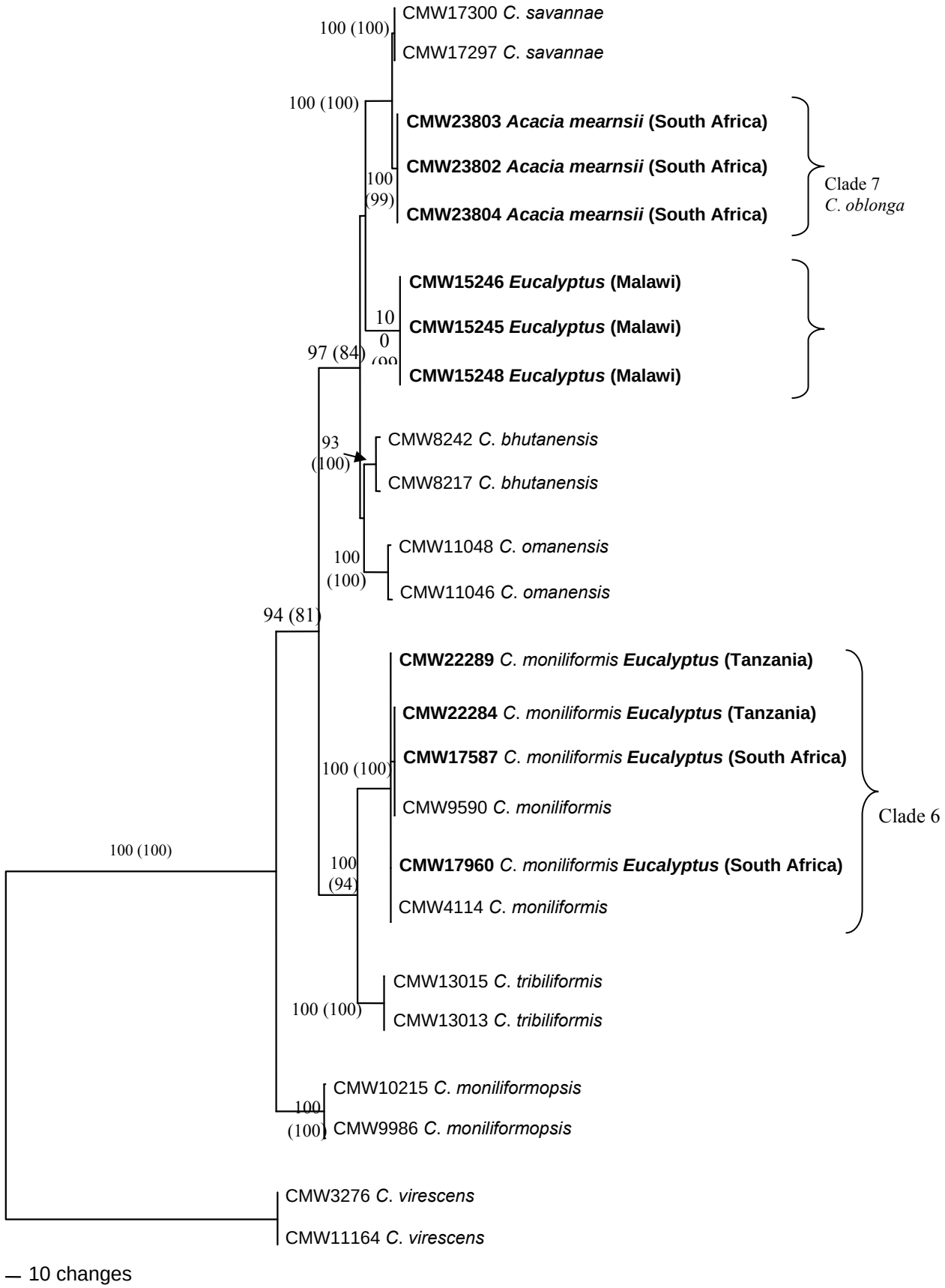

Fig. 3. Phylogenetic tree based on the combined ITS, *β*-tubulin and EF1-α gene regions of the *Ceratocystis moniliformis sensu lato* group. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated above the branches with posterior probability values in brackets. *Ceratocystis virescens* represents the out-group taxon. Clades 6-8 (Bold) indicate isolates identified in this study.

20-25°C. Minimal growth at 5°C and 35°C. Fast growing, reaching 81 mm in 7 days at 25°C. *Ascomatal bases* dark brown to black, globose, (121-) 153–221 (-269) µm long, (133-) 153–223 (-277) µm wide. No ornamentation. *Ascomatal necks* dark brown to black at bases becoming lighter brown to hyaline towards apices, (326-) 389–429 (-694) µm long, (20-) 26–34 (-39) µm wide at bases of necks, (11-) 13–19 (-23) µm wide at tips of necks. *Ostiolar hyphae* divergent, hyaline, (36-) 39–47 (-57) µm long. *Asci* not observed. *Ascospores* accumulating in round, white to yellow (yellow-buff 19d) masses at the apices of the ascomatal necks, cucullate in side view, (3-) 3–4 (-5) µm long, (4-) 4–5 (-6) µm wide without sheath and (4-) 4–5 (-6) µm wide with sheath, doliiform in top view.

Thielaviopsis anamorph: Primary and secondary *conidiophores* occurring singly on mycelium, conidiophores phialidic, hyaline, primary conidiophores flask-shaped, (72-)74–117 (-128) µm long, (4-) 4–6 (-7) µm wide at bases, (6-) 6–8 (-8) at the widest point, (4-) 4–4 (-6) µm wide at tips. Secondary conidiophores flaring at apices, (38-) 52–87 (-105) µm long, (4-) 5–7 (-7) µm wide at bases, (5-) 6–8 (-9) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, or in chains, primary conidia cylindrical, bacilliform, (14-) 18–25 (-28) µm long, (4-) 4–6 (-6) µm wide, secondary conidia barrel-shaped, (8-) 9–11 (-13) µm long, (5-) 6–8 (-8) µm wide. *Chlamydospores* terminal, single, thick walled, sub-globose, argus brown (13m), (9-) 9–11 (-13) µm long, (8) 11–14 (-16) µm wide.

Material examined: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stumps of *Acacia mearnsii*. Collected: R.N. Heath, 2006. PREM59788 (**holotype**), CMW23809 = CBS 122289 (**culture ex-type**).

Additional specimens: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stumps of *Acacia mearnsii*. Collected: R.N. Heath, 2006. PREM59789 (**paratype**), CMW23818 = CBS 122290; CMW23819 = CBS(122821) = PREM59790; CMW 23817 = PREM59791, CMW23810 = PREM 59863 (**culture ex-type**).

Ceratocystis tanganyicensis R.N. Heath & Jol. Roux**, sp. nov.** (Fig. 6)

MycoBank: 511247

Etymology: The name refers to the famous lake Tanganyika in Tanzania, not far from where this fungus was first isolated.

Coloniae eburneo-atrovirides. *Colla ascomatum* atrobrunnea vel nigra, apicem versus pallescentia vel hyalina (302-) 366–484 (-558) µm longa, apicibus (13-) 14–18 (-21) µm latis. *Ascosporae* lateraliter visae cucullatae vel pileiformes (3-) 3–5 (-6) x (4-) 4–6 (-8) µm vagina exclusa, e supra visae doliiformes. *Anamorpha Thielaviopsis* cum conidiophoris phialidicis. Conidiophorae primariae ampulliformes. Conidiophorae secondariae expansae. Conidia primaria cylindrica baciliformia; secondaria doliiformia. *Chlamydosporae* in hyphis singulae, globosae.

