Celoporthe dispersa gen. et sp. nov. from native *Myrtales* in South Africa

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Abstract: In a survey for *Cryphonectria* and *Chrysoporthe* species on *Myrtales* in South Africa, a fungus resembling the stem canker pathogen *Chrysoporthe austroafricana* was collected from native *Syzygium cordatum* near Tzaneen (Limpopo Province), *Heteropyxis canescens* near Lydenburg (Mpumalanga Province) and exotic *Tibouchina granulosa* in Durban (KwaZulu-Natal Province). The fungus was associated with dying branches and stems on *S. cordatum*, *H. canescens* and *T. granulosa*. However, morphological differences were detected between the unknown fungus from these three hosts and known species of *Chrysoporthe*. The aim of this study was to characterise the fungus using DNA sequence comparisons and morphological features. Pathogenicity tests were also conducted to assess its virulence on *Eucalyptus* (ZG 14 clones), *H. natalensis* and *T. granulosa*. Plants of *H. canescens* were not available for inoculation. Results showed distinct morphological differences between the unknown fungus and *Chrysoporthe* spp. Phylogenetic analysis showed that isolates reside in a clade separate from *Chrysoporthe* and other related genera. *Celoporthe dispersa* gen. et sp. nov. is, therefore, described to accommodate this fungus. Pathogenicity tests showed that *C. dispersa* is not pathogenic to *H. natalensis*, but that it is a potential pathogen of *Eucalyptus* and *Tibouchina* spp.

Taxonomic novelties: *Celoporthe* Nakab., Gryzenh., Jol. Roux & M.J. Wingf. gen. nov., *Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf. sp. nov.
Key words: *Chrysoporthe*, *Heteropyxis*, *Holocryphia*, Phylogeny, *Syzygium*, *Tibouchina*.

INTRODUCTION

The taxonomy of *Cryphonectria* (Sacc.) Sacc. species associated with cankers of *Eucalyptus* spp. and the worldwide distribution of these fungi have undergone numerous revisions and changes in recent years (Venter *et al*. 2002, Gryzenhout *et al*. 2004, 2006a, 2006b). Studies have shown that the important *Eucalyptus* canker pathogen, *Cryphonectria cubensis* (Bruner) Hodges (Sharma *et al*. 1985, Hodges *et al.* 1986, Wingfield *et al*. 1989, Roux *et al.* 2003, Wingfield 2003), is different from other *Cryphonectria* spp. and has been placed in a new genus, *Chrysoporthe* Gryzenh. & M.J. Wingf., that includes at least two distinct species, *C. cubensis* (Bruner) Gryzenh. & M.J. Wingf. and *C. austroafricana* Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2004). Similarly, the opportunistic *Eucalyptus* canker pathogen, *Cryphonectria eucalypti* M. Venter & M.J. Wingf., formerly known as *Endothia gyrosa* (Schwein.: Fr.) Fr. (Venter *et al*. 2002), now resides in the new genus *Holocryphia* Gryzenh. & M.J. Wingf. as *H. eucalypti* (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf. (Gryzenhout *et al*. 2006a).

Chrysoporthe cubensis occurs in South America on native *Psidium cattleianum* (Hodges 1988), and on exotic *Eucalyptus* spp. and *Syzygium aromaticum* (Boerboom & Maas 1970, Hodges *et al.* 1976, 1986, Van der Merwe *et al*. 2001), all of which reside in the family *Myrtaceae*, as well as on native *Miconia rubiginosa* and *M. theaezans* belonging to the family *Melastomataceae* (Rodas *et al*. 2005). In South East Asia and Australia the pathogen has been reported from *Eucalyptus* spp. (Sharma *et al*. 1985, Hodges *et al.* 1986, Davison & Coates 1991, Myburg *et al.* 1999) and *S. aromaticum* (Hodges *et al.* 1986, Myburg *et al.* 2003). In Africa, *C. cubensis* has been reported from Cameroon, Republic of Congo, Democratic Republic of Congo and Unguja Island, Zanzibar on *Eucalyptus* spp. and *S. aromaticum* (Nutman & Roberts 1952, Gibson 1981, Hodges *et al.* 1986, Micales *et al.* 1987, Roux *et al*. 2000, Myburg *et al*. 2003, Roux *et al.* 2003).

Chrysoporthe austroafricana has, until recently, been known only from South Africa. In this country, it has been reported from both native South African tree species and non-native ornamental and plantation forest trees (Wingfield *et al*. 1989, Myburg *et al*. 2002, Heath *et al*. 2006). The fungus was the cause of an important disease of *Eucalyptus* spp. in the 1990's (Wingfield *et al*. 1989) and has recently also been reported from the non-native ornamental tree *Tibouchina granulosa* (*Melastomataceae*) (Myburg *et al*. 2002) and native *Syzygium cordatum* and *S. guineense* (*Myrtaceae*) (Heath *et al.* 2006) in South Africa.

Holocryphia eucalypti is an opportunistic pathogen of *Eucalyptus* spp. in South Africa, mostly resulting in only superficial bark cankers on trees (Van der Westhuizen *et al.* 1993, Gryzenhout *et al.* 2003). The fungus is also known to occur in Australia on *Corymbia* and *Eucalyptus* spp. (Walker *et al.* 1985, Old *et al*. 1986), where it has been associated with cankers and tree death (Walker *et al*. 1985, Davison & Coates 1991, Wardlaw 1999).

Chrysoporthe spp. can be confused with *Holocryphia* because species in both genera have orange stromatal tissue in their teleomorph states (Venter *et al*. 2002, Gryzenhout *et al.* 2004, Myburg *et al*. 2004, Gryzenhout *et al.* 2006a) and they share the same hosts and geographical distributions (Old *et al*. 1986, Wingfield *et al*. 1989, Davison & Coates 1991, Van der Westhuizen *et al.* 1993).

Table 1. Isolates included in this study.

