Ceratocystis and *Ophiostoma* species, including three new taxa, associated with wounds on native South African trees

Kamgan, N.G.[1*], Jacobs, K.[2], de Beer, Z.W.[1], Wingfield, M.J.[1] and Roux, J.[1]

[1]Department of Microbiology and Plant Pathology, DST/NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa
[2]Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa

Kamgan, N.G., Jacobs, K., de Beer, Z.W., Wingfield, M.J. and Roux, J. (2008). *Ceratocystis* and *Ophiostoma* species including three new taxa, associated with wounds on native South African trees. Fungal Diversity 29: 37-59.

Fungal species in *Ceratocystis* and *Ophiostoma* include important pathogens, associated with insects that typically infect wounds visited or made by their vectors. There are few reports of these fungi from the African continent and little is known regarding their relative importance in the area. In this study, species of *Ceratocystis* and *Ophiostoma* were collected from wounds on native tree species in selected areas of South Africa and they were identified based on morphological and DNA sequence comparisons. *Ceratocystis* and *Ophiostoma* species were collected from eight different native trees including six different families. These included *Ophiostoma quercus*, *Pesotum fragrans*, *Ceratocystis albifundus* as well as an undescribed *Ophiostoma* sp. and two undescribed *Ceratocystis* spp. The new *Ceratocystis* spp. are described here as *Ceratocystis savannae* sp. nov. and *Ceratocystis tsitsikammensis* sp. nov. and the *Ophiostoma* sp. as *Ophiostoma longiconidiatum* sp. nov. In pathogenicity tests, *C. tsitsikammensis* sp. nov. resulted in significant lesions on *Rapanea melanophloeos* trees, while *C. savannae* sp. nov. produced very small lesions on *Acacia nigrescens* and *Sclerocarya birrea* trees.

Key words: Africa, fungal pathogens, ophiostomatoid fungi

Article Information
Received 23 August 2007
Accepted 10 January 2008
Published online 31 March 2008
*Corresponding author: G. Kamgan Nkuekam; e-mail: gilbert.kamgan@fabi.up.ac.za

Introduction

Species of *Ceratocystis* Ellis & Halst. and *Ophiostoma* Syd. & P. Syd. and their anamorph genera are collectively referred to as the ophiostomatoid fungi. This name arises from their morphological similarities and particularly convergent evolution of structures adapted to insect dispersal (Wingfield *et al*., 1993). Fungi in these genera are characterized by their dark, globose ascomata with elongated necks giving rise to sticky spores at their apices. Asci generally disappear early in the development and are seldom seen (Upadhyay, 1981). It is now widely accepted that *Ceratocystis* and *Ophiostoma* are distinct genera, in separate orders of the Ascomycetes. *Ceratocystis* spp. have *Thielaviopsis* anamorphs with enteroblastic conidiogenesis (Paulin-Mahady *et al*., 2002) and reside in the order *Microascales* Luttr. ex Benny & Kimbr. (Hausner *et al*., 1993; Spatafora and Blackwell, 1994; Paulin-Mahady *et al*., 2002). *Ophiostoma sensu lato* is recognized as a generic aggregate in the order *Ophiostomatales* Benny & Kimbr. (Hausner *et al*., 1993; Spatafora and Blackwell, 1994). *Ophiostoma* s. l. includes *Ophiostoma* Syd. & P. Syd. *sensu stricto* with *Pesotum* J.L. Crane & Schokn. and *Sporothrix* Hektoen & C.F. Perkins anamorphs, *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. with *Hyalorhinocladiella* H.P. Upadhyay & W.B. Kendr. anamorphs, and *Grosmannia* Goid. with *Leptographium* Lagerb. & Melin anamorphs (Upadhyay, 1981; Zipfel *et al*., 2006). These genera, although phylogenetically distinct, have clearly evolved similar morphologies in response to the similar niches and the survival strategies that they have adapted (Hausner *et al*., 1993; Spatafora and Blackwell, 1994; Wingfield *et*

al., 1993), leading to long-standing confusion in their taxonomy.

Many *Ceratocystis* and *Ophiostoma* species are responsible for significant economic losses to both agricultural and forest crops worldwide. Well-documented examples of tree pathogens are *O. ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, responsible for the Dutch Elm disease pandemics in Europe and North America, *C. fagacearum* (Bretz) J. Hunt, a damaging wilt pathogen of *Quercus* spp. in the USA (Sinclair and Lyon, 2005) and species in the *C. fimbriata sensu lato* complex (Kile, 1993). On agricultural crops, *C. fimbriata* Ellis & Halst. *sensu stricto* is a well-known pathogen, causing black rot of sweet potato and other species in the group cause canker stain and wilt diseases, especially of trees (Kile, 1993; Baker Engelbrecht and Harrington, 2005).

Reports of *Ceratocystis* species from Africa are very limited. Where there are reports of these fungi, they are typically unconfirmed and voucher specimens or cultures have not been retained for them. Some of the better known *Ceratocystis* species from Africa include *C. albifundus* M.J. Wingf., De Beer & M.J. Morris from native South African trees including *Protea* spp. (Gorter, 1977; Wingfield *et al*., 1996; Roux *et al*., 2004a, 2007) and from non-native *Acacia mearnsii* De Wild. trees (Morris *et al*., 1993; Wingfield *et al*., 1996), and *C. moniliformis* (Hedgc.) C. Moreau and *C. pirilliformis* I. Barnes & M.J. Wingf. reported from *Eucalyptus* spp. in South Africa (Roux *et al*., 2004b).

Reports of *Ophiostoma* species from Africa are very few and essentially confined to South Africa. Examples include *O. quercus* that infect both hardwoods and softwoods of both native and non-native trees (De Beer *et al*., 2003) and *L. eucalyptophilum* K. Jacobs, M.J. Wingf. & Jol. Roux infesting *Eucalyptus* trees in the Republic of Congo (Jacobs *et al*., 1999). Other reports of *Ophiostoma* from South Africa are from non-native bark beetle-infesting *Pinus* species (Zhou *et al*. 2001, 2006).

Various ophiostomatoid fungi occur in the flower heads of native South African *Protea* spp. Seven *Ophiostoma* spp. have been reported from this unusual niche and very little is known regarding their ecology (Wingfield *et al*., 1988; Wingfield and Van Wyk, 1993; Marais and Wingfield, 1994; Marais and Wingfield, 1997; Marais and Wingfield, 2001; Roets *et al*., 2006a). It has, however, recently been shown that they are associated with insects that visit the infructescences (flower heads) of these unusual plants (Roets *et al*., 2006b).

The vegetation of South Africa is essentially a woodland savannah with little indigenous forest, covering only 0.56% of the total surface of the country (Lawes *et al*., 2004). These forests are dispersed around the country in an archipelago-like fashion, especially along the southern and eastern seaboard (Lawes *et al*., 2004). Very little information is available regarding diseases of native trees in South Africa and until recently, no *Ceratocystis* and *Ophiostoma* species were known from native trees in the country. In recent studies *C. albifundus*, the cause of wattle wilt of non-native *A. mearnsii* trees in South Africa was reported from seven native tree genera (Roux *et al*., 2004b; Roux *et al*., 2007). This was consistent with the view that the fungus is native to South Africa (Roux *et al*., 2001; Barnes *et al*., 2005) and has provided motivation to determine whether other ophiostomatoid fungi occur on native trees in the country.

The aim of this study was to expand the knowledge base regarding the biodiversity of *Ceratocystis* and *Ophiostoma* species on native trees in South Africa. This is consistent with the goals of the DST/NRF Centre of Excellence in Tree Health Biotechnology (www.fabinet.up.ac.za/cthb/index). Fungi were identified based on their morphology and by means of DNA sequence comparisons for various gene regions. The ability of some of the isolated fungi to cause disease of their hosts under greenhouse conditions was also considered.

Materials and methods

Collection of isolates

Surveys of naturally occurring and artificially made wounds were conducted during 2004 and 2005 in the Kruger National Park (Mpumalanga Province), Leeuwfontein

Table 1. List of *Ceratocystis* isolates and their accession numbers sequenced in this study (*) and used for DNA sequence comparisons.

Isolate designation	Isolate number	Genbank	Gene regions	Other numbers	Hosts	Collectors	Origin
C. albifundus	CMW5329	AF388947	ITS	NA	*Acacia mearnsii*	J. Roux	Uganda
	CMW4068	DQ520638	ITS	NA	*A. mearnsii*	J. Roux	South Africa
	CMW5364	DQ371650	BT	NA	*A. mearnsii*	J. Roux	South Africa
		AY528977	EF				
	CMW2473	DQ371648	BT	NA	*A. dealbata*	M. Morris	South Africa
		AY528976	EF				
C. bhutanensis	CMW8399	AY528959	ITS	CBS115772, BH 8/8	*Picea spinulosa*	T. Kirisits & DB. Chhetri	Bhutan
		AY528964	BT				
		AY528954	EF				
	CMW8215	AY528958	ITS	CBS114290, PREM57805	*P. spinulosa*	T. Kirisits & DB. Chhetri	Bhutan
		AY528963	BT				
		AY528953	EF				
C. fimbriata	CMW1547	AF264904	ITS	NA	*Ipomoea batatas*	NA	Papua N. Guinea
		NA	BT	NA			
		NA	EF	NA			
	CMW15049	DQ520629	ITS	CBS141.37	*I. batatas*	CF. Andrus	USA
		NA	BT				
		NA	EF				
C. moniliformis	CMW9590	AY431101	ITS	CBS116452	*Eucalyptus grandis*	J. Roux	South Africa
		AY528985	BT				
		AY529006	EF				
	CMW8379	AY528995	ITS	NA	*Cassia fistula*	MJ. Wingfield	Bhutan
		AY529005	BT				
		AY529016	EF				
	CMW8240	AY529000	ITS	NA	*C. fistula*	MJ. Wingfield, T. Kirisits & DB. Chhetri	Bhutan
		AY528989	BT				
		AY529010	EF				
C. moniliformopsis	CMW10214	AY528999	ITS	CBS115792, ORB 33	*E. sieberi*	MJ. Dudzinski	Australia
		AY528988	BT				
		AY529009	EF				
	CMW9986	AY528998	ITS	CBS109441	*E. obliqua*	ZQ. Yuan	Australia
		AY528987	BT				
		AY529008	EF				

Table 1 (continued). List of *Ceratocystis* isolates and their accession numbers sequenced in this study (*) and used for DNA sequence comparisons.

Isolate designation	Isolate number	Genbank	Gene regions	Other numbers	Hosts	Collectors	Origin
C. omanensis	CMW11048	DQ074742	ITS	CBS115780, PREM57815	*Mangifera indica*	AO. Al-Adawi	Oman
		DQ074732	BT				
		DQ074737	EF				
	CMW3777	DQ074740	ITS	NA	*M. indica*	AO. Al-Adawi	Oman
		DQ074730	BT				
		DQ074735	EF				
	CMW11046	DQ074739	ITS	CBS118112, PREM57814	*M. indica*	AO. Al-Adawi	Oman
		DQ074729	BT				
		DQ074734	EF				
C. pirilliformis	CMW6569	AF427104	ITS	PREM57322, DAR75993	*E. nitens*	MJ. Wingfield	Australia
		DQ371652	BT				
		AY528982	EF				
	CMW6579	AF427105	ITS	PREM57323, DAR75996	*E. nitens*	MJ. Wingfield	Australia
		DQ371653	BT				
		AY528983	EF				
C. polychroma	CMW11455	AY528973	ITS	CBS115774, PREM57822	*Syzygium aromaticum*	ECY. Liew & MJ. Wingfield	Indonesia
		AY528969	BT				
		AY528981	EF				
	CMW11436	AY528971	ITS	CBS115777, PREM57819	*S. aromaticum*	ECY. Liew & MJ. Wingfield	Indonesia
		AY528967	BT				
		AY528979	EF				
	CMW11449	AY528972	ITS	CBS115775, PREM57821	*S. aromaticum*	ECY. Liew & MJ. Wingfield	Indonesia
		AY528968	BT				
		AY528980	EF				
C. savannae	*CMW17300	EF408551	ITS	PREM59423	*Acacia nigrescens*	G. Kamgan & J. Roux	South Africa
		EF408565	BT				
		EF408572	EF				
	*CMW17297	EF408552	ITS	NA	*Combretum zeyheri*	G. Kamgan & J. Roux	South Africa
		EF408566	BT				
		EF408573	EF				
	*CMW17298	EF408553	ITS	NA	*Terminalia sericea*	G. Kamgan & J. Roux	South Africa
		EF408567	BT				

Table 1 (continued). List of *Ceratocystis* isolates and their accession numbers sequenced in this study (*) and used for DNA sequence comparisons.