Colonies dark ivory green (25"m), reverse ivory green (25"m). *Mycelium* mostly superficial and smooth, sparse tawny olive (19"i) aerial mycelium. *Optimal temperature* for growth 20°C. No growth at 5°C or at 35°C. Slow growing, reaching 27 mm in 7 days at 20°C. *Ascomatal bases* dark brown to black, subglobose, (127-) 149–190 (-216) µm long, (119-) 138–177 (-205) µm wide. No ornamentation. *Ascomatal necks* dark brown to black at base becoming lighter brown to hyaline towards apices, (302-) 366–484 (-558) µm long, (19-) 24–32 (-37) µm wide at bases of necks, (13-) 14–18 (-21) µm wide at tips of necks. *Ostiolar hyphae* divergent, hyaline, (17-) 39–47 (-47) µm long. *Asci* not observed. *Ascospores* accumulating in round, white to yellow (yellow-buff 19d) masses at the apices of the ascomatal necks, cucullate in side view, (3-) 3–5 (-6) µm long, (4-) 4–6 (-8) µm wide without sheath and (5-) 6–7 (-8) µm wide with sheath, doliiform in top view.

Thielaviopsis anamorph: Primary and secondary *conidiophores* occurring singly on mycelium, conidiophores phialidic, hyaline, primary conidiophores flask shaped, (49-) 60–116 (-179) µm long, (4-) 5–7 (-8) µm wide at bases, (4-) 6–8 (-9) at the widest point, (3-) 4–5 (-8) µm wide at tips. Secondary conidiophores flaring at the apices, (43-) 55–85 (-98) µm long, (4-) 5–7 (-8) µm wide at bases, (5-) 6–8 (-9) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, bacilliform, (12-) 14–19 (-24) µm long, (3-) 4–5 (-5) µm wide, secondary conidia barrel-shaped, (3-) 3–9 (-13) µm long, (6-) 7–10 (-12) µm wide. *Chlamydospores* developing singly on

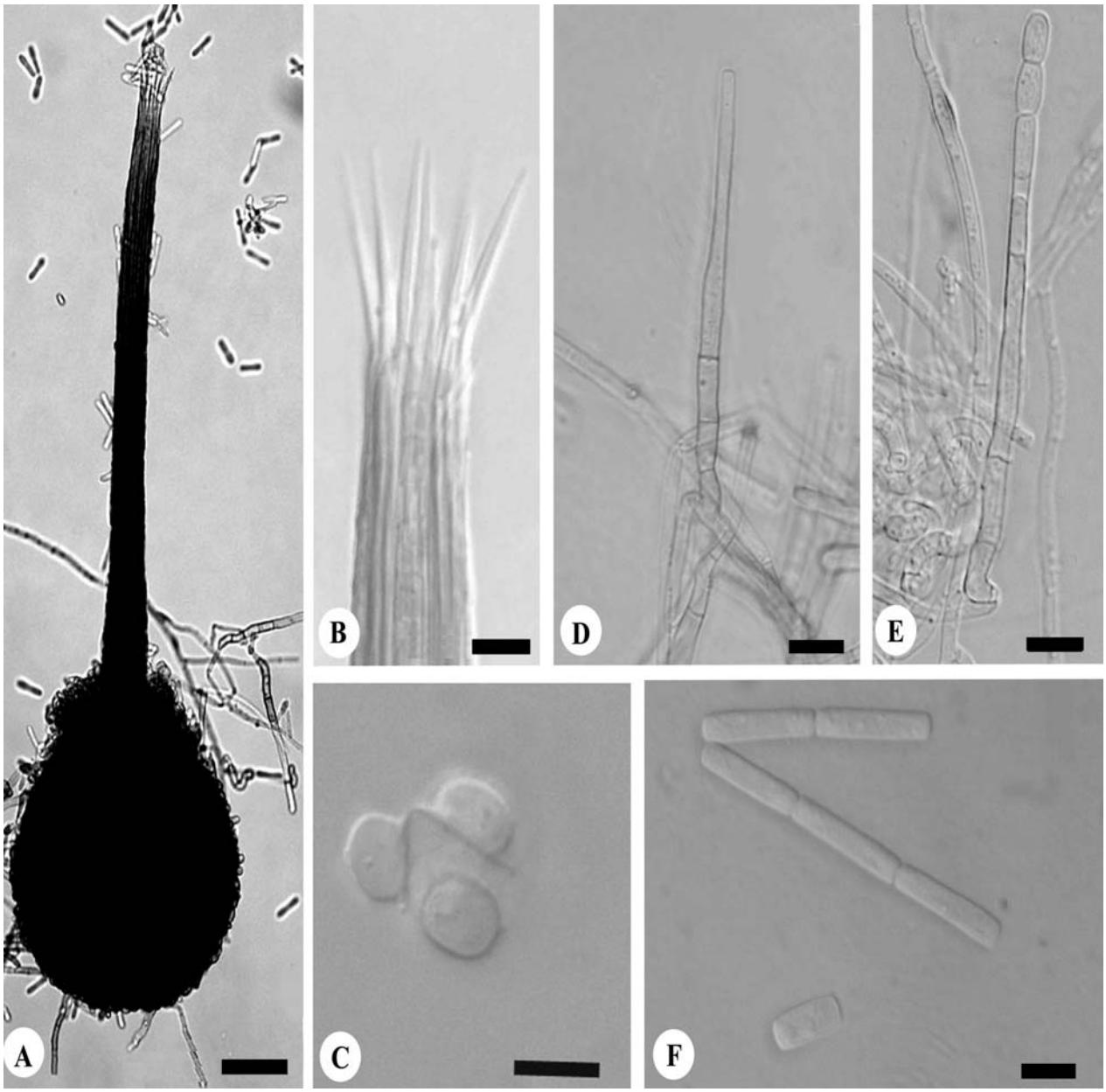

Fig. 4. Morphological characteristics of *Ceratocystis zombamontana*: A. Obpyriform ascomata. Scale bar = 25 µm B. Divergent ostiolar hyphae. Scale bar = 5 µm C. Hat-shaped ascospores. Scale bar = 2.5 µm D. Flask shaped primary phialide Scale bar = 10 µm E. Secondary phialides producing barrel-shaped conidia. Scale bar = 10 µm F. Primary conidia, cylindrical (above) and secondary conidium, barrel-shaped (below) Scale bar = 10 µm.

hyphae, argus brown (13m), globose, (10-) 10–13 (-14) µm long, (3-) 10–12 (-13) µm wide.

Specimens examined: Tanzania, Njombe area (S 09° 16.366, E 034° 38.765). Isolated from cutstumps of *Acacia mearnsii*. Collected R.N. Heath and J. Roux, 2004. PREM59800 (**holotype**), CMW15992 = CBS 122293 (**culture ex-type**).

Additional specimens: Tanzania, Njombe area (S 09° 16.366, E 034° 38.765). Isolated from cutstumps of *Acacia mearnsii*. Collected R.N. Heath and J. Roux, 2004. PREM59801 (**paratype**), CMW15999 = CBS 122294; CMW15991 = CBS122295 = PREM 59802; CMW15993 = PREM59803, CMW15988 = PREM59864 (**culture ex-type**).

Ceratocystis obpyriformis R.N. Heath & Jol. Roux, **sp. nov.** (Fig. 7)

MycoBank: 511248

Etymology: The name refers to the distinctly obpyriform shape of the ascomatal bases.

