System. Appl. Microbiol. 19, 191–202 (1996)

A New *Ceratocystis* Species Defined Using Morphological and Ribosomal DNA Sequence Comparisons

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Received September 15, 1995

Summary

A *Ceratocystis* Ell. & Halst. species has recently been reported from *Acacia mearnsii* De Willd. in South Africa showing symptoms of die-back and gummosis. The fungus was tentatively identified as *Ceratocystis fimbriata* Ell. & Halst. that is a well-known and notorious pathogen of many plants of the world. The fungus from *A. mearnsii*, however, differs morphologically from authentic *C. fimbriata* isolates. In this study, isolates from *A. mearnsii* in South Africa were compared with *C. fimbriata* isolates from different geographic areas on the basis of morphology and ribosomal RNA (rRNA) sequence data. Morphological comparisons showed that the *Ceratocystis* isolates from *A. mearnsii* have light-coloured perithecial bases and divergently arranged ostiolar hyphae compared with entirely black perithecia and convergently arranged ostiolar hyphae of typical *C. fimbriata* isolates. Comparisons of rDNA confirmed the morphological differences and grouped the South African isolates apart from *C. fimbriata* originating from other areas. This important pathogenic fungus is consequently described as new.

Key words: *Ceratocystis* – *Chalara* – *Acacia* Wilt disease – *C. fimbriata* – Ribosomal RNA sequence

Introduction

Ceratocystis sensu lato includes the genera, *Ceratocystis sensu stricto*, *Ceratocystiopsis* Upad. & Kendr. and *Ophiostoma* H. & P. Sydow (*De Hoog* and *Scheffer*, 1984; *Wingfield* et al., 1993). These fungi are known to be closely associated with insects (*Whitney*, 1982) and include important plant pathogens (*Wingfield* et al., 1993). Pathogens range from being weak; such as *C. paradoxa* (Dade) C. Moreau to aggressive primary pathogens as in the case of *O. ulmi* (Buism.), *O. novo-ulmi* Brasier and *C. fimbriata* (*Kile*, 1993).

Ceratocystis fimbriata is a well known pathogen with a world-wide distribution and causes diseases on a wide range of hosts. It is sometimes referred to as the sweet potato black rot fungus (*Kojima* and *Uritani*, 1974; *Kojima* et al., 1982; *Kawakita* and *Kojima*, 1983; *Kawakita* and *Kojima*, 1986; *Yasuda* and *Kojima*, 1986) due to the fact that it was first isolated from sweet potatoes (*Ipomoea batatas* [L.] Lam.), causing dark circular spots on the roots (*Halsted*, 1890). Other hosts include, rubber (*Olson* and *Martin*, 1949), coffee (*Pontis*, 1951), quaking aspen (*Wood* and *French*, 1963), prune, apricot and peach trees (*De Vay* et al., 1963; *De Vay* et al., 1968), apsen (*Zalasky*, 1965; *Manion* and *French*, 1967), pimento (*Leather*, 1966), poplar (*Gremmen* and *De Kam*, 1977), plane (*Anonymous*, 1986; *Grosclaude* and *Olivier*, 1988), mango (*Ribeiro* et al., 1986), syngonium (*Vogelzang* and *Scott*, 1990) and almond (*Teviotdale* and *Harper*, 1991).

A fungus tentatively identified as *C. fimbriata* has recently been isolated from *A. mearnsii* (black wattle) in South Africa (*Morris* et al., 1993). In this situation, the fungus causes gummosis and die-back of trees. This was the first report of *C. fimbriata* attacking *A. mearnsii* but was not the first record of the fungus in South Africa. The fungus was previously isolated from a diseased *Protea* L. plant in the Transvaal Province (*Gorter*, 1977).

Isolates of the purported *C. fimbriata* are being obtained from black wattle in South Africa regularly. These, however, differ morphologically from authentic isolates occurring elsewhere in the world and from various descriptions of the fungus. The aim of this study was, therefore, to compare South African isolates of *C. fimbriata* from black wattle with those from other parts of the world using conventional mycological techniques as well as partial ribosomal RNA sequence comparisons.

Materials and Methods

Sources of isolates

Isolates used for morphological and molecular comparisons are listed in Table 1. Cultures of *C. fimbriata* from plane trees (PREM 51642, PREM 51644) were supplied by Prof. C. Grosclaude, INRA, Station de Pathologie Vègètale, 84143 Montfavet cedex, France. The isolate from sweet potato in Papua New Guinea (PREM 51643) was obtained from the culture collection of the DSIR, Auckland New Zealand (8579); isolates from Italy were supplied by Dr. P. Capretti, Instituto di Patologi E Zoologia Forestale, Piazzale Delle Casine 28, Firenze and isolated by Dr. Panconesi. The isolate of *C. moniliformis* (Hedgc.) C. Moreau from Japan originates from the culture collection of the Institute for Fermentation, Osaka (IFO 8667).

Morphological comparisons

Isolates PREM 51641 and PREM 51645 of the *Ceratocystis* sp. from black wattle in South Africa and PREM 51643 and PREM 51642 from Papua New Guinea and France respectively, were cultured on malt extract agar (20 g Difco malt extract; 20 g Difco Bacto agar/1000 ml water) at 25 °C and studied by means of light microscopy. Measurements were taken for the different structures and analyzed with SAS/Stat system for personal computers (SAS Institute Inc. SAS Campus Drive, Cary, NC 27513). These were then compared with morphological characteristics from a herbarium specimen identified as *C. fimbriata* from a *Protea* sp. in South Africa (PREM 48263) as well as characteristics of this fungus previously recorded in the literature.

For scanning electron microscopy (SEM), cultures were grown on 2% malt extract agar. Agar blocks were cut from cultures and fixed in glutaraldehyde (3%) for 4 h and then in osmium (1%) for 1,5 h. Dehydration of the material followed via a graded acetone series. The material was critical point dried, mounted on stubs, coated with gold/palladium and examined with a *JEOL WINSEM* (JSM 6400) scanning electron microscope.