Isolate designation	Isolate number	Genbank	Gene regions	Other numbers	Hosts	Collectors	Origin
		EF408574	EF				
	*CMW17575	EF408554	ITS	NA	*T. sericea*	G. Kamgan & J. Roux	South Africa
		EF408568	BT				
		EF408575	EF				
C. tribiliformis	CMW13015	AY529004	ITS	CBS115949	*Pinus merkusii*	MJ. Wingfield	Indonesia
		AY528994	BT				
		AY529015	EF				
	CMW13013	AY529003	ITS	CBS115866	*P. merkusii*	MJ. Wingfield	Indonesia
		AY528993	BT				
		AY529014	EF				
C. tsitsikammensis	*CMW14276	EF408555	ITS	PREM59424	*Rapanea melanophloeos*	G. Kamgan & J. Roux	South Africa
		EF408569	BT				
		EF408576	EF				
	*CMW14278	EF408556	ITS	NA	*R. melanophloeos*	G. Kamgan & J. Roux	South Africa
		EF408570	BT				
		EF408577	EF				
	*CMW14280	EF408557	ITS	NA	*Ocotea bullata*	G. Kamgan & J. Roux	South Africa
		EF408571	BT				
		EF408578	EF				
C. virescens	CMW3276	DQ061281	ITS	NA	*Quercus* sp.	T. Hinds	USA
		AY528990	BT				
		AY529011	EF				

Table 2. List of *Ophiostoma* isolates and their accession numbers sequenced (*) and used for DNA sequence comparisons.

Isolate designation	Isolate number	Genbank	Gene regions	Other numbers	Hosts	Collectors	Origin
O. arduennense	NA	AY573242	ITS	MUCL44867	*Fagus sylvatica*	FX. Carlier & T. Defrance	NA
	NA	AY573247	ITS	MUCL45367	"	"	NA
O. floccosum	C1086	AF198231	ITS	CBS799.73	NA	A. Käärik	Sweden
	CMW7661	AF493253	ITS	NA	*Pinus elliottii*	ZW. de Beer	South Africa
O. himal-ulmi	C1183	AF198233	ITS	CBS374.67; ATCC36176; ATCC36204	*Ulmus* sp.	HM. Heybroek	India
	C1306	AF198234	ITS	HP27	*Ulmus* sp.	CM. Brasier	India
O. kryptum	NA	AY304434	ITS	DAOM229702 (IFFFBW/1)	*Larix decidua*	T. Kirisits & MJ. Wingfield	Austria
	NA	AY304437	ITS	IFFFHasd/1	*L. decidua*	T. Kirisits & MJ. Wingfield	"
O. longiconidiatum	CMW17574	EF408558	ITS	NA	*Terminalia sericea*	G. Kamgan & J. Roux	South Africa
	CMW14265	EF408560	ITS	NA	*Faurea saligna*	G. Kamgan & J. Roux	"
	CMW17688	EF408559	ITS	NA	*T. sericea*	G. Kamgan & J. Roux	"
	CMW17684	EF408561	ITS	NA	*F. saligna*	G. Kamgan & J. Roux	"
O. multiannulatum	NA	NA	ITS	CBS357.77	*Pinus* sp.	RW. Davidson	NA
	NA	AY934512	ITS	CBS124.39	*Pinus* sp.	RW. Davidson	NA
O. novo-ulmi	C510	AF198236	ITS	NA	*Ulmus* sp.	NA	Iowa, USA
	C1185	AF198235	ITS	CBS298.87; WCS637	*Ulmus* sp.	H. M. Heybroek	Russia
O. piceae	NA	AF198226	ITS	C1087; CBS108.21	*Abies or Picea*	E. Münch	Germany
	CMW7648	AF493249	ITS	C967; H2181	*Picea sitchensis*	DB. Redfern & JF. Webber	United Kingdom
O. piliferum	NA	AF221070	ITS	CBS129.32	*Pinus sylvestris*	H. Diddens	"
	NA	AF221071	ITS	2/97	NA	NA	NA
O. pluriannulatum	NA	AY934517	ITS	MUCL18372	conifer	NA	USA
	C1567	DQ062972	ITS	UAMH9559; WIN(M)869	*Podocarpus* sp.	Reid	New Zealand
O. quercus	CMW7656	AF493250	ITS	NA	*Q. robur*	MJ. Wingfield	South Africa
	CMW2520	AF493241	ITS	NA	*Eucalyptus* sp.	ZW. de Beer	NA
	CMW7658	AF493251	ITS	NA	*Olinia ventosa*	MJ. Wingfield	NA
	CMW3119	AF493244	ITS	NA	*Pinus* sp.	ZW. de Beer	NA
	CMW2534	AF493242	ITS	NA	*E. grandis*	GHJ. Kemp	NA
	CMW7645	AF493246	ITS	*W3; HA367*	*Q. robur*	T. Kirisits & E. Halmschlager	Austria
	CMW7650	AF198238	ITS	C969; CBS102352; H1042	*Quercus* sp.	PT. Scard & JF. Webber	United Kingdom
	*CMW17573	EF408562	ITS	NA	*T. sericea*	G. Kamgan & J. Roux	South Africa
	*CMW20452	EF408563	ITS	NA	*R. melanophloeos*	J. Roux	"
	*CMW14279	EF408564	ITS	NA	*R. melanophloeos*	G. Kamgan & J. Roux	"
O. subannulatum	NA	AY934522	ITS	CBS188.86	*Pinus* sp.	BWH. Livingston	NA

Table 2 (continued). List of *Ophiostoma* isolates and their accession numbers sequenced (*) and used for DNA sequence comparisons.

Isolate designation	Isolate number	Genbank	Gene regions	Other numbers	Hosts	Collectors	Origin
	CMW518	DQ294364	ITS	CBS118667	*P. ponderosa*	BWH. Livingston	NA
O. ulmi	C1182	AF198232	ITS	CBS102.63; IMI101223; JCM9303	*Ulmus* sp.	WF. Holmes & HM. Heybroek	Netherlands
Pesotum fragrans	NA	AF198248	ITS	CBS279.54	*P. sylvestris*	A. Mathiesen-Käärik	Sweden
	NA	AY194518	ITS	NLC348	*Abies balsamifera*	G. Warren	NA
	CMW19357	DQ396790	ITS	NA	*P. patula*	XD. Zhou	NA
P. fragrans-like	NA	DQ062977	ITS	C1496	*P. radiata*	R. Farrell	NA
	*CMW20673	NA	NA	NA	*R. melanophloeos*	J. Roux	South Africa
	*CMW20671	NA	NA	NA	*Rhus chirindensis*	J. Roux	"

Table 3. List of *Ceratocystis* and *Ophiostoma* isolates collected from native South African hosts in this study.

Isolate designation	Isolate number	Hosts	Area
Ceratocystis	CMW14287	*Terminalia sericea*	Kruger National Park
albifundus	CMW14289	*T. sericea*	"
	CMW14288	*Combretum zeyheri*	"
	CMW14290	*C. zeyheri*	"
C. savannae	CMW17300	*Acacia nigrescens*	"
	CMW17297	*C. zeyheri*	"
	CMW17298	*T. sericea*	"
	CMW17301	*Sclerocarya birrea*	"
	CMW17306	*S. birrea*	"
	CMW17302	*S. birrea*	"
	CMW17575	*T. sericea*	Leeuwfontein Collaborative Nature Reserve
	CMW17576	*Burkea africana*	"
C. tsitsikammensis	CMW14276	*Rapanea melanophloeos*	Groenkloof Forest, Tsitsikamma
	CMW14278	*R. melanophloeos*	"
	CMW14280	*Ocotea bullata*	"
	CMW14275	*R. melanophloeos*	"
	CMW14274	*R. melanophloeos*	"
	CMW13981	*R. melanophloeos*	"
	CMW13982	*R. melanophloeos*	"
Ophiostoma	CMW17574	*T. sericea*	Leeuwfontein Collaborative Nature Reserve
longiconidiatum	CMW14265	*Faurea saligna*	"
	CMW17688	*T. sericea*	"
	CMW17684	*F. saligna*	"
	CMW17685	*F. saligna*	"
	CMW17689	*F. saligna*	"
O. quercus	CMW17573	*T. sericea*	"
	CMW20452	*R. melanophloeos*	Groenkloof Forest, Tsitsikamma
	CMW14279	*R. melanophloeos*	"
Pesotum fragrans-	CMW20673	*R. melanophloeos*	"
like	CMW20671	*Rhus chirindensis*	"

All isolates were collected by G. Kamgan and J. Roux or in the case of (CMW20671 and CMW20673) by J. Roux.

Collaborative Nature Reserve (Gauteng Province) and Groenkloof Forest (Tsitsikamma Forests, Western Cape Province). Wounds from which samples were collected included damage caused by elephants, kudu, eland, wind as well as wounds made artificially (Barnes *et al*., 2003; Roux *et al*., 2004a) using axes or masonry chisels. Pieces of bark and wood were examined using a 10× magnification hand lens and those showing signs of fungal growth and discoloration were collected and stored, separately for each tree, in paper bags. All the samples were then transported to the laboratory in plastic bags to retain moisture. Dry samples were sprayed with water, sealed in plastic bags and incubated to induce sporulation of fungi.

Samples were observed regularly for the presence of *Ceratocystis* and *Ophiostoma* species. As they appeared, these fungi were isolated, plated onto 2% malt extract agar (MEA: 20gl^{-1} malt extract and 15gl^{-1} agar, Biolab, Midrand, South Africa and 1000 ml deionised water) containing 0.05g/l of the antibiotic streptomycin (SIGMA-ALDRICH, Steinheim, Germany) and incubated at 24ºC. Isolates were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, for long term preservation. Representative specimens have also been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands. Dried specimens of representative isolates were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

Morphological characterization

Fruiting structures (ascomata and ascospores; synnemata and conidia) were mounted in 80% lactic acid on microscope slides and studied using a Zeiss Axiocam light

microscope (München-Hallbergmoos, Germany). *Ceratocystis* and *Ophiostoma* species were initially identified based on morphology. Fifty measurements of all characteristic morphological features were made for isolates chosen as the types of new species and ten measurements were made for additional isolates. Measurements were noted as (minimum -) mean minus st. dev. - mean plus st. dev. (- maximum). The means were then calculated for relevant morphological structures.

Growth in culture

A disk of agar (9 mm diam.) bearing mycelium of isolates selected to be tested for their growth in culture, was transferred from the actively growing margins of seven-day-old cultures and placed upside down at the centres of 90 mm Petri dishes containing 2% MEA. The plates were incubated in the dark for 10 days at temperatures ranging from 5ºC to 35ºC at 5 degree intervals. Five replicate plates were used for each isolate at each temperature considered. Two diameter measurements, perpendicular to each other, were taken daily for each colony and the ten diameter measurements were averaged for each temperature.

