Chrysoporthe, a new genus to accommodate *Cryphonectria cubensis*

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Abstract: *Cryphonectria cubensis* is an important canker pathogen of tree species residing in the *Myrtaceae* and *Melastomataceae*. Recent phylogenetic studies based on multiple gene sequence comparisons have revealed that isolates of *C. cubensis* group separately from other *Cryphonectria* species. Within the *C. cubensis* clade, isolates formed three distinct sub-clades that include isolates mainly from South America, South Africa and South East Asia, respectively. In this study, we establish a new genus, *Chrysoporthe*, for this species. *Chrysoporthe* is characterized by superficial, blackened conidiomata, limited ascostromatic tissue, and blackened perithecial necks protruding from the orange stromatal surface. Although specimens of *C. cubensis* from South East Asia and South America reside in two distinct phylogenetic sub-clades, they could not be separated or distinguished from the type specimen, originating from Cuba, based on morphological characteristics. For the present, these specimens are collectively transferred to *Chrysoporthe* as a single species, *Chrysoporthe cubensis*. Specimens previously treated as *C. cubensis* from South Africa reside in a discrete phylogenetic clade and could be distinguished from those in the other sub-clades based on having longer asci and ascospores with rounded apices as opposed to tapered apices. The South African fungus is described as *Chrysoporthe austroafricana*. Isolates from *Tibouchina* spp. in Colombia resided in a fourth sub-clade of *Chrysoporthe*. Isolates in this phylogenetic assemblage grew optimally at 25 °C in contrast to those in the other groups that grew optimally at 30 °C. No sexual state is known for the fungus in this fourth sub-clade and a new anamorph genus and species name, *Chrysoporthella hodgesiana*, is provided for it.

Taxonomic novelties: *Chrysoporthe* Gryzenhout & M.J. Wingf. gen. nov., *Chrysoporthella* Gryzenhout & M.J. Wingf. anam. gen. nov., *Chrysoporthe cubensis* (Bruner) Gryzenhout & M.J. Wingf. comb. nov., *Chrysoporthe austroafricana* Gryzenhout & M.J. Wingf. sp. nov., *Chrysoporthella hodgesiana* Gryzenhout & M.J. Wingf. sp. nov.

Key words: *Cryphonectria cubensis*, *Chrysoporthe cubensis*, *Chrysoporthe austroafricana*, *Chrysoporthella hodgesiana*, *Diaporthales*, phylogeny.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is a serious and often deadly canker pathogen of commercially grown plantation *Eucalyptus* spp. (Fig. 1A) (Boerboom & Maas 1970, Hodges *et al.* 1976, Hodges 1980, Florence *et al.* 1986, Wingfield *et al.* 1989). This pathogen causes cankers on the trunks of trees (Fig. 1B–D) that reduce growth and can lead to stem breakage (Fig. 1E) or tree death (Hodges *et al.* 1976, 1979, Sharma *et al.* 1985). The fungus generally occurs in countries that are situated between the 30° North and South latitudes where rainfall and temperatures are high (Boerboom & Maas 1970, Hodges *et al.* 1976, 1979, Sharma *et al.* 1985). More specifically, *C. cubensis* has been reported from countries in South America (Boerboom & Maas 1970, Hodges *et al.* 1976), the Caribbean (Bruner 1917, Hodges *et al.* 1979), Africa (Gibson 1981, Hodges *et al.* 1986, Micales *et al.* 1987, Wingfield *et al.* 1989, Roux *et al.* 1999, 2003), South East Asia (Sharma *et al.* 1985, Florence *et al.* 1986, Van Heerden *et al.* 1997), Australia (Davison & Coates 1991), Western Samoa, Florida, Puerto Rico and Hawaii (Hodges *et al.* 1979).

Cryphonectria cubensis occurs on hosts other than *Eucalyptus* species. This fungus has been shown to be synonymous with *Endothia eugeniae* (Nutman & Roberts) J. Reid & C. Booth, which is the causal agent of clove (*Syzygium aromaticum* (L.) Murr. & Perry) die-back (Fig. 1F, G) (Hodges *et al.* 1986, Micales *et al.* 1987, Myburg *et al.* 2003). *Cryphonectria cubensis* has also been recorded on strawberry guava (*Psidium cattleianum* Sabine) in Brazil (Hodges 1988). The pathogen was recently discovered causing cankers on native *Tibouchina lepidota* Baill. (Fig. 1H) and *T. urvilleana* Cogn. (*Melastomataceae*) in Colombia (Wingfield *et al.* 2001). There have been subsequent reports of *C. cubensis* on *T. granulosa* (Fig. 1I) in South Africa (Myburg *et al.* 2002a) and Brazil (Seixas *et al.* 2004).

The appropriate generic placement of *C. cubensis* has been problematic ever since its discovery. It was originally described as *Diaporthe cubensis* Bruner (Bruner 1917), but was transferred to *Cryphonectria* because the orange stromatal tissue surrounding the

perithecia, single-septate ascospores and cultural characteristics resembled those of *Cryphonectria* species (Hodges 1980). Walker *et al.* (1985) noted that *C. cubensis* possibly belonged in a genus other than *Cryphonectria* due to the limited stromatic development, superficial pycnidia and simple to slightly convoluted pycnidial cavities, which are different from the highly convoluted cavities in other *Cryphonectria* spp. Roane (1986) suggested that *C. cubensis* should be accommodated in *Cryptodiaporthe*.

Recent studies employing DNA sequence data have clearly shown that isolates of *C. cubensis* are only distantly related to species of *Cryphonectria*. In phylogenetic analyses of the ribosomal LSU and SSU region, isolates of *C. cubensis* grouped separately from *C. parasitica* (Murrill) M.E. Barr, and more closely to *Cryptodiaporthe corni* (Wehm.) Petr. (Zhang & Blackwell 2001, Castlebury *et al.* 2002), suggesting that the fungus does not reside in *Cryphonectria*. This, however, did not imply that *C. cubensis* should reside in *Cryptodiaporthe*, since the *Cryptodiaporthe corni* isolate included in the study of Castlebury *et al.* (2002) was not representative of *Cryptodiaporthe*. This species grouped separately from the type species, *Cryptodiaporthe aesculi* (Fuckel) Petr., as well as from other species of *Cryptodiaporthe* (Castlebury *et al.* 2002).

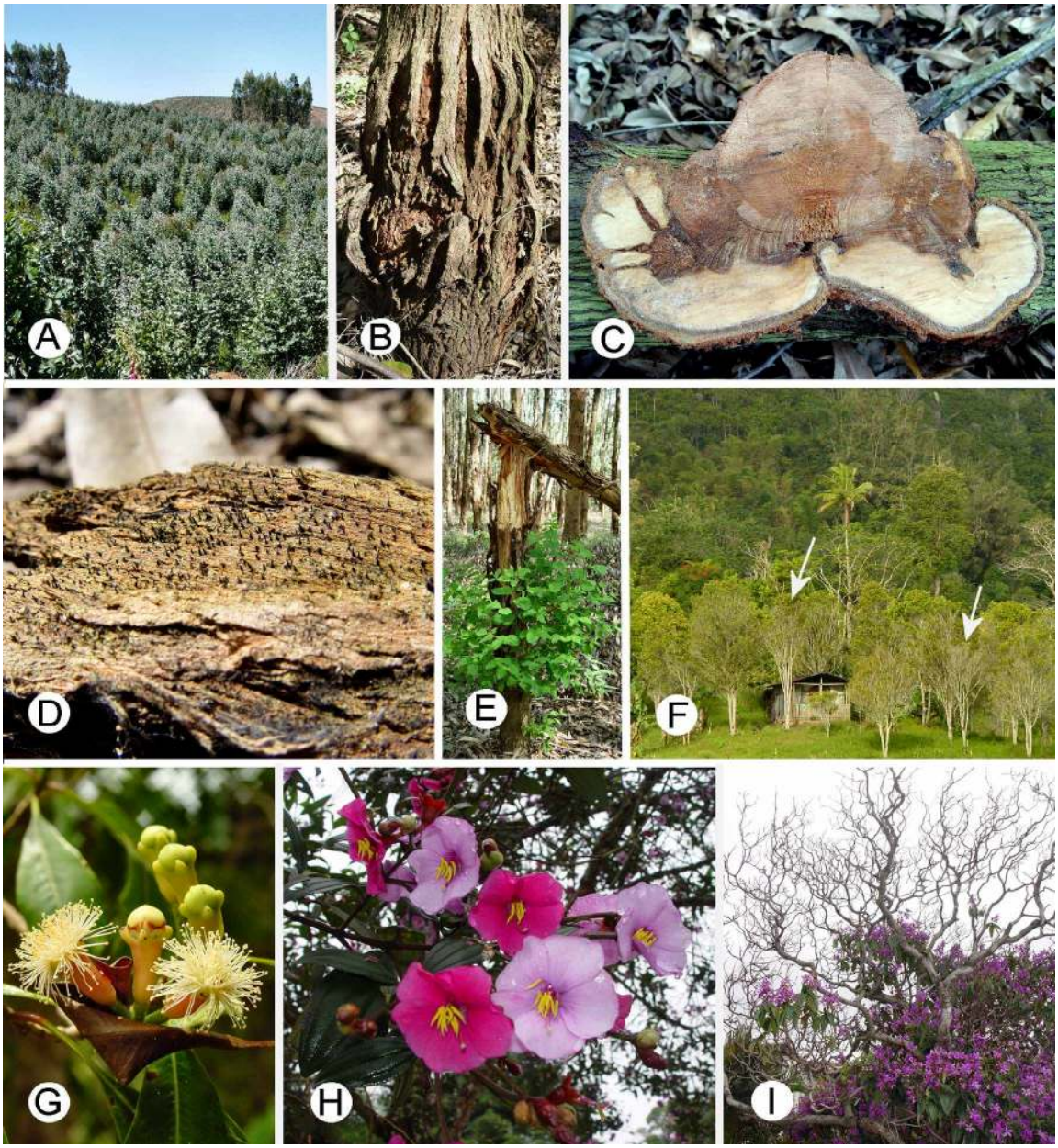


Fig. 1. Hosts and disease symptoms associated with species of *Chrysoporthe*. A. Plantation of *Eucalyptus globulus*. B. Canker caused by *Chrysoporthe cubensis* on the trunk of *Eucalyptus grandis* in Colombia. C. Section through trunk canker caused by *Chr. cubensis* on *E. grandis*. D. Conidiomata of *Chr. cubensis* around canker margin on *E. grandis*. E. Stem of *E. grandis* broken at canker caused by *Chr. cubensis*. F. Die-back caused by *Chr. cubensis* on *Syzygium aromaticum* (clove) in Indonesia. G. Flowers of *S. aromaticum*. H. Flowers of *Tibouchina lepidota*. I. Die-back caused by *Chrysoporthe austroafricana* on *Tibouchina granulosa* in South Africa.

More variable sequence data of the ribosomal ITS region and two regions within the β-tubulin genes (Myburg *et al.* 2004) confirmed conclusively that *C. cubensis* is phylogenetically distinct from *Cryphonectria*. The separate phylogenetic grouping of *C. cubensis* isolates was supported by distinct morphological features such as superficial, blackened conidiomata, ascomata with reduced stromatic development and blackened perithecial necks extending beyond the stromatal surface (Myburg *et al.* 2004). Species belonging to *Cryphonectria* have orange conidiomata, well-developed ascostromata and orange perithecial necks where they extend beyond the stromatal surface (Myburg *et al.* 2004).

Phylogenetic analyses based on DNA sequences of the ITS region of the ribosomal operon (Myburg *et al.* 1999), two regions within the β-tubulin genes and one region of the histone *H3* gene (Myburg *et al.* 2002b) have made it possible to compare isolates of *C. cubensis* collected from a wide range of geographic locations and hosts. These isolates thus grouped in three related but phylogenetically discrete clades within the *C. cubensis sensu lato* group. One of these clades included isolates from a number of South American countries (Myburg *et al.* 1999, 2002b, 2003) as well as isolates from the Democratic Republic of Congo (Zaire) and the Republic of Congo (Myburg *et al.* 2003, Roux *et al.* 2003). A second group included isolates from South East Asian countries as well as Australia (Myburg *et al.* 1999, 2002b, 2003), Zanzibar and Hawaii (Myburg *et al.* 2003). The third group accommodated isolates from South Africa (Myburg *et al.* 2002a, 2002b, 2003).

Myburg *et al.* (2002b) were not able to find obvious morphological differences between specimens representing the three phylogenetic sub-clades of *C. cubensis sensu lato*. This was consistent with previous reports suggesting that *C. cubensis* from different regions are identical (Hodges *et al.* 1979, Hodges 1980, Hodges *et al.* 1986, Wingfield *et al.* 1989). However, the study of Myburg *et al.* (2002b) was limited by the rare occurrence of the teleomorph of *C. cubensis* on *Eucalyptus* spp. in South Africa (Wingfield *et al.* 1989), and comparisons based on this state could thus not be made.

Teleomorph specimens for the fungus known as *C. cubensis* in South Africa have now become available. The objectives of this study were to undertake morphological comparisons of *C. cubensis* specimens from different parts of the world. Consistent with morphological and phylogenetic differences, a new genus is provided for the fungus that has been known as *C. cubensis*. Furthermore, characteristics were sought to determine whether isolates residing in different phylogenetic groups of this new genus could be described as different species.