The growth rates of isolates of the South African *Ceratocystis* sp. (PREM 51641, PREM 51645) were compared with two authentic isolates from Papua New Guinea (PREM 51643) and France (PREM 51642). Three plates containing 2% MEA were inoculated with 4 mm discs from two week old cultures of each and were incubated at temperatures ranging from 15 °C to 30 °C at 5 °C intervals. Growth rates were recorded after eight days and two measurements of the colony diameter were made at right angles to each other. For each isolate, the average of six measurements was therefore taken.

Molecular comparisons

Cultures used for molecular comparisons included PREM 51639, PREM 51829, PREM 51645, PREM 51643, IFO 8667, PREM 51644, PREM 51830, PREM 51831 (Table 1). Sterile cellophane discs (8 cm diameter) were placed on 2% MEA in Petri dishes and inoculated with the test fungi. Strains were grown at 25 °C until the mycelia covered the cellophane discs which were then removed and lyophilised.

Fungal mycelium was scraped from the cellophane surface and the DNA isolated according to the methods described by *Viljoen* et al. (1993). The internal transcribed spacer regions (ITS1 and ITS2), as well as the 5.8S gene of the ribosomal RNA operon were amplified using the Polymerase Chain Reaction (PCR) [*Saiki* et al., 1988] with primers ITS1 and LR1 (Table 2). DNA fragments were purified using Magic PCR Preps (Promega Cor-

Table 1. Isolates of *Ceratocystis* spp. used in morphological and molecular comparisons[1]

Species[2]	Source	Origin
Ceratocystis sp. (PREM 51641)	*Acacia mearnsii*	South Africa
Ceratocystis sp. (PREM 51639)	*A. mearnsii*	South Africa
Ceratocystis sp. (PREM 51829)	*A. mearnsii*	South Africa
Ceratocystis sp. (PREM 51645)	*A. mearnsii*	South Africa
C. fimbriata (PREM 48263)	*Protea* sp.	South Africa
C. fimbriata (PREM 51643)	*Ipomoea batatas*	Papua New Guinea
C. fimbriata (PREM 51642)	*Platanus hybrida*	France
C. fimbriata (PREM 51644)	*P. hybrida*	France
C. fimbriata (PREM 51830)	*P. orientalis*	Italy, Sicily
C. fimbriata (PREM 51831)	*P. orientalis*	Italy
C. moniliformis (IFO 8667)	–	Japan

[1] The isolate from *Protea* sp. represents a dried culture used in light microscopy

[2] PREM is the official designation of the National Collection of Fungi, Pretoria, South Africa and IFO refers to the culture collection of the Institute for Fermentation, Osaka, Japan

Table 2. Primers used to amplify and to sequence the desired region of the rDNA operon

Primers	Sequence	Source
ITS1	TCCGTAGGTGAACCTGCGG	*White* et al., 1990
LR1	GGTTGGTTTCTTTTCCT	*Vilgalys* and *Hester*, 1990
5.8SR	GCATCGATGAAGAACGCAGC	*Vilgalys* and *Hester*, 1990
CS2[1]	CGAATCTTTGAACGCACATG	Dr. B. D. Wingfield

[1] Based on the 5.8S gene of several ophiostomatoid fungi sequenced in our laboratory

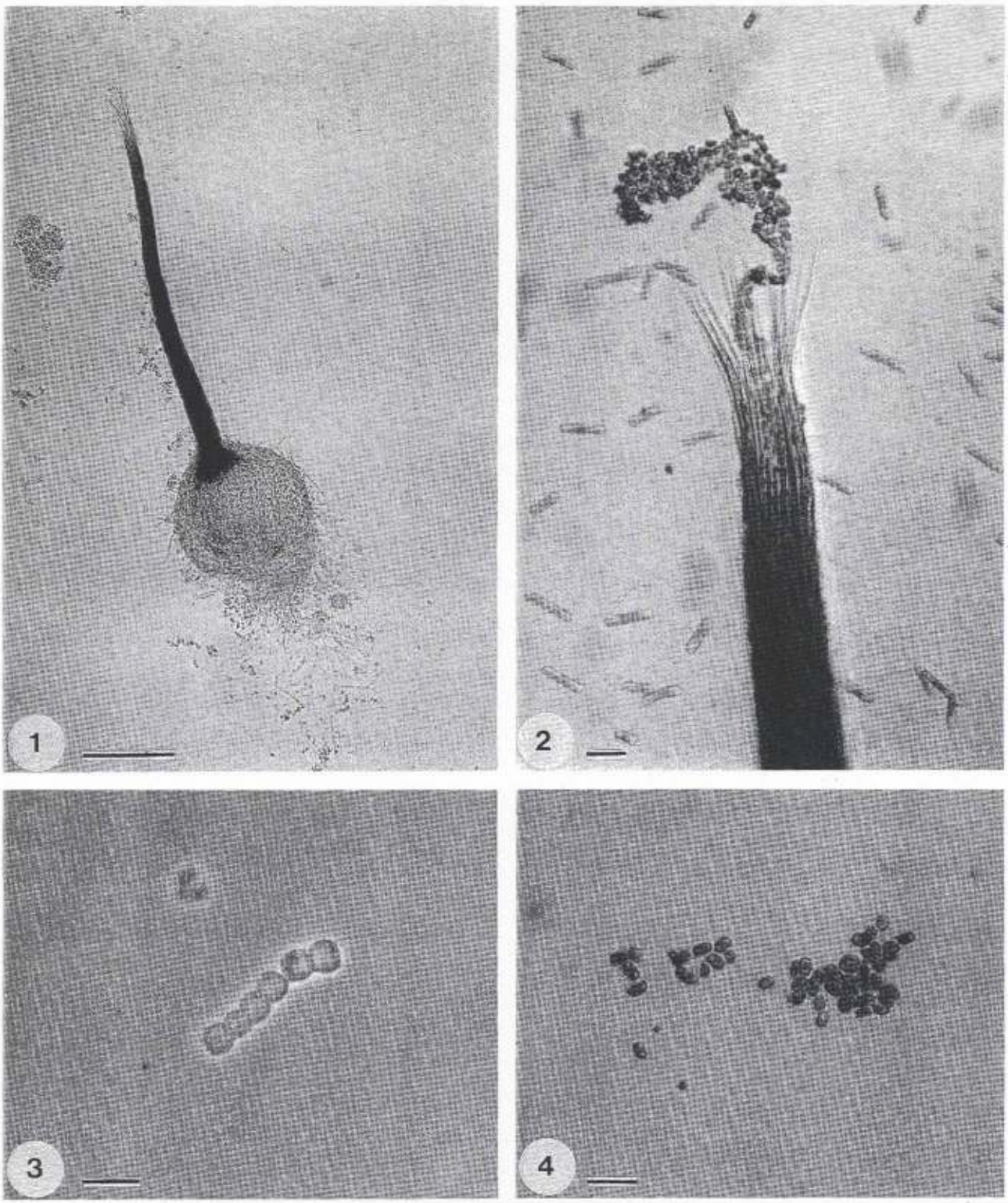
poration, Madison, USA). Both strands of the purified PCR fragment were sequenced using the *f*mol DNA Sequencing System (Promega Corporation, Madison, USA). The ITS1, CS2, 5.8SR and LR1 primers (Table 2) were used for sequencing.