Species	Isolate number[a]	Alternative isolate number[b]	Host	Origin	Collector	GenBank accession numbers[c]
Amphilogia gyrosa	CMW 10469	CBS 112922	*Elaeocarpus dentatus*	New Zealand	G.J. Samuels	AF452111, AF525707, AF525714
	CMW 10470	CBS 112923	*El. dentatus*	New Zealand	G.J. Samuels	AF452112, AF525708, AF525715
Celoporthe sp.[d]	CMW 10781	CBS 115844	*Syzygium aromaticum*	Kalimantan, Indonesia	M.J. Wingfield	AY084009, AY084021, AY084033
	CMW 10779		*S. aromaticum*	Indonesia	M.J. Wingfield	AY084007, AY084019, AY084031
	CMW 10780		*S. aromaticum*	Indonesia	M.J. Wingfield	AY084008, AY084020, AY084032
Celoporthe dispersa[d]	CMW 9978	CBS 118781	*Syzygium cordatum*	Tzaneen, South Africa	M. Gryzenhout	AY214316, DQ267135, DQ267141
	CMW 9976	CBS 118782	*S. cordatum*	Tzaneen, South Africa	M. Gryzenhout	DQ267130, DQ267136, DQ267142
	CMW 13936	CBS 118785	*Tibouchina granulosa*	Durban, South Africa	M. Gryzenhout	DQ267131, DQ267137, DQ267143
	CMW 13937	CBS 119118	*T. granulosa*	Durban, South Africa	M. Gryzenhout	DQ267132, DQ267138, DQ267144
	CMW 13646		*Heteropyxis canescens*	Lydenburg South Africa	G. Nakabonge, J. Roux & M. Gryzenhout	DQ267133, DQ267139, DQ267145
	CMW 13645	CBS 119119	*H. canescens*	Lydenburg South Africa	G. Nakabonge, J. Roux & M. Gryzenhout	DQ267134, DQ267140, DQ267146
Cryphonectria parasitica	CMW 13749	MAFF 410158	*Castanea mollisima*	Japan	Unknown	AY697927, AY697943, AY697944
	CMW 7048	ATCC 48198	*Quercus virginiana*	USA	F.F. Lombard	AF368330, AF273076, AF273470
Cryphonectria radicalis	CMW 10455	CBS 238.54	*Castanea dentata*	Italy	A. Biraghi	AF452113, AF525705, AF525712
	CMW 10477	CBS 240.54	*Quercus suber*	Italy	M. Orsenigo	AF368328, AF368347, AF368346
	CMW 10436	CBS 165.30	*Q. suber*	Portugal	B. d'Oliviera	AF452117, AF525703, AF525710
	CMW 10484	CBS 112918	*Castanea sativa*	Italy	A. Biraghi	AF368327, AF368349, AF368349
Chrysoporthe austroafricana	CMW 2113	CBS 112916	*Eucalyptus grandis*	South Africa	M.J. Wingfield	AF046892, AF273067, AF273462
	CMW 9327	CBS 115843	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF273473, AF273060, AF273455
Chrysoporthe cubensis	CMW 10639	CBS 115747	*E. grandis*	Colombia	C.A. Rodas	AY263419, AY263420, AY263421
	CMW 10669	CBS 115751	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF535122, AF535124, AF535126
	CMW 8651	CBS 115718	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY084002, AY084014, AY084026
	CMW 11288	CBS 115736	*S. aromaticum*	Indonesia	M.J. Wingfield	AY214302, AY214230, AY214266
Chrysoporthella hodgesiana	CMW 9994	CBS 115729	*Tibouchina semidecandra*	Colombia	R. Arbelaez	AY956968, AY956975, AY956976
	CMW 10641	CBS 115854	*T. semidecandra*	Colombia	R. Arbelaez	AY692322, AY692326, AY692325
Diaporthe ambigua	CMW 5288	CBS 112900	*Malus domestica*	South Africa	W.A. Smit	AF543817, AF543819, AF543821
	CMW 5587	CBS 112901	*M. domestica*	South Africa	W.A. Smit	AF543818, AF543820, AF543822
Endothia gyrosa	CMW 2091	ATCC 48192	*Quercus palustris*	U.S.A.	R.J. Stipes	AF046905, AF368337, AF368336
	CMW 10442	CBS 118850	*Q. palustris*	U.S.A.	R.J. Stipes	AF368326, AF368339, AF368338
Holocryphia eucalypti	CMW 7037	CRY 45, CBS 119477	*Eucalyptus delegatensis*	Australia	K.M. Old	AF232880, AF368343, AF368342
	CMW 14546	CRY 287, CBS 115838	*Eucalyptus* sp.	South Africa	H. Smith	AF232879, DQ368732, DQ368733

Table 1. (Continued).

Species	Isolate number[a]	Alternative isolate number[b]	Host	Origin	Collector	GenBank accession numbers[c]
Rostraureum tropicale	CMW 9971	CBS 115725	*Terminalia ivorensis*	Ecuador	M.J. Wingfield	AY167426, AY167431, AY167436
	CMW 10796	CBS 115757	*Te. ivorensis*	Ecuador	M.J. Wingfield	AY167428, AY167433, AY167438

[a]CMW **and** CRY= Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

[b]ATCC = American Type Culture Collection, Manassas, USA; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MAFF, Microorganisms Section, MAFF GENEBANK, National Institute of Agrobiological Sciences (NIAS), MAFF Gene Bank, Ibaraki, Japan.

[c]Accession □

[d]Isolates sequenced in this study.

However, there are distinct morphological differences between the genera. For example, the conidiomata of *Chrysoporthe* are superficial, fuscous-black, pyriform to orange with attenuated necks (Gryzenhout *et al.* 2004, Myburg *et al.* 2004), whereas those of *Holocryphia* are semi-immersed, orange and globose without necks (Venter *et al.* 2002, Myburg *et al.* 2004, Gryzenhout *et al.* 2006a). Furthermore, the ascospores of *Chrysoporthe* are septate, whereas those of *Holocryphia* are aseptate. Phylogenetic analyses have also shown that the two genera form distinct, well-supported groups (Myburg *et al.* 2004, Gryzenhout *et al.* 2006a, 2006b), separate from each other and from the genus *Cryphonectria,* in which both had been placed previously.

Like *C. cubensis*, *C. austroafricana* is an economically important pathogen of commercially grown *Eucalyptus* spp. (Wingfield *et al.* 1989, Wingfield 2003). In South Africa, *C. austroafricana* has caused substantial damage to clonal plantation forestry, which has been partially mitigated through the selection and planting of disease-resistant clones (Wingfield *et al.* 1989, Wingfield 2003). The recent discovery of *C. austroafricana* on native *S. cordatum* and *S. guineense* in South Africa has led to a change of view regarding its possible origin. Where it was once thought to be an introduced pathogen (Wingfield *et al.* 1989, Van Heerden & Wingfield 2001, Wingfield 2003), there is now substantial evidence to suggest that it is a native pathogen that could have moved from native South African *Syzygium* spp. to exotic species such as *Eucalyptus* and *Tibouchina* (Hodges *et al.* 1986, Myburg *et al*. 2002, Slippers *et al*. 2005, Heath *et al*. 2006).

Although only two species of *Syzygium* are known as hosts of *C. austroafricana*, it is highly likely that this fungus occurs on other *Myrtales* in South Africa. For this reason surveys were conducted in the country to establish the occurrence of *Chrysoporthe* spp. on indigenous tree species belonging to this plant order (Roux *et al*. 2005). These surveys yielded a fungus similar to *C. austroafricana* that was collected from three hosts in three geographic areas of the country. The aims of this study were to characterise the unknown fungus based on morphology and DNA sequence comparisons and to assess its pathogenicity in greenhouse inoculations on plants of *Heteropyxis, Eucalyptus* and *Tibouchina*.

MATERIALS AND METHODS

Isolates and specimens

Isolates were obtained from symptomatic bark material that was collected from *S. cordatum* from Tzaneen, *Heteropyxis canescens* from Lydenburg and *T. granulosa* from Durban (Table 1; Fig. 1). Fungal cultures for all isolates have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and duplicates in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Bark specimens have been deposited in the National Collection of Fungi, Pretoria, South Africa (PREM).

Table 2. Comparison of morphological characteristics between *Celoporthe* and *Chrysoporthe* spp.

Character	*Celoporthe*	*Chrysoporthe*[a]
Perithecia	Black, valsoid, embedded in bark tissue	Similar to *Celoporthe*
Perithecial necks	Short (50 µm)	Long (240 µm)
Stromatic tissue	Limited cinnamon to orange prosenchymatous to pseudoparenchymatous stromatic tissue	Similar to *Celoporthe*
Asci	8-spored, fusoid to ellipsoid	Similar to *Celoporthe*
Ascospores	1-septate, hyaline, oblong to ellipsoidal	Similar to *Celoporthe*
Conidiomata	Pulvinate to conical, superficial, mostly without a neck	Pyriform to pulvinate with attenuated necks
Conidia	Oblong to cylindrical to ovoid	Oblong
Conidiophores	Basal cells not prominent	Basal cells prominent
Stromatic tissue	Stromatic tissue of the base of conidiomata is pseudoparenchymatous	Tissue of the base consists of larger cells of *textura globulosa*
Cultures	White with grey patches, eventually becomes umber to hazel to chestnut	White with cinnamon to hazel patches

[a]From Gryzenhout *et al*. (2004).