Coloniae olivaceae. *Colla ascomatum* atrobrunnea vel nigra, apicem versus pallescentia (477-) 569–675 (-708) µm longa, apicibus (13-) 16–21 (-26) µm latis. *Ascosporae* lateraliter visae cucullatae vel pileiformes (3-) 3–4 (-6) µm longa, apicibus (3-) 4–5 (-

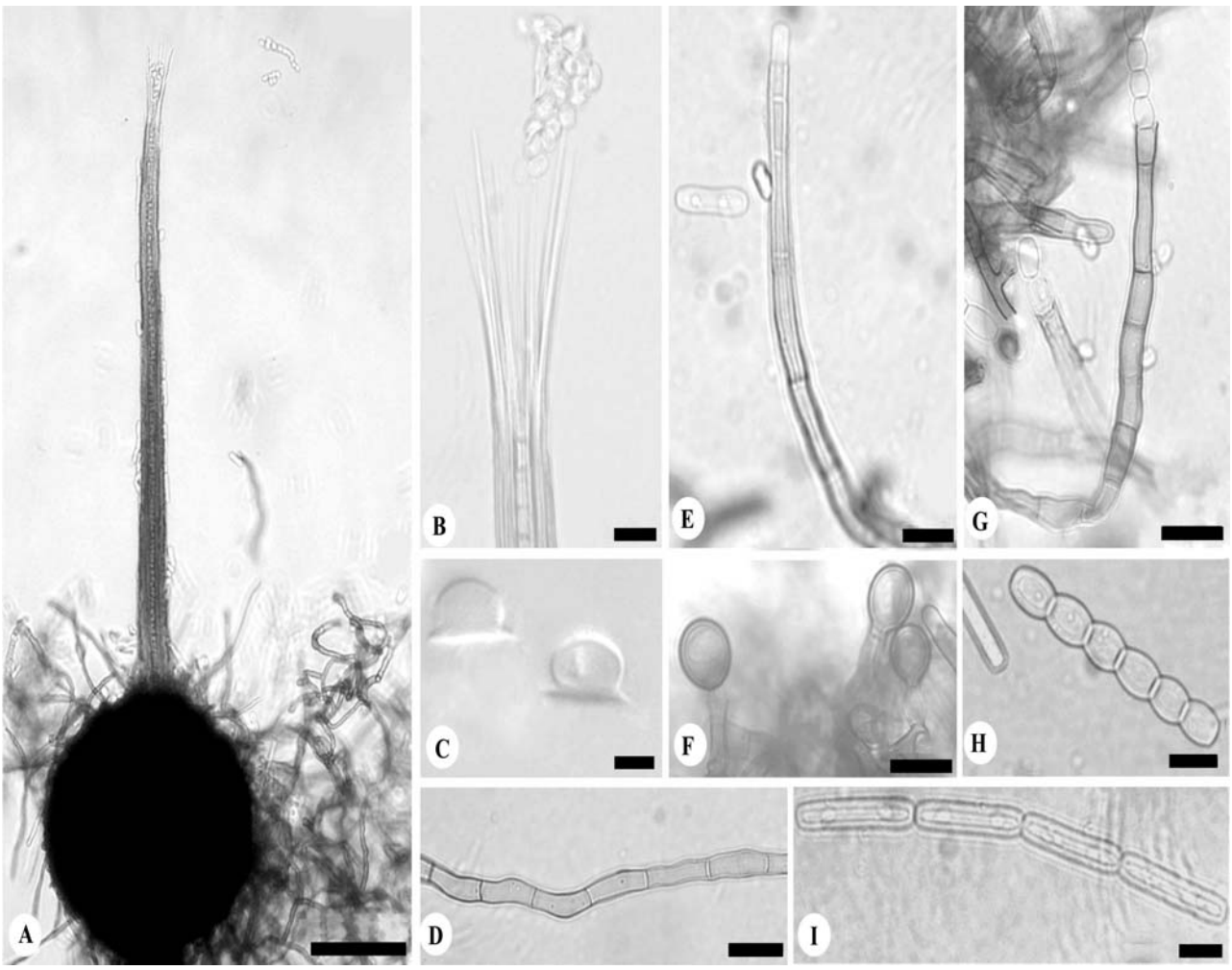

Fig. 5. Morphological characteristics of *Ceratocystis polyconidia*: A. Globose ascomatal base. Scale bar = 100 µm B. Divergent ostiolar hyphae with emerging hat-shaped ascospores. Scale bar = 10 µm. C. Hat-shaped ascospore. Scale bar = 2.5 µm. D. Segmented hyphae. Scale bar = 10 µm. E. Flask-shaped primary phialide with emerging cylindrical conidia. Scale bar = 20 µm. F. Subglobose chlamydospores. Scale bar = 10 µm. G. Secondary phialide producing barrel-shaped conidia in chains. Scale bar = 20 µm. H. Barrel-shaped conidia in chains. Scale bar = 10 µm. I. Cylindrical conidia in chains. Scale bar = 5 µm.

7) µm latis vagina exclusa, e supra visae doliiformes. *Anamorpha Thielaviopsis* cum conidiophoris phialidicis in mycelio singulis, hyalinis. Conidiophorae primariae ampulliformes. Conidiophorae secondariae expansae. Conidia primaria cylindrica baciliformia; secondaria doliiformia. *Chlamydosporae* absunt.

Colonies olivaceous (21"k), reverse olivaceous (21"k). *Mycelium* mostly superficial and smooth, sparse white aerial mycelium. *Optimal temperature* for growth at 25°C. No growth at 5°C or at 35°C. Fast growing, reaching 52 mm in 7 days at 25°C. *Ascomatal bases* dark brown to black, obpyriform, (152-) 177-217 (-233) µm long, (149-) 166–206 (-228) µm wide. No ornamentations. *Ascomatal necks* dark brown to black at base becoming lighter brown to hyaline towards apices, (477-) 569–675 (-708) µm long , (26-) 28–36 (-45) µm wide at bases of necks, (13-) 16–21 (-26) µm wide at tips of necks. *Ostiolar hyphae* divergent, hyaline, (34-) 37–47 (-54) µm long. *Asci* not observed. *Ascospores* accumulating in round, white to yellow (yellow-buff 19d) masses at the apices of the ascomatal necks, embedded in sheath, aseptate, hyaline, cucullate in side view, (3-) 3–4 (-6) µm long, (3-) 4–5 (-7) µm wide without sheath and (4-) 5–8 (-8) µm wide with sheath, doliiform in top view.

Thielaviopsis anamorph: Primary and secondary *conidiophores* occurring singly on mycelium, conidiophores phialidic, hyaline, primary conidiophores flask shaped, (57-) 75–124 (-157) µm long, (4-) 5–6 (-7) µm wide at bases, (3-) 6–8 (-8) µm at the widest point, (3-) 4–5 (-5) µm wide at tips. Secondary conidiophores flaring at apices, (54-) 56–74