MATERIALS AND METHODS

Isolates and specimens examined

Isolates have been assembled over the course of approximately 15 years and represent a wide diversity of hosts and origins (Table 1). These include collections from known areas and hosts, as well as reports from new areas and hosts, for instance collections from Mexico and *Tibouchina semidecandra* Cogn. in Colombia, respectively. Isolates are preserved on oatmeal agar (OA; 30 g/800 L water, extract added to 20 g/L Biolab agar, Merck, Midrand, South Africa) slants at 5 °C in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Representative isolates have also been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands. Bark specimens with fruiting structures, collected from diseased trees, were used for the morphological comparisons. The bark specimens (Table 2) have been deposited with the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM). A number of these specimens are linked to or originated from the same areas as some of the isolates included in the phylogenetic study (Table 2).

DNA sequence comparisons

DNA sequence data of the ribosomal ITS region and two regions of the β-tubulin genes that are currently available from previously characterised *C. cubensis* isolates, were included in this study. These isolates originated from *Eucalyptus* spp. (Myburg *et al.* 2002b, 2003, Roux *et al.* 2003), *S. aromaticum* (Myburg *et al.* 1999, 2003) and *Tibouchina* spp. (Wingfield *et al.* 2001, Myburg *et al.* 2002a) from different parts of the world (Table 1). Sequences were also generated for additional isolates specifically required for this study (Table 1). Three species of *Cryphonectria*, namely *C. parasitica*, *C. nitschkei* (G.H. Otth) M.E. Barr and *C. macrospora* (Tak. Kobay. & Kaz. Itô) M.E. Barr, were chosen as outgroups in the phylogenetic analyses. This was justified because it has previously been shown that *Cryphonectria* spp. are phylogenetically related but clearly separate from *C. cubensis* (Myburg *et al.* 2004).

Prior to DNA extraction, isolates were grown in 2 % malt extract broth [20 g/L Biolab malt extract]. DNA was extracted from the mycelium following the method used by Myburg *et al.* (1999). Using the primer pair ITS1 and ITS4 (White *et al.* 1990), the internal transcribed spacer (ITS) regions ITS1 and ITS2, as well as the conserved 5.8S gene of the ribosomal RNA (rRNA) operon, were amplified (Myburg *et al.* 1999).

Table 1. Isolates included in this study.

Isolate number[a]	Alternative isolate number[a]	Species identity	Host	Origin	Collector	GenBank accession numbers[c]
CMW 1856		*Chrysoporthe cubensis*	*Eucalyptus* sp.	Kauai, Hawaii		AY 083999, AY 084010, AY 084022
CMW 9903		*Chr. cubensis*	*Syzygium aromaticum*	Kalimantan, Indonesia	C.S. Hodges	AF 292044, AF 273066, AF 273461
CMW 11288[b]	CBS 115736	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214302, AY 214230, AY 214266
CMW 11289[d]	CBS 115737	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214303, AY 214231, AY 214267
CMW 11290[d]	CBS 115738	*Chr. cubensis*	*Eucalyptus* sp.	Indonesia	M.J. Wingfield	AY 214304, AY 214232, AY 214268
CMW 8650[b]	CBS 115719	*Chr. cubensis*	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084001, AY 084013, AY 084024
CMW 8651	CBS 115718	*Chr. cubensis*	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	AY 084002, AY 084014, AY 084026
CMW 10774		*Chr. cubensis*	*S. aromaticum*	Zanzibar, Tanzania		AF 492130, AF 492131, AF 492132
CMW 2631		*Chr. cubensis*	*Eucalyptus marginata*	Australia	E. Davison	AF 543823, AF 543824, AF523825
CMW 2632		*Chr. cubensis*	*E. marginata*	Australia	E. Davison	AF 046893, AF 273078, AF 375607
CMW 10453	CBS 505.63	*Chr. cubensis*	*Eucalyptus saligna*	Republic of Congo		AY 063476, AY 063478, AY 063480
CMW 10669[b]	CBS 115751	*Chr. cubensis*	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 535122, AF 535124, AF 535126
CMW 10671	CBS 115752	*Chr. cubensis*	*Eucalyptus* sp.	Republic of Congo	J. Roux	AF 254219, AF 254221, AF 254223
CMW 10639[b]	CBS 115747	*Chr. cubensis*	*Eucalyptus grandis*	Colombia	C.A. Rodas	AY 263419, AY 263420, AY 263421
CMW 8757		*Chr. cubensis*	*Eucalyptus* sp.	Venezuela	M.J. Wingfield	AF 046897, AF 273069, AF 273464
CMW 1853		*Chr. cubensis*	*S. aromaticum*	Brazil		AF 046891, AF 273070, AF 273465
CMW 10777		*Chr. cubensis*	*S. aromaticum*	Brazil	C.S. Hodges	AY 084005, AY 084017, AY 084029
CMW 10778	CBS 115755	*Chr. cubensis*	*S. aromaticum*	Brazil	C.S. Hodges	AY 084006, AY 084018, AY 084030
CMW 9432[d]	CBS 115724	*Chr. cubensis*	*E. grandis*	Mexico	M.J. Wingfield	AY 692321, AY 692324, AY 692323
CMW 62		*Chr. austroafricana*	*E. grandis*	South Africa	M.J. Wingfield	AF 292041, AF 273063, AF 273458
CMW 2113[b]	CBS 112916	*Chr. austroafricana*	*E. grandis*	South Africa	M.J. Wingfield	AF 046892, AF 273067, AF 273462
CMW 8755		*Chr. austroafricana*	*E. grandis*	South Africa	M.J. Wingfield	AF 292040, AF 273064, AF 273458
CMW 9327	CBS 115843	*Chr. austroafricana*	*Tibouchina granulosa*	South Africa	M.J. Wingfield	AF 273473, AF 273060, AF 273455
CMW 9328[b]		*Chr. austroafricana*	*T. granulosa*	South Africa	M.J. Wingfield	AF 273474, AF 273061, AF 273456
CMW 9932		*Chr. austroafricana*	*T. granulosa*	South Africa	M.J. Wingfield	AF 273472, AF 273062, AF 273457
CMW 9927		*Chrysoporthella hodgesiana*	*Tibouchina urvilleana*	Colombia	C.A. Rodas	AF 265653, AF 292034, AF 292037
CMW 9928		*Chrysoporthella hodgesiana*	*T. urvilleana*	Colombia	C.A. Rodas	AF 265654, AF 292035, AF 292038
CMW 9929		*Chrysoporthella hodgesiana*	*T. urvilleana*	Colombia	C.A. Rodas	AF 265656, AF 292036, AF 292039
CMW 10641[b, d]	CBS 115854	*Chrysoporthella hodgesiana*	*Tibouchina semidecandra*	Colombia	R. Arbelaez	AY 692322, AY 692326, AY 692325
CMW 1652	CBS 112914	*Cryphonectria parasitica*	*Castanea dentata*	U.S.A.	–	AF 046902, AF 273075, AF 273468
CMW 10518	CBS 112919	*C. nitschkei*	*Quercus* sp.	Japan	T. Kobayashi	AF 452118, AF 525706, AF 525713
CMW 10463	CBS 112920	*C. macrospora*	*Castanopsis cupsidata*	Japan	T. Kobayashi	AF 368331, AF 368351, AF 368350

[a]CMW = Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. [b]Isolates used in growth studies. [c]Accession numbers refer to sequence data of the ITS, β-tubulin 1 (primers Bt1a/1b) and β-tubulin 2 (primers Bt2a/2b) regions respectively. [d]Isolates sequenced in this study.

Table 2. Herbarium specimens examined in this study.

Species identity	Herbarium number[a]	Isolate[b]	Host	Origin	Collector	Date
Chrysoporthe cubensis	BPI 631857 (holotype)		*Eucalyptus botryoides*	Santiago de las Vegas, Cuba	S.C. Bruner	1916
	BPI 631858 (slides only)		*E. botryoides*	Santiago de las Vegas, Cuba	S.C. Bruner	1916
	PREM 57294	CMW 10639	*Eucalyptus grandis*	Vanessa, Colombia	M.J. Wingfield	2000
	PREM 58017		*Eucalyptus urophylla*	Colombia	M.J. Wingfield	2000
	PREM 57295	CMW 9432	*Eucalyptus* sp.	Tabasco, Mexico	M.J. Wingfield	2000
	PREM 58016	CMW 9432	*Eucalyptus* sp.	Tabasco, Mexico	M.J. Wingfield	2000
	IMI 284438		*E. grandis/ Eugenia* sp.	Uverito, Venezuela	C.S. Hodges	1983
	MASS		*E. grandis*	Minas Gerais, Brazil	C.S. Hodges	1973
	IMI 184653		*Eucalyptus maculata*	Minas Gerais, Brazil	C.S. Hodges	1974
	IMI 184652		*Eucalyptus propinqua*	Minas Gerais, Brazil	C.S. Hodges	1974
	IMI 172718		*Eucalyptus* sp.	Minas Gerais, Brazil	C.S. Hodges	1973
	IMI 173960		*Eucalyptus* sp.	São Paulo, Brazil	L. May	1973
	IMI 285983		*Syzygium aromaticum*	Espirito Santo, Brazil	C.S. Hodges	1983
	IMI 285982	**CMW 10777, CMW 10778**	*S. aromaticum*	Valenca, Brazil	C.S. Hodges	1983
	IMI 177647		*Eucalyptus citriodora*	Paramaribo, Surinam	P.A. Tennissen	1973
	IMI 202849		*E. grandis*	Florida, U.S.A.	C.S. Hodges	1976
	CUP 58722		*E. grandis*	La Belle, Florida, U.S.A.	W. Sinclair	1981
	IMI 351788		*Psidium cattleianum*	Santa Catarina, Brazil	C.S. Hodges	1988
	IMI 263717		*Eucalyptus* sp.	Hong Kong, China	C.S. Hodges	1981
	IMI 45450	CMW 10774	*S. aromaticum*	Zanzibar, Tanzania	–	1951
	IMI 45440	CMW 10774	*S. aromaticum*	Zanzibar, Tanzania	J. Nutman	1951
	IMI 279035	CMW 10774	*S. aromaticum*	Zanzibar, Tanzania	A. Dabek	1983
	IMI 249406		*E. urophylla*	Edea, Cameroon	F.B. Armitage	1980
	IMI 261569		*E. grandis*	Kerala, India	J.K. Sharma	1981
	IMI 58569		*S. aromaticum*	Malaysia	A. Johnston	1954
	IMI 58388		*S. aromaticum*	Malaysia	A. Johnston	1954
	IMI 58567		*Eugenia* sp.	Malaysia	A. Johnston	–
	IMI 58568		*Eugenia* sp.	Malaysia	A. Johnston	1954
	IMI 304273		*S. aromaticum*	Malaysia	Low Chow Fong	1986
	PREM 57470	**CMW 8650 CMW 8651**	*S. aromaticum*	Sulawesi, Indonesia	M.J. Wingfield	2001
	PREM 58018	**CMW 8650, CMW 8651**	*S. aromaticum*	North Sulawesi, Indonesia	M.J. Wingfield	2003
	PREM 58019	**CMW 8650, CMW 8651**	*S. aromaticum*	North Sulawesi, Indonesia	M.J. Wingfield	2003
	PREM 58020	**CMW 8650, CMW 8651**	*S. aromaticum*	Utard, Sulawesi, Indonesia	M.J. Wingfield	2003
	IMI 231648		*Eugenia* sp.	Bankals, Indonesia	C.P.A. Bennett	–
	PREM 57297	CMW 11289, CMW 11290	*Eucalyptus* sp.	Sumatra, Indonesia	M.J. Wingfield	2001
	IMI 231649		*Eugenia* sp.	Sumatra, Indonesia	C.P.A. Bennett	–
	PREM 58021	CMW 11289, CMW 11290	*Eucalyptus* sp.	Sei Kabaro, Indonesia	M.J. Wingfield	2001
	IMI 350626		*S. aromaticum*	Istana grounds, Singapore	C.P. Yik	1991
	DAR 35434	CMW 1856	*E. saligna*	Kauai, Hawaii	C.S. Hodges	1978
Chrysoporthella hodgesiana	PREM 58022 (holotype)	**CMW 10641**	*Tibouchina semidecandra*	Darien, Colombia	R. Arbeleaz	2001
	PREM 56914	**CMW 9927, CMW 9928, CMW 9929**	*Tibouchina urvilleana*	Buga, Colombia	M.J. Wingfield	1999

	PREM 56915	**CMW 9927, CMW 9928, CMW 9929**	*T. urvilleana*	Buga, Colombia	M.J. Wingfield	1999
	PREM 56913		*Tibouchina lepidota*	Buga, Colombia	M.J. Wingfield	1999
Chr. austroafricana	PREM 58023 (holotype)	**CMW 2113**	*E. grandis*	KwaMbonambi, South Africa	M.J. Wingfield	1989
	PREM 49377		*E. grandis*	KwaMbonambi, South Africa	M.J. Wingfield	1986
	PREM 49378		*E. grandis*	KwaMbonambi, South Africa	M.J. Wingfield	1987
	PREM 49379		*E. grandis*	KwaMbonambi, South Africa	M.J. Wingfield	1988
	PREM 57293		*E. grandis*	Dukuduku, South Africa	M. Venter	2001
	PREM 58024	**CMW 2113**	Inoculation of CMW 2113	KwaMbonambi, South Africa	J. Roux	2003
	PREM 57357	CMW 9327, CMW 9328	*Tibouchina granulosa*	KwaMbonambi, South Africa	J. Roux	1999
	PREM 57358	CMW 9327, CMW 9328	*T. granulosa*	KwaMbonambi, South Africa	J. Roux	1999
	PREM 57359	CMW 9327, CMW 9328	*T. granulosa*	KwaMbonambi, South Africa	J. Roux	1999
	PREM 57360		*T. granulosa*	Durban, South Africa	J. Roux	2000
	PREM 57361		*T. granulosa*	Durban, South Africa	J. Roux	2000
Cryphonectria gyrosa	K 109807 (holotype)		Bark	Sri Lanka	–	1868
	K 109809		Bark	Mount Eliya, Sri Lanka	G.H.K. Thwaites	–
	BPI 614797		*Elaeocarpus glandulifer*	Hakgala, Sri Lanka	T. Petch	1913
C. parasitica	CUP 2926	CMW 10790	*Castanea dentata*	New York, USA	W.A. Murrill	1907
C. nitschkei	TFM: FPH 1045 (holotype)	CMW 10518	*Quercus grosseserrata*	Japan	T. Kobayashi	1954
C. macrospora	TFM: FPH 1057 (holotype)	CMW 10463	*Shiia sieboldii*	Japan	T. Kobayashi	1954