DNA sequence comparisons

Actively growing mycelium of each isolate was scraped from the surface of one plate each containing MEA (20 g/L malt extract and 20 g/L agar, Biolab, Midland, Johannesburg) and 100 mg/L streptomycin sulfate (Sigma-Aldrich, Chemie, Gmbh, Steinheim, Germany) using a sterile scalpel, and transferred to 1.5 mL Eppendorf tubes. DNA was extracted as described by Myburg *et al.* (1999). Using primers ITS1 and ITS4 (White *et al*. 1990), the rDNA (ITS 1, 5.8S and ITS 2) regions were amplified, while primer pairs Bt1A/Bt1B and Bt2A/Bt2B (Glass & Donaldson 1995) were used to amplify the β-tubulin 1 and 2 gene regions respectively. The reactions were performed in a volume of 25 µL comprising of 2 ng DNA template, 800 µM dNTPs, 0.15 µM of each primer, 5 U/µL *Taq* polymerase (Roche Diagnostics, Mannheim, Germany) and sterile distilled water (17.4 µL). Polymerase chain reactions (PCR) and purification of the PCR products were carried out as described by Nakabonge *et al.* (2005).

The purified PCR products were sequenced in a reaction volume of 10 µL consisting of 5× dilution buffer, 4.5 µL H_2O, DNA (50 ng PCR product), 10× reaction mix BD (ABI Prism Big Dye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA), and ~ 2 pmol/µL of one of either the reverse or forward primers that were used in the PCR reactions. The PCR sequencing products were cleaned by using 0.06 g/mL Sephadex G-50 (Sigma-Aldrich, Amersham Biosciences Limited, Sweden) according to the manufacturer's protocol. The products were sequenced in both directions using the Big Dye Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an ABI Prism™ 3100 DNA sequencer (Applied Biosystems).

The gene sequences were analysed and edited using Sequence Navigator v. 1.0.1™ (Perkin-Elmer Applied BioSystems, Foster City, CA). Sequences were compiled into a matrix using a modified data set (S1128, M1935) of Myburg *et al*. (2004) as template. Additional sequences that included those of *Chrysoporthe* (Gryzenhout *et al*. 2004), *Holocryphia* (Venter *et al*. 2002, Gryzenhout *et al*. 2006a), *Cryphonectria* (Venter *et al*. 2002, Myburg *et al.* 2004), *Endothia* Fr. (Venter *et al*. 2002, Myburg *et al.* 2004), *Rostraureum* Gryzenh. & M.J. Wingf. (Gryzenhout *et al.* 2005a) and *Amphilogia* Gryzenh., Glen & M.J. Wingf. (Myburg *et al*. 2004, Gryzenhout *et al.* 2005b) species were added to the data matrix. Sequences representing an undescribed genus identified by Myburg *et al*. (2003) and originating from clove in Indonesia were also added. The alignment was executed using the web interface (http://timpani.genome.adjp/%7Emafft/server/) of the alignment program MAFFT v. 5.667 (Katoh *et al*. 2002), and deposited with TreeBASE as S1488 and M2673.

Phylogenetic analysis was performed using the software package PAUP (Phylogenetic Analysis Using Parsimony) v. 4.01b (Swofford 1998). A partition homogeneity test (Huelsenbeck *et al*. 1996) to determine the similarity and combinability of the data for the ITS and the β-tubulin 1 and 2 regions, was run. The most parsimonious trees were obtained with heuristic searches using simple stepwise addition and tree bisection and reconstruction (TBR) as the branch swapping algorithms. All equally parsimonious trees were saved and all branches equal to zero were collapsed. Gaps were treated as a fifth character. Bootstrap replicates (1000) were done on consensus parsimonious trees (Felsenstein 1985). Two isolates of *Diaporthe ambigua* Nitschke (CMW 5288 and CMW 5587) were used as outgroup to root the tree (Myburg *et al*. 2004).

Morphology

Fruiting structures of the unknown fungus were cut from the bark under a dissection microscope, boiled for 1 min and sectioned (12 µm thick) using a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa) as described by Gryzenhout *et al.* (2004). Fruiting structures were also crushed on microscope slides in 85 % lactic acid or 3 % KOH in order to study the asci, ascospores, conidia, conidiophores and conidiogenous cells. Measurements were then taken for the above-mentioned structures. For the holotype

specimen PREM 58896 50 measurements were made for each character. Only 20 measurements per character were made for the remaining specimens (PREM 58897–58901). A HRc Axiocam digital camera with Axiovision 3.1 software (Carl Zeiss Ltd., Germany) was used to capture digital images and to compute measurements. Characteristics of specimens were compared with those published for *Chrysoporthe* and *Holocryphia* (Gryzenhout *et al*. 2004, 2006a).

Two representative isolates from *H. canescens* (CMW 13645 and CMW 13646), *T. granulosa* (CMW 13936 and CMW 13937) and *S. cordatum* (CMW 9976 and CMW 9978) were used for studies of cultural characteristics. Discs (4 mm diam) taken from the margins of actively growing young cultures were placed onto the centres of 90 mm diam Petri dishes containing MEA. The cultures were grown in the dark in incubators set at temperatures ranging from 15 to 35 °C in 5 ° intervals. Four plates per isolate were inoculated and two measurements perpendicular to each other were taken daily until the fastest growing culture covered the plate. For each isolate, the colony diameter was calculated as an average of eight readings. Colour notations of Rayner (1970) were used for the descriptions of cultures and fruiting bodies.

Pathogenicity tests

The pathogenicity of two isolates of the unknown fungus, one from *H. canescens* (CMW 13645) and one from *T. granulosa* (CMW 13936), was tested on 25 trees each of an *E. grandis* clone (ZG14) that is known to be highly susceptible to fungal pathogens (Van Heerden & Wingfield 2001), and *T. granulosa* seedlings respectively, in a greenhouse set at 25 °C. The *Eucalyptus* clones were approximately 2 m tall while the *Tibouchina* seedlings were approx. 1 m tall. In order to expose the cambium, wounds were made in the bark using a cork borer (4 mm diam). Discs of the same size from the actively growing edges of 4-d-old colonies were inserted into the wounds with the mycelium facing the xylem. To prevent desiccation and contamination, wounds were covered with parafilm (Pechiney plastic packing, Chicago, USA). Twenty-five trees each of the *E. grandis* clone (ZG14) and *T. granulosa* served as negative controls and were inoculated with sterile water agar (WA: 20 g agar Merck, South Africa / 1 L water). Lesion development was evaluated after 8 wks by taking measurements of the lengths of lesions in the xylem. The trial was repeated after four months. Re-isolations were made from lesions by plating small pieces of discoloured xylem onto MEA.

Regeneration of *Heteropyxis* trees such as *H. canescens* in nurseries is seldom achieved. Only three trees (~1 m tall) of a related species, *H. natalensis*, could be obtained for pathogenicity tests. Two isolates (CMW 13645 and CMW 13646) of the unknown fungus from *H. canescens* were inoculated into the stems of two *H. natalensis* trees respectively. The third tree was inoculated with a sterile agar disc to serve as a negative control. The inoculation procedure was the same as that used when inoculating *Eucalyptus* and *Tibouchina* plants, except that each of the three trees had two inoculation points, with the same isolate, on opposite sides of the stem at the same height. Lesion lengths were measured 8 wks after inoculation and re-isolations were made using the same procedures as with the *Eucalyptus* and *Tibouchina* inoculations.

Data were analysed using the general linear model of analysis of variance (ANOVA). Means were separated using the Least Significant Difference (LSD) method available in STATISTICA for Windows (StatSoft 1995).