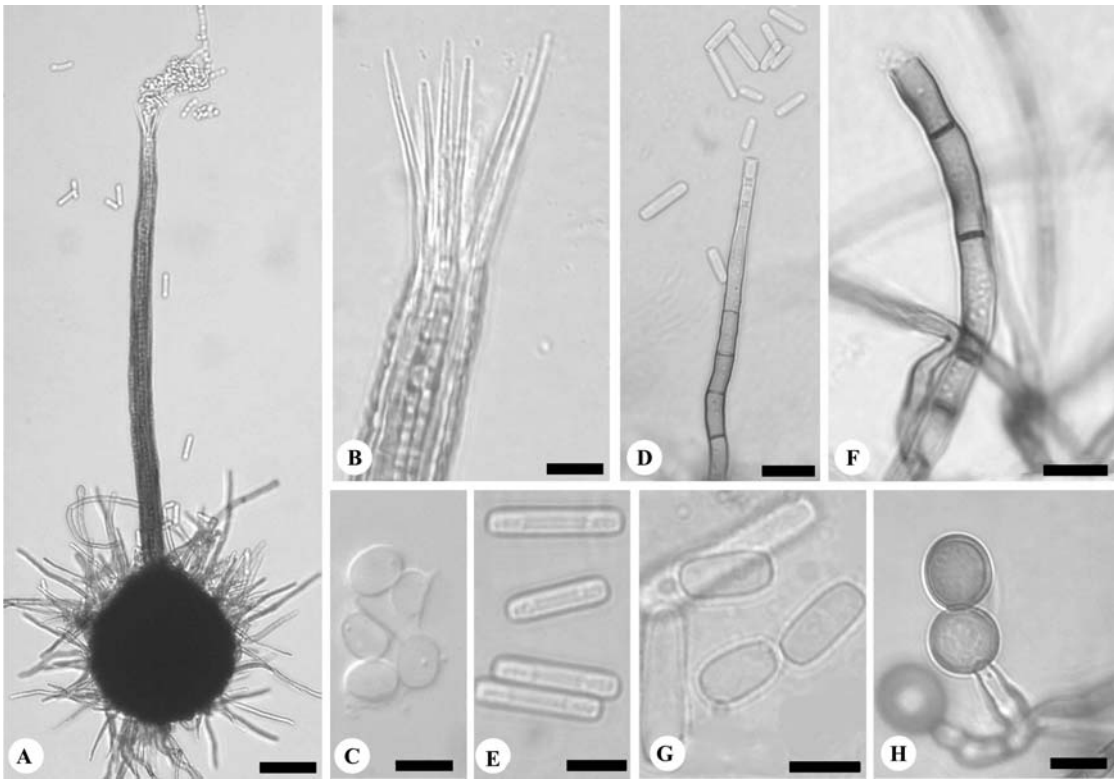

Fig. 6. Morphological characteristics of *Ceratocystis tanganyicensis*: A. Subglobose ascomatal base. Scale bar = 100 µm. B. Divergent ostiolar hyphae. Scale bar = 10 µm. C. Hat-shaped ascospores. Scale bar = 5 µm. D. Flask-shaped primary phialide producing cylindrical conidia. Scale bar = 20 µm. E. Cylindrical conidia. Scale bar = 10 µm. F. Secondary phialide producing barrel shaped conidia. Scale bar = 5 µm. G. Barrel shaped conidia. Scale bar = 5 µm. H. Globose chlamydospores produced singly or in chains. Scale bar = 10 µm.

(-83) µm long, (4-) 4–6 (-7) µm wide at bases, (5-) 6–8 (-9) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, bacilliform, (14-) 15–20 (-24) µm long, (3-) 3–5 (-6) µm wide, secondary conidia barrel-shaped, (8-) 10–12 (-13) µm long, (5-) 6–8 (-9) µm wide. *Chlamydospores* absent.

Material examined: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stumps of *Acacia mearnsii*. Collected: R.N. Heath, 2006. PREM59796 (**holotype**), CMW23808 = CBS122511 (**culture ex-type**).

Additional specimens: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stumps of *Acacia mearnsii*. Collected: R.N. Heath, 2006. **Paratype**, PREM59797 (**paratype**), CMW 23807 = CBS122608; CMW23806 = CBS12609 = PREM59798; CMW27862 = PREM59799 (**culture ex-type**).

Ceratocystis oblonga R.N. Heath & Jol. Roux **sp. nov.** (Fig. 8)

MycoBank: 511249.

Etymology: The name refers to the oblong shape of the secondary conidia.

Coloniae iuvenes albae, fuscantes. *Colla ascomatum* atrobrunnea vel nigra, apicem versus pallescentia (405-) 5025–721 (-881) µm longa, apicibus (12-) 13–18 (-23) µm latis. *Ascosporae* lateraliter visae cucullatae vel pileiformes (3-) 3–4 (-4) x (4-) 5–6 (-6) µm vagina exclusa, e supra visae doliiformes. *Anamorpha Thielaviopsis* cum conidiophoris phialidicis in mycelio singulis, hyalinis. Conidiophorae secondariae expansae. Conidia primaria cylindrica baciliformia; secondaria oblonga, apicibus truncatis. *Chlamydosporae* absunt.

Colonies white when young, becoming deep colonial buff (21"b), reverse grayish sepia (15""i). *Mycelium* superficial producing aerial mycelia. *Hyphae* granular. *Optimal temperature* for growth at 20-25°C. Minimal growth at 5°C and 35°C. Fast growing, reaching 83 mm in 7 days at 25°C. *Ascomatal bases* dark brown to black, obpyriform, (149-) 206–329 (-372) µm long, (130-) 180–254 (-315) µm wide with conical spines, (8-) 11–16 (-19) µm long. *Ascomatal necks* dark brown to black at base becoming lighter brown to hyaline towards apices,

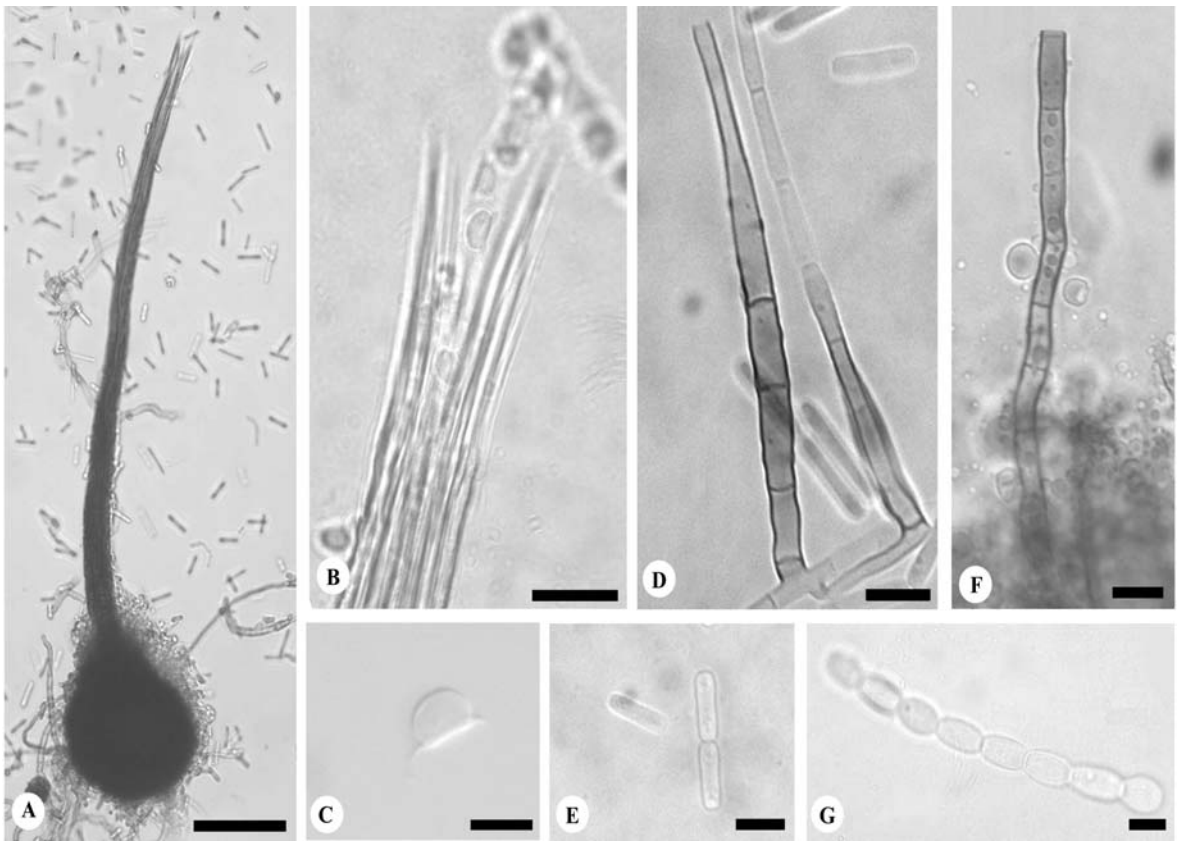

Fig. 7. Morphological characteristics of *Ceratocystis obpyriformis*. A. Obpyriform ascomatal base. Scale bar = 100 µm. B. Divergent ostiolar hyphae with emerging hat-shaped ascospores. Scale bar = 20 µm. C. Hat-shaped ascospores. Scale bar = 5 µm. D. Primary, flasked shaped phialide producing cylindrical conidia. Scale bar = 50 µm. E. Cylindrical conidia. Scale bar = 5 µm. F. Secondary phialides. Scale bar = 20 µm. G. Barrel shaped conidia in chains. Scale bar = 20 µm.