DNA sequence comparisons

Representative isolates of each *Ceratocystis* and *Ophiostoma* species collected in this study were selected for DNA sequence comparisons (Tables 1, 2). Single spore drops collected from the apices of ascomata or conidiophores in pure cultures were grown on 2% MEA for 7-10 days. Mycelium was then transferred to 1.5 ml Eppendorf tubes using a sterile scalpel. Mycelium was freeze-dried and ground into a fine powder with a sterile toothpick after addition of liquid nitrogen. DNA was extracted using the protocol described by Möller *et al*. (1992) except that 10 µl of RnaseA were added at the final step and incubated overnight at room temperature to digest RNA. The presence of DNA was verified by separating an aliquot of 5 µl on 1% agarose gels stained with ethidium bromide and visualized under Ultraviolet light (UV).

The internal transcribed spacer regions (ITS1, ITS4) and 5.8S gene of the ribosomal RNA operon were amplified on an Eppendorf Mastercycler (Merck, Germany) using primers ITS1 (3'-TCCGTAGGTGAACCTGCGG-5') and ITS4 (3'-TCCTCCGCTTATTGATATGC-5') (White *et al*., 1990). Part of the β-tubulin gene and the transcription elongation factor-1α gene were also amplified using the primers βt1a (5'-TTCCCCCGTCTCCACTTCTTCATG-3') and βt1b (5'-GACGAGATCGTTCATGTTGAACTC-3') (Glass and Donaldson 1995), EF1F (5'-TGCGGTGGTATCGACAAGCGT-3') and EF2R (5'-AGCATGTTGTCGCCGTTGAAG-3') (Jacobs *et al*., 2004) respectively.

The PCR reaction (25 µl) mixtures were prepared using 60 ng of the DNA template, 2.5 µl of 10x reaction buffer with $MgCl_2$ (25 mM) (Roche), 2.5 µl $MgCl_2$ (25 mM) (Roche), 1U of Taq polymerase (Roche), 2.5 µl of deoxynucleotide triphosphate mix (DNTP) (10 mM) and 0.5 µl of each primer (10 mM). The conditions used for the thermal cycling were as follows: an initial denaturation of the DNA at 96ºC for 2 min, followed by 35 cycles consisting of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s for the ITS and β-tubulin genes and 60ºC for the elongation factor-1α gene, primer extension at 72ºC for 1 min and a final extension at 72ºC for 10 min. An aliquot of 5 µl of the PCR products were separated on a 1% agarose gel stained with ethidium bromide and visualized under UV light.

PCR products were purified using Sephadex G-50 Gel (Sigma-Aldrich), following the manufacturer's instructions. Subsequently, the concentrations of the purified PCR products were determined using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Rockland, USA). Sequencing reactions were performed using the Big Dye cycle sequencing kit with Amplitaq DNA polymerase, FS (Perkin-Elmer, Warrington, UK) following the manufacturer's protocol on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Between 60-100 ng of PCR product was used to prepare 10 µl sequencing PCR that also contained 2 µl of ready reaction mixture (Big dye), 2 µl of 5x reaction buffer, 1 µl of primer (10 mM) and sufficient sterile water to bring the volume to 10 µl. The same primers were used for sequencing as those described for the PCR

amplifications. Both DNA strands were sequenced.

A preliminary identity for the isolates was obtained by performing a similarity search (standard nucleotide BLAST) against the GenBank database (http://www.ncbi.nlm.nih.gov). Sequences of both strands for each isolate were examined visually and combined using the programme Sequence Navigator. Sequences were then aligned automatically using Mafft ver.5.851 (Katoh *et al*., 2002) and analyzed using PAUP 4.0b10 (Swofford, 1998). Additional sequences of related *Ceratocystis* and *Ophiostoma* species were obtained from the GenBank database. PAUP 4.0b10 was used to construct phylogenetic trees from the distance matrices by pair-wise alignment of the sequences, using the neighbour-joining method (Saitou and Nei, 1987). Confidence levels of the phylogenies were estimated with the bootstrap method (Felsenstein, 1985).

Pathogenicity tests

Pathogenicity tests were conducted in a greenhouse using three different tree species, native to South Africa and which were natural hosts for the test strains. Twenty trees, approximately two-years-old, were inoculated with each test strain and ten other trees of the same age were inoculated with a sterile agar disc to serve as controls. Test strains collected in Kruger National Park (CMW17300) and Leeuwfontein Collaborative Nature Reserve (CMW17575) were used to inoculate *Acacia nigrescens* Oliv. and *Sclerocarya birrea* (A. Rich.) Hochst., while test strains collected in Groenkloof (CMW14276, CMW14278) were used to inoculate *Rapanea melanophloeos* (L.) Mez.

Inoculations were done by growing test strains on MEA for ten days and inoculating 6 mm diam. discs, overgrown with the fungi, or sterile agar in the case of the controls, into wounds of equal size. These were made by removing the bark to expose the cambial layer, using a sterile metal cork borer. The wounds inoculated with the agar discs were sealed with Parafilm (Pechiney, Chicago, USA) to protect them from desiccation. Sixty days after inoculation, the lengths of lesions on the bark surface and in the cambium were measured. Re-isolations were made from the lesions to meet the requirements of Koch's postulates. Lesion lengths were then analysed using SAS/STAT in SAS (SAS Institute Inc. 1999).

Results

Collection of isolates

A wide diversity of *Ceratocystis* and *Ophiostoma* species were isolated from wounds on trees during the course of this study. Isolates were obtained from bark, cambial and wood samples collected from wounds on native tree species spanning eight different genera and six families, growing in three geographical areas of South Africa, including the Kruger National Park, Leeuwfontein Collaborative Nature Reserve and Groenkloof Forest (Tables 1, 2, 3). Tree species from which *Ceratocystis* and *Ophiostoma* isolates were obtained included: *Acacia nigrescens* (*Leguminosae*), *Combretum zeyheri* Sond. (*Combretaceae*), *Sclerocarya birrea* (*Anacardiaceae*), *Burkea africana* Hook. (*Leguminosae*), *Faurea saligna* Harv. (*Proteaceae*), *Ocotea bullata* (Burch.) Baill. (*Lauraceae*), *Rapanea melanophloeos* (*Myrsinaceae*) and *Terminalia sericea* Burch. ex DC. (*Combretaceae*) (Table 3). In most cases, the fungi were associated with and isolated from wood showing signs of xylem discolouration.

Morphological characterization

Ceratocystis spp. collected during the course of this study could be assigned to three morphotypes based on colony colour and the production of ascomata on MEA. The first group, representing isolates from Groenkloof produced grey to green-coloured colonies with black ascomata lacking spines on their bases. Isolates of the second morphotype were collected in the Kruger National Park. These fungi produced light-coloured, slow-growing colonies and ascomata with light-coloured bases and black necks. Based on these characteristics, the isolates resembled *C. albifundus*. Isolates of the third morphotype were collected in both the Kruger National Park (KNP) and Leeuwfontein Collaborative Nature Reserve and they produced fluffy colonies with white mycelium when young, turning dark brown as they became older.

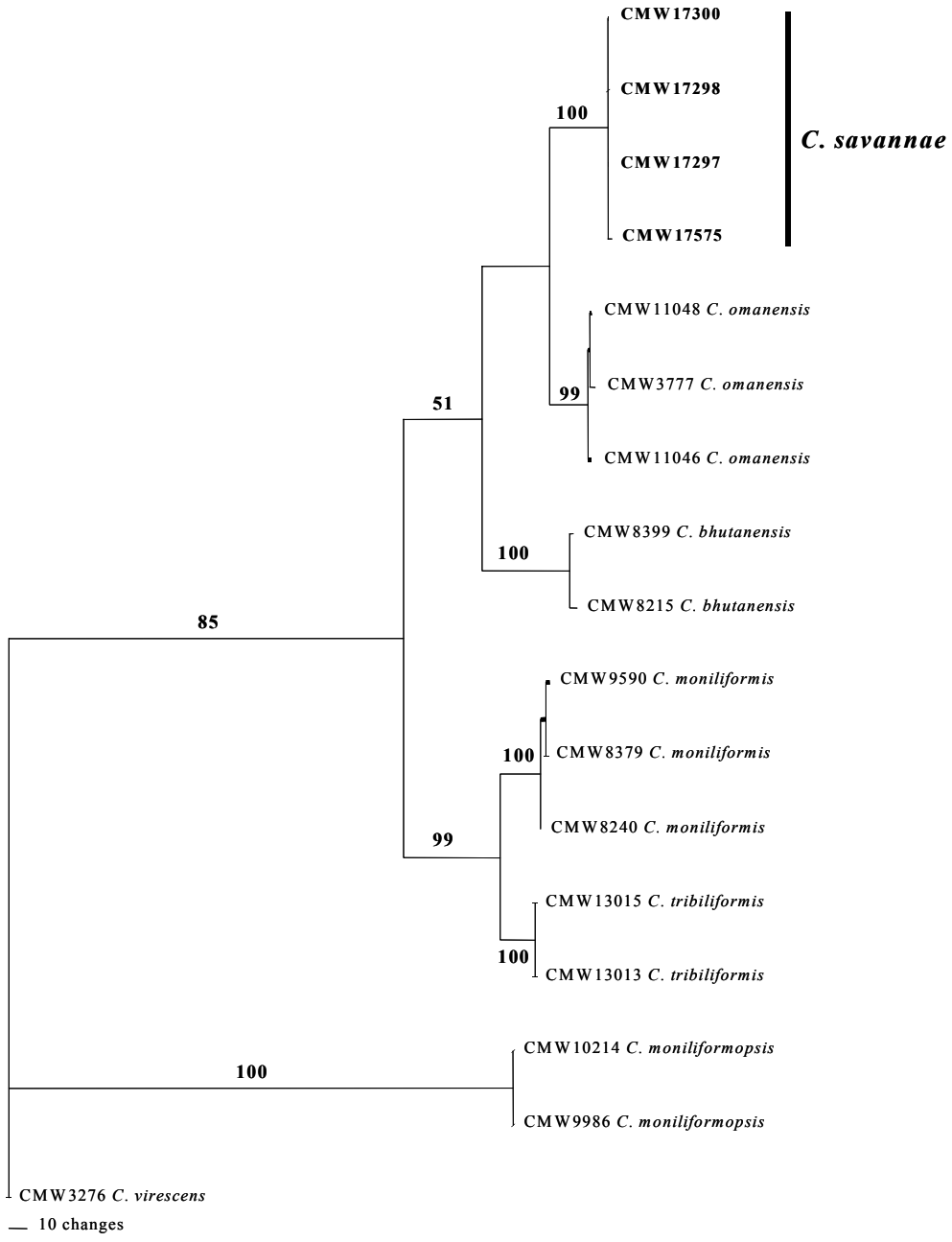

Fig. 1. Phylogenetic tree produced from a heuristic search of the combined ITS, β-tubulin and Elongation factor-1α sequence data, showing the relationship between *C. savannae* sp. nov. from native tree species in the Kruger National Park and Leeuwfontein Collaborative Nature Reserve and other *Ceratocystis* spp. resembling *C. moniliformis*. *C. virescens* was used as out-group taxon. Bootstrap values were derived from 1000 replicates and are indicated next to each clade.

Ascomata were produced abundantly in these cultures, had bases with spines and a strong fruity odour. These characteristics are similar to those of *C. moniliformis* and led us to assign this third group of isolates to the *C. moniliformis sensu lato* species complex.

Ophiostoma spp. that were collected could be assigned to two morphotypes based on colony colour and the production of sexual fruiting structures. The first group, originating from Leeuwfontein, produced pale to grey and fluffy colonies. Ascomata had small, light

coloured bases and very long black necks, in most cases bearing single annuli, and they were produced abundantly in culture. The asexual state of this species was also produced abundantly in cultures and could be identified easily under the light microscope as a *Sporothrix* sp. The second morphotype similar in colour and appearance to the first morphotype produced only a *Pesotum* anamorph.