Fig. 1. Symptoms associated with *Celoporthe dispersa* infection. A. Dying *Heteropyxis canescens*. B. Fruiting structures of *C. dispersa* on *H. canescens*. C. Cross section through trunk canker on *H. canescens*. D. Cracks and cankers on *Tibouchina granulosa*.

RESULTS

Isolates and specimens

Specimens of the unknown fungus were collected from cracked stems of two *S. cordatum* trees near Tzaneen in the Limpopo Province. Fruiting structures occurred between structures of *C. austroafricana* that were also fruiting profusely on these trees. A similar fungus was collected from six native *H. canescens* trees exhibiting severe cankers and die-back growing in the private Buffelskloof Nature Reserve near Lydenburg in Mpumalanga Province. Some of the trees were dying or dead (Fig. 1A). Additional collections were made from the stems of two non-native *T. granulosa* trees from the Durban Botanic Gardens in KwaZulu-Natal Province. These trees displayed symptoms of branch die-back (Fig. 1D).

DNA sequence comparisons

PCR amplicons for the two regions of the β-tubulin gene were approximately 500 bp in size. Those for the ITS rDNA region amplified were approximately 600 bp in size. Results obtained from the partition homogeneity test showed that the data for each gene region were significantly congruent (p-value = 0.02). The aligned sequences of the combined regions generated 1532 characters of equal weight, with 812 constant characters, 32 parsimony-uninformative characters and 688 parsimony-informative characters. Five most parsimonious trees were generated with similar branch lengths and topology and one was chosen for presentation. This tree had a length of 1725, a consistency index (CI) of 0.737 and retention index (RI) of 0.922 (Fig. 4).

Isolates representing species of *Amphilogia, Chrysoporthe, Cryphonectria*, *Endothia, Holocryphia* and *Rostraureum* formed distinct and well-supported clades reflecting the different genera. The isolates of the unidentified fungus from *H. canescens, S. cordatum* and *T. granulosa* in South Africa grouped separately from these genera (100 % bootstrap support), specifically separate from isolates of *C. austroafricana* and *H. eucalypti*, which also occur on *Myrtales* in South Africa. The isolates of the unidentified fungus formed a clade with the isolates of an undescribed fungus from *S. aromaticum* from Indonesia (Myburg *et al.* 2003). However, within this clade, isolates formed sub-clades linked to the collections from different hosts. These were based on constant single base pair differences between isolates from the different hosts. These sub-clades include the Indonesian *Syzygium* sub-clade (100 % bootstrap support), the South African *Syzygium* sub-clade (96 % bootstrap support), the *Heteropyxis* sub-clade (100 % bootstrap support), and the *Tibouchina* sub-clade (96 % bootstrap support) from South Africa. Differences were most pronounced between the South African isolates and those from Indonesia (100 % bootstrap support), strongly suggesting that they represent different species.

Morphology

The fungus on *H. canescens, S. cordatum,* and *T. granulosa* in South Africa is characterised by fruiting structures (Table 2; Figs 2–3) that are morphologically very similar to those of *Chrysoporthe* species and the *Chrysoporthella* anamorph of *Chrysoporthe* (Gryzenhout *et al.* 2004). In the teleomorph states of both genera, the perithecial necks are covered in umber tissue as they extend beyond the bark surface (Fig. 2A–B) and limited orange to cinnamon stromatic tissue can be seen at the bases of the necks (Fig. 2A–B). Ascospores are 1-septate, hyaline, and oblong to ellipsoidal (Fig. 2C, F). In the anamorph of the unknown fungus, conidiomata are pulvinate to conical, fuscous-black and superficial (Figs 1G, 2D), similar (Table 2) to the conidiomata of the same shape and colour in *Chrysoporthella* (Gryzenhout *et al.* 2004).

The fungus characterised in this study differs from *Chrysoporthe* in several morphological characters (Table 2). Perithecial necks of the fungus are about 50 µm long (Figs 2A–B, 3A–B), while *Chrysoporthe* spp. have long necks extending up to 240 µm (Gryzenhout *et al.* 2004). Conidiomata are often without a neck or have necks with slightly attenuated apices (Figs 2G, 3D), differing from those of *Chrysoporthella* spp. that have long attenuated necks (Gryzenhout *et al.* 2004). The basal cells of the conidiophores in the unknown fungus (Figs 2J–K, 3F) are not as prominent as those of members of *Chrysoporthe*. Conidia are oblong to cylindrical to ovoid and occasionally allantoid (Figs 2L, 3F), differing from those of *Chrysoporthe* spp. that are typically oblong (Gryzenhout *et al.* 2004). The stromatic tissue at the base of the conidiomata is pseudoparenchymatous (Fig. 2I), differing from that of *Chrysoporthe*, which consists of larger cells of *textura globulosa* (Gryzenhout *et al.* 2004). Lastly, cultures of the unknown fungus are white with grey patches, eventually becoming umber to hazel to chestnut. This is different from cultures of *Chrysoporthe* spp., which are white with cinnamon to hazel patches (Gryzenhout *et al.* 2004).

Phylogenetic analyses suggested that the collections from *H. canescens, S. cordatum* and *T. granulosa* might represent three related but cryptic species. However, no significant morphological differences were found for fruiting structures among specimens linked to the isolates used in the phylogenetic analyses. These included specimens from *H. canescens* (PREM 58898 and PREM 58899), *S. cordatum* (PREM 58896 and PREM 58897) and *T. granulosa* (PREM 58900 and PREM 58901). There were also no clear differences in cultural morphology.

Phylogenetic analyses showed that an unnamed fungus previously treated by Myburg *et al*. (2003) from clove in Indonesia is related to the unknown fungus from South Africa, which formed the focus of the present study. It was, however, not possible to compare the South African and the Indonesian fungus based on morphology, because the latter fungus is known only from culture without any connection to morphological structures on the bark (Myburg *et al*. 2003). Some poorly formed conidiomata obtained for the Indonesian fungus by artificially inoculating it into *Eucalyptus* twigs (Myburg *et al*. 2003), however, suggested that the fungus is similar to the South African collections and probably represents the same genus.

Taxonomy

Morphological characteristics combined with DNA sequence data show that the unknown fungus collected from *H. canescens*, *S. cordatum* and *T. granulosa* in South Africa can be distinguished from *Chrysoporthe*, *Cryphonectria* and other closely related genera. Based on morphology, the fungus most closely resembles *Chrysoporthe* but clearly represents an undescribed genus. The taxon also appears to include an unnamed fungus previously collected from clove in Indonesia (Myburg *et al*. 2003). Based on these differences, a new genus is thus established for the fungi from South Africa and Indonesia.

DNA sequence data showed that more than one species exists for the new genus. The sub-clade

representing the Indonesian isolates was distinctly different from the South African isolates, but could not be described because there are insufficient structures on which to base a meaningful description. The isolates from the different hosts in South Africa formed a closely related group in the genus, although three possibly cryptic species, representing the isolates from three areas (Mpumalanga, Limpopo and KwaZulu-Natal Provinces) and hosts (*H. canescens, S*. *cordatum* and *T*. *granulosa*), respectively, could be identified based on sequence differences. However, no morphological differences could be observed for these apparent cryptic species, and at present there is insufficient material or ecological information available regarding these groups to support the separation of three species. For the present, we have chosen to retain the South African collections in a single species. The isolates from Indonesia most likely do not belong to this species, but must remain undescribed until fresh host material bearing fungal structures can be collected.