(405-) 502–721 (-881) µm long, (30-) 46–69 (-76) µm wide at bases of necks, (12-) 13–18 (-23) µm wide at tips of necks with disciform bases. *Ostiolar hyphae* divergent, hyaline, (22-) 22–27 (-31) µm long. *Asci* not observed. *Ascospores* accumulating in round, white to yellow(yellow-buff 19d) masses at the apices of the ascomatal necks, embedded in sheath, aseptate, hyaline, cucullate (hat-shaped) in side view, (3-) 3–4 (-4) µm long, (4-) 5–6 (-6) µm wide without sheath and (6-) 7–8 (-8) µm with sheath, doliiform in top view.

Thielaviopsis anamorph: Primary and secondary *conidiophores* occurring singly on mycelium, conidiophores phialidic, hyaline, primary conidiophores flask shaped, (19-) 21–35 (-41) µm long, (2-) 3–4 (-4) µm wide at bases, (2-) 3–4 (-5) µm at the widest point, (2-) 2–3 (-3) µm wide at tips. Secondary conidiophores flaring at apices, (23-) 29–50 (-59) µm long, (3-) 3–4 (-5) µm wide at bases, (4-) 4–6 (-6) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, bacilliform, (12-) 14–19 (-23) µm long, (3-) 3–5 (-5) µm wide, secondary conidia oblong, apices truncate, (5-) 6–7 (-9) µm long, (3-) 4–5 (-6) µm wide. *Chlamydospores* absent.

Material examined: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stump of *Acacia mearnsii*. Collected: R.N. Heath, 2006. PREM59792 (**holotype**), CMW23803 = CBS122291 (**culture ex-type**).

Additional specimens: South Africa, Piet Retief (S 26° 58' 68.5", E 030° 54' 28.3"). Isolated from cut stumps of *Acacia mearnsii*. Collected: R.N. Heath, 2006. PREM59793 (**paratype**), CMW23802 = CBS (122820); CMW23804 = CBS122292 = PREM59794; CMW23805 = PREM59795 (**culture ex-type**).

Thielaviopsis ceramica R.N. Heath & Jol. Roux. **sp. nov.** (Fig. 9)

MycoBank: 511250.

Etymology: The name originates from the historic name of the geographical region (Zomba, Malawi) where the fungus was found. Historically, the name Zomba originnated from the fact that early settlers used the area to produce clay pots (called

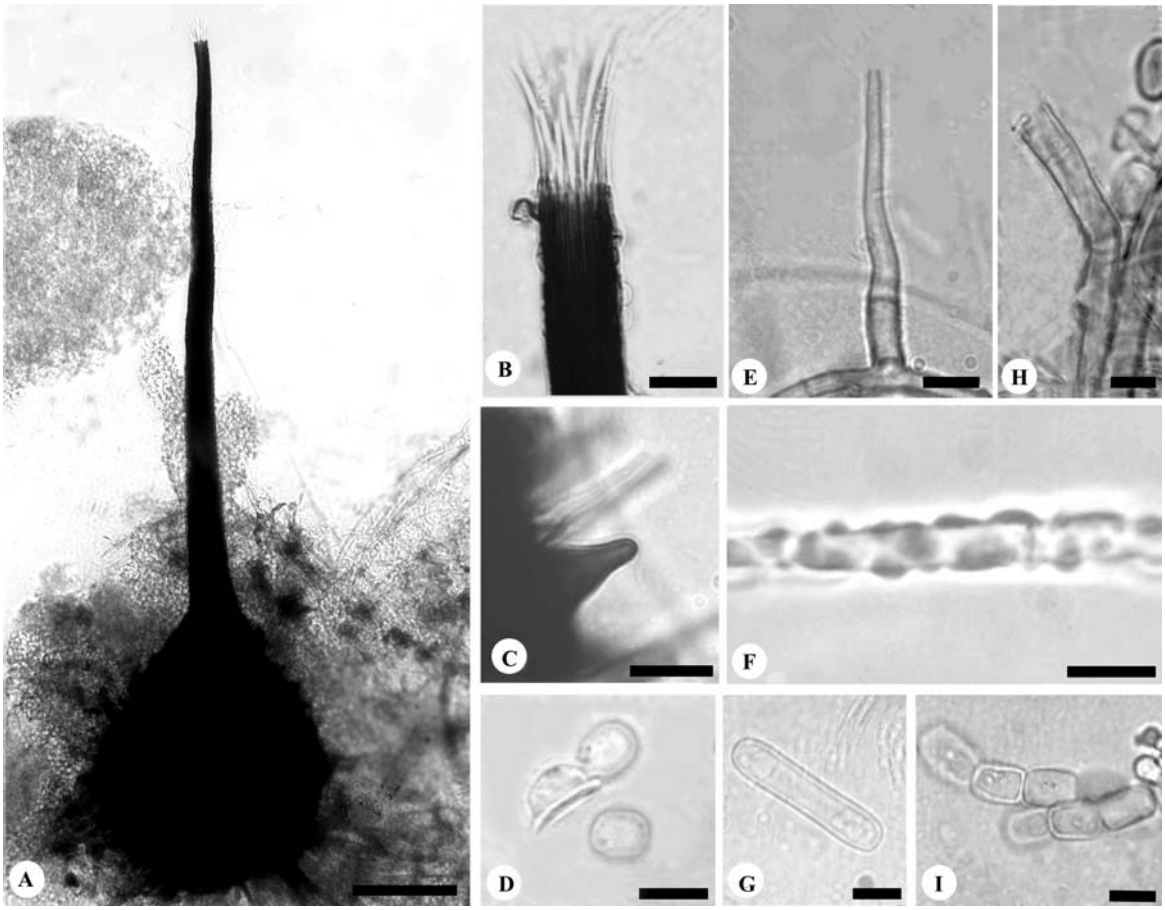

Fig. 8. Morphological characteristics of *Ceratocystis oblonga*: A. Obpyriform ascomatal base. Scale bar = 100 µm. B. Divergent ostiolar hyphae. Scale bar = 20 µm. C. Ascomatal base with short conical spines. Scale bar = 5 µm. D. Hat-shaped ascospores. Scale bar = 5 µm. E. Flask shaped primary phialide producing cylindrical conidia. Scale bar = 10 µm. F. Hyphae with rough walls. Scale bar = 5 µm. G. Cylindrical conidia. Scale bar = 10 µm. H. Secondary phialide producing oblong-shaped conidia. Scale bar = 10 µm. I. Oblong-shaped conidia. Scale bar = 5 µm.