The nucleotide sequences were aligned using CLUSTAL (PC-Gene, IntelliGenetics, Inc., Mountain View, California). Phylogenetic relations of the fungal isolates were determined with PAUP (Phylogenetic Analysis Using Parsimony, *Swofford*, 1993) and UPGMA (unweighted group-pair method with arithmetic mean, *Sneath* and *Sokal*, 1973). The DNA sequence of *Neurospora crassa* Shear & Dodge was used as an outgroup for the phylogenetic analyses (*Chambers* et al., 1986). *C. moniliformis* was included in this study to provide a measure of the relative distance from the other *Ceratocystis* species.

Table 3. Comparison of morphological characteristics of the *Ceratocystis* sp. from black wattle in South Africa, authentic isolates of *C. fimbriata* from various geographic regions, herbarium material from *Protea* sp. in South Africa and published descriptions[1]

Character	*Halsted* and *Fairchild* 1891	*Hunt* 1956	*Upadhyay* 1981	PREM 51641	PREM 51645	PREM 51642	PREM 51643	PREM 48263
Perithecial base width	96–224	130–200	121–255	104–200 (158)d	160–368 (245)b	208–320 (270)a	88–200 (157)d	112–280 (176)c
Neck length	395–608	–800	950–1200	208–840 (510)d	440–1000 (685)a	495–688 (595)c	480–736 (628)bc	416–848 (645)b
Neck width, base	24–34	20–35	18–40	20–32 (27)c	32–64 (44)a	32–40 (33)b	24–40 (31)b	16–32 (26)c
Neck width, tip	14–20	10–20	10–18	16–24 (17)c	16–32 (25)a	16–32 (24)a	16–20 (17)c	16–32 (19)b
Ascospore length	–	4.5–8	3.5–8	4–6.5 (5.2)a	5–6 (5.6)ab	4–6 (5.1)ab	5–7 (5.8)ab	3.5–5 (4.6)b
Ascospore width	–	2.5–5.5	2–2.5	3.5–5 (4.2)a	3–5 (3.7)b	3–5 (3.7)b	3–5 (4)a	3–3.5 (3)c
Cylindrical conidia, length	16–30	11–16	9–21	8–24 (14.6)bc	7–20 (13.8)c	12–32 (18)a	9–23 (14.3)bc	9–19 (15)b
Cylindrical conidia, width	4–9	4–5	2–6.5	3–4 (3.5)a	3–4 (3.3)a	2.5–4 (3.8)a	3–5 (3.9)a	2–4 (3.1)a
Barrel conidia, length	12–19	9–16	9–17	6–10 (7)b	6–12 (9)a	–	5–13 (8)b	–
Barrel conidia, width	–	6–13	5–13	4–8 (6)a	4–10 (7)a	–	4–9 (8)a	–
Hyphal diameter	2.0–6.0	2.0–6.0	1.5–5	2–5	2.3–3.1	1.5–2	2–3	–
Perithecial colour	–	brown-black	brown-dark brown/ black	yellowish with black neck	yellowish with black neck	black	black	yellowish with black neck
Colony colour	–	hyaline – light brown, greenish brown	hyaline – subhyaline, greyish brown – dark brown	creamy – light brown	creamy – light brown	greenish brown	greenish brown	–
Hyphal colour	–	aerial: hyaline, submerged: darker	hyaline – pale brown	hyaline	hyaline	hyaline	hyaline	hyaline
Ostiolar hyphae arrangement	–	–	straight/ flexuous	divergent	divergent	convergent	convergent	–
Odour	–	banana oil/ fruit-like	–	fruit-like	fruit-like	banana oil	banana oil	–

[1] For each isolate, the ranges of measurements are given in µm, followed by the mean value of 50 measurements in brackets. Corresponding alphabetical letters in rows indicate no significant difference for that particular character



Figs. 1–4. Light micrographs of teleomorph and anamorph characteristics of *Ceratocystis albofundus*. Fig. 1. Perithecium with light coloured base (Bar = 100 μm). Fig. 2. Divergent ostiolar hyphae with hat-shaped ascospore extruding from the tip. Rectangular conidia in the background (Bar = 10 μm). Fig. 3. Barrel-shaped conidia (Bar = 10 μm). Fig. 4. Hat-shaped ascospores (Bar = 10 μm).

Results

Morphological comparisons

Morphological comparisons of the purported *C. fimbriata* isolates from black wattle with those from other sources and with characteristics reported in the literature are presented in Table 3. Characteristics of isolates from *A. mearnsii* in South Africa (Figs. 1–5) differed morphologically from *C. fimbriata* (Figs. 6–9) in various ways. South African isolates including those from *Protea* had light coloured colonies that were distinguishable from the dark colonies of the *C. fimbriata* isolates from other geographic areas. This was to some extent influenced by differences in peripheral colour between the sets of isolates. The South African isolates including herbarium specimens from *Protea* had light perithecial bases with black necks that formed flattened areas where the necks extruded from the bases (Figs. 1, 5a). In contrast, the *C. fimbriata* isolates had entirely black perithecia (Fig. 8). Isolates from *A. mearnsii* in South Africa also differed from authenticated isolates in that they had divergent ostiolar hyphae (Fig. 9) compared with the convergent ostiolar hyphae for the authenticated isolates of *C. fimbriata* (Fig. 7). Measurements of various structures varied greatly in all isolates and there were also significant differences amongst isolates known to represent the same fungus (Table 3). These were thus not considered as taxonomically useful.