The specimens from *S. cordatum* in Tzaneen include both the anamorph and teleomorph, while specimens from *Heteropyxis* and *Tibouchina* have only the anamorph present. For the purpose of this study, a single species is described in a new genus, and this is based on specimens from *S. cordatum* as the holotype. Descriptions of the new genus and species follow:

Celoporthe Nakab., Gryzenh., Jol. Roux & M.J. Wingf., **gen. nov.** MycoBank MB500886.

Etymology: Latin, *celo*, to hide, referring to the fact that the fungus is difficult to find deliberately, and *porthe*, destroyer, referring to its pathogenic nature.

Ascostromata e peritheciis nigris facta, collis textura umbrina tectis, textura stromatica limitata cinnamomea vel aurantiaca praesens. *Ascosporae* uniseptatae, oblongo-ellipsoideae. *Conidiomata* superficialia, juventia aurantiaca, matura fusco-nigra, pulvinata vel conica, collis brevibus vel absentibus. *Textura stromatica* pseudoparenchymatosa. *Conidiophora* cylindrica, ramosa. *Conidia* non septata, oblonga, cylindrica vel ovoidea, interdum allantoidea.

Ascostromata consisting of black, valsoid perithecia embedded in bark tissue, with the cylindrical perithecial necks covered with umber tissue as they protrude through the bark surface. Limited cinnamon to orange prosenchymatous to pseudoparenchymatous stromatic tissue present around the upper parts of the perithecial bases, usually beneath the bark or erumpent through the bark surface. *Asci* 8-spored, fusoid to ellipsoid. *Ascospores* hyaline, with one median septum, oblong-ellipsoidal.

Conidiomata superficial, orange to scarlet when young, fuscous-black when mature, pulvinate to conical with or without short attenuated necks, unilocular with even inner surface. *Stromatic tissue* pseudoparenchymatous. *Conidiophores* hyaline, branched irregularly at the base or above into cylindrical cells, separated by septa or not. *Conidiogenous cells* phialidic, apical or lateral on branches beneath the septa. *Conidia* hyaline, non-septate, oblong to cylindrical to ovoid, occasionally allantoid, exuded as bright luteous tendrils or droplets.

Type species: *Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf., sp. nov.

Celoporthe dispersa Nakab., Gryzenh., Jol. Roux & M.J. Wingf., **sp. nov**. MycoBank MB500887. Figs 2–3.

Etymology: Latin, *dispersus*, scattered, referring to the conidiomata scattered on the bark surface.

Ascostromata perithecia nigra continentia, collis perithecialibus brevibus extensis textura umbrina tectis, textura stromatica limitata aurantiaca vel umbrina composita. *Ascosporae* uniseptatae, oblongo-ellipsoideae. *Conidiomata* superficialia, pulvinata vel conica collis brevibus vel absentibus, fusco-nigra. *Textura stromatica* pseudoparenchymatosa. *Conidiophora* cylindrica, ramosa, cellulae conidiogenae apicibus attenuatae. *Conidia* non septata, oblonga, cylindrica vel ovoidea, interdum allantoidea.

Ascostromata semi-immersed in bark, recognizable by short, extending, umber, cylindrical perithecial necks, occasionally erumpent, limited, orange to umber ascostromatic tissue covering the tops of the perithecial bases; ascostromata extending 100–400 µm high above the bark, 320–505 µm diam (Figs 2A, 3A–B). Stromatic tissue cinnamon and pseudoparenchymatous at the edges, prosenchymatous in the centre (Fig. 2D). *Perithecia* valsoid, 1–6 per stroma, bases immersed in the bark, black, globose to subglobose, 100–300 µm diam, perithecial wall 30–50 µm thick (Figs 2B–C, 3B). *Perithecial necks* black, periphysate, 80–100 µm wide (Figs 2B, 3B), emerging through the stromatal surface, covered in umber stromatic tissue of *textura porrecta* (Fig. 2A), extended necks up to 50 µm long, 100–150 µm wide. *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoidal, (19.5–)23.5–29.5(–33.5) × (4.5–)5.5–7(–7.5) µm (Figs 2E, 3C). *Ascospores* hyaline, with one median septum, oblong-ellipsoidal, with rounded ends, (4.5–)6–7(–8) × (2–)2.5–3(–3.5) µm (Figs 2F, 3C).

Conidiomata eustromatic, superficial to slightly immersed, pulvinate to conical without necks, occasionally with a neck that is slightly attenuated (Figs 2G, 3D), orange to scarlet when young, fuscous-black when mature, conidiomatal bases above the bark surface 300–500 µm high, 200–1000 µm diam. *Conidiomatal locules* with even to convoluted inner surfaces, occasionally multilocular, locules 100–550 µm diam (Figs 2H, 3E). *Stromatic tissue* pseudoparenchymatous (Fig. 2I). *Conidiophores* hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, (9.5–)12–17(–19.5) × 1.5–2.5 µm (Figs 2J, 3F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical with or without attenuated apices, (1.5–)2–3 µm wide, collarette and periclinal thickening inconspicuous (Figs 2K, 3F). *Conidia* hyaline, non-septate, oblong to cylindrical to ovoid, occasionally allantoid, (2.5–)3–4(–5.5) × (1–)1.5(–2.5) µm (Figs 2L, 3F), exuded as bright luteous tendrils or droplets.

Cultural characteristics: On MEA, *C. dispersa* appears white with grey patches, eventually becoming umber to hazel to chestnut, fluffy with an uneven margin, fast-growing, covering a 90 mm diam plate in a minimum of 5 d at the optimum temperature of 25 ºC. Cultures rarely sporulate after sub-culturing and teleomorph structures are not produced in culture.

Substrates: Bark of *Heteropyxis canescens, Syzygium cordatum* and *Tibouchina granulosa*.

Distribution: South Africa

Specimens examined: **South Africa**, Limpopo Province, Tzaneen, *Syzygium cordatum*, 2003, M. Gryzenhout, **holotype** PREM 58896, culture ex-type CMW 9976 = CBS 118782, PREM 58897, living culture CMW 9978 = CBS 118781; KwaZulu-Natal Province, Durban, Durban Botanic Gardens, *Tibouchina granulosa*, M. Gryzenhout, May 2004, PREM 58900, living culture CMW 13936 = CBS 118785, PREM 58901, living culture CMW 13937 = CBS 119118; Mpumalanga Province, Lydenburg, Buffelskloof private nature reserve, *Heteropyxis canescens*, G. Nakabonge, J. Roux & M. Gryzenhout, Oct. 2003, PREM 58899, living culture CMW 13645 = CBS 119119, PREM 58898, living culture CMW 13646.

Pathogenicity tests

Eight wks after inoculation with *C. dispersa*, lesions were observed on the stems of the *Eucalyptus* clone (ZG 14) and on those of *T. granulosa* (Fig. 5). These lesions were light to dark brown, and stretched up and down the stems from the inoculation points. Similar results were obtained in both repeats of the inoculation study. Mean lesion lengths were 106 mm for *Eucalyptus* and 29 mm for *Tibouchina* in the first experiment and 104 mm and 25 mm, respectively, in the second experiment. The differences observed between hosts were significant ($P < 0.001$) and were similar in both trials. *Celoporthe dispersa* was re-isolated from the lesions. No lesions