Zoomba omba), where after the region was called Zomba. "*ceramica*" means clay in Greek.

Coloniae iuvenes albae, cum aetate atrobubalinae. Mycelium granulare. Conidiophorae phialidicae in mycelio singulae, hyalinae. *Conidiophorae* primariae ampulli-formes (20-) 22–30 (-36) µm longae, basi (2-) 3–4 (-5) µm, apicibus (2-) 2 (-3) µm latae. *Conidiophorae* secondariae expansae (23-) 33–46 (-53) µm longae, basi (1-) 2–3 (-3) µm, apicibus (2-) 2–3 (-3) µm. *Conidia* primaria cylindrica baciliformia; secondaria doliiformia. *Chlamydosporae* absunt.

Colonies white when young, becoming deep colonial buff (21"b). *Mycelium* super-ficial producing aerial mycelia, granular. *Optimal temperature* for growth at 25°C. No growth at 5, 10 or at 35°C. Fast growing, reaching 90 mm in four days at 25°C. *Coni-diophores* occurring singly on mycelium, co-nidiophores phialidic, hyaline, primary coni-diophores flask shaped, (20-) 22–30 (-36) µm long, (2-) 3–4 (-5) µm wide at bases, (3-) 3–4 (-5) µm wide at middle, (2-) 2 (-3) µm wide at tips. Secondary conidiophores flaring at apices, (25-) 35–46 (-53) µm long, (1-) 2–3 (-3) µm wide at bases, (2-) 2–3 (-3) µm wide at tips. Phialidic *conidium* development through ring wall building, *conidia* of two types, formed singly or in chains, primary conidia cylindrical, bacilliform, (4-) 6–8 (-10) µm long, (1-) 2 (-3) µm wide, secondary conidia barrel-shaped, (2-) 2–3 (-4) µm long, (3-) 4–6 (-8) µm wide. *Chlamydospores* absent.

Material examined: Malawi, Zomba Mountain (S 15° 21.269, E 035° 18.163). Isolated from wounds on *Eucalyptus grandis*. Collected: R.N. Heath and J. Roux, 2004. PREM59808 (**holotype**), CMW15245 = CBS122299, CMW15251 (**culture ex-type**).

Additional specimens: Malawi, Zomba (S 15° 21.269, E 035° 18.163). Isolated from wounds on *Eucalyptus grandis*. Colected: R.N. Heath and J. Roux, 2004. PREM59809 (**paratype**), CMW15248 = CBS

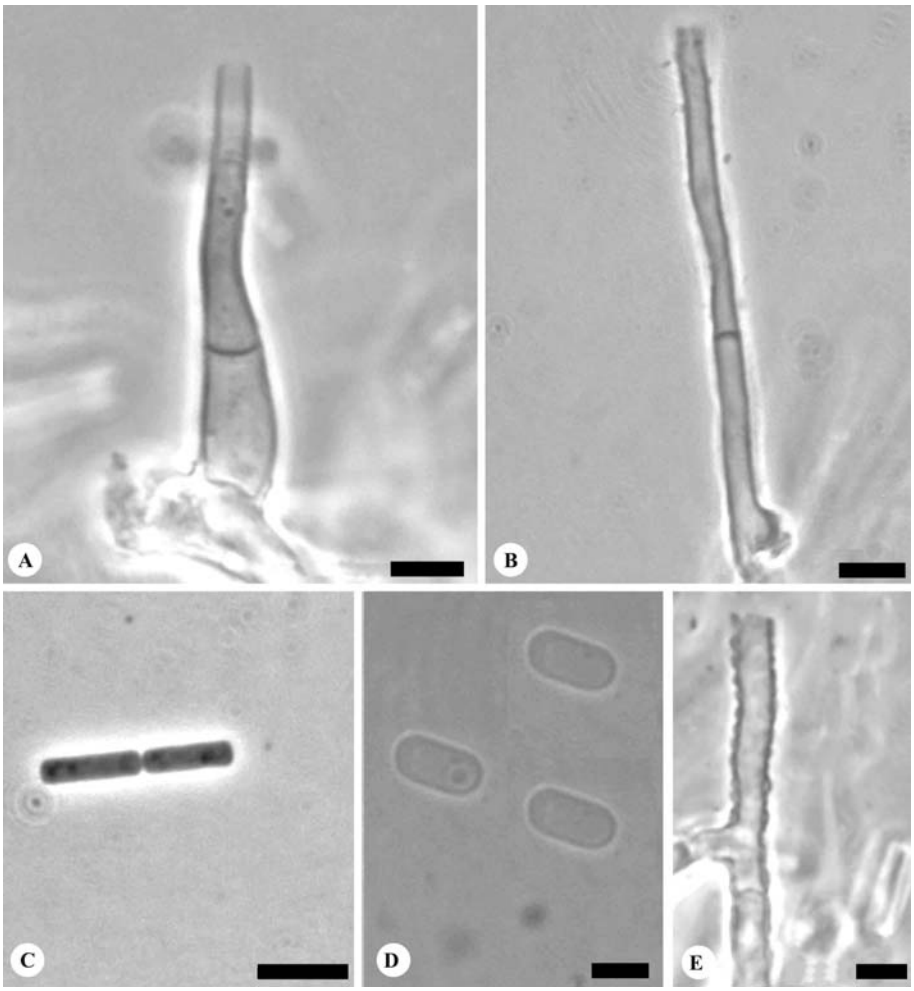

Fig. 9. Morphological characteristics of *Thielaviopsis ceramica*: A. Primary phialide Scale bar = 5 µm. B. Flask shaped secondary phialide. Scale bar = 5 µm. C. Primary cylindrical conidia. Scale bar = 5 µm. D. Secondary barrel-shaped conidia. Scale bar = 2.5 µm. E. Hypha with granulated wall. Scale bar = 5 µm.

122300; CMW15246 = CBS122624 = PREM59810; CMW15238 = PREM59811, CMW15249 = PREM 59865 (**culture ex-type**).

Pathogenicity tests

Greenhouse inoculations on *A. mearnsii* trees with *Ceratocystis* isolates collected in this study resulted in distinct lesions, whereas the control inoculations produced no lesions (Fig. 10). Lesions associated with all isolates differed significantly from the controls ($P<0.0001$). Although *C. polyconidia* (CMW23809, CMW23818) produced the longest lesions, and *C. obpyriformis* (CMW23807, CMW23808) produced the shortest lesions, there were no statistical differences between the lesion lengths produced by the test isolates. After six weeks, the wounds of the control inoculations had begun to recover and to produce callus tissue. All test organisms were consistently re-isolated from the lesions after six weeks. Both replicates of the experiments produced similar results. Greenhouse inoculations on the *E. grandis* (ZG14) trees with all *Ceratocystis* and *Thielaviopsis* isolates resulted in distinct lesions, whereas the control inoculations produced no lesions (Fig. 11). *Ceratocystis zombamontana* (CMW15235, CMW15236) produced significantly larger lesions than *T. ceramica* (CMW15248, CMW 15245) or the control ($P<0.0001$). However, *T. ceramica* also produced significantly larger lesions than the control inoculations ($P<0.0001$). Both the test organisms were consistently isolated from the lesions. Both replicates of the experiments produced the same results. produced no lesions (Fig. 11). *Ceratocystis zombamontana* (CMW15235, CMW15236) produced significantly larger lesions than *T. ceramica*