DNA sequence comparisons

Selected isolates (CMW17297, CMW 17298, CMW17300, CMW17575) of the *Ceratocystis* sp. resembling *C. moniliformis*, collected in the KNP and Leeuwfontein and that represented the third morphotype amongst the *Ceratocystis* isolates collected in the study, generated amplicons of about 600, 550, 850 bps for part of the ITS, β-tubulin and translation elongation factor-1α genes (EF-1 α) respectively. Partition homogeneity tests using 1000 replicates for sequence data of these three gene regions resulted in a P-value of 0.714, suggesting that the data from the three gene regions could be combined. Comparison of these isolates with those from GenBank and automatic alignment using Mafft ver.5.851 (Katoh *et al*., 2002), followed by analysis in PAUP resulted in a total of 1896 characters including gaps, with 1546 constant characters, 188 variable characters (parsimony-uninformative) and 162 parsimony informative characters. Phylogenetic analysis using parsimony and the heuristic search option resulted in 455 best trees with a consistency index (CI) and retention index (RI) value of 0.886 and 0.902 respectively. Isolates formed a well-resolved clade (Fig. 1), supported by a bootstrap value of 100%, separate from any of the described species in the *C. moniliformis* species complex, suggesting that they represent an undescribed species. The closest phylogenetic neighbors of these isolates were *C. bhutanensis* M. van Wyk, M.J. Wingf. & Kirisits and *C. omanensis* Al-Subhi, M.J. Wingf., M. van Wyk & Deadman.

Selected isolates (CMW14276, CMW 14278, CMW14280) of the *Ceratocystis* sp. resembling *C. fimbriata* collected from Groenkloof generated amplicons of about 600, 550 and 900 bp for part of the ITS, β-tubulin and EF-1α gene regions respectively. Partition homogeneity tests using 1000 replicates of sequence data for these three gene regions resulted in a P-value of 0.699, suggesting that the data from the three regions could be combined. Comparison of these isolates with those from GenBank and automatic alignment using Mafft ver.5.851, followed by analysis in PAUP resulted in a total of 1955 characters including gaps, with 1550 constant characters, 176 variable characters (parsimony-uninformative) and 229 parsimony informative characters. Phylogenetic analysis using parsimony and the heuristic search option resulted in 511 best trees of which one was retained for representation (Fig. 2). The consistency index (CI) and retention index (RI) values were 0.908 and 0.902, respectively. The isolates from Groenkloof formed a well-resolved clade, separate from any of the described species in the *C. fimbriata* species complex and supported by a bootstrap value of 100%, suggesting that they represent a previously undescribed species. The closest phylogenetic neighbors of these isolates were *C. polychroma* M. van Wyk, M.J. Wingfield & E.C.Y. Liew and *C. pirilliformis*.

The *Pesotum* and *Ophiostoma* isolates (CMW14279, CMW20452, CMW14265, CMW17574, CMW17684, CMW17688) from Groenkloof and Leeuwfontein, for which DNA sequence comparisons were made, produced fragments of approximately 650 bp, using the primers ITS1 and ITS4. Preliminary blast searches suggested that the isolates represent three distinct taxa. Comparison of these isolates with those from GenBank in PAUP resulted in a total of 681 characters including gaps, 303 characters were constant, 19 characters were parsimony-uninformative and 359 characters were parsimony informative. Phylogenetic analysis using parsimony and the heuristic search option resulted in 753 best trees. Six of these were retained, of which one (Fig. 3) was selected for representation. The consistency index (CI) and the retention index (RI) values were 0.783 and 0.949, respectively. Isolates from native trees in South Africa could be separated into three distinct taxa. The first group clustered with strains of *O. quercus*, supported by a bootstrap value of 82%. The second group formed a clade close to *Pesotum*

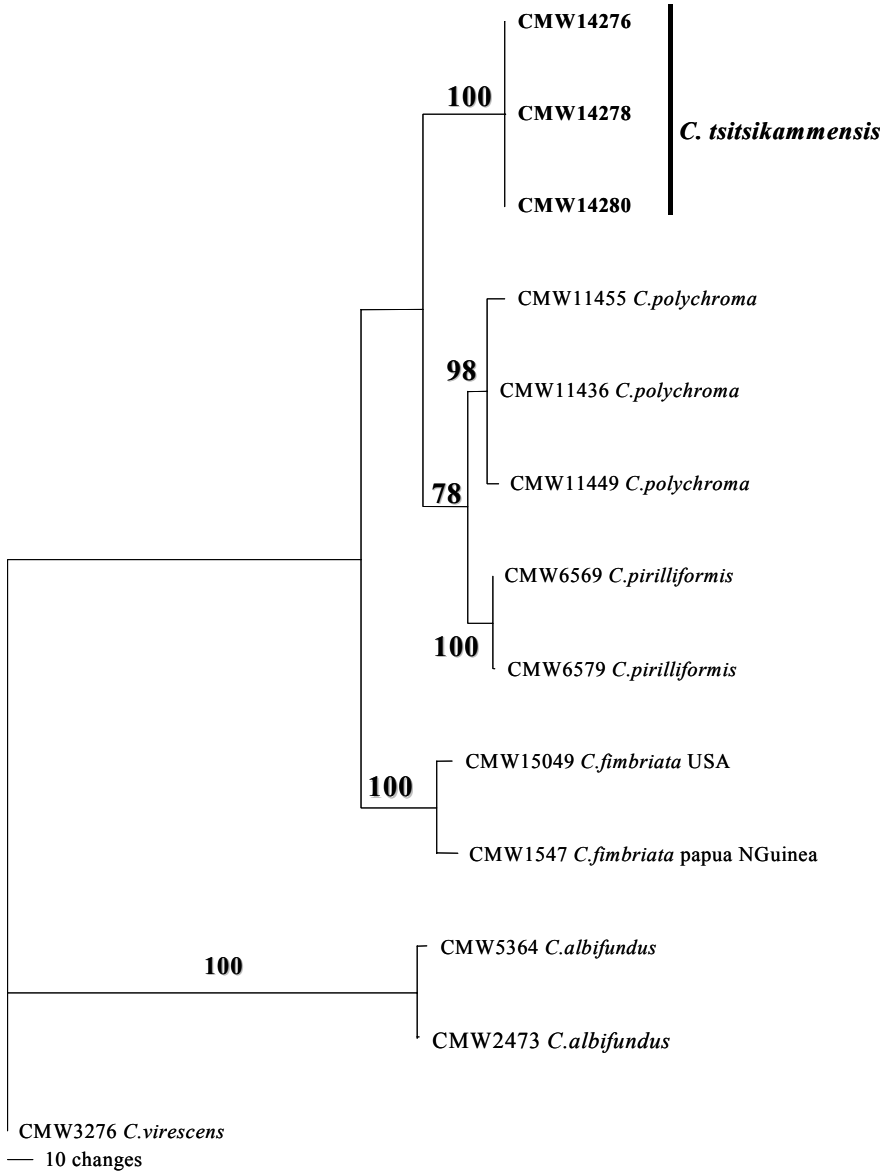

Fig. 2. Phylogenetic tree produced from a heuristic search of the combined ITS, β-tubulin and Elongation factor-1α sequence data, showing the relationship between *C. tsitsikammensis* sp. nov. from native tree species in Groenkloof and other *Ceratocystis* spp. resembling *C. fimbriata*. *C. virescens* was used as out-group taxon. Bootstrap values were derived from 1000 replicates and are indicated next to each clade.

fragrans, but clustering with a *P. fragrans*-like isolate with a bootstrap value of 89%. A number of isolates from Leeuwfontein formed a separate third clade, clearly different to other known strains, suggesting that they represent an undescribed species of *Ophiostoma*.

Taxonomy

Based on morphological and DNA sequence comparisons, isolates of the *Ceratocystis* sp. in the *C. fimbriata* species complex from Groenkloof (CMW14276, CMW14278, CMW14280), and those from Leeuwfontein (CMW17575) and the Kruger National Park (CMW17300, CMW17297, CMW17298) residing in the *C. moniliformis* species complex, represent two undescribed species. Likewise, one of the *Ophiostoma* sp. (CMW14265, CMW17574, CMW17684, CMW17688) isolated from Leeuwfontein represents a new taxon. The following descriptions are, therefore, provided for them.

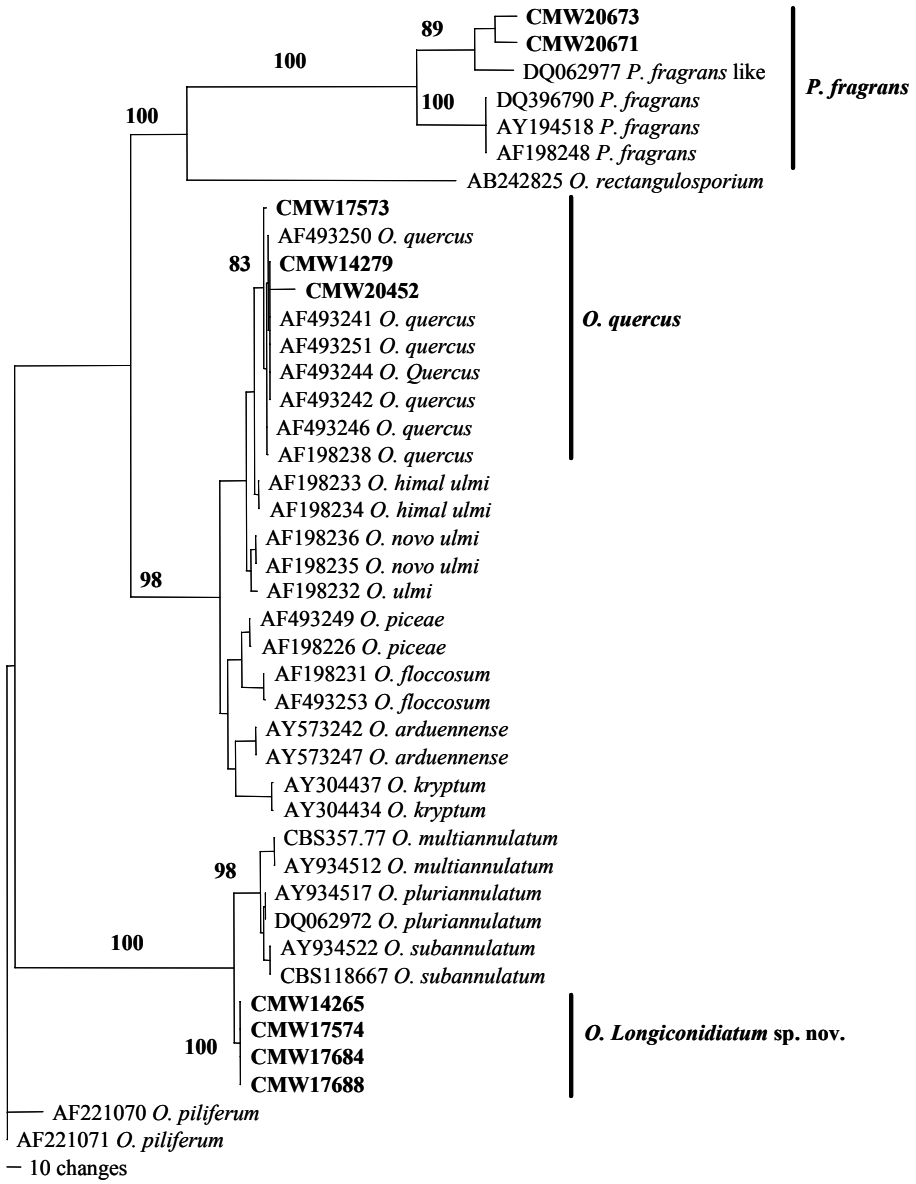

Fig. 3. Phylogenetic tree produced from a heuristic search of the ITS sequence data. *Ophiostoma piliferum* was used as out-group taxon. Bootstrap values were derived from 1000 replicates and are indicated next to each clade.