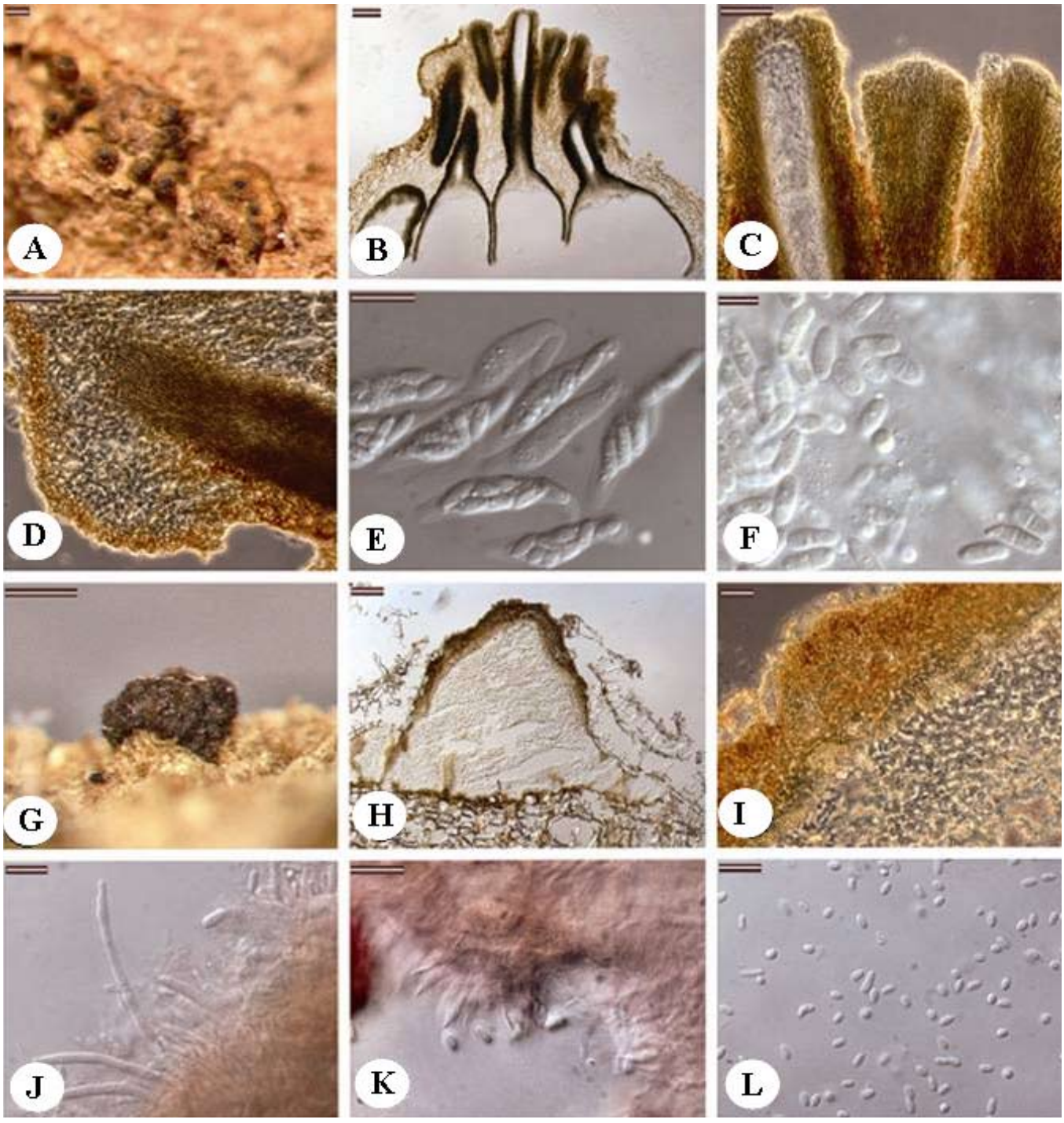

Fig. 2. Fruiting structures of *Celoporthe dispersa*. A. Ascoma on bark. B. Longitudinal section through ascoma. C. Perithecial neck tissue. D. Stromatic tissue. E. Asci with ascospores. F. Ascospores. G. Conidioma on the bark. H. Longitudinal section through conidioma. I. Stromatic tissue of conidioma. J. Conidiophores. K. Conidigenous cells. L. Conidia. Scale bars: A–B, G–H = 100 µm; C–D, I = 20 µm; E–F, J–L = 10 µm.

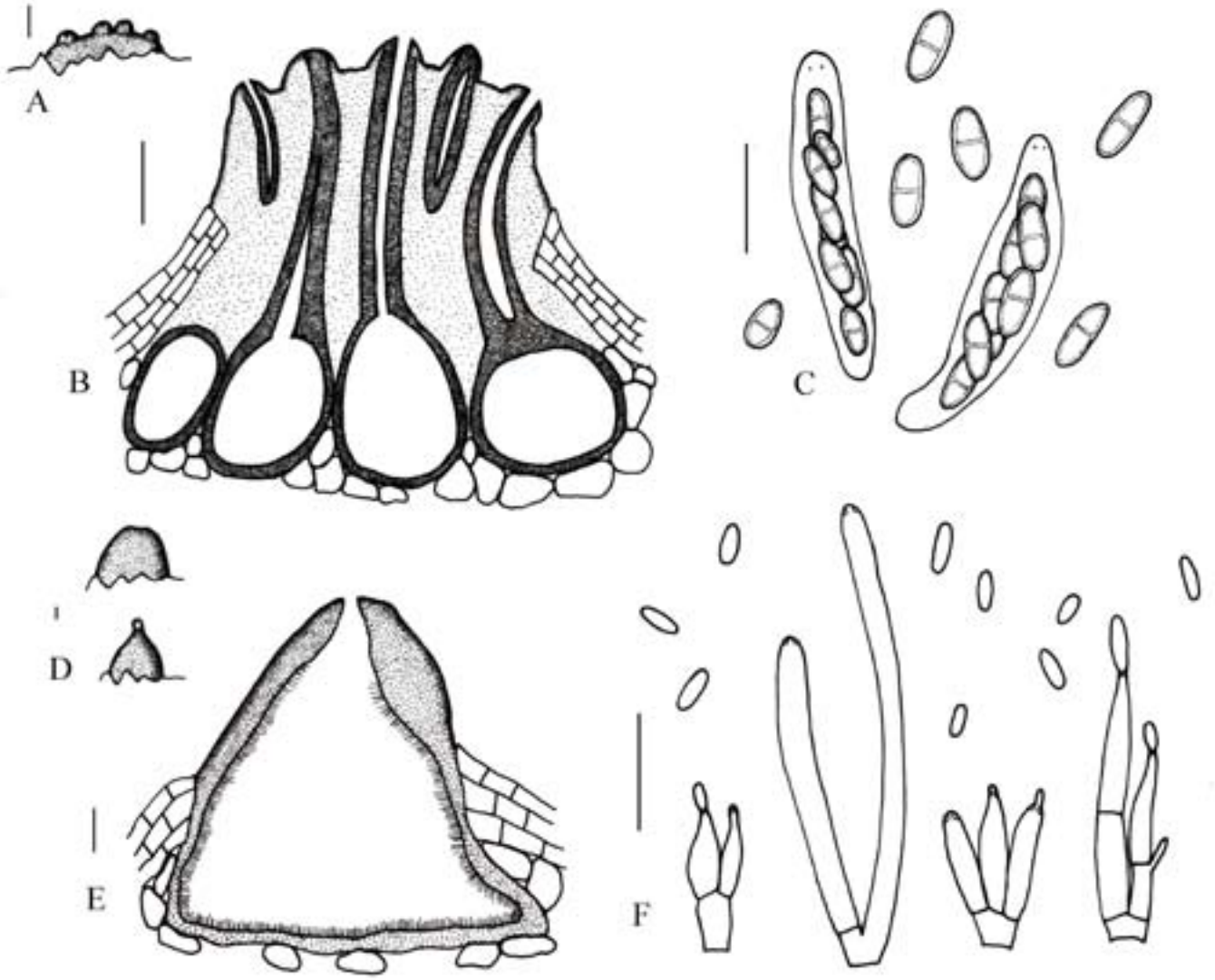

Fig. 3. Line drawings of *Celoporthe dispersa*. A. Shape of ascoma. B. Section through ascoma. C. Asci and ascospores. D. Shapes of conidiomata. E. Section through conidioma. F. Conidiophores and conidia. Scale bars: A–B, D–E = 100 μm; C, F = 10 μm.

developed on the negative controls, and the margins of the points of inoculation were closed by callus tissue (Figs 5D, 6).