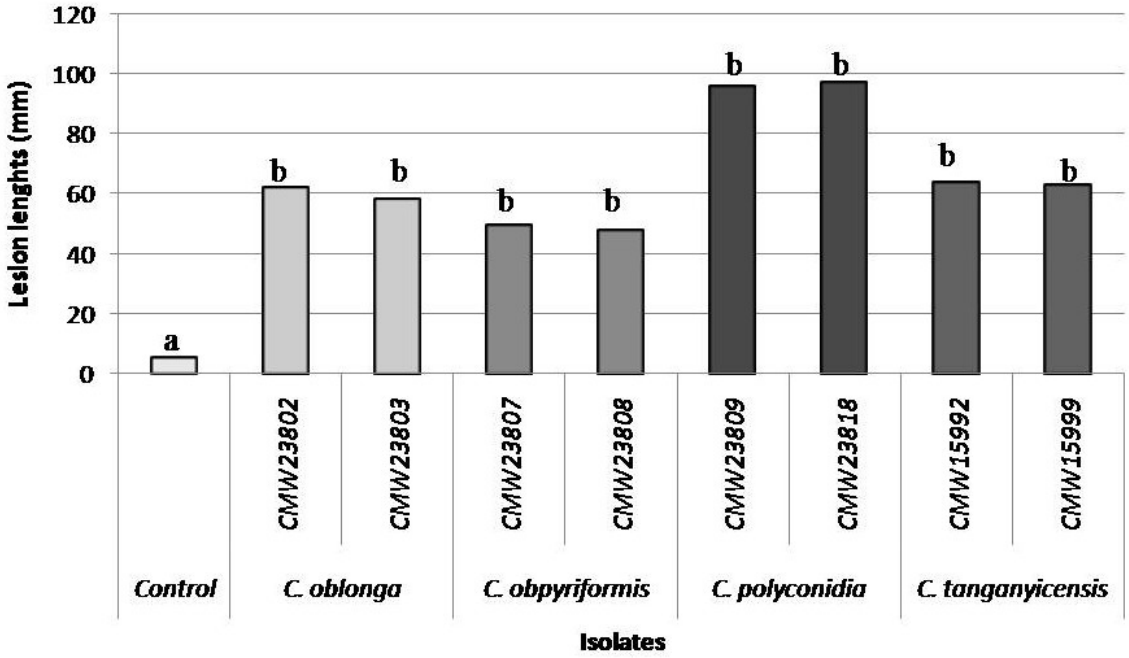

Fig. 10. Bar chart indicating the average lesion length (in millimetres) resulting from inoculation trials with *Ceratocystis oblonga* (CMW23802, CMW23803), *C. obpyriformis* (CMW23807, CMW23808), *C. polyconidia* (CMW23809, CMW23818), and *C. tanganyicensis* (CMW15992, CMW15999) onto *Acacia mearnsii*. Different letters above the bars indicate treatments that are statistically different based on a 95% confidence limit.

(CMW15248, CMW 15245) or the control (P<0.0001). However, *T. ceramica* also produced significantly larger lesions than the control inoculations (P<0.0001). Both the test organisms were consistently isolated from the lesions. Both replicates of the experiments produced the same results.

Discussion

This study focused on identifying *Ceratocystis* spp. from two non-native plan-tation tree species in southern and eastern Africa, led to the discovery of six previously undescribed *Ceratocystis* spp. In addition, *C. albifundus* and *C. moniliformis s.s.* were commonly encountered. Identification of the species arose from a combination of morphological characteristics and comparisons of DNA sequence data, the latter of which were important in recognizing new species for fungi that are morphologically similar.

Two of the previously undescribed species encountered in this study are related to *C. moniliformis* and reside in a group that we refer to as the *C. moniliformis s.l.* species complex. Fungi in the *C. moniliformis s.l.* group can easily be distinguished from other *Ceratocystis* spp. based on the presence of conical spines on their ascomatal bases (Hedgcock, 1906; Hunt, 1956; Upadhyay, 1981). This group also produces disc-like structures at the bases of the ascomatal necks (Bakshi, 1951; Hunt, 1956). The two species described in this study have been provided with the names *C. oblonga* and *T. ceramica*. *Ceratocystis oblonga* grouped close to *C. savannae* within the *C. moniliformis s.l.* species complex, but in a discrete clade with strong bootstrap support. It can also be distinguished from *C. savannae* based on colony colour. In this regard, *C. oblonga* produces white colonies when young, turning colonial buff with age, whereas *C. savannae* produces smokey gray cultures (Kamgan *et al*., 2008). *Ceratocystis oblonga* also has hyphae that have a granular appearance, whereas *C. savannae* has smooth-walled hyphae. Furthermore, *C. savannae* does not produce secondary phialides, but these structures are common in *C. oblonga*. *Ceratocystis oblonga* produces significantly smaller primary and seconddary conidiophores than those of *C. savannae* and it produces secondary conidia

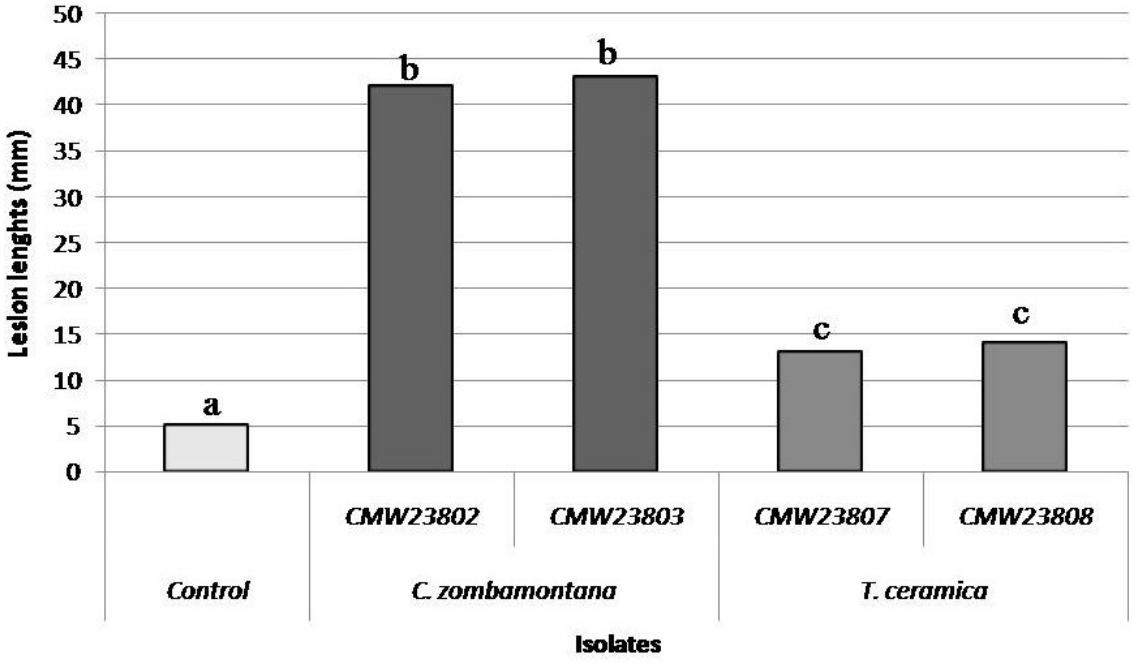

Fig. 11. Bars chart indicating the average lesion length (in millimetres) resulting from inoculation trials with *C. zombamontana* (CMW15235, CMW15236) and *T. ceramica* (CMW15245, CMW15248) onto *Eucalyptus grandis* (ZG 14). Different letters above the bars indicate treatments that are statistically different based on a 95 % confidence limit.

that are oblong with truncate apices, which separates it from all other *Ceratocystis* spp. Another clear distinction between *C. oblonga* and *C. savannae* is found in the shapes of their ascomatal bases. *Ceratocystis oblonga* produces obpyriform ascomatal bases in contrast to the globose ascomatal bases produced by *C. savannae*. *Ceratocystis oblonga* also differs from *C. savannae* and most other species in the *C. moniliformis s.l.* group, other than *C. bhutanensis* (Van Wyk *et al*., 2004; Kamgan *et al*., 2008), in the fact that it is able to grow at 5°C, producing colonies of up to 5mm diameter after four days of growth. Together with *C. bhutanensis*, it is one of two species in this group adapted to growth at low temperatures.