Scanning electron microscopy showed that the hat-shaped ascospores of the South African isolates (Fig. 6) had double brims. The same was true for the authentic *C. fimbriata* isolates as has previously been observed (*De Beer* et al., 1995). One of these brims was around the spore whereas the other was discontinuous and occurred only at opposite ends of the ascospores.

Growth characteristics on agar of the South African *Ceratocystis* sp. from *A. mearnsii* differend from the authentic *C. fimbriata* isolates (Table 4). South African isolates had an optimum growth at 30 °C. Isolates of *C. fimbriata* grew optimally between 20–25 °C.

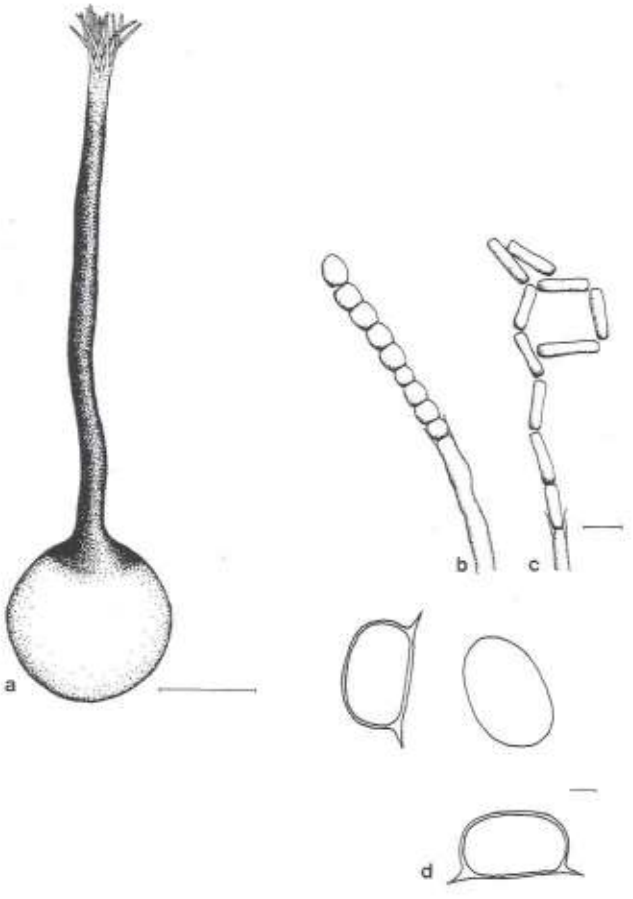


Fig. 5. Perithecium, conidiophores with conidia and ascospores of *Ceratocystis albofundus*. Fig. 5a. Perithecium with light coloured base and dark neck terminating in divergent ostiolar hyphae (Bar = 100 µm). Figs. 5b, c. Barrel-shaped and cylindrical conidia produced enteroblastically through ring wall building (Bar = 10 µm). Fig. 5d. Hat-shaped ascospores from side and end view (Bar = 10 µm).

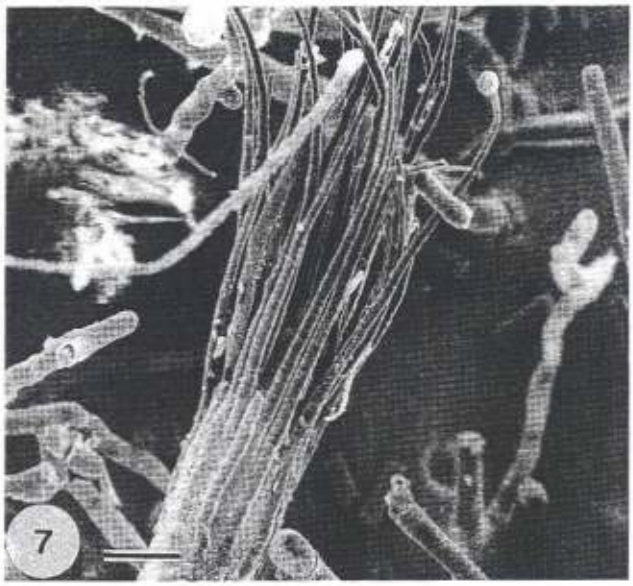
Table 4. Comparative growth rates of *Ceratocystis albofundus* from *Acacia mearnsii* in South Africa and *C. fimbriata* at different temperatures[1]

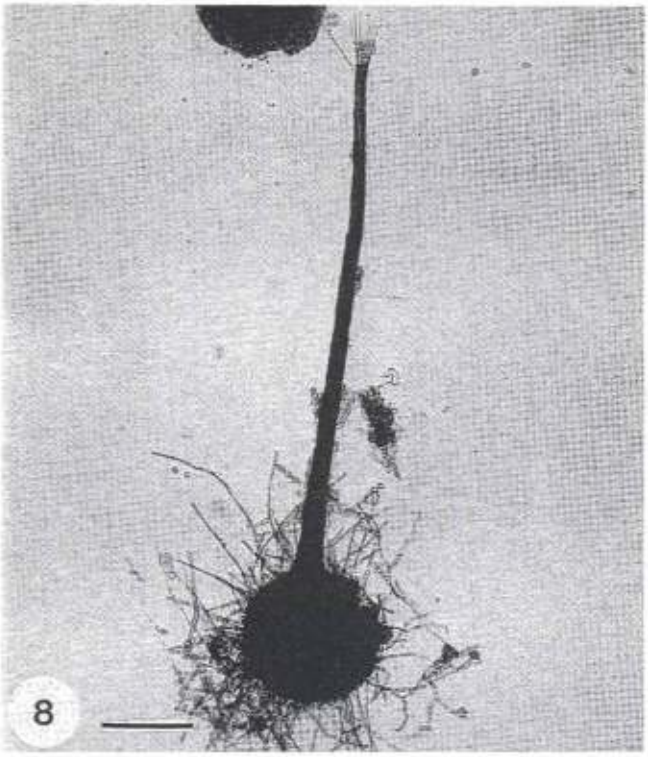
Source of isolates		Temperature (°C)			
		15	20	25	30
		Colony diameter (mm) after 8 days			
Platanus hybrida	(PREM 51642)	16	22	20	13
Ipomoea batatas	(PREM 51643)	10	24	25	19
Acacia mearnsii	(PREM 51641)	10	18	20	27
A. mearnsii	(PREM 51645)	9	11	20	24