[a]**BPI**, U.S. National Fungus Collections, Systematic Botany and Mycology, Beltsville, U.S.A.; **PREM**, National Collection of Fungi, Pretoria, South Africa; **DAR**, Plant Pathology Herbarium, Orange Agricultural Institute, N.S.W., Australia; **CUP**, Plant Pathology Herbarium, Plant Pathology Department, Cornell University, Ithaca, New York, U.S.A.; **IMI**, Herbarium, CABI Bioscience, Egham, Surrey, U.K.; **MASS**, Herbarium, Biology Department, University of Massachusetts, Amherst, Massachusetts, U.S.A.; **K**, Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, England, U.K.; **TFM: FPH**, Forestry and Forest Products Research Institute, Norin Kenkyu, Danchi-Nai, Ibaraki, Japan. [b]Isolates in bold are directly linked to the specimens. Other isolates were only collected from the same country and host. CMW refers to the culture collection of the Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Table 3. Summary of polymorphic nucleotides found within the ribosomal ITS region and the two regions in the β-tubulin genes generated for the phylogenetic groups of *Chrysoporthe cubensis, Chr. austroafricana* and *Chrysoporthella hodgesiana*. Only polymorphic nucleotides occurring in all of the isolates are shown, and not alleles that occur in a single or small number of individuals per phylogenetic group. Fixed polymorphisms for each group are highlighted and in bold, single nucleotide polymorphisms that also occur in other groups are only highlighted and those fixed but shared between groups are in italics. Numerical positions of the nucleotides in the DNA sequence alignments are indicated, and those nucleotides occurring in exons are in bold.

Group	**Isolate number**	**β-tubulin (Bt1a/1b)**										**ITS1/5.8S/ITS1** [a]				
		141	**162**	**164**	**167**	**185**	**189**	**201**	**209**	**256**	**274**	**1023**	**1309**	**1316**	**1317**	**1342**
Asia/Hawaii/ Zanzibar	CMW 1856, CMW 2631, CMW 2632, CMW 8650, CMW 8651	C	G	A	A	A	T	T	T	T	C	A	**G**	**T**	**T**	*G*
	CMW 9903, CMW 10774	C	G	A	A	A	T	T	T	T	C	A	**G**	**T**	**T**	*G*
South Africa	CMW 9327, CMW 9328, CMW 9932, CMW 62	C	G	**C**	**C**	A	**C**	**C**	**C**	*C*	C	A	A	C	-	A
	CMW 2113	C	G	**C**	**C**	A	**C**	**C**	**C**	*C*	C	A	A	**T**	-	A
	CMW 8755	C	G	A	A	A	**C**	**C**	**C**	*C*	C	A	A	C	-	A
South America	CMW 9432, CMW8757, CMW 10639, CMW1853, CMW 10777, CMW 10778	**T**	**A**	A	A	A	T	T	T	T	*T*	A	A	C	-	A
Congo	CMW 19776, CMW 10453, CMW 10669, CMW 10671	**T**	**A**	A	A	A	T	T	T	T	*T*	A	A	C	-	A
Colombia, *Tibouchina*	CMW 9927, CMW 9929, CMW 9928, CMW 10641	C	G	A	A	**G**	T	T	T	*C*	*T*	**G**	A	C	-	*G*

Table 3. Continued.

Group	Isolate number	β-tubulin (Bt2a/2b)							
		537	**546**	**585**	**669**	**789**	**810**	**819**	**859**
Asia/Hawaii/ Zanzibar	CMW 1856, CMW 2631, CMW 2632, CMW 8650, CMW 8651	C	G	**C**	**T**	**T**	T	**T**	G
	CMW 9903, CMW 10774	C	G	**C**	G	**T**	T	**T**	G
South Africa	CMW 9327, CMW 9328, CMW 9932, CMW 62	**T**	G	T	G	C	T	C	G
	CMW 2113	**T**	G	T	G	C	T	C	G
	CMW 8755	**T**	G	T	G	C	T	C	G
South America	CMW 9432, CMW 8757, CMW 10639, CMW 1853, CMW 10777, CMW 10778	C	G	T	G	C	T	C	G
Congo	CMW 19776, CMW 10453, CMW 10669, CMW 10671	C	G	T	G	C	T	C	G
Colombia, *Tibouchina*	CMW 9927, CMW 9929, CMW 9928, CMW 10641	C	**A**	T	G	C	**C**	C	**A**

The primer pairs Bt1a/Bt1b and Bt2a/Bt2b (Glass & Donaldson 1995) were used to amplify two regions within the β-tubulin gene using the reaction conditions outlined in Myburg *et al.* (2002b). PCR products were visualised with UV light on 1 % agarose (ethidium bromide-stained) gels. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were sequenced with the same primers used in the PCR reactions. Sequencing reactions were as specified by the manufacturers of the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.). Nucleotide sequences were determined on an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, U.K.).

The nucleotide sequences were edited using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software. Sequences were manually aligned and analysed in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). A 500 replicate partition-homogeneity test (PHT) was applied to the rRNA and β-tubulin gene sequence data sets (after the exclusion of uninformative sites) to determine whether they could be analysed collectively in PAUP (Farris *et al.* 1994).

A phylogenetic tree was inferred from maximum parsimony (MP) using the heuristic search option with the tree-bisection-reconnection (TBR) branch swapping and MULTREES options (saving all optimal trees) effective. Gaps inserted to achieve sequence alignment were treated as fifth character (NEWSTATE) in the heuristic searches, and nucleotides were defined as unordered and unweighted. The phylogenetic signal from the dataset was computed and compared against values obtained by Hillis & Huelsenbeck (1992). A 1000 replicate bootstrap analysis (Felsenstein 1985) was executed to assess the confidence levels of the branch nodes in the phylogenetic tree. MODELTEST version 3.5 (Posada & Crandall 1998) were used to determine the appropriate distance model for the datasets. Distance analyses were thus executed using the HKY85 model (Hasegawa *et al.* 1985) with the gamma distribution shape parameter set to 0.1717 (HKY+G). DNA sequences have been deposited in GenBank and accession numbers are listed in Table 1. The sequence alignment and phylogenetic tree have been deposited in TreeBase as SN1966.

Morphology

Fruiting structures were cut from bark and boiled in water for 1 min to rehydrate the cells. The structures were embedded in Leica mountant (Setpoint Premier, Johannesburg, South Africa) and sectioned with a Leica CM1100 cryostat (Setpoint Technologies) at –20 ºC. The 12 µm thick sections were mounted in lactophenol. Sectioning was also done by hand and the sections were mounted in lactophenol or 3 % KOH to specifically observe conidiophore and ascus morphology. For the holotype specimen (BPI 631857) of *C. cubensis* and a representative specimen for each phylogenetic group (PREM 57297, PREM 57294, PREM 58023, PREM 58022), 50 measurements were taken of ascospores, asci, conidia and conidiogenous cells, and the range of measurements was calculated from at least ten anamorph and ten teleomorph stromata, respectively. Twenty measurements were taken for other structures on the remaining specimens. Measurements and digital photographs were made using an HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd., Germany). Standard colour notations given by Rayner (1970) were used.

Two isolates representing each of the phylogenetic groups making up *C. cubensis sensu lato* (Table 1) were selected for comparisons of growth in culture. Culture growth was assessed on MEA in 90 mm diam Petri dishes. The studies were conducted in the dark at temperatures from 15 to 35 ºC at 5 º intervals. Discs, 6 mm diam, were taken from the edge of actively growing colonies, and placed at the center of the Petri dish. Four plates were inoculated for each isolate. Two measurements, perpendicular to each other, were taken of colony diameter (mm) each day until the mycelia of the fastest growing isolates had covered the plates. Colony diameter of each isolate was computed as an average of eight readings per isolate (two meas-

urements for each of four replicates). The growth comparisons were repeated twice.

RESULTS

DNA sequence comparisons

DNA amplicons for the ITS region of the ribosomal operon were approximately 600 bp in size, and those for the two regions amplified in the β-tubulin genes were approximately 550 bp each. The results of the PHT showed that DNA sequences for the two partitions (β-tubulin and ITS1/ITS2) were significantly incongruent (P-value = 0.016). This was a result of the South African isolates that grouped with South American isolates in the ITS tree (Myburg *et al.* 1999), but formed a distinct group in the β-tubulin dataset (Myburg *et al.* 2002b). After the exclusion of the South African isolates from the dataset, the data from the β-tubulin and ITS1/ITS2 partitions were fully congruent (P-value = 0.13). Since it is known that isolates from South Africa can be distinguished from isolates in other parts of the world based on β-tubulin and Histone *H3* genes (Myburg *et al.* 2002b), we have thus combined the ITS and β-tubulin datasets in order to present our data in a compact way. The DNA sequence of the partial ITS1/ITS2 region (538 bp) consisted of 414 constant characters, 41 parsimony-uninformative and 83 parsimony-informative characters, while the sequence of the β-tubulin gene regions (894 bp) consisted of 658 constant characters, 55 parsimony-uninformative and 181 parsimony-informative characters.

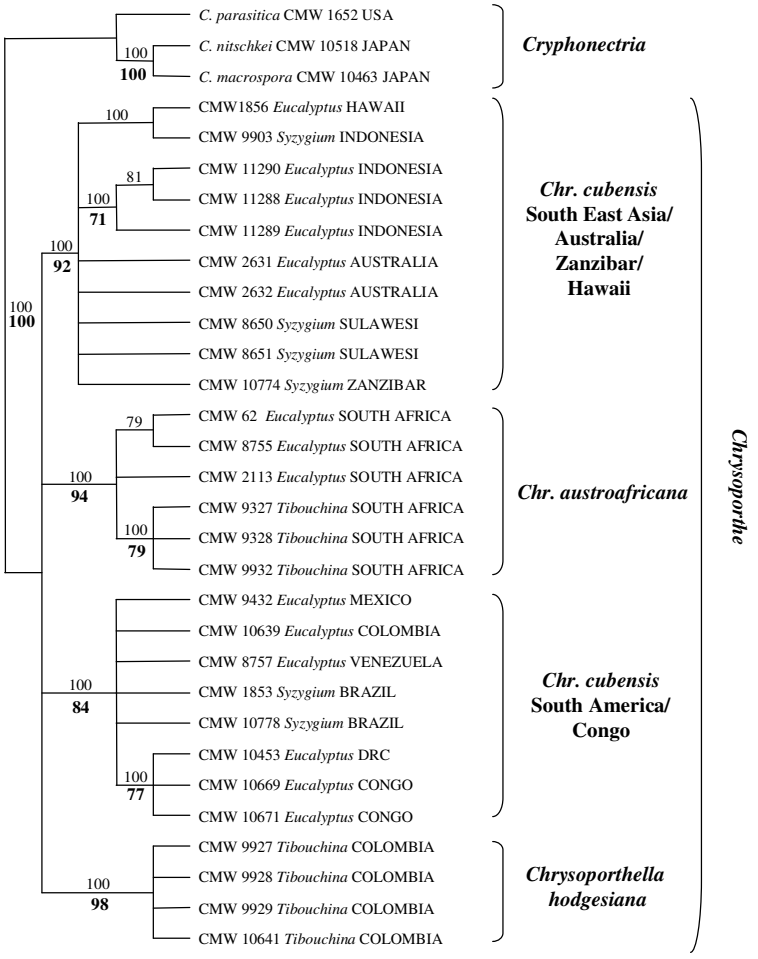


Fig. 2. Majority rule (70 %) consensus tree (tree length = 459 steps, consistency index/CI = 0.939, retention index/RI = 0.951, g1 = –3.6) obtained from 100 trees produced with the TBR algorithm of a heuristic search on a combined data set of ribosomal DNA and β-tubulin gene sequences. Group frequencies and bootstrap values (1000 replicates) of branches are indicated, with bootstrap values in bold. *Cryphonectria parasitica*, *C. nitschkei* and *C. macrospora* were used as outgroups.

After combination, the data set was comprised of 1432 characters, of which 1072 were constant, 96 parsimony-uninformative and 264 parsimony-informative. Of these, three ambiguous characters were excluded.

A 70 % majority consensus tree (tree length = 459 steps, consistency index/CI = 0.939, retention index/RI = 0.951, g1 = –3.6) was computed from 100 trees obtained with the TBR algorithm (Fig. 2). The 100 trees differed in the way the different clades were related. All of the trees (Fig. 2) showed that isolates of *C. cubensis* from the different geographic regions and hosts (*Myrtaceae* and *Melastomataceae*) grouped with

strong bootstrap support in the three previously identified sub-clades (Myburg *et al.* 2002a, b, 2003, 2004). Isolates from *Tibouchina* spp. in Colombia grouped separately from all other phylogenetic groups (bootstrap support 98 %) and were distinct from a *C. cubensis* isolate (CMW 10639) collected on *E. grandis* in Colombia. Isolate CMW 10639 resided in the South American group. The distance tree obtained using the HKY85 parameter model (Fig. 3) showed the same four phylogenetic groups generated using parsimony.