Inoculation of *C. dispersa* on stems of *H. natalensis* showed no obvious lesion development after eight wks. Similarly, no lesions developed on the controls.

DISCUSSION

In this study, we have shown that the fungus isolated from *H. canescens, S. cordatum* and *T. granulosa* in South Africa represents a new genus and species related to, but distinctly different from, *Chrysoporthe*. Description of this new taxon, *C. dispersa*, is supported by both morphological characteristics and DNA sequence data. These have clearly shown that isolates of *C. dispersa* form a clade distinct from *Chrysoporthe*, *Holocryphia* and other taxa, which it resembles morphologically.

Celoporthe dispersa most closely resembles species of *Chrysoporthe* and may appear indistinguishable from *Chrysoporthe* spp. when it is observed macroscopically in the absence of light microscopy. Species of both genera have black conidiomata of similar shape. The ascostromata are in both cases semi-immersed, with limited orange to cinnamon stromatic tissue and perithecial necks covered in umber tissue as they extend beyond the bark surface. Both genera have conidia and ascospores that are expelled as bright luteous spore tendrils. The ascospores of both *Celoporthe* and *Chrysoporthe* are 1-septate, hyaline and oblong to ellipsoidal. Furthermore, *C. dispersa* occurs on the same hosts as *Chrysoporthe*. The fungus was isolated from *T. granulosa* and *S. cordatum,* two hosts on which the morphologically similar *C. austroafricana* also occurs (Myburg *et al.* 2002, Heath *et al*. 2006). However, to the best of our knowledge this is the first fungus belonging to the group that has been collected from a species of *Heteropyxis*.

Although *Celoporthe* resembles *Chrysoporthe*, distinct morphological differences separate these two fungi. The presence of short perithecial necks, pulvinate to conical conidiomata without necks, conidia that are oblong to cylindrical to ovoid, and pseudoparenchymatous stromatic tissue in the conidiomatal base, distinguish *Celoporthe* from *Chrysoporthe* spp.

Chrysoporthe spp. have long cylindrical perithecial necks, the conidiomata are pyriform to pulvinate with attenuated necks, conidia are oblong and uniform in shape, and stromatic tissue of the conidiomatal base is of *textura globulosa* and that of the neck of *textura porrecta* (Gryzenhout *et al.* 2004). *Celoporthe dispersa* produces cultures that are white with grey to chestnut-coloured patches, in contrast to *Chrysoporthe* spp. that have white to cinnamon-coloured cultures with hazel patches. Careful morphological and cultural comparisons thus make it relatively easy to distinguish *C. dispersa* from *Chrysoporthe* spp.

Three distinct but closely related and morphologically similar pathogenic fungi occur on exotic and native *Myrtales* in South Africa. These are *C. austroafricana*, which is a highly pathogenic fungus on *Eucalyptus* spp. grown in South Africa (Wingfield *et al.* 1989, Conradie *et al*. 1990) and which also occurs on *T. granulosa* (Myburg *et al.* 2002) and native *S. cordatum* (Heath *et al*. 2006). *Celoporthe dispersa* has been described in this study and occurs on native *S. cordatum*, *H. canescens* and exotic *T. granulosa* in South Africa. The third fungus, *H. eucalypti*, has been recorded only from *Eucalyptus* spp. in South Africa (Van der Westhuizen

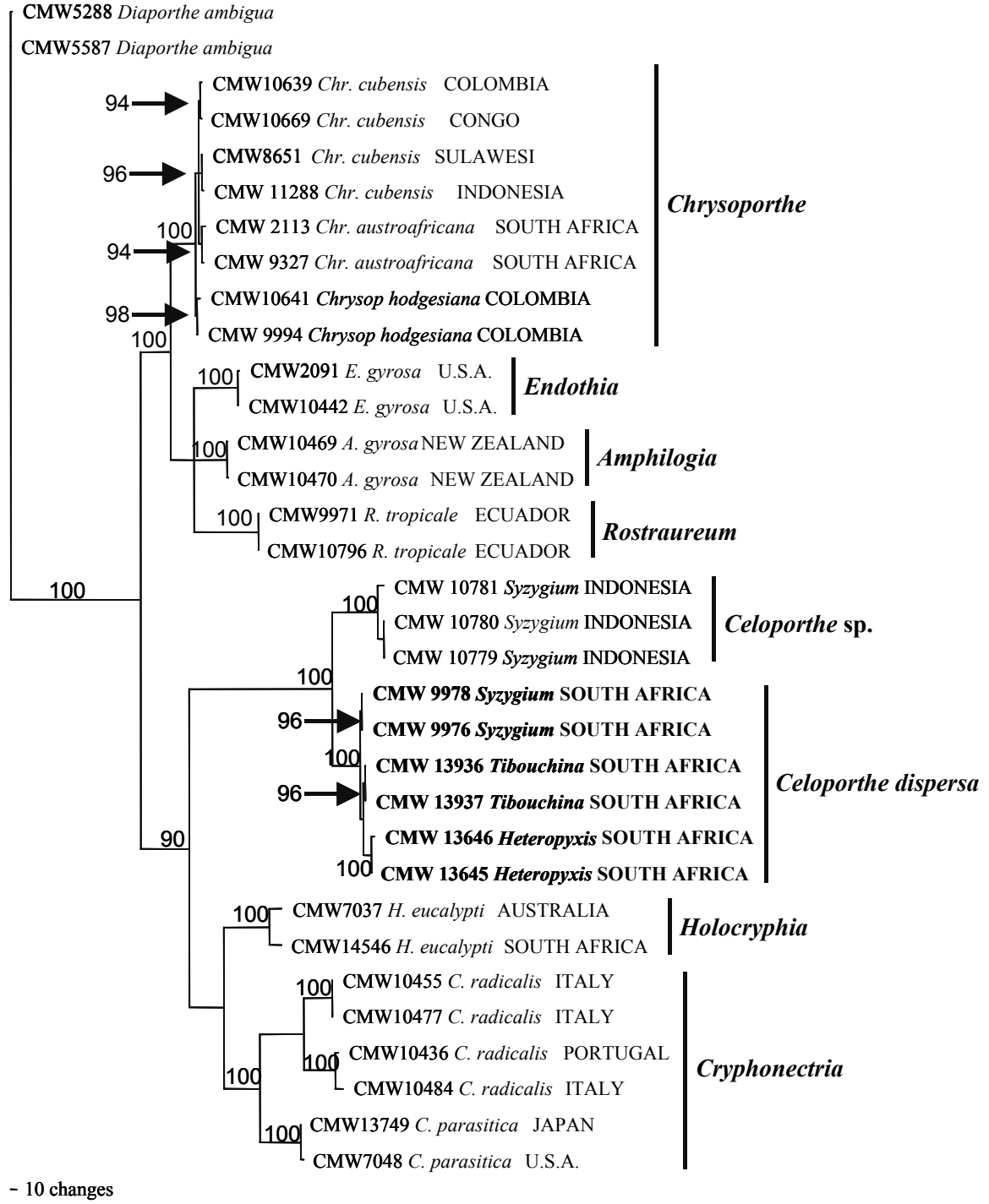

Fig. 4. A phylogenetic tree generated from combined sequence data of the ITS ribosomal DNA and β-tubulin gene sequence data and generated from heuristic searches performed on the combined data set (tree length of 1725, CI of 0.737 and RI of 0.922). Bootstrap values (1000 replicates) above 50 % are indicated on the branches. Isolates sequenced in this study are in bold. *Diaporthe ambigua* sequences were used as outgroup.