Ceratocystis tsitsikammensis Kamgan & Jol. Roux, **sp. nov.** (Fig. 4)
MycoBank: 510950

Etymology: The name describes the Tsitsikamma forests of South Africa (Groenkloof) where this fungus was found. The word <<Tsitsikamma>> comes from the Khoi words of <<tse-tsesa>> (meaning clear) and <<gami>> (meaning water). It is possible that the name Tsitsikamma refers to the clear water in the Tsitsikamma River which runs through the forest.

Crescit optime in 25°C; infra 10°C et supra 30°C non crescit. *Bases* ascomatum nigrae, globosae vel obpyriformes (105-) 129-211 (-279) µm longae (124-) 143-175 (-186) µm latae sine spinis ornamentisque. *Colla* ascomatum nigra (217-) 321-425 (-465) µm longa, hyphis ostiolaribus divergentibus (22.5-) 27.7-37.5 (-41.7) µm longis. *Ascosporae* piliformes. Forma anamorpha *Thielaviopsis*, conidiophoris singulis, *conidiis* bacillariformibus (15.4-) 18.3-22.7 (-28.5) × (2.6-) 3.3-4.5 (-5.5) µm, in catenis factis. *Chlamydo-sporae* ovoideae laeves, singulae factae, terminales, iuventute hyalinae, maturitate atrescentes (9.91-) 11.27-14.01 (-15.61) × (7.49-) 8.57-10.78 (-11.95) µm.

Colonies greenish olivaceous (23’’’i) on MEA, reverse grey olivaceous (23’’’’i) almost dark coloured. Colony diameter reaching 19 mm in 10 days on MEA at 25ºC. Optimal growth at 25ºC, no growth below 10ºC and above 30ºC. Colony surfaces scattered with black coloured ascomata. Mycelium immersed and superficial, with white-grey aerial myce-

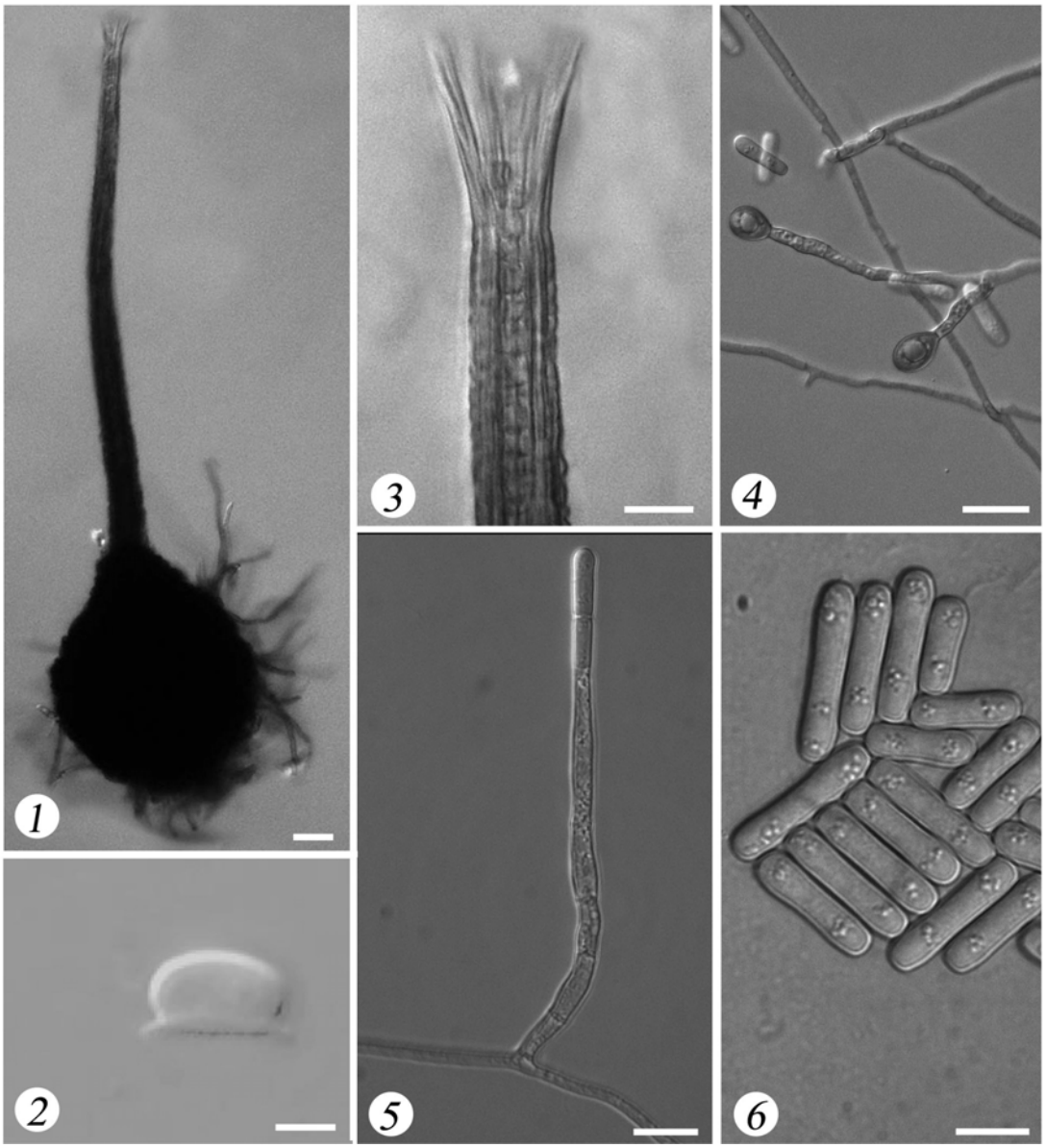

Fig. 4. Morphological characteristics of *Ceratocystis tsitsikammensis* sp. nov. **1.** Globose to obpyriform ascomatal base. **2.** Hat-shaped ascospores in side view. **3.** Divergent ostiolar hyphae. **4.** Ovoid chlamydospores. **5.** Phialidic conidiogenous cell with emerging bacilliform conidia. **6.** Bacilliform shaped conidia. Scale bars: 1, 3 = 10 µm, 2, 4-6 = 5 µm.

lium. Hyphae smooth, not constricted at septa. *Ascomatal* bases black, globose to obpyriform (105-) 129-211 (-279) µm long and (124-) 143-175 (-186) µm wide. Spines or ornamentations absent. *Ascomatal* necks black (217-) 321-425 (-465) µm long, bottom of necks smooth (31-) 32.1-47.1(-62) µm wide, middle of necks (23.4-) 25.3-29.7 (-32.5) µm wide, tips of necks (14.3-) 16.75-20.9 (-23.4) µm wide. *Ostiolar hyphae* present, divergent (22.5-) 27.7-37.5 (-41.7) µm long. *Asci* evanescent. *Ascospores* hat-shaped, invested in sheath, aseptate (4.4-) 5-6.3 (-6.7) µm long and (2.4-) 3.1-4 (-4.5) µm wide. Ascospores accumulating in round, hyaline spore drops when fresh, turning pale luteous (19d) when old.

Anamorph state: *Thielaviopsis*.

Conidiophores occurring singly, phialidic (34.2-) 46.9-110.2 (-162.1) × (3.6-) 4.4-6.7 (-8.7) µm, tubular with slight thin bases making them almost constricted at septa, hyaline, colarettes absent. *Conidia* bacilliform-shaped

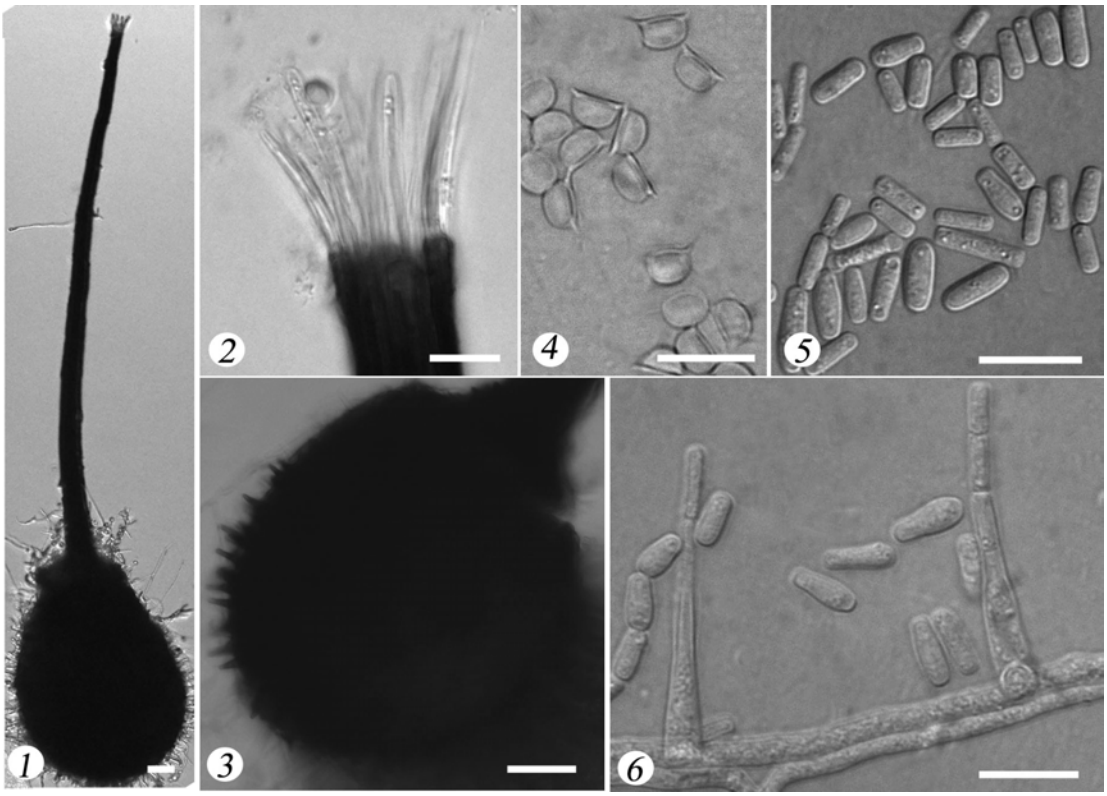

Fig. 5. Morphological characteristics of *Ceratocystis savannae* sp. nov. **1.** Globose to obpyriform ascomatal base. **2.** Divergent ostiolar hyphae. **3.** Ascomatal base with conical spines. **4.** Hat-shaped ascospores. **5.** Oblong and Bacilliform shaped conidia. **6.** Phialidic conidiogenous cell with emerging bacilliform conidia. Scale bars: 1-3 = 10 µm, 4-6 = 5 µm.

(15.4-) 18.3-22.7 (-28.5) × (2.6-) 3.3-4.5 (-5.5) µm, produced in chains. *Chlamydospores* (aleuroconidia) ovoid, smooth, formed singly, terminal, hyaline when young, becoming dark when mature (9.91-) 11.27-14.01 (-15.61) × (7.49-) 8.57-10.78 (-11.95) µm.

Specimens examined: SOUTH AFRICA, Groenkloof. Isolated from wounds on *Rapanea melanophloeos*, 28/01/2005, G. Kamgan Nkuekam, holotype PREM 59424, living culture CMW14276, CBS:121018.

Additional specimens: **South Africa**, Groenkloof. Isolated from wounds on *Rapanea melanophloeos*, 28/01/2005, G. Kamgan Nkuekam, **paratype**, living culture CMW14278/PREM 59658/CBS:121019 CMW 14280/PREM 59660/CBS:121020, CMW14274/PREM 59659.

Ceratocystis savannae Kamgan & Jol. Roux, **sp. nov.** (Fig. 5)

MycoBank: 510949

Etymology: Name refers to the Savanna vegetation type where the trees from which the fungus was collected are found.