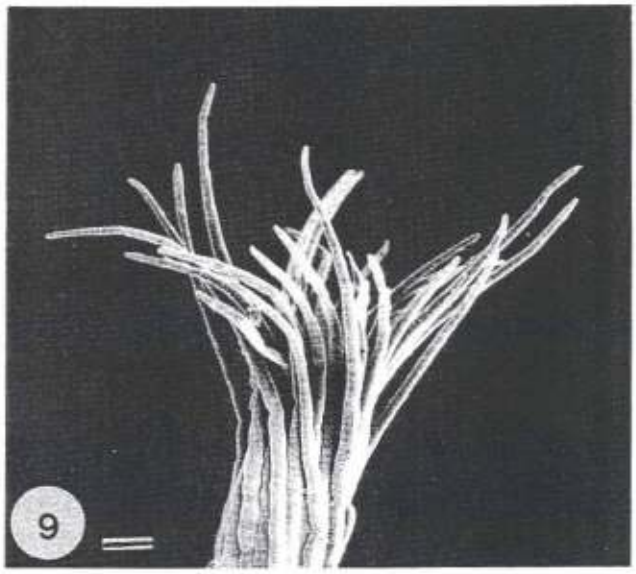
[1] Growth rates determined on 60 mm Petri dishes containing 15 ml 2% MEA

Molecular comparisons

The amplified fragments obtained for the South African isolates from *A. mearnsii* and the authentic *C. fimbriata* isolates included approximately 620 and 520 base pairs respectively (Fig. 10). Rooted phylogenetic trees from PAUP analysis (Fig. 11) grouped the South African isolates apart from authentic *C. fimbriata* isolates. *Ceratocystis moniliformis* which is morphologically similar to *C. fimbriata* grouped apart from all other isolates. The *C. fimbriata* isolate from Papua New Guinea, although grouping in the greater *C. fimbriata* group, was more distant from the European isolates of *C. fimbriata* than they were from each other. Bootstrap values gave 67–100% confidence intervals for branching patterns obtained with PAUP. Distance matrix analysis [UPGMA] (unweighted pair group method using arithmetic averages) yielded the same results as obtained using PAUP analysis and are therefore not presented.







Figs. 6–9. Perithecial and ascospore characteristics of *Ceratocystis albofundus* and *C. fimbriata*. Fig. 6. Scanning electron micrograph of hat-shaped ascospores of *C. albofundus* with double brims above the continuous brims and on opposite ends of the spores (Bar = 1 μm). Fig. 7. Scanning electron micrograph of convergent arrangement of ostiolar hyphae for *C. fimbriata* (Bar = 1 μm). Fig. 8. Light micrograph of the entirely black perithecium of *C. fimbriata* (Bar = 100 μm). Fig. 9. Scanning electron micrograph of divergent ostiolar hyphae in *C. albofundus* (Bar = 1 μm).

Discussion

Based on detailed morphological and molecular comparisons in this study, we conclude that the *Ceratocystis* species from black wattle in South Africa and tentatively identified as *C. fimbriata* (*Morris* et al., 1993) is a distinct taxon. The following description is therefore provided for the fungus:

Ceratocystis albofundus Wingfield, De Beer and Morris, sp. nov.

Coloniae in 2% MEA tarde crescentes, incremento optimo ad 30 °C, attingentes diametrum 2.7 cm 8 diebus, initio albae, maturae cremescentes. Nullum incrementum ad 10 °C. *Perithecia* nata superficialiter in superficie texti hospitis et in laminis primarii secernendi in cultura in laminis 2% MEA. Bases peritheciales 104–200 (158) μm, fulvae, globosae, inornatae; colla perithecialia, 208–840 (510) μm longa, 20–32 (27) μm lata ad basim, 16–24 (17) μm lata proxime infra hyphas ostiolares, atra ad basim pallescentia prope apicem, glabro-tunicata, extendentia in basim perithecialem cum collari (modo lato). *Hyphae os-*

tiolares 40–60 (49) µm, hyalinae, dispositione digrediente. *Asci* evanescentes. *Ascosporae* unicellulares, hyalinae, ellipsoideae visae a fronte, similes petasorum visae a latere 4–6 (5.2) µm longae, 3.5–5 (4) µm latae accumulatae in guttulis mucosis ad apicem colli, costula gemina praesente ad extrema opposita ascosporarum supra costulam continuam, sine vagina gelatinosa. *Conidia* nata et in hospite et in 2% MEA. Duae in mycelio superficiali formae conidiorum, vel cylindrica 8–24 (15) µm longa, 3–4 (3.5) µm lata; vel doliiformia 6–10 (7.4) µm longa, 4–8 (6.2) µm lata; ambo hyalina, extremis truncatis, nata parietibus annuliformibus constructis, unicellularia, nata concatenata, aliquando aggregatis massis mucosis. *Conidiophora* 24–104 (54) µm longa, 3–5 (3.8) µm lata, septata, mononematosa, hyalina, glabro-tunicata. Hyphae 2–5 (3.2) µm, hyalinae, septatae.

Specimina explorata: In ramis *A mearnsii* monstrantibus in Valle Fluminis "Mkomasi", Provinica Natali, Africa Australi, 1992, M. Morris, PREM 51641 HOLOTYPUS. Paratypi in *A. mearnsii*, in Valle Fluminis "Mkomasi", Provincia Natali, Africa Australi, 1992, M. Morris, PREM 51637, PREM 51639; Isotypi in *A. mearnsii*, "East London" 1993, M. Morris, PREM 51645.

Ceratocystis albofundus Wingfield, De Beer and Morris, sp. nov.