Thielaviopsis ceramica is phylogenetically most closely related to *C. bhutanensis*. These species are, however, fungi with vastly different ecologies with one occurring on wounds on *Eucalyptus* spp. in Africa and the other associated with conifer infesting bark beetles in Bhutan (Van Wyk *et al*., 2004). The two species can further be distinguished based on various morphological characteristics. *Thielaviopsis ceramica* produces colonial buff-coloured colonies whereas those of *C. bhutanensis* are cream-buff to dark olive to black in colour. *Thielaviopsis ceramica* also produces slightly longer (4-6 µm) barrel shaped conidia than that of *C. bhutanensis* (3-5 µm). As no teleomorph structures could be induced for *T. ceramica* no other morphological comparisons were possible.

Ceratocystis moniliformis was isolated from *Eucalyptus* stumps in South Africa and Tanzania. This fungus has previously been reported from artificially induced wounds on *Eucalyptus* spp. in South Africa (Roux *et al*., 2004*)* but this is the first confirmed report of its occurrence elsewhere in Africa. Isolation of *C. moniliformis* was not surprising as it is a fungus with a broad global distribution and host range (Davidson, 1935; Bakshi, 1951; Hunt, 1956; Van Wyk *et al*., 2006b). It is not known to be a pathogen of *Eucalyptus* spp. or other tree species.

Five of the species collected in this study reside in the *C. fimbriata s.l.* species complex. Of these, four were of previously undescribed species which we have provided with the names *C. tanganyicensis*, *C. zombamontana*, *C. polyconidia* and *C. obpyriformis*. Fungi in the *C. fimbriata s.l.* species complex are generally known as virulent pathogens and they include species such as *C. fimbriata s.s.*, *C. platani*, *C. cacaofunesta* and *C. albi-*

fundus. Although species in this group are aggressive pathogens, they are not as fast growing as species in the *C. moniliformis s.l.* species complex and in this respect, they can generally be distinguished from the later group based on culture morphology.

Ceratocystis zombamontana is most closely related to *C. pirilliformis* within the *C. fimbriata s.l.* species complex, but it resides in a distinct phylogenetic clade. This species produces hair brown cultures in contrast to the pale olivaceous grey cultures found in *C. pirilliformis*. *Ceratocystis zombamontana* has shorter ascomatal necks that are wider at the tip compared to that of *C. pirilliformis* and it has significantly larger ascospores than those of the former species. Furthermore, *C. zombamontana* produces flask-shaped primary phialides compared to the cylindrical to lageniform primary phialides of *C. pirilliformis*. Another distinct morphological difference between these two phylogeneticaly closely related species is that *C. pirilliformis* produces clamydospores (Barnes *et al*., 2003), whereas these structures have not been found in *C. zombamontana*.

Ceratocystis tanganyicensis, isolated from *A. mearnsii*, resides in a large clade, with *C. tsitsikammensis*, but forming a distinct clade with strong bootstrap support. Although this species is phylogenetically closest to *C. tsitsikammensis*, it can clearly be distinguished from that species based on culture morphology. In this regard, *C. tanganyicensis* produces ivory green cultures, whereas those of *C. tsitsikammensis* are a greenish olivaceous colour. *Ceratocystis tanganyicensis* also grows optimally at 20°C while *C. tsitsikammensis* grows optimally at 25°C. *Ceratocystis tanganyicensis* can be distinguished from *C. tsitsikammensis* based on the sub-globose ascomatal bases in *C. tanganyicensis* compared to the globose ascomatal bases of *C. tsitsikammensis*. Furthermore, *C. tanganyicensis* produces longer (39-47µm) ostiolar hyphae than *C. tsitsikammensis* (23-38 µm) (Kamgan *et al*. 2008).

Ceratocystis polyconidia and *C. obpyriformis*, from *A. mearnsii* in South Africa reside in distinct clades, however, both grouped closely with *C. pirilliformis* and *C. tanganyicensis* in phylogenetic analyses. *Ceratocystis polyconidia* could be distinguished from *C. pirilliformis* based on culture morphology as it produces dark ivory grey cultures compared to those of *C. pirilliformis* which are pale olivaceous grey. *Ceratocystis polyconidia* can be distinguished from *C. obpyriformis* and *C. pirilliformis* by its globose ascomatal bases, compared to the pyriform ascomatal bases in the latter species and the obpyriform ascomatal bases in *C. obpyriformis*. *Ceratocystis obpyriformis* can further be distinguished from *C. pirilliformis* and *C. polyconidia* as these species produce chlamydospores (Barnes *et al*., 2003) whereas these structures are absent in *C. obpyriformis*.

Ceratocystis albifundus was isolated from *A. mearnsii* stumps in three of the countries in which collections were made. Identification of this species can easily be achieved using morphology, as it is the only species in the group with light coloured ascomatal bases. Isolation of *C. albifundus* from *A. mearnsii* from Tanzania, Kenya and South Africa was expected as this species has previously been reported from these countries causing wilting and death of trees (Roux and Wingfield 1997; Roux *et al*., 2005). In South Africa it is considered the most important pathogen of *A. mearnsii* and an important factor in plantation health (Roux and Wingfield, 1997).

Pathogenicity tests showed that all the species described in this study can give rise to lesions on the host plants from which they were isolated. Under field conditions all the species were isolated from wounds and although inoculation trials gave rise to lesions, it is unknown whether they are able to cause disease in nature. As a number of species in the *C. fimbriata s.l.* group are known to be pathogens, it is possible to speculate that isolates obtained in this study, and residing in the *C. fimbriata s.l.* species complex, could be important pathogens. In contrast, based on knowledge of the isolates residing in the *C. moniliformis s.l.* complex, these are probably not pathogenic in nature, but predominantly saprobic.

This study presents the most comprehensive consideration of wound-infecting

Ceratocystis spp. on non-native plantation grown tree species in Southern and Eastern Africa. Recently it has been estimated that there are approximately 171 500 fungal species in South Africa (Crous *et al*., 2006). The fact that six new fungal species were found in a limited study in three countries emphasizes the distinct lack of knowledge regarding microfungi in Africa. This is even more so if one considers that this study reports three previously undescribed species for South Africa, a country in which research on the health of plantation forestry trees has a significant history.

Acknowledgements

We thank Mr. Chris Bekker of the Tanganyika Wattle Company, Tanzania, Mr. William Sagona of the Malawian Forestry Research Institute, Mr. Linus Mwangi of the Forestry Research Institute, Kenya and Dr. Pia Barklund of The International Centre for Research in Agroforestry for logistical assistance and support. We are also grateful to Dr. Lawrie Wright, Ms. Almuth Hammerbacher and Mr. Leon Labuschagne for assistance in collecting samples. We thank Ms. Marelize van Wyk for assistance in the phylogenetic analyses and Prof. Goeneveld and Dr. Van der Linde from the Department Statistics, University of Pretoria for their assistance with the statistical analyses. The South African Department of Trade and Industry's THRIP initiative, the National Research Foundation of South Africa (NRF), the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) and the Tree Protection Co-operative Programme (TPCP) are gratefully acknowledged for funding this research.

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