Coloniae ad 58 mm diametro in 4 diebus in MEA in 30°C crescentes. Crescit optime in 30°C, in 35°C coloniae ad 30 mm in 4 diebus crescit, infra 5°C non crescit. *Mycelium* superficiale et inclusum, in agaro tegem grassam formans. *Bases* ascomatum atrobrunneae globosae vel obpyriformes (155-) 181-227.3 (-248) µm longae (155-) 178-217 (-248) µm latae, cum spinis atris conicis (1.42-) 2.9-8.1 (-13.4) µm longis et indumento hyphali. *Colla* ascomatum atrobrunnea (359.6-) 455-703 (-775) µm longa, *hyphis ostiolaribus* divergentibus (17-) 24.5-39.8 (-46) µm longis cum vinculis ad basin disciformibus, basi (37.2-) 48.2-59.3 (-62) µm latis. *Asci* evanescentes, *ascosporae* pileiformes hyalinae non septatae, vaginatae (4.5-) 4.6-5.3 (-5.8) × (2.2-) 2.6-3.2 (-3.7) µm. Forma anamorpha *Thielaviopsis*. *Conidiophorae* in mycelio singulae phialidicae hyalinae tubulosae basi leviter incrassatae (15.9-) 19.5-31.3 (-52.2) × (2.4-) 2.6-3.8 (-5) µm; collulis manifestis (0.59-) 0.98-1.97 (-2.7) µm. *Conidia* hyalina non septata biformia, oblonga (3.84-) 4.6-5.7 (-6.16) × (2.2-) 2.6-3.4 (-3.7) µm et bacilliformia basibus rotundatis (5.35-) 6.04-8.1 (-10.27) × (1.71-) 2-2.9 (-3.94) µm.

Colonies smoke grey (21’’’’d), fluffy on MEA, reverse smoke grey (21’’’’d) almost pale. Colony diameter reaching 58 mm in 4 days on MEA at 30ºC. Optimal growth at 30ºC, growth at 35ºC with colony diameter reaching 30mm in 4 days. No growth below 5ºC. *Mycelium* forming thick mat on agar. *Hyphae* smooth, not constricted at septa. *Ascomata*

scattered over the surface of the colonies or embedded in mycelium. *Ascomatal* bases dark brown, globose to obpyriform (155-) 181-227.3 (-248) µm long and (155-) 178-217 (-248) µm wide, with dark conical spines (1.42-) 2.9-8.1 (-13.4) µm and hyphal hair. *Ascomatal* necks dark brown (359.6-) 455-703 (-775) µm long, middle of necks (23.9-) 29.9-37.1 (-39.3) µm wide, tips of necks (13.1-) 16.2-20.9 (-23.6) µm wide, producing sticky and hyaline spore drops at the tips of divergent *ostiolar hyphae* (17-) 24.5-39.8 (-46) µm long and with disc-like (disciform) bases (37.2-) 48.2-59.3 (-62) µm wide at bases. *Asci* rarely seen, evanescent, deliquescing early in the development. *Ascospores* hat-shaped, hyaline, aseptate, invested in sheaths (4.5-) 4.6-5.3 (-5.8) × (2.2-) 2.6-3.2 (-3.7) µm, accumulating in round, straw yellow (21'd) spore drops, becoming creamy with age.

Anamorph: *Thielaviopsis*. *Conidiophores* singly on mycelium, phialidic, hyaline, tubular with a slight swelling at bases (15.9-) 19.5-31.3 (-52.2) × (2.4-) 2.6-3.8 (-5) µm; colarettes visible (0.59-) 0.98-1.97 (-2.7) µm. *Conidia* hyaline, aseptate, two types, oblong (3.84-) 4.6-5.7 (-6.16) × (2.2-) 2.6-3.4 (-3.7) µm and bacilliform with rounded bases (5.35-) 6.04-8.1 (-10.27) × (1.71-) 2-2.9 (-3.94) µm. *Chlamydospores* (aleuroconidia) not observed.

Specimen examined: SOUTH AFRICA, Mpumalanga Province, Kruger National Park, isolated from wound on *Acacia nigrescens*, 09/02/2005, G. Kamgan Nkuekam, **holotype** PREM 59423, living culture CMW17300, CBS:121151.

Additional specimens: SOUTH AFRICA, Mpumalanga Province, Kruger National Park, from wound on *Combretum zeyheri*, 09/02/2005, G. Kamgan Nkuekam, paratype, living culture CMW17297/PREM. 59738/CBS:121021, CMW17298/PREM.59739/CBS: 121022, CMW17575/PREM.59740.

Ophiostoma longiconidiatum Kamgan, K. Jacobs & Jol. Roux, **sp. nov.**

(Fig. 6)

MycoBank: 510951

Etymology: The name refers to the unusually long conidia found in the anamorph state.

Coloniae ad 20 mm diametro in 10 diebus in MEA in 25°C crescunt; infra 10°C et supra 30°C non crescunt. *Ascomata* in annulis concentricis in mediis artefactis dispositis, guttas hyalinas mucilagineas sporarum in apicibus collorum ascomatum facientes. *Colla* ascomatum atrobrunnea (279-) 352-698 (-868) µm longa saepe cum annulis singulis. *Bases* ascomatum globosae (267-) 415-797 (-992) µm longae (62-) 74-115 (-155) µm latae, laete flavescentes, sine ornamentis. Basis colli laevis (31-) 30-43 (-50) µm lata. *Hyphae ostiolares* desunt. *Asci* evanescentes, in evolutione praecoque deliquescentes. *Ascosporae* allantoideae non septatae hyalinae (3-) 3.5-4 (-4.4) × (1.09-) 1.1-1.4 (-1.6) µm. Anamorpha *Sporothrix, conidiophoris* tubulosis hyalinis (3.7-) 5.5-9.3 (-10.8) × (0.8-) 0.9-1.3 (-1.6) µm. Conidia oblonga vel cylindrica basibus rotundatis obtusis hyalinis (6.3-) 7.7-15.9 (-21) × (1.5-) 1.8-2.4 (-2.8) µm.

Colonies pale mouse grey (15'''''d) almost brown, fluffy on MEA. Reverse mouse grey (15'''''I). Colony diameter reaching 20 mm in 10 days on MEA at 25˚C. Optimal growth at 25˚C. No growth below 10˚C and above 30˚C. *Ascomata* arranged in concentric rings on agar surface producing hyaline, slimy spore drops at the neck apices. *Ascomatal* necks dark brown (279-) 352-698 (-868) µm long, often with single annuli. *Ascomatal bases* globose (267-) 415-797 (-992) µm long and (62-) 74-115 (-155) µm wide, light-yellowish without ornamentations. Neck base smooth, (31-) 30-43 (-50) µm wide. *Ostiolar hyphae* absent. *Asci* rarely seen, evanescent, deliquescing early in the development. *Ascospores* allantoid, aseptate, hyaline (3-) 3.5-4 (-4.4) × (1.09-) 1.1-1.4 (-1.6) µm.

Anamorph: *Sporothrix,* conidiophores, hyaline, cylindrical tapering towards the apex, (3.7-) 5.5-9.3 (-10.8) × (0.8-) 0.9-1.3 (-1.6) µm, prominent denticles present. *Conidia*, aseptate, hyaline, oblong, occasionally acerose, proximal end distinctly foot-shaped in some cases (6.3-) 7.7-15.9 (-21) × (1.5-) 1.8-2.4 (-2.8) µm.

Specimens examined: SOUTH AFRICA, Gauteng Province, Leeuwfontein Collaborative Nature Reserve, isolated from wound on *Terminalia sericea*, 03/02/2004, G. Kamgan Nkuekam, holotype PREM 59425, living culture CMW17574, CBS:121023.

Additional specimens: SOUTH AFRICA, Gauteng Province, Leeuwfontein Collaborative Nature Reserve, from wound on *Terminalia sericea*, 03/02/2004, G. Kamgan Nkuekam, paratype, living culture CMW17688/PREM 59661/CBS:121024, CMW17684/PREM 59662.

Pathogenicity tests

Two months after inoculation, trees were assessed for disease development based on the length of lesion seen on the bark or at the cambial surface. *Ceratocystis tsitsikammensis* (Fig. 7) and *C. savannae* (Fig. 8) produced

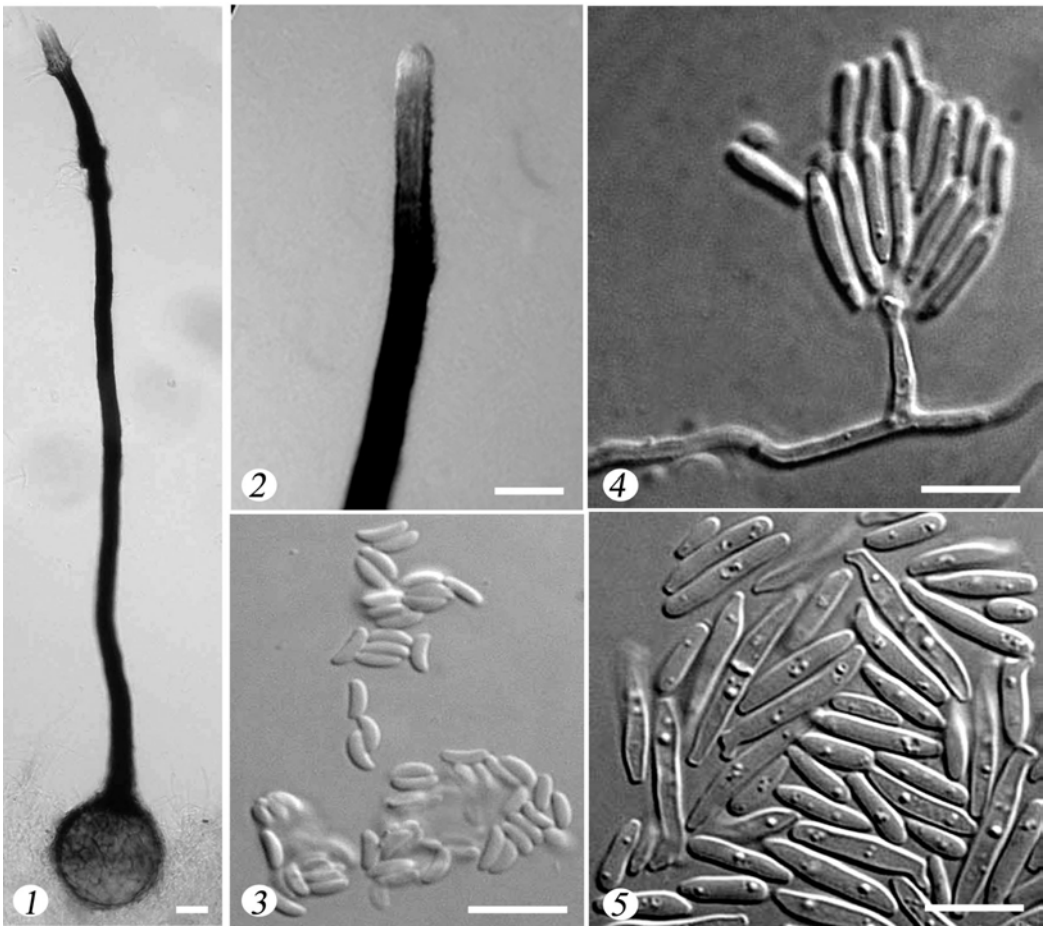

Fig. 6. Morphological characteristics of *Ophiostoma longiconidiatum* sp. nov. **1.** Globose ascomatal base. **2.** Ostiolar hyphae absent. **3.** Allantoid ascospores. **4.** Conidiogenous cell with emerging conidia. **5.** Conidia, oblong, acerose, proximal end distintly foot-shaped in some cases. Scale bars: 1-2 = 10 µm, 4-6 = 5 µm.

distinct lesions on the stems of inoculated *A. nigrescens*, *S. birrea* and *R. melanophloeos*, respectively. Some *R. melanophloeos* trees inoculated with *C. tsitsikammensis* produced epicormic shoots below the inoculation points and lesions reached up to 20 cm or longer in six weeks. *Ceratocystis tsitsikammensis* was re-isolated from a large number of trees while re-isolation of *C. savannae* from lesions was not successful. Significant differences ($P < 0.0001$) in lesion lengths were found for the two strains of *C. tsitsikammensis* as well as *C. savannae* when compared to the control inoculations (Fig. 7, 8).