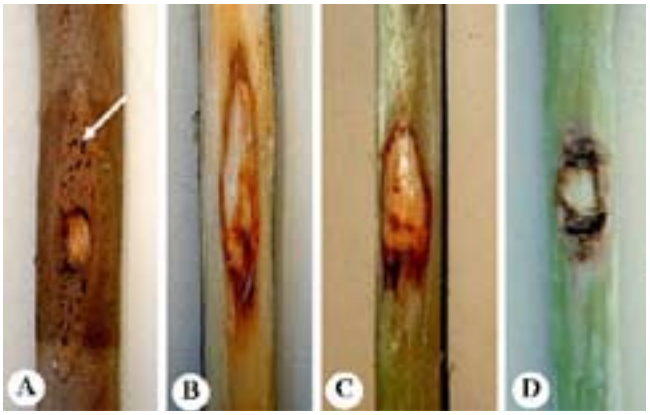

Fig. 5. Lesions associated with inoculation of *Celoporthe dispersa* on a clone of *Eucalyptus grandis* (ZG 14) and *Tibouchina granulosa*. A. Fruiting structures formed on host as a result of inoculation (arrow). B. Lesion on *Eucalyptus* sp. C. Lesion formed on *T. granulosa*. D. Control inoculation on *T. granulosa* showing callus formation and the absence of lesion development.

et al. 1993, Gryzenhout *et al.* 2003), but is common in and probably originates from Australia (Old *et al*. 1986). *Holocryphia eucalypti* can easily be distinguished from *C. dispersa* and *C. austroafricana* based on differences in the colour and shape of conidiomata as well as cultural morphology (Venter *et al.* 2002, Gryzenhout *et al*. 2004, Myburg *et al*. 2004, Gryzenhout *et al*. 2006a).

DNA-based comparisons in this study have shown that different phylogenetic groups are represented by the isolates now treated as the single species *C. dispersa.* Thus, *C. dispersa* is represented by isolates from *Heteropyxis*, *Tibouchina* and *Syzygium* spp. in South Africa, and these isolates form three closely related sub-clades. A fourth sub-clade represents isolates from clove in Indonesia and was previously studied by Myburg *et al.* (2003). Based on DNA sequence data, this fungus clearly represents a distinct species, which could not yet be described because of insufficient material available to characterize it. The fact that the unknown Indonesian fungus is now known to reside in *Celoporthe* should facilitate the collection of additional samples from clove in Indonesia.

The three closely related sub-clades consisting of isolates of *C. dispersa* from South Africa, were correlated with their three different host genera (*Heteropyxis*, *Syzygium* and *Tibouchina*) and areas of collection (Lydenburg, Tzaneen and Durban). These sub-clades are, however, represented by a limited number of isolates and a larger collection of isolates will be required to better understand the relationship among them. We were unable to detect clear morphological differences between the fungi in these three sub-clades and the comparison was also hindered by the absence of teleomorph structures on the specimens from *H. canescens* and *T. granulosa*. Description of different species for the three phylogenetic sub-clades contained in *C. dispersa* must await the acquisition of additional material and isolates. The ecological data and distribution of these fungi in South Africa is also largely unknown, and such information would be useful in studying the taxonomic status of these three sub-clades of *C. dispersa.*

Heteropyxis canescens is a rare and endangered tree species in South Africa. Currently it is found only in Mpumalanga Province (John Burrows, pers. comm., Lawes *et al.* 2004). Fruiting structures of *C. dispersa* were collected from dying trees in the Buffelskloof Nature Reserve near Lydenburg and it was thought that the fungus might be responsible for the death of the trees. However, pathogenicity tests conducted using a limited number of trees of a closely related species, *H. natalensis*, showed that *C. dispersa* is not pathogenic to that species. Although it is possible that *H. canescens* is more susceptible to *C. dispersa* than is *H. natalensis*, the fungus might not be the cause of tree death at Buffelskloof. However, in order to understand the pathogencity of *C. dispersa* more clearly, the fungus will need to be inoculated on *H. canescens* and on a larger number of trees than was possible in this study. This will be difficult to achieve because *H. canescens* is endangered and is extremely difficult to propagate artificially. The cause of tree mortality in the Buffelskloof Nature Reserve thus remains unclear. The possibility that another organism is responsible for the death of the trees must also be investigated.

Pathogenity trials conducted on *E. grandis* and *T. granulosa* showed that *C. dispersa* is pathogenic on both these hosts. In these trials, the *Eucalyptus* clone was more susceptible than *T. granulosa*. *Celoporthe dispersa* is thus a newly discovered pathogen of these trees and it could become important on commercially grown *Eucalyptus* trees in South Africa.

Celoporthe dispersa and *C. austroafricana* are present on both native and non-native *Myrtales* in South Africa. This raises many important issues pertaining to the origin and distribution of these fungi. Both fungi are currently known only from southern Africa, and they also occur on native African trees. It has already been suggested that *C. austroafricana* is native to South Africa (Wingfield 2003, Heath *et al*. 2006) and the same is probably true for *C. dispersa*. These fungi are virulent pathogens of exotic *Eucalyptus* trees and their

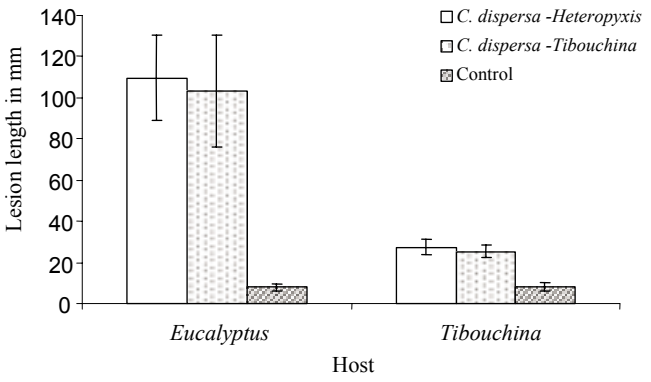

Fig. 6. Comparison of lesion lengths associated with inoculation of *Celoporthe dispersa* on a *Eucalyptus* (ZG 14) clone and *Tibouchina granulosa* plants under greenhouse conditions. The trees were inoculated with *C. dispersa* isolated from *Heteropyxis canescens* (CMW 13645) and *T. granulosa* (CMW 13936). Mean lesion lengths were determined with 98 % confidence limits ($P < 0.001$).

accidental introduction into Australia, where *Eucalyptus* spp. and many other *Myrtales* are native, could result in an ecological disaster. This view is based on the fact that similar canker pathogens, such as *Cryphonectria parasitica* (Murrill) M.E. Barr, have caused devastating losses to trees after being introduced into new environments (Anagnostakis 1987, Slippers *et al*. 2005). Both *C. austroafricana* and *C. dispersa* also potentially threaten plantation *Eucalyptus* trees wherever they are grown commercially.

Additional surveys are necessary to expand the host and geographic ranges of *Celoporthe* and *Chrysoporthe* spp. on *Myrtales* in South Africa and on other parts of the African continent. The fact that these fungi are almost indistinguishable in the field will complicate such surveys, and laboratory studies will be required for reliable identifications. New collections and associated isolates of *C. dispersa* might also lead to the subdivision of this species into additional taxa. Additional material will thus add knowledge to the relatively poorly studied fungal biodiversity on the African continent and especially on native African tree species.

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