The sub-clades in the phylogenetic trees resulted from single-nucleotide differences that were characteristic for each phylogenetic group. The majority of these nucleotide polymorphisms was fixed between the four phylogenetic groups and were distributed throughout both DNA regions considered (Table 3).

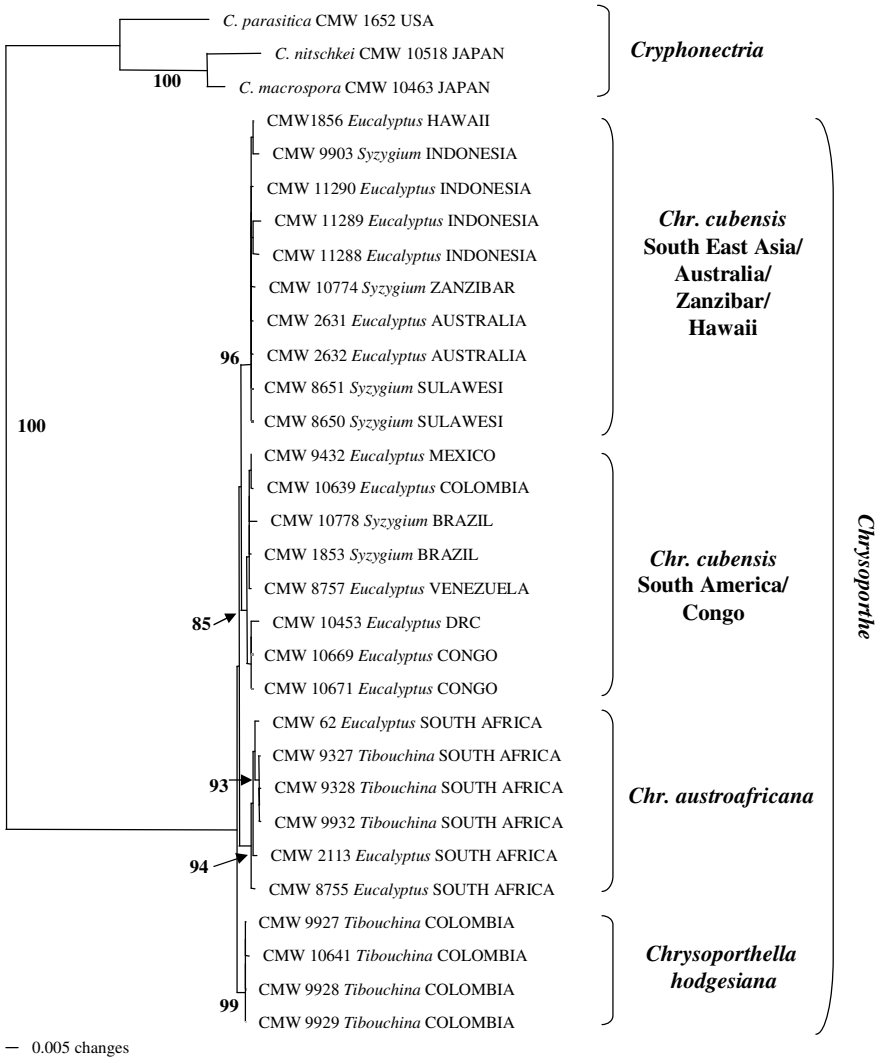


Fig. 3. Distance phylogram obtained with the HKY85 parameter model (G = 0.1717) on the combined data set of ribosomal DNA and β-tubulin gene sequences. Bootstrap values (1000 replicates) of branches are indicated in bold. *Cryphonectria parasitica*, *C. nitschkei* and *C. macrospora* were defined as outgroups to root the tree.

In the combined data set, 23 bases were polymorphic of which 16 were fixed for one of the groups (70 %) and three were shared between two of the groups (Table 3). The fixed polymorphisms occurred in all four phylogenetic groups (Table 4). Four additional sites represented a nucleotide that occurred in all isolates of a group, except for one isolate of that group that shared an allele with the other groups (Table 3). Some of the isolates also showed individual variation and had substitutions that were unique to the isolate (data not shown).

Morphological comparisons

Differences between C. cubensis specimens and other Cryphonectria spp.: Morphological characteristics, previously noted by Myburg *et al.* (2004), that distinguish specimens of *C. cubensis*, are mainly based on stromatal differences. These characters differentiate *C. cubensis* specimens from the type species of *Cryphonectria*, *C. gyrosa* (Berk. & Broome) Sacc. (K 109807, K 109809, BPI 614797), as well as other species of *Cryphonectria* such as *C. parasitica*, *C. radicalis*, *C. macrospora* and *C. nitschkei* (Table 2). The conidiomata of *C. cubensis* specimens are super-

ficial, fuscous-black, pyriform to globose with attenuated necks (Fig. 4G–I), while those of *Cryphonectria* spp. are semi-immersed, orange and globose with no necks (Myburg *et al.* 2004). Perithecial necks of *C. cubensis* are covered with umber tissue as they extend through the stromatal surface, thus appearing fuscous-black (Fig. 4A, D), whereas the extending necks of *Cryphonectria* spp. are covered with orange tissue. Teleomorph structures of *C. cubensis* have limited, orange to cinnamon stroma tissue forming a clypeus around the upper parts of the perithecial bases and bases of the perithecial necks (Fig. 4A–C, I), thus not completely surrounding the perithecial bases.

This stromatic development can vary depending on the host. For instance, on clove stromatic development is more extensive, whereas in some cases stromatic tissue is absent on *Eucalyptus* bark (Hodges *et al.* 1986). *Cryphonectria* spp. have well-developed, orange ascostromata usually covering the greater part of the perithecial bases (Shear *et al.* 1917, Kobayashi 1970, Myburg *et al.* 2004). Necks of *C. cubensis* perithecia commonly extend beyond the stroma surface (Fig. 4I) whereas those of *Cryphonectria* spp. often fail to develop beyond the tops of the stromata.

The ascospores, conidiophores and conidia of *C. cubensis* are different from those of *Cryphonectria* spp. For *C. cubensis*, ascospores are septate in the center or somewhat off the center to a variable extent (Fig. 4F). Although the ascospore septa in *Cryphonectria* spp. have been known to deviate slightly from the center of the ascospores, they are not known to occur near the apex of the spores (Shear *et al.* 1917, Kobayashi 1970). Conidiophores of *C. cubensis* also consist of a basal cell of variable shape, with conidiogenous cells branching from it radially and irregularly (Figs 4M, N, 9–11). The conidiophore basal cells of *Cryphonectria* spp. usually are not easily discernable. Furthermore, the conidia of *C. cubensis* are oblong (Fig. 4O), while those of *Cryphonectria* spp. are more cylindrical (Shear *et al.* 1917, Kobayashi 1970, Myburg *et al.* 2004).

Differences between different phylogenetic groups of C. cubensis: Limited morphological differentiation existed for the *C. cubensis* specimens representing the four phylogenetic groups emerging from the DNA sequence comparisons. Specimens in only two of these groups could be distinguished from each other based on morphology. Specimens representing the South African group could be distinguished from those in other groups. This distinction was based on ascospore and ascus morphology. Asci of a *C. cubensis* specimen (PREM 58023) collected from South African *E. grandis* (Figs 5E, 6C) were (25–)27–32(–34) µm long, although no asci could be observed for the South African specimen (PREM 57359) from *T. granulosa* (Myburg *et al.* 2002a). Asci from specimens in the other phylogenetic groups were typically smaller. For example, asci for specimen PREM 58017 (Colombia) were (19–)22–26.5(–28) µm in length and those for specimen PREM 57297 (Indonesia) were (20.5–)22.5–25.5(–27) µm in length. Asci for the type specimen of *C. cubensis* from Cuba were reported to be 24.9–34 µm long (Bruner 1917), but such long asci were not observed for specimens linked to the South American and South East Asian phylogenetic groups.

Apices of ascospores from South African specimens (PREM 58023, PREM 57359) were rounded (Figs 5F, 6C) while those for the specimens representing the other phylogenetic groups (Table 2) were more tapered (Fig. 4F). This included ascospores from the type specimen of *C. cubensis* (BPI 631857). Morphological characteristics, such as size and shape of the conidia, were similar to those on specimens representing other phylogenetic groups and the type specimen.

The phylogenetic group from *Tibouchina* in Colombia differed from isolates in the other phylogenetic groups based on optimal colony growth. The Colombian isolate from *T. semidecandra* (CMW 10641) grew optimally at 25 ºC and covered the 90 mm plates on day 6. Isolates representing the other three phylogenetic groups had a temperature optimum of 30 ºC, and covered the plates within five days.

Few differences other than those observed in colony growth, were found associated with the fungal structures on the bark specimens for the phylogenetic group from *Tibouchina*. Conidia (Figs 7H, 8D) were slightly longer [(3–)3.5–5(–5.5) µm] than those of the other *C. cubensis* specimens [(3–)3.5–4.5(–5) µm]. These measurements were, however, based on relatively few, sporadically occurring conidia and could not be used with confidence. No sexual state was found for this fungus.

Table 4. Number of fixed alleles between different phylogenetic groups of *Chrysoporthe cubensis, Chr. austroafricana* and *Chrysoporthella hodgesiana.*

	South East Asian/Zanzibar/Hawaiian group of *Chr. cubensis*	South American/Congolese group of *Chr. cubensis*	*Chrysoporthella hodgesiana*
Chrysoporthe austroafricana	11	8	11
Chrysoporthella hodgesiana	13	9	
South American/Congolese group of *Chr. cubensis*	10		

Specimens of *C. cubensis* representing the remaining phylogenetic groups (South East Asia / Zanzibar / Hawaii and South America / Congo), could not be distinguished from each other. The only possible differences between specimens in these two phylogenetic groups were in conidiophore morphology. We could, however, not use these features with confidence because of potential variation within groups. The distinguishing characteristic of the fungi in these two groups was that basal cells for the South East Asian group (Fig. 9) were more variable in shape than those of the South American group (Fig. 10). Furthermore, conidiogenous cells of the South East Asian specimens were simple or septate, usually with a single lateral branch at the septum (Fig. 9). The conidiogenous cells of South American specimens were also simple or septate, but were often branched irregularly into two to three conidiogenous cells at the septa, which often branched again (Fig. 10).

The type specimen of *C. cubensis* could not be distinguished from the South East Asian / Zanzibar / Hawaiian and South American / Congolese phylogenetic groups based on morphology. The phylogenetic position of the type specimen from Cuba could not be established because there are no isolates linked to the type specimen of this species. In the original description of Bruner (1917), asci of *C. cubensis* from the Cuban sample are given as 24.9–34.03 ″ 4.15–6.64 µm. This is longer than the asci measured for the South East Asian and South American samples in this study. The difference in ascus length could not be verified, because no intact asci were present on the type specimen and no additional specimens of this fungus from Cuba are available. Morphology of the conidiophores was similar to those of the South East Asian group, although conidiophores were more irregular and secondary branching also occurred (Fig. 11).

Taxonomy

Extensive comparisons based on DNA sequence data (Myburg *et al.* 2004) have shown that *C. cubensis* and other *Cryphonectria* spp. represent distinct groups. Results of the present and previous (Myburg *et al.* 2004) studies have also shown that these groups can be clearly distinguished based on morphology. It is thus appropriate to establish a new genus to accommodate isolates and specimens referred to as *C. cubensis*. Fruiting structures of *C. cubensis* do not resemble those of any other member of the *Diaporthales* because of the orange colour of the stromatic tissue (Barr 1978). Other than *Cryphonectria*, the only genus in the *Diaporthales* having orange stromatic tissue, is *Endothia*. Specimens of *C. cubensis* can be distinguished from those of *Endothia* by the septate ascospores and weakly developed ascostroma in the former genus (Barr 1978, Micales & Stipes 1987, Venter *et al.* 2001, Myburg *et al.* 2004). Based on this justification, a new genus in the *Diaporthales* is provided for *C. cubensis* as follows:

Chrysoporthe Gryzenhout & M.J. Wingf., **gen. nov.** MycoBank MB500032.

Anamorph: *Chrysoporthella* Gryzenhout & M.J. Wingf., anam. gen. nov.

Etymology: Greek, *chrysous*, golden, referring to the orange stromatic tissue, and *porthe*, destroyer, referring to its pathogenic nature.

Ascostromata perithecia nigra valsoidea in contextu corticis inclusa; colla peritheciorum longa, cylindrica, per superficiem corticis protrudentia, contextu umbrino tecta, itaque atrofusca apparentia. Contextus ascostromaticus prosenchymatosus parcus, cinnamomeus vel aurantiacus, oculo nudo aurantiacus, plerumque infra corticem adest vel per superficiem erumpens. Asci fusoidei vel ellipsoidei. Ascosporae hyalinae, uno solo septo positione variabili, plerumque centrali, fusoideae vel ovales.
Conidiomata ad *Chrysoporthellam* pertinentia, superficialia, atrofusca, pyriformia vel pulvinata, 1–4 collis attenuatis praedita, uni- vel multilocularia, superficie interna levi vel subconvoluta. Textura stromatica, basim versus textura globulosa, in collo textura porrecta. Conidiophora hyalina, cellulae basales irregulariter ramosae, phialides cylindricas proferentes, septis divisas an non. Conidiorum massa in cirrhis vel guttis laete luteis exudata, conidia hyalina, non septata, oblonga.

Ascostromata consisting of black, valsoid perithecia embedded in bark tissue, long, cylindrical, perithecial necks covered with umber tissue as they protrude through the bark surface, thus appearing fuscous-black. Limited cinnamon to orange prosenchymatous stromatic tissue present around the upper part of the perithecial bases, appearing orange to the naked eye, usually present beneath or erumpent through the bark surface. *Asci* 8-spored, fusoid to ellipsoid. *Ascospores* hyaline, with one septum in variable, usually median, position, fusoid to oval.

Conidiomata of *Chrysoporthella* occurring separately or on top of the ascostroma, superficial, fuscous-black, pyriform to pulvinate with one to four attenuated necks, single to multilocular with even to slightly convoluted inner surface. *Stromatic tissue* of base tissue of *textura globulosa* and that of neck cells of *textura porrecta*. *Conidiophores* hyaline, consisting of a basal cell, branched irregularly at the base or above into cylindrical cells, separated by septa or not. *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum. *Conidia* hyaline, non-septate, oblong, exuded as bright luteous spore tendrils or droplets.

Typus: *Chrysoporthe cubensis* (Bruner) Gryzenhout & M.J. Wingf., comb. nov.

Notes: Various species of *Chrysoporthe* predominantly occur as anamorphs (Wingfield *et al*. 1989, Myburg *et al.* 2002a, Seixas *et al*. 2004) and there is a strong practical reason to provide anamorph names for these fungi. This is further necessitated by the fact that some species, such as the fungus from *Tibouchina* spp. in Colombia, have no known teleomorph and thus cannot be described in *Chrysoporthe* (ICBN, Art. 59.2, Greuter *et al.* 2000). We, therefore, describe a new anamorph genus for *Chrysoporthe*, with the anamorph of *Chr. cubensis* as type species. The fungus from *Tibouchina* in Colombia represents a second species. This new anamorph genus could equally be used for other species of *Chrysoporthe,* but we are not providing anamorph names for these fungi following ICBN recommendation 59.A.3 (Greuter *et al.* 2000).

Chrysoporthella Gryzenhout & M.J. Wingf., **anam. gen. nov.** MycoBank MB500033.

Etymology: diminutive of *Chrysoporthe*, referring to the anamorph structures that commonly occur independently from the teleomorph.

Conidiomata sparsa vel in summo ascostromate reperta; ab ascostromatibus forma pyriformi, collis attenuatis, dispositione superficiali, loculis conidiis repletis texturaque stromatica distinguenda. Conidiomata superficialia, atrofusca, pyriformia vel pulvinata, 1–4 colla attenuate proferentia, uni- vel plurilocularia, intus levia vel convoluta. Basis stromatis e textura globulosa, collorum e textura porrecta composita. Conidiophora hyalina, e cellula infima basilari vel supra ramificata in acervos irregulares phialidum cylindricarum vel ampulliformium, sursum attenuatarum, ad basim septis divisarum an non, collari et inspissatione periclinali inconspicuis. Conidiorum massa cirrhis vel guttis laete luteis exudata; conidia hyalina, non septata, oblonga.

Conidiomata occurring separately or on top of an ascostroma, distinguishable from ascomata by their pyriform shape, attenuated necks, conidiomatal locules and characteristic stromatic tissue. Conidiomata superficial, fuscous-black, pyriform to pulvinate, with one to four attenuated necks, single to multilocular, with even to slightly convoluted inner surface. *Stromatic tissue* of base of *textura globulosa* and neck tissue of *textura porrecta*. *Conidiophores* hyaline, consisting of a basal cell, irregularly branched into cylindrical cells, with or without separating septa. *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath septum. *Conidia* hyaline, non-septate, oblong, masses exuded as bright luteous tendrils or droplets.

Typus: *Chrysoporthella* anamorph of *Chrysoporthe cubensis* (Bruner) Gryzenhout & M.J. Wingf.

The following key is provided to aid in differentiation between *Chrysoporthe* with its *Chrysoporthella* anamorph, and the closely related genera *Cryphonectria* and *Endothia*:

1a. Ascostromata reduced, orange; perithecial necks long, fuscous-black; conidiomata usually pyriform, superficial, with attenuated necks, fuscous-black..... ***Chrysoporthe***
1b. Ascostromata erumpent, orange; perithecial necks short, orange; conidiomata pulvinate, orange 2

2a. Ascostromata and conidiomata semi-immersed; ascospores uniseptate ..... ***Cryphonectria***
2b. Ascostromata and conidiomata superficial; ascospores non-septate ..... ***Endothia***

No distinct morphological differences could be found for specimens that represented the South East Asian/Zanzibar/Hawaiian and South American/Congolese phylogenetic groups. These specimens were also indistinguishable from the type specimen. No isolates are available connected with the type specimen from Cuba and it is unknown where Cuban isolates would group with respect to the other phylogenetic groups. Nevertheless, it can be assumed that the South American group is closest to the type. The fungi in the South East Asian/Zanzibar/Hawaiian and South American/Congolese phylogenetic groups, including the type specimen from Cuba are thus retained as a single species. A more detailed description for *C. cubensis* to supplement the original description of Bruner (1917) is provided below to enable comparison with the other new species:

Chrysoporthe cubensis (Bruner) Gryzenhout & M.J. Wingf., **comb. nov**. MycoBank MB500034. Fig. 4.

Basionym: *Diaporthe cubensis* Bruner, Estac. Exp. Agron., Cuba, Bull. 37: 15–16. 1917.

≡ *Cryphonectria cubensis* (Bruner) Hodges, Mycologia 72: 547. 1980.

= *Cryptosporella eugeniae* Nutman & Roberts, Ann. Appl. Biol. 39: 607. 1952.

≡ *Endothia eugeniae* (Nutman & Roberts) J. Reid & C. Booth, Mycologia 78: 347. 1986.

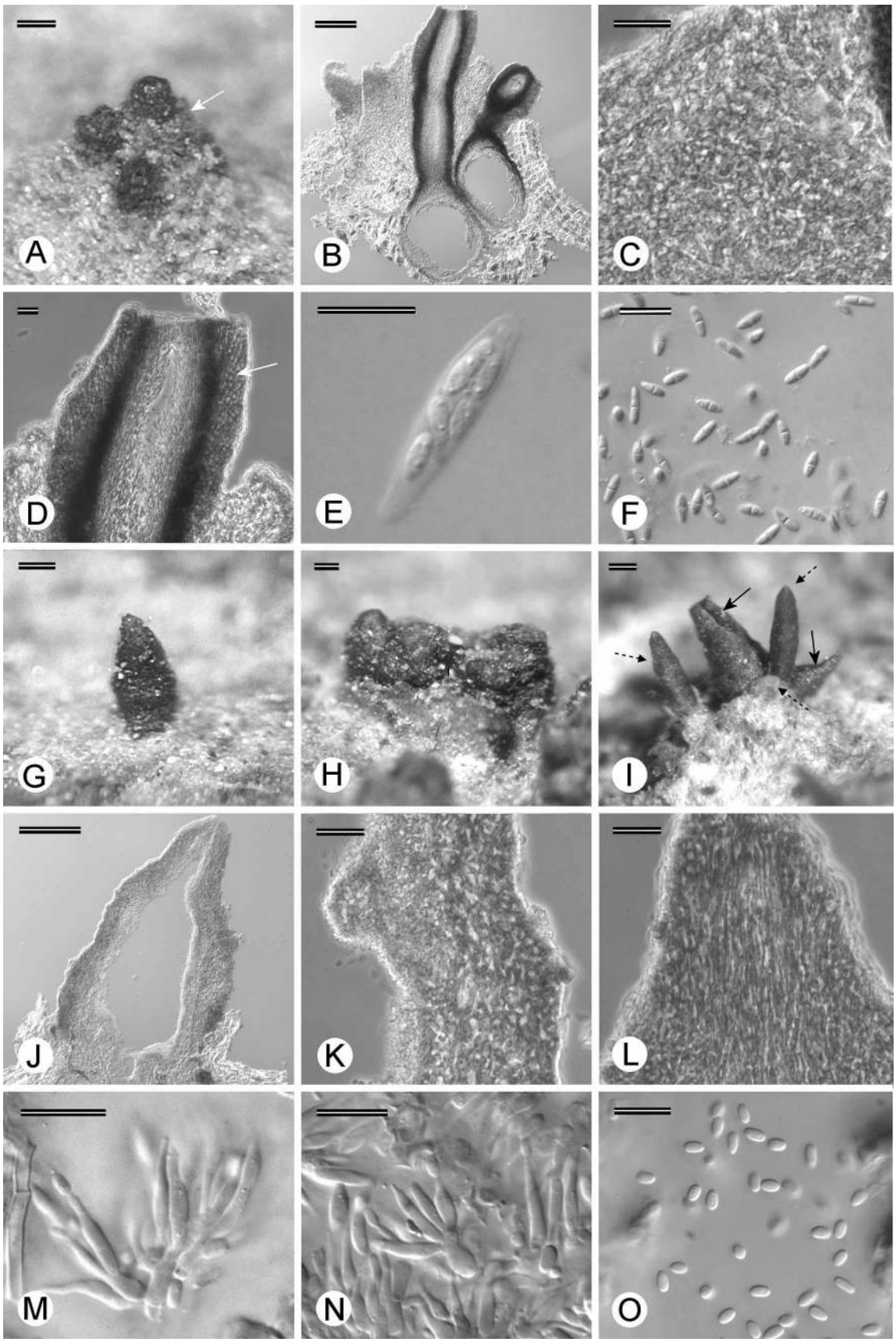


Fig. 4. Micrographs of *Chrysoporthe cubensis*. A. Black perithecial necks and orange stromatic tissue (arrow) of ascostroma on bark. B. Vertical section through ascoma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus from specimen PREM 57297 collected in Indonesia. F. Ascospores. G, H. Conidiomata of different shapes on bark. I. Ascoma (dashed arrows) with conidiomata (arrows) on top. J. Vertical section through conidioma. K. Tissue of the conidiomal base. L. Tissue of conidiomal neck. M–N. Conidiophores from a Colombian specimen (PREM 57294) and Cuban specimen (BPI 631857), respectively. O. Conidia. Scale bars A–B, G–J = 100 µm; C–D, K–L = 20 µm; E–F, M–O = 10 µm.

Typus: BPI 631857, *Eucalyptus botryoides*, Cuba, Santiago de las Vegas, 1916, C.L. Shear.

Ascomata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks, and in some cases, erumpent, limited, orange ascostromatic tissue; ascostroma 120–230 µm high above level of bark, 280–490 µm diam (Fig. 4A). *Perithecia* valsoid, 1-9 perithecia per stroma, bases immersed in bark, black, globose, 170–250 µm diam, perithecial wall 17–22 µm thick (Fig. 4B). Top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue forming a clypeus of variable extent, which is occasionally visible above the bark surface (Fig. 4A–C). *Perithecial necks* black, periphysate, 80–170 µm wide (Fig. 4B). Necks emerging through bark covered in umber stromatic tissue of *textura porrecta*, thus appearing fuscous-black, extending necks up to 240 µm long, 110–610 µm wide (Fig. 4D, I). *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate, with a non-amyloid refractive ring, fusoid to ellipsoid (Fig. 4E), 25–34 ″ 4–6.5 µm (Bruner 1917). *Ascospores* hyaline, one-septate, with septum variously placed in the spore but usually central, fusoid to oval, with tapered apices, (5.5–)6.5–7.5(–8) ″ 2–2.5(–3) µm (Fig. 4F).

Conidiomata occurring separately (Fig. 4G, H) or on the top of an ascostroma (Fig. 4I), distinguishable from ascomata by their pyriform shape, attenuated necks, conidiomatal locules and distinct stromatic tissue (Fig. 4G–L). Conidiomata eustromatic, superficial to slightly immersed, pyriform to clavate, sometimes pulvinate, with one to four attenuated necks per structure (Fig. 4G, I, J), fuscous-black, with an umber interior when young, conidiomatal base above the bark surface 130–740 µm high, 100–950 µm diam, necks up to 230 µm long, 90–240 µm wide. *Conidiomatal locules* with even to convoluted inner surface, occasionally multilocular, single locule connected to one or several necks, locules 110–500 µm diam (Fig. 4J). *Stromatic tissue* of base of *textura globulosa* with walls of outer cells thickened (Fig. 4K), and neck cells of *textura porrecta* (Fig. 4L). *Conidiophores* hyaline, with a globular to rectangular basal cell, (2.5–)4–7(–8.5) ″ (2–)3–4.5(–5.5) µm, branched irregularly at the base or above into cylindrical cells, cells delimited by septa or not, total length of conidiophore (12–)13.5–19(–24.5) µm (Figs 4M, N, 9, 10, 11). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath the septum, cylindrical to flask-shaped with attenuated apices, (1.5–)2–2.5(–3) µm wide, collarette and periclinal thickening inconspicuous (Figs 4M, N, 9–11).

Conidia hyaline, non-septate, oblong, (3–)3.5–4.5(–5) ″ (1.5–)2(–2.5) µm (Fig. 4O), exuded as bright luteous spore tendrils or droplets.

Cultural characteristics: No cultures from the type location are available. Cultures from Indonesia (CMW 11288, CMW 8650), Colombia (CMW 10639) and the Republic of Congo (CMW 10669) on MEA white with cinnamon to hazel patches, fluffy, with a smooth margin, fast-growing, covering a 90 mm diam plate after a minimum of five days at the optimum temperature of 30 ºC. Cultures rarely sporulating, especially after sub-culturing, teleomorphs not produced.

Substrate: Bark of *Eucalyptus* spp. and *Syzygium aromaticum* (clove). Also reported from *Psidium cattleianum*.

Distribution: Cuba, Mexico, Colombia, Venezuela, Brazil, Surinam, U.S.A. (Florida, Hawaii, Puerto Rico), Democratic Republic of Congo (Zaire), Republic of Congo, Cameroon, Tanzania (Zanzibar), Indonesia, Malaysia, Singapore, China, India, Australia, Western Samoa.

Specimens examined: **Cuba**, Santiago de las Vegas, *Eucalyptus botryoides*, 1916, C.L. Shear, **holotype** BPI 631857. **Colombia**, Cali, Vanessa farm, *Eucalyptus grandis*, 2000, M.J. Wingfield, PREM 57294, culture from same area CMW 10639 = CBS 115747; Cali, Vanessa farm, *Eucalyptus urophylla*, 2000, M.J. Wingfield, PREM 58017. **Mexico**, Tabasco, *Eucalyptus* sp., 2000, M.J. Wingfield, PREM 57295, PREM 58016, culture from same area CMW 9432 = CBS 115724. **Venezuela**, Uverito, host given as *Eucalyptus grandis*/ *Eugenia* sp., 1983, C.S. Hodges, IMI 284438. **Brazil**, Minas Gerais, *Eucalyptus grandis*, 1973, C.S. Hodges, MASS; Minas Gerais, Dionisio, *Eucalyptus maculata* Hook., 1974, C.S. Hodges, IMI 184653; Minas Gerais, Dionisio, *Eucalyptus* sp., 1973, C.S. Hodges, IMI 172718; Minas Gerais, Coronel Fabriciano, *Eucalyptus propinqua* H. Deane & Maiden, 1974, C.S. Hodges, IMI 184652; Santa Catarina, Ilha de Santa Catarina, *Psidium cattleianum*, 1988, C.S. Hodges, IMI 351788; São Paulo, *Eucalyptus* sp., 1973, L. May, IMI 173960; Espirito Santo, Fundão, *Syzygium aromaticum* (as *Eugenia caryophyllata* Thunb.), 1983, C.S. Hodges, IMI 285983; Bahia, Valenca, *Syzygium aromaticum* (as *Eugenia caryophyllata*), 1983, C.S. Hodges, IMI 285982, cultures CMW 10777, CMW 10778 = CBS 115755. **Surinam**, Paramaribo, *Eucalyptus citriodora* Hook., 1973, P.A. Tennissen, IMI 177647. **U.S.A.**, Florida, La Belle, *Eucalyptus grandis*, 1981, W. Sinclair, CUP 58722; Florida, *Eucalyptus grandis*, 1976, C.S. Hodges, IMI 202849; Hawaii, Kauai, *Eucalyptus saligna* Sm., 1978, C.S. Hodges, DAR 35434, culture from same area CMW 1856. **China**, Hong Kong Island, Botanical Gardens, *Eucalyptus* sp., 1981, C.S. Hodges, IMI 263717. **India,** Kerala Forest Research Institute, *Eucalyptus grandis*, 1981, J.K. Sharma, IMI 261569. **Singapore,** Istana grounds, *Syzygium aromaticum*, 1991, C.P. Yik, dried

culture IMI 350626. **Malaysia,** Johar, Kluang, *Eucalyptus aromatica* (Salisb.) Domin, 1986, Loh Chow Fong, IMI 304273; Serdang, Fe. Exp. Stn., *Syzygium aromaticum* (as *Eugenia caryophyllata*), 1954, A. Johnston, IMI 58569; *Eugenia* sp., 1954, A. Johnston, IMI 58567, IMI 58568; Jelok Bahang, *Syzygium aromaticum* (as *Eugenia caryophyllata*), 1954, A. Johnston, IMI 58388. **Indonesia,** Sulawesi, *Syzygium aromaticum*, 2001, M.J. Wingfield, PREM 57470, cultures CMW 8650 = CBS 115719, CMW 8651 = CBS 115718; Sulawesi, *Syzygium aromaticum*, 2003, M.J. Wingfield, PREM 58018, PREM 58019; Sulawesi, Utard, *Syzygium aromaticum*, 2003, M.J. Wingfield, PREM 58020; Bankals, Selindung, *Eugenia* sp., C.P.A. Bennett, IMI 231648; Sumatra, Kurai, Taji, *Eugenia* sp., C.P.A. Bennett, IMI 231649; Sumatra, *Eucalyptus* sp., 2001, M.J. Wingfield, PREM 57297, cultures from the same area CMW 11288 = CBS 115736, CMW 11289 = CBS 115737, CMW 11290 = CBS 115738; Sumatra, Sei Kabaro, *Eucalyptus* sp., 2001, M.J. Wingfield, PREM 58021, cultures from same area CMW 11289, CMW 11290. **Tanzania**, Zanzibar, Mkaje district, *Syzygium aromaticum* (as *Eugenia caryophyllata*), 1951, J. Nutman & F.M. Roberts, IMI 45440, IMI 45450, culture from same area CMW 10774; Zanzibar, *Syzygium aromaticum* (as *Eugenia caryophyllata*), 1983, A. Dabek, IMI 279035, culture from same area CMW 10774. **Cameroon**, Cellucam, Edea, *Eugenia urophylla* S. T. Blake, 1980, F.B. Armitage, IMI 249406.

Notes: Specimens residing in the South African phylogenetic group were distinguishable from those representing the other three phylogenetic groups based on ascospore shape and ascus size. This fungus is thus described as a new species of *Chrysoporthe* as follows:

Chrysoporthe austroafricana Gryzenhout & M.J. Wingf., **sp. nov.** MycoBank MB500035. Figs 5, 6.

Etymology: Latin, from Southern Africa.

Ascomata in cortice semi-immersa, collis peritheciorum protrudentibus atrofuscis cylindricis, et textura erumpente ascostromatica limitata aurantiaca visibilibus. Perithecia valsoidea nigra, basibus globosis in cortice immersis, textura ascostromatica prosenchymatosa cinnamomea vel aurantiaca, interdum supra superficiem corticis visibili tecta. Colla peritheciorum per corticem emergentia, contextu stromatico textura porrecta umbrino tecta, ita ut atrofusca videantur. Asci octospori, fusoidei vel ellipsoidei. Ascosporae hyalinae, uniseptatae, septo positione variabili sed plerumque centrali, fusoideae vel ovales, utrinque rotundatae.
Conidiomata ad *Chrysoporthellam* pertinentia, sparsa vel in summo ascostromate aggregata; eustromatica, superficialia vel sub-immersa, pyriformia vel pulvinata, 1–4 collis in quaque structura, atrofusca, juvenia intus umbrina. Loculi conidiomatum intus leves vel convoluti, interdum multiloculares, quisque loculus uno vel pluribus collis junctus. Basis stromatis e textura globulosa, cellulis superficialibus inspissatis, colla e textura porrecta composita. Conidiophora hyalina, cellula infima forma irregulari, supra irregulares greges phialidum cylindricarum vel ampulliformium, sursum attenuatarum proferentes; rami ad basim septati an non; collare et inspissatio periclinalis inconspicuae. Conidiorum massa cirrhis vel guttis laete luteis exudata; conidia hyalina, non septata, oblonga. Coloniae in MEA albae cinnamomeo- vel avellaneo-maculatae, celeriter crescentes, patellam 90 mm diam in minime quinque diebus temperatura optima 30 ºC tegentes.

Ascomata semi-immersed in bark, recognizable by extending, fuscous-black, cylindrical perithecial necks and in some cases, erumpent, limited ascostromatic tissue appearing orange, 70–260 µm high above the bark, 220–740 µm diam (Figs 5A, 6A, B). *Perithecia* valsoid, 1–11 per stroma, bases immersed in the bark, black, globose, 320–505 µm diam, perithecial wall 19–23 µm thick (Figs 5B, 6B). Top of perithecial bases covered with cinnamon to orange, predominantly prosenchymatous, stromatic tissue forming a clypeus of variable extent, which is occasionally visible above the bark surface (Figs 5A–C, 6B). *Perithecial necks* black, periphysate, 75–100 µm wide (Figs 5A, 6B). Necks emerging through the bark covered in umber, stromatic tissue of *textura porrecta*, thus appearing fuscous-black (Figs 5D, 6B), extending necks up to 1900 µm long, 100–150 µm wide. *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoid, (25–)27–32(–34) × (4–)5.5–7(–7.5) µm (Figs 5E, 6C). *Ascospores* hyaline, one-septate with septum variously placed in the spore but usually central, fusoid to oval, with rounded apices, (5.5–)6–7 × (2–)2.5 µm (Figs 5F, 6C).

Conidiomata occurring separately or on the top of an ascostroma, distinguishable from the ascomata by their pyriform shape, attenuated necks, conidiomatal locules and distinct stromatic tissue (Figs 5I, J, 6A, D, E). Conidiomata eustromatic, superficial to slightly immersed, pyriform to clavate, sometimes pulvinate, with one to four attenuated necks per structure (Figs 5G, 6D, E), fuscous-black, inside umber when young, conidiomatal base above the bark surface 100–220 µm high above level of bark, 80–210 µm diam, necks up to 200 µm long, 30–80 µm wide. *Conidiomatal locules* with even to convoluted inner surface, occasionally multilocular, single locule connected to 1 or several necks, locules 90–380 µm diam (Figs 5H, 6E). *Stromatic tissue* of base of *textura globulosa*, the walls of outer cells thickened (Fig. 5I), neck tissue of *textura porrecta* (Fig. 5J).

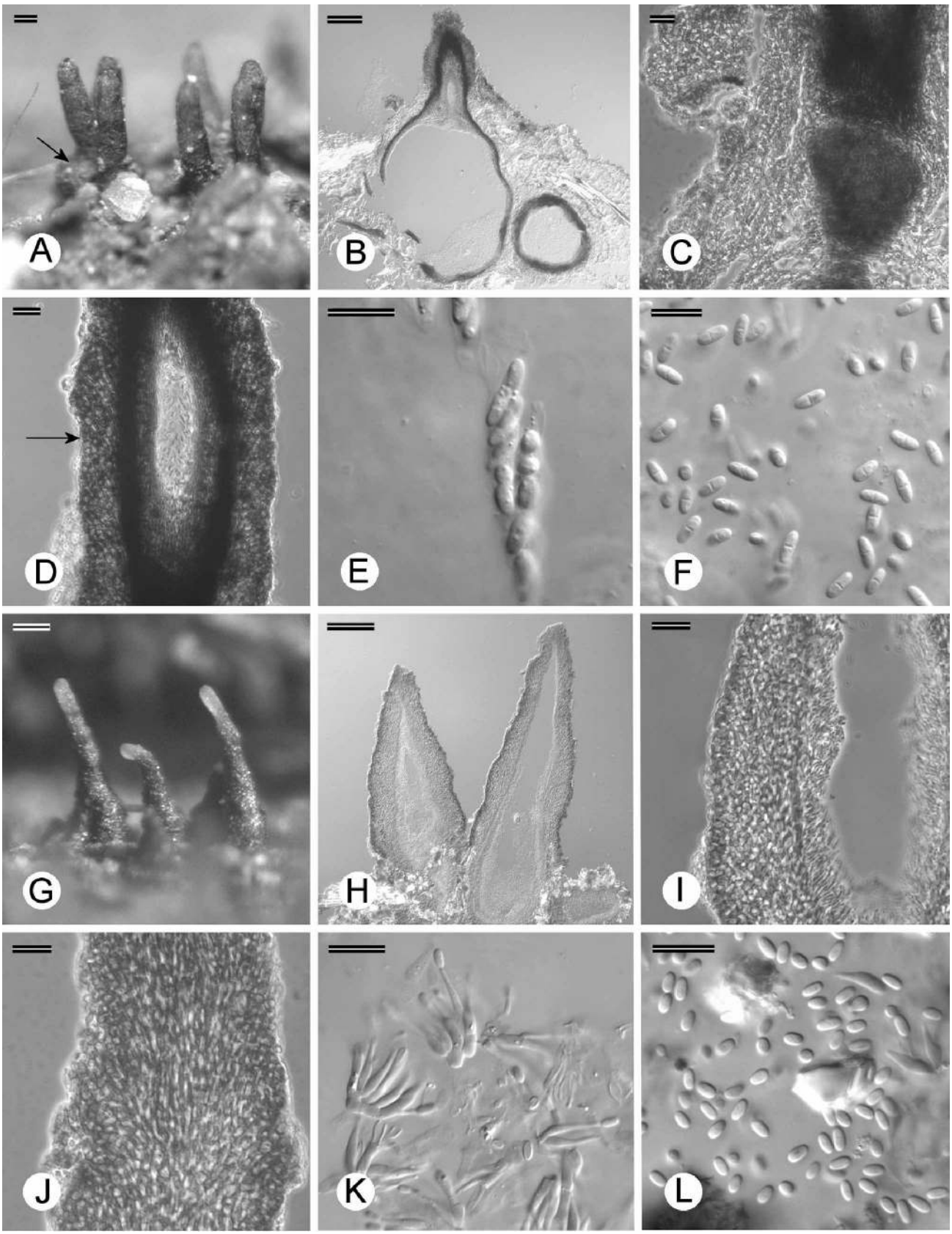


Fig. 5. Micrographs of fruiting structures of *Chrysoporthe austroafricana*. A. Ascostromata on bark showing black perithecial necks and orange stromatic tissue (arrow). B. Vertical section through ascoma. C. Stromatic tissue of ascostroma. D. Perithecial neck and surrounding tissue (arrow). E. Ascus. F. Ascospores. G. Pyriform conidiomata. H. Vertical section through conidiomata. I. Tissue of the conidiomal base. J. Tissue of conidiomal neck. K. Conidiophores. L. Conidia. Scale bars A–B, G–H = 100 µm; C–D, I–J = 20 µm; E–F, K–L = 10 µm.

Conidiophores hyaline, with basal cells of irregular shape, (2.5–)3.5–6(–8)˝ (2–)2.5–4.5(–6) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (11.5–)14.5–21(–28) µm (Figs 5K, 6F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical to flask-shaped with attenuated apices, (1–)1.5–2.5(–3.5) µm wide, collarette and periclinal thickening inconspicuous (Figs 5K, 6F). *Conidia* hyaline, non-septate, oblong, 3–4(–4.5)˝ 1.5–2 µm (Figs 5L, 6G), masses exuded as bright luteous tendrils or droplets.

Typus: PREM 58023 **holotype**, *Eucalyptus grandis*, South Africa, KwaZulu-Natal, KwaMbonambi, 1989, M.J. Wingfield (culture ex-type CMW 2113 = CBS 112916).

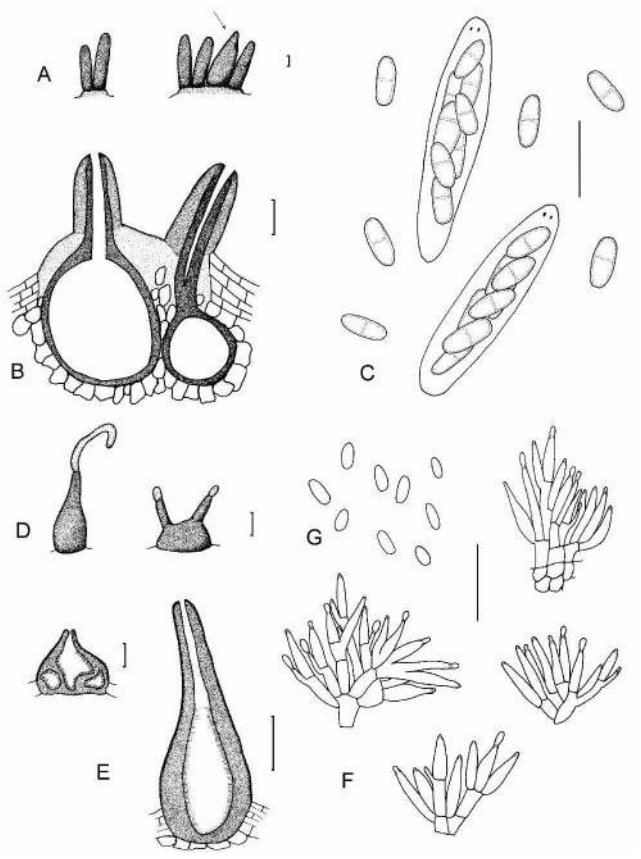


Fig. 6. Line drawings of *Chrysoporthe austroafricana*. A. Shapes of ascomata and conidiomata (indicated with arrow) on bark. B. Section through ascoma. C. Asci and ascospores. D. Shapes of conidiomata. E. Section through conidiomata. F. Conidiophores and conidiogenous cells. G. Conidia. Scale bars A–B, D–E = 100 µm; C, F–G = 10 µm.

Cultural characteristics: On MEA (CMW 2113, CMW 9328) white with cinnamon to hazel patches, fluffy with a smooth margin, fast-growing, covering a 90 mm diam plate after a minimum of five days at the optimum temperature of 30 ºC. Cultures rarely sporulating after sub-culturing, teleomorphs not produced.

Substrate: Bark of *Eucalyptus* spp. and *Tibouchina granulosa.*

Distribution: South Africa

Specimens examined: **South Africa**, KwaZulu-Natal, KwaMbonambi, on *Eucalyptus grandis*, 1989, M.J. Wingfield, **holotype** PREM 58023, ex-type culture CMW 2113 = CBS 112916; KwaZulu-Natal, KwaMbonambi, *Eucalyptus grandis*, 1986-88, M.J. Wingfield, PREM 49377, PREM 49378, PREM 79379; KwaZulu-Natal, KwaMbonambi, *Eucalyptus grandis* clone inoculated with isolate CMW 2113 during artificial inoculations, 2003, J. Roux, PREM 58024, culture CMW 2113 = CBS 112916; KwaZulu-Natal, Mtubatuba, Dukuduku estate, *Eucalyptus grandis*, 2001, M. Venter, PREM 57293; KwaMbonambi & Richardsbay, *Tibouchina granulosa*, 1999, J. Roux, PREM 57357, PREM 57385, PREM 57359, cultures from same area CMW 9327 = CBS 115843, CMW 9328; KwaZulu-Natal, Durban, *Tibouchina granulosa*, 2000, J. Roux, R. Heath & L. Lombard, PREM 57360, PREM 57361.

Notes: Specimens representing the Colombian *Tibouchina* group contained only the anamorph and no teleomorph has been found for this fungus. The anamorph structures could not be distinguished from those of *Chr. cubensis* or *Chr. austroafricana*. However, isolates of this fungus grew optimally at 25 °C, which was different from isolates representing *Chr. cubensis* and *Chr. austroafricana* that grew optimally at 30 °C.

Chrysoporthella hodgesiana Gryzenhout & M.J. Wingf., **sp. nov.** MycoBank MB500036. Figs 7, 8.

Etymology: Latin, in honour of Dr Charles S. Hodges recognizing his fundamental research on the distribution, host range, pathology and taxonomy of *Cryphonectria cubensis.*

Conidiomata eustromatica, superficialia vel subimmersa, plerumque pulvinata, interdum pyriformia, 1–4 collis brevibus attenuatis in quaque structura, atrofusca. Loculi conidiomatum intus leves vel convoluti, interdum multiloculares, quisque loculus uno vel pluribus collis junctus. Basis stromatis e textura globulosa, cellulis superficialibus incrassatis, colla e textura porrecta composita. Conidiophora hyalina, cellula infima forma irregulari, supra greges irregulares phialidum cylindricarum vel ampulliformium, sursum attenuatarum proferentes; rami ad basim septati an non, collare et inspissatio periclinalis inconspicuae. Conidiorum massa cirrhis vel guttis laete luteis exudata; conidia hyalina, non septata, oblonga. Coloniae in MEA albae, cinnamomeo- vel avellaneo-maculatae, celeriter crescentes, patellam 90 mm diam in minime sex diebus temperatura optima 25 ºC tegentes.

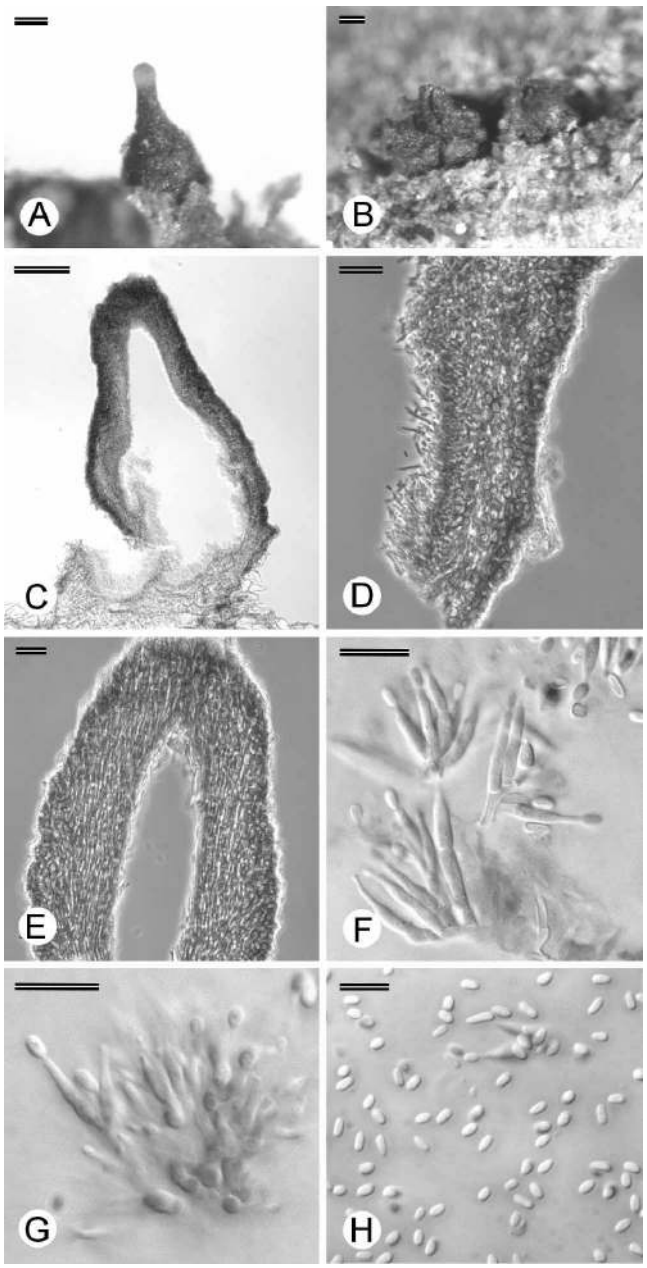


Fig. 7. Micrographs of fruiting structures of *Chrysoporthella hodgesiana*. A, B. Conidiomata of different shapes on bark. C. Vertical section through conidioma. D. Tissue of the conidiomal base. E. Tissue of conidiomal neck. F–G. Conidiophores. H. Conidia. Scale bars A–C = 100 µm; D–E = 20 µm; F–H = 10 µm

Conidiomata eustromatic, superficial to slightly immersed, generally pulvinate, sometimes pyriform, with one to four short attenuated necks per structure (Figs 7A, B, 8A, B), fuscous-black, with an umber interior when young, conidiomatal base above the bark surface 85–310 µm high, 145–635 µm wide, necks up to 380 µm long, 65–170 µm wide. Conidiomatal locules with even to convoluted inner surface, occasionally multilocular, a single locule connected to one or several necks, locules 125–410 µm diam (Figs 7C, 8B). *Stromatic tissue* of base of *textura globulosa*, the outer cells with thickened walls (Fig. 7D), neck tissue of *textura porrecta* (Fig. 7E). *Conidiophores* hyaline, with a basal cell of irregular shape, (1.5–)3–6.5(–9.5) ″ (2–)2.5–4(–5.5) µm, branched irregularly at the base or above into cylindrical cells, with or without separating septa, total length of conidiophore (12–)13–21(–33) µm (Figs 7F, G, 8C). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical to flask-shaped with attenuated apices, (1.5–)2–2.5 µm wide, collarette and periclinal thickening inconspicuous (Figs 7F, G, 8C). *Conidia* hyaline, non-septate, oblong, (3–)3.5–5(–5.5) ″ 1.5–2(–2.5) µm (Figs 7H, 8D), masses exuded as bright luteous tendrils or droplets.

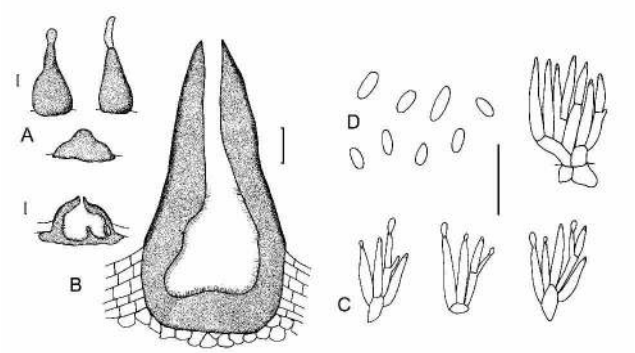


Fig. 8. Line drawings of *Chrysoporthella hodgesiana*. A. Shapes of conidiomata on bark. B. Section through conidiomata. C. Conidiophores and conidiogenous cells. D. Conidia. Scale bars A–B = 100 µm; C–D = 10 µm.

Typus: PREM 58022 **holotype**, *Tibouchina semidecandra*, Colombia, Darien, 2001, R. Arbelaez (culture ex-type CMW 10641 = CBS 115854).

Cultural characteristics: On MEA (CMW 10641) white with cinnamon to hazel patches, fluffy with a smooth margin, fast-growing, covering a 90 mm diam plate after a minimum of 6 d at the optimum temperature of 25 ºC. Cultures rarely sporulating especially after sub-culturing, teleomorphs not produced.

Substrate: Bark of *Tibouchina semidecandra*, *T. urvilleana* and *T. lepidota*.

Distribution: Colombia.