Discussion

Three new fungal species from native South African trees were discovered in this study. Two of these were species of *Ceratocystis* and one is a new *Ophiostoma* sp., for which the names *C. tsitsikammensis*, *C. savannae* and *O. longiconidiatum* have been provided. In addition to these new species, *C. albifundus*, *O. quercus* and a fungus similar to *P. fragrans* were found. Amongst the isolated fungi considered in terms of their pathogenicity, *C. tsitsikammensis* gave rise to distinct lesions in inoculation trials on *R. melanophloeos* and it could be an important pathogen.

The genus *Ceratocystis* as it currently stands, represents an aggregate of species that includes species in very distinct monophyletic lineages (BD. Wingfield *et al*., 2006; www.fabinet.up.ac.za/ophiostoma/abstracts). Species of *Ceratocystis sensu lato* as they are currently treated, reside in two large phylogenetic groups. One of these accommodates *C. fimbriata* and many related species that have

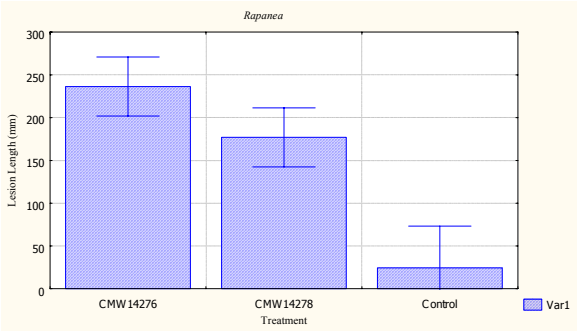

Fig. 7. Histogram showing results of 2nd inoculation trial (xylem lesion) with *C. tsitsikammensis* (CMW14276, 14278) on *R. melanophloeos* trees. Lsmean = 170.26, R = 0.52, CV = 45.07, P<0.0001, Confidence limit = 95%. Average lesion lengths (177 – 236.4) mm

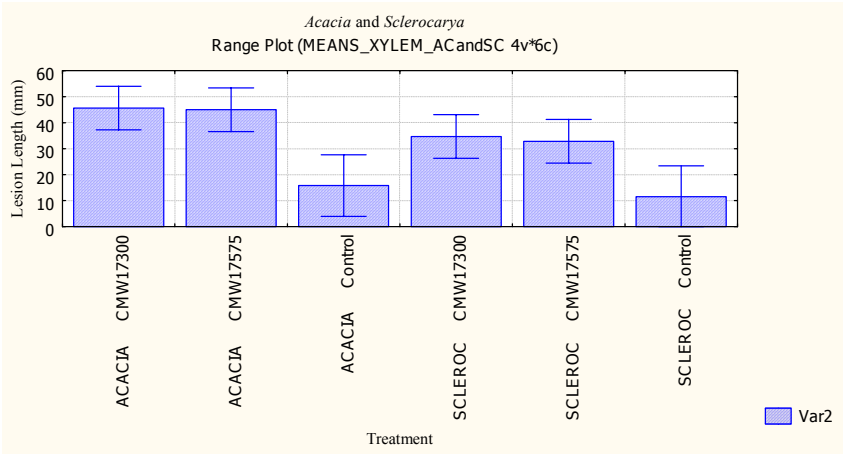

Fig. 8. Histogram showing results of 2nd inoculation trial (xylem lesion) with *C. savannae* (CMW17300, 17575) on *A. nigrescens* and *S. birrea* trees. Lsmean = 34.37, R = 0.28, CV = 55.07, P<0.0001, Confidence limit = 95%. Average lesion lengths (34.7 – 45.65) mm.

hat-shaped ascospores and of which many are important pathogens (Kile 1993, Witthuhn *et al*., 1999, Wingfield *et al*., 2006). The other clade includes *C. coerulescens* (Münch) B.K. Bakshi and its relatives (Witthuhn *et al*., 1999; Wingfield *et al*., 2006). The latter group might be further sub-divided (BD. Wingfield *et al*., 2006; www.fabinet.up. ac.za/ophiostoma/ abstracts) to include species related to *C. coerulescens* (Withuhn *et al*., 1999) and *C. moniliformis sensu lato* (Van Wyk *et al*., 2006). *Ceratocystis tsitsikammensis* sp. nov., one of the new species discovered in this study was most closely related to species in the *C. fimbriata sensu lato* species complex. *Ceratocystis savannae* the other new species of *Ceratocystis*, was most closely related to species in the *C. moniliformis sensu lato* species complex.

Ceratocystis tsitsikammensis resembles species in the *C. fimbriata sensu lato* species complex, producing hat-shaped ascospores and with a colony morphology very similar to that of *C. pirilliformis* and *C. fimbriata sensu stricto.* This new species can be distinguished from others in the *C. fimbriata s.l.* species complex based on a number of morphological characteristics. It differs from *C. pirilliformis* in that it produces divergent ostiolar hyphae. Furthermore, *C. pirilliformis* has distinct pear-shaped ascomatal bases, different to the globose bases of other species in this group (Barnes *et al*., 2003). *Ceratocystis tsitsikam-mensis* differs from *C. polychroma* in that its ascomata are smaller (217-465 µm long) than those of the latter species (837-1187 µm long). *Ceratocystis polychroma* also grows much faster (90 mm / 16d at 25˚C) than *C.*

tsitsikammensis (19 mm / 10d at 25˚C), and the conidiophores of *C. tsitsikammensis* are tubular to almost obpyriform, while those of *C. polychroma* are cylindrical. Also, *C. pirilliformis* and *C. polychroma* produce two types of conidiophores and two types of conidia (Barnes *et al.* 2003, Van Wyk *et al*., 2004a), in contrast to *C. tsitsikammensis* and *C. fimbriata* (Baker Engelbrecht and Harrington 2005) that produces only one type of conidiophore and one conidial form.

Multiple T-rich regions were present in DNA sequence data for the ITS and 5.8S gene regions of *C. tsitsikammensis.* This also serves to confirm that this fungus represents a species distinct from any other species in the larger *C. fimbriata* species complex. For phylogenetic analyses, we used sequences for the ITS, β-tubulin and EF-1α gene regions. Comparisons of sequences for these regions showed that *C. tsitsikammensis* is different from morphologically similar *Ceratocystis* spp. *Ceratocystis tsitsikammensis* falls within a sub-clade comprising *C. fimbriata*, *C. pirilliformis* and *C. polychroma*. However, within this group, *C. tsitsikammensis* forms a well-resolved clade clearly separated from the other three taxa. The closest phylogenetic neighbor of *C. tsitsikammensis* in a combined tree is *C. polychroma*, while in non-combined trees, its closest phylogenetic neighbor was a strain of *C. fimbriata s.s.* from Papua New Guinea.

Ceratocystis savannae, described as new in this study, is morphologically similar to species in the *C. moniliformis* species complex, within the larger *C. coerulescens* (Witthuhn *et al*., 1999) clade. Species in the *C. moniliformis* species complex are morphologically similar to each other with hat-shaped ascospores, disk-shaped attachment points at the bases of the ascomatal necks, short conical spines on the ascomatal bases and the production of both cylindrical and barrel shaped conidia (Yuan and Mohammed, 2002; Van Wyk *et al*., 2004b; Al-Subhi *et al*., 2006; Van Wyk *et al*., 2006). Other than *C. moniliformis* (Hedgcock, 1906), this complex includes *C. bhutanensis* M. van Wyk, M.J. Wingf. & Kirisits (Van Wyk *et al*., 2004b), *C. moniliformopsis* Z.Q. Yuan & C. Mohammed (Yuan & Mohammed, 2002), *C. omanensis* Al-Subhi, M.J. Wingf., M. van Wyk & Deadman (Al-Subhi *et al*., 2006) and *C. tribiliformis* M. van Wyk & M.J. Wingf (Van Wyk *et al*., 2006). Based on phylogenetic comparisons, *C. savannae* is different from other species in the *C. moniliformis* complex, residing in a separate clade and most closely related to *C. bhutanensis* and *C. omanensis* (97% bootstrap value).

Ceratocystis savannae can be distinguished from closely related species by a few phenotypic traits. This fungus produces tubular phialides while *C. omanensis* and *C. tribiliformis* produce phialides with characteristically swollen bases (Al-Subhi *et al*., 2006; Van Wyk *et al*., 2006). Colonies of *C. savannae* are a smokey grey colour while those of *C. omanensis* and *C. tribiliformis* are white to wood-brown (Al-Subhi *et al*., 2006, Van Wyk *et al*., 2006). The mycelium of *C. tribiliformis* is nearly embedded in agar, not as abundant and fluffy as *C. savannae*. *Ceratocystis savannae* differs from *C. bhutanensis* in that it has globose to obpyriform ascomatal bases, oblong and bacilliform conidia, and smoke grey colonies while *C. bhutanensis* has globose ascomatal bases, cylindrical and barrel-shaped conidia and cream-buff to dark olive colonies. Among species in the *C. moniliformis* complex, these morphological differences are not always reliable characteristics for identification, therefore, comparison of identities based on DNA sequence comparisons are important.

Isolation of *C. albifundus* from wounds on native hardwood trees in this study was not surprising. The fungus has previously been reported from many native tree genera in South Africa (Roux *et al*., 2004b; Roux *et al*., 2007). *Ceratocystis albifundus* is also well-known as a pathogen of non-native *A. mearnsii* trees in South Africa (Morris *et al*., 1993; Roux and Wingfield, 1997; Wingfield *et al*., 1996). Its occurrence in the Kruger National Park, on native trees in isolation from non-native hosts such as *A. mearnsii*, supports the view (Barnes *et al*., 2005; Roux *et al*., 2001) that it is most likely native to South Africa.

Three *Ophiostoma* spp. were collected from native hardwood tree species in this study. These included three known species and the newly described *O. longiconidiatum*. Based on DNA sequence comparisons, *O. longiconidiatum* is closely related to species in the *O. pluriannulatum* complex, such as *O. plurian-*

nulatum (Hedgc.) Syd. & P. Syd., *O. multiannulatum* (Hedgc. & R.W. Davidson) Hendr. and *O. subannulatum* Livingston & R.W. Davidson. Morphologically, *O. longiconidiatum* can, however, be distinguished from other species in the complex by its light coloured ascomatal bases and lack of ostiolar hyphae. Also, the ascomatal necks of *O. longiconidiatum* are much shorter than those of other species in the *O. pluriannulatum* complex.

Ophiostoma quercus and a species resembling *P. fragrans* were also found in this study. *Pesotum fragrans* was first described in Sweden from the galleries of *Ips sexdentatus* Boerner, infesting *Pinus sylvestris* (Mathiesen and Käärik, 1953). The fungus was later also reported from Australia, California, Canada and New Zealand (Harrington *et al*., 2001; Jacobs *et al*., 2003). In South Africa, *P. fragrans* was first reported from *Pinus patula* Schiede ex Schltdl. & Cham., where it was consistently isolated from an introduced conifer-infesting bark beetle, *Hylastes angustatus* Herbst. (Zhou *et al*., 2006). In this study we report strains of a *P. fragrans*-like isolate that were collected from *R. melanophloeos* and *Rhus chirindensis*. These isolates grouped with a *P. fragrans*-like reference strain in a major clade that also includes the true *P. fragrans*. Additional research will be required to clarify the species delimitation for these isolates.