Colonies on 2% MEA slow growing, with optimum growth at 30 °C, reaching a diameter of 2.7 cm in 8 days, white at first, becoming creamy at maturity. No growth at 10 °C. *Perithecia* (Figs. 1, 5a) produced superficially on surface of host tissue and on primary isolation plates in

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                    10         20         30         40         50
PREM 51639    CTGTNTTTTG GTGAAGACGG AAAGCTGCCT TGGTGGGTGT NTGTAGTGGT
PREM 51829    ....G..... .......... .......... .......... C.........
PREM 51645    ....T..... ....G..... .......... .......... C.........
PREM 51644    ...AT.C... TA.TGAGAT. ..T.G..TT. ......TA.- ----------
PREM 51831    T..AT.C... TA.TGAGAT. ..T....TT. ......TA.- ----------
PREM 51830    T..AT.C... TA.TGAGAT. ..T....TT. ......TA.- ----------
PREM 51643    T..AT.C... TA.TGAGAT. ..T....TT. ......TA.- ----------
IFO 8667      TGTAA.CCAT T..TGA.--- ---------- ---------- ----------
N. crassa     .--------- -----.TAC. GTT..CT.GG C.C...CG.. CC.G.AA..-

                    60         70         80         90        100
PREM 51639    GTTAACCT-C TTTTTATAAG GGGGCAGCCC ACTACCGCTA GCCACCAGCA
PREM 51829    --------.- .......... .......... .......... ..........
PREM 51645    --------.- .......... .......... .......... ..........
PREM 51644    ---GG..--. ..C.G.AGG. ----..---- ----.....G ----......
PREM 51831    ---GG..--. ..C.G.AGG. ----..---- ----.....G ----......
PREM 51830    ---GG..--. ..C.G.AGG. ----..---- ----.....G ----......
PREM 51643    ---GG..--. ..C.G.AGG. A.....---- ----.....G ----......
IFO 8667      -..T...--- -----.A.CA T.AA.T..-- --AT.G..GG .T.T..----
N. crassa     ------.--. ..C------- -....CT... GGAT..T.GG .T.T..C..-

                   110        120        130        140        150
PREM 51639    GCATACAAGT CTTTTACCAC TATAAACCTT CTGTATATTT TTTAAAATTT
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    .......... .......... .......... .......... ...N......
PREM 51644    .T..---... ..C--G.... .G.....--. ..TATAT.-. ..CC.G....
PREM 51831    .T..---... ..C--G.... .G.....--. ..TATAT.-. ..CC.G....
PREM 51830    .T..---... ..C--G.... .G.....--. ..TATAT.-. ..CC.G....
PREM 51643    .T..T--... ..C--..... ........--. ..T.TAT.A. ...CT.GA..
IFO 8667      -.GAG..GTA ..C..G---- ----...--. .GT.T.TA.A .AA.G.....
N. crassa     ----T.GC.G .--.GC..G. CGG.GTG.-- .GAA.CTAAA C.CTTG..A.

                   160        170        180        190        200
PREM 51639    TTAAAAATTG CTGAGTGGCA TAACTATAAA AAAAGTTAAA ACTTTCAACA
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    ....N..... .......... ......NNNN N......... ..........
PREM 51644    ..TC.--... .......... .......... ...-...... ..........
PREM 51831    ..TC.--... .......... .......... .N.-...... ..........
PREM 51830    ..TC.--... .......... .......... ...-...... ..........
PREM 51643    ..TC.--... .......... .......... .......... ..........
IFO 8667      A.TC.--... ......A... .T--.TAT.. .TGTA..... ..........
N. crassa     ..T---..GT ..CTC..AGT A....T.T.. .T....C... ..........
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                    210         220         230         240         250
PREM 51639    ACGGATCTCT TGGCTCTAGC ATCGATGAAG AACG--CAGC GAAATGCGAT
PREM 51829    .......... .......... .......... ....--.... ..........
PREM 51645    .......... .......... .......... ....--.... N.........
PREM 51644    .......... .......... .......... ....--.... ..........
PREM 51831    .......... .......... .......... ....--.... ..........
PREM 51830    .......... .......... .N........ ....--.... ..........
PREM 51643    .......... .......... .......... ....--.... ..........
IFO 8667      ......N... .......... .......... ....AG..N. N....N.N..
N. crassa     .......... ...T...G.. .......... ....--.... ..........

                    260         270         280         290         300
PREM 51639    AAGTAATGTG AATTGCAGAA TTCAG-TGAA TNATCGAATN TTTGAACGCA
PREM 51829    .......... .......... .....-.... .C.......C ..........
PREM 51645    .......... .......... .....-.... .C.......C ..........
PREM 51644    .......... .......... .....-.... .C.......C .N........
PREM 51831    .......... ......N... .....-.... .C.......C ..........
PREM 51830    .......... .......... .....N.... .C....NN.C .N........
PREM 51643    .......... .......... .....-.... .C.......C .N........
IFO 8667      .......... .......... .....-.... .C.......C .N........
N. crassa     .G........ .......... .....-.... .C.......C ..........

                    310         320         330         340         350
PREM 51639    CATTGNNCCT GGTAGTATTC TGCCAGGCAT GCCTGTNCGA GCGTCATTTC
PREM 51829    .....NN... ..N....... ...'...... ......N... ..........
PREM 51645    .....CN... ..N....... .......... ......N... ..........
PREM 51644    .....CC... ..N....... .......... ......C... ..........
PREM 51831    .....CN... ..N....... .......... ......N... ..........
PREM 51830    .....CG... ..C....... .......... ......C... ..........
PREM 51643    .....NN... ..N....... .......... ......N... ..N.......
IFO 8667      .....CN..C A.C....C.. ...TG..... ......C... ....G.....
N. crassa     .....NCG.. C.CC...... ...GC.A... ......N.T. ....G.....

                    360         370         380         390         400
PREM 51639    ACCACTCAAG ACTTGCTTTA GTTTTGGTGT TGGAGGTCCT GTTCTTACCC
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    .......... .......... .......... .......... ..........
PREM 51644    .......... GAC.C....G NNC....C.. .......... .....--...
PREM 51831    .......... GAC.C....G T.C....C.. .......... .....--...
PREM 51830    .......... GAC.C....G T.C....C.. .......... .....--...
PREM 51643    .......... .T.A--..CT T.C....C.. .......... .....--...
IFO 8667      .......... CTC..---- ---...T..N ......AG.C TG.GC..TG.
N. crassa     .....CA... CTC.....-- -------GC. ..-.....G. A...G-----

                    410         420         430         440         450
PREM 51639    TTCCGAACAG GCCGCCGAAA TGCATCGGCT GTTATTTTTA CTTGCCAACT
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    ...T...... .......... .......... .......... ..........
PREM 51644    --.T...... .......... ..T....... .....----. ..........
PREM 51831    --.T...... .......... ..T....... .....----. ..........
PREM 51830    --.T...... .......... ..T....... .....----. ..........
PREM 51643    --.T...... .......... .......... .....----. ..........
IFO 8667      -------GC. .G.CT.TG.. ATGCATC.GC TG.N..---. .A..-..GT.
N. crassa     --------C. .--CT.TG.G AT.CATAT.- -CGT..---- ------.GTG

                    460         470         480         490         500
PREM 51639    CCCCTGTGTA GTACAAGATT TTTTAAATTT TTACGCTTTG GAGTGCTTGT
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    .......... .......... .......... .......... ..........
PREM 51644    .......... ...T..A... .C.--..... ....A..... A...T.....
PREM 51831    .......... ...T..A... .C.--..... ....A..... A...T.....
PREM 51830    .......... ...T..A... .C.--..... ....A..... A...T.....
PREM 51643    .......... ...T..A... .C.--..... ....A..... A...T.....
IFO 8667      T......... ...A..CT.. -------...G ..G.A..... A.A.T.....
N. crassa     .T.TCCGTC. CAGT------ ---A.GCG.G G.GACTC.AC ACTG.AA..
```

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                    510         520         530         540         550
PREM 51639    GTAACATGCC GTTAAAGGTT ACAGAAGGGC TTATAGTGGG TGGTGATAGA
PREM 51829    ........G. .......... .......... .......... ..........
PREM 51645    ........G. .......... .......... .......... ..........
PREM 51644    ......C.G. .C....---- ---------- ---------- ----------
PREM 51831    ......C.G. .C....---- ---------- ---------- ----------
PREM 51830    ......C.G. .C....---- ---------- ---------- ----------
PREM 51643    ......C.G. .C....---- ---------- ---------- ----------
IFO 8667      ACT.....G. ......---- -..------- ---------- ----------
N. crassa     ---------- --.---C... .------... -------... .---------

                    560         570         580         590         600
PREM 51639    AGATGGAGGG AATCTCTTTC CTTCTTTCTA CTTTCCCCAT CCCCCTATAA
PREM 51829    .......... .......... .......... .......... ..........
PREM 51645    .......... ...N...... .N........ .......... ..........
PREM 51644    ---------- ---------- ---------C .AAC....-. .AA.T.T.GT
PREM 51831    ---------- ---------- ---------C .AAC....-. .AA.T.T.GT
PREM 51830    ---------- ---------- ---------C .AAC....-. .AA.T.T.GT
PREM 51643    ---------- ---------- ---------- ---C..G.-. .AA.T.T.GT
IFO 8667      ---------- ---------- ---------- ---------- ---------.
N. crassa     ---------- ----....G. .G.------. AAAC...... TT-.T-----

                    610         620
PREM 51639    CCCTCCTCAA CTCACAAGGT TGACC
PREM 51829    .......... .......... .....
PREM 51645    .......... ..N....... .....
PREM 51644    ---.GAA.TT T-........ .....
PREM 51831    ---.GAA.TT T-.N...... .....
PREM 51830    ---.GAA.TT T-........ .....
PREM 51643    ---.GAA.TT T......... .....
IFO 8667      ...--AATTT T.TT--.... .....
N. crassa     ---------- -----..... .....
```

Fig. 10. Alignment of bases of the ITS1 and ITS2 regions and 5.8r RNA gene for three isolates of *Ceratocystis* sp. from black wattle in S.A., four authentic isolates of *C. fimbriata* from various other areas, one isolate of *C. moniliformis* and *Neurospora crassa* which was used as an outgroup. N indicates unknown bases; a dot indicates bases identical to the corresponding base in the *Ceratocystis* sp. from South Africa (PREM 51639); dashes represent deletions in the sequence.

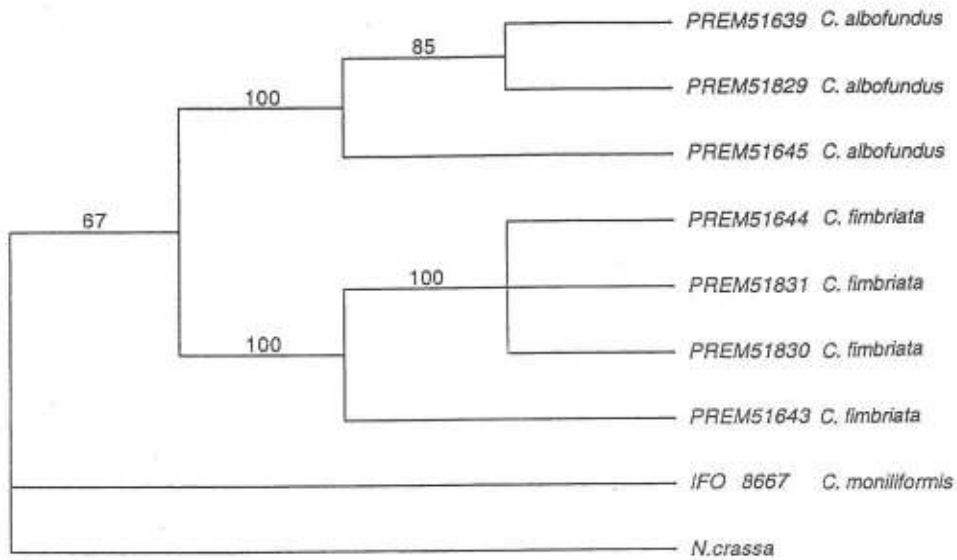

Fig. 11. Dendrogram obtained with PAUP analysis from ITS1 and ITS2 regions and the 5.8S gene of the rRNA operon, showing the phylogenetic relationships between the *Ceratocystis* sp. from black wattle in South Africa, *C. fimbriata* isolates from other areas and *C. moniliformis*. The bootstrap values are expressed as % confidence intervals.

culture on 2% MEA plates. Perithecial bases 104–200 (158) µm in diameter, yellowish brown, globose, unornamented; perithecial necks, 208–840 (510) µm long, 20–32 (27) µm wide at base, 16–24 (17) µm wide just below ostiolar hyphae, black at base becoming lighter near tip, smooth-walled, extending into perithecial base with a "collar". *Ostiolar hyphae* 40–60 (49) µm, hyaline, divergent arrangement (Figs. 2, 9). *Asci* evanescent. *Ascospores* (Figs. 2, 4, 5d, 6) one-celled, hyaline, elliptical in face view, hat-shaped in side view, 4–6 (5.2) µm long, 3.5–5 (4) µm wide, accumulating in slimy droplet at tip of neck, double brim (Fig. 6) present at opposite ends of ascospores above continuous brim, without gelatinous sheath. *Conidia* produced on both host and 2% MEA. Two conidial forms on superficial mycelium, cylindrical 8–24 (15) µm long, 3–4 (3.5) µm wide (Figs. 2, 5c); barrel-shaped 6–10 (7.4) µm long, 4–8 (6.2) µm wide (Figs. 3, 5b), hyaline, truncated ends, produced by ring wall building, 1-celled, produced in chains, sometimes forming aggregated slimy masses. *Conidiophores* 24–104 (54) µm long, 3–5 (3.8) µm wide, septate, mononematous, hyaline, smooth walled. Hyphae 2–5 (3.2) µm, hyaline, septate.

Specimens examined: On branches from *A. mearnsii* with die-back symptoms, Mkomasi River Valley, Natal Province, South Africa, 1992, M. Morris, PREM 51641 HOLOTYPE. Paratypes from *A. mearnsii*, Mkomasi River Valley, Natal Province, South Africa, 1992, M. Morris, PREM 51637, PREM 51639. Paratypes from *A. mearnsii*, East London, Cape Province, South Africa, 1992, M. Morris, PREM 51645.

The most obvious morphological characters distinguishing *C. albofundus* occurring on black wattle in South Africa from *C. fimbriata*, are the perithecial base colour and arrangement of ostiolar hyphae. The former isolates are characterized by light bases and dark, black necks as opposed to dark bases in *C. fimbriata*. Isolates of *C. albofundus* have divergent ostiolar hyphae in contrast to the convergent arrangement of the ostiolar hyphae of *C. fimbriata*.

The herbarium material of *C. fimbriata* from *Protea* in South Africa shares the characteristic light perithecial bases and dark necks with the *C. albofundus* isolates from *A. mearnsii* in South Africa. Significant differences in sizes of various structures were observed between isolates from black wattle and those from *Protea* sp. However, differences were also observed amongst the isolates from *A. mearnsii*. We believe that perithecial colour is the most stable and reliable morphological character. It is thus likely that the collections from *Protea* in South Africa represent *C. albofundus* and are thus distinct from *C. fimbriata*.

Comparisons of rRNA gene DNA sequence in this study supported morphological observations that *C. albofundus* from black wattle in South Africa represents a distinct taxon. Analysis of sequence data from ITS1 and ITS2 regions has shown variability for the different isolates while sequences of the 5.8S gene correlate well for the different isolates. Based on the variability of the ITS regions it is possible to distinguish between the isolates from *A. mearnsii* in South Africa and authentic *C. fimbriata* isolates in phylogenetic trees. If the sequences of the different isolates were too conserved, no distinction could have been made.

Sequencing of ribosomal DNA holds the advantages that the rRNA operon is repeated and easily detectable (*Metzenberg*, 1991). The rRNA operon includes both conserved and variable regions (*Hillis* and *Dixon*, 1991; *Metzenberg*, 1991) and depending on the level of comparison, different regions can be used for analysis. The 5.8S rRNA gene is very small and well conserved (*Blanz* and *Unseld*, 1988) while intergenic transcribed spacers (ITS) on either side of the 5.8S gene are highly variable (*Chambers* et al., 1986; *Nazar* et al., 1988). This region has proved useful in previous taxonomic studies (*Otsuka* et al., 1983; *Viljoen* et al., 1993; *Seifert* et al., 1995).

Ceratocystis fimbriata is the type species of *Ceratocystis s.s.* and has been known to science for more than 100 years (*Halsted*, 1890). It has a worldwide distribution and a wide range of hosts to which the fungus has developed considerable specificity (*De Vay* et al., 1963; *De Vay* et al., 1968; *Kile*, 1993; *Kojima*, 1993). It is possible that *C. fimbriata* represents a species complex representing numerous distinct species that are morphologically and ecologically similar. *C. albofundus* might then represent one of these genetic entities and studies on allied species are necessary to resolve this question.

At this stage, very little is known concerning the distribution or losses associated with *C. albofundus* in South Africa. *Acacia mearnsii* is, however, a commercially important forest tree and the appearance of this virulent pathogen in recent years is of understandable concern. The distinct differences between *C. albofundus* and *C. fimbriata* shown in this study suggest that the fungus might have been present in the country for a considerable time. Indeed, the occurrence of this fungus associated with native *Protea* spp. adds further credence to the hypothesis that the fungus is native to South Africa. If this is the case, we must assume that the fungus has adapted to pathogenicity on the exotic *A. mearnsii*.

In addition to being an important commercial forest species in South Africa, *A. mearnsii* is also a serious invader weed that particularly contaminates river courses (*Boucher*, 1978). Any pathogen with the potential to reduce the negative impact of the tree but without resulting in losses to commercial operations would be of interest. If *C. albofundus* is native to South Africa, as results of the present study suggest, it would be more acceptable to use the fungus for biological control of weed *A. mearnsii*. Additional studies, particularly on the infection biology and host range of *C. albofundus* are required before decisions can be taken on this issue.

Acknowledgements. We are grateful to Dr. *M. Morris* for first bringing this fungus to our attention; to Mr. *L. Van Ryneveld* for providing the Latin diagnosis, Prof. *J. P. van der Walt* for suggesting a name for the new species, Mr. *G. Kemp* for assistance various colleagues for supplying cultures and Mr. *Mike Fair* for assistance with statistical analyses. We also acknowledge the financial support of the South African Forestry Council, South African Wattle Growers Union and Foundation for Research Development (FRD), South Africa.

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