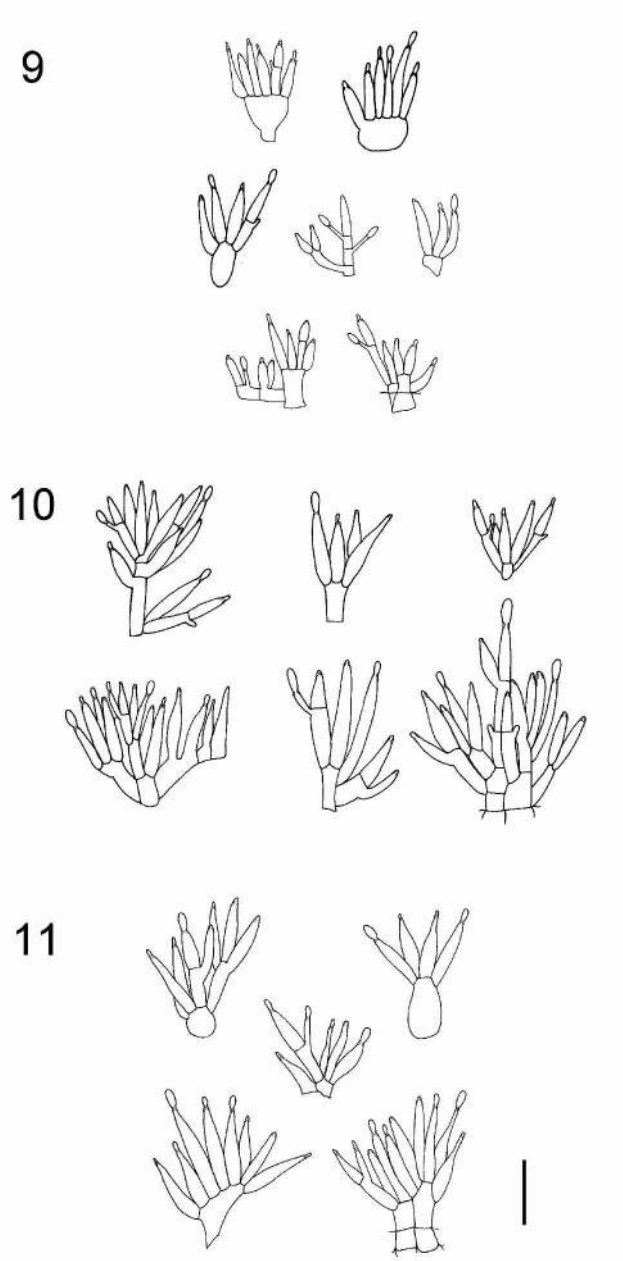
Specimens examined: **Colombia**, Darien, *Tibouchina semidecandra*, 2001, R. Arbelaez, **holotype** PREM 58022, ex-type culture CMW 10641 = CBS 115854; Buga, *Tibouchina lepidota*, 1999, M.J. Wingfield, PREM 56913; Buga, *Tibouchina urvilleana*, 1999, M.J. Wingfield, PREM 16914, PREM 56915.

The following key, based on morphological characteristics, is provided to aid in differentiation between the three *Chrysoporthe* species. This key will not help to identify specimens with confidence in the absence of a teleomorph and should, as far as possible, be used in conjunction with DNA sequence data of the regions sequenced in this study. This is especially true since a teleomorph is not known for *Chrysoporthella hodgesiana*, and its anamorph is virtually indistinguishable from that of *Chr. cubensis* and *Chr. austroafricana*.

Key to species of *Chrysoporthe*

1a. Teleomorph not known; optimal growth at 25 ºC ..... ***Chrysoporthella hodgesiana***
1b. Teleomorph present; optimal growth at 30 ºC.....2

2a. Ascospores with tapered apices ..... ***Chrysoporthe cubensis***
2b. Ascospores with rounded apices..... ***Chrysoporthe austroafricana***



Figs 9–11. Line drawings of the conidiophores of *Chrysoporthe cubensis*. 9. South East Asian specimens. 10. South American specimens. 11. Type specimen (BPI 631857). Scale bar = 10 µm.

DISCUSSION

The new genus *Chrysoporthe* (*Diaporthales*) is described to accommodate the fungus previously known as *Cryphonectria cubensis*. This study and the work of Myburg *et al.* (2004) have shown that *Chrysoporthe* can easily be distinguished from *Cryphonectria* based on morphological characters. In addition, isolates in *Chrysoporthe* were shown to be phylogenetically distinct from *Cryphonectria* and *Endothia*, based on comparisons of DNA sequences of the ITS1/ITS2 region of the ribosomal operon and β-tubulin genes (Myburg *et al.* 2004). Recognition of a new genus to accommodate *C. cubensis* is consistent with previous suggestions (Walker *et al.* 1985, Roane 1986, Venter *et al.* 2001) that this species was atypical of *Cryphonectria*.

We have described the new anamorph genus *Chrysoporthella*, residing in the *Diaporthales*, to accommodate the asexual structures of *Chrysoporthe*. *Chrysoporthella* is clearly distinct from *Endothiella*, the currently recognised anamorph of *Cryphonectria* and *Endothia* (Barr 1978). *Chrysoporthella* has blackened, pyriform and superficial conidiomata while the conidiomata of *Endothiella* are orange, pulvinate and semi-immersed (Myburg *et al.* 2004).

In addition to *Endothiella*, Barr (1978) described the anamorph of *Cryphonectria* as dendrophoma-like, an observation most probably based on the anamorph of *Chrysoporthe*. *Dendrophoma* is, however, currently a synonym of *Dinemasporium*, which is an anamorph for *Phomatospora* (Hawksworth *et al.* 1995). This is thus no appropriate anamorph genus for *Chrysoporthe,* since *Phomatospora* is of uncertain position in the *Xylariales* (Hawksworth *et al.* 1995, Kirk *et al.* 2004). This further justifies our description of a new anamorph genus for *Chrysoporthe*.

Previous DNA sequence comparisons have shown that isolates of *C. cubensis* represent three phylogenetic lineages (Myburg *et al.* 2002a, 2002b, 2003, 2004). Two of these, representing isolates mainly from South East Asia and South America, can be distinguished based on sequences of the ITS region, β-tubulin and the Histone *H3* genes (Myburg *et al.* 1999, 2002a, b, 2003, 2004). South African isolates were shown to be distinct from the former two groups using β-tubulin and Histone *H3* genes (Myburg *et al.* 2002b), although DNA sequences of the ITS region

grouped these isolates together with South American isolates (Myburg *et al.* 1999). Results of this study recognized the same three phylogenetic lineages, and a fourth clade has also emerged accommodating isolates from *Tibouchina* in Colombia. The four lineages are characterised by a number of fixed alleles found in the ITS1/ITS2 regions and two regions of the β-tubulin gene (Table 3) that can be used to identify each group. These characteristic alleles were also noted for the ITS region by Van der Merwe *et al.* (2001).

Specimens representing the South African phylogenetic clade can be distinguished from the others based on morphological characters. These include ascospores with rounded apices and seemingly longer asci. Consequently we have described this fungus as the new species *Chr. austroafricana*. In addition to the morphological differences, *Chr. austroafricana* also differs from *Chr. cubensis* in various other respects. *Chr. austroafricana* rapidly invades the cambium and gives rise to girdling cankers (Wingfield *et al.* 1989). In contrast, *Chr. cubensis* invades the wood more deeply (Fig. 1C) and gives rise to swollen cankers on infected stems (Wingfield 2003), although this could be due to different host reactions (C.S. Hodges, pers. comm.). Furthermore, teleomorph structures of *Chr. austroafricana* are rarely encountered on *Eucalyptus* (Wingfield *et al.* 1989, Van Heerden & Wingfield 2001) or *Tibouchina* spp. (Myburg *et al.* 2002a) in South Africa. In contrast, teleomorph structures of *Chr. cubensis* are common on the surfaces of cankers on *Eucalyptus* stems in South East Asia, Hawaii and South America (Hodges *et al.* 1976, 1979, Van Heerden *et al.* 1997, Van Zyl *et al.* 1998, Van der Merwe *et al.* 2001, Van Heerden & Wingfield 2001).

The fourth phylogenetic clade recognized in this study includes isolates, previously treated as *C. cubensis*, from *Tibouchina* in Colombia. This fungus occurs only in its asexual morph. Based on phylogenetic data, we would ideally have chosen to provide a name for this fungus in *Chrysoporthe*. This is, however, contrary to Art. 59.2 of the ICBN. We have consequently erected the anamorph genus *Chrysoporthella* to accommodate this fungus and provided the species name *Chrysoporthella hodgesiana* for it. This fungus is morphologically indistinguishable from the anamorphs of *Chr. cubensis* and *Chr. austroafricana*. Other than by its origin and DNA sequence, *Chrysoporthella hodgesiana* can only be distinguished from the latter two species based on its optimum growth at 25 ºC. Little additional information is, however, available to aid in its identification.

Although they represent two distinct phylogenetic groups, no significant morphological or biological differences could be found to separate the remaining two clades of isolates previously treated as *C. cubensis*. These two clades contain isolates mainly from South America and South East Asia. Where they have been tested, isolates in both groups were shown to be homothallic (Hodges *et al.* 1976, 1979, Van Heerden *et al.* 1997, Van Zyl *et al.* 1998). They also give rise to the same disease symptoms (Boerboom & Maas 1970, Florence *et al.* 1986, Hodges *et al.* 1976, 1979, Sharma *et al.* 1985). Furthermore, fungi representing both groups have been found on clove (Hodges *et al.* 1986, Micales *et al.* 1987, Myburg *et al.* 2003).

Whether isolates representing the two phylogenetic groups within *Chr. cubensis* represent distinct species remains to be decided. They are clearly closely related and yet, based on DNA sequences, equally different to each other as they are from *Chr. austroafricana* and *Chrysoporthella hodgesiana*. For the present, we have chosen not to provide different names for these fungi, and these isolates will thus represent *Chr. cubensis*. However, we believe that with more data, and particularly with additional specimens from areas such as Cuba, it will be possible to decide whether the remaining phylogenetic groups represent reproductively isolated species, or whether gene flow occurs between them. Progress in resolving this question has already been made with the development of Simple Sequence Repeats (SSR) markers to study the population structure of *Chr. cubensis* and *Chr. austroafricana* (Van der Merwe *et al.* 2003).

Various hypotheses have been presented to explain the origin of *Chr. cubensis* (Hodges *et al.* 1986, Wingfield *et al.* 2001, Wingfield 2003, Seixas *et al.* 2004). These have tended to focus on the occurrence of the fungus on hosts other than *Eucalyptus*. This is because *Eucalyptus* spp. have not been considered as native hosts of *Chr. cubensis* since there has only been a single report of *Chr. cubensis* on *Eucalyptus* spp. in their native range (Davison & Coates 1991). One view is that *Chr. cubensis* originated on cloves in Indonesia and that the fungus has moved from this native tree to infect exotic *Eucalyptus*, via the movement of cloves around the world through the spice trade (Hodges *et al.* 1986). Wingfield *et al.* (2001) showed that the fungus occurs commonly on native *Tibouchina* spp. in Colombia and that it could equally have originated on this continent. Isolates from *Tibouchina* studied by Wingfield *et al.* (2001) have now been shown to represent a species related to but different from *Chr. cubensis*. However, other native Colombian *Melastomataceae* such as *Miconia theaezans* (Bonpl.) Cogn. and *M. rubiginosa* (Bonpl.) DC., have been recognized as hosts of *Chr. cubensis* in Colombia (Rodas 2003). Thus, the hypothesis that *Chr. cubensis* has a South American origin retains strong support. Resolution of the hypotheses relating to the origin of *Chr. cubensis* is, however, obscured by the fact that isolates from the two regions form distinct phylogenetic groups (Myburg *et al.* 1999, 2002b, 2003). Another hypothesis based on this fact, states that *Chr. cubensis* could have a world-wide distribution with isolates occurring in Asia and South America (Seixas *et al.*

2004). Before studies on the origin of *Chr. cubensis* are pursued further, the exact relationship between the two phylogenetic groups within *Chr. cubensis* should, however, be clarified.

Chrysoporthe austroafricana (as *C. cubensis*) was first described as a pathogen on *Eucalyptus* in South Africa in 1989 (Wingfield *et al.* 1989). This fungus was assumed to have been introduced into South Africa from areas where the *Eucalyptus* canker pathogen was known to occur (Van Heerden & Wingfield 2001). The view that *Chr. austroafricana* was introduced into South Africa was based on a relatively low phenotypic diversity amongst isolates on *Eucalyptus* spp. (Van Heerden & Wingfield 2001). Van der Merwe (2000) has recently shown, using microsatellite markers, that despite a low genetic diversity, genetic recombination occurs among isolates of *Chr. austroafricana* on *Eucalyptus* in South Africa. Perithecia are seldom seen on *Eucalyptus* trees in South Africa and one possible explanation has been that genetic recombination in *Chr. austroafricana* occurs on hosts other than *Eucalyptus* (Conradie *et al.* 1990, Swart *et al.* 1991, Van der Merwe 2000). Recently this fungus was discovered on *T. granulosa* (Myburg *et al.* 2002a) and preliminary surveys have suggested that the fungus also occurs on native *Syzygium* spp. (Heath *et al.* 2002). The question of the host range and host of origin of *Chr. austroafricana* is currently being actively pursued.

Chrysoporthe represents one of the most important genera of tree pathogens. All three species recognized in this genus are highly pathogenic. *Chrysoporthe cubensis* is an important pathogen that infects *Eucalyptus* spp. and clove in tropical and sub-tropical areas of South America and South East Asia (Boerboom & Maas 1970, Hodges *et al.* 1976, 1979, Sharma *et al.* 1985). The fungus also occurs in Central Africa (Gibson 1981, Hodges *et al.* 1986, Myburg *et al.* 2003, Roux *et al.* 2003) and Hawaii (Hodges *et al.* 1979, Myburg *et al.* 2003), where it has probably been introduced. *Chrysoporthe austroafricana* is one of the most important pathogens of *Eucalyptus* in South Africa (Wingfield *et al.* 1989). This fungus also causes a serious canker disease on ornamental *Tibouchina* spp. (Myburg *et al.* 2002a). *Chrysoporthella hodgesiana* causes a serious stem canker disease of *Tibouchina* spp. in Colombia (Wingfield *et al.* 2001). Many of these fungi pose serious threats to *Myrtaceae* and *Melastomataceae* in countries where they do not currently occur (Wingfield 2003). In the past, they have been recognised under a single name, and their relative threat has certainly been underestimated. This situation serves as an example of the importance of in-depth taxonomic studies including DNA sequence comparisons that expose differences that cannot be discerned based on morphology alone.

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