It was not surprising to find *O. quercus* on native hardwood trees in this study. The fungus has previously been reported from South Africa on native *Olinia* sp. and on non-native *E. grandis* and *Quercus robur* L. (De Beer *et al*., 1995). Its occurrence on wounds on *R. melanophloeos* and *T. sericea* in this study has expanded the host range of the fungus in South Africa. *Ophiostoma quercus* has a cosmopolitan distribution on hardwoods and is found both in the northern and southern Hemisphere (Harrington *et al*., 2001; De Beer *et al*., 2003). Its occurrence in South Africa, on a number of native tree species further expands the already wide host range of this fungus. Hypotheses regarding its origin include both the northern and southern Hemisphere (Brasier and Kirk, 1993; Harrington *et al*., 2001; De Beer *et al*., 2003) but additional research is required to clarify this question. Nonetheless, studies such as the one presented here, contribute to unravelling these questions.

Inoculation studies with *C. savannae* showed that this fungus can cause small lesions on young *A. nigrescens* and *S. birrea* trees. This is likely a native fungus with a very low level of pathogenicity to the tree from which it was isolated. In contrast, pathogenicity tests conducted with strains of *C. tsitsikammensis* have showed that it is highly pathogenic. Artificial inoculations on *R. melanophloeos* trees resulted in severe lesions in both the bark and the xylem, within eight weeks after inoculation. At the time when the lesion lengths were recorded, many trees had developed epicormic shoots below the inoculation points as a result of stem-girdling. *Ceratocystis tsitsikammensis* was also consistently re-isolated from the lesions. Pathogenicity tests on trees growing in the field are now required to determine the impact of this fungus under natural conditions.

This study represents the most comprehensive consideration of *Ceratocystis* and *Ophiostoma* species on native hardwood trees in Africa, ever to have been undertaken. The number of new taxa encountered, clearly emphasizes the importance of expanding these surveys to include additional tree species and a wider geographic area within South Africa as well as the rest of the African continent. The high level of pathogenicity found in *C. tsitsikammensis*, and that of the better-known *C. albifundus* also supports the view that the group includes important pathogens that have yet to be discovered.

Acknowledgements

We thank the DST/NRF Center of Excellence in Tree Health Biotechnology (CTHB), National Research Foundation of South Africa (NRF), the THRIP Initiative of the Department of Trade and Industry (THRIP/DST), members of the Tree Protection Co-operative Programme (TPCP) and the University of Pretoria for funding and the facilities to undertake this study. We also thank the late Dr. B.E. Eisenberg for assistance with statistical analyses, as well as Dr. Hugh Glen for assistance with Latin translations. In addition, we thank the South African National Parks and the Gauteng Department of Agriculture, Conservation and Environment for field sites and assistance with the

surveys, particularly Me. Thembi Khoza, Dr. Coert Geldenhuys and Mr. Leon Labuschagne of Leeuwfontein Collaborative Nature Reserve.

References

Al-Subhi, A.M., Al-Adawi, A.O., Deadman, M.L., Van Wyk, M. and Wingfield, M.J. (2006). *Ceratocystis omanensis*, a new species from diseased mango trees in Oman. Mycological Research 110: 237-245.

Baker 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.

Barnes, I., Dudzinski, M.J., Old, K.M., Roux, J., Wingfield, B.D. and Wingfield, M.J. (2003). *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia 95: 865-871.

Barnes, I., Nakabonge, G., Roux, J., Wingfield, B.D. and Wingfield M.J. (2005). Comparison of populations of the wilt pathogen *Ceratocystis albifundus* in South Africa and Uganda. Plant Pathology 54: 189-195.

Brasier, C.M. and Kirk, S.A. (1993). Sibling species within *Ophiostoma piceae*. Mycological Research 97: 811-816.

De Beer, Z.W., Wingfield, M.J. and Kemp, G.H.J. (1995). First report of *Ophiostoma querci* in South Africa. South African Journal of Science 91: 6.

De Beer, Z.W., Wingfield, B.D. and Wingfield, M.J. (2003). The *Ophiostoma piceae* complex in the southern hemisphere: a phylogenetic study. Mycological Research 107: 469-476.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

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.

Gorter, G.J.M.A. (1977). *Index of plant pathogens and the diseases they cause in cultivated plants in South Africa*. Science Bulletin no. 392. Pretoria, South Africa: Plant Protection Research Institute, Department of Agricultural Technical Services.

Harrington, T.C; Farrell, R; Hofstra, D; McNew, D. and Steimel, J. (2001). Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93: 111-136.

Hausner, G., Reid, J. and Klassen, G.R. (1993). On the subdivision of *Ceratocystis* s.l., based on partial ribosomal DNA sequences. Canadian Journal of Botany 71: 52-63.

Hedgcock, G.G. (1906). Studies upon some chromogenic fungi which discolor wood. Missouri Botanical Garden 17: 59-114.

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.

Jacobs, K., Seifert, K.A., Harrison, K.J. and Kirisits, T. (2003). Identity and phylogenetic relationships of ophiostomatoid fungi associated with invasive and native *Tetropium* species (Coleoptera, Cerambycidae) in atlantic Canada. Canadian Journal of Botany 81: 316-329.

Jacobs, K., Wingfield, M.J. and Roux, J. (1999). *Leptographium eucalyptophilum*, a new species from *Eucalyptus* in the Congo. South African Journal of Botany 65: 388-391.

Katoh, K., Misawa, K., Kuma, K.I. and Miyata, T. (2002). MAFFT: a novel method for rapid sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059-3066.

Kile, G.A. (1993). Plant diseases caused by species of *Ceratocystis* sensu stricto and *chalara*. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity: (eds. M.J. Wingfield, K.A. Seifert and J.F. Webber). American Phytopathological Society Press, St. Paul, Minnesota: 173-183.

Lawes, M.J., Eeley, H.A.C., Shackleton, C.M. and Geach, B.G.S. (2004). *Indigenous Forests and Woodlands in South Africa*. University of KwaZulu-Natal Press.

Mathiesen, A. and Käärik, A. (1953). Eine Ubersicht Uber die gewohnlichsten mit Borkenkafern assoziierten Blauepilze in Schweden und einige fur Schweden neue Blauepilze. Meddelanden fran Statens. Skogsforskningsinstitutut 43: 1-74.

Marais, G.J. and Wingfield M.J. (1994). Fungi associated with infructescences of *Protea* species in South Africa, including a new species of *Ophiostoma*. Mycological Research 98: 369-374.

Marais, G.J. and Wingfield, M.J. (1997). *Ophiostoma protearum* sp. nov. associated with *Protea caffra* infructescences. Canadian Journal of Botany 75: 362-367.

Marais, G.J. and Wingfield, M.J. (2001). *Ophiostoma africanum* sp. nov., and a key to ophiostomatoid species from *Protea* infructescences. Mycological Research 105: 240-246.

Möller, E.M., Bahnweg, G., Sandermann, H. and Geiger, H.H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research 20: 6115-6116.

Morris, M.J., Wingfield, M.J. and De Beer, C. (1993). Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathology 42: 814-817.

Paulin-Mahady, A.E., Harrington, T.C. and McNew, D.L. (2002). Phylogenetic and taxonomic evaluation of C*halara, Chalaropsis* and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia 94: 62-72.

Roux, J. and Wingfield, M.J. (1997). Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management 99: 327-336.

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

Roux, J., Harrington, T.C., Steimel, J.P. and Wingfield, M.J. (2001). Genetic variation in the wattle pathogen *Ceratocystis albifundus*. Mycoscience 42: 327-332.

Roux, J., Heath, R.N., Labuschagne, L., Kamgan Nkuekam, G. and Wingfield, M.J. (2007). Occurrence of the wattle wilt pathogen, *Ceratocystis albifundus* on native South African trees. Forest Pathology (In press).

Roux, J., Labuschagne, L., Heath, R.N., Nkuekam, G.K. and Wingfield, M.J. (2004b). The Occurrence of the wilt pathogen, *Ceratocystis albifundus* on native South African trees. Proceedings of the American Phytopathological Society Meeting, July 31-August 4, Anaheim, California. Phytopathology 94: S89.

Roets, F., Crous, P.W., De Beer, Z.W., Dreyer, L.L., Wingfield, M.J. and Zipfel, R. (2006a). Multigene phylogeny for *Ophiostoma* spp. reveals two new species from *Protea* infructescences. Studies in Mycology 55: 199-212.

Roets, F., Bellstedt, D.U., Crous, P.W., Dreyer, L.L. and Wingfield, M.J. (2006b). A PCR-based method to detect species on *Gondwanamyces* and *Ophiostoma* on surfaces of insects colonizing Protea flowers. Canadian Journal of Botany 84: 989-994.

SAS Institute Inc., SAS/STAT Users Guide, Version 8, Cary NC: SAS Institute Inc., 1999. ISBN 1-58025-494-2.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sinclair, W.A. and Lyon, H.H. (2005). Diseases of trees and shrubs 2nd Edition. Cornell University Press: Ithaca, London: 232-241.

Spatafora, J.W. and Blackwell, M. (1994). The polyphyletic origins of Ophiostomatoid fungi. Mycological Research 98: 1-9.

Swofford, D.L. (1998). PAUP. Phylogenetic analysis using parsimony (and other methods). Version 4. Sinaur Associates, Sunderland, Massachusetts.

Upadhyay, H.P. (1981). A monograph of the genus *Ceratocystis* and *Ceratocystiopsis*. Athens: University of Georgia press.

Van Wyk, M., Assa, B., Barnes, I., Liew, E.C.Y., Roux, J., Summerell, B.A., Wingfield, B.D. and Wingfield, M.J. (2004a). *Ceratocystis polychroma* sp. nov., a new species from *Syzygium aromaticum* in Sulawesi. Studies in Mycology 50: 273-282.

Van Wyk, M., Barnes, I., Chhetri, D.B., Roux, J., Kirisits, T., Wingfield, B.D. and Wingfield, M.J. (2004b). *Ceratocystis bhutanensis* sp. nov., associated with the bark beetle *Ips schmutzenhoferi* on *Picea spinulosa* in Bhutan. Studies in Mycology 50: 365-379.

Van Wyk, M., Barnes, I., Roux, J., Wingfield, B.D. and Wingfield, M.J. (2006). Molecular phylogeny of the *Ceratocystis moniliformis* complex and description of *C. tribiliformis* sp. nov. Fungal Diversity 21: 181-201.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A sequencing guide to methods and applications*. (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White). Academic Press, San Diego: 315-322.

Wingfield, M.J., Van Wyk, P.S. and Marasas, W.F.O. (1988). *Ceratocystiopsis proteae* sp. nov., with a new anamorph genus. Mycologia 80: 23-30.

Wingfield, M.J. and Van Wyk, P.S. (1993). A new species of *Ophiostoma* from *Proteae* infructescences in South Africa. Mycological Research 97: 709-716.

Wingfield, M.J., Seifert, K.A. and Webber, J.F. (1993). *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity. American Phytopathological Society Press, St. Paul, Minnesota.

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.

Wingfield, B.D., Roos, H., Van Wyk, M. and Wingfield, M.J. (2006). Species of *Ceratocystis*: Emerging evidence for discrete generic boundaries. In: Ophiostomatoid fungi: Expanding frontiers, 16-18 August 2006, North Stradbroke Island Brisbane, Australia, Abstract PP 19. (www.fabinet.up.ac.za/ophiostoma/abstracts).

Witthuhn, R.C., Harrington, T.C., Wingfield, B.D. and Wingfield, M.J. (1999). PCR-based identification and phylogeny of species of *Ceratocystis sensu stricto*. Mycological Research 103: 743-749.

Yuan, Z-Qing and Mohammed, C. (2002). *Ceratocystis moniliformopsis* sp. nov., an early coloniser of *Eucalyptus oblique* logs in Tasmania, Australia. Australian Systematic Botany 15: 125-133.

Zhou, X.D., De Beer, Z.W., Wingfield, B.D. and Wingfield, M.J. (2001). Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. Sydowia 53: 290-300.

Zhou, X.D., De Beer, Z.W. and Wingfield, M.J. (2006). DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa. Studies in Mycology 55: 269-277.

Zipfel, R.D., De Beer, Z.W., Jacobs, K., Wingfield, M.J. and Wingfield, B.D. (2006). Multigene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Studies in Mycology 55: 